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Transparent-Scaled Variant of the Rosy Bitterling, *Rhodeus ocellatus ocellatus* (Teleostei: Cyprinidae)

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ABSTRACT—Transparent-scaled variant (TSV) of the rosy bitterling *Rhodeus ocellatus ocellatus* (Kner) was observed on both morphology and heredity. Compared with the normal-scaled type (NST), TSV is characterized by the blackish coloration in both eyes and peritoneum, and the luminescent one over the whole body. Histologically, the density of guanophores containing reflecting platelets was conspicuously low, especially in scale, iris, choroid and peritoneum, while the increase in the number of goblet cells (mucous cells) was recognized all over the dermal/epidermal regions. The heredity of TSV was recessive and supposed to be controlled by a single pair of genes unrelated to sex, judging from the result of crossbreeding experiments between NST and TSV. In growth and reproduction, no difference was seen between these two phenotypes. Transparent-scaled variant of the rosy bitterling can be competent for a genetic marker in experimental and developmental biology.

INTRODUCTION

The rosy bitterling *Rhodeus ocellatus ocellatus* is one of the bitterling forms, widely distributed in the temperate regions of Euracia (Nichols, 1943; Okada, 1960). Originally it was not distributed in Japan, but its subspecies *R. o. kurumeus*, endemic to Japan, was distributed. However, *R. o. ocellatus* was accidentally introduced into Japan from China during the World War II, contaminated in the seedlings of the grass carp *Ctenopharyngodon idellus* and the silver carp *Hypophthalmichthys molitrix* (Nakamura, 1955). In consequence of expulsion of *R. o. kurumeus*, *R. o. ocellatus* inhabits many small lakes, ponds and creeks all over Japan (Nagata *et al*., 1996)

Rhodeus o. ocellatus is a colorful ornamental fish, which is excellent in growth and reproduction, in addition to the less susceptibility to disease. Owing to the extensive distribution in Japan, it is easily obtainable in field. As an experimental material in fishes, the medaka *Oryzias latipes* and the zebradanio *Brachydanio rerio* are well-known and used widely in biological study. These fishes are excellent especially in growth and reproduction. Contrarily *R. o. ocellatus* has an inherent and great property in reproduction, lacking in these fishes. It is easily reproduced by artificial insemination, and ovulation timing in female is exactly presumed by the length of ovipositor (Ohta, 1982). The developmental and morphological features in *R. o. ocellatus* were described in detail by Nakamura (1969). *Rhodeus o. ocellatus* seems suited not only for appreciation but also for laboratory research, especially in developmental biology (Ueda *et al*., 1990; Kawamura, 1991).

A color variant of *R. o. ocellatus* was found in a small pond in the northern part of Osaka, Japan. The color variant was easily discriminated from the normal color type in appearance. Color variants have often been found in many cyprinid fishes. However, the morphological trait and the hereditary basis have not been well studied except for some ornamental fishes, e.g. the goldfish *Carassius auratus* (Kajishima, 1977) and *O. latipes* (Iwamatsu, 1993). In this paper, the morphological and hereditary traits in the color variant of *R. o. ocellatus* were examined and the possibility of its utility in biological study was also discussed.

MATERIALS AND METHODS

Color variant of *R. o. ocellatus* used in this study (Fig. 1), was sampled in a small pond adjacent to the Expo '70 commemoration park in Suita City, located in the northern part of Osaka, Japan. Matsui (1934) called the same color variant in the goldfish “transparent-scaled”, based upon its feature in appearance. Then, the color variant of *R. o. ocellatus* was termed transparent-scaled variant (TSV), contrary to the normal-scaled type (NST) in this paper.

For the clarification of the morphological trait in TSV, histological observation was carried out over the whole body using NST and TSV, both of which were in the same clutch and at the same age of three months (young). Samples were fixed in Bouins solution. Each body

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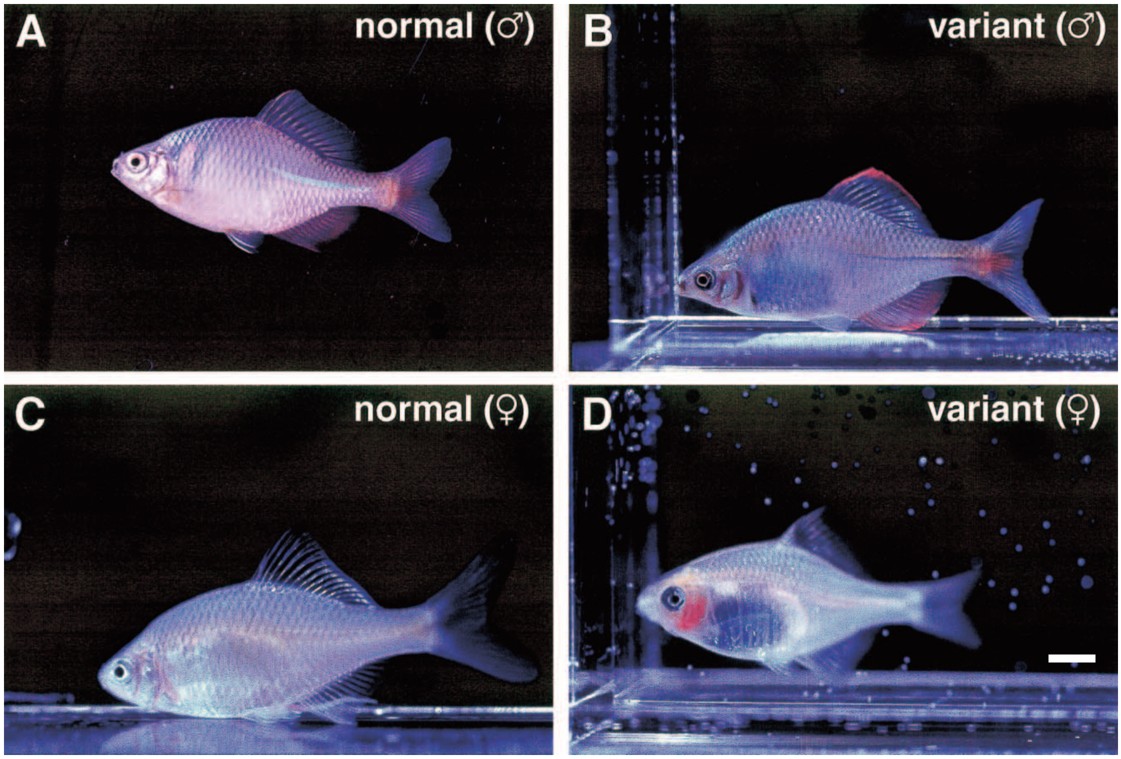

Fig. 1. Color variant of *Rhodeus ocellatus ocellatus*. Normal-scaled type (NST) (**A**, **C**) and transparent-scaled variant (TSV) (**B**, **D**). Scale bar indicates 10 mm.

part was dehydrated, embedded in wax, and sectioned to a thickness of 7 µm. The sections were stained with hematoxylin and eosin. Chromatophores in scale were observed by optical microscopy after dipping scales in 0.1 M NaCl or 0.1 M KCl for a few minutes, according to Ohta (1983). The heredity of TSV was inferred by the crossbreeding between NST and TSV, which was composed of the following five experiments: 1) TSV × TSV, 2) NST × TSV, 3) F_1 × NST, 4) F_1 × TSV and 5) F_1 × F_1 (Fig. 2). F_1 were all offspring between NST (♂) and TSV (♀). The rearing experiments were performed in fish tanks (45 cm in width × 30 cm in depth × 30 cm in height) under the conditions at the water temperature of 21-24°C and the photoperiod of 14:10 hr LD cycle. Survival rate, sex ratio and phenotype in offspring were examined once a week.

RESULTS

Field observation

Transparent-scaled variant of *R. o. ocellatus* was sampled together with NST and the Japanese minnow *Pseudorasbora parva* with fish net. They inhabited a small pond, which was 20 m and 2 m at the largest diameter and depth. Approximately, the appearance rate of TSV was below 2% in the captured samples of *R. o. ocellatus*. Transparent-scaled variant was easily distinguished from NST, based upon the difference in external coloration. The intermediate phenotype between the two color was not recognized at all.

Morphology

In appearance, TSV was primarily characterized by its specific coloration (Fig. 1). The body surface was so luminescent that the branchial arches and the air bladder could be clearly seen without dissection. In matured male, nuptial coloration was prominent, especially the reddish color on the unpaired fins. Contrary to the luminescence of the body surface, the coloration on the orbital and abdominal regions was conspicuous in black. Concerning the condition of the body surface, TSV was softer and slimier than NST. In behavior, TSV seemed somewhat slower and timider with the tendency of settlement on the bottom.

Histological traits of TSV were recognized in the orbital, abdominal, dermal/epidermal regions and scale.

Orbital region: In NST, iris and choroid, which were silvery in color and easily distinguished from the neighboring connective tissues, were developed between the inner retina and the outer cartilaginous sclera (Fig. 3A). In TSV, iris was hardly recognized, and the development of choroid was remarkably poor (Fig. 3B).

Abdominal region: In the transverse section of the abdominal region in NST, the peritoneum in black color was lined between the abdominal cavity and the musculature (Fig. 3C). In TSV, the thickness of peritoneum was thinner than that of NST (Fig. 3D).

Dermal/epidermal regions: Both the two phenotypes had goblet cells, secreting mucus over the whole body. In TSV, the density and the size of goblet cell were much higher and larger than those in NST (Fig. 3E, F).

Scale: Four kinds of chromatophores (melanophore, xanthophore, erythrophore and guanophore), as known in teleostei, were recognized in both the two phenotypes. The density of guanophore in TSV is much lower than that in NST

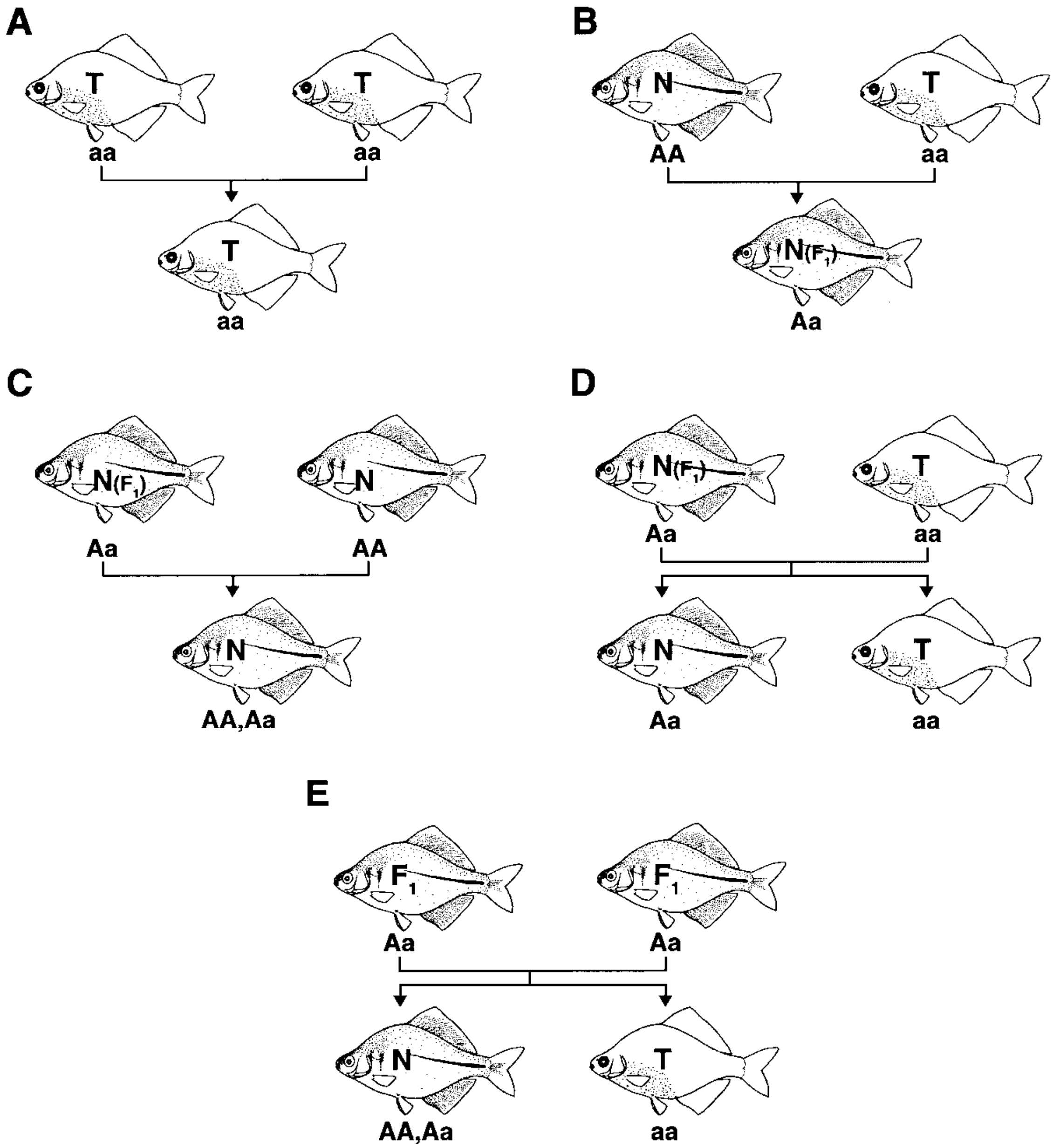

Fig. 2. Schematic illustration of crossbreeding between NST and TSV of *R. o. ocellatus*. (**A**) TSV × TSV, (**B**) NST × TSV, (**C**) F_1 × NST, (**D**) F_1 × TSV and (**E**) F_1 × F_1. T, TSV; N, NST; F_1, offspring between NST (♂) and TSV (♀). "A" means a gene determinant to NST, while "a" to TSV.

(Fig. 3G, H).

Hereditary examination

The results in the crossbreeding between NST and TSV are as follows (Table 1). The discrimination in both phenotype and sex was possible only in appearance at 100th day after hatching, when all reared fish reached maturation. In all crossbreeding, no intermediate phenotype between the two was seen at all.

TSV × TSV: Offspring were all TSV, the same as their parents, not containing even a single NST in four experiments (Table 1A). Hatchability was high and ranged from 66.7% (a-2) to 100.0% (a-3) (84.0 ± 15.8%, Mean ± SD). The survival rate was 45.3 ±16.7% (Mean ± SD). The sex ratio in offspring was almost 1:1 (♂:♀) except for a-3 (only two females). The low survival rate in a-1 and 2 was owing to a white spot disease, caused by protozoon *Ichthyophthirius*.

NST × TSV: Offspring were all NST in the reciprocal crossbreeding (Table 1B). Hatchability was high (79.1 ± 25.4%, Mean ± SD), independent of the phenotype of parents, except for b_1-4 (25%). The survival rate at 100th day was 49.0 ± 17.6% (Mean ± SD). The sex ratio in offspring was about 1:1 (♂:♀) in almost all experiments, except for b_1-2 (only three females).

F_1 × NST: Offspring were all NST (Table 1C). TSV did not appear in the least. Hatchability ranged from 63.3% (c-1) to

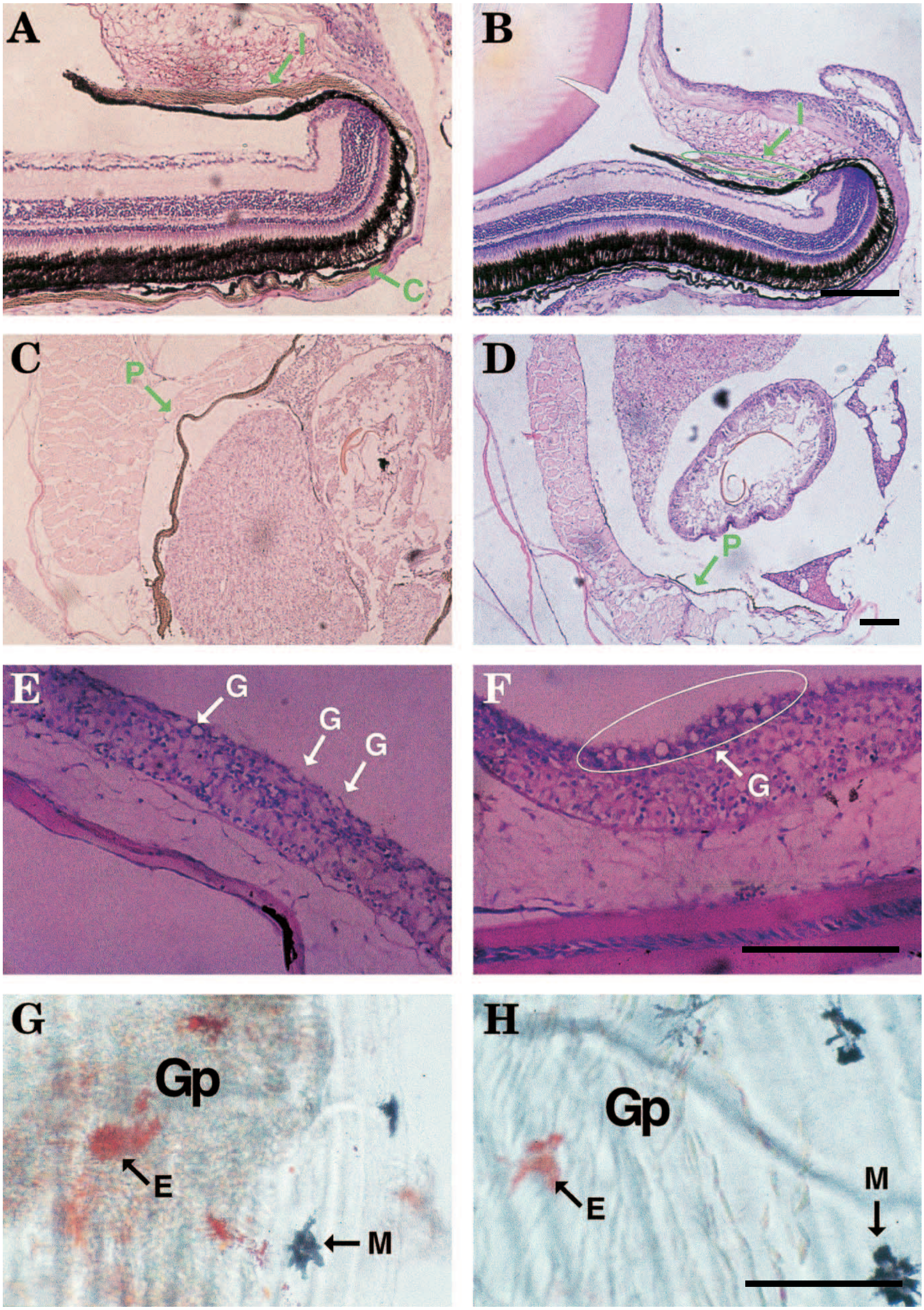

Fig. 3. Morphological comparison between NST and TSV of *R. o. ocellatus*. (**A**, **B**) Sagittal sections of orbital region. (**C**, **D**) Sagittal sections of abdominal region. (**E**, **F**) Sagittal sections of dermal/epidermal regions. (**G**, **H**) Scale. **A**, **C**, **E** and **G** from NST, while **B**, **D**, **F** and **H** from TSV. C, choroid; E, erythrophore; G, goblet cell; I, iris; Gp, guanophore; M, melanophore; P, peritoneum. Scale bars indicate 60 µm.

Table 1. Results in crossbreeding between NST and TSV of *R. o. ocellatus*, as shown in Fig. 2

					Fish at 100th day after hatching				
			Eggs treated	Embryos hatched (%)*	NST		TSV		Total (%)***
					♂(%)**	♀(%)**	♂(%)**	♀(%)**	
A	TSV × TSV (♂) (♀)	a-1	18	17 (94.4)	0 (0.0)	0 (0.0)	3 (50.0)	3 (50.0)	6 (35.3)
		a-2	36	24 (66.7)	0 (0.0)	0 (0.0)	3 (42.9)	4 (57.1)	7 (29.2)
		a-3	4	4 (100)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	2 (50.0)
		a-4	16	12 (75.0)	0 (0.0)	0 (0.0)	4 (50.0)	4 (50.0)	8 (66.7)
B	NST × TSV (♂) (♀)	b_1-1	12	10 (83.3)	2 (40.0)	3 (60.0)	0 (0.0)	0 (0.0)	5 (50.0)
		b_1-2	12	10 (83.3)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	3 (30.0)
		b_1-3	13	11 (84.6)	2 (50.0)	2 (50.0)	0 (0.0)	0 (0.0)	4 (36.4)
		b_1-4	28	7 (25.0)	2 (66.7)	1 (33.3)	0 (0.0)	0 (0.0)	3 (42.9)
	TSV × NST (♂) (♀)	b_2-1	8	8 (100.0)	1 (33.3)	2 (66.7)	0 (0.0)	0 (0.0)	3 (37.5)
		b_2-2	27	21 (77.8)	7 (46.7)	8 (53.3)	0 (0.0)	0 (0.0)	15 (71.4)
		b_2-3	8	8 (100.0)	3 (50.0)	3 (50.0)	0 (0.0)	0 (0.0)	6 (75.0)
C	F_1**** × NST (♂) (♀)	c-1	11	7 (63.3)	2 (40.0)	3 (60.0)	0 (0.0)	0 (0.0)	5 (71.4)
		c-2	34	29 (85.3)	6 (42.9)	8 (57.1)	0 (0.0)	0 (0.0)	14 (48.3)
		c-3	21	18 (85.7)	5 (45.4)	6 (54.6)	0 (0.0)	0 (0.0)	11 (61.1)
D	F_1**** × TSV (♂) (♀)	d-1	32	24 (75.0)	2 (16.7)	1 (8.3)	4 (33.3)	5 (41.7)	12 (50.0)
		d-2	42	11 (26.2)	1 (33.3)	1 (33.3)	0 (0.0)	1 (33.3)	3 (27.3)
		d-3	45	34 (75.6)	3 (33.3)	1 (11.1)	3 (33.3)	2 (11.1)	9 (26.5)
E	F_1**** × F_1**** (♂) (♀)	e-1	40	16 (40.0)	3 (50.0)	1 (16.7)	1 (16.7)	1 (16.7)	6 (37.5)
		e-2	36	24 (66.7)	6 (50.0)	4 (33.3)	1 (8.3)	1 (8.3)	12 (50.0)

* Embryos hatched (eggs treated).
** Ratio of each sex to total individuals at 100th day after hatching.
*** Total individuals at 100th day after hatching (embryos hatched).
**** Offspring between NST (♂) and TSV (♀) in experiment b_1.

85.7% (c-3) (78.1 ± 12.8%, Mean ± SD). The survival rate at 100th day was 60.3 ± 11.6% (Mean ± SD). The sex ratio in offspring was about 1:1 (♂:♀).

F_1 × TSV: Contrary to the result in crossbreeding between F_1 and NST (Table 1C), not only NST but also TSV was observed in this crossbreeding (Table 1D). Hatchability and the survival rate at 100th day were 58.9 ± 28.3% and 34.6 ± 13.3% (Mean ± SD) respectively. The ratio between NST and TSV was 1:3 (d-1), 2:1 (d-2) and 4:5 (d-3). The sex ratio ranged from 1:1 (♂:♀) (d-2) to 3:1 (d-3) in NST and from 3:2 (d-3) to 4:5 (d-1) in TSV. The low survival rates in all the crossbreeding were caused by white spot disease.

F_1 × F_1: Both NST and TSV appeared (Table 1E). Hatchability was 40.0% (e-1) and 66.7% (e-2). The survival rate at 100th day was 37.5% (e-1) and 50.0% (e-2). The ratio between NST and TSV was 2:1 (e-1) and 5:1 (e-2). In the sex ratio, though male and female appeared in both NST and TSV, NST male dominantly outnumbered the opposite sex or the other phenotype (50% in both e-1 and 2).

DISCUSSION

Morphological traits in TSV of the rosy bitterling

Kajishima (1960a-c, 1977) tried to clarify the morphological trait in TSV of the goldfish. He found in TSV, that not only the reflecting guanine layer in eye but also melanophores and xanthophores degenerate over the whole body at the early developmental stage. Especially, the decrease in the number of guanophores was conspicuous in iris, peritoneum and operculum. In *R. o. ocellatus*, the discrimination of phenotype was impossible in larvae just after hatching (at second day after fertilization at 20°C). However, in juvenile at about 20th day after hatching, the phenotype was visibly discriminated. This finding suggests that, in TSV of *R. o. ocellatus* as well as the goldfish, the degeneration of each chromatophore occurs during the larval stage. In the morphological observation in TSV of the adult *R. o. ocellatus*, three characteristics were recognized, which were the degeneration of iris, choroid and peritoneum, the increase in the number of goblet cell and the low density of guanophores in scales (Fig. 3). In NST, iris, choroid and peritoneum remarkably contain many guanophores, where there are many reflecting platelets of guanine, giving fish a shiny appearance (Kajishima, 1960a; Hawks, 1983). Therefore, the low content of guanine on the body surface is considered to create the transparent body coloration, as well as the blackish color in eye and peritoneum.

Contrary to the goldfish, however, such a remarkable difference was not seen in the other chromatophores except guanophore in *R. o. ocellatus*. The increase in the number of goblet cell on the dermal/epidermal regions in TSV has not been reported in other fishes so far. It remains unknown whether this trait was caused by either genetic or environmental factors. As one of the possibilities, the inner body parts in TSV are directly exposed to the influence of UV rays, because of the low content of guanine. Therefore, on behalf of guanine, many goblet cells develop and excrete much mucus for the purpose of body protection. In behavior, TSV of *R. o. ocellatus* seemed cowardlier and stagnanter than NST. Possibly, such a behavior in TSV has some relation with the degeneration of the iris and choroid in eye.

Hereditary traits in TSV of the rosy bitterling

Berndt (1925) considered that the character "hypolepidose" (transparent-scaled) in the goldfish is dominant against the normal character. However, Chen (1928) demonstrated that the genetic dominance of this character is imperfect. He also distinguished the homozygous and the heterozygous types in TSV of the goldfish. The former is all transparent and the latter mosaic transparent. In the genetic and developmental analyses of the goldfish, Kajishima (1960a, 1977) concluded that the recessive transparent-scaled character is incompletely dominant to the normal-scaled character and determined by a single autosomal gene.

In the crossbreeding experiments of *R. o. ocellatus*, the transparent-scaled character is obviously recessive to the normal-scaled character, because the offspring between NST and TSV were all NST (Table 1B). In the backcross between F_1 and TSV and the crossbreeding in F_1, however, TSV appeared again (Table 1D, E), while in the backcross between F_1 and NST, only NST did (Table 1C). The ratio between TSV and NST is about 1:1 in the backcross between F_1 and TSV, and about 1:3 in the crossbreeding in F_1. Judging from the results in crossbreeding, the transparent-scaled character in *R. o. ocellatus* is thought to be controlled by a single gene ("a" in Fig. 2), which is recessive to a gene determinant to the normal-scaled character ("A" in Fig. 2). In addition, the fact that the sex ratio always converges on 1:1 in both TSV and NST in all crossbreeding, indicates that a gene determinant to the transparent-scaled character is unrelated to sex.

The hereditary manner of the transparent-scaled character in *R. o. ocellatus* is very similar to that in the goldfish (Kajishima, 1977). In both fishes, the transparent-scaled character is controlled by a single gene, unrelated to sex. *R. o. ocellatus* differs from the goldfish in that the former has only one type in TSV, contrary to two types (all transparent and mosaic transparent-scaled) in TSV of the latter. This means that, in *R. o. ocellatus*, a gene determinant to the transparent-scaled character is completely recessive, not incompletely dominant to the normal-scaled character, different from that of the goldfish.

Utility in TSV of the rosy bitterling

The color variant in fish has often been reported in many freshwater fishes. The well-known examples are the medaka (Iwamatsu, 1993), the Guppy *Poecilia resticulata* (Winge, 1927), the platyfish *Xiphophorus maculatus* (Borowski, 1984) and the goldfish (Matsui, 1934). As for TSV of *R. o. ocellatus*, the most outstanding morphological trait is the low density of guanophores in the whole body. The same color variant as seen in *R. o. ocellatus* has already been reported in other fishes e.g. *O. latipes* (Takeuchi, 1969) and *C. auratus* (Chen, 1928). Therefore, the occurrence of TSV in fish does not seem to be so rare and restricted to certain taxa.

In general, color variants in fishes, including albino, are inferior to the normal type in growth and reproduction (Kirpichnikov, 1981). In TSV of *R. o. ocellatus*, however, such deficiency has not seen at all in this study. In addition, the simple heredity and the conspicuous morphological character in TSV of *R. o. ocellatus* suggest that TSV in *R. o. ocellatus*, which is excellent in growth and reproduction, can be a good material in experimental biology. Particularly, the recessive transparent-scaled character is useful as a genetic marker for experimental and developmental biology and genetics.

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