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Published in:
Optics Express

DOI:
10.1364/OE.14.004880

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2006

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Download date: 15-11-2018
Reflectance and transmittance of light scattering scales stacked on the wings of pierid butterflies

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Abstract: The colors of butterfly wings are determined by the structural as well as pigmentary properties of the wing scales. Reflectance spectra of the wings of a number of pierid butterfly species, specifically the small white, Pieris rapae, show that the long-wavelength reflectance of the scales in situ, on the wing, is distinctly higher than that of single, isolated scales. An optical model explains that this is due to multiple scattering on overlapping scales by treating the layers of scales on both sides of the wing as a stack of incoherently scattering plates. The model sheds new light on the adaptive significance and evolution of butterfly wing patterns.

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OCIS codes: (290.0290) Scattering; (290.5880) Scattering, rough surfaces

References and links

1. Introduction

Butterflies are generally strikingly colored, due to light reflected by the wing scales, which are arranged on the wing surface like shingles on a roof. The reflected light commonly results from scattering on the scale structures, the prominent longitudinal ridges and the connecting cross-ribs. A distinct color is observed when the scales contain a pigment that absorbs part of the scattered light in a restricted spectral band. Butterflies thus combine physical and chemical coloration methods. The scattered light usually is random and spectrally indistinct, but interference can create striking, intense iridescent colors, as occurs in the multilayers in the scales of the blue Morpho and other nymphaid butterflies [1-4]. Similar multilayers in the wing scales of male sulphurs cause a strong UV reflectance [5-7]. Photonic crystals in the scales of lycanids (blues and hairstreaks) result in a blue or green color [8].

Many pierids apply a specific method of structural coloration in order to achieve brightly-colored wings. The scales are studded with beads [9,10] that act as strong scatterers, which enhance the reflectance [11,12]. The wing scales of the yellow sulphurs (Coliadiine) contain pigments that absorb in the ultraviolet as well as blue wavelength ranges. The distinct yellow wing color thus is due to the combined effect of strong scattering in the long wavelength range and strong absorption at the shorter wavelengths. The scales of male whites (Pierinae) contain an ultraviolet-absorbing pigment, causing a low UV-reflectance, which is well recognized by butterflies, but not by humans [13,14].

The scales on each side of the wing are usually arranged in two layers, a layer of ground scales and an overlapping layer of cover scales. In order to clarify the optical consequences of the scale layering and their biological significance, we have studied the optics of the butterfly wing scale assembly by measuring reflectance and transmittance spectra of intact wings, of partially denuded wings, and of single, isolated scales of pierid butterflies, and we have analyzed the results with a simple model that treats the scales on the wing as a stack of layers, assuming random scattering. We conclude that pierid butterflies realize their bright wing colorations with stacks of strongly scattering scales, which contain short wavelength absorbing pigments.

2. Materials and methods

Animals All investigated butterflies belong to the family Pieridae. The small white, *Pieris rapae*, was obtained from a culture maintained by Dr J. J. van Loon, Entomology department of the Agricultural University in Wageningen, the Netherlands. The autumn leaf vagrant, *Eronia leda*, was studied in the butterfly collection of the latter department and in the Royal Museum of Central Africa, Brussels (curator Dr U. Dall’Asta). The common jazabel, *Delias nigrina*, was obtained from Dr M. Braby, Australian National University, Canberra.

Anatomy The wing anatomy was investigated with standard scanning-electron-microscopical methods.

Spectrophotometry Reflectance spectra of intact wings were measured with a reflection probe connected to a fiber optic spectrometer (SD2000, Avantes, Eerbeek, the Netherlands). Reflectance and transmittance spectra of intact as well as denuded wings were measured with an integrating sphere (AvaSphere-50-Refl), connected to the fiber optic spectrometer. Spectra of single scales were measured with a home-built microspectrophotometer consisting of a Leitz Ortholux microscope and the fiber optic spectrometer. A white reflectance standard (Spectralon, Labsphere, North Sutton, NH, USA) served as a reference.
Modeling

The optical model that describes light propagation in a stack of layers assumes non-coherent scattering. Each layer is characterized by reflectances $r$ and $s$, and transmittances $t$ and $u$, depending on the direction of the incident light (Fig. 1). The reflected and transmitted light fluxes in a two-layer stack with incident light flux $I_1$ from one side and $I_6$ from the opposite side are then described by the following set of equations [Fig. 1(a)]:

$$
I_2 = r_1 I_1 + u_1 I_4; \quad I_3 = t_1 I_1 + s_1 I_4; \quad I_4 = r_2 I_3 + u_2 I_6; \quad I_5 = t_2 I_3 + s_2 I_6.
$$

(1)

When the incident light flux is only from medium 1, then $I_6 = 0$, or $I_4 = r_2 I_3$, so that $I_3 = t_1 I_1 / (1 - s_1 r_2)$ and $I_4 = t_2 I_3 / (1 - s_1 r_2)$. The transmittance, $T = I_2/I_1$, and reflectance, $R = I_2/I_1$, of the two layer stack then are:

$$
T = t_2 I_1 / (1 - s_1 r_2) \quad \text{and} \quad R = r_1 + t_1 u_1 r_2 / (1 - s_1 r_2).
$$

(2)

A stack consists in general of $n$ layers. Equation (1) (where $n = 2$) can be generalized as:

$$
\begin{bmatrix}
I_{2i} \\
I_{2i+1}
\end{bmatrix} = A_i
\begin{bmatrix}
I_{2i-1} \\
I_{2i+2}
\end{bmatrix}, \quad \text{with} \quad A_i =
\begin{bmatrix}
r_i & u_i \\
t_i & s_i
\end{bmatrix}
$$

for $i = 1...n$,

(3)

where layer $i$ is characterized by matrix $A_i$ [Fig. 1(b)]. By introducing $\tau_i = I_{2i+1} / I_{2i-1}$ (for $i = 1...n$) and $\rho_i = I_{2i} / I_{2i-1}$ (for $i = 1...n+1$), Eq. (3) is equivalent to:

$$
\tau_i = t_i / (1 - s_i \rho_{i+1}) \quad \text{and} \quad \rho_i = r_i + u_i \rho_{i+1} \tau_i = t_i + u_i \rho_{i+1} / (1 - s_i \rho_{i+1}).
$$

(4)
With only incident light \( I_1 \), \( L_{2n+2} = 0 \) or \( \rho_{2n+1} = 0 \), which allows calculation of the values of \( \rho_i \) and \( \tau_i \) by recursion. Starting from \( i = n \), it follows that \( \tau_n = t_n \), \( \rho_n = r_n \), \( \tau_{n-1} = t_{n-1} / (1 - s_{n-1} \rho_n) \), etc. The multilayer transmittance and reflectance are thus directly derived from:

\[
T = \prod_{i=2}^{n} \tau_i \quad \text{and} \quad R = \rho_1. \tag{5}
\]

It is easy to see that Eq. (5) is identical to Eq. (2) for \( n = 2 \).

3. Results

3.1. Reflectance spectra of intact wings of two pierids

The male autumn leaf vagrant, *Eronia leda*, provides a characteristic example of the coloration of pierid butterflies (Fig. 2). The dorsal forewings are marked by a prominent orange tip, which highly reflects ultraviolet light (Fig. 2, location and spectrum 1). The remaining parts of the dorsal forewings and hindwings have a yellow color (Fig. 2, location and spectrum 2). The orange as well as yellow colors are due to pigments that selectively absorb short-wavelength light scattered at the scale structures, the ridges, cross-ribs as well as the beads that adorn the scales [10-12]. The high reflectance of the tips in the UV results from iridescence in the ridge lamellae [5-7]. The ventral wings of the male *Eronia leda* are overall yellow, except for a minute UV-reflecting spot in the ventral forewing. *Eronia leda* has a distinct sexual dichroism, as the female has pale yellow dorsal and ventral wings and only ventrally a few scattered UV-reflecting spots.

![Fig. 2. Reflectance spectra of the dorsal forewing of the male autumn leaf vagrant, *Eronia leda* (inset: upper – UV, lower – RGB), measured with a fiber optic spectrometer. Spectrum 1 is from the orange tip, which exhibits a pronounced UV band, due to interference reflectors in the scale ridges. The orange color results from scattered light, filtered by a pigment absorbing in the UV, blue and green wavelength range. Spectrum 2 is from the dorsal forewing area outside the orange tip. The yellow color results from scattered light filtered by a pigment absorbing in the UV and blue wavelength range.](image-url)
The dorsal wings of the male common jezabel, *Delias nigrina*, have a simple color pattern (Fig. 3). The tips of the forewings are black, characteristic of a melanin pigment, and they lack iridescence. The remaining parts of the dorsal forewings and hindwings are white, due to strongly scattering scales. The reflectance is low in the ultraviolet, due to a pigment that absorbs exclusively in the UV (Fig. 3, spectra and locations 1-3). The ventral forewings and hindwings are marked by yellow and red bands (spectra and locations 4 and 5) in a mainly brown-black background (spectrum and location 6). Interestingly, the red bands can be seen at the dorsal side as a slight red sheen, which is reflected in the increase in reflectance in the wavelength range above 560 nm (spectra and locations 1 and 3), corresponding to the wavelength range where the reflectance of the red bands increases (spectrum and location 5).

This observation demonstrates that the ventral scales contribute to the dorsal wing reflectance. The opposite also holds, namely the white spots in the tip of the dorsal scales contribute to the ventral wing reflectance, as can be seen from the slight foot at wavelengths above 400 nm in spectrum 4 of Fig. 3. The scales in the white spots at the dorsal side add to the reflectance of the scales in the yellow bands.

![Reflectance spectra of various parts of the wings of the male common jezabel, *Delias nigrina* (inset: upper – dorsal, and lower – ventral), measured with a fiber optic spectrometer. Spectra 1-3 were measured from the dorsal side, and spectra 4-6 were measured from the ventral side. The reflectance of the dorsal hind wing is high and at most places virtually constant in the visible wavelength region, which is reflected in the white color (spectrum and location 2). The low reflectance at wavelengths below 400 nm demonstrates that the white scales contain a UV absorbing pigment. The dorsal hind wing has bands with a very slight red sheen (locations 1 and 3), which is reflected in the increased reflectance of spectra 1 and 3 at wavelengths above 560 nm. The red sheen is due to bands of red scales at the ventral hindwing (5), which contain pterin pigments absorbing throughout the visible wavelength region, except the red. The yellow bands in the ventral forewing (4) contain UV and blue absorbing pterins. Spectrum 4 has a small foot between 400 and 470 nm due to the contribution of white scales that occur in white spots at the dorsal side. The ventral wings are mainly covered by brown-black scales, due to strongly absorbing melanin pigment (6).](image-url)
3.2. Reflectance and transmittance spectra of intact and denuded wings of the small white, Pieris rapae

We have attempted to assess the relative contributions of the scales to the reflectance on both wing sides by considering the butterfly wing as a stack of plates (Fig. 4), using the formalism described in the Methods. The reflectance was measured with an integrating sphere from both sides of intact wings [DWV, VWD; Fig. 5(a)], from wings with scales removed on one side [DW, WD, WV, VW; Fig. 5(a), and 5(b)] or from fully denuded wings [Wd, Wv; Fig. 5(b)]. The reflectance of the wings with scales is low in the UV and high at wavelengths above 450 nm. The peak reflectance, around 500 nm, is about 0.6 for the intact wing, but the reflectance of the scaleless wing is about 0.1, virtually independent of wavelength [Fig. 5(b)]. The overall spectral dependence of the transmittance, \( T(\lambda) \), resembles that of the reflectance, \( R(\lambda) \); the transmittance is low in the UV and high in the visible wavelength range [Fig. 5(c), and 5(d)].

Accordingly the absorptance, \( A(\lambda) = 1 - R(\lambda) - T(\lambda) \), is high in the ultraviolet and negligible at the visible wavelengths [Figs. 5(e) and 5(f)]. The scaleless wing absorbs only slightly in the ultraviolet [Fig. 5(f)]. The absorptance spectra of denuded wing and wings with scales differ in shape, indicating that the pigments in wing substrate and scales differ.

The wing covered by scales can be considered as a stack of layers, each with a specific reflectance and transmittance, depending on the direction of the incident light. The intact wing thus is a three layer stack, consisting of a layer of scales at the dorsal side, D, the wing substrate, W, and the layer of scales at the ventral side of the wing, V (Fig. 5). The reflectances \( r \) and \( s \) and the transmittances \( t \) and \( u \) (Fig. 1) can be calculated for each of the three layers with the formalism of the Methods. The wing reflectances \( r_w \) and \( s_w \) [Fig. 5(b)] as well as the transmittances \( t_w \) and \( u_w \) [Figure 5(d)] were measured directly, and further spectral
data were obtained for various two layer combinations, DW, WD, VW, or VW, where only one layer of scales was present.

Fig. 5. Reflectance (a, b) and transmittance (c, d) spectra measured from wings of the small white butterfly, *Pieris rapae*, in various conditions, together with the resulting absorptance spectra (e, f). The measurements were performed with an integrating sphere. The wing was intact for the conditions DWV and VWD, where D indicates the dorsal side of the wing, W is the wing substrate, and V is the ventral side. The order of the letters indicates the direction of the incident light. For DW and WD, the wing scales were removed at the ventral side, and for VW and WV, the wing scales were removed at the dorsal side. For Wv and Wd, both dorsal and ventral scales were removed, and the incident light came from the ventral (v) and dorsal side (d), respectively. The scales contain a strongly UV absorbing pigment, resulting in a very low transmittance, or, a very high absorptance, in the ultraviolet. The wing scales strongly scatter in the visible wavelength range. The wing reflectance is virtually constant throughout the whole spectral range, with amplitude about 0.11 (b), and the wing substrate contains a small amount of pigment that absorbs in the UV (f).
The reflectance and transmittance spectra measured from the intact wing [Figs. 5(a) and 5(c), DWV], $R_n$ and $T_n$, are compared with the calculated spectra, $R_c$ and $T_c$. The calculated reflectance (transmittance) is somewhat smaller (larger) than the measured reflectance (transmittance). The absorptances are calculated with $A_{m,c} = 1 - R_{m,c} - T_{m,c}$.

3.3. Reflectance and transmittance spectra of single scales

Butterfly scales are more or less flat structures (Fig. 4), but they are quite asymmetrical. The face toward the side of the wing (adwing) is rather smooth, but the opposite side (abwing) is highly structured [inset Fig. 7(b)]. We have measured the reflectance of single scales, glued to the tip of a thin glass rod, with a microspectrophotometer [Figs. 7(a) and 7(b)]. We investigated both faces of ground and cover scales from both the dorsal and ventral side of the scale. The reflectance of both scale faces slightly differ, and the reflectance spectrum also depends somewhat on the type of scale. The peak reflectance is roughly 0.3 in both cases, at about 500 nm, but the reflectance in the UV is lower for the scale surface not facing the wing [Fig. 7(a)].

The reflectance clearly depends on the scattering on the scale structures as well as on the ultraviolet-absorbing pigment. To assess the amount of absorption in a single scale, we measured the transmittance of various scales embedded in xylene, an immersion fluid with a refractive index approximating that of insect cuticle [11] [Fig. 7(c)]. Figure 7(c) also contains a transmittance spectrum calculated from the absorptance spectra of Figs. 5 and 6. The calculation was performed as follows. It was assumed that the absorption process in the layers can be approximated with Lambert-Beer’s law. The absorbance then is $D(\lambda) = -\log_{10}[1 - A(\lambda)]$. 

#69197 - $15.00 USD
Received 22 March 2006; revised 8 May 2006; accepted 14 May 2006
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29 May 2006 / Vol. 14, No. 11 / OPTICS EXPRESS 4887
Fig. 7. Reflectance and transmittance spectra measured with a microspectrophotometer of single cover and ground scales isolated from the dorsal as well as the ventral side of the forewing of a male *Pieris rapae*. a Reflectance spectra measured abwing, that is, with the illumination coming from the side of the ridges, which do not face the wing (see inset of b). The bold curve is the mean of the measured curves. b Reflectance spectra measured adwing, that is, with the illumination coming from the side of the scale that does not have ridges. The bold curve is the mean of the measured curves. The maximum scale reflectance, both abwing and adwing, is roughly 0.3. In the short wavelength range, the abwing reflectance (a) is more affected by the absorbing pterin pigment than the adwing reflectance (b). c Transmittance spectra measured with the scales immersed in xylene, to reduce scattering. The transmittance is low due to pterin pigment that absorbs strongly in the UV. The transmittance at 375 nm is about 0.15, meaning a peak absorbance of about 0.8. The bold curve (LB) is the transmittance spectrum calculated assuming Lambert-Beer’s law, taking the absorption spectrum derived from the average normalized absorptance curves of Figs. 5(e) and 5(f). The distinct difference between the measured curves and the bold curve demonstrates that the immersion fluid did not fully annihilate the refractive index differences. Inset b Transmission electron microscopic image of a wing scale of *Pieris rapae*. The abwing side has prominent ridges that are connected by cross-ribs. Beads adorn the latter structures. The adwing side is rather smooth, although regularly spaced protrusions exist, resembling minor ridges (see Ref. 11).

The absorbance spectra of Figs. 5(e) and 5(f) thus yielded absorbance spectra, which after normalization appeared to be very similar. The mean of the normalized absorbance spectra was therefore used to calculate a transmittance spectrum (Fig. 7(c), bold curve, LB) with an amplitude in the ultraviolet of 0.15, about equal to the transmittance in the UV of the immersed scales. The calculated transmittance spectrum deviates from the measured transmittance spectra. Presumably xylene does not fully annihilate the difference in refractive index of the scale structures with that of the surrounding medium, which can be understood, because probably the refractive index of pierid scales is not constant. The transmittance then remains affected by scattering. Another reason for the deviation may be the limited applicability of the model, which assumes perfectly diffuse light.

### 3.4. Reflectance spectra of a stack of scales

We have used the model for a stack of reflecting and absorbing plates (Methods) to calculate the reflectance of a multilayer of butterfly wing scales on the wing. The measurements of Fig. 7 demonstrate that the reflectance slightly depends on the type of scale (cover or ground). The reflectance also depends on which side of the scales faces the incident beam. We nevertheless assumed a stack of identical scales, with abwing (r) and adwing (s) reflectances equal to the mean of the measured spectra [bold curves of Figs. 7(a) and 7(b), respectively]. The scale
transmittances $t$ and $u$ (Fig. 1) were calculated from $t = 1 - r - a$ and $u = 1 - s - a$, where the absorptance is $a = 1 - t_{LB}$, with $t_{LB}$ the transmittance given by the bold curve in Fig. 7(c).

The resulting reflectance spectra of various stacks of scales on the wing are given by Fig. 8. The reflectances of both sides of the denuded wing were assumed to be equal to the average (W) of the two wing reflectance spectra given in Fig. 5(b). Spectrum SW of Fig. 8 presents the reflectance spectrum of a wing with one layer of scales on the side of the incident beam. At a glance it may seem strange that the reflectance spectrum SW is lower than reflectance spectrum W in the UV. The reason is the low reflectance as well as low transmittance of a single scale (S) at short wavelengths [Fig. 7(a)], resulting in a low reflectance of the assembly, SW. Spectrum SWS presents the case with one layer of scales on each side of the wing, etc. The reflectance in the visible wavelength range steadily increases with an increase in the number of scale layers, but the increment in reflectance gradually decreases. The reflectance in the ultraviolet is completely governed by the reflectance of the first layer of scales.

![Fig. 8. Reflectance spectra calculated for a set of identical scales stacked on the wing. The reflectance of the scaleless wing (W) is the average of the spectra Wd and Wv of Fig. 5(b). SW is the reflectance calculated for a single layer of scales at the wing, with the reflectance $r$ taken to be the mean abwing reflectance of Fig. 5(b) and $s$ is the mean adwing reflectance of Fig. 5(b). The illumination is from the side of the scale, S. SWS is the reflectance when single layers of scales exists on both sides of the wing. SSWS has two layers of scales on the side of the wing from which the illumination comes, and one layer of scales on the other side of the wing. SSSWS has two layers of scales on both sides of the wing, and SSSSWS has three layers of scales on the illuminated wing side and two layers on the other side.]

4. Discussion

Butterfly scales are extremely thin, in the order of hundreds of nanometer. Not surprisingly, multilayer interference plays therefore a dominant role in many cases of butterfly coloration. This is specifically the case in the cover scales on the dorsal wings of male sulphur butterflies [5-7], and in the tips of male pierid butterflies of the Colotis group (Fig. 2).

Stokes [15] gave the first theoretical treatment of light reflected and transmitted by a pile of plates where interference effects are negligible, which was followed by a few more elaborate studies on stacks of thick layers [16,17]. We have presented here a simple recursion procedure for calculating the incoherent light flux in a stack of plates. For the modeling we have assumed that the layers are sufficiently rough so that interference effects are averaged out. The reflectance and transmittance spectra are then determined by light scattering on the scale structures as well as by the absorbing pigments. Using spectra measured on wings of the small white butterfly [Fig. 5(a)-5(d)], we have derived the reflectance and transmittance of the scale layers on each side of the wing [Fig. 6(a), 6(b)]. The calculated spectra for the
reflectance and transmittance of an intact wing did not completely match the measured spectra, however [Fig. 6(c)]. This implies that the assumption of random scattering is not fully valid. In addition, coherence may be not fully negligible. The general features are nevertheless very clear. The wing reflectance of the small white is not exclusively determined by the scales on one side, but also the scales on the other side of the wing contribute.

The maximal wing reflectance of the small white is about 0.6 [Fig. 5(a)]. This corresponds to the reflectance value obtained for a stack of two scale layers on each side of the wing (Fig. 8, SSWSS). Light and scanning electron microscopical observations indeed show that in average about two scales overlap at the wings of the small white (Fig. 4). The wing reflectance of the large white, *Pieris brassicae*, can go up to 0.8, in agreement with our observation that stacks of effectively three to four scales overlap in this species. Similarly, the dorsal wings of the common jezabel (Fig. 3) have a high reflectance as well as several layers of overlapping scales. In the latter case, the white reflectance is mainly due to scales on the dorsal side of the wings, because most scales at the ventral side contain a dense brown-black pigment. The scales in the striking red bands at the ventral wing noticeably contribute to the dorsal reflectance in the long-wavelength region (Fig. 3, spectra 1 and 3).

Enhancement of reflectance by multilayers of scales have been recently reported for Morpho butterflies by Yoshioka and Kinoshita [18,19]. They have used a three layer wing model similar to that used above, but the procedure presented here is easier and more generally applicable.

The wing reflectance is only a few percent in the ultraviolet for all cases presented here. This is due to pigments absorbing in the short-wavelength range, even in the white wings. Stacking several scales at the wing enhances the reflectance at the long-wavelengths, meanwhile the short-wavelength reflectance stays low. The pierid butterflies achieve a strong, saturated color by combining a distinct short-wavelength absorption with strong long-wavelength scattering, which is realized by a multitude of nanometer-sized beads [11]. The strong coloration plays an important role in mutual, intersexual recognition [13]. This will be especially the case where the male adds a strong direction-depending iridescence in the ultraviolet [10, 12] to a yellow or orange pigment color (Fig. 2).

In conclusion, we have demonstrated that the reflectance of butterfly wings results from the coordinated effect of reflectances of single scale that are arranged in stacks on the wings. This insight will be of considerable significance for biologists working to understand the adaptive significance and evolution of butterfly wing patterns.

**Acknowledgments**

We thank three anonymous referees for constructive criticisms. Drs R. Rutowski and H. Ghiradella read earlier versions of this paper. Financial support was given by the EOARD (Grant 063027) and the EU via SYNTHESYS.