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Inheritance of Golden Coloration in the Zebrafish, *Danio rerio*

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Zebrafish (*Danio rerio*)의 체색 변이에 관한 유전 분석

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The study has been conducted in order to understand the inheritance of body color in the wild type zebrafish (zebra danio), *Danio rerio*, and its golden mutant (golden danio). The body color was also studied to determine the effect of golden coloration on the survival rate of zebrafish eggs and larvae up to 15 days after fertilization. Reciprocal monohybrid crosses between the wild and the golden type of zebrafish indicated that golden coloration was controlled by a single gene which had two alleles. Transmission of these alleles from parents to their progenies followed the principles of dominance and segregation based on Mendelian inheritance. Similar results from the reciprocal crosses implied that a locus for golden coloration was located on an autosomal chromosome. On the other hand, average survival rates from four different types of mating between, and within, zebra and golden danio suggested that golden coloration seemed to be associated with the survival rate of zebrafish, especially in its early embryonic stage. This indicated that homozygous recessive golden mutation was likely to weaken the golden danio's chance of survival.

Key words : Inheritance, Golden coloration, Zebrafish, *Danio rerio*

Introduction

Genetic analysis on observable and measurable phenotypes has been performed in order to discover the biological potential of various populations such as fish, domestic sheep, goats, horses and cattle. In some case it was performed to achieve improvement in productivity. Such phenotypic variation can be classified into two categories, qualitative and quantitative variances, which are known to be dependent on the number, and behavior, of the genes (Tave, 1986). The former can be dealt with by using Mendelian genetics ; the latter with quantitative genetics. Since the body color of fish is a qualitative characteristic and has an advantage of being easily identified by the naked eye, it has long been used to understand the underlining genetic mechanisms for coloration inheritance in various fish species such as the guppy (Gordon, 1953), rainbow trout (Bridges and Limbach, 1972), sailfin molly (Angus, 1983), eye-spot rasbora (Frankel, 1987) and three-spot gourami (Frankel, 1992). Regarding the genetics of pigmentation in the genus *Danio*, Frankel (1979) reported the inheritance of spotting in the leopard danio through a mating experiment between *Brachydanio* (=*Danio*) *nigrofasciatus* and *B. frankei*. Streisinger et al. (1986) have demonst-

rated that four unlinked gene loci (*gol-1, gol-2, alb-1 and spa-1*), operating through epistasis, affect the pigmentation of the zebrafish.

On the other hand, the segregation ratio of a certain coloration estimated from the progenies can be influenced either by environmental factors such as temperature (Angus, 1983) or by genetic factors which may be associated with the survival rate of individuals having such a coloration. Thus, if golden coloration was related to an individual weakness, such as a decrease in fertility or early survival rate of zebrafish as shown in the study of inbreeding depression (Mrakovcic and Haley, 1979), we may draw incorrect conclusions about segregation patterns of body color observed in the progenies. When looking at the study to determine the segregation ratios of body color in the wild and blond guppies, Gordon (1953) also mentioned the importance of noting the survival rates at the egg and larval stage.

Therefore, the present study has been conducted in order to understand the inheritance of body color through reciprocal monohybrid crosses and backcrosses between zebra danio (*Danio rerio*) and its "golden danio" (mutant). Also, the effect of golden coloration has been investigated in regard to the survival rates of the zebrafish eggs and larvae.

Materials and Methods

Zebra and golden danio were obtained from a local pet store in Champaign, Illinois. Zebra danios possess vertical metallic blue stripes on their body as well as on the caudal and ventral fins. Golden danios are morphologically the same as the zebra danios except for the fact they lack the black pigment cells (Fig. 1).

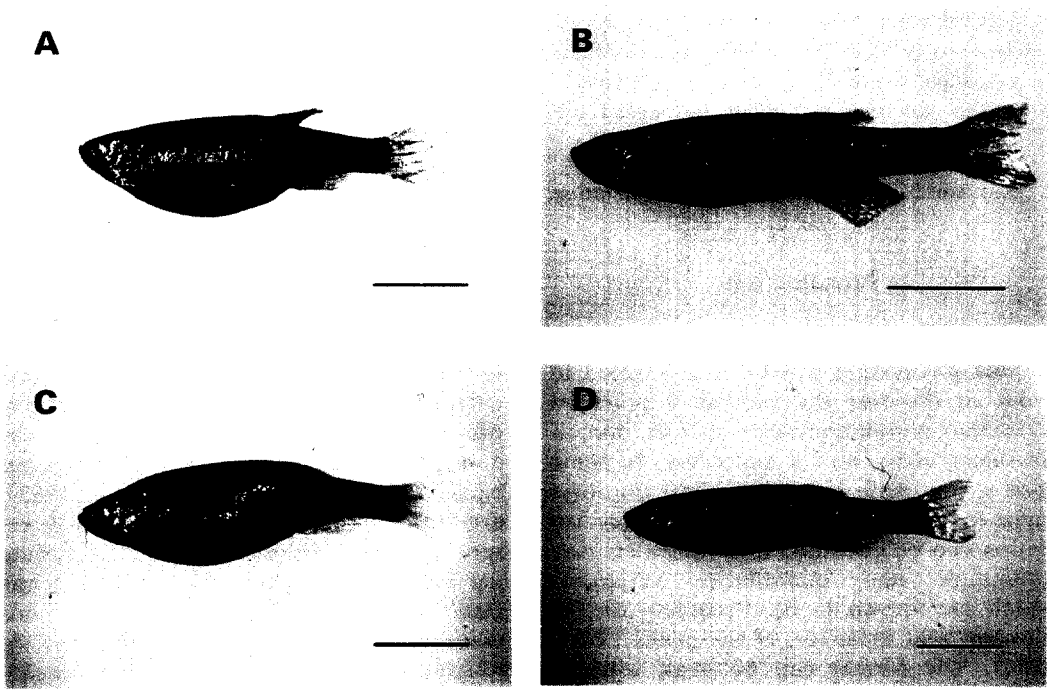

Fig. 1. The wild (A,B) and golden (C,D) types of zebrafish used in this study. A : Zebra danio female ; B : Zebra danio male ; C : Golden danio female ; D : Golden danio male. *Bar 1 cm*

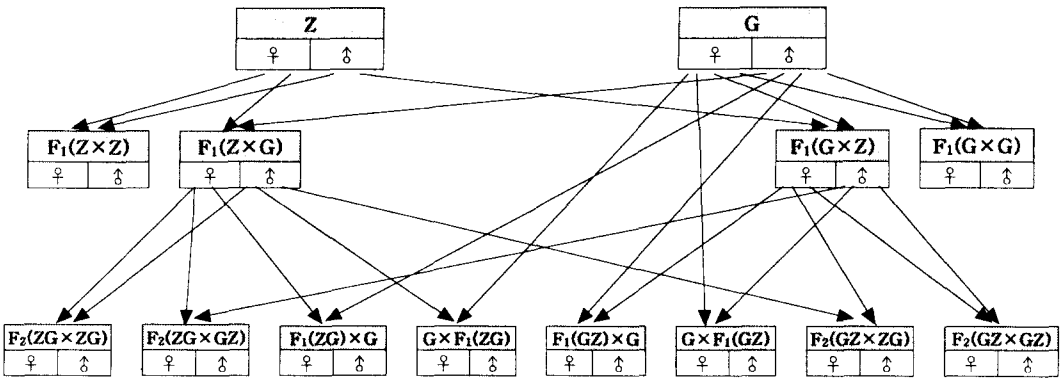

Fig. 2. A diagram of the mating program within, and between, zebra danios, golden danios, and its F_1 progenies. Z : Zebra danio ; G : Golden danio ; F_1(Z×Z) : F_1 progeny obtained from the mating of female and male zebra danio ; F_1(Z×G) : F_1 progeny of female zebra danio and male golden danio ; F_1(G×G) : F_1 progeny of female golden danio and male golden danio ; F_1(Z×G) : F_1 progeny of female zebra danio and male golden danio ; F_2(ZG×ZG) : F_2 progeny of F_1(Z×G) female and F_1(Z×G) male ; F_2(ZG×GZ) : F_2 progeny of F_1(Z×G) female and F_1(G×Z) male ; F_1(ZG)×G : Progeny of F_1(Z×G) female and golden danio male ; G×F_1(ZG) : Progeny of female golden danio and F_1(Z×G) male ; F_1(GZ)×G : Progeny of F_1(G×Z) female and male golden danio ; G×F_1(GZ) : Progeny of female golden danio and F_1(G×Z) male ; F_2(GZ×ZG) : F_2 progeny of F_1(G×Z) female and F_1(Z×G) male ; F_2(GZ×GZ) : F_2 progeny of F_1(G×Z) female and F_1(G×Z) male.

The maintenance and spawning of parental zebrafish and the nursing of their eggs and larvae were conducted according to the procedures suggested by Westfield (1989). The mating plan is shown in Fig. 2.

Single-pair and its reciprocal matings were set up within and between each type of danio using 10-l breeding tanks at 26-28℃. Sometimes pooled eggs collected from several matings were used to increase the number of specimens for each replicate. When the male zebrafish actively followed the female, both of them were anesthetized with 5% MS-222 in order to collect the sperm and eggs from their gonads. This artificial insemination procedure was performed with the aid of Hank's solution. One minute after fertilization, the eggs were washed with distilled water and then incubated at 28℃ in petri-dishes containing egg water which consisted of 100 ppm methylenblue, distilled water and seawater (1 : 1 : 18). After hatching, the free-swimming larvae were provided with artificial feed (250 μl in grain size) from day 2 to day 12, and then brine shrimp were added until day 15. After this, only brine shrimp was used as feed until the fries were old enough to take a larger size of artificial feed. The difference between survival rates was tested using Duncan's multiple range test (Duncan, 1955), while the difference between the expected and observed segregation ratio was done again using the χ^2-test.

Results

The types of reciprocal crosses between parental zebra and golden danio, as well as the mating results and probable genotypes are shown in Table 1. All progenies from the mating of the female and male zebra danio were the wild type, while all progenies of the parental golden danios were the golden type. Reciprocal crosses between parental zebra and golden danio produced all wild type offspring showing the zebra danio phenotype. Each mating was performed two times.

putative parental genotypes, phenotypic frequency of progeny and their observed and expected ratio. Mating F_1(ZG)×F_1(ZG)

Table 1. Results of progeny tests from matings between zebra and golden danio showing putative parental genotypes and phenotypic frequency of progeny

Cross (♀×♂)	Trial no.	Putative parental genotype		Phenotypic frequency of progeny	
		Female	Male	Wild type	Golden type
Z×Z	1	GG	GG	391	0
	2	GG	GG	266	0
Z×G	1	GG	gg	461	0
	2	GG	gg	296	0
G×Z	1	gg	GG	418	0
	2	gg	GG	199	0
G×G	1	gg	gg	0	231
	2	gg	gg	0	255

Z : Parental zebra danio ; G : Parental golden danio.

Table 2. Results of progeny tests from matings between F_1 progenies showing putative parental genotypes, phenotypic frequency of progeny, and their observed and expected ratio

Cross (♀×♂)	Trial no.	Putative parental genotype		Phenotypic frequency of progeny		Observed ratio	Expected ratio	*P*
		Female	Male	Wild type	Golden type			
$F_1(ZG)\times F_1(ZG)$	1	Gg	Gg	1115	407	2.74 : 1	3 : 1	0.25−0.10
	2	Gg	Gg	245	77	3.18 : 1		0.75−0.50
	3	Gg	Gg	1022	353	2.90 : 1		0.75−0.50
	Total			2832	837	2.85 : 1		0.25−0.10
$F_1(GZ)\times F_1(GZ)$	1	Gg	Gg	946	351	2.70 : 1	3 : 1	0.50−0.10
	2	Gg	Gg	1265	384	3.30 : 1		0.50−0.10
	3	Gg	Gg	769	258	2.98 : 1		0.50−0.10
	Total			3062	1019	2.99 : 1		0.50−0.10
$F_1(GZ)\times F_1(ZG)$	1	Gg	Gg	999	420	2.38 : 1***	3 : 1	<0.001
	2	Gg	Gg	621	172	3.60 : 1*		0.05−0.025
	3	Gg	Gg	764	243	3.14 : 1		0.75−0.70
	Total			2384	835	2.90 : 1		0.50−0.25
$F_1(ZG)\times F_1(GZ)$	1	Gg	Gg	347	142	2.63 : 1	3 : 1	0.05−0.10
	2	Gg	Gg	862	205	4.03 : 1***		<0.001
	3	Gg	Gg	1081	404	2.68 : 1*		0.05−0.025
	Total			2254	404	3.04 : 1		0.75−0.50

$F_1(ZG)$: F_1 progeny from the mating of female zebra danio and male golden danio ; $F_1(GZ)$: F_1 progeny of female golden danio and male zebra danio.
* : $P<0.05$; *** : $P<0.001$.

Table 2 shows the results of progeny tests from matings between F_1 progenies showing gave offspring showing two phenotypes, zebra danio (wild type) and golden danio (golden type) in the ratios of 2.74 : 1, 3.18 : 1, 2.90 : 1. Those ratios were not significantly different from the expected ratio of 3 : 1 ($p>0.05$). In the mating of $F_1(GZ)\times F_1(GZ)$, the observed segregation ratios were 2.70 : 1, 3.30 : 1, 2.98 : 1, which were not different from the expected ratio of 3 : 1 ($p>0.05$). On the other hand, two out of the three replicate matings of $F_1(ZG)\times F_1(GZ)$ and $F_1(GZ)\times F_1(ZG)$ gave rise to the significantly different observed segregation ratios from the expected ratio of 3 : 1 ($p<0.05$).

Progeny tests between each type of parental zebrafish and its F_1 generations were performed to confirm segregation patterns of gol-

Table 3. Results of progeny tests from the reciprocal backcrosses showing putative parental genotypes, phenotypic frequency, observed ratios, and its probability of fit against expected ratios

Cross (♀×♂)	Trial no.	Putative parental genotype		Phenotypic frequency of progeny		Observed ratio	Expected ratio	*P*
		Female	Male	Wild type	Golden type			
F_1(ZG)×G	1	Gg	gg	760	569	1.34 : 1***	1 : 1	<0.001
	2	Gg	gg	610	531	1.15 : 1*		0.025−0.010
	3	Gg	gg	444	318	1.40 : 1***		<0.001
	Total			1814	1418	1.28 : 1***		<0.001
G×F_1(ZG)	1	gg	Gg	415	419	0.99 : 1	1 : 1	0.95−0.90
	2	gg	Gg	528	590	0.89 : 1		0.10−0.05
	3	gg	Gg	693	569	1.22 : 1***		<0.001
	Total			1636	1578	1.04 : 1		0.50−0.25
F_1(GZ)×G	1	Gg	gg	411	379	1.08 : 1	1 : 1	0.25−0.10
	2	Gg	gg	402	430	0.93 : 1		0.50−0.25
	3	Gg	gg	566	477	1.19 : 1**		0.01−0.001
	4	Gg	gg	268	283	0.95 : 1		0.75−0.50
	Total			1647	1569	1.05 : 1		0.25−0.10
G×F_1(GZ)	1	gg	Gg	555	493	1.13 : 1	1 : 1	0.10−0.05
	2	gg	Gg	542	411	1.32 : 1***		<0.001
	3	gg	Gg	583	571	1.02 : 1		0.75−0.50
	Total			1680	1475	1.14 : 1***		<0.001

* : $P<0.05$; ** : $P<0.01$; *** : $P<0.001$.

Table 4. Results of progeny tests from matings between F_1 progenies and reciprocal backcrosses showing putative genotypes, phenotypic frequency of progeny, and their observed and expected ratio parental during embryonic stage

Cross (♀×♂)	Trial no.	Putative parental genotype		Phenotypic frequency of progeny		Observed ratio	Expected ratio	*P*
		Female	Male	Wild type	Golden type			
F_1(ZG)×F_1(GZ)	1	Gg	Gg	189	59	3.20 : 1	3 : 1	0.75−0.50
	2	Gg	Gg	216	67	3.22 : 1	3 : 1	0.75−0.50
F_1(GZ)×F_1(ZG)	1	Gg	Gg	192	57	3.37 : 1	3 : 1	0.75−0.50
	2	Gg	Gg	75	21	3.57 : 1	3 : 1	0.50−0.25
F_1(ZG)×G	1	Gg	gg	172	175	0.98 : 1	1 : 1	0.975−0.95
	2	Gg	gg	237	251	0.94 : 1	1 : 1	0.75−0.50
F_1(GZ)×G	1	Gg	gg	194	179	1.08 : 1	1 : 1	0.50−0.25
	2	Gg	gg	212	196	1.08 : 1	1 : 1	0.50−0.25

den coloration (Table 3). Test cross F_1(GZ)×G produced progenies having two phenotypes, zebra danio (wild type) and golden danio (golden type) in the ratios of 1.08 : 1, 0.93 : 1, 1.19 : 1, and 0.95 : 1, which were not significantly deviant from the expected ratio of 1 : 1 ($p>0.05$). The mating results from F_1(ZG)×G turned out to have ratios of 1.40 : 1, 1.34 : 1, 1.15 : 1, which were all different from the expected ratio of 1 : 1 ($p<0.05$). One out of the three replicate matings of G×F_1(ZG) was different from 1 : 1 ($p<0.05$), and the same result was observed in G×F_1(GZ).

Most of the deviations from the expected segregation ratios occurred due to the relatively high frequency of wild type zebrafish caused by the differential survival rates between the wild and golden type. Thus, each body color type was determined at an early

Table 5. Average survival rates (%) of progenies from the cross (♀×♂) within, and between, zebra and golden danio for the first 15 days after natural and artificial fertilization

Days after fertilization	Matings by natural fertilization				Matings by artificial fertilization			
	Z×Z[a]	Z×G[b]	G×Z[b]	G×G[c]	Z×Z[a]	Z×G[a]	G×Z[b]	G×G[b]
1	95.1± 2.2	90.4± 4.6	93.9± 1.5	91.7± 2.2	90.0± 1.7	91.9± 1.8	91.3± 2.6	87.6± 2.7
2	92.2± 3.3	88.7± 3.9	87.6± 3.2	84.0± 6.2	88.8± 2.7	88.2± 4.9	86.5± 1.7	85.2± 2.3
3	90.6± 3.8	87.2± 3.6	85.1± 2.9	80.5± 6.4	88.7± 2.8	87.0± 5.4	84.0± 3.4	82.6± 3.5
4	89.4± 4.3	86.3± 3.8	84.5± 3.4	78.6± 6.4	87.9± 2.9	86.4± 5.6	83.3± 3.0	82.6± 3.5
5	88.0± 5.3	85.2± 4.7	83.7± 3.6	78.2± 6.4	87.9± 2.9	86.3± 5.5	82.0± 2.9	80.3± 4.1
6	87.9± 5.1	84.8± 5.0	82.7± 4.0	77.0± 6.7	87.5± 2.6	84.9± 4.9	81.4± 2.6	78.4± 2.9
7	87.7± 5.0	84.2± 5.1	82.6± 3.9	76.3± 6.1	86.8± 2.2	84.9± 4.9	80.4± 2.2	76.0± 2.5
8	87.7± 5.0	83.2± 5.4	82.0± 4.3	75.6± 5.7	86.8± 2.1	84.9± 4.9	78.5± 2.5	76.0± 2.5
9	86.2± 5.4	83.2± 5.4	81.3± 3.9	75.6± 5.7	86.2± 3.3	84.8± 5.0	78.0± 2.7	75.8± 2.7
10	85.7± 5.4	82.4± 5.5	79.4± 3.5	75.0± 6.9	86.2± 2.6	84.0± 5.1	77.7± 2.9	73.2± 2.4
11	85.4± 5.2	82.4± 5.5	78.3± 2.8	74.2± 6.2	85.9± 2.8	84.0± 5.1	77.2± 2.9	72.5± 2.6
12	85.4± 5.2	82.0± 5.6	78.2± 3.1	73.8± 5.9	85.7± 2.8	82.8± 5.8	75.9± 4.0	72.5± 2.6
13	83.8± 6.7	81.6± 6.1	77.3± 2.4	73.1± 6.4	85.7± 2.4	82.6± 6.0	74.3± 4.8	71.8± 2.4
14	83.8± 6.7	81.0± 5.9	76.9± 2.2	72.8± 6.0	85.5± 2.6	81.6± 5.3	74.1± 5.1	71.8± 2.4
15	83.8± 6.7	81.0± 5.9	76.9± 2.2	71.8± 5.1	85.5± 2.6	81.6± 5.3	73.7± 5.1	71.4± 2.9

Each survival rate (mean± SD) was calculated based on 6 replicates ; the columns that have the same superscript in each fertilization type were not significantly different from each other ($p>0.05$).

embryonic stage, normally 48 hours after fertilization when the melanophore could be discerned. As shown in Table 4, both matings, F_1(ZG)×F_1(GZ) and F_1(GZ)×F_1(ZG), gave rise to the segregation ratios which were not different from the expected ratio of 3 : 1 ($p>0.05$). The results from test crosses, F_1(ZG)×G and F_1(GZ)×G, turned out not to be different from the expected ratio of 1 : 1 ($p>0.05$).

To know and prove the effect of golden coloration on the survival rates of the zebrafish eggs and larvae, 6 replicate experiments had been carried out for the first 15 days after fertilization. Average survival rates, estimated from the crosses between the zebra and golden danio, seemed to be reduced due to the mutational effect caused by golden coloration (Table 5). With natural fertilization, the survival rate for the cross between golden danios (G×G) were 71.8± 5.1 %, 76.9± 2.2% between golden female and wild type male (G×Z), 81.0± 5.9% between wild type female and golden male (Z×G), and 83.8± 6.7% between wild type zebrafish (Z×Z). With artificial fertilization, the survival rate for the cross between golden danios (G×G) were 71.4± 2.9%, 73.7± 5.1% between G×Z, 81.6± 5.3% between Z×G, and 85.5± 2.6% between Z×Z. When survival rates were compared with each other, there were no statistically significant difference between natural and artificial fertilization ($p>0.05$).

Discussion

Since many fish species are appreciated for their beauty or as food, a higher commercial value may be gained if they have more attractive colors and patterns. Also slightly modified color patterns may be convenient for artificial marking, which can help us easily distinguish artificially released or cultured fish from their natural populations. We can therefore manage these populations more appropriately (Fujii, 1993). In addition, fish coloration can be used to indicate chromosomally manipulated fish such as the triploid rainbow trout (Thorgaard et al., 1995).

Like other vertebrates, fish coloration is governed by three primary types of chroma-

tophores all of which are neural crest derivatives : i.e., melanophores which are black or brown ; xanthophores or lipophores which are yellow ; and guanophores or iridophores which are silver in color. Various combinations and arrangements of these pigmented cells create the pigment patterns (Lehman and Youngs, 1959). In regard to the golden coloration in fish, Golden (1953) mentioned that the golden mutant reduced the number of melanophores by about fifty percent and this loss of black pigment cells uncovered and revealed the yellow pigment which were always present in the skin of the wild gray guppy. When dealing with Professor H. B. Goodrich's data on the inheritance of golden coloration in guppies, Golden (1953) seemed to be convinced that golden coloration was inherited through a simple recessive Mendelian factor. Although a similar result is expected, no study on the inheritance of golden coloration in zebrafish has been attempted so far.

In this breeding experiment, reciprocal monohybrid crosses between the wild and the golden type of zebrafish indicated that body color was controlled by a single gene which had two alleles designated by G and g. The result also indicated that the golden danio was a recessive homozygous mutant (gg). Despite some deviated segregation ratios that were observed and counted with approximately 30-day old fish, the transmission of these alleles from parents to their progenies followed the principle of dominance and segregation based on Mendelian inheritance. Almost the same results were found from the reciprocal crosses which implied that a locus for golden coloration was located on an autosomal chromosome. Concerning the inheritance of a spotting pattern, an investigation with the teleosts *Brachydanio nigrofasciatus* (leopard danio) and *B. frankei* (spotted danio) revealed that the leopard and spotted phenotypes were inherited by way of single-factor inheritance, showing complete dominance of the leopard type (Frankel, 1979). Tan and Phang (1995) also reported that the inheritance of pigmentation pattern from *Brachydanio rerio*, the zebra danio and the leopard danio, was determined by a single autosomal locus, with the gene for striped pigmentation pattern, S, being dominant to the spotted allele, s. These results on body pigmentation were very similar to those found in the inheritance pattern of golden coloration.

Golden (1953) pointed out that the actual data sometimes deviated widely from the expected result due to a weakness in guppies, particularly embryos, having a certain phenotype such as homozygous recessive albinism. In this study, since most of the deviations from the expected segregation ratios occurred due to the relatively higher frequency of wild type zebrafish than the expected, we suspected that the difference in the survival rates between the wild and golden zebrafish would produce such deviations. When the four different mating types were compared to each other, the average survival rate was ranked from highest to lowest : $Z\times Z > Z\times G > G\times Z > G\times G$. This indicated that homozygous recessive golden mutant would weaken the zebrafish during the early embryonic stages. Thus, each type of body color was determined at the early embryonic stage, normally 48 hours after fertilization when the melanophore could be discerned. As a result, the matings, $F_1(ZG)\times F_1(GZ)$, $F_1(GZ)\times F_1(ZG)$, $F_1(ZG)\times G$ and $F_1(GZ)\times G$, did not deviate from the expected segregation ratios this time (for more detail, see Table 5). These results strongly supported our understanding that the weakness of the zebrafish was effected by homozygous recessive golden coloration.

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