THE ROYAL SOCIETY

Received 21 August 2003
Accepted 17 October 2003

Wavelength-selective and anisotropic light-diffusing scale on the wing of the *Morpho* butterfly

Shinya Yoshioka* **and Shuichi Kinoshita**

Graduate School of Frontier Biosciences, Osaka University, Toyonaka, Osaka 560-0043, Japan

We have found that cover scales on the wing of the butterfly *Morpho didius* possess specially designed microscopic structures for wavelength-selective reflection and contribute considerably to the brilliant blue colour of the wing. In addition, the cover scale functions as an anisotropic optical diffuser which diffuses light only in one plane, while it makes the range of reflection narrower in the orthogonal plane. The quantitative analyses for the wavelength-selection mechanism and the peculiar optical diffuser are given and the role of such a special optical effect is discussed from physical and biological viewpoints.

Keywords: structural colour; *Morpho* butterfly; diffuser; interference; butterflies

1. INTRODUCTION

The brilliant blue wings of the *Morpho* species are some of the most well-known examples of structural colour (Ghiradella 1991; Parker 2000). The surprisingly minute structure hidden in their scales was first revealed by Anderson and Richards with the use of an electron microscope, and many scientific investigations have been performed to clarify the mechanisms of their coloration (Mason 1927; Anderson & Richards 1942; Bingham *et al.* 1995; Tabata *et al.* 1996; Vukusic *et al.* 1999; Kinoshita *et al.* 2002*a*,*b*). However, among many species in the *Morpho* genus, there still exists a great deal of variation in the tone of the blue colour, which has not been fully understood. In particular, some species, e.g. *Morpho rhetenor*, *M. cypris* and *M. sulkowskyi*, have strongly glossy wings as if the surface is drenched with liquid. However, the other species, e.g. *M. didius*, *M. menelaus* and *M. deidamia*, have somewhat whitish-blue wings and do not look so glossy. From a microscopic observation, it is easily noticed that the arrangement of scales is markedly different among those species. Namely, glossy wings are almost covered with just one kind of scale (ground scale in *M. rhetenor* and *M. cypris*) or two very similar kinds of scale (*M. sulkowskyi*) having a brilliant blue colour, whereas on the other whitish-blue wings, there exists an additional layer of highly transparent scales called cover or glass scales above the layer of blue ground scales (Ghiradella 1994). These facts suggest that cover scales have some optical effects on the appearance of the wing. However, their optical role in these butterflies is rather mysterious because their high transparency seemingly makes little contribution to the coloration of the whole wing. Detailed optical study of the cover scales has not been reported except for the proposed optical effect as diffraction-assisted angle broadening (Vukusic *et al.* 1999).

In this paper, we will show that cover scales of *M. didius* play a significant role in the coloration of the blue wing through their wavelength-selective mechanism and anisotropic light-diffusing character. These characteristics are realized by the specially designed microscopic structures within the cover scale.

2. MATERIAL AND METHODS

Samples of male *M. didius* and *M. rhetenor* were purchased from Mushi-sha, Japan. The dorsal side of the wings was observed by using an Olympus BX50 fluorescence microscope to investigate the arrangement of ground and cover scales. Microscopic structures of cover scales were observed by a JEOL JSM-5800 scanning electron microscope (SEM). The sample was sputtered with gold for the SEM observations.

Optical reflection was characterized by the following four methods.

(i) The spatial pattern of reflection was qualitatively observed by colour photography. The intact wing of the two species was illuminated by loosely focused white light from a xenon lamp. A screen was placed *ca*. 10 cm away from the sample. The reflection patterns of a single cover and ground scale of *M. didius* were also examined. In this case, the incident light was first focused on a pinhole having a diameter of 50 μm and the transmitted light was collected and focused by a camera lens, Canon EF 100 mm F2.8, on a single scale. The sample was attached to a needle tip by an adhesive. A similar method has been reported previously (Vukusic *et al.* 1999).

(ii) The angular dependence of the reflected light intensity was measured under monochromatic light illumination. One end of an optical fibre was put on a rotating stage to pick up the light as a function of angle. The other end was placed before a photomultiplier as a detector.

(iii) The spectrum of the reflected light was measured by a spectrometer, Ocean Optics USB2000, equipped with an optical fibre.

(iv) The total reflectivity of a single cover scale was measured by collecting the diffusely reflected light with an integrating sphere. In this measurement, the white light from a xenon lamp was first monochromatized by the use of a spectrometer, Nikon P250, and then focused on a pinhole having a diameter of 25 μm. The transmitted light was collected by a camera lens, Nikon lens series E f35, and focused onto a small spot which lies completely within the sample. The incident light passed through an integrating

---

[*] Author for correspondence (syoshi@mph.phys.sci.osaka-u.ac.jp).

DOI 10.1098/rspb.2003.2618

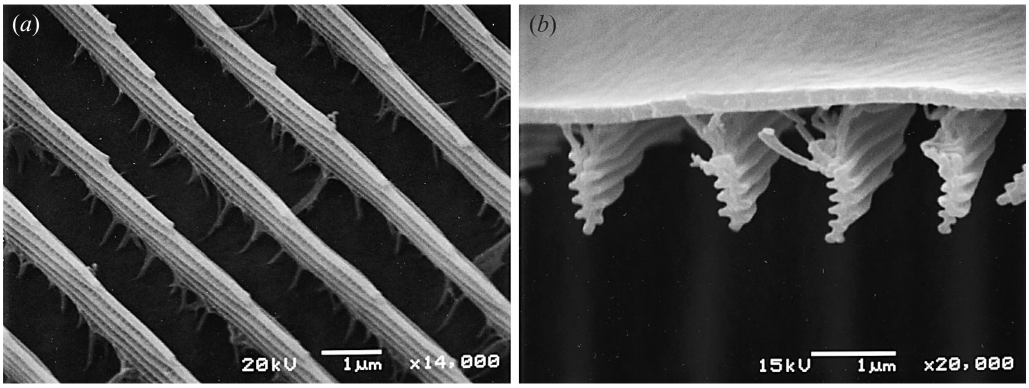

Figure 1. SEM images of (*a*) a top view from a slightly oblique direction and (*b*) a cross-section of a cover scale of male *Morpho didius*. The scale is upside down in (*b*) so that the bottom surface is viewed.

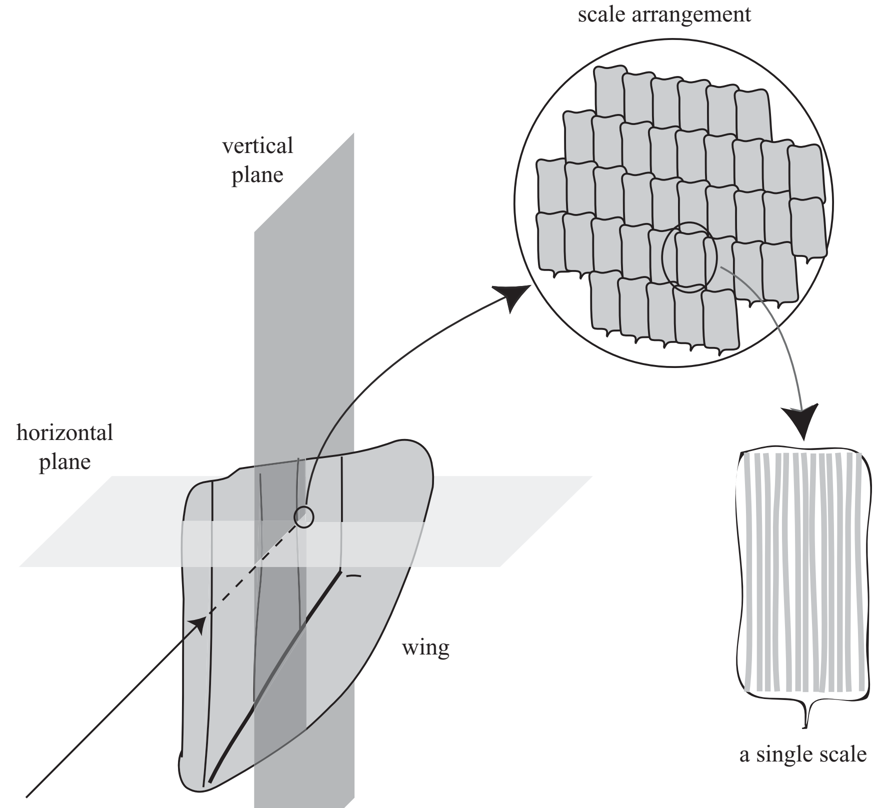

Figure 2. The geometry of the observation is schematically depicted. The vertical and horizontal directions roughly correspond to the directions along and perpendicular to the ridges of the ground scale, respectively. Although the cover scales are not shown in this figure, their ridges are almost parallel to those of ground scales.

sphere having a diameter of 6 cm to illuminate the sample, which was placed on the plane of the exit hole of the sphere so as not to collect the transmitted light. A camera lens was employed to avoid spherical astigmatism and chromatic aberration. The reflectivity was obtained by dividing the observed spectrum by the reference spectrum of a $BaSO_4$ plate having a comparable size with the scale to avoid a multiple reflection.

3. RESULTS

First, we performed the microscopic investigations of cover scales by using the SEM. As shown in figure 1, there are a lot of ridges on a scale, which are formed from a series of longitudinally overlapping cuticle layers like ordinary *Morpho* scales (Ghiradella 1994, 1998; Vukusic *et al.* 1999). In the cross-section, we can see that four to six layers of cuticle are stacked forming an air–cuticle multilayer structure. The cuticle layers have a width of *ca.* 300 nm and thickness of 100–120 nm with the air gap of 60–90 nm. These layers are found to run obliquely to the base plate with the tilt angle 7–10°, which is estimated from the length of the layer and height of the ridge. The separation between adjacent ridges is 1.5 μm, which is more than twice the width of 0.7 μm of the ground scale (Kinoshita *et al.* 2002*a*). As a consequence, the base plate of a cover scale is notably exposed to a view unlike a ground scale. The thickness of the base plate is found to be 190–230 nm.

Next, we compared the reflection pattern from the intact wing of the two *Morpho* species, *M. didius* and *M. rhetenor*, to characterize their qualitatively different appearance depending on the presence of the cover scale. Figure 2 illustrates the geometry of the observation schematically. It was found that the two butterflies have markedly different patterns from each other as shown in figure 3*a*,*b*. Namely, the glossy wing of *M. rhetenor* has a highly anisotropic reflection forming a narrow band spreading horizontally on the screen, whereas the whitish-blue wing of *M. didius* has a rather isotropic pattern. The narrow band pattern of *M. rhetenor* is thought to result directly from the anisotropic reflection from each ground scale, which has already been reported (Vukusic *et al.* 1999). To clarify the origin of this difference, we removed the cover scales of *M. didius* with an adhesive tape and found that the reflection pattern is largely changed into an anisotropic one as shown in figure 3*c*. Furthermore, this pattern change qualitatively affects the appearance of the wings to human eyes; the region of the removal looks glossier than the other intact part of the wing.

Then, we quantitatively characterized the reflection pattern of *M. didius* by measuring the angular dependence of the reflected light intensity. The measurements have been performed for the following four cases: within the two orthogonal planes for the intact wing and the wing without cover scales. For the wing without cover scales, we can read the anisotropic reflection by comparing the angular range between figure 4*a*,*b*. The characteristic line shapes in figure 4*b* have already been explained by the simple model (Kinoshita *et al.* 2002*a*). However, for the intact wing, the reflection intensity ranges from −60° to +30° in the vertical plane (figure 4*c*) and the line shapes are asymmetric with the one-sided tail in the direction towards the distal part of the wing. In the horizontal plane, the line shapes are almost symmetrical and seem to converge towards the centre regardless of wavelength (figure 4*d*). Further, they have the sharp component around 0°. The maximum intensities do not appear at 0° in the vertical plane for both cases (figure 4*a*,*c*) because of the tilt of the scale plate to the wing membrane and of the air–cuticle multilayer structure within ridges to the scale plate (Ghiradella *et al.* 1972).

Next, we examined the single-scale optical properties of *M. didius* to clarify the contribution of each kind of scale to the reflection of the whole wing. In figure 3*d*, it is shown that the ground scales have a narrow-band reflection pattern which is quite similar to that of *M. rhetenor*. This result is quite reasonable because the ground scales of the two species have very a similar structure to each other (Vukusic *et al.* 1999). However, we have found that the cover scale reflects the incident light into two planes, so that two narrow bands are formed on the screen as shown in figure 3*e*. These bands have different hues from each other: deep blue (lower band) and light blue (upper band). Each band has the angular broadening of *ca.* 90° in the horizontal plane and the angular separation between two bands in the vertical direction is 16°. We have carefully checked the direction of the reflected light and found that the light-blue band includes the direction of the specular reflection from the base plate of the scale. Because the scale is mounted with its root down in this observation, it is deduced that the deep-blue band appears in the direction 16° tilted towards the root of the scale. Then, we measured the spectrum to quantitatively characterize their hues. It was found that the two bands have the maximum reflectivity at almost the same wavelength of 460–470 nm as shown in figure 5*a*,*b*. However, the difference appears in the bandwidth: the full width at half maximum of 110 nm for the light-blue band is broader than 75 nm of the deep-blue band. It is also noticed that the spectrum of the light-blue band has a rising feature in the longer wavelength region accompanied by the minimum at 670 nm. Finally, we measured the total reflectivity of a single cover scale by collecting all the reflected light by the use of the integrating sphere. The result, shown in figure 5*d*, indicates that the reflectivity is 30% at the maximum wavelength of 470 nm.

4. ANALYSES

(a) *Optical properties of a single cover scale*

The most peculiar characteristic of cover scales is the reflection pattern forming two bands having different colours to each other. Because of the relatively large separation between adjacent ridges, the incident light directly irradiates two layer structures, the air–cuticle multilayer within ridges and a single layer of the base plate. Thus, it is suggested that these two reflection bands come from those two layer structures by a consideration of the oblique nature of the multilayer to the base plate. Actually, the angular separation of 16° between the two bands is in the range of twice the oblique angle of 7–10°. Because the light-blue band includes the specular direction for the reflection from the base plate, we compare the observed spectrum with the reflectivity, which is theoretically calculated by the transfer matrix method (Born & Wolf 1975) for a single thin layer of cuticle having a thickness of 215 nm and refractive index of 1.56. The result, shown in figure 5*c*, reasonably reproduces the line shape of the spectrum (figure 5*b*) in three points: (i) the peak at 470 nm; (ii) the minimum at 670 nm; and (iii) the rising feature in the longer wavelength. Although the spot pattern of reflection is expected for this flat layer, the origin of the broadened band observed is not fully clear at the present stage. However, the ridges on the base plate may disturb the phase of the reflected light to result in optical diffraction in a broad angular range. In fact, when the scale is irradiated from the back, the distinct light-blue spot appears on the screen instead of the band. As a consequence, it is deduced that the remaining deep-blue band originates from the reflection by the multilayer structure. Actually, the optical length of a layer, estimated by thickness of the air gap, 70 nm, and cuticle layer, 110 nm,

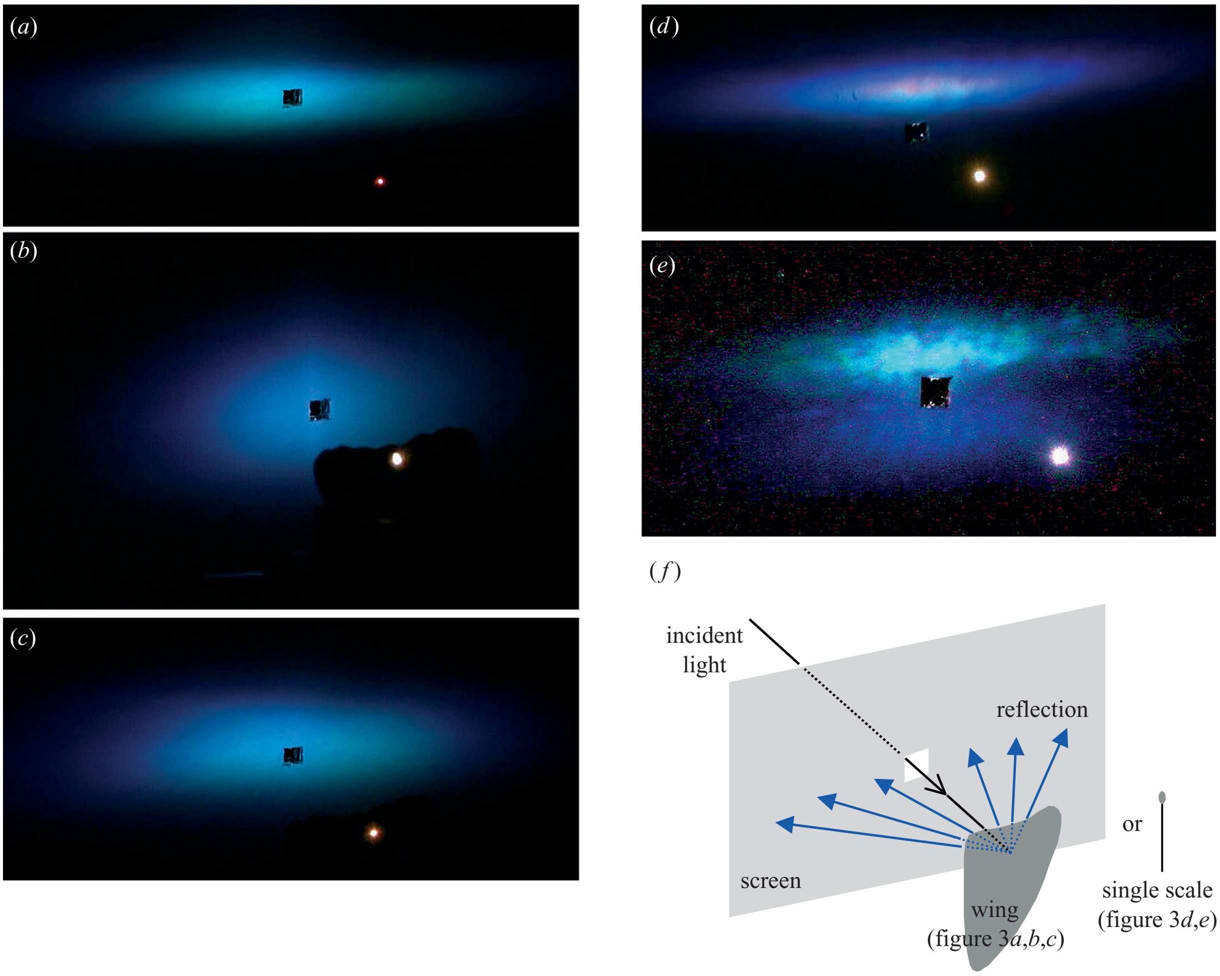

Figure 3. The spatial pattern of the reflection from the wing of the two *Morpho* species and single scales of *M. didius*. (*a*) Pattern for an intact wing of *M. rhetenor*. The other images are all for *M. didius*: (*b*) an intact wing; (*c*) a wing without cover scales; (*d*) a single ground scale; and (*e*) a single cover scale. The white spots in the images are the illuminated position of the sample. (*f*) This shows the experimental set-up for these observations. The incident light goes through the square hole of the screen and illuminates the sample; the wing for (*a*)–(*c*) and a single scale on a needle tip for (*d*) and (*e*).

having a refractive index of 1.56, is 240 nm, which causes constructive interference in blue light. The narrower bandwidth of reflection is qualitatively consistent for a general character of multilayer reflection: the bandwidth becomes narrower as the number of layers is increased. In the analyses above, we ignore the optical absorption, because the cover scales of *M. didius* have been reported to have a negligible amount of melanin (Ghiradella 1994; Vukusic *et al.* 1999).

Owing to its highly transparent appearance, the reflectivity of a cover scale has not been paid so much attention. However, the present experiment indicates that they have a rather high reflectivity of 30% at the peak wavelength. On the basis of the model discussed above, we have simply estimated that the total reflectivity amounts to 31% for a single cover scale. This estimation was made by calculating the sum of effective reflectivity for two layer structures. The effective reflectivity is the product of the reflectivity and the ratio of area of each layer structure to the total exposure. The following values are used in the estimation. For the single layer part, the reflectivity is assumed to be 17% at 470 nm (figure 5*c*). For the multilayer part, assuming the infinitely large area of layer, we expect 87% of reflectivity at 470 nm by means of the transfer matrix method for five cuticle layers having the thickness and air gap mentioned above. The area ratio of each structure to the total exposure is 0.8 and 0.2 for the single and multilayer part, respectively, which is calculated by the ridge separation and width. As a consequence, the sum of effective reflectivity for two layer structures gives 31% as the total reflectivity, which is in good agreement with the experimental result. Here, we should emphasize that these two layer structures have the maximum reflectivity at almost the same wavelength, 470 nm (figure 5*a*,*b*). It is quite interesting that the butterfly adjusts the optical length of the completely different parts of the scale structures.

(b) *Contributions to the optical properties of the whole wing*

To analyse the mechanism for the isotropic reflection caused by the presence of the cover scales, we schematically depict the directions of reflected and transmitted light in a plane containing a ridge in figure 6. Ignoring all factors that cause the angular broadening, we expect that the reflected light goes into three directions with the intensity ratio of 0.14 : 0.54 : 0.03. In this estimation, we consider the multiple reflection between the scales up to three

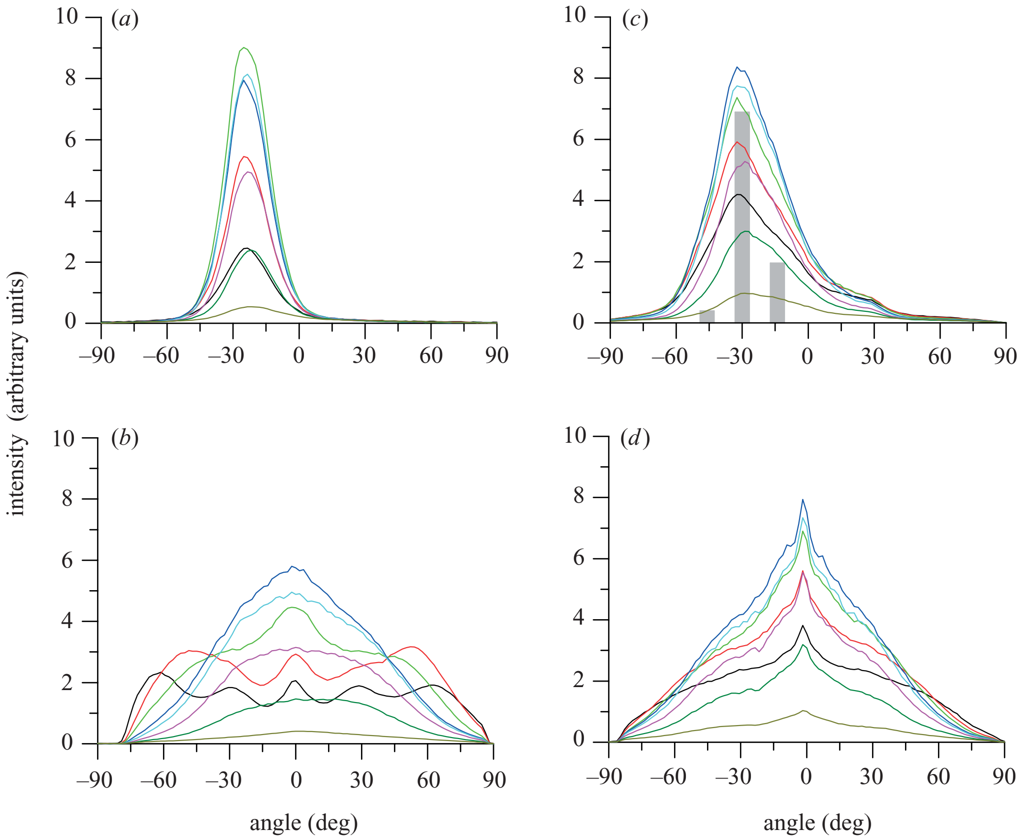

Figure 4. The angular dependence of the reflected light intensity under monochromatic illumination. The observation plane is a vertical one, shown in figure 2, for (*a*) and (*c*) for the wing without cover scales and the intact wing, respectively, whereas (*b*) and (*d*) show the results in the horizontal plane for the wing without cover scales and the intact wing, respectively. The observation plane is not rigorously perpendicular to ridges on scale in (*b*) and (*d*), because of the tilt of the air–cuticle multilayer within ridges and of the scale plate to the wing membrane (Ghiradella *et al.* 1972). The incident direction is 0°. Key for (*a*), (*b*) and (*d*): black, 380 nm; red, 420 nm; light green, 440 nm; dark blue, 480 nm; light blue, 500 nm; purple, 520 nm; dark green, 540 nm; brown, 580 nm. Key for (*c*): black, 400 nm; red, 420 nm; light green, 460 nm; dark blue, 480 nm; light blue, 500 nm; purple, 520 nm; dark green, 540 nm; brown, 580 nm.

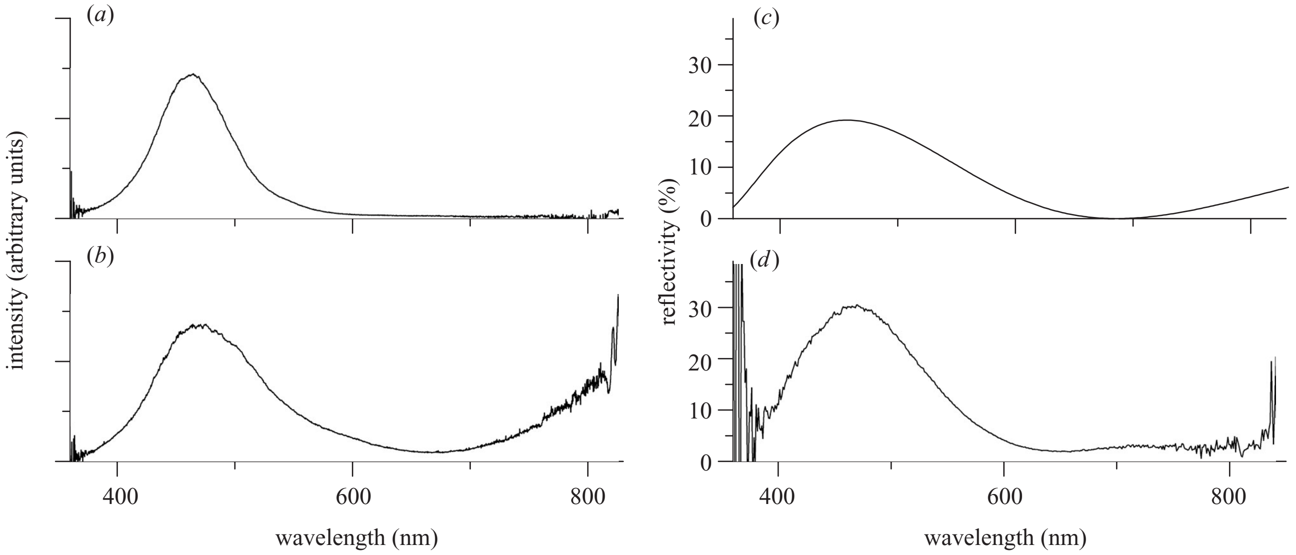

Figure 5. The observed spectra of the reflected light for (*a*) the deep-blue band and (*b*) the light-blue band of a single cover scale. The reflected light around the centre of each band was picked up by the use of an optical fibre. The curve (*c*) shows the reflectivity calculated theoretically for a thin layer of cuticle with thickness 215 nm and refractive index 1.56 by the transfer matrix method. The structural parameter is determined by the SEM observations within the experimental resolution. The curve (*d*) is the total reflectivity from a single cover scale experimentally obtained by the use of an integrating sphere.

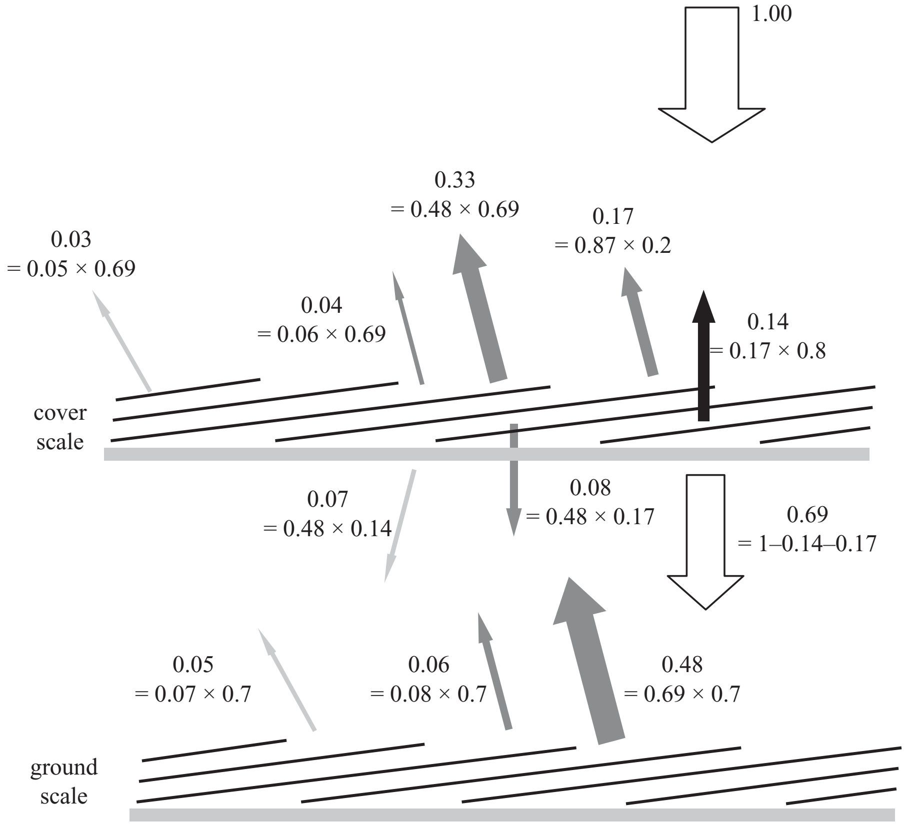

Figure 6. Schematic description of the direction and intensity of the reflected and transmitted light in a cross-section of cover and ground scales along ridges under normal incidence of light having an intensity of unity. The intensities of the reflected lights are simply calculated as the product of reflectivity and ratio of each layer structure to total exposure. The rest of the light is assumed to transmit. For the calculation, the reflectivity of 0.87 and 0.17 and the ratio of 0.2 and 0.8 are used for the multilayer and single layer parts, respectively. For the ground scale, the reflectivity of 0.7 (Kinoshita *et al.* 2002*b*) and the same oblique angle of the multilayer are assumed. In total, the incident light is reflected into three directions with the intensity ratio of 0.14 : 0.54 : 0.03 from the normal to the left direction.

times and ignore the contribution from the other side of the wing. This intensity distribution is shown as the three bars depicted in figure 4*c* with an angular separation of 16°, which is the angle between two reflection bands. We can see that the distribution partly explains the one-sided tail in the angular dependence. Further broadening may be explained by the loose bend of the cover scales. In fact, the scales are found to be bent *ca*. 20° from the root to the distal part by the microscopic observation of the longitudinal cross-section.

In the orthogonal plane, which is perpendicular to the ridges, the different effect of the cover scales is observed; they make the distribution of light intensity narrower, having smooth line shapes (figure 4*d*). Further, the sharp component appears at 0°. An effect of multiple scattering called coherent back scattering (Van Albada & Lagendijk 1985; Wolf & Maret 1985) may account for that sharp component, because it appears even under a 30° inclined incidence. The observed changes in line shapes are partly explained by the slightly narrower angular range of the reflection of the cover scale. However, we still need further studies, especially on the transmission properties of cover scales such as diffraction efficiency and its wavelength dependence, to quantitatively explain this optical effect.

The total reflectivity of the wing has already been reported to be 55% (Kinoshita *et al.* 2002*a*). This value includes contributions from many parts of the wing structure such as the cover scales, ground scales, wing membrane and the scales on the other side. However, the contribution purely coming from the cover scales is the first reflection as depicted in figure 6 and its efficiency is found to be 30% by the present experiment. Thus, it is concluded that the contribution of the cover scales amounts to about half of the total reflectivity of the blue wing.

5. DISCUSSION

As we see above, the cover scale of *M. didius* considerably contributes to the reflection pattern and reflectivity of the whole wing. In particular, the spatial pattern is drastically changed into the isotropic one owing to its peculiar optical characteristics. In this sense, we can say that the cover scales have a role as an optical diffuser. However, this diffuser is specially designed to cooperate with the ground scales of this butterfly and is remarkably different from an ordinary diffuser in the following two aspects. First, its function is quite anisotropic: it broadens the range of reflection in one plane while it makes the light intensity higher around the centre in the orthogonal plane. This anisotropic nature is quite in contrast to isotropic reflection of an ordinary diffuser and is very efficient in producing an isotropic pattern for the present case, because the narrow band pattern of the ground scales is reshaped anisotropically in two orthogonal planes.

Second, the cover scales function as an optical diffuser only for blue light owing to the selective reflection, and they have a high transmissivity for unnecessary red light. This wavelength-selection mechanism is achieved by carefully designed microscopic structures. Actually, the single layer of the base plate has the optimized thickness which is a unique solution to simultaneously achieve high and low reflection for blue and red light, respectively. Owing to optical interference, a single layer has the maximum and minimum reflectivity at the wavelength $\lambda = 2nd/(m + 1/2)$ and $2nd/m$, respectively, where d is the real thickness, n is refractive index and m is the integer. As shown in figure 5*c*, the optical thickness of the layer, 335 nm, realizes the interference condition $m = 1$ for this wavelength-selection mechanism. If the layer were thicker and a different order of the condition were satisfied, the difference between maximum and minimum wavelength would be too narrow for blue and red light and this wavelength selectivity would be lost. Not only the single layer part, but also the multilayer structure, has the optical length that is excellently adjusted to reflect the same wavelength of light. Namely, one pair of the cuticle and air gap has the appropriate optical length, 240 nm, for the reflection of the blue light.

These carefully designed structures within the cover scale strongly suggest that they have been developed through evolution under selection pressures rather than just coincidence. The strong brilliance of the *Morpho* wings is considered to serve conspecific long-range communication. It has already been pointed out that the angular spread of reflection of a ground scale has a function to broaden observable directions (Vukusic *et al.* 1999). Likewise, *M. didius* may use cover scales to widen the solid angle of observable direction further in the orthogonal direction. However, the angular spread is inevitably accompanied by the reduction of light intensity per solid angle. Consequently, the range of communication is limited and, at the same time, the risk of conspicuousness to predatory birds may be lowered as well. In contrast to *M. didius*, wings of *M. rhetenor* do not employ cover scales and do keep anisotropic reflection. Thus, these various optical properties in different species may reflect their individual strategies for communication and survival.

To summarize, we have found that the cover scales of *M. didius* have carefully designed microscopic structures that function as the selective optical diffuser for blue light. Further, the cooperation of the ground and cover scales makes possible the high reflectivity in a wide range of solid angles. When we look at these butterflies from the viewpoint of application, they may give a good hint in clarifying the optical origin of gloss, because the different patterns in reflection result in the different appearance of their glossy wings to human eyes. Thus, the *Morpho* butterflies are interesting research objects from the aspects of biology, physics and application.

We thank Professor Helen Ghiradella at the State University of New York at Albany for the helpful comments. This work is partly supported by The Sumitomo Foundation and Kato Memorial Foundation.

REFERENCES

Anderson, T. F. & Richards Jr, A. G. 1942 An electron microscope study of some structural colours in insects. *J. Appl. Phys.* **13**, 748–758.

Bingham, L., Bingham, I., Geary, S., Tanner, J., Driscoll, C., Cluff, B. & Gardener, J. S. 1995 SEM comparison of *Morpho* butterfly dorsal and ventral scales. *Microsc. Res. Tech.* **31**, 93–94.

Born, M. & Wolf, E. 1975 *Principle of optics*, 5th edn. Oxford: Pergamon.

Ghiradella, H. 1991 Light and colour on the wing: structural colours in butterflies and moths. *Appl. Optics* **30**, 3492–3500.

Ghiradella, H. 1994 Structure of butterfly scales: patterning in an insect cuticle. *Microsc. Res. Tech.* **27**, 429–438.

Ghiradella, H. 1998 Hairs, bristles, and scales. In *Microscopic anatomy of invertebrates*, vol. 11A (*Insecta*) (ed. F. N. Harrison & M. Locke), pp. 257–287. New York: Wiley–Liss.

Ghiradella, H., Aneshansley, D., Eisner, T., Silberglied, R. E. & Hilton, H. E. 1972 Ultraviolet reflection of a male butterfly: interference colour caused by thin-layer elaboration. *Science* **178**, 1214–1217.

Kinoshita, S., Yoshioka, S. & Kawagoe, K. 2002*a* Mechanisms of structural colour in the *Morpho* butterfly: cooperation of regularity and irregularity in an iridescent scale. *Proc. R. Soc. Lond.* B **269**, 1417–1421. (DOI 10.1098/rspb.2002.2019.)

Kinoshita, S., Yoshioka, S., Fujii, Y. & Okamoto, N. 2002*b* Photophysics of structural colour in the *Morpho* butterfly. *Forma* **17**, 103–121.

Mason, C. W. 1927 Structural colors in insects. II. Iridescent colors. *J. Phys. Chem.* **31**, 321–354.

Parker, A. 2000 515 million years of structural colour. *J. Opt.* A **2**, R15–R28.

Tabata, H., Kumazawa, K., Funakawa, M., Takimoto, J. & Akimoto, M. 1996 Microscopic structures and optical properties of scales of butterfly wings. *Opt. Rev.* **3**, 139–149.

Van Albada, M. P. & Lagendijk, A. 1985 Observation of weak localization of light in a random medium. *Phys. Rev. Lett.* **55**, 2692–2695.

Vukusic, P., Sambles, J. R., Lawrence, C. R. & Wootton, R. J. 1999 Quantified interference and diffraction in single *Morpho* butterfly scales. *Proc. R. Soc. Lond.* B **266**, 1403–1411. (DOI 10.1098/rspb.1999.0794.)

Wolf, P.-E. & Maret, G. 1985 Weak localization and coherent backscattering of photons in disordered media. *Phys. Rev. Lett.* **55**, 2696–2699.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.