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Different colors reveal different information: how nutritional stress affects the expression of melanin- and structurally based ornamental plumage

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Summary

Avian plumage colors have emerged recently as model systems for investigating the types of information that can be signaled by showy sexual displays in animals. In many species, the brightness of carotenoid-based plumage reflects the health and condition of individuals and is used in mate selection. The information contained in melanin-based and structurally based ornamental colors in birds is less well resolved, however. We subjected male house sparrows *Passer domesticus* and brown-headed cowbirds *Molothrus ater* to stressful nutritional conditions during molt to test the hypothesis that melanin- and structurally based plumage colors are nutritionally condition-dependent. We restricted food access for treatment males during randomized 6 h periods on 4 days per week, while allowing control birds access to food *ad libitum* throughout the course of the molt. We found that the size and brightness of the melanin-based throat badges in male house sparrows were not affected by nutritional stress. Similarly, there were no differences between treatment and control male cowbirds in the size or brightness of the melanin-based brown hood. However, the structurally based iridescent plumage of cowbirds was indicative of the nutritional condition of males during molt. Nutritionally stressed cowbirds grew significantly less colorful plumage than did males with access to food *ad libitum*. These results are consistent with observations in other avian species that different types of plumage color communicate different sets of information. Melanin ornaments are less sensitive to nutritional conditions during molt and instead may reflect the hormonal status and/or competitive ability of males, whereas structural coloration appears to be an accurate signal of health and condition.

Key words: brown-headed cowbird, house sparrow, *Molothrus ater*, *Passer domesticus*, condition-dependent, ornamental trait, plumage.

Introduction

Avian plumage ornaments are some of the best-studied sexually selected traits in animals. From the elongated tails of long-tailed widowbirds *Euplectes progne* (Andersson, 1982) to the ornate forehead crests of crested auklets *Aethia cristatella* (Jones et al., 2000), particular attention has been paid to understanding the types of information that birds communicate with these extravagant displays. The bright plumage colors of male birds have emerged recently as ideal models for investigating the different types of information that can be signaled by ornamental traits (Owens and Hartley, 1999; McGraw and Hill, 2000).

Plumage coloration in most birds comes in three distinct forms: carotenoid-based, melanin-based and structurally based colors (Fox and Vevers, 1960). The most thoroughly investigated of all three ornament types, from both the proximate and ultimate perspectives, are carotenoid-based signals, which include most of the red, orange and yellow colors seen in animals (Brush, 1978). Carotenoid pigments must be obtained in the diet before being deposited in the integument (Goodwin, 1984). Because carotenoids are thought to be scarce in nature (Grether et al., 1999), variation in the expression of carotenoid ornaments typically reflects the nutritional condition of male birds at the time of molt (e.g. Hill and Montgomerie, 1994; Hill, 2000). Females from many species prefer to mate with males that exhibit the brightest carotenoid-based displays (for a review, see Olson and Owens, 1998; Hill, 1999).

Melanin-based color displays (e.g. blacks, browns) have recently begun to receive equal empirical consideration. Historically, most studies have emphasized the link between social aggression and the degree to which melanin ornaments are exaggerated (e.g. Rohwer, 1975, 1977). In a variety of avian species, males displaying the largest melanin-based badges are behaviorally dominant to those having smaller color patches (for a review, see Senar, 1999). More recent work has focused on the physiological constraints that may be associated

with producing melanin pigments. Early research suggested that there might be nutritional limitations to growing large patches of feathers pigmented with melanin (Veiga and Puerta, 1996). Since then, there has been little experimental support for the idea that melanin pigments are nutritionally or energetically expensive to produce (Gonzalez et al., 1999; McGraw and Hill, 2000). Moreover, there is equivocal evidence that females use variation in the expression of melanin ornaments in their mating decisions (e.g. Møller, 1988; Veiga, 1993; Kimball, 1996; Cordero et al., 1999; Griffith et al., 1999). Clearly, more research is needed to better understand the costs associated with the production of melanin-based ornamental traits.

The function of structurally based color ornaments in birds remained virtually unstudied until the last few years. Structural colors include the blue, violet, ultraviolet and iridescent patches of feathers and skin (Auber, 1957; Dyck, 1974). In contrast to the pigment-based systems, structurally based plumage produces bright colors *via* constructive interference of light at the interfaces between keratin, air and melanocytes in feather barbs and barbules (for a review, see Prum, 1999). As is the case for most carotenoid-based ornamental displays, females generally prefer to mate with males having the brightest structurally colored plumage ornaments (Bennett et al., 1997; Andersson and Amundsen, 1997; Andersson et al., 1998; Hunt et al., 1999). Moreover, variability in structural coloration appears to be related to the nutritional condition of males during molt (Keyser and Hill, 1999, 2000; Doucet, 2002). However, these findings that point to a proximate mechanism underlying variation in structural plumage ornaments are only correlational; as of yet, no experiments have been conducted to assess the environmental or physiological factors that affect the expression of structurally based color displays in birds.

Here we investigate the effect of nutritional constraints during molt on the expression of both melanin-based and structurally based ornamental plumage coloration. We studied these two forms of plumage coloration in two sexually dichromatic songbird species: the house sparrow *Passer domesticus* and the brown-headed cowbird *Molothrus ater*. Males of both species grow colorful plumage in the fall through a pre-basic molt and display the ornament throughout the year. Male house sparrows exhibit a melanin-based black throat patch (Lowther and Cink, 1992), and male cowbirds have structurally based iridescent green-black plumage coloration on the breast and back (Lowther, 1993). Male cowbirds also possess a deep brown, melanin-based hood that extends down to the nape and throat (Lowther, 1993). Female sparrows and cowbirds are drab brown in coloration.

The house sparrow is one of the best-studied of all songbird species in the context of sexual selection, and certainly the most common subject in work on melanin-based plumage. Males with larger black badges are behaviorally dominant to males having smaller badges (Møller, 1987a,b). Males with the largest patches also experience the highest reproductive success in most populations (Møller, 1988, 1992; Veiga, 1993; but see Griffith et al., 1999). In contrast, little is known of the function of bright plumage in male brown-headed cowbirds. Visual displays accompany courtship vocalizations prior to mating (Lowther, 1993), and in other cowbird species the extent of sexual dichromatism is positively associated with variance in male mating success (Hauber et al., 1999). However, more work is needed to determine whether or not ornamental coloration is a sexually selected trait in this species.

To test the hypothesis that the expression of melanin-based and structurally based plumage coloration is dependent on nutritional condition in male house sparrows and brown-headed cowbirds, we restricted access to food during randomized time intervals for captive groups of treatment birds, while allowing control males access *ad libitum* to the same diet throughout the course of molt. We followed the experimental methods of Hill (2000), who found that nutritional stress has a significant impact on the brightness of carotenoid-based plumage in male house finches *Carpodacus mexicanus*. We housed captive cowbirds individually and sparrows in triads to minimize pseudoreplication from housing all birds within a treatment group in the same cage (e.g. Brawner et al., 2000; Hill, 2000; McGraw and Hill, 2000). At the end of molt, we compared the brightness of ornamental plumage between control and experimental groups.

Materials and methods

House sparrows

42 male house sparrows *Passer domesticus* L. were trapped in mistnets and baited Potter traps in Tompkins County, NY, USA between 28 February and 1 May 2000. At capture, we marked birds with a unique combination of colored leg bands for individual identification, and measured tarsus length to the nearest 0.1 mm with calipers and body mass to the nearest 0.1 g with a scale. Because all birds were captured in their after-hatch-year (AHY) plumage, we were unable to determine their precise age in years. Veiga (1993) found age-related differences in badge size between hatch-year (HY) and AHY male sparrows; thus, we reduced the confounding effects of age on plumage by using only AHY males in our study. We arranged birds by capture date into groups of three and housed them in 14 stainless steel cages (0.6 m long × 0.45 m wide × 0.8 m tall) in an animal-approved indoor room (4.6 m × 4 m × 2.4 m) at Cornell University. Each cage contained wooden dowels as perches and had an open top, front and side that were covered in hardware cloth. Steel grates covered the cage bottoms so that feces and discarded seed would not collect on the cage floor. Fluorescent light timers maintained natural day/night cycles. Daily temperatures were 20–25°C and humidity 40–70%.

Brown-headed cowbirds

15 male cowbirds *Molothrus ater* Boddaert of known age were used in this study, which spanned 2 years (2000 and 2001). In 2000, we housed ten cowbirds in individual hardware cloth cages (0.6 m × 0.3 m × 0.4 m) in an indoor room

(2.9 m × 4 m × 2.4 m) that was separate from the house sparrows. Eight of these birds were previously banded adults (mean=2.6 years, range=2–4) who were removed as nestlings from the nests of song sparrows *Melospiza melodia* and eastern phoebes *Sayornis phoebe* from 1997–1999 and hand-raised to independence in captivity (Hauber et al., 2000). The remaining two birds were juveniles that we captured from the wild in Tompkins County, NY, USA using baited Potter traps in July 2000. In 2001, we housed five juveniles in a free-flying indoor group that also were obtained as nestlings from phoebe nests. All of these males were part of a behavioral study during the year in which they hatched (Hauber et al., 2000), but were never manipulated physiologically or nutritionally prior to this experiment. All other housing conditions follow those described above for house sparrows.

General procedures

Before birds began molting in captivity, we quantified the melanin- and structurally based ornamental plumage coloration that males displayed prior to the experiment. We scored males from each species with an Ocean Optics, Inc. S2000 fiber-optic spectrometer (Dunedin, FL, USA) illuminated by a tungsten–halogen/deuterium light source (Analytical Instrument Systems, Inc., Flemington, NJ, USA) to determine the degree to which both ornament types reflected in the ultraviolet (for detailed methods, see McGraw et al., 1999). Neither the melanin pigmentation of male house sparrow badges and cowbird hoods nor the iridescent plumage of male cowbirds exhibited a UV reflectance peak (Fig. 1). Consequently, we quantified only the visible light reflectance from the plumage of all males, which should serve as a reliable assay of short-, medium- and long-wave light reflectance, despite the fact that it may not truly capture what the birds see (Cuthill et al., 2000).

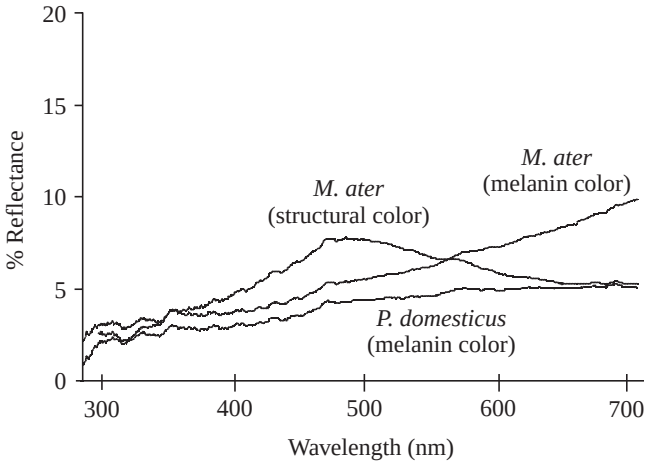

Fig. 1. Representative reflectance spectra for the types of ornamental color exhibited by male house sparrows *P. domesticus* and brown-headed cowbirds *M. ater*. Data were collected following McGraw et al. (1999). Note the absence of discrete peaks in the ultraviolet portion of the spectrum (300–400 nm) for all plumage types. Also note that we have described the spectral profile of iridescent cowbird plumage at only one angle (90° to the reading surface); the general height of the curve for this plumage region would be expected to vary, depending on the angle at which the feather is analyzed.

To score house sparrow plumage, we digitally photographed the ventral sections of each male against a grayboard (at an image resolution of 1760×1168 pixels) and imported these images into Adobe® Photoshop® (Adobe Systems Inc., San Jose, CA, USA). We measured badge size by outlining the melanin-based pigmented area using the 'lasso' marquee and determining the number of pixels occupied with the 'histogram' function (*sensu* Dale, 2000). Although freshly molted badges are partially concealed by buff feather tips, a number of studies have shown that measuring the area of black-pigmented feathers from the base of the bill to the lowest point on the breast to which black feathers extend is a reliable and meaningful scoring method (Møller and Erritzøe, 1992; Gonzalez et al., 1999; Griffith et al., 1999). Because photos may have differed slightly in distance from the subject, badge area (in cm^2) was calculated relative to an area standard that was photographed next to each bird. We measured melanin-based coloration as the brightness (also known as lightness or tone) of the badge at its center using the 'HSB scale' on Photoshop's 'color picker' function. To control for any lighting differences among photos, we scored badge brightness as a percentage relative to a standard black color chip that also was photographed with each male. Repeatability of both badge size and brightness, as measured using separate photographs of the same birds, was high using these scoring methods (area: r=0.91, $F_{22,23}$=20.67, P<0.0001; brightness: r=0.81, $F_{22,23}$=9.25, P<0.0001; Lessells and Boag, 1987).

We scored the color of cowbird plumage with a hand-held Colortron™ reflectance spectrophotometer (Light Source Inc., San Rafael, CA, USA; Hill, 1998). Although this unit does not gather UV-reflectance data, it does quantify spectral reflectance from 390–700 nm and derives tristimulus scores (hue, saturation and brightness, using the Colorshop™ 2.6 software package) from the generated reflectance curves (Light Source, 1996). Thus, as in other studies of avian structural coloration (e.g. Andersson 1999; Keyser and Hill, 1999), hue represents the wavelength at maximum reflectance, for which Colorshop™ assigns numerical values around a 360° color wheel (with red starting at 0°). In this cowbird species with dark green-black plumage, higher hue values correspond to shorter wavelengths of light (toward blue/violet light). Saturation captures spectral purity and is measured by Colorshop™ as a percentage relative to black and white standards provided by Light Source Inc. (100%=fully saturated, or comprised entirely of one light-wavelength). Lastly, brightness is a measure of the total amount of light reflected by a surface (or area under the spectral curve), and again is represented by Colorshop™ as a percentage (with 100% being total reflectance, or white). For all measurements, the Colortron™ was held perpendicular to the reading surface and the foot lever containing the 9 mm^2 reading area depressed firmly against the feather patches. We measured the hue,

saturation and brightness of six colorful body regions (upper, middle and lower portions of both the dorsum and venter) and averaged these values to obtain mean hue, saturation and brightness scores for each bird. All three color variables were moderately to highly repeatable using this method (hue: r=0.93, $F_{9,10}$=29.9, P<0.0001; saturation: r=0.94, $F_{9,10}$=30.3, P<0.0001; brightness: r=0.71, $F_{9,10}$=5.96, P=0.01). We quantified the color of melanin-based hoods in cowbirds by taking four brightness measurements, on the top, back and two sides of the head, with the Colortron™ and averaging these values to obtain a mean brightness score for each male. Because male cowbirds also vary in the extent to which the brown hood extends down the neck, we measured the length of the melanin patch to the nearest 0.1 mm with digital calipers. Scoring the extent (r=0.89, $F_{9,10}$=26.8, P<0.001) and brightness of melanin plumage (r=0.97, $F_{9,10}$=66.5, P<0.0001) was also highly repeatable.

Prior to and during the experiment, captive males from both species were fed *ad libitum* a 50:50 diet of unmedicated game starter (26% protein, Agway® Inc., Batavia, NY, USA) and white millet. Water was treated with 6.6 drops l^{-1} of Premium Multi-Drops™ high-potency multivitamins (Eight in One® Pet Products, Inc., Hauppauge, NY, USA) and 0.26 g l^{-1} of sulfadimethoxine (Sigma® Chemicals, St Louis, MO, USA), a drug that effectively controls coccidial endoparasitism in these and other passerine species (Brawner et al., 2000; Hill, 2000; McGraw and Hill, 2000). Throughout our study, one food dish and one water dish were provided for each bird, and spaced evenly on the floor of the sparrow cages, to ensure that all individuals had equal access. At no point were any males infected with obvious ectoparasites (e.g. ticks, avian pox, feather mites/lice).

The pre-basic molt period spans the late summer and fall for both species (Lowther and Cink, 1992; Lowther, 1993), so we ran our food-deprivation experiments from 15 July until all birds completed molt by 15 October. Following the protocol developed by Hill (2000), we removed the food dishes from experimental groups for randomly selected 6 h periods of daylight (a range of 42–52% of total daylight hours throughout the study) on 3 out of every 4 days during molt. This randomized design was used to prevent birds from tracking food removal and ingesting large quantities of food prior to deprivation periods. As Hill (2000) found with house finches, food-stressed cowbirds and sparrows were noticeably hungry after the food-stress period and would descend from their perches to the floor of the cage to consume food while dishes were still being replaced elsewhere in the room. For control groups, who had access *ad libitum* to the aforementioned diet throughout the study, we inserted our hands into the cages to raise and lower the dishes to provide similar levels of disturbance when dishes were removed and replaced in food-stressed cages. With 14 cages of house sparrows, we randomly assigned 7 to the food-deprived group and 7 as controls (N=21 males in both groups). In our 2-year cowbird study, we divided the 10 individually housed cowbirds in 2000 into 5 treatment birds and 5 control birds (with one juvenile in each group), whereas in 2001 all 5 birds were in the control group.

To ensure that food-deprived males consumed less food than did unstressed individuals, we measured food intake on 5 separate days during molt for house sparrows. At the start of the stress period on these days, we removed all old food from the dishes and provided 10 g $dish^{-1}$ of fresh millet to each control cage. Food was available in the normal dishes, but these were placed inside larger containers that prevented birds from spilling seed out of the cage. In nutritionally deprived cages, dishes were empty to start and the same amount of millet was added after the 6 h deprivation period; control birds were similarly disturbed at this time. 24 h after the start of the stress period, we measured the amount of food consumed by each group using an electronic balance. Birds in both groups never consumed more than half of the seed that we provided during these trials, indicating that food intake reflected the feeding capacity of the birds rather than the gross quantity of millet available. We used non-parametric Mann–Whitney U-tests to analyze food intake data because of small sample sizes (7 cages per day per treatment group) and because not all variables were normally distributed (Shapiro–Wilk W-tests, P<0.05).

Once all males had completed their prebasic molt, we again measured the body mass of each bird and quantified melanin- and structurally based ornamental plumage coloration, following the protocols listed previously. We also measured tarsus length for all cowbirds because it had not been determined before the experiment. As a measure of nutritional stress on feather growth and to ensure that treatment males were more stressed than control birds, we plucked all six pairs of tail feathers from each sparrow at the end of molt and measured the fluctuating asymmetry of rectrices with calipers to the nearest 0.1 mm (*sensu* Swaddle and Witter, 1994). Rectrix-length measurements were highly repeatable (r=0.997, F=749.7, P<0.0001) for a random subset of feathers (N=25) measured twice (on 4 March and 8 October 2001), and differences in tail symmetry (1.05±0.14%) far exceeded measurement error (0.19±0.03%).

Statistical analyses

All analyses were performed using the statistical program StatView® 5.0.1 (SAS Institute 1998). To comprehensively analyze the predictors of plumage color in our study, we used both univariate and multivariate statistical procedures. We tested for normality (Shapiro–Wilk W-test) and equality of variance (Equality-of-variances F-test) in house sparrow data and used univariate, unpaired t-tests when these assumptions of parametric statistics were met; when assumptions were violated, we used non-parametric Mann–Whitney U-tests. Because of small sample sizes, we used Mann–Whitney tests in all cowbird analyses. We used analyses of covariance (ANCOVA) to investigate the effect of nutritional stress on plumage expression; tarsus length, body mass and pre-molt plumage scores were entered as covariates in the models, and we added year and age into cowbird analyses. All data for

Table 1. *Food consumption rate, rectrix asymmetry, tarsus length, body mass and plumage colors for individual male house sparrows* P. domesticus *and brown-headed cowbirds* M. ater

	Passer domesticus		*Molothrus ater*	
	Food-stressed	Food-unstressed	Food-stressed	Food-unstressed
Food intake (g bird^{-1} day^{-1})[a]	2.40±0.13	3.13±0.25	–	–
Rectrix asymmetry[b]	1.31±1.03	0.78±0.49	–	–
Tarsus length (mm)	17.24±0.70	16.85±1.09	22.36±1.16	21.49±2.15
Pre-molt body mass (g)	26.74±1.62	26.13±1.83	–	–
Post-molt body mass (g)	27.67±2.45	26.57±2.86	44.88±3.32	44.72±3.74
% change in body mass (g)	1.73±1.49	0.84±2.28	–	–
Pre-molt melanin patch size[c,d]	6.47±0.85	6.62±0.96	24.29±1.74	23.13±0.56
Pre-molt melanin darkness (%)[d,e]	–3.29±4.09	–3.70±2.30	16.57±1.08	16.87±2.15
Pre-molt structural hue (degrees)[d]	–	–	201.3±2.50	207.0±7.75
Pre-molt structural saturation (%)[d]	–	–	26.12±8.21	26.86±8.94
Pre-molt structural darkness (%)[d]	–	–	15.49±3.37	15.59±3.75

Values are means ± 1 S.D. For *P. domesticus*, N=21; for *M. ater*, N=5 (food-stressed), N=10 (unstressed).

[a]Represents the average amount of food consumed over five 24-h periods by all birds within the treatment group.

[b]Percentage length difference (mm) in six pairs of rectrices; measured with calipers to the nearest 0.1 mm.

[c]Measured from digital photographs in cm^2 for sparrows (relative to an area standard); measured with calipers to the nearest 0.1 mm for cowbirds.

[d]Only four birds per treatment group were used in these comparisons for cowbirds because juveniles did not display ornamental plumage prior to molt.

[e]For sparrows, calculated as a percentage relative to a black standard included in each photograph. More negative values correspond to blacker plumage.

house sparrows were analyzed using both individual birds and individual cages as units of analysis.

Results

Food intake and nutritional condition

Male house sparrows who had restricted access to food during molt consumed significantly less food than did males who had access *ad libitum* to the same type and amount of food, with no overlap between the groups; this was true when we analyzed food intake separately for all five measurement periods (all U>40, all P<0.02) and as a per-day average (U=49, P=0.002; Table 1). Moreover, food-limited males grew significantly more asymmetrical rectrices than birds in the food-unlimited group, using data both for individual birds (U=119, P=0.05; Table 1) and cages (U=132, P=0.04). Thus, food-restricted individuals were under a higher degree of nutritional stress than food-unrestricted males.

Plumage expression and nutritional condition in house sparrows

Prior to the experiment, there were no significant differences between nutritionally deprived and undeprived sparrows in tarsus length (per individual: U=155.5, P=0.16; per cage: U=39, P=0.07) or body mass (individuals: U=166, P=0.17; cages: U=34, P=0.22), nor were there initial differences in the size (individuals: t=0.54, P=0.60; cages: U=22, P=0.75) or brightness (individuals: t=0.74, P=0.46; cages: U=19, P=0.48) of melanin-based throat badges (Table 1). We found that food-unlimited birds did not gain significantly more mass during the experiment than did food-limited birds (individuals: U=170, P=0.20; cages: U=34, P=0.22; Table 1). Overall, nutritionally stressed sparrows grew plumage badges that did not differ in size or brightness from those of nutritionally unstressed birds (Fig. 2; using cage means for area: U=21, P=0.65; using cage means for brightness: U=16.5, P=0.30). Using ANCOVA, we also found no effects of nutritional deprivation, tarsus length, body mass or pre-molt badge area on either badge size (all P>0.2 for both per-individual and per-cage analyses) or badge brightness (all P>0.1).

Because of these non-significant results, we evaluated the statistical power of our tests by considering the likelihood of detecting results similar to those for species in which a significant effect of food stress on plumage color expression had been previously established. Hill's experiments with house finches used sample sizes (N=7–13 per treatment) that were smaller than those in our studies, but found much higher effects (r^2=0.33) than those for house sparrows in the present study (all r^2<0.07) (Hill, 2000). With N=21 in each experimental group, we had the statistical power to detect effects ≤0.6 at α=0.05 (Cohen, 1988). Thus, the development of melanin-based ornamental coloration in house sparrows appears to be much less sensitive to nutritional deprivation than is carotenoid pigmentation in house finches.

Plumage expression and nutritional condition in brown-headed cowbirds

Within the control group, juvenile males did not differ significantly from adults in either melanin- (hood size: U=5, P=0.43; brightness: U=3.5, P=0.24) or structurally based

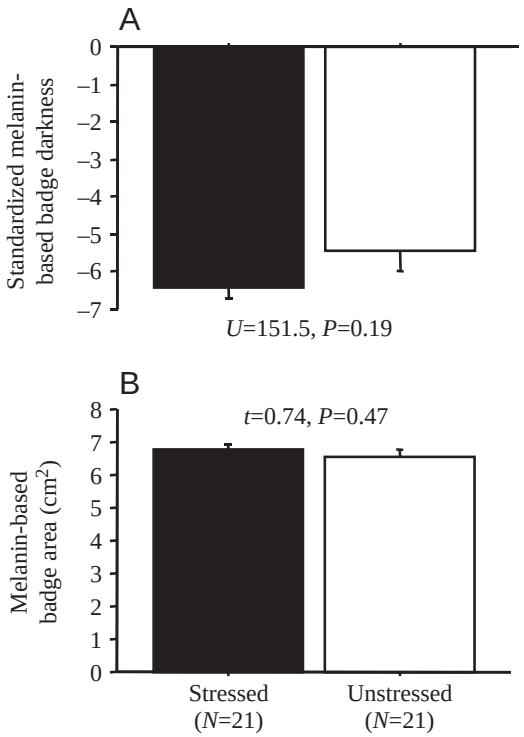

Fig. 2. Effect of nutritional stress on the (A) brightness and (B) size of melanin-based ornamental plumage in male house sparrows. Nutritionally stressed males (dark bars) were deprived of food for randomly assigned 6 h periods on 3 out of every 4 days (*sensu* Hill, 2000), while unstressed males (white bars) were allowed access *ad libitum* to the same type and amount of food. Badge size and brightness were measured with Adobe® Photoshop® from digital photographs. Values are means + 1 S.E.M. For badge brightness, more negative values indicate blacker plumage.

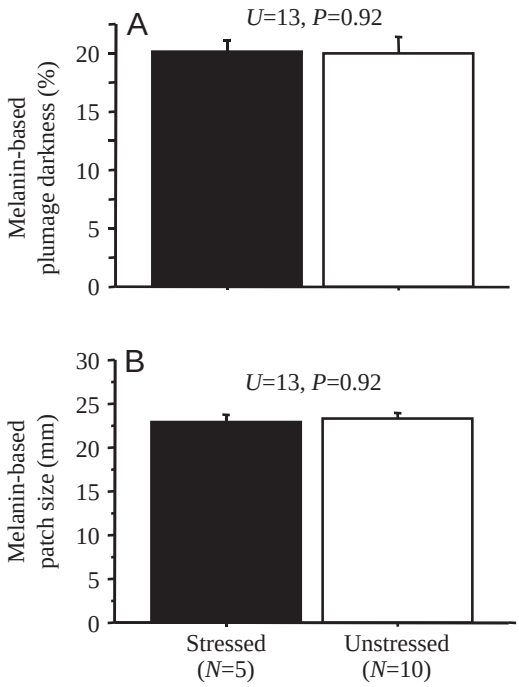

Fig. 3. Effect of food deprivation on the (A) brightness and (B) size of melanin-based ornamental coloration in male brown-headed cowbirds. Plumage brightness was scored with the Colortron™ reflectance spectrophotometer; hood length was measured with calipers. See Fig. 2 for description of nutritional treatment and bar charts.

plumage coloration (hue: U=18, P=0.45; saturation: U=21, P=0.42; brightness: U=15.5, P=0.16), nor was there any effect of year on our measures of ornamental color (all P>0.25). Thus, we pooled birds across age classes and years for analysis. Before the experiment, we found no significant differences in tarsus length (U=9.5, P=0.53; Table 1) between nutritionally stressed and unstressed birds, nor were there initial differences between treatment groups in the size (U=3.5, P=0.25) or brightness (U=7, P=0.77) of the melanin-based brown hood, or in the hue (U=5, P=0.37), saturation (U=7, P=0.77), or brightness (U=7.5, P=0.88) of structurally colored plumage (Table 1). Food-limited birds did not differ in body mass from food-unlimited birds after the experiment (U=12, P=0.92; Table 1). Thus, for both sparrows and cowbirds, and as Hill (2000) found for house finches, this food-deprivation protocol did not impose unreasonably high levels of nutritional stress on the animals.

We detected no significant effect of nutritional stress during molt on the size and brightness of the melanin-based brown hoods (Fig. 3). However, in these same birds, nutritionally deprived males grew significantly less green, less saturated, and less bright iridescent plumage than did undeprived birds (Fig. 4). These results are consistent if we exclude all birds from 2001 (hue: U=5, P=0.10; saturation: U=1, P=0.02; brightness: U=1, P=0.01) and if we exclude all juveniles from the analyses (hue: U=0, P=0.02; saturation: U=1, P=0.04; brightness: U=0.5, P=0.03). ANCOVA results again supported those from univariate analyses. Neither treatment nor any of the covariates (year, age, tarsus, mass and pre-molt color) significantly predicted melanin-based hood size (all P>0.3) or brightness (all P>0.25). However, food-limitation did have a significant main effect on the hue, saturation, and brightness (all P<0.05) of iridescent coloration in male cowbirds (all other P>0.15).

As with house sparrows, effect sizes for melanin color in cowbirds were very low (r^2=0.01). We had the power to detect effects up to r^2=0.35 in this experiment (Cohen, 1988), even with our small sample sizes. All of the significant effects for structural color were large in this study (r^2=0.65), but we encourage the use of more birds in future studies to detect weaker effects. It is also worth pointing out that, although iridescent plumage reflects light differently at different incident angles, this color difference was detectable in our study by measuring feather reflectance at only one angle (90° from surface) with the Colortron™.

Discussion

By studying the effects of mild nutritional constraints on the expression of melanin- and structurally based ornamental

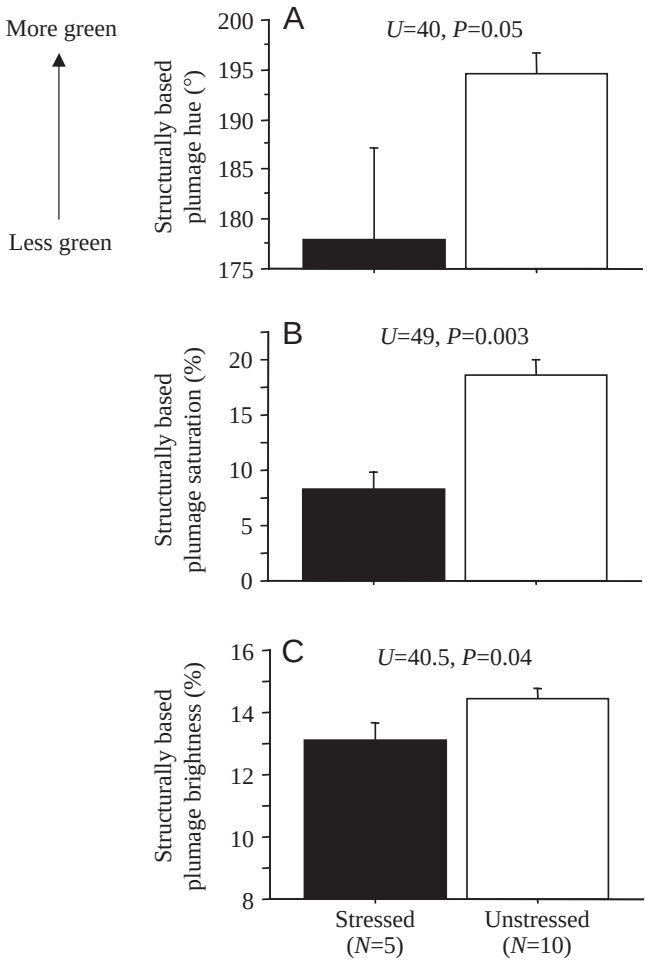

Fig. 4. Influence of nutritional stress on the expression of structurally based plumage (A) hue, (B) saturation and (C) brightness in male brown-headed cowbirds. Structural color was scored with the Colortron^{TM}. See Fig. 2 for other details.

coloration in male house sparrows and brown-headed cowbirds, we investigated whether these two types of plumage can signal the condition of male birds at the time of molt. We found that the melanin-based throat badges of nutritionally stressed male house sparrows were no different in size or brightness from the black plumage patches grown by unstressed males. Under the same nutritional-stress regime, food-stressed cowbirds failed to develop melanin-based brown hoods that differed in size or brightness from unstressed birds. However, in these same cowbirds, there was a significant difference in the color of structurally based iridescent plumage between treatment and control males, with nutrient-limited birds growing less saturated plumage than individuals who had unlimited access to food. These results indicate that these two plumage color types do not respond in the same way to nutritional limitations and thus do not reveal similar types of information about the nutritional condition of molting males.

Although certain correlative studies have linked melanin ornaments to condition (e.g. Slagsvold and Lifjeld, 1988; Veiga and Puerta, 1996), our data are consistent with more recent experimental studies on the physiological constraints of melanin-based plumage ornaments. Gonzalez et al. (1999) manipulated protein content in the diet of molting male house sparrows and failed to detect any influence on the development of badge size and color. McGraw and Hill (2000) studied the effects of coccidial infections on plumage displays in male American goldfinches *Carduelis tristis*, and, despite significant effects of endoparasitism on carotenoid-based ornamentation, found no differences in melanin pigmentation between infected and uninfected birds. Why might the expression of melanin coloration be uncoupled from the nutritional state of an animal? The amino acid tyrosine serves as the precursor to the production of melanin granules that are deposited in feathers (Fox, 1976). Although tyrosine can be obtained in the diet through protein degradation or direct uptake, it is a nutritionally dispensable (non-essential) amino acid (Meister, 1965), and can be synthesized *de novo* from phenylalanine as long as there is organic nitrogen in the diet (Moldawer et al., 1983). Thus, it seems that producing melanin pigments should not be especially demanding energetically for birds.

Other physiological factors may be more likely to control the expression of melanin-based ornamental coloration. Central to melanin production is increased activity of the enzyme tyrosinase that acts at the cellular organelles (melanosomes) of the epidermal melanocytes (color-producing cells) (Fitzpatrick and Kukita, 1959). Certain hormones (e.g. estradiol, luteinizing-hormone) are known to have stimulatory effects on tyrosinase activity and subsequent biosynthesis of melanin granules in feather tracts (Hall, 1966, 1969; Ralph, 1969). Evans et al. (2000) and Gonzalez et al. (2001) recently found that experimentally elevated levels of circulating testosterone increased the size of the melanin badge in house sparrows. Testosterone also influences aggressive behavior in birds (Wingfield et al., 1987), and thus may represent the proximate means by which melanin ornaments can accurately signal social dominance and competitive ability in animals.

To our knowledge, our investigation and documentation of the nutritional condition-dependence of structurally based ornamental coloration is the first experiment of its kind in any avian species. Fitzstephens and Getty (2000) recently manipulated the diet of male black-winged damselflies *Calopteryx maculata* who, like male brown-headed cowbirds, display striking glossy green-black coloration, and found that nutritionally deprived males were less colorful than males provided with more food. Keyser and Hill (1999) detected a correlation between the rate at which male blue grosbeaks *Guiraca caerulea* grew tail feathers in the wild and the blueness of their structurally based ornamental plumage. Doucet (2002) also observed a positive relationship between feather growth and structural plumage coloration in blue-black grassquits *Volatinia jacarina* from Mexico.

The relationship between structurally based ornamental coloration and nutritional condition reported here is much like that found for carotenoid-based ornamentation (Hill, 2000). Although based on only a few observations for structural colors, both signals most often relay the condition

of males during the time at which the ornament is developed (e.g. Hill, 1999; Keyser and Hill, 1999; but see Dale, 2000) and are used in female mate choice (e.g. Hill, 1999; Hunt et al., 1999; but see Pryke et al., 2001). However, the means by which structural coloration is produced is very different from that of carotenoid pigmentation. Carotenoid-based colors have a direct tie to nutrition because vertebrates must ingest these pigments through the diet to become colorful (Brush, 1978). In contrast, structural colors in animals are produced by the physical interaction of light with biological tissues (Fox, 1976). Cowbirds produce perhaps the most simple of structural colors, different from other previously studied species exhibiting structural coloration (e.g. blue grosbeak), but much like starlings (Family Sturnidae) and other birds with iridescent plumage. Their glossy, green-black sheen is generated by the interference of light scattered by the keratin at the surface of the feather and a single layer of melanin granules below (Durrer, 1986; Prum, 1999). So why might this form of structurally based coloration, which contains melanin pigments, be affected by an individual's nutritional condition?

Recent studies of plumage ultrastructure indicate that structurally colored feathers are composed of highly organized matrices of keratin, air and pigments, such that nanoscale variation in the orientation of melanin granules or the uniformity and thickness of the tissue matrix may all contribute to the directionality and intensity of light reflected (Prum et al., 1998, 1999; Andersson, 1999, 2000). Thus, in this case, fine control over tissue and pigment arrangement during feather growth may be particularly sensitive to perturbations in a bird's health or condition. As there are a number of types of structural color in animals, depending on the type (e.g. feathers, scales, skin, eye) and composition (amount and arrangement of keratin, air and melanin) of the reflectance medium as well as the type of light scattering (incoherent *versus* coherent; Prum, 1999), careful examination of the potential condition-dependence for each of these forms is warranted.

Although we have identified a link between nutrition and a form of structurally based plumage ornamentation in this study, it will be important in future research to investigate the full signal content of structural coloration in brown-headed cowbirds and other species displaying this ornament type. First, it is not known to what degree hormones may influence the expression of structurally colored ornaments. Testosterone affects the speed of acquiring structurally based breeding plumage in male superb fairy wrens *Malurus cyaneus* (Peters et al., 2000) and satin bowerbirds *Ptilonorhynchus violaceus* (Collis and Borgia, 1992, 1993). However, much as for melanin pigmentation, it is unclear if individual differences in plumage brightness reflect variation in circulating androgens during molt *per se*. Similarly, it is uncertain whether structural colors function in male/male competitive situations as well as in female mate-attraction. As of yet, no test of the status-signaling hypothesis has been conducted for structurally colored ornaments in birds.

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