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Authors: Yoshida, Akihiro, Motoyama, Mayumi, Kosaku, Akinori, and Miyamoto, Kiyoshi

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Antireflective Nanoprotuberance Array in the Transparent Wing of a Hawkmoth, *Cephonodes hylas*

Akihiro Yoshida[1]*, Mayumi Motoyama[1], Akinori Kosaku[2] and Kiyoshi Miyamoto[3]

[1]*Biohistory Research Hall, 1-1 Murasaki-cho, Takatsuki, Osaka 569-11, Japan*
[2]*Laboratory of Clinical Sciences, Dokkyo University School of Medicine, 880 Kitakobayashi, Mibu, Tochigi 321-02, Japan*
[3]*Laboratory of Medical Sciences, Dokkyo University School of Medicine, 880 Kitakobayashi, Mibu, Tochigi 321-02, Japan*

ABSTRACT—A transparent wing of a hawkmoth, *Cephonodes hylas*, has a highly ordered array of nanosized protuberances in its surface. This protuberance array has almost the same morphology as that of the "corneal nipple array", which is suggested to function as an antireflective device to light, using a scaled model experiment, a theoretical calculation, and a comparative way. For the direct study of the function of this protuberance array, we prepared an artificial wing without protuberances and compared it with an intact one with protuberances. Directly measuring the light reflectance spectra of the intact wing and the artificial one, we demonstrated that the nanocomposite in the *Cephopnodes* wing decreases the light reflectance by the wing into 29-48% in the broad wavelength range. This antireflective effect is also showed by the color difference between the wings, coated by gold, with and without protuberances.

INTRODUCTION

The optical characteristics of various kinds of cuticular nanostructures of insect body surfaces have long been studied by biologists and physicists. It has been reported that arrays of nanoordered cuticular structures in the butterfly scales and the beetle bodies generate colors by interference, diffraction, and scattering (Onslow, 1921; Mason, 1927; Anderson and Richards, Jr., 1942; Ghiradella *et al.*, 1972; Morris, 1975; Huxley, 1976; Kosaku and Miyamoto, 1994), and that those in the corneal surfaces of some insect eyes and in butterfly scales function as anti-reflective devices by impedance matching (Bernhard and Miller, 1962; Bernhard *et al.*, 1965; Huxley, 1975).

A hawkmoth, *Cephonodes hylas*, has transparent wings without scales, which is a rare case among lepidopteran (butterfly and moth) wings. Most lepidopteran wings have colored scales on their surfaces; scale colors are generated by pigments and/or cuticular nanostructures. If scales are removed, most wings lose color but do not become as transparent as the *Cephonodes* wing. This means that the *Cephonodes* wing is structurally and/or chemically different from the other lepidopteran wings. We previously reported that a highly ordered nanocomposite is located on the *Cephonodes* wing surface (Yoshida *et al.*, 1996). This nanocomposite is morphologically closely similar to those discovered in the corneal surfaces of some insect eyes by Bernhard and Miller (1962) over thiry years ago. They showed that the corneal nanocomposite functions as an antireflective device to light, by three methods: a scaled model experiment, a theoretical calculation, and a comparative way. In this report, we shows that the nanocomposite in the *Cephonodes* wing also functions as an antireflective device to light, by direct measurement of reflected light, which was not performed with a cornea.

* Corresponding author: Tel. +81-726-81-9753;
FAX. +81-726-81-9757.

MATERIALS AND METHODS

Materials

Dried wings of adult hawkmoths *Cephonodes hylas* were used for the studies described below.

Morphology

Wing pieces cut with scissors were coated with thin membranes of gold (about 8 nm in thickness) using an ion sputtering device (JEOL, JFC-1500), and examined with a scanning electron microscope (JEOL, JSM-5300).

Preparation for wings without protuberances

A wing put on copying paper was pressed and rubbed with a piece of styrofoam, until the wing glitters. This artificial wing without protuberances is called "smooth" wing in this report, while the intact wing with protuberances is called "rough" wing.

Spectrophotometry

Spectral reflectance in visible and near ultraviolet light range scattered by the dorsal forewing surface was obtained using a spectrophotometer equipped with an integrating sphere (diameter, 60 mm; aperture ratio, 0.078), digital data acquisition and analysis system (HITACHI, U-3300). The specimen was held on the auxiliary white board, made of barium sulfate, of the integrating sphere and illuminated vertically by the incident light of about 5×10 mm width; this size of the light spot is larger than that of the forewing. The wings having fairly high transparency at least in visible light range, the light passed through the wing and reflected by the auxiliary white board will drown out the light with a relatively weak intensity reflected by the wing surface. To reduce the light intensity due to the white board behind the specimen, the wing surfaces opposite to those for illumination of both intact and smooth wings were painted black with a felt-tipped pen several times. It was confirmed that the efficiency of reflectance caused by this backing was lowered uniformly all over the spectral range. Optical spectra were recorded from 200 to 800 nm at a 1 nm interval. Before measuring the intensity reflected by the wing, the intensity by the white board (with no wing) was used to normalize unity throughout the full spectrum range. All the dots comprising the trace in the figure were the averages of every ten nm (that is, ten values of reflectance), and they were plotted at every 10 nm from 205 nm to 795 nm.

RESULTS

Artificial "smooth" wing

The adult *Cephonodes* wing is transparent except for fairly small parts: the wing margin and veins (Fig. 1). This transparent part has no scales, since they are shed immediately after eclosion. In the preceding report (Yoshida *et al.*, 1996), we illustrated that the protuberances, about 250 nm in height, are regular-hexagonally arranged in the transparent part, the center-to-center distance between neighboring protuberances is about 200 nm, and spaces intervening the neighboring protuberances present concaves like reversed protuberances. These morphologies are indistinguishable between dorsal and ventral surfaces, and male and female animals.

To study the function of the wing nipple array, we made an artificial "smooth" wing with much lower protuberances by pressing the wing between pieces of paper and styrofoam (Fig. 2C), and compared it with the "rough" one with intact protuberances (Fig. 2A, B). No change other than protuberance deformation was observed in the wing (Compare between Fig. 2B and 2C). Dorsal and ventral surfaces in the

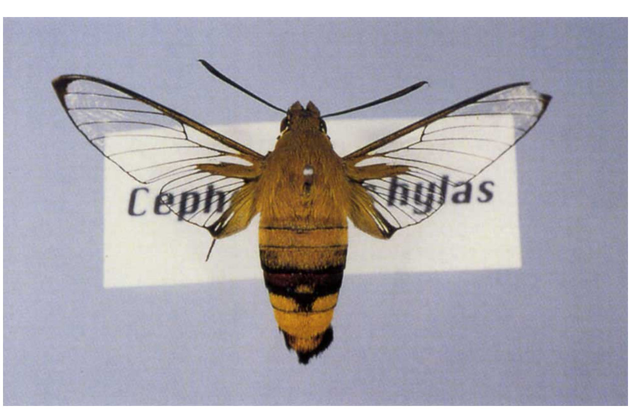

Fig. 1. A hawkmoth, *Cephonodes hylas*, male. Letters under the animal are visible through the transparent part of the wing.

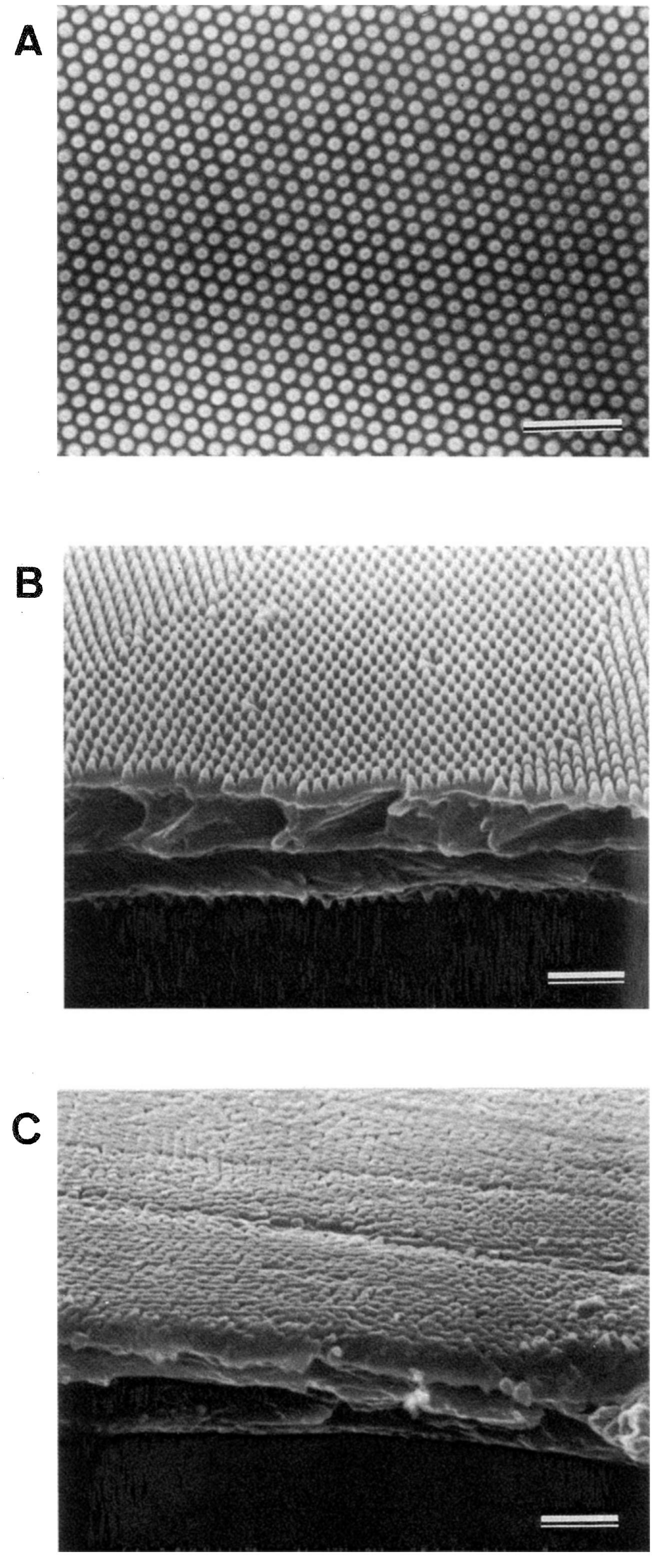

Fig. 2. (**A**) A scanning electron micrograph of the transparent part of the *Cephonodes* wing. An upper view presenting the regular-hexagonal array of circles. (**B**, **C**) Scanning electron micrographs of the oblique views of the rough wing with intact protuberances (**B**) and the smooth one with much lower protuberances (**C**), cut with scissors. The dorsal surfaces and the cut ones are observed. Scale bar: 1 μm.

smooth wing are morphologically indistinguishable, as in the rough wing. Although the *Cephonodes* wing is mainly composed of transparent cuticles like those of bees and flies, the intact rough wing of *Cephonodes* is not glossy unlike them. The smooth wing of *Cephonodes* was, however, as glossy as the other transparent wings. That is, light reflectance is larger in the smooth wing than in the rough one. The smooth wing is slightly opaque, which may be caused by greater reflectance than that of the rough wing.

Reflectance spectrophotometry

For studies on the extent and wavelength dependence of antireflection, we measured the reflectance spectra of the rough wings and the smooth ones in the wavelength range from near ultraviolet (200 nm) to red (800 nm). To sum up the energy of lights reflected in various directions on the wing surface, we used a spectrophotometer equipped with an integrating sphere. Energy of the reflected light measured by the spectrophotometer is the sum of that of the light reflected by the dorsal surface and that by the ventral one. It is assumed that the light passed through the wing is absorbed by the black paint on the ventral surface. Reflectance spectra of the wing before and after crushing its protuberances are illustrated in Fig. 3A. At all wavelengths, reflectance increased more in the smooth wing (after crushing the protuberances) than in the rough wing with the intact protuberances. This indicates that the protuberance array of the *Cephonodes* wing functions as a broad-band antireflective device at least in the wavelength range from 200 nm to 800 nm. The reflectance ratio of the smooth wing to that of the rough one is around 2.1-3.5 throughout the entire wavelength measurement range (Fig. 3B), which means that the protuberance array decreases the reflectance into 29-48% (the inverse of the reflectance ratio described above). Our preliminary measurement of the light transmittance spectra showed that this protuberance array increases the transmittance of the *Cephonodes* wing in the same wavelength range as in the reflectance measurement, probably due to decreased reflectance (data not shown).

After the surface of the wing was coated with thin layer of gold (about 8 nm in thickness) by ion sputtering, the smooth wing and the rough one were different in color as well as in brightness; Fig. 4 shows that the smooth part looks clear and golden due to metallic reflection, while the rough one looks dark and greenish blue. One wing was applied for the reflectance measurement. At all wavelengths, reflectance is larger in the gold-coated smooth wing than in the gold-coated rough one (Fig. 5A). Reflectance in the smooth wing increases as the wavelength exceeds 500 nm, that is, selective metallic reflection of gold gives a glittering brown. Reflectance in the rough wing, however, does not increase in the same wavelength range, that is, selective metallic reflection does not occur. The reflectance ratio of the smooth wing to that of the rough one is shown in Fig. 5B. Metallic reflection color is dominant in the smooth metal surface, while absorption color

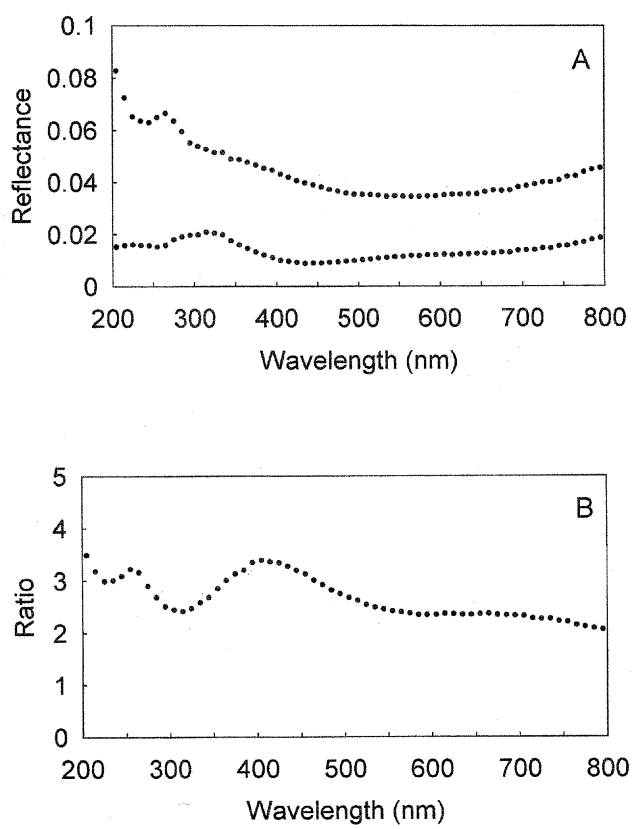

Fig. 3. (**A**) Representative reflectance spectra of the rough wing with intact protuberances (lower trace) and the smooth wing after crushing its protuberances (upper trace). (**B**) A spectrum of the reflectance ratio of the smooth wing to the rough one. All dots are the averages of six wings (four male wings and two female ones), and there is a rough tendency for the standard deviation to increase as the wavelength decrease; e.g. the ratios are 3.49 ± 1.17 at 205 nm, while 2.06 ± 0.49 at 795 nm.

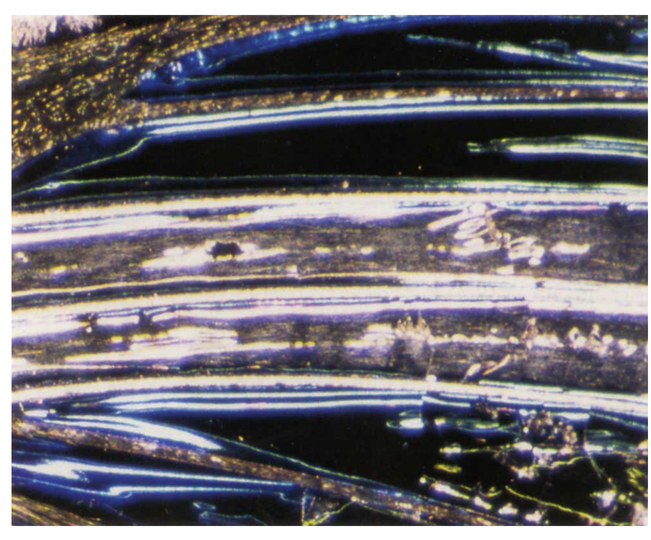

Fig. 4. A hindwing surface coated with a thin layer of gold by an ion sputtering device (JEOL, JFC-1500). The smooth part looks clear and golden, while the rough one dark and greenish-blue. In the marginal part and the vein, golden scales are seen. Scale bar: 1 mm.

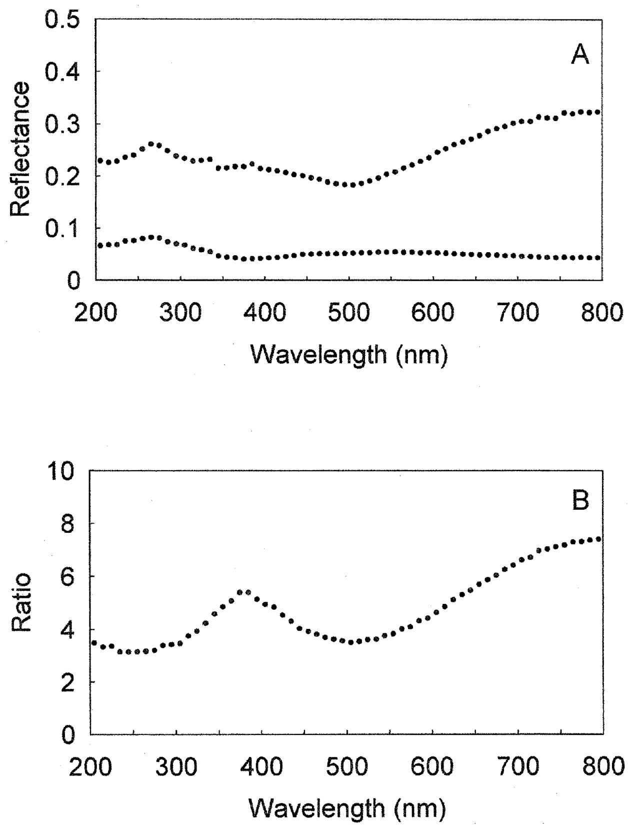

Fig. 5. (**A**) Representative reflectance spectra of the rough (male) wing (lower trace) and the smooth wing (upper trace) coated with gold. (**B**) A spectrum of the reflectance ratio of the smooth wing to the rough one.

(complementary to the reflection one) is dominant in the rough metal surface on the protuberance array (Born and Wolf, 1970).

DISCUSSION

In the preceding report (Yoshida *et al.*, 1996), we showed that a highly ordered array of nanosized protuberances is in the transparent wing of *Cephonodes*. In the present report, we indicated that this protuberance array is an antireflective device in the broad wavelength range of light, by preparing an artificial smooth wing by crushing its protuberances without damaging the wing shape, and by comparing the reflectance spectra of the smooth wing to that of the rough one having intact protuberances. The comparison between the rough and smooth wings is likely to be the most appropriate way to study the function of the protuberance array. This protuberance array in the *Cephonodes* wing is closely similar to those in the corneal surfaces of some insect eyes, termed corneal nipple arrays, discovered by Bernhard and Miller (1962). Bernhard *et al.* (1965) studied the function of the corneal nipple array using different methods: a scaled model experiment, a theoretical calculation, and a comparative way, since the corneal nipple array could not be removed without large damage to the cornea. Their studies also showed that a corneal nipple array functions as an antireflective device in the broad-band wavelengths from near ultraviolet to red as the *Cephonodes* wing. They attributed the antireflective function of the protuberance array to a mechanism of impedance matching between cuticle and air, as described followingly. Individual protuberances are not detectable by visible light, since each protuberance is smaller than the shortest visible light (See Fig. 2A). The protuberance array can be viewed as homogeneously transparent coating on the corneal surface. A refractive index of the protuberance array is, however, inhomogeneous along the axis perpendicular to the corneal surface, since the proportion of cuticle to air changes along this axis. That is, a refractive index is 1.0 at the top of the protuberance where the proportion of cuticle to air is 0%, 1.5-1.6 at the base where the proportion is 100%, and smoothly changes along the top-to-base axis due to nipple-like shape of the protuberance. Since both wing and corneal nipples are cuticular protuberances with closely similar morphologies, it is probable that the wing protuberance array has the same mechanism for antireflection to light as that of the corneal nipple array.

Thus, each protuberance from the top to the bottom is assumed to be a unit of an antireflective device. Viewing the bottoms as the boundaries between neighboring protuberances, the protuberances arranged regular-hexagonally are thought to be most closely packed in two-dimensional space (Fig. 6). The protuberance array of the *Cephonodes* wing is likely to be the most efficient antireflective coating that the anti-reflective units are most closely packed in two-dimensional space.

An antireflective transparent wing such as the *Cephonodes* wing is hard to be distinguished from its back-

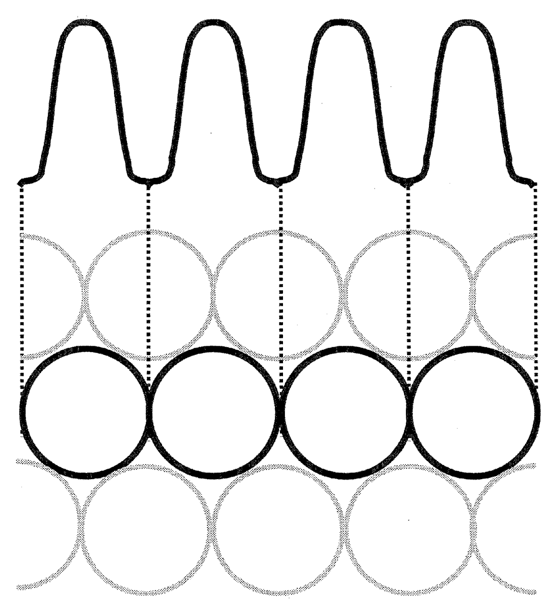

Fig. 6. Schematic figure of the protuberance arrangement. The upper part of the figure shows the side views of the protuberances in a row, and the lower one shows the upper views of the circumferences of the protuberance bases. Faded circles in the lower part are those of the protuberances in adjacent rows.

ground. Since the antireflective wavelength range of the wing protuberance array is at least 200-800 nm which includes the visible ranges of insects (Autrum and Burkhardt, 1961; Langer *et al.*, 1979; Arikawa *et al.*, 1987; Shimohigashi and Tominaga, 1991) and vertebrates (Neumeyer, 1986; Schnapf *et al.*, 1987), the antireflective transparent wing may be nearly invisible to the other insects and vertebrates. This concealing effect might make the insect less likely to be found by other animals including parasites and predators.

From the studies with gold-coated wings, we also showed that this nanocomposite functions as an excellent device for decreasing selective metallic reflection, and that it accordingly changes the surface color of the metal from a metallic reflective color to a dark absorptive one. Designs and fabrications of nanocomposites have recently been intensively studied, paying particular attention to applications of optical and microelectronic devices (Tonucci *et al.*, 1992; Huber *et al.*, 1994; Masuda and Fukuda, 1995). The array of nanoprotuber-ances in the *Cephonodes* wing with its simple morphology and interesting optics would present a model (or template) for a new optical device consisting of nanosized structures.

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