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FORUM

Deliberate rusty staining of plumage in the bearded vulture: does function precede art?

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Body coloration plays a central role in sexual selection for numerous animal species that rely principally on visual cues for mate choice (Darwin 1871; Andersson 1994). In birds, carotenoids are the pigments most frequently involved in the coloration of egg yolk, plumage, skin or iris (Brush 1990). With several exceptions (e.g. Kennard 1918; Höhn 1955), the majority of species exhibiting colours from yellow and orange to brown and red obtain these pigments from their diet (for a review see Møller et al. 2000). Carotenoids are used as the basis for coloration of signals, but they also have physiological functions as antioxidants and enhancers of immune function (reviews in von Schantz et al. 1999; Møller et al. 2000; Surai et al. 2001). These physiological mechanisms suggest that the message of carotenoid-based signals may be directly related to the physiological function.

The bearded vulture, *Gypaetus barbatus*, is possibly unique among birds of prey in that it deliberately stains the pale underparts of its body and head with iron oxides found in its natural environment (Jaubert & Barthelemy-Lappomeraye 1859; Berthold 1967; Brown & Bruton 1991; Houston et al. 1993). It meticulously deposits oxides on its feathers by bathing in rusty water or mud (Frey & Roth-Callies 1994; Margalida & Pelayo 1999) and perhaps by rubbing its feathers on to reddish rocky surfaces.

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Negro et al. (1999) discussed the possible function of this behaviour and speculated about its evolutionary origins. In their opinion, the self-staining behaviour of bearded vultures using reddish pigments could play a major role as a signal in dominance, sexual selection and mate choice. According to Negro et al. (1999), the preference for rusty or rufous rather than any other pigment would have evolved from the key role played in social interactions by the inflatable scleric ring around the eye, which is pure red, and perhaps by the iris itself, which is yellowish. In other words, the preference of bearded vultures for reddish pigments and rufous plumage would be ‘innate’. Apart from the fact that the rusty or rufous colour of stained feathers is different from the pure red of the scleric eye ring and the pale yellow of the iris, this proposed ‘cosmetic’ hypothesis is similar to the sensory exploitation hypothesis with preferences arising from perceptual biases of the sensory system and properties of the stimuli (e.g. Ryan & Rand 1993). We would not deny that animals may have evolved kinds of aesthetic preferences based on sensory exploitation, but we propose here alternative functional explanations about the significance of this behavioural trait.

Brown & Bruton (1991) first suggested that active deposition of iron oxides on feathers in the bearded vulture may serve primarily to deter ectoparasites. Although this hypothesis is appealing, the mechanism by which parasites could be removed by this behaviour is unclear, making this possibility unlikely. In particular, it remains unclear why iron oxides would have such a function while other chemical components of the soil would not.

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We hypothesize first that bearded vultures use iron oxides to reduce the negative effects of bacteria on egg and nestling mortality. Iron oxides are likely to have pro-oxidative effects. For example, poultry eggs are commonly treated with chlorine dioxide and ozone (both strong pro-oxidants) before incubation to kill bacteria (see also Sheldon & Brake 1991 as regards hydrogen peroxide). Several studies have suggested that Fe_2O_3 can cause severe oxidative stress conditions that can damage lipidic constituents of bacteria (Gosset et al. 1996; Haguenoer et al. 1996; Garçon et al. 2001). Egg infertility caused by bacteria can be common in birds (e.g. Pinowski et al. 1991), and there is thus strong selection on birds to reduce this effect. Scavengers have evolved particularly strong immune defences (relative spleen size; total leukocyte concentration) compared with non-scavenging relatives (Blount 1999). Scavenging has been hypothesized to be a lifestyle that is associated with particularly virulent parasites because of the horizontal transmission of parasites at cadavers, and because of the multiple genetic strains of parasites arising from the presence of scavengers of many different origins and species (Blount 1999). Bearded vultures may be particularly affected by such virulent parasites because they are the last species of scavenger to exploit a carcass. Hence, different kinds of parasites will already have been deposited by the other scavengers, producing a deadly cocktail for eggs and nestlings of the bearded vulture with relatively naïve immune systems.

The use of chemical substances by hosts to reduce the fitness costs of parasitism has been reported repeatedly in birds and mammals (review in Lozano 1998). The pro-oxidation hypothesis predicts that the use of iron oxides should directly reflect the impact of parasite-mediated mortality caused by bacteria. It also predicts that removal (or the natural absence) of iron oxides will result in reduced hatching and fledging success. However, recent breeding programmes (supplementation with iron oxides in bath water is now the rule in the aviaries involved in the international bearded vulture breeding programme) cannot be used to test the effectiveness of iron oxides in managing virulent parasites because there will be no or few parasites present in these artificial conditions. Only comparisons of hatching success and growth of nestlings from eggs experimentally impregnated with iron oxides coupled with infection with virulent pathogens and nestlings from control eggs would permit an evaluation of the validity of our hypothesis.

Iron oxides may also play a role both in mobilizing vitamin A, thus improving growth and health, and/or in scavenging tissue-damaging free radicals. In many bird species, reddish plumage is due to carotenoids; carotenoid-based signals are usually assumed to be honest because carotenoids are believed to be required, rare or even risky (toxic) (Olson & Owens 1998). Because bearded vultures have no access to carotenoids through their diet, Negro et al. (1999) proposed that bathing in soils may be an alternative way of acquiring a red signal of dominance. In the light of the multiple physiological functions of carotenoids (scavenging of free radicals, stimulation of the immune function, precursors of vitamin A) for both embryo and chick growth discovered in recent decades (Lozano 1994; Olson & Owens 1998; von Schantz et al. 1999; Møller et al. 2000; Surai et al. 2001), we can also hypothesize two other functional explanations for active rusty staining in the bearded vulture. Like other species that have no access to carotenoids through their diets (essentially bones in the present case) bearded vultures would benefit from being able to find alternative substances with a similar role. For instance, in a species closely related to the bearded vulture, the scavenging Egyptian vulture, *Neophron percnopterus*, regular consumption of bird eggs (yolk is rich in carotenoids; Blount et al. 2000) may be a way to compensate for the lack of these essential substances. However, bearded vultures are not known to consume eggs. Iron substantially increases the mobilization of vitamin A stores within the organism, which improves growth and health (Jang et al. 2000; Muñoz et al. 2000). Furthermore, we speculate that, owing to their chemical structure, iron atoms, as part of antioxidant enzyme catalase, also play a role in preventing damaging effects of oxygen radicals. In bearded vultures, iron oxides may be passed from parents to the embryo, either through the egg shell and membranes or through the chick's skin, or adults and chicks may ingest iron oxides when preening or manipulating feathers.

These two hypotheses assume that bearded vultures will generally have relatively low concentrations of circulating carotenoids compared with related species. They predict that supplementation of captive bearded vultures with iron oxides in bath water would enhance embryo and chick survival. Because bearded vultures lay eggs from December to February, a period when soils are frozen or covered by snow in mountain areas, access to bathing sites may be strongly limited during incubation. Thus, plumage may represent a convenient means of storage of iron oxides to reduce the risk of not being able to find them at times of critical need. Accordingly, we also predict that iron oxide deposition on feathers might be, on average, more regular, that is reddish plumage coloration would be more intense, during the reproductive period, in particular incubation and chick rearing.

We believe that our hypotheses are consistent with several observations both on free-ranging and captive birds. First, eggs and chicks of bearded vultures have a strong reddish coloration which is due to contact with breeding parents at the eyrie (Terrasse 2001). Frey (personal communication) describes how captive breeding parents go immediately to their nest after having had a bath in ferruginous water, and delicately rub their ventral feathers impregnated with pigments on to their eggs or offspring. Therefore, transfer of iron oxides from adults to the egg or young seems to be a realistic scenario. Second, females are, on average, more highly coloured than males, both in captivity (Frey & Roth-Callies 1994) and in nature (Bertrán & Margalida 1995). This may be linked to greater nest attendance by females than by males, particularly at night as shown for captive and free-living individuals (Heuret & Rouillon 1999; Margalida & Bertrán 2000; H. Frey, personal communication), and thus greater rates of iron oxide transfer to eggs or offspring. Similarly, Negro et al. (1999) noted that subordinate males in polyandric trios are less coloured than dominant males. Negro et al.'s (1999) cosmetic hypothesis suggests that subordinate males are less coloured than dominant males, which in turn are less coloured than females, because of their relative social ranks. However, dominance of females over males is inferred merely from the observation that males are, on average, slightly smaller than females, whereas biometrical data appear to be equivocal (Brown 1989), and, to our knowledge, behavioural evidence is lacking. In our interpretation, the paler coloration of subordinate males versus dominant males, and of males versus females, might simply reflect differential investment in reproduction, depending primarily on one individual's strategic decision and/or need to transfer iron oxides to the next generation.

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References

Andersson, M. 1994. *Sexual selection*. Princeton, New Jersey: Princeton University Press.

Berthold, P. 1967. Über Haftfarben bei Vögeln: Rostfärbung durch Eisenoxid beim Bartgeier (*Gypaetus barbatus*) und bei anderen Arten. *Zoologische Jahrbücher Systematik*, **93**, 507–595.

Bertrán, J. & Margalida, A. 1995. Copulatory behavior of the bearded vulture. *Condor*, **101**, 164–168.

Blount, J. D. 1999. Parasite virulence and host immune defence: are scavenging birds more immunocompetent than non-scavenging birds? M.Sc. thesis, University of Wales.

Blount, J. D., Houston, D. C. & Møller, A. P. 2000. Why egg yolk is yellow. *Trends in Ecology and Evolution*, **15**, 47–49.

Brown, C. J. 1989. Plumages and measurements of the bearded vulture in southern Africa. *Ostrich*, **60**, 165–171.

Brown, C. J. & Bruton, A. G. 1991. Plumage colour and feather structure of the bearded vulture (*Gypaetus barbatus*). *Journal of Zoology*, **223**, 627–640.

Brush, A. H. 1990. Carotenoids in wild and captive birds. In: *Carotenoids as Colorants and Vitamin A Precursors* (Ed. by J. C. Bauerfeind), pp. 539–562. New York: Academic Press.

Darwin, C. 1871. *The Descent of Man and Selection in Relation to Sex*. London: John Murray.

Frey, H. & Roth-Callies, N. 1994. Zur Genese der Haftfarbe (Rostfärbung durch Eisenoxid) beim Bartgeier, *Gypaetus barbatus*. *Egretta*, **37**, 1–22.

Garçon, G., Gosset, P., Garry, S., Marez, T., Hannothiaux, M.-H. & Shirali, P. 2001. Pulmonary induction of proinflammatory mediators following rat exposure to benzo(a)pyrene-coated onto Fe_2O_3 particles. *Toxicology Letters*, **121**, 107–117.

Gosset, P., Shirali, P., Marez, T., Boutin, A. C., Balduyck, M., Huet, G., Venembre, P. & Haguenoer, J. M. 1996. Toxicity of ferric oxide and benzo[a]pyrene alone or in combination in respiratory tract of Sprague Dawley rats. *Central European Journal of Public Health 1996*, **4**, 56–57.

Haguenoer, J. M., Shirali, P., Hannothiaux, M.-H. & Nisse-Ramond, C. 1996. Interactive effects of polycyclic aromatic hydrocarbons and iron oxide particles. Epidemiological and fundamental aspects. *Cententral European Journal of Public Health 1996*, **4**, 41–45.

Heuret, J. & Rouillon, A. 1999. Première reproduction réussie de Gypaètes barbus *Gypaetus barbatus* issus de réintroduction dans les Alpes (Haute-Savoie, France): observations comportementales du couple et du jeune. *Nos Oiseaux*, **45**, 199–207.

Höhn, E. O. 1955. Evidence of iron staining as the cause of rusty discoloration of normally white feathers in Anserine birds. *Auk*, **72**, 414.

Houston, D. C., Hall, A. & Frey, H. 1993. The characteristics of the cosmetic soils used by bearded vultures *Gypaetus barbatus*. *Bulletin of the British Ornithologists' Club*, **113**, 260–263.

Jang, J. T., Balmer Green, J., Beard, J. L. & Green, M. H. 2000. Kinetic analysis shows that iron deficiency decreases liver vitamin A mobilization in rats. *Journal of Nutrition*, **130**, 1291–1296.

Jaubert, J. B. & Barthelemy-Lappomeraye, C. J. 1859. *Richesses Ornithologiques du Midi de la France*. Marseille: Presses du Sud.

Kennard, F. H. 1918. Ferruginous stains on waterfowl. *Auk*, **35**, 123–132.

Lozano, G. A. 1994. Carotenoids, parasites, and sexual selection. *Oikos*, **70**, 309–311.

Lozano, G. A. 1998. Parasitic stress and self-medication in wild animals. *Advances in the Study of Behavior*, **27**, 291–317.

Margalida, A. & Bertrán, J. 2000. Breeding behaviour of the bearded vulture *Gypaetus barbatus*: minimal sexual differences in parental activities. *Ibis*, **142**, 225–234.

Margalida, A. & Pelayo, R. 1999. Observation of a Pyrenean bearded vulture (*Gypaetus barbatus*) bathing in a ferruginous spring. In: *Bearded Vulture, Annual Report 1998* (Ed. by H. Frey, G. Schaden & M. Bijleveld), pp. 89–90. Wassenaar, The Netherlands: International Foundation for the Conservation of the Bearded Vulture.

Møller, A. P., Biard, C., Blount, J. D., Houston, D. C., Ninni, P., Saino, N. & Surai, P. F. 2000. Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? *Poultry Avian Biology Review*, **11**, 137–159.

Muñoz, E. C., Rosado, J. L., López, P., Furr, H. C. & Allen, L. H. 2000. Iron and zinc supplementation improves indicators of vitamin A status of Mexican preschoolers. *American Journal of Clinical Nutrition*, **71**, 789–794.

Negro, J. J., Margalida, A., Hiraldo, F. & Heredia, R. 1999. The function of cosmetic coloration of bearded vultures: when art imitates life. *Animal Behaviour*, **58**, F14–F17: http://www.academicpress.com/anbehav

Olson, V. A. & Owens, I. P. F. 1998. Costly sexual signals: are carotenoids rare, risky or required? *Trends in Ecology and Evolution*, **13**, 510–514.

Pinowski, J., Kavanagh, B. P. & Górski, W. 1991. *Nestling Mortality of Granivorous Birds due to Microorganisms and Toxic Substances.* Warszaw, Polish Scientific Publishers.

Ryan, M. J. & Rand, A. S. 1993. Sexual selection and signal evolution: the ghost of biases past. *Philosophical Transactions of the Royal Society of London, Series B*, **340**, 187–195.

von Schantz, T., Bensch, S., Grahn, M., Hasselquist, D. & Wittzell, H. 1999. Good genes, oxidative stress and condition-dependent sexual signals. *Proceedings of the Royal Society of London, Series B*, **266**, 1–12.

Sheldon, B. W. & Brake, J. 1991. Hydrogen-peroxide as an alternative hatching egg disinfectant. *Poultry Science*, **70**, 1092–1098.

Surai, P. F., Speake, B. K. & Sparks, N. H. C. 2001. Carotenoids in avian nutrition and embryonic development. 2. Antioxidant properties and discrimination in embryonic tissues. *Journal of Poultry Science*, **38**, 117–145.

Terrasse, J. F. 2001. *Le Gypaète Barbu*. Lausanne: Delachaux & Niestlé.