Structural or pigmentary? Origin of the distinctive white stripe on the blue wing of a *Morpho* butterfly

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A few species of *Morpho* butterflies have a distinctive white stripe pattern on their structurally coloured blue wings. Since the colour pattern of a butterfly wing is formed as a mosaic of differently coloured scales, several questions naturally arise: are the microstructures the same between the blue and white scales? How is the distinctive whiteness produced, structurally or by means of pigmentation? To answer these questions, we have performed structural and optical investigations of the stripe pattern of a butterfly, *Morpho cypris*. It is found that besides the dorsal and ventral scale layers, the wing substrate also has the corresponding stripe pattern. Quantitative optical measurements and analysis using a simple model for the wing structure reveal the origin of the higher reflectance which makes the white stripe brighter.

Keywords: structural colour; *Morpho* butterfly; butterfly wing colour; colour pattern

1. INTRODUCTION

The brilliant blue colour of the wing of *Morpho* butterflies is one of the most famous examples of so-called structural colour (Srinivasarao 1999; Parker 2000; Vukusic & Sambles 2003). To clarify the microstructure inside their iridescent scales, electron microscopy was first applied in 1942 by Anderson and Richards. Later, the tree-like microstructure was clearly observed in a cross-section of the scale (Lippert & Gentil 1959), and it is now well known that the structure is essential to producing the brilliant blue colour (Ghiradella 1991; Tabata *et al.* 1996). However, detailed optical study has been performed for the first time only very recently. In 1999, Vukusic and co-workers performed spectroscopy of individual scales and characterized the single-scale optical properties of two species of *Morpho* butterflies (Vukusic *et al.* 1999). Subsequently, a theoretical model has been successfully proposed to explain the observed extraordinary reflective properties by introducing the cooperation of the regularity and irregularity of the structure (Kinoshita *et al.* 2002a). Further, an inter-scale mechanism of blue light reflection was found in a species of *Morpho* butterfly (Yoshioka & Kinoshita 2004).

Among many species of genus *Morpho*, a few, e.g. *M. cypris* and *M. rhetenor helena*, have a white stripe pattern on their brilliantly coloured blue wings. Since its whiteness looks very distinctive, several questions naturally arise: what kind of structure exists inside the white scale? What causes the optical difference between the blue and white scales? In general, a white colour originates from wavelength-independent light scattering caused by randomly oriented surfaces of a material having a refractive index different from that of the surrounding media. Therefore, the brightness of the white is directly associated with the magnitude of reflectance. In fact, it is easily observed that common white materials, such as a piece of copy paper, have a rather high reflectance of several dozen percent. However, this higher reflectance is obviously

related to the substantial thickness of the material. For example, the thickness of a copy paper is typically of the order of 100 µm. On the other hand, the thickness of a butterfly scale is only several micrometres, although its upper surface is finely sculpted (Ghiradella 1991, 1994, 1998). Thus, it is seemingly difficult for such thin scales to possess higher reflectance causing a distinctly white appearance.

We have investigated the stripe pattern of the wing of *M. cypris* to clarify the origin of its whiteness. In particular, we have performed detailed optical measurements of almost all parts of the butterfly wing structure, the dorsal blue and white scales, the ventral brown and white scales and the wing substrate itself. What we have found is a thoroughly controlled pigmentation throughout the wing structure which realizes the bright white stripe.

2. MATERIAL AND METHODS

A male specimen of *M. cypris*, collected in 1997 in Columbia, was purchased from Mushi-sha Japan. The scale arrangement was observed with an Olympus BX50 fluorescence microscope and a Nikon SMZ1000 stereoscopic microscope. The microscopic structure of the scale was observed with a JEOL JSM-5800 or Hitachi S-800 scanning electron microscope (SEM) after the surface was sputtered with gold or platinum.

The quantitative optical properties of the single scales were characterized by using an optical system which was specially designed for a butterfly scale (Yoshioka & Kinoshita *in press*). Briefly, monochromatized light was focused on a small spot, which lay completely within the scale. The total amounts of diffuse reflection and transmission were measured by using an integrating sphere. The resulting reflectance and transmittance was subtracted from 100%, and the remaining percentage was assigned to be the absorbance (the fluorescence contribution was confirmed to be negligible).

To check the colour pattern of the wing substrate, almost all the scales were removed by the use of the liquid polyvinyl alcohol. The butterfly wing was first sandwiched by two pieces of paper, which were fully drenched with the liquid. After the liquid had dried, the paper was trimmed to the

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Figure 1. Wing colour pattern of the (a) ventral and (b) dorsal sides of *M. cypris* and (c) the naked wing membrane, where the scales of both sides are almost removed. Note that a number of remaining blue scales causes some blue patches in (c).

outline of the wing. Then, the two pieces of the paper to which the scales were attached were carefully separated from the naked wing membrane. For the optical measurements of the substrate, the scales were removed simply mechanically, with a brush.

3. RESULTS

(a) Correspondence in colour pattern

We first compare the colour patterns of the dorsal and ventral sides of the wing of *M. cypris*. As shown in figure 1a,b, it is quite interesting that both sides exhibit a similar white stripe pattern, which is almost, although not perfectly matching. To check the colour pattern of the wing substrate, we have removed the scales on both sides almost completely. Surprisingly, the naked wing membrane also has the same stripe pattern formed by transparent and brown areas (figure 1c). Careful inspection of the colour pattern at the wing membrane reveals that it is more similar to the colour pattern of the ventral wing scales rather than to that of the dorsal scales; the substrate has several ocelli corresponding to those of the scale pattern on the ventral wing. Since the substrate stripe pattern looks the same when viewed from both the dorsal and ventral sides, we consider the substrate as one layer, although it is developed as a flattened sac to form a two-layered structure. The good correspondence observed in the stripe patterns among these three layers (the two scale layers on the both sides, and the wing substrate) suggests that the whole of the wing structure is designed to make the white stripe.

(b) Microscopic observations

First, we observed the scale arrangement. Many species of *Morpho* butterflies have two kinds of the scales, called ground and cover (or glass) scales, which form two distinctive layers (Ghiradella 1994). However, the dorsal wing surface of *M. cypris* is almost completely covered with only one layer of scales. The stripe pattern is formed as a mosaic consisting of white and blue scales, which have similar dimensions of $85 \times 200 \mu\text{m}$. On the ventral surface, brown and white scales form the stripe. In both brown and white areas, two kinds of scales, ground and cover scales, are present, which slightly overlap by *ca* 20–30% of their width. These scales have similar dimensions of $70 \times 200 \mu\text{m}$.

Figure 2. Scanning electron microscope images of a cross-section of the (a) blue and (b) white scales on the dorsal wing, the upper surface structure of the (c) brown and (d) white ground scales of the ventral side, and the surface structure of the dorsal (e) transparent and (f) brown areas of the wing substrate.

We further investigated the microstructure inside the scales. As shown in figure 2a,b, both the blue and white dorsal scales have in cross-section a tree-like air-cuticle multilayer structure (Anderson & Richards 1942; Lippert & Gentil 1959) like other *Morpho* species. Despite the large difference in their colours, the structures of the blue and white scales are very similar, and no distinctive difference is noticed in the ridge separation of $0.7 \mu\text{m}$, number of cuticle layers of 10–13, and layer thickness of 60–90 nm. We have also investigated the ventral scales. The observed structures of the brown and white ground scales, shown in figure 2c,d, respectively, constitute an unspecialized ridge-lamella connected by crossribs, which is common for butterfly scales (Ghiradella 1991). The ridge separation of the brown scale is slightly wider than that of the white scale, and the pillars under the ridges look more disordered in the white scale. However, their overall

Figure 3. Experimentally determined optical properties of the (a) blue and (b) white areas of the intact dorsal wing and (c) the white stripe area after the ventral scales are removed.

structures are not very different. The microstructures of both the brown and white cover scales are similar to those of the ground scale. Finally, the surface structure of the wing substrate was observed. As shown in figure 2*e,f*, an array of many sockets, into which the scales are inserted, is observed. However, we can see no particular difference, at least, on the surface between the brown and transparent areas. At a higher magnification, small bumps are observed in both areas.

(c) Optical characteristics of the wing

We have quantitatively determined three optical characteristics: the reflectance, transmittance and absorbance. Figure 3*a,b* shows those three parts of the blue and white areas of the dorsal intact wing, respectively. As is immediately seen, the most prominent differences appear in the absorbance, indicating the difference in the amount of pigmentation, and in the reflectance of wavelengths greater than 550 nm. A large reflectance peak is commonly observed in the wavelength region below 500 nm. The presence of this peak is quite reasonable, because both the blue and white scales have the tree-like microstructure, which is known to cause constructive optical interference in blue light. Focusing our attention now on the difference in the reflectance in the longer wavelength region, we can see that the blue area has a minimum reflectance of only 5% around 580 nm, while that of the white one is more than 30%. Since the reflectance determines what we see, this quantitative difference in the longer wavelength region should explain the difference in colour. The reason the white area looks white in spite of the large reflectance peak in the blue part of the spectrum is related to the special reflective properties of *Morpho* butterflies, which are discussed in §5.

The reflectance may be determined not only by the dorsal scales, but also by the wing substrate, and also the ventral scales. To investigate the different contributions to the reflection, we removed the ventral scales. As shown in figure 3*c*, the reflectance is decreased by *ca* 10% over the whole wavelength region. This fact clearly indicates that reflectance is not simply attributed to the dorsal scales, but to the whole wing structure, including the ventral scales.

(d) Optical characteristics of the single scales and the wing substrate

To further analyse the optical properties of the wing, the individual scales on both sides were examined. As shown in figure 4*a,b*, the results for the dorsal blue and white

Figure 4. Optical characteristics of the four kinds of single scales; (a) blue and (b) white scales on the dorsal side, (c) brown and (d) white ground scales on the ventral side. The ventral scales are illuminated from their back (facing the substrate) to analyse the optical properties of the intact wing illuminated from the dorsal side.

scales have a similar tendency to the blue and white areas of the intact wing, respectively. However, there are three quantitative differences. First, the absorbance of the blue scale is less than that of the blue wing area. Second, the absorbance of the white scale is negligible. Third, the reflectance of the white scale in the longer wavelength region is not more than 12%, which is much less than the *ca* 30% of the intact wing. As for the scales on the ventral side, the results, shown in figure 4*c,d*, are very different from those of the dorsal scales. The large peak in blue light region does not exist any more, and no characteristic wavelength-dependence can be seen. The absorption of the white scale is negligible, just like that of the dorsal white scale. The absorption of the brown scale is larger in the shorter wavelength region, as is expected from its brown colour.

Finally, we examined the wing substrate. The optical difference between the brown and transparent areas

Figure 5. Optical characteristics of the (a) brown and (b) transparent areas of the substrate.

mainly appears again in the amount of absorbance (or transmittance), as shown in figure 5a,b. However, the transparent part also absorbs to some extent, although the percentage is much smaller than that of the brown area. These results confirm that the wing substrate is responsible for the optical absorption observed in the white area of the intact wing.

The absorption is attributed to melanin pigment in the scales of *Morpho* butterflies (Ghiradella 1998; Vukusic *et al.* 1999). Thus, we can say that this butterfly thoroughly controls the pigmentation in both the scales and substrate to form the stripe pattern.

4. ANALYSIS

To analyse how much each part of the wing contributes to the reflectance of the intact wing, we consider a simple model which consists of three layers. The model, shown in figure 6, treats the multiple reflection between the three layers as corresponding to the scale layers on both sides and the wing substrate. Although the reflection of the scale is actually diffuse, we assume specular reflection for simplicity and just calculate the total reflectance by accumulating an infinite series of multiple reflections. A similar calculation for the two layer system has been already discussed in Appendix B of Yoshioka & Kinoshita (*in press*). In the present three-layer case, we first consider the upper two layers, which correspond to the dorsal scale and the substrate, paying attention to the fact that the dorsal blue scale has a very different reflectance depending on whether it is illuminated from upper or lower sides (Yoshioka & Kinoshita *in press*). After the net reflectance and transmittance for the upper two layers is obtained, the same calculation method is then applied to the pair of the upper two layers and the lowest third layer.

Figure 7a shows the optical properties of the three-layer system calculated using the experimental data of the white stripe part. It is clearly demonstrated that the reflectance is increased step by step as the wing components are added owing to the multiple reflection. The reflectance for the three-layer system reaches almost 30%, which nearly reproduces that of the intact wing in longer wavelength region. The present model does not take into account the overlap of the ventral ground and cover scales. Including the overlap will increase the reflectance and yield a better agreement with the experimental results.

On the other hand, as for the blue area having the large absorption, the effect of multiple reflection is very limited

as shown in figure 7b. The reflectance is increased only slightly in the longer wavelength region, where the absorption is relatively small. In contrast to this small effect on the reflectance, the lowering in transmission is reproduced fairly well as shown in figure 7c. It is decreased down to 30% at 700 nm for the three-layer system from 57% of the single blue scale.

The above analysis qualitatively confirms that all three layers contribute to a reflectance increase by multiple reflection, which originates from the absence or reduction of the pigmentation of the scales and the substrate.

5. DISCUSSION

First, we consider the relation between the spectral shape of reflectance and the colour. In spite of the large peak in the blue wavelength range, the stripe area of the wing still looks white. This is explained by the special reflection properties of *Morpho* wing; the blue light is reflected into a very narrow angular range owing to the anisotropy in the ridge-lamella structure (Vukusic *et al.* 1999; Kinoshita *et al.* 2002a,b). As a result, when the wing is illuminated by a narrow beam of light, reflected blue light will be seen only from a limited angle of observation. From other directions, only randomly scattered white light will be seen. Similarly, under an ambient illumination, the higher reflectance for the blue light is only applicable to the limited angular range of the incident light and the other major part is just diffusely reflected toward the observing direction. We have experimentally determined the angular spread of the reflected light using a He–Ne laser of 633 nm according to the method described in Yoshioka & Kinoshita (*in press*). At that wavelength, the dorsal white scale was found to reflect the light almost isotropically, while the wing substrate yields a rather specular reflection. Thus, we conclude that the *Morpho* scale functions to produce diffuse reflection. However, further study is necessary to clarify the origin of the diffuse nature and wavelength-independence of the reflection.

In general, the random structure of a bright white object causes the multiple scattering, and the relatively large thickness results in a high reflectance and the brightness. The brightness of snow, clouds in the sky and a common white paper is all produced in such a way. On the other hand, the butterfly wing should be thin and lightweight to serve for a tool of locomotion. Thus, a butterfly wing of white colour includes two essentially contradictory factors. To reconcile these, the butterfly *M. cypris* adopts a quite simple strategy. It locally removes the pigment in the scales as well as the wing substrate. This method fully exploits the three-layer structures of the wing, and the multiple reflections among them produce a high reflectance in the area of the white stripe. Nevertheless, the microscopic structures of the scales are hardly modified across the wing. It may be easier for the butterfly to control the pigmentation than to change the scale structures. As for the blue part of the wing, the enhanced optical absorption makes the blue colour more vivid by reducing the unnecessary white light.

Other species of butterflies adopt a totally different strategy to obtain the white wing. The small white butterfly, *Pieris rapae*, is one case. The scale of a male butterfly is adorned with many tiny beads, 100 nm in size (Yagi 1954). It is thought that the beads scatter light

Figure 6. A three-layer model considering the multiple reflection between the dorsal and ventral scales and the wing substrate. The illumination-direction dependence is considered for the dorsal scale; R_{du} (R_{dl}) and T_{du} (T_{dl}) are reflectance and transmittance of the dorsal scale, respectively, illuminated from the upper (lower) side. R_m (R_v) and T_m (T_v) are reflectance and transmittance of the substrate (the ventral scale), respectively. Arrows show a series of reflections and transmissions between the layers.

Figure 7. Calculated reflectance and transmittance of the three-layer model. (a), (b) Reflectance of a dorsal scale only (experimental), a dorsal scale on the substrate (calculated), and the scales of both sides on the substrate (calculated), from bottom to top, for the white and blue areas, respectively. (c) Transmittance of a dorsal blue scale only (experimental), a dorsal scale on the substrate (calculated), and the scales of both sides on the substrate (calculated), respectively, from top to bottom.

efficiently and serve to make the wing brighter (Stavenga *et al.* 2004). In fact, our preliminary measurements show that nearly 30% of incident light is reflected by a single white scale (Yoshioka & Kinoshita *in press*). The nymphalid butterfly *Neptis sappho* utilizes another structural variation to produce the white stripe pattern. On the wing substrate of the stripe area, not in the scale, there exists a randomized structure like a frosted glass, and the stripe area is observed to be brighter under an optical microscope.

It is commonly assumed that the colour pattern of the butterfly wing is mainly due to the colour of the scales (Nijhout 1991). However, the colour white may be an exception, since, as discussed above, it is advantageous to employ the whole wing structure to obtain higher reflectance. In fact, we can see a lot of species having the corresponding white stripe pattern on both dorsal and ventral sides. On the other hand, the situation is opposite when a saturated colour is necessary. Pigmented dark scales are usually present under the coloured scales to

increase the colour contrast. For example, the papilionid butterfly *Trogonoptera brookiana* has a structurally produced green stripe pattern, where matt black scales are present below the green scales.

Our findings add a new dimension to the question of control of pattern formation, the process by which cells control the architecture of the larger structures—tissues, organs and bodies—in which they reside. A butterfly wing has to determine several patterns at once: the shape of the wing, the venation pattern, the distribution of different scale types (often quite different on the two sides of the wing), and finally the detailed architecture and pigmentation of the individual scales. Complex as they are, all these patterns usually ‘work together’, implying some sort of as yet unknown mechanism for global control of their development. Our results provide more immediate support for this view, as they are an example of two sets of scales and the underlying wing membrane all working precisely together to provide an optical effect.

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