Photonic nanoarchitectures in butterfly scales allowing species identification

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INTRODUCTION

Vision and color constitute a very important communication channel in the living world. Chemical colors are based on the selective absorption of certain wavelengths by intramolecular processes while structural colors are arising from the interaction of light with photonic crystal (PhC) type nanoarchitectures with periodicity comparable with the wavelength of light. If the characteristic size of the nanostructures building up the PhC is in the range of tens to hundreds of nanometers, the color of the PhC will be in the visible range.

In the present work we investigated the quasiordered photonic nanoarchitectures of nine *Lycaenid* butterfly species living in similar habitats with overlaps of their flying period (Figure 8.). The dorsal side of the wings of these male butterflies has blue coloration of different hues. Entomologists distinguish these and other blue *Lycaenid* species on the basis of complex patterns occurring on the ventral sides of their wings. It is very unlikely that butterflies in flight may have enough "computing power" for mate / competitor recognition using these intricate patterns. On the other hand, the blue sexual signaling color of different hues may constitute a clear enough discriminating signal.

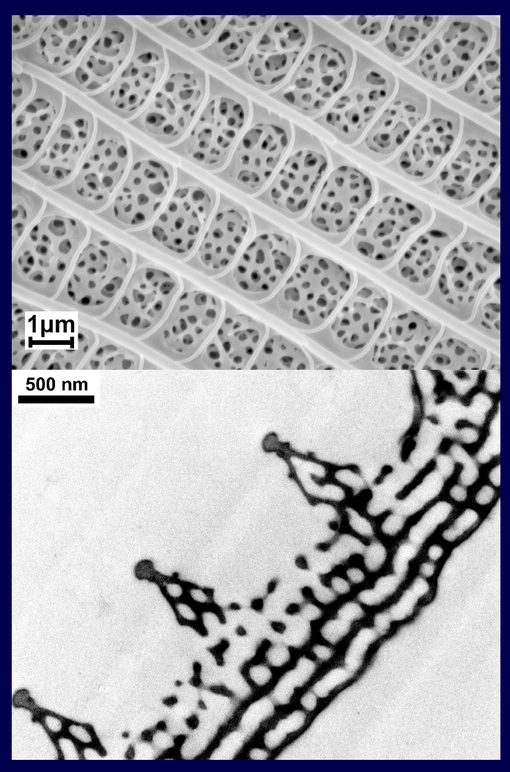

Figure 1. Scanning and cross-section transmission electron microscope image of *Polyommatus thersites* dorsal scale. Between longitudinal ridges and cross-ribs linking these there is the pepper-pot type nanoarchitecture. From 5 parallel perforated chitin layers only the upper 2 are seen in SEM images. The ridges and the area under the ridges also contain nanostructures.

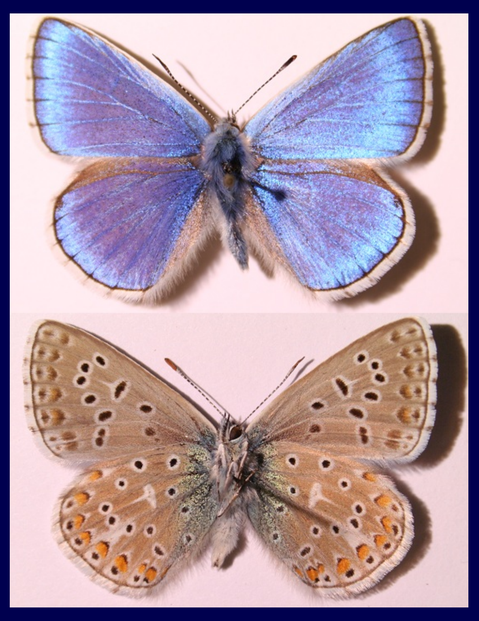

Figure 2. Dorsal (top) and ventral (bottom) image of a *Polyommatus icarus* butterfly. Mean wingspan is 3 – 3.5 cm. The position of the shadow indicates the light incidence angle.

EXPERIMENTAL

All the examined specimens were collected in the proximity of Budapest (Hungary) hills and originated from the Lepidoptera collection of the Hungarian Natural History Museum (www.nhmus.hu), this made possible to have reliable information both on the location and time of the capture.

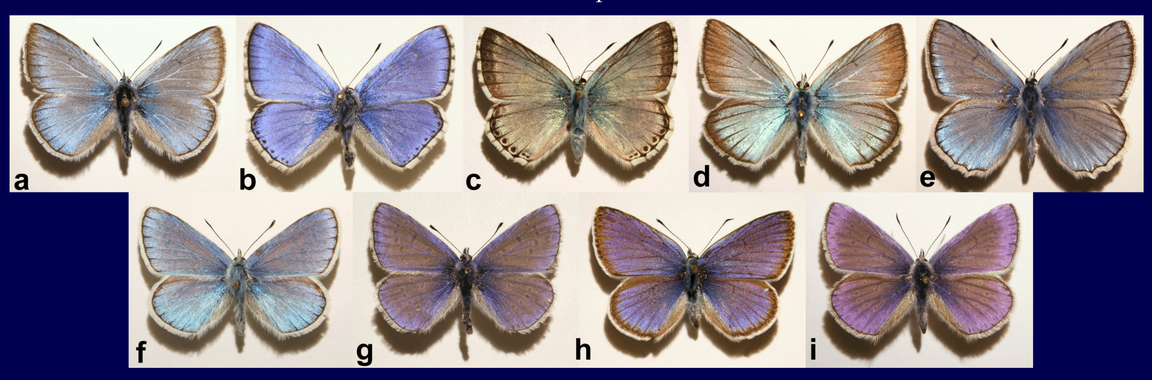

Figure 3. Dorsal side perpendicular view photographs of the nine investigated *Polyommatus* species: (a) *P. amandus*, (b) *P. bellargus*, (c) *P. coridon*, (d) *P. damon*, (e) *P. daphnis*, (f) *P. dorylas*, (g) *P. icarus*, (h) *P. semiargus*, and (i) *P. thersites*.

Optical spectroscopy was carried out using an Avantes 2048-2 modular fiber optic spectrometer. Due to the large number (110 pc.) of individuals used in the study it was necessary to elaborate a measurement method that allowed the reproducible spectral characterization of the butterflies without harming the museum specimens. We developed an instrument called "spectroboard" constituted from a basis reminiscent of the setting board used by the entomologists with additional mechanical parts that allows the illuminator and pick-up fiber to move over butterfly wings at a fixed distance [2]. The light incidence and detection are perpendicular to the wing. This setup offers reproducible and characteristic spectra while it does not necessitate physical destruction of the specimens.

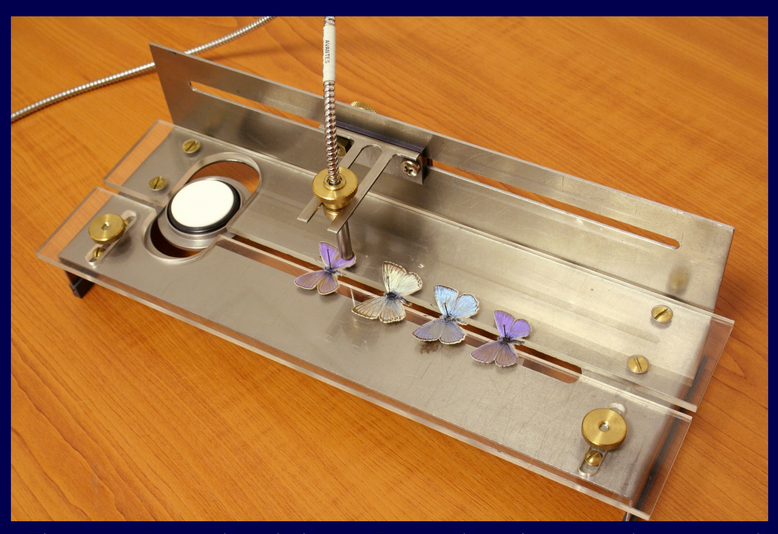

Figure 4. "Spectroboard" in use. Prepared specimens can be measured rapidly and safely in reproducible conditions.

RESULTS AND DISCUSSION

The nanoarchitecture in the wing scale acts as a photonic band gap composite, the color is determined by the physical dimensions, arrangement and refractive index contrast of the components. The wing is mainly constituted of chitin, the structural differences should be responsible for different hues. To register well comparable spectra, with the "spectroboard", all specimens were measured in the same region of the right forewing. As a proof of reproducibility a good concordance of spectral shapes is obtained from conspecific exemplars, a characteristic spectrum might be derived by averaging the curves. Normalized to the highest peek, the averaged curves for the investigated species are presented in Figure 5. The differences are significant enough to discriminate them by their shape.

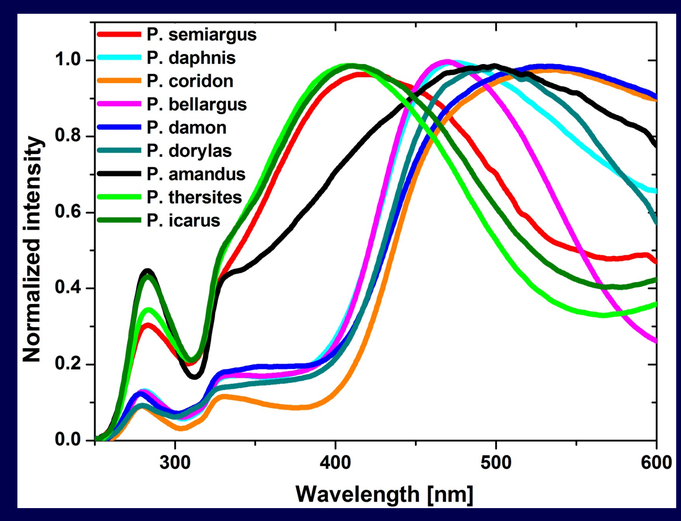

Figure 5. Normalized average spectra of the nine *Polyommatus* species measured with the "spectroboard".

For an automated classification with artificial neural network (ANN), a feature extraction is needed for every single spectra of all exemplars. Raw data preprocessing was done with "Origin 8" software (www.originlab.com) through automated routines. We defined characteristic parameters describing the curve shapes with discrete values that are used as inputs for the ANN. Half of all of the specimens were used for the teaching process of the ANN with error backpropagation algorithm. After successful teaching we tested the network with the remaining 55 specimens. 53 of them were classified correctly, which means 96% accuracy. This shows that the studied nine blue *Polyommatus* species have a characteristic hue.

By measuring the visible reflectance of the wing, a properly trained neural network can identify an unknown specimen from a "library" of previously stored species characteristic spectra [3].

expected / result	*Daphnis*	*Coridon*	*Icarus*	*Thersites*	*Dorylas*	*Semiargus*	*Damon*	*Amandus*	*Bellargus*
Daphnis	**5**	0	0	0	0	0	0	0	0
Coridon	0	**4**	0	0	0	0	0	0	0
Icarus	0	0	**5**	**1**	0	0	0	0	0
Thersites	0	0	0	**4**	0	0	0	0	0
Dorylas	0	0	0	0	**10**	0	0	0	0
Semiargus	0	0	0	0	0	**5**	0	0	0
Damon	0	**1**	0	0	0	0	**5**	0	0
Amandus	0	0	0	0	0	0	0	**5**	0
Bellargus	0	0	0	0	0	0	0	0	**10**

Figure 6. The artificial neural network identification results. Among the 55 tested exemplars only two were missed which means 96% accuracy.

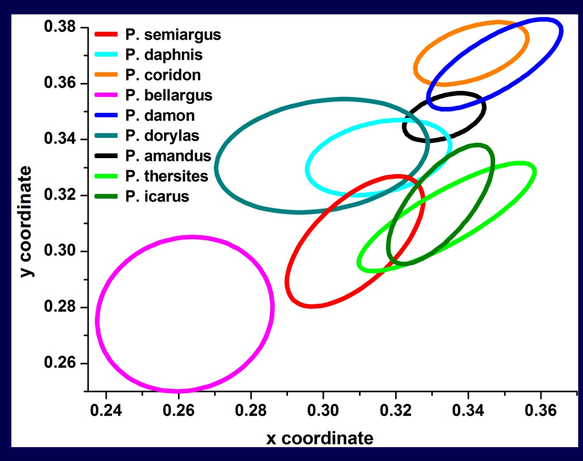

Figure 7. Zoomed in CIE chromaticity diagram for the blue range. Inside the ovals are the xy plots for every specimen. Single plots are not marked for clarity.

In a further step, we compared the interval of time when the butterflies fly in their habitat and their wing color. The spectra were first transformed into CIE XYZ tristimulus values, then the derived x and y parameters were represented in CIE 1931 color space chromaticity diagram. Each point in this diagram represents a specimen; the points assemble in domains marked with colored elliptical shapes (Figure 7.). The determination of flying time intervals was based on data stored in museum collection: we represented the exact time in days (month noted on x axis) when the specimens were collected for each species (Figure 8.).

Considering the butterfly species of the year in the order of their appearance, *P. icarus, P. amandus, P. dorylas* and *P. bellargus*, their ellipses in the chromaticity diagram are separate. In other words the simultaneously flying butterflies possess different hues of blue. In the same way, we can prove that two coincident ellipses e.g. orange and blue on Figure 7. belong to two species that are not flying in the same time: *P. icarus* and *P. thersites*. Therefore the mating of the butterflies is going to be successful: the further generations will inherit the proper genes [3].

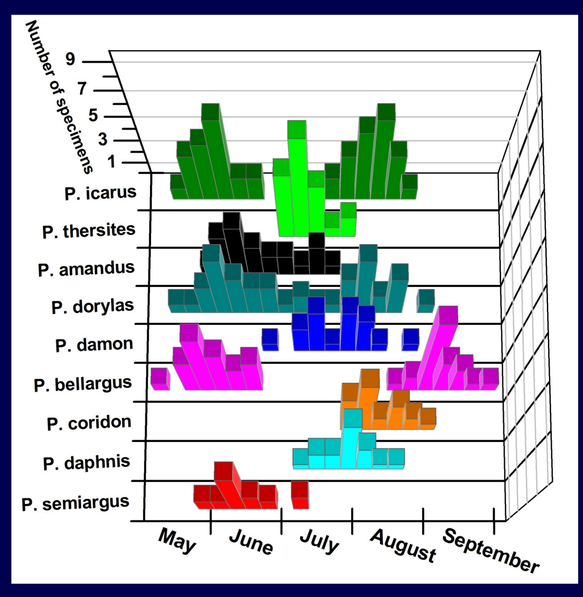

Figure 8. Flying intervals diagram.

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