

# Roaming of Materials Scientists in Biology: Structural Colours of Butterfly Wings

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## Abstract

The photonic nanoarchitectures occurring in the wing scales of Lycaenid butterflies were investigated by scanning electron microscopy (SEM), transmission electron microscopy (TEM) and UV-VIS spectroscopy. We found that the males of all the nine investigated species possess photonic nanoarchitectures built according to the same general “plan”, but each species exhibits species-specific features which results in species-specific colours reproduced generation by generation with a high degree of accuracy.

**Keywords:** *butterfly wing scales, photonic nanoarchitecture, electron microscopy, UV-Vis spectroscopy.*

## 1. Introduction to photonic nanoarchitectures

### 1.1. As seen from physics

Physics discovered about thirty years ago that it is possible for light waves to produce forbidden bands as is well-known for semiconductors [1, 2]. To achieve this, we have to produce a composite from two transparent media with sufficiently different refractive indexes, in such a way that the local value of the refractive index changes in a periodic way in space, and on a scale comparable with the wavelength of the radiation that is not able to propagate in the composite. This last condition dictates that in the case of visible light, the composite in fact has to be a nanocomposite, in other words the changing of the refractive index takes place, on scale of the order of 100 nm. If taken strictly the above conditions define a photonic crystal, in which the light falling within the forbidden gap will not be able to propagate in any direction, therefore it will be reflected from the surface of the photonic crystal. If we relax the condition of a perfectly ordered structure, the photonic band gap will still be present, its spectral position will be also conserved, its width decrease and the complete band gap may transform into a partial band gap [3].

### 1.2. As seen from biology

Around 50 million years ago, biological evolution gave rise to the possibilities of photonic nanoarchitectures [4]. Since then various organisms have used this colour generation method to accomplish various biological functions. The most well-known examples can be found between beetles and butterflies [5]. In the case of butterflies, the blue and green colours are of structural origins, in other words they originate from photonic nanoarchitectures. The blue colour and its various shades are particularly well suited, for example, in front of a green background to make coloured objects conspicuous [6]. The males of the Lycaenid butterflies living in such types of habitat have their dorsal colours in various hues of blue, while the dorsal colour of the females is pigment (melanin) generated brown. This allows the wings of the females to absorb heat from solar radiation in a more efficient way [7], which is very useful for the development of their eggs.

### 1.3. As seen from materials science

As we examine the nanoarchitectures generating structural (physical) colour, the first factor we have to emphasize is the sufficient contrast in the magnitude of refractive index between the two transparent media. The larger this contrast,

the easier it is to produce the photonic band gap, if the two components alternate with each other with an adequate periodicity. For butterflies – the insects in the focus of this paper – the two components are chitin  $n_1 = 1.56$  and air  $n_2 = 1$ . Chitin is a biopolymer, a polysaccharide, with a chemical formula and properties very close to cellulose, the material used for paper manufacturing.

From the discussion above it follows that chitin and air have to be “mixed” with each other on a 100 nm scale if we want the resulting nanocomposite to generate colour.

Of course, this mixing can be carried out in various ways: a) resulting in a length scale much larger than 100 nm in a perfectly periodic mixing, which is called a photonic crystal [8]; b) a photonic polycrystal built of micron sized grains, each of which individually constitutes a photonic crystal [8]; c) a so-called photonic amorphous, which exhibits only a short range order between its building elements, for example in the distance of the first neighbours [9]. This last case is presented in Figure 1.

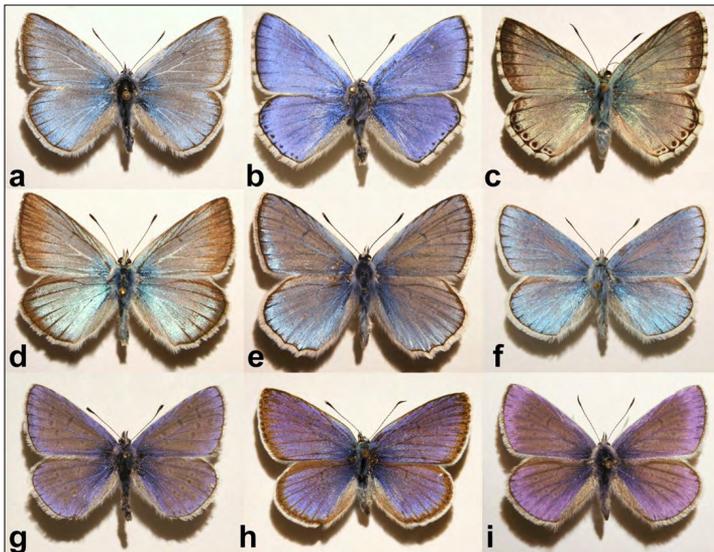
One may well note in Figure 1. that the scales are arranged in regular rows, resembling the arrangement of the tiles on a roof. Their characteristic size is  $100 \times 50 \mu\text{m}^2$ , their shape is like a flattened sac with a thickness of  $1 \mu\text{m}$ . This is the volume which is filled by the photonic nanoarchitecture.



**Figure 1.** Photograph of the dorsal wing surface of a male *Polyommatus icarus* (left); scanning electron microscopy image of the scales on the wing membrane (right upper); scanning electron micrograph of the nanoarchitecture filling the volume of the blue coloured scale (right lower).

## 2. Sexual signalling colour

Between the Lycaenids and especially between the Blues, sexual dimorphism, when the male and the female have very different appearance, is very frequent. The dorsal wing surface of the males usually has a blue colouration, which can be attributed to structural colour (Figure 2. [6]), while the dorsal wing surface of the females is brown coloured, due to the presence of melanin. The ventral wing surfaces of both sexes have an identical complex pattern of dots generated by pigment coloured scales. The pattern is species-characteristic, but there are many similarities between the patterns of different species.



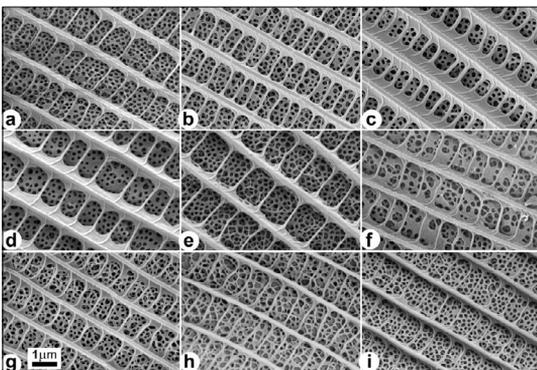
**Figure 2.** Males of nine closely related Lycaenid species, living in the same habitat. Photographs taken under artificial illumination. a) *Polyommatus amandus*; b) *Polyommatus bellargus*; c) *Polyommatus coridon*; d) *Polyommatus damon*; e) *Polyommatus daphnis*; f) *Polyommatus dorylas*; g) *Polyommatus icarus*; h) *Polyommatus semiargus*; i) *Polyommatus thersites*.

### 3. The nanoarchitectures generating the colours

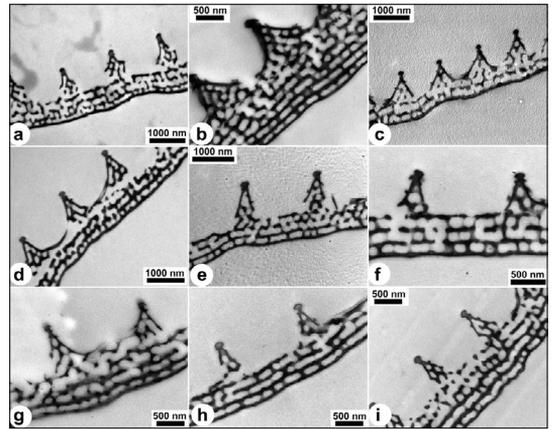
The species-specific sexual signaling colours are generated by nanoarchitectures built according to the same “ground plan”, but which have enough species-specific characteristics to generate specific colours. Due to their characteristic sizes the structural details of these nanoarchitectures can be revealed only by electron microscopic methods (Figure 3). The surface structure of individual scales is revealed by scanning electron microscopy (SEM), while the cross-sectional details of the scale structure can be revealed by transmission electron microscopy (TEM) (Figure 4). To be able to use this last method, one has to incorporate the pieces of wings in special resin and to cut slices of 70 nm thickness with a diamond ultramicrotome knife.

The TEM images (Figure 4) show that all the nanostructures are built from the alternation of chitin (dark in the TEM) and air regions (light in the TEM). The differences are found in the number of layers and in the order/disorder characterizing the individual layers. In the interpretation of the TEM images one has to keep in mind that the images are taken from slices only 70 nm thick. Therefore, features exceeding this size may not be completely present in the image.

One may well observe in the SEM images (Figure 3) that all the scales have similar structural features: all have a system of longitudinal ridges interlinked by cross-ribs, and below the network of these, one finds a perforated layer.



**Figure 3.** SEM micrographs of the scale surface of the males of the nine butterfly species presented in Figure 2. a) *Polyommatus amandus*; b) *Polyommatus bellargus*; c) *Polyommatus coridon*; d) *Polyommatus damon*; e) *Polyommatus daphnis*; f) *Polyommatus dorylas*; g) *Polyommatus icarus*; h) *Polyommatus semiargus*; i) *Polyommatus thersites*. The scale bar given in the micrograph in the lower left corner is valid for all the micrographs.

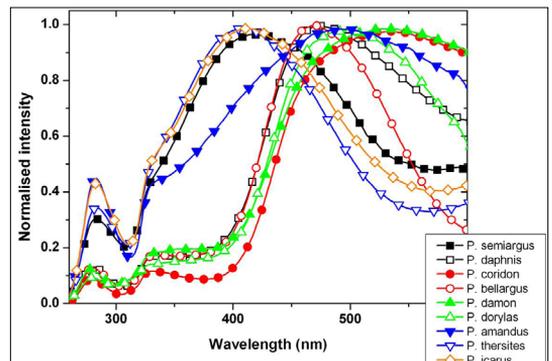


**Figure 4.** TEM micrographs of the cross-sectional scale structure for the males of the nine butterfly species presented in Figure 2. Dark regions correspond to chitin, while the light areas represent voids filled with air. a) *Polyommatus amandus*; b) *Polyommatus bellargus*; c) *Polyommatus coridon*; d) *Polyommatus damon*; e) *Polyommatus daphnis*; f) *Polyommatus dorylas*; g) *Polyommatus icarus*; h) *Polyommatus semiargus*; i) *Polyommatus thersites*.

### 4. Spectral characterization of the colours

To characterize the light reflected by the wing of the investigated butterflies we used a modular fiber optic spectrophotometer. The results are shown in Figure 5.

It can be observed in Figure 5. that all the spectra have distinct characteristic features. Because of this an artificial neural network based software is able to identify the butterfly species on the basis of the wing reflectance of the males with an accuracy of 96% [6].



**Figure 5.** Reflectance of the dorsal wing surface of the males of the nine investigated butterfly species. To facilitate the comparison of the spectra all curves have been normalized to 1

## 5. Conclusions

We have shown that the blue and green colours of butterflies are of structural origin. These colours are generated by nanocomposites built from the spatially periodic arrangement of two transparent materials with different refractive indexes: chitin and air, which generate a photonic band gap. The photonic nanoarchitecture is found in the volume of the wing scales. The scales cover the wing membrane in a regular arrangement.

The colours generated in this way are species specific and play an important role in sexual communication. Because of this they are reproduced with a high degree of precision from generation to generation [10].

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