〈Research Article〉

Discrimination of thin-film interference color of bird feathers using circular polarized light

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Summary Circularly polarized light (CPL) is reflected in a mirror in the opposite direction. As thin-film interference is caused by light specularly reflected from a thin-film surface and a basement surface, thin-film interference also causes CPL to be reflected in the opposite direction. When mallard and rock dove feathers were illuminated with right- or left-handed CPL and viewed through an opposite-handed CPL cut filter, their structural green color at approximately 480 nm in the reflectance spectrum disappeared. Based on these results, it is clear that the structural color owing to thin-film interference in bird feathers can be easily distinguished using CPL illumination and a CPL cut filter. The color of the peacock feathers is considered to be structural as it is derived from the lattice structure of melanin rods. However, the green color around the eye pattern of peacock feathers and two peaks of reflection spectral at 420 and 520 nm disappeared when the CPL illumination method was applied. Furthermore, when the cortical thickness of mallard, rock dove, and peacock feathers (287 ± 11.0, 528 ± 34.9, and 261 ± 24.0 nm, respectively) measured using electron microscopy were used to simulate the thin-film interference with Fresnel formula, their simulated spectral peaks were coincident to the actual peaks reflected from their feathers. It was confirmed that the color of the mallard, rock dove, and peacock feathers is structural and is derived from thin-film interference.

Key words: Bird feather, Thin-film interference, Spectrum, Circular polarization

1. Introduction

Bird feathers come in various beautiful colors, signaling individual characteristics to other individuals, particularly members of the opposite sex, or representing species characteristics[1-3]. One of the origins of these colors is pigments, e.g., melanin, which is common to many bird feathers; carotenoids, found in flamingos; protoporphyrin, found in bokmakieries; psittacofulvin, found in parakeets; and spheniscin, found in penguins[4-6]. Another source

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of color is the nanolevel structure of feathers, including the thin layer that creates interference in mallard and dove feathers, lattice structure of melanin granules in peacock feathers, and the spongy layer of the feathers of parakeets and kingfishers[7-15]. Colors originating from pigments are known as pigment colors, and those originating from feather structure are known as structural colors. However, the colors of bird feathers do not always fit neatly into either one of these categories. For example, in parakeets, various colors are created by a combination of a blue structural color derived from the sponge layer of the feathers and a yellow-red pigment color derived from cortical psittacofulvin[4,11].

Right- or left-handed circularly polarized light (CPL) irradiated on the thin film is reflected from the mirror surface as the opposite-handed CPL[16,17]. Therefore, I hypothesized that color derived from thin-film interference in bird feathers would be changed by a cut filter on the opposite side of the irradiated CPL and that I could use this technique to distinguish thin-film interference color from other colors derived from pigments, spongy layers, and photonic crystal-like structures such as the lattice structure of melanin granules, because structural colors other than thin film interference are composed of scattered light[18]. In this study, this method was used to compare the colors of the mallard and rock dove feathers reflecting the structural color derived from thin-film interference[15,18,19] with that peacock and parakeet feathers reflecting the other structural colors[4,7-11]. This method can be a very simple and convenient approach for distinguish thin-film interference color from surfaces of various materials such as beetles[20], pearls[21] and biomimetic materials[22,23] other than bird feathers.

2. Materials and Methods

Materials

Rock dove (*Columba livia*) feathers were gathered in the field surrounding the Yamazaki University of Animal Health Technology. Mallard (*Anas platyrhynchos*) and male peacock (*Pavo cristatus*) tail feathers were obtained from Kamatac Co., Ltd., Tokyo, Japan. Red and blue parakeet (*Psittacidae*) feathers were obtained from Fuji Kachoen, Fujinomiya City, Shizuoka Prefecture, Japan.

Discrimination of bird feather coloration using the CPL illumination method

CPL is formed by providing a phase difference of λ1/4 to the wavelength of linearly polarized light. Therefore, the CPL filter is constructed so that the plane of incidence of linearly polarized light is incident at an angle of 45 degrees with respect to the fast axis or the slow axis of the wave plate possessing birefringence. Using the above CPL filters, each bird feather was illuminated with either right- or left-handed CPL, and the reflected color of the feather was observed and photographed using an opposite-handed CPL cut filter.

Spectrophotometric analysis

Reflectance spectrum analysis of bird feathers was performed using a CPL cut filter and a mini-spectrometer C11009MA (Hamamatsu Photonics, Hamamatsu, Japan) under visible light from an incandescent lamp, JOR 110V 75W E11X1 (Tokyo Metaru Kogyo, Tokyo, Japan). The reflectance A/D ratio was calculated using light from a light-reflective sheet of microcellular foam (MCPET; Furukawa Electric Co., Ltd., Tokyo, Japan) as a control.

Transmission electron microscopic observation of transverse cross-sectioned bird feathers

Samples were prepared as previously described[10] prior to conducting transmission electron microscopic (TEM) observations of the transverse sections of dove feather barbules. Barbules were then observed and imaged using a TEM (JEM-1400Plus; JEOL Ltd., Tokyo, Japan) with a CCD camera (EM-14830RUBY2; JEOL Ltd., Tokyo, Japan) in the Tokyo Metropolitan Industrial Technology Research Institute. The thickness of the cortex of each bird feather and the diameter and density of the melanin granules were measured using TEM images. The melanin granule density was measured as the number of melanin granules/1 μm length under the cortex.

3. Results

Discrimination of bird feather coloration originating from thin-film interference using the CPL illumination method

The mallard, rock dove, peacock, and parakeet feathers were illuminated with either right- or left-handed CPL and observed through an opposite-handed CPL cut filter. The green color of the mallard feathers and the pinkish purple or green color of dove feathers disappeared when observed through the CPL cut filter. Additionally, the green color around the eye pattern of the male peacock tail feathers also disappeared, and the blue color at the center of the eye pattern appeared purple (Fig. 1A-C). However, the color of the red and blue parakeet feathers remained unchanged using the CPL cut filter (Fig. 1D).

Spectral analysis of right- or left-handed CPL reflected from bird feathers

When the reflection spectra of the feathers shown in Fig. 1 were measured without and with the CPL cut filter, the peak at 480 nm disappeared from the mallard feather reflection spectrum (Fig. 2A), and the broad-band peak at 460 nm and shoulder-shaped small peak near 600 nm disappeared from the dove feather reflection spectrum. However, rock dove feathers had a high reflectance of 700 nm or more and did not disappear even through the CPL cut filter (Fig. 2B). Two peaks at 420 and 520 nm disappeared from the reflection spectrum of green part around the eye pattern of peacock feathers (Fig. 2C-a), and the peak at 460 nm disappeared from the reflection spectrum of blue part at the center of the eye pattern and appeared at the shifted peak at 440 nm (Fig. 2C-b). In the reflection spectrum of the parakeet feathers, the peaks remained without disappearing even when viewed through the CPL cut filter (Fig. 1D-a and b).

TEM observation of barbules exhibiting structural color derived from thin-film interference

When the barbules of the mallard, rock dove, and peacock (green part) feathers whose color and reflection spectrum were changed using the CPL illumination method (Fig. 1, 2) were embedded for

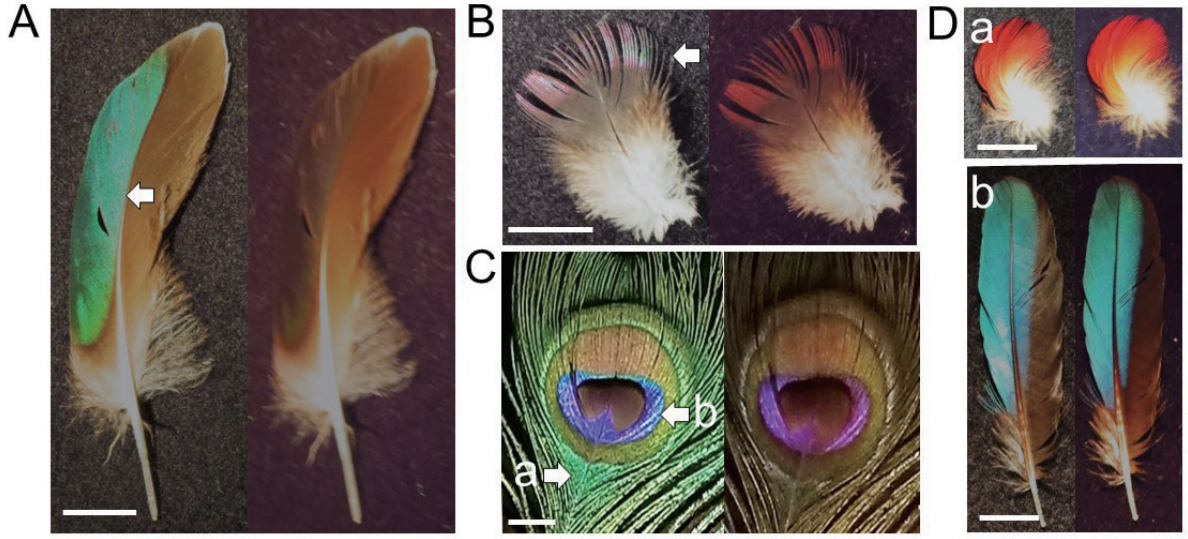

Fig. 1 Discrimination of bird feather coloration derived from thin-film interference. Feathers were illuminated with right- or left-handed circularly polarized light (CPL) and viewed with an opposite-handed CPL cut filter. A: mallard feather; B: rock dove feather; C: peacock feather (a: the green part around the eye pattern b: the blue part at the center of the eye pattern); D: parakeet feathers (a: red feather; b: blue feather). Left: with no filter; right: through a CPL cut filter. Arrows indicate the green parts of mallard and rock dove feathers and the green and blue parts of peacock feathers. These colors disappeared or changed when viewed through the filter. White bar = 10 mm.

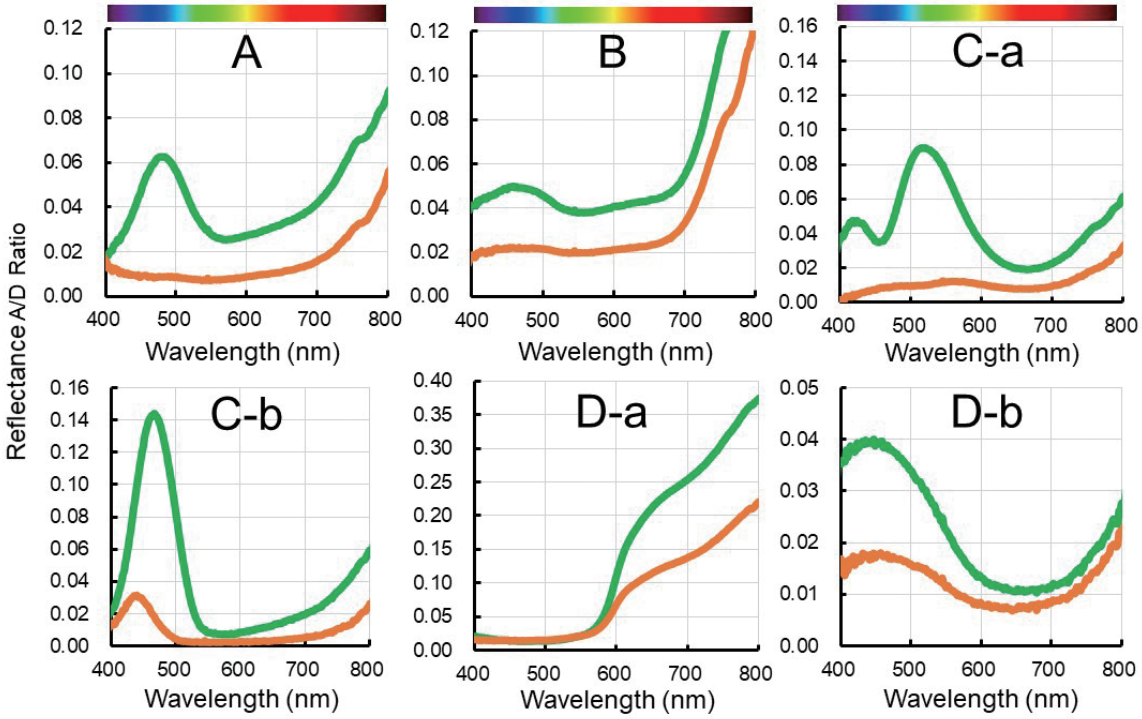

Fig. 2 Spectrum analysis of right- or left-handed circularly polarized light (CPL) reflected from bird feathers without (green) and with (brown) an opposite-handed CPL cut filter. A: mallard feather; B: rock dove feather; C: peacock feather (a: green part; b: blue part); D: parakeet feathers (a: red feather; b: blue feather).

TEM observation, many melanin granules were observed under the cortex (Fig. 3). Cortical thickness was lowest for peacocks, followed by mallards and then rock doves. The diameter of the melanin granules was also smallest for peacocks, again followed by mallards and then rock doves. The density of the melanin granule distribution was highest in mallards and lowest in rock doves. The thickness of the cortex of each bird feather and the diameter and density of their melanin granules were measured using the TEM images shown in Fig. 3. The cortical thickness of the mallard, rock dove and peacock were 287 ± 11.0, 528 ± 34.9, and 112 ± 12.0 nm, respectively, and the diameter of their melanin granules were 148 ± 13.4, 422 ± 68.9, and 114 ± 13.8 nm, respectively. Because peacock melanin granules were small and gaps were observed between them, the distance to the cavity surface under the melanin granule layer was also measured, and found to be 261 ± 24.0 nm. The number of melanin granules/μm of mallard, dove, and peacock was approximately 6, 2 and 6, respectively. These measurement results are listed in Table 1.

4. Discussion

It is well known that the beautiful, brilliant colors of bird feathers can be pigmented or structural. The sources of pigment colors include carotenoids, protoporphyrin, psittacofulvin, and spheniscin[4-6], and the sources of structural colors include sponge layers, cortical thin layers, and lattices of medullary melanin granules[7-15]. However, the characteristic colors of certain birds do not necessarily originate from any one of these pigments or structures but rather from a combination. For example, the green color of parakeet feathers is formed through a combination of a pigment color derived from psittacofulvin and a structural color derived from a spongy layer[5,11,12]. When the surface of the pigment or spongy layer of parakeet feathers was irradiated with either right- or left-handed CPL, red or blue incoherent diffuse light was reflected. However, when the mallard and rock dove feathers were irradiated with CPL, the opposite-handed CPL was reflected (Fig. 1). This phenomenon occurs

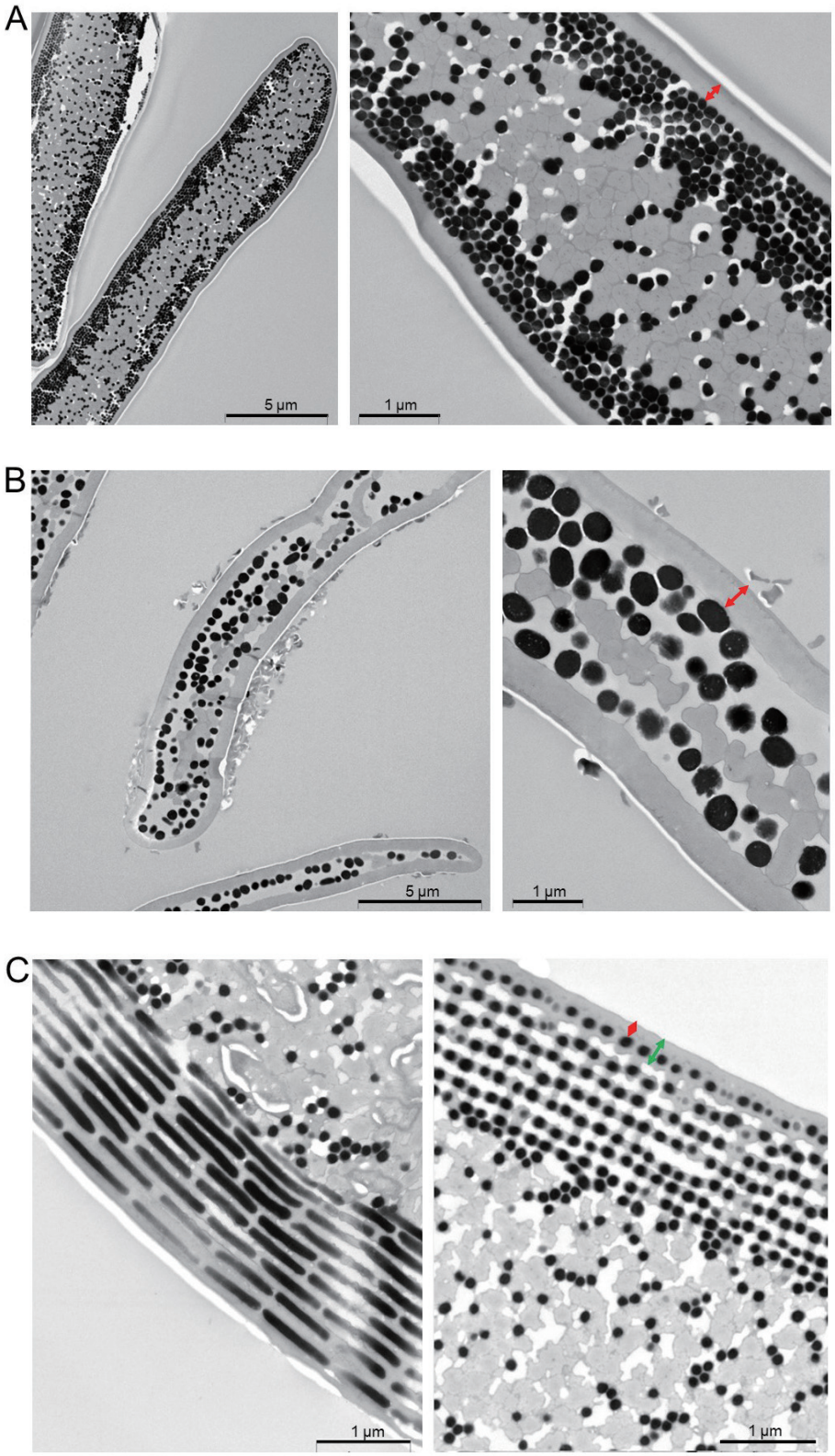

Fig. 3 TEM observation of feather barbules. A. Mallard feather. B. Rock dove feather. C. Peacock feather. Red double arrows indicate the distance between the cortical surface and melanin granule surface, and a green double arrow in C indicates the distance between the cortical surface and the air dome surface in the cortex. Black bars = 1 or 5 μm.

Table 1 Comparison of cortex and melanin structure in the feathers of different birds

Bird	Cortical thickness (nm) (n = 6)	Melanin granule	
		Diameter (nm) (n = 6)	Density (number of melanin granules/μm)
A. mallard	287 ± 11.0	148 ± 13.4	6
B. rock dove	528 ± 34.9	422 ± 68.9	2
C. peacock	112 ± 12.0 (261 ± 24.0)⋆	114 ± 13.8	6

Data of cortical thickness and diameter of melanin granules are means ± SD.
⋆Values in parenthesis indicate the distance from the cortex surface to the air dome surface below the first melanin layer.

because the structural color resulting from thin-film interference is created by the interference of specularly reflected light from the thin-film surface and substrate. The thin-film interference color reflected from the bird feathers can be distinguished by irradiating the feathers with right- or left-handed CPL light and observing the irradiated areas through an opposite-handed CPL cut filter, as shown in Fig. 1. This method was termed the CPL illumination method and the outline is summarized in Fig. 4.

The color of the eye pattern of the peacock tail feather is known to be derived from the lattice structure of rod-shaped melanin granules[8,9]. However, the green color around the eye pattern disappeared using the CPL illumination method, and was clearly caused by thin film interference (Fig. 1). Using the CPL illumination method, the blue part of the eye pattern turned purple, and the reflection spectrum was shifted to the short wavelength side (Fig. 2). It was presumed that this part was colored by the combination of thin-film interference and photonic crystals. Thus, it was revealed for the first time in this study that thin-film interference is involved in the appearance of the color of the eye pattern of the peacock tail feathers (Fig. 4).

Using the thickness data in Table 1, calculated from the TEM images in Fig. 3, and the refraction index of β-keratin (1.6) and eumelanin (1.7), the thin-film interference colors of the mallard, rock dove, and peacock feathers were simulated using the following simulation software: https://www.filmetricsinc.jp/reflectance-calculator, based on the Fresnel formula, similar to previous studies[11,14]. The mallard structural color simulation showed a spectrum very similar to the reflection spectrum of the actual feathers, whose peak was the CPL opposite to the irradiated CPL (Fig. 2 and 5A). The reflection spectrum of the rock dove feathers showed two small broad CPL peaks opposite to the irradiated CPL at approximately 460 and 600 nm. By observing the structure of the rock dove feathers, the melanin granules below the cortex were large and sparsely distributed. When the thin-film interference was simulated between the cortex surface and melanin granule surface, the simulation spectrum showed two peaks that were similar to peaks in the spectrum of the actual rock dove feather (Fig. 2 and 5B). Peacock feathers have a thin cortex, and no interference peaks within the visible light range were obtained in the simulation. There was an air dome between the first melanin layer and the second melanin layer, and the gap between the granules in the melanin layer was wide, so it was thought that thin-film interference due to reflection from the cortical surface and the air dome surface might occur in peacock feathers. When the simulation was run using a distance of 261 ± 24.0 nm between the cortical surface (refractive index: 1.6) and the air dome surface (refractive index: 1.0), an interference peak was observed at 557 nm, which corresponds to

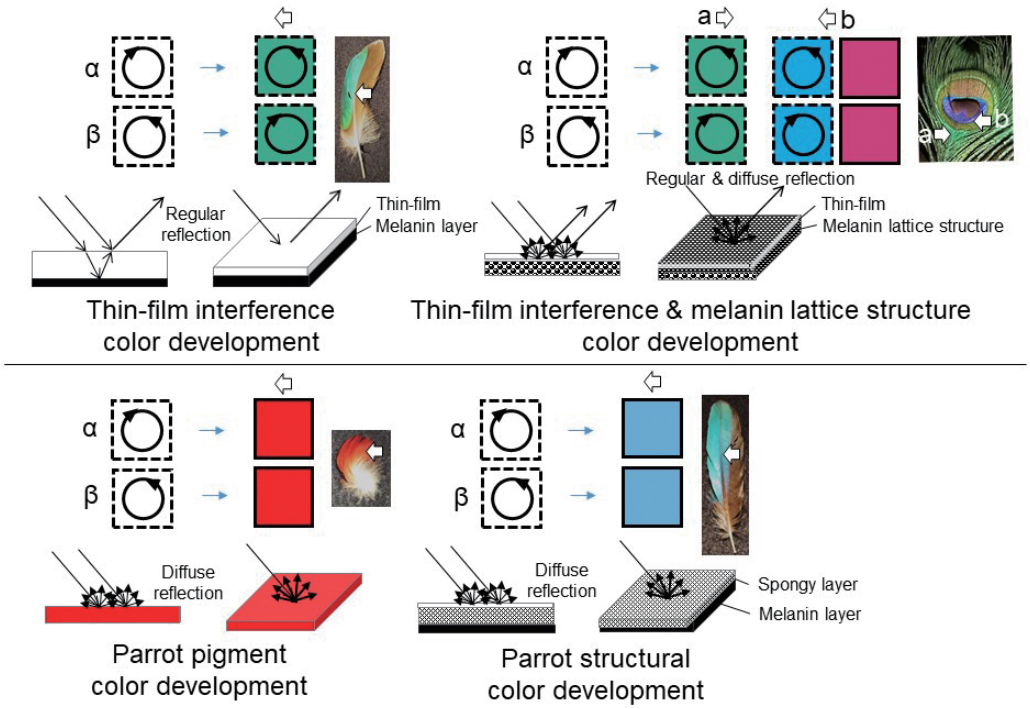

Fig. 4 Methods and principles to distinguish thin-film interference colors from pigment and other structural colors. α: left-handed CPL illumination; β: right-handed CPL illumination. Bold white arrows on the feather images indicate the areas observed using the CPL illumination method. Upper: specular reflection from the thin layer of mallard and peacock feathers. The bold white arrows a and b of the peacock feather insertion photograph show the peripheral and center parts of the eyeball pattern, respectively. Lower: diffuse reflection from parakeet feathers estimated from reference 11 and 12. Black arrows indicate the direction of reflected light. The direction of the circular arrows in the boxes bordered by broken lines indicates the direction of circular polarization of the irradiated or reflected light, and the color of the box represents the approximate color of the reflected light. A box bordered by a solid line without a circular arrow indicates incoherent diffuse light. CPL: circularly polarized light.

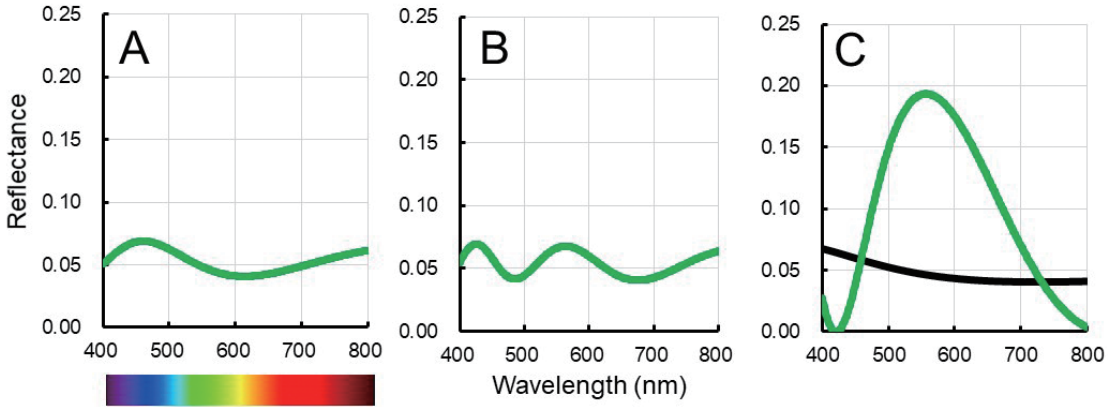

Fig. 5 Simulation of reflectance spectra from bird feathers. A. Mallard feather. B. Rock dove feather. Green line: from the melanin granule surface. C. Peacock feather. Black line: from the first melanin layers; green line: from the air dome surface. To simulate the reflection spectra of bird feathers, the cortical thickness data in Table 1 was used. The refractive indices of β-keratin and eumelanin were 1.6 and 1.7, respectively.

green light (Fig. 2 and 5C). From the above results, it became more clear that the green color around the eye pattern of the peacock feathers was formed by the thin- film interference of the cortex.

It was confirmed that the structural color of bird feathers derived from thin-film interference could be easily identified, and that the thin-film interference was also observed to be involved in the eye pattern of peacock tail feathers using the CLP illumination method. Biomimetic technology is being actively developed to imitate the structural color of the peacock feathers with resin granules coated with melanin[22], and shiny cuticle of beetles by cholesteric films[23]. It is expected that thin-film interference in various materials will be confirmed using the CPL illumination method to contribute to the development of biomimetic materials.

Conflicts of interest

The author has no conflicts of interest.

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