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A Study on the Inheritance of Body Color and Chromatophores in the Guppy *Poecilia reticulata*

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Summary

There are a number of body colors and color patterns of the guppy, and several strains have been established for them. The chromatophores in the scales of this fish were examined, and three types of chromatophores were detected, melanophore, xanthophore and erythrophore.

Cross experiments observing the chromatophores revealed that alleles B and b controlled the formation of melanin in melanophores of non-albino types. The types carrying B possessed well developed melanophores with full melanin formation and those having the recessive b in the homozygote had small melanophores with little melanin formation. The autosomal allele i permited the suppression of melanin formation in melanophores in albino types and was epistatic to the B and b.

The autosomal alleles R and r controlled the deposition of the yellow carotenoid in xanthophores. The types carrying R possessed well developed xanthophores and in those having the recessive r in the homozygote we failed to detect xanthophores. The autosomal alleles E and e controlled the deposition of red carotenoids in erythrophores. The types carrying E possessed well developed erythrophores and in those having the recessive e we failed to detect the erythrophores. The autosomal allele Ex controlled the extent of erythrophore distribution and was epistatic to the E and e.

The guppy (*Poecilia reticulata*) is useful as a pilot fish for study of genetics and breeding, because of its short life cycle, capacity to reproduce and ease of breeding as an aquarium fish. Various breeds of the guppy, differing in body color types and color patterns as well as in the shape of fins, have been created by aquarists. Laboratory strains with varying size, fin shapes, body color and color patterns have been maintained and/or produced in this laboratory and genetically characterized at several allozyme loci (1-3).

Moreover, sexual dimorphism in which the males show striking color patterns can be used to study the mode of inheritance of sex-linked or autosomal genes (4).

They demonstrated that most of the genes responsible for the color patterns are concentrated in the sex chromosomes X and Y, and some of these genes are always located in the Y-chromosome. Mutations affecting all types of pigment cells (chromatophores); melanophores, erythrophores, xanthophores and as well as guanophores (iridocytes), have been described by Schroder (5). In the medaka, the inheritance of body colors has been reported by Aida (6), who determined phenotypically as follows; brown (wild type) *BR*; blue *Br*; orange red *bR*; white *br*. The alleles *B* and *b* control the formation of melanin in melanophores and are autosomal. The alleles *R* and *r* govern the deposition of carotinoid in xanthophores and are sex-linked.

In the present work, chromatophores of the scales were examined in several body color types of the male guppy and the inheritance of body color was demonstrated by cross experiment.

Materials and Methods

Animals

Nine guppy strains, A, D, D1, D2, F, R, T, T1 and Y were used in this work. Both sexes of each strain are represented in Fig. 1. They have been maintained in closed colony in 60 liter aquaria at a density of 300-500 individuals per aquarium depending on the average size of the strain. The fish are maintained at a temperature of $23\pm2^{\circ}C$ and fed with ground carp pellets twice a day; dried *Daphnia* is given as a supplementary diet. The history and development as well as body color and color patterns of nine strains are summarized as follows:

A—F_1 hybrid between King Cobra and Albino was purchased in 1991. Albino appeared in the F_2 generation at a ratio of 1 (albino) : 3 (non-albino) and these albino individuals were propagated as an albino strain. Both sexes have a pinkish-orange body and the body size was small. Males displayed an orange-red delta tail.

D—The large body size guppy, characterized by males displaying a metallic blue body with delta tail was purchased in 1991 as a commom breed name, Diamond.

D1—This strain was produced in this laboratory, by selecting from the offspring of the original Diamond, those that did not exhibit the typical metallic blue body. The males display a red mosaic red delta tail. The body color is grey in both sexes.

D2—This strain was also produced in this loboratory by selecting from the offspring of the original Diamond those that did not exhibit the typical metallic blue body. The males display a black delta tail. The body color is the light grey in both sexes.

F—The large body size guppy, characterized by a male displaying a flamingo-

red delta tail was purchased in 1982 as a common breed name, Flamingo. The body color is pale yellow in both sexes.

R—This strain was purchased in 1996 as a common breed name Real red-eye albino. The body color is white in both sexes. Males have a delta tail with no color and the body size is large in both sexes.

T—The large body size guppy, characterized by its black deltatail, was purchased in 1985, under a common breed name, Tuxedo. Males display the black color from the latter half of the body to the caudal fin and the females usually display a drab grey in the body with a black caudal fin.

T1—This strain was produced in this laboratory by selecting from the offspring of the original Tuxedo, those that did not exhibit the typical black tail but a pale yellow body and a light blue delta tail.

Y—This strain was purchased in 1995 as a common breed name Micarif Super Yellow. The large body size guppy was characterized by the male displaying a yellow body color and yellow delta tail and the females displaying a pale yellow.

Observation of chromatophores in the scales

The presence or absence, and the number of chromatophores were examined by observation of the scales which were picked from the left side of the male body. The scales picked from the body were enclosed with the Ringer's solution of Medaka (128.1 mM NaCl, 2.6 mM KCl, 1.8 mM $CaCl_2$ adjusted to pH 7.3 by $NaHCO_3$) (7). From the pictures of the scale, the chromatophores were counted and the number of chromatophores on the surface of the scales were caluclated per surface area. The average numbers of chromatophores per scale and per mm^2 of area on the scale were calculated. For distribution, chromatophores were also examined in the scales from the three longitudinal scale rows of the dosal midline, from the scales which contact the shoulder girdle to the scales on the posterior end of the hypurol bone in the male guppy.

Cross experiment

The cross experiment was done to elucidate the mode of inheritance for the body color and chromatophores. For the albino, the cross between T and A strains were used. For the pale yellow, the cross between T and T1 strains were used. For the light grey, the cross between T and D2 strains were used. For the red tail, the cross between F and T1 strains were used.

Results and Discussion

Detection of chromatophores in the scales

The chromatophores were examined in the scales from the males of nine

strains in the guppy. The melanophores, xanthophores and erythrophores responsible for body colors of this fish were observed as shown in Fig. 2. The formation of melanin in melanophores was observed in the non-albino type ; D, D1, D2, F, T, T1 and Y strains, and not observed in albino type ; A and R strains (Table 1). The deposition of yellow pigments in xanthophore was observed in all strains (Table 1). The deposition of yellow pigments in xanthophore was observed in all

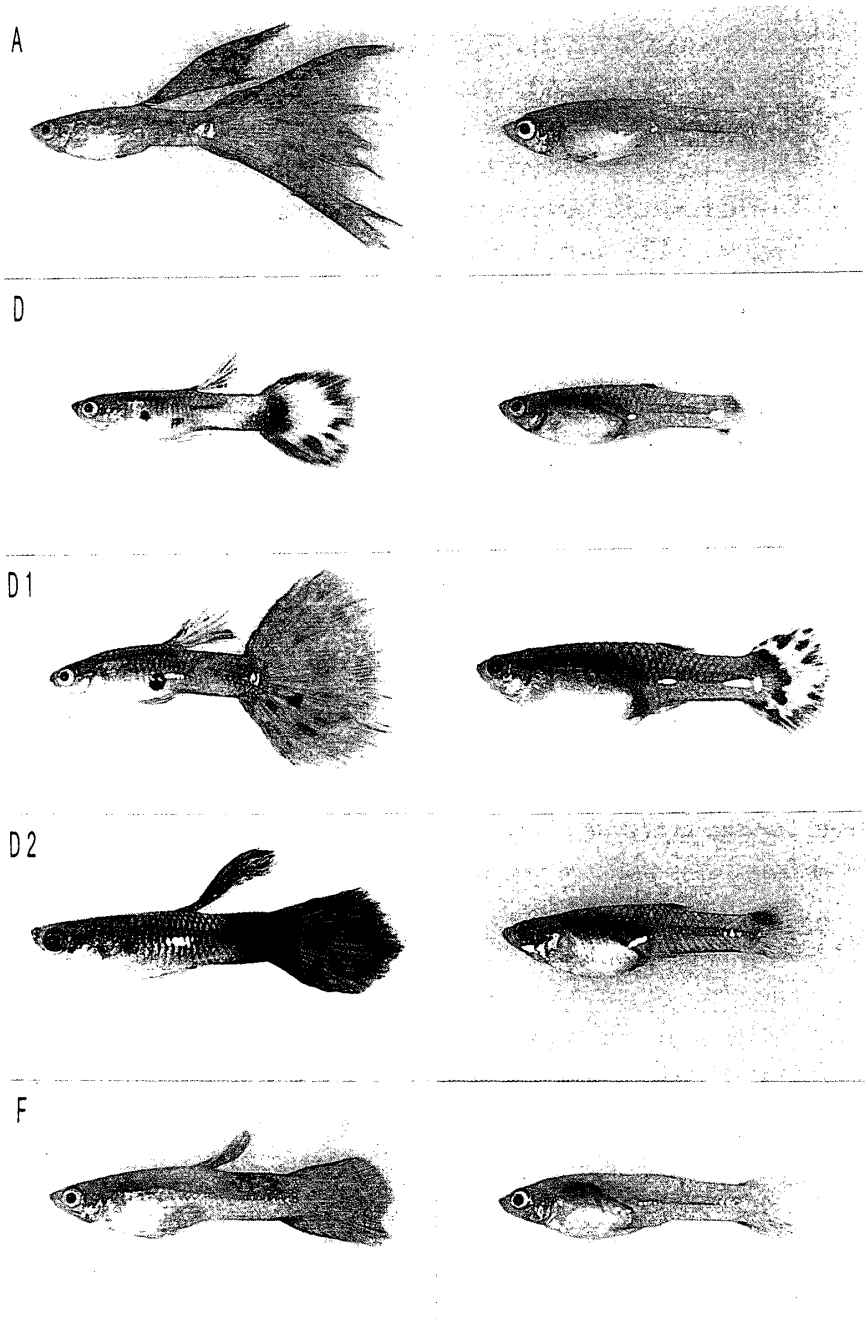
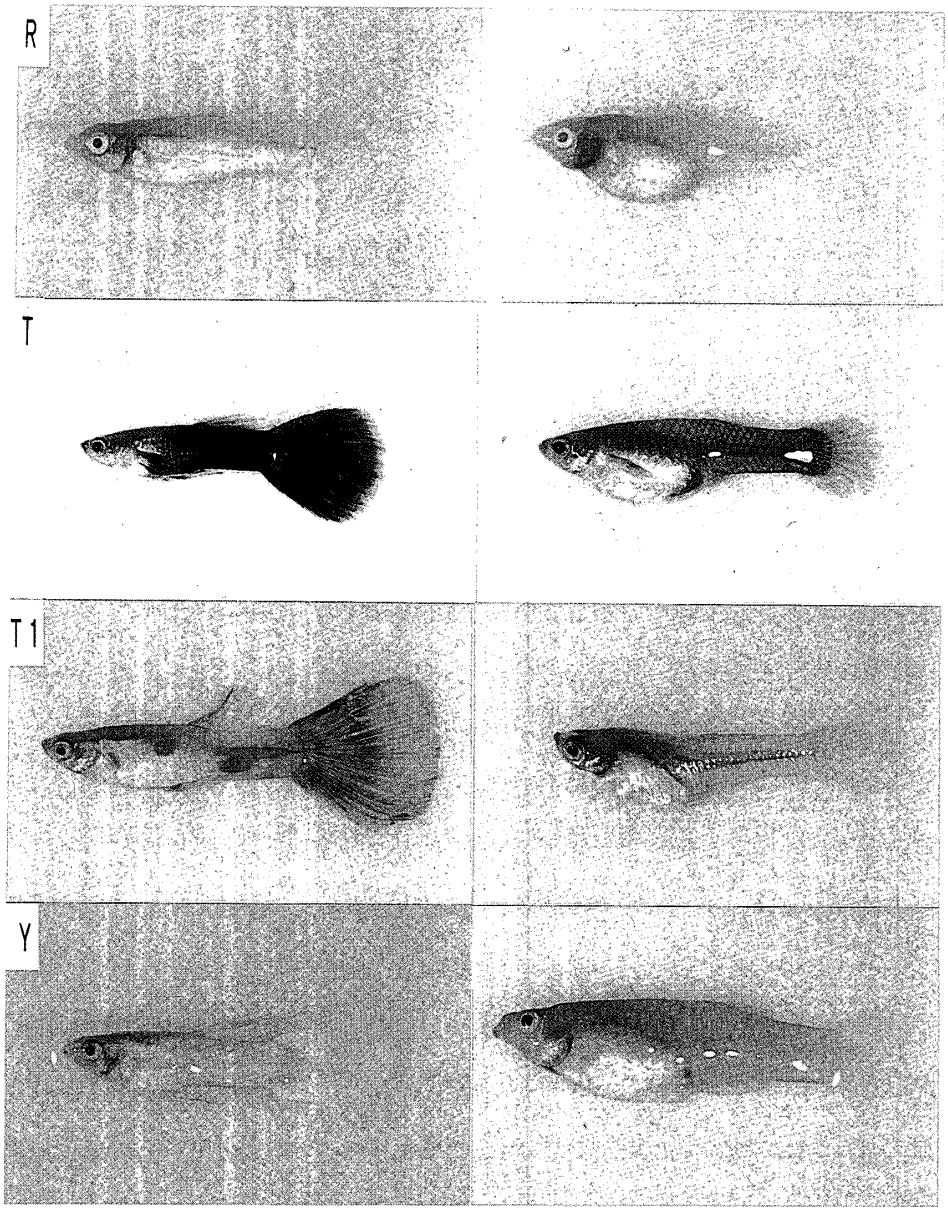


FIG. 1. Body color and color pattern in nine guppy strains.

strains except D2 and R strains. The deposition of red pigments in erythrophore was found in the red-tail type; A, D1, and F, but was not found in the non-red-tail type; D, D2, R, T, T1 and Y strains.

The F_1 of the albino A strain which mated with the non-albino T strain showed the presence of melanophores and the segregation of F_2 offspring was a typical 3 : 1 for the presence and absence of the melanophores, as shown in Table 2. Albino Medaka was produced by recessive autosomal gene, *i*. The albino Medaka have a melanotic melanophore and show almost no tyrosinase activity



Left side is males and right side is females.

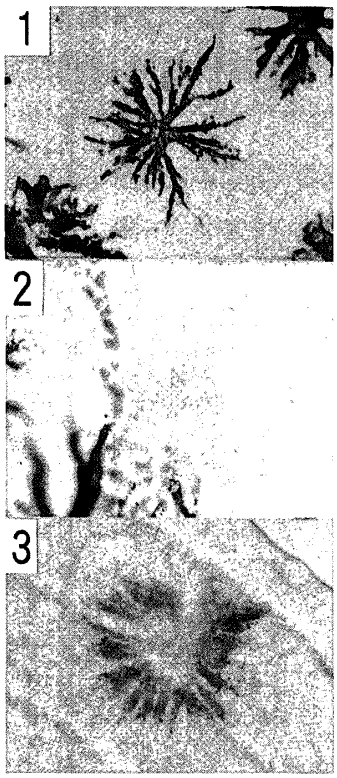


FIG. 2. The chromatophores observed in the scale of the guppy.
(1) Melanophore, (2) Xanthophore, (3) Erythrophore

TABLE 1. *Melanophores, xanthophores and erythrophores in scales from nine strain of the guppy*

Strain	Body color	Meranophores	Xanthophores	Erythrophores
A	Pinkish orange	−	+	+
D	Metalic blue	+	+	−
D1	Grey	+	+	+
D2	Light grey	+	−	−
F	Pale yellow	+	+	+
R	White	−	−	−
T	Black	+	+	−
T1	Pale yellow	+	+	−
Y	Yellow	+	+	+

+ : presence, − : absence

except for the expression of xanthophores (8, 9). The *i* gene is epistatic to the *B* and *b* in the production of melanin in the melanophores. Similar expression would be applicable to the guppy.

TABLE 2. *F_2 segregation of "albino" in the cross experiment*

Cross	Melanophores		Expected ratio of segregation	χ^2
	+ ($I-$)	− (ii)		
T×T	254	0	1 : 0	
A×A	0	188	0 : 1	
F_1 (T×A)	284	0	1 : 0	
F_1 (A×T)	357	0	1 : 0	
F_2 (T×A)	285	85	3 : 1	0.811
F_1 (T×A)×T	378	0	1 : 0	
F_1 (T×A)×A	154	146	1 : 1	0.213

The parentheses represent the presumed genotype

TABLE 3. *F_2 segregation of "pale yellow" in the cross experiment*

Cross	pale yellow		Expected ratio of segregation	χ^2
	non-pale yellow ($B-$)	pale yellow (bb)		
T×T	40	0	1 : 0	
T1×T1	0	102	0 : 1	
F_1 (T×T1)	146	0	1 : 0	
F_1 (T1×T)	20	0	1 : 0	
F_2 (T×T1)	176	58	3 : 1	0.006
F×F	0	106	0 : 1	
F_1 (F×T1)	0	207	0 : 1	
F_1 (T1×F)	0	240	0 : 1	
F_2 (F×T1F)	0	141	0 : 1	

The parentheses represent the presumed genotype

Gene for formation of melanin in melanophores

The formation of melanins in melanophore was observed in the non-albino type strains. However, the body color of F, T1 and Y strains exhibited a pale yellow color and melanophores were observed in these strains. From the cross experiment, the pale yellow was controlled by a recessive gene as shown in Table 3. The average number of melanophores were examined in T, D2, F, T1 and their F_1 hybrids (Table 4). There were no differences in the number of melanophores, but the melanophore size in F, T1 and F_1 (F×T1) was smaller than that in T, D2, F_1 (T×T1) and F_1 (T×D2) as shown in Fig. 3.

For the melanin formation in melanophores, the autosomal alleles B and b were considerables as demonstrated in medaka (6). The B gene which was dominant to the b, permitted full melanin formation in melanophores but the b

TABLE 4. *Average number of melanophore with standard error and size of melanophores in scales*

Strain	Number/scale	Numbers/mm^2	size
D2	69.4± 3.5	140.8±15.9	large
F	89.9±17.7	105.4±22.8	small
T	86.8±19.7	159.6±12.9	large
T1	83.9±13.1	158.4±11.7	small
F_1 (T×T1)	83.9± 6.6	138.7± 7.2	large
F_1 (F×T1)	98.2± 7.9	136.7±10.7	small
F_1 (T×D2)	79.4± 7.8	141.7±10.5	large

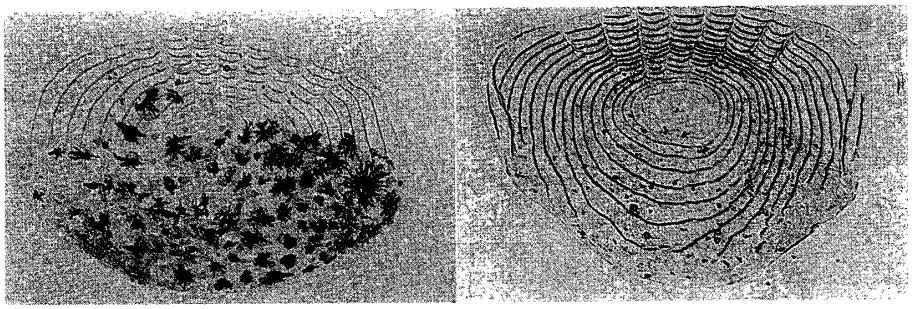


FIG. 3. Two types of melanophores observed in the scales of the guppy. Left side shows the large size of the melanophore in the T strain and the right side shows the small size.

gene caused incomplete melanin formation in melanophores. In the orange-red (*bR*) and white (*br*) Medaka, melanin formation was so scanty that melanophores were almost colorless in adults and the tyrosinase reaction was revealed in "colorless" melanophores (10).

Gene for deposition of yellow pigment in xanthophore

The D2 strain showed a scanty deposition of yellow carotinoid in xanthophores. The F_1 of the T strain which mated with the D2 strain showed the presence of xanthophores and the segregation of the F_2 offspring was a typical 3 : 1 for the presence and absence of xanthophores as shown in Table 5. The cross experiment demonstrated that the type carrying *R* gene possessed well developed xanthophores and those having the recessive *r* in the homozygote had xanthophores with no pigment. The average number of xanthophores were examined in T, D2, F_1 (T× T1) and F_1 (T×D2). The average numbers of xanthophores in F_1 (T×D2) was smaller as compared with that of others, suggesting the gene dosage (Table 6).

In Medaka, the gene *R* and *r* govern the deposition of carotenoid in xanthophores and are partially sex-linked (6). Both the X and Y chromosome have a locus for either *R* and *r*. The *r* gene in the domesticated medaka is usually

TABLE 5. *F_2 segregation of "light grey" in the cross experiment*

Cross	Body color		Expected ratio of segregation	χ^2
	non-light grey ($R-$)	light grey (rr)		
T×T	40	0	1 : 0	
D2×D2	0	23	0 : 1	
F_1 (T×D2)	135	0	1 : 0	
F_1 (D2×T)	0	4	0 : 1	
F_2 (T×D2)	65	16	3 : 1	1.189

The parentheses represent the presumed genotype

TABLE 6. *Average number of xanthophores with standard error of xanthophores in scales*

Strain	Number/scale	Numbers/mm²
D2	0.0±	0.0±
T	276.3±12.3	507.8±52.2
T1	293.0±58.1	533.3±62.5
F_1 (T×T1)	306.2±17.2	506.0±28.3
F_1 (T×D2)	197.7±13.2	352.4±27.2

restricted to the X chromosome while the *R* is carried by either X or Y. According to Kirpichnikov (11), however, the body color of the guppy is determined by two autosomal genes; *B* and *R*, and the F_2 segregation corresponds to the classical ratio 9 : 3 : 3 : 1 for grey (*BR*), blue (*Br*), pale (*bR*) and white (*br*).

Gene for deposition of red pigment in erythrophores

The presence of erythrophores was detected in the scales of D1, F, and A

TABLE 7. *F_2 segregation of "red tail" in the cross experiment*

Cross	Red tail		Expected ratio of segregation	χ^2
	+ ($Ex-/E-$)	− ($Ex-/ee$, $exex/E-$, $exex/ee$)		
F×F	106	0	1 : 0	
T1×T1	0	102	0 : 1	
F_1 (F×T1)	207	0	1 : 0	
F_1 (T1×F)	24	0	1 : 0	
F_2 (F×T1)	59	36	9 : 7	1.323

The parentheses represent the presumed genotype

strains, which have a red tail. The F_1 male of the F strain which mated with the T1 strain showed the presence of erythrophores. In the F_2 segregation, there were 59 males with red tail to 36 males with non-red tails with a ratio of approximately 9 : 7, as shown in Table 7. The segregation in the F_2 corresponding to the classical ratio of 9 : 3 : 3 : 1 was assumed for the two genes of the extension of erythrophores in distribution (*Ex*) and the deposition of red pigment in erythrophores (*E*). The F_2 segregation was phenotically four types ; *ExE*, *Exe*, *exE* and *exe*. The four types were classified into red tail (*ExE*) and non-red tail (*Exe*, *exE* and *exe*). These color patterns were expressed in adult males but not in adult females. However, the same color pattern was observed in teststerone treated females, suggesting that the two genes, *Ex* and *E* are autosomal.

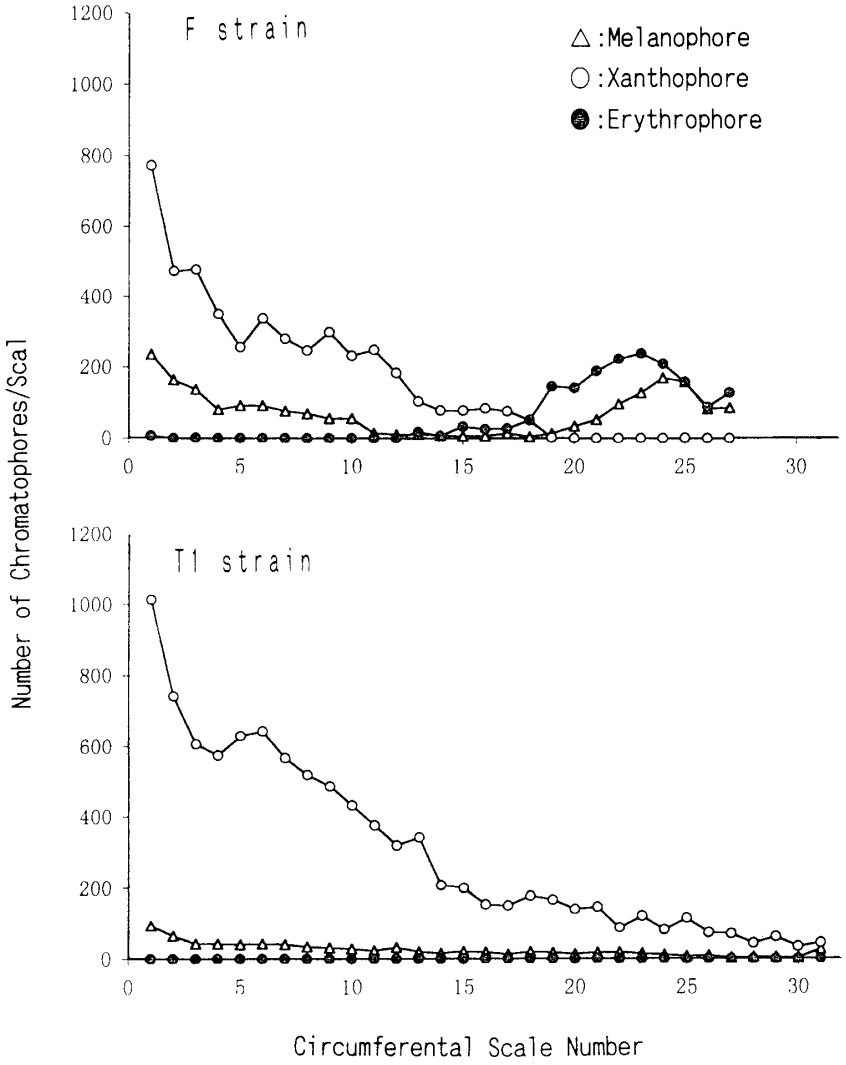


FIG. 4. The distribution of chromatophores per scale from the scales which contact the shoulder girdle to the scales on the posterior end of the hypural bone in F and T1 strain.

TABLE 8. *Presumed genotypes for body color in nine strians of the guppy*

strain	Genotype
A	*ii/BB/RR/ExEx/EE*
D	*II/BB/RR/exex/ee*
D1	*II/BB/RR/ExEx/EE*
D2	*II/BB/rr/exex/ee*
F	*II/bb/RR/ExEx/EE*
R	*ii/−−/rr/−−/ee*
T	*II/BB/RR/−−/ee*
T1	*II/bb/RR/exex/ee*
Y	*II/bb/RR/exex/ee*

The number of chromatophores per scale were counted on the scales which contact the shoulder girdle to the scales on the posterior hypurol bone in F and T1 males (Fig. 4). The xanthophores showed the most number in the scales which contact the shoulder girdle, however, the number decreased towad the tail in both of F and T1 males. The erythrophores were inversely less or not detected in the scales which contact the shoulder girdle but number increased towad the tail in the F male and the melanophores also increased with the increase of erythrophores. The relation of erythrophore and melanophore suggested that the *Ex* gene might be affects on the extension of erythrophore and melanophore in the distribution.

The genotypes of body color and color pattern in each strain of the guppy were summarized as shown in Table 8.

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