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DIETARY CALCIUM NEGATIVELY AFFECTS THE SIZE OF A STATUS SIGNAL IN JUVENILE MALE HOUSE SPARROWS (*PASSER DOMESTICUS*)

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Abstract.—The production of some types of signals is often assumed to be cost-free, yet we know relatively little about the factors that affect signal development. In birds, many plumage signals are produced by the pigment melanin being deposited into growing feathers. Birds synthesize melanin in a complex pathway involving many potentially limiting resources, one of which may be dietary calcium. We tested the effect of dietary calcium on the development of the black throat patch (bib) of juvenile male House Sparrows (*Passer domesticus*). Subjects were maintained throughout their molt on artificial diets containing either a low, medium, or high level of calcium, or a bicarbonate control diet with a low level of calcium but an acid–base balance equivalent to that of the high-calcium diet. Dietary calcium affected the size of the bib, but not in the predicted direction: birds on the low-calcium diet produced the largest bibs. Birds on the bicarbonate control diet produced bibs intermediate in size that were significantly smaller than those on the low-calcium diet, which suggests that acid–base balance may also influence melanin-based signals. Dietary calcium also had a broader physiological effect; birds on the low-calcium diet were in better condition than those on other diets, but only the calcium treatment explained significant variation in bib size. Although our treatments affected bib size, our results indicate that access to dietary calcium is not a general mechanism for condition dependence of melanin-based ornaments. *Received 10 December 2009, accepted 15 March 2010.*

Key words: calcium, condition dependence, melanin, *Passer domesticus*, plumage, signal.

El Calcio de la Dieta Afecta Negativamente el Tamaño de una Señal de Estatus en los Machos Jóvenes de *Passer domesticus*

Resumen.—Usualmente se supone que la producción de algunos tipos de señales no tiene costos, pero conocemos relativamente poco sobre los factores que afectan el desarrollo de las señales. En las aves, muchas señales del plumaje son producidas por los depósitos del pigmento melanina en las plumas en crecimiento. Las aves sintetizan melanina mediante un circuito complejo que involucra muchos recursos potencialmente limitantes, uno de los cuales puede ser el calcio de la dieta. Evaluamos el efecto del calcio de la dieta sobre el desarrollo del babero negro de los machos jóvenes de *Passer domesticus*. Los individuos fueron mantenidos a lo largo de toda su muda con dietas artificiales que contenían niveles de calcio bajos, medios y altos, o una dieta control basada en bicarbonato con bajo nivel de calcio pero con un balance acido-básico equivalente a aquel de la dieta alta en calcio. El calcio de la dieta afectó el tamaño del babero, pero no en la dirección predicha: las aves con una dieta baja en calcio produjeron los baberos más grandes. Las aves con la dieta control de bicarbonato produjeron baberos de tamaño intermedio, que fueron significativamente menores que aquellos de individuos con la dieta baja en calcio, lo que sugiere que el balance ácido-básico también puede influenciar las señales basadas en melanina. El calcio de la dieta también tuvo un efecto fisiológico más amplio; las aves con una dieta baja en calcio estuvieron en mejores condiciones que aquellas sometidas a otras dietas, pero sólo el tratamiento de calcio explicó una variación significativa en el tamaño del babero. Aunque nuestros tratamientos afectaron el tamaño del babero, nuestros resultados indican que el acceso al calcio de la dieta no es un mecanismo general que explique la dependencia de la condición en los ornamentos basados en melanina.

In many birds, males display conspicuous patches of colorful feathers that function as signals in mate choice, dominance interactions, or both (e.g., Searcy and Nowicki 2005). The coloration of these feathers is usually a result of their infusion with one of two common pigment types: yellow or red feathers contain carotenoids, and brown or black feathers contain melanins (McGraw 2006a, b). Birds cannot produce carotenoids and therefore must acquire them from their diet (McGraw 2006a). It is primarily this limitation that regulates the information content of carotenoid-based signals, although carotenoid allocation to feathers rather than to other physiological functions could also affect the signal (e.g., Alonso-Alvarez et al. 2004).

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By contrast, melanins are synthesized endogenously from the amino acid tyrosine through a complex process that includes a variety of biochemical interactions (Lerner and Fitzpatrick 1950, Jawor and Breitwisch 2003, Galván and Alonso-Alvarez 2008). Some authors have suggested that melanin-based signals are cost-free (e.g., Badyaev and Hill 2000), and several studies have supported a lack of production costs for melanin-based traits. For example, the extent of black or brown breast spots in Barn Owls (*Tyto alba*) has a strong genetic basis (Roulin and Djikstra 2003), and several studies that manipulated condition during molt found no effect on the expression of melanin-based traits (e.g., Hill and Brawner 1998). However, the melanin-synthesis pathway contains an array of potential steps that could involve condition dependence. These range from variation in availability or allocation of tyrosine (e.g., Poston et al. 2005) to variation in dietary components that support the synthesis or deposition of melanin (McGraw 2003). Several micro- and macro-minerals are known to be involved in melanin synthesis (Prota 1992), and elemental analyses of feathers have produced correlative evidence of an association between melanin pigmentation and certain minerals. First, Niecke et al. (1999) found that the black base of the white tail feathers of White-tailed Eagles (*Haliaeetus albicilla*) was enriched in calcium, zinc, and manganese compared with the white distal portion, whereas the levels of several other elements did not differ between the two. Second, Niecke et al. (2003) found that the levels of calcium and zinc were 5× higher in the black breast-spot feathers of Barn Owls than in the adjacent pale breast feathers, and Roulin et al. (2006) found that owls with more calcium in their bones had a greater proportion of melanistic plumage.

These observations, coupled with the known role of certain minerals in melanogenesis, led McGraw (2003) to hypothesize that access to specific dietary minerals limits the expression of melanin-based plumage traits. McGraw (2007) tested this hypothesis using calcium, a macromineral that is essential for many physiological functions (Sigel 1984). Calcium may also have a positive effect on several stages of melanin production, such as regulation of the activity of tyrosinase, the enzyme involved in the initial conversion of the amino acid tyrosine to dopaquinone (Buffey et al. 1993). McGraw (2007) provided male Zebra Finches (*Taeniopygia guttata*) with either a control diet of seed or an experimental diet of seed supplemented with calcium carbonate and found that males maintained on the supplemented diet produced a larger patch of black feathers on their breast than males on the seed-only diet.

We investigated the role of calcium in the production of a melanin-based plumage signal in the House Sparrow (*Passer domesticus*), a small passerine commensal with humans throughout much of the world (Anderson 2006). The species is sexually dimorphic, the females being pale brown whereas the males have a suite of striking plumage features, the most conspicuous of which is a patch of melanin-containing black feathers on the throat and chest known as the bib. The bib develops during the complete annual molt that House Sparrows undergo in the autumn of each year. Its size is thus fixed until the next molt, but it becomes more exposed throughout the year as the pale tips present on the melanin-containing feathers wear away to reveal more of the underlying black feathers. However, apparent bib size is positively correlated with absolute bib size (Jensen et al. 2006). An array of studies of the function of the bib in both captive and free-living populations have produced variable results, but the most consistent finding is that males with large bibs are dominant over those with small bibs and, in some populations, have higher reproductive success (for a review of the function of bib size, see Nakagawa et al. 2007).

The prevailing evidence is that variation in the House Sparrow's bib is not attributable to genetic variation, because an individual's bib size changes considerably between molts (Morrison et al. 2008), a cross-fostering study found no correlation between the bib size of juvenile males and their genetic father (Griffith et al. 1999), and a pedigree analysis produced low estimates of heritability (Jensen et al. 2008). Several studies have also found that bib size is influenced by endocrinological or nutritional conditions before or during molt. Manipulations of testosterone levels in adult birds (Evans et al. 2000) and eggs (Strasser and Schwabl 2004) affect bib size, but what causes variation in hormone levels and whether that explains natural variation in bib size is not clear. Veiga and Puerta (1996) provided captive juvenile and adult male House Sparrows with ad libitum food during their molt. Although juvenile males possessed smaller bibs than adults in wild populations (Veiga 1993, Morrison et al. 2008), there was no age-related difference under these conditions, which suggests that wild juvenile males produced smaller bibs because of nutritional limitations. Several subsequent studies have examined whether bib size has a nutritional component by varying the protein content of food (Gonzalez et al. 1999, Buchanan et al. 2003, Poston et al. 2005), the regularity with which food or food supplements were available (McGraw et al. 2002), or the availability of the amino acid precursors of melanin (Poston et al. 2005). None of these studies detected an effect of diet on bib size, although Poston et al. (2005) found that juvenile males on a diet deficient in tyrosine produced lighter-colored bib feathers than males on control diets. Thus, despite evidence that the bib is environmentally flexible, the mechanism responsible for size variation is not clear.

We performed an experiment similar to that of McGraw (2007) to test whether access to calcium in the diet is a general condition-dependent mechanism with the potential to influence melanin-based signals. We predicted that male House Sparrows provided with diets containing greater amounts of calcium would produce the largest bibs and that the bib feathers of these males would be darker than those of males provided with diets containing lower levels of calcium.

Methods

We captured 72 juvenile male House Sparrows from 12 locations around Lexington, Kentucky, in July 2007, before the annual molt, which occurs from late August through October. Juvenile House Sparrows can be sexed reliably in the field because males have a dusky tinge to the throat whereas females have a pale throat (I. Stewart and D. Westneat pers. obs.). We recorded each bird's mass and tarsus length on the day it was captured, fitted it with a numbered metal band, and placed it into an individual metal cage (25 × 25 × 40 cm). The cages were arranged in racks within two large outdoor aviaries. Each cage was outfitted with perches and a plastic roost tube, and a water dispenser and hopper of millet were fitted to the front of the cage. Birds were supplied with millet and water ad libitum. An increasing amount of artificial diet (0.65%

calcium; see below) was mixed in with the millet on successive days so that by the end of the week the birds subsisted solely on the artificial diet.

The birds were switched to their experimental diets over a 2-day period in early August, at which point we examined them to ensure that they had not begun to molt into their adult plumage. The bib feathers are among the first feathers to be molted (I. Stewart and D. Westneat pers. obs.), and 26 birds had at least one emerging bib feather. These birds were removed from the experiment and released at their site of capture. The remaining 46 birds were randomly allocated to one of four diets as a first block, and then 26 replacement birds were captured in the next 10 days. These birds were immediately supplied with a mix of millet and artificial diet, which was increased to pure diet over a 2-day period. Two days later, these birds were allocated to their experimental diets as a second block. The birds introduced in the second block thus had less time to acclimate to the artificial diet, but we did not observe any negative effects of this.

Diet formulations.—The birds were provided with artificial diets based on those used by Murphy and King (1982, 1991) to maintain captive White-crowned Sparrows (*Zonotrichia leucophrys gambelii*), which are granivorous passerines approximately the same size as House Sparrows. The diet is composed mostly of corn starch (67%) and protein (12%) in the form of casein supplemented by crystalline amino acids (see Poston et al. 2005: tables 1 and 2) but also includes cellulose, calcium-free salt mix, vitamins, and grit in the form of silica sand. One kilogram of each diet was mixed in a food blender with ~300 mL cold water and then spread over a foil tray and dried overnight at 55°C (following Koutsos et al. 2001). The final diets resembled a coarse crumble and were stored in a sealed container at 4°C.

The birds were maintained on one of four diets. Three of these diets varied in calcium content, and the fourth served as a treatment control. In McGraw's (2007) experiment, the diets provided to the control and supplemented Zebra Finches contained 0.65% and 1.2% calcium, respectively. We repeated these two levels but added a third treatment containing 0.1% calcium. This is the concentration of calcium present in the types of grain commonly consumed by House Sparrows (Earle and Clarke 1991, Klasing 1998) and is therefore probably more representative of the level they obtain in the wild. For these first three diets, we added increasing amounts of calcium carbonate ($CaCO_3$) to achieve calcium levels of 0.1%, 0.65%, and 1.2%. The increase in $CaCO_3$ was balanced by a corresponding decrease in the amount of cellulose, a non-nutritive, nonreactive bulking agent. We refer to these three treatments hereafter as low-, medium-, and high-calcium.

Calcium carbonate is a strong base (Sigel 1984), and hence it is possible that the positive effect that supplementary $CaCO_3$ had on the size of a melanin-based trait in Zebra Finches (McGraw 2007) was caused by a change in acid–base balance rather than the increase in calcium, given that changes in acid–base balance produce significant physiological effects in poultry (e.g., Keshavarz and Austic 1990, Klasing 1998). We therefore included a fourth diet in which we used sodium bicarbonate ($NaHCO_3$) to control for the increased alkalinity caused by adding $CaCO_3$. The acid–base balance of a diet is calculated by the equation *meq* (Na + K + Ca – P – Cl) (see Koutsos et al. 2001: table 1, where *meq* = milliequivalents or molar mass/ionic charge). We prepared the bicarbonate control diet by following the recipe used for the 0.1% calcium diet but then added sufficient *meq* of sodium ($Na_{meq} = 23$) to equal the additional *meq* of calcium ($Ca_{meq} = 20$) present in the high-calcium diet (1.2%). The *meq* of sodium added was thus the equivalent of 1.1% Ca (the difference between the 1.2% present in the high-calcium and the 0.1% present in the low-calcium diet).

The logic behind the bicarbonate control is as follows. If calcium alone regulates melanin synthesis, the birds on the bicarbonate control diet would produce bibs similar in size to those on the low-calcium diet because both of their diets contained the same amount of calcium (0.1%). If acid–base balance regulates melanin synthesis, however, the birds on the bicarbonate control diet would produce bibs similar in size to those on the high-calcium diet because both of their diets had the same acid–base balance, even though they contained very different levels of calcium (0.1% vs. 1.2%).

During a 1-week period early in the experiment, three of the birds on the bicarbonate control diet died and were found to have lost ~4 g in mass, which suggests that they were physiologically stressed. We immediately reformulated the bicarbonate diet to make it less basic by balancing the milliequivalents of the entire compounds ($CaCO_{3meq} = 100$, $NaHCO_{3meq} = 84$) rather than just the sodium and calcium ions, following the method used to calculate the amount of $CaCO_3$ needed to control for the effects of $NaHCO_3$-induced alkalosis in horses (Frey et al. 2001). The birds remained on this diet for the rest of the experiment.

The hoppers in each cage were filled with the appropriate diet mix at the start of each week and then topped up with fresh mix every 1 or 2 days. At the end of the week, the remaining food was weighed and subtracted from the amount added initially plus the amount added during the week to determine how much had been consumed. We collected spilled food in a tray below the cage, which was weighed every 2 weeks and then averaged to get a weekly total. True weekly consumption was thus apparent consumption minus spillage. To measure water consumption, we filled each bird's dispenser with 96 mL of water at the start of each week and 48 h later recorded how much remained using a 100-mL measuring cylinder.

Over the 5-month experiment (July–November), 12 of the 72 birds died: 3 from the low-calcium group, 2 from the high-calcium group, and 7 from the bicarbonate control group (of which 3 died before we reduced the alkalinity of the diet). Although these latter three deaths almost certainly resulted from the initial formulation of that diet, and one bird died as the result of a handling accident, the cause of the remaining mortality was unknown. The level of mortality in the present experiment is much lower than that in previous studies that have housed juvenile House Sparrows in captivity (e.g., Gonzalez et al. 1999, Poston et al. 2005).

Measuring birds post-experiment.—We ended the experiment 13 weeks after the first block of birds had been placed on their experimental diets. We weighed each bird and then measured its absolute bib size by holding the bird with its bill perpendicular to its body and used calipers to measure the length and width of the feathers that contained some black. Bib size was calculated using the following equation: bib size (mm^2) = 167 + (0.45 × length × width) (Møller 1987). We selected three of the black feathers at the lower extent of the bib and measured the length of their pale tips (Anderson 2006), and we also measured the length of the

white tip of the leading three median covert feathers. The latter, which compose the white wing bar (Poston et al. 2005, Bókony et al. 2006), have a white tip but a dark brown basal region. We carefully plucked the third wing-bar feather and a feather from the lower third of the bib for later spectrometric analysis. The birds were moved into aviaries in small flocks, provided with seed and water ad libitum for several weeks, and then released at their site of capture.

We taped the bib and wing-bar feather from each bird to a black index card and estimated their lightness by obtaining their reflectance spectra from 300–700 nm using a USB2000 spectrometer (Ocean Optics, Dunedin, Florida) standardized to both white and dark standards, with the probe and light source at 45° to the plane of the feather. Each feather's reflectance was measured twice. The two values were significantly repeatable (Lessells and Boag 1987; bib analysis of variance [ANOVA]: $r_{IC} = 0.86$, $F = 12.4$, df = 59 and 60, $P < 0.001$; black base of wing bar ANOVA: $r_{IC} = 0.60$, $F = 4.6$, df = 60 and 61, $P < 0.001$; white tip of wing bar ANOVA: $r_{IC} = 0.83$, $F = 10.7$, df = 60 and 61, $P < 0.001$) and so were averaged for the analyses.

We compared the plumage traits produced by the captive birds maintained on artificial diets with those from a comparable number of wild male House Sparrows captured at approximately the same time of year at our nearby longitudinal study site. The latter were measured with the same techniques used for the captive individuals.

Determination of calcium content of feathers.—We measured the amount of calcium present in single bib feathers taken from 8 birds from each diet treatment, 4 from each of the two blocks, using inductively coupled plasma emission spectrometry (ICP). Each feather was weighed on a microbalance to the nearest 0.001 mg and then rinsed in distilled water, dried overnight at 55°C, and digested by boiling in concentrated nitric acid and hydrogen peroxide, before being analyzed on a Varian Vista Pro spectrometer together with standards, method blanks, and blanks spiked with known amounts of calcium. We also measured the calcium content of a tail feather from 20 birds, 5 from each treatment, to test the prediction that nonmelanized feathers contain less calcium than melanized feathers (McGraw 2003). We also compared the calcium content of the bib feathers plucked from our captive juvenile (i.e., hatch-year) males with those of wild birds by analyzing bib feathers plucked from 16 free-living House Sparrows captured at our nearby longitudinal study site, where many recaptured birds can be assigned to age classes because they were originally banded as either nestlings or adults. This sample comprised 5 hatch-year males, 5 after-hatch-year males, and 6 males of unknown age. Finally, we analyzed the calcium content of upper breast feathers plucked from 4 hatch-year females, 4 after-hatch-year females, and 4 females of unknown age, to test whether these uniformly nonmelanized feathers also contain low levels of calcium.

Statistical analyses.—We used parametric statistics in SYSTAT, version 10 (SPSS, Chicago, Illinois), and SAS, version 9.1 (SAS Institute, Cary, North Carolina), for the majority of our analyses. For most analyses, we included block as a covariate, but we present statistics on block only if it had an effect. To test for effects of diet treatment on phenotypes at the end of the experiment, we directly tested three *a priori* predictions. The first prediction was that increasing calcium in the diet would increase bib size linearly. We used a general linear model with block as a covariate. Despite the directionality of this prediction, we did a two-tailed test using Type III sums of squares and the Satterthwaite method for calculating denominator degrees of freedom. Two other, nonorthogonal comparisons were made. The effect of the bicarbonate diet was specifically tested against data from the low-calcium diet (which had the same level of calcium) and the high-calcium diet (which presumably had the same acid–base balance) using *t*-tests. Data that were not normally distributed were analyzed using nonparametric tests. All tests were two-tailed, and we present means ± SE except where stated otherwise.

Results

Food and water consumption.—There was no difference in the weekly rate of food consumption of birds on each diet (repeated-measures ANOVA: $F = 1.27$, df = 3 and 56, $P = 0.29$ for gross consumption, and $F = 1.86$, df = 3 and 49, $P = 0.15$ after accounting for spillage; analysis restricted to the 60 birds present during the entire trial). The birds on the bicarbonate control diet drank more water than the birds on the other three diets, which consumed similar volumes (repeated measures ANOVA: $F = 4.35$, df = 3 and 55.5, $P < 0.01$). However, the difference became marginally nonsignificant when one outlier from the bicarbonate control group was deleted (repeated-measures ANOVA: $F = 2.73$, df = 3 and 54.2, $P = 0.052$).

Diet and morphology.—Bib size was significantly negatively related to dietary calcium concentration (general linear model [GLM]: slope = –25.3 ± 8.7, $F = 8.5$, df = 1 and 46, $P = 0.006$), and block had no effect (difference = 4.9 ± 8.7, $F = 0.3$, df = 1 and 46, $P = 0.58$). Birds on the bicarbonate control diet produced bibs that did not differ significantly from either comparison calcium treatment (Fig. 1); they were similar in size to the birds on the high-calcium diet ($t = 0.61$, df = 25, $P = 0.55$) and tended to be smaller than the birds on the low-calcium diet, but this difference was marginally nonsignificant ($t = 2.02$, df = 24, $P = 0.053$). We also found that birds on the low-calcium treatment had significantly larger bibs than wild hatch-year males ($t = 4.82$, df = 28, $P < 0.001$), but there was no size difference between the bibs produced by birds on the high-calcium diet and those produced by wild males ($t = 1.63$, df = 29, $P = 0.11$).

There was no difference in mass when first captured among birds in the four experimental groups nor between the two blocks (ANOVA by group, $F = 0.2$, df = 3 and 56, $P = 0.92$; by block, $t = 0.4$, df = 58, $P = 0.58$). However, dietary calcium had a significant and negative effect on post-experiment mass (GLM: slope attributable to calcium level = –1.7 ± 0.4, $F = 19.1$, df = 1 and 46, $P < 0.0001$), with birds on the low-calcium diet weighing the most. The mass change over the course of the experiment was also negatively and significantly associated with calcium level (GLM: slope attributable to calcium level = –1.3 ± 0.5, $F = 6.2$, df = 1 and 46, $P = 0.02$). The birds on the low-calcium diet gained mass, and those on the high-calcium diet lost mass (Table 1). Consequently, the birds on the low-calcium diet were in better body condition, as estimated from the residuals of a regression of body mass on tarsus length (GLM with block included; effect of calcium level = –1.5 ± 0.4, $F = 17.7$, df = 1 and 46, $P < 0.0001$). The birds on the bicarbonate diet were in significantly poorer condition (body condition index

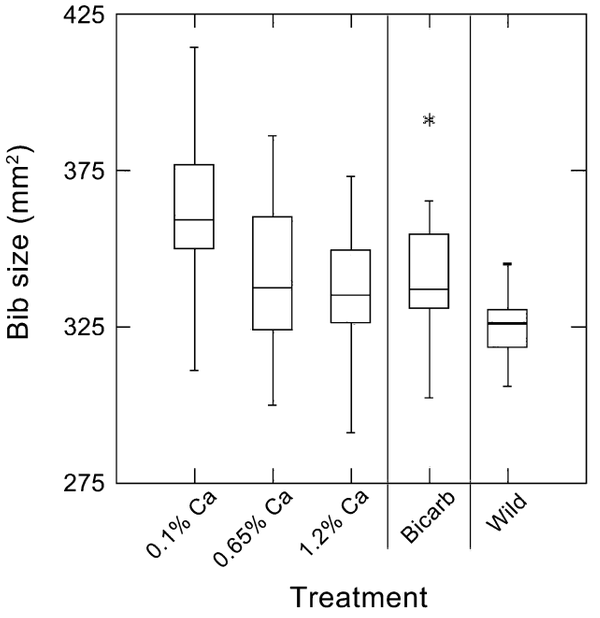

FIG. 1. Boxplot illustrating postmolt bib sizes (mm^2) of juvenile male House Sparrows maintained on artificial diets containing low (0.1%), medium (0.65%), or high (1.2%) levels of calcium, or low calcium with a bicarbonate control (Bicarb), as well as bib sizes of wild juvenile males. Horizontal bars in the plot indicate the 10th, 25th, 50th, 75th, and 90th percentiles, and asterisks represent values outside these ranges. Birds on the low-calcium diet produced significantly larger bibs than birds on each of the other diets except those on the bicarbonate control. Wild birds had significantly smaller bibs than birds on each of the experimental diets except those on the high-calcium diet.

[BCI] = −0.26 ± 0.3 g) than those on the low-calcium diet (BCI = 0.99 ± 0.4 g, $t = -2.5$, df = 24, $P = 0.02$) and were not significantly different from those of the high-calcium diet (BCI = −0.70 ± 0.2 g; $t = 1.2$, df = 25, $P = 0.25$). Both mass and body condition were significantly associated with calcium level (mass, effect size = −1.6 ± 0.4; $t = -4.3$, df = 45, $P < 0.0001$; body condition, effect size = −1.5 ± 0.4; $t = -4.1$, df = 45, $P = 0.0002$) and not with weekly food consumption (mass, effect size = 0.06 ± 0.04; $t = 1.5$, df = 45, $P < 0.13$; body condition, effect size = 0.06 ± 0.04; $t = 1.6$, df = 45, $P = 0.12$).

The negative effect of calcium on juvenile mass could explain the negative effect of calcium on bib size. To test this, we analyzed the relationship between bib size and calcium level with condition as a covariate. In an analysis that included both diet and mass, the effect of calcium on bib size remained large (effect size = −20.4 ± 10.2, $t = -2.0$, df = 46, $P = 0.05$), whereas the effect of body condition was not significant (effect size = 3.1 ± 3.5, $t = 0.90$, df = 46, $P = 0.37$). Furthermore, separate analyses of birds in each diet treatment revealed that there was no correlation between bib size and body condition in any group ($r = 0.02–0.29$, $P > 0.28$, $n = 11–18$).

No other aspects of plumage were affected by the treatments. Dietary calcium level had no effect on the reflectance of a bib feather, controlling for block (GLM: slope = −0.4 ± 0.6, $F = 0.3$, df = 1 and 45, $P = 0.56$); the reflectance of the white tip of the wing-bar feather (GLM: slope = 1.7 ± 1.2, $F = 1.9$, df = 1 and 38, $P = 0.17$); the reflectance of the dark base of the wing-bar feather (GLM: slope = −0.6 ± 0.6, $F = 1.1$, df = 1 and 46, $P = 0.30$); or the contrast in reflectance between the dark base and the white tip of the wing-bar feather (GLM: slope = −1.7 ± 2.2, $F = 0.6$, df = 1 and 46, $P = 0.44$; Table 1). Dietary calcium level did not influence the length of the white terminal portion of the wing-bar feather (GLM: slope = 0.02 ± 0.22, $F = 0.01$, df = 1 and 46, $P = 0.93$) or the proportion of the wing-bar feather that was white ($H = 3.14$, df = 2 and 43, $P = 0.21$). None of these variables differed between the bicarbonate control and either the low-calcium (all $P > 0.35$) or high-calcium (all $P > 0.08$) diets.

Calcium analysis of bib feathers.—When we restricted the analysis to the three main diets, we found a significant positive relationship between the concentration of calcium in the bib feathers after molt and that present in the diet (GLM: slope = 0.04 ± 0.01, $F = 7.9$, df = 1 and 21, $P = 0.01$; Fig. 2). The calcium content of the bib feathers produced by birds on the bicarbonate control diet (0.22 ± 0.04 [SD]) was closer to that found in birds on the high-calcium diet (0.23 ± 0.04 [SD]) than to that found in birds on the low-calcium diet (0.19 ± 0.04 [SD]; Fig. 2), but neither comparison was significant (high-calcium, $t = -0.71$, df = 14, $P = 0.49$; low-calcium, $t = 1.6$, df = 14, $P = 0.13$).

The calcium content of nonmelanized tail feathers taken from 14 birds was less than that of their bib feathers (paired t-test: $t = 4.8$, df = 14, $P < 0.001$), although the two levels were not correlated ($r = 0.40$, $P = 0.22$, $n = 14$). When the analysis was restricted to birds on the three main diets, there was no relationship between

TABLE 1. Postmolt phenotypic variables of juvenile male House Sparrows maintained on artificial diets containing low (0.1%), medium (0.65%), or high (1.2%) levels of calcium or on a bicarbonate control treatment containing low calcium (0.1%) but an acid–base balance equivalent to that of the high-calcium diet. Values are means ± SE.

	Low ($n = 15$)	Medium ($n = 18$)	High ($n = 16$)	Bicarbonate ($n = 11$)
Bib size (mm^2)	364.1 ± 7.7	340.4 ± 5.9	336.1 ± 6.0	342.0 ± 7.3
Wing bar (mm)	5.6 ± 0.2	5.6 ± 0.2	5.6 ± 0.2	5.8 ± 0.2
Final mass (g)	26.4 ± 0.3	25.4 ± 0.3	24.6 ± 0.2	25.2 ± 0.4
Mass change (g)	0.64 ± 0.44	−0.17 ± 0.44	−0.77 ± 0.32	−0.36 ± 0.50
Bib reflectance (%)	3.0 ± 0.5	3.7 ± 0.5	2.6 ± 0.4	3.6 ± 0.4
Wing-bar reflectance (%)	13.9 ± 0.7	15.0 ± 1.0	15.5 ± 1.0	13.5 ± 1.4

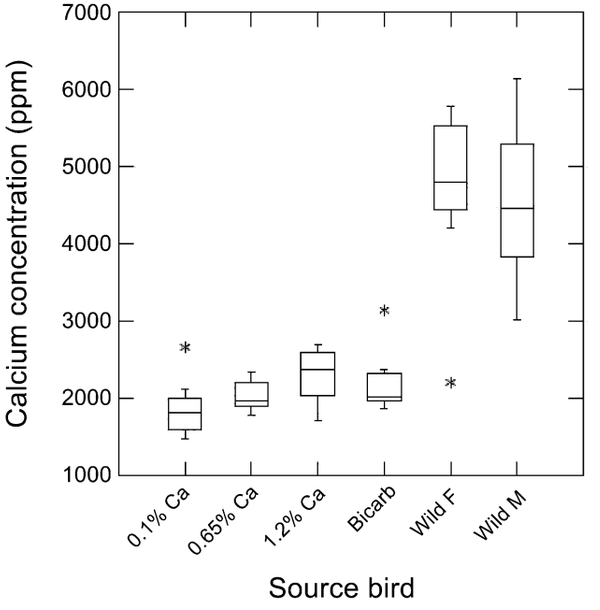

Fig. 2. Boxplot illustrating the calcium concentration (ppm) of bib feathers taken from juvenile male House Sparrows maintained on diets containing low (0.1%), medium (0.65%), or high (1.2%) levels of calcium, or low calcium with a bicarbonate control (Bicarb), and also bib feathers taken from wild males and wild females. Horizontal bars in the plot indicate the 10th, 25th, 50th, 75th, and 90th percentiles, and asterisks represent values outside these ranges. Note that a feather from one wild female contained 11,000 ppm calcium. This datum was deleted from the graph to enhance clarity.

calcium concentration in the diet and in the tail feathers (regression: $t = 0.7$, df = 1 and 13, $P = 0.48$). The level of calcium in the tail feathers of birds on the bicarbonate control diet was comparable to that in birds on the low-calcium diet ($t = 0.63$, df = 10, $P = 0.54$).

The calcium level in bib feathers from wild hatch-year males was approximately twice that in bib feathers from the captive hatch-year males used in the diet experiment (Fig. 2). There was no difference in calcium level between the bib feathers of 16 free-living males and feathers plucked from the equivalent region of 12 free-living females ($t = 1.61$, df = 16, $P = 0.13$), nor between feathers from hatch-year birds and those from birds in at least their second year ($t = 0.45$, df = 18, $P = 0.66$).

Discussion

The possibility that the expression of melanin-based plumage signals depends on access to minerals in the diet has circumstantial and experimental support (McGraw 2003, 2007). We tested that idea directly in juvenile House Sparrows and obtained a contrary result; high levels of calcium in the diet resulted in the production of smaller melanin-based ornaments, even after we controlled for treatment-induced differences in body mass. We also found little or no effect of the diet treatment on the development of other plumage traits. These results do not support the hypothesis that the House Sparrow's bib acts either as a signal of the bearer's ability to acquire a key mineral required for melanin synthesis or as a calcium-mediated, condition-dependent signal.

The contrast in results between our study and McGraw's (2007) is intriguing but perplexing. Why should increasing the level of dietary calcium increase the size of a melanin-based breast patch in one bird species and yet decrease it in another? There are two main differences between our study and McGraw's (2007): the study species and the nature of the calcium supplementation. First, it is possible that dietary calcium has a different effect on melanin synthesis in Zebra Finches than in House Sparrows. For example, Zebra Finches might have an optimal calcium level close to 1.2%, but House Sparrows an optimum closer to 0.1%. This seems unlikely, however, because the melanin pathway appears to be highly conserved, even across taxa (McGraw 2006b), and physiological studies across an array of birds indicate that 0.1% is close to the minimum requirement for survival (Klasing 1998). It also seems unlikely that Zebra Finches and House Sparrows would have such different calcium physiologies, given that both are small, granivorous, sexually dimorphic passerines that evolved in arid zones (Anderson 2006, McGraw 2007).

Second, McGraw (2007) increased dietary calcium by coating millet with powdered $CaCO_3$, whereas we thoroughly mixed the $CaCO_3$ into an artificial diet. It seems unlikely that the House Sparrows had undergone an unusual molt simply because of the artificial diet itself. There were no differences between the experimental birds and wild House Sparrows with regard to a range of plumage variables: bib feather reflectance ($t = 0.3$, df = 85, $P = 0.77$), wing bar length ($t = 1.3$, df = 125, $P = 0.20$), length of the pale tip of a representative bib feather ($t = 0.02$, df = 124, $P = 0.99$), and wing length ($t = 0.29$, df = 121, $P = 0.77$). Murphy and King (1982) also showed that the artificial diet did not affect the progress of molt in captive White-Crowned Sparrows compared with wild birds. However, the $CaCO_3$ may have been absorbed and assimilated more readily by the Zebra Finches than by the House Sparrows, although it is unclear why. Both studies found that the calcium content of the black breast feathers increased with dietary calcium, which suggests qualitatively similar assimilation patterns. Furthermore, the calcium level in feathers produced by birds on diets containing 0.65% calcium was similar in both studies (1,810 ppm in Zebra Finches; 2,040 ppm in House Sparrows). However, there was a dramatic difference in the calcium content of feathers from birds in the enhanced calcium (1.2%) treatment; the level in Zebra Finches was 12,485 ppm, approximately 7× higher, whereas the level in House Sparrows was only 2,300 ppm, a much more modest increase. Although we do not think that the differences in how the diet was delivered dramatically influenced assimilation, there may be some physiological difference between Zebra Finches and House Sparrows in how dietary minerals accumulate in feathers or in exactly how diet composition affects calcium uptake. In partial support of this idea, we found that the House Sparrows on the bicarbonate control diet produced feathers with considerably higher levels of calcium than the birds on the low-calcium diet despite identical levels of dietary calcium, potentially implicating acid–base balance in the uptake of calcium. This effect appears to be restricted to melanized feathers, however, because the tail feathers from the bicarbonate control birds contained a low level of calcium commensurate with the amount present in the diet.

The results from the bicarbonate control group also illustrate the potential importance of interactions among dietary components. This treatment contained the same amount of calcium as the low-calcium diet but the same acid–base status as the high-calcium diet. The fact that birds on the bicarbonate control diet produced bibs that were more similar to those of birds on the medium-calcium diet than to those of birds on the low-calcium diet suggests that calcium alone was not responsible for the differences in bib size between treatments. Although we did not measure plasma pH in our study, calcium carbonate and phosphate supplements provided to poultry led to an increase in blood pH (Keshavarz and Austic 1990) that could conceivably increase cellular pH. This could partly explain why the addition of bicarbonate had a positive effect on bib size, because near-neutral to basic melanosomal pH appears to be optimal for tyrosinase activity in humans (Ancans et al. 2001; see also the exchange between Ramaiah [2002] and Ancans and Thody [2002]). However, acid–base balance issues alone cannot explain the difference between our result and McGraw's (2007), because the $CaCO_3$ supplementation would have increased alkalinity in both studies.

The results from the bicarbonate control also highlight a potential complexity to the role of minerals in pigment deposition: calcium uptake, and perhaps its influence on the bib, could be affected by chemical reactions between the $CaCO_3$ and other ingredients in the diet that affect their digestibility. If food containing higher levels of calcium passes through the intestines more rapidly, the assimilation of several key nutrients also present in the diet, such as protein and carbohydrates, would be reduced. This could explain why birds on the medium- and high-calcium diets lost mass during the experiment whereas birds on the low-calcium diet gained mass, even though there was no difference in their mean consumption rates.

However, $CaCO_3$ is virtually insoluble in water and is relatively nonreactive (Sigel 1984), which suggests that the patterns of mass change may be more than an artifact of interactions between dietary components. For example, $CaCO_3$ may be more easily absorbed at low concentrations. Also, there is considerable evidence in mammals that dietary calcium affects energy metabolism. For example, mice maintained on a low-calcium diet (0.4%) showed marked increases in body mass because of deposition of adipose tissue, whereas mice on high-calcium diets (1.2%) lost mass (Zemel 2003). Papakonstantinou et al. (2003) found that rats on high-calcium diets excreted more fat in their feces than those on control diets. The patterns of mass change that we observed in our experiment may thus be related to differences in the physiological response elicited by each diet and not differences in digestibility.

A final consideration is that varying the levels of dietary $CaCO_3$ may interact with other physiological responses in complex ways. Calcium may interact with stress hormones, which are known to affect several of the steps in melanogenesis (e.g., Roulin et al. 2008). More importantly, calcium levels may influence feedback systems designed to regulate calcium in the bloodstream, which then indirectly affect plumage. Parathyroid hormone and vitamin D both cause blood calcium levels to increase because they stimulate the breakdown of bone tissues and promote absorption by the intestine, respectively (Klasing 1998). The thyroid hormone calcitonin causes calcium levels to decrease by reducing its absorption by the intestine and kidney (Sigel 1984, Klasing 1998). Consequently, any changes in melanin-based plumage that are produced by manipulating dietary calcium levels could actually be the result of homeostatic changes in the level of parathyroid hormone, vitamin D, or calcitonin, rather than calcium per se. Moreover, a recent experiment by Galván and Alonso-Alvarez (2008) illustrates how compensatory physiological responses could affect melanin production. Nestling Great Tits (*Parus major*) were treated with an inhibitor of glutathione, an essential antioxidant that inhibits melanin production. Treated nestlings developed larger melanistic breast patches and also higher levels of alternate antioxidants, which suggests that only high-quality individuals can afford to maintain the low glutathione levels required for enhanced melanin production. This result is relevant to the current study because Staal et al. (1994) found a strong positive correlation between levels of intracellular glutathione and calcium.

An ecological context for calcium-dependent production of plumage traits.—Although several studies have examined patterns of calcium intake in wild birds, almost all have focused on eggshell formation by breeding females (e.g., Schifferli 1977, Graveland 1996). However, these data are useful for assessing the bioavailability of calcium for molting males. The average clutch size in House Sparrows is ~5 eggs, 1 egg being laid on each of several successive days (Anderson 2006). Each eggshell weighs ~0.2 g and is almost entirely composed of $CaCO_3$ (Krementz and Ankney 1995). The $CaCO_3$ required for the first egg is derived from endogenous sources such as medullary bone, but the female must rely on exogenous sources for the remaining eggs. The bioconversion rate of dietary $CaCO_3$ into eggshell $CaCO_3$ varies considerably (e.g., Reynolds 1997, Klasing 1998), but even if it approaches 100%, a female must acquire 0.8 g of $CaCO_3$ in its diet for the 4 remaining eggs. In our experiment, the juveniles that produced the largest bibs were those maintained on the low-calcium diet (0.1%), and their dietary intake of $CaCO_3$ was ~0.1 g $week^{-1}$. If a breeding female can acquire 0.8g of $CaCO_3$ in just 4 days, it seems reasonable to expect that a juvenile male foraging in the same general location could acquire the 0.1 g $week^{-1}$ required to produce a large bib.

Although these comparisons are basic, they highlight the need for careful studies on the acquisition and assimilation of calcareous materials by males in the period leading up to and during the molt. One simple prediction that could be tested using choice experiments is that males, but not females, should show a preference for foods that provide the appropriate level or type of calcium as they approach molt, in the same way that breeding females undergo a dietary shift toward calcareous materials in the prelaying period (Schifferli 1977, Krementz and Ankney 1995).

Surprisingly, the calcium content of bib feathers from wild males was almost twice that of bib feathers from birds on the high-calcium diet, even though there was no difference in the size of their bibs. Because adult and juvenile House Sparrows subsist primarily on seeds, which contain only 0.1–0.2% calcium (Earle and Clarke 1991, Klasing 1998), the feather-content data suggest that wild House Sparrows obtained relatively large amounts of calcium in their diet. House Sparrows may occasionally find and consume items that contain extremely high concentrations of calcium, such as snail shells and limestone grit (Anderson 2006), and this may be particularly likely in Kentucky, where 60% of the underlying rock is calcium-rich limestone (Noger 1988).

Hence, we conclude that the ability to acquire dietary calcium does not limit bib production per se because calcium appears to be readily available in the natural environment. High dietary calcium could in fact inhibit melanin production, so the key variable may be how birds utilize calcium once it has been ingested, because it could be subject to a tradeoff against other physiological functions. A hint about such tradeoffs appears in the poultry literature, which contains many studies of nutrition and plumage coloration (e.g., Maynard et al. 1979, Grau et al. 1989) that are relevant to an understanding of plumage signals. For example, McGinnis and Carver (1948) reported abnormal blackening of the feathers of young chickens provided with diets deficient in calcium, as did Glazener and Briggs (1948). Glazener and Briggs (1948) also reported that vitamin D deficiency produced abnormal feather blackening in certain breeds of chicks, which is interesting because of the link between vitamin D and calcium homeostasis.

These reports, when combined with the present study and that of McGraw (2007), indicate that there is a link between dietary calcium and melanin synthesis, although it may not be direct and may not be the same across all species. This provides interesting challenges at several levels, including an assessment of individual differences in the ability to forage for calcareous materials, and also a better understanding of the complexity of nutritional physiology once calcium or other minerals have been ingested. Thus, while melanin-based status signals may have production costs that influence their honesty, in contrast to the direct link between input and expression in carotenoid-based signals, they may have a more complex condition-dependent mechanism. Such complexity creates empirical challenges but also raises some intriguing theoretical issues about how production and use costs might interact to maintain a stable signaling system.

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