Original Paper *Forma*, **17**, 123–132, 2002

The Origin of the Iridescent Colors in Coleopteran Elytron

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(Received April 29, 2002; Accepted August 1, 2002)

Keywords: Coleoptera, Optical Thickness, Epicuticle, Exocuticle, Structural Color

Abstract. The physical mechanisms responsible for the wide variation of metallic color within a single species, *Plateumaris sericea*, were investigated by the structural observations and the spectral analysis of the reflected light. The surface of elytra consisted of epicuticle and exocuticle, both having high and low refractive index layers. Only the epicuticle had the understandable optical thicknesses of approximately one-quarter of the wavelength of the light. The results of theoretical analysis were compared with the measured spectral reflections.

1. Introduction

Colors manifested by the majority of natural objects have their origin in the absorption of the light-rays which do not emerge by reflection or transmission. The true colors which result from selective absorption are the unabsorbed residue of the incident light. Besides the chemical processes described above, there exists a color which is caused by physical phenomena. The chief physical events which affect incident light are as follows; (1) simple reflection of unbroken white light, (2) refraction into spectral colors by prismatic structures, (3) scattering brought about by discontinuities, (4) diffraction into spectra by gratings, minute openings, (5) interference which yields iridescent or changeable colors (FOX, 1953).

Concerning the interference color, there are several well-known reports that the surfaces of several beetles and other arthropods (NEVILLE, 1975, 1977; HINTON, 1976) develop metallic colors. Most of the reports discussed extensively the structure composed of multilayer of alternative high and low refractive index materials within the exocuticle in their integument (PARKER *et al.*, 1998; PARKER, 2000).

Leaf beetles, *Plateumaris sericea*, are widely distributed in the Palaearctic region from Europe to Japan, and show a wide variation of metallic color within a single species, from blue individual to red one. Recently, we reported the structural basis for producing such a wide range of wavelength of color variation using several techniques and showed that only five layers within epicuticle of elytron worked as an interference reflector (KURACHI *et al.*, 2002).

Here we investigate the origin of the iridescent colors in Coleoptran elytron using the leaf beetles, and show the simulation result of the colors both from the epi- and exo-cuticle.

2. Materials and Methods

2.1. Animals

Beetles, *Plateumaris sericea*, were collected randomly from a single population at the highland marsh (Tanbara marsh in Gunma Pref., Honshu, Japan).

2.2. Reflectance

Reflectance of the dorsal surfaces of *P. sericea* was measured in the UV and visible wavelengths from 340 nm to 740 nm using a spectrophotometer (Shimadzu MPS-5000, Japan). Magnesium oxide was used as a white standard for reference. For microspectrophotometry, the reflectance from the elytron was measured in range of wavelength from 380 nm to 760 nm with a microspectrophotometer (Carl Zeiss, MPM800, Germany), equipped with dark field epi-illumination and an Epiplan-Neofluar 5× objective lens (NA = 0.15). The measured area in each sample was 0.2×0.2 mm^2. Barium sulphate was used as a white standard for reference. A part of the elytron was scraped off using the sliver of razor blade, and the reflectance of its area was measured with the same microspectrophotometer.

2.3. Electron microscopy

For the transmission electron microscopy, the elytra of individuals were removed in cold first-fixative solution (2% paraformaldehyde, 2% glutaraldehyde in 0.1M cacodylate buffer, pH7.2) and kept in a refrigerator for overnight. The samples were then rinsed with a 0.1M cacodylate buffer solution, and post-fixed for 2 h in 1% OsO_4 buffered with 0.1M cacodylate. After rinsing with the cacodylate buffer solution, the samples were dehydrated with an ethanol series and embedded in Araldite resin. Observations were performed using a Hitachi H-300 transmission electron microscope.

For the scanning electron microscopy, the elytra were fixed with the same first-fixative solution. The dehydrated samples were dried with a critical point drying apparatus (Hitachi HCP-1) and coated with gold vapors (Eico ion coater IB-3), and glued onto aluminum stubs. The observations were carried out with a Hitachi-S310 scanning electron microscope.

2.4. Simulation

The reflectance of each elytron was calculated theoretically using the matrix method for a multilayer stack of thin films (MACLEOD, 1969), making use of the measured thickness of each unit layer within the epicuticle and the exocuticle.

3. Results and Discussion

3.1. Macroscopic appearance of each elytron

The color appearance of individual leaf beetles, *P. sericea*, showed the ranges across the visible spectrum from blackish-blue to red with several variations. Individual spectral reflectance from the dorsal surface of these beetles was measured. One side of elytron was cut off from the body and measured by a spectrophotometer (Fig. 1). Several characterized spectral reflectances peaking from blue (*ca*. 400 nm) to red (*ca*. 710 nm) were recorded. The elytra which mainly reflected longer wavelength had troughs in the shorter wavelength region, whereas one reflected shorter wavelength had no troughs in the longer wavelength.

3.2. Grained substances of elyton

The natural object manifests its color physically or chemically. The chemical color is caused by the substance which is either produced by biochemical pathway in each animal or received from their food. The chemical coloration substances are easily damaged when

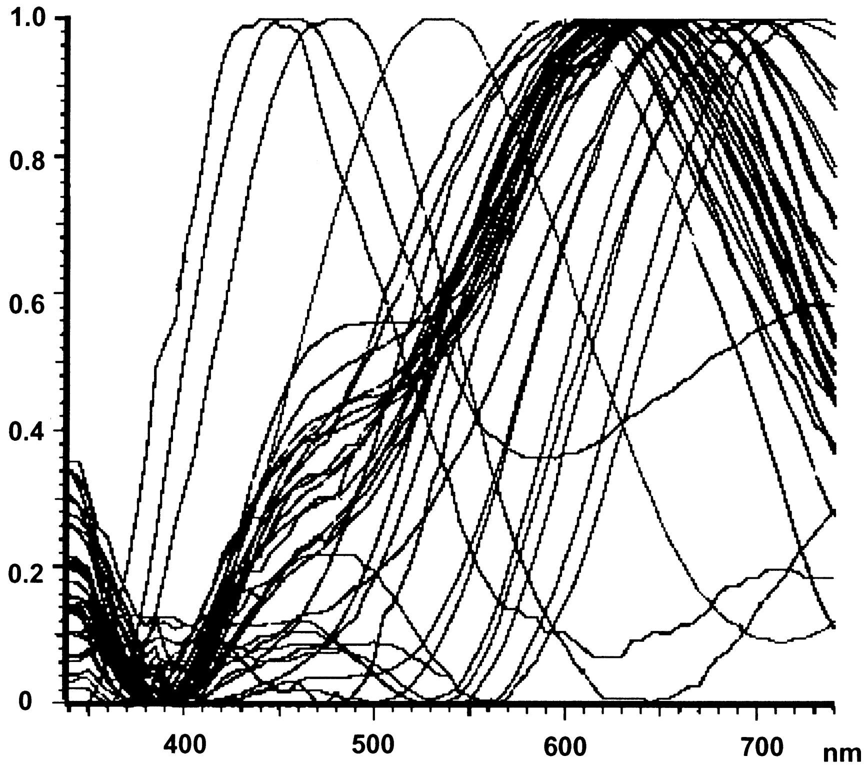

Fig. 1. Spectral reflectance variation of the beetles *Plateumaris sericea*. The beetles display various colors over whole visible region.

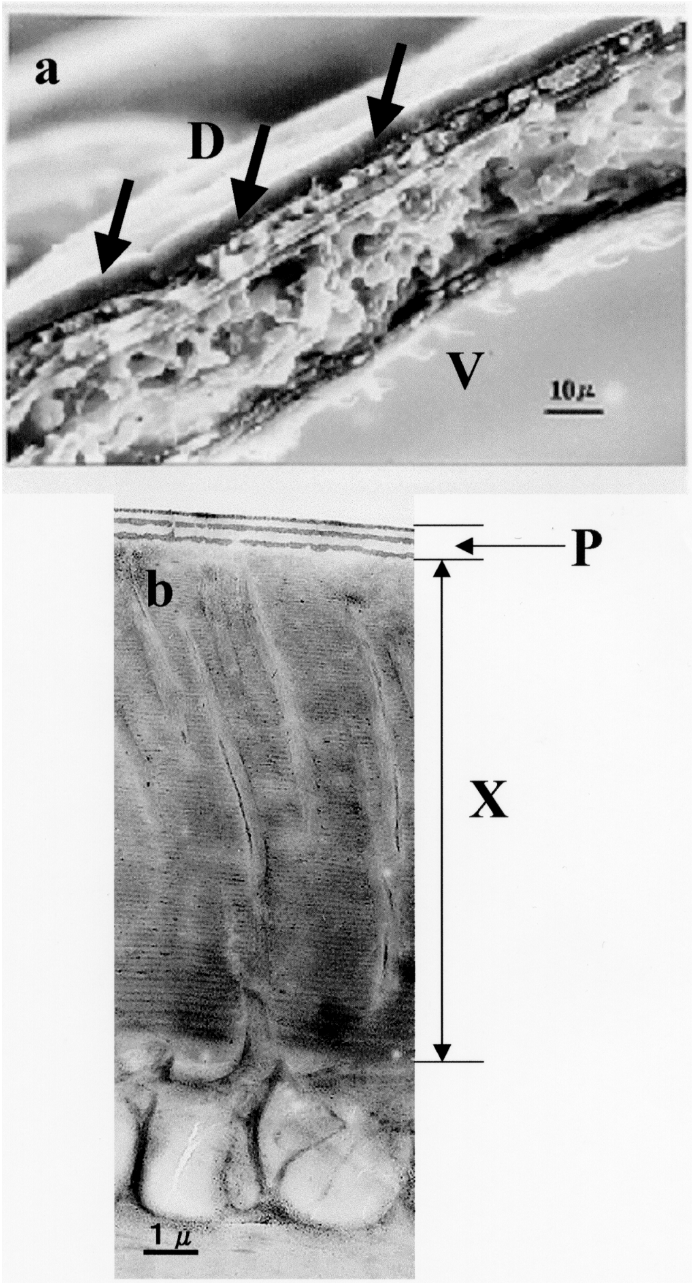

Fig. 2. Scanning (a) and transmission (b) electron micrographs sectioned with the sagittal plane. The epicuticle (P) just below the dorsal surface was distinguished from the exocuticle (X) by the difference of thickness of each lamination. Five layers were observed in the epicuticle, and a multilayer in the exocuticle. Arrows indicate the cuticle which includes the epi- and exo-cuticle. D; dorsal side, V; ventral side, P; epi-cuticle layer, X; exo-cuticle layer.

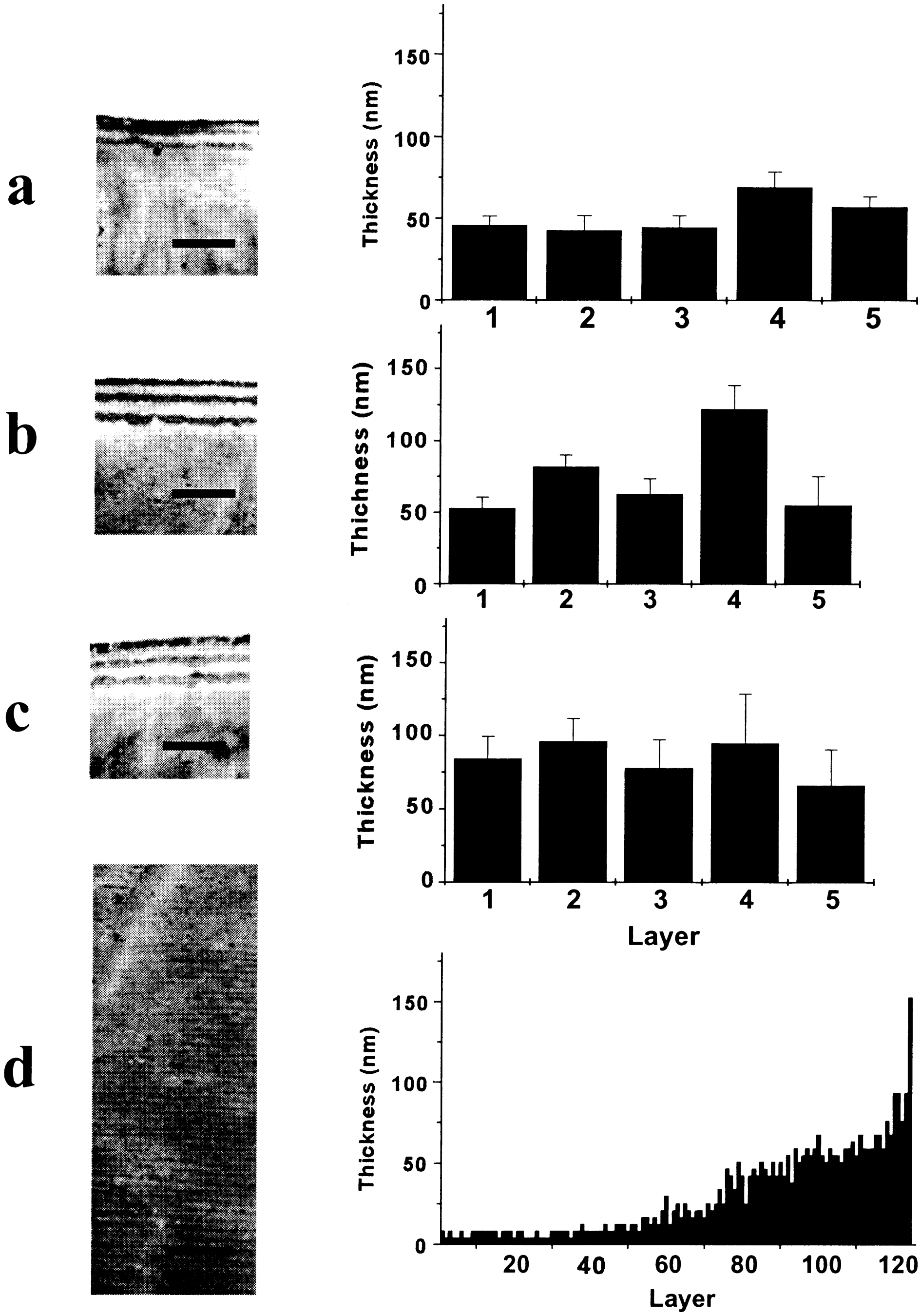

Fig. 3. The transimission micrographs of epi- (a, b, c left side) and exo-cuticle (d left). The thickness of each layer of epicuticle was shown according to the color appearance in a, b, c; right side, and the thickness of each layer of exocuticle was in d; right. Scale bars, 500 nm.

the animals are dead. According to those particular origins of the chemical properties, those colors or complementary colors should be observed under the microscope when the substances of elytron are grained. When the cuticle of each elytron was scraped and/or grained, no visible color was observed but gray. In addition, the color of each elytron remained for a certain period of time after their death, suggesting that the colors were originated from physical foundations in the cuticle of each elytron.

3.3. Structural basis of physical color

Physical color is based either on interference, diffraction or scattering light (HERRING, 1994). In order to elucidate the structural basis of physical color development in the electron, the light and electron microscopic observations were performed using transverse sections of the representative specimens from each color group. No color was observed by light microscope when the incident light was applied transversely to the semi-thin sections (*ca*. 2–5 μm) of the elytron in all cases (data not shown). Figure 2a shows the surface of the elytron which was cut following the sagittal plane by a razor blade. Black transverse line which is indicated by arrows is the cuticle of the dorsal side of elytron. The transimission electron microscopic observations revealed that two kinds of laminated structures were common in all color types, consisting of five layers in epicuticle and of multilayers in

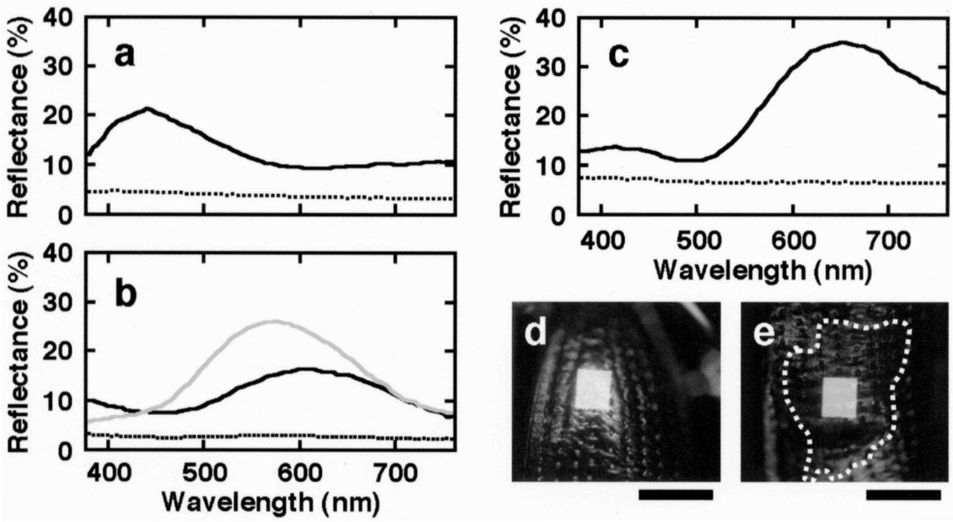

Fig. 4. Spectral reflectance from small surface area of elytron was shown by solid line (a, b, c), and that from scraped surface of each color type of elytron was shown by dotted line. Reflectances from the scraped areas (dotted) were lower than those from non-scraped areas (solid), and had no peak (a, blue-type; b, copper-type; c, red-type). d, measured area of the copper type elytron. e, scraped area of the copper-type elytron. A limited area was scraped up to about 0.5 μm in depth from the surface, and the exocuticle was exposed. Border of scraped area in e retouched with dotted white lines. Yellow squares in d and e indicate windows measured with a microscpecrophotometer. The color of scraped area was black, which was quite different from that of non-scraped area. Scale bar, 0.5 mm.

exocuticle, both of which were composed of alternate electron-dense and -lucent layers (Fig. 2b). The multilayer consisting of numerous thin layers was about 10 μm thick in total. The thickness of the electron-dense layer was *ca*. 10 nm and it was almost the same throughout the whole exocuticle. However that of the electron-lucent layer was 10 nm at the distal end and gradually increased following the depth, finally reached 80 nm at the proximal end (Fig. 3d). The epicuticle, consisting of five alternate electron-dense and electron-lucent layers just below the smooth surface, showed a total thickness of 0.3–0.5 μm. The thickness of each layer was different among the color of types (Figs. 3a, 3b and 3c). The elytra had no repeated striations on the surface (potential diffraction gratings), or particles that might cause scattering. It is therefore assumed that the coloration of *P. sericea* comes from the laminated structures in the epicuticle and/or exocuticle.

3.4. Spectral reflectance measured by microspectrophotometer

We removed a small piece of surface that was about 0.5 μm thick and included the epicuticle by scraping with a sliver of razor blade. The scraped areas exposing the multilayered exocuticle appeared black in all color types of animals: quite different from the surrounding surface (Fig. 4e). The spectral reflectance of the scraped area measured by microspectrophotometry was extremely low, with no peaks within the measured range (dotted lines in Figs. 4a, 4b and 4c). These results confirm that the five layers within the epicuticle are solely responsible for the development of the spectrum of interference colors observed in this beetle species.

3.5. Simulations

Thin films or laminations yield iridescent or changeable colors, interference, as a result of asynchrony between the wave-trains reflected from the upper and the lower surface of the layers. It could be postulated that these multilayer reflectors in exocuticle were responsible to manifest the metallic coloration in *P. sericea* as in the case of *Aspidimorpha tecta* (PARKER *et al*., 1998). When this multilayer structure is assumed to contribute to manifest the visible interference color, the optical thickness (the product of the actual thickness and the refractive index) of each layer must be roughly equal to the quarter-wavelength of visible light. Calculation was made to obtain the refractive index of each thin unit layer in multilayer; the refractive index should be over 10, which was thought to be the least probable in the case of biological materials.

On the other hand, the epicuticle just below the smooth surface of elytra consisted of only five layers and was 0.3–8.5 μm thick totally, and the thickness of each layer was different among color types; 60–100 nm in blue, 70–160 nm in copper and 100–140 nm in red color elytra (Figs. 3a, 3b and 3c). Following the increase in reflective wavelength, the thickness of each layer increased corresponding to the color of elytra. Assuming that the each refractive indices of more- and less-dense layers are ~1.7 and ~1.4, respectively, as previously reported (LAND, 1972), the optical thicknesses of five layers within the epicuticle correspond roughly with the quarter-wavelength of visible light.

We calculated the reflectance in each color types of elytron theoretically using the matrix method for a multilayer stack of thin films. The simulated spectral reflectance calculated from the five layers of the red group showed the peak at around 670 nm and the trough at 400 nm, beside the shoulder peak at around 470 nm. The calculated spectrum from

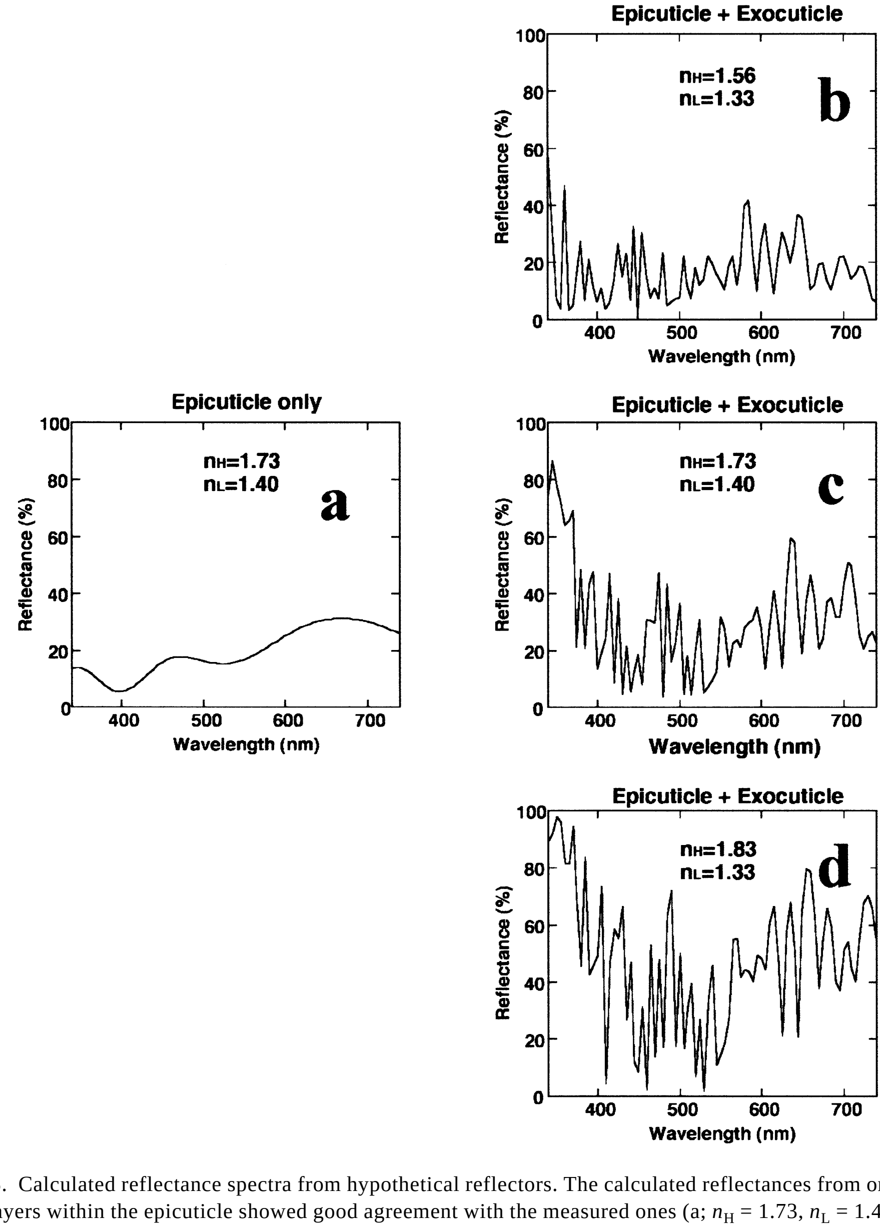

Fig. 5. Calculated reflectance spectra from hypothetical reflectors. The calculated reflectances from only five layers within the epicuticle showed good agreement with the measured ones (a; n_H = 1.73, n_L = 1.40). The calculated reflectances of the multilayer within the exocuticle (b; n_H = 1.56, n_L = 1.33, c; n_H = 1.73, n_L = 1.40, d; n_H = 1.83, n_L = 1.33) showed notched shape, but the reflectance curve was fit for the measured one when we used the values of n_H = 1.56, and n_L = 1.33 for high-dense and low-dense layers, respectively.

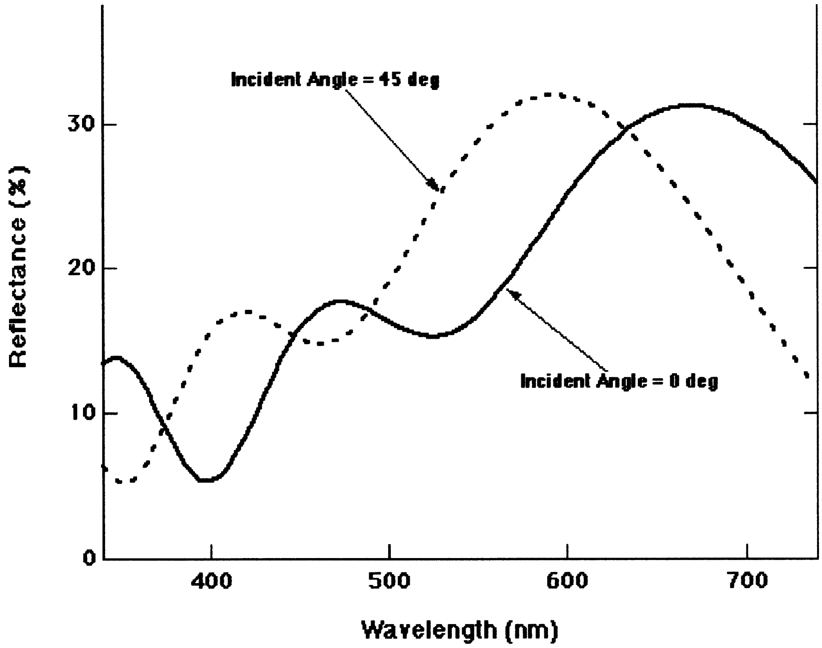

Fig. 6. Calculated reflectances obtained on the assumption that the angle of incident light is 0 degree (solid line) or 45 degree (dotted line).

only five layers showed good agreement with the measured ones in each color group (cf. Figs. 1 and 5a) (see KURACHI *et al.*, 2002). The multilayer within the exocuticle showed, however, quite different spectrum from measured one from the dorsal surface. These spectra showed many peaks and troughs. When we adopt 1.56 and 1.33 as the refractive indices for high- and low-dense layers, respectively, the reflection level was decreased (Fig. 5b) as the measured reflection spectrum (cf. dotted lines in Fig. 4). These results indicate again that only five layers within the epicuticle produce the interference colors of the elytra extending over whole visible spectrum and that the multilayer within the exocuticle does not contribute to their visible colors.

3.6. Simulation of reflectance from epi- and exo-cuticle layers and the angle of incident light

Recently, KURACHI *et al.* (2002) reported by theoretical analyses that the reflectors, consisting of only five layers within the epicuticle, are responsible for all the different colors observed in *P. sericea*. The spectral reflections are fit well for the measured ones when 1.73 and 1.40 are used as the refractive indices for high-dense and low-dense layers, respectively. Figure 5a shows an example of simulation in the red elytron, and is fit well for one of the curves shown in Fig. 1. However, the calculated reflection from the epicuticle and exocuticle using the same values of refractive indices as those for the epicuticle did not fit well the measured ones (Fig. 5c). When we simulated with different refractive indices

(1.56 for high dense layers and 1.33 for low-dense layers), the reflectance was fit well for the measured one (Fig. 5b). There is a possibility that the refractive index values for electron-dense and -lucent layers are different between the epicuticle and the exocuticle.

When the angle of the incident light was changed from 0° to 45° in simulation, the spectral reflection peak has changed from 680 nm to 590 nm (Fig. 6). It seems that the metallic view for human vision is caused by those physical foundations. In general, the beetles have developed, through their evolution, the lamination within their elytra for hardening against a variety of stress from outside world and for reducing weight to fly. In addition to these mechanical properties, some beetles have developed the lamination in their elytra for appearing their interference colors, as it were, metallic colors. The reason why the beetle *P. sericea* needs such various metallic colors is still unknown. Interestingly, all blue-type beetles were male, but both sexes were observed in the red- and copper-type beetles. The difference of color depending on their sexes suggests that the appearance of their colors may be in the genetic background. Their colors must play an important role in their behavior and their life history.

We thank Dr. Hubert Nässe (Carl Zeiss, Oberkochen, Germany) and Junzo Kawaguchi (Carl Zeiss, Sendai, Japan) for microspectrophotometry. This study is partly supported by Grant-in-Aid for Scientific Research (C) 14540625 for T.H.

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