The evolution of eu- and pheomelanic traits may respond to an economy of pigments related to environmental oxidative stress

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Dear Sir,

Vertebrate animals produce two chemically distinct melanin pigments, eumelanin and pheomelanin, often simultaneously in the same cells but one usually prevailing on the other (e.g., Ozeki et al., 1997). The production of these pigments takes place in melanosomes, the specialized organelles of melanocytes. The first step in the melanogenesis pathway consists in the hydroxylation of L-tyrosine by tyrosinase to produce dopaquinone. Dopaquinone then undergoes a cyclization to form leukodopachrome leading to eumelanins. Alternatively, dopaquinone can react with thiol groups to form thioldopa conjugates, the precursors of pheomelanins (e.g., Ozeki et al., 1997).

The switch from eu- to pheomelanogenesis is controlled by hormones such as the melanocyte-stimulating hormone (alpha-MSH) and the agouti signalling protein, which stimulate and inhibit, respectively, the action of tyrosinase. High tyrosinase activity favours eumelanogenesis as opposed to pheomelanogenesis (Ozeki et al., 1997). Because of the dependence of pheomelanins on thiol groups that react with dopaquinone, cysteine and cysteine-containing peptides also act as regulatory agents of the production of these melanins. Pheomelanogenesis preferentially occurs under conditions of high thiol concentrations and low tyrosinase activity, while opposite conditions are required for eumelanogenesis (Benathan et al., 1999). The fact that the thiol groups interact with the tyrosinase active site, thus inhibiting its activity (Jara et al., 1988), increases the opposed nature of conditions under which eu- and pheomelanogenesis occur.

Whether thiol groups that are used during pheomelanogenesis are obtained from free cysteine or from reduced glutathione (GSH) is still under debate, but the most likely explanation is that free cysteine is transported from cytosol into melanosomes through a membrane transport mechanism (Potterf et al., 1999). In any case, GSH is the main physiological reservoir of cysteine, and thus it influences cysteine levels and potentially the process of pheomelanogenesis (Benedetto et al., 1981). GSH is also the most important intracellular antioxidant (Anderson, 1998; Wu et al., 2004). This means that, if eumelanogenesis takes place under conditions of low levels of thiol groups, this process requires proceeding with a diminished antioxidant capacity as compared to pheomelanogenesis. Therefore, oxidative stress (i.e. the imbalance between production of oxygen reactive species and availability of antioxidant compounds) might be higher in melanocytes where eumelanogenesis prevails on pheomelanogenesis because, although the latter requires cysteine consumption to proceed, there are generally lower levels of free thiol groups during eumelanogenesis. From kinetic considerations, tyrosine exclusively produces cysteinyldopas as long as cysteine is present since addition of thiol groups on dopaquinone is much faster than cyclization to leukodopachrome (Ozeki et al., 1997).

Evidence that eumelanogenesis increases oxidative stress as a consequence of the low GSH levels it requires has recently come from birds, as great tit (*Parus major*) nestlings with experimentally decreased GSH levels developed more intensively an eumelanin-based plumage trait than controls, while they were forced to increase the levels of alternative, circulating antioxidants (Galván and Alonso-Alvarez, 2008). In mice, it is known that a mutation in the Slc7a11 gene decreasing extracellular cystine transport into melanocytes and thus GSH levels is responsible for a notably high susceptibility to oxidative stress in the individuals that present it (Chintala et al., 2005).

Melanin-based phenotypic traits often evolve as signals of individual quality in vertebrate animals, an issue that has been mainly studied in birds (McGraw, 2008). Here we propose that, as eu- and pheomelanogenesis occur under conditions of low and high levels, respectively, of the key intracellular thiol-containing antioxidant (GSH), eumelanic and pheomelanic traits have the potential to signal different, even opposite, physiological information on the individuals that exhibit them, irrespective of

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genetic factors such as mutations in the MC1R receptor (Haitina et al., 2007). Eumelanic and pheomelanic traits are generally of distinctive colours, the formers being responsible for black and grey colours and the latters for yellowish, reddish, chestnut and brown colours (Toral et al., 2008). This apparently clear differentiation of the two groups of coloured traits may favour that their potentially different information is perceived and used by conspecifics or heterospecifics. Therefore, eumelanin-based colour traits may act as signals related to high oxidative stress conditions, while the expression of pheomelanin-based colours may be related to low stress levels. This could have important consequences for our understanding of the evolution of melanin-based traits, as the differential information conferred by eumelanic and pheomelanic colours would arise as a consequence of a physiological constraint imposed by the different conditions under which these pigments are produced. Other models proposed to explain the evolution of these traits, like that of Ducrest et al. (2008) that explains how relationships between melanism and several behavioural attributes of organisms can arise because of pleiotropic effects caused by melanocortins interacting with different melanocortin receptors, are not mutually exclusive with that proposed here.

Although the mechanism explaining this potential capacity of eu- and pheomelanin to produce phenotypic traits with different information content has, to our knowledge, never been suggested before, some recent studies performed with birds provide results in the direction of our hypothesis. Roulin et al. (2008a) have found that offspring reared by barn owls (*Tyto alba*) and tawny owls (*Strix aluco*) exhibiting more intense pheomelanic plumage colouration grew faster than offspring reared by less pheomelanic parents in rich environments where brood size had been experimentally reduced (oxidative stress levels of nestlings increase with brood size; Costantini et al., 2006; Alonso-Alvarez et al., 2007). By contrast, offspring reared in poor environments (brood size enlarged) by more intensely coloured Alpine swifts (*Apus melba*), a species that presents eumelanic colouration, grew faster than offspring reared by less eumelanic parents. In barn owls, which exhibits both eumelanic and pheomelanic colourations, more eumelanic individuals are less sensitive to the physiological stress (measured as differences in parental investment capacity) caused by corticosterone than less eumelanic birds, while the degree of pheomelanism did not affect that response (Almasi et al., 2008). These authors interpreted their results as more eumelanic individuals being more resistant to physiological stress and as pheomelanin being a pigment unable to signal that resistance capacity, although they have also shown that experimental injections of corticosterone decrease the deposition of pheomelanin into barn owl feathers (Roulin et al., 2008b). On the other hand, Dauwe and Eens (2008) have found in a correlative study that great tits exposed to high pollution stress levels by heavy metal exposure exhibit larger eumelanic traits than those exposed to lower metal levels. It must be noted that exposure to heavy metals decreases GSH levels (e.g. Congiu et al., 2000).

In sum, there is some evidence that eumelanin production prevails in wild birds under stressful conditions, and that pheomelanin production prevails under more favourable conditions. This has been interpreted as a differential signalling information of eu- and pheomelanin about the capacity to cope with stress levels (Almasi et al., 2008; Roulin et al., 2008a), but the exact mechanism leading to the evolution of those strategies had not been explained. We propose that the different biochemical bases of eu- and pheomelanogenesis, in particular the dependence on GSH of the latter process but not of the former, is responsible for the differential environmental regulation of the production of both types of melanins once natural selection has favoured the origin of melanin deposition in the integument as a consequence of the diverse adaptive benefits of these pigments (Bortolotti, 2006). The evolutionary maintenance of melanic traits may thus respond to an economy of pigments.

Additional predictions can be formulated for the particular case of melanic traits that act as honest signals of quality (Hill, 2006; Hoi and Griggio, 2008; Kingma et al., 2008; McGraw, 2008). Following the signal theory, the reliability of signals is mediated by the costs that signallers experience during their production or maintenance (i.e. honesty is conferred by costs and/or by design; Hasson, 1997). In the case of melanin-based signals, their honesty seems to be mediated by production costs rather than by signal design (Galván and Alonso-Alvarez, 2008). In that sense, only high-quality individuals might be able to afford the decrease of GSH that allows eumelanization, as they should have sufficient alternative antioxidant resources (Galván and Alonso-Alvarez, 2008). This argument can be applied to eumelanin-based signals, because eumelanin is the melanin form that is produced in conditions of low GSH levels. Because of the opposed nature of eu- and pheomelanogenesis, the latter explanation cannot be applied, however, to pheomelanin-based signals, but the honesty of these traits may be indeed understood by an opposite explanation: under environments generating high oxidative stress, where the evolution of general (i.e. non-signalling) eumelanic traits should be favoured, pheomelanin may be more costly to produce than eumelanin because maintaining high levels of GSH in stressful environments should be costly (see above). Therefore, in those kinds of environments pheomelanic traits may have a higher potential to act as quality signals. In contrast, under less stressful environments, in which high levels of GSH are not costly to maintain because this antioxidant is less required for vital processes, presenting low GSH levels may be the mechanism with a higher signalling potential, as

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alternative antioxidants should be movilized (Galván and Alonso-Alvarez, 2008). Therefore, eumelanic and pheomelanic signals may inform about the capacity of signalers to present a physiological state contrary to that imposed by environmental conditions.

Most studies regarding the role of melanic traits as signals have been performed with birds, but given the similarity of the avian and mammalian melanocortin systems (Boswell and Takeuchi, 2005) it seems reasonable that any advance in the understanding of the evolution of melanic traits can be applied to both groups. In that sense, it may be worthy to speculate on the gradient of melanization of human skin from the equator (eumelanic humans) to northern zones of the planet (pheomelanic humans), as the spectral distribution of sunlight at the earth's surface does not seem enough to explain the distribution of human skin colour variation (Blum, 1961). Thus, in addition to a trade-off between UV protection and vitamin D synthesis (Jablonski and Chaplin, 2000), lower levels of thermal stress experienced by humans at high latitudes could contribute to explain why pheomelanin production prevails on eumelanin production in those areas because GSH levels in human erythrocytes decrease with thermal stress, probably as a consequence of oxidizing agents that are generated at high temperatures and then cause GSH depletion (Ohtsuka et al., 1994). Another, not-excluding possibility is that higher selective pressures on maintaining MC1R function act, and hence eumelanogenesis prevails, at low latitudes, as this melanocortin receptor enhances the repairing capacity of UV-induced DNA damage (Hauser et al., 2006). Given that UV incidence on skin can be partially avoided by other endotherms with dense hair and feather covering, this last mechanism may exclusively serves to explain the geographic distribution of human races regarding skin colour. However, the mechanism that associates thermal stress to GSH levels may be also applied to other vertebrates if other factors like the radiation penetration capacity into feathers or hairs are considered too (Bortolotti, 2006). The influence of GSH on melanogenesis thus opens new research avenues to understand geographical distributions of humans and other animals.

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References

Almasi, B., Roulin, A., Jenni-Eiermann, S., and Jenni, L. (2008). Parental investment and its sensitivity to corticosterone is linked to melanin-based coloration in barn owls. Horm. Behav. *54*, 217–223.

Alonso-Alvarez, C., Bertrand, S., Faivre, B., and Sorci, G. (2007). Increased susceptibility to oxidative damage as a cost of accelerated somatic growth in zebra finches. Funct. Ecol. *21*, 873–879.

Anderson, M.E. (1998). Glutathione: an overview of biosynthesis and modulation. Chem. Biol. Interact. *111–112*, 1–14.

Benathan, M., Virador, V., Furumura, M., Kobayashi, N., Panizzon, R.G., and Hearing, V.J. (1999). Co-regulation of melanin precursors and tyrosinase in human pigment cells: roles of cysteine and glutathione. Cell. Mol. Biol. *45*, 981–990.

Benedetto, J.P., Ortonne, J.P., Voulot, C., Khatchadourian, C., Prota, G., and Thivolet, J. (1981). Role of thiol compounds in mammalian melanin pigmentation. Part I. Reduced and oxidized Glutathione. J. Invest. Dermatol. *77*, 402–405.

Blum, H.F. (1961). Does the melanin pigment of human skin have adaptive value? Q. Rev. Biol. *36*, 50–63.

Bortolotti, G.R. (2006). Natural selection and coloration: protection, concealment, advertisement, or deception? In Bird Coloration, Vol II: Function and Evolution, G.E. Hill, and K.J. McGraw, eds (Cambridge, MS: Harvard University Press), pp. 3–35.

Boswell, T., and Takeuchi, S. (2005). Recent developments in our understanding of the avian melanocortin system: Its involvement in the regulation of pigmentation and energy homeostasis. Peptides *26*, 1733–1743.

Chintala, S., Li, W., Lamoreux, L. et al. (2005). *Slc7a11* gene controls production of pheomelanin pigment and proliferation of cultured cells. Proc. Nat. Acad. Sci. U.S.A. *102*, 10964–10969.

Congiu, L., Chicca, M., Pilastro, A., Turchetto, M., and Tallandini, L. (2000). Effects of chronic dietary cadmium on hepatic glutathione levels and glutathione peroxidase activity in starlings (*Sturnus vulgaris*). Arch. Environ. Con. Tox. *38*, 357–361.

Costantini, D., Casagrande, S., de Filippis, S., Brambilla, G., Fanfani, A., Tagliavini, J., and dell'Omo, G. (2006). Correlates of oxidative stress in wild kestrel nestlings (*Falco tinnunculus*). J. Comp. Physiol. B. *176*, 329–337.

Dauwe, T., and Eens, M. (2008). Melanin- and carotenoid-dependent signals of great tits (*Parus major*) relate differently to metal pollution. Naturwissenschaften *95*, 969–973.

Ducrest, A.-L., Keller, L., and Roulin, A. (2008). Pleiotropy in the melanocortin system, coloration and behavioural syndromes. Trends Ecol. Evol. *23*, 502–510.

Galván, I., and Alonso-Alvarez, C. (2008). An intracellular antioxidant determines the expression of a melanin-based signal in a bird. PLoS ONE *3*, e3335.

Haitina, T., Ringholm, A., Kelly, J., Mundy, N.I., and Schiöth, H.B. (2007). High diversity in functional properties of melanocortin 1 receptor (MC1R) in divergent primate species is more strongly associated with phylogeny than coat color. Mol. Biol. Evol. *24*, 2001–2008.

Hasson, O. (1997). Towards a general theory of biological signaling. J. Theor. Biol. *185*, 139–156.

Hauser, J.E., Kadekaro, A.L., Kavanagh, R.J., Wakamatsu, K., Terzieva, S., Schwemberger, S., Babcock, G., Rao, M.B., Ito, S., and Abdel-Malek, Z.A. (2006). Melanin content and MC1R function independently affect UVR-induced DNA damage in cultured human melanocytes. Pigment Cell Res. *19*, 303–314.

Hill, G.E. (2006). Female mate choice for ornamental coloration. In Bird Coloration, Vol II: Function and Evolution, G.E. Hill, and K.J. McGraw, eds (Cambridge, MS: Harvard University Press), pp. 137–200.

Hoi, H., and Griggio, M. (2008). Dual utility of a melanin-based ornament in bearded tits. Ethology *114*, 1094–1100.

Jablonski, N.G., and Chaplin, G. (2000). The evolution of human skin coloration. J. Hum. Evol. *39*, 57–106.

Jara, J.R., Aroca, P., Solano, F., Martínez, J.H., and Lozano, J.A. (1988). The role of sulfhydryl compounds in mammalian melano-

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genesis: effects of cysteine of glutathione upon tyrosinase and the intermediates of the pathway. Biochim. Biophys. Acta *967*, 296–303.

Kingma, S.A., Szentirmai, I., Székely, T., Bókony, V., Bleeker, M., Liker, A., and Komdeur, J. (2008). Sexual selection and the function of a melanin-based plumage ornament in polygamous penduline tits *Remiz pendulinus*. Behav. Ecol. Sociobiol. *62*, 1277–1288.

McGraw, K.J. (2008). An update of the honesty of melanin-based color signals in birds. Pigment Cell Melanoma Res. *21*, 133–138.

Ohtsuka, Y., Yabunaka, N., Fujisawa, H., Watanabe, I., and Agishi, Y. (1994). Effect of thermal-stress on glutathione metabolism in human erythrocytes. J. Appl. Physiol. *68*, 87–91.

Ozeki, H., Ito, S., Wakamatsu, K., and Ishiguro, I. (1997). Chemical characterization of pheomelanogenesis starting from dihydroxyphenylalanine or tyrosine and cysteine. Effects of tyrosinase and cysteine concentrations and reaction time. Biochim. Biophys. Acta *1336*, 539–548.

Potterf, S.B., Virador, V., Wakamatsu, K., Furumura, M., Santis, C., Ito, S., and Hearing, V.J. (1999). Cysteine transport in melanosomes from murine melanocytes. Pigment Cell Res. *12*, 4–12.

Roulin, A., Gasparini, J., Bize, P., Ritschard, M., and Richner, H. (2008a). Melanin-based colorations signal strategies to cope with poor and rich environments. Behav. Ecol. Sociobiol. *62*, 507–519.

Roulin, A., Almasi, B., Rossi-Pedruzzi, A., Ducrest, A.-L., Wakamatsu, K., Miksik, I., Blount, J.D., Jenni-Eiermann, S., and Jenni, L. (2008b). Corticosterone mediates the condition-dependent component of melanin-based coloration. Anim. Behav. *75*, 1351–1358.

Toral, G.M., Figuerola, J., and Negro, J.J. (2008). Multiple ways to become red: pigment identification in red feathers using spectrometry. Comp. Biochem. Physiol. B *150*, 147–152.

Wu, G., Fang, Y.Z., Yang, S., Lupton, J.R., and Turner, N.D. (2004). Glutathione metabolism and its implications for health. J. Nutr. *134*, 489–492.

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