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Are there different requirements for trace elements in eumelanin- and pheomelanin-based color production? A case study of two passerine species

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ABSTRACT

Melanin is the most common pigment in animal integuments including bird plumage. It has been shown that several trace elements may play roles in the production and signaling function of melanin-colored plumage. We investigated coloration and content of various metal elements in the rectrices of two insectivorous passerines, Common Redstarts (*Phoenicurus phoenicurus*) and Blackcaps (*Sylvia atricapilla*), which have eumelanin- and pheomelanin-based coloration, respectively. We hypothesized that 1) the two species would differ in concentrations of metals important in melanin synthesis (Ca, Fe, Cu, Zn), 2) differences in metal concentration levels would be related to feather coloration. Our study confirmed the first prediction and provides the first evidence that selected elements may play a greater role in pheomelanin than in eumelanin synthesis. Concentrations of three elements considered as important in melanin synthesis (Ca, Fe, Zn) were 52% to 93% higher in rusty colored Common Redstart feathers compared to the dark gray Blackcap feathers. However, element concentrations were not correlated with feather coloration or sex in either species. Our study suggests that, of the two melanin forms, pheomelanin synthesis may bear higher costs associated with the acquisition of specific elements or limited elements may create trade-offs between ornamentation and other physiological functions. Our findings warrant further investigations designed to better understand the roles of macro- and microelements in the synthesis of both forms of melanin.

1. Introduction

The main component of avian feathers is keratin, a polypeptide common in integuments of other vertebrates like mammalian fur, hoofs and horns (Dullaart and Mousquès, 2012). It has been known for decades, however, that plumage also contains small quantities of various elements. These elements include macrominerals (e.g., Ca, Na, K and Cl) that are present in large quantities and microminerals or trace minerals (e.g. Se, Fe, Zn, Cu, and Mn) that are present in small quantities in the body (McGraw, 2003). The origin of minerals in feathers could be explained in several ways. First, they play broad and important physiological functions in organisms (Bogden and Klevay, 2000) so their presence in integuments may simply reflect their content in the entire body. Secondly, toxic ions of heavy metals acquired by birds through their diet may also accumulate in various tissues including plumage (e.g., Burger, 1993; Dauwe et al., 2000).

McGraw (2003) proposed an intriguing hypothesis, which linked metal elements with condition signaling properties of melanin-based ornaments of avian plumage. Earlier studies demonstrated that elements including Ca, Cu, Fe and Zn are used in the formation of intermediate products in both eumelanin and pheomelanin syntheses. Moreover, metal ions may also modulate activity of tyrosinase — a key enzyme that catalyzes production of melanins from amino-acid precursors. On the other hand, acquiring and metabolizing metal ions bear clear costs for birds. Both macro- and microminerals are rare in the diets of most species. Simultaneously, some of them play important roles in many physiological functions. For example, Ca is crucial in skeletal mineralization and eggshell formation, while microminerals are common enzyme cofactors and components. As outlined by McGraw (2003), despite the importance of metals for health maintenance, excess amounts might be toxic for organisms. The same is true for small amounts of other microelements like Cd, Pg or Hg. Feather melanin may prevent this toxic effect by binding ions with carboxyl functional groups that serve as cation chelators. Thus, melanin-based ornaments in birds should

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serve as honest advertisements, because they signal the ability to accumulate high amounts of important elements and simultaneously indicate capability to cope with them after they exceed potentially toxic levels (McGraw, 2003).

To date, this hypothesis has been tested only in few species (reviewed in McGraw, 2008). This is surprising considering the vast number of species with melanin coloration and the current interest in melanin-based signaling in birds (McGraw, 2008). Studies of Barn Owls (*Tyto alba*) showed that among four metals (Ca, Zn, Fe and Cu), calcium and zinc are positively related to eumelanin-based coloration (breast plumage spottiness; Niecke et al., 2003). Moreover, zinc was positively correlated with reddish-brown pheomelanin-based coloration (Niecke et al., 2003). In a subsequent study Roulin et al. (2006) demonstrated that Barn Owl eumelanin-based spottiness reflects calcium concentration in humerus bones. These results support the idea that eumelanin-based traits signal a bird's general ability to absorb calcium from its diet (Roulin et al., 2006). Dauwe and Eens (2008) found positive correlations between the width of the black breast stripe of the Great Tit (*Parus major*) and the level of lead and cadmium contamination in their habitat. In another correlative study, positive correlation was found between plumage melanization and zinc (but not lead) concentrations in feathers of feral pigeons (*Columba livia*, Chatelain et al., 2014). In this study, however, effects of both forms of melanin were not studied separately. Diet manipulation experiments performed to date demonstrate contradicting results. In males Zebra Finches (*Taeniopygia guttata*), Ca supplementation caused the size of the black breast patch to become enlarged (McGraw, 2007), while the opposite effect was found in black breast patches of males House Sparrows (*Passer domesticus*; Stewart and Westneat, 2010, 2013). Moreover, it is important to emphasize that even if experimental manipulations of metal content in diet alter the expression of melanin-based traits, it does not necessarily mean that between-individual variation in coloration observed in nature is due to differential access to metals.

The mechanism of condition-dependent signaling by melanin-based coloration proposed by McGraw (2003) depends, to great extent, on species-specific life histories. For example, calcium could be expected to be a less limiting factor in owls or birds of prey, which feed on vertebrates containing calcium rich skeletons. However, the diets of insectivorous and grainivorous species are generally expected to be calcium deficient and therefore clearer trade-offs between coloration and Ca should be expected. Moreover, melanin-based coloration results from the co-occurrence of the two melanin forms: eumelanin and pheomelanin (McGraw, 2006). The color of eumelanin-dominant plumage is gray or black, while higher relative content of pheomelanin produces brown, rufous and buff colors. In addition to color differences, the biochemical synthesis pathways of the two forms differ (McGraw, 2006). Although it never has been tested, eumelanins and pheomelanins may also differ in the amount of metal elements needed for synthesis. To date, McGraw's (2003) hypothesis has been tested on only five species with mainly eumelanin-based coloration, and results have been equivocal.

Here, we investigated associations between rectrices coloration and the content of various metal elements in two insectivorous passerines: Common Redstarts (*Phoenicurus phoenicurus*) and Blackcaps (*Sylvia atricapilla*). These species differ in the mechanism of melanin coloration that is pheomelanin-dominated in Common Redstarts and eumelanin-dominated in Blackcaps. We made the following hypotheses: 1) the species will differ with regard to the concentrations of metals important in melanin synthesis (Ca, Fe, Cu, Zn), 2) within-species differences in metal concentration levels will be related to feather coloration.

2. Material and methods

2.1. Feather collection

The study was carried out in the Kızılırmak delta that stretches along the Black Sea coast from 41°30′N and 41°45′N to 35°43′E and 36°08′E. The delta is one of the largest wetlands in Turkey and an important area for migratory birds as it forms the last/first stopover site before/after crossing the Black Sea (Erciyas et al., 2010). Common Redstarts (*P. phoenicurus*) and Blackcaps (*S. atricapilla*) are common passerines of the western Palearctic (Cramp, 1998) and cross the Kızılırmak delta on their migration twice a year.

Birds were caught during autumn migration in 2012 using mist-nets. All individuals were ringed with standard aluminum rings and age and sex were determined based on plumage characteristics (Svensson, 2006). All birds were aged first-year of their life (juveniles on their first migration, EURING code 3) and we included only rectrices that represented to the first generation of plumage grown during nestling period. We used only tail feathers from first year birds to standardize birds according to the age, plumage region and diet. Previous studies indicated that feather metal content might vary due to body region (e.g., Adout et al., 2007; Rodriguez-Ramos Fernandez et al., 2011; Seco Pon et al., 2011). Moreover, birds' diet at nestling stage, when tail feathers grown, is much more similar in both species compared to their adult diet (Cramp, 1998). From each bird, we collected two tail feathers (T3 — third feathers numbered from the central pair). Samples were stored in zip-lock bags in dark at −18 °C until further analysis. In total, samples from 50 Common Redstarts (25 males and 25 females) and 30 Blackcaps (15 males and 15 females) were used in the study.

2.2. Color analysis by reflectance spectrophotometry

We measured the spectral reflectance of feathers using an Ocean Optics spectrometer (USB 4000, in the range of 200–1100 nm, Dunedin, Florida, USA) and a PX-2 pulsed xenon lamp (Ocean Optics, Dunedin, FL, USA) that measures both UV and visible light (220–750 nm). We used a bi-furcated fiber-optic measuring probe (R 200-7-UV/VIS, Ocean Optics, Dunedin, Florida, USA) that provided illumination from the lamp and transferred reflected light from the feather to the spectrometer. To avoid ambient light and to standardize measuring distance (1.5 mm), a black plastic tip was mounted on the ferrule of the probe. The probe was held at a 90° angle to the feather surface and illuminated an area of ca. 2 mm diameter. All reflectance data were generated relative to a white standard (WS-1-SL, Labsphere). Spectral measurements were expressed as percent reflectance of light per wavelength in relation to a white standard reflectance (100%). From each feather we took five readings distributed evenly along the outer side of feather shaft. Feathers were laid on black velvet during measurements.

We processed spectral data using RCLR v0.9.28 software (Montgomerie, 2008). First, we averaged all reflectance measures obtained from both feathers of the same individual. Then, we calculated two variables typically used in studies on melanin-based coloration: brightness and red chroma. Brightness is the mean reflectance for each wavelength (1 nm) between 300 and 700 nm (B2 in RCLR software). This variable is a good predictor of the amount of both eumelanin and pheomelanin deposited in feathers (McGraw et al., 2005). Chroma (S1R in RCLR software) is a measure of spectral purity and is expressed as the proportion of reflectance in red region of spectra (600–700 nm) to the total reflectance (300–700 nm). Red chroma is commonly used in studies on pheomelanin-based colors, which have the greatest reflectance within long-waved range of spectrum (e.g. Surmacki et al., 2011).

2.3. Element analyses

2.3.1. Sample preparation

To remove any external contamination, feathers were washed vigorously in deionized water (Smart 2 Pure, TKA, Germany) alternated with 1 mol/L acetone (99.5% pure p.a. basic, POCH, Poland) and then were rinsed with deionized water. Next, samples were dried at room temperature for 48 h (Costa et al., 2013), until stabile mass was achieved and stored in sterile 15 mL capped polypropylene centrifuge test tube (VWR