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ORIGINAL ARTICLE

A link between eumelanism and calcium physiology in the barn owl

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Abstract In many animals, melanin-based coloration is strongly heritable and is largely insensitive to the environment and body condition. According to the handicap principle, such a trait may not reveal individual quality because the production of different melanin-based colorations often entails similar costs. However, a recent study showed that the production of eumelanin pigments requires relatively large amounts of calcium, potentially implying that melanin-based coloration is associated with physiological processes requiring calcium. If this is the case, eumelanism may be traded-off against other metabolic processes that require the same elements. We used a correlative approach to examine, for the first time, this proposition in the barn owl, a species in which individuals vary in the amount, size, and blackness of eumelanic spots. For this purpose, we measured calcium concentration in the left humerus of 85 dead owls. Results showed that the humeri of heavily spotted individuals had a higher concentration of calcium. This suggests either that plumage spottiness signals the ability to absorb calcium from the diet for both eumelanin production and storage in bones, or that lightly spotted individuals use more calcium for metabolic processes at the expense of calcium storage in bones. Our study supports the idea that eumelanin-based coloration is associated with a number of physiological processes requiring calcium.

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Introduction

Melanin pigments are involved in the production of many color patterns. Interestingly, the degree of melanism is often under strong genetic control (Buckley 1987; Hearing and Tsukamoto 1991; Majerus 1998), which provides a wonderful opportunity to investigate how genetically inherited color variants can coexist in the same population. In a number of species, the extent to which individuals are melanic covaries with growth rate (Monney et al. 1996; Roulin et al. 2004), immunocompetence (Gonzalez et al. 1999; Roulin et al. 2000; Galeotti and Sacchi 2003), resistance to parasites (Roulin et al. 2001a; Armitage and Siva-Jothy 2005), stressful factors (Rohwer and Wingfield 1981; Senar et al. 2000; Roulin et al. 2003), parental care (Roulin et al. 2001b), dominance in social interactions (Senar 1999; West and Packer 2002), sexual attractiveness and activity (Osawa and Nishida 1992; Van Gossum et al. 2001; Horth 2003), and habitat use (review in Roulin 2004a). This indicates that the degree of melanism is often associated with various fitness components.

The fact that melanin-based coloration covaries with fitness components and plays a role in mating success raises the possibility that these traits may be involved in sexual selection. However, it has been argued that melanin-based traits are less likely to be sexually selected than carotenoid-based traits (Badyaev and Hill 2000). Carotenoid-based traits are costly to produce because these pigments are gathered from the diet, metabolically transformed, and deposited in the skin, feathers, or cuticle (Hill et al. 1994). In contrast, because the production of melanin pigments is usually under strong genetic control and weakly sensitive to environmental factors (e.g., Hill and Brawner 1998; Majerus 1998; Roulin and Dijkstra 2003; Siefferman and Hill 2005), individuals with different degrees of melanin-based pigmentation may pay similar costs. According to the handicap principle (Zahavi 1975), females should therefore not assess melanin-based coloration to obtain information on individual quality because only costly traits may honestly signal phenotypic or genotypic quality.

Two recent studies (McGraw 2003; Niecke et al. 2003) proposed that the cost of producing different types of melanin-based coloration could be more intricate than previously believed. The barn owl (*Tyto alba*) displays a eumelanic ornament in the form of black spots on the under parts of the body, with individuals varying continuously in the extent of spottiness. Chemical analyses of feathers showed that black spots are 5.4 times more concentrated in calcium (Ca) than neighboring unspotted feather parts, and that large spots are more concentrated in Ca than small spots (Niecke et al. 2003). These observations are important because they suggest that the expression of melanin-based coloration is associated with major physiological processes requiring Ca. This association may take one of two forms. First, the production of eumelanin pigments may be traded-off against the development of other morphological traits that also require Ca for their full expression. Because large quantities of Ca are stored in bones (Heany 2003), this hypothesis predicts that heavily spotted barn owls have lower Ca concentrations in their bones than lightly spotted owls. However, because owls can absorb Ca from the bones of small mammals that are rich in Ca, and because lightly and heavily spotted owls have similar diets (Roulin 2004b), they are unlikely to be limited in their access to Ca. Second, the amount of eumelanin pigmentation may reflect the physiological ability to absorb Ca from the diet to be stored in bones. This second hypothesis predicts that more heavily spotted barn owls have higher bone concentrations of Ca. As a first correlative investigation of these hypotheses, we collected dead barn owls along French highways, quantified the extent to which these owls were spotted, and measured calcium concentration in one of their humeri. Our aim was to examine whether the degree of eumelanism is positively or negatively correlated with the amount of Ca stored in the bones.

Materials and methods

Bone collection and assessment of individual characteristics

Bones were collected from the same individuals used to measure feather asymmetry among adults in another study (Roulin et al. 2003). Between January 2000 and April 2001, 93 dead barn owls were collected in the Champagne and Lorraine regions of France and frozen at −20°C. Bodies were collected daily, and hence, stayed no more than 1 day on the roadside. In April 2001, the bodies were thawed and A. Roulin counted the number of spots within 60×40-mm areas of the breast, belly, one flank, and the underside of one wing. The mean number of spots from these four body parts (mean±SD, 42±16) was used in the statistical analyses. Five birds were discarded because their plumage was in bad condition and the spots could not be counted; the plumage of all other birds was in very good condition. Because barn owls vary in spot size as well as spot number, the diameter of one to 18 (mean±SD, 6.1±2.7) spots was measured on each of the same four body parts to the nearest 0.1 mm. The mean value from these four body parts (mean±SD, 1.26±0.36 mm) was used in the statistical analyses. To estimate pigment concentration in the black spots, we collected one flank feather from each bird, stuck it onto some black paper with adhesive tape, and placed it in a black box equipped with a fluorescent tube (8w/20-640 bl-super). A picture of this feather was taken with a digital camera (Konika Minolta, Dimage A200) from a fixed distance of 27 cm. The picture was imported into Adobe Photoshop to measure the amount of light reflected (i.e., brightness) from five randomly chosen locations of one spot. For each individual, we calculated a mean brightness value (mean±SD 40.4±4.2%), lower values corresponding to darker coloration (Siefferman and Hill 2003). Birds displaying more (Pearson correlation: $r=-0.40$, $n=81$, $P=0.0002$) and larger ($r=-0.54$, $n=81$, $P<0.0001$) black spots had lower brightness values, indicating that spots of these birds were more concentrated in eumelanin pigments, as already suggested by Niecke et al. (2003). To obtain an index of the amount of eumelanin pigments produced, we extracted the first component from a principal components analysis including three variables: number (loading=0.59), size (0.63), and brightness (−0.50) of black spots. This component, referred to as “plumage spottiness,” explained 72.2% of the total variance (eigenvalue=2.17), positive values indicating that birds are blacker. Eigenvalues for the second and third principal components were 0.64 and 0.20, respectively.

Barn owls also vary in plumage coloration between reddish-brown and white. Because this trait is genetically correlated with spot number, and to a lower extent with spot diameter (darker reddish-brown individuals display on average more and larger spots; Roulin 2003; Roulin and Dijkstra 2003), A. Roulin compared plumage with eight color chips ranging from I for reddish-brown to VIII for white. Color scores from the four body regions were averaged (mean±SD, 5.1±1.4). The methods of assessing plumage coloration and number and size of black spots are known to be reliable (Roulin and Dijkstra 2003; Roulin 2004c).

The sexes of the dead owls were determined after gonad inspection. Individuals with a bursa of Fabricius were assigned to the age category "juvenile," while others were classed as "adult" (Glick 1983). We measured bill length to the nearest 0.1 mm (19.1±0.66 mm), a trait that reflects reliably the size of the skeleton (Roulin et al. 2001a). Body mass (288±23 g) was calculated as the mass measured on the day of collection minus the mass of the stomach contents (11±11 g; range: 0–49 g). We removed stomach contents to minimize variation in body mass due to recent meals.

The humerus was extracted from the left wing of 85 of the 93 individuals (in eight cases, the bone was broken). We did not choose this bone for any particular reason except that it could be easily extracted from the dead bodies. Without knowing which individual each humerus came from, M. Beaud put the bones in a colony of dermeste hide beetle (*Dermestes maculates*) for 2 days to clean them from any remaining flesh. The bones were then sent to T. Dauwe, who analyzed calcium concentration blind to plumage spottiness.

Assessment of calcium concentration in bones

For each individual, we took a section of the diaphysis of the humerus. The resulting bone samples were put in an oven at 60°C for 24 h to determine the dry weight to the nearest 0.01 mg. To digest the samples, we added 1 ml of ultrapure 70% HNO_3 and 1 ml of ultrapure 10% H_2O_2. We speeded up digestion by heating the samples in a microwave oven (De Wit and Blust 1998). Once the samples were completely digested, we added 10 ml of ultrapure water. We then diluted the samples 1,000 times and analyzed them by flame atomic absorption spectrometry (Perkin Elmer). Based on standard calibration with solutions of known Ca content, we could determine the Ca concentration of the bones. Samples were run with reagent blanks and spiked samples as quality control.

Statistical procedure

Statistical analyses were carried out with the software package JMP (Sall and Lehman 1996). We performed a stepwise analysis of covariance (ANCOVA) using backward elimination, omitting factors with a *P* value greater than 0.10. Ca concentration was the dependent variable, sex and age were entered as two factors, and plumage spottiness, plumage coloration, body mass, bill length and collection date were entered as five covariates. In the text, we report statistics for all seven independent variables, even though five of them were rejected from the final model. We also included in the model the interaction between sex and plumage spottiness, to examine whether the correlation between bone Ca concentration and the degree of eumelanism is significantly stronger in one of the two sexes. We had information on all variables for 35 juvenile and seven adult males, and for 30 juvenile and nine adult females. Mean plumage spottiness was similar in juveniles and adults in both males (Student's *t* test: t_{40}=0.02, P=0.85) and females (t_{37}=0.64, P=0.53). Means are quoted ±SD. All tests are two-tailed and *P* values smaller than 0.05 are considered significant.

Results

Mean Ca concentration in the humerus was 227.3±34.6 (mg/g dry weight), with values ranging between 135.5 and 326.0. Ca concentration was positively associated with plumage spottiness (stepwise ANCOVA: $F_{1,75}$=6.26, P=0.015; Fig. 1) and date of collection ($F_{1,75}$=6.84, P=0.011; Fig. 2), but not with plumage coloration ($F_{1,75}$=0.16, P=0.69), sex ($F_{1,75}$=1.88, P=0.17), age ($F_{1,75}$=2.93, P=0.09), bill length ($F_{1,75}$=1.42, P=0.24), and body mass ($F_{1,75}$=0.04, P=0.84). The interaction between sex and plumage spottiness was not significant ($F_{1,75}$=1.28, P=0.28).

The positive correlation between plumage spottiness and Ca concentration in the humerus (Fig. 1) may be confounded if calcium deposited in the shell of eggs is associated with female plumage spottiness. To avoid this potential problem we excluded the females. The relationship between Ca concentration and plumage spottiness for males only was significant (Pearson correlation: r=0.35, n=42, P=0.022).

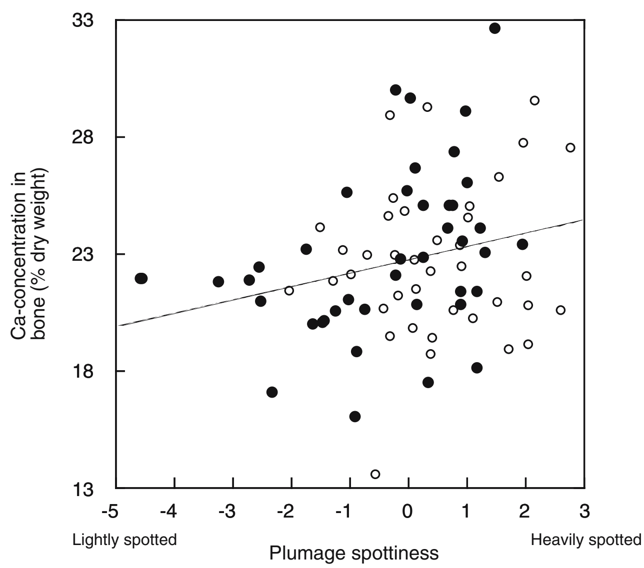

Fig. 1 Relationship between plumage spottiness and calcium concentration in the left humerus of dead French barn owls. *Filled circles* represent males and *open circles* females. Pearson correlation: r=0.25, n=81, P=0.026

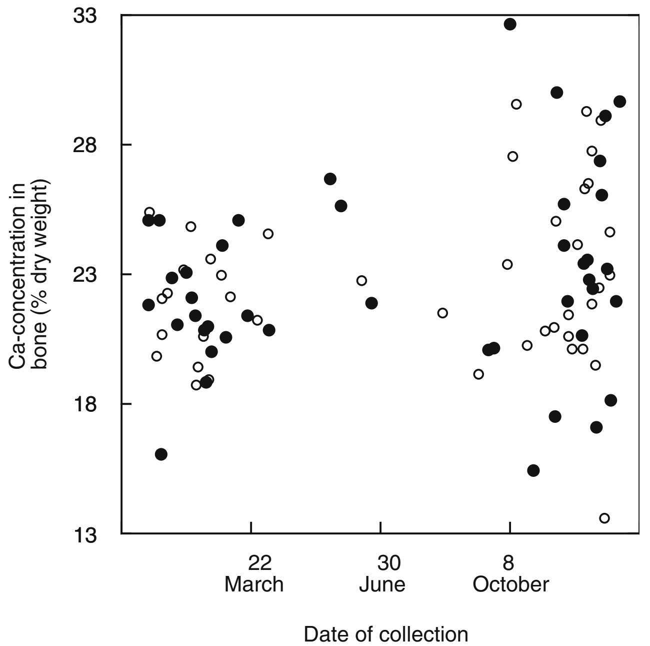

Fig. 2 Relationship between calcium concentration in the left humerus and date when barn owl bodies were collected along French highways. *Filled circles* represent males and *open circles* females

Discussion

Based on the observation that eumelanic feathers have a higher Ca concentration than neighboring noneumelanic feather parts, Niecke et al. (2003) proposed that the production of eumelanin-based traits is traded off against the production of other Ca-dependent morphological traits. An evaluation of this hypothesis is essential to understand better the adaptive value of melanin-based coloration. Indeed, interindividual variation in the amount of melanin pigments deposited in feathers typically has a strong genetic component (review in Roulin 2004a) and is apparently insensitive to environmental factors such as food supply (Hill and Brawner 1998; Roulin et al. 1998; Roulin and Dijkstra 2003; Siefferman and Hill 2005). This implies that the production of different amounts of melanin may entail the same costs. Following the handicap principle, melanin-based colorations may therefore not honestly signal individual quality (Badyaev and Hill 2000). However, because the production of eumelanic black spots requires relatively large amounts of Ca, we examined whether more heavily spotted individuals had bones with lower concentrations of Ca. Such a result would indicate that eumelanin pigments are more costly to produce than previously believed. The present study shows that this may not be the case because spottiness was positively, and not negatively, associated with Ca concentration measured in one humerus (Fig. 1). Assuming that Ca concentration measured in dead owls is correlated with Ca concentration at the time when black spots are synthesized, we were unable to demonstrate that the production of eumelanin pigments is costly in terms of extra use of Ca at the expense of Ca storage in bones. Even if there is a trade-off between Ca deposition in feathers and in bones within individuals, this trade-off is not sufficiently pronounced to lead to a negative correlation among individuals between plumage spottiness and bone Ca concentration. This presents a paradox: if the expression of eumelanin-based traits has negligible costs, how can such traits be so frequently associated with aspects of individual quality?

Several experimental and correlative studies have demonstrated that the degree of melanism covaries with a number of phenotypic attributes (reviews in Jawor and Breitwisch 2003 and Roulin 2004a). The present study adds new elements to our understanding of how these covariances can arise. To our knowledge, we report the first evidence that the production of eumelanin pigments is linked to a major physiological process, namely, Ca storage in bones, with the humeri of blacker barn owls containing more Ca. This finding may explain why, in the barn owl, more heavily spotted individuals have higher immunocompetence (Roulin et al. 2000), are more resistant to parasites (Roulin et al. 2001a), show a better developmental homeostasis (Roulin et al. 2003), and apparently have a mating advantage (Roulin 1999). Even though we did not measure Ca-concentration in bones at the specific time when owls produce black spots, our observation indicates that the production of eumelanin pigments is associated with aspects of Ca metabolism. This chemical element is crucial for many physiological processes that ultimately affect fitness (e.g., Dawson and Bidwell 2005) and bone function as a mineral reserve to be called upon in times of Ca shortage and replenished in times of Ca surplus (Heany 2003). Whereas insectivorous and granivorous birds are limited in the amount of Ca they can obtain from the diet (Graveland et al. 1994), raptors and owls have plentiful access to Ca from the bones of their prey. Interestingly, these predatory birds show large interspecific variation in the extent to which they digest bones (Barton and Houston 1993). The barn owl is among the species showing the lowest efficiency of digestion, which is why this bird is a perfect model organism to analyze diet based on prey remains found in regurgitated pellets. Interindividual differences in bone Ca concentration may therefore arise if digestive efficiency is more pronounced in barn owls displaying a blacker plumage. For instance, the degree of plumage spottiness may be associated with vitamin D, which plays a role in Ca absorption from ingested food, or with parathyroid hormone, which increases Ca resorption from bones (Heany 2003). Finally, even if differently spotted owls are similarly efficient in absorbing Ca from the food, they may differ in their metabolism, with lightly spotted individuals using more Ca for metabolic processes at the expense of Ca storage in bones. Clearly, several different mechanisms could be responsible for our results. We hope that they will stimulate further research aimed at understanding the full range of phenotypic qualities associated with melanin-based coloration.

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References

Armitage SAO, Siva-Jothy MT (2005) Immune function responds to selection for cuticular colour in *Tenebrio molitor*. Heredity 94:650–656

Badyaev AV, Hill GE (2000) Evolution of sexual dichromatism: contribution of carotenoid- versus melanin-based coloration. Biol J Linn Soc 69:153–172

Barton NWH, Houston DC (1993) A comparison of digestive efficiency in birds of prey. Ibis 135:363–371

Buckley PA (1987) Mendelian genes. In Cooke F, Buckley PA (eds) Avian genetics, a population and ecological Approach. Academic, Orlando, pp 1–44

Dawson RD, Bidwell MT (2005) Dietary calcium limits size and growth of nestling tree swallows *Tachycineta bicolor* in a non-acidified landscape. J Avian Biol 36:127–134

De Wit M, Blust R (1998) Determination of metals in saline and biological matrices by axial inductively coupled plasma atomic emission spectrometry using microconcentric nebulization. J Anal At Spectrom 13:515–520

Galeotti P, Sacchi R (2003) Differential parasitaemia in the tawny owl (*Strix aluco*): effects of colour morph and habitat. J Zool 261:91–99

Glick B (1983) Bursa of Fabricius. In Farner DS, King JR, Parkes KC (eds) Avian biology, vol 7. Academic, New York, pp 400–443

Gonzalez G, Sorci G, de Lope F (1999) Seasonal variation in the relationship between cellular immune response and badge size in male house sparrows (*Passer domesticus*). Behav Ecol Sociobiol 46:117–122

Graveland J, van der Wal R, van Balen JH, van Noordwijk AJ (1994) Poor reproduction in forest passerines from decline of snail abundance on acidified soils. Nature 368:446–448

Heany RP (2003) How does bone support calcium homeostasis? Bone 33:264–268

Hearing VJ, Tsukamoto K (1991) Enzymatic control of pigmentation in mammals. FASEB J 5:2902–2909

Hill GE, Brawner WR (1998) Melanin-based plumage coloration in the house finch is unaffected by coccidial infection. Proc R Soc Lond B 265:1105–1109

Hill GE, Montgomerie R, Inouye CY, Dales J (1994) Influence of dietary carotenoids on plasma and plumage colour in house finch: intra- and intersexual variation. Funct Ecol 8:343–350

Horth L (2003) Melanic body colour and aggressive mating behaviour are correlated traits in male mosquitofish (*Gambusia hotbrooki*). Proc R Soc Lond B 270:1033–1040

Jawor JM, Breitwisch R (2003) Melanin ornaments, honesty, and sexual selection. Auk 120:249–265

McGraw KJ (2003) Melanins, metals, and mate quality. Oikos 102:402–406

Majerus MEN (1998) Melanism, evolution in action. Oxford University Press, Oxford

Monney JC, Luiselli L, Capula M (1996) Body size and melanism in *Vipera aspis* in the Swiss Prealps and central Italy and comparison with different Alpine populations of *Vipera berus*. Rev Suisse Zool 103:81–100

Niecke M, Rothlaender S, Roulin A (2003) Why do melanin ornaments signal individual quality? Insights from metal element analysis of barn owl feather. Oecologia 137:153–158

Osawa N, Nishida T (1992) Seasonal variation in elytral colour polymorphism in *Harmonia asyridis* (the ladybird beetle): the role of non-random mating. Heredity 69:297–307

Rohwer S, Wingfield JC (1981) A field study of social dominance, plasma levels of luteinizing hormone and steroid hormones in wintering Harris' sparrows. Ethology 57:173–183

Roulin A (1999) Nonrandom pairing by male barn owls *Tyto alba* with respect to a female plumage trait. Behav Ecol 10:688–695

Roulin A (2003) Geographic variation in sexually selected traits: a role for direct selection or genetic correlation? J Avian Biol 34:251–258

Roulin A (2004a) The evolution, maintenance and adaptive function of genetic colour polymorphism in birds. Biol Rev 79:815–848

Roulin A (2004b) Covariation between plumage colour polymorphism and diet in the barn owl *Tyto alba*. Ibis 146:509–517

Roulin A (2004c) Proximate basis of the covariation between a melanin-based female ornament and offspring quality. Oecologia 140:668–675

Roulin A, Bize P, Ravussin P-A, Broch L (2004) Genetic and environmental effects on the covariation between colour polymorphism and a life history trait. Evol Ecol Res 6:1253–1260

Roulin A, Dijkstra C (2003) Genetic and environmental components of variation in eumelanin and phaeomelanin sex-traits in the barn owl. Heredity 90:359–364

Roulin A, Richner H, Ducrest A-L (1998) Genetic, environmental and condition-dependent effects on female and male plumage ornamentation. Evolution 52:1451–1460

Roulin A, Jungi TW, Pfister H, Dijkstra C (2000) Female barn owls (*Tyto alba*) advertise good genes. Proc R Soc Lond B 267:937–941

Roulin A, Riols C, Dijkstra C, Ducrest A-L (2001a) Female plumage spottiness and parasite resistance in the barn owl (*Tyto alba*). Behav Ecol 12:103–110

Roulin A, Riols C, Dijkstra C, Ducrest A-L (2001b) Female- and male-specific signals of quality in the barn owl. J Evol Biol 14:255–267

Roulin A, Ducrest A-L, Balloux F, Dijkstra C, Riols C (2003) A female melanin-ornament signals offspring fluctuating asymmetry in the barn owl. Proc R Soc Lond B 270:167–171

Sall J, Lehman A (1996) JMP start statistics. A guide to statistical and data analysis using JMP and LMP in software. Duxbury, Wadsworth, London

Senar JC (1999) Plumage coloration as a signal of social status. In: Adams N, Slotow R (eds) Proceedings of the 22nd International Ornithology Congress, Durban. BirdLife South Africa, Johannesburg, pp 1669–1686

Senar JC, Polo V, Uribe F, Camerino M (2000) Status signalling, metabolic rate and body mass in the siskin: the cost of being a subordinate. Anim Behav 59:103–110

Siefferman L, Hill GE (2003) Structural and melanin coloration indicate parental effort and reproductive success in male eastern bluebirds. Behav Ecol 14:855–861

Siefferman L, Hill GE (2005) Evidence for sexual selection on structural plumage coloration in female eastern bluebirds (*Sialia sialis*). Evolution 59:1819–1828

Van Gossum H, Sotks R, de Bruyn L (2001) Reversible frequency-dependent switches in male mate choice. Proc R Soc Lond B 268:83–85

West PM, Packer C (2002) Sexual selection, temperature, and the lion's mane. Science 297:1339–1343

Zahavi A (1975) Mate selection—a selection for a handicap. J Theor Biol 53:205–214