Metal exposure influences the melanin and carotenoid-based coloration in great tits

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Short communication

Metal exposure influences the melanin and carotenoid-based colorations in great tits

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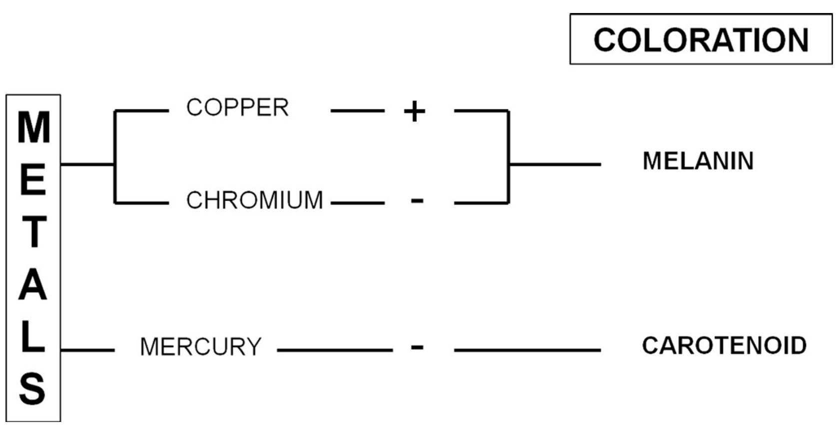
HIGHLIGHTS

- We measured the concentrations of metals in feathers collected on great tits.
- Melanin pigmentation was positively associated with the concentration of copper.
- Melanin pigmentation was negatively related with the concentration of chromium.
- Carotenoid-based coloration was negatively related with the concentration of mercury.

GRAPHICAL ABSTRACT



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ABSTRACT

Metals are naturally found in the environment but are also emitted through anthropogenic activities, raising some concerns about the potential deleterious effects of these elements on wildlife. The potential effects of metals on bird coloration have been the focus of several recent studies since animal colored-signals often reflect the physiology of their bearers and are thus used by animals to assess the quality of another individual as a mate or competitor. These studies have shown that the melanin pigmentation seems to be positively associated and the carotenoid-based coloration negatively associated with metal exposure in wild birds. Although these studies have been very useful to show the associations between metal exposure and coloration, only few of them have actually quantified the levels of metal exposure at the individual level; always focusing on one or two of them. Here, we measured the concentrations of eight metals in great tits' feathers and then assessed how these levels of metals were associated with the carotenoid and melanin-based colorations. We found that the melanin pigmentation was positively associated with the copper concentration and negatively correlated with the chromium concentration in feathers. In addition, we have shown that the carotenoid-based coloration was negatively

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associated with the feather's mercury concentration. This study is the first one to identify some metals that might affect positively and negatively the deposition of melanin and carotenoid into the plumage of wild birds.

1. Introduction

Metals are naturally found in the environment (Nriagu, 1989) but are also emitted through anthropogenic activities (Azimi et al., 2005; Roux and Marra, 2007). As a consequence, the levels of metals have increased in urban areas raising some concerns about the potential deleterious effects of these elements on wildlife (Dauwe et al., 2004; Hsu et al., 2006). Metals are commonly absorbed by wild animals through their diet and some of the metals (zinc, iron, copper, cobalt and manganese) are involved in the control of various metabolic and signaling pathways (Bogden and Klevay, 2000; Valko et al., 2005). However, at high concentrations, these metals are known to have toxic effects, partly by the induction of high levels of oxidative stress (Ercal et al., 2001). Other metals like cadmium and lead do not have any known biological function but have various toxic effects (Ercal et al., 2001).

Animal colored-signals often reflect the recent nutrition, health, endocrinology, oxidative stress, or other resource-based attributes of their bearers (McGraw, 2006) and are thus involved in sexual contexts because they can be used by animals to assess the quality of another individual as a mate (Hill, 2006) or competitor (Senar, 2006). The potential effects of metals on animal coloration have been the focus of several studies in the last 15 years, with a special interest on the relationships between exposure to these metals and the deposition of the two most common pigments of the vertebrate tegument: the carotenoid and melanin pigments (Chatelain et al., 2014; Dauwe and Eens, 2008; Geens et al., 2009; McGraw, 2003, 2007, 2008, Niecke et al., 2003; Roulin et al., 2006). So far, melanin pigmentation seems to be positively associated with metal exposure in wild birds since (1) more pigmented individual feral pigeons (*Columba livia*) are found in urban compared to rural environments (Jacquin et al., 2013; Obukhova, 2007), (2) darker feral pigeons had higher concentrations of zinc in their feathers compared to paler ones after one year in standardized conditions (Chatelain et al., 2014), (3) copper deficiency influences plumage color in poultry species (Leeson, 2009; Leeson and Walsh, 2004) and (4) melanin pigmentation in great tits (*Parus major*) increases along a gradient of metal pollution caused by a lead smelter in Belgium (Dauwe and Eens, 2008). Two main hypotheses have been proposed to explain this association. First, the tyrosinase enzyme activity, a crucial component of the melanogenesis process, might be stimulated in metal-rich environment due to its copper-containing molecular structure (McGraw, 2003; Prota, 1993). Second, highly pigmented individuals might be more able to store metals in feathers due to the capacity of melanin pigments to bind metal ions (Chatelain et al., 2014; McGraw, 2003).

To the contrary, the carotenoid-based coloration was negatively affected along the same gradient of metal pollution Belgium (Dauwe and Eens, 2008; Eeva et al., 1998; Geens et al., 2009) and in urban areas (Isaksson et al., 2007). In this case, the detrimental effect of metal exposure on coloration might be explained by a decrease in the dietary availability of carotenoid pigments in polluted environments (Isaksson and Andersson, 2007) and/or the allocation of carotenoid pigment to the antioxidant and immune systems, at the detriment of coloration, since pollutants such as metals are known to induce oxidative stress in animals (Blount et al., 2003; Faivre et al., 2003; McGraw and Ardia, 2003).

Although the studies published on this topic have been very useful to show the associations between metal exposure and both melanin and carotenoid-based colorations, only few of them have actually quantified the levels of metal contamination at the individual level; always focusing on one or two metals (Chatelain et al., 2014, Niecke et al. 2003, Roulin et al. 2006). Here, we measured the concentrations of eight metals in great tits' feathers (metal concentrations in feathers are known to reflect blood metal levels during feather growth, Burger and Gochfeld, 1992; Janssens et al., 2001) and then assessed how these levels of metals were associated with the carotenoid and melanin based colorations, allowing us to determine which specific metals might have a positive or a negative effect on both colorations.

2. Material and methods

2.1. Field procedure

From 9th February to 6th May 2009, we use baited funnel traps to capture 32 great tits (13 females and 19 males) in city parks in Barcelona (Spain, see Björklund et al. (2010) for a detailed description of the parks). At capture, sex and age were determined according to Svensson (1992) and the second left and right outermost tail rectrices (R5 and L5) were collected for later analyses of pollutant levels (see below). Feathers were stored in dry and dark conditions in individually marked polyethylene bags.

We also photographed each bird to measure the size of the melanin-based black breast-tie using Image Tool 3.0© (University of Texas, USA, Figuerola and Senar, 2000). We drew a line around the black-tie and then measured the black area within 3 cm, starting 2 cm after the junction of the black-tie and the white cheek (Järvi and Bakken, 1984; Norris, 1990, 1993; Pöysä, 1988; Quesada and Senar, 2007; Wilson, 1992). The use of digital photography standardized to a metrical reference allowed a high repeatability in the measurement of the black-tie area (Figuerola and Senar, 2000).

The breast carotenoid-based coloration (brightness, hue and chroma) was measured with a portable colorimeter Minolta CR200 (Minolta Corporation 1994). Three color measurements were taken at 90°, making contact with the surface of the feathers patch, and the mean was then calculated for each of the three components of the carotenoid-based coloration (brightness, hue and chroma). Repeatability of these measures was high ($r_i = 0.85$–0.92, $p < 0.001$) (Figuerola et al., 1999). Values of brightness, chroma and hue were collapsed into a single variable using a Principal Components Analysis. The first eigenvalue from PCA ($\lambda = 1.89$) accounted for 63% of the variance in plumage coloration. The three values (brightness, hue and chroma) of plumage coloration loaded positively to the first component (PC1), with all the values >0.70 (Brightness = 0.79, Chroma = 0.72, Hue = 0.86). Hence, PC1 is hereafter called yellow coloration. We have shown elsewhere that there is no correlation between tie size and the carotenoid-based coloration in great tits (Senar et al., 2003).

2.2. Trace element analysis

Feathers were first rinsed with a 0.25 M NaOH solution for 1 min and then with deionized water for another minute. Feathers (ca. 20 mg) were oven dried at 60° for 24 h and then digested in H_2NO_3 (0.5 ml) and H_2O_2 (0.5 ml) in Savilles Teflon digestion vessels for 12 h at 100 °C. Levels of mercury, copper, lead, chrome, arsenic, cadmium, antimony and zinc were measured using a ICP-MS Perkin Elmer ELAN 6000. Accuracy of analysis was checked by measuring certified reference tissue: human hair (BCR 397, Community Bureau of Reference, Commission of the European Communities). All trace element concentrations were expressed on a dry weight basis ($ng \cdot g^{-1}$ dw). Mean recoveries ranged 96–100% for total mercury, selenium, copper, lead, chromium and arsenic; and no corrections were done. Analyses were performed at the Serveis Científico-Tècnics from Universitat de Barcelona (UB).

2.3. Statistical methods

Values of trace element concentrations were routinely checked for normal distributions using Q–Q plots. Metal levels data were log transformed to meet the assumptions of parametric statistics. Variance inflation factor (VIF) was high (>3) for three of the elements (Cd, Pb, Sb), emphasizing the presence of colinearity and we thus assessed for potential relationships between metal concentration and coloration using ridge regression (Hill and Lewicki, 2007; Press et al., 2007).

Black tie size and yellow coloration were used as dependent variables, and the concentration of metals in feathers were our independent variables. Since sex and age could have an effect on our dependent variables (Senar et al., 2003), we introduced sex and age as collateral variables, to control for their effect. We used stepwise backward selection to eliminate non-significant variables ($p > 0.05$) in the final model. We used SPSS 15.0 and Statistica 9. The level of significance was fixed at 0.05.

3. Results

The concentration of metals in feathers was not influenced by age (Wilk's test, $F_{8,21} = 1.42$, $p = 0.25$), but we found an effect of sex (sex: Wilk's test, $F_{8,21} = 3.04$, $p = 0.02$), which was restricted to zinc, males presented higher values of zinc than females ($F_{1,28} = 18.2$, $p < 0.001$).

We found that the sum of all the metals measured was positively correlated with tie size ($F_{1,30} = 10.93$, $p = 0.002$) but not with the carotenoid-based coloration ($F_{1,30} = 2.07$, $p = 0.16$).

When regressing tie size in relation to the concentration of heavy metals in feathers, we found that tie size was positively related to copper concentration in feathers and negatively associated to chromium concentration (Table 1, Fig. 1). We also found that the yellow breast coloration (PC1) was negatively correlated with the mercury concentration in feathers (Table 1, Fig. 2).

4. Discussion

Metal exposure has been known to be positively associated with melanin pigmentation in birds (Chatelain et al., 2014 (zinc and lead), Dauwe and Eens, 2008 (along a metal pollution gradient), McGraw, 2003, 2007 (calcium), 2008, Niecke et al. 2003 (calcium and zinc), Roulin et al. 2006 (calcium)) and here, we found a significant positive relation between the feather' copper concentration and the melanin-based coloration in great tits. This result supports the hypothesis proposed by McGraw (2003) which stipulates that the tyrosinase enzyme might be stimulated in metal-rich environments due to its copper-containing molecular structure (Prota, 1993); tyrosinase being a crucial component of the melanogenesis process where it catalyzes the oxidative conversion of tyrosine to the intermediate product, dopaquinone (Hearing and Tsukamoto, 1991; Sanchez-Ferrer et al., 1995). Alternatively, our results might also be explained by an increased capacity to store copper in feathers in highly pigmented individuals due to the capacity of the melanin to bind metal ions (Chatelain et al., 2014; McGraw, 2003). However, under this last hypothesis, we predict that

Table 1
Results of the ridge regressions examining the relationships between metal exposure and coloration.

	Partial r	t	p
Tie size			
Cu	0.49	3.0	0.01
Cr	−0.39	2.3	0.03
Sex	0.83	7.8	0.00
Yellow PC1			
Hg	−0.42	2.5	0.02
Sex	0.45	2.7	0.01

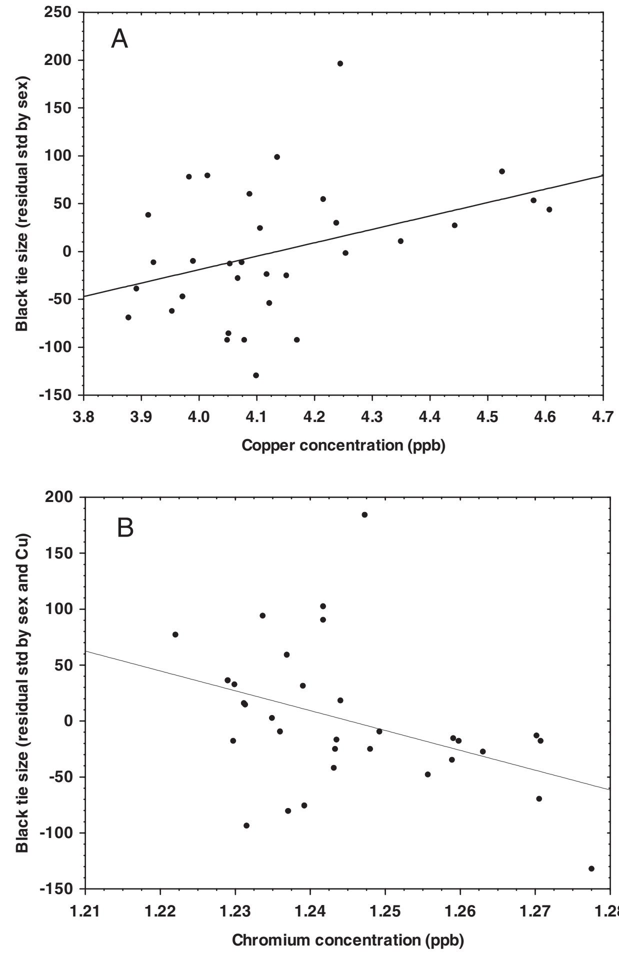

Fig. 1. Relationships between the feather (A) copper and (B) chromium concentrations and the size of the black tie. Residuals are presented to account for the effect of sex (A) and sex and copper levels (B) on tie size.

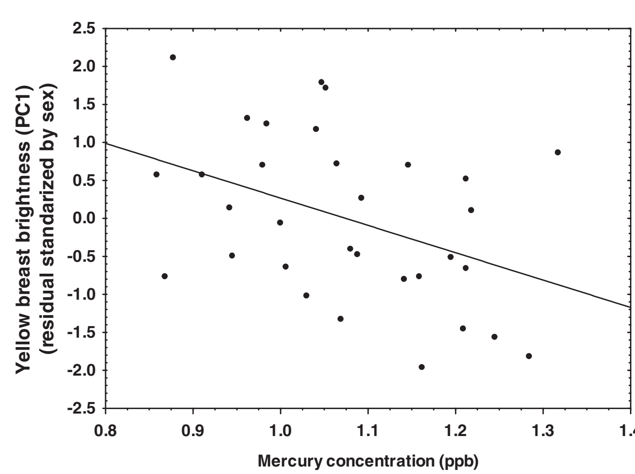

Fig. 2. Relationships between the feather concentration of mercury and the plumage carotenoid-based coloration.

the binding property of the melanin would not only be directed toward the copper element and thus, more than one metal would be positively associated with the melanin pigmentation, which was not the case in our study. Inversely, our data also show that the melanin-based coloration was negatively associated with the feather concentration of chromium. Our study is the first one to show this negative association, but the physiological mechanisms by which this metal might alter the melanin pigmentation remain unexplored for the moment. Chromium can have mutagenic, teratogenic and carcinogenic effects and has the ability to produce reactive oxygen species at high concentrations (Koivula and Eeva, 2010; Stohs and Bagchi, 1995; Valko et al., 2005). In offspring black ducks (*Anas rubripes*), elevated levels of dietary chromium can alter the growth pattern and reduce survival (Eisler, 1986). Thus, the toxic effects of high levels of chromium are already known but future studies should experimentally test the potential effect of this element (at the concentration found in the wild) on the melanin coloration and on the physiological mechanisms known to influence the development of this coloration in birds (oxidative stress, testosterone (Galvan and Alonso-Alvarez, 2010), corticosterone (Roulin et al., 2008)).

We have also shown for the first time a significant negative relationship between one of the metals measured, mercury, and the carotenoid-based coloration in a wild bird species. Our result is in accordance with the study of Geens et al. (2009) where a reduction of carotenoid-based coloration have been shown along a gradient of metal pollution in adult and nestling great tits. However, plasmatic carotenoid concentrations do not seem to be affected in metal polluted environments in the same species or others (Eeva et al., 2012), suggesting that the dietary availability of carotenoid is not reduced by metal pollution (but see also Isaksson and Andersson, 2007). The toxic effects of mercury on the neurology, physiology and reproduction of wild animals have been well documented in the literature (Depew et al., 2012; Wolfe et al., 1998). Interestingly, a positive correlation between mercury levels and oxidative stress related gene expression has been recently found in wild double-crested cormorant (*Phalacrocorax auritus*, Gibson et al., 2014), suggesting that carotenoids in the body may be drained to be used by the immune and/or antioxidant systems and thus comparatively less available for use in ornate coloration in birds exposed to high levels of mercury.

To conclude, our results agree with the general view that scarce transition and alkaline earth metals obtained from the diet could act as critical regulatory factors in the biosynthesis of melanin pigments (McGraw, 2003, 2006, 2008). However, it seems that the melanogenesis process is stimulated only by the copper element in great tits. In addition, we show for the first time that another metal, the chromium, can inversely be negatively associated with the melanin pigmentation. Finally, we have shown that the reduction of the carotenoid-based coloration in metal-contaminated areas might be explained by the toxic effect of mercury.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.06.021.

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