
Melanins, metals, and mate quality

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Many animals display large patches of black or brown color in their integument, which contain melanin pigments and function as important sexually selected indicators of mate quality. To date, however, the particular means by which melanin-based color ornaments are costly to produce and serve as honest advertisements have remained elusive. Here, I propose a novel biochemical mechanism by which melanin-based coloration may serve as a reliable indicator of quality, involving the interplay of metal ions. In animals and other biochemical systems, a series of scarce macro- and microminerals (e.g. Ca, Zn, Cu, Fe) obtained from the diet act as critical regulatory factors in the biosynthesis of eumelanin and phaeomelanin pigments, but can also be toxic to the body when accumulated in high concentrations. As large polymers with many functional (carboxyl) groups, melanin granules also bind these metals and store them in pigmented cells. Thus, by sequestering large deposits of melanin, often in dead tissue (as in hair or feathers), animals may directly reveal dietary access to these rare elements and the physiological protection they have afforded themselves from initially beneficial, but eventually damaging, high mineral concentrations.

Elaborate sexual ornaments in animals (e.g. antlers, long tails, brilliant colors) persist over evolutionary time because they are costly to produce or maintain and provide reliable information to conspecifics about the mate quality of individuals (Zahavi 1975, Kodric-Brown and Brown 1984). For some types of ornaments, the costs are quite clear; animals that use carotenoid pigments as yellow, orange, or red integumentary colorants, for example, must acquire sufficient pigments from the diet and properly process these chemicals physiologically before displaying maximum coloration (Hill 2002, Hill et al. 2002). For other sexual signals, the specific mechanistic requirements for developing the most exaggerated ornament have been more difficult to elucidate.

Many animals display conspicuous patches of black, brown and gray coloration, such as the dark wings of insects, black skin patches in fishes and lizards, and the dark feathers of birds (Fox and Vevers 1960). These colors serve important sexual-signaling functions in a variety of taxa (reviewed by Andersson 1994) and are derived from melanin pigments, which come in two primary forms (gray to black eumelanins and brown to

red phaeomelanins; Protá 1992). The black patch of throat plumage found in male house sparrows (*Passer domesticus*) is a classic example. Some of the nutritional (Gonzalez et al. 1999, McGraw and Hill 2000) and endocrinological (Evans et al. 2000, Korzan et al. 2000) components of melanin-ornament expression have been investigated in animals, but there is currently no consensus regarding the factor(s) that critically regulate how much melanin animals incorporate into their sexual ornaments. Moreover, these studies have largely overlooked the biochemical properties of melanin pigments and of the particular molecules that facilitate melanin-pigment production.

Here, I propose a novel biochemical mechanism by which melanin-based color ornaments may represent a costly and honest signal of quality, involving the accumulation, stimulatory action, and eventual detoxification of metal ions. It should be noted from the outset that nearly all of the hypotheses and data presented here that link metals and melanins are based on *in vitro* biochemical studies, or from *in vivo* studies of humans and other mammals. As such, there is currently no empirical support for any of these ideas in animal species that display sexually selected melanin pigmentation. However, I see this as both an opportunity to integrate information across disciplines on the biochemical and molecular properties of melanins as well as to offer ideas for future work on this new line of research.

Metals and the synthesis of melanin pigments

Animals manufacture melanins from amino-acid precursors, such as L-tyrosine, within specialized pigment cells known as melanocytes (Protá 1992, Riley 1997). The critical, rate-limiting steps of melanin biosynthesis involve the enzyme tyrosinase, which catalyzes the initial events of melanogenesis, namely the oxidative conversion of tyrosine to the intermediate product, dopaquinone (Hearing and Tsukamoto 1991, Sanchez-Ferrer et al. 1995). Among non-human animals, the

central role of tyrosinase activity has been demonstrated for the melanins in mammalian fur (Wierzbicki 2000), fish scales (e.g. medaka; Inagaki et al. 1994), and feathers in chickens (Ferguson and Kidson 1997) and other colorful birds (e.g. weaverbirds, Hall 1966). Recently, however, other non-enzymatic regulatory factors have been identified, specifically involving the stimulatory action of metal ions (Prota 1993).

A variety of metals are found in animal diets and perform a suite of critical, biological functions (Bogden and Klevay 2000). These metals can be divided into two main categories: macrominerals (such as Ca, Na, K, and Cl) and microminerals (or trace minerals, such as Se, Fe, Zn, Cu, and Mn), distinguished by the relative amounts needed by the body (large and small, respectively). In addition to their very broad, homeostatic and health functions, these metals can have more subtle effects on cellular activity. Relevant to this article, the *in vitro* formation of intermediate products leading up to eumelanin (e.g. dopachrome) and pheomelanin (e.g. 5-S-cysteinyl-dopa) pigments is activated by the presence of metal ions, such as Ca, Fe, Zn, and Cu (Gallas et al. 1999, Di Donato et al. 2002). The mode of action is thought to be via the molecular rearrangement of dopachrome to other intermediates (5,6-dihydroxyindoles; Napolitano et al. 1985a, Palumbo et al. 1987) and the subsequent oxidative polymerization of these indoles to melanins (Napolitano et al. 1985b, Pezzella et al. 1996). Metal-ion status may also modulate tyrosinase activity itself (Palumbo et al. 1985, 1990), which may be due to its copper-containing molecular structure (Prota 1992) or to a Ca-mediated cyclic AMP pathway (Buffey et al. 1993). While the general effect of trace minerals on melanin pigmentation was noted over 50 years ago (Lerner and Fitzpatrick 1950), this process is now known to occur specifically within human (Carsberg et al. 1995), murine (Shibita et al. 1993), and avian melanocytes (Bowers et al. 1997).

Costs of metal-ion accumulation

So while the benefits of acquiring minerals for pigimentary purposes are clear (directly for stimulating melanin production, and perhaps indirectly for maintaining overall health), might there be difficulties in or risks with acquiring high concentrations of these metal ions? In fact, these elements cannot be produced by animals, are often in high physiological demand, and yet occur in relatively low concentrations in the diet. The alkaline earth metal, Ca, for example, is perhaps the most challenging mineral in the body, as it plays a critical role in processes such as skeletal mineralization, egg-shell formation, and cell-to-cell signaling, but is typically deficient in grains and insects and abundant only in inorganic matter (e.g. limestone, grit) and calcified

animal parts (e.g. bones, shells; Sigel 1984a, Granger 1986, Schulkin 2001). Although animals require less of the microminerals Zn, Cu, Fe to serve their several physiological functions (e.g. enzyme cofactors and components), they too can be rare dietary components and poorly bioavailable where they do exist, especially for herbivorous, granivorous, and frugivorous species (Sigel 1983, 1984b, Adriano 2001). Thus, it seems that, by seeking out particular mineral-enriched foods in the environment, animals could use melanin ornaments to signal their dietary access to the scarce elements that help produce these dark pigments.

Accumulation of these essential minerals may come at a physiological cost, however. When ingested in high concentrations, these minerals can be toxic (reviewed in NRC 1980, Sigel 1986). For example, elevated exposure to transition metals such as Zn or Cu can induce anemia and gastroenteritis, impair thyroid and liver function, and slow food intake, growth, and reproductive output, while prolonged Ca intake can lead to rickets, gout, and nephrosis (Goyer et al. 1995, Klasing 1998). Trace-metal toxicity is most commonly demonstrated among captive animals (Harper and Skinner 1998, Casteel 2001, Peterson 2001), but wild animals also experience metal poisoning when subjected to high levels (Sorensen 1991, Adriano 2001). For example, there is an interesting case of a marine diatom (genus *Pseudo-nitzschia*) that produces a substance (domoic acid) that selectively binds metal ions (Cu and Fe) in seawater and consequently results in shellfish poisoning in humans as well as mass marine mammal and bird mortality (Rue and Bruland 2001).

Thus, where selection might favor steadily increasing metal-ion accumulation, to promote melanin-pigment production, the detrimental effects of high mineral concentration on the health and survival of individuals should counteract such a benefit. How might animals circumvent this potential cost of metal-ion accumulation?

Melanin polymers as chemoprotective reservoirs

This question might be answered with a thorough understanding of the biochemical properties of melanin polymers. Among the structural units of melanins are several negatively charged, carboxyl (COOH⁻) functional groups that serve as cation chelators (Riley 1997). These substituents allow melanin pigments to selectively bind positively charged particles, like the transition metals, and sequester them in the pigmented cells of hair, skin, and feathers (Borovansky et al. 1976, Borovansky 1994, Bilinska 2001). As a result, these minerals accumulate in high concentrations within melanized tissues, specifically within the pigment-cell

organelles (melanosomes; Shibita et al. 1993), such as the mammalian retina (Salceda and Sanchez-Chavez 2000) and hair (Tobin and Paus 2001), the eyes of fishes and frogs (Bowness and Morton 1952), and bird feathers (Niecke et al. 1999). For example, Niecke et al. (1999) showed that black, melanin-pigmented feathers in white-tailed eagles (*Haliaeetus albicilla*) were particularly enriched with Ca, Mn, and Zn compared to white feathers. In fact, reservoirs of melanin pigments may serve as a sink for potentially harmful transition-metal ions, adsorbing and harboring the very minerals that helped produce them and thereby offering chemoprotection to adjacent cells and tissues (Larsson 1993, Tobin and Paus 2001). Riley (1992) advanced a similar view for the inactivation of orthoquinones, which are biochemicals that are generated intrinsically by tyrosinase, generally serve as antibiotics, tanning agents, and melanin precursors in many plants and invertebrates, but like metals may have damaging effects unless detoxified by melanin polymers.

Thus, I hypothesize that the size or darkness of melanin ornaments in animals may serve as an honest signal of quality because individuals that accumulate higher mineral concentrations are able to produce more melanins and subsequently store away or 'excrete' (in dead tissues like feathers or hair) the potentially toxic mineral levels needed to make large or dark patches of pigment. It is the metal-binding capacity of these pigments that keep melanin signals honest; otherwise, the toxic costs of mineral accumulation would offset their pigimentary benefits.

Implications

This hypothesis generates several testable predictions regarding the relationship between melanin ornaments and metal ions in animals: 1) ornamental patches of melanin, like other melanized morphological traits, should be comparatively enriched with minerals relative to other tissues, 2) individual animals with higher intracellular metal-ion status should develop larger or darker patches of melanin, 3) experimentally increased dietary-mineral supplies should elevate melanin-based ornamental displays, and 4) experimentally inhibiting melanogenesis (e.g. by impairing tyrosinase function, independent of mineral cofactors) should result in metal accumulation (because they cannot be sequestered by melanins) and produce toxic effects in animals.

It should be made clear that the proposed link between metals and melanins is by no means the only mechanism by which the honesty of melanin ornaments may be reinforced, however. Although previous studies on melanin-based ornamental traits in birds show that factors such as food quantity (McGraw et al. 2002), protein content (Gonzalez et al. 1999), and endopara-

sitic infections (McGraw and Hill 2000) have no effect on ornamental melanin pigmentation, at least four alternative physiological or behavioral mechanisms have been proposed for the maintenance of melanin ornaments in animals: 1) several steroid (e.g. estrogen, testosterone), protein/peptide (e.g. LH, α -MSH), and monoamine (e.g. epinephrine) hormones have powerful stimulatory effects on melanin pigmentation and coloration in animals (Ralph 1969, Aroca et al. 1993, de Oliveira et al. 1996), 2) patches of melanin pigment may be behaviorally regulated via social-status signaling (Senar 1999, Korzan et al. 2000, McGraw et al. 2003), 3) increased melanization might offer superior protection from parasites, bacteria, or UV radiation. Melanins increase tissue durability (Bonser 1995, Fitzpatrick 1998), quench harmful UV rays (Krol and Liebler 1998), ward off macroparasites (Vey 1993, Kose and Møller 1999, Sugumaran 2002), and resist microbial activity in a number of taxa (Fogarty and Tobin 1996, Mackintosh 2001, Burt and Ichida, in press), and 4) because of their conjugated-bond system, melanins may serve as cellular antioxidants, scavenging potentially damaging free radicals when in circulation or within melanocytes (Rozañowska et al. 1999, Shcherba et al. 2000). These hypothesized honesty-reinforcing mechanisms should be considered carefully in all future tests of the costs and signal content of melanized animal traits.

Because of their direct link to metal-exposure, melanin ornaments should also serve as useful biomarkers of environmental quality to humans. Other sexually selected animal features, such as the carotenoid-derived colors of birds and fishes, have been touted as sensitive indicators of environmental stress, because of their ability to reveal honestly the health, condition, and reproductive state of individuals to conspecifics interested in finding a mate or fighting off competitors (Hill 1995). In this case, the metal-binding properties of melanin pigments should allow us to track the distribution of hazardous heavy metals (e.g. Cd, Pb, Hg) in polluted environments using changes in the melanized body tissues of animals. Conservation biologists have in fact used bird feathers, for example, to assay metal contamination (Burger and Gochfeld 1992, Pilastro et al. 1993), and many of these feathers are black and contain melanin pigments (Eens et al. 1999, Janssens et al. 2001). In certain environments, however, such as acidified aquatic ecosystems, some of these catalytic minerals may be severely limited (e.g. Ca; Scheuhammer 1991), and thus a spatial or temporal decrease in the expression of melanized traits may provide critical information about the chemistry and health of local habitats.

In the end, this paper should serve as only a very general and preliminary review of the potential link between melanin coloration and metal ions. Animal species may use two different forms of melanin as

signals, and subtle differences in the production pathways (Land and Riley 2000) and metal-binding capacities (Cesarini 1996) of pheomelanins and eumelanins may have important implications for their relative signal content. Moreover, animals vary in the acquisition and utilization of various dietary minerals, such that different metals may have different effects on melanin pigmentation across species. Only after more detailed biochemical studies of melanin ornaments are completed will we gain a better understanding of how and why animals use these pigment-based color traits as social and sexual signals.

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