Study of structural colour of *Hebomoia glaucippe* butterfly wing scales

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Abstract. Structural colours of *Hebomoia glaucippe* butterfly wing scales have been studied experimentally using high resolution scanning electron microscopy. Visualization of scales structures and computer simulation allowed distinguishing correlation between nanostructures on the scales and their colour.

1. Introduction

The butterfly colour depends on the interaction of light with the material and spatial structure of wing scales. Three typical mechanisms of wing scales colouration are distinguished: (1) pigmentary colour, (2) structural colour, and (3) a combination of the two [1]. Pigments usually determine the colour of the scales. Additional colouration sometimes occurs because of the light interaction with complicated scale structures. The structural colours have been attributed to various physical mechanisms, such as multilayer interference, diffraction, Bragg scattering, and other [2]. The real colour of the butterfly wings can be understood by analysis of the inputs of the pigment absorption and light interaction with the structure of the wing scales.

In the present work, we investigate the origin of the different colourations of the wing scales of *Hebomoia glaucippe* butterfly both experimentally and by computer simulation.

2. Experimental methods

Samples of *Hebomoia glaucippe* butterfly (Fig. 1) have been received from Ekaterinburg Butterfly Garden, Russia. Butterfly wing scales were visualized using optical microscope (OM) Olympus BX51 (Olympus, Japan) and scanning electron microscope (SEM) with focused ion beam AURIGA™ Crossbeam Workstation (Carl Zeiss, Germany). For visualization of scales structure by SEM, the samples consisting of a butterfly wing or a part of the wing were mounted on a sample holder by a conductive carbon or a copper sticky tape. The samples were covered with 25-nm-thick Au/Pd thin film by magnetron sputtering to remove residual charge induced by electron beam. The scale structures were visualized using in-lens secondary electron detector at 5 kV electron accelerating energy. Cross-sections of wing scales were made by focused ion beam with liquid Ga emitter and ions accelerating energy 30 kV.

The reflectance spectra of butterfly wings were measured by spectrophotometer Agilent Cary 5000 (Agilent Technology Inc., USA) in the spectral range from 175 to 3300 nm. The samples were taped on microscope slide and placed perpendicular to a light path.
3. Results

The wings of *Hebomoia glaucippe* have orange, white, and black colours (Fig. 1). The outer part of the dorsal forewings is bordered by narrow band of the black scales. Orange scales cover the main part of the dorsal forewings. White scales cover the rest of forewings and the whole hindwings.

SEM images of the wing scales from the dorsal side are shown in Figure 2. The studied scales of all colours have the similar sizes about 120x50 μm² and consist of ridges and crossribs. Three kinds of scales have been separated. The black and white scales have wavy ends (Fig. 2a,b), whereas the end of orange scale is rounded (Fig. 2c). Ridges extend longitudinally from one end of the scale to the other. The periods of ridges are 2.2 ± 0.1 μm for black scales, 2.3 ± 0.1 μm for white ones, and 1.1 ± 0.1 μm for orange ones. The periods of crossribs are 750 ± 50 nm and 1.2 ± 0.1 μm for black and white scales, respectively.

The structure of black and white scales can be attributed to unspecialized microstructure according to classification presented in [1]. The distinctive feature of the white scales is the presence of pigment beads that cover free space between ridges and crossribs (Fig. 2e).

![Figure 1. Hebomoia glaucippe butterfly.](image)

![Figure 2. SEM images of the structures of *Hebomoia glaucippe* butterfly wing scales of different colours: (a,d) black; (b,e) white; (c,f) orange.](image)
Figure 3. Measured reflectance spectra of the wing scales: (a) black and white colour; (b) orange colour.

The colouration of orange scales can be attributed to a ridge microstructure [1]. The ridges of these scales are folded into overlapped lamellae that run parallel to the scale surface. The ridge period is $1.1 \pm 0.1 \mu m$. The crossribs are very irregular and their period is difficult to estimate (Fig. 2f). The orange scales contain also the pigment beads but in essentially smaller portion than in the white ones.

The reflectance spectra were measured by spectrophotometer in spectral range from 200 to 800 nm. The black scales show very low reflectance in the whole spectral range (Fig. 3a), which can be attributed to the presence of melanin pigment contained in ridges and crossribs, which absorbs ultraviolet as well as visible light [3].

The white scales consist of ridges, crossribs, and pigment bead, which can play role of the scattering elements. Their reflectance is low for ultraviolet light (wavelength below 400 nm) and high for visible light (wavelengths above 450 nm) (Fig. 3). The low ultraviolet reflectance can be attributed to absorption by pigment located in the beads of the scales [3].

The reflectance spectrum from orange scales shows high intensity in the ultraviolet (from 300 to 450 nm) and in the visible wavelength range (above 550 nm) (Fig. 3b).

The computer simulation has been done for understanding, which elements of orange scales make contribution to reflection spectrum. The detailed information about ridge structure was obtained from its cross-section by focused ion beam. The platinum thin film was deposited on top surface of the scales to eliminate ridge damage by heavy ions during cutting. The ridge structure can be easily observed at the cross-section of the butterfly wing scale (Fig. 4a) due to its dark contrast in secondary electron image, as compared to lighter contrast of surrounding Pt film. It has been found that the ridge consists of six overlapped lamellae with thickness $55 \pm 5$ nm and period $255 \pm 5$ nm.

Figure 4. (a) Cross-section of the ridge scale obtained using focused ion beam cutting. (b) Multilayer model used for computer simulation of the reflectance spectrum. (c) Comparison of normalized measured (EXP) and simulated (CS) reflectance spectra.
During simulations, the parallel ridge lamellae structure was approximate with multilayer thin film structure (Fig. 4b). The layer thicknesses correspond to the respective values of lamellae. It was taken in mind that the ridges of butterfly wing scales consisted of chitin with refractive index about 1.5 [1], which was distinctly higher than the refractive index of air. The reflective spectrum was calculated using commercial finite-element modelling software COMSOL Multiphysics™. The obtained qualitative correlation of simulated spectrum with experimental one (Fig. 4c) allows concluding that the parallel ridge lamellae structure makes the main contribution to orange colour of the wing scales.

4. Conclusion
The microstructure of wing scales of the *Hebomoia glaucippe* butterfly was investigated. Three kinds of scales were found in butterfly dorsal wings. The cross-section of orange coloured scale allowed us to obtain the detail information about the ridge structure. Experimentally obtained parameters were used for computer simulation of the reflectance spectrum of orange scale using the two-dimensional planar multilayer model. The obtained qualitative correlation of simulated spectrum with experimental one allows concluding that the parallel ridge lamellae structure makes the main contribution to the orange colour of the wing scales.

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