

REVIEW

The antioxidant function of many animal pigments: are there consistent health benefits of sexually selected colourants?

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Several classes of pigments impart flamboyant colours on animals, including the red, orange and yellow carotenoids. Carotenoid-based colours have served as ideal models for studying the honesty-reinforcing mechanisms underlying sexually selected traits, because the very pigments used to become colourful also have antioxidant and immunoregulatory properties that allow individuals to signal their superior health to prospective mates. It is unclear, however, whether other chemical colourants in animals confer similar physiological benefits. Here I make the observation that the other major groups of animal pigments, including melanins, pterins, porphyrins, psittacofulvins and flavonoids, also exhibit antioxidant activity in living systems. Thus, many types of pigment-based colour ornaments in animals have the potential to honestly reveal health state via the immunomodulatory action of the pigments themselves. To evaluate this hypothesis, the extent to which these sets of pigments serve antioxidant functions in colourful animals and to which animals face a trade-off shunting pigments to physiological functions versus colour displays should be investigated.

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Animals use a variety of signals, including elaborate vocalizations, weaponry and bright coloration, to advertise their quality and to attract potential mates. For signals to contain reliable information, they must be costly to produce (Zahavi 1975; Grafen 1990). There is now ample evidence that the most elaborate forms of animal signals incur survival, physiological, or health-related costs (reviewed in Johnstone 1995).

In certain instances, however, animals also reap benefits, beyond direct mating advantages, from components of their sexual displays. This can be the case for biochemically based signals, like certain pigmentary colours, where the pigments used to generate conspicuous and sexually attractive colours also serve important physiological functions. A good example of this is the red, orange and yellow carotenoid-based coloration systems of many fish and birds (Olson & Owens 1998). Many male fish and birds are more colourful than females of their species, and females prefer to mate with the most colourful males (reviewed in Hill 1999). Carotenoid pigments are known to have antioxidant properties, scavenging potentially harmful free radicals and protecting cells and tissues (including

the immune system) from oxidative damage (reviewed in Lozano 1994; Møller et al. 2000; Krinsky 2001). Recent experimental studies have demonstrated the immunomodulatory action of carotenoids in several species of colourful birds and fish (reviewed in McGraw & Ardia 2003; Saino et al. 2003). Thus, carotenoid coloration stands as a model system in which to study the honesty-reinforcing mechanisms of sexual trait expression; those individuals that accumulate the most carotenoids achieve superior health and communicate their carotenoid and health status to females directly with their bright colours.

Carotenoids are by no means the only class of pigments that bestow sexual colours on animals, however. Melanin pigments confer black, brown, grey, chestnut and buff hues on insect cuticles, fish scales, bird feathers and mammalian skin, hair and fur (Ralph 1969; Bagnara & Hadley 1973; Riddiford & Hiruma 1988; Prota 1992). Pterin pigments (also called pteridines) are responsible for the red, orange and yellow colours of lepidopteran wings, fish/amphibian/reptile skin, and avian irides (Bagnara & Obika 1965; Pfliegerer 1992, 1994; Oliphant & Hudon 1993). Porphyrin pigments also colour the reddish-brown feathers and blue and brown eggshells of birds (Baird et al. 1975; Kennedy & Vevers 1976; Miksik et al. 1994). Psittacofulvins are the rare class of red, orange and yellow biochromes found only in the feathers of

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parrots (Völker 1937; Stradi et al. 2001). Lycaenid butterflies use flavonoids from plant foods to acquire bluish and ultraviolet hues on their wings (Knüttel & Fiedler 2001). In contrast to the work on carotenoid pigments, very little is known of the physiological action of these pigment classes in animals that use them as integumentary colourants. With knowledge of the antioxidant functions of carotenoid pigments, I was interested in determining whether these other classes of chemical colourants in animals have similar beneficial physiological properties.

Here, I review the literature and show that all of these other major classes of sexual colourants in animals (melanins, pterins, porphyrins, psittacofulvins and flavonoids) also show antioxidant activity in living systems. Note that these observations were made across a broad range of organisms, from fungi to humans, and in none of the cases was a sexual-signalling function ascribed to pigment-based coloration for the species examined. However, much like Lozano's (1994) original hypothesis for carotenoid coloration, these observations should serve as a foundation upon which several hypothesis tests can be built and performed on animals with sexually attractive pigmentary colours.

MELANINS AS ANTIOXIDANTS

Melanin pigments are large polymers synthesized from amino acids that have strong light-absorbing capabilities across the ultraviolet-visible spectrum (Prota 1992). They occur in two main forms: (1) eumelanin, which confers black and grey colours, and (2) phaeomelanin, which bestows chestnut and buff colours. Animals like mosquitofish, *Gambusia holbrooki* (Horth 2003), house sparrows, *Passer domesticus* (reviewed in McGraw et al. 2003), fence lizards, *Sceloporus undulatus* (Cooper & Burns 1987), and African lions, *Panthera leo* (West & Packer 2002) all use melanins as display pigments in feathers, scales, or hair to attract mates or to repel rivals (reviewed in Jawor & Breitwisch 2003).

Among the many potential physiological functions of melanins (reviewed in Riley 1997), which include tissue strengthening (e.g. Bonser 1995), photoprotection (e.g. Zeise et al. 1995) and thermoregulation (e.g. Cloudsley-Thompson 1999), is their role as antioxidants and immunostimulants (McGinness et al. 1970; Rózanowska et al. 1999). As molecules that contain both oxidizing (*o*-quinone) and reducing (*o*-hydroquinone) functional groups, both eu- and phaeomelanins have the ability to quench potentially damaging reactive oxygen or nitrogen species (e.g. singlet oxygen, hydroxyl or peroxy radical, superoxide anion) via either electron donation or capture (reviewed in Borovansky 1996). Melanins have been touted as valuable free-radical scavengers in several organisms, including fungi (Shcherba et al. 2000), amphibians (Geremia et al. 1984) and humans (Rózanowska et al. 1999; Kasraee et al. 2003). The dermal and epidermal tissues (e.g. skin, retina) of animals are often heavily melanized, and most have speculated that this is to protect individuals from harmful solar UV rays (Robins 1991). However, several types of extracutaneous cells also accumulate melanin (e.g. oesophagus, neuromelanin in neurons, thyroid, melanomas, phagocytes), and in these instances

(and potentially for all melanin-containing cells), melanins may also exhibit direct immuno-enhancing or antimicrobial activity as well as defend cells from oxidative stress (Riley 1992; Mackintosh 2001). Melanin is an integral component of the immune responses of many invertebrates (Sugumaran 2002), and there are several mechanisms and lines of evidence linking melanins and immunity in vertebrates (e.g. phagocytosis, lysosomal enzyme activity, cytokine regulation, nitric oxide production; reviewed in Mackintosh 2001). The ability of melanins to bind and sequester toxic metals may be yet another line of physiological defence against oxidative stress in animals (reviewed in McGraw 2003).

PTERINS AS ANTIOXIDANTS

Pterin pigments are a group of nitrogenous, heterocyclic compounds that are catabolic by-products of purines, the group of fundamental building blocks (nucleotides, including adenine and guanine) of DNA and RNA (Hurst 1980). They occur as red, orange and yellow pigments, which orange sulphur butterflies, *Colias eurytheme* (Watt 1964), guppies, *Poecilia reticulata* (Grether et al. 2001) and green anoles, *Anolis* sp. (Ortiz et al. 1962; Macedonia et al. 2000), as well as many other insects, fish, amphibians and reptiles incorporate into their sexual colour displays. Pterins have also been identified in the colourful red, orange and yellow eyes of birds such as starlings, blackbirds, owls and pigeons (Oliphant 1987; Hudon & Oliphant 1995; Hudon & Muir 1996).

Like melanins, pterins can also be synthesized by non-integumentary tissues, most notably by immune cells (e.g. monocytes, macrophages) (Gieseg et al. 1995). In fact, activation of the immune system (e.g. by lymphokines like gamma interferon) is known to stimulate the release of pterins (such as neopterin and 7,8-dihydroneopterin) from primate macrophages and monocytes (Huber et al. 1984). Immune cells recruited to inflammation sites are often exposed to a series of potentially damaging reactive oxygen species, and the efflux of pterins functions to scavenge free radicals and reduce oxidative stress on the immune system (Gieseg et al. 2001; Duggan et al. 2002; Gieseg & Cato 2003; reviewed in Oettl & Reibnegger 2002). The role of pterins as immune cell protectants is so robust that their concentrations in serum and urine are used as clinical biomarkers for immune performance in humans (e.g. allograft rejection, autoimmune disease, viral/bacterial/parasitic infections; Margreiter et al. 1983; Fuchs et al. 1984; Reibnegger et al. 1984; Wachter et al. 1989). Certain pterins, like tetrahydrobiopterin, are also known to modulate receptor-binding affinity of interleukin 2, which is an integral part of the T-cell-mediated immune response (Boyle et al. 1993). No data are yet available on the antioxidant or immunopromissive action of coloured pterins in animal tissues (e.g. drosoperins in fruit flies and guppies).

PORPHYRINS AS ANTIOXIDANTS

As a class of pigments, porphyrins are a biochemical potpourri. Porphyrins are generally united by their

heterocyclic, pyrrolic molecular structure but often divided by the type of metal ion found at the core of the super-ring. The most familiar representatives include chlorophyll as well as haem (Marks 1969), which colours the fleshy, blood-engorged red parts of many birds (e.g. wattles and combs: Laruelle et al. 1951; Lucas & Stettenheim 1972; nestling gapes: Swynnerton 1916; Götmark & Ahlström 1997; Kilner 1997). A different set of porphyrins has been classified from the eggshells and feathers of birds (reviewed in With 1973, 1978). Most if not all porphyrins, which include reddish-brown coproporphyrin and uroporphyrin in the feathers of goatsuckers, bustards and owls (Völker 1938; With 1978) and protoporphyrin and biliverdin (a bile pigment) in brown and blue eggs, respectively (Kennedy & Vevers 1976; Miksik et al. 1994, 1996), are derivatives of haem from erythrocytes (Thiel 1968; but see With 1973 for discussion of eggshell pigment synthesis in oviducts). One of the earliest pigments described from bird feathers was the unusual uroporphyrin-copper complex known as turacin in the red feathers of turacos (Church 1869; With 1957).

There is an extensive literature for humans and other mammals on the antioxidant functions of porphyrins and their derivatives (e.g. Gardner et al. 1996; Day et al. 1999; Kachadourian et al. 2003). Much of the work has traditionally focused on metalloporphyrins, including complexes with iron (e.g. in mice, Wu et al. 2003) and manganese (e.g. Ohse et al. 1999; in mice: Milano & Day 2000; Oury et al. 2001; in rats: Vujaskovic et al. 2002), but recent work has also emphasized the radical-trapping abilities of some of the porphyrins and porphyrin-derivatives found in birds, such as protoporphyrin (e.g. Sahoo et al. 2002; in humans: Williams et al. 1995; in rats: Williams et al. 1994; Imai et al. 2000) and biliverdin (e.g. Asad et al. 2001; Kaur et al. 2003; in rats: Bauer & Bauer 2002; also see the literature on the biliverdin derivative, bilirubin: Llesuy & Tomaro 1994; Dore et al. 1999; Noreiga et al. 2003). Haem (Gaffron 1960) and haemoglobin (Giulivi & Davies 1990; Otterbein et al. 1995) themselves have been touted as potent antioxidants. Moreno & Osorno (2003) have also recently discussed the antioxidant properties of porphyrins as they relate to eggshell coloration and maternal quality in birds.

PSITTACOFULVINS AS ANTIOXIDANTS

For over a century, ornithologists have known that parrots use an unusual class of pigments to colour their plumage red, orange and yellow (Kruckenberg 1882). It was only recently, however, that the biochemical structure of these compounds was elucidated (Veronelli et al. 1995). Stradi et al. (2001) identified the red pigments in the plumage of scarlet macaws, *Ara macao*, as a suite of endogenously synthesized linear polyenes. A larger survey of red feather pigments in parrots uncovered the same set of polyenes in 45 species spanning the three main parrot families (McGraw & Nogare, in press), including species with sexually dichromatic plumage (e.g. *Electus* parrot, *Electus roratus*). These noncarotenoid lipochromes apparently are found in nature only in parrot feathers.

To date, only a single study has been conducted on the antioxidant action of psittacofulvins. Morelli et al. (2003) used electron paramagnetic resonance to investigate the ability of these colourful molecules to quench free radicals in vitro. They found that these pigments can act as potent antioxidants, exhibiting strong inhibition of hydroxyl (OH) radical formation.

FLAVONOIDS AS ANTIOXIDANTS

Blue butterflies from the family Lycaenidae acquire blue and UV hues on their wings not from a structural phenomenon that is typical of such colours, but from the presence of flavonoids (Knuttel & Fiedler 2001). Flavonoids, which include the anthocyanins, flavonols, flavones and flavanones, are one of the primary classes of colourants in plants, bestowing bright colours on flowers, fruits and berries (Brouillard & Dangles 1993). These butterflies acquire host plant flavonoids as larvae and sequester these in wing scales as adults (Burghardt 2000). In the common blue butterfly, *Polyommatus icarus*, males show strong mate preferences for flavonoid-rich, highly UV-reflective females (Knuttel & Fiedler 2001).

Beyond their role as natural reflectors, flavonoids exist as valuable antioxidants in plant foods. There is an enormous literature on the human health benefits of flavonoids in fruits, vegetables and plant extracts (e.g. herbal tea, wine) (reviewed in Pietta 2000; Nijveldt et al. 2001; Prior 2003). There is abundant evidence from in vitro biochemical studies that flavonoids defend cells from lipid peroxidation (Aviram & Fuhrman 1998; Croft 1998; Bergman et al. 2003), and from human intervention studies that dietary supplements decrease incidence of cancer and atherosclerosis (Horvathova et al. 2001; Wiseman et al. 2001). This is true for several different forms and subclasses of flavonoids as well (Milde et al. 2004), including the commonly known flavonol quercetin from wine (Hertog & Hollman 1996). Quercetin is also the flavonoid stored in the wings of *P. icarus* (Burghardt 2000; Knuttel & Fiedler 2001).

SYNTHESIS

From these disparate bodies of literature on the antioxidant properties of various natural pigments, I offer the hypothesis that melanins, pterins, porphyrins, psittacofulvins and flavonoids produced for coloration purposes may also serve intrinsic health functions and allow animals with sexually selected pigment-based coloration to signal their superior health state. This idea was previously formalized for carotenoid-based colours in animals like birds and fish (Lozano 1994; Møller et al. 2000; Blount et al. 2003), but what has largely been viewed as a characteristic unique to carotenoids may in fact apply to most if not all systems of pigment-based colour communication.

A basic structural attribute shared by all of these aforementioned classes of pigments seems to underlie their dual function as natural colourants and antioxidants. All are molecules that have highly conjugated double bond (or pi electron) systems. Conjugated molecules are potent chromophores because their series of coplanar valence

electrons are shared freely throughout the resonant system, allowing them to readily absorb incoming solar electromagnetic energy (via the small energy requirement to change transition states) and hence impart bright colours (Needham 1974). This very same bond arrangement is responsible for the quenching of free radicals (e.g. unpaired electrons, singlet oxygens) by carotenoids, melanins, pterins, porphyrins, psittacofulvins and flavonoids, again because excess energy can be readily absorbed and dissipated throughout the conjugated molecule (Needham 1974).

Experimental studies are now needed to determine whether animals can reap health benefits by producing more of these colourants (see more below). For several of these types of colour ornaments, there already are some correlational indications that increased pigmentation is tied to improved health. Male house sparrows, *Passer domesticus*, for example, that develop large patches of melanized throat feathers are in good body condition and show lower levels of parasitic infection (Møller et al. 1996). Heavily melanized lepidopterans more effectively resist parasitoid colonization and viral and fungal infections (reviewed in Wilson et al. 2001). Male guppies that acquire and display more immuno-enhancing carotenoids (Grether et al. 2004) also manufacture and deposit more pterins in orange colour spots (Grether et al. 2001). To date, very little is known about the variation in or fitness correlates of porphyrin (sensu Moreno & Osorno 2003) or psittacofulvin coloration in animals.

Several experimental studies have probed aspects of ornamental colour development in animals (particularly for melanins), but rather than address the benefits of pigmentation, they instead have targeted the costs of coloration (reviewed in McGraw et al. 2002, 2003). Researchers have been interested in whether environmental or physiological factors such as quantity or quality of food (Gonzalez et al. 1999; McGraw et al. 2002), incidence of parasitism (McGraw & Hill 2000; Fitze & Richner 2002), levels of hormones (Evans et al. 2000; Korzan et al. 2000; Buchanan et al. 2003), or social interactions (McGraw et al. 2003) affect colour expression. Unfortunately, these reveal little about the health consequences of pigment production and accumulation because the experimental manipulations themselves compromise or confound health state. Thus, to truly pinpoint the antioxidative potential of melanins, porphyrins, pterins and psittacofulvins, the challenge now is to develop a manipulation that elevates pigment production independent of health status. However, unlike carotenoids, which can be manipulated dietarily, these pigments are manufactured endogenously by animals. Thus, manipulation may need to involve sophisticated alterations of the enzymes that regulate biochrome synthesis (e.g. tyrosinase for melanins; Ralph 1969).

While several of these ideas for melanins, pterins, porphyrins, psittacofulvins and flavonoids are in line with those previously offered and supported for carotenoids (e.g. Lozano 1994; McGraw & Ardia 2003), there are a few notable differences that should receive special attention in future tests of this hypothesis. For example, a critical tenet of the hypothesized link between carotenoids and immunity in colour-signalling systems is that animals face

a trade-off when allocating pigments to antioxidant or coloration functions (Lozano 1994). Those individuals that acquire the most carotenoids from the diet can shunt sufficient amounts both to scavenge harmful radicals and to develop integumentary coloration, and thus are the most preferred mates. Because of the different natures of pigment acquisition and the uses for these other types of colourants, it is unclear how melanin-, pterin-, porphyrin-, or psittacofulvin-coloured animals might balance this trade-off. Because they are synthesized internally, and often at peripheral sites where pigments are displayed in the integument (e.g. at maturing feather/hair follicles; Ralph 1969; Wierzbicki 2000), how accessible are these pigments for distribution to needy, oxidatively stressed cells and tissues elsewhere in the body? How potent are the antioxidant effects of pigments at these localized sites? Related to this is the notion that other tissues and organs can often produce these pigments (see above), perhaps directly to quench free radicals. So, do animals that make more of these pigments at the integument also produce more at internal tissues, such that they would not face a direct trade-off? Clearly, obtaining a better understanding of the physiological distribution, accessibility and costliness of all of these pigment types will be an important step towards determining how likely they are to serve as immunomodulators and honest signals of quality.

In conclusion, there may be very good health-related reasons why animals use the pigments they do to acquire their colourful sexual ornaments. By developing flashy pigmentary colours, animals may signal to rivals and suitors their ability to complete difficult environmental and physiological challenges to obtain pigments (e.g. Hill 1996, 2002) and to improve their health by doing so. In general, ornamental pigment-based colours may provide an ideal and unique opportunity to investigate the molecular costs and benefits of signal development in animals.

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