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Source: Zoological Science, 18(2) : 165-174

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.18.165

ZOOLOGICAL SCIENCE **18**: 165–174 (2001)

Coloration and Chromatophores of the Domino Damsel, *Dascyllus trimaculatus*

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ABSTRACT—The general background hue of the skin of the domino damsel (*Dascyllus trimaculatus*; Pomacentridae) is velvet-black, but three white spots, one on the median parietal region and the other two around the base of the dorsal fin, form a characteristic, conspicuous pattern for this species. In the dark background region, dermal melanophores form a dense monolayer, while in the white spots, a thick accumulation of non-dendritic iridophores is present in the dermis, which is lined with a layer of melanophores. The melanophores in the velvet-black area extend their processes parallel to the plane of the skin, and respond to various stimuli by aggregating or dispersing their melanosomes, which result in the hue changes there. Iridophores in the white spots contain many piles of thin light-reflecting platelets which are responsible for the whiteness of the spots through the multilayered thin-film interference phenomenon. In response to certain stimuli, the spectral reflectance peak shifts to some extent, and such responses may be due to simultaneous changes in the distance of platelets in those piles. The melanophores underlying the white spots extend their cellular processes through spaces among iridophores in order to cover them. When melanosomes migrate peripherally into the tips of the dendrites, they cover the underlying iridophores and the spot darkens; the whiteness reappears when the melanosomes move back down the dendrites into the perikarya of the melanophores.

INTRODUCTION

Many coastal and coral-reef fish display a wide variety of beautiful colors. Indeed, they attract many humans, including hobbyists, scuba divers and also zoologists. Nowadays, illustrated encyclopedia of marine fishes or of tropical fishes are easily available and many of them are in color. Live specimens of such species can of course be appreciated in aquaria, and many of those which can be reared easily are now frequently kept in home aquaria. Notwithstanding this trend of the hobbyistic world, relatively few scientific studies have been carried on their coloration, and most of those have been concerned with species gorgeously colored with red, yellow or blue (cf. Fujii, 1993a, b, 2000; Oshima, 2000).

As can easily be supposed, red-to-yellow hues are generated by the presence of corresponding colored materials in chromatophores that can be classified as erythrophores or xanthophores (Fujii, 1993a, b). We have also recently clarified the mechanisms by which the cobalt blue of blue damselfish (Kasukawa *et al*., 1987), the greenish hue of blue-green damselfish (Fujii *et al*., 1989) and the sky blue of surgeonfish (Goda *et al*., 1994; Goda and Fujii, 1998) are generated. To our surprise, such bluish hues are not dependent on a blue pigment, but are essentially due to multilayered thin-film interference phenomena of the non-ideal type that occur in iridophores. That is, they are physical colors. Further, as exceptional cases, we have recently found blue chromatophores (cyanophores) that actually contain bluish chromatosomes in two callionymid species (Goda and Fujii, 1995).

However, not all fishes inhabiting tropical waters are gorgeously colored, and some of them are rather restrained in their hues. Among such plainly colored fish is the subject of this study, the domino damsel. Their general background coloration is velvet-black, and three white spots form a pattern characteristic of this species. Actually however, this species is one of the most popular aquarium fish, primarily because of this odd coloration pattern, although the clownish movements of these fish resemble domesticated varieties of goldfish and may also be part of the reason for their popularity. In the present work, we have investigated the mechanisms of coloration of the two contrasting hues displayed by this species. The system by which they control the motile responses of their chromatophores has also been examined, because we have found that their integument shows some changes in coloration patterns.

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MATERIALS AND METHODS

Materials

The fish used in this study was the domino damsel (also known as the three-spot humbug) *Dascyllus trimaculatus*, which belongs to the family Pomacentridae, the order Perciformes. The Japanese name of this species is "mitsuboshi-kuro-suzumedai", which means the "three-star", "black", "pomacentrid". They are very common around coral reefs in the Indo-Pacific waters and also in the Red Sea. They are frequently found symbiotic with large sea anemones, although their dependence on the latter is not as high as many anemone fishes.

Their general background hue is mostly a lusterless velvet-black, with three conspicuous white spots (one on the median parietal region and the other two around the base of the dorsal fin on both sides) which form the characteristic pattern of this species. According to descriptions in illustrated encyclopedia and other sources, they can be up to 15 cm long in their natural habitat (e.g., Okamura and Amaoka 1997). The descriptions further indicate that when they are young, the white spots are very conspicuous against the velvet-black background, but in adults the darkness decreases and these white spots become less prominent. This may be the main reason why young specimens are so popular, and thus easily obtainable commercially. For this study, young fish with body lengths ca. 3 cm were purchased from a local dealer in Tokyo, and prior to experimentation, they were maintained in a seawater aquarium in our facility.

Observations of Live Fish

Observations were made on individual live fish kept alone in small upright plastic tissue culture flasks (Corning Glass Works, Corning, NY). To more easily introduce fish, the mouths of the flasks were enlarged. The water was continuously and gently aerated, and hovering or resting fish were observed through the side (10×8 cm) of the flask, and photographed with a 35-mm camera (OM-4; Olympus, Tokyo) fitted with a close-up lens (Zuiko Auto-Macro, 50 mm, 1:3.5; Olympus). Color negative film (Agfacolor, SXG 100, ISO 100, Agfa-Gevaert, Leverkusen) was used.

Morphological Observations

The general morphology of the skin was studied with an industrial light microscope (Optiphot XT-BD, Nikon, Tokyo). After the scales were removed, skin pieces excised either from the black area or from the white spots were immediately immersed in a fixative solution of 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2). Two hr afterwards, the specimens were washed with 0.1 M phosphate buffer (pH 7.2), dehydrated in a graded ethanol series, and embedded in Epon. Semi-ultrathin sections were made vertically to the plane of the skin with glass knives on a Porter-Blum MT-1 ultramicrotome (Ivan Sorvall, Newtown, CT). The sections were stained with toluidine blue for transmission optics, and were observed and photographed on color negative film as used for macrophotography.

To examine the structural details of chromatophores and of the tissues surrounding them, transmission electron microscopy was employed as partly modified from that previously described (Goda *et al*., 1994). Excised skin pieces were immersed in a fixative solution of 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) for 2 hr. The specimens were then cut into small pieces, and washed with 0.1 M phosphate buffer. After dehydration through a graded ethanol series, the tissues were treated with methyl glycidyl ether, and embedded in epoxy resin (Quetol 812; Nisshin EM). Ultrathin sections were cut vertically to the plane of the skin using an MT-1 ultramicrotome with a diamond knife. Sections were stained with uranyl acetate and Reynolds' lead citrate, and viewed with an electron microscope (JEM-1210, JEOL, Tokyo) operated at 80 kV.

Physiological Experiments *in vitro*

After pieces of skin or fins were excised as detailed above, they were immediately immersed in a physiological saline solution which had the following composition (in mM): NaCl 125.3, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.8, D-(+)-glucose 5.6, and Tris-HCl buffer (5.0; pH 7.2). By employing the industrial light microscope (Optiphot XT-BD, Nikon, Tokyo), changes in the state of chromatophores were observed and photomicrographed. The responses of melanophores in excised trunk skin pieces or in split caudal-fin pieces (Fujii, 1959) were examined using transmission optics, while those of melanophores and iridophores in the white spots were observed under epi-illumination optics.

In some experiments, the motile responses of melanophores in the velvet-black integument were measured photoelectrically. In those cases, split fin pieces from caudal fins were employed, and the transmission photometric technique used has been described in detail elsewhere (Fujii *et al*., 2000). In the present study, the light transmission through a circular area (150 μm in diameter) of the skin was measured. Since melanophores of this species are somewhat smaller than those of common fishes, the motile activities of several melanophores were usually included within each area measured.

At the end of each series of measurements, a sufficiently strong solution (2.5 μM) of norepinephrine hydrochloride (NE; racemic modification; Sankyo, Tokyo) in physiological saline was applied for a few min to bring about full aggregation of melanosomes for reference. The NE concentration is expressed in terms of the active L-isomer, i.e. half the concentration of its synthetic racemic modification. In every measurement, we set the apparatus to record the increase in light transmission as an upward trace on the record, i.e. an upward deflection indicates the aggregation of melanosomes. In all cases, the magnitude of melanosome aggregation is expressed as a percentage of the maximal response observed, with the fully dispersed state set as zero.

Prior to the examination of effects of various agents, a K^+-rich saline solution in which the concentration of K^+ ions was raised to 50 mM, was commonly applied to the skin to confirm that the chromatophores to be examined would show normal motile responsiveness. In this solution, the concentration of Na^+ was compensatorily decreased to 78 mM in order to keep its osmolarity the same as that of normal saline. Incidentally, such an increase in the concentration of K^+ ions is known to liberate the neurotransmitter from presynaptic elements of nerves that control melanophores, thus acting as a sympathetic stimulus (Fujii, 1959). A positive response to the heightened K^+ concentration indicated that the cells were normally innervated, in addition to the normal responsiveness of the cells themselves.

In a few measurements, sympathetic fibers that function to aggregate melanosomes were electrically stimulated. In such cases, skin pieces were stimulated in a field of sine-wave alternating current generated by a CR oscillator (AG-203, Kenwood, Tokyo). Such an electrical field stimulates sympathetic fibers to liberate catecholaminergic transmitter to control chromatophores (Fujii and Novales, 1968). The stimulating waves were monitored by a storage oscilloscope (5111A, Tektronix, Beaverton, OR).

In order to study the spectral characteristics of the light reflected from the white spots quantitatively, a commercial 1024-channel photodiode-array spectrophotometric system (MCPD-100; Otsuka Electronics, Osaka) was employed (Kasukawa *et al*., 1987; Fujii *et al*., 1989). The area to be measured for reflectance was ca. 2 mm^2, and the spectral reflectance was measured from 400 to 700 nm every 1 nm. Data were recorded on floppy disks and were processed using a personal computer. Spectra were graphically displayed on a CRT monitor, and were further processed for representation and printed on an X–Y plotter (MC-920, Otsuka Electronics).

Chemicals and Drugs Used

In addition to NE, melatonin (Sigma Chemical, St. Louis, MO), adenosine (Kohjin, Tokyo), α-melanophore-stimulating hormone (MSH; Sigma Chemical), melanin-concentrating hormone (MCH; Peninsula Lab., Belmont, CA) and acetylcholine (chloride salt, Daiichi

Seiyaku, Tokyo) were employed as common agents known to induce motile responses of chromatophores. As an α-adrenolytic agent, phentolamine mesylate (Ciba-Geigy, Basel) was used. Stock solutions of these agents were diluted with physiological saline immediately before use.

All physiological and pharmacological measurements were made at room temperature (20–25°C).

RESULTS

Observations on Live Fish

Fig. 1 shows photographs of a young specimen of the domino damsel, *Dascyllus trimaculatus*, hovering during the day (A), and resting at night (B). In the daytime, the velvet-black color covers most of the body surface, and three white spots, one on the parietal part and the other two around the base of the dorsal fin on both sides, are conspicuously seen as a characteristic feature of this species. This state is the same as that illustrated in encyclopedia of marine, tropical fishes. During the night or in the dark, the velvet-black skin faded to some degree, while the whiteness of the spots also decreased at the same time. We can also see that under such conditions, the fins became transparent to a certain extent.

Morphological Observations

Fig. 2 shows photomicrographs of semi-ultrathin sections that were cut vertically across the integument of the white spot of a domino damsel. In both panels, a vast number of non-dendritic iridophores that form a stack in the dermis are observed. Panel A shows the histological architecture of the integument which had been equilibrated in physiological saline before fixation. Melanosomes in the melanophores were mostly deposited above the stack of iridophores, which indicates that melanosomes had migrated from the perikarya to the peripheries of dendritic processes that penetrated spaces among iridophores and covered them.

Panel B of the same figure shows integument that had been treated with 2.5 μM NE before fixation. The two large dark masses located under the accumulation of iridophores are the cell bodies of the melanophores. Melanosomes had aggregated into the cell bodies, leaving their dendritic processes devoid of pigment.

Fig. 3A shows an electron-micrograph of the velvet-black portion of the integument. In the uppermost part of the dermis, melanophores exist in a monolayer, and cross-sections of their cellular processes are abundant. By contrast, xanthophores and/or erythrophores containing xanthosomes and/or erythrosomes were not found in these sections, although we could find parts of iridophores in some places.

Panel B of the same figure shows an electron-micrograph of an accumulation of iridophores in a white spot. The thickness of the accumulation was often more than 30 μm. In the cytoplasm of these iridophores, thin light-reflecting platelets are arranged to form piles, which took various angles to the plane of the skin.

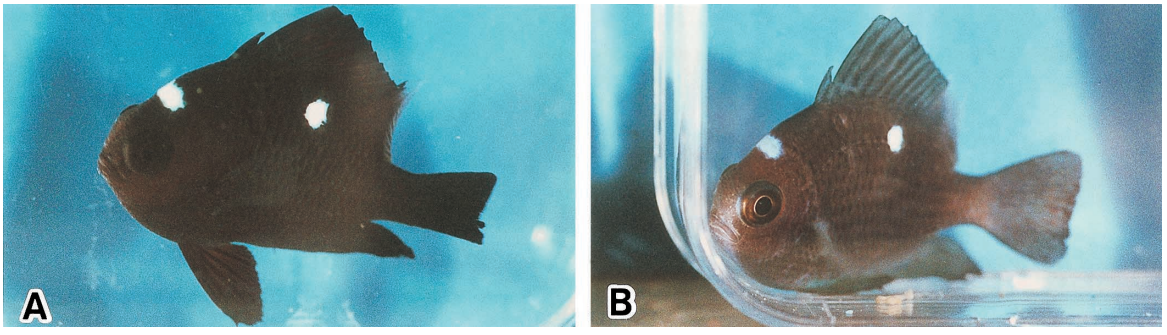

Fig. 1. Photographs of a young domino damsel, *Dascyllus trimaculatus*. **A**: In the daytime; the body exhibits a velvet-black hue except for three bright white spots. **B**: At night; the dark skin becomes paler, and the spots become less distinct. Note that the fins become rather transparent. Approximately actual size.

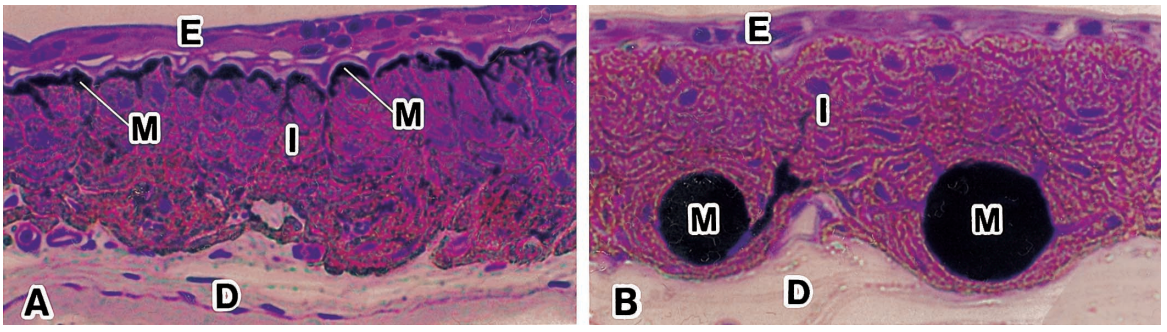

Fig. 2. Photomicrographs of semi-ultrathin sections cut vertically across the integument of a white spot. **A**: Specimen equilibrated in physiological saline before fixation. **B**: Specimen treated with 2.5 μM NE before fixation. D: dermis composed of loose connective tissue, E: epidermis, I: thick layer of iridophores, M: aggregates of melanosomes. ×530.

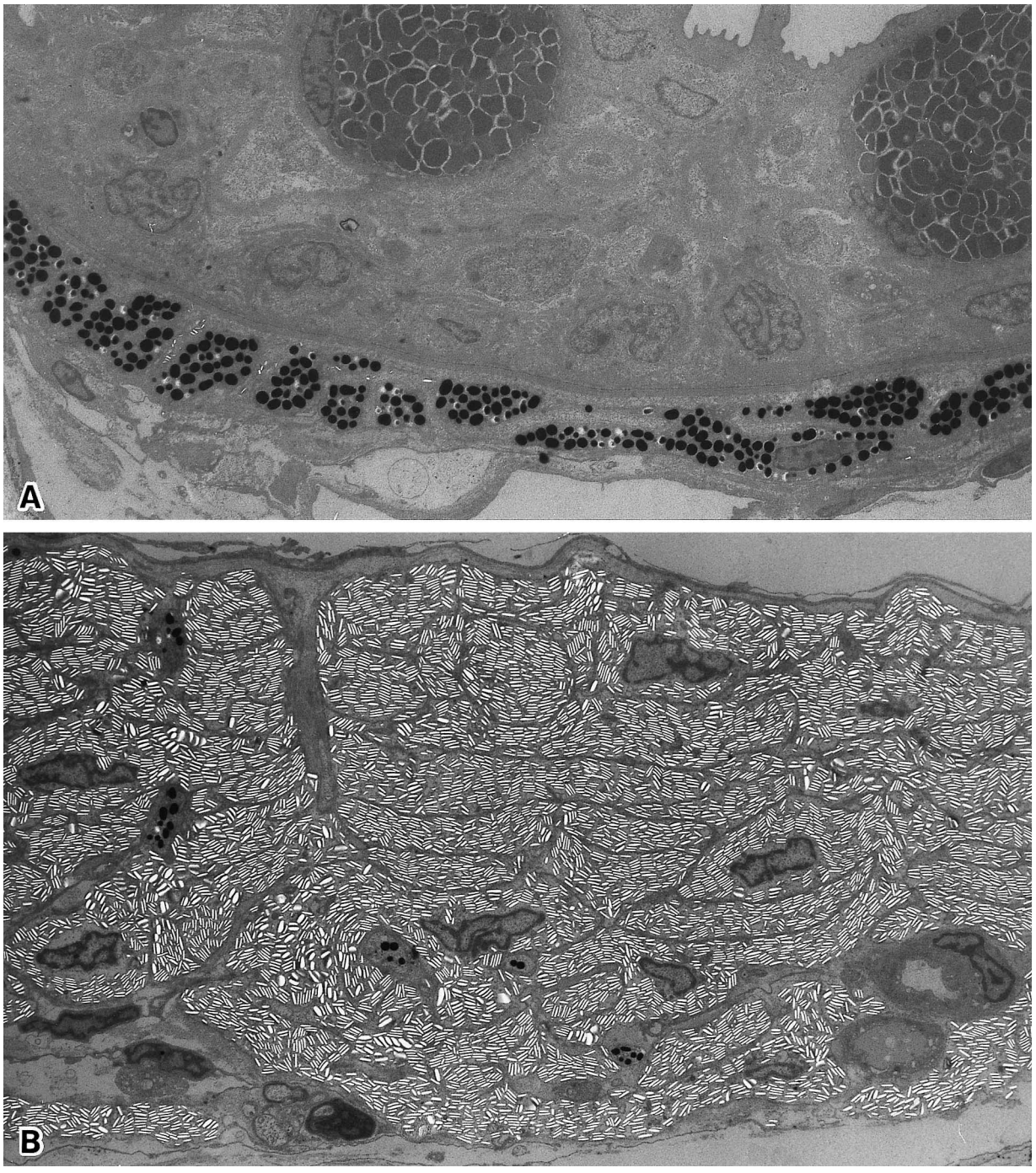

Fig. 3. Electron-micrographs of vertical sections across an excised piece of skin that had been equilibrated in physiological saline. **A**: General background, velvet-black region. Under the epidermis, processes of melanophores with numbers of melanosomes are densely distributed. X3,800. **B**: Part of a white spot. A vast number of iridophores are seen densely accumulated. The light-reflecting platelets have fallen off the section, and look like tiny flat holes. Note that the direction of the piles of platelets is rather random. ×3,200.

Owing to the fact that practically all the light-reflecting platelets had fallen off the ultrathin sections, we could not measure their thickness. However, we can roughly estimate the periodicity of the platelet piles in electron micrographs such as that shown in Fig. 4. In this way, we could recognize that the periodicity is not so far from 160 nm.

Motile Responses of Melanophores *in Vitro*

Fig. 5 shows a typical series of photomicrographs that followed the motile responses of melanophores in a split caudal-fin preparation. When equilibrated in physiological saline (A), melanosomes are almost fully dispersed in melanophores, such as occurs in many teleosts. Upon application of 2.5 μM NE, melanosomes rapidly aggregated (B and C). After re-equilibration in physiological saline (D), 1 μM melatonin (MT)

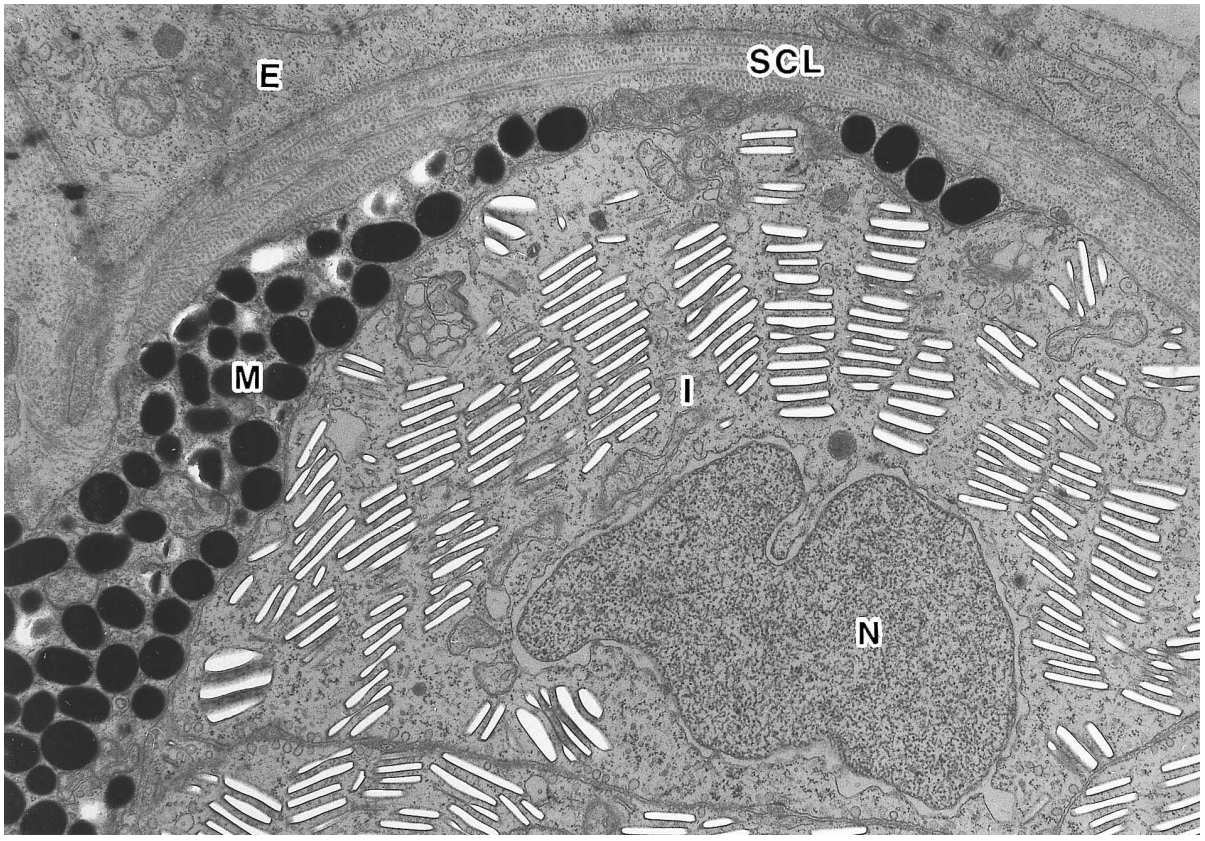

Fig. 4. Electron-micrographs of vertical sections across a piece of skin excised from the parietal white area that had been equilibrated in physiological saline. Note that the direction of the piles of platelets is radiating from the nucleus (N). Part of a branch of a melanophore (M) extending up into the space between the uppermost iridophore (I) and the subepidermal collagenous space (SCL). E: epidermis. ×15,000.

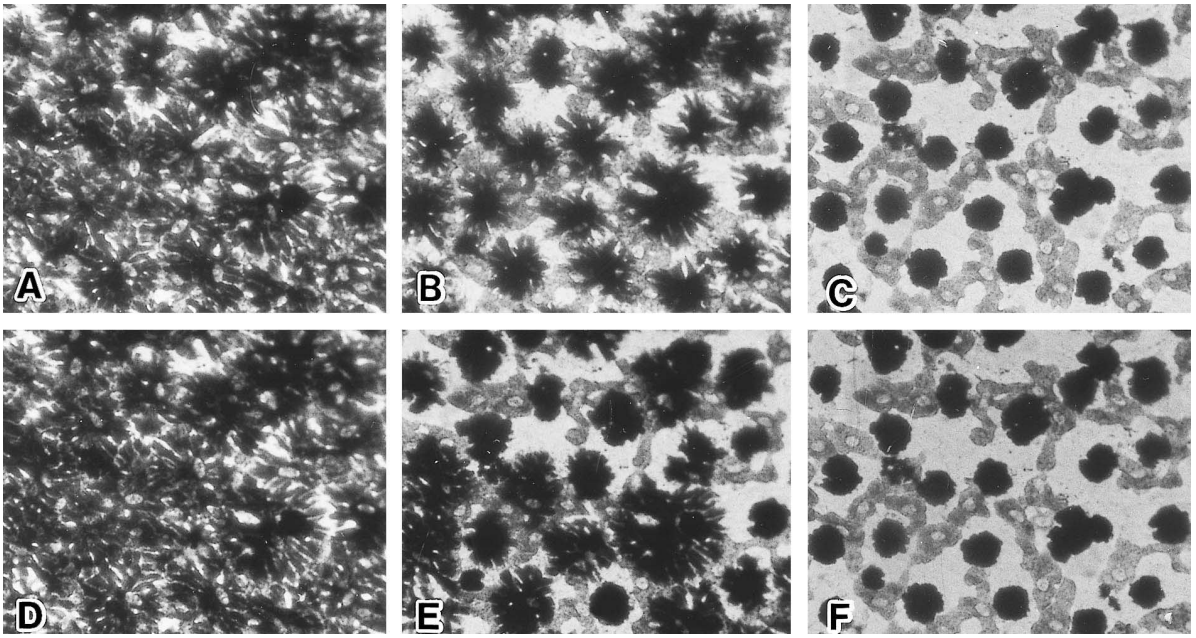

Fig. 5. Serial photomicrographs showing the motile responses of melanophores in a split caudal-fin piece. **A**: Equilibrated in physiological saline; melanosomes in the melanophores are fully dispersed. **B**: 10 sec after the application of K^+-rich saline (K^+: 50 mM); melanosomes began to aggregate. **C**: 60 sec after the application of the K^+ saline; melanosomes are almost fully aggregated. **D**: The melanosome dispersed state after re-equlibration with physiological saline. **E**: 60 sec after the application of 1 μM melatonin: in some melanophores, the amine moderately aggregated pigment, while in others the effect was only partial or had no response. **F**: 5 min after the application of 2.5 μM NE: melanosomes are completely aggregated. The semi-transparent cells that can clearly be seen when the melanosomes are aggregated are iridophores. ×300.

was applied. The melanosome-aggregating action of MT differed among melanophores and some of them were practically refractory to the amine (E). Finally, NE was added to the solution containing MT. It elicited complete aggregation of melanosomes in all melanophores (F).

In order to examine the action of various agents that affect the state of chromatophores, the photoelectric method was used to record melanophore responses in split caudal fin preparations (Fig. 6). AC-field stimulation (A), K^+-rich saline (B), NE (A, B, C), melatonin (MT; C) or melanin-concentrating hormone (MCH; D) elicited the aggregation of melanosomes as seen by the upward traces. In the presence of an α-adrenergic blocker, phentolamine (PA), the effects of AC (A) and of NE (B) were effectively antagonized. During the melanosome aggregation induced by MCH, α-melanophore-stimulating hormone (D) or adenosine (data not shown) was applied. In either case, the distinct dispersion of melanosomes was recorded. Acetylcholine (ACh), which has been shown to aggregate melanosomes in the melanophores of some fish (cf. Fujii, 1993a, 2000; Fujii and Oshima, 1994), did not affect

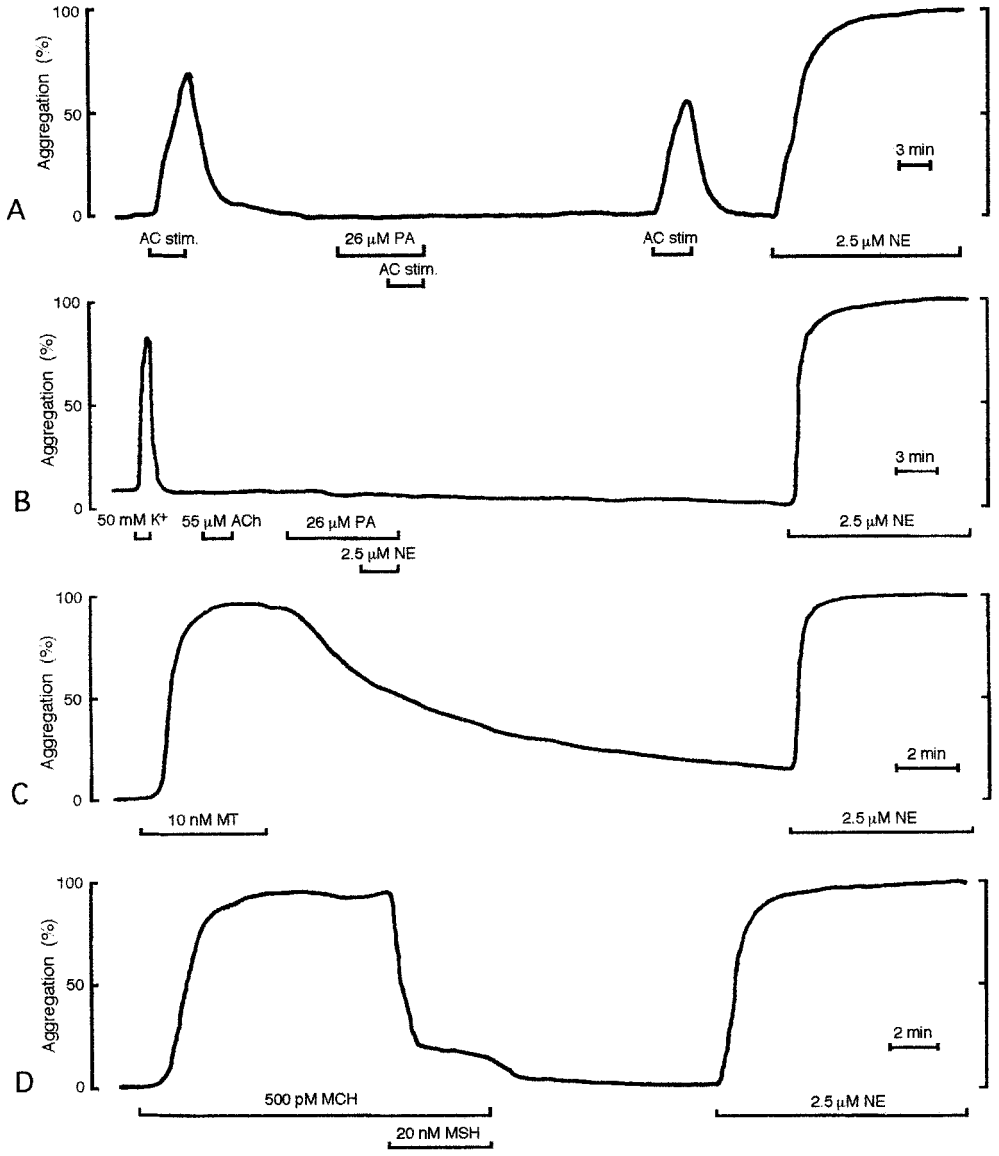

Fig. 6. Photoelectric recordings of the responses of melanophores in the velvet-black integument of split tail-fin preparations. At the end of all measurements, 2.5 μM NE was applied to induce the maximal aggregation of melanosomes for reference. **A**: Recording showing the effects of electrical stimulation of nerves, and the effect of phentolamine (PA) on that. Alternating field stimulation (AC; sine wave, 10 Hz, 0.7 V/mm) potently elicited aggregation of melanosomes, but the effect was effectively antagonized by PA. Upon removal of the blocker, the action of AC was restored. **B**: Recording showing the effects of increased K^+ concentration (K^+: 50 mM) in the bathing saline, acetylcholine (ACh) and NE. The K^+ increase aggregated melanosomes, while ACh had no effect. PA effectively blocked the action of NE. **C**: Recording showing the effect of melatonin (MT), which effectively aggregated melanosomes. **D**: Recording showing the effects of melanin-concentrating hormone (MCH) and α-melanophore-stimulating hormone (MSH). First, 500 pM MCH was applied which potently aggregated melanosomes. Then, 20 nM MSH was added in the presence of MCH, and induced the rapid dispersion of melanosomes.

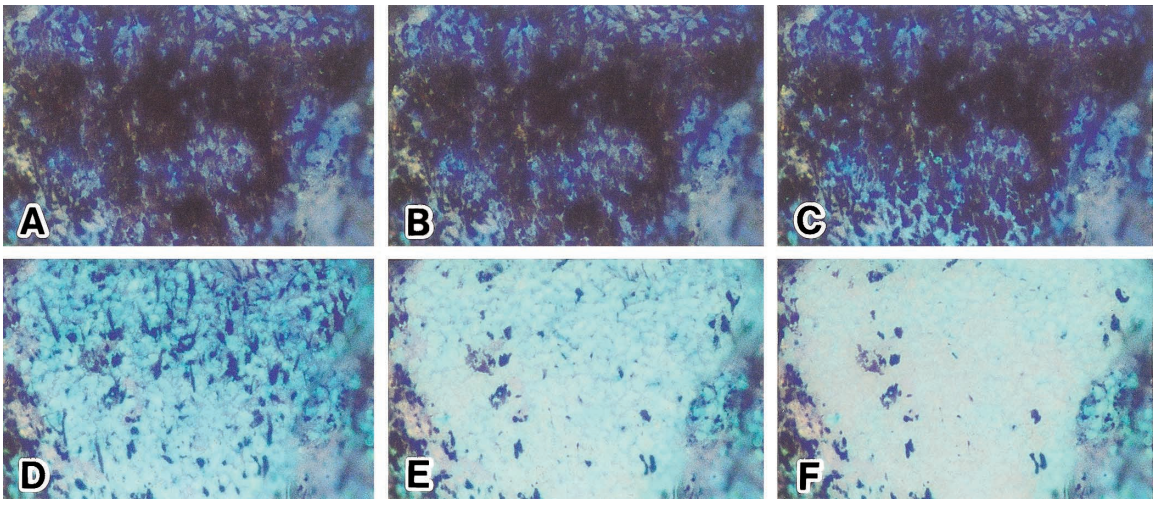

Fig. 7. Serial photomicrographs showing color changes of the integument from a white spot. Epi-illumination optics. **A**: Equilibrated in physiological saline; the skin exhibited a dark purplish hue. The dark patches seen are small accumulations of melanosomes in peripheral parts of the dendritic processes of melanophores. **B**, **C**, **D**, **E** and **F**: 15, 30, 45, 60 and 75 sec after the application of K^+-rich saline (K^+: 50 mM), respectively. The overall coloration of the integument becomes greenish white, and the black patches gradually disappear. ×160.

the state of melanophores in this system (B).

Optical Changes of White Spots *in Vitro*

Changes in the coloration of a skin piece excised from a white spot were examined by photomicrography under epi-illumination optics (Fig. 7). When equilibrated in physiological saline, the excised skin exhibited a dark purplish hue (A). In addition, small black islets were recognizable on the surface of the white spot. Along with the histological observations mentioned before, we recognized these islets to be small accumulations of melanosomes within the tips of the dendritic processes of melanophores (Figs. 2A and 4). When the irrigating solution was changed to K^+-rich saline (K^+: 50 mM), the coloration of the integument became greenish white, and the black islets gradually became invisible (Fig. 7B–F). The former hue changes may result from the motile response of iridophores, while the latter results from the disappearance of melanosomes from the surface of the stack of iridophores.

Spectral reflectance from the white skin was then measured by the multichannel spectrophotometric system (Fig. 8). When the skin piece was immersed in physiological saline, a spectral peak was detected at around 450 nm, although it was rather broad (A). When the perfusing medium was changed to 50 mM K^+, the overall reflectance increased greatly, and the spectral reflectance peak shifted to a longer wavelength (B).

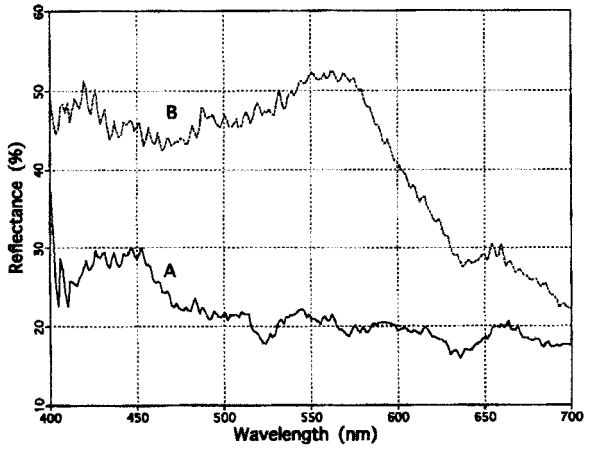

Fig. 8. Changes in the spectral reflectance from the surface of an isolated skin piece from a white spot. **A**: Spectrum recorded when the skin was equilibrated in physiological saline. The spectral peak stands at around 450 nm, although it is rather broad. **B**: 75 sec after the application of K^+-rich saline (K^+: 50 mM), the overall reflectance greatly increased, and the spectral reflectance peak shifted towards a longer wavelength region.

DISCUSSION

In the dark skin of the domino damsel, *Dascyllus trimaculatus*, melanophores are densely present in the uppermost layer of the dermis. Their cellular processes extend parallel to the plane of the skin, and they respond to electrical nervous stimulation, NE and some hormonal substances that are known to affect chromatophore movements. Thus, they seem to be controlled by the nervous and the endocrine systems in an orthodox manner (Fujii, 1993a, 2000; Fujii and Oshima, 1986), that regulates the color changes, including those observed during the day-night cycle and also in response to various environmental and ethological cues (Fujii, 1993b). In the skins of other fish species, xanthophores and/or erythrophores commonly exist along with melanophores. In the present species however, there were no such brightly-colored chromatophores, and possibly their lack may result in the characteristic velvet black appearance.

Extending their dendritic processes parallel to the plane of the skin, chromatophores in the dendritic types in teleosts generally assume a two dimensional configuration, and the

melanophores of velvet-black part of the present material assume such a form. By contrast, melanophores in the white spots extend their cellular projections three-dimensionally to invade the spaces among the non-dendritic iridophores that overlie them. Incidentally, a similar configuration of melanophores has already been reported in some amphibians and reptiles (Bagnara and Hadley, 1973) and also in some teleostean species, for example, the blue damselfish (*Chrysiptera cyanea*; Kasukawa *et al*., 1985), the blue green damselfish (*Chromis viridis*; Fujii *et al*., 1989) and the common surgeonfish (*Paracanthurus hepatus*; Goda *et al*., 1994; Goda and Fujii, 1998). When melanosomes are dispersed in the cytoplasm of these melanophores, scattered lightrays from the iridophores are absorbed by melanosomes, resulting in the higher "chroma" and lower "value" of the reflected colors in terms of the Munsell Color Notation System. Conversely, the aggregation of melanosomes into the perikarya may induce lower chroma and higher value colorations. In this way, the motile activities of melanophores may dramatically influence the whitish coloration of the spots expressed by the iridophores.

In the present study, we have found that the white spots are primarily composed of large accumulations of iridophores in the dermis, and that their thickness frequently exceeds 30 μm. In each iridophore, piles of very thin light-reflecting platelets are present. Due to the large difference in refractive indices that exist between the flat platelets and the intervening cytoplasm, incident light rays are reflected very effectively. Thus, the multilayered thin-film interference phenomenon takes place in the piles of platelets (Land, 1972; Fujii, 1993a). Without the presence of pigmentary materials, such an interference system can produce different colors, and the resultant hues are categorized as "physical colors" or "structural colors". If such an interference system is close to the ideal type, the spectral reflectance becomes very wide, namely, very white. Therefore, it may rather safely be said that the optical characteristics of the white spots are in accordance with the conditions mentioned above, although the thickness of the platelets could not be determined owing to their loss from the ultrathin sections. In consideration of past results on iridophores of a few teleost species (Kawaguti and Kamishima, 1966; Kawaguti and Takeuchi, 1966) and the discussions about iridophore inclusions (Land, 1972; Fujii, 1993a), we presume the thickness of the platelets in the skin of the domino damsel to be 60–80 nm. In the present study, we have estimated the periodicity of the platelet arrangement in those piles to be about 160 nm. If we assume that the thickness of the platelets is 70 nm and that the cytoplasmic space between the platelets is 90 nm, the peak of the spectral reflectance can be calculated to be about 500 nm (cf. Fujii, 1993a). Under such conditions, the multilayer interference system becomes very close to the ideal type.

The axes of the piles of reflecting platelets in an iridophore seem to be arranged around the nucleus as if it were radiating from it (Fig. 4). When many iridophores are present, the platelets are more randomly arranged (Fig. 3B), namely, the directions of the axes of light-reflecting units in the stack of iridophores vary, assuming various angles to the plane of the skin. Thus, the incident light rays are very randomly reflected within the accumulation of iridophores, and the spots look very whitish from every direction. Such a situation is quite different from the case of the mirror-like skin that can very often be observed on the flanks of many oceanic migratory fishes, where the iridophore platelets are usually arranged almost parallel to the sagittal plane of the fish as it swims (Denton and Nicol, 1965; Land, 1972; Fujii, 1993b).

It may be appropriate to note here that the whiteness of the spots of these fish is tinged with blue. We further know that the peak of the spectral reflectance of the interference system shifts towards a shorter wavelength when light rays are irradiated obliquely to the axis of a pile of platelets (Huxley, 1968; Fujii, 1993a). In the present system, the incidence is commonly oblique, since the piles are arranged at various angles as described above. Actual measurements of the spectral reflectance from skin in the white spots indicate that the spectral curve is higher in the shorter wavelength region (Fig. 8).

We remember that highly light-scattering white skins can commonly be found on the abdominal trunks of vertebrates, including fishes. Such a chromatic organization is thought to produce the so-called "countershading" of the body that is widely observed within the animal kingdom. In a watery environment in particular, animals are exposed by light rays mostly from above, and naturally, their ventral regions tend to look darker. However, if those regions are lighter, the animals look flatter, and such coloration functions for the animal to minimize its risk of being detected as a three-dimensional entity. As cryptic or concealing coloration, such patterning is therefore crucial for the survival of the possessors, who are always in danger of being captured by predators, or who need to maximize their ability to capture prey (Denton and Nicol, 1965; Land, 1972, Fujii, 1993b). Using an electron microscope, Kawaguti and Kamishima (1966) actually showed the presence of such layers of iridophores in the silvery sides of the Japanese shad, *Sardinella* (synonyms: *Herklotsichthys*, *Harengula*) *zunasi* (Clupeidae, Clupeiformes). Kawaguti and Takeuchi (1966) further found similar stacks of iridophores in the peritoneal wall of the medaka, *Oryzias latipes* (Cyprinidontidae, Cyprinodontiformes).

In consideration of their distribution on the trunk, by contrast, it is rather difficult to consider that the white spots of the domino damsel take some part in the cryptic coloration. Since they are not located on the abdominal area, but are rather on the more receptible portions of the trunk, the characteristic bright spots against a black background are indeed very conspicuous, and may function as a type of signal coloration. More strictly speaking, this pattern may work as a recognition coloration that transmits the chromatic information to individuals of the same species. Namely, it may be useful during schooling behavior or for mating.

That the whiteness of the spots is mingled with a fluorescent bluish tint may also be related to the conspicuousness of the pattern. So far as we are aware, such a conspicuous blu-

ish white color is not expressed by fish that require their whiteness for protective purposes. In other words, the lack of bluish shades may be an indispensable condition for the protective role of the whiteness in these animals.

Here, we can refer to some species of fish which possess very similar whitish parts of skin with a tint of blue. For example, the interzonal white areas between the black stripes of the Ryukyu three-stripe damsel (*Dascyllus aruanus*.) and the Ryukyu four-stripe damsel (*Dascyllus melanurus*) also exhibit very similar hues. The similar structural organization of chromatophores as well as a similar mechanism for color revelation can be supposed, because these species belong to the same genus as the domino damsel. Anemone fish, represented by *Amphiprion clarkii,* are famous symbia with sea anemones, and are extraordinary. Their conspicuous white stripes are also tinged with blue. They belong to the same family Pomacentridae, and they are also systematically close to the present material.

We found in this study that the dark general background regions became lighter during the night. Since the melanophores in the skin were responsive to melatonin (MT) and aggregated their pigment, we can naturally conclude that MT secreted from the pineal is responsible for the blanching, as occurs in many teleosts (Fujii, 2000; Fujii and Oshima, 1986). The present study further disclosed that a monolayer of large melanophores lines the white spot. The cell bodies of these melanophores always reside under the mass of iridophores. During the night the spots darken to a certain degree. When excised skin pieces were equilibrated in physiological saline, melanosomes actually migrate into the dendrites of these melanophores that extend among iridophores, and further cover the surface of the white spots. The cell bodies of the melanophores thus function to store the dark material needed to cover the white spots. The dispersion of melanosomes in melanophores might be ascribable to the action of MT, because the darkening takes place during the night or in the dark. Such a rather contradictory action of MT has lately been reported in melanophores in the dark spots of some pencilfish species, and that action is thought to be mediated by the β-MT receptor on melanophores (Nishi and Fujii, 1992; Masagaki and Fujii, 1999). Until the present time however, we have been unable to detect either an active melanosome-aggregating or a dispersing action of MT on melanophores of the domino damsel. We presume that the melanophores in the white spots lack both α- and β-MT-receptors, and that the melanosome dispersion is due to the lowered activity of the sympathetic nervous system responsible for the aggregation of pigment (Fujii, 2000; Fujii and Oshima, 1986).

Finally, electron micrographs show that, when compared with other species of a similar size (Fujii, 1968, 1993b; Goda *et al*., 1994, Goda and Fujii, 1998), both the epidermis and the subepidermal collagenous lamella of the domino damsel are quite thin (cf. Fig. 4). Thus, the chromatophores that underlie the lamella can be observed very clearly, which results in an effective transmission of chromatic information to other individuals.

ACKNOWLEDGMENTS

We thank Professor N. Oshima who kindly allowed us to use facilities available in the Animal Physiology Laboratory at Toho University, and Dr. M. Sugimoto for his interest. We also like to thank Professor Y. Fujiyoshi, Department of Biophysics, Faculty of Science, Kyoto University, for interest and encouragement.

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(Received September 27, 2000 / Accepted October 28, 2000)