

Melanin-based color variation in the Bearded Vulture suggests a thermoregulatory function

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Abstract

We document a case of intraspecific variation in plumage colouration in Bearded Vultures that may have arisen as a functional response to climate conditions. Two subspecies, *Gypaetus barbatus barbatus* (Eurasia and North Africa populations) and *Gypaetus barbatus meridionalis* (Eastern and Southern Africa), have been described on the basis of plumage colour differences. The plumage of *G. b. barbatus* tends to be darker in comparison with that of *G. b. meridionalis*. The plumage of the two subspecies differ in the feathering of the tarsi (more abundant in *G. b. barbatus*) and presence/absence of dark ear tufts (only present in *G. b. barbatus*, and this being the most useful character to distinguish adult specimens of both subspecies). When exposing skins under the sun or to electric bulbs in a cold room, temperature increases were significantly higher in the black ear tufts than in the frontal region of the head, suggesting that the melanized ear patch of *G. b. barbatus* serves, at least, to heat up the air entering the ear channel and perhaps also the underlying skin. In addition, *G. b. barbatus*, which inhabits mountainous regions with harsh and long winters, would benefit more from feathered tarsi to prevent heat loss through the legs.

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1. Introduction

Bearded Vultures (*Gypaetus barbatus*) are large scavengers widely distributed in mountains ranges in Eurasia and Africa (Del Hoyo et al., 1994). They can be considered aberrant in relation to the remaining Old World vultures, as they have specialized on eating bones of large and medium-size ungulates (Margalida et al., 2007) which deliberately and repeatedly throw from the air into rocky surfaces (bone-breaking sites or ossuaries) until they become fragmented or disjointed (Boudoint, 1976; Margalida and Bertran, 2001), have feathered heads and because some populations (Crete and Balkans) hunt a relatively large proportion of live prey (i.e., land tortoises, Terrasse, 2001). Bearded Vultures are also peculiar for bathing in red soils for cosmetic purposes (Negro

et al., 1999), or for their well developed nest-building abilities (Margalida and Bertran, 2000). Recent molecular phylogenies based on the sequencing of both mitochondrial and nuclear DNA suggest a close relationship of Bearded Vultures, Egyptian Vultures (*Neophron percnopterus*), Palm-nut Vultures (*Gypohierax angolensis*), and an almost extinct Snake-eating Eagle from Madagascar (*Eutriorchis astur*) (Lerner and Mindell, 2005). This mostly African clade, the Gypaetinae, is only distantly related to the clade including the remaining Old World vultures.

Up to five subspecies of Bearded Vultures have been described in the past, but currently only the two subspecies proposed by Hiraldo et al. (1984) are upheld (Fergusson-Lees and Christie, 2001). These two subspecies are based on morphological evidence, including plumage colour and patterns in the adult plumage: *G. b. barbatus*, encompassing all Bearded Vulture populations of Eurasia and North Africa, and *G. b. meridionalis* that includes the Bearded Vultures

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of Eastern and Southern Africa. The main features of *meridionalis*, distinguishing it from the nominate subspecies, are its small size, absence of black ear tufts, absence of black bristles on the chin, interciliar region (crown) of pure white color or only slightly spotted, pectoral band usually absent or incomplete, darker back plumage, and featherless legs (Hirraldo et al., 1984). Size, however, shows gradual geographic variation.

A recent genetic study based on the sequencing of the mitochondrial control region (Godoy et al., 2004) has found a larger genetic distance among extreme populations of *G. b. barbatus* in Eurasia, than between populations of *G. b. barbatus* and *G. b. meridionalis*. The subspecies status of both African and Eurasian populations, and thus the existence of northern and southern independent lineages, is not supported. This finding also suggests that plumage differences have, at most, a shallow genetic origin and that they may represent recent adaptations to different local environments.

Eurasian Bearded Vultures live in high mountains with harsh and long winters. They have feathered tarsi, thicker plumage overall and larger size (Delibes et al., 1984), and thus we concur with these authors that the differences with the African birds living in more temperate mountain ranges can be explained as thermoregulatory adaptations to cold weather in a classical example of Bergmann's rule. Delibes et al. (1984), however, failed to provide insights into the possible adaptive value of the differences in plumage traits. They suggested instead that the different patterns could simply be incidental by-products of genes involved in different physiological processes (Mayr, 1963). Black is produced by melanin (McGraw, 2006) and represents the most heat-absorbing colour (Hamilton and Heppner, 1967; Heppner, 1970; Lustick, 1971; Ward et al., 2007), and several authors evidenced that large dark-plumaged birds face important thermoregulatory constraints in torrid environments (Walsberg, 1982; Cloudsley-Thompson, 1999; Ward et al., 2002; Negro et al., 2006). Noting that the main plumage differences among African and Eurasian morphs are in the degree of melanization, we here hypothesize that black colour markings, and particularly the ear tufts and the frontal patch diagnostic of Eurasian birds, improve heat absorption. This would be advantageous for individuals inhabiting high mountains in the northern hemisphere, which could have favoured the appearance of that trait only in those areas.

Although the most common selective pressures invoked to explain intraspecific colour variation deal with sexual selection, the pressures that natural selection exerts on plumage colouration has received relatively less attention (Bortolotti, 2006). Our aim in this paper is to document a case of intraspecific variation in plumage colouration that may have arisen as a functional response to climate conditions. Numerous avian subspecies have been described on the basis of plumage colour differences, but in most cases these differences remain only as diagnostic characters for taxonomy. Here we aimed at describing taxonomic diagnostic traits while giving reasons that explain why they could have evolved through their adaptive function.

2. Material and methods

We have re-analysed plumage patterns described in Delibes et al. (1984), who examined numerous specimens in museums all across Europe belonging to the two currently recognized subspecies (see Appendix): (a) *Gypaetus barbatus barbatus*, including specimens from Central Asia, India, Caucasus–Persia, Balkans, Alps, Sardinia, Iberian Peninsula, North Africa and Yemen; and (b) *Gypaetus barbatus meridionalis*, including specimens from Ethiopia and South Africa.

Plumage traits and levels of variation (Fig. 1) were those described by Delibes et al. (1984): (a) *central portion of the crown* (all white, dirty white or slightly marked with black, black and white); (b) *black tuft in the ear* (well developed, visible but little-developed, absent); (c) *density of black bristles on the chin* in a 30×30 mm area on each individual (0, 1–8 bristles, 9–19, and >20 bristles); (d) *pectoral band* (complete or almost complete, traces of pectoral band or intermediate and absent).

The temperature of different feather tracts in the head (“b” and “e”, Fig. 1) of a stuffed Bearded Vulture of the *barbatus* subspecies was registered with an electronic thermometer (precision of ±0.1 °C) incorporating a flexible wire probe that could be placed between head feathers over the skin. Temperature was measured on a summer day in the morning (air temperature 28 °C). In parallel, we measured the temperature in a control sample that consisted in a synthetic white cotton ball. Measurements were taken in a shaded area and also exposing the stuffed skin to the sun different lengths of time (5 and 10 min). The specimen was an adult (i.e., bird in definitive plumage) from the Pyrenees (Spain–France) deposited in the vertebrate collection of the Estación Biológica de Doñana (Sevilla).

In addition to the temperature measures taken in the outdoor, we used a similar procedure but under laboratory conditions inside a cold-storage room with the skins of another two adult birds with definitive plumage collected in the Pyrenees and deposited in the collection of the National Museum of Natural Sciences (Madrid). This way we were able to record the increase

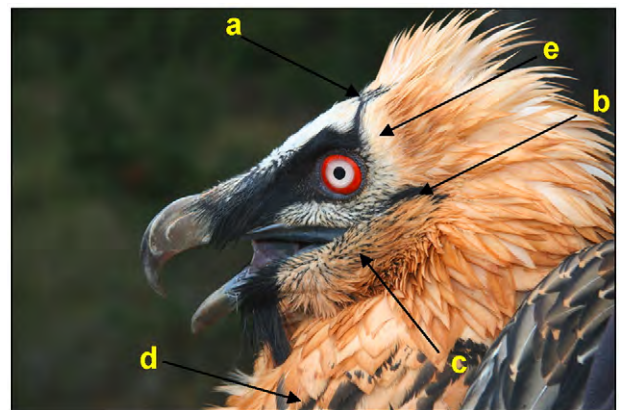


Fig. 1. Detail of the different feather tracts in the head and body of a *Gypaetus barbatus barbatus* in which plumage traits and levels of variation were analyzed (see Materials and methods) and temperature was measured: control (i.e., white cotton ball) b) ear tufts e) frontal region.

in temperature of the skins starting at low temperatures. The heat source was a daylight 100 W lamp located 15 cm from the skins. The electronic thermometer was placed on the plumage patch, and the lamp was not turned on until the temperature reached a constant value. Although the cold-storage room maintains an almost constant temperature of 4 °C, some variation exists due to technical reasons and this made that the starting temperatures slightly differed between plumage patches and specimens. The temperature was recorded during 10 min at 5 s intervals.

Plumage differences between subspecies were tested by comparing observed frequencies of the plumage characteristics with Chi-square tests and applying the Yates correction (Sokal and Rohlf, 1995). Statistical comparisons were carried out using NCSS 2000 and PASS Trial (Hintze, 2001).

3. Results

3.1. Plumage differences between G. b. barbatus and G. b. meridionalis

3.1.1. Crown

Out of 67 *G. b. barbatus* examined, 80.6% showed a black and white crown, 19.4% dirty white and 0% completely white. On the contrary, out of 34 *G. b. meridionalis*, 64.7% showed a completely white crown area, 26.5% dirty white and 8.8% black and white. The differences between the two subspecies are highly significant ($\chi^2_2=64.46, P<0.0001$).

3.1.2. Ear tufts

Out of 69 *G. b. barbatus*, 75.4% showed well developed ear tufts, 21.7% little developed and none (0%) had it absent. Contrarily, all 34 (100%) *G. b. meridionalis* had no ear tufts.

3.1.3. Pectoral band

G. b. barbatus (n=69): 78.3% showed a complete pectoral band, 18.8% an intermediate pectoral band and 2.9% no band.

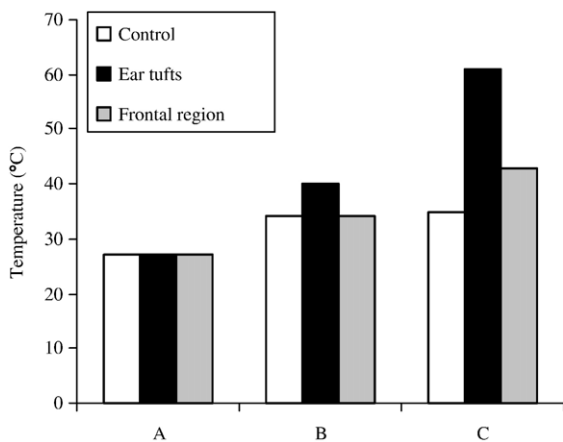


Fig. 2. Changes of the temperature measured according the different feather tracts in the head of the skin adult Bearded Vulture and in the control sample. A — Shaded area, B — after 5 min of sun exposure, C — after 10 min of sun exposure.

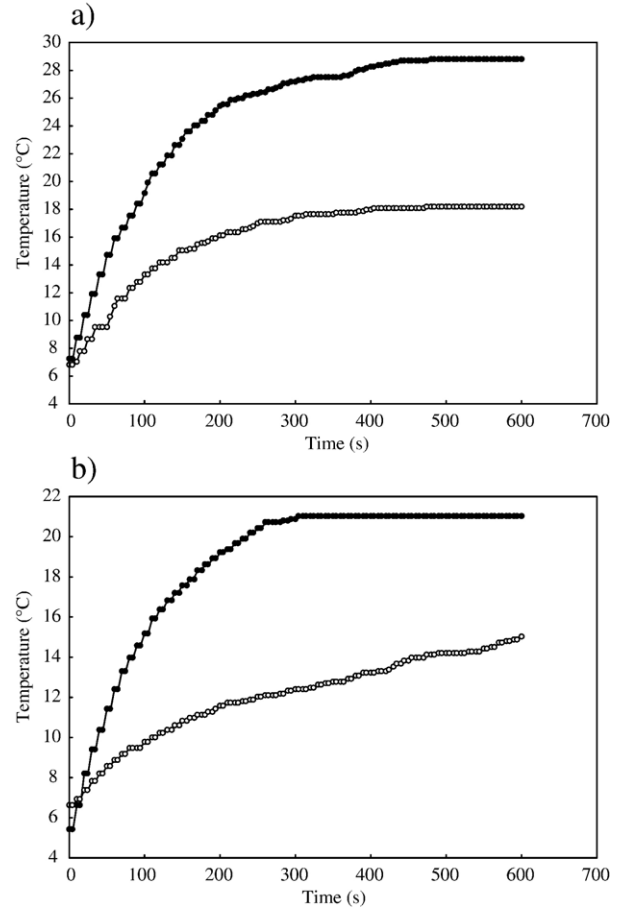


Fig. 3. Temperature increment curves obtained after exposing the black ear tufts (filled symbols) and the frontal region (open symbols) of two museum skins of adult Bearded Vultures (individuals “a” and “b”) to a heat source inside a cold-storage room during 10 min.

G. b. meridionalis (n=34) showed a complete pectoral band (17.6%), intermediate (52.94%) or absent (29.4%). The differences between the two subspecies are significant ($\chi^2_2=36.91, P<0.0001$).

3.1.4. Black bristles on the chin

Out of 59 *G. b. barbatus* specimens examined, 5.1% had no bristles on the chin, 3.4% 1–8 bristles, 32.2% 9–20 bristles and 59.3% >20 bristles. On the contrary, out of 33 *G. b. meridionalis* examined, 93.9% had no bristles, 6.1% 1–8 bristles and none of them (0%) >9 bristles ($\chi^2_3=75.76, P<0.0001$).

3.2. Temperature increments

As shown in Fig. 2, there were no differences between control and feather’s head (ear tufts and frontal region) in the temperature values obtained in a shaded area located outdoors. On the contrary, after exposing the stuffed skin 5 min to the sun, the temperature of the ear tufts increased with respect to the frontal region and control sample (white cotton ball). Finally, after exposing the stuffed skin 10 min to the sun, the temperature again increased substantially in the ear’s tuft with respect to the frontal region and the control sample.

The curves representing the increments in temperature of the different plumage patches obtained in the cold-storage room are shown in Fig. 3. The final temperature values were much higher in the ear tufts than in the frontal region of the head, with a difference of 10 °C and 6 °C for both specimens respectively. Furthermore, the higher increments in temperature observed, measured as the maximum slope of the curves, were noticeably higher in the ear black tufts (0.32 after 20 s of light exposure in both adult specimens) than in the adjacent white frontal region (0.18 after 25 s and 0.1 after 20 s in both specimens respectively). This means that the main changes in temperature occur below 11 °C and 9 °C, though due to this rapid increase we cannot discard the possibility that the slope of the curves were even higher if we would have been able to start measuring at lower temperatures.

4. Discussion

Considering wing length as indicative of overall body size, the largest Bearded Vultures live in central Asia, the size gradually diminishing towards the south and west until the lowest values are reached in Ethiopia, ascending slightly in South Africa (Delibes et al., 1984). The negative correlation between the size of the animal and the annual average of minimum temperatures found by Delibes et al. (1984) suggests a bionergetic motivation according to the interpretation of the Bergmann's ecogeographical rule (Mayr, 1963; Kendeigh, 1969; James, 1970) and this has been interpreted as a selective pressure in favour of an optimal surface to volume ratio. It seems that the density of plumage is related to the temperature, since the individuals with fewer feathers on the tarsi are found in Africa. The lighter colour of the back feathers in central Asia specimens and the darker colour of the African ones could perhaps be considered in the light of the debated Gloger's rule in which "races in warm and humid areas are more heavily pigmented than those in cool and dry areas" (Mayr, 1963).

Our results suggest, however, that the plumage of *G. b. barbatus* tends to be darker in comparison with that of *G. b. meridionalis*. Considering that the two subspecies differ in the feathering of the tarsi it can be argued that an adaptive strategy to improve thermoregulation has evolved. The tarsi in birds have been said to be of great importance for heat loss during flight or when submerged in water (Burt, 1986; Elkins, 1988). In addition, species such as Mallards (*Anas platyrhynchos*) or Herring Gulls (*Larus argentatus*) are capable of losing a high proportion of heat produced thanks to the adjustments of blood circulation in its legs. In this sense, it seems that different bird species defecate on their toes apparently to increase cooling by evaporation (Elkins, 1988). Our results agree with observations of several bird species in which unfeathered areas are greater at low latitudes because heat dissipation may be of greater importance there (Crowe, 1979; Bucholz, 1996; Negro et al., 2006). *G. b. barbatus*, which inhabits mountainous regions with harsh and long winters, would benefit more from feathered tarsi to prevent heat loss through the legs. In addition, and although

the higher radiation absorption of black feathers does not necessarily imply a higher skin temperature because we do not know the penetration capacity of radiation in the ear tufts and the frontal patch (see Stoutjesdijk, 2002), which greatly depends on wind speed (Walsberg et al., 1978; Wolf and Walsberg, 2000), it can be said that the melanized ear patch of *G. b. barbatus* serves, at least, to heat as much as possible the air entering the ear channel. It would be interesting to explore whether, in addition to colour differences, an especial structure of feathers comprising this patch with respect to those covering other body parts has also arisen as a consequence of the need to heat the air around the ear and/or the surrounding skin.

The proportion of solar radiation has the capacity to influence thermal balance and metabolism (Wolf and Walsberg, 1996; Wolf et al., 2000). Under windless conditions and high levels of insolation, radiative heat gains produced by dark plumage (Hamilton and Heppner, 1967; Walsberg et al., 1978; Ellis, 1980) may be costly to birds. The rapid increase of temperature experienced by the black ear tufts (the most useful character which allowed to distinguish 100% of the adult specimens, $n=103$, examined by Hiraldo et al., 1984) of Bearded Vultures in comparison with the white plumage patch makes us wonder about the possibility that this property of the black plumage could represent in certain environments (particularly low wind speeds; see Ward et al., 2002 and cited references) a thermoregulatory cost of melanization in birds with extensive melanized plumage patches that would be traded-off against its adaptive benefits. Although the risk of overheating is a well-known constraint for the production of melanin in insects (Windig, 1999; Talloen et al., 2004) and also seems to exist in mammals (West and Packer, 2002), the implications of this cost have not been investigated in birds in a signalling context albeit having been already proposed by Ward et al. (2002), which is noticeable given the current debate on the possible physiological and social costs that could act as control mechanisms of the honesty of melanin-based signals (Moreno and Møller, 2006; Senar, 2006). The consideration of this possible cost could help us to understand the evolution of these traits.

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Appendix A

a) *Gypaetus barbatus barbatus* (© Antoni Margalida)



b) *Gypaetus barbatus meridionalis* (© Chris van Rooyen)



References

- Bortolotti, G.R., 2006. Natural selection and coloration: protection, concealment, advertisement, or deception? In: Hill, G.E., McGraw, K.J. (Eds.), Bird Coloration. Funct. Evolution, vol. II. Harvard University Press, Cambridge, pp. 3–35.
- Boudoint, Y., 1976. Techniques de vol et de cassage d'os chez le gypaète barbu *Gypaetus barbatus*. *Alauda* 44, 1–21.
- Bucholz, R., 1996. Thermoregulatory role of the unfeathered head and neck in male wild turkeys. *Auk* 113, 310–318.
- Burt Jr., E.H., 1986. An analysis of physical, physiological, and optical aspects of avian coloration with emphasis on wood-warblers. *Ornithological Monographs*, 38. American Ornithologists' Union, Washington D.C.
- Cloudsley-Thompson, J.L., 1999. Multiple factors in the evolution of animal coloration. *Naturwissenschaften* 86, 123–132.
- Crowe, T.M., 1979. Adaptive morphological variation in Helmeted Guineafowl *Numida meleagris* and Crested Guineafowl *Guttera pucherani*. *Ibis* 121, 313–320.
- Del Hoyo, J., Elliott, A., Sargatal, J., 1994. *Handbook of the Birds of the World*, vol. 2. Lynx Edicions, Barcelona.
- Delibes, M., Hiraldo, F., Calderón, J., 1984. Age and geographic variation in the Bearded Vulture *Gypaetus barbatus* (Linnaeus, 1758). *Bonn. Zool. Beitr.* 35, 71–90.
- Elkins, N., 1988. *Weather and Bird Behaviour*. T. and A. D. Poyser, Colton.
- Ellis, H.I., 1980. Metabolism and solar radiation in dark and white herons in hot climates. *Physiol. Zool.* 53, 358–372.
- Fergusson-Lees, J., Christie, D.A., 2001. *Raptors of the World*. Helm, London.
- Godoy, J.A., Negro, J.J., Hiraldo, F., Donazar, J.A., 2004. Phylogeography, genetic structure and diversity in the endangered Bearded Vulture (*Gypaetus barbatus*, L) as revealed by mitochondrial DNA. *Mol. Ecol.* 13, 371–390.
- Hamilton, W.J., Heppner, F., 1967. Radiant solar energy and the function of black homeotherm pigmentation: an hypothesis. *Science* 155, 196–197.
- Heppner, F., 1970. The metabolic significance of differential absorption of radiant energy by black and white birds. *Condor* 72, 50–59.
- Hintze, J., 2001. NCSS and PASS. Number Cruncher Statistical Systems. Kaysville, Utah.
- Hiraldo, F., Delibes, M., Calderón, J., 1984. Comments on the taxonomy of the Bearded Vulture *Gypaetus barbatus* (Linnaeus, 1758). *Bonn. Zool. Beitr.* 35, 91–95.
- James, F.C., 1970. Geographic size variation in birds and its relationship to climate. *Ecology* 5, 365–390.
- Kendeigh, S.C., 1969. Tolerance of cold and Bergmann rule. *Auk* 86, 13–25.
- Lerner, H.R.L., Mindell, D.P., 2005. Phylogeny of eagles, Old World vultures, and other Accipitridae based on nuclear and mitochondrial DNA. *Mol. Phylogenet. Evol.* 37, 327–346.
- Lustick, S.I., 1971. Plumage colour and energetics. *Condor* 73, 121–122.
- Margalida, A., Bertran, J., 2000. Nest-building behaviour of the Bearded Vulture (*Gypaetus barbatus*). *Ardea* 88, 259–264.
- Margalida, A., Bertran, J., 2001. Function and temporal variation in the use of ossuaries by the Bearded Vulture (*Gypaetus barbatus*) during the nestling period. *Auk* 118, 785–789.
- Margalida, A., Mañosa, S., Bertran, J., García, D., 2007. Biases in studying the diet of the Bearded Vulture. *J. Wildl. Manage.* 71, 1621–1625.
- Mayr, E., 1963. *Animal species and evolution*. Belknap Press, Harvard University Press, Cambridge.
- McGraw, K.J., 2006. Mechanics of melanin-based coloration. In: Hill, G.E., McGraw, K.J. (Eds.), *Bird Coloration. Mech. Meas.*, vol. I. Harvard University Press, Cambridge, pp. 243–294.
- Moreno, J., Møller, A.P., 2006. Are melanin ornaments signals of antioxidant and immune capacity in birds? *Acta Zool. Sin.* 52, 202–208.
- Negro, J.J., Margalida, A., Hiraldo, F., Heredia, R., 1999. The function of the cosmetic colouration of Bearded Vultures: when art imitates life. *Anim. Behav.* 58, F14–F17.
- Negro, J.J., Sarasola, J.H., Fariñas, F., Zorrilla, I., 2006. Function and occurrence of facial flushing in birds. *Comp. Biochem. Physiol. Part A* 143, 78–84.
- Senar, J.C., 2006. Bird colors as intrasexual signals of aggression and dominance. In: Hill, G.E., McGraw, K.J. (Eds.), *Bird Coloration. Funct. Evolut.*, vol. II. Harvard University Press, Cambridge, pp. 125–193.
- Sokal, R.R., Rohlf, F.J., 1995. *Biometry*, 2nd edn. W.H. Freeman, San Francisco.
- Stoutjesdijk, F., 2002. The ugly duckling: a thermal viewpoint. *J. Therm. Biol.* 27, 413–422.
- Talloon, W., Van Dyck, H., Lens, L., 2004. The cost of melanization: butterfly wing coloration under environmental stress. *Evolution* 58, 360–366.
- Terrasse, J.F., 2001. *El Quebrantahuesos*. Omega edicions, Barcelona.
- Walsberg, G.E., 1982. Coat color, solar heat gain, and conspicuousness in the phainopepla. *Auk* 99, 495–502.
- Walsberg, G.E., Campbell, G.S., King, J.R., 1978. Animal coat color and radiative heat gain: a re-evaluation. *J. Comp. Physiol.* 126, 211–222.
- Ward, J.M., Blount, J.D., Ruxton, G.D., Houston, D.C., 2002. The adaptive significance of dark plumage for birds in desert environments. *Ardea* 90, 311–323.
- Ward, J.M., McCafferty, D.J., Ruxton, G.D., Houston, D.C., 2007. The thermal consequences of turning white in winter: a comparative study of red grouse (*Lagopus lagopus scoticus*) and Scandinavian willow grouse (*L. l. lagopus*). *Wildlife Biol.* 13, 120–129.
- West, P.M., Packer, C., 2002. Sexual selection, temperature, and the lion's mane. *Science* 297, 1339–1343.
- Windig, J.J., 1999. Trade-offs between melanization, development time and adult size in *Inachis io* and *Araschnia levana* (Lepidoptera: Nymphalidae)? *Heredity* 82, 57–68.
- Wolf, B.O., Walsberg, G.E., 1996. Thermal effects of wind and radiation on a small bird and implications for microsite selection. *Ecology* 77, 228–2236.
- Wolf, B.O., Walsberg, G.E., 2000. The role of the plumage in heat transfer processes of birds. *Am. Zool.* 40, 575–584.
- Wolf, B.O., Wooden, K.M., Walsberg, G.E., 2000. Effects of complex radiative and convective environments on the thermal biology of the white-crowned sparrow (*Zonotrichia leucophrys gambelii*). *J. Exp. Biol.* 203, 803–811.