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PRODUCTION OF MELANIN-BASED PLUMAGE TRAITS IN MALE HOUSE SPARROWS: NO EFFECT OF DIETARY COPPER

ABSTRACT

In many bird species, males possess conspicuous patches of black feathers on their head or breast, the size of which is often positively related to their success in intra- and intersexual interactions. The production of these plumage signals is presumed to depend upon one or more limiting factors which prevent low quality males developing large signals in order to gain the associated benefits. The coloration of these feathers is due to their infusion with the pigment melanin, which birds synthesize endogenously during a series of conversion steps beginning with the enzymatic oxidation of the amino acid tyrosine. Copper is the cofactor of the enzyme responsible for this process, and it is possible that copper level limits the activity of the enzyme and therefore limits melanin production. We tested this hypothesis by manipulating the level of dietary copper available to individually caged juvenile and adult house sparrows (*Passer domesticus*) throughout their moult. Birds were provided with artificial diets containing either 100%, 50% or 10% of the recommended level of copper. Dietary copper did not affect the size of the black breast patch, the most obvious melanin-based plumage trait in this species, nor did it affect the reflectance of the black feathers. In sum, we reject the hypothesis that dietary copper limits the size or blackness of the breast patch of the male house sparrow.

Key words: Melanin, copper, diet, plumage, *Passer domesticus*

Running page headline: Dietary copper and plumage in house sparrows

INTRODUCTION

A striking feature of male birds from a variety of species is the presence of distinct patches of black or brown feathers, particularly around their face or breast (McGraw 2006). Males with large patches are often dominant to males with small patches in contests over resources, and may also be favored as social mates by females (see Searcy and Nowicki 2005 for a review). The coloration of these feathers is usually due to their infusion with one of two classes of the pigment melanin; eumelanins are responsible

for the appearance of black feathers while phaeomelanins are responsible for the appearance of a range of brown feathers (Jawor and Breitwisch 2003; McGraw 2006).

Several authors have speculated that there must be some cost to melanin production which prevents low quality individuals producing large black patches in order to reap the associated rewards (e.g. Jawor and Breitwisch 2003). The most parsimonious of these production costs is nutritional. Melanins are synthesized endogenously from the amino acid tyrosine (Lerner and Fitzpatrick 1950; Prota 1992), and thus a simple hypothesis is that the production of melanin-based traits is limited by the amount of protein or overall food available during moult. This hypothesis has received several tests, particularly in the house sparrow (*Passer domesticus*), a species in which the male possesses a conspicuous 'bib' of black feathers (Anderson, 2006). Veiga and Puerta (1996) found that juvenile male house sparrows kept in captive flocks and given ad libitum access to seed during moult produced black bibs of a comparable size to adult males, whereas in the wild, juvenile males have smaller bibs than adults. However, Gonzalez et al. (1999), Buchanan et al. (2003) and Poston et al. (2005) varied the level of protein provided to moulting sparrows but found no effect of diet quality on bib size. McGraw et al. (2002) manipulated food availability by varying the regularity with which food was provided to captive sparrows but found no difference in bib size between experimental birds and those provided with ad libitum food. More specifically, Poston et al. (2005) provided individually caged juvenile sparrows with artificial diets containing reduced levels of phenylalanine and tyrosine, the amino acid precursors of melanin, but found no effect on bib size, although they did produce bib feathers that were lighter in colour than control birds.

It is possible, however, that the production of melanin-based traits is limited by specific nutritional components rather than the gross amount of protein or food consumed (McGraw 2003, 2008). Several micro- and macro-minerals are involved in melanin production (Prota 1992), with copper having the most clearly defined role. Melanin synthesis begins with the oxidation of the amino acid tyrosine to dihydroxyphenyl L-alanine (DOPA) by the enzyme tyrosinase (Lerner and Fitzpatrick 1950; Prota 1992). Copper is the cofactor for this enzyme, and the essentiality of copper for melanin production has long been recognized by breeders of pets and agricultural animals, since diets deficient in copper produce depigmentation in rats, cats, rabbits, cattle and chickens (Lerner and Fitzpatrick 1950; Maynard et al. 1979; Scott et al 1982). Furthermore, melanin synthesis can be blocked by providing animals with chemicals which bind to copper and thus inhibit the action of tyrosinase (Maynard et al. 1979). Tyrosinase activity and thus melanin production can be restored by adding cupric salts, while adding salts of a range of other metals has no effect (Maynard et al. 1979). Also, one of the albino mutations in chickens is caused by a deletion in the copper-binding site of the tyrosinase gene (Tobita-Teramoto et al. 2000).

Since the presence of copper is demonstrably essential for melanin production, it is conceivable that the amount present is a rate-limiting step for the production

of melanin-based plumage traits. In this study we tested whether dietary copper affects the expression of melanin-based plumage traits in the house sparrow. The house sparrow is a small (≈ 30 g), socially monogamous, multi-brooded passerine common around human settlements throughout the world (Anderson 2006). It is sexually dichromatic, with the females being plain brown while the males have a chestnut head and back, a gray crown, white cheeks, a white wing bar, and a bib of black feathers which extends over the throat and upper breast. The bib feathers initially have pale tips which occlude the underlying black feathers, but these are gradually abraded so that the majority of the bib is visible by early spring to coincide with mate attraction and breeding (Anderson 2006). The size of the bib varies considerably between males, and the functional significance of this variation has received much attention. Males with large bibs are dominant over those with small bibs in both free-living and captive populations, and, in some populations, have higher seasonal and lifetime reproductive success (reviewed by Nakagawa et al. 2007).

We maintained individually caged male house sparrows throughout their moult on artificial diets containing different levels of copper. We predicted that there would be a positive correlation between the level of dietary copper and the size of the bib produced, and also the blackness of the feathers which comprise the bib. We also measured the spectral reflectance of other melanistic and non-melanistic male plumage traits and predicted that dietary copper would have a positive effect on the former but no effect on the latter.

MATERIAL AND METHODS

We caught 32 juvenile male house sparrows from several sites around Lexington, Kentucky during July 2004. Juvenile sparrows can be reliably sexed in the field because males have a dusky throat and chestnut head flecks whereas females have a pale throat and no flecks (personal observation). Birds were given a unique combination of colour bands then we measured their weight to the nearest 0.1g on an electronic balance and used calipers to measure the length of their left tarsus and the length of their dusky throat patch. In addition, we obtained 32 adult male sparrows from outdoor aviaries at the University of Kentucky's Ecological Research Facility in Lexington where they had been housed in small single-sex groups during the summer as part of a behavioural study. We fitted them with color bands, recorded their weight, and used calipers to measure the length of their left tarsus and the length and breadth of their black bib. We also plucked a feather from the lower third of their bib for later analysis of spectral reflectance (see below for details). All 64 birds were placed into individual metal cages (25 x 25 x 40 cm) arranged in racks within two large outdoor aviaries at the aforementioned research facility. Each cage was outfitted with perches, a plastic tube used for roosting, a water dispenser and a feed hopper attached to the front of the cage.

The hoppers were initially filled with a mix of millet and chicken feed but in the third week of July we began to add an increasing proportion of an artificial diet each day, so that by the end of the week the birds were subsisting purely on this diet. The artificial diet was based on that used to maintain captive white-crowned sparrows, *Zonotrichia leucophrys*, a granivorous passerine similar in size to the house sparrow (Murphy and King 1982). This diet was also used to maintain moulting house sparrows in a previous experiment (Poston et al. 2005). Briefly, the diet contains 67% corn starch and 8% protein, in the form of casein and amino acids, then a suite of other ingredients including oils, vitamins and minerals (see Tables 1 and 2 in Poston et al., 2005 for the overall recipe and the amino acid content of the protein mix respectively). The diet resembles a coarse powder and was readily consumed by the birds.

On July 29th we randomly assigned birds to one of three diets which were identical apart from their copper content. We adjusted copper content by varying the ratio of two mineral mixes included in the diet, one of which contained copper while the other did not (both obtained by Dyets, Inc, Bethlehem PA, USA). The first of these, the Fox-Briggs N mineral mix, provides a level of dietary copper of 4 mg/kg when added at the manufacturer's suggested rate of 60 g per kg of diet, which is the copper level recommended for domestic fowl chicks (*Gallus gallus domesticus*) by the Nutrition Research Council. The other mineral mix was identical but contained no copper. We generated three dietary treatments supplying either 100% of the recommended level of dietary copper (mineral content comprised of pure Fox-Briggs N mix), 50% of the recommended level (1 part Fox-Briggs N mix: 1 part copper-free mix) or 10% of the recommended level (1 part Fox-Briggs N mix: 9 parts copper-free mix).

The recipe we used to make our artificial diets (Murphy and King 1982) uses slightly less mineral mix than the manufacturer's recommended level, at 55 g per kg of diet. Thus, the actual copper content of our 10%, 50% and 100% copper diets was 0.37, 1.85, and 3.7 mg/kg respectively. We did not have a treatment that was entirely copper-free because some copper is essential for normal bodily function (Maynard et al. 1979; Klasing 1998). To prevent birds obtaining extra copper through their water they were only provided with distilled water during the experiment.

We started the experiment with 64 birds; 22 on the 100% copper diet (11 adults, 11 juveniles), 20 on the 50% copper diet (10 adults, 10 juveniles), and 22 on the 10% copper diet (11 adults, 11 juveniles). At the start of the first week we filled each feed hopper with a weighed amount of diet then added more diet every second day. At the end of the week we weighed how much diet was left uneaten in the hopper, then subtracted this from the sum of the amount added initially and the amount added during the week to determine weekly consumption. Spillage was negligible.

The birds were maintained on their artificial diets for 14 weeks, at which point they were removed from their cages and weighed, then one of us (IRKS) used calipers to measure the length (L) and breadth (B) of their black bib, excluding the pale tips of the

bib feathers, and calculated its size according to the equation $\text{Bib size (mm}^2\text{)} = 167 + (0.45 \times \text{L mm} \times \text{B mm})$ (Møller 1987). Unfortunately, the repeatability of these bib size measurements was not assessed. However, IRKS had been measuring house sparrow bibs for several years at our nearby long-term field site with significant repeatability ($r_{IC} = 0.61$, $F_{32,35} = 4.22$ $P < 0.001$) and thus we feel confident that the bibs were measured reliably. We also divided the birds into two categories according to whether the whole of their freshly-moulted bib was covered by pale feather tips or whether they had a distinct region of untipped bib feathers directly below the bill, since this dichotomy had previously been noted in wild sparrows (personal observation). We measured the length of the pale tip of a black feather originating at the lowest point of the bib, and also the length of the terminal white portion of the third wing bar feather.

We measured the spectral reflectance of the white wing bar, the grey crown, and the grey cheek in situ using a USB2000 spectrometer (Ocean Optics, Inc., Dunedin, FL, USA) standardized to both white and dark standards and with the probe/light source at 45° to the plane of the feather. We measured the reflectance of each region twice and used mean reflectance over the range 350-700 nm as our estimate of brightness. To reduce handling time, we plucked a bib feather from each bird then later estimated its reflectance after taping it to a white index card. The reflectance measures from each bird were significantly repeatable ($r_{IC} = 0.63$, $F_{29,30} = 4.41$ $P < 0.001$ for wing bar, $r_{IC} = 0.66$ $F_{29,30} = 4.87$ $P < 0.001$ for crown, $r_{IC} = 0.47$ $F_{29,30} = 2.74$ $P < 0.005$ for cheek and $r_{IC} = 0.66$ $F_{31,32} = 5.18$ $P < 0.001$ for bib) (Lessells and Boag 1987), and so were averaged for the analyses.

We punctured the brachial vein of each bird after they were measured at the end of the trial, and collected about 100 μl of blood into an eppendorf tube which was immediately centrifuged and the plasma separated and stored at -60°C . We measured the amount of copper present in plasma samples obtained from 5 birds in each of the 3 diet treatments using inductively-coupled plasma emission spectrometry at the Environmental Research Training Laboratory at the University of Kentucky. 50 μl plasma was digested in 3 ml concentrated nitric acid at 100°C , boiled with 2 ml hydrogen peroxide to remove excess organic content, allowed to cool and then diluted to 25 ml with ultrapure water and analyzed on a Varian Vista Pro spectrometer together with five standards, method blanks, and blanks spiked with known amounts of copper. The intra-assay coefficient of variation was 14%. We analyzed the data using parametric statistics in SYSTAT 10 (SPSS 2000). We report means \pm standard errors throughout.

RESULTS

Mortality and food consumption

30 of the 64 birds died during the 14 week experiment. The birds that died were approximately evenly spread across diet and age categories although mortality was particularly

high among juveniles on the 10% copper diet, with 9 of the 12 birds dying. We ended the experiment with 34 birds, of which 20 were adults and 14 were juveniles. There were 11 birds from the 100% copper treatment (6 adults, 5 juveniles), 11 from the 50% copper treatment (5 adults, 6 juveniles), and 12 from the 10% copper treatment (9 adults, 3 juveniles).

The average weekly food consumption did not differ between birds on the three diets (Repeated Measures ANOVA $F_{2,31} = 0.58$ $P = 0.57$). A two-way ANOVA showed that weight change across the experiment was not related to diet treatment ($F_{2,27} = 1.31$ $P = 0.29$) although it was related to age ($F_{1,27} = 4.89$ $P = 0.036$), with adults losing weight during the experiment while juveniles showed no change in weight.

Plumage trait size

The data from both age classes were pooled when analyzing the size of each plumage trait in relation to diet since there was no difference between those of adult males and juvenile males ($t = 0.37$ to 1.3 , $P = 0.20$ to 0.71). The average bib size ($F_{2,28} = 0.07$ $P = 0.94$, Fig. 1), length of the pale tip of a bib feather ($F_{2,28} = 1.68$ $P = 0.21$), and length of a white wing bar feather ($F_{2,28} = 0.18$ $P = 0.83$) did not vary with dietary copper. These results were qualitatively unchanged if the data were reanalyzed using a two-way ANOVA with age and dietary copper as categorical variables. After accounting for the repeatability of the bib size measurement, we would have detected a major effect of dietary copper with a power of 0.40.

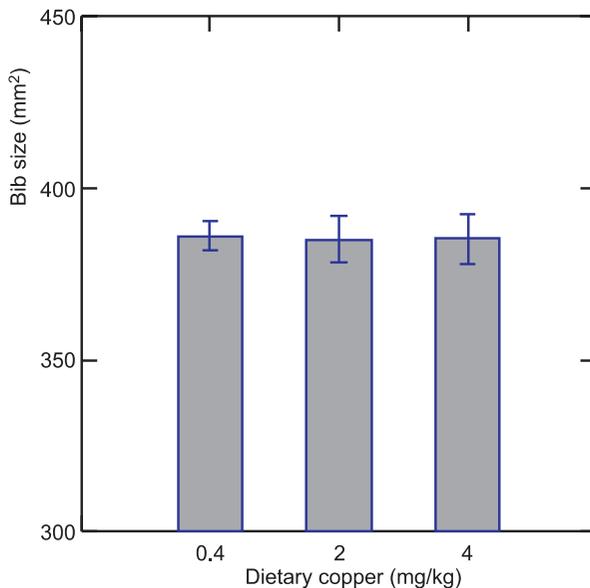


Figure 1. Mean bib sizes (mm²) produced by male house sparrows maintained throughout their moult on artificial diets containing 0.4, 2 or 4 mg/kg of copper.

The length of the dusky throat patch males possessed as juveniles was not related to the length of the bib they possessed after moult ($r = -0.38$, $n = 12$, $P = 0.2$). Pooling all diets, adult males had significantly larger bibs after the moult than before (paired $t = 4.0$ $df = 16$ $P = 0.001$). Data on bib size before and after the experiment were not available for all 20 males, as two had not been measured beforehand and another two did not moult (one on the 10% copper diet and one on the 50% copper diet). Adult males were significantly more likely to have a distinct region of untipped bib feathers below their bill after the moult than juvenile males (16/17 v 2/14, Fisher's exact test $P < 0.001$).

Spectrometry

The reflectance data from both adult and juvenile males were pooled for the analysis as the means did not differ between age classes ($t = 1.08$ to 1.46 , $P = 0.16$ to 0.29). The reflectance of the black bib ($F_{2,25} = 0.311$ $P = 0.74$, Fig. 2), the grey crown ($F_{2,26} = 0.455$ $P = 0.64$) and the grey cheek ($F_{2,26} = 0.11$ $P = 0.89$) did not vary with dietary copper. However, there was a non-significant tendency for wing bar reflectance to vary with diet ($F_{2,26} = 2.48$, $P = 0.1$). In both age classes, birds on the 50% copper diet had brighter wing bars than birds on either the 10% or 100% copper diets. Repeating the analysis using two-way ANOVAs produced qualitatively similar results. After accounting for the repeatability of the measurements, we would have detected a major effect of dietary copper upon the reflectance of these traits with a power of 0.28-0.44.

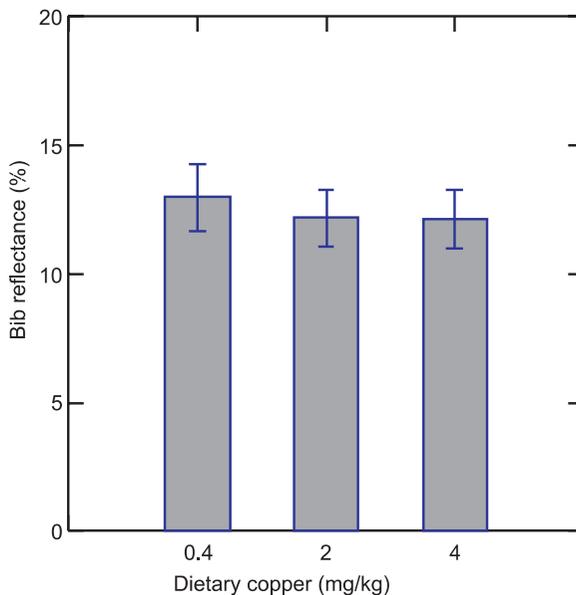


Figure 2. Mean reflectance (%) of bib feathers produced by male house sparrows maintained throughout their moult on artificial diets containing 0.4, 2 or 4 mg/kg of copper.

The reflectance of freshly-moulted bib feathers was negatively correlated with the size of the bib from which they were taken ($r = -0.391$ $n = 32$ $P = 0.027$). The reflectance of bib feathers taken from adult males after their moult was not correlated with those taken from the bib they possessed prior to moult ($r = 0.18$, $n = 18$, $P = 0.46$). There was a significant negative correlation between the reflectance of the bib and cheek ($r = -0.38$ $n = 32$ $P < 0.05$), and a non-significant positive correlation between the reflectance of the wing bar and cheek ($r = 0.32$ $n = 32$ $P < 0.1$).

Plasma copper levels

Plasma copper concentrations varied considerably between birds, with an average of 0.35 ppm (± 0.19 SE) and a range of 0 – 2.85 ppm. The levels in four birds (1 on the 10% copper diet, 2 on the 50% copper diet, and 1 on the 100% diet) were below the detection limit of the apparatus (~ 0.02 ppm), and were counted as zeros for the analysis. Plasma copper concentration did not vary with dietary copper ($F_{2,12} = 0.72$ $P = 0.5$), and was not related to bib size ($r = 0.004$, $P > 0.9$)

DISCUSSION

Several of the plumage signals displayed by male birds appear to be condition-dependent (Griffith et al. 2006), which has important implications because ecological or socially-mediated variation in access to a limiting resource would thus provide the developmental variation necessary for signals to be informative. However, establishing a condition-dependent mechanism for melanin-based signals has proved challenging (McGraw 2006, 2008). We performed the first explicit test of the hypothesis that dietary copper affects the size of melanin-based plumage traits by maintaining individually caged male house sparrows throughout their moult on one of three artificial diets which were identical apart from their copper content. We did not support the hypothesis, however, since there was no difference in bib size between birds maintained on different levels of dietary copper, nor was there a difference in the mean reflectance of their bib feathers. Dietary copper had no significant effects on other plumage traits that are sexually dimorphic in house sparrows, including the size or reflectance of the white wing bar, or the reflectance of the gray crown or cheek patch. While the power of our experiment cannot eliminate small effects of copper, our results indicate it is not the main explanatory variable for natural variation in bib size.

We found a significant negative relationship between bib size and reflectance, as did Vaclav (2006), which suggests males either allocate a relatively large proportion of melanin to relatively few feathers or vice versa. There was no correlation between the reflectance of the bib feathers the adult males produced during the experiment and the reflectance of the bib feathers they had produced in the wild during the previous molt (i.e. the bib they had at capture). This suggests that bib blackness, like bib size, does not have a strong genetic component (see also Griffith et al. 1999).

We tested the assumption that circulating levels of copper would be positively correlated with dietary levels by assaying the concentration of copper in plasma samples obtained from a subset of birds on each diet. In chickens, for example, increasing the concentration of copper in the diet results in a higher concentration of copper in the plasma (Ewing et al. 1998; Mondal et al. 2007). Surprisingly, however, plasma copper concentrations were strikingly variable between individuals and did not differ between sparrows consuming ~ 0.4mg, 2mg or 4mg of copper per kilogram of diet. This was not because birds on the reduced copper diets had compensated by increasing their intake, since consumption rates did not differ among treatments. Instead, this may have been caused by the differential release of copper stored in labile pools in the blood or other organs (Evans and Wiederanders 1967), especially the liver (Haarakangas et al. 1974). Copper is essential for several bodily functions other than melanin production, including the activity of several other enzymes and the formation of haemoglobin, bone and connective tissue (Maynard et al. 1979, reviewed in Frank 1998). Consequently, homeostatic mechanisms likely exist which release copper from loosely-bound fractions in response to a variety of physiological requirements.

The experiment produced relatively few effects on plumage, which raises several concerns about our methodology. The first of these is whether the levels of copper we used in our artificial diets were in the appropriate range to encompass natural variation. We are unaware of any studies of dietary copper in passerines, or indeed any wild bird, so we had to base our uppermost diet (100% of the required amount of copper) on the level recommended for domestic chicks by the poultry industry (\approx 4 mg/kg of diet, see Maynard et al. 1979; Klasing 1998). This seems reasonable, for two reasons. First, young chicks are of a similar size to fully grown house sparrows. Second, house sparrows are omnivorous but subsist mostly on grain, particularly in agricultural settings (Anderson 2006). The concentration of copper in cereal grains is marginal (4-8 mg/kg, Klasing 1998) and reasonably similar to that found in our 100% copper diet (4 mg/kg). We therefore believe that our 100% copper diet is comparable to the amount of copper obtained by the wild sparrows which are most successful at foraging, and that the 50% and 10% copper diets represent the levels obtained by sparrows which have intermediate and poor foraging success, respectively, or perhaps an impaired ability to assimilate copper from their diet.

A second methodological concern is the mortality. We experienced a comparable level of mortality in a previous experiment where individually caged sparrows were maintained on artificial diets (Poston et al. 2005), as well as in a more recent experiment involving seed-based diets (unpublished data), and Gonzalez et al. (1999) also observed significant mortality among captive juvenile house sparrows, suggesting that some house sparrows do not adapt well to solitary confinement, possibly because of their typically gregarious nature. We were careful to regularly clean beneath the cages to avoid the build-up of droppings and other waste, and we also monitored the appearance of each bird while refilling their feed hoppers so that we could quickly remove any

birds that were visibly suffering or inactive. However, mortality was never preceded by outward signs of distress or the appearance of discolored droppings. It is possible that the birds had died from West Nile Virus. Biting insects are prevalent at the research facility, probably because it contains a network of small marshy ponds and streams, and mosquitoes are regularly found inside the aviaries, which are outdoors and have large-gauge mesh sides. Although the mortality was unfortunate, we are confident that it does not bias or invalidate our results, since there were no differences in body size or initial mass between the birds which died and those which survived, and the birds that survived until the end of the experiment were similar to wild sparrows measured around the same time of year with regard to several morphological indices (e.g. body mass, size and condition, reflectance of plumage traits etc, unpublished data).

The patterns of mortality may even provide an insight into the importance of mineral and metal reserves to molting birds. For instance, 9 of the 12 juveniles on the 10% copper diet died, but only 3 of the 11 adults. One explanation for this difference is that the adults may have had relatively large reserves of copper which they were able to call upon for other essential bodily functions, whereas the juveniles may have had relatively little time to acquire copper in the interval (likely 1-2 months) between fledging and being placed into captivity. Hence, their low copper reserves may have been adequate for melanin production but not enough to sustain their other physiological requirements (Frank 1998), which suggests that the threshold level of copper required for the production of melanin-based plumage traits per se may be low. Haarakangas et al. (1974) measured the level of copper and three other elements in the liver of house sparrows collected throughout the year in Finland and found that it was highest at the start of moult, which is consistent with birds storing copper in advance of molt. However, the level of copper in the liver was not higher in males than in females, as would be expected if males needed additional copper to aid melanogenesis during moult.

Our study joins several others which have failed to support the intuitively appealing hypothesis that intraspecific size variation in melanin-based plumage traits is due to nutritional differences at the time of moult (see McGraw 2006 for a review). The interest in nutritional studies waned following this series of null results and the focus of developmental studies shifted toward endocrinological (Buchanan et al. 2003) or social factors (McGraw et al. 2003). Recently, however, evidence has emerged for a role of specific micronutrients in melanin production. Firstly, a correlational study by Dauwe and Eens (2008) found that great tits (*Parus major*) in an area contaminated with several metals including lead, zinc and copper, developed black breast stripes which were wider than males in uncontaminated areas. Secondly, McGraw (2007) found that zebra finches (*Taeniopygia guttata*) maintained on a diet of millet coated with calcium carbonate developed larger patches of black breast feathers than those fed on millet alone.

It would therefore seem premature to rule out a nutritional component to the production of melanin-based plumage signals in birds, though such components are

likely to be specific rather than general and may interact in complex ways. A systematic set of controlled experiments such as the current study would be an ideal approach through which to test the importance of additional minerals and metals, either alone or in concert. However, the studies that are likely to be most informative are those which consider the ecology of the animal in its environment and evaluate the likelihood that the elements concerned are indeed limiting in the wild. Almost nothing is known about the rates of consumption, storage, and excretion of most elements by wild birds (Klasing 1988; McGraw 2008) and yet these are exactly the types of data that are required for an understanding of how dietary physiology during the moult period affects the production of plumage traits.

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