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Iron oxides in the plumage of bearded vultures. Medicine or cosmetics?

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We have hypothesized (Negro et al. 1999) that bearded vultures, *Gypaetus barbatus*, deliberately stain their plumage with iron oxides to signal dominance status. The conceptual frame to which we ascribe our hypothesis is the handicap principle (Zahavi & Zahavi 1997). For a signal to convey reliable information, and thus to evolve, it has to be costly (Zahavi 1975), and we proposed that the associated cost of the red colour signal for bearded vultures is that adequate ferruginous springs are a limited resource.

Arlettaz et al. (2002) propose alternative explanations, namely that iron oxides have two functions that help bearded vultures in reproduction. One function (hypothesis 1) is that iron oxides have pro-oxidant effects that may kill bacteria, which in turn are detrimental for embryo and nestling development. The second function (hypothesis 2) is that iron oxides might increase mobilization of vitamin A or scavenge free radicals (a typical job for antioxidants such as the carotenoids, Surai et al. 2001), and this would enhance embryo and chick survival.

Previous functional hypotheses for cosmetic coloration in bearded vultures (see review in Negro et al. 1999) include protection against feather wear or protection against mallophaga (Brown & Bruton 1991). However, Frey & Roth-Callies (1994) found no experimental support for any of them. That iron oxides may be toxic to feather ectoparasites is essentially the same idea as in hypothesis 1 in Arlettaz et al. However, these authors state that ectoparasite avoidance is unlikely because it remains unclear why iron oxides would be detrimental for ectoparasites (and we agree with this).

Effects of Iron on Bacteria

Arlettaz et al. cite three papers seemingly suggesting that Fe_2O_3 can cause severe stress conditions that can damage lipidic constituents of bacteria (Gosset et al. 1996; Heguenoer et al. 1996; Garçon et al. 2001). However, none of these studies dealt with bacteria, and all three described the potential negative effects of ferric oxides in tissues of humans or rats. Yet another study by the same French team (Boutin et al. 1996) stated that Fe_2O_3 alone is not very toxic for cultured cells.

Iron is an essential element for almost all living organisms, including bacteria, which require it for growth and replication (Miller & Britigan 1997). However, the most common forms of iron, including Fe_2O_3 in the plumage of bearded vultures, are very insoluble at about neutral pH and in oxic environments. To increase the solubility of iron oxides and get iron, many microorganisms use special ligands (Hersman et al. 200110). Given that bacteria actively seek iron, it is unlikely that they are severely affected by iron oxides in the way suggested by Arlettaz et al.

Iron Oxides and Carotenoids

As for Arlettaz et al.'s second hypothesis, that iron oxides may help mobilization of vitamin A, or scavenge free radicals in the embryo or in nestlings, it assumes a transfer of iron oxides through the eggshell or through the chick's skin. It remains to be demonstrated that such transference is possible, particularly in the case of the eggshell. If that were true, one might also expect absorption of iron oxides through the skin of the adult birds. This possibility, for which there is no current evidence, opens the door to alternative explanations for iron bathing, such as the adults medicating themselves.

Arlettaz et al. also assume that bearded vultures will have relatively low concentrations of circulating carotenoids compared with related species, and would use iron oxides as substitutes with the same function. We said in our Forum paper that carotenoids are absent or rare in bearded vulture diets, but this is so in the diets of the

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majority of avian scavengers, except the Egyptian vulture, *Neophron percnopterus*, which may eat bird eggs and insects (Mundy et al. 1992), as well as faeces of ungulates containing high amounts of lutein (Negro et al. 2002). We have measured circulating levels of total carotenoids in adult birds of several vulture species (J. L. Tella & J. J. Negro, unpublished data) including cinereous vulture, *Aegypius monachus*, (median 0.44 μg/ml, N=15), griffon vulture, *Gyps fulvus* (median 1.01 μg/ml, N=5) and turkey vulture, *Cathartes aura* (median 1.87 μg/ml, N=11). These are low values for plasma carotenoids and are the lowest recorded for wild birds: Tella et al. (1998) reported a range between 0.43 and 74.16 μg/ml of total plasma carotenoids in a survey of 26 bird species. Carotenoids are therefore likely to be limited for bearded vultures, but this is also true for the majority of other scavengers, which do not seem to have special problems in this respect, except that they have not evolved any visual signals based on carotenoid pigments.

Breeding Biology

There are certain aspects of bearded vulture breeding biology named by Arlettaz et al. that require clarification. They argue that bathing sites are strongly limited during incubation, from December to February, because soils are frozen or covered in snow. This may be so in some parts of the species' range, but it is unlikely for African populations and those in the Mediterranean. They also argue that sex-biased differences in coloration reflect differential parental investment. However, only two studies have investigated nest attendance in this species (Heuret & Rouillon 1999; Margalida & Bertrán 2000), there is considerable variation between pairs and it has not been conclusively shown that there is a sex bias in this parameter. As for evidence of dominance by female bearded vultures, Bertrán & Margalida (2002) reported that within trios, females exert control over the two males, and actively refuse unwanted copulations. In captivity, females tend to win agonistic encounters and sometimes injure their male mates, whereas the opposite is rare (A. Llopis, personal communication). Finally, juvenile bearded vultures also stain their feathers, although to a lesser extent than the adults (Berthold 1967; Frey & Roth Callies 1994). If iron oxides were useful in a context of reproduction, bathing 5–6 years before the first breeding attempt makes little sense, particularly given that ferruginous sources seem to be limited.

Concluding Remarks

What would happen to our hypothesis, that its red plumage conveys a signal, if a utilitarian function were eventually found for iron oxides? We would have a different starting point (no need to invoke sensory preferences), but a visual signal may still evolve. If iron oxide were a bactericide, individuals showing redder colours would have an advantage over paler individuals to disinfect themselves or their progeny, and would be preferred by others as mates. A tendency towards redder hues in the plumage, or, in other words, towards more frequent or more elaborate bathing sessions, may spread through sexual selection. The sex- or status-dependent variation in colour that we have reported for birds sharing the same territory (i.e. couples and trios) cannot be easily explained on the grounds of natural selection: individuals would all converge on the same coloration or differences would reflect only time passed since the last bathing session.

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