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Blue Variants in *Hyla arborea japonica*

By

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(With 7 Text-figures and 1 Plate)

INTRODUCTION

BERNS and NARAYAN (1970) have reported two blue variants in *Rana clamitans*. As these variants had blue and green areas on the dorsal surfaces of the body or legs, the dermal chromatophores of the blue integument were compared with those of the green integument under a light and an electron microscope. NISHIOKA (1977) produced many blue variants by exposing spermatozoa or eggs of *Rana nigromaculata* to X-rays or neutrons. She confirmed that these variants are mutants due to a recessive gene. In the Japanese tree-frog, *Hyla arborea japonica*, blue variants are occasionally collected from the field, although it is not evident whether the blue color is a heritable character or not.

In the present study, two blue variants collected from the field in 1977 were examined in terms of their genetics and the minute structure of the dermal chromatophores in their dorsal skin.

MATERIALS AND METHODS

A blue variant of *Hyla arborea japonica* at the juvenile stage was discovered in Konosu, Saitama Prefecture. This was donated to the Laboratory for Amphibian Biology on July 25, 1977 by Mrs. Yoriko KANEKO, the chief of a kindergarten, after reared for about one year at the kindergarten. When this variant was brought to our laboratory by herself, it was already 24 mm in body length. Another blue variant was collected in July of 1977 from Tottori situated about 500 km west of Konosu. This variant was a juvenile frog immediately after metamorphosis, being about 15 mm in body length, and was before long donated to our laboratory from Tottori Natural History Museum. Thereafter, these two blue variants reared in our laboratory attained sexual maturity in the breeding season of 1978. Six mature wild-type tree-frogs which had been reared from the egg stage in our laboratory were used for comparison with the blue variants.

The two blue and six wild-type tree-frogs were adapted for 24 hours to a light environment under a fluorescent lamp. A piece of skin, about 5 mm square in size, was cut off from the dorsal surface of each of these tree-frogs, cut into minute pieces 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 were dehydrated in an ethanol series and embedded in Epon 812. Sections were made on a Porter-Blum MT-1 ultramicrotome at the thickness of silver or silver-gold and double stained with saturated uranyl acetate and alkaline lead citrate. Observation was made under a Hitachi Hs-8 electron microscope.

OBSERVATION

I. Normal tree-frogs

Normal tree-frogs are green or yellowish green in most cases and become gray, grayish brown, or dark brown in accordance with change of environment (Plate

Fig. 1. Electron microphotograph of dermal chromatophores in the dorsal skin of a green wild-type *Hyla arborea japonica*. ×4500

X, xanthophore I, iridophore M, melanophore

I, 1, 2). When they are adapted for 24 hours to a light environment under a fluorescent lamp, their dorsal surfaces usually become bright yellowish-green.

The dermal chromatophores of six normal tree-frogs adapted to a light environment were observed under an electron microscope (Fig. 1). Three kinds of chromatophores, upper xanthophores, middle iridophores and under melanophores, construct the dermal chromatophore unit which has been designated as an integral, functional unit by BAGNARA, TAYLOR and HADLEY (1968).

a. Xanthophore

Xanthophores are convex lens-shaped cells expanded horizontally and have short dendritic processes. The convex undersurface of each xanthophore is usually suited close to the concave surface of an underlaid cup-like iridophore. When the median sections of 20 xanthophores were measured, they were 13.8～32.3 μ, 19.20 μ on the average, in width and 3.8～8.3 μ, 6.0 μ on the average, in thickness.

The cytoplasm of xanthophores is filled with pterinosomes and carotenoid vesicles. The carotenoid vesicles are nearly spherical. When 50 of them were measured, they were 0.18～0.35 μ, 0.26 μ on the average, in diameter. However, a few large carotenoid vesicles, being 0.40～0.63 μ in diameter, were found here and there. Pterinosomes are ellipsoids in shape. Fifty of them were 0.73～1.16 μ, 0.93 μ on the average, in major axis and 0.53～0.89 μ, 0.73 μ on the average, in minor axis (Fig. 2a).

In expanded xanthophores, pterinosomes are mingled with carotenoid vesicles and evenly distributed in the cytoplasm, although the most part of the cytoplasm is occupied by carotenoid vesicles. On the other hand, the two kinds of granules are separately distributed in contracted xanthophores, when the dorsal surfaces of tree-frogs are grayish brown or dark brown. In this case, carotenoid vesicles are gathered in the center of the cytoplasm and surrounded by pterinosomes.

b. Iridophore

Iridophores are cup-shaped and closely attached to the convex undersurfaces of xanthophores. These two kinds of chromatophores have many short processes which are intertwined. When the median sections of 20 iridophores were measured, they were 17.3～22.8 μ, 20.20 μ on the average, in width and 4.5～7.3 μ, 6.04 μ on the average, in thickness. Adjacent iridophores are usually separated from each other at an interval of about 1 μ on the average, 3.3 μ at the widest. Except a narrow area surrounding the nucleus, each iridophore is filled with reflecting platelets. In electron microphotographs, only the traces of reflecting platelets are observable, as the crystalline structures of reflecting platelets have fallen out. About 10 reflecting platelets are arranged in parallel to one another and form a group. The groups of reflecting platelets are mostly arranged almost parallel to the cell surface. When 42 traces of reflecting platelets were measured, they were 1.3～2.7 μ, 1.85 μ on the average, in length and 0.05～0.13 μ, 0.09 μ on the average, in thickness. The spaces between the limiting membranes of

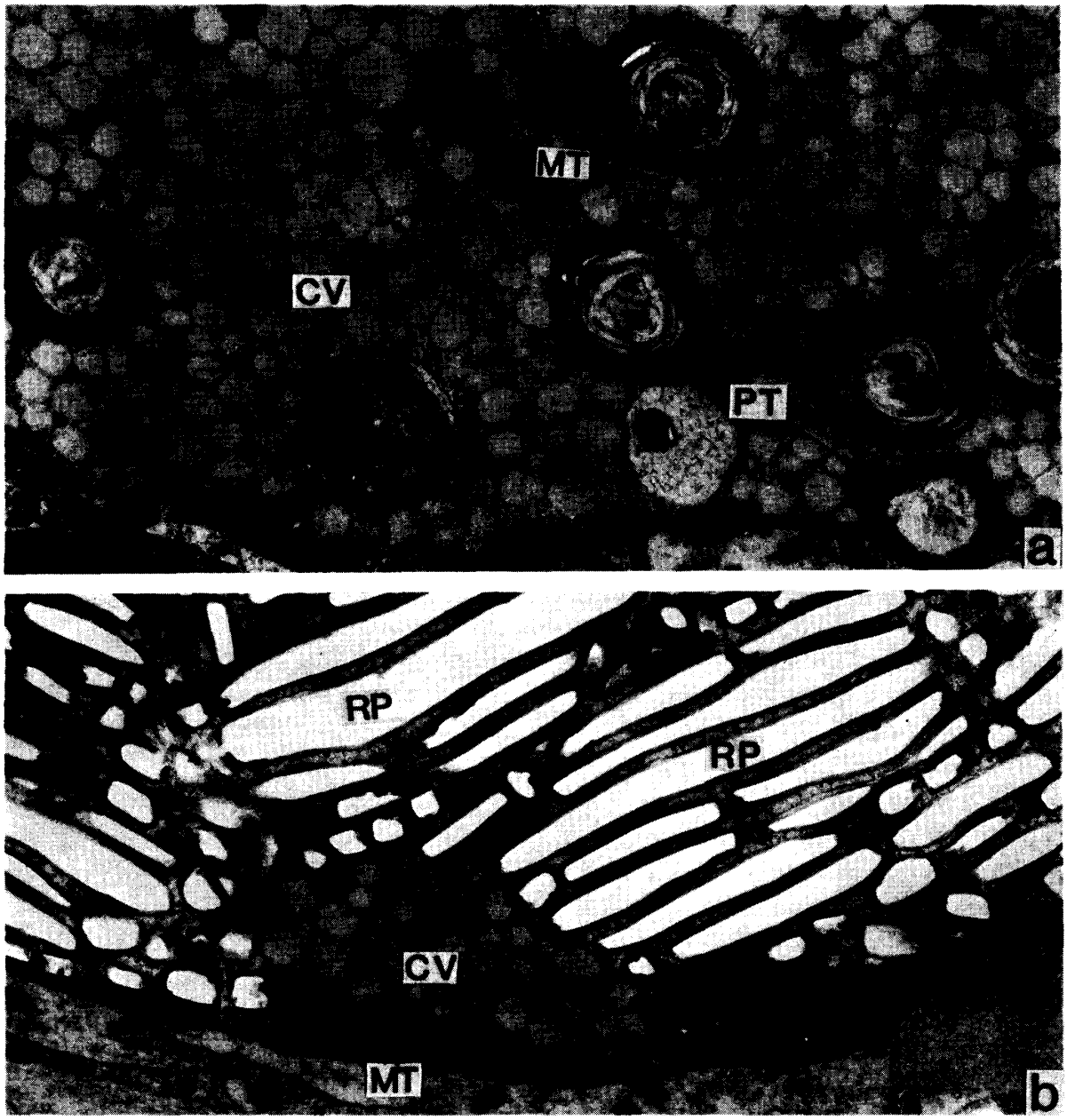

Fig. 2. Electron microphotographs of dermal chromatophores in the dorsal skin of a green wild-type *Hyla arborea japonica*. ×22000

a. Xanthophore. b. Iridophore.
CV, carotenoid vesicles PT, pterinosomes
RP, reflecting platelets MT, mitochondria

reflecting platelets arranged in a group were 0.03～0.06 μ wide, while the intervals between the holes were 0.09～0.11 μ wide.

There are some iridophores which include a few carotenoid vesicles in the cytoplasm (Fig. 2b).

c. Melanophore

Melanophores have long dendritic processes which constitute a thick layer under

the iridophores. The processes of adjacent melanophores are complicatedly overlapped with one another. Each melanophore and its processes are filled with spherical or ellipsoidal melanosomes. When 20 ellipsoidal melanosomes were measured, they were 0.50～0.73 μ, 0.68 μ on the average, and 0.30～0.35 μ, 0.31 μ on the average, in major and minor axes, respectively. When 14 nearly spherical melanosomes were measured, they were 0.32～0.71 μ, 0.47 μ on the average, in diameter (Fig. 1).

II. Blue variants

1. Production of offspring

a. Blue variant from Tottori

As the blue variant from Tottori was a female, ovulation was accelerated by injecting frog pituitaries. Offspring were produced by mating with normal males as well as by diploid gynogenesis (cf. NISHIOKA and UEDA, 1977). The results of experiments are presented in Table 1.

Of 284 eggs inseminated with sperm of two normal males, Nos. 1 and 2, 276 (97.2%) cleaved normally, 260 (91.5%) hatched normally and 229 (80.6%) metamorphosed normally. All these froglets were normal in coloration. On the other hand, only five of 1044 eggs hatched normally by diploid gynogenesis and four attained completion of metamorphosis. These froglets were all normal in coloration.

TABLE 1

Offspring of two kinds of blue variants in *Hyla arborea japonica*

Year	Parents		No. of eggs	No. of normal cleavages	No. of hatched tadpoles	No. of frogs		
	Female	Male				Total	Wild	Blue
1978	T. Blue No. 1	W. 78, Nos. 1, 2	284	276	260	229	229	0
		GD	1044	1019	5	4	4	0
	K. Blue No. 1	W. 78, Nos. 1, 2	210	173	146	131	131	0
		GD	380	323	33	17	17	0
1979	T. Blue♀ × W♂ No. 1	T. Blue♀ × W♂ No. 1	379	375	343	303	303	0
		K. Blue♀ × W♂ No. 1	277	275	243	164	164	0
	K. Blue♀ × W♂ No. 1	K. Blue♀ × W♂ No. 2	429	422	403	311	311	0
		T. Blue♀ × W♂ No. 2	158	156	142	97	97	0

T, Tottori K, Konosu W, field-caught GD, diploid gynogenesis

In the breeding season of 1979, the offspring of the female blue variant from Tottori mated with normal males were sexually matured. Then, a brother and sister mating between a female and a male offspring was made to produce F_2 offspring. It was found that 375 (98.9%) of 379 eggs cleaved normally, 343

(90.5%) hatched normally and 303 (79.9%) metamorphosed normally. All these froglets were normal in coloration. Thus, it is evident that the blue color is not a heritable character (Table 1).

b. Blue variant from Konosu

This tree-frog was a female. Ovulation was accelerated by injecting frog pituitaries in May, 1978. Eggs were inseminated with sperm of two normal males. As presented in Table 1, 173 (82.4%) of 210 eggs cleaved normally, 146 (69.5%) hatched normally and 131 (62.4%) metamorphosed normally. All these froglets were normal in coloration. On the other hand, 33 (8.7%) of 380 eggs hatched normally by diploid gynogenesis and 17 (4.5%) attained completion of metamorphosis. These 17 froglets were all normal in coloration.

In June, 1979, the offspring of the female blue variant from Konosu mated with normal males were sexually matured. Then, a brother and sister mating between a female and a male offspring was made to produce F_2 offspring. It was found that 422 (98.4%) of 429 eggs cleaved normally, 403 (93.9%) hatched normally and 311 (72.5%) metamorphosed normally. All these froglets were normal in coloration. Thus, it is evident that the blue variant collected from Konosu is not a mutant.

Reciprocal matings between offspring of the blue variant from Tottori and those of the blue variant from Konosu were made. As presented in Table 1, 164 froglets originated from Tottori blue variant and 97 froglets from Konosu blue variant were all normal in coloration, as expected.

2. Dermal chromatophores

a. Blue variant from Tottori

The dorsal surfaces of this variant were bright blue when it was adapted to a light environment (Plate I, 3), while they became brownish gray under a dark environment. This state of affairs was kept from the juvenile stage in summer of 1977 to the sexually mature stage in summer of 1978. Thereafter, three small green spots suddenly appeared in the central part of the blue back. Such spots increased very slowly in number and size and became green areas on the back, trunk and hind legs in summer of 1979 (Plate I, 4).

i) Blue area

On August 22, 1978, the dermal chromatophores of this variant which had still no green spots on the dorsal surfaces were examined under an electron microscope. It was found that there were no xanthophores in any part of the dorsal skin. The place of xanthophores under the epidermis in normal tree-frogs was occupied by iridophores alone (Fig. 3).

The median sections of 10 iridophores were measured. The results showed that they were 15.10~23.23 μ, 19.74 μ on the average, in width and did not remarkably differ from those of the normal tree-frogs in this respect. In contrast, they were 9.30~13.60 μ, 10.98μ on the average, in thickness, that is, about twice thicker than the latter or nearly equal in thickness to the sum of xanthophores and

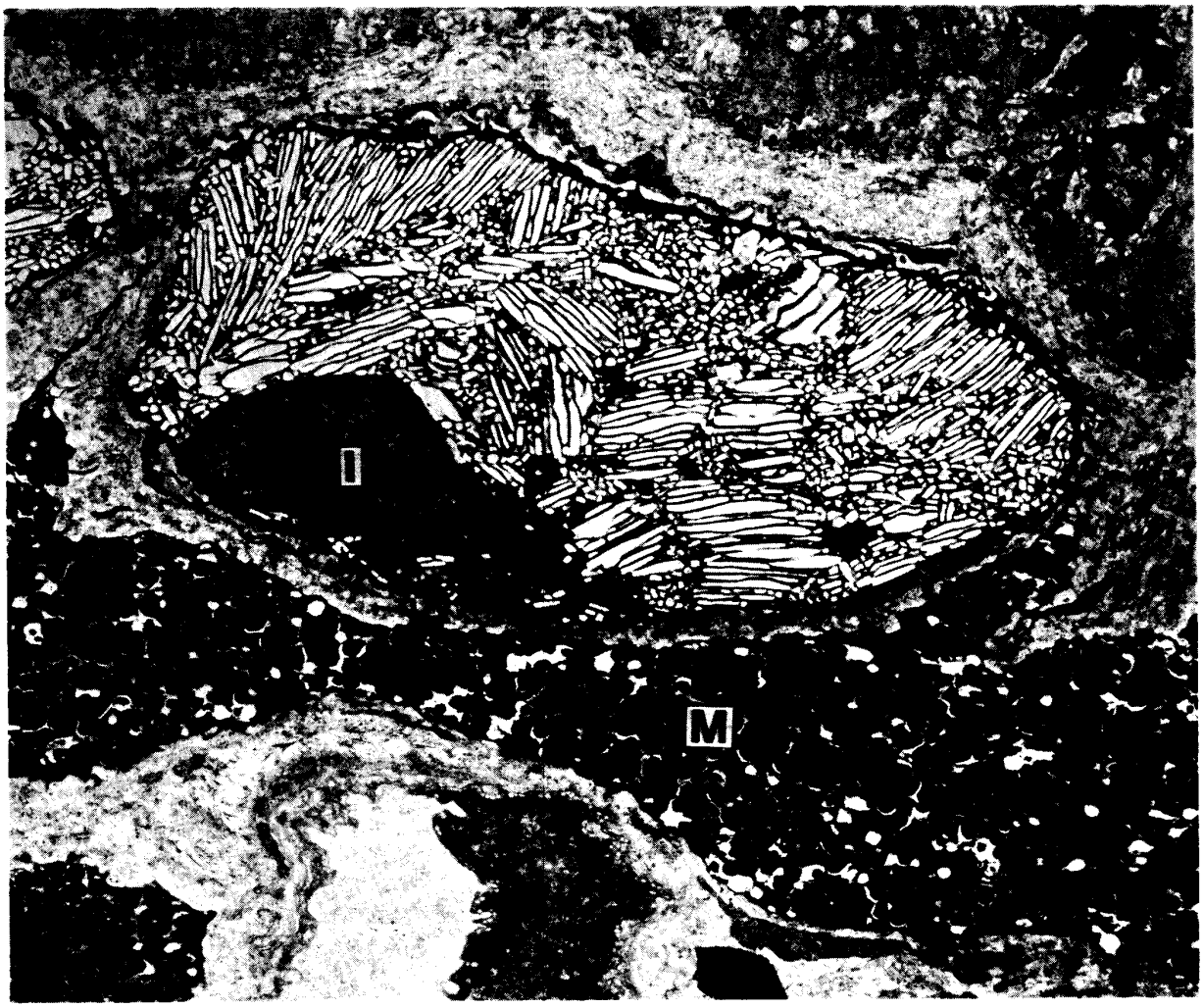

Fig. 3. Electron microphotograph of dermal chromatophores in a blue area of the dorsal skin of a blue variant from Tottori. ×4500

I, iridophore M, melanophore

iridophores of the normal tree-frogs. Moreover, the iridophores of the blue variant differed from those of the normal tree-frogs in shape, as the former were semicircular in the median section, while the latter were somewhat crescent-shaped.

The reflecting platelets contained in the iridophores of the blue variant did not differ in size and shape from those of the normal tree-frogs. Twenty-five of them were 0.06～0.10 μ, 0.09 μ on the average, in thickness and 1.0～2.3 μ, 1.83 μ on the average, in length. However, they differed in arrangement from those of the normal tree-frogs. While the reflecting platelets contained in the lower one-third or two-thirds of each iridophore were arranged parallel to the cell surfaces as found in the iridophores of the normal tree-frogs, those contained in the remaining upper part were arranged almost perpendicular to the upper flat surface of the iridophore.

The melanophores of this blue variant were more largely developed and had thicker processes than those of the normal frogs. The processes of adjacent melanophores were complicatedly overlapped with one another. Thus, the

Fig. 4. Electron microphotograph of dermal chromatophores in a green area of the dorsal skin of a blue variant from Tottori. ×4500

X, xanthophore I, iridophore M, melanophore

melanophores constructed a continuous thick layer. The melanosomes did not differ from those of the normal tree-frogs in size and shape, while they were distinctly more numerous than the latter (Fig. 3).

ii) Green area

On July 24, 1979, the dermal chromatophores in the dorsal green areas were examined after the blue variant had been adapted to a light environment under a fluorescent lamp (Plate I, 4). It was found that there were some xanthophores lying upon iridophores here and there. However, these xanthophores were remarkably inferior in development, especially in thickness, as compared with those of the normal tree-frogs (Fig. 4). When the median sections of 15 xanthophores were measured, they were 12.3~34.0 μ, 17.10 μ on the average, in width and 1.7~6.0 μ, 3.01 μ on the average, in thickness. The cytoplasm was almost completely filled with carotenoid vesicles, which were remarkably lower in electron density than those of the normal tree-frogs. When 20 carotenoid vesicles were measured, they were 0.21~1.14 μ, 0.36 μ on the average, in diameter (Figs. 4,

5a). The pterinosomes were very few and, moreover, their inside was scarcely constructed of concentric lamellae.

Iridophores and melanophores were the same in size, shape and inner structure as those found in the skin of the blue area.

b. Blue variant from Konosu

When this variant arrived at the Laboratory for Amphibian Biology and was

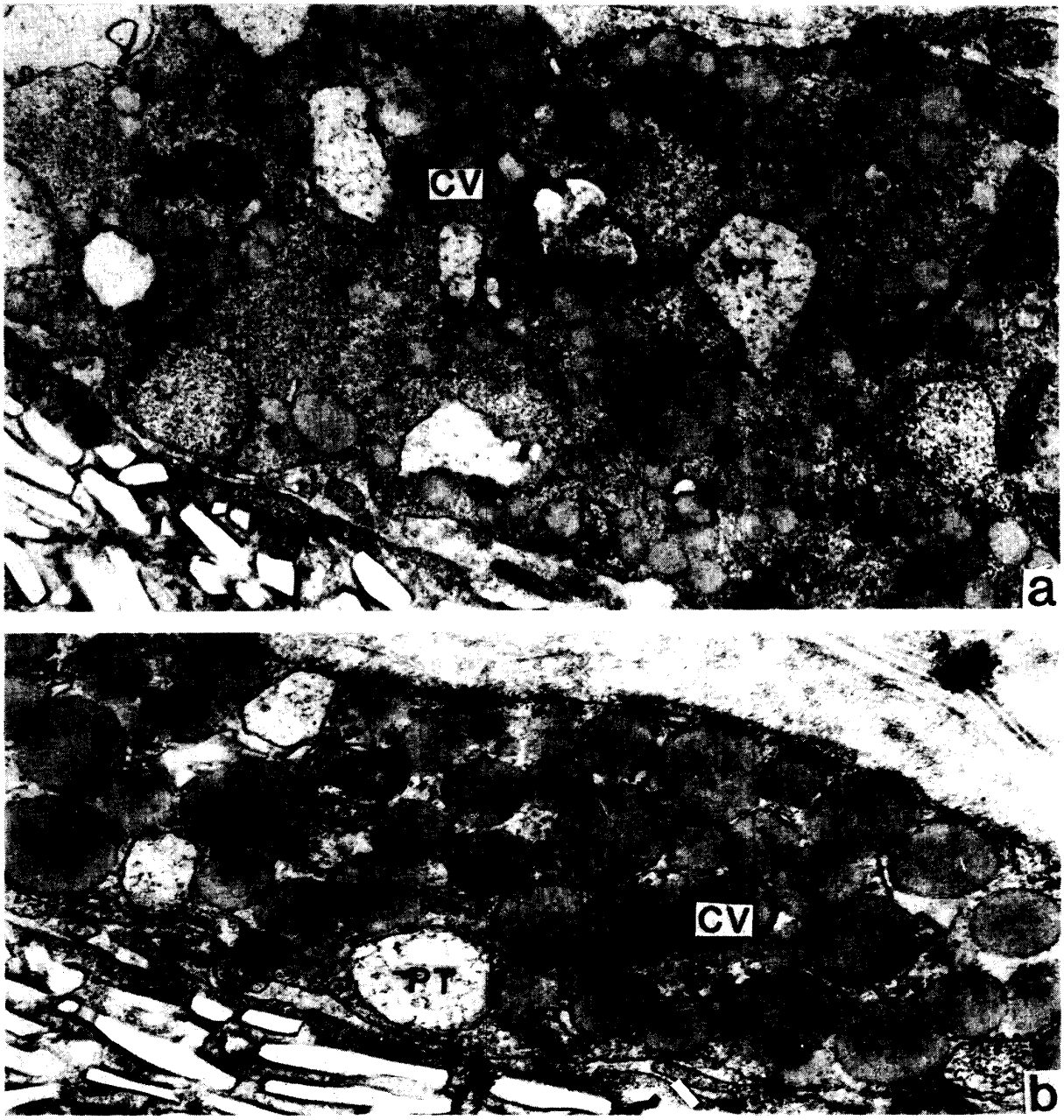

Fig. 5. Electron microphotographs of xanthophores in green areas of the dorsal skins of blue variants. ×22000

a. Abnormal xanthophore in a blue variant from Tottori.
b. Abnormal xanthophore in a blue variant from Konosu.

PT, pterinosomes CV, carotenoid vesicles

observed after adapted to a light environment, the dorsal surfaces were bright blue except narrow areas above the left eye and the left and right tympanic membranes and on the posterior part of the back (Plate I, 5). These exceptional areas were yellowish green and about 2 mm in length and breadth. In June, 1979, they became somewhat larger (Plate I, 6).

The general color of the dorsal surfaces of the variant from Konosu changed from sky blue to brownish white with the change of environment. When compared with the variant from Tottori, this was always more whitish.

On June 22, 1978, the dermal chromatophores in the dorsal skin of this variant which had been adapted to a light environment were examined. On July 24, 1979, the dermal chromatophores of the green area in the posterior part of the back were also examined after the variant had been adapted to a light environment.

i) Blue area

As found in the blue variant from Tottori, this variant had no xanthophores in all the blue areas. The dermal site for both xanthophores and iridophores in the normal tree-frogs was occupied by iridophores alone. These iridophores were nearly the same in size and shape as those of the variant from Tottori (Fig. 6).

The median sections of 11 iridophores were 14.3～25.5 μ, 20.70 μ on the average, in width and scarcely differed from those of iridophores found in the normal tree-frogs in this respect. However, they were 6.1～14.0 μ, 10.13 μ on the average, in thickness and nearly twice as thick as the latter. In this respect, the variant from Konosu was the same as that from Tottori.

The reflecting platelets contained in iridophores did not differ in size and shape from those of the normal tree-frogs as well as from those of the variant from Tottori. When 25 reflecting platelets were measured, it was found that they were 0.05～0.10 μ, 0.09 μ on the average, in thickness and 1.3～2.5 μ, 1.85 μ on the average, in length. As found in the variant from Tottori, the reflecting platelets remarkably differed in arrangement from those of the normal tree-frogs. While the reflecting platelets contained in the lower part of each iridophore were arranged in almost parallel to the cell surfaces, those contained in the upper part were generally arranged almost perpendicular to the upper flat surface of the cell.

There were some iridophores which included a few carotenoid vesicles. Dermal melanophores of the variant from Konosu were distinctly smaller and fewer than those of the variant from Tottori as well as those of the normal tree-frogs. While the melanophores of the variant from Tottori and of the normal tree-frogs were widely expanded and rather difficult to be counted, each iridophore was always backed with one melanophore in the variant from Konosu. In contrast to the melanophores which constituted a continual thick layer in the variant from Tottori, the melanophores of the variant from Konosu were sparsely distributed and there were some iridophores which were not backed with a melanophore. This seems to be the cause of the finding that the blue color of the variant from Konosu was somewhat whitish as compared with that of the variant from Tottori.

Melanosomes contained in the melanophores were nearly the same as those of

Fig. 6. Electron microphotograph of dermal chromatophores in a blue area of the dorsal skin of a blue variant from Konosu. ×4500

I, iridophore M, melanophore

the normal tree-frogs in size, shape and density (Fig. 6).

ii) Green area

On July 24, 1979, the dermal chromatophores in the green area situated in the posterior part of the back were examined after the variant was adapted to a light environment. The results showed that they were completely the same as those of the variant from Tottori in that there were three kinds of chromatophores, xanthophores, iridophores and melanophores. Both iridophores and melanophores were the same as those found in the blue area of this variant in size, shape and inner structure. The reflecting platelets contained in the iridophores as well as the melanosomes in the melanophores were also the same as those found in the blue area in size, shape and arrangement. However, xanthophores were remarkably inferior in development as compared with those of the normal tree-frogs (Fig. 7). They were smaller and thinner than those of the latter.

The median sections of 10 xanthophores were 11.0~21.4 μ, 15.50 μ on the average, in width and 1.4~4.0 μ, 3.10 μ on the average, in thickness. The

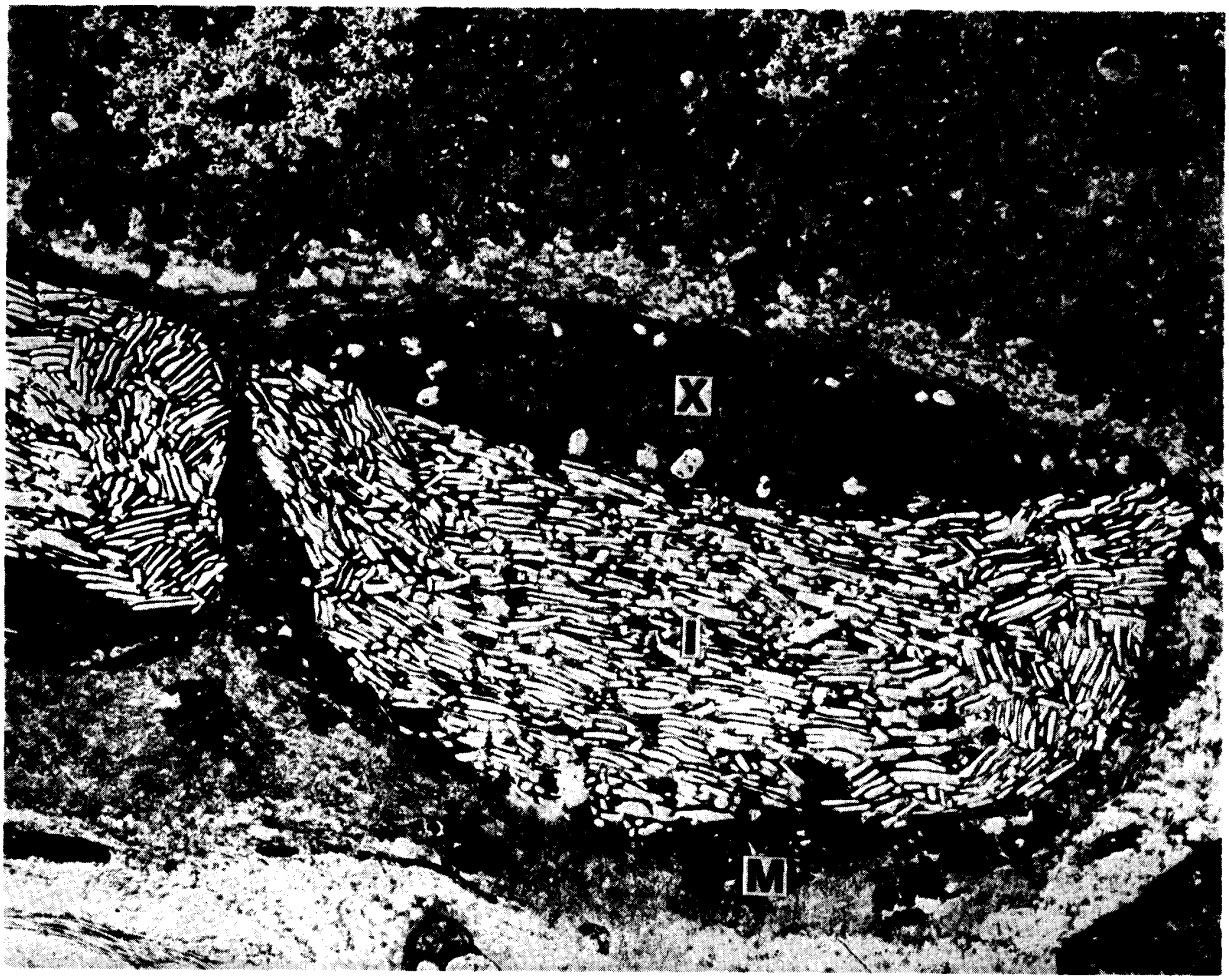

Fig. 7. Electron microphotograph of dermal chromatophores in a green area of the dorsal skin of a blue variant from Konosu. ×4500

X, xanthophore I, iridophore M, melanophore

pigment granules contained in xanthophores were very inferior in development as compared with those of the normal tree-frogs. Especially, the pterinosomes were extremely few. There were many xanthophores which scarcely contained pterinosomes. Moreover, the pterinosomes revealed no lamellar structure. The xanthophores were almost completely filled with carotenoid vesicles, which were inferior in development as compared with those of the normal tree-frogs. The carotenoid vesicles contained in the xanthophores of the blue variant from Konosu were remarkably larger than those of the variant from Tottori as well as those of the normal tree-frogs. When 20 of them were measured, they were 0.42～1.36 μ, 0.62 μ on the average, in diameter (Fig. 5b).

In the surroundings of the green area, dermal parts including three kinds of chromatophores, xanthophores, iridophores and melanophores, and those including two kinds of chromatophores, iridophores and melanophores, were intermingled. The xanthophores found in these areas were the same as those of the green area in size, shape and inner structure.

DISCUSSION

Blue variants of anurans have occasionally been found in the field (LIU, 1931; MARTOF, 1962; BERNS and UHLER, 1966; BLACK, 1967; BERNS and NARAYAN, 1970). However, the difference between the blue and green skins was clarified for the first time under an electron microscope by BERNS and NARAYAN (1970). The specimens used by these investigators were two blue variants of *Rana clamitans*.

NISHIOKA (1977) found 26 blue variants in the diploid gynogenetic offspring of seven females raised from irradiated gametes of *Rana nigromaculata*. These gametes were spermatozoa exposed to 90 or 170 rads of X-rays or 130 rads of neutrons and eggs exposed to 50 rads of neutrons. It was evident that the blue variants are induced mutants due to a recessive gene *e*. The dorsal surfaces of these mutants were blue in ground color. The dermal chromatophores of the blue integument were observed under a light and an electron microscope by NISHIOKA and UEDA (1977a). Under a light microscope, xanthophores were invisible by lighting with transmitted light. When examined under an electron microscope, the three kinds of dermal chromatophores, xanthophores, iridophores and melanophores, were very similar to those of green wild-type frogs, except that the xanthophores contained no carotenoid vesicles.

In the present study, two female blue variants of *Hyla arborea japonica* collected from the field were genetically and morphologically examined. The blue color of both variants was not a heritable character, as the gynogenetic diploids produced from eggs of these blue variants as well as the F_2 of the latter mated with wild-type males were all of the wild-type in coloration. Thus, it became evident that blue variants found in the field are not always spontaneous mutants. The two blue variants described by BERNS and NARAYAN (1970) seem also not to be spontaneous mutants, as the breeding experiments performed by BERNS and UHLER (1966) between blue adults, and blue and green adults failed to yield blue offspring.

While the dorsal surfaces of the blue mutants induced by irradiation of gametes were uniformly blue and had no green areas, the two blue variants collected from Konosu and Tottori had several small green areas on the back. The blue variants described by BERNS and NARAYAN were similar to the above variants in that there were narrow green areas at different positions. In contrast with the blue mutants produced by irradiation of gametes, the two blue variants collected from Konosu and Tottori had no xanthophores in the blue area. The site for xanthophores was occupied by iridophores which were about twice as thick as those of normal tree-frogs. In the green area, the xanthophores were remarkably smaller and thinner than those of normal tree-frogs, although they were normally arranged above the iridophores. These xanthophores were almost completely filled with carotenoid vesicles, while the pterinosomes were extremely few and scarcely revealed a lamellar structure.

The blue area of these variants was very similar to that of the variants described by BERNS and NARAYAN, in which neither pterinosomes nor carotenoid vesicles

were found in the region normally containing xanthophores. There was an abnormal cell type in place of xanthophores. On the other hand, the green area showing an abnormal structure of the xanthophores remarkably differed from that of the variants described by BERNS and NARAYAN, in which the xanthophores were of apparently normal structure.

Two other kinds of non-inheritable variants have been reported by the present authors (1985d). They are a white and a blackish variants found in *Rana brevipoda porosa*. The existence of non-heritable black-eyed variants has also been confirmed by the present authors in *Rana nigromaculata* and *Rana brevipoda porosa* (unpublished). In contrast with these, albinic variants in anurans have been confirmed to be due to a recessive gene by many investigators (EALES, 1933; SMALLCOMBE, 1949; TOKUNAGA, 1949; BROWDER, 1967, 1972; SMITH-GILL, RICHARDS and NACE, 1970, 1972; HOPERSKAYA, 1975; NISHIOKA, 1977; NISHIOKA and UEDA, 1977b, 1985a, b). Heritable black-eyed variants also have been reported in *Rhacophorus schlegelii* and *Hyla arborea japonica* by the present authors (1985b, c).

It is interesting to note that the heritable variants such as the albinos and some of the blue and the black-eyed variants are deficient in formation of melanosomes, carotenoid vesicles or reflecting platelets, while non-heritable variants such as some of the blue and black-eyed variants and the white and blackish variants are abnormal as a whole in formation of one or more kinds of dermal chromatophores.

It seems strange that heritable blue variants have not yet been collected from the field hitherto. It is very desirable to confirm the existence of heritable blue variants in nature. Natural occurrence of such heritable variants seems to be very probable, as they can be produced artificially by the method of irradiating gametes (NISHIOKA, 1977).

SUMMARY

1. Two female blue variants in *Hyla arborea japonica* were discovered in 1977 in Konosu, Saitama Prefecture and Tottori situated about 500 km west of Konosu. The blue color of these variants was not a heritable character, because gynogenetic diploids produced from eggs of the blue variants as well as the F_2 of these variants mated with wild-type males were all of the wild-type in coloration.

2. When these two blue variants were adapted to a light environment, their dorsal surfaces were bright blue, although the variant from Konosu was somewhat whitish as compared with that from Tottori. Both variants had several small green areas on the back at the sexually mature stage. The dermal chromatophores in the blue and green areas on the dorsal surface of the body were observed under an electron microscope.

3. Both blue variants from Tottori and Konosu had no xanthophores in the blue area. The site for xanthophores was occupied by iridophores which were about twice thicker than those of normal tree-frogs. Iridophores and melanophores were almost normal in inner structure.

4. While the melanophores constructed a continuous thick layer under the

iridophores in the blue area of the blue variant from Tottori, those of the variant from Konosu were sparsely distributed and there were some iridophores which were not backed with a melanophore. This seems to be the cause of the finding that the blue color of the variant from Konosu was somewhat whitish as compared with that of the variant from Tottori. Some iridophores in the blue area of the variant from Konosu contained a few carotenoid vesicles.

5. In the green areas of both variants from Tottori and Konosu, there were three kinds of chromatophores, xanthophores, iridophores and melanophores. The iridophores and melanophores were nearly the same in size, shape and inner structure as those found in the blue area. The xanthophores were remarkably smaller and thinner than those of normal tree-frogs. In the xanthophores of both variants, the pterinosomes were extremely few and scarcely revealed a lamellar structure. The cytoplasm of xanthophores was almost completely filled with carotenoid vesicles. The carotenoid vesicles of the variant from Konosu were remarkably larger than those of the variant from Tottori as well as those of normal tree-frogs.

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LITERATURE

BAGNARA, J. T., J. D. TAYLOR and M. E. HADLEY 1968. The dermal chromatophore unit. J. Cell Biol., **38**: 67–79.

BERNS, M. W. and K. S. NARAYAN 1970. An histochemical and ultrastructural analysis of the dermal chromatophores of the variant Ranid blue frog. J. Morph., **132**: 169–180.

BERNS, M. W. and L. D. UHLER 1966. Blue frogs of the genus *Rana*. Herpetologica, **22**: 181–183.

BLACK, J. H. 1967. A blue leopard frog from Montana. Herpetologica, **23**: 314–315.

BROWDER, L. W. 1967. Pigmentation in *Rana pipiens*. A study in developmental genetics. Ph. D. Thesis, University of Minnesota.

———— 1972. Genetic and embryological studies of albinism in *Rana pipiens*. J. Exp. Zool., **180**: 149–155.

EALES, N. B. 1933. Albinism in the common frog. Nature, **132**: 278–279.

HOPERSKAYA, O. A. 1975. The development of animals homozygous for a mutation causing periodic albinism (a^p) in *Xenopus laevis*. J. Embryol. exp. Morph., **34**: 253–264.

LIU, C. 1931. The occurrence of blue frogs in the Peiping region. China J., **15**: 248–249.

MARTOF, B. S. 1962. An unusual color variant of *Rana pipiens*. Herpetologica, **17**: 169.

NISHIOKA, M. 1977. Color variants induced by radiation and their inheritance in *Rana nigromaculata*. Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ., **2**: 25–89.

NISHIOKA, M. and H. UEDA 1977a. An electron-microscopic study on six kinds of color variants induced by radiation in *Rana nigromaculata*. Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ., **2**: 91–102.

———— 1977b. Genetic and morphologic studies on ten albino stocks in *Hyla arborea japonica*. Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ., **2**: 103–163.

———— 1985a. Genetics and morphology of 13 albino stocks in the *Rana nigromaculata* group. Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ., **7**: 1–121.

———— 1985b. Electron-microscopic observation on the dermal chromatophores of normal frogs and three kinds of color variants in *Rhacophorus schlegelii*. Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ., **7**: 123–155.

———— 1985c. Two kinds of black-eyed variants in *Hyla arborea japonica*. Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ., **7**: 157–179.

———— 1985d. Non-inheritable color variants in *Rana brevipoda porosa*. Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ., **7**: 199–217.

SMALLCOMBE, W. A. 1949. Albinism in *Rana temporaria*. J. Genet., **49**: 286–290.

SMITH-GILL, S. J., C. M. RICHARDS and G. W. NACE 1970. Biochemical and developmental studies on albinism in *Rana pipiens*. Amer. Zool., **10**: 529.

———— 1972. Genetic and metabolic bases of two "albino" phenotypes in the leopard frog, *Rana pipiens*. J. Exp. Zool., **180**: 157–167.

TOKUNAGA, C. 1949. Albino frogs produced by artificial parthenogenesis. J. Hered., **40**: 279–281.

EXPLANATION OF PLATE

PLATE I

Two kinds of blue variants and wild-type tree-frogs. ×1

1. Green wild-type tree-frog.
2. Brown wild-type tree-frog.
3. Blue variant from Tottori. Photographed in May, 1978.
4. The same individual as shown in Fig. 3. Photographed in June, 1979. A large green area is found on the dorsal surface.
5. Blue variant from Konosu. Photographed in May, 1978.
6. The same individual as shown in Fig. 5. Photographed in June, 1979. Some green spots are found on the dorsal surface.

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