Chapter 7
Optical and structural properties of the silver scales of the Silverspot Butterfly, *Dione juno*

Spectrophotometry as well as structural analyses were performed on the scales that form the silver spots in the ventral wings of fritillary butterflies, specifically *Dione juno*. Electron microscopy shows that the scales have a very thin body and a rather smooth upper surface, in large contrast with most butterfly wing scales, which have a highly structured upper lamina. The silver scales have clear ridges, and the spaces in between the ridges form longitudinal, semi-cylindrical channels. Transmitted light through a single scale forms a linear diffraction pattern perpendicular to the direction of the ridges. The diffraction pattern agrees with calculations using the anatomical ridge distance. The far-field scattering profile of light reflected by a single silver scale is a non-uniform, coloured stripe, also perpendicular to the direction of the ridges. This pattern resembles the scattering profile of *Morpho* butterflies, but the physical basis is very different, because the *Morpho* scales have multilayered ridges. The scattering pattern of *D. juno* scales is primarily determined by reflection at the semi-cylindrical channels. The far-field distribution of light reflected by silver spot areas on the wing is a broadened white stripe, which results from the summed scattering by multiple single scales.

7.1 Introduction

Butterfly wings have perhaps the most diverse colorations and patterning in biological nature. Different purposes have presumably driven the formation of such patterns, including sexual selection, camouflage, conspecific recognition and mimicry. A butterfly wing is formed by a so-called wing substrate, which is a two-cell layer of transparent cuticular material, and rows of scales stacked at both
sides of the wing substrate. Different wing areas have distinct colours, depending on the structure and/or pigmentation of the scales. To understand the coloration of the wing as a whole, it is therefore necessary to understand first the mechanisms of coloration of single scales. In general, two mechanisms are distinguished. One is due to pigments that absorb light in a restricted wavelength range and therefore is called chemical or pigmented coloration. The other is due to regular structures that cause coherent scattering of certain wavelengths. This is called physical or structural coloration. The scale structures are usually not developed uniformly throughout the scale. In Pierids, the bead density increases from the base to the tip. This is a morphological characteristic that has functional consequences (Giraldo & Stavenga 2007b).

In a study of single butterfly scales of different species, Giraldo et al. (2007) showed that the spatial profile of the light reflected by the scales is highly dependent on the scale structure. In pierids, the intricate upper lamina of the scales is usually beset with pigment granules, which scatter light incoherently, similar to scattering by a Lambertian reflector. In *Morpho* butterflies, the scales scatter light coherently, due to the multilayered ridges of their upper lamina. Some scales, as in the purple tip of the dorsal forewings of *C. regina* combine blue coherent scattering with pigment absorption and incoherent scattering of the pigment granules (Giraldo et al. 2007).

In the present study we describe the anatomical and optical properties of the silver scales of fritillary butterflies, specifically the Silverspot butterfly, *Dione juno*. We analysed the optical properties of single scales as well as that of large wing areas so to increase our understanding of wing coloration.

### 7.2 Materials and methods

**Animals**

We studied the silver spots of various fritillary butterflies belonging to the nymphalid tribe Heliconini (Simonsen (2006, 2007). *Dione juno* (Silverspot butterfly) was a donation of Dr. Marta Wolf from the Entomology Group of the University of Antioquia, Medellin, Colombia, *Dione moneta* (Mexican Silverspot) was a gift from Andrés Vélez from the Ecological Park of Piedras Blancas, Santa Elena, Colombia, and *Agraulis vanillae* (Gulf Fritillary) was donated by Mr Ric Wehling, Eglin, Florida.

**Spectrophotometry**

Single scale reflectance was studied with the microspectrophotometer described in Section 2.2.1. The microscope objective used was an Olympus 20x, NA 0.46.
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The angular distribution of the reflected light was studied with the optical system described in Section 2.2.3. In brief, a light beam passed through a pinhole and was imaged on a scale. The reflected light was collected with a fiber optic and relayed to a spectrometer (SD2000, Avantes, Eerbeek, the Netherlands). The scattering pattern appearing on a white screen, either put between the imaging lens and the scale (reflected light) or behind the scale (transmitted light) was photographed. The reflectance and transmittance spectra of semi-denuded silver wing areas were measured with an integrating sphere (AvaSphere-50-Refl; Section 2.2.2). The dorsal orange and brown scales, opposite to the silver spots, were therefore fully removed by using adhesive tape. A white reflectance standard (Spectralon, Labsphere, North Sutton, NH, USA) served as a reference.

### 7.3 Results

The Silverspot butterfly, *Dione juno*, derives it name from the metallic shining spots on the ventral wings (Fig. 1a). The scales at the opposite, dorsal side of the...
wings of this species are rather inconspicuously orange-brown coloured. The specular properties of the silver spot areas are prominently clear when observed with a light microscope (Fig. 1b). To understand the silver scale optics we performed scanning electron microscopy. Fig. 1c shows a cross section of a silver scale and the wing substrate. The thickness of the scale is approximately the same as that of the wing substrate (~0.5 µm), which is about half the thickness of pierid scales (for example, Giraldo and Stavenga 2007c). The scale is composed of two closely spaced laminae. The windows, which are the areas between ridges and crossribs, are closed (Fig. 1d), in contrast with the orange and brown scales of the same wing of *D. juno* as well as the scales of most butterflies, which have open windows.

For comparison, we also performed scanning electron microscopy on two other fritillaries, *Dione moneta* and *Agraulis vanillae*. The scales of silverspot areas of *D. moneta* were indistinguishable from those of *D. juno*, as also their windows were fully closed. The silver scales of *A. vanillae* were slightly different in that a small percentage of the window panes appeared to have small pores. Similar observations were very recently reported by Simonsen (2007).

The specular characteristics of the silver scales are presumably derived from the smoothness of the upper scale lamina. In order to verify this hypothesis, we have studied single scales as well as scales on the wing under transmitted and reflected light conditions. The reflectance spectrum of Fig. 2a was measured with a microspectrophotometer from a single silver scale, The spectrum increases monotonically, is high throughout the visible wavelength range, and suggests the presence of an ultraviolet-blue absorbing pigment.

In addition to the single scale microspectrophotometry, we performed experiments on semi-denuded wings with an integrating sphere (see Section 2.2.2). We removed the orange and brown scales from the dorsal wing to evaluate the optical properties of the silver spots without the contaminating effect of the scales on the opposite side of the wing. Fig. 2b shows the reflectance and transmittance measured from a silver spot illuminated with a white light beam of about 2 mm diameter. The absorptance was calculated by subtracting the summed reflectance and transmittance from 1. The absorptance spectrum shows that the cuticular material possesses a UV-blue absorbing pigment.

Because the scales on the dorsal wing were removed, the experimental spectra correspond to a stack of approximately two overlapping scales plus the wing substrate. The reflectance of the semi-denuded wing reaches a maximum of 0.45 at long wavelengths, considerably lower than the single scale reflectance measured with the microspectrophotometer. This difference must be due to the different apertures of the experimental arrangements. The measurements were in
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Fig. 2. Reflectance spectra of silver scales. a Reflectance spectrum of a single scale measured microspectrophotometrically. b Reflectance and transmittance measured with an integrating sphere, together with the calculated absorptance of a semi-denuded silver spot. The absorptance curve shows that the cuticular material contains a UV-blue absorbing pigment.

both cases done with a white diffuser as a reference. The integrating sphere collects all the back-scattered light, from all angles, but the microspectrophotometer objective has a limited numerical aperture and therefore collects preferentially specularly reflected light.

Knowledge of the spatial profile of the reflected light hence is clearly of crucial importance to understand the optical properties of single silver scales, and therefore we have studied the angular distribution of the reflected and transmitted light into further detail. For measurements of the reflected light profile, the setup described in Section 2.2.3 was used. A single scale, with the ridges positioned vertically, was illuminated with a narrow light beam (Fig. 3a). The far-field spatial profile of the reflected light then is a stripe perpendicular to the ridges (Fig. 3b). The stripe is not uniform, but rather consists of coloured spots with the highest intensity for longer wavelengths. The reflectance as a function of angle of a single scale was measured for seven different wavelengths, chosen throughout the visible spectrum. The curves, which have been normalized to the maximum reflectance (reached at 680 nm), slightly vary for the different wavelengths (Fig. 3c).

The reflectance spectra like that of Fig. 3c showed some fine structure, indicating interference effects, and therefore we performed measurements of light transmitted by single scales with the angular distribution setup of Fig. 4a, which is a slight modification of the optical system used to study the angular reflection. The transmitted light appeared to display a diffraction pattern perpendicular to the direction of the ridges (Fig. 4b), similarly as the linear profile of reflected light.
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(Fig. 3b). The angular distribution of the maxima was studied for five different wavelengths throughout the visible range. The grating constant was calculated for each wavelength and yielded a value $2.1 \pm 0.2 \ \mu m$, similar as the anatomical distance between the scale ridges.

Since the actual observed coloration of a silver spot depends upon the stack of scales, we have also photographed the scattering pattern of an intact silver spot area. The diameter of the illumination beam was about $400 \ \mu m$. The resulting scattering profile retained roughly its linear shape, although it was considerably broadened (Fig. 3d), as expected from the cumulative effect of a large number of scales that are not perfectly aligned. The latter consideration also explains why the resulting stripe is no longer spotted, but uniform and white.

7.4 Discussion

A single scale from a silver spot of a fritillary butterfly scatters incident light in approximately a plane, perpendicular to the scale ridges (Fig. 3b). The assembly of scales on the intact wing scatters light in a broader angle (Fig. 3d), resulting in a white, silvery colour. The physical mechanism of the silver spots displayed by

Fig. 3. a Microphotograph of a single silver scale attached to a glass micropipette. The dashed circle depicts the illumination area and the approximated size of the light spot (~30 µm); bar 50 µm. b Spatial reflection pattern of a single scale of D. juno. The dashed circles represent angles in degrees. The dark spot in the centre is the hole in the screen through which the light beam passed. The vertical dark line below the hole is the glass pipette holder. c Angular reflectance for seven wavelengths along the visible spectrum. d Spatial reflection pattern of an intact silver spot area illuminated with a light beam of approximately 400 µm diameter. e Diagram showing the mechanism that produces a linear pattern of the light reflected by a Silverspot Butterfly scale. Incident light is reflected into a plane due to the cylindrical shape of the area between the ridges.
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Fig. 4. a) Diagram of the optical system used to study the transmission of a scale. The setup described in Section 2.2.3 was modified. A semitransparent screen was put behind the illuminated scale and then photographed. b) Photograph of the semitransparent screen showing the diffraction pattern produced when light is transmitted through the scale.

several fritillary species of the nymphaeid tribes Argynnini and Heliconiini (Simonsen 2007) has so far not been elucidated. Mason (1926) suggested, based on immersion experiments, that the pearl white of the silver scales is produced by a multiple-film system. Similarly, Simonsen (2007), who performed an extensive comparative survey of silverspot scales using scanning electron microscopy, also stated that the specular properties might be caused by a fine structured scale lumen. He regarded this nevertheless unlikely, because his anatomy favoured as the most parsimonious explanation that the only difference between normal and silver scales exists in the windows, which are usually open but are closed in the silver scales. Our scanning electron microscopical images agrees with that interpretation and, furthermore, do not provide any evidence for multilayers. The silver spot scales indeed appear to be similar to the classical bilayer scales with a smooth lower lamina and an elaborated upper lamina, with ridges like in the orange and brown coloured scales of D. juno. However, the closed windows create between the ridges a smooth, semicircular cylindrical surface, which acts as a cylindrical mirror. A cylindrical mirror reflects a small-aperture incident light beam into a stripe pattern in the far-field. Because the upper lamina of a silver scale forms a series of parallel cylindrical mirrors, the reflection pattern is a combined result from the specular and diffraction properties of the scale, resulting in a somewhat irregular stripe pattern.

The reflectance properties of single scales have previously been studied for a number of butterfly species (Vukusic et al. 1999; Yoshioka & Kinoshita 2004; Giraldo et al. 2007) Especially scales of Morpho butterflies have received most of
the attention, presumably due to their eye catching characteristics and special anatomy. The multilayered ridges that characterize their structure have also been found in other species and thus are called Morpho type scales. The light reflected by the Morpho scale type forms a linear pattern, similar to the one reported here for D. juno, but the reflectance is restricted to ultraviolet or blue wavelengths (Vukusic et al. 2000; Yoshioka & Kinoshita 2004; Giraldo et al. 2007). A light beam hitting the narrow multilayers in the ridges of a Morpho scale is diffracted in a plane determined by the mirror law. As we argued above, although the far field reflection pattern of the Morpho scale type resembles that of D. juno silver scales, the optical mechanism of the silver scales is probably that of a cylindrical mirror with diffraction at the ridge lattice, rather than that of an assembly of slender multilayers, which is the case in a Morpho scale. The narrow coloured stripe seen in the far-field reflection pattern of a single silver scale of D. juno contrasts with the broader white stripe created by an intact silver spot (Figs. 3b and d). A similar broadening of the reflection pattern was also reported by Giraldo et al. (2007) for Morpho aega.

The reflectance of a single scale was determined to be of the order of 0.5-0.6 (Fig. 2a), which is extremely difficult to reconcile with a simple bilayered scale. Measurements with a microscope objective that samples the light reflected by a single scale inevitably overestimates the reflectance when using a random diffuser as the reference, because of the directionality of the light back-scattered by a single scale. The reflectance of a semi-denuded wing, being the wing substrate with only on one side rows of two overlapping silver scales, was determined to be of the order of 0.4 (Fig. 2b). Assuming that the reflectance of the wing substrate is about 0.1, as in pierids (Stavenga et al. 2006; Giraldo & Stavenga 2007b), the reflectance of a single scale still has to be higher than (0.4-0.1)/2 = 0.15 (see Stavenga et al. 2005). This is about what might be expected for two layers of cuticular material with refractive index ~1.6 (Vukusic et al. 1999). However, a proper optical analysis of the reflectance of silver scales will require a more detailed analysis, as the two layers of a scale have thin film properties, which may have distinct wavelength effects. Furthermore, SEM micrographs indicate that the upper and lower lamina approach each other in the midplane between two adjacent ridges. In fact, there the two layers could form a multilayer, where wave optical interference effects may occur. Transmission electron microscopy is hence necessary to acquire precise geometrical data about scale layer thicknesses and distances.
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