BRIEF COMMUNICATION

Complex Plumage Patterns Can Be Produced Only with the Contribution of Melanins

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ABSTRACT

Birds exhibit an extraordinary diversity of plumage pigmentation patterns. It has been overlooked, however, that complex patterns can be produced only with the contribution of melanins because these are the only pigments under direct cellular control. We tested this hypothesis for the first time examining the plumage patterning of all species of extant birds. Thirty-two percent of species show complex plumage patterns, the vast majority (98%) including the contribution of colors produced by melanins. Only 53 species show complex patterns that do not contain melanin-based colors, and these species display unusual colorations and belong to three families where innovative metabolic modifications of conventional carotenoid pigments have been described. While the adaptive functions of complex plumage patterns remain poorly understood and in most cases are ascribed to fulfill camouflage, our findings indicate that such functions will be understood only by considering the synthesis pathway of melanins.

Keywords: feathers, melanins, pigmentation patterns, plumage coloration.

Introduction

Attempts to explain the extraordinary diversity in animal pigmentation across species have been made since the beginning of studies on evolution and adaptation, especially in birds (Darwin 1871). Yet few rules applicable to a broad taxonomic scale have been elucidated in birds (Galván et al. 2013; Dale et al. 2015). The most striking variation in coloration concerns differences in the appearance (hue) of colors (e.g., black, orange), and, not surprisingly, most studies have dealt with the occurrence of colors among species. However, the combination of different colors within or between feathers produces complex pigmentation patterns that are also extraordinarily diverse even if the pigment palette is very restricted. Pigmentation patterns predominantly occur as mottles, scales, bars, and spots, with different degrees of saturation in the rather achromatic scale of the melanins (mainly black and different shades of brown and gray; Somveille et al. 2016). Although being traditionally assumed to have evolved in response to camouflage needs (Cott 1940), recent analyses show that their diversity is not explained by common evolutionary pressures, and thus, it remains a mystery (Somveille et al. 2016).

The evolution of pigmentation patterns may be better understood by the mechanisms of color production, as these determine the physiological constraints that lead to natural selection's favoring or limiting different color types (Galván and Solano 2009). Plumage coloration is mainly achieved by two classes of pigments (melanins and carotenoids) and by specialized feather nanostructures (structural colors; Maia et al. 2011). Particular groups of birds, such as the Psittaciforms or the Turacos (family Musophagidae), have also evolved exclusive pigments (psittacofulvins and turacin, respectively). Plumage pigmentation patterns, however, do not seem to comprise colors produced by all these mechanisms. Here we postulate a largely overlooked observation regarding plumage patterns: they are apparently produced by colors conferred by melanins (for a description of colors produced by natural melanins, see Galván and Wakamatsu 2016) but not by colors conferred by carotenoids (McGraw 2006), nanostructures (Maia et al. 2011), or the group-specific pigments such as psittacofulvins, turacin, or porphyrins (Galván et al. 2016). This difference is key for understanding the pressures that allow or constrain the development of plumage patterns, as while carotenoids are photosynthetic pigments that birds obtain from dietary sources and are transported with lipoproteins through the bloodstream to the follicles of growing feathers (McGraw 2006), melanins are endogenously synthesized in specialized cells (i.e., melanocytes; Lin et al. 2013). This means that the expression of melanin-based pigmentation is more directly controlled by birds than the expression of carotenoid-based pigmentation, as the former depends on a cellular control that produces the pigments while the latter depends on the capacity to uptake circulating pigments. Although birds are able to chemically transform uptaken carot-

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enoids at feather follicles to change their optical properties (McGraw 2004), this metabolization may occur mainly in the liver and not at the target tissues (Del Val et al. 2009), and, most importantly, birds are always limited by the form and abundance of the ingested carotenoids, as no carotenoids are synthesized de novo. The organization of specialized feather nanostructures that produce structural colors is not under direct cellular control, either, but responds to an entropically driven self-assembly during feather development (Maia et al. 2011). Therefore, melanin-based pigmentation is the only mechanism of bird color production that occurs under direct cellular control, hence holding the greatest potential (or possibly the only way) to generate complex pigmentation patterns. In fact, it has been shown that birds produce complex plumage patterns by modulating the presence, arrangement, or differentiation of melanocytes in the developing feather follicles (Lin et al. 2013).

However, it has never been clearly suggested that the developmental control of melanin-based pigmentation leads to the observed diversity of bird plumage patterns at a broad taxonomic scale, contrary to all other mechanisms of color production that alone cannot lead to similar patterns and just broadly stain relatively large areas of individual feathers, with no fine modulation and thus precluding delineation or spotting within feather vanes. Our aim here is to test, by examining the appearance of all species of extant birds, the hypothesis that complex plumage patterns are produced only with the contribution of melanin-based pigmentation.

Methods

Definition of Complex Plumage Pattern

Although bird plumage patterns converge on four types of spatial arrangement of pigmentation (Somveille et al. 2016), a particular definition of complex pattern must be defined to differentiate from simpler combinations of colors. We defined a complex plumage pattern as a combination of two or more discernible colors (to the human eye) that occur spatially more than two times uninterrupted. These patterns can occur entirely within single feathers or arise by the combination of more than two adjacent feathers. We therefore excluded combinations of similarly colored adjacent feathers if the spatial arrangement of the distinct color patches makes them appear as a single combination of colors, not a repetition of this combination (fig. 1). Although other definitions of complex plumage patterns are probably possible, our definition certainly refers to plumage patterns that are not simple and is largely similar to the definition in the recent study by Somveille et al. (2016) and appropriate for comparative purposes.

Data Collection

We examined illustrations of 9,049 species of birds in *Handbook of the Birds of the World* (del Hoyo et al. 1992–2002, 2003–2010), thus comprising the entire class Aves according to these authors (although the total number of extant species is probably higher according to other sources). Only illustrations of adult birds were examined. For each species, we recorded the presence or absence of complex plumage patterns in both males and females. If present, we determined whether the colors that the complex patterns are composed of are produced by melanins. The palette of plumage colors generated by the different chemical forms of melanins (eumelanin and pheomelanin) is known to comprise different intensities of black, gray, brown, and orange (Galván and Wakamatsu 2016); thus, we considered that a complex plumage pattern had the contribution of melanin-based pigmentation when it contained any of these colors or was based on any of the two other main mechanisms of color production in birds (i.e., carotenoid based or structural) when other colors were present. This human-based visual recognition of plumage colors produced by melanins reports little error in comparisons between species (Galván and Wakamatsu 2016). It must be noted that structural colors (i.e., those produced by ordered feather nanostructures) can be produced with no contribution of melanins (Igic et al. 2016), but most structural colors require layers of melanin granules interacting with the feather nanostructures (Li et al. 2005). This means that our exclusion of structural colors from the category of complex plumage patterns produced by the contribution of melanins is conservative, as many structural colors are actually produced by the contribution of melanins.

In addition to melanins and carotenoids, bird feathers possess other less common classes of pigments, most notably, psittacofulvins and porphyrins. However, psittacofulvins are limited to parrots (order Psittaciformes; McGraw and Nogare 2005), among which we did not find any complex plumage pattern (table S1), and porphyrins do not produce permanent plumage coloration (Galván et al. 2016). Thus, our results are not influenced by the presence of pigments different from melanins and carotenoids.

The species in which we found complex plumage patterns with no contribution of melanins (see below) exhibit unusual colorations among birds (fig. 2). For one of these species (the yellow-billed stork [*Mycteria ibis*]), we obtained two scarlet wing feathers of the complex wing pattern (fig. 2) from a specimen (reference MNCN-A1290) deposited in the bird collection of the National Museum of Natural Sciences–Consejo Superior de Investigaciones Científicas (MNCN, Madrid, Spain), to test whether this color was produced by porphyrins or carotenoids. We first examined the feathers under UV light and found no red fluorescence, suggesting that porphyrins were absent (Galván et al. 2016). Then we cut the portion of the feathers exhibiting scarlet coloration, separated the barbs from the rachis, and treated them with cold N,*N*-dimethylformamide. From this treatment of feathers we obtained a yellow extract, indicating that the scarlet color is produced by carotenoids (Del Val et al. 2009).

Results

We found complex plumage patterns (fig. 1) in 2,918 out of 9,049 species examined (32.2%), comprising 16 different orders and 137 families (table S1). Among those species with complex patterns, 2,866 (98.2%) were species in which patterns had the contribution of melanin-based colors, while 53 species had complex patterns in which melanin-based colors were absent. These 53 spe-

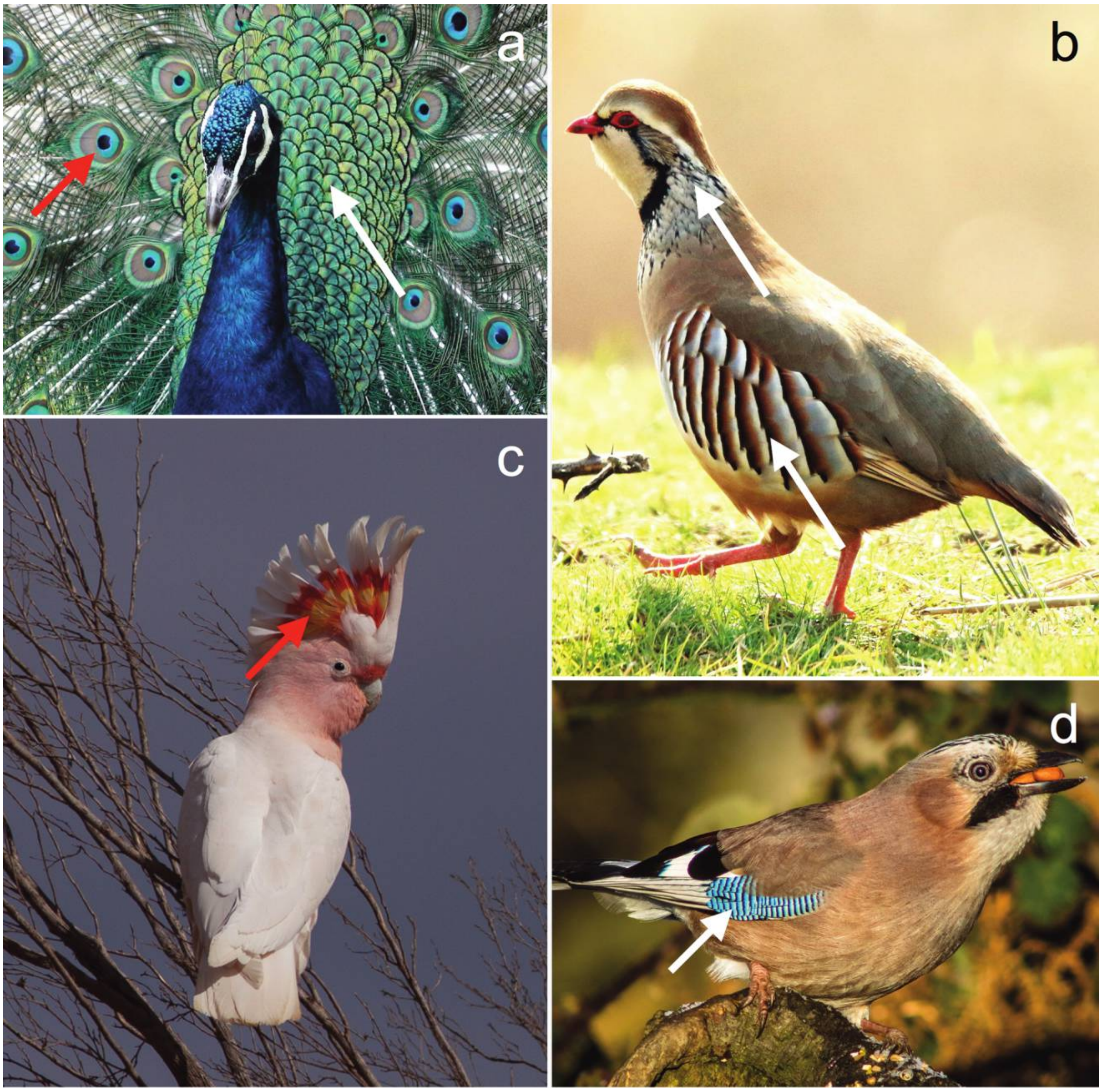

Figure 1. We defined a complex plumage pigmentation pattern as a combination of two or more discernible colors that spatially occur more than two times uninterrupted, arising within single feathers or by the combination of more than two adjacent feathers. Shown are examples of complex and noncomplex pigmentation patterns. *a*, In the Indian peacock (*Pavo cristatus*), each uppertail covert feather (white arrow) is composed of a brown patch around the central rachis, a green inner patch, and a black border. Brown and black colors are produced by melanins (Galván and Wakamatsu 2016), while the green color is produced by feather nanostructures (Maia et al. 2011). It must be noted that the brown color of peacocks is produced by an ordered feather nanostructure but including layers of melanin granules (Li et al. 2005). Thus, the succession of adjacent uppertail covert feathers forms a complex plumage pattern produced by melanin-based colors (brown and black) in combination with a structural color (green). Therefore, this is a melanin-based complex pattern. A similar pattern exclusively composed of different structural colors would be complex but not melanin based. By contrast, the combinations of colors in the rectrix feathers (ocelli; red arrow) do not form a complex pattern, as each combination appears in spatially separated feathers (i.e., there is no uninterrupted repetition of color combinations). Photograph credit: John Clouston (https://flic.kr/p/9ZqRoE). *b*, In the red-legged partridge (*Alectoris rufa*), each flank feather (bottom white arrow) is composed of the following combination of colors (from base to tip): gray, white, black, and brown. Gray, black, and brown colors are all produced by melanins, and therefore the succession of adjacent flank feathers forms a melanin-based complex plumage pattern. Similarly, the succession of black-and-white neck and breast feathers (upper white arrow) also forms a melanin-based complex pattern. Photograph credit: Nick Goodrum (https://flic.kr/p/EZ9ecn). *c*, In the major Mitchell's cockatoo (*Lophochroa leadbeateri*), each crest feather (red arrow) is composed of the following combination of colors (from base to tip): red, yellow, red, and white. Red and yellow colors are produced by carotenoids, but the spatial arrangement of these feathers forms a continuous succession of color patches between feathers. Thus, there is no repetition of the red-yellow-red-white combination, as all crest feathers appear as a single combination of these colors. Photograph credit: Ron Knight (https://flic.kr/p/diY3sm). *d*, The blue-and-black bands in the covert wing feathers (white arrow) of the Eurasian jay (*Garrulus glandarius*) form a complex pattern. Although blue is a structural color, black is produced by melanins, and therefore this complex pattern is melanin based. Photographs *a*–*c* are under a CC BY 2.0 license (https://creativecommons.org/licenses/by/2.0/).

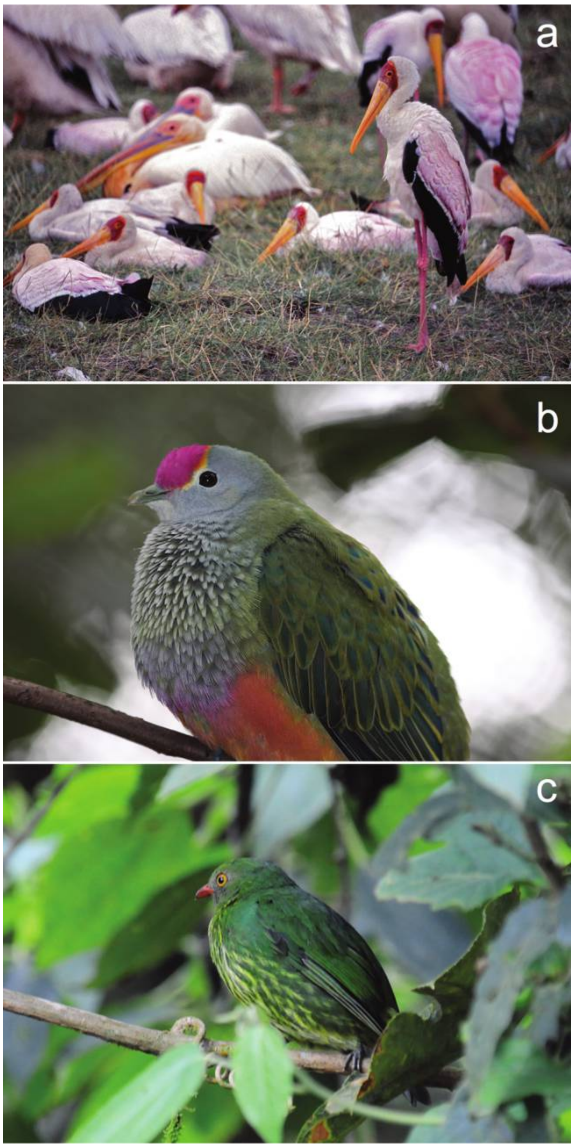

Figure 2. Species with complex plumage patterns produced without the contribution of melanins: *a*, yellow-billed stork (photograph credit: Bernard Dupont; https://flic.kr/p/dCJkVo); *b*, rose-crowned fruit dove (*Ptilinopus regina*; photograph credit: David Lochlin; https://flic.kr/p/bNqVbT); *c*, orange-breasted fruiteater (*Pipreola jucunda*; photograph credit: Vince Smith; https://flic.kr/p/82VV8P). Photograph *a* is under a CC BY-SA license (https://creativecommons.org/licenses/by-sa/2.0/), and photographs *b* and *c* are under a CC BY 2.0 license.

cies belong to three families: Ciconiidae (one species; yellow-billed stork), Columbidae (37 species; genera *Ptilinopus* and *Drepanoptila*), and Cotingidae (15 species; genera *Pipreola* and *Procnias*; table S1). Given this limitation of complex plumage patterns with no contribution of melanins to three families of birds, phylogenetic analyses are not necessary.

Discussion

As predicted, we found that virtually all complex pigmentation patterns in the plumage of birds have colors that are produced by melanins. We found complex plumage patterns produced by mechanisms other than melanins in only 53 species from three families, most of them being fruit doves. These birds present very unusual plumage colorations such as purple or scarlet of nonmetallic appearance (del Hoyo et al. 1992–2002, 2003–2010; fig. 2), not likely to be produced by ordered feather nanostructures (Maia et al. 2011). A similar purple coloration is exhibited by the yellow-billed stork, the only species of the family Ciconiidae with a complex plumage pattern (fig. 2). It has been reported for other species with such unusual purple or scarlet colorations that they are produced by some modifications of the vibrational characteristics of the interaction between conventional carotenoid molecules and feather proteins (Mendes-Pinto et al. 2012). Indeed, we obtained evidence that the scarlet feathers of the yellow-billed stork contain a yellow carotenoid. Last, we also found complex plumage patterns where melanin-based colors were absent in 15 species of cotingas, which exhibit combinations of green and yellow colors typically conferred by carotenoids (fig. 2). However, carotenoid-based plumage coloration in cotingas is also due to some metabolic modifications of conventional carotenoids that produce unusual vivid colors (Prum et al. 2012). Therefore, it is likely that the few exceptions of complex plumage patterns with no contribution of melanin-based colors are also due to exceptional innovations in the metabolism of carotenoids. This suggests that these evolutionary innovations allow birds to produce complex plumage patterns with pigments (i.e., carotenoids) that normally cannot form such patterns.

With the exceptions mentioned above, the vast majority of complex plumage patterns are produced with the contribution of melanins. The proportion of species with complex plumage patterns produced with the contribution of melanins is so large (98.2%) and the exceptions are so limited taxonomically (three families) that no statistical or phylogenetic analyses are necessary to conclude that melanins form the basis of complex plumage patterns in birds. As other authors have shown (Lin et al. 2013), birds are able to take precise control of the pigmentation by melanins in growing feathers. This is possible because melanins are synthesized in specialized cells; thus, modulating the spatial arrangement, the activation, and the activity (i.e., melanin forms that are synthesized) of melanocytes allows them to exert a direct cellular control of melanin deposition in each individual feather follicle (Lin et al. 2013). Our study shows that this mechanism is in fact essential to produce complex pigmentation patterns, as these cannot be produced by exclusively using carotenoids or specialized nanostructures, which generate colors through processes that are not under direct cellular control (McGraw 2006; Maia et al. 2011).

Our findings imply that any functions that complex plumage patterns may fulfill in birds will be related to the synthesis of melanins. These functions are poorly understood, but recent analyses show that these do not play a role in crypsis or camouflage as previously thought (Somveille et al. 2016). Alternatively, complex plumage patterns may convey information about individual attributes and have a role as sexual signals (Bortolotti et al. 2006). This potential signaling function of complex plumage patterns deserves further investigation, and our study indicates that this investigation should consider how the synthesis pathway of melanins determines the information content of melanin-based signals (Galván et al. 2015).

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