Not all Red, Orange, and Yellow Colors are Carotenoid-based: The Need to Couple Biochemical and Behavioral Studies of Color Signals in Birds

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Introduction
Ornamental traits in animals, including elaborate vocalizations and bright coloration, have long been known to serve valuable roles in communicating an individuals worth as a rival or mate (reviewed in Andersson 1994). Behavioral ecologists and evolutionary biologists are now faced with the challenges of determining what types of information animals communicate with their signals, how this information is kept reliable, and to what set(s) of individuals the information is being communicated (Maynard Smith & Harper 2003). The colors of animals, particularly in birds and fishes, have emerged as a model system for investigating the information content, honesty, and efficacy of biological signals. Different color types, from pigment-based carotenoid and melanin coloration to structurally based color traits, are produced by a suite of biochemical and physical mechanisms, each of which entails unique means and costs of production and maintenance and thus presents the opportunity to reveal disparate sets of information to different receivers (McGraw & Hill 2000, McGraw et al. 2002, Senar et al.
example of an exaggerated sexual trait that confers mating advantages (Møller 1994, 2001). Male and female barn swallows also display rufous patches of plumage (figure 1a) that have received recent attention as reliable signals of quality. Males are more colorful than females (Ninni 2003), and males with darker color patches have longer tail streamers, high concentrations of carotenoids in blood (Saino et al. 1999, Camplani et al. 1999), and are more likely to find a mate than less colorful males (Ninni 2003). There also is reduced color intensity (Camplani et al. 1999) and an increase in partial albinism of throat plumage from swallow populations exposed to the Chernobyl nuclear disaster (Møller & Mousseau 2001, 2003). In sum, these lines of evidence have been used to support the idea that plumage color is a condition-dependent sexually selected trait (Saino et al. 1999). In doing so, researchers have pointed to the carotenoid-based origin of feather coloration as the hallmark of this honest signaling system. Carotenoids act as free-radical scavengers and immunostimulants (Møller et al. 2000) and thus can be used to fend off environmental challenges like radioactive contamination, maintain good condition, as well as signal health in the form of bright coloration to prospective mates (Camplani et al. 1999, Saino et al. 1999).

The problem with this inference, however, was that evidence for the predominance or presence of carotenoids in rufous barn swallow feathers was equivocal. Saino et al. (1999) claimed that melanin pigments conferred most of the brown hue in plumage, but that small amounts of a yellow carotenoid, lutein, were also present. Yet in a book published a year earlier, Stradi (1998) reported a suite of yellow and red carotenoids from these feathers. Until recently, this uncertainty over the pigmenary basis of barn swallow coloration remained unresolved. McGraw et al. (2004a) subsequently analyzed feathers of males and females from a free-ranging population of *H. r. rustica* in Scotland and detected no carotenoids. Instead, high concentrations of the two main types of melanin pigments in birds—phaeomelanin and eumelanin—were found. Barn swallows from the North American subspecies (*H. r. erythrogaster*) also pigment their sexually selected ventral plumage (Safran & McGraw 2004) with melanins only (McGraw et al. 2004b). These new results force us to re-visit the honesty-reinforcing mechanisms of rufous coloration in barn swallows and ask what costs and benefits are reaped from accumulating melanin pigments, not carotenoids, in the body and depositing them in feathers for signaling.

**Yellow and Orange Plumage Coloration in Penguins**

Beyond the basic black-and-white plumage appearance
that typifies penguins, some of the world’s penguin species also display patches of yellow or orange feathers. King (Aptenodytes patagonicus) and emperor (Aptenodytes forsteri) penguins, for example, acquire yellow-orange auricular and breast plumage (Figure 1b). Macaroni (Eudyptes chrysolophus) and yellow-eyed penguins (Megadyptes antipodes) grow yellow or orange post-orbital plumes (figure 1c). Historically, the bright colors of penguins have been largely ignored in studies of sexual selection and communication. Studies where ornamental plumage features were blackened (e.g. in A. patagonicus) or trimmed (e.g. in E. chrysolophus) showed reduced mating success for less colorful penguins (Jouventin 1982), but only recently have studies of natural color variation been conducted. Massaro et al. (2003) investigated the fitness correlates of colorful postocular feathers in M. antipodes and found that yellower male and female penguins were older, in better body condition, assortatively mated with respect to their color, and fledged more chicks in a year. They went on to discuss these colorful ornaments as being ‘carotenoid-based’ and interpreted their costliness and signaling roles based on the environmental limitations and health functions of carotenoid pigments (Møller et al. 2000). This was despite the fact that several other classes of pigments can confer these same colors in birds (e.g. pterins, phaeomelans; Needham 1974) and that they provided no biochemical evidence for the use of carotenoids in feathers.

More recent work on the biochemical nature of yellow and orange plumage in other penguins has shown that carotenoids do not confer such plumage colors (McGraw et al. 2004b). In king and macaroni penguins, yellow color in feathers is derived from an as-yet undescribed class of pigments in plumage that fluoresces under UV light and can be extracted from feathers with a mild acid or base (McGraw et al. 2004b). These pigments likely are or are related to pterin pigments that color the eyes of certain birds (Oliphant 1987, see more below), the skin of poikilothermic vertebrates (Bagnara & Hadley 1973), and the wings and eyes of butterflies and other insects (Pfleiderer 1994). These findings beg the question of whether yellow-eyed penguins also do not pigment their plumes with carotenoids. To test this, I obtained a sample of yellow postocular feathers from an adult male yellow-eyed penguin skin housed at the American Museum of Natural History (specimen # 525847) and subjected these feathers to biochemical tests used to identify carotenoid pigments (see methods in McGraw et al. 2003b). High-performance liquid chromatographic (HPLC) analyses showed that lipid extracts from yellow feathers of M. antipodes contained no carotenoids (at a detection limit of 5 mg pigment/g pigmented feather barbule). Instead, as in other penguins, yellow plumage pigments were soluble in mild acids (e.g. acidified ethanol) and bases (e.g. sodium hydroxide), showed strong ultraviolet-light absorbance, and in particular fluoresced under long-wave ultraviolet light (figure 1d). Ultimately, this new information on feather pigmentation does not invalidate the basic correlations found between color expression and mate quality by Massaro et al. (2003), but it does shift our focus away from the characteristics of carotenoids and instead towards the production mechanisms and biochemical action of these new yellow compounds to understand the function of and information contained within the colorful traits of penguins.

**Red and yellow epaulet coloration in red-winged black birds**

The red-winged blackbird (Agelaius phoeniceus), like the barn swallow, is one of the most studied avian species on the topic of sexual selection (Searcy and Yasukawa 1995, Beletsky 1996). Their territorial behavior, complex vocalizations, and colorful epaulets have been the targets of most such studies. Compared to females, males have larger and brighter patches of shoulder plumage that contains a primary region of red feathers (the upper marginal coverts) that is bordered by yellow upper lesser coverts. Many researchers have attributed these colors to the presence of carotenoids (Miskinnen 1980, Irwin 1994, Johnson and Lanyon 2000, Pryke and Andersson 2003), but the pigments in red and yellow A. phoeniceus feathers were not yet characterized at the time these claims were made. This is notable because the signaling information and function of the redwing epaulet have stood out as clear exceptions to those for avian carotenoid-based colors generally (Hill 2002). Epaulet characteristics are uncoupled from an individual’s health state (Weatherhead et al. 1993, Westneat et al. 2003), and females consistently fail to show mating preferences for the most colorful males (Searcy & Yasukawa 1996).

McGraw et al. (2004c) undertook a biochemical investigation into the pigments that color redwing epaulets and in fact described substantial concentrations of hydroxy- and o xo-carotenoids from red feathers (e.g. lutein, zeaxanthin, canthaxanthin, astaxanthin). However, these red feathers retained a deep brown hue after carotenoids were extracted (figure 1e) and were found to contain comparable amounts of brown melanins that contribute color to the feathers. In contrast, yellow feathers derived none of its base color from carotenoids and instead contain unusual amounts of

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phaeomelanin, to give them the buff appearance that is much like human blonde hair and yellow mouse fur (Ito and Wakamatsu 2003). Ultimately, this fresh perspective on epaulet pigmentation sheds new light on the value and control of color elaboration in this species. With the occurrence of melanin comes the notion that many sexually dichromatic melanic colors in birds function as valuable signals of aggression and social status (Senar 1999), which is unlike many purely carotenoid-colored traits. It is interesting then that, rather than serve a role in mate attraction, epaulet coloration in red-winged blackbirds successfully mediates competitive interactions between males (Røskaft and Rohwer 1987). The co-occurrence of carotenoids and melamins in these feathers also makes me wonder how many other color traits are similarly derived, and for which perceptions of signal utility and content are clouded because of it.

Figure 1. Photographs illustrating some of the colorful traits described in this article: (a) rufous plumage in barn swallows, (b) orange auricular and breast plumage in king penguins, (c) orange forehead plumes in macaroni penguins, (d) UV-fluorescent characteristics of orange feathers from yellow-eyed penguins, as determined at l = 366 nm using a Minerelight lamp, model UVCL-58, UVP Inc., San Gabriel, CA, and (e) red epaulet feathers from red-winged blackbirds before carotenoid extraction (left) and after (right; note the retained brown hue).
Yellow to red mouth flanges and flushes of nestling birds

Though most of the bright colors we study in the context of signaling are found in adults, in certain situations immature birds may also benefit by developing colorful traits for use in communication. Nestling birds in many species often show richly colored and patterned mouths. This can include keratinized mouth parts like the rictal flanges and the roof of the mouth, to the more fleshy parts containing skin. Colors can even change over a rapid time-course, like the well-known mouth flush that occurs as many nestlings vigorously beg for food from parents. Biologists have been interested in the information encoded in plastic traits like the mouth flush, as offspring may communicate to parents their fluctuating levels of hunger or health (Kilner 1997, Saino et al. 2003). Remarkably, no studies to my knowledge have biochemically identified the pigments that give rise to these red-, orange-, or yellow-colored mouth parts. Still, many have been quick to assign a pigment class to nestling mouth colors, as either carotenoid-based for some of the yellow features or derived from the red color of blood and hemoglobin from vascularized tissues (Hunt et al. 2003), again so that inferences can be drawn about the costs and benefits of coloration from the underlying physiological roles of the pigments. Some studies have even interleaved these two color- and pigment categories, suggesting that carotenoids determine red gape color (Saino et al. 2000). This assignment in nestling barn swallows was based on a feeding study where dietary supplementation of lutein reddened the gape; however, without chemical analyses one cannot disentangle a health-mediated effect of this treatment on coloration from a direct pigmenting effect. Ultimately, it is due time that we carefully and separately analyze the pigments from all regions and types of colorful mouth parts in birds so that we can begin to draw informed conclusions about their nature and function.

Conclusions
From this collection of recent examples and studies, it is clear that, despite over a century of work on the chemical and physical basis of animal coloration (e.g. Fox and Veveres 1960, Fox 1976), we still have quite a rudimentary understanding of and appreciation for the diversity of pigment-based mechanisms by which birds can become colorful. New analytical methods such as HPLC that have been made more accessible to many behavioral ecologists and evolutionary biologists now provide the opportunity to prevent further misuses in pigment classification and to advance our understanding of color production and evolution at unprecedented rates. This is true not only for the few main, well-known groups of integumentary colorants in birds, but also for the several other pigment types that confer some of the very same hues that the common carotenoids and melamins do. These chromatographic techniques can also diagnose the presence of red to yellow pterin pigments in bird eyes, fish, amphibian, and reptile skin, and invertebrate wings (Oliphant and Hudon 1993), the novel red to yellow psittacofulvins that parrots (Aves, Psittaciformes) manufacture and deposit in feathers (Stradi et al. 2001, McGraw and Nogare 2004), as well as the unique copper-containing turacins used by turacos (Aves, Musophagiformes) to color their plumage red (With 1957, Moreau 1958). Even the historically well-studied chromatophore systems of poikilo therm should be revisited to improve our grasp of the pigments contained within the cell types we still only classify based on color appearance (e.g. xanthophores, erythrophores). It is from these and now other systems that we gain an appreciation for the ability of multiple pigment types to confer color within a single patch of tissue (Grether et al. 2004), and this notion should keep us from being so quick to assign pigment types, let alone a single pigment type, to colors without ample chemical evidence.

In conclusion, the excitement over the evolutionary and physiological significance of colorful traits at the level of the individual molecule is much welcomed, but it must come with a hint of caution and a vested biochemical approach if we are to progress and not regress in our levels of understanding of color signals in birds.

Reference
Camplani A, Saino N and Møller A P 1999 Carotenoids, sexual signals and immune function in barn swallows from Chernobyl; Proc. R. Soc. Lond. B 266 1111-1116
Fox D L 1976 Animal biochromes and structural colors; (Berkeley, CA: University of California Press)
Fox H M and Veveres G 1960 The nature of animal colours; (London: Sidgwick and Jackson Ltd.)
Grether G F, Kolluru G R and Nersissian K 2004 Individual colour patches as multicomponent signals; *Biol. Rev.* 79 583-610


Hunt S, Kilner R M, Langmore N E and Bennett A T D 2003 Conspicuous, ultraviolet-rich mouth colours in begging chicks; *Proc. R. Soc. Lond. B (Suppl.)* 270 S25-S28

Irwin R 1994 *The evolution of plumage dichromatism in the New World blackbirds: social selection on female brightness; Am. Nat.* 144 890-907

Ito S and Wakamatsu K 2003 Quantitative analysis of eumelanin and phaeomelanin in humans, mice, and other animals: a comparative review; *Pigment Cell Res.* 16 523-531

Johnson K P and Lanyon S M 2000 Evolutionary changes in color patches of blackbirds are associated with marsh nesting; *Behav. Ecol.* 11 515-519

Jouventin P 1982 *Visual and vocal signals in penguins: their evolution and adaptive characters*; (Berlin: P. Parey)

Kilner R 1997 Mouth colour is a reliable signal of need in begging canary nestlings; *Proc. R. Soc. Lond. B* 264 963-968

Massaro M, Davis L S and Darby J T 2003 Carotenoid-derived ornaments reflect parental quality in male and female yellow-eyed penguins (*Megadyptes antipodes*); *Behav. Ecol. Sociobiol.* 55 169-175


McGraw K J, Hill G E and Parker R S 2003b Carotenoid pigments in a mutant cardinal: implications for the genetic and enzymatic control mechanisms of carotenoid metabolism in birds; *Condor* 105 587-592

McGraw K J, Safran R J, Evans M R and Wakamatsu K 2004a European barn swallows use melanin pigments to color their feathers brown; *Behav. Ecol.* 15 889-891

McGraw K J, Wakamatsu K, Clark A B and Yasukawa K 2004c Red-winged blackbirds *Agelaius phoeniceus* use carotenoid and melanin pigments to color their epaulets; *J. Avian Biol.* (in press)


Møller A P and Mousseau T A 2001 Albinism and phenotype of barn swallows (*Hirundo rustica*) from Chernobyl; *Evolution* 55 2097-2104

Møller A P and Mousseau T A 2003 Mutation and sexual selection: a test using barn swallows from Chernobyl; *Evolution* 57 2139-2146


Moreau R E 1958 Some aspects of the Musophagidae. Part 3; *Ibis* 100 238-270

Needham A E 1974 The Significance of Zoochromes; (New York: Springer-Verlag)


Oliphant L W 1987 Pteridines and purines as major pigments of the avian iris; *Pigm. Cell Res.* 1 129-131

Oliphant L W and Hudon J 1993 Pteridines as reflecting pigments and components of reflecting organelles in vertebrates; *Pigm. Cell Res.* 6 205-208

Pfleiderer W 1994 *Nature pteridine pigments—pigments found in butterflies wings and insect eyes*; *Chimia* 48 488-489

Pryke S R and Andersson S 2003 Carotenoid-based status signalling in red-shouldered widowbirds (*Euplectes axillaris*): epaulet size and redness affect captive and territorial competition; *Behav. Ecol. Sociobiol.* 53 393-401

Roskaft E and Rohwer S 1987 An experimental study of the function of the red epaulets and the black body colour of the male red-winged blackbirds; *Anim. Behav.* 35 1070-1077
Safran R J and McGraw K J 2004 Plumage coloration, not length or symmetry of tail-streamers, is a sexually selected trait in North American barn swallows; Behav. Ecol. 15 455-461


Saino N, Stradi R, Ninni P, Pini E and Møller A P 1999 Carotenoid plasma concentration, immune profile, and plumage ornamentation of male barn swallows (Hirundo rustica); Am. Nat. 154 441-448


Senar J C 1999 Plumage colouration as a signal of social status; Proc. Int. Ornithol. Congr. 22 1669-1686

Senar J C, Figuerola J and Domenech A J 2003 Plumage coloration and nutritional condition in the Great Tit Parus major: the roles of carotenoids and melanins differ; Naturwiss. 90 234-237

Stradi R 1998 The Colour of Flight: Carotenoids in Bird Plumage; (Milan, Italy: Solei Gruppo Editoriale Informatico)


Weatherhead P J, Metz K J, Bennett G F and Irwin R E 1993 Parasite faunas, testosterone, and secondary sexual traits in male red-winged blackbirds; Behav. Ecol. Sociobiol. 33 13-23

Westneat D F, Hasselquist D and Wingfield J C 2003 Tests of association between the humoral immune response of red-winged blackbirds (Agelaius phoeniceus) and male plumage, testosterone, or reproductive success; Behav. Ecol. Sociobiol. 53 315-323