

*Original Article*

## Color Expression on Dorsal Skin of Japanese Tree Frogs

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Japanese tree frogs are known to change their body color readily according to external (ambient) or internal (mental) conditions. The coloration of the frog skin is shown by pigment cells distributed under the epidermis. Pigment cells in amphibians are classified into three types: melanophores, iridophores and xanthophores. These pigment cells in the dorsal skin are formed chromatophore units, each of which consists of three different types of pigment cells. In the chromatophore unit, these pigment cells are layered one on the other: that is, a melanophore on the innermost layer, a xanthophore on the outermost and an iridophore in the middle. Various colors of the chromatophore unit combines spectra from each pigment cell, and the total skin color of a certain area results from a combination of colors from chromatophore units in the area. In this study, we examined how the chromatophore unit can show various colors, and to what extent it can exhibit a color range in relation to its component pigment cells. We also examined the total skin coloration in relation to the chromatophore unit deduced by computer calibration. It was also determined that the various patterns of brown or black stripes, which appeared against the green background skin, were caused by differences of MSH sensitivities of melanophores in the skin area.

**Key Words:** Tree frogs, skin color, chromatophore unit, color exhibition, pattern formation

### Introduction

Vertebrates exhibit various colors on their body surface. The body colors in poikilotherms, such as teleosts, frogs and lizards are known to change according to external environmental factors or internal conditions of the animal, while homeotherms, such as mammals and birds have fixed and unchanged

coloration on their body surfaces. Of all amphibians and reptiles, coloration of tree frogs and Anole lizards show remarkable color change in a fairly short time and the mechanisms that regulate color change have been investigated (1~5). The color change in these animals is produced by three different types of pigment cells that are located under the epidermal layer of the skin. The pigments cells are melanophores, that contain black melanin granules, xanthophores, that contain yellow carotenoids granules and iridophores, that contain colorless purine crystal platelets which refract incoming light into spectra and reflect shorter wave lengths, such as blue backwards. In anuran

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amphibians, these pigment cells are known to form chromatophore units in which one of the three pigment cell types is placed one on another so as to form layers with xanthophore, iridophore and melanophore aligned in order, from surface to innermost layer (1 and 3). Each pigment cell in the chromatophore unit change its coloration so that the coloration of the unit is the summation of colors from the three types of pigment cells that comprise the unit. In other words, the body color in tree frogs is an overall expression of the chromatophore units in the skin rather than the color of any pigment cell itself.

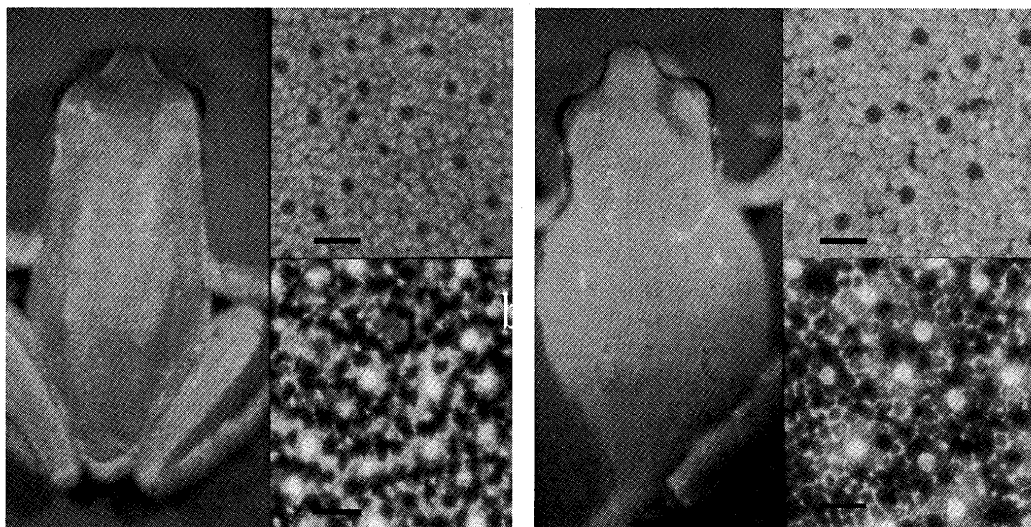
In the ordinary environments of bushes and shrubs, Japanese tree frogs show green on their dorsal skin. Under unfavorable conditions, they show remarkable color variations ranging from light brown to pure black, green to yellow green or blue-green, and grey to silver-grey or creamy white; along all the variations of these hues. However, mechanisms to elucidate these variable color expressions on Japanese frog skin have not yet been described. Since each pigment cell is controlled by hormonal and/or neuronal agents (1, 2 and 5), it must be understood how these variable and delicate colorations of the tree frog skin are produced in relation to each pigment cell and to the chromatophore units. This experiment demonstrates

that each type of cell in the unit continuously reflects its proper spectrum from full strength to almost transparent (no color is shown). Color expression of the chromatophore unit is calibrated as an integration of the colors of the three pigment cells in the unit. Overall skin coloration is simulated by feeding colors from chromatophore units, whose colors are the combined results of pigment cells that consist of the unit, into computer calibration.

The color change in Japanese tree frog skin does not mean only color variations on the skin. The expression of characteristic patterns, such as black bands or stripes with various gradations on the green or brown background skin, are also analyzed in relation to the melanophore modulating agent, MSH.

### Materials and methods

Japanese tree frogs (*Hyla japonica*) used in the experiment were collected from nearby fields at Okayama University (Okayama, Japan). The animals were kept in a laboratory condition with ambient temperature of  $25 \pm 5^\circ\text{C}$  and a photoperiod of 12 h light and 12 h dark with one hour transition time with gradual increases on the start and decrease on the end of the light period. The sexuality or size of the frogs was not considered in the experiment, although newly metamorphosed froglets were



**Fig. 1** Japanese tree frogs show various coloration on their dorsal skin. This depicts a green specimen (Fig. 1a) and a pale brown one (Fig. 1b). The right half of the each figure portrays a microscopic enlargement of the skin. The top ones are taken under reflected light and the bottom ones through transmitted light. The scale bar shows  $50\ \mu\text{m}$ . The dark or white spots about  $15\ \mu\text{m}$  in diameter on the reflected or transmitted picture, respectively, show openings of the mucous glands. Original color figures can be supplied on request to the corresponding author.

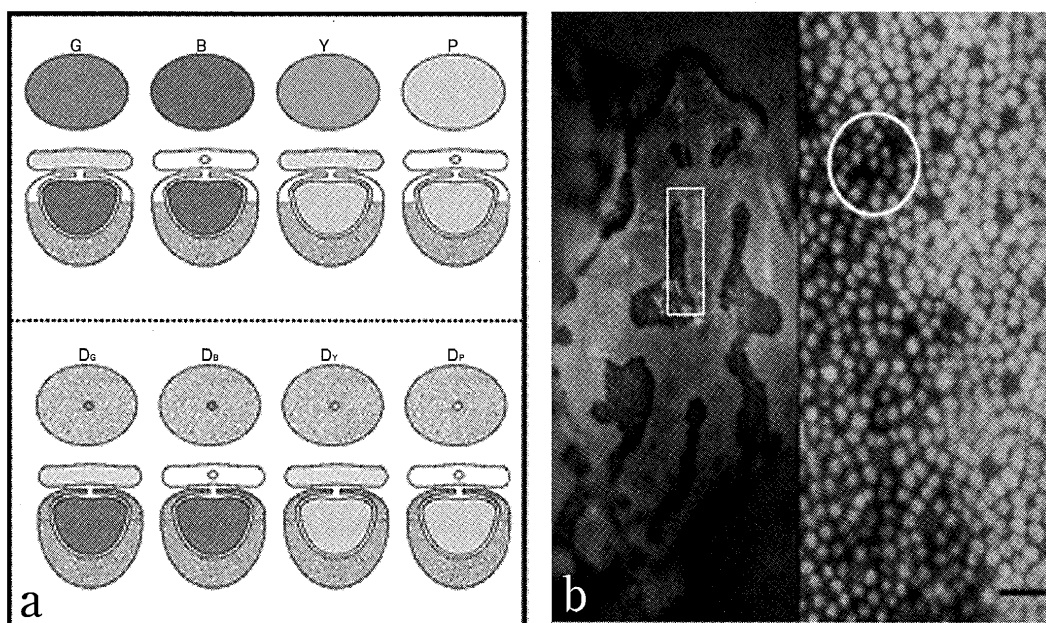
excluded. The animals were fed mealworms and had access to water, *ad libitum*.

The frogs were double pithed and decapitated before the dorsal skin was dissected. The skin was normalized for 30 min in the Ringer solution before being processed for each experiment. Usually white fluorescent tubes were used to induce color change on animals under an intensity of 5000 lux. Hormones (MSH and noradrenalin, both from Sigma) were dissolved in the Ringer solution and used at concentrations as indicated on the text.

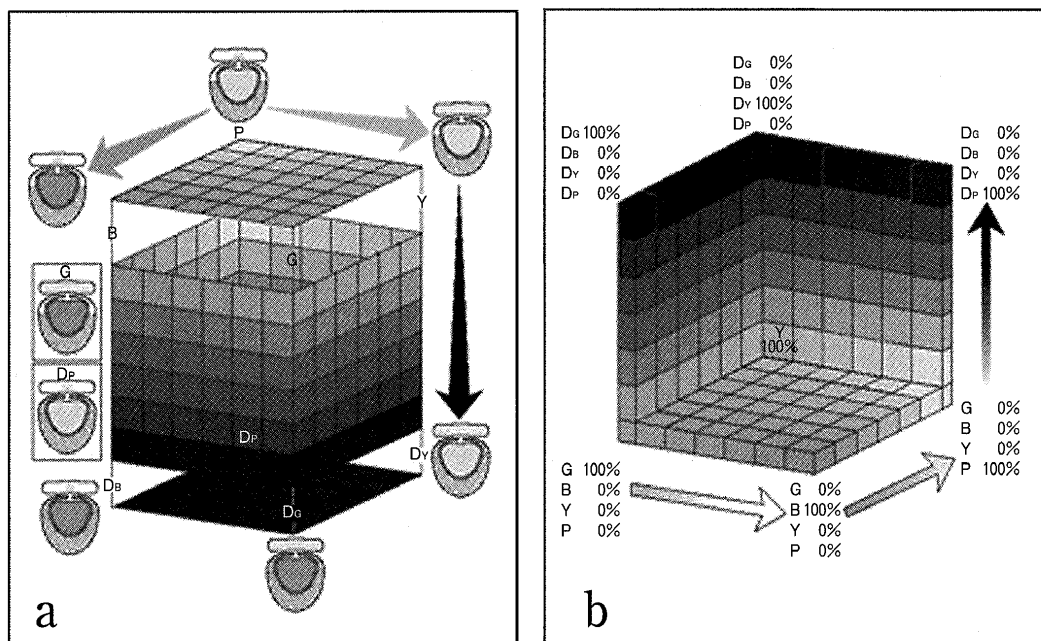
## Results and Discussion

In Japanese tree frogs, dorsal skin color was produced by chromatophore units (Fig. 1a) which are distributed under the epidermis. The chromatophore unit consisted of three different types of pigment cells, each of which was layered one on the other from the inside, in the order of xanthophore, iridophore and melanophore. About 3000

chromatophore units were distributed in one mm<sup>2</sup> of the dorsal skin (Figs. 1a and 1b). The xanthophore layer contained carotenoid pigments that produced a yellow color. The melanophore layer contained melanin granules that produced a black or brown color. The iridophore layer contained thin guanine platelets that reflected a strong blue interference color or a pale dull white diffusion light. Melanophore extended its dendrites outward to cover the side and top of the chromatophore unit. Each pigment cell changed its color due to the state of pigment granules it contained, that is, due to expansion or contraction of the pigment granules in the cytoplasm. While pigments in a cell were expanded fully in the cytoplasm, the cell showed its own proper coloration, such as the xanthophore was yellow, the iridophore blue and the melanophore black or brown. On the other hand, when pigment granules were contracted, the cell showed a pale or dull color, or even a transparency irrelevant to the type of cell. This observation summarizes that the



**Fig. 2** A schematic representation of three types of pigment cells in the chromatophore unit in fully expanded and contracted states of pigment granules (Fig. 2a). The upper oval figures show the color expression of the chromatophore unit under a combination of pigment cells represented in the lower figure and observed from the outside. The lower figures represent a lateral cross-section of the chromatophore unit consisting of three parts representing each pigment cell. A flat shape on the top of each figure represents a xanthophore, a cup shaped one in middle an iridophore and a curved one at the bottom melanophore. A frog with dark bands on light brown skin is showing (Fig 2b) and the enclosed (rectangle) portion of skin is microscopically enlarged at the far right. In the banded area, melanin granules are expanded into dendrites that cover the top of chromatophore unit which appears small in diameter and the interspace is widened (circled area in Fig. 2b). Appearance of the chromatophore unit is shown by abbreviation. G: green, B: blue, Y: yellow, P: pale and D: dark.



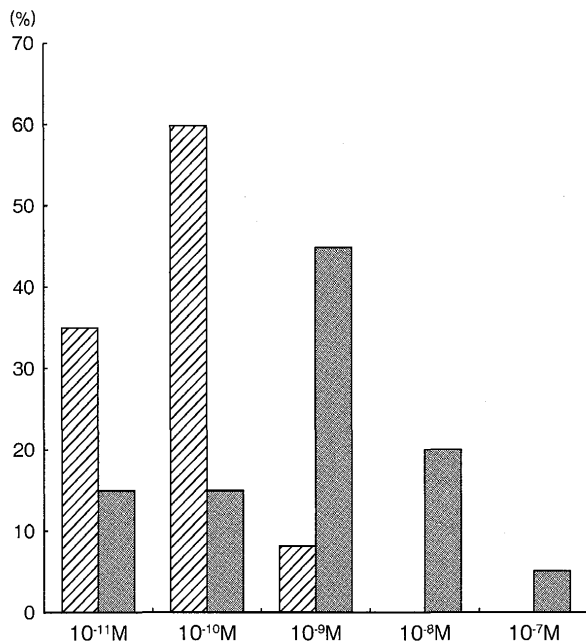
**Fig. 3** A schematic representation of colors expressed on the chromatophore unit by various states of pigment cells (Fig. 3a). The color expression is calibrated by computer with the eight states of pigment cells presented in Fig. 2a placed on each corner of the schematic cube. A final representation of skin colors is expressed by various states of chromatophore units, in which results are calibrated as states of chromatophore units placed at the corners of the cube (Fig. 3b).

chromatophore unit logically shows eight patterns according to each of three types of pigment cells in full expansion or full contraction (Fig. 2a). When the melanophore in the chromatophore unit is contracted and iridophore expanded, it shows green (G on Fig. 2a) or blue (B on Fig. 2a) due to the expansion or contraction of xanthophore pigments, or yellow (Y on Fig. 2a) or pale (P on Fig. 2a) when iridophore is contracted according to the expansion or contraction of the xanthophore. No matter what states the xanthophore and iridophore take, the chromatophore unit shows black when melanophore is fully expanded (lower figures on Fig. 2a). However, each cell in the unit can take any states between expansion and contraction of pigment granules, so that the chromatophore unit can exhibit various colors between these colors exhibited on Fig. 2a. These variable colorations of the chromatophore unit are logically simulated by computer calibration (Fig. 3a).

Since each chromatophore unit was about  $10\ \mu\text{m}$  in diameter and the color from it could not be discernible, so that the overall color in certain skin area was expressed as mixed colors from each chromatophore unit in the area. Overall skin

coloration according to the distribution of chromatophore units was schematically represented by computer calibration (Fig. 3b). The ordinary green coloration of tree frog skin under the arboreal environment such as shown in Fig. 1a was produced by 95% yellow chromatophore units (Y in Fig. 2a) and 5% of blue ones (B in Fig. 2a), respectively. Black melanophore underlying basal and lateral sides in the unit worked as an absorbing material for stray light scattered by tissue components, so that the reflected light from the skin surface was visually enhanced. Black coloration of various shades was expressed by an amount of melanin granules in dendrites covering top portions of the chromatophore units. Since apparent coloration of skin was an overall expression of all chromatophore units in the area, the body color was shown as distribution density of the each chromatophore unit in the area as shown in Fig. 3b.

Japanese tree frogs show proper color patterns of stripes or bands on their back. Since the color patterns were black or brown in color and always appeared on particular portion of skin (Fig. 2b), we examined and analyzed behavior of melanophores on banded and non-banded background area of skin to



**Fig. 4** The distribution of melanophores reacted to MSH in banded (striped) and background (grey) areas of a Japanese tree frog skin. Melanophores in the banded area are more sensitive to the hormone ( $10^{-10}$  M~ $10^{-11}$  M), than those in the background area where they are less sensitive melanophores ( $10^{-7}$  M~ $10^{-9}$  M). For abbreviations refer to Fig. 2.

melanophore stimulating hormone (MSH), an agent known to induce expansion of melanin granules (Fig. 4).

When excised skin was treated with MSH, melanin granules in a larger part of melanophores in the banded area were expanded at low concentration ( $10^{-10}$  M~ $10^{-11}$  M), while in melanophores in non-

banded areas (background area), the granules expanded at a higher concentration ( $10^{-7}$  M~ $10^{-9}$  M) of the agent. This indicates that melanophores in the banded area are extremely sensitive to MSH, while those in background area (non-banded area) are less sensitive to the hormone. By changing their MSH secretion, the tree frogs can produce various patterns of bands with various hues on their back skin. When melanin granules are expanded into dendrites that cover the top of a chromatophore unit which was decreased in diameter and the inter-space is widened, so that the overall appearance in the area are darkened (refer to rectangle and circled areas in Fig. 2b).

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**PS:** On request to the corresponding author, original color figures are supplied.

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