THE STRUCTURAL COLORATION MECHANISMS OF MORPHO

BUTTERFLY WING SCALES

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THE STRUCTURAL COLORATION MECHANISMS OF *MORPHO*

BUTTERFLY WING SCALES

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SUMMARY

Many bright colors in nature are generated by the optical effects of biological structures. These intricate structures, combined with the absorption and reflection effects of the chemical pigments within, provide the observed color with high visibility and some other startling optical properties. A prominent example comes from the iridescent colors observed on the wing scales of *Morpho*, a family of subtropical butterflies. Iridescent color refers to the color which changes with varying viewing angle. It is proposed that a layered structure alternating in refractive indices produces the observed colors on the butterfly wing scales, but this generalized idea does not explain some optical effects observed through a variety of methods. This research suggests that the structures in the lower lamina also contributes to the macroscopic optical effects. The observation methods used in this research include optical microscopy, spectroscopy, integrating sphere analysis, and scatterometry. The scatterometry visualizes the far field optical effects from all viewing angles simultaneously. Elementary explanations are proposed for the unexpected patterns observed in the experiments.
CHAPTER 1

INTRODUCTION

Colors created by physical mechanics, such as interference and diffraction, rather than by the absorption of chemical pigments, are categorized as *structural colors* [1]. Coloration mechanism by structure, due to its high visibility and stability, has captured researchers’ interest since the time of Hooke and Newton. Various characterization techniques have made it possible to study the structures responsible for these colors observed in biological systems, such as the wing scales of butterfly, the exocuticles of beetles, and the bird feathers [2]. In the past decade, an extensive study has been dedicated to the bright, iridescent colors found on butterfly wing scales. Iridescent color refers to the color which varies with different viewing angles. A structure such as thin-film reflector, which prefers to transmit or reflect light of a certain wavelength range at a particular angle, creates the iridescent phenomenon. Three different structural components of a scale have been proposed to explain the observed iridescent colors on butterfly wings: the ridges that function as a thin-film reflector, the flats between the ridges which produce colors by scattering, and a lamella/microrib system [3].

The cross section of ridges is a “pine-tree” shape structure, in which the refractive indices of the tree branches alternate, and in the case of butterfly wing scales, these layers are consisted of lamellae of air pockets and biological compounds. This structure, which is also named as Bragg’s reflector in the physics literature, is widely used in optical switching and limiting applications. The thickness of the air pocket layers is roughly 0.1-0.2 μm. On the same order of the wavelength range of visible light, this structure does not propagate diffraction patterns of orders higher than zero. Similar to total internal reflection,
diffraction light with higher orders renders the sine value of the constructive interference angle higher than 1, which does not have a physical meaning. Most of the studies explained the iridescent color on the butterfly wing scales by the model of alternating lamellae with different refractive indices, but few of them has taken into account the effect of zero-order gratings. The effect of zero-order diffraction gratings could be visualized in the back-focal plane of an optical microscope. Similar far-field effect is observed in the scatterometer with more precise control on the incident angle.

Several characterization methods will be used to observe the structure of butterfly wing scales. The resolution power of optical microscopy is not enough to characterize the details of the sub-micron structures on the wing scales due to the diffraction limit[2]. Methods that can visualize the structures include transmission and scanning electron microscopy. An optical microscope equipped with spectrometer is the most accessible and convenient method to measure the reflectance spectrum of individual wing-scales. A scatterometer is used to observe the far-field optical effect of individual scales, which processes the information of the wing scales with an elliptical mirror. The mirror focuses the sample placed on the first focal point onto the second focal point. The advantage of the scatterometer over a gonio-spectrophotometer is that the scatterometer receives the real-color signals from all directions simultaneously and overlaps them onto one image, while the gonio-spectrophotometer detects the intensity of only one wavelength in a set of experiment, and a considerable number of measurements need to be done for one image by adjusting the positions of the light source and the spectrometer. An integrating sphere is used to collect the macroscopic spectra of individual scales. The highly reflective diffuse PTFE material inside the integrating sphere brings a reflectance above 98% over the range of the visible wavelength though the averaged results ignore the angle dependence of the iridescence effect.

Potential applications of the unfolded mechanism of coloration includes reflective displays,
iridescent pigments for and automobiles, and anti-reflection coatings for optical devices. Pigments based on structural color, durable and brilliant, will be preferred over traditional chemical pigments once commercially available.
CHAPTER 2

LITERATURE REVIEW

Walking into any of the Museum of Natural History, one would be surprised by the bright, iridescent colors created by nature. Iridescent color, referring to the color which changes as the viewing angle is varied, is widely observed in a number of butterflies, beetles, and gemstones. The study of the mechanism of the iridescent color formation has focused on structural color formation. Unlike artificial colors which are generated by absorption of chemical pigments, structural colors result from the interaction between the light and the structures through interference, diffraction, scattering, and/or dispersion; these colors are not only highly visible, but also reliably stable with the passage of time. Structural colors are observed in the butterfly wing scales, in the exocuticles of beetles, and in moth eyes, among which the iridescence of butterfly wing scales has long attracted the attention of researchers [2]. Most of the studies on butterfly wing scales focus on the model of thin-film reflectors, and in recent years, the computational simulation of wing scales as thin-film reflectors yields agreement with experimental observation [1], but structures on the sub-micron dimension suggest that other mechanisms can contribute to the coloration effects [2]. This research explores the mechanism of iridescent color formation on butterfly wing scales from a comprehensive aspect. This review will contain a brief discussion of the structures of butterfly wing scales, the angle dependence of thin-film interference model, the diffraction model of zero-order gratings, the role of scattering in color formation, and other optical effects culminating in the observed color on butterfly wing scales.
Six distinct structural variations are identified on butterfly wing scales as the potential generators of color. The structures responsible for the metallic blue color of *Morpho* wing scales are the ridges with a spacing less than a micron, the flats present between the ridges that are intertwined with air bubbles, and the lamella or microrib extended from the ridges that behave as thin film reflectors [3-5]. As in *Morpho rhetenor*, the high-magnification image from a scanning electron microscope shows that the ridge has a cross section with “pine-tree” shape. The chitin on the branches and the empty space are filled with air. Studies have concentrated on the simulation of this structure as a thin-film reflector with alternating refractive index [1, 6]. In certain species, other structures such as the air perforations in the case of *Papilio palinurus*, are characterized and proved to be responsible for the perceived color [7]. This model has been re-constructed with oxide multi-layers in laboratory and the observed color from the reconstructed material is coherent with that of the *P. palinurus* [8]. A simple experiment can confirm that the color is indeed created by structure. If a blue scale of *Morpho* is immersed in a solution of acetone, or any other solution of a refractive index different than that of the air, the observed color of the scale will change; after the acetone is evaporated, the original color will recover [2, 5]. This simple experiment cannot determine the type of the light interaction attributing to the structural color because the colors produced both by scattering and by interference vary in response to the change in the refractive index of the medium.

**Thin Film Interference**

A thin-film reflector refers to a film with a thickness on the order of the wavelength of visible light, about a few hundred nanometers. If the thin-film reflector has a thickness of exactly a quarter of the incident light wavelength, the reflected rays will interfere constructively and create a color depending on the viewing angle. The phase shift of reflected light from a thin-film reflector comes from two sources: a) a 180° phase shift from the air-liquid interface (the top face of the thin-film); and, b) a path difference of
$2n_{\text{liquid}}d\cos\alpha$ created by the extra path for the light traveling in the secondary medium. For a thin film of which the thickness equals $\lambda/4$, the phase shift between the reflected light from the top interface and the bottom interface equals to an integer multiple of incident wavelength, which satisfies the condition of constructive interference[2]. An equation is derived for the thin-film reflector to predict the wavelength of the interfered light as a function of viewing angle and the refractive index of the thin-film,

$$\lambda_{\text{max}} = \left(\frac{4n_{\text{liquid}}d}{2n+1}\right)\sqrt{1 - \frac{n_{\text{air}}^2}{n_{\text{liquid}}^2}\sin^2\alpha_1}$$

(1)

where $n$ is an integer (usually equals to 1 in the system of butterfly), and $\alpha_1$ is the incident angle [9]. The equation shows that the observed color shifts to a lower wavelength as the incident angle increases, which is consistent with what is observed in nature. Early studies have found the similarities between the optical effects generated by thin-film reflector and the iridescence of butterfly wing scales [10]. Literature has modeled the ridges of butterfly wing scales as a stack of thin-film reflectors with alternating refractive index [1, 6]. The biological compound present in the stack is mainly chitin, with a refractive index around 1.56 [6]. The thickness of the thin-film is around 90 nm and the thickness of the air lamella is also approximately 90 nm, resulting in a vertical periodicity of around 180 nm. The horizontal periodicity is 675±75 nm [1, 2]. Computational simulation for the wing scales of *Morpho rhetenor* based on these dimensions has shown that a model of a rectangular lattice with rectangular dielectric elements produces a spectrum in accordance with the experimental result. Variations on the model, such as a centered rectangular lattice, or a tapered version of the rectangular lattice create a smoother spectrum with a narrower peak range [1].

**Diffraction**
Diffraction is another mechanism of angle-dependent color formation. Diffraction refers to the spectrum observed when the light confronts an obstacle. While always discussed separately, interference could be viewed as a result of geometric addition of two diffracted lights from a coherent source. A white light diffraction spectrum consists of a white band in the central area and symmetric rainbow bands on both sides of the incident axis. The central white band is referred to as the zero-order diffraction. In a zero-order diffraction grating, of which the periodicity is comparable to the visible wavelength, the first-order diffraction and the diffractions of orders beyond become evanescent, and therefore the zero-order diffraction is the only band perceived. The reflection diffraction grating is widely used in antireflection coatings. The antireflective characteristic of a moth eye is achieved by arrays of conical protuberances on the surface of the eyes [11]. This dimpled structure provides a graded refractive index layer that will suppress the reflection. The reflectance non-linearly decreases as the ratio of the diameter of the hemisphere to the incident light wavelength increases. The protuberance must be fine enough to reduce the reflected diffraction; practically, the diameter of the hemisphere should be less than the shortest visible wavelength divided by the refractive index of the substrate. A dimension of 250 nm gives an excellent anti-reflection coating by “moth-eye” principle [12]. From the SEM images, the length between the air pockets on the ridges of the butterfly wing scale is around 0.1-0.2 micron [2]; this sub-wavelength dimension falls into the range of the dimension for zero-order diffraction gratings. Therefore, the repeated ridges on the butterfly wing scale could also be viewed as zero-order diffraction gratings [2]. Formulation of the angle-dependence of zero-order diffraction is still needed and further accommodation to the structure of the ridges of butterfly wing scales needs to be studied. In recent years, interest has grown in the simulation based on the sub-wavelength structures [1], but a systematic study devoted to explain the iridescence of the butterfly wing scale is still needed in this area.
Scattering

Scattering is another mechanism to produce structural colors. Linear scattering is subdivided into Rayleigh scattering and Mie scattering. Lord Rayleigh argued that the blue color of the sky results from the scattering of air molecules instead of external particles. The intensity of the Rayleigh scattered light is inversely proportional to the fourth order of the incident wavelength \([13]\). The scattered light within blue range has a high intensity due to the small wavelength of blue light. Even though violet has the lowest wavelength on the visible light spectrum, the human eyes are more sensitive to blue than to violet, and therefore the sky is perceived as blue instead of the stronger violet \([2, 14]\). Mie scattering occurs when the light is obstructed by a larger particle, and the intensity of the scattered light depends less on the incident light wavelength, but more on the size of the particle. A particle of size around 500 nm will effectively scatter red light, while more particles, or particles of size larger than 500 nm will scatter white light \([15]\). Scattering creates the color for the feather of the blue jay. The feather consists of rachis, which is the main shaft of the feather, barbs, which are the lateral components extending on either side of the rachis, and barbules, which are the hooked overlapping structures between barbs. The barb has three layers which are the transparent outer layer, the box cell containing a mixture of keratin and irregular shaped air bubbles, and a dark layer rich in melanin. The box cell is the entity that scatters light and gives out the blue light; the melanin layer absorbs the light transmitted through the box cell which accentuates the color of the scattered light. If a trace of yellow from carotene is reflected, the bird feather will show a color of green, and if hemoglobin is present near the surface, as in the case of some species of monkeys, the observed color will be purple \([16, 17]\). Though scattering explains the coloration effects for some birds, constructive interference also contributes to the colors of bird feathers \([18, 19]\). Though the explanation for the iridescent butterfly wing scales usually exclude it, scattering can arise from the space between two ridges on the scales and contribute to the
overall coloration [2]. Interesting polarization properties of the reflected light from the wing scales should follow from the polarization effects of scattering.

**Scatterometer**

A scatterometer visualize the far-field view of any micro-scale objects. The core component of the scatterometer is an elliptical mirror. The light that comes through a hole from the center of the mirror illuminates the sample which is place at the first focal point of the mirror. The mirror will reflect the image onto the second focal point. Subsequent optical path includes two convex lenses converging the image onto the receiver. A glass slide with a dark dot is placed between the two convex lenses which forbids the light directly from the hole of the elliptical mirror. The image includes the reflected view of the sample from all viewing angles. Different viewing angles correspond to circles of different radii on the image [20]. Besides qualitative measurements, the scatterometer is capable of conducting quantitative measurements. A spectrometer at the receiver position can analyze the spatial spectrum distribution of the sample, which is useful in butterfly wing scale simulation [20, 21]. The scatterogram of a diffraction grating is the Fourier transform of its aperture function, which includes the information of periodicity. An additional light path is also available in the scatterometer used for this research. The aperture position of this additional light path dictates the incident angle of the illumination which is helpful in studying the iridescence effect of the sample.

In conclusion, computational simulation has confirmed that the thin-film reflector model could generate the spectrum in agreement with the experimental observation. Other minor optical effects need explanation from other perspectives. Further study requires intricate characterization techniques and a fundamental understanding of optics. The potential applications include novel painting pigments, iridescent coating for construction and automobiles, and other bio-mimicry materials.
CHAPTER 3

METHODS AND MATERIALS

Butterflies

The samples of 13 species of male *Morpho* butterflies were collected from insect retailers. For microspectrometry and scatterometry, single scales were glued onto the tip of micropipettes. Micropipettes were dipped into a transparent drying-adhesive, and the end of a single wing scale was picked up by a piezo stage under a stereo microscope. For spectrometry with integrating sphere, wing patches were used for measurements. The single scales were brushed off with tweezers from the dorsal wing (upper side of the wing), where the refined microstructures had been widely observed.

Photography

The photos of the wing scale were taken with a DCM 510 camera and a Zeiss Universal Microscope (Zeiss, Oberkochen, Germany). The bright field images were taken under an Olympus 16X objective. The dark field images were taken under a Zeiss Epiplan dark field 8X objective.

Spectrometry

The spectra of single scales were recorded with a microspectrometer. The microspectrometer is built on a Leitz Ortholux microscope (Leitz, Wetzlar, Germany), combined with an AvaSpec 2048-2 CCD detector array spectrometer (Avantes, Eerbeek, Netherlands). The maximum reflectance angle of the wing scale could be reached by rotating a manipulator attached to the working stage of the microscope. All the spectra
were retrieved at the scales’ highest reflectance angle, under an Olympus LUCPlanFL N 20X, 0.45 objective (Olympus, Tokyo, Japan). Both the abwing (facing away from the wing substrate) and adwing (facing toward the wing substrate) sides were recorded.

The spectra of wing patches were recorded with an AvaSphere-50-REFL integrating sphere (Avantes, Eerbeek, Netherlands) and the spectrometer mentioned above.

**Scatterometry**

The sample was positioned onto the first focal point of the elliptical mirror. The images were retrieved by an Olympus DP70 camera. A Matlab script was used to add the contour of the spatial angles onto the image. For more explanations and detailed calculations, the author refers to the paper by D. G. Stavenga, et. al [20].

**SEM**

A scanning electron microscope (Philips XL-30 ESEM; Philips, Eindhoven, Netherlands) was applied to observe the detailed structures on the butterfly wing scales.
In 8 species among all the samples being investigated, a substantial area of the ground scales is overlapped by the cover scales. In *M. zephyritis* and *M. sulkowski*, two types of scales are observed, different in the width but same from the optical and structural aspects. In *M. rhetenor*, *M. cypris*, and *M. catenia*, the cover scales are atrophied [22] and only the ground scales contribute to the color of the butterfly wings. Three *Morphos* are discussed in detail in the following section.

*Morpho rhetenor*

The dorsal side of *M. rhetenor* has a metallic blue color (Figure 1.a). This blue color results from a stack of lamellae on top of the trabeculae, or as in a cross-section view of the ridges, from a ‘pine tree’ shape structure. The microribs on each lamellae extending from the ridges form a layer of thin film, which selectively reflect light within the blue range. The multi-layered structure increases the saturation of the color, and produces a well-defined peak in the range from 460 nm to 500 nm on the reflectance spectrum. This model is extensively studied and accurately simulated [1, 23].

In Figure 1.d, a blue stripe exists across the scatterogram of a single wing scale on *M. rhetenor*, perpendicular to the longitude of the ridges. The spacing between two ridges is on the order of 0.1μm. The repeated ridges therefore creates the diffraction pattern perpendicular to the longitude of the ridges [2]. The blue color of the diffraction line is a result of thin-film interference as discussed before.
As seen in Figure 1.e, the peak reflectance wavelengths of the spectra are 460.73 nm and 500.71 nm for the integrating sphere spectrometry and for the microspectrometry (MSP) respectively. The iridescent property of interference contributes to this phenomenon. The reflected wavelength shifts to a smaller value when the incident angle increases [9]. When the thin films in the upper lamina is under normal incidence, the objective collects all the reflected light and the reflectance intensity is the highest among all incident angles. At this angle, the peak reflectance wavelength is also the highest according to Eq (1), but because the thin film structure is tilted with respect to the substrate of the wing, the peak wavelength is lower than its highest value when the wing scale lays horizontally. The results from the integrating sphere lose the spatial information of the structure and therefore the peak wavelength in the integrating sphere spectrum is an average value of the peak wavelengths under all angles. The results from the integrating sphere is lower than the peak wavelength in MSP because the peak reflected wavelength from thin-film interference shifts to the lower end of visible light spectrum as the incident angle increases,. The angle-dependence effect is visualized in the scatterogram. In Figure 1.d, the color of the stripe shifts from cyan to violet from the center to the ends of the stripe.

The reflectance of the single scale spectrum has a value over 100% due to an artifact of the spectrometer. A reference spectrum is taken with a diffusive white standard, but the numerical aperture of the objective limits the amount of light that reaches the spectrometer, but the spectrometer still records this value as 100% reflectance. When a scale is normally incident, the objective cone receives all the light that has been emitted from the light source, which exceeds the intensity value of the reference. Therefore, the peak reflectance intensity is higher than 100%. This problem does not exist in the spectrum retrieved with integrating sphere because all reflected signals are collected by the PTFE material inside the sphere.
Figure 1. Measurements were made with a male *M. rhetenor* specimen. (a) the macroscopic view of a male *M. rhetenor*; (b) a photograph of the specimen under dark field illumination; (c) a photograph of the abwing side of a single scale under bright field illumination; (d) the scatterogram of a single scale, with the red contour lines indicating 5, 30, 60, 90 degrees of incident angles from inner to outer side respectively; (e) the spectra of the sample measured with an integrating sphere and with a microspectrophotometer. The scale bar in (b) is 100 μm. The photograph of the single scale (c) and the scatterogram (d) were rotated by the same angle and it is revealed that the diffraction line in the scatterogram is perpendicular to the ridges on the upper lamina, which are parallel to the longitude of the scale.

This study went on to investigate the adwing side of the scale. The adwing side of *M. rhetenor* scale, as observed in most ground scales of *Morpho* butterflies, preserves a different color from the abwing side, as seen in Figure 2.a. This difference arises from the pigment in the wing scales. When the *M. rhetenor* scales were drenched in an immersion oil with a refractive index of 1.515 at 23 Celsius degrees, the scale revealed the color of the pigment (see Figure 2.c). The similar absorbance spectra suggest that the pigment layer
mainly consists of melanin. Melanin will absorb the transmitted visible light from the upper lamina, which emphasizes on the reflected blue interference color.

Interestingly, the color of the adwing side varies from green to blue, and eventually to purple on a dark background. This pattern is also observed in the ground scales of *M. deidamia, M. richardus, M. menelaus alexadrovna*, and *M. godarti assarpai*. Seven spectra were taken in different spots along the central longitude of a ground scale in *M. rhetenor*, which are presented in Figure 2.e. The peak value of the spectrum gradually shifts to the lower wavelength, which arises from a thin-film layer with a thickness gradient. The reflectance spectrum of a thin-film has a blue shift as the thickness decreases based on the model of interference. The purple emerges due to the coexistence of two peaks within the visible wavelength. Usually only one peak of reflectance spectrum from thin-film interference is observed within the visible wavelength range, but as the thickness continues to decrease, the first peak shifts to the violet, and another peak appears in the red range. The additive effect of violet and red produce the purple color. The orange in background may come from the gradually increased reflectance of melanin in the high visible wavelength.

*Morpho sulkowski*

Two types of scales different in width and length are observed in *M. sulkowski*. The broad scale has a width around 68 μm while the thin scale has a width around 45μm. As seen in Figure 3.b, these two types of scales alternate in sequence on the wing substrate. The broad and thin scales, though different in width, are identical from the optical aspect. From the immersion oil experiment (Figure 3.m and Figure 3.n), both types of scales are mainly consisted of chitin, a transparent biological compound often found in *Morpho* wing scales. The abwing scatterograms have a clearly defined blue diffraction line while the adwing scatterograms obtains a diffuse blue pattern in the
The transparent scales allow the light illuminated from the adwing side to interact with upper lamina and produce the diffraction line on the scatterograms. A rough lower lamina would generate the diffuse reflectance pattern but as seen from SEM, the lower lamina is rather smooth, which leaves the origin of the diffuse pattern unknown. One suggestion is

**Figure 2.** (a) the adwing side of a *M. rhetenor* scale taken with a longer exposure time; (b) the adwing scatterogram; (c) the scale drenched in an immersion oil with refractive index of 1.515 at 23 °C; (d) the spectra of the melanin and the immersed scale; (e) seven spectra taken along the central longitude of the scale. The numbers in (a) indicate the positions where the reflectance spectra in (e) were taken.
that the trabeculae in the middle layer of the scale extend their roots and crinkle the lower lamina. More observations are needed to explain the diffuse pattern.

Similar scales are found in Morpho zephyritis, except for the broad scales in the blue area, which have pigment layer between the upper and lower lamina. The photograph of these broad scales are The adwing scatterogram of the pigmented scales has an orange diffraction line, of which the origin is left unclear.

Figure 3. (a) a photograph of a male M. sulkowski; (b) the dark field photograph of the wing scales; (c), (f), (h), (j) are the photographs of a broad scale abwing side, a broad scale adwing side, a thin scale abwing side, and a thin scale adwing side respectively; (d), (g), (i) and (k) are the scatterograms from the side of the scales to the left; (e) and (l) are the spectra of the broad and thin scales; (m) the photograph of the scales drenched in the immersion oil with refractive index of 1.515 at 23°C; (n) spectra of a transparent M. sulkowski scale and chitin. The scale bars in (b) and (m) are 100 μm.

*Morpho deidamia*
In *M. deidamia*, the cover scales completely overlaps with the ground scales (see in Figure 4.b). The optical effects of the ground scales are similar to that of the scales in *M. rhetenor*, except for that the diffraction line in the abwing scatterogram is wider than that of *M. deidamia*. As in Figure 4.a, the transparent cover scale of *M. deidamia* has a widely open hand-held fan shape, which curves at the tip toward the wing substrate. The abwing scatterogram in Figure 4.d has two discrete diffraction lines of different widths, while the adwing scatterogram in Figure 4.f has a diffraction line and a specular reflection point. The scale, based on these observations, is proposed to have an upper lamina equipped with ‘Christmas tree’ structure, and a lower lamina that is flat without pigment. The wider diffraction line in the abwing scatterogram originates from the direct reflection of the multilayer structure on the upper lamina. The light transmitted through the upper lamina is usually absorbed by the melanin in the case of most ground scales, but when the melanin is not present in the wing scale, the transmitted light reaches to the multilayered structure in the upper lamina and the reflected light will then again possess the blue diffraction line. The second diffraction line is shorter in length and thinner in width as the intensity of the light is reduced upon each encounter of an interface. Besides the difference in dimension, two diffraction lines are otherwise the same. The bright point in the adwing scatterogram arises from the specular reflection of the flat lower lamina, the blue color from the thin-film interference; the light transmitted through the lower lamina reaches to the refined multilayer structure, and creates the subtle diffraction line in the abwing scatterogram. Similar fan-shaped cover scales are observed in *M. richardus* and *M. patroclus orestes*. Other *Morpho*, such as *M. peleides, M. menelaus alexandrovna, M. godarti assarpai*, and *M. helenor violaceus*, have transparent, fan-shaped cover scales with a smaller opening angle, among which *M. helenor violaceus* and *M. godarti assarpai* also curve at the tip while the other two have a less announced effect. Similar scatterograms are observed in all cover scales with a noticeable exception of *M. adonis*.
Figure 4. (a) a photograph of a male *M. deidamia*; (b) the dark field image of the wing scales on *M. deidamia*; (c) and (e) the photographs of the abwing and adwings side of a cover scale; (d) and (f) scatterograms from the two sides of the cover scale; (g) the spectra of the two sides of the cover scale. The scale bar in (b) is 100 μm.

The diffraction lines on the scatterograms of *M. adonis* are segmented into three parts from either side of the wing scales. The cover scales of *M. adonis* have an oval shape with one axis of symmetry. The scale has a wavy surface along the direction perpendicular to the ridges. The spectra of a single scale possess two peaks that are well-defined and comparable in reflectance value. Interestingly, the ridges of *M. adonis* do not have a multilayer structure as observed from SEM images; the cross-section of the ridges resembles the shape of a crown. More explanations are awaited for the observations of *M. adonis*. 
Similarities are easily to be found among the species of *Morpho* butterflies. The majority of the *Morphos* adopt the same arrangement of their scale structure, i.e., an upper lamina with ridges, a thin-film lower lamina with or without melanin. For abwing scatterograms, the ridges along the longitude of the scale produce a diffraction line in the far-field image; the multilayered structure on the ridges serves as a reflection filter with a pass band in blue range. The melanin, if existed, absorbs the transmitted light, and emphasizes the blue reflection. When a scale is lack of the melanin, a double diffraction line is observed when the second line is produced by the reflection of lower lamina. For adwing scatterograms, a specular reflection point is expected resulted from the flat lower lamina. With pigmented layer, an iridescent pattern in the range of violet-blue-purple is observed; without a pigmented layer, a subtle diffraction line in addition to the blue specular point is observed.

*Mopho* family has a rich variation in structure and optical appearance. *M. sulkowski* differs from other species due to its transparent ground scale; its adwing scatterogram has a diffuse pattern which may arise from the roughness of the lower lamina. The broad scale on *M. zephyritis* shows a yellow diffraction line in the adwing scatterogram instead of a specular point as seen from other ground scales. *M. adonis* adopts a different ridge structure from other *Morphos*, which engenders a segmented diffraction line specific to this species. Table.1 categorizes the observed optical effects from *Morpho* family.

More study is needed before the optical effects of *Morpho* family could be fully understood. The scatterometer collected some unexpected optical effects, for which
generalized thin-film model are insufficient to explain. Research on structural coloration will progress along with the improvement on optical instrument.

Table 1. A summary of the scatterograms of different species in *Morpho* family

<table>
<thead>
<tr>
<th></th>
<th>Abwing scatterogram</th>
<th>Adwing scatterogram</th>
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</thead>
<tbody>
<tr>
<td>Transparent scales</td>
<td>Double blue diffraction line</td>
<td>A single blue diffraction line and a specular reflection point</td>
</tr>
<tr>
<td>Pigmented scales</td>
<td>A single blue diffraction line</td>
<td>A specular reflection point, usually brown or purple</td>
</tr>
<tr>
<td><em>M. sulkowski</em> scales</td>
<td>A single blue diffraction line</td>
<td>A single blue diffraction line with a blue diffuse circle in the background</td>
</tr>
<tr>
<td>(supposedly rough scales)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. zephyritis</em> blue</td>
<td>A single blue diffraction line</td>
<td>A yellow diffraction line</td>
</tr>
<tr>
<td>broad scales</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. adonis</em> cover</td>
<td>A segmented diffraction line</td>
<td>A segmented diffraction line</td>
</tr>
<tr>
<td>scales</td>
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REFERENCES


