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Spatial organization of the structural color system in the quetzal, *Pharomachrus mocinno* (Aves: Trogonidae) and evolutionary implications

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Abstract: The quetzal, *Pharomachrus mocinno,* is a Neotropical bird whose structural green appears to be cryptic. The electron microscope shows that green barbules are thick segmented ribbons that twist slightly along the main axis and end in a bifid or trifid filament. Pigmented barbules are simpler and may have lateral flaps. Barbules from the brown- green interfase change gradually. Even though the color mechanism is internal, it has a significant influence on feather shape, limited by selection for strength and thermoregulation. These results and computer simulations support the interference air-keratin-melanin model. Irregular organization of the melanin and reduced barbule interlocking are expected when dull coloration is favored, for example, by high predation. The capacity to produce iridescence appears to be an ancient metazoan character lost in amphibians and mammals. Evolutionary convergence of avian structural color is suggested because the specific mechanisms do not parallel phylogeny.

Key words: Structural color, quetzal, evolutionary ecology.

Animal coloration has a variety of functions which may depend on habitat; for example the same color pattern may be cryptic under some light conditions and conspicuous elsewhere (Endler 1993). This is also true in birds, whose colors are mostly on their plumage.

Feather colors are of two basic kinds: pigmentary and structural. In the former, certain light wavelengths are absorbed by specific pigments and those reflected produce the observed color. In contrast, the structural color is caused by a single pigment, melanin, which produces a variety of colors, chiefly green and blue, in a iridescent spectrum. How a single pigment can produce many colors is explained by the interaction of light with air and feather structures (see Gill 1990 for a recent review). At least in *insects,* some iridescence is invisible *to humans* (Silberglied 1984).

In fact, the structural nature of some avian colors has long been known (e.g. Gadow 1882) and the first use of the transmission electron microscope (TEM) on organic material was a study of structural color in feathers (Frank and Ruska 1939). Nevertheless, the exact physical mechanism involved has been the subject of much debate. For example, three mechanisms have been proposed for the "spongy tissue" of barbs: diffraction (Gadow 1882), scattering (HAcker and Meyer 1902) and interference (Raman 1935). A later detailed study with TEM supported the hypothesis of interference for the rose-faced lovebird *Agapornis roseicollis* (Dyck 1971).

When predation pressure overrides sexual selection, birds are likely to become sexually monomorphic for color (Shutler and Weatherhead 1990). This may explain why ma'e quetzals *(Pharomachrus mocinno)* are only slightly more brilliant than their mates and suggests that their structural green is basically a cryptic adaptation (LaBastille *et* at. 1972).

The color mechanism of the quetzal was studied with TEM by Durrer and Villiger (1966) and with the same technique but in less detail by La Bastille et al. (1972). Both supported the idea that the color of the species, which iridesces around the yellow-green-blue range, is produced by interference of light in a complex of keratin-melanin-air interactions chiefly within the barbules. Structural green may have a cryptic function for quetzals in their habitat of moist folliage (La Bastille *et al.* 1972).

A study of the spacial organization of the whole color system, though, had to wait until a new technique was applied: scanning electron microscopy (SEM). This paper reports the resuits of the first SEM study of the southern subspecies of the quetzal, *Pharomachrus mocinno costaricensis.* The morphology of structurally colored barbules is compared with that of pigmented barbules, and evolutionary theory is applied to structural colors in quetzals and other birds.

MATERIAL AND METHODS

Green feathers from the head, back and breast, and red and white feathers from throat and tail, respectively, were extracted from museum specimens of *P. in. costaricensis.* Barbs from each feather were removed and washed with 100% ethanol, air dryed, fixed on aluminium stubs for SEM and coated with about 15 nm thick platinum in an ion-sputtering apparatus. Some barbs from green feathers were treated with 10% NaOH for 2-5 mm at room temperature, washed with distilled water and treated as described above. Barbs were also mounted for light microscopy using Permount.

RESULTS

Green feathers: Green feathers from all body parts were similar and most had a brown basal part. Those from the breast and back were almost completely green, while those of the head were green only in the distal section. The unexposed parts of the feather lack structural coloration. The green barbule is a thick segmented ribbon which twists slightly along its axis (Figs. 1-4) and ends in a bifid or trifid filament (Fig. *5).* The segments, Ca. 15.4 un in length, were obvious only when the sample was tilted more than 50, otherwise the surface appears smooth. Partially digested samples showed a regular lattice of 0.02X0.17 im rod- shaped bodies (Fig.6-7); these were aligned with the major axis along the barbule.

The overall structure of the green feather is shown in Fig.8.

Pigmented feathers: Brown barbules (Fig.9) had a smooth cylindrical body 650 μm long with a hooked terminal filament. The cylinder had a lateral flap along the basal half (Fig. 10).

Barbules from the brown-green interfase (Figs. 11-12) change gradually from one type to the other: the flap is reduced in length to a vestige at mid height and the segmentation becomes increasingly marked towards the green part (Fig. 13).

The white barbules (Figs. 14-15) had a tubular body with a wide flap along the mid basal part and hooklets in the distal half. Red barbules (Figs. 16-17) were the simplest and were composed of a smooth cylinder swollen at the - base and with no hooklets in the terminal section.

DISCUSSION

General structure: The general structure of quetzal green feathers greatly resembles the drawings of the iridescent barbules of "bronze turkeys" published by Lucas and Stettenheim (1972: Fig. 255). The difference is that turkey barbules have hooklets in the mid section.

The similarities of structurally colored feathers in these unrelated species suggests that even though the basic color mechanism is internal and of small size, it has a significant influence on general feather morphology. Our results are in agreement with Dick's (1971) findings that barbules are more important than barbs for structural color, and that their color is correlated with barbule transversal shape, thicknes, site of insertion, length and cortex thickness, and with shape and organization of air, keratin and melanin. The twisting of the barbule may improve iridescence by reflecting light in more angles, Dick (1971) mentioned that the surface was rougher in structurally colored barbules than in pigmented barbules but offered no explanation.

Barbule segments probably represent dead medullary cells (Lucas and Stettenheim 1972) and their nuclei are supposed to fill with air (Dick 1971). Although there seem to be no studies showing that air is really part of the system, computer simulations based on the air-keratin-melanin model of Durrer and Villiger (1966) produced the correct color for quetzals (J. Soley 1993 pers. corn.). The simulations al so indicate that melanin grains are relatively homogeneous, a fact considered uncertain by Dick (1971).

Pigment colored barbules also resemble their equivalents in the turkey (Lucas and Stettenheim 1972). The gradual morphological change in the green-brown interface is not reported in the literature we have seen and may reflect a gradual change during the ontogeny of the barbule, which is discussed by Lucas and Stettenheim (1972).

The rod-shaped bodies of the digested sample could be the melanin granules observed with transmission electron microscopy (Durrer and Villiger 1966).

Structural color regulation and evolution: The development of brilliant structural colors is counteracted by the need for physical strength, which could explain why only exposed parts have structural color (Dick 1971). Another opposing force could be that thermoregulation and structural colors should be rare in species from cold habitats where a brilliant plumage would reduce heat absorption.

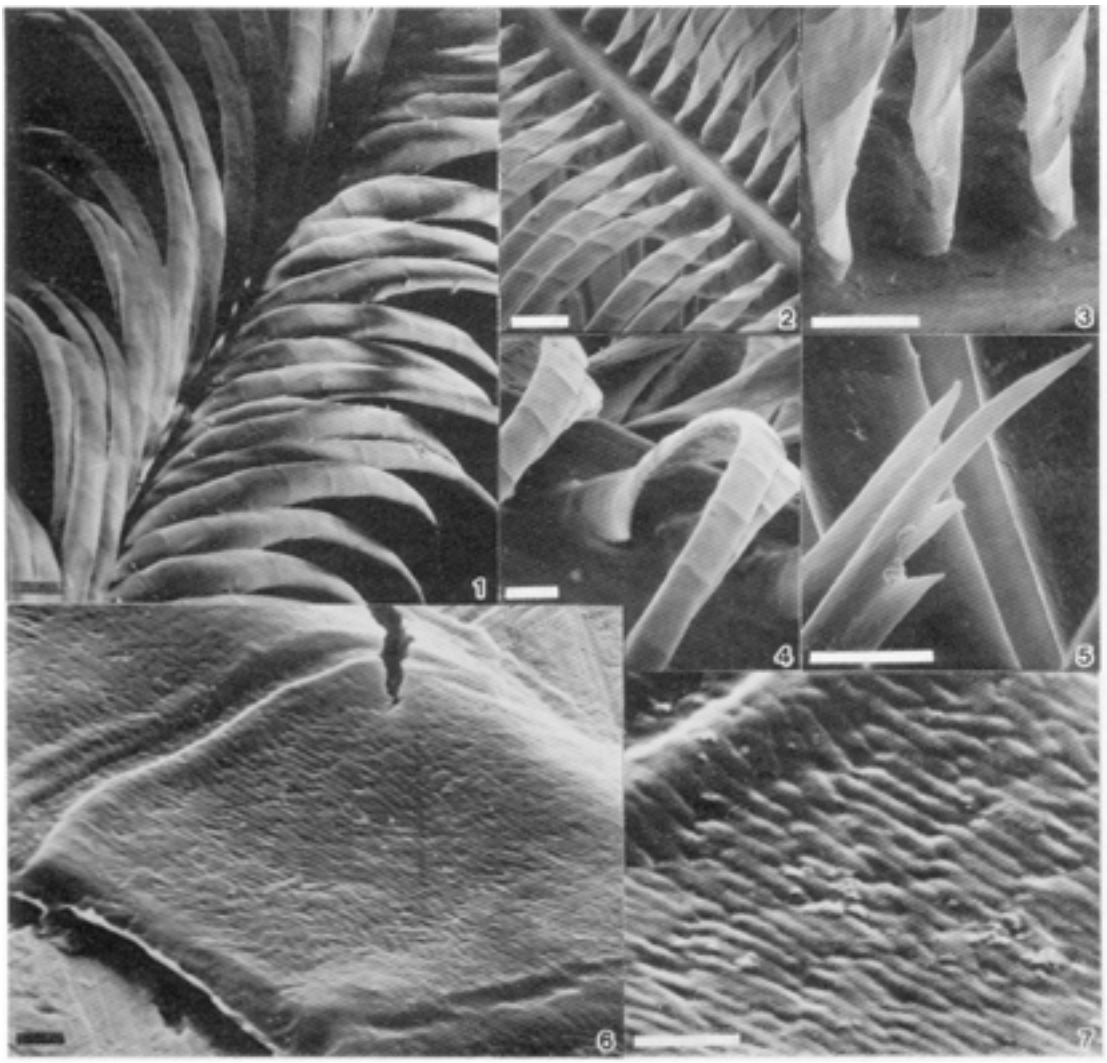
Thus, natural selection must regulate the type (iridescent versus non-iridescent) and brightness of structural color. A list of regulauon mechanisms should include changes in the thicknes of melanin layers, in number of layers (Simon 1971), fusion or disorganization of melanin grains (Dick 1971), changes in the compactness of the barbule interlocking vane system (Durrer and Villiger 1966) and covering barbules with oil or other material, as occurs in some lepidopterans (Lucas and Stettenheim 1971, Simon 1971).

We used evolutionary theory to make the following general predictions. Irregular organi1 Nation of the melanin and reduced barbule interlocking are expected when dull coloration is favored, for example, as a result of high predatory pressure or when young males mimic females to trespass territories (see Shutler and Weatherhead 1990, Slagsvold and Saetre 1991). In contrast, low morphometric variation, regulai melanin arrangement and tightly interlocked barbules should characterize species in which sexual selection, imprinting, or low habitat light levels favor brilliant plumage (e.g. Durrer and Villiger 1966, Cate and Bateson 1988, Marchetti 1993).

Taxonomic distribution: The capacity to produce iridescence, the most outstanding type of structural coloration, may be ancient in the Metazoa, because it exists in molluscs, insects, fishes, reptiles and birds, according to Simon (1971). If that hypothesis is correct, the capacity apppears to have been lost in two major taxa: amphibians and mammals (Fig. 18). Among the Ibirds, the mechanisms of structural coloration do not follow a clear phylogenetic pattern; for example, quetzals resemble hummingbirds more than they resemble other members of their own family (Durrer and Villiger 1966). Although this could be interpreted as evidence of frequent convergent evolution, it may also represent modification of an ancient and widespread air-keratin-melanin system, as suggested by the occurrence of structural coloration in most avian orders (Fig. 19).

Auber (1957) hypothetized that iridescent and non-iridescent structural color almost never occur in the same individuals or species because they are ontogenetically incompatible, but his idea cannot explain Coua cerulea, a bird which has both (in different parts of the body, see Lucas and Slettenheim 1972). Ecological factors may be a better explanation and deserves further study.

Research should stress at least four aspects which have not been properly treated in this and previous papers: (1) if there is an association between thin skin and structural color, as suggested by Simon (1971), (2) the interrelation of barbules of different barbs, (3) the possible anti-halo function of disorganized melanin grains, and (4) the ecological correlates of iridescent and non-iridescent plumage.



Figs. 1-7. Organization of green barbules in the quetzal. 1. General view (bar 20 μm). 2. Barbules' insertion in barb (bar 25 μm). 3. The barbule base is wider and twisted (bar 15 μm). 4. Cell segments (bar 15 μm). 5. Distally the barbule is bifid or tnfid (bar 5 μm). 6. Partially digested cell with probable melanin grains (bar 1 μm). 7. The rod-shaped bodies inside the cell may be rows of melanin grains.

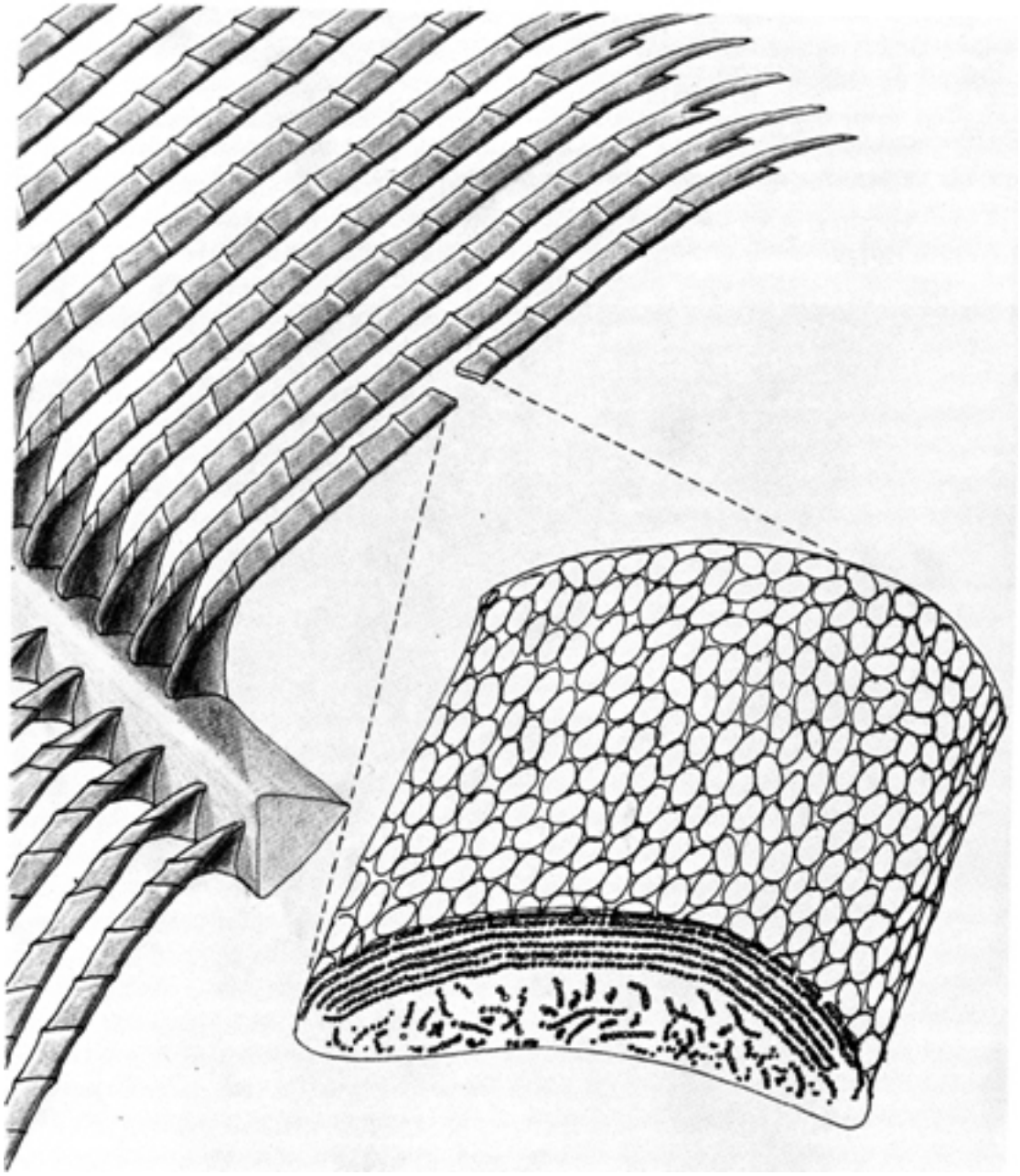
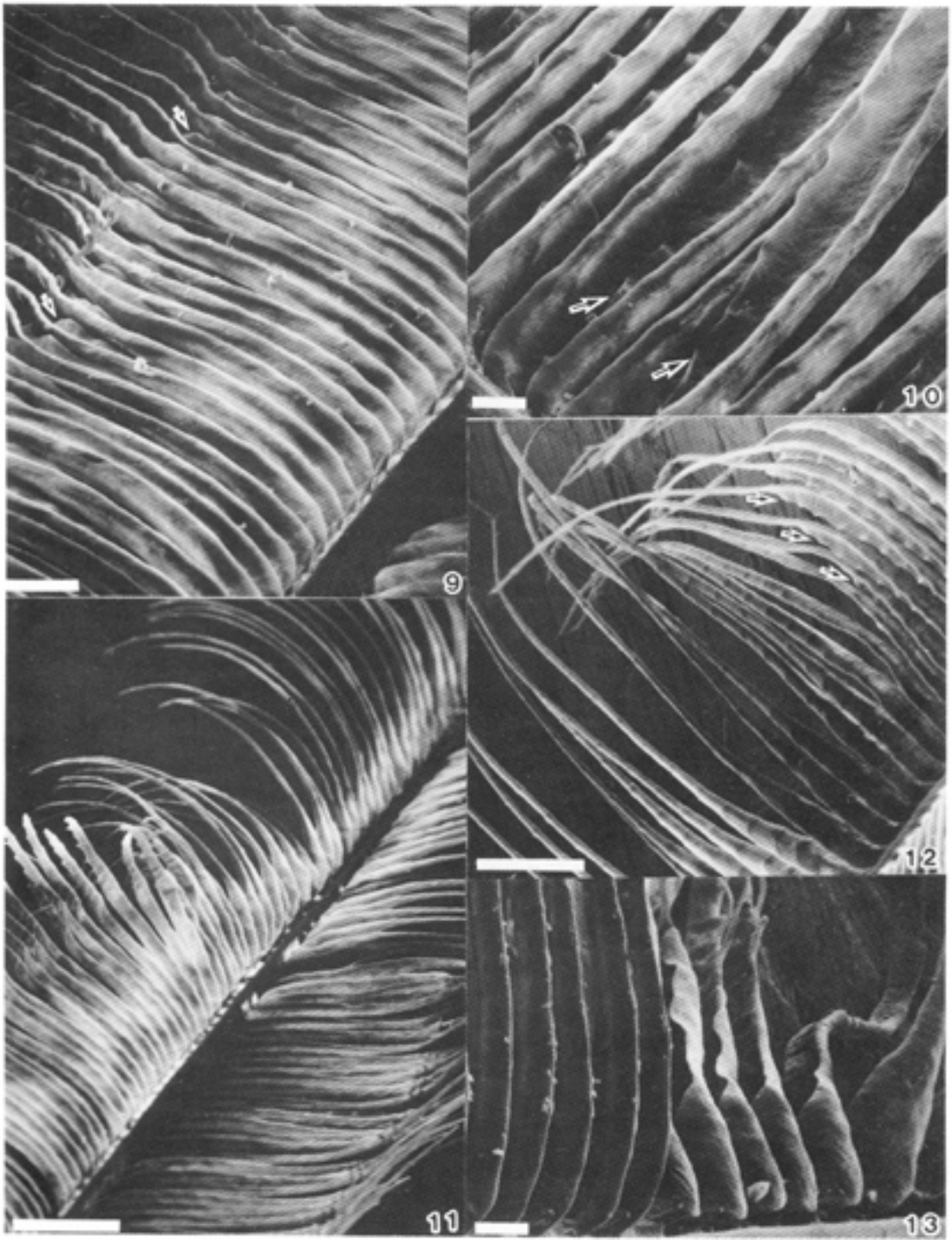
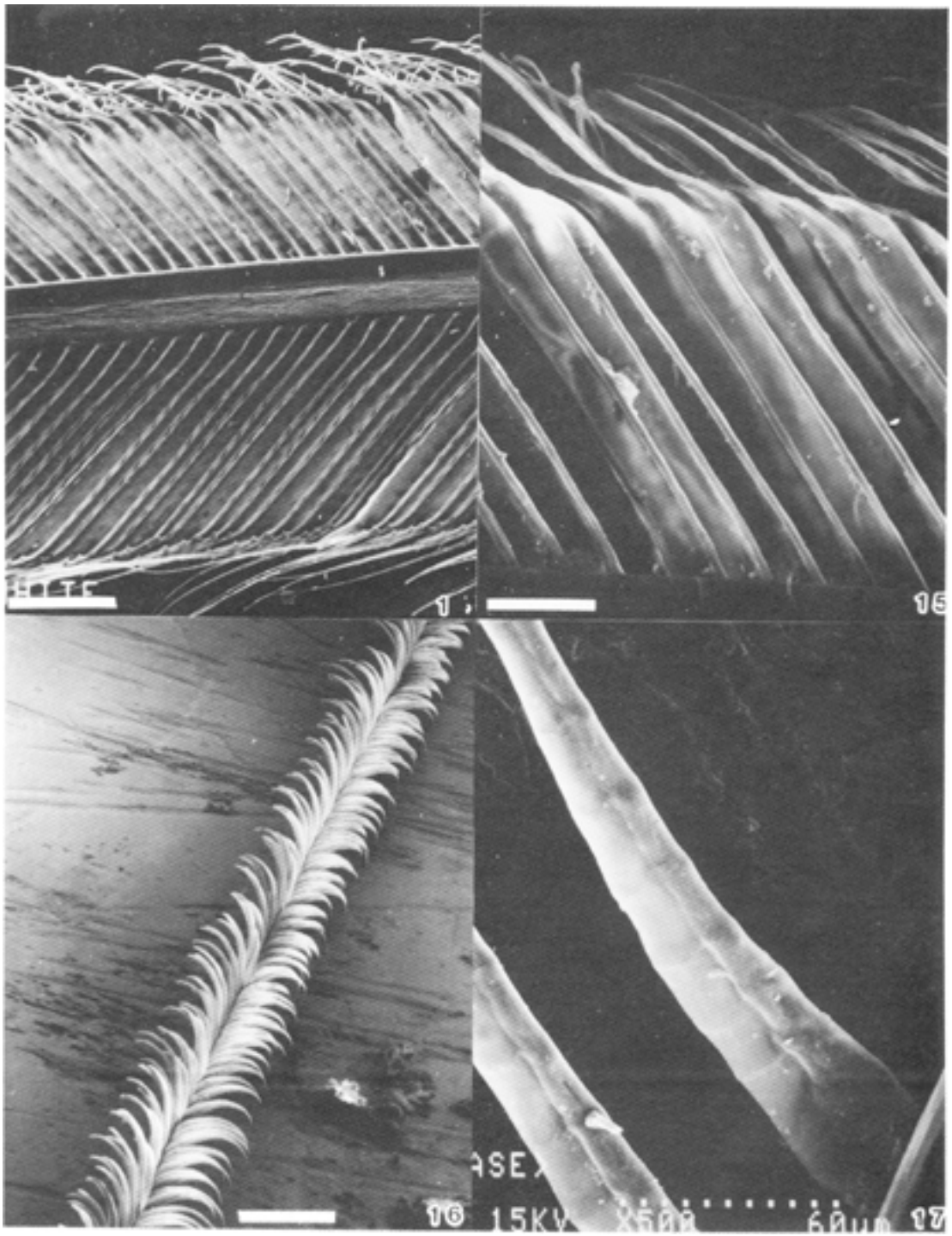


Fig. 8. Schematic reconstruction of the three-dimensional organization of structural color in the quetzal (based on Figs. 1-7 of this paper and on transmission electron micrographs by Durrer and Vifliger 1966). The external face of each barb1e has six subjacent layers of oval melanin platelets. The platelets are conposed of ordered melanin grains with an air core. The back of the barbule (below in detail at right) has scattered melanine grains which prevent additional light from polluting the green coloration.



Figs. 9-13. Brown (pigment colored) barbules and interface with green (stnicturally colored) barbules. 9. Brown barbules (bar 30 μm). 10. Detail of flaps in brown barbules (bar 15 μm). 11. In the interface, the flapped brown barbules (lower half) change gradually (middle) to the segmented green barbule shape (upper half) (bar 0.1 mm). 12. Detail of interface (bar 20 μm). 13. Change of barbule shape in the interface (bar 15 μm). Arrows indicate lateral flaps in Figs. 9, 10 and 12.



Figs. 14-17. Other pigment colored barbules. 14. Overview of white barbules (bar 30 μm). 15. Detail of white barbule flaps (bar 60 μm). 16. Overview of red barbules (bar 02 mm). 17. Detail of red barbules near base.

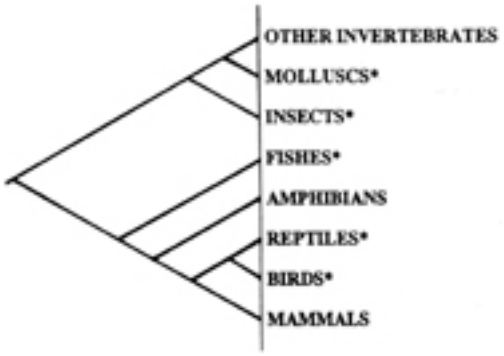


Fig. 18. Occurrence of iridescent coloration (asterisks) in the Metazoa (from data in Simon 1971).

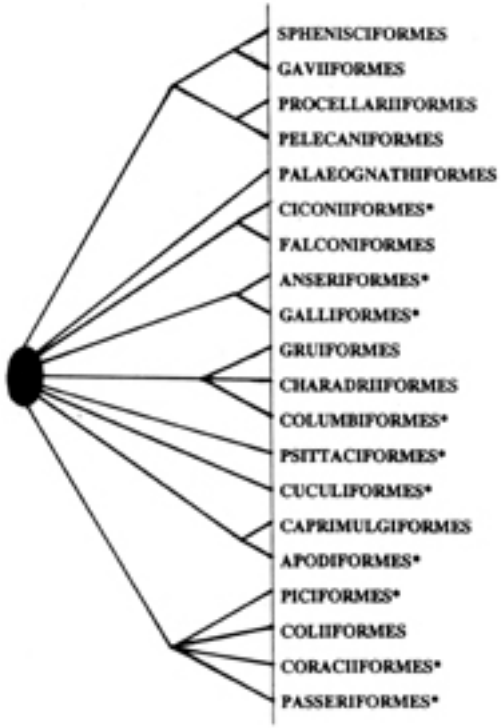


Fig. 19. Occurrence of structural coloration (asterisks) in the Neomithes (Class Ayes). Cladogram based on a classification by Cracraft (1981). Occurrence from Durrer and Villiger (1966), Dick (1971), Simon (1971), Lucas and Slettenheim (1972) and Gill (1990), and from personal communications by F.G. Stiles and G. Barrantes.

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RESUMEN

El quetzal, *Pharomachrus mocinno,* es un ave neotropical con coloración estructural verde, la cual parece ser críptica. Un estudio con microscopio electrónico de barrido mostró que las bárbulas verdes tienen forma de gruesas cintas segmentadas que se vuelven ligeramente sobre el eje longitudinal y terminan en un filamento bífido o de tres puntas. Las bárbulas pigmentadas son más simples y pueden tener aletas laterales. En la interfase cafe-verde, las barbulas cambian gradualmente de un tipo a otro. Aunque el mecanismo de coloración es interno, tiene una influencia significativa en la forma general de la pluma, la cual está limitada por la selección hacia mayor resistencia y termorregulación. Estos resultados y simulaciones de computadora apoyan el modelo de interferencia de aire-queratina-melanina. Se esperaría que exista una organización regular de la melanina y poco entrelazamiento entre bárbulas cuando la selección natural favorezca coloración opaca, por ejemplo, debido a fuerte presión de los depredadores. La capacidad de producir iridiscencia parece ser antigua en los metazoos y haber sido perdida en anfibios y mamíferos. Se sugiere que hay convergencia evolutiva en el color estructural de las aves porque los mecanismos específicos no corresponden con la filogenia.

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