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Structural Color Production by Constructive Reflection From Ordered Collagen Arrays in a Bird (*Philepitta castanea:* Eurylaimidae)

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ABSTRACT Ordered hexagonal arrays of parallel collagen fibers produce the brilliant green structural color of the fleshy, supraorbital caruncles of male Velvet Asity (*Philepitta castanea;* Aves: Eurylaimidae). The collagen arrays are organized in larger macrofibrils that are packed irregularly within cone-shaped papillae that cover the surface of the caruncle. The color of the caruncle conforms closely to the wavelengths predicted by applying Bragg's Law of constructive reflection to measurements of the size and spatial organization of the collagen arrays. These observations constitute a novel mechanism of structural color production in animals. These collagen arrays are convergently similar to the smaller, highly structured collagen arrays in the mammalian cornea, which exploit the same physical mechanism to produce optical transparency.

Structural colors of animals are produced by the interaction of light with organic and inorganic materials of different optical densities. The known mechanisms of structural color production in animals include diffraction from colloids of liquids, solids, or gases, and constructive reflection from ordered layers or films of mucus, keratin, chitin, melanin granules, or intracellular purine crystals (Fox, '76). In birds, the only documented structural colors are produced by ordered layers of melanin granules or air vacuoles within the keratin of feathers (Durrer, '62, '77, '84; Durrer and Villiger, '66; Lucas and Stettenheim, '71; Dyck, '76, '87). However, structural color production in the dermis of birds has not been analyzed in detail. Structural blue colors in bird skin have been hypothesized to be produced by Tyndall scattering, but the anatomical basis of these colors has never been described (Rawles, '60: 223; Lucas and Stettenheim, '72:406, 410; Fox, '76; Durrer, '84).

This investigation documents a novel mechanism of structural color production based on the optical properties of ordered collagen fibers. Collagen is one of the most abundant and structurally important proteins in animals, but it is not presently known to contribute to production of any wavelength-specific structural color. However, the optical properties of collagen do contribute to its functional role in many organisms. Whiteness can be produced by reflection of incident light on unordered collagen fibers which vary in diameter and spacing. Structures such as the sclera of the human eye and the dermis of white axolotls appear white by this mechanism (Fox, '76; Vaezy and Clark, '91). Conversely, transparency of the vertebrate cornea is achieved by destructive interference among reflections from small, uniformly sized parallel collagen fibers in quasi-ordered arrays (Maurice, '57; Hart and Farrell, '69; Cox et al., '70; Benedek, '71; Vaezy and Clark, '91).

The novel structural color described here is found in the Velvet Asity (*Philepitta castanea;* Aves: Eurylaimidae; formerly Philepittidae): a frugivorous, suboscine perching bird in a small subfamily (Philepittinae) that is endemic to the island of Madagascar (Amadon, '79; Langrand, '91; Prum, '93). We first describe the color and anatomy of the caruncles of *Philepitta castanea* and then present a model for the production of the ob-

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served color based on the ultrastructure of the caruncle.

MATERIALS AND METHODS

These observations are based on caruncles of five museum spirit specimens of *Philepitta castanea* which were examined using a dissecting microscope, light microscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM). The museum specimens were collected in Madagascar by S.M. Goodman in 1989, preserved in the field in buffered 10% formalin, and later transferred to 70% ethanol for permanent storage at the Field Museum of Natural History (FMNH 345696-7, 345708-10). Additional photographs were taken under the dissection microscope of caruncle tissue from three other specimens of *P. castanea* which were collected in 1993 by S.M. Goodman, and preserved in the field in 2.5% glutaraldehyde for 1 hour and stored in 0.1 M cacodylate buffer (0.1 M sodium cacodylate, 0.0025 M calcium chloride, 4% sucrose).

Caruncle tissue from the museum specimens was critical point dried in CO_2 in preparation for SEM. Specimens were then coated with 30 nm of gold/palladium. Images and micrographs were produced using a Philips 501 on the 157.2 kV setting. For light microscopy, caruncle tissue was embedded in paraffin, cut in 10 μm sections, and stained with Masson's trichrome, which includes fast-green, a collagen-specific stain.

Caruncle tissue observed by TEM was taken from the cadual margin of the caruncle, which was bright green in life but violet to blue in the preserved museum specimens. Tissues were prepared for TEM using a standard protocol (Hayat, '81; Morrison and Frost-Mason, '91). Tissues were diced into 1 mm^2 pieces and placed in 2.5% glutaraldehyde fixative for 1 hour at 4–6°C. They were then post-fixed in 2% osmium tetroxide for 1 hour at 4–6°C, and then en bloc stained with 2% uranyl acetate for 1 hour at room temperature. Tissue pieces were then dehydrated through an ethanol series and embedded in Epon. Thin sections were cut and stained with 2% uranyl acetate and 0.4% lead citrate. Specimens were viewed and photographed in a Jeol JEM-1200 TEM. Fixation of all caruncle tissues observed by TEM was relatively poor due to the effects of formalin used to preserve the museum specimens when originally collected in the field.

Measurements of fibril dimensions and spacing were made with calipers on transmission electron micrographs. Equations for the calculation of Bragg reflections from fibril arrays were taken from Durrer's ('77, '84; Durrer and Villiger, '66) adaptation of Bragg's Law for constructive reflection in matrices composed of materials of different optical densities. Although the collagen arrays in *Philepitta castanea* are not perfectly ordered like a crystal lattice, Benedek ('71) and others (Hart and Farrell, '70) have demonstrated theoretically that Bragg wavelengths constitute the only constructive reflections from an array in which interfibril distances are regular over the distance of the wavelengths of incident light. These conditions are met by the collagen arrays in the caruncles of *Philepitta castanea,* confirming the suitability of the application of Bragg's Law to this system (see Discussion below).

RESULTS

Color and anatomy

Female *Philepitta castanea* have largely olive green plumage and lack supraorbital caruncles (Langrand, '91). Adult male *Philepitta castanea* have velvety black plumage with large, fleshy supraorbital facial caruncles (Langrand, '91) in the breeding season (September–February). In the nonbreeding season (April–August), adult males have black plumage with olive-green barring, and lack well-developed, colorful caruncles (Langrand, '91; S.M. Goodman, S. Zack, personal communication). The annual cycle of development of the caruncles and their structural color has not been documented. Further, male *Philepitta castanea* have delayed plumage maturation (S.M. Goodman, personal communication), and first year males that are sexually mature have female-like plumage and lack completely developed caruncles (e.g., FMNH 345697). Early in development, the caruncles of immature males appear as uneven, whitish thickenings of the supraorbital skin.

The caruncles of adult males are featherless, fleshy excrescences of the dermis. They are attached at their base to the dermis above the eye. The caruncles are approximately 25 mm long and 7 mm wide, and they extend from the base of the bill to behind and below the eye (Fig. 1). The lateral surface of the fully developed caruncle is brilliant green with bright blue immediately above the eyes (Figs. 1, 2). The medial surface of the caruncle is black. In life, the color of the caruncle does not change with the angle of incident light or the angle of observation (S.M.

Fig. 1. *Philepitta castanea.* An adult male Velvet Asity showing supraorbital caruncle. (Photo courtesy of S. Zack).

Goodman, personal communication; R.O. Prum, personal observation). The color of the fleshy caruncle of adult male *Philepitta castanea* has been described as "fluorescent green" (Langrand, '91). Color photographs of caruncles of living specimens of *P. castanea* were compared to published color references and appeared closest to yellow-green of

Smithe ('75; Munsell 5.0 GY 8.0/10.0) and viridine yellow of Ridgway ('12; Munsell 1.0 GY 7.8/8.0). The color of the caruncle in life is very similar to the color of caruncle tissue preserved directly in glutaraldehyde (Figs. 1, 2). Following initial fixation in buffered 10% formalin and storage in 70% ethanol, the caruncles appear violet blue or blue with bright iridescent highlights (Fig. 2). The museum specimens fixed in formalin have been used for all the observations in this analysis.

The color of the adult supraorbital caruncle is produced by cone-shaped papillae that cover its lateral surface (Figs. 1, 2, 3A). The papillae have smooth surfaces, and are largely perpendicular to the surface of the skin (Fig. 2, 3A). Each papilla is composed of a keratinized epidermal sheath and a capsule filled with irregularly oriented, cylindrical structures that stain densely with fast-green, a collagen-specific stain (Fig. 2). These collagen macrofibrils are hundreds of micrometers long and more than 50 μm wide. The base of the papilla rests on the upper dermis, which contains a dense layer of melanophores and unstructured collagen fibers (Fig. 2). The rest of the caruncle is composed of typical dermal tissue with blood vessels, nerves, and fine muscle fibers. In immature males, the papillae are not well developed and do not include large collagen macrofibrils.

Transmission electron microscopy reveals that the cylindrical collagen macrofibrils in the core of the papillae of adults are composed of highly ordered, hexagonal arrays of parallel collagen fibers (Fig. 3B,C). The average diameter of the fibers is 98 nm (n = 60, s.d. = 14) and the average spacing between the centers of the fibers is 185 nm (n = 60, s.d. = 22). In contrast, the collagen fibers present in the white caruncles of an immature male are not organized in macrofibrils, vary greatly in size, and do not form ordered arrays (Fig. 3D).

Mechanisms of color production

The color of the caruncles of *Philepitta castanea* is not produced by the keratinized sheathes of the papillae, because the color remains when the outer sheaths are removed (Fig. 2). Rather, the color emanates from the collagenous structures inside of the papillae. Tyndall scattering is an unlikely mechanism for the production of the observed color, as the collagen macrofibrils are much too large to produce color by that mechanism (Fig. 2). Structures that cause Tyndall scattering must have a mean diameter of < 0.7 μm (Fox, '76). Furthermore, Tyndall scattering structures would produce only a blue to violet color and would require an additional pigment to produce the green observed in the caruncles of living *Philepitta castanea* (e.g., Fox, '76). No pigment cells, however, occur in the core of the papillae where the color is produced. The hexagonal arrays in the collagen macrofibrils are the only potential color-producing structures inside the papillae.

The physical model for constructive reflection from a lattice of independent reflectors is Bragg's Law (Bragg and Bragg, '15), which has been adapted for application to constructive reflection by arrays of biological materials that differ in optical density (Durrer, '62, '77; Durrer and Villiger, '66; Benedek, '71). According to the Bragg model, light reflected by fibrils in independent parallel planes of a lattice travels different distances to any observation point, resulting in a shift in the phase of light waves reflected from different planes. The summation of these phase-shifted reflections results in destructive interference of most wavelengths, but produces constructive reflection of light with a wavelength that is equal to the addition in path length (Fig. 4). This wavelength, L, is given by the equation for the Bragg reflection condition:

$$L = n\,2d \sin \alpha$$

where n is the refractive index of the medium between the reflectors, d is the space lattice dimension (the minimum distance between parallel planes of reflection), and α is the angle of incident light (Bragg and Bragg, '15;

Fig. 2. *Philepitta castanea*. **Upper left:** The surface of a caruncle of an adult male viewed under a dissection scope by reflected light. This specimen was fixed in 2.5% glutaraldehyde in the field, and the colors preserved are similar to those of the tissue in life. Most of the caruncle is green but a small portion that is usually hidden within a wrinkle above the eye is blue. **Upper right:** View of the collagenous material inside a papilla from the caruncle of an adult male (FMNH 345708) that was fixed in formalin and transferred to ethanol, viewed by reflected light. The keratin sheath that covers the papilla has been removed. **Lower left:** Light micrograph of a cross-section of caruncle papillae stained with Masson's trichrome stain. Each papilla is composed of a keratinized sheath, a capsule filled with collagenous macrofibrils, and a basal layer of melanophores in the dermis. Collagen is stained with fast-green and appears blue. The gap below the collagen macrofibrils on the right is an artifact. Bar = 175 μm. **Lower right:** Light micrograph of the core of a single papilla showing irregularly packed collagen macrofibrils. Bar = 75 μm. S, keratinized sheath; CM, collagen macrofibrils; M, melanophores; UC, normal, unordered collagen.

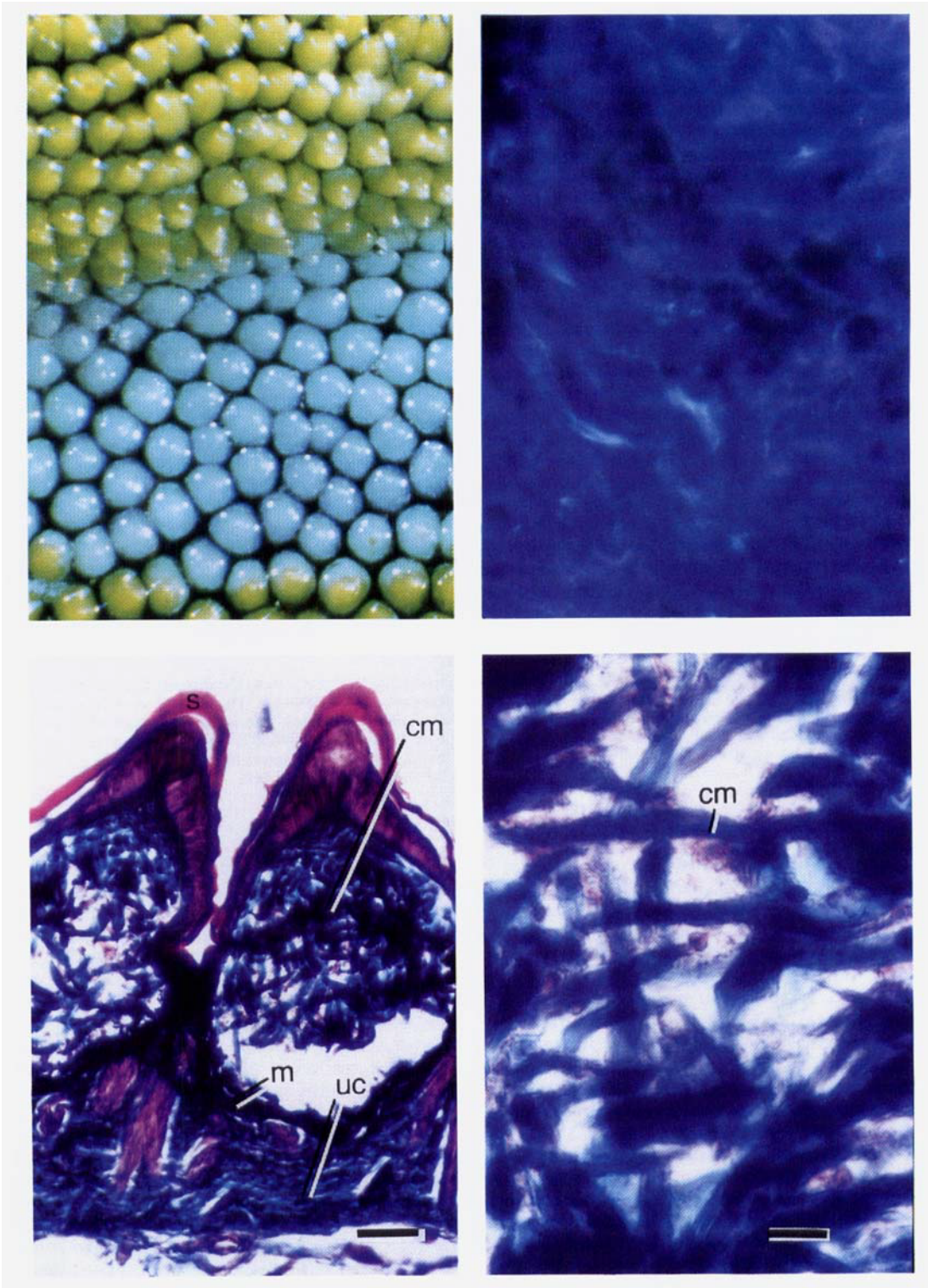

Figure 2

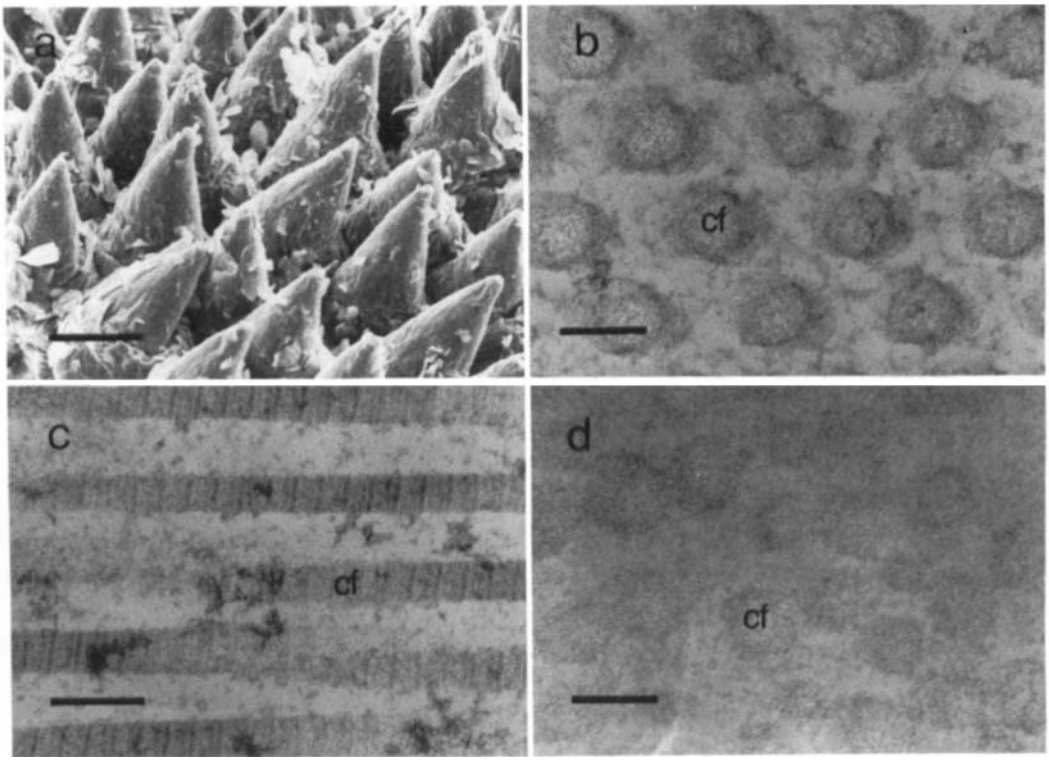

Fig. 3. *Philepitta castanea.* **A:** Scanning electron micrograph of the surface of the caruncle of an adult male (FMNH 345708). Surfaces of the caruncle papillae are smooth. Bar, 200 μm. **B:** Transmission electron micrograph (TEM) of a cross-section of an array of collagen fibrils in a collagen macrofibril. Bar = 100 nm. **C:** TEM of a longitudinal section of collagen fibrils within a caruncle papilla. Fibrils show the distinct banding pattern of collagen. Bar = 200 nm. **D:** TEM of a cross-section of collagen fibrils within the whitish caruncle of an immature male (FMNH 345697). Collagen fibrils vary greatly in diameter, and the interfibril spacing is irregular. Bar = 100 nm. CF, collagen fibril.

Durrer, '66, '77, '84). In a hexagonal array of fibrils 185 nm apart, the primary family of parallel planes has a space lattice dimension of 160.5 nm (Fig. 5A). The potential angles of incidence on these planes are limited by the distribution and size of the fibrils (Durrer, '66, '77). In this hexagonal array, the potential angles of incidence range from 68–90° and provide minimum and maximum values for potential Bragg reflections (Fig. 5A). The matrix between the collagen fibrils in the caruncle and its refractive index is unknown; however, Maurice ('57) determined the refractive index of the mucopolysaccharide between collagen fibrils in the mammalian cornea to be 1.35. We used this value as an approximation of the actual refractive index.

Based on these values, the wavelengths of potential Bragg reflections from the collagen arrays in the caruncles of *Philepitta castanea* range from 401 nm ($\alpha = 68°$) to 433 nm ($\alpha = 90°$). These wavelengths range from violet to blue in color, and conform closely to the observed color of the preserved caruncle tissue (Fig. 2). Shallow angle Bragg reflections could potentially occur at a few limited angles ($\approx 31°$), but these reflections would be nonvisible ultraviolet wavelengths (≈ 223 nm). Owing to the symmetry of the hexagonal array, incident light at these shallow angles is also incident on other planes of reflection that have the same space lattice dimension and will produce visible Bragg reflections in the above wavelengths (Fig. 5B). The space lattice dimensions of other families of parallel planes in the array are ≤ 80 nm. These planes have first order Bragg reflection wavelengths entirely in the portion of the ultraviolet spectrum (< 259 nm) that is not visible to birds or humans, and reflections from these planes should not contribute significantly to the observed color of the caruncle.

The Bragg model predicts that the change in color from green to blue following fixation in 10% formalin and storage in 70% ethanol was produced by the shrinking of the interfibril distances of the collagen arrays. The distance between adjacent collagen fibrils would have to be approximately 230 nm in life (as opposed to 185 nm in the preserved tissue of museum specimens) to produce the green color observed in nature by constructive reflections from the primary space lattice. This amount of tissue shrinkage (approximately 20%) is explicable and would be expected as an artifact of dehydration during fixation and preparation for microscopy (Hayat, '81; R.L. Morrison unpublished observations).

The Bragg reflection model is further corroborated by the observation that the whitish caruncle of an immature male *Philepitta castanea* is composed of collagen fibrils that vary tremendously in size and interfibril distance (Fig. 3D). Irregular fibril size and interfibril distance on the scale of the wavelengths of visible light will result in a lack of phase-correlations among reflections from different fibrils (Maurice, '57; Benedek, '71; Vaezy and Clark, '91). As a result, most wavelengths of light will be reflected by the tissue, and it will appear white.

DISCUSSION

The color of the preserved caruncles of adult male *Philepitta castanea* conforms well to the wavelengths predicted by the application of the Bragg equation for constructive reflection to the dimensions of collagen arrays in the caruncle papillae. The color produced by the caruncles of *Philepitta castanea* is the summation of constructive reflections from thousands of collagen arrays arranged in macrofibrils within each papilla. The change in color of the caruncles from green to blue with preservation is accounted for by shrinking of the tissue due to the preservatives used. Wavelength-specific structural color production is an entirely novel function for collagen in animals.

The first physical model for the production of wavelength-specific colors by constructive reflection from an ordered lattice was developed by W.H. and W.L. Bragg ('15) who studied the interaction of X-rays and crystals. Subsequent studies of arrays of melanin granules in a keratin matrix in bird feathers have expanded the application of the Bragg model to ordered arrays of fibrils in media of a different optical density (Durrer, '62, '77; Durrer and Villiger, '66). Experimental and theoretical investigations of arrays of collagen fibrils in mucopolysaccharide matrices in the vertebrate eye have also demonstrated that Bragg's Law is an appropriate model for analyzing constructive reflection by regular arrays of collagen fibers (Maurice, '57; Benedek, '71).

Color production in *Philepitta* differs in some interesting ways from other known mechanisms of reflective color production. Many reflective structural colors are iridescent, or change hue with angle of observation. For example, iridescent bird feathers produce color by constructive reflection from square lattices of melanin granules that are oriented parallel to the surface of the feather barb (Durrer, '62, '77, '84; Lucas and Stettenheim, '71; Dyck, '76). The color of these feathers varies with angle of observation because changes in the orientation of the observer alter the addition to the path length of light waves reflected from the planes of melanin granules. In one genus of birds, the *Ptilinopus* pigeons, this color variation is eliminated by having curved arrays of melanin that maintain the same space lattice dimension and path length addition from any point of observation (Dyck, '87).

In contrast to iridescent structural colors, two aspects of the structure of the caruncles of *Philepitta* contribute to the creation of a reflective color that is unchanged with the angle of incident light or the point of observation. First, each unit hexagon in a collagen array includes three sets of parallel planes with the appropriate space lattice dimension for visible Bragg reflections (Fig. 5B). Each set of planes intersects the others at 60° angles. Light incident on a collagen array from any direction, except exactly parallel with these planes of reflection, should produce visible Bragg reflections within a narrow band of wavelengths (approximately 30 nm wide) from one of these three sets of planes. Second, at a larger level of organization, the macrofibrils within the papillae are composed of numerous collagen arrays. They are irregular in shape and orientation, and are packed together irregularly within the papillae (Fig. 2). Light incident on the papillae from any angle will interact with thousands of collagen arrays within these irregularly packed macrofibrils. The reflected light is the summation of the constructive reflections from different angles of incidence on thousands of arrays.

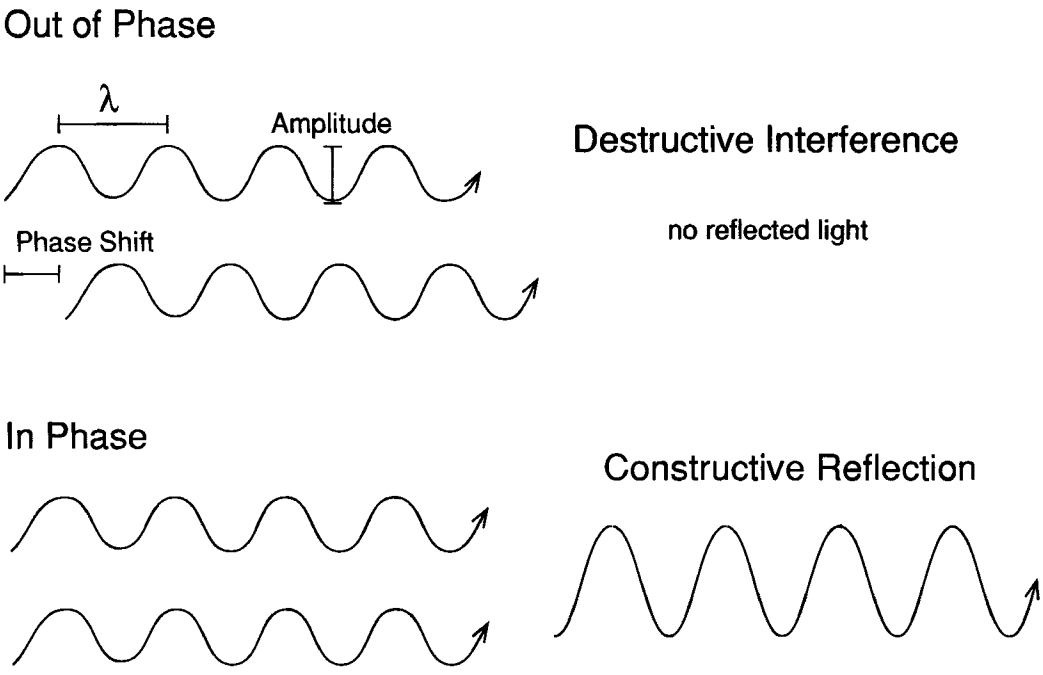

Fig. 4. *Philepitta castanea.* Diagram of the summation of independently reflected light waves. Reflected waves that have traveled different distances, or path lengths, may be out of phase, or at different stages in the cycle of the wave (**upper left**). The difference in path length between the waves contributes to the phase shift, the amount by which the two waves are out of phase. Summation of two completely out-of-phase reflections of the same wavelength and amplitude results in complete, destructive interference; no light wave is propagated (**upper right**). Reflected waves that have traveled different path lengths may have shifted back in phase if the path length addition is an integer multiple of the wavelength (**lower left**). Summation of two in-phase reflections will result in constructive reflection of a light wave with the same wavelength and twice the amplitude (**lower right**). λ, wavelength.

In the caruncles of *Philepitta castanea,* the symmetrical structure of the hexagonal arrays and the irregular packing of the collagen macrofibrils combine to produce a reflective color that is noniridescent, uniform, and brilliant from any angle of observation. Changes in the orientation of an observer to the caruncle of **Philepitta** do not predictably alter the angle of observation to the color producing arrays, because each array presents multiple opportunities for Bragg reflections from different directions, the arrays are so numerous, and the macrofibrils are irregularly oriented within the papillae.

Like many structures that produce colors by either reflection or Tyndall scattering, the caruncle papillae of *Philepitta castanea* are backed by a dense layer of melanophores (Fig. 2). Highly melanized dermal tissue eliminates reflections of white light that is transmitted entirely through the papillae. Irregular reflections from the tissue below the papillae would be out of phase with the Bragg

Fig. 5. *Philepitta castanea.* **A:** Diagram of the Bragg model of constructive reflection showing an array of parallel collagen fibrils, the space lattice dimension of the array (d), and the path length addition to reflected light at different angles of incident light (α), in cross-section. Arrows indicate the paths of incident and reflected light from adjacent fibrils in neighboring, parallel planes of reflection. The planes of reflection and space lattice dimension are indicated by the shaded planes on the right. The wavelength of Bragg reflections varies with the path length addition which is determined by the angle of incident light (α) and the spatial conformation of the fibrils. Values of α for this array vary from a minimum of ≈ 68° (**left**) to a maximum of 90° (**right**). The primary space lattice dimension in the preserved material is ≈ 160.5 nm, and incident light on these planes should produce visible Bragg reflections between violet (401 nm) and blue (433 nm) at α values from 68 to 90°. **B:** Diagram of the three sets of parallel planes with the maximum space lattice di- mension (≈ 160.5). Each set of planes (shown in different stippling) is parallel with two flat sides of the basic, hexa- gonal unit cell of the array. Each set of planes intersects the others at a 60° angle. Incident light from any angle except parallel with these planes will produce visible Bragg reflections from one of these three sets of planes. α, angle of incident light; d, space lattice dimension.

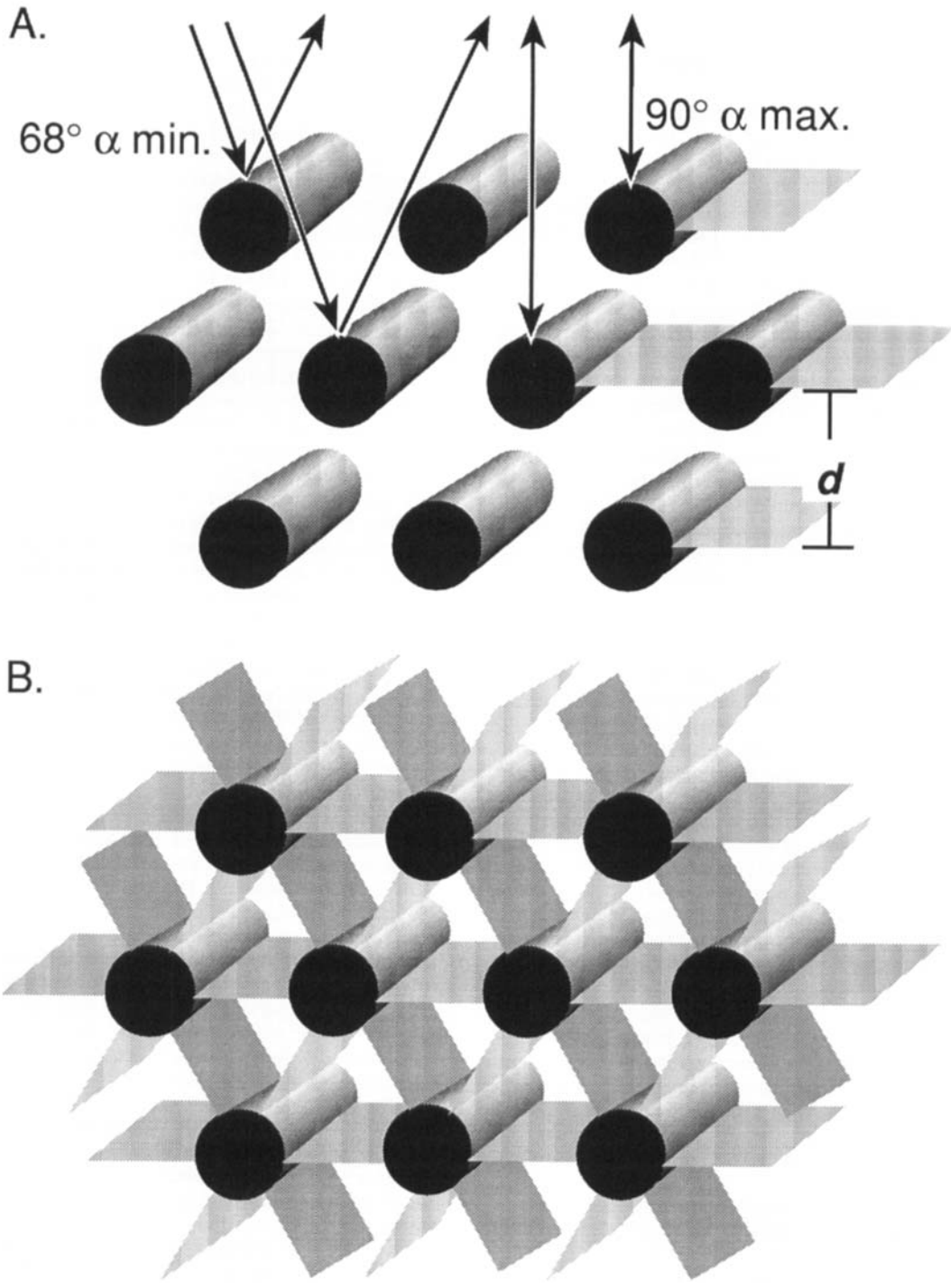

Figure 5

reflections from the collagen lattices and would dim the color of the constructive reflections (Lucas and Stettenheim, '71; Fox, '76; Fujii et al., '89).

The brilliance of the color of the caruncles of *Philepitta castanea* may be related to the number of reflective arrays within each papilla. In bright green species of *Ptilinopus* pigeons, Dyck ('87) has shown a linear relationship between the peak percent reflectance of light incident on the feathers and the number of ordered layers of the constructively reflecting melanin granules within the feather barbules. A maximum of over 60% reflectance was observed in *Ptilinopus victor* which has 20 layers of melanin granules. In comparison, any cross-section of a papilla of *Philepitta castanea* intersects dozens of collagen macrofibrils; each macrofibril ($\sim$50 μm in diameter) includes hundreds of reflective planes of collagen fibers ($\sim$160 nm apart). This enormous number of independent reflective planes within the papillae apparently contributes to the brilliance of the color produced by the caruncle. Spectral reflectance measurements from the caruncles of *Philepitta castanea* should be made to quantify the efficiency of this color production mechanism.

The production of wavelength-specific structural color is a unique and unexpected function for collagen. However, investigations of the mammalian cornea demonstrate that corneal collagen fibrils produce transparency by essentially the same physical mechanism. The cornea of mammals is composed of parallel fibrils of collagen (20–30 nm in diameter) in a quasi-ordered array with an average center-to-center fibril spacing of 60 nm (Maurice, '84). Maurice ('57) was the first to hypothesize that corneal collagen fibrils behave like a crystal lattice, and that corneal transparency is the result of destructive interference among correlated, phase-shifted reflections from different collagen fibrils. The corneal collagen fibrils and interfibril spacing are so small that the only resulting reflections would be Bragg wavelengths in the nonvisible ultraviolet spectrum (Maurice, '57).

Subsequent theoretical physical models of the optical properties of arrays of parallel fibrils by Benedek ('71) and others (Hart and Farrell, '69) demonstrate that the Bragg reflection phenomena can occur even when the array of fibrils is not a perfect lattice. Benedek's models demonstrate that constructive reflections at Bragg frequencies should occur when predictable spatial variation in optical density occurs over distances comparable to the wavelength of incident light. In specific, an array of fibers will scatter or reflect wavelengths related to the Fourier components of the spatial variation in optical density. Experimental work by Vaezy and Clark ('91) has confirmed Benedek's model of transparency for the human cornea. The Fourier components of the spatial variation in optical density of the cornea are very low at all wavelengths related to visible light, but there was a large peak in fourier components at 80 nm, close to the interfiber distance and resulting in constructive reflections in the nonvisible ultraviolet spectrum. In comparison to the cornea, the mammalian sclera appears white, and is composed of collagen fibrils that are highly variable in diameter (40–200 nm) and larger interfibril spacing ($\sim$250 nm; Maurice, '84; Vaezy and Clark, '91). The Fourier components for spatial variation in the reflective index of the human sclera are high across all wavelengths with a peak in the 300–400 nm range corresponding to frequencies of visible light (Vaezy and Clark, '91). These irregularly organized scleral fibrils do not produce predictable correlations in path length addition among reflections from different fibrils, resulting in nearly complete reflection of incident light and the white color (Maurice, '57; Benedek, '71; Vaezy and Clark, '91).

Theoretical and experimental results from the mammalian eye support our application of Bragg's Law to the analysis of constructive reflection from collagen lattices in a bird. Although the collagen arrays in the caruncles of *Philepitta* do not constitute a perfect lattice, the fibril size and interfibril spacing are regular and highly predictable over distances greater than all wavelengths of visible light (Fig. 3B). Under these conditions, the array should produce Bragg reflections at the observed wavelengths (Benedek, '71). Furthermore, the irregularly sized and spaced collagen fibrils in the whitish caruncle of an immature male *Philepitta castanea* apparently function like the similarly structured collagen of the mammalian sclera. Observations of immature males confirm that the regularity in size and spacing of collagen in the adult caruncles is responsible for the vivid green structural color.

The vertebrate eye has evolved through natural selection for visual perception (Williams, '92). The supraorbital caruncle of

Philepitta castanea is a secondary sexual structure that has probably evolved through sexual selection (e.g., Darwin, 1871). Natural and sexual selection on the optical properties of these two very different structures has lead to convergent evolution in ultrastructure of their collagen fibrils. Each structure exploits the same physical mechanism to achieve its own distinct optical quality, transparency on the one hand, and vivid, multidirectional color production on the other.

Much remains to be learned about the function, development, and evolution of collagenous color-producing arrays. We have made observations of the caruncles of a single immature *Philepitta castanea,* and additional observations should be made of the development of the caruncle in young males. Furthermore, nothing is known about the process of annual caruncle development and atrophy in adult males, and additional observations should be made of the annual variation in caruncle size, color, and ultrastructure. Unfortunately, material of these poorly known rain forest birds is very rare.

Nothing is known about the possible distribution of this color production mechanism in other birds. However, all three other species of asities have colorful facial caruncles (Langrand, '91), and the caruncles of these species also differ in color and morphology from *Philepitta castanea. Philepitta schlegeli* has a larger caruncle that is "pearly light green below and in front of the eyes, blue above the eyes, and turquoise behind the eyes" (Langrand, '93:249). From observations of museum study skins, the surface of the caruncle in *schlegeli* appears to be covered with broad, round, bubble-like tubercles instead of the small, erect papillae as in *castanea.* In *Neodrepanis coruscans,* the caruncle is light blue above the eye and ultramarine blue below with a turquoise eye ring (Langrand, '93: 250). The surface of the caruncles of both species of *Neodrepanis* are smooth, and entirely lack papillae or tubercles (R.O. Prum, personal observation). The caruncles of other asities are homologous to those of *Philepitta castanea* (Raikow, '87; Prum, '93), and we would predict that the vivid blues, greens, and purple colors of the caruncles of other asities are produced by collagen arrays. However, the color and morphology of the caruncles of these other species are different from those of *Philepitta castanea,* indicating that various anatomical aspects of this color production mechanism have continued to evolve since its origin.

The constructive reflection model predicts that variation in hue in different parts of a single caruncle and among the caruncles of different species of asities is a result of differences in the space lattice dimensions of the collagen arrays. Future investigations should focus on testing this critical prediction. Observations of other species of asities should provide additional opportunities to examine the association between collagen ultrastructure and color production, and to investigate the evolution of this novel color production mechanism.

More generally, detailed anatomical investigations of structural color production in the avian dermis are necessary. The repeated suggestions in the literature that the vivid blues and greens of the facial skin of the cassowaries (Casuariidae), peafowls (Numididae), and toucans (Ramphastidae) are produced by Tyndall scattering are entirely unsupported by any direct observations (Rawles, '60:223; Lucas and Stettenheim, '72:406, 410; Fox, '76). No detailed studies of structural color production in the avian dermis have been published previously (Lucas and Stettenheim, '72:406, 410; Fox, '76; Durrer, '84). The preoccupation of Ornithologists with the brilliant feathers of birds have resulted in an entire literature on structural color production in these dermal derivatives, but may have contributed to the dearth of information about color production in the avian skin.

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