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Form Approved OMB No. 0704-0188

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1. REPORT DATE (DD-MM-YYYY) 22-03-2007	2. REPORT TYPE Final Report	3. DATES COVERED (From – To) 15 March 2006 - 08-Jun-07
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4. TITLE AND SUBTITLE Butterflies: Photonic Crystals on the Wing	5a. CONTRACT NUMBER FA8655-06-1-3027
	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S) Professor Doekele G Stavenga	5d. PROJECT NUMBER
	5d. TASK NUMBER
	5e. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Groningen Nijenborgh 4 Groningen NL-9747 AG The Netherlands	8. PERFORMING ORGANIZATION REPORT NUMBER N/A
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9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) EOARD PSC 821 BOX 14 FPO AE 09421-0014	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S) Grant 06-3027

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT
In this project we focus on the optical properties of the wing scales of butterflies. The scales of numerous butterflies, specifically several Papilionidae and Lycaenidae, are very likely structured in the form of three-dimensional (3D) photonic crystals to achieve vivid coloration of the wings. It is not uncommon for the same butterfly to have more than one type of structural color on the same wing. Current interpretations of the optics of butterfly wings are in terms of light reflected from volume diffraction gratings or multiple layers of thin films, but these explanations are far from satisfactory. The modeling of the 3D sub-wavelength structures has been hampered partly due to lack of detailed 3D structure characterization and measurement of the optical properties of the wing scales. We propose to use a combination of SEM and TEM to quantitatively determine the 3D structure of the wing scales. Angle-dependent reflection and transmission microspectrophotometry data obtained from isolated, single scales will be interpreted in terms of the photonic band structure of these complex 3D structures. The main objective is to demonstrate that the band folding and singularities at the intersecting points in k-space give rise to the angle and wavelength dependence of the light scattering from butterfly wing-scales. Most of the 3D structures observed in the scales of the wings of a butterfly appear to be much more complex than the photonic structures presently produced artificially. Since self-assembly methods are currently unable to reproduce these complex natural 3D structures, the butterfly wing-scales are expected to be extremely valuable for analyzing angle and wavelength dependent light scattering.

15. SUBJECT TERMS
EOARD, metamaterials, Organic Optical Materials, optics

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UL	18. NUMBER OF PAGES 7	19a. NAME OF RESPONSIBLE PERSON DONALD J SMITH
a. REPORT UNCLAS	b. ABSTRACT UNCLAS	c. THIS PAGE UNCLAS			19b. TELEPHONE NUMBER (Include area code) +44 (0)20 7514 4953

Fourth and concluding report

[FA8655-06-1-3027] – Butterflies: photonic crystals on the wing

Doekele G. Stavenga

Department of Neurobiophysics, University of Groningen, the Netherlands

Summary

We investigated the optics of the wings of various butterfly species with both experimental and theoretical techniques. The focus has been on unraveling the methods of coloration used by butterflies of the family Pieridae. Both structural (physical) and pigmentary (chemical) coloring exists among males, whilst females apply virtually exclusively pigmentary coloring. The genetic expression of key pigments, leucopterin, xanthopterin and erythropterin, appears to be intimately linked to the subfamily. The analysis of pierid coloration has created a solid framework for studies of other butterflies, notably lycaenids and papilionids, which display striking colors due to intricate photonic crystal (PC) structures. A combined theoretical and computational study has yielded the insight that butterfly PCs are structured as gyroids, infinitely connected triply periodic minimal surfaces. Elucidating the butterfly PC structure will allow the calculation of reflectance spectra and polarization properties of butterfly wings.

Introduction

The color of a butterfly wing depends on the interaction of light with the material of the wing and their spatial structure. Butterfly wings consist of a wing substrate, upon which stacks of light-scattering scales are arranged. When the scales contain pigment, it selectively absorbs part of the incident light spectrum, resulting in a characteristic color. Additional, iridescent coloration sometimes occurs because of the interaction of light with periodic structures, for instance when the scales contain multilayers. The wing colors can be understood by analyzing the different contributions of light scattering, pigment absorption, iridescence and other wave-optical effects.

The butterfly family Pieridae has two main subfamilies, the Coliadinae and the Pierinae, appropriately called the sulfurs and whites, respectively. Pieridae are attractive for developing our understanding of butterfly wing coloration, because their coloration methods are relatively simple compared to the often complex and as yet ill-understood optical phenomena encountered in lycaenids and papilionids. In these latter cases the wing scales are often periodically structured with dimensions in the nanometer range, causing reflection phenomena, typical for so-called photonic crystals.

Results

The colors of pierid butterflies

Many butterfly species are marked by a distinct sexual dichroism (see 2nd report). For understanding the complexity of butterfly coloration it is instructive to compare the males of different pierids (Fig. 1). Butterflies of the subfamily Coliadinae generally have an overall orange or yellow color, underlying the common name sulfurs. Members of the subfamily Pierinae, called the whites, indeed often are prominently white, but they exhibit not rarely colorful (orange or red) wing tips, and exceptionally their main wing areas can be yellow (for instance *Eronia leda*; Fig. 1).

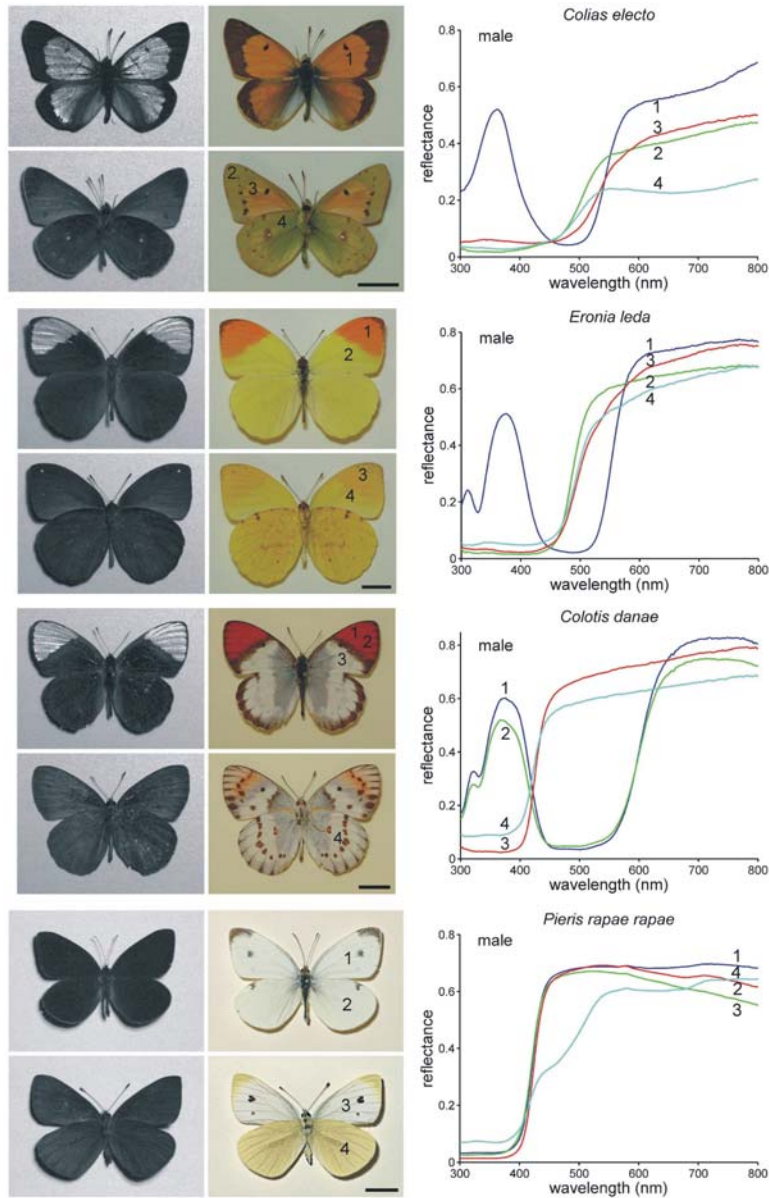


Fig. 1. Photographs and reflectance spectra of male pierid butterflies: *Colias electo* (Coliadinae), *Eronia leda*, *Colotis danae*, and *Pieris rapae rapae* (all Pierinae), respectively. The left and right column of the photographs are taken with ultraviolet and visible light. Of each species the top and bottom rows of the photographs represent the upper (dorsal) and lower (ventral) side, respectively. The numbers in the photographs indicate the location where the reflectance spectrum with the corresponding number was taken.

The dorsal wings of males of the Coliadinae usually have a distinct ultraviolet reflectance, which is also present in certain members of the Pierinae, but then exclusively in the dorsal wing tips. The reflectance properties correlate with the phylogenetic tree of the Pieridae, as it was established by morphological and molecular-biological methods (Fig. 2). The pierids depicted as an example in Fig. 1 are arranged in the same way as the phylogenetic tree: *Colias electo* belongs to the Coliadinae, *Eronia leda* and *Colotis danae* belong to the *Colotis* group, and *Pieris rapae rapae* to the Pierini.

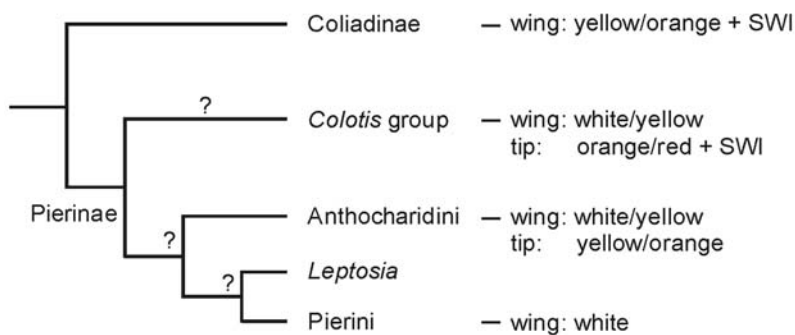


Fig. 2. Phylogenetic tree of the butterfly family Pieridae. The subfamily Pierinae consists of the *Colotis* group, and the tribes Anthocharidini and Pierini. The Coliadinae are mainly yellow or orange, and the dorsal wings of the males have a short-wavelength iridescence (SWI). The wings of males of the *Colotis* group are characterized by dorsal wing tips that are orange or red and short-wavelength iridescent. Males of the Anthocharidini are generally non-iridescent. The wings of Pierini are also non-iridescent and are white colored with some black dots, and they have occasional black wing margins.

We have studied the pigments responsible for the colors of pierid butterflies, by applying a novel, though simple method, allowing very local extraction of the pigment (Fig. 3). Older studies have demonstrated that pigments of the pterin family determine the color of pierid wings. Pterins are easily extracted with a solution of ammonium hydroxide with isopropylalcohol. Application of a local drop, and measuring the transmittance of the drop with two light guides, connected to a broad-spectrum light source and a photodiode array, yielded the absorbance spectra of the wing pigments.

The pigments, erythropterin, xanthopsin, and leucopterin, have absorption peaks at about 450, 390 and 340 nm, respectively, and they are located in small granules (so-called beads) attached to the crossribs of the scales (Fig. 4, 5). The pigments absorb scattered light, resulting in orange/red, yellow and white wing scales, respectively. All pterin pigments absorb ultraviolet light, causing a low wing reflectance in the UV. The high UV reflectance, observed in several male wings (Fig. 1) is caused by the fact that the scales have multilayered ridges (Fig. 5, 6).

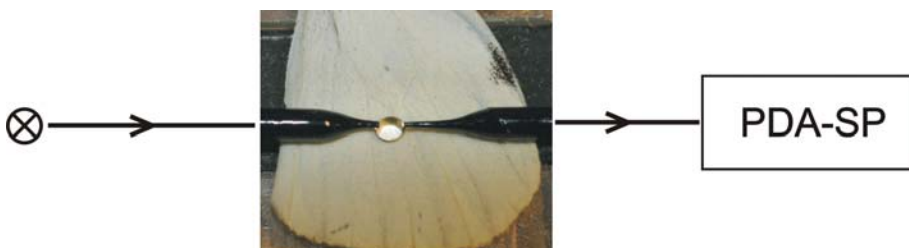


Fig. 3. Local extraction of pterins from pierid wings. **a** A droplet of a 0.1 % ammonium hydroxide solution is held between the tips of two quartz light guides that are coated with black paint. Light from a deuterium/halogen light source is focused into the light guide at the left, and the light transmitted by the droplet is guided by the light guide at the right to a photodiode-array-spectrophotometer (PDA-SP). At the start of an experiment the droplet is brought into contact with a piece of wing (here of a large white, *Pieris brassicae*).

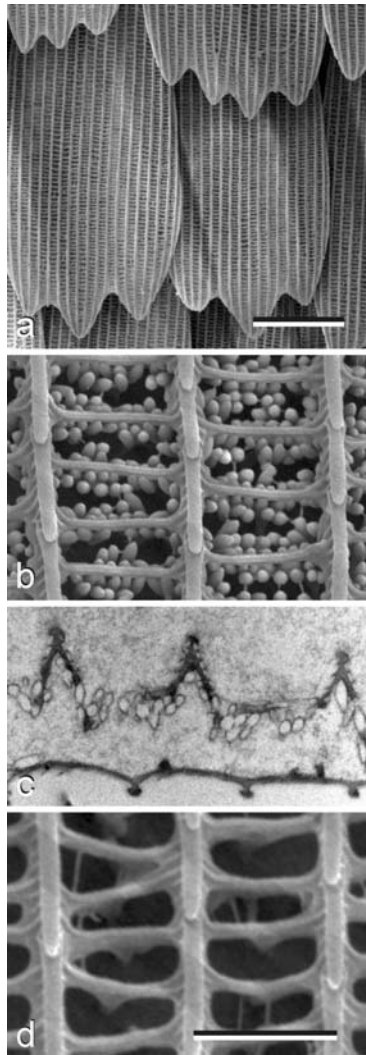


Fig. 4. Electron microscopic photographs of scales of a male small white, *Pieris rapae*. **a** Cover scales partly overlap ground scales (scanning electron microscopy, SEM). The upper lamina of the scales features longitudinal ridges. **b** Part of a white scale (SEM). The ridges are connected by crossribs, which are adorned by numerous oval-shaped beads. **c** Transmission electron microscopic (TEM) section of a white scale, showing the beads at the upper lamina of the scale. The lower lamina of the scale is more or less flat. **d** A scale from one of the black spots (SEM), showing that in this case the crossribs do not have beads (**a**, bar: 25 µm; **b-d**, bar: 2 µm).

In photonic crystal (PC) theory, multilayers are called 1D-PCs, because of the repetitive refractive index changes along one coordinate. In the pierid wing scales, the multilayers are extremely slender piles, and thus rather form 2D-PCs. The structures result in narrow-band reflectance spectra, peaking in the ultraviolet (and sometimes in the blue, in the case of the male purpletip, *Colotis regina*).

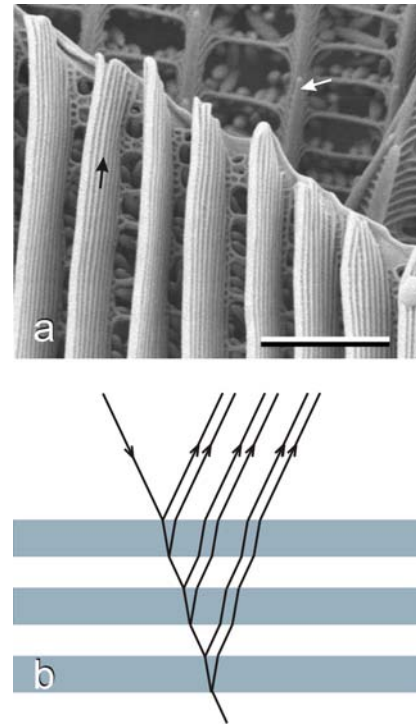


Fig. 5. **a** Part of a cover scale, overlapping a ground scale, from the orange tip of a male *Hebomoia glaucippe* (SEM). Both cover and ground scales have crossribs with beads. The ridges of the cover scale (black arrow) are more densely spaced than the ridges of the ground scale (white arrow) and are strongly folded into lamellae (bar: 2 µm). **b** The lamellae form a multilayer stack, so that incident light arriving at a surface of a lamella is partly reflected and transmitted. Because light behaves as a wave for structures with nanosized dimensions, the reflected light waves can interfere constructively depending on the distances of the surfaces. The lamellae act together as an interference reflector for short-wavelength, ultraviolet light.

The color of Morphos

The same multilayered ridges as in male pierids are found in the strikingly blue reflecting Morpho butterflies (Fig. 6, 7). The ridges function as reflecting multilayers, but because of the narrowness, light is diffracted in a plane (Fig. 8). With oblique illumination from a large angle with respect to the ridge axis, interference reflection is no longer possible, and then the brown pigment located below the ridges, which serves as a contrast medium, becomes visible (Fig. 7).

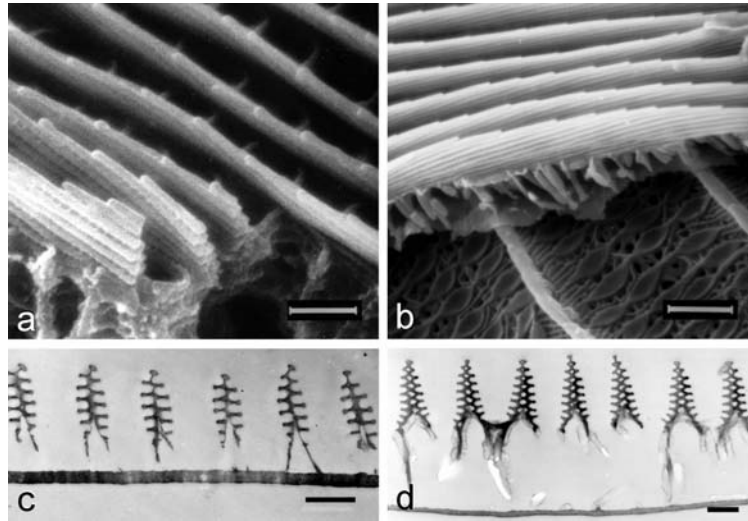


Fig. 6. SEM (a, b) and TEM (c, d) of wing scales of the butterflies *Morpho menelaus* (a, c) and *Colias eurytheme* (b, d). The wing ridges are structured into lamellae that cause a distinct blue reflection in *Morpho menelaus* and ultraviolet reflection in *Colias eurytheme*. Scale bars 1 μm (courtesy of Dr. H. Ghiradella).

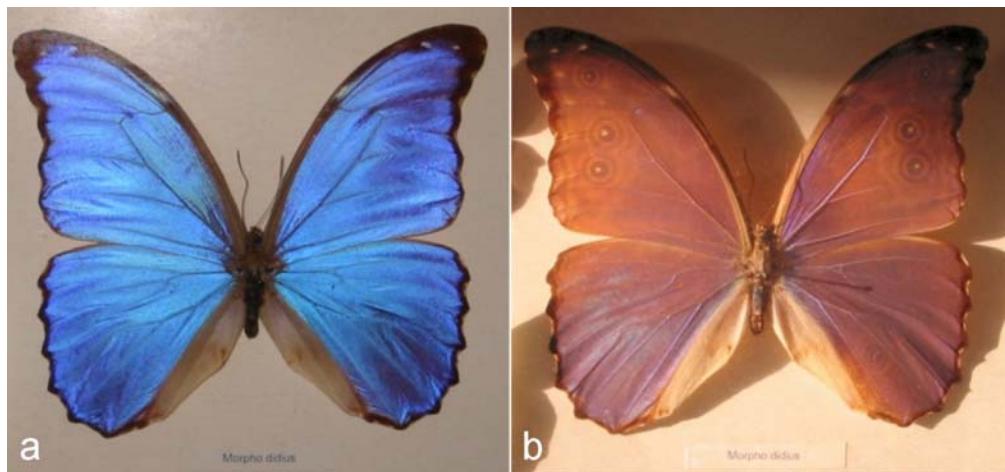


Fig. 7. The dorsal wings of the tropical butterfly *Morpho didius* have a striking blue color (a) due to multilayer interference reflection. Upon oblique illumination (b), outside the angle of interference reflection, a light brown pigmentation in the wing scales becomes visible; note the so-called eyes at the under wings, which are often quite marked in other butterflies, but with natural, wide-angle illumination they are completely swamped by the interference reflection in Morpho butterflies.

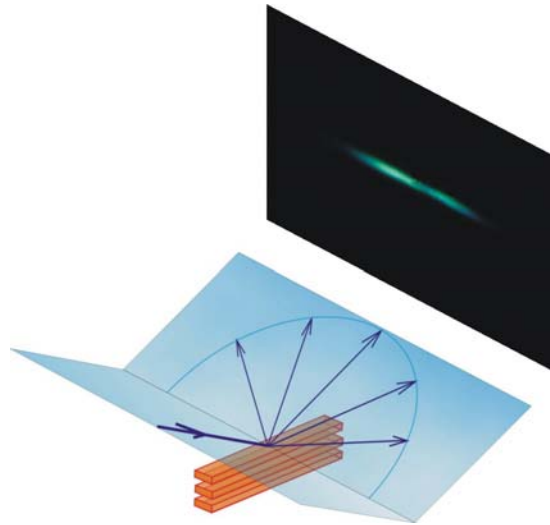


Fig. 8. Diagram explaining the blue iridescent coloration of Morpho wings (similar phenomena occur in pierid wings in the ultraviolet). A scale ridge forming a multilayer stack reflects an incident light wave in principle according to Snell's law, but the light wave is diffracted in a plane, because the width of the ridge is in the order of the wavelength of light.

The color of lycaenids and papilionids

Lycaenidae, comprising the blues, hairstreaks and coppers, are often marked by highly iridescent colors. Early transmission electron microscopic work on the green hairstreak, *Callophrys rubi*, suggested that the scales have a 3D cubic network organization (Fig. 9). An extensive analysis of the scales of a variety of scales, of lycaenids as well as of papilionids has now revealed that the anatomy of all known scale types can be described by gyroid structures, as based on the theory of cubic minimal surfaces. This analysis has led to the proposal to use the scales of lycaenids, like *Callophrys rubi*, as a biotemplate to produce three-dimensional optical photonic crystals, since these scales are nearly optimal for obtaining a large photonic band gap.

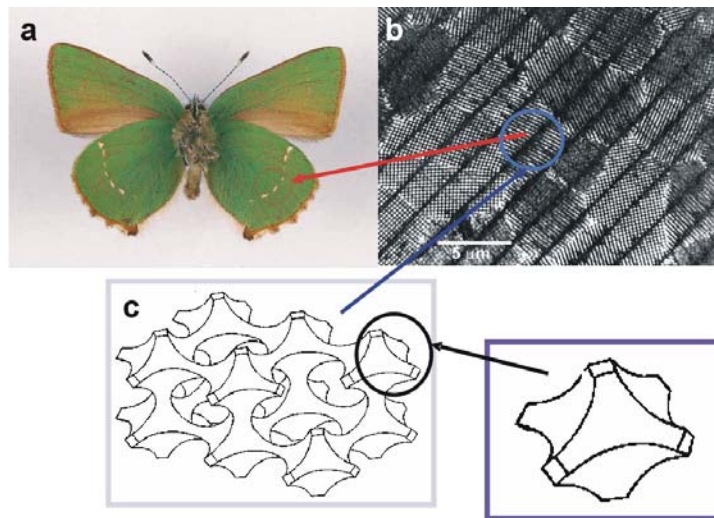


Fig. 9. **a** Ventral side of the wings of the green hairstreak, *Callophrys rubi*. **b** Transmission electron micrograph of a small area of a single scale of the ventral side of the wing. **c** Simple cubic network model of the structure of a single wing scale.

From the known 3D structure, we can embark on further studies of the complicated biological photonic crystals of butterfly wing scales. In a combined experimental and theoretical approach, we will measure reflectance spectra of single butterfly wing scales and their dependence on polarization, and we will calculate reflectance spectra for various gyroid structures and compare them with the experimental results. This study should lead to a deeper understanding of butterfly wing coloration, and ultimately this will lead to technical applications.

When we have progressed further with the analysis of single scales, we can return to the coloration of intact wings. We then can bolster our recently published optical model for the reflectance and transmittance of a stack of scales at the wing.

Acknowledgements

The financial support provided by the EOARD has (obviously) been instrumental for the work here described.

Publications during the preceding year

- Stavenga DG, Foletti S, Palasantzas G, Arikawa K (2006) Light on the moth eye corneal nipple array of butterflies. *Proc Roy Soc B* 273:661-667
- Stavenga DG (2006) Invertebrate superposition eyes – structures that behave like metamaterial with negative refractive index. *JEOS – Rapid publications* 1, 06010
- Stavenga DG (2006) Invertebrate photoreceptor optics. In: *Invertebrate vision* (Warrant E, Nilsson D-E, eds), Cambridge University Press, Cambridge, pp 1-42
- Stavenga DG (2006) Partial coherence and other optical delicacies of lepidopteran superposition eyes. *J Exp Biol* 209:1904-1913
- Stavenga DG, Giraldo MA, Hoenders BJ (2006) Reflectance and transmittance of incoherently scattering scales stacked at the wings of pierid butterflies. *Optics Express* 14: 4880-4890.
- Stavenga DG, Arikawa K (2006) Evolution of color and vision of butterflies. *Arthropod Struct Dev* 35:307-318
- Giraldo MA Stavenga DG (2007) Sexual dichroism and pigment localization in the wing scales of *Pieris rapae* butterflies. *Proc R Soc B*, 274, 97–102
- Wakakuwa M, Stavenga DG, Arikawa K (2007) Spectral organization of ommatidia in flower-visiting insects. *Photochem Photobiol* 83:27-34
- Stavenga DG (2007) Insect eyes and surface colors. In: *Functional Surfaces in Biology* (Gorb S, ed). Springer: Berlin Heidelberg New York, in press
- Stavenga DG, Hironaka M, Horiguchi H, Pirih P, Hariyama T (2007) Sexual dichroism and iridescence of calopterygid damselflies. *J Exp Biol*, submitted, February 2007
- Michielsen K, Stavenga DG (2007) Gyroid cuticular structures in lepidopteran scales: Biological photonic crystals. *J. R. Soc. Lond. Interface*, to be submitted, March 2007
- Wijnen B, Leertouwer HL, Stavenga DG (2007) Wing coloration, pteridine pigmentation and phylogeny of pierid butterflies. *Proc R Soc B*, to be submitted, April 2007