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PROXIMATE CORRELATES OF CAROTENOID-BASED MOUTH COLORATION IN NESTLING HOUSE SPARROWS

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Abstract. The mouth coloration of passerine nestlings is hypothesized to attract parental care by increasing the visual conspicuousness of begging chicks and/or by signaling the reproductive value of nestlings. Specifically, carotenoids are often hypothesized to mediate the latter relationship. In House Sparrow (*Passer domesticus*) nestlings, we confirmed both the presence of carotenoids in rictal flanges and a positive relationship between carotenoid concentration and the intensity of yellow coloration. This carotenoid-based coloration was positively associated with nestling mass and with plasma carotenoid concentration. Red gape coloration also revealed titers of circulating carotenoids. Carotenoids reduced the overall brightness and the UV reflectance of flanges, an effect that may limit the detectability of carotenoid-rich mouth colors and the ability of brightness and UV coloration to function in communication. For example, flange brightness, likely the primary determinant of conspicuousness, was positively related to nestling mass and levels of circulating carotenoids but only when the reflectance effect of carotenoids was removed statistically. We found no evidence that UV coloration positively reflected nestling condition. Most aspects of mouth coloration were influenced by Julian date and differed among broods, suggesting that colors can capture information about temporal and nontemporal features of the environment experienced by nestlings and, furthermore, could have a genetic component.

Key words: *begging, carotenoids, communication, mouth color, UV,* Passer domesticus.

Correlatos Probables de la Coloración Bucal Basada en Carotenoides en Polluelos de *Passer domesticus*

Resumen. Se ha hipotetizado que la coloración bucal de pichones de paserinos atrae el cuidado parental al aumentar la visibilidad de los polluelos que solicitan comida y/o al indicar el valor reproductivo de los pichones. Específicamente, se ha hipotetizado que los carotenoides son los que intermedian la segunda relación. Utilizando extracciones bioquímicas tomadas de polluelos de *Passer domesticus*, confirmamos la presencia de carotenoides en los bordes peribucales y una relación positiva entre la concentración de carotenoides y la intensidad de la coloración amarilla. Esta coloración basada en carotenoides estuvo asociada positivamente con el peso de los polluelos y con la concentración de carotenoides en plasma. La coloración roja de la boca abierta también reveló concentraciones de carotenoides en circulación. Los carotenoides redujeron el brillo total y la reflectancia de rayos UV de los bordes peribucales, un efecto que puede limitar la detectabilidad de colores bucales ricos en carotenoides y la capacidad del brillo y de la coloración UV de funcionar en la comunicación. Por ejemplo, el brillo de los bordes peribucales, probablemente los elementos más llamativos, estuvo relacionado positivamente con el peso de los pichones y los niveles de carotenoides en circulación sólo cuando el efecto de la reflectancia de los carotenoides fue removido estadísticamente. No encontramos evidencia de que la coloración UV refleje positivamente la condición de los pichones. La mayor parte de los aspectos de la coloración bucal estuvo influenciada por la fecha Juliana y difirió entre nidadas, sugiriendo que los colores pueden brindar información acerca de las características temporales y no temporales del ambiente experimentado por los polluelos y podrían además tener un componente genético.

INTRODUCTION

Traits that increase the receipt of parental care are often adaptive for dependent offspring (Trivers 1974, Clutton-Brock 1991). In altricial birds, the morphology and coloration of the nestling's mouth are hypothesized to be such traits, reflecting selective pressures imposed by reliance on post-hatching parental care (e.g., Swynnerton 1916, Kilner and Davies 1998, Gil et al. 2007). The gapes of most passerine nestlings are bordered by fleshy rictal flanges, and both the flanges and gape are often colorful (Harrison and Castell 1998, Baicich and Harrison 2005). Typically, flanges regress and mouth coloration diminishes after the nestling fledges (Clark 1969), suggesting that if these traits are advantageous, it is when offspring are dependent upon parents.

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Although alternative interpretations exist (reviewed in Dugas 2010), the evolution of nestling mouth colors is typically considered in the context of visual communication between offspring and parents during begging (e.g., Swynnerton 1916, Kilner 1997, Avilés et al. 2008). Early functional explanations highlighted the need for nestlings, particularly those in dark nests, to present visually conspicuous targets to provisioning parents (Pycraft 1907, Swynnerton 1916). This visual ecology approach has more recently been complemented by the hypothesis that nestling mouth colors communicate not only the presence and position of nestlings but also their potential fitness value to parents (Kilner 1997, Saino et al. 2000). This hypothesis is supported by relationships between color and nestling hunger (Kilner 1997, Kilner and Davies 1998), immune status (Saino et al. 2000, 2003), and size and/or age (de Ayala et al. 2007, Ewen et al. 2008, Loiseau et al. 2008, Dugas and Rosenthal 2010).

Both within and among species, the reflectance of mouth parts varies in three visually relevant ways that may be important for both the detectability and signaling hypotheses. Brightness, or the overall intensity of reflected light, is probably the primary mediator of visual conspicuousness (Dugas and Rosenthal 2010, Holveck et al. 2010) and may be positively associated with nestling size (de Ayala et al. 2007). Mouth parts, especially flanges, often also feature an ultraviolet (320–400 nm; UV) reflectance peak (Fig. 1; Hunt et al. 2003) that has been suggested, but not demonstrated, to reveal condition and/or increase detectability (Hunt et al. 2003, Jourdie et al. 2004, de Ayala et al. 2007, Soler et al. 2007). Finally, mouth parts vary qualitatively in color. In passerines, flanges range from white to pale yellow to orange, and gapes range from yellow to orange or pink/red; within a species, individuals vary around a species-typical mean (Harrison and Castell 1998, Baicich and Harrison 2005, Kilner 2006). Flange and some gape colors are probably carotenoid based; positive effects of dietary carotenoid supplementation and carotenoid-typical reflectance suggest this mechanism, although it has yet to be confirmed biochemically (Saino et al. 2000, Hunt et al. 2003, Loiseau et al. 2008, Thorogood et al. 2008). Blood probably also determines or contributes to the coloration of some gapes (but not flanges; Wetherbee 1961, Hunt et al. 2003). Blood-based coloration could offer parents information about nestlings by directly revealing traits associated with fitness prospects (e.g, extent of vascularization, the volume of blood in the tissue, or properties of the blood itself; reviewed by Negro et al. 2006). Although carotenoids are unlikely to enhance detectability (Andersson 2000, Dugas and Rosenthal 2010), they commonly produce colorful ornaments in adult birds (reviewed by Hill 2006, McGraw 2006a) and may serve a similar ornamental function in nestlings, attracting parental care rather than matings (Saino et al. 2000, Ewen et al. 2008, Dugas 2009).

The use of carotenoids as colorants may have visual consequences aside from conferring reflectances rich in long wavelengths (i.e., yellow, orange, red). Although numerous

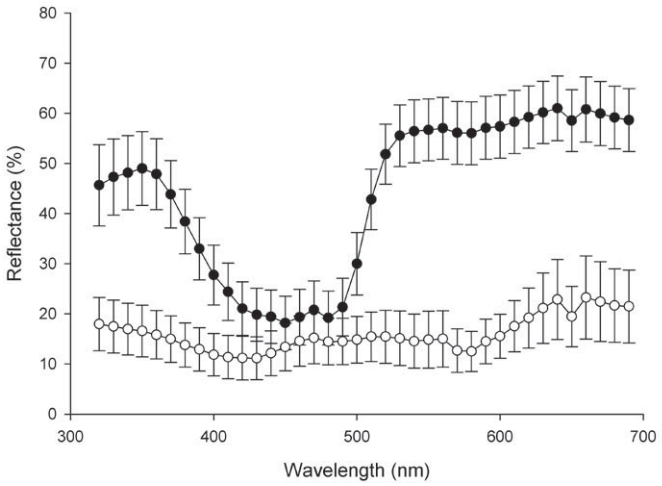

FIGURE 1. Mean ± SD reflectance, at 10-nm intervals, of flange (solid circles) and gape (unfilled circles) tissue of nestling House Sparrows.

mechanisms have been proposed to maintain the information content of carotenoid-based colors (reviewed in Olsen and Owens 1998, Møller et al. 2000, McGraw 2006a), all predict a positive relationship between the quantity of pigments allocated, color intensity, and the putative quality of the signaler. Because carotenoids produce colors rich in long wavelengths by disproportionately absorbing light of medium wavelengths and also absorb moderately in the UV (Shawkey and Hill 2005, Bleiweiss 2005, Andersson and Prager 2006), carotenoid richness should, *ceteris paribus*, be negatively associated with the intensity of both overall and UV reflectance. By extension, carotenoids may have secondary effects; specifically, these pigments may (1) reduce the visual conspicuousness of carotenoid-rich colors (Andersson 2000, Dugas and Rosenthal 2010), (2) mask relationships between brightness/UV intensity and other aspects of a nestling's phenotype (e.g., those that reflect reproductive value), and/or (3) constrain the possible combinations of short- and long-wavelength coloration animals display (Bleiweiss 2008).

Here, we examined the signaling potential of several aspects of mouth coloration in nestling House Sparrows (*Passer domesticus*). In nestlings of this species, the intensity of yellow flange coloration is positively associated with nestling mass (Loiseau et al. 2008, Dugas and Rosenthal 2010) and influences parental food allocation (Loiseau et al. 2008, Dugas 2009), but the potential for other aspects of coloration to function in a similar way has not been assessed. We explored the role of carotenoids in flange coloration by confirming that carotenoids are present in flanges, then testing the prediction that their concentration is associated positively with the intensity of yellow coloration and negatively with brightness and UV coloration. Predictions drawn from the absorptive properties of carotenoids are likely to be met if carotenoid-rich

and carotenoid-poor flanges are otherwise identical (e.g., structurally). We then examined relationships among measures of mouth color and other aspects of nestling phenotype as an initial assessment of the potential of each to function in offspring–parent communication. We used nestling mass as a proxy for the reproductive value of offspring, assuming that, at any stage, heavier chicks are more likely to fledge and be recruited into the breeding population than are lighter chicks (Schwagmeyer and Mock 2008, Mock et al. 2009). We also considered two other aspects of nestling phenotype: circulating carotenoids and hematocrit. Any relationships between these latter two variables and nestlings' fitness prospects should be positive (Saino et al. 2000, 2003, Cuervo et al. 2007), but we chose these measures primarily because of the a priori prediction that they should be mechanistically linked to blood or carotenoid-based coloration.

METHODS

We studied nestling House Sparrows in a free-living population in Norman, Oklahoma (see Schwagmeyer et al. 2002 for details), from April to July 2008. We monitored nests regularly to establish day of hatching (day 0), and we sampled nestlings (n = 94 from 26 broods) on day 6 post-hatching. At this age, slightly less than mid-way through the 2-week nestling period (Anderson 2006), parents still control food allocation (Dugas 2009), as required for offspring–parent signaling (Royle et al. 2002). Because parents were not banded, we used each nest box only once. We likely avoided using the same parents twice, as in this population banded pairs typically occupy just one box per season (172/182 pairs; P.L. Schwagmeyer and D.W. Mock, unpubl. data; see Mock et al. 2009 for details).

On day 6, we weighed each chick to the nearest 0.01 g on an electronic balance, sampled mouth coloration (details below), then drew a small (~75 μL) blood sample from the brachial vein. Blood samples were centrifuged in the field (within 5 min), and plasma was transferred to cryovials. Samples were stored on ice in the field, then, typically within 1 hr, moved to storage at –80 °C. In all but four nests, we sampled all chicks in the brood, but we left at least one chick in the nest at all times to prevent parents from deserting the nest. We could not obtain blood samples from four individuals, and the quantity of blood collected from one was sufficient for carotenoid analysis but not for hematocrit reading.

For direct measurement of the carotenoid content of flange tissue, we collected one nestling from each of 10 broods (mean age ± SD = 4.7 ± 2.2 days) not included in the above sample. As a compromise between ethical concerns and the need for representative samples, we collected the majority of nestlings from broods that appeared abandoned (i.e., nestlings were cold on two checks of the nest within ~2 hr and parents were not observed during ~30 min of observation). To preserve carotenoids in tissue, we euthanized nestlings by immersion in liquid nitrogen (Grether et al. 1999). Samples thawed briefly during color measurement but were otherwise stored at –80 °C until analyzed for carotenoid content. For carotenoid extraction and color measurement, we dissected the right side of the flange bordering the mandible. Reflectance of frozen samples was similar to that of live birds, and a similar study found no effect of freezing on soft-part coloration (Mougeot et al. 2007).

Using a USB4000 spectrometer, light produced by a deuterium–tungsten halogen lamp (DT-MINI-2-GS), and a 600-μm bifurcated fiber-optic cable (Ocean Optics, Dunedin, FL), we measured reflectance (% relative to a white standard, WS-1) at 90° to the tissue (Andersson and Prager 2006) and recorded the measurements with SpectraSuite software (Ocean Optics). For flange samples from sacrificed nestlings, we sampled color two to four times (at the most points we could be sure were unique). We also photographed flange samples digitally under standardized lighting conditions (details available upon request) and scored their color at four points with the color-sampler tool in Adobe Photoshop version CS (Adobe Systems, Inc., San Jose, CA). In the field, we sampled reflectance of flange and gape color four times each; flanges were sampled once from each quadrant of the mouth (right and left sides of both the mandible and maxilla), gapes twice each from the surfaces of the maxilla and mandible, on either side of the *papillae palatinae* and tongue, respectively. We used the median of these four reflectance measurements from each tissue for further analyses.

QUANTIFYING COLOR

In nestling House Sparrows, a pink to red gape is bordered by clearly defined flanges that vary from pale to intense yellow. At hatching, flanges are nearly white and there is little variation among nestlings or broods (MBD, pers. obs.); the intensity of yellow then increases as nestling age (Loiseau et al. 2008, Dugas and Rosenthal 2010), with variation among individuals and broods increasing. On the basis of previous work in this species (Hunt et al. 2003, Loiseau et al. 2008, Dugas and Rosenthal 2010), we began with the working assumption that in nestling House Sparrows flange coloration is carotenoid based and gape coloration is determined primarily by vascularization (see also Wetherbee 1961). We recovered carotenoids from flanges (see Results), and blood-based gape coloration was further indicated by the rapid draining of color with applied pressure and its rapid return when pressure was released. For subsequent analyses, we quantified color with measures appropriate for these mechanisms of coloration. Although not equivalent to color as perceived by parents (which depends on ambient light), objective measures of color are visually relevant (Endler 1990), correspond to specific phenotypic traits (e.g., structural or pigmentary colors; Grether et al. 2004), and, perhaps most importantly, are amenable to experimental manipulation (e.g., Endler and Day 2006, de Ayala et al. 2007, Dugas 2009).

We estimated the intensity of yellow coloration of flange tissue with chroma (sensu Endler 1990), calculated as

$$\sqrt{(R-G)^2+(Y-B)^2}$$

where R, Y, G, and B equal the proportion of total reflectance of red (625–699 nm), yellow (550–624 nm), green (475–549 nm), and blue (400–474 nm) light, respectively. A variety of color variables have been used in the literature to estimate the intensity of colors assumed to be carotenoid based (see Andersson and Prager 2006, Montgomerie 2006 for reviews); we chose chroma because it has been previously used in studies of the House Sparrow (Dugas 2009, Dugas and Rosenthal 2010) and is calculated independently of brightness and UV coloration (Endler 1990).

We estimated the brightness of flange tissue as average reflectance (%) from 320 to 700 nm (sensu Endler 1990). To estimate the intensity of UV coloration, we compared the average reflectance of the UV peak (320–350 nm; Fig. 1a) to the average reflectance from 600 to 699 nm (see Bleiweiss 2005 for similar metric). Reflectance at 600–699 nm should not be influenced by the absorptive action of lutein (Mays et al. 2004), the primary pigment in flanges (see Results); a higher UV score, then, is associated with a higher level of UV reflectance relative to what we assume maximum tissue reflectance would be if carotenoids were absent. Whether UV reflectance is actually an independent trait or, instead, is generated by the same proximate mechanism as overall brightness is unclear, but it has been treated this way in the literature (see Andersson and Prager 2006 for review) and has been manipulated separately in experimental studies (e.g., Jourdie et al. 2004, de Ayala et al. 2007).

Typical of gape colors presumed to be blood based (Hunt et al. 2003), the House Sparrow's gape color features three broad peaks in reflectance (Fig. 1). We first quantified total gape brightness (as above); in addition to being visually relevant, we expected that this color variable might be negatively associated with levels of circulating hemoglobin (estimated with hematocrit) and carotenoids, both of which absorb light. Other authors, using photographic analysis, have reported relationships between gape "redness" and nestling state (hunger: Kilner 1997; immune response: Saino et al. 2000; temperature: Clotfelter et al. 2003). We used chroma, as above, to quantify this feature of gape color (we initially approximated redness as the proportion of reflectance from 580 to 699 nm, corresponding to the intuitive red peak in reflectance (Fig. 1, see also Mougeot et al. 2007), but this measure was highly correlated with chroma ($r = 0.95$, $n = 94$, $P < 0.001$), so we used the latter for consistency). In the gape, chroma is likely to also be a composite variable, positively revealing the amount of blood in the tissue, the level of vascularization, and perhaps the levels of circulating carotenoids (Kilner 1997, McGraw 2006b). Gape brightness and chroma were not significantly correlated ($r = -0.16$, $n = 94$, $P = 0.120$). Because the House Sparrow's gape coloration does not feature a prominent UV peak, there was no reason to consider relative UV intensity as a separate color variable. Repeatability (sensu Lessells and Boag 1987) of flange brightness and gape chroma were low (Table 1), so null results must be interpreted with some caution.

TABLE 1. Repeatability (r) of color measurements used to calculate medians for flange and gape tissue of nestling House Sparrows (on the basis of one randomly selected chick per brood). For flanges, repeatability is presented for both field observations and those from which carotenoids were extracted.

	Extracted samples			Field observations		
	$F_{9,25}$	P	r	$F_{26,27}$	P	r
Flange						
Brightness	2.94	0.02	0.69	3.46	<0.001	0.38
Chroma	24.45	<0.001	0.96	10.15	<0.001	0.70
Relative UV intensity	18.03	<0.001	0.95	8.18	<0.001	0.64
Gape						
Brightness				15.39	<0.001	0.78
Chroma				3.45	<0.001	0.38

IDENTIFYING AND QUANTIFYING CAROTENOIDS

Plasma carotenoid extraction and high-performance liquid chromatography (HPLC) analyses followed the ethanol + tert-butyl methyl ether (TBME) method described by McGraw et al. (2008). For extractions of carotenoids from flanges, we first ground tissue samples in a ball mill for 30 min in the presence of 1 mL TBME. The resulting solutions were centrifuged for 2 min at 10 000 revolutions min^{-1}, and supernatants were then transferred to fresh tubes for analysis (see below). We compared resolved HPLC peaks to purified reference carotenoids and identified lutein and zeaxanthin in both plasma and flange tissue, with lutein being dominant (see Results). Pilot tests of flange tissue, however, indicated the presence of esterified forms of the xanthophylls (typical of avian bare parts); because we did not want to lose samples from sacrificed nestlings to develop a saponification procedure (which might also damage any carotenoids present), we instead used absorbance spectrophotometry to quantify total xanthophyll concentration in flanges (sensu Steffen and McGraw 2007). We determined carotenoid concentration by comparison to external standard curves created separately for lutein and zeaxanthin on the HPLC (for plasma) and for lutein (λ_{max} = 447 nm) on the spectrophotometer (for flanges).

STATISTICAL ANALYSES

For the 10 flange samples for which we measured carotenoid content directly, we used linear regressions to test the prediction that carotenoid content should be positively associated with chroma and negatively associated with brightness and relative UV intensity. The carotenoid richness of color has been estimated in a number of other ways in the literature, so we have provided similar analyses of our directly measured samples with commonly used metrics, especially those from previous studies of nestling mouth coloration. To test relationships among

mouth-color measurements, environmental variables, and nestling phenotype, we used linear mixed models with a single color value entered as the dependent variable, with mass, hematocrit, total plasma carotenoid concentration (see Results for details), brood size, and date (days after April 1) as fixed effects and with brood as a random effect. Fixed effects that were nonsignificant ($P > 0.05$) in all models were dropped before presentation, and the significance of all fixed effects was tested by sequentially dropping nonsignificant terms from the model. We also ran models for flange brightness and relative UV intensity with flange chroma included as a fixed effect. Without chroma included, these analyses test whether a reflectance property, as it would be visually available to parents (i.e., actually expressed in tissue), reveals information about the fixed effects. The inclusion of chroma offers a further test of the prediction that carotenoid content of flange tissue is negatively associated with total brightness and UV intensity and tests whether there is a relationship between these color features and the nestling's other traits, independently of the effect of carotenoids. In other words, only by including chroma as a covariate can we appropriately test whether the physical attributes of the flange tissue contributing to total brightness and UV intensity (e.g., gross anatomical or nanostructures) are related to the fixed effects. As detailed earlier, the esterification of flange carotenoids did not allow pigments to be removed while leaving tissue otherwise intact, the method typically used to accomplish the assessment of pigment-free reflectance in feathers (e.g., Shawkey and Hill 2005).

To test the null hypothesis that the random effect of brood did not contribute to color differences, we used a –2 residual log-likelihood ratio test in which a full model including the random effect of brood is compared to that of a reduced model not including this random effect (Sokal and Rohlf 1995, Quinn and Keough 2002, Agresti 2007, Dickey 2008). Following Quinn and Keough (2002), we refer to this test statistic as G^2 and used a chi-squared distribution with 1 degree of freedom to estimate a P value (Quinn and Keough 2002, Agresti 2007, Dickey 2008).

To allow for clearer presentation of β values, all were multiplied by 10^3. To meet the assumption of normality, total plasma carotenoid concentration was square-root transformed and brightness was $\log_{10}$ transformed. We ran mixed models with the PROC MIXED procedure in SAS version 9.2 (SAS Institute, Cary, NC); all other analyses were performed with SPSS version 15. Throughout, α is set at 0.05 and means are presented ± SD unless otherwise noted.

RESULTS

CAROTENOID ANALYSES

Carotenoids were recovered from 9 of the 10 flange samples from sacrificed nestlings. Under the assumption that our sample (one flange quadrant) was representative of all flange tissue, flanges were colored by a total of 0.50 ± 0.37 μg (range 0.00–1.06 μg) of carotenoids per bird (19.72 ± 14.11 μg g^{-1}, range 0.00–40.93 μg g^{-1}). As predicted, flange carotenoid content was positively associated with chroma ($r^2 = 0.67$, $F_{1,8} = 18.1$, $P = 0.003$) and negatively associated with relative UV intensity ($r^2 = 0.41$, $F_{1,8} = 5.5$, $P = 0.05$); total brightness, however, was unrelated to carotenoid content ($r^2 = 0.01$, $F_{1,8} = 0.1$,

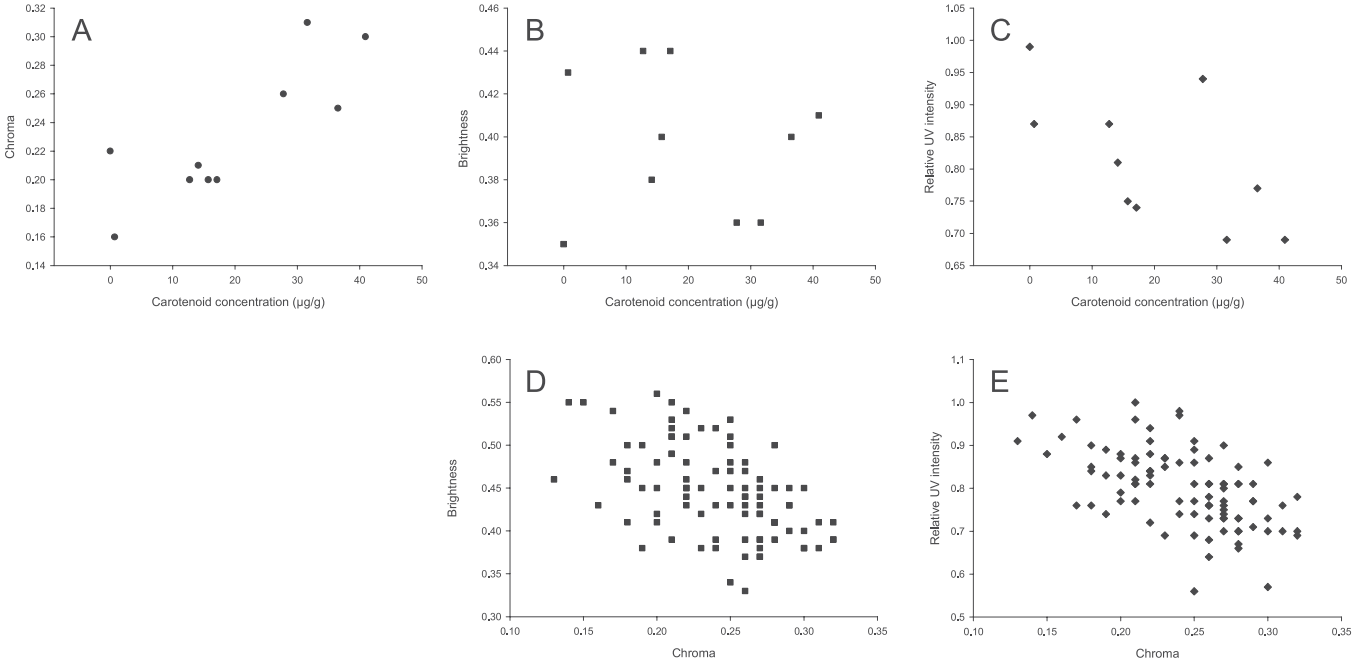

FIGURE 2. The relationship between the carotenoid content of flange tissue of nestling House Sparrows with chroma (A), brightness (B), and relative UV intensity (C) and the relationship between chroma, a carotenoid proxy, and brightness (D) and relative UV intensity (E) of flanges measured in the field.

TABLE 2. Results of linear mixed models assessing the relationship between flange and gape color and body mass, circulating carotenoids (square-root transformed), and hematocrit (gape only) of nestling House Sparrows at day 6. Chroma was included as a covariate in the analysis of flange UV coloration and brightness to control for the negative influence of carotenoids on overall brightness and UV reflectance. Differences in degrees of freedom for flange and gape colors reflect one individual from which hematocrit was not measured.

	Fixed effects			Random effect		
	F	P	β(SE) × 10^3		G^2	P
Flange						
Chroma						
Mass	8.5[a]	0.005	2.73 (0.96)	brood	18.7	<0.001
Total carotenoids	6.8[a]	0.01	14.72 (5.81)			
Date	4.3[a]	0.04	0.70 (0.34)			
Brightness						
Mass	9.1[b]	0.004	3.38 (1.11)	brood	0.3	0.60
Total carotenoids	4.1[b]	0.05	13.19 (6.49)			
Date	13.2[b]	0.001	1.09 (0.30)			
Chroma	68.3[b]	<0.001	–10.28 (1.24)			
Relative UV intensity						
Mass	1.5[b]	0.23	2.50 (2.08)	brood	16.6	<0.001
Total carotenoids	0.4[b]	0.52	–8.12 (12.40)			
Date	4.9[b]	0.03	1.64 (0.74)			
Chroma	52.3[b]	<0.001	–16.20 (2.24)			
Gape						
Chroma						
Mass	1.3[c]	0.26	–1.13 (1.01)	brood	7.0	0.01
Total carotenoids	5.3[c]	0.03	2.37 (1.03)			
Hematocrit	1.6[c]	0.22	75.45 (60.40)			
Date	0.2[c]	0.69	–0.11 (0.27)			
Brightness						
Mass	0.03[c]	0.87	–0.74 (4.66)	brood	10.6	0.001
Total carotenoids	0.0[c]	1.00	0.004 (0.48)			
Hematocrit	0.8[c]	0.37	–253.30 (281.80)			
date	0.1[c]	0.76	–0.41 (1.31)			

[a]df = 1, 64.
[b]df = 1, 63.
[c]df = 1, 62.

$P = 0.76$; Fig. 2). In nestlings for which color was measured in the field, chroma (a carotenoid proxy) was negatively associated with both flange brightness and relative UV intensity (Table 2, Fig. 2). Supplementary analyses with other color estimates generally suggest that the ratio of long-wavelength reflectance to short-wavelength reflectance is a good predictor of the carotenoid content of flanges (Table 3). Chroma was associated with all other color variables significantly correlated with carotenoid content (absolute value of all $r > 0.91$, all $P < 0.001$).

Photographs may be appropriate for some questions in future work; chroma and saturation (from photographs) were significantly correlated ($r = 0.92$, $n = 9$, $P < 0.001$), as were spectral (sensu Endler 1990) and photographic estimates of hue ($r = 0.77$, $n = 9$, $P = 0.02$).

PHENOTYPIC CORRELATES OF COLOR

Lutein and zeaxanthin were the two carotenoids detected in nestlings' plasma, with lutein accounting for 89 ± 5% of total plasma carotenoids. Levels of these two pigments were positively correlated ($r = 0.83$, $n = 93$, $P < 0.001$), so we used total carotenoid concentration for further analysis. Brood size was not a significant predictor of any measure of color (all $P > 0.21$), so we removed it from all models. Hematocrit was not a significant predictor of any measure of flange (all $P > 0.64$) or gape color; although these results are nonsignificant, we present them for the gape in Table 2 because of our a priori expectation that this aspect of nestling phenotype would be revealed by gape color.

Flange chroma was positively associated with nestling mass, plasma carotenoid concentration, and date (Table 2). Flange brightness was unrelated to date ($F_{1,64} = 1.4$, $P = 0.25$), mass ($F_{1,64} = 1.2$, $P = 0.27$), or total carotenoid concentration ($F_{1,64} = 0.6$, $P = 0.46$). However, after flange chroma was controlled for, brightness was positively associated with nestling mass, total plasma carotenoid concentration, and date (Table 2). The relative UV intensity of flanges was not associated

TABLE 3. The relationship between the carotenoid content of flanges of nestling House Sparrows and measures of color commonly used to estimate the carotenoid content of tissues, especially nestlings' mouth parts. Color variables are divided into those that estimate saturation and those that estimate spectral location. These include estimates calculated from tissue reflectance (see text for details) and estimated from photographs taken under standardized lighting conditions (one sample was not photographed) with Adobe Photoshop (version CS). Relationships based on the segment-classification method proposed by Endler (1990) are included, here extended to include UV reflectance, as such divisions of reflectance are common precursors to estimates of carotenoid concentrations. Equations are shown for variables for which $P < 0.15$. Repeatability of each variable, the formula used for calculation, and a representative reference are also shown. Reflectance is abbreviated as "R" and wavelength ranges are shown in subscript.

			Repeatability			Relationship with carotenoid content			
Variable	Calculation	Reference	F	P	r	r^2	F	P	Equation
Spectral reflectance									
Saturation estimates									
Carotenoid chroma	$(R_{700} - R_{450})/R_{700}$	Andersson and Prager 2006	17.7[a]	< 0.001	0.95	0.74	23.0[b]	0.001	$y = 139.17x - 73.29$
Yellow-chroma	$R_{550-625}/R_{300-700}$	Thorogood et al. 2008	25.4[a]	< 0.001	0.97	0.74	23.0[b]	0.001	$y = 486.15x - 182.07$
Yellow	mean $R_{552-570}$	Mays et al. 2004	4.0[a]	0.003	0.77	0.27	2.9[b]	0.13	$y = 1.63x - 60.20$
Spectral location									
Hue	arcsin[$(Y - B^e)$/chroma[f]]	Endler 1990	1.6[a]	0.17	0.40	0.10	0.9[b]	0.38	
λR_{vis50}	λ = mean $R_{400-700}$	Bleiweiss 2008	15.8[a]	< 0.001	0.94	0.57	10.7[b]	0.01	$y = 2.63x - 1332.03$
Segment classification									
R	$R_{625-699}/R_{325-699}$	Endler 1990	9.5[a]	< 0.001	0.91	0.64	14.5[b]	0.005	$y = 656.38x - 164.22$
Y	$R_{550-624}/R_{325-699}$	Endler 1990	50.0[a]	< 0.001	0.98	0.68	16.9[b]	0.003	$y = 793.09x - 175.08$
G	$R_{475-549}/R_{325-699}$	Endler 1990	19.0[a]	< 0.001	0.95	0.51	8.4[b]	0.02	$y = -966.62x + 183.46$
B	$R_{400-474}/R_{325-699}$	Endler 1990	19.7[a]	< 0.001	0.96	0.64	14.4[b]	0.005	$y = -697.15x + 88.73$
UV	$R_{325-399}/R_{325-699}$	Endler 1990	18.3[a]	< 0.001	0.95	0.29	3.3[b]	0.11	$y = -527.74x + 126.52$
Photographs									
Saturation		Kilner and Davies 1998	24.1[c]	< 0.001	0.85	0.501	7.03[d]	0.03	$y = 0.61x + 74.72$
Hue		Kilner and Davies 1998	9.8[c]	< 0.001	0.69	0.002	0.01[d]	0.92	
R			12.6[c]	< 0.001	0.74	0.149	1.23[d]	0.30	
G			7.7[c]	< 0.001	0.63	0.019	0.13[d]	0.73	
B			21.8[c]	< 0.001	0.84	0.486	6.62[d]	0.04	$y = -0.63x + 29.52$

[a] df = 9,25.
[b] df = 1,8.
[c] df = 8,27.
[d] df = 1,7.
[e] Y and B ranges/$R_{400-699}$.
[f] See text for details.

with mass ($F_{1,64} = 0.1$, $P = 0.72$) or date ($F_{1,64} = 0.49$, $P = 0.49$) but was negatively associated with plasma carotenoid concentration ($F_{1,64} = 5.4$, $P = 0.02$, $\beta \pm SE = -3.33 \pm 1.44$). Only date was associated with relative UV intensity (Table 2) once the negative effects of carotenoids on UV intensity were controlled. Gape brightness was not associated with date or any nestling trait, while chroma, a measure of the intensity of red coloration, was positively associated with circulating carotenoids only (Table 2). All mouth-color variables except flange brightness differed among broods (Table 2).

DISCUSSION

Nestling mouth coloration has the potential to provide a parent House Sparrow with information about its offspring by revealing aspects of phenotype that may be associated with reproductive value. All aspects of flange coloration were affected by Julian date, suggesting that they capture temporal variation in the pre- and/or post-hatching environment nestlings experience. All features of gape and flange color except flange brightness differed among broods even when date was controlled for, suggesting that colors might also reveal nontemporal features of the environment and, furthermore, could have a genetic component.

Flange coloration was generally better predicted by proxies for nestling condition than was gape color, but gape redness (chroma) was positively associated with levels of circulating carotenoids, perhaps because this property of the blood was directly revealed through the blood's color. Blood-based colors in the mouths of other birds have been shown to vary rapidly with hunger (Kilner 1997, but see Kilner and Davies 1998) or

temperature (Clotfelter et al. 2003), which might explain both the lack of associations with relatively more stable aspects of individual phenotype like mass and hematocrit and the significant differences we found among broods. Previous authors suggesting a signaling capacity of blood-based gape colors have quantified color from photographs taken during voluntary begging (e.g., Kilner 1997, Kilner and Davies 1998, Clotfelter et al. 2003). Photographs might better capture natural expression of gape coloration, as handling could alter stress and blood flow, but the use of photographs also presents logistical and methodological challenges (e.g., for visual modeling).

While physical features of the flange (e.g., structural coloration) probably determine maximum UV intensity and overall brightness, the level of these traits actually expressed is negatively influenced by the deposition of carotenoids (Mougeot 2007, Thorogood et al. 2008). These effects may limit both the detectability of carotenoid-rich colors and the capacity of brightness and UV coloration to carry information about nestling phenotype or environmental conditions. For example, we found that flange brightness was positively associated with nestling mass and circulating carotenoids once the absorbance effects of carotenoids were controlled. However, as it would actually be visible to parents (i.e., with the effects of carotenoids not controlled), flange brightness was unrelated to either. Similarly, a principal-components score associated with brightness was found by de Ayala et al. (2007) to be positively associated with mass, tarsus length, and feather growth in nestling Barn Swallows (*Hirundo rustica*). This may suggest that high-quality, carotenoid-rich nestlings can somewhat compensate for any detectability constraints imposed by carotenoid-rich coloration via increased brightness of the underlying tissue. Additionally, highly reflective (i.e., bright) flanges may serve as an amplifier (sensu Hasson 1989), making information about carotenoid richness more available to parents (Grether et al. 2004). Both visual modeling and behavioral studies of parents will be needed to establish any functional significance of these effects.

We found UV coloration, as visible to parents, to be negatively associated with the carotenoid content of flanges and with circulating carotenoids (see also Mougeot et al. 2007) but unrelated to any measured aspect of nestling phenotype once the effects of carotenoids were controlled. Although high UV reflectance of body skin may have the potential to signal an individual's immune status (Jourdie et al. 2004, Bize et al. 2006, Soler et al. 2007), there is little support yet for the condition dependence of UV mouth coloration (de Ayala et al. 2007, Soler et al. 2007). However, UV coloration of flanges has been shown experimentally to influence parental food allocation in the Barn Swallow (de Ayala et al. 2007), so it may be too early to dismiss the hypothesis that UV reflectance plays some role in detectability or signaling (see also Dugas 2010).

Within broods, we found carotenoid-based flange coloration to be associated with nestling mass, a result generally consistent with previous findings suggesting the condition dependence of these colors in nestling birds, including the House Sparrow (Saino et al. 2000, 2003, Ewen et al. 2008, Loiseau et al. 2008). Both the intensity of yellow flange and red gape coloration also revealed circulating carotenoid levels (see also Loiseau et al. 2008). While the relative mass of offspring might be a trait accessible to parents without the use of mouth color, levels of circulating carotenoids per se are almost certainly inaccessible to parents without carotenoid-based colors. To the extent that nestlings rich in carotenoids more efficiently translate parental care into growth (Hall et al. 2010) or are better able to maintain growth under stressful conditions (e.g., parasites; Ewen et al. 2009), parental allocation based on these traits may be advantageous.

While flange coloration reveals total carotenoids allocated to tissue, the extent to which mouth coloration represents a major (i.e., costly) carotenoid sink for nestling House Sparrows remains unclear (see also Hill 1999). On the basis of rough estimates of blood (Hoysak and Weatherhead 1991) and yolk (Anderson 2006) volume, day-6 nestlings probably circulate ~12 times the quantity of carotenoids used for coloration; yolks contained ~56 times this amount in a sample of five second-laid eggs in the population we studied (40.7 ± 22.7 μg g^{-1}, range 12.5–72.9 μg g^{-1}, unpubl. data; see also Cassey et al. 2005). The fact that relatively small quantities of carotenoids are found in flanges is consistent with the finding that only the flange surface displayed during begging is colorful (Dugas 2010). However, experimental manipulations of corticosterone levels caused House Sparrow flanges to lose color (Loiseau et al. 2008), which may suggest that flange carotenoids are either drawn upon in times of physiological stress (as are gape colors; Saino et al. 2000, 2003) or must be regularly replenished, either of which could raise the total carotenoid cost of maintaining colorful flanges.

Although color can be considered as a single visual trait, the reflectance of tissues is typically a product of several physical and chemical traits, including the reflectance properties of the tissue itself and the visual properties of any pigments present (Grether et al. 2004). These contributors to color may result from different proximate mechanisms, may reflect different physiological processes (and thus potential information content), and may evolve under different selective pressures (e.g., detectability and signaling; Grether et al. 2004). In future comparative studies of nestling mouth coloration, treating color as a multicomponent signal (rather than simply a visual phenomenon) may promote more accurate identification of the effect of signaling environment on signal design and better reveal the ecological, social, and physiological constraints on signaling.

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