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RESEARCH ARTICLE

Glass scales on the wing of the swordtail butterfly *Graphium sarpedon* act as thin film polarizing reflectors

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SUMMARY

The wings of the swordtail butterfly *Graphium sarpedon* (the Common Bluebottle) have blue/green-colored patches that are covered on the underside by two types of scales: white and glass scales. Transmission and scanning electron microscopy revealed that the white scales are classically structured: the upper lamina, with prominent ridges and large open windows, is well separated by trabeculae from a flat, continuous lower lamina. In the glass scales, the upper lamina, with inconspicuous ridges and windows, is almost flat and closely apposed to the equally flat lower lamina. The glass scales thus approximate ideal thin films, in agreement with the observation that they reflect light directionally and are iridescent. Reflectance and transmittance spectra measured from the glass scales with a microspectrophotometer agree with spectra calculated for an ideal non-absorbing thin film. Imaging scatterometry of single, isolated glass scales demonstrated that the reflected light can be strongly polarized, indicating that they function as polarizing reflectors.

Key words: iridescence, wing coloration, imaging scatterometry, microspectrophotometry, Papilionidae.

INTRODUCTION

The striking coloration of butterflies is generally determined by the scales that cover the wings like shingles on a roof. A rare exception to this rule is the swordtail butterfly *Graphium sarpedon*, the Common Bluebottle (Allyn et al., 1982; Nijhout, 1991). The wings of this butterfly are marked by dark-brown margins covered on both the upper and undersides by scales that contain melanin. The wings have a brightly colored midband consisting of a row of blue/green patches where the wing membrane contains bile pigment. On the upper side of the wings, the patches have arrays of long, hair-like bristles but they are otherwise devoid of scales. On the wing underside, the patches do have scales. The patches near the wings’ inner margin are covered by white scales but most other patches have glass scales (Stavenga et al., 2010). Reflectance spectra measured from the white patches were broad-band and virtually polarization- and angle-independent but the reflectance spectra measured from the patches with glass scales were strongly polarization- and angle-dependent. The latter spectra were undulating, indicating thin film or multilayer interference effects (Stavenga et al., 2010).

We have further investigated the white and glass scales with transmission and scanning electron microscopy, microspectrophotometry and imaging scatterometry, and we found that the glass scales are uniquely structured and approximate ideal thin films. The transmittance and reflectance spectra of single scales could be well explained with classical thin film optics. Furthermore, the hypothesis that the glass scales can have a function in polarization signaling (Stavenga et al., 2010) has been reinforced.

MATERIALS AND METHODS

Animals

The optical and structural measurements were performed on the Japanese subspecies *nipponum* of the swordtail butterfly *Graphium sarpedon* Linnaeus 1758 (Common Bluebottle; Papilionidae). Specimens were captured around the Sokendai Hayama campus, Kanagawa, Japan. Additional pinned *G. sarpedon* specimens were obtained from Robert Goodden (Worldwide Butterflies, www.wwb.co.uk). Photographs of the intact animal were made with a Nikon D70 camera, which, when needed, was equipped with a linear polarization filter. Photographs of small wing areas were made with an epi-illumination microscope (Zeiss Universal Microscope, www.wwb.co.uk). For light and transmission electron microscopy (TEM) of the scales, wing parts were prefixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 mol l⁻¹ sodium cacodylate buffer (CB, pH 7.3) or sodium phosphate buffer (PB, pH 7.3) for ~45 min at 20–25°C. Embedding in Epon followed dehydration with a graded series of ethanol and infiltration with propylene oxide. For light microscopy (LM), the tissues were cut into ~1 μm sections and observed with a BX51 (Olympus) microscope. For TEM, the tissues were cut into 70–80 nm sections, which were observed with an H7650 transmission electron microscope (Hitachi, Tokyo, Japan). For scanning electron microscopy (SEM), the wing pieces were sputtered with platinum and observed with a JSM-6490LV (JEOL, Tokyo, Japan).
Microspectrophotometry
Reflectance and transmittance spectra of isolated scales were acquired with a microspectrophotometer (MSP), consisting of a xenon light source, a Leitz Ortholux microscope (Wetzlar, Germany) and an AvaSpec-2048 spectrometer (Avantes, Eerbeek, The Netherlands). The microscope objective was an Olympus ×10 (NA 0.30). A white diffuse reference tile (WS-2, Avantes) served as a reference.

Imaging scatterometry
The spatial scattering characteristics of single white and glass scales were investigated with an imaging scatterometer. Briefly, a single scale was isolated from the wing and glued to the slender tip of a glass micropipette. The scale was positioned in the first focal plane of an ellipsoidal mirror and illuminated, with either a narrow-aperture (~5 μm) or a wide-aperture (180 deg) beam, at a spot with diameter ~15 μm. The scatter diagram, i.e. the spatial distribution of the scattered light at infinity, was collected, via a diaphragm in the second focal plane and projection lenses, by an Olympus DP-70 digital camera (see also Stavenga et al., 2009).

Optics of thin films
The propagation of a light wave in a thin film with thickness \( d \) and refractive index \( n_1 \) between two media with refractive indices \( n_0 \) and \( n_2 \), is described by Snell’s law: 

\[
\frac{n_0 \sin \theta_0}{n_1} = \frac{n_1 \sin \theta_1}{n_2},
\]

where \( \theta_0 \) and \( \theta_1 \) are the angles of incidence and refraction, respectively. The reflection coefficients \( r_i \) at the interfaces are given for TE- (s-)polarized light (i.e. light polarized perpendicular to the plane of light incidence) (Eqn 1) and TM-(p)-polarized light (i.e. light polarized parallel to the plane of light incidence) (Eqn 2) by the Fresnel formulas (Yeh, 2005):

\[
r_{i-1,i} = \frac{n_{i-1} \cos \theta_{i-1} - n_i \cos \theta_i}{n_{i-1} \cos \theta_{i-1} + n_i \cos \theta_i}, \quad (1)
\]

and

\[
r_{i-1,i} = \frac{n_{i-1} \cos \theta_i - n_i \cos \theta_{i-1}}{n_{i-1} \cos \theta_i + n_i \cos \theta_{i-1}}, \quad (2)
\]

with \( i = 1, 2 \). The reflectance \( R \) of the thin film is given for both TE- and TM-polarized light by the Airy formula:

\[
R = \frac{\left( \frac{1}{n_2} + \frac{1}{n_1} \right) \cos \theta_2 - n_2 \cos \theta_1}{\left( \frac{1}{n_2} + \frac{1}{n_1} \right) \cos \theta_2 + n_2 \cos \theta_1}, \quad (3)
\]

with \( \varphi = (2\pi/\lambda)n_1 \cos \theta_1 \), where \( \lambda \) is the light wavelength.

RESULTS
The blue/green bands in the wings of G. sarpedon (Fig. 1A), when observed at low magnification, have a few patches with white scales at the underside; most patches have glass scales (Stavenga et al., 2010). Observed at high magnification with an epi-illumination microscope, the white scales have a mixture of colors (Fig. 1B); the glass scales brightly reflect blue/green (Fig. 1C).

As noted above, in the green bands of the wings, scales exist only on the wing underside. Light microscopic sections of the wing patches with the two scale types (Fig. 2A,B) showed that the anatomical structure of the white and glass scales distinctly differs. We recall here that a butterfly wing scale generally consists of two laminae, of which the lower lamina is approximately flat and the upper lamina is elaborated into ridges, running parallel to the scale long axis; the ridges are connected by so-called cross ribs, with open spaces in between, known as the windows. TEM demonstrated that the lower lamina, i.e. the layer facing the ~4-μm-thick wing, of both the white (Fig. 2C) and glass (Fig. 2D) scales approximates a flat layer with a thickness of ~200 nm. SEM further showed that the upper lamina of the white scale has a quite irregular structure, with prominent ridges (Fig. 2C) and large open windows (Fig. 2E) but the glass scale has very minor ridges (Fig. 2D) and virtually no windows (Fig. 2F). Because the thickness of the upper lamina is also ~200 nm, the glass scale approximates an ideal thin film with a thickness of ~400 nm (Fig. 2D).

The optics of thin films is a fundamental and widely studied part of physics. We therefore have attempted to interpret the optical properties of the glass scales with standard thin film theory (Yeh, 2005). We first measured transmittance spectra (Fig. 3A; upper two curves) of single glass scales with an MSP. The spectra clearly demonstrate that the scales are almost fully transparent, except for small periodic oscillations. Reflectance spectra also measured with the MSP (Fig. 3A; lower two curves) feature oscillations mirroring those in the transmittance spectra. Because a white diffuser served as a reference, the amplitude of the reflectance spectra presented in Fig. 3A has to be corrected, however.

The reflectance of a thin film depends on its thickness and the refractive indices of the film and the surrounding media. Applying Jamin–Lebedeff interference microscopy, Leertouwer et al. (Leertouwer et al., 2011) measured the effective thickness of the glass scales to be \( d = 404 ± 28 \) nm and the refractive index was shown to be well described by the Cauchy equation: 

\[
n(\lambda) = A + B/\lambda^2,
\]

with...
A=1.517 and \( B=8.80 \times 10^{-3} \text{nm}^2 \). Using the thin film reflectance formula (Eqn 3), we have calculated the reflectance spectrum of a glass scale with thickness \( d=400 \text{nm} \) in air (Fig. 3B).

For an ideal thin film the reflectance in the minima vanishes (Fig. 3B, curve for standard deviation \( \sigma=0 \)), while this does not occur in the measured spectra (Fig. 3A). We therefore assumed that the thickness in the measured area of the glass scale was not perfectly constant or that the measured spectra are a superposition of spectra resulting from a range of thicknesses (see also Stavenga et al., 2011a). For a Gaussian distribution of the thickness with standard deviation \( \sigma \), oscillating reflectance spectra result with an amplitude that rapidly diminishes with increasing \( \sigma \); an almost flat spectrum results for \( \sigma=80 \text{nm} \) (Fig. 3B). The shape of the experimental reflectance spectra of Fig. 3A is very similar to the reflectance spectrum of Fig. 3B calculated for \( \sigma=20 \text{nm} \), and therefore we conclude that the actual scale thickness in the measured area slightly varies, with a standard deviation of \( \approx 20 \text{nm} \). The transmittance spectra of Fig. 3A show transmittance values near 500 nm and 600 nm of 0.86 and 0.97, respectively, whereas for the thin film with \( \sigma=20 \text{nm} \) the calculated reflectance at 500 nm and 600 nm is 0.16 and 0.02, respectively. Taking the latter values together with those of the measured transmittance spectra, this means that the sum of transmittance and reflectance is \( \approx 1 \) or that absorption is negligible. The experimental reflectance values at 500 nm and 600 nm are 0.45 and 0.05, respectively, meaning that the experimental spectra shown are too large by a factor of 2.8, due to using a diffuser as a reference.

It is well known that thin films are iridescent, i.e. their reflectance spectrum depends on the angle of light incidence. To investigate this effect for a thin film with \( d=400 \text{nm} \) and \( \sigma=20 \text{nm} \), we calculated reflectance spectra for angles of incidence \( \theta=0 \text{deg}, 20 \text{deg}, 40 \text{deg} \) and 60 deg for TE-polarized light as well as for TM-polarized light (Fig. 3C, D). With increasing \( \theta \), the peak wavelengths decrease, i.e. shift to shorter wavelengths. The reflectance peak values increase for TE-polarized light (Fig. 3C) but decrease for TM-polarized light (Fig. 3D). The reflectance of TM-polarized light virtually vanishes for a Brewster’s angle of \( \approx 60 \text{deg} \).

The different anatomy of the white and glass scales not only has distinct consequences for their spectral properties but also for their spatial reflection characteristics. We investigated the scale optics in more detail with an imaging scatterometer by locally illuminating single, isolated white and glass scales and then observing the distribution of reflected and scattered light (Fig. 4). Illumination of a white scale with a narrow-aperture (5 deg), white-light beam caused a prominent, spotty colored band, superimposed on a spatially diffuse scatter pattern (Fig. 4A). The band is immediately understood from light diffraction on the ridges in a plane perpendicular to the ridges, and the diffuse pattern results from the irregular structure of the white scale. Hence, illumination with a wide-aperture beam will spread both the diffraction and diffuse scattering effects throughout the whole hemisphere above the scale, resulting in a very diffuse, white scatter pattern (Fig. 4C). Adding a linear polarizer to the wide-aperture light beam caused a strongly depolarized, diffuse light distribution (Fig. 4E).

The reflection behavior of the glass scales is quite different. A narrow-aperture, white beam incident about normally on a glass scale resulted in a reflected beam that was spatially limited to a very small angle and spectrally limited to the green-wavelength range (Fig. 4B). A glass scale thus approximates a colored mirror. Because of this mirror behavior, obliquely incident light will be reflected in the opposite oblique direction. From about normally incident white light the green-wavelength range is reflected but from white light incident at a large angle, the blue and red wavelengths remain, creating purplish-colored reflections (Fig. 4D). Applying polarized incident light showed that the reflection properties strongly depend on the light polarization. For large angles of incidence (and reflection), TE-polarized light was strongly reflected but the reflection of TM-polarized light was then low (Fig. 4F).

The glass scales are closely juxtaposed on the underside of the wings of *G. sarpedon* and there form an almost continuous shiny layer (Fig. 1C) (Stavenga et al., 2010). We thus conjectured that the angle dependence of the reflection of polarized light might be directly visible on the intact animal. To investigate this, we illuminated a pinned butterfly with a white-light beam in the body symmetry plane at an angle of incidence \( \theta=58 \text{deg}, \approx 60 \text{deg} \), about Brewster’s angle. We then photographed the butterfly with the camera axis coinciding with the direction of reflection (Fig. 5A). A linear polarizing filter positioned in front of the camera lens was directed either horizontally (TE, Fig. 5B) or vertically (TM, Fig. 5C). TE-polarized light appeared to be reflected well, with a pinkish tone, by the patches of the wing undersides that have glass scales (Fig. 5B). For TM-polarized light, the reflection from the patches with glass scales was distinctly less; however, a greenish-colored reflection remained. The angle of light incidence was about Brewster’s angle, and then the reflection of TM-polarized light is minimal (Fig. 5D and Fig. 4F). The remaining green light is therefore not due to
reflections of the glass scales but it is backscattered light from the wing proper, as can be understood by repeating the experiment for the upper side of the wings with TE- and TM-reflected light, photographed again at Brewster’s angle, ~58 deg (Fig. 5D,E). The reflected light of the wing upper side is green, due to the bile pigment in the wing (Stavenga et al., 2010). The reflections only very slightly depend on the polarization, because the upper side only has bristles and the wing surface is rather rough. Finally, the reflectance of the few patches with white scales, near the wing’s inner margin, is also virtually independent of the light polarization (Fig. 5B,C), as expected from the single scale scatterometry (Fig. 4C,E).

**DISCUSSION**

The basic structure of a butterfly scale is a more or less flat lower lamina and a highly elaborate upper lamina, marked by longitudinal ridges, usually with fine flutes or microribs, running down their
Butterfly glass scale polarizers

Butterfly glass scale polarizers sides. Ridges are generally connected by cross ribs (netted in form in the Papilionidae), and the scale upper and lower laminae are joined by trabeculae, which run through the lumen or hollow scale interior (Ghiradella and Radigan, 1976; Ghiradella, 1989; Ghiradella, 1998; Nijhout, 1991; Ghiradella, 2010). On this theme a wide variety of modifications exists, however (Ghiradella, 1984; Vukusic et al., 2000). The organization of the white scales at the wing underside of G. sarpedon closely conforms with the basic structure of butterfly scales (Ghiradella, 2010). The glass scales, however, appear to be unique in that the two laminae have collapsed, the ridges have become minimal, the trabeculae are absent and the windows, so prominent in the white scales, have virtually completely vanished. The resulting structure closely approximates a simple plate, and thus the scale features optical properties characteristic for an optical thin film. We have found that the experimentally measured reflectance spectra approximate the spectrum of a thin film with a variable thickness, with mean ~400 nm and σ≈20 nm. Nakamura et al. similarly explained the coloration of pigeon feathers with thin film interference in a layer with variable thickness (Nakamura et al., 2008). The latter authors also discuss the effect of the surface roughness, which possibly additionally contributed to deviations from the reflectance spectrum of an ideal, perfectly flat thin plate. Although the glass scales are certainly neither smooth nor perfectly flat (Fig. 2D), for explaining the joint reflectance and transmittance measurements on isolated glass scales it is sufficient to assume an almost ideal thin film with a slightly varying thickness.

An intriguing question is why G. sarpedon butterflies have the uniquely flattened glass scales. We previously demonstrated that they enhance the green color of the midband patches for light incident from the wing upper side (Stavenga et al., 2010). For light incident from the underside the function of the glass scales is quite different. The reflectance of the glass scales at normal illumination is small but with increasing angle of incidence the reflectance of TE-polarized light becomes substantial, whilst the reflectance of TM-polarized becomes minimal. Consequently, obliquely incident light on the patches with glass scales in the midband of the wing undersides of G. sarpedon becomes highly polarized after reflection (for angle-dependent reflectance spectra of the wing patches, see Stavenga et al., 2010). The strongly polarized reflected light is potentially an important signal for conspecifics, because many insects can detect polarized light (Rossel, 1989; Labhart and Meyer, 1999). Butterflies discriminate between vertically and horizontally polarized light of the same color in the contexts of oviposition and feeding (Kelber et al., 2001; Kinoshita et al., 2011) and for mate detection (Sweeney et al., 2003). Horseflies detect the polarized light reflected by the body surface of horses (Horváth et al., 2010). Presumably, polarized light reflected by surfaces can in general be a crucial signal for insects (Horváth et al., 2007; Stavenga et al., 2011b) and therefore insects, including butterflies, most likely recognize each other by their polarization signals (Douglas et al., 2007; Stavenga et al., 2011b). Because their main predators, birds, probably cannot use polarization vision for object recognition (Greenwood et al., 2003), polarization can serve as a secret signaling channel for insects.

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