Has removal of excess cysteine led to the evolution of pheomelanin?

Pheomelanogenesis as an excretory mechanism for cysteine

Ismael Galván[1)*], *Ghanem Ghanem*[2)] *and Anders P. Møller*[1)]

Introduction

Melanins are the most common pigments in animals, and are mostly found in the integument. The color traits they generate fulfill a diversity of functions that range from protection against damaging ultraviolet (UV) radiation to crypsis and signaling of individual quality [1, 2]. Higher vertebrates (birds and mammals) synthesize two chemically distinct forms of melanin, eumelanin, and pheomelanin, in melanosomes, specialized organelles of melanocytes [3]. Eumelanin is a polymer of dihydroxyindole and carboxylic acid units that generate traits responsible for black and gray (i.e. darker) colors, while pheomelanin is produced by benzothiazine derivatives and leads to the production of yellowish, reddish and chestnut, and brown (i.e. lighter) colors [4].

Most of the research into melanins has focused on eumelanin [4]. However, there is increasing knowledge of the physical and chemical properties of pheomelanin and pheomelanogenesis, and it is known that most natural melanins are mixed polymers of eumelanin and pheomelanin [3, 4]. Furthermore, recent studies of birds suggest that the production of pheomelanin is involved in physiological trade-offs, as judged by negative relationships between the extent of integument colored by pheomelanin and brain size [5] and interspecific differences in the capacity to resist the effects of ionizing radiation linked to pheomelanic color [6]. It has also been reported that more-pheomelanic individual barn owls (*Tyto alba*) are particularly sensitive to physiological stress caused by corticosterone compared to less-pheomelanic birds [7], and that tawny owls *Strix aluco* belonging to the pheomelanic morph have lower viability during adverse environmental conditions than conspecifics belonging to the eumelanic morph [8]. In humans and mice, pheomelanin increases the photosensitization of cells to UV-induced oxidative damage [9], its content being positively related to cancer risk in humans [10]. Overall, these results indicate that pheomelanin is a limiting pigment that may have important evolutionary consequences.

The limiting nature of pheomelanin is probably caused by the chemical pathway of pheomelanogenesis. The first steps of the process are shared with eumelanogenesis and consist of the hydroxylation of the amino acid L-tyrosine by the enzyme tyrosinase to produce dopaquinone. Dopaquinone then reacts with thiol groups to synthesize pheomelanin, or, in the absence of thiol groups, suffers a cyclization that leads to the synthesis of eumelanin [3]. Thiol groups that react with dopaquinone are provided by free cysteine or by the cysteine-containing tripeptide glutathione (GSH) [11], which is the main physiological reservoir of cysteine and the most important intracellular antioxidant [12]. This means that pheomelanogenesis requires a constant supply of cysteine via GSH, thus lowering the levels of this important antioxidant [13]. Furthermore, pheomelanin is phototoxic because it produces reactive oxygen species when exposed to UV radiation [9, 14]. In contrast, the biological benefits conferred by pheomela-

Keywords:
excess amino acids; oxidative stress; pheomelanogenesis; pigmentation

DOI 10.1002/bies.201200017

[1)] Laboratoire d'Ecologie, Systématique et Evolution, Université Paris-Sud 11, Orsay, France
[2)] Laboratoire d'Oncologie et de Chirurgie Expérimentale (L.O.C.E.), Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium

***Corresponding author:**
Ismael Galván
E-mail: ismael.galvan@u-psud.fr

Abbreviations:
CDO, cysteine dioxygenase; **GSH,** glutathione.

nin are unknown. For example, the above-mentioned phototoxicity of pheomelanin suggests that any photoprotective role of melanins is better fulfilled by eumelanin. Therefore, if pheomelanin production is associated with so important physiological costs and its biological benefits are not evident, what are the factors that have promoted the evolution of pheomelanin?

As far as we know, this question has only been tangentially addressed. Indeed, pheomelanin has been considered ''an accident of nature'', its adaptive value being obscure [10]. One answer may be related to the potential of pheomelanin-based traits to signal ability to cope with oxidative stress (i.e. the imbalance between the production of pro-oxidative compounds and the availability of antioxidant substances, tipped toward the former). Since pheomelanogenesis consumes GSH [3, 13], which cannot then be used to combat oxidative stress, only individuals with a high antioxidant capacity should be able to produce large amounts of pheomelanin to color their integument under high environmental oxidative stress [15]. Pheomelanin-based color is also necessary to produce skin, coat and plumage patterns that confer camouflage or concealment, and in fact it has been suggested that the persistence of pheomelanin is mainly due to its camouflage and esthetic properties [10]. These properties may favor the evolution of pheomelanin, but certainly not all pheomelanin-based color traits act as quality signals [16], nor are they involved in concealment patterns [17]. Thus, these properties can only partly, at best, explain the evolutionary origin and the maintenance of pheomelanin.

Here we propose a new hypothesis that may help to understand the evolution of pheomelanin. Since pheomelanogenesis occurs as long as cysteine/GSH are present in melanocytes and the thiol group of cysteine is incorporated in the structure of pheomelanin [3], the synthesis of this pigment implies a removal of cysteine (Fig. 1). Although cysteine used for GSH synthesis can be obtained from protein breakdown, its availability mainly depends on the cysteine content of the diet [18]. However, cysteine also causes toxicity related to its autoxidation of the corresponding disulfide, which can even decrease GSH levels [19, 20]. Some enzymes can convert the oxidized form of cysteine back to its unmodified form, but the presence of cysteine residues in proteins makes them susceptible to oxidative stress and associated damage [21]. Indeed, cysteine metabolism fulfils an important function by keeping cysteine levels below the threshold of toxicity [22]. This is achieved by cysteine sulfoxidation, a process mainly catalyzed by cysteine dioxygenase (CDO) in which molecular oxygen is added to the sulfhydryl group of cysteine to form less toxic products than cysteine such as sulfate and taurine [22] (Fig. 1). Despite the existence of this process, excess cysteine can occur when its content in diet is higher than needed for protein synthesis, or probably also as a reaction to constant oxidative stress. In birds, excess cysteine contributes to metabolic acidosis and a variety of associated problems such as thinning of egg shells and poor growth [23]. In mammals, excess cysteine has been associated with rheumatoid arthritis, Parkinson's disease, Alzheimer's disease, systemic lupus erythematosus, increased risk of cardiovascular disease, and adverse pregnancy outcomes in humans (ref. [22] and cited references). Any mechanism that contributes to the removal of excess cysteine might thus be of adaptive value; we propose that pheomelanogenesis represents such a mechanism. In the case of human diseases that manifest themselves long after the reproductive age has been reached, such as Parkinson's and Alzheimer's diseases, natural selection may still indirectly affect a mechanism that contributes to the removal of excess cysteine via selection for grand-parental care of offspring. This is because grandparental care increases child survival in many societies [24], and individuals with Parkinson's or Alzheimer's disease cannot normally provide high quality grand-parental care.

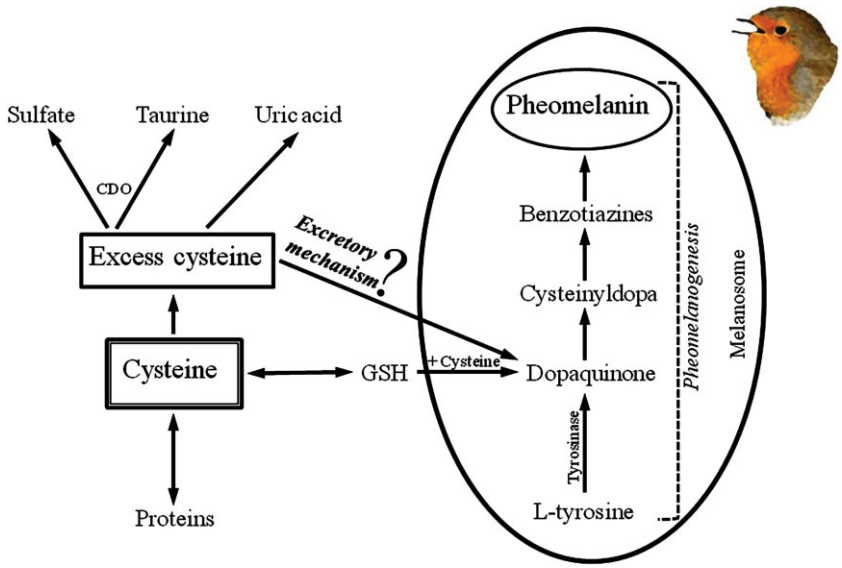

Figure 1. Chart showing the physiological activity of dietary cysteine. This amino acid is used for protein synthesis, and can be recovered by protein breakdown. It is also used for the synthesis of reduced glutathione (GSH), which is the main storage of cysteine and thus also acts as a source of cysteine. When the levels of cysteine are higher than required for these functions, especially for protein synthesis, excess cysteine occurs. This excess, which can be toxic, is partly eliminated by cysteine sulfoxidation, a process mainly catalyzed by cysteine dioxygenase (CDO) in which less toxic products than cysteine such as sulfate and taurine are formed. In birds, excess dietary amino acids are also diverted to the synthesis of uric acid, the main product of excretion in birds. Cysteine also reacts with dopaquinone thus participating in the synthesis of pheomelanin that takes place in melanosomes. If cysteine incorporated into the pheomelanogenesis pathway comes from an excess pool, then pheomelanogenesis could represent an excretory mechanism of cysteine and an adaptive process that may explain the evolution of pheomelanin.

Pheomelanogenesis as an excretory mechanism

It is likely that some adaptive mechanisms have evolved in vertebrates to avoid the toxicity of excess sulfur-containing amino acids. It would not be surprising, however, if the adaptive mechanisms to avoid the toxicity of excess cysteine are more diverse than those previously mentioned. Although our proposed mechanism implies that excess cysteine is removed in melanocytes, where pheomelanogenesis takes place, and melanocytes only represent a small portion of all cells of organisms, this adaptive benefit may contribute to the efficient performance of the detoxification activity of animals.

Despite the huge advances made in the fields of melanocyte biology and melanogenesis in recent decades, the evolution of pheomelanin remains a mystery. This pigment is phototoxic [9] and its production consumes GSH, a key intracellular antioxidant [13]. Probably as a consequence, different studies conducted on birds have shown that individuals or species producing large amounts of pheomelanin present a limited capacity to cope with physiological processes or environmental conditions that generate high levels of oxidative stress [5–8]. Under our current understanding of evolutionary theory it is difficult to explain the evolution of a pigment whose production is associated with the above-mentioned physiological costs without the existence of any adaptive benefit. Our proposal represents a novel hypothesis that, together with the role of pheomelanin in camouflage, may explain the persistence of this pigment in higher vertebrates. Thus, the synthesis of pheomelanin may be favored in individual animals that, due to environmental conditions, are susceptible to excess cysteine.

Implications for understanding human pigmentation patterns

Our hypothesis has implications for all higher vertebrates, including humans. In humans, large amounts of pheomelanin and small amounts of eumelanin are characteristic of red hair, fair skin, freckling, and green irides [25], phenotypes that are more common at high latitudes [10]. If the removal of excess cysteine has adaptive value, this should be reflected in human pigmentation patterns.

Currently, the most accepted explanation for the geographical distribution of human skin pigmentation patterns refers to two natural selection pressures that create a trade-off between the protection of UV radiation and the cutaneous synthesis of vitamin D_3 [26]. Eumelanin protects skin from UV radiation, which generates skin cancer, but UV radiation also stimulates vitamin D_3 synthesis [26]. However, this view ignores older studies highlighting the fact that the spectral distribution of sunlight at the earth's surface is insufficient to explain the distribution of human skin color classes [27], as well as the occurrence of two chemically distinct melanins (i.e. eu- and pheomelanin) in the skin that cause its color variation not just to be a continuous gradient from light to dark. Our hypothesis may serve as a complement for a general explanation of the geographical distribution of human skin color.

Thermal stress depletes GSH levels in human erythrocytes [28]. As thermal stress caused by high temperatures is likely to decrease with latitude, and cysteine-GSH promotes pheomelanogenesis and inhibits eumelanogenesis [3], conditions favoring the production of pheomelanin may prevail at high latitudes, as is indeed observed [15]. A higher prevalence of human parasites at low latitudes [29] may also contribute to this, as parasites generate oxidative stress in their hosts [30]. Indeed, it has been suggested that high levels of human skin eumelanization have evolved as a response to high parasite abundance and diversity in the tropics [31]. While this may contribute to explain the origin of pheomelanin-based traits in human populations, it alone does not explain the maintenance of such traits given the physiological costs of pheomelanin (phototoxicity, carcinogenesis, and GSH consumption). We propose that our view of pheomelanogenesis as an excretory mechanism of excess cysteine may provide an explanation for how pheomelanic traits have prevailed in northern human populations, as excess cysteine is likely to occur more frequently at high latitudes where thermal stress and parasite loads may be relatively low. Humans with a pheomelanic phenotype may thus benefit from a better capacity to remove excess cysteine, which would potentially confer a greater ability to avoid diseases caused by excess cysteine such as Parkinson's and Alzheimer's diseases or systemic lupus erythematosus (ref. [22] and cited references). As these diseases might obviously affect both reproductive success and survival, our hypothesis suggests that human integument coloration may be under natural selection, something that some authors have disputed [26].

Challenges

To formally test our hypothesis, future studies should provide direct measurements of excess cysteine and pheomelanin content in feathers or hair. Particular environmental factors increasing the susceptibility to excess cysteine should be identified. We do not propose our hypothesis as the only explanation for the evolution of pheomelanin, as this is likely more complex than the background behind our hypothesis. For example, it may be suggested that all excess cysteine could be transferred to GSH synthesis so that no alternative mechanisms of removal such as pheomelanogenesis would be required. However, it may also be argued that GSH serves as both a storage mechanism and a continuous source of cysteine (i.e. it cannot be a real excretory mechanism of cysteine; ref. [18]). Thus, pheomelanogenesis may be the only excretory mechanism that is specific to cysteine, thus making its evolution likely because of this function.

An important challenge is to test our hypothesis specifically in humans. In particular, if pheomelanogenesis has adaptive value because it removes excess cysteine, do pheomelanic humans (i.e. phenotypes typically having red hair, fair skin, freckles, and green irides) avoid risk of diseases associated with excess cysteine more easily than eumelanic individuals?

Acknowledgments

Funding agencies: IG was supported by a Marie Curie Intra-European Fellowship (PIEF-GA-2009-252145) within the 7th European Community Framework Program.

The European robin image shown in Figure 1 was made on the basis of a photograph courtesy of Rafael Palomo Santana.

Three anonymous reviewers commented on the manuscript.

References

1. **Tadokoro T**, **Kobayashi N**, **Zmudzka BZ**, **Ito S**, et al. 2003. UV-induced DNA damage and melanin content in human skin differing in racial/ethnic origin. *FASEB J* **17**: 1177–9.
2. **Bortolotti GR.** 2006. Natural selection and avian coloration: protection, concealment, advertisement or deception? In Hill GE, McGraw KJ, eds; *Avian Coloration*. Vol. **2**, Function and Evolution. Harvard: Harvard University Press. p. 3–35.
3. **García-Borrón JC**, **Olivares Sánchez MC.** 2011. Biosynthesis of melanins. In Borovansky? J, Riley PA, eds; *Melanins and Melanosomes: Biosynthesis, Biogenesis, Physiological, and Pathological Functions*. Weinheim: Wiley-Blackwell. p. 87–116.
4. **Ito S**, **Wakamatsu K**, **d'Ischia M**, **Napolitano A**, et al. 2011. Structure of melanins. In Borovansky? J, Riley PA, eds; *Melanins and Melanosomes: Biosynthesis, Biogenesis, Physiological, and Pathological Functions*. Weinheim: Wiley-Blackwell. p. 167–85.
5. **Galván I**, **Møller AP.** 2011. Brain size and the expression of pheomelanin-based color in birds. *J Evol Biol* **24**: 999–1006.
6. **Galván I**, **Mousseau TA**, **Møller AP.** 2011. Bird population declines due to radiation exposure at Chernobyl are stronger in species with pheomelanin-based coloration. *Oecologia* **165**: 827–35.
7. **Almasi B**, **Roulin A**, **Jenni-Eiermann S**, **Jenni L.** 2008. Parental investment and its sensitivity to corticosterone is linked to melanin-based coloration in barn owls. *Horm Behav* **54**: 217–23.
8. **Karell P**, **Ahola K**, **Karstinen T**, **Valkama J**, et al. 2011. Climate change drives microevolution in a wild bird. *Nat Commun* **2**: 208.
9. **Samokhvalov A**, **Hong L**, **Liu Y**, **Garguilo J**, et al. 2005. Oxidative potentials of human eumelanosomes and pheomelanosomes. *Photochem Photobiol* **81**: 145–8.
10. **Hill HZ**, **Hill GJ.** 2000. UVA, pheomelanin and the carcinogenesis of melanoma. *Pigment Cell Res* **13**: 140–4.
11. **Potterf SB**, **Virador V**, **Wakamatsu K**, **Furumura M**, et al. 1999. Cysteine transport in melanosomes from murine melanocytes. *Pigment Cell Res* **12**: 4–12.
12. **Wu G**, **Fang YZ**, **Yang S**, **Lupton JR**, et al. 2004. Glutathione metabolism and its implications for health. *J Nutr* **134**: 489–92.
13. **Meyskens FL**, **Buckmeier JA**, **Mcnulty SE**, **Tohidian NB.** 1999. Activation of nuclear factor-kappa B in human metastatic melanoma cells and the effect of oxidative stress. *Clin Cancer Res* **5**: 1197–202.
14. **Greco G**, **Wakamatsu K**, **Panzella L**, **Ito S**, et al. 2009. Isomeric cysteinyldopas provide a (photo)degradable bulk component and a robust structural element in red human hair pheomelanin. *Pigment Cell Melanoma Res* **22**: 319–27.
15. **Galván I**, **Solano F.** 2009. The evolution of eu- and pheomelanic traits may respond to an economy of pigments related to environmental oxidative stress. *Pigment Cell Melanoma Res* **22**: 339–42.
16. **Hoekstra HE**, **Hirschmann RJ**, **Bundey RA**, **Insel PA**, et al. 2006. A single amino acid mutation contributes to adaptive beach mouse color pattern. *Science* **313**: 101–4.
17. **Bradley BJ**, **Mundy NI.** 2008. The primate palette: the evolution of primate coloration. *Evol Anthropol* **17**: 97–111.
18. **Lu SC.** 1999. Regulation of hepatic glutathione synthesis: current concepts and controversies. *FASEB J* **13**: 1169–83.
19. **Viña J**, **Saez GT**, **Wiggins D**, **Roberts AFC**, et al. 1983. The effect of cysteine oxidation on isolated hepatocytes. *Biochem J* **212**: 39–44.
20. **Munday R.** 1989. Toxicity of thiols and disulphides: involvement of free-radical species. *Free Radic Biol Med* **7**: 659–73.
21. **Berlett BS**, **Stadtman ER.** 1997. Protein oxidation in aging, disease, and oxidative stress. *J Biol Chem* **272**: 20313–6.
22. **Stipanuk MH**, **Dominy JE Jr**, **Lee J-I**, **Coloso RM.** 2006. Mammalian cysteine metabolism: new insights into regulation of cysteine metabolism. *J Nutr* **136**: 1652S–9S.
23. **Klasing KC.** 1998. *Comparative Avian Nutrition*. Wallingford: CAB International.
24. **Coall DA**, **Hertwig R.** 2010. Grandparental investment: past, present, and future. *Behav Brain Sci* **33**: 1–59.
25. **Ito S**, **Wakamatsu K.** 2003. Quantitative analysis of eumelanin and pheomelanin in humans, mice, and other animals: a comparative review. *Pigment Cell Res* **16**: 523–31.
26. **Jablonski NG**, **Chaplin G.** 2010. Human skin pigmentation as an adaptation to UV radiation. *Proc Natl Acad Sci USA* **107**: 8962–8.
27. **Blum HF.** 1961. Does the melanin pigment of human skin have adaptive value? *Q Rev Biol* **36**: 50–63.
28. **Ohtsuka Y**, **Yabunaka N**, **Fujisawa H**, **Watanabe I**, et al. 1994. Effect of thermal-stress on glutathione metabolism in human erythrocytes. *J Appl Physiol* **68**: 87–91.
29. **Guégan J-F**, **Thomas F**, **Hochberg ME**, **de Meeûs T**, et al. 2001. Disease diversity and human fertility. *Evolution* **55**: 1308–14.
30. **Erel O**, **Kocyigit A**, **Avci S**, **Aktepe N**, et al. 1997. Oxidative stress and antioxidative status of plasma and erythrocytes in patients with vivax malaria. *Clin Biochem* **30**: 631–9.
31. **Mackintosh JA.** 2001. The antimicrobial properties of melanocytes, melanosomes and melanin and the evolution of black skin. *J Theor Biol* **211**: 101–13.