

## Non-inheritable Color Variants in *Rana brevipoda porosa*

By

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(With 6 Text-figures and 1 Plate)

### INTRODUCTION

Numerous color variants of amphibians have been found in the field. Although most of them have not been genetically studied, their color and pattern were indefinitely considered to be controlled by mutation genes. The only color variants in which the coloration is confirmed to be non-inheritable are blue variants of *Hyla arborea japonica* reported by the present authors (1985d).

A blackish and a white variants of *Rana brevipoda porosa* were collected from Niigata Prefecture located in the west side of the central part of Japan in 1977 and 1979, respectively. As these variants were females, gynogenetic diploids were produced from their eggs. Contrary to expectation, no variants were found in the offspring raised from these eggs. Then, F<sub>2</sub> offspring were produced from the female variants. The results of experiments also showed that no color variants appeared in the F<sub>2</sub> offspring, except that only one blackish variant was produced from a mating of the female blackish variant with a wild-type male.

Electron-microscopic observations on the dorsal skin of the blackish and white variants seemed to indicate participation of phagocytes in formation of these color variants.

In this paper, the results of morphological and experimental studies on the blackish and white variants of *Rana brevipoda porosa* will be reported.

### MATERIALS AND METHODS

A blackish variant was found out in six male and 23 female *Rana brevipoda porosa* (COPE) collected from Maki-cho, Nishikambara-gun, Niigata Prefecture by Mr. M. KINEBUCHI, a teacher of Maki Senior High School, on August 15, 1977. This variant was a female, 30 mm in body length, whose body was semitransparent and appeared to be wholly blackish. A white variant was discovered at a rice field in Shitada-mura, Minamikambara-gun, Niigata Prefecture by a farmer in September, 1979. It was a mature female *Rana brevipoda porosa* whose body was semitransparent and whitish. The eyes were normal and had black pupils and golden irises. The discovery of this color variant was first reported to a newspaper office of Niigata Nipposha and then the animal was brought to Dr. K. SEKIYA,

Faculty of Science, Niigata University. Finally, it came to our laboratory through the good offices of Niigata Nipposha on November 7, 1979. At that time, this variant was 77 mm in body length.

In order to determine whether these two color variants are inheritable, gynogenetic diploids were produced from their eggs and also offspring were obtained from them by mating with normal male *Rana brevipoda porosa*. The gynogenetic diploids were produced by the following method.

Ovulation of these female color variants was accelerated by injecting frog pituitary suspension into the coelomic cavity. Sperm suspension obtained by crushing the testes of a male *Rana clamitans* in a small quantity of tap-water was exposed for 2 minutes to UV emitted from a mercury lamp, GUL-5·J Type of Toshiba Co., 2537 Å in the main wave length at a distance of 20 cm (2400 erg/cm<sup>2</sup>/sec). Although the nuclei of spermatozoa become incompetent by such an exposure, the spermatozoa themselves are motile and enter into eggs. Twenty minutes after the eggs of the color variants were inseminated with UV-irradiated sperm and left at room temperature (20~25°C), they were exposed to low temperature (1~2°C) for 2.5 hours. By this treatment, the nucleus of the second polar body was retained in the egg and fused with the egg pronucleus. Then the eggs developed as gynogenetic diploids.

Matings between the female color variants and normal males were performed by artificial fertilization. Eggs taken out of the cloaca were put on a glass plate and covered with a small quantity of sperm suspension.

Electron-microscopic observation of the skin of each of the color variants and normal frogs was made as follows. A piece of skin, about 5 mm square in size, was cut off from each of the dorsal, lateral and ventral surfaces, cut into smaller pieces at once in cold 0.1 M phosphate buffer (pH 7.4) containing 4% glutaraldehyde and then kept in the same solution for 2 hours after renewal of the fluid. The minute pieces were washed and then postfixed in 0.1 M phosphate buffer (pH 7.4) containing 2% osmic acid for 2 hours. These fixing procedures were performed at 2~4°C. The fixed pieces of skin were then dehydrated in an ethanol series and embedded in Epon 812. Sections were made on a Porter-Blum MT-1 ultramicrotome with a glass knife, double stained with saturated uranyl acetate and alkaline lead citrate and photographed with a Hitachi Hs-8 electron microscope.

## OBSERVATION

### I. Production of offspring

#### 1. Blackish variant

i) The mature female blackish variant (Blk. 77♀, No. 1) of *Rana brevipoda porosa* was injected with suspension of bullfrog pituitaries in order to accelerate ovulation in the breeding season of 1978. The results showed that this female

laid many eggs which were completely normal in color and size. When 265 eggs were inseminated with sperm of a wild-type male (W. 77♂, No. 1) collected from the same place as that where the blackish variant was collected, 192 (72.5%) of them cleaved normally, 155 (58.5%) hatched normally and 134 (50.6%) attained completion of metamorphosis. On the other hand, 271 eggs of the female variant were refrigerated after pseudofertilization with UV-irradiated sperm of *Rana clamitans*. It was found that 204 (75.3%) of them cleaved normally, 56 (20.7%) hatched normally and 36 (13.3%) became normally metamorphosed diploids. Among the froglets produced by normal fertilization and diploid gynogenesis, there were no color variants (Table 1).

TABLE 1  
Offspring of a female blackish variant in *Rana brevipoda porosa*

Year	Parents		No. of eggs	No. of normal cleavages	No. of normally hatched tadpoles	No. of metamorphosed frogs		
	Female	Male				Total	Wild	Variants
1978	Blk. 77, No. 1	W. 77, No. 1	265	192	155	134	134	0
		GD	271	204 (75.3%)	56 (20.7%)	36 (13.3%)	36	0
1979	Blk. 77, No. 1	(Blk ♀ × W ♂)78, No. 1	226	190 (84.1%)	173 (76.5%)	152 (67.3%)	152	0
1981	(Blk ♀ × W ♂)78, Nos. 1~3	(Blk ♀ × W ♂)78, Nos. 2~4	316	235 (74.4%)	213 (67.4%)	168 (53.2%)	168	0

Blk, blackish variant    W, field-caught    GD, diploid gynogenesis

When these froglets were continuously reared, one of the frogs which had been raised from eggs fertilized with sperm of a normal male began to become semi-transparent. One year later, this frog was completely semitransparent except the irises and became a blackish variant (Blk. 78♀, No. 2). All the other frogs were of the wild-type.

ii) In 1979, a wild-type male obtained in 1978 from the mating between the female blackish variant (Blk. 77♀, No. 1) and the wild-type male (W. 77♂, No. 1) was backcrossed with the female blackish variant. The results showed that 190 (84.1%) of 226 eggs cleaved normally, 173 (76.5%) hatched normally and 152 (67.3%) attained completion of metamorphosis. These froglets were all of the wild-type and thereafter did not change into blackish variants until the stage of sexual maturity.

iii) In 1981, brother and sister matings were made between three males and three females obtained in 1978 from the mating, Blk. 77♀, No. 1 × W. 77♂, No. 1. It was found that 235 (74.4%) of 316 eggs cleaved normally, 213 (67.4%) hatched normally and 168 (53.2%) completed metamorphosis. The froglets produced were all of the wild-type and thereafter did not change into blackish

variants until the stage of sexual maturity (Table 1).

These findings described above indicate that the female blackish variant (Blk. 77♀, No. 1) is not a mutant. However, the fact that a single blackish variant (Blk. 78♀, No. 2) was produced from the original blackish variant by mating with a wild-type male collected from the same place should not be ignored.

## 2. White variant

i) In the breeding season of 1980, the female white variant (Wh. 79♀, No. 1) of *Rana brevipoda porosa* collected in 1979 was mated with a wild-type male (W. 77♂, No. 2). The results showed that 358 (95.7%) of 374 eggs cleaved normally, 329 (88.0%) became normally hatched tadpoles and 191 (51.1%) attained completion of metamorphosis. These froglets and tadpoles produced were all of the wild-type and indistinguishable from normal ones. On the other hand, 176 (46.3%) of 380 eggs refrigerated after pseudofertilization with UV-irradiated sperm of *Rana clamitans* cleaved normally, 105 (27.6%) became normally hatched tadpoles, and 57 (15.0%) became gynogenetic diploid froglets. In these froglets and tadpoles, no white variants were found.

TABLE 2  
Offspring of a female white variant in *Rana brevipoda porosa*

Year	Parents		No. of eggs	No. of normal cleavages	No. of normally hatched tadpoles	No. of metamorphosed frogs		
	Female	Male				Total	Wild	Variants
1980	White 79, No. 1	W. 77, No. 2	374	358 (95.7%)	329 (88.0%)	191 (51.1%)	191	0
		GD	380	176 (46.3%)	105 (27.6%)	57 (15.0%)	57	0
1984	(White ♀ × W ♂)80, No. 1	(White ♀ × W ♂)80, No. 1	582	382 (65.6%)	343 (58.9%)	314 (54.0%)	314	0
	(White ♀ × W ♂)80, No. 2	(White ♀ × W ♂)80, No. 2	566	291 (51.4%)	263 (46.5%)	237 (41.9%)	237	0
	(White ♀ × W ♂)80, No. 3	(White ♀ × W ♂)80, No. 3	580	241 (41.6%)	208 (35.9%)	141 (24.3%)	141	0
	Total		1728	914 (52.9%)	814 (47.1%)	692 (40.0%)	692	0

W, field-caught    GD, diploid gynogenesis

The froglets produced from the female white variant by mating with the wild-type male as well as by diploid gynogenesis were continuously reared. The frogs which could attain sexual maturity were all of the wild-type. No white variants were produced (Table 2).

ii) In 1984, brother and sister matings were made between three females and three males obtained in 1980 from the mating, Wh. 79♀, No. 1 × W. 77♂, No. 2. It was found that 914 (52.9%) of 1728 eggs cleaved normally, 814 (47.1%) became normally hatched tadpoles and 692 (40.0%) attained completion of meta-

morphosis. No white variant was found in these froglets and tadpoles (Table 2). The above findings show that the female white variant is not a mutant.

## II. Electron-microscopic structure

### 1. Chromatophores of wild-type frogs

*Rana brevipoda porosa* is intermediate between *Rana nigromaculata* and *Rana brevipoda brevipoda* in color and pattern. There are two types of frogs in dorsal coloration, brown and yellowish green (Plate I, 1). When the dorsal skin of the brown type is examined under an electron microscope, there are three kinds of dermal chromatophores, xanthophores, iridophores and melanophores, under the epidermis. These three kinds of chromatophores are arranged in upper, middle and lower layers and constitute the chromatophore units coined by BAGNARA, TAYLOR and HADLEY (1968) (Fig. 1).

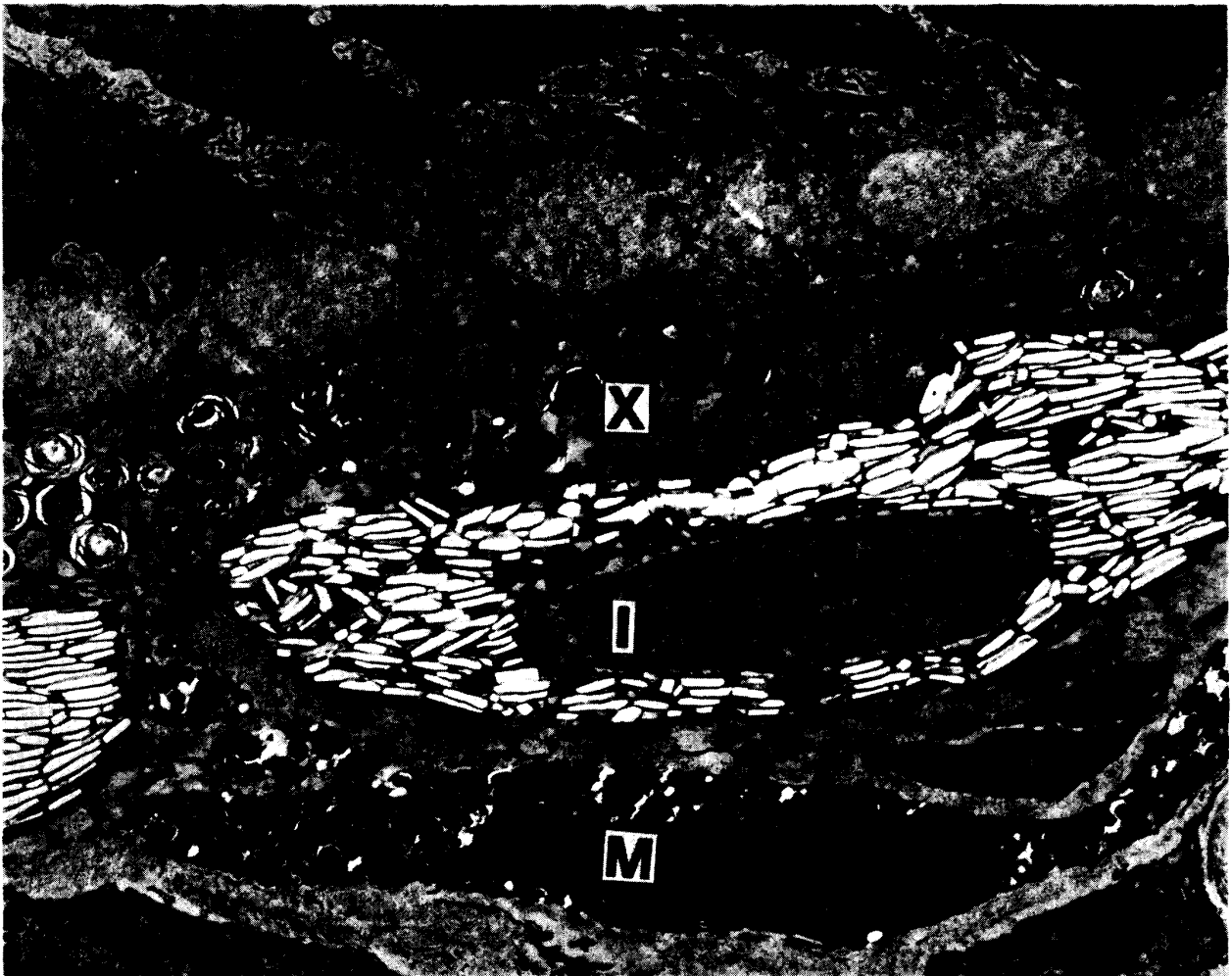


Fig. 1. Electron microphotograph of dermal chromatophores in the dorsal skin of a wild-type *Rana brevipoda porosa*. × 5000

X, xanthophore

I, iridophore

M, melanophore

### a. Xanthophore

Xanthophores are flat cells, being  $4\sim 10\ \mu$  in thickness, and horizontally arranged directly below the basal lamina of the epidermis. Each xanthophore is usually separated from the neighbors by some space. A small part of the cytoplasm is occupied by masses of carotenoid vesicles, while the other large part is filled with pterinosomes having a concentric structure.

When a piece of the dorsal skin,  $5\text{ mm}\times 5\text{ mm}$  in size, is cut off and directly observed under an optic microscope, the masses of carotenoid vesicles in xanthophores are recognized as yellow spots, while the other parts which are filled with pterinosomes are almost colorless. Thus, the pterinosomes do not seem to participate in manifestation of the dorsal color in brown-colored frogs. The carotenoid vesicles are minute spheroids. When 10 of them were measured, they were  $0.18\sim 0.27\ \mu$ ,  $0.23\ \mu$  on the average, in diameter. When 10 pterinosomes were measured, they were  $1.0\sim 1.4\ \mu$ ,  $1.2\ \mu$  on the average, and  $0.8\sim 1.0\ \mu$ ,  $0.9\ \mu$  on the average, in major and minor axes, respectively.

### b. Iridophore

Iridophores are somewhat flat cells situated under xanthophores. The median cross-section of an iridophore is a long ellipsoid concaved upward. When 10 iridophores were measured, they were  $20.0\sim 25.3\ \mu$ ,  $23.0\ \mu$  on the average, in length and  $5.6\sim 7.5\ \mu$ ,  $6.4\ \mu$  on the average, in thickness at the median part containing the nucleus.

The iridophores are horizontally arranged at intervals of  $3\sim 10\ \mu$ . The cytoplasm of each iridophore is almost filled with reflecting platelets. When 20 reflecting platelets were measured by the holes from which they had fallen off, they were rod- or die-shaped, being  $0.1\sim 1.9\ \mu$  in length and  $0.1\sim 0.2\ \mu$  in width. The reflecting platelets are mostly arranged in parallel with the dorsal surface of the body.

### c. Melanophore

Melanophores are situated under iridophores with some space. Under a light environment, they are contracted and their melanosomes are gathered into a mass. Each melanosome is observable as a black spheroid under an electron microscope owing to its high electron density without being dyed. When 20 melanosomes were measured, they were  $0.3\sim 0.8\ \mu$ ,  $0.49\ \mu$  on the average, and  $0.3\sim 0.5\ \mu$ ,  $0.35\ \mu$  on the average, in major and minor axes, respectively.

A few phagocytes are very rarely found under melanophores and between iridophores and melanophores.

## 2. Chromatophores of the blackish variants

### a. Blackish variant collected from the field

The dorsal surface of the blackish variant, Blk. 77♀, No. 1, was brownish gray, when collected in 1977. The spots on the back were brown black and distinct. A part of the visceral organs was seen through the semitransparent

body wall. The dorso-median and dorso-lateral stripes were yellowish. The irises were partially light yellowish-brown and revealed the presence of some iridophores.

In the breeding season of 1978, the ground color of the back of this blackish variant was still brownish-gray and the brown-black spots were distinct on the back and trunk (Plate I, 2). Thereafter, these spots were gradually faded away from the trunk. They decreased in number and the outline of each spot became obscure.

In the breeding season of 1979, all the spots on the back disappeared, while the ground color of the back was dark brownish-gray in the middle part and gradually whitish in the lateral parts toward the trunk (Plate I, 3, 4). The dorso-median stripe was scarcely found, while the dorso-lateral stripes were white and their outlines were somewhat obscure.

In May of 1978, small pieces of the skin, 5 mm × 5 mm in size, were removed from the dorsal, lateral and ventral surfaces of the body of the blackish variant, 52 mm in body length. When these pieces were directly observed under an optic microscope, no iridophores were observed in each of the skin pieces.

The dermal chromatophores in the dorsal skin were examined under an electron microscope. No iridophores could be found, while there were xanthophores and melanophores. In the neighborhood of the site which should be occupied by iridophores, numerous phagocytes were found (Fig. 2) in contrast to wild-type frogs, in which phagocytes were scarcely found in the neighborhood of iridophores. The phagocytes found in the skin of the blackish variant appeared to be very active in physiological function, as they had abundant cytoplasm, including well-developed mitochondria, lysosomes, both smooth and rough endoplasmic reticula and GOLGI bodies. Such phagocytes were also abundantly found in the area under melanophores.

Some phagocytes were in contact with xanthophores and melanophores by their pseudopodia (Fig. 3a). It was often found that phagocytes were digesting pieces of melanophores in their cytoplasm (Fig. 3b).

The xanthophores of this blackish variant were smaller than those of wild-type frogs and remarkably emaciated. Thus, adjacent xanthophores were separated from each other by a wide space. The pterinosomes included in the cytoplasm

Fig. 2. Electron microphotographs of dermal chromatophores in the dorsal skin of a blackish variant (Blk. 77 ♀, No. 1). × 5000

- |                                |                              |
|--------------------------------|------------------------------|
| a. Well-developed melanophore. | b. Degenerating melanophore. |
| X, xanthophore                 | M, melanophore               |
|                                | PC, phagocytes               |

Fig. 3. Electron microphotographs of phagocytes in the dorsal skin of a blackish variant (Blk. 77 ♀, No. 1). × 22000

- |   |              |                  |
|---|--------------|------------------|
| a. Phagocyte in contact with a xanthophore and a melanophore. |              |                  |
| Enlargement of the rectangle shown in Fig. 2b.                |              |                  |
| b. Phagocyte digesting pieces of a melanophore.               |              |                  |
| ER, endoplasmic reticulum                                     | L, lysosomes | MT, mitochondria |
| Arrows, degenerating melanosomes                              |              |                  |

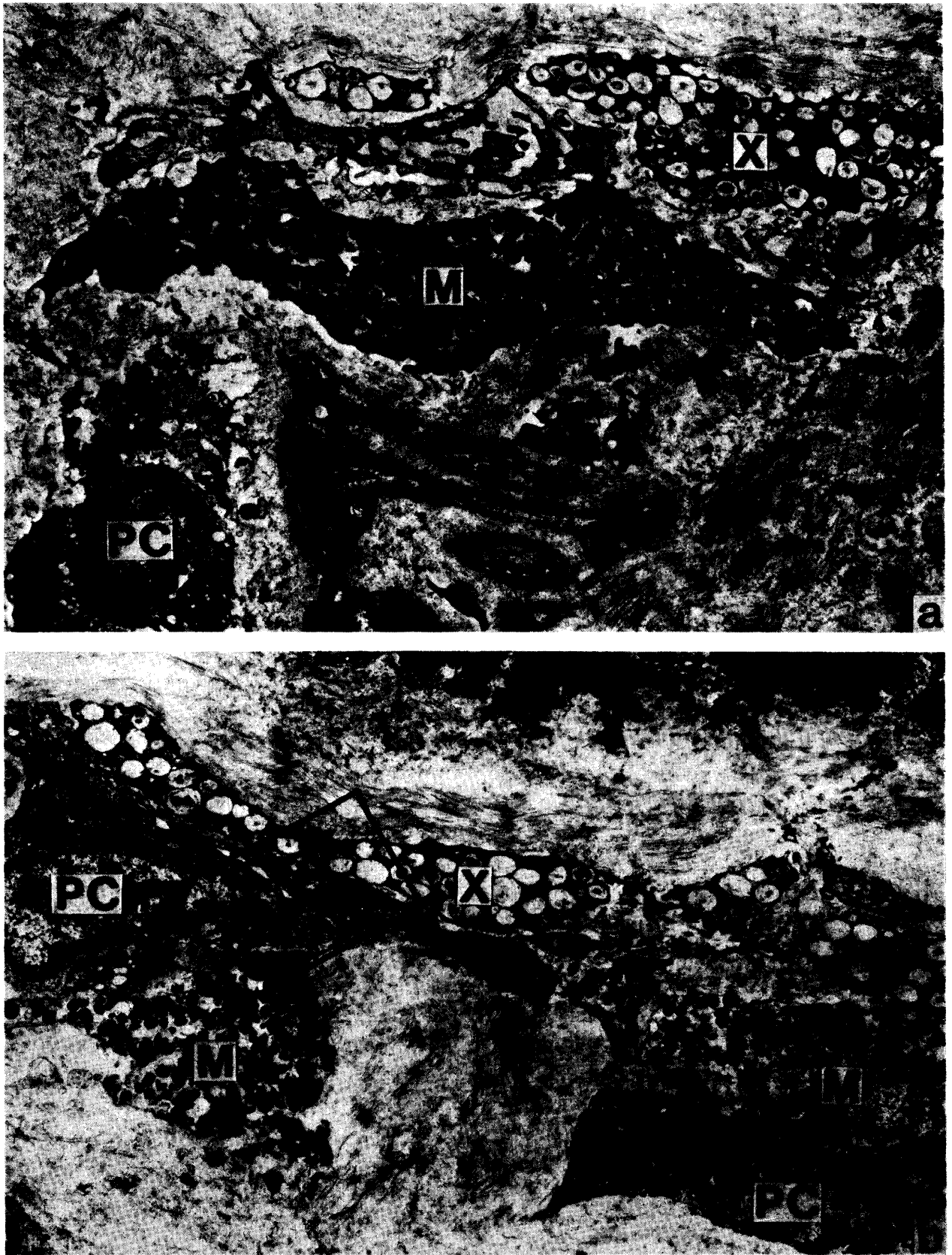


Fig. 2



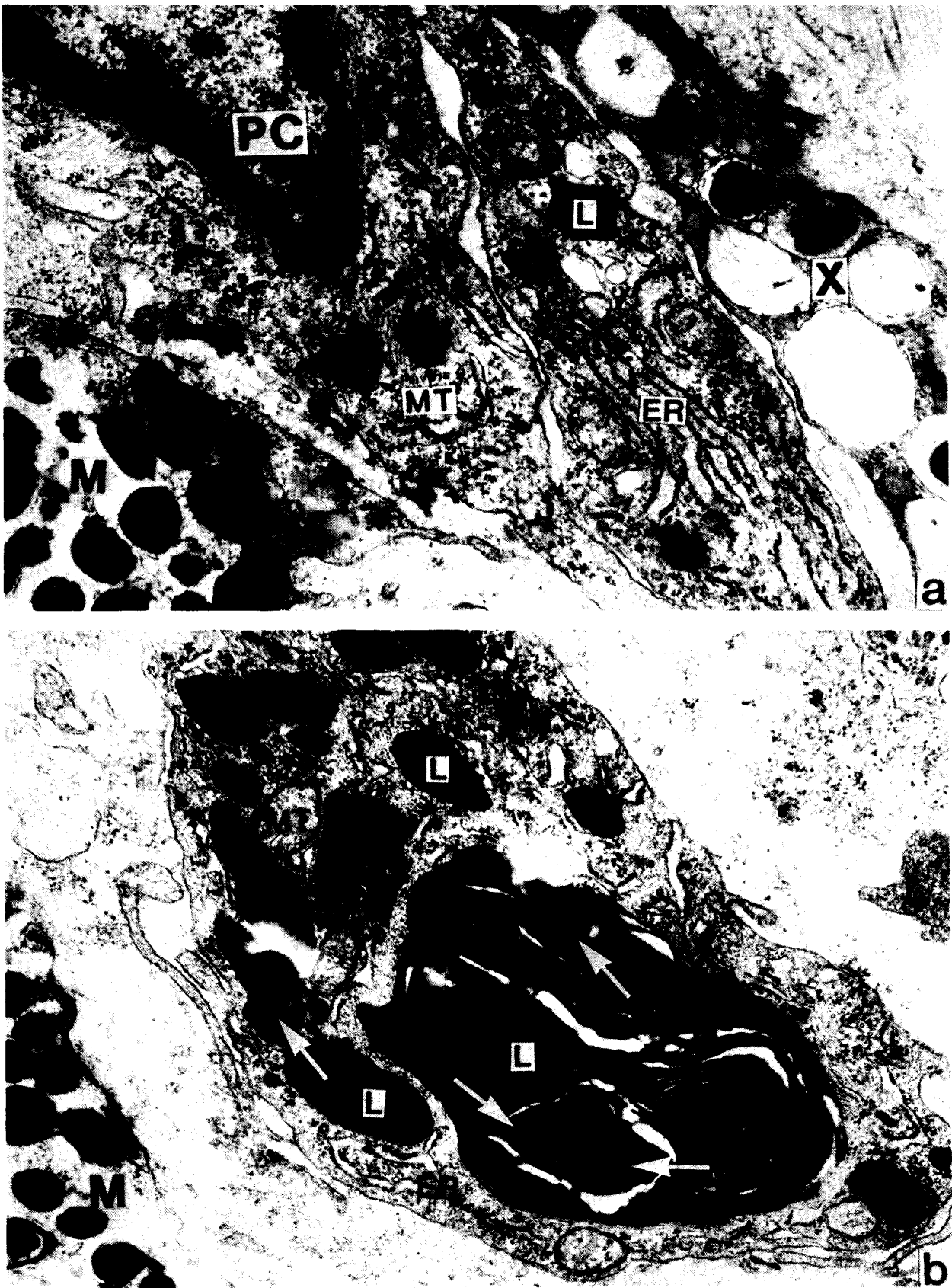


Fig. 3

of these xanthophores were generally ellipsoids near spheres and comparatively small. When 20 of them were measured, they were  $0.6\sim 1.1\ \mu$ ,  $0.9\ \mu$  on the average, and  $0.5\sim 0.9\ \mu$ ,  $0.7\ \mu$  on the average, in major and minor axes, respectively. Most of the pterinosomes were abnormal in inner structure. The concentric structure of the inside was indistinct, as it was collapsed as a whole or congregated in the center. The carotenoid vesicles in the xanthophores were normal, being  $0.14\sim 0.56\ \mu$ ,  $0.23\ \mu$  on the average, in diameter.

The dermal melanophores did not remarkably differ from those of wild-type frogs in distribution as well as in size and shape. However, the melanosomes contained in these melanophores were remarkably low in density. Moreover, there were some places where the melanophores were poorly developed in the dorsal skin.

The above findings seem to show that multiplied phagocytes first digest the iridophores and then invade the xanthophores and melanophores. The condition of this blackish variant in the breeding season of 1979 (Plate I, 4), where the spots have disappeared and the ground color is whitish, seems to indicate an increase of such an invasion by phagocytes.

#### b. Blackish variant obtained in the laboratory

The second blackish variant, Blk. 78♀, No. 2, was discovered in the offspring produced in 1978 from the mating, Blk. 77♀, No. 1 × W. 77♂, No. 1. This variant was dark brownish-gray and semitransparent. There were no spots on the back. The dorso-median and dorso-lateral stripes were whitish. A part of the visceral organs was seen through the semitransparent body wall. The eyes did not remarkably differ from those of the wild-type frogs. The pupils were black and the irises glittered with a golden color (Plate I, 5).

In January of 1980, the second blackish variant was 49 mm in body length. In order to examine the dermal chromatophores, small pieces of the skin, 5 mm × 5 mm in size, were cut off from the dorsal, lateral and ventral skins of the body. When these pieces were directly observed under an optic microscope, neither xanthophore nor iridophore was observed in each of the skin pieces. When the dermal chromatophores in the dorsal skin were examined under an electron microscope, neither xanthophore nor iridophore was found anywhere, while there were only melanophores (Fig. 4a). The site for the xanthophores and iridophores was occupied by increased melanophores. Thus, the number of melanophores was far larger than that in the wild-type frogs. Around the melanophores, there were more numerous phagocytes than those in the wild-type frogs. The cytoplasm of some phagocytes contained well-developed mitochondria,

Fig. 4. Electron microphotographs of dermal chromatophores in the dorsal skin of a blackish variant (Blk. 78♀, No. 2).

- |  |               |                                  |
|--|---------------|----------------------------------|
| a. Cross-section of the dorsal skin.                                   |               | × 5000                           |
| b. A part of phagocyte. Enlargement of the rectangle shown in Fig. 4a. |               | × 22000                          |
| M, melanophore   | PC, phagocyte | L, lysosomes                     |
| MT, mitochondria   | V, vacuoles   | Arrows, degenerating melanosomes |

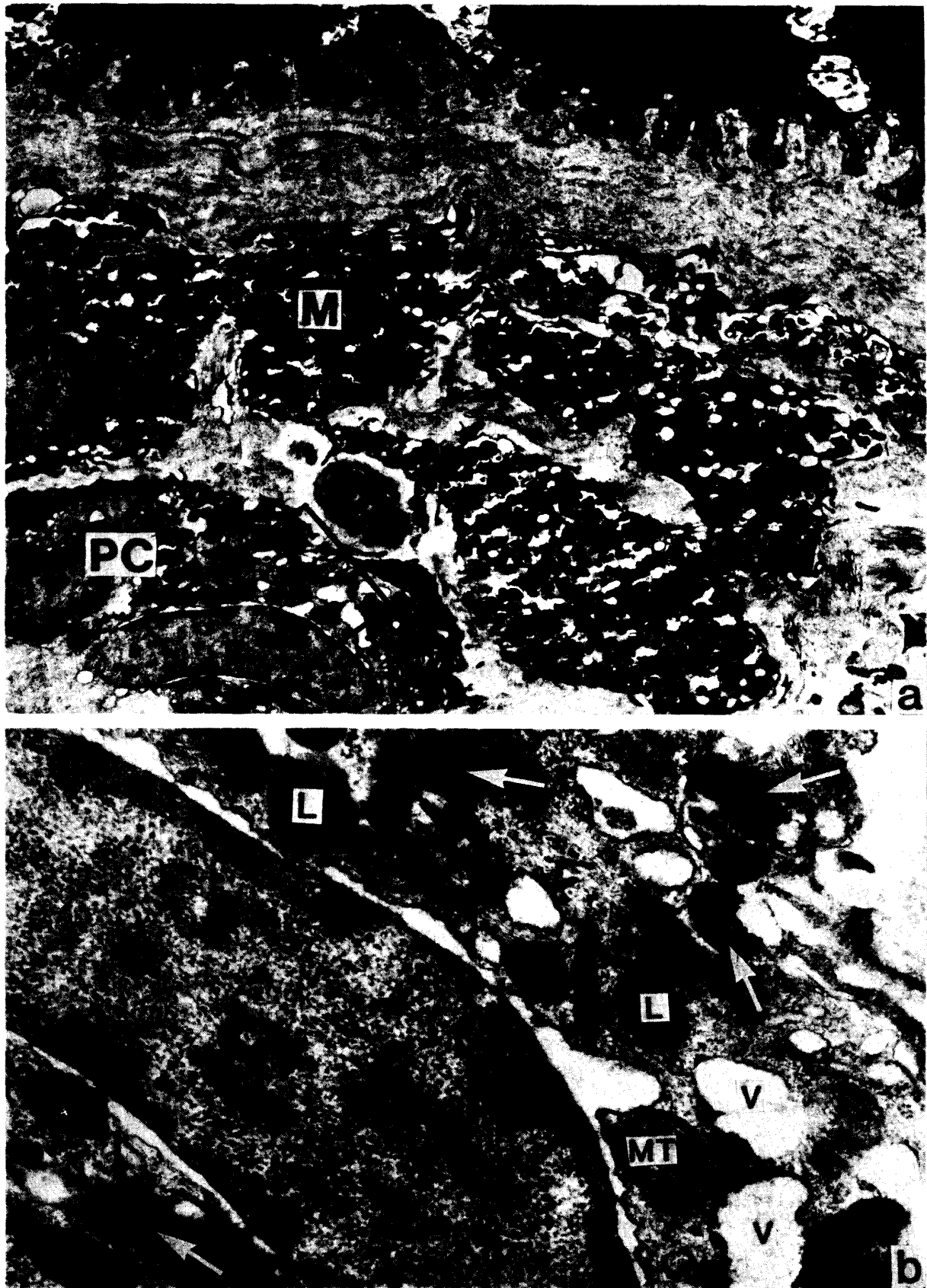


Fig. 4

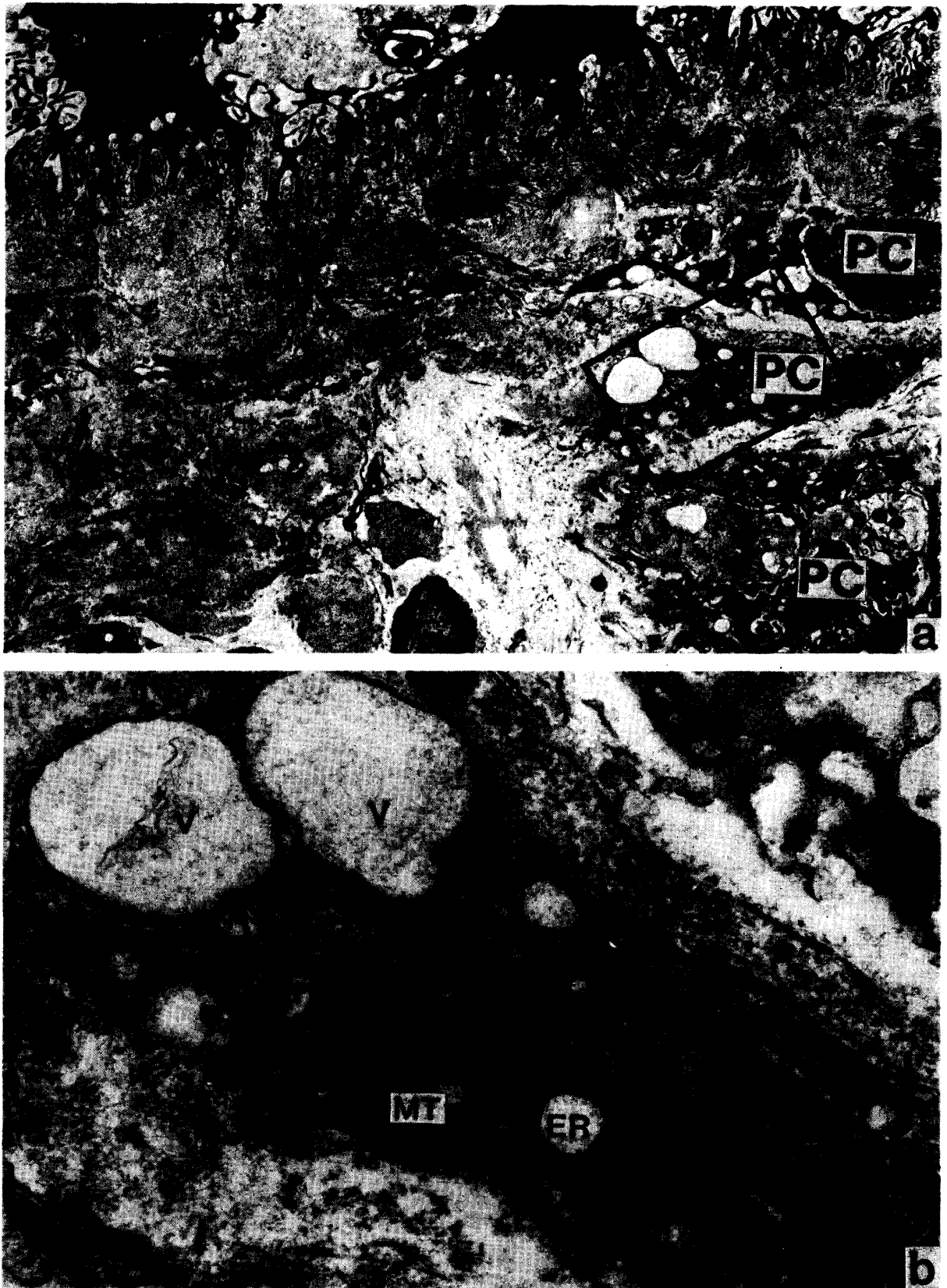


Fig. 5. Electron microphotographs of a cross-section of the dorsal skin of a white variant.  
 a. Phagocytes. × 5000  
 b. Enlargement of the rectangle shown in Fig. 5a. × 22000  
 PC, phagocytes MT, mitochondria ER, endoplasmic reticulum V, vacuoles

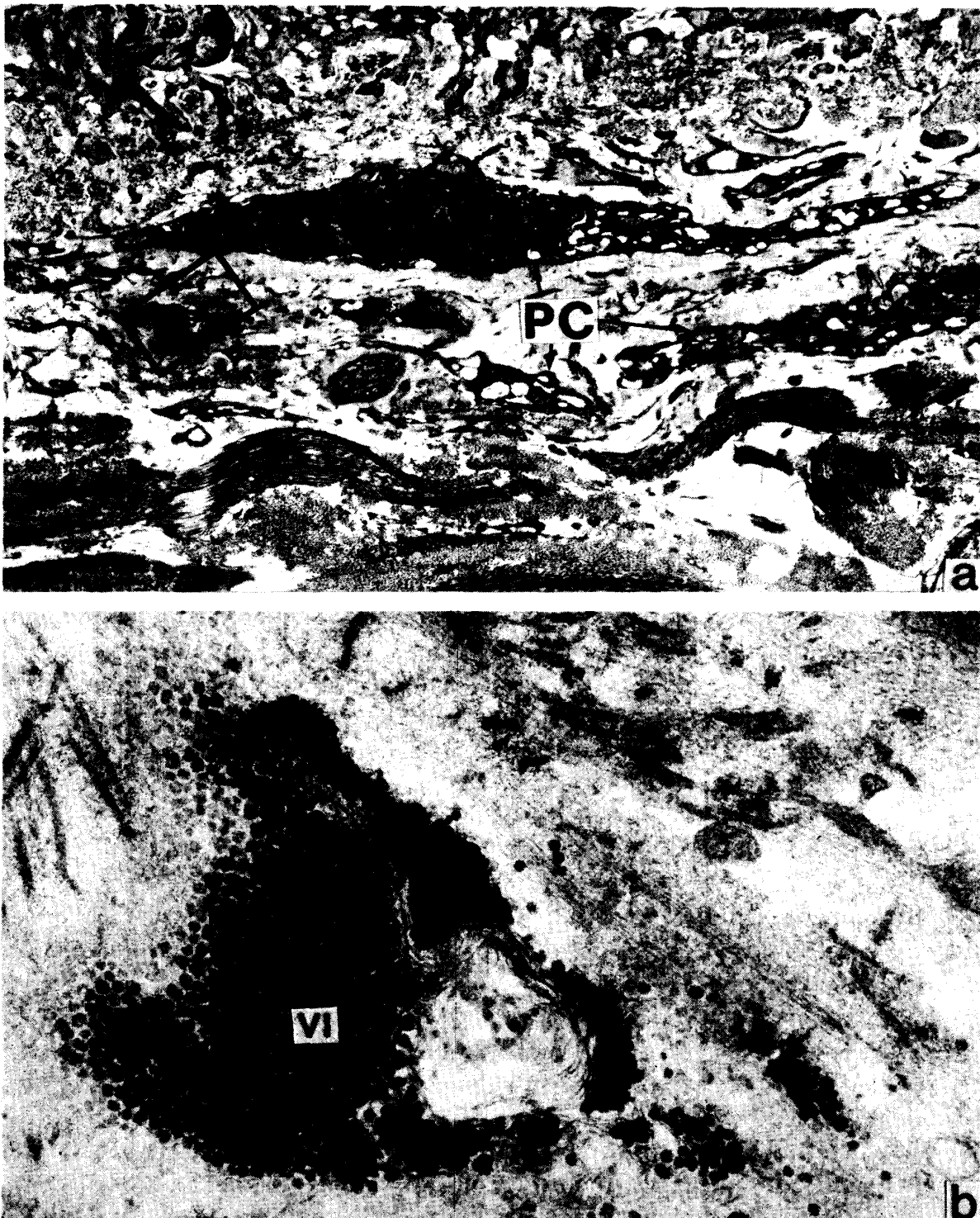


Fig. 6. Electron microphotographs of a cross-section of the dorsal skin of a white variant.

a. Phagocytes and virus-like particles. × 5000

b. Enlargement of the rectangle shown in Fig. 6a. × 44000

PC, phagocytes                      VI, virus-like particles

lysosomes, endoplasmic reticula and many vacuoles of various sizes. Throughout the cytoplasm, there were melanosomes which were ingested by the phagocyte (Fig. 4b).

These findings seem to show that the degeneration of melanophores follows that of iridophores and xanthophores.

### 3. Chromatophores of the white variant

The whole body of the white variant was white tinged with red and was semi-transparent. A part of the visceral organs was seen through the ventral body wall. The eyes did not remarkably differ from those of normal frogs. The pupils were black and the irises glittered with a golden color (Plate I, 6). When this variant was brought to our laboratory, normal-colored eggs were seen through the semitransparent body wall.

When small pieces of the skin, 5 mm × 5 mm in size, were cut off in May of 1980 from the dorsal, lateral and ventral surfaces of the white variant, 80 mm in body length, and directly observed under an optic microscope, no dermal chromatophores of any kind could be detected.

When cross-sections of the dorsal skin were examined under an electron microscope, no dermal chromatophores were also detectable. In the area where dermal chromatophores should be distributed, collagenous fibers, connective tissue cells and a small number of phagocytes having large or small vacuoles were observable (Fig. 5). These phagocytes had a small quantity of cytoplasm and their mitochondria and endoplasmic reticula (ER) were underdeveloped. Thus, they seemed to have already lost their activity (Figs. 5a, 6a). It was remarkable that there were cells including masses of electron-dense granules, being about 45 m $\mu$  in diameter. These granules were considered to be viruses which might participate in destruction of the dermal chromatophores (Fig. 6b).

## DISCUSSION

In amphibians, color and pattern have been studied by a large number of investigators. The main problems of these studies were individual and local variations, origin and development, light- and electron-microscopic structures of chromatophores such as xanthophores, iridophores and melanophores, synthesis of melanin that is the most important pigment, color change and adaptation, etc. Of these problems, variations in color and pattern were the most interesting for zoologists. Especially, there are numerous reports on albinism (GILBOA and DOWLING, 1974). The present authors (1977) have published genetic and morphologic studies on ten albinic stocks in *Hyla arborea japonica*. They (1985a) have also performed similar studies on 13 albinic stocks in *Rana nigromaculata*. The senior author has obtained various kinds of color variants by irradiating gametes with X-rays or neutrons in *Rana nigromaculata* (NISHIOKA, 1977). Besides, the present authors (1985b, c) have reported on black-eyed mutants in *Hyla arborea japonica*, and on albinos and black-eyed mutants in *Rhacophorus schlegelii*.

While color variants in amphibians are usually mutants due to a recessive gene in the homozygous condition, the blue variants reported by the present authors (1985d) in *Hyla arborea japonica* were not inheritable. Such color variants

which were confirmed to be non-inheritable by mating experiments have been scarcely known in amphibians, although it has been assumed that non-inheritable color variants are not very few in the field.

A blackish and a white variants of *Rana brevipoda porosa* reported in this paper were confirmed to be non-inheritable, as similar color variants did not reappear in gynogenetic diploids as well as in the  $F_2$  offspring produced from them. However, a single blackish variant was produced from the original blackish variant by mating with a normal male. This situation does not always seem to be an accident, as such a blackish variant has not yet been found from the other matings. It seems possible that some factor was introduced into the egg from the parental blackish variant.

The dorsal surfaces of the two blackish variants are dark brownish-gray. The body wall is semitransparent. The iris is light yellowish-brown or has a glittering golden color. The blackish variant collected from the field has the dorso-median and dorso-lateral stripes on the back. These stripes were light yellow in May of 1978. When examined under an electron microscope, the dorsal skin had xanthophores and melanophores, while no iridophores were found anywhere. In June of 1979, the dark spots on the back and trunk as well as the dorso-median and dorso-lateral stripes were in process of fading out. Especially, the dorso-lateral stripes were wide and obscure in outline and white in color, showing degeneration of xanthophores.

When the dorsal skin of the second blackish variant obtained in the laboratory was examined under an electron microscope, iridophores and xanthophores were nowhere to be found. Numerous phagocytes were found in the neighborhood of the site which should be occupied by these two kinds of dermal chromatophores. As some phagocytes were in contact with melanophores by their pseudopodia and there were several phagocytes digesting pieces of melanophores in their cytoplasm, it is very probable that all the iridophores and xanthophores were already destroyed and the melanophores were destined to be destroyed.

The whole body of the white variant was white tinged with red and semitransparent, while the eyes did not remarkably differ from those of the wild-type frogs. When examined under an electron microscope, the dorsal skin had no dermal chromatophores. In the area where dermal chromatophores should be distributed, there were a small number of phagocytes and some cells including masses of electron-dense granules which are considered to be viruses.

The skin color of the white variant seems to be a kind of vitiligo brought about by destruction of all the chromatophores contained in the skin. The black variants appear to be at an earlier stage of vitiligo. Of the three kinds of dermal chromatophores, the iridophores seem to be destroyed at the start and then the xanthophores are destroyed. The melanophores appear to be lastly destroyed. Although the cause for the destruction of chromatophores is unknown, viruses might have been involved. It is probable that the phagocytes eventually ingest and digest all the chromatophores and that the wild-type color and pattern of the dorsal surface change into white through blackish color. Thus, the blackish

and white colors of these variants are no doubt acquired characters.

### SUMMARY

1. A blackish and a white variants in *Rana brevipoda porosa* were collected from Niigata Prefecture in 1977 and 1979, respectively. They were mature females and produced gynogenetic diploids from their eggs by refrigeration after inseminating with UV-irradiated sperm of male *Rana clamitans*. They also produced first-generation offspring by mating with wild-type males and then F<sub>2</sub> offspring by brother and sister matings. As the gynogenetic diploids and the F<sub>2</sub> offspring were all of the wild-type, it is evident that the two variants were not mutants.

2. The dermal chromatophores in the dorsal skin of wild-type *Rana brevipoda porosa* consist of xanthophores, iridophores and melanophores. These three kinds of chromatophores constitute the chromatophore units.

3. The dorsal surface of the blackish variant was brownish gray and the spots on the back were distinct immediately after collection. The body wall was semitransparent. The dorso-median and dorso-lateral stripes were yellowish. The irises were partially light yellowish-brown. When examined under an electron microscope, the dorsal skin had xanthophores and melanophores, while no iridophores were found anywhere. There were numerous phagocytes in the neighborhood of the site where iridophores had probably occupied. Some phagocytes were in contact with xanthophores and melanophores by their pseudopodia. There were often phagocytes which were digesting pieces of melanophores in their cytoplasm.

In 1979, all the spots on the back disappeared. The ground color of the back was dark brownish-gray in the middle portion and gradually whitish toward the trunk. The dorso-lateral stripes were white.

4. The second blackish variant was discovered among the offspring produced in 1978 from the first blackish variant by mating with a wild-type male. The dorsal surface of this variant was dark brownish-gray and the body wall was semitransparent. There were no spots on the back. The eyes were almost normal. The dorso-median and dorso-lateral stripes were whitish. When the dermal chromatophores in the dorsal skin were examined under an electron microscope, neither xanthophore nor iridophore was found anywhere, while there were only melanophores. Around the melanophores, there were numerous phagocytes.

5. The whole body of the white variant was white tinged with red and semitransparent. The eyes were almost normal. The eggs were normal-colored. The dorsal skin had no dermal chromatophores. In the area where dermal chromatophores had been distributed, collagenous fibers, connective tissue cells and a small number of phagocytes having large or small vacuoles were observed. There were some cells including masses of electron-dense granules which are considered to be viruses.

6. In the blackish and white variants, the dermal chromatophores seemed to have been destroyed in the sequence from iridophores to melanophores through



xanthophores by invasion of phagocytes. It is possible that viruses take part in the destruction of chromatophores.

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## EXPLANATION OF PLATE

## PLATE I

Blackish and white variants and a wild-type frog in *Rana brevipoda porosa*.

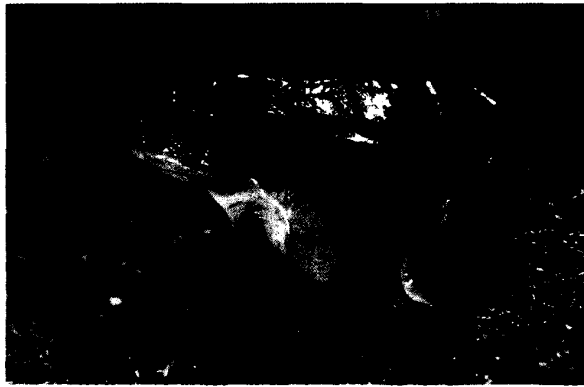
1. Wild-type *Rana brevipoda porosa*.  $\times 0.6$
2. Blackish variant (Blk. 77 ♀, No. 1). Photographed in May, 1978.  $\times 0.8$
- 3, 4. The same individual as shown in Fig. 2. Photographed in June, 1979.  $\times 0.6$
5. Blackish variant (Blk. 78 ♀, No. 2).  $\times 0.8$
6. White variant (Wh. 79 ♀, No. 1).  $\times 0.6$



1



2



3



4



5



6