UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

ORNAMENTAL NESTLING MOUTH COLORATION AND PARENTAL CARE IN HOUSE SPARROWS

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

Degree of

DOCTOR OF PHILOSOPHY

By

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Norman, Oklahoma
2010

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A DISSERTATION APPROVED FOR THE
DEPARTMENT OF ZOOLOGY

BY

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Acknowledgements

First and foremost, I thank Doug Mock for serving as my advisor and P.L. Schwagmeyer, Ingo Schlupp, Rosemary Knapp and Jorge Mendoza for serving on my committee. I am grateful to my committee, and the OU Zoology Department as a whole, for treating me as an equal. I also thank my collaborators Kevin McGraw (Chapter I) and Gil Rosenthal (Chap IV). Funding was provided by support from the George Miskch Sutton Scholarship in Ornithology, the American Ornithologists' Union and a Doctoral Dissertation Improvement Grant from the National Science Foundation. I was also supported by an Alumni Fellowship in Zoology and Adams Summer Scholarships. Perhaps most importantly, Doug Mock generously purchased the spectrometer I have come to love and hate, and handled the resulting explosions (literal and metaphorical) with magnanimity. The contribution of my teachers can't be overstated. I especially thank Ralph Bruno for giving me the tools to enjoy science, Debra Sansone for teaching me to take pride in good writing, Jim Whitson and Joe Wagner for teaching me to follow rules (well, the potential value of doing so anyway), and Lorna Bonner for thinking I was worth a little extra patience. It was a more pleasant trip for the company, and I wouldn't have made it without the friends I've made. I would be remiss if I failed to single out Jon Shik, who was with me since day one (this acknowledgement serves as advance re-payment for the fact that he will hear for the rest of his life that I beat him to the finish-if only by a few days). Chris Leary, Don Shepard and Adrian Garda taught me what graduate school was about, and Ben Leslie put it all in perspective by pointing out that finishing a PhD would make re-

enacting the “doctor” scene from Spies Like Us more appropriate. Of course, Hardin Dr. will always be home, and my roommates will always be family. Stephanie Strickler reminded me that a day could be pleasant, not just productive. And of course, Bella and Mills made it impossible to be depressed after getting home. Last and the opposite of least, I thank my parents. My father took me outside and taught me the value of finding a job I wouldn’t hate, the two reasons I’m here. My mother’s attention to my schoolwork made up for my own lack of motivation, and I’m still pondering her outlandish suggestion that I might not always be right.

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Abstract

Dependent offspring across taxonomically-diverse lineages use behavioral, vocal, chemical, and morphological traits to attract parental care. Such offspring solicitations are often hypothesized to evolve as a means of offspring-parent communication, where offspring traits furnish information about aspects of offspring phenotype of potential interest to parents (e.g., hunger, body size, immune status), and parents use these offspring traits to make adaptive decisions about the level and/or division of investment they provide. While offspring solicitations are widely interpreted as indicative of offspring "need" (more formally, the contribution that a unit of parental investment will make to an offspring's personal fitness), this interpretation seems at odds with the observation that dependent offspring solicit parental care with traits that, when found in adult animals, are typically interpreted as signals of high quality. For example, while soliciting food from provisioning parents, altricial nestling birds commonly reveal elaborately colored mouth parts, including colorful rictal flanges that border the gape. Several lines of evidence suggest that the mouth coloration of nestling birds may be a trait reflecting selective pressures imposed by reliance upon parental care. For example, flanges are present only during the nestling period, and their coloration is restricted to the portion of tissue revealed to parents during begging. I addressed the possibility that the yellow flange coloration of nestling house sparrows may serve in offspring-parent communication by examining i) the potential information content of this trait and ii) parental response to variation. I measured tissue color using reflectance spectrometry and, most often, quantified three features

of flange reflectance: i) overall brightness (total reflected light), ii) relative intensity of ultraviolet reflectance (UV peak / an estimate of pigment-free reflectance), and iii) chroma, an estimate of the saturation of yellow coloration. With biochemical extractions, I demonstrated that the yellow flange coloration of nestling house sparrows is carotenoid-based, and that chroma positively reflects the amount of carotenoids present. While the maximum brightness and UV intensity of flange coloration is likely structural in origin, carotenoids limit the expression of these traits through their absorptive properties (i.e., all else being equal, carotenoid-richness is negatively associated with brightness and UV intensity). To account for this effect, I typically analyzed structural features of color with chroma included as a covariate. At days three and six post-hatching, both the carotenoid-richness and brightness (controlling for the effects of carotenoids) of flange tissue have the potential to provide parents with information about their offspring. These features of reflectance were positively associated with nestling mass, tarsus length and circulating carotenoid levels. Carotenoid-richness increased with nestling age, although brightness did not change significantly. Between days three and six post-hatching, the magnitude of ontogenetic changes in both color parameters was positively associated with the amount of mass gained by nestlings, suggesting that food intake influences the development of coloration. There was little evidence that UV coloration contained information about individual phenotype. Even after these individual-level associations among colors and other aspects of nestling phenotype were accounted for statistically (i.e., are included as covariates in models), broods were different from each other. A cross-fostering study revealed that most among-brood variation was explained by

factors shared by parents breeding contemporaneously (presumably reflecting environmental variation or similarities among parents themselves); this result was consistent with seasonal differences in color revealed by the descriptive study. Carotenoid-based coloration was influenced by both pre- and post-hatching parental effects, while structural colors (brightness and UV) were not. These parental effects on chick coloration most likely result from differences in carotenoid supply (via yolk or solid food) or physiological consumption (e.g., via immune responses), although genetic differences among parents (captured by nest-of-origin effects) are also possible. In summary, within-brood variation seems likely to capture within-brood status, while among-brood variation likely reflects aspects of the conditions in which broods are reared rather than intrinsic qualities of the brood members themselves. When parents were presented with similarly-sized nestlings with mouth colors manipulated to appear carotenoid-rich or carotenoid-poor, they allocated more resources to the nestlings that appeared carotenoid-rich; this effect was significant only for females, although the trend was similar for males, and the non-significant effect likely reflected low statistical power. These preferences themselves did not indicate that parents were responding to color, in the ultimate sense, because of their information content. If carotenoid-rich colors are more visually conspicuous, parental responses might simply reflect limitations of their sensory systems. To distinguish between these alternatives, I used a model of house sparrow vision to estimate the conspicuousness of flanges under a suite of realistic ambient light conditions, and compared carotenoid-richness (chroma) to conspicuousness (contrast between the flange and interior of the mouth and the flange and nesting material). The achromatic

contrast, probably the primary mediator of detectability, was either unaffected by chroma or negatively associated with this proxy for carotenoid richness, depending on ambient light conditions. Overall, these results suggest that carotenoid-based flange coloration plays a functional role in mediating the allocation of parental care, that within-brood parental preferences favor offspring of relatively high value, and that these parental preferences probably (proximately and/or in evolutionary time) exploit the information content of offspring traits to make adaptive life-history decisions. More broadly, these suggest that offspring solicitations may evolve under pressure to signal an individual's status as a promising target for future investment.

Chapter I

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 nestlings, we confirmed both the presence of carotenoids in rictal flanges (using biochemical extractions) and a positive relationship between carotenoid concentration and the intensity of yellow coloration. This carotenoid-based coloration was positively associated with individual nestling mass and with plasma carotenoid concentration for both individuals and broods. Red (probably blood-based) gape coloration also revealed circulating carotenoid titers. Carotenoids reduced the overall brightness and the intensity of UV reflectance of flanges, an effect that may limit the detectability of carotenoid-rich mouth colors and the ability of brightness and UV reflectance to function in communication. For example, flange brightness, likely the primary determinant of conspicuousness, was positively related to nestling mass and circulating carotenoid levels, 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 time-of-year and differed among broods, suggesting that colors can capture information about temporal and non-temporal features of the environment experienced by nestlings and, furthermore, could have a genetic component.

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 mouth are hypothesized to be such traits, reflecting selective pressures imposed by reliance on 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 period (Clark 1969), suggesting that if these traits are advantageous, it is when offspring are dependent upon parents.

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, Loiseau et al. 2008, Dugas and Rosenthal 2010).

Both within- and among-species, the reflectance of mouth parts varies in three

general ways that may be relevant to 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 to reveal condition (Jourdie et al. 2004, de Ayala et al. 2007, Soler et al. 2007). Because nesting material (the background against which mouths are presented) typically reflects little UV light, UV flange coloration has additionally been suggested as an important mediator of detectability via contrast; however, an equally strong case might be made that the relative dearth of UV in the ambient light at dark nests (e.g., cavities) makes this feature of color especially unimportant (Hunt et al. 2003). Finally, mouth parts vary in qualitative color. In passerines, flanges range from white to pale yellow to orange, and gapes from yellow to orange or pink/red; within 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 (although this has yet to be confirmed biochemically; Ficken 1965, Hunt et al. 2003, Thorogood et al. 2008), while 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 quantity of blood in the tissue, or properties of the blood itself: reviewed in Negro et al. 2006). While carotenoids are unlikely to enhance detectability (Andersson 2000, Dugas and

Rosenthal 2010), they commonly produce colorful ornaments in adult birds (reviewed in 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, Loiseau et al. 2008, Dugas 2009).

The use of carotenoids as colorants may have visual consequences aside from conferring long-wavelength-rich reflectance (e.g., yellow, orange, red) to tissues. Although numerous 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 long-wavelength rich colors via disproportionate absorbance of medium-wavelength light 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 might have secondary effects, specifically these pigments can: (i) reduce the visual conspicuousness of carotenoid-rich colors (Andersson 2000, Dugas and Rosenthal 2010); (ii) mask relationships between brightness/UV intensity and other aspects of nestling phenotype (e.g., those that reflect reproductive value); and/or (iii) constrain the possible combinations of short- and long-wavelength coloration displayed by animals (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 remains unclear. We explored the role of carotenoids in flange coloration by first testing the primary assumption that carotenoids are present in flanges, and then the prediction that their concentration is associated positively with the intensity of yellow coloration and negatively with brightness and UV coloration. We then examined relationships among mouth color parameters 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 offspring reproductive value, assuming that heavier chicks at any stage are more likely to fledge and recruit than lighter chicks (Schwagmeyer and Mock 2008, Mock et al. 2009), and also considered two other aspects of nestling phenotype: circulating carotenoids and hematocrit. Any relationships between these latter two parameters and nestling fitness prospects should be positive (Saino et al. 2000, 2003, Cuervo et al. 2007), but these measures were chosen primarily based on the *a priori* prediction that they would be mechanistically linked to blood- or carotenoid-based coloration.

METHODS

General Methods.—We studied nestling House Sparrows in a free-living population in Norman, OK, USA (see Schwagmeyer et al. 2002 for details) in April–July 2008. Nests were regularly monitored 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 two-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, each nest box was used only once. We likely avoided using the same parents twice, as banded pairs in this population use at most two boxes per season and typically occupy just one (172/182 pairs; P.L. Schwagmeyer and D.W. Mock *unpublished 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), and then drew a small (~75 μl) blood sample from the brachial vein. Blood samples were centrifuged in the field (within 5 min), and plasma transferred to cryovials. Samples were stored on ice in the field, and then, typically within 1 hr, moved to -80° C storage. In all but 4 nests, all chicks in the brood were sampled, but at least one chick remained in the nest at all times to avoid potential desertion by parents. For 4 individuals, we could not obtain blood samples, and for one, the quantity of blood collected 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. To preserve carotenoids in tissue, nestlings were euthanized by immersion in liquid nitrogen (Grether et al. 1999). Samples were thawed briefly for color measurement, but 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).

Reflectance (% relative to a white standard, WS-1) was measured at a 90° angle to the tissue (Andersson and Prager 2006) using a USB4000 spectrometer, light produced by a deuterium-tungsten halogen lamp (DT-MINI-2-GS) and a 600 μm bifurcated fiberoptic cable (Ocean Optics, Dunedin, FL, USA). Measurements were recorded using SpectraSuite software (Ocean Optics). For flange samples from sacrificed nestlings, we sampled color 2–4 times (at the most points we could be sure were unique). In the field, we sampled 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), and 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 (M.B.D. personal observations); the intensity of yellow coloration then increases as nestling age (Loiseau et al. 2008, Dugas and Rosenthal 2010), with among-individual and -brood variation increasing. Based on previous work in this species (Hunt et al. 2003, Loiseau et al. 2008, Dugas and Rosenthal 2010), we began with the working assumption that House Sparrow flange coloration is carotenoid-based and gape coloration is primarily determined by vascularization (see also Wetherbee 1961). Carotenoids were recovered 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 parameters appropriate for these mechanisms of coloration.

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 parameters 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 is visually-relevant (Endler 1990), has been previously used in studies of House Sparrows (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 320–700 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 600–699 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), and was not associated with chroma (a carotenoid proxy) in our sample of day 6 nestlings (r = -0.074, n = 94, P = 0.479). 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.

Typical of gape colors presumed to be blood-based (Hunt et al. 2003), House Sparrow 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 parameter 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 580–699 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 ($r = 0.952$, $n = 94$, $P < 0.001$) and 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 ($r = -0.158$, $n = 94$, $P = 0.120$). Because gape coloration of House Sparrows does not feature a prominent UV peak, there was no reason to consider relative UV intensity as a separate color parameter (see also results of PCA below). Repeatability (Lessels and Boag 1987) of all color parameters is shown in Table 1.

Both to confirm the appropriateness of our color parameters and to identify other possible features of color that might be highly variable among individuals, we

used a Principal Components Analysis (PCA) to summarize variation in reflectance at 10 nm intervals and compared this method of color quantification to that detailed above. While color quantification using PCA is common (e.g., de Ayala et al. 2007) and offers some advantages, it has been criticized because results are not easily interpreted with respect to the biological basis or visual consequences of color variation, and are not comparable between studies (Endler 1990). A comparison of PCA to standardized color quantification methods, therefore, is a useful step in the verification of chosen color parameters.

Identifying and quantifying carotenoids.— Plasma carotenoid extraction and high-performance liquid chromatography (HPLC) analyses follow 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 10000 RPM, 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). Concentration was determined 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 would be positively associated with chroma and negatively associated with brightness and relative UV intensity. To test relationships among mouth color parameters, environmental variables, and nestling phenotype, we used linear mixed models with a single color parameter 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 non-significant ($P > 0.05$) in all models were dropped before presentation, and the significance of all fixed effects was tested by sequentially dropping non-significant 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, 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 other nestling 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 nano-

structures) 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 methodology 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 (Quinn and Keough 2002, Agresti 2007, Dickey 2008). Following Sokal and Rohlf (1995), we refer to this test statistic as G^2 and used a chi-square distribution with 1 degree of freedom to estimate a *P* value (Quinn and Keough 2002, Agresti 2007, Dickey 2008).

Brood is typically a significant predictor of color in such analyses (e.g. de Ayala et al. 2007, Soler et al. 2007). To examine among-brood differences, we analyzed brood means for each color parameter (Soler et al. 2007). We used general linear models (GLMs) with each color parameter entered as the dependent variable; we initially included average mass, total plasma carotenoid concentration, hematocrit, brood size and date as covariates. We then sequentially removed covariates until all remaining were significant ($P < 0.05$). We did not include the 4 broods sampled incompletely in the analysis of brood means (final $n = 22$).

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. Mixed models were performed using the PROC MIXED procedure in SAS version 9.2 (SAS), while all

other analyses were performed with SPSS version 15. Throughout, means are presented ± standard deviations unless otherwise noted.

RESULTS

Principal components analyses of mouth coloration.— The first PC explained 73.9% and 95.6% of variance in flange and gape reflectance respectively, and was equivalent to total brightness of both tissues ($r = 1.00$, $P < 0.001$, $n = 94$ throughout; Fig. 2). Two other PCs with eigenvalues >1 were extracted for flange tissue, explaining 18.9% and 4.5% of variance respectively (Fig 2). Loadings from PC2 were consistent with the predicted effects of carotenoid-richness (Fig. 2), and this was reflected in a strong association between flange PC2 and chroma ($r = -0.835$, $P < 0.001$). Flange PC3 had negative loadings at short wavelengths and positive at long (Fig 2), and was associated with UV coloration ($r = -0.686$, $P < 0.001$) and not with chroma ($r = -0.077$, $P = 0.462$). Because this analysis was exploratory, we also considered a second gape PC (eigenvalue = 0.96) that explained 2.5% of the variance. Gape PC2 was associated with colors that were relatively long-wavelength rich (Fig. 2), and was correlated with gape chroma ($r = 0.843$, $P < 0.001$). This analysis suggests that our color parameters, generated using *a priori* predictions from mechanisms of coloration, also identified the most variable aspects of coloration.

Carotenoid analyses.—Carotenoids were recovered in 9 of the 10 flange samples from sacrificed nestlings. Assuming that our sample (one flange quadrant) was representative of all flange tissue, flanges were colored by a total of 0.50±0.37 µg of carotenoids (range = 0.00–1.06 µg) per bird. As predicted, flange carotenoid

content was positively associated with chroma (r^2 = 0.674, F = 18.11, df = 1 and 8, P = 0.003), and negatively associated with relative UV intensity (r^2 = 0.407, F = 5.48, df = 1 and 8, P =0.047); total brightness, however, was unrelated to carotenoid content (r^2 = 0.012, F = 0.10, df = 1 and 8, P = 0.764; Fig. 3). 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. 3). Carotenoid-based colors have been quantified in a number of other ways in the literature, and so we have provided similar analyses of our directly-measured samples using commonly-used parameters, especially those from previous studies of nestling mouth coloration (Appendix A). These results generally suggest that the ratio of long- to short-wavelength reflectance is a good predictor of the carotenoid content of flanges.

Individual-level analyses.—Lutein and zeaxanthin were the two carotenoids detected in nestling plasma, with lutein accounting for 89±5% of total plasma carotenoids. Levels of these two pigments were positively correlated (r = 0.828, n = 93, $p < 0.001$), and so we used total carotenoid concentration for further analysis. Brood size was not a significant predictor of any color parameter (all $P > 0.212$) and so was removed from all models. Hematocrit was not a significant predictor of any flange (all $P > 0.640$) or gape color parameter; we retained this term in the model and present these results for the gape only (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 individual nestling mass, plasma carotenoid concentration and date (Table 2). Flange brightness was unrelated to date (F = 1.37, df = 1 and 64, P = 0.246), mass (F = 1.24, df = 1 and 64, P = 0.270) or

total carotenoid concentration (F = 0.56, df = 1 and 64, P = 0.456). However, after controlling for flange chroma, brightness was positively associated with nestling mass, total plasma carotenoid concentration and date (Table 2). The relative UV intensity of flanges was not associated with mass (F = 0.13, df = 1 and 64, P = 0.721) or date (F = 0.49, df = 1 and 64, P = 0.487), but was negatively associated with plasma carotenoid concentration (F = 5.35, df = 1 and 64, P = 0.024, $\beta \pm$ SE = -3.33 ± 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 parameters except flange brightness differed among broods (Table 2).

Brood average analyses.—For brood averages, brood size was initially included in all models, but was significant in none (all $P > 0.190$). Again, hematocrit was not a significant predictor of any flange (all $P > 0.303$) or gape color parameter, but results are presented for gape color (Table 3) to explore the *a priori* prediction that this trait would be revealed by blood-based coloration. Both flange and gape chroma were positively associated with plasma carotenoid concentration (Table 3). Flange brightness was not associated with average mass (F = 2.85, df = 1 and 18, P = 0.109), date (F = 1.43, df = 1 and 18, P = 0.248) or plasma carotenoid titers (F = 2.85, df = 1 and 18, P = 0.440) when analyzed alone, but was positively associated with both average mass and date when chroma was included as a covariate (Table 3). The relative UV intensity of flanges was negatively associated with plasma carotenoid levels (F = 8.21, df = 1 and 18, P = 0.010, $\beta \pm$ SE = -9.21 ± 3.21) but not with mass (F

= 1.39, df = 1 and 18, $P = 0.254$) or date ($F = 0.37$, df = 1 and 18, $P = 0.552$). However, with chroma included as a covariate, relative UV intensity was unrelated to any predictor (Table 3). Gape brightness was not associated with any predictor considered here.

DISCUSSION

Nestling mouth coloration has the potential to provide House Sparrow parents with information about their offspring by revealing aspects of phenotype that may be associated with the reproductive value of both individuals and broods. All aspects of flange coloration were affected by time-of-year, suggesting that they capture temporal variation in the pre- and post-hatching environment experienced by nestlings. All gape and flange color features except flange brightness differed among broods even when controlling for date, suggesting that colors can also reveal non-temporal features of the environment and, furthermore, could have a genetic component.

Flange coloration was generally more well-predicted by proxies for nestling condition than was gape color at both the brood and individual level, but gape redness (chroma) was positively associated with circulating carotenoid levels, perhaps because this property of the blood was directly revealed through the blood's color. Blood-based colors in the mouths of other avian taxa have been shown to vary rapidly with nestling 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 fixed aspects of individual phenotype like mass and hematocrit and the significant between-brood differences we found. Previous authors suggesting a

signaling capacity of blood-based gape colors have quantified color from photographs taken during voluntary begging bouts (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 photographs also present 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, 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 available to parents (i.e., with the effects of carotenoids not controlled), flange brightness was unrelated to either. Similarly, a PC score associated with brightness was positively associated with mass, tarsus length and feather growth in Barn Swallow nestlings (*Hirundo rustica*: de Ayala et al. 2007). 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. Both visual modeling and detailed behavioral studies of parents will be needed to establish any functional significance of these effects.

UV coloration was negatively associated with the carotenoid content of flanges

and with circulating carotenoids as expressed (see also Mougeot et al. 2007), but was unrelated to any other 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 individual 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 to influence parental food allocation in Barn Swallows (de Ayala et al. 2007), and so it may be too early to dismiss the hypothesis that UV reflectance of passerine nestling mouths plays some role in detectability or signaling (see also Dugas 2010).

Within-broods, carotenoid-based flange coloration was associated with nestling mass, a result generally consistent with previous findings for nestling birds, including those for House Sparrows (Saino et al. 2000, 2003, Ewen et al. 2008, Loiseau et al. 2008). This relationship with mass was notably absent from the brood-level analysis, suggesting that among-brood color differences are driven by factors other than those that explain mass. Color differences could arise, for example, from the carotenoid content, rather than the quanitity, of food provided by parents (Hõrak et al. 2000). Both the intensity of yellow flange and red gape coloration revealed circulating carotenoid levels at both the within- and among-brood level (see also Loiseau et al. 2008). While the relative mass of offspring might be a trait accessible to parents without the use of mouth colors, circulating carotenoid levels *per se* are almost certainly inaccessible to parents without these ornaments. 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 adaptive.

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). Using 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, while yolks contained ~56 times this amount in a sample of 5 second-laid eggs from this population (40.7±22.7μg/g, range = 12.5–72.9 μg/g, *unpublished 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 for 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 (Andersson and Prager 2006, Jacot et al. 2010). These contributors to color may result from different proximate mechanisms (Shawkey and Hill 2005, Mougeot et al. 2007), may reflect different physiological processes (and thus potential information

content; Jacot et al. 2010), and may evolve under different selective pressures (e.g., detectability and signaling). In future comparative studies, considering specific features of color, rather than reflectance as a whole, 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.

ACKNOWLEDGMENTS

Jennifer Place assisted with fieldwork, and Ted Gibbons developed a program to aid with the processing of spectrometer files. We thank D.W. Mock, R. Knapp, J. Mendoza, I. Schlupp, and especially P.L. Schwagmeyer for comments that greatly improved the quality of this manuscript. This work was funded by a research grant from the American Ornithologists' Union and a George Miksch Sutton Scholarship in Ornithology from the University of Oklahoma (MBD). The University of Oklahoma IACUC approved all protocols (RM6-012, RM6-012B) and all required permits were obtained from Oklahoma Department of Wildlife Conservation.

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TABLES

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

	Extracted samples			Field observations		
	$F_{9,25}$	*P*	*r*	$F_{26,27}$	*P*	*r*
Flange						
Brightness	2.94	0.016	0.52	3.46	< 0.001	0.38
Chroma	24.45	< 0.001	0.93	10.15	< 0.001	0.70
Relative UV intensity	18.03	< 0.001	0.90	8.18	< 0.001	0.64
Gape						
Brightness				15.39	< 0.001	0.78
Chroma				3.45	< 0.001	0.38

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 day 6 nestling House Sparrows. 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) x 10^3		G^2	*P*
Flange						
Chroma						
mass	8.47[a]	0.005	2.73 (0.96)	brood	18.70	<0.001
total carotenoids	6.80[a]	0.011	14.72 (5.81)			
date	4.29[a]	0.042	0.70 (0.34)			
Brightness						
mass	9.13[b]	0.004	3.38 (1.11)	brood	0.30	0.600
total carotenoids	4.14[b]	0.046	13.19 (6.49)			
date	13.24[b]	0.001	1.09 (0.30)			
chroma	68.32[b]	<0.0001	-10.28 (1.24)			

Relative UV intensity						
mass	1.45[b]	0.233	2.50 (2.08)	brood	16.60	<0.001
total carotenoids	0.43[b]	0.515	-8.12 (12.40)			
date	4.94[b]	0.030	1.64 (0.74)			
chroma	52.32[b]	<0.0001	-16.20 (2.24)			
Gape						
Chroma						
mass	1.27[c]	0.264	-1.13 (1.01)	brood	7.00	0.010
total carotenoids	5.31[c]	0.025	2.37 (1.03)			
hematocrit	1.56[c]	0.216	75.45 (60.40)			
date	0.16[c]	0.693	-0.11 (0.27)			
Brightness						
mass	0.03[c]	0.874	-0.74 (4.66)	brood	10.60	0.001
total carotenoids	0.00[c]	0.999	0.004 (0.48)			
hematocrit	0.81[c]	0.372	-253.30 (281.80)			
date	0.10[c]	0.759	-0.41 (1.31)			

[a] df = 1 and 64
[b] df = 1 and 63
[c] df = 1 and 62

TABLE 3. Results of GLMs assessing the relationship between brood mean flange and gape colors and mass, circulating carotenoids (square-root transformed) and hematocrit (gape only) of day 6 nestling House Sparrows. 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. Results of step-down models in which non-significant terms ($p > 0.05$) were removed sequentially are also shown.

	Initial Model			Reduced Model		
	F	P	B (SE) x 10^3	F	P	B (SE) x 10^3
Flange						
Chroma						
mass	<0.01[a]	0.996	0.01 (2.29)			
total carotenoids	6.27[a]	0.022	4.25 (1.70)	12.12[c]	0.002	5.49 (1.57)
date	3.16[a]	0.092	0.72 (0.41)			
Brightness						
mass	5.78[b]	0.028	4.09 (1.7)	6.85[a]	0.017	4.46 (1.7)
total carotenoids	1.61[b]	0.222	1.86 (1.46)			
date	10.66[b]	0.005	1.07 (0.03)	10.88[a]	0.004	1.09 (0.33)
chroma	19.35[b]	0.000	-769.72 (175.00)	18.38[a]	0.000	-656.70 (153.25)
Relative UV intensity						
mass	1.41[b]	0.252	5.12 (4.31)			
total carotenoids	3.68[b]	0.072	-7.11 (3.71)			
date	0.99[b]	0.335	0.82 (0.83)			
chroma	1.24[b]	0.281	-493.62 (443.36)			

Gape						
Chroma						
mass	0.16[b]	0.691	0.92 (2.27)			
total carotenoids	3.96[b]	0.063	2.74 (1.38)	5.51[c]	0.029	2.70 (1.15)
hematocrit	0.42[b]	0.524	-76.26 (117.29)			
date	0.04[b]	0.848	-0.06 (0.33)			
Brightness						
mass	0.56[b]	0.466	8.12 (10.88)			
total carotenoids	0.00[b]	0.985	-0.12 (6.59)			
hematocrit	0.17[b]	0.682	234.01 (561.05)			
date	1.14[b]	0.301	-1.68 (1.57)			

[a] df = 1 and 18, [b] df = 1 and 17, [c] df = 1 and 20

FIGURE CAPTIONS

FIG. 1. Mean ± SD reflectance, at 10 nm intervals, of nestling House Sparrow flange (solid circles) and gape (open circles) tissue.

FIG. 2. Loadings of reflectance at each 10nm interval on principal components for nestling House Sparrow mouth parts.

FIG. 3. The relationship between the carotenoid content of nestling House Sparrow flange tissue 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.

Fig. 1

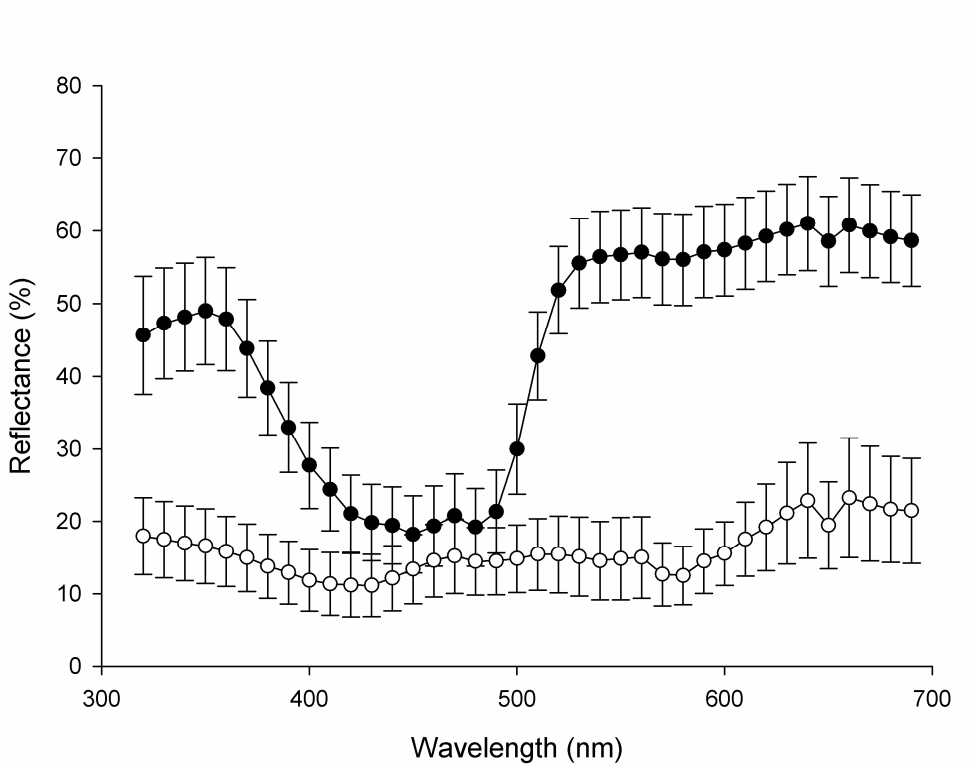

Reflectance (%)
Wavelength (nm)
0
10
20
30
40
50
60
70
80
300
400
500
600
700

Fig. 2

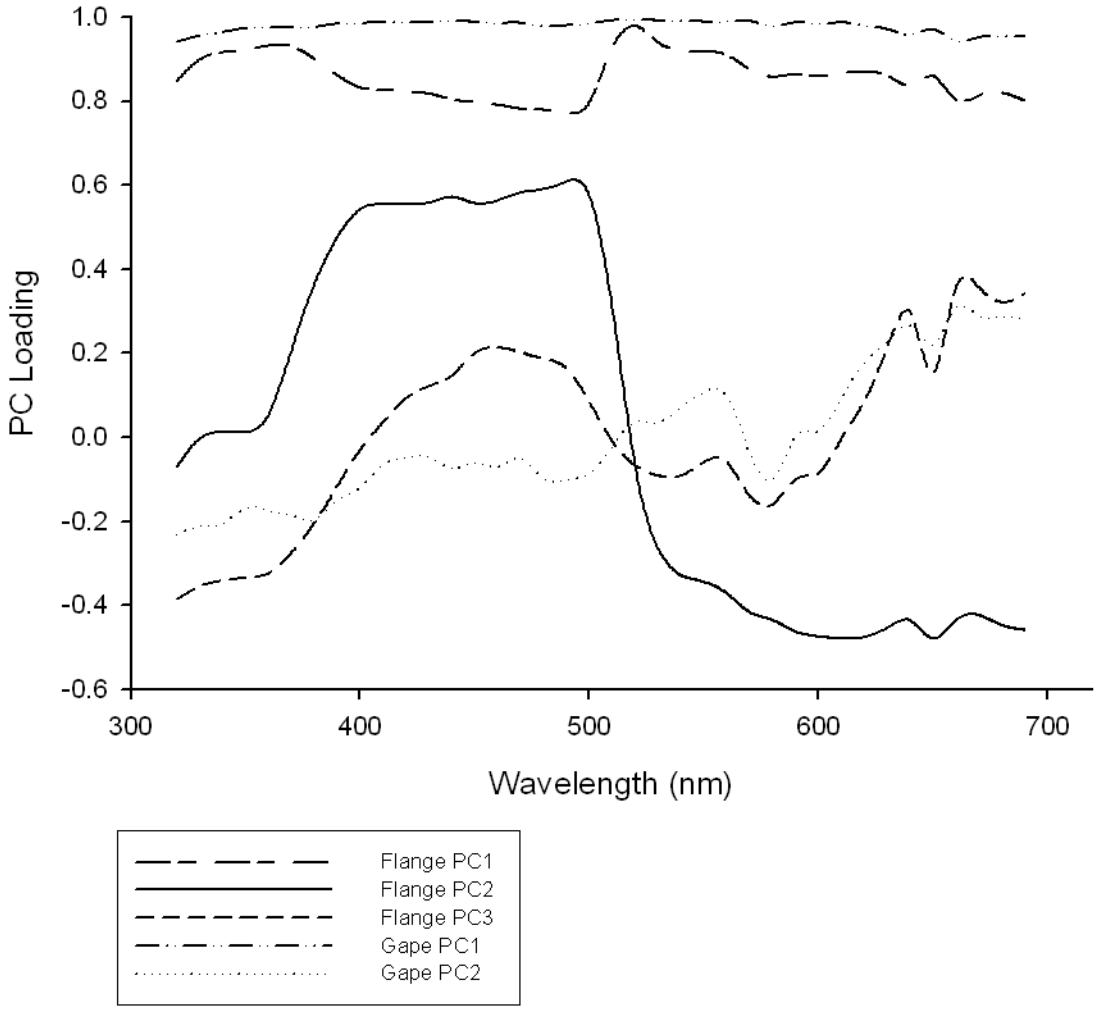
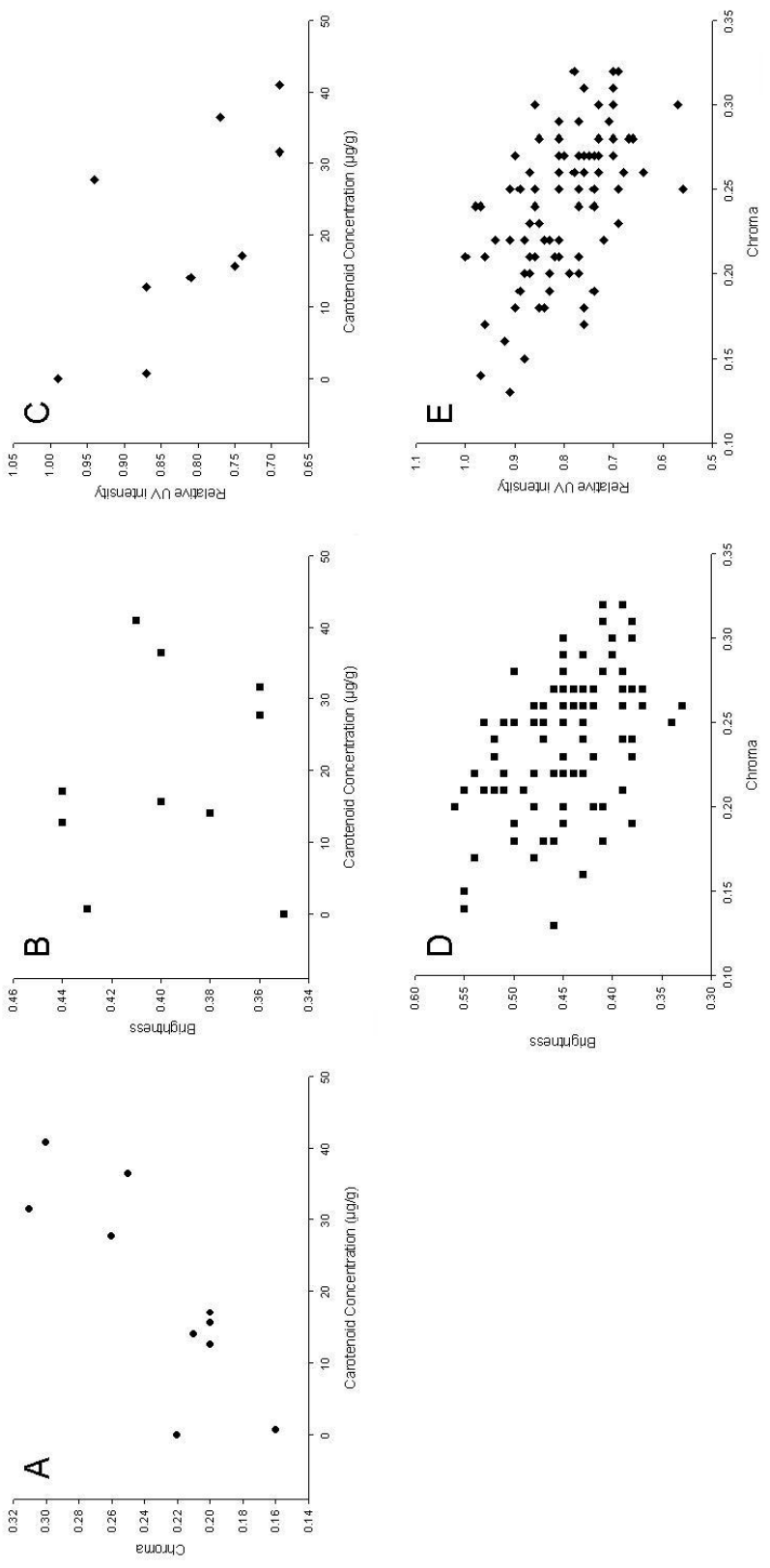

1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
PC Loading
300
400
500
600
700
Wavelength (nm)
Flange PC1
Flange PC2
Flange PC3
Gape PC1
Gape PC2

Fig. 3


A
Chroma
Carotenoid Concentration (µg/g)
B
Brightness
Carotenoid Concentration (µg/g)
C
Relative UV Intensity
Carotenoid Concentration (µg/g)
D
Brightness
Chroma
E
Relative UV Intensity
Chroma

Appendix A

The relationship between the carotenoid content of nestling House Sparrow flanges and color parameters commonly used to estimate the carotenoid content of tissues, especially nestling mouth parts. Color parameters are divided into those that estimate saturation and those that estimate spectral location, and include estimates calculated from tissue reflectance (see text for details) and estimated from photographs taken under standardized lighting conditions (one sample was not photographed) using Adobe Photoshop (version CS). Relationships using 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 carotenoid estimates. Repeatability of each parameter and the formula used for calculation. Reflectance is abbreviated as "R" and wavelength ranges are shown in subscript.

Variable	Calculation	Repeatability				Relationship with carotenoid content		
		F	*P*	*r*	r^2	*F*	*P*	Equation
Spectral reflectance								
Saturation Estimates								
carotenoid chroma	$(R_{700} - R_{450}) / R_{700}$	17.7^a	< 0.001	0.90	0.74	23.0^b	0.001	y = 139.2x - 73.3
yellow-chroma	$R_{550–625} / R_{300–700}$	25.4^a	< 0.001	0.93	0.74	23.0^b	0.001	y = 486.2x -182.1
yellow	mean $R_{552–570}$	4.0^a	0.003	0.62	0.27	2.9^b	0.127	y = 1.6x - 60.2

Spectal Location								
Hue	arcSin((Y-B)/chroma))[e]	1.6[a]	0.172	0.10	0.10	0.9[b]	0.381	y = -145.6x + 154.4
λR_{vis50}	λ = mean $R_{400-700}$	15.8[a]	< 0.001	0.89	0.57	10.7[b]	0.011	y = 2.6x - 1332.0
Segment Classification								
R	$R_{625-699}$ / $R_{325-699}$	9.5[a]	< 0.001	0.83	0.64	14.5[b]	0.005	y = 656.4 -164.2
Y	$R_{550-624}$ / $R_{325-699}$	50.0[a]	< 0.001	0.96	0.68	16.9[b]	0.003	y = 793.1 - 175.1
G	$R_{475-549}$ / $R_{325-699}$	19.0	< 0.001	0.91	0.51	8.4[b]	0.020	y = -966.6 + 183.5
B	$R_{400-474}$ / $R_{325-699}$	19.7[a]	< 0.001	0.91	0.64	14.4[b]	0.005	y = -697.2 + 88.7
UV	$R_{325-399}$ / $R_{325-699}$	18.3[a]	< 0.001	0.91	0.29	3.3[b]	0.105	y = -527.7 + 126.5
Photographs								
Saturation		24.1[c]	< 0.001	0.85	0.50	7.0[d]	0.033	y = 0.6 + 74.7
Hue		9.8[c]	< 0.001	0.69	0.00	0.0[d]	0.917	
R		12.6[c]	< 0.001	0.74	0.15	1.23[d]	0.304	y = 0.9 - 95.2
G		7.7[c]	< 0.001	0.63	0.02	0.13[d]	0.725	
B		21.8[c]	< 0.001	0.84	0.49	6.62[d]	0.037	y = -0.6x + 29.5

[a] df = 9 and 25, [b] df = 1 and 8, [c] df = 8 and 27, [d] df = 1 and 7, [e] see text for details

Chapter II

Environmental and parental effects explain among-brood differences in ornamental mouth coloration of nestling house sparrows

(formatted for *Functional Ecology*)

Summary

1. Dependent offspring use specialized traits to attract parental care. In birds, this includes morphological ornaments (e.g., plumage, mouth and skin colouration) that are often associated with nestling condition (e.g., mass, immune status) and influence the allocation of parental care.

2. The intensity of ornament expression often differs among broods, even after differences in individual condition are accounted for statistically. Among-brood differences could result from environmental effects and/or parental effects (either pre- or post-hatching).

3. I used a cross-fostering experiment to assess the relative contributions of parental effects to among-brood differences in ornamental mouth colouration in nestling house sparrows (specifically, the carotenoid-richness, overall brightness, and relative UV intensity of rictal flanges).

4. The expression of carotenoid-based colouration was explained by dyad, nest-of-rearing and nest-of-origin. Features of colour that are likely physical in origin (brightness and relative UV intensity) were explained by dyad, but not by parents.

5. At the individual level, ontogenetic changes in the carotenoid-richness and brightness of flanges positively reflected mass gain (a proxy for food intake). Within broods, larger and yellower chicks gained more mass, suggesting parental preferences for these traits.

6. Brood-level variation in mouth colouration may primarily contain information not about the intrinsic quality of offspring themselves, but about the environment in which they are reared.

Introduction

Dependent offspring across taxonomically-diverse lineages use behavioural, vocal, chemical, and morphological traits to attract parental care (e.g., Bell 2007, Mas, Haynes & Kölliker 2009, reviewed in Wright & Leonard 2002). Such offspring solicitations are often hypothesized to evolve as a means of offspring-parent communication, where offspring traits furnish information (albeit not entirely honest information; Trivers 1974) about aspects of offspring phenotype of potential interest to parents (e.g., hunger, body size, immune status), and parents use these offspring traits to make adaptive decisions about the level and/or division of investment they provide (Godfray 1991, 1995, see reviews in Mock & Parker 1997, Royle, Hartley & Parker 2002 for alternatives). Offspring ornaments (e.g., elaborate plumage; Lyon, Eadie & Hamilton 1994), for example, have been hypothesized to signal the quality (reproductive value) of offspring in much the same way sexually-selected ornaments reveal adult quality (Grafen 1990, Saino et al. 2000). Consistent with this signalling hypothesis, the ornaments of nestling birds have been shown both to reveal condition proxies (e.g., body size, immune status) and to influence the distribution of parental care (Saino et al. 2000, Jourdie et al. 2004, Bize et al. 2006, Ewen et al. 2008, Loiseau et al. 2008, Tanner & Richner 2008, Dugas 2009).

Population-level variation in the intensity of offspring ornament expression is, however, often explained not only by individual phenotype, but also at the level of the brood. In other words, even when variation in individual condition is accounted for statistically, broodmates tend to resemble each other (plumage colouration: Johnsen et al. 2003, skin colouration: Bize et al. 2006, Soler et al. 2007, mouth colouration: de

Ayala et al. 2007, Dugas & McGraw *unpublished data*). Among-brood differences suggest that ornaments are influenced by factors beyond those associated with individual phenotype, and presumably arise from broad environmental (e.g., seasonal) factors and/or pre- or post-hatching parental effects (Tschirren, Fitze & Richner 2003). Understanding how among-brood differences arise is a useful first step in decoding what information ornaments might contain, and thus how they might have evolved.

Here, I used a cross-fostering experiment to estimate the contributions of environmental variation, post-hatching and pre-hatching parental effects to among-brood differences in the rictal flange colouration of nestling house sparrows (*Passer domesticus*). I considered one pigment-based and two structural features of flange reflectance that have been hypothesized to function in parent-offspring interactions: i) carotenoid-richness, a trait positively associated with nestling condition and with the share of parental care obtained by nestlings in house sparrows (Loiseau et al. 2008, Dugas 2009) and other passerines (Saino et al. 2000, Ewen et al. 2008), ii) brightness, a trait positively associated with nestling condition (de Ayala et al. 2007, Dugas & McGraw *unpublished data*, but see Soler et al. 2007) and likely the primary determinant of visual conspicuousness, especially in dark (e.g., cavity) nests (Götmark & Ahlström 1998, Avilés et al. 2008, Dugas & Rosenthal 2010, Holveck et al. 2010), and iii) ultraviolet (UV) colouration, a trait that does not appear to covary positively with nestling condition (de Ayala et al. 2007, Soler et al. 2007, Dugas & McGraw *unpublished data*), but one that has been shown experimentally to influence parental allocation patterns in barn swallows (*Hirundo rustica*; de Ayala et al. 2007). Descriptive work in house sparrows has revealed seasonal effects on all three colour

parameters and among-brood differences in carotenoid-richness and UV colouration (Dugas & McGraw *unpublished data*).

The potential for carotenoid-based colouration to be influenced by the environment and/or parents is well-established (e.g., Tschirren et al. 2003), and inferences about structural colours (brightness and UV colouration) can be drawn from studies of other avian soft parts and feathers. Nestling birds, like all vertebrates, must obtain carotenoids from exogenous sources (Fox & Vevers 1960); for nestlings, these are provided by parents in the form of yolk (Blount, Houston, & Møller 2000) and parental food deliveries (Fizte, Tschirren, & Richner 2003a). Among-brood differences in carotenoid budgets might then arise from variation in parental feeding behaviour and/or in carotenoid abundance in available foods (Bortolotti et al. 2000, Hõrak et al. 2000, Johnsen et al. 2003, Fitze et al. 2003a, Fitze, Kölliker & Richner 2003b, Isaksson, Uller & Andersson 2006). Natural variation, however, might not result exclusively from differences in carotenoid intake; genetic or developmental differences may regulate carotenoid uptake or transport and hence, the amount of pigment available for ornamentation (Hadfield & Owens 2006). In chickens (*Gallus g. domesticus*), an enzyme that breaks down carotenoids in the integument is responsible for among-strain differences in integument colour (Eriksson et al. 2008). The proximate mechanisms underlying variation in the brightness and UV intensity of flanges are unclear, but almost certainly arise from physical features of the tissue itself (i.e., structural colouration: Prum & Torres 2003, Mougeot et al. 2007, Thorogood et al. 2008). In nestling plumage, structural colouration varies among-broods (Johnsen et al. 2003), and experimental brood-size increases reduce its expression (Jacot et al.

2010).

I sampled nestling house sparrow flange colouration at two ages, primarily to test how the relative contributions of parental and environmental effects to among-brood differences might change as a function of time spent in a common rearing environment. This design also allowed me to test several hypotheses about the signalling function of mouth colouration at the individual (i.e., within-brood) level. I first examined ontogenetic changes in colour expression, further testing the signalling potential of these ornaments. I then tested the hypothesis that the relationship between colour and condition is mediated by nutrition (Soler et al. 2007) by comparing the magnitude of ontogenetic colour shifts with the magnitude of nestling mass gain (a commonly-used proxy for food intake, e.g., Götmark & Ahlström 1998). Finally, I tested if parental preferences for carotenoid-rich mouth colours (revealed by a short-term behavioural experiment; Dugas 2009) were consistent in longer-term samples by examining the relationship between within-brood colour rank at day 3 and mass gain from days 3–6.

Materials and methods

CROSS-FOSTERING EXPERIMENT

I studied a free-living house sparrow population in Norman, OK, USA (see Schwagmeyer, Mock & Parker 2002 for details) in April–July 2009 and April–June 2010. Parents were not banded, and so to avoid sampling the same pairs twice, I used each nest box only once per year; in an earlier study of banded pairs in this population, 172/182 used only one box per year, and 170/182 bred on the study site in only one

year (P.L. Schwagmeyer & D.W. Mock *pers. comm.*; see Mock, Schwagmeyer & Dugas 2009 for details). Nests were checked twice weekly to allow estimation of hatch date, and then visited daily beginning one day before hatching was expected; pairs of nests (hereafter, dyads) were used for cross-fostering only if both broods began hatching on the same day. When possible, partial broods were swapped between nests before 15:00 CST on the day of first hatching (day 0). However, if hatching began late in the day or only one nestling had hatched in one or both nests (i.e., I anticipated being able to transfer more nestlings if I waited), I completed transfers before 11:00 CST the next day. The number of nestlings swapped was based on the size of the smaller brood of each pair: when the smaller brood size was two, I swapped one nestling; when three, I alternately swapped one or two nestlings; when four, I swapped two nestlings, and when five, I alternately swapped two or three nestlings. When transferring only one nestling, I avoided the smallest brood members because these are most often the victims of brood reduction (Mock et al. 2009). Otherwise, I chose nestlings for transfer based on similarity of mass (exchanged nestlings differed by, mean±SD, 0.5±0.6 g, or 16±19% of the smaller chick's body mass). At transfer, nestlings were marked on the tarsus with non-toxic ink to identify nest-of-origin. Nestlings were also fitted with colour bands for individual identification on day 3, if they were sufficiently large.

Three and six days after transfer (hereafter, days 3 and 6), nestlings were briefly (<20 min) removed to a nearby location, where I weighed each to the nearest 0.01g on an electronic balance, measured left and right tarsus to the nearest 0.5mm, and sampled mouth colour (details below). Nestlings were kept warm when away from

the nest, and at least one nestling remained in the nest at all times to prevent parental desertion. Day 3 is the day before peak brood reduction in this population (Mock et al. 2009) and the youngest age (i.e., smallest size) at which nestlings can be reliably handled for colour measurement. Day 6 is slightly before the midpoint of the 14 d nestling period, and is an age at which parents still control food allocation (Dugas 2009) as required for offspring-parent signaling (Royle et al. 2002).

I limited analysis to dyads in which at least one nestling from each nest-of-origin survived in both rearing nests. In 2009, this criterion was met at day 3 in 10 pairs of nests (73 chicks) and in 8 (58 chicks) of these again at day 6; in 2010, 13 dyads (110 chicks) met this criterion at day 3 and in 12 (97 chicks) of these again at day 6. In broods used at both ages, brood reduction (1 chick) occurred between days 3 and 6 in 2 of 16 rearing nests in 2009 and 5 of 24 rearing nests in 2010; brood reduction occurred only in rearing nests of 5 chicks.

MEASUREMENT AND QUANTIFICATION OF COLOUR

I measured the reflectance of flanges (% relative to a white standard, WS-1-SL) with a USB4000 spectrometer (Ocean Optics, Dunedin, FL, USA). Light produced by a deuterium-tungsten halogen lamp (DT-MINI-2-GS) was directed through a 600µm bifurcated fiberoptic cable to a reflectance probe held at 90° to the tissue (Andersson & Prager 2006). Reflected light was processed by the spectrometer and data captured using Spectra Suite software (Ocean Optics). During colour measurement, nestlings were placed in a portable "dark box" that excluded ambient light, and their mouths were held open gently so that flange tissue that would be

visible to parents during begging (see Dugas 2010 for details) could be measured. Flange reflectance was sampled four times, once from each quadrant of the mouth (left and right side of the maxilla and mandible), and median reflectance curves were used for further analysis.

To estimate the intensity of yellow flange colouration, I used chroma, calculated as:

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

where R, Y, G and B equal the proportion of total reflectance of red (625–699nm), yellow (550–624nm), green (475–549nm) and blue (400–474nm) light, respectively. This colour parameter is visually relevant (Endler 1990), calculated independently of brightness and UV colouration (Endler 1990), and positively associated with the carotenoid content of house sparrow flanges (Dugas & McGraw *unpublished data*). Flange brightness was estimated as the mean reflectance (%) from 320–700 nm (*sensu* Endler 1990). To estimate the intensity of UV colouration, I compared the mean reflectance of the UV peak (320–350 nm) to the mean reflectance from 600–699 nm, a spectral region unlikely to be influenced by carotenoids (Mays et al. 2004, Jacot et al. 2010, Dugas & McGraw *unpublished data*). Repeatability (*R*; *sensu* Lessells & Boag 1987), estimated using one randomly-selected nestling from each day 6 rearing brood, was highest for chroma ($F_{39,120} = 41.77$, $P < 0.001$, $R = 0.911$), followed by relative UV intensity ($F_{39,120} = 28.48$, $P < 0.001$, $R = 0.873$) and brightness ($F_{39,120} = 12.72$, $P < 0.001$, $R = 0.746$).

In avian soft parts, both maximum brightness and relative UV intensity are probably determined by physical (i.e., pigment-independent) properties of the tissue,

with carotenoid (or other pigment) deposition negatively influencing these features of reflectance (Mougeot 2007, Thorogood et al. 2008, Dugas & McGraw *unpublished data*). Because colour was of interest here as a measure of the physical and chemical phenotype of nestlings (i.e., not as a visual phenomenon), chroma was included as a covariate in all analyses of brightness and UV intensity to allow for estimation of the properties of pigment-free tissue (Dugas & McGraw *unpublished data*).

DATA ANALYSIS

For analysis of cross-fostering data, I used linear mixed models with an individual colour parameter entered as the dependent variable, with mass as a fixed effect (details below), and with four random effects included: i) 'dyad' (pair of nests), which reflects both pre- and post-hatching environmental variation experienced by parents and offspring (e.g., weather, abundance and/or quality of available food); ii) 'nest-of-origin', which reflects pre-hatching parental effects (i.e., maternal effects and/or incubation) and any genetic effects; iii) 'nest-of-rearing', which reflects variation in post-hatching parental effects (e.g., the quantity and/or quality of parental care); and iv) 'nest-of-origin by nest-of-rearing', which accounts for differences between chicks from the same nest-of-origin that are reared in their own vs. a foster nest. Nest-of-origin, nest-of-rearing and the interaction were all nested within dyad (see Fitze et al. 2003b, Biard, Surai & Møller 2006, Isaksson et al. 2006 for similar analyses). In this population, 20% of nestlings are unrelated to at least one rearing parent, with 17% sired by extra-pair males (Whitekiller et al. 2000, Edly-Wright et al. 2007); 'nest-of-origin', therefore, probably underestimates genetic differences, but

captures maternal effects fairly, at least to the extent they are shared by all members of a nest-of-origin (Saino et al. 2002, Bertrand et al. 2006).

Mass was included as a fixed effect in the above model because I was interested in the among-brood differences not explained by individual phenotype (Loiseau et al. 2008, Dugas & Rosenthal 2010, Dugas & McGraw *unpublished data*). Although swapped nestlings were matched for mass, similar-sized nestlings may have differed with respect to within-brood rank in each nest-of-rearing (e.g., a 2g chick might be the heaviest in one brood and the lightest in another). Therefore, mass was also a necessary covariate to avoid overestimating nest-of-origin effects. Furthermore, including mass as a covariate provided an additional test of the relationship, reported elsewhere, between each colour parameter and nestling mass (Dugas & Rosenthal 2010, Dugas & McGraw *unpublished data*). In a supplementary analysis, I replaced mass with tarsus length, an aspect of nestling condition not yet examined in this system. As detailed earlier, chroma was also included as a fixed effect in analyses of brightness and relative UV intensity.

To estimate the significance of parameter estimates of random effects, I used a -2 residual Log likelihood ratio test in which a full model was compared to a reduced model not including a given random effect (Quinn & Keough 2002, Agresti 2007, Dickey 2008). With this test statistic (G^2; *sensu* Quinn & Keough 2002 pg 364), I estimated a *P* value using a chi-square distribution with 1 degree of freedom (Sokal & Rohlf 1995, Quinn & Keough 2002, Agresti 2007, Dickey 2008). Degrees-of-freedom for fixed effects were calculated with Satterthwaite's approximation.

To examine ontogenetic changes in mouth colouration and the relationship

between colouration and nestling growth, I used a reduced data set containing only nestlings from 12 dyads in which all chicks were re-identified on day 6 (two (15 chicks) from 2009 and 10 (85 chicks) from 2010). I tested for differences between day 3 and 6 colour values of individuals using linear mixed models with random effects as above but with age instead of mass as a fixed effect (unstructured covariance); as before, chroma was included as a fixed effect in analyses of brightness and relative UV intensity. Using this same reduced data set, I calculated the magnitude of ontogenetic changes (day 6 - day 3) in colour and nestling mass to examine potential associations between the two. First, I tested the prediction that the magnitude of colour change would be predicted by mass gained (used as a proxy for food consumed) using linear mixed models with colour change as the dependent variable, mass change and day 3 colour values as fixed effects, and random effects as described earlier; in analyses for brightness and relative UV intensity, the change in chroma was this time included as a fixed effect. The inclusion of day 3 colour value as a covariate accommodates the possibility that colour increases in a non-linear fashion.

Finally, I used the smaller 12-dyad data set to test the prediction that, within broods, more colourful nestlings are fed preferentially by parents (Saino et al. 2000, Dugas 2009). Within rearing broods, I ranked day 3 nestlings according to mass and chroma (separately), and then used linear mixed models with mass gain as the dependent variable, random effects as described earlier and, as fixed effects, either i) chroma rank, ii) mass rank, or iii) both chroma and mass rank (three different analyses). Because colour variables were inter-correlated (Dugas & McGraw *unpublished data*), assessing the physical aspects of colouration independently of

carotenoid-richness was impractical (and, given that the actual display to parents was of interest, perhaps uninformative). Because chroma is the aspect of colouration previously shown to influence parents in this species (Loiseau et al. 2008, Dugas 2009), it was given priority. Mass gain over days 3 to 6 was squared to meet the assumption of normality; all other variables were normally distributed. SAS version 9.2 was used for all analyses (SAS Institute, Cary, NC).

Results

The total variation explained by random effects (dyad, nest-of-origin, nest-of-rearing and the nest-of-origin by nest-of-rearing interaction) was similar on days 3 and 6, with ~80% of chroma, ~50% of brightness and ~40% of relative UV intensity variation explained (Table 1). Chroma was significantly explained by dyad, nest-of-rearing and nest-of-origin, with the amount of variation explained declining in that order (Table 1). Between days 3 and 6, the contribution of dyad and nest-of-rearing both increased slightly whereas that of nest-of-origin fell slightly (Table 1). Brightness, on the other hand, was explained only by dyad, which accounted for slightly more variation on day 3 than day 6 (Table 1). Relative UV intensity was explained significantly by dyad on day 3, and the effect of nest-of-rearing was marginal; on day 6, no random effects were significant (Table 1).

The analyses detailed above revealed positive relationships between nestling mass and chroma and brightness, but not relative UV intensity (Table 1). These relationships were similar for tarsus length (Table 2), as were results for random effects (not shown). Individuals had higher chroma values on day 6, lower relative UV

intensity values, and brightness did not differ significantly (Table 3). The magnitude of these ontogenetic changes between days 3 and 6 was negatively associated with the day 3 value for all three colour parameters, and for brightness and chroma, was positively with nestling mass gain over the same period (Table 4). Most random effects included in the above models were non-significant, but brood-of-rearing explained significant variation in chroma increase, and there was a marginal (P = 0.06) effect of dyad on the change in relative UV intensity (Table 4). Within broods, nestlings with higher day 3 mass ranks gained more mass from day 3 to 6 ($F_{1,70.1}$ = 6.39, P = 0.0138, β±SE = -4.73±1.87), and there was a marginal tendency ($F_{1,68.7}$ = 3.36, P = 0.071, β±SE = -3.43±1.87) for nestlings with higher day 3 chroma ranks to gain more mass than lower-ranked broodmates. When both mass and chroma rank were included in the model, the effect of mass rank was significant ($F_{1,69}$ = 5.00, P = 0.029, β±SE = -4.21±1.19), while the effect of chroma rank was not ($F_{1,69.9}$ = 1.91, P = 0.172, β±SE = -2.59±1.87).

Discussion

Most among-brood variation in the physical (brightness and relative UV intensity) and chemical (carotenoid-richness) aspects of nestling house sparrow flange colouration was explained by dyad, a term that captured similarities between contemporaneous broods. Beyond that, parents influenced the carotenoid-based colouration of their offspring both before and after hatching; post-hatching parental effects were stronger, and this difference increased with nestling age. These parental effects, as well as their absolute and relative magnitudes, are consistent with cross-

fostering studies of carotenoid-based nestling plumage in other passerines (Johnsen et al. 2003, Fitze et al. 2003b, Isaksson et al. 2006). However, one such study revealed no variation in carotenoid-based colouration attributable to contemporaneous breeding (i.e., dyad; Fitze et al. 2003b), a difference that may stem from relatively high temporal fluctuations in carotenoid availability for this house sparrow population.

Among-brood differences in the brightness and relative UV intensity of nestling house sparrow flanges were explained only by dyad. This result is similar to that of a cross-fostering study of great tit plumage (*Parus major*; Fitze et al. 2003b), and consistent with a seasonal increase in these reflectance parameters revealed by a descriptive study of nestling mouth colouration in this house sparrow population (Dugas & McGraw *unpublished data*). Results for UV colouration were ambiguous, and suggest that among-brood variation arises from small and variable contributions of the environment and parents. While brightness variation at the individual level seems linked to nestling mass and food consumption (see also Jacot et al. 2010), any brood-level variation seems to reflect only differences in the timing of breeding attempts (Dugas & McGraw *unpublished data*). The proximate mechanism through which this effect emerges is unclear; it might reflect differences in developmental rates and thus the arrangement of physical structures responsible for bright reflectance (Fitzpatrick 1998). Alternatively, the availability of specific nutrients required for the generation of bright reflective base tissue might vary seasonally but not among families (Peters et al. 2007).

For carotenoid-based colouration, brood-level variation attributable to dyad, rearing nest and nest-of-origin could all reflect differences in the quantity of

carotenoids available to nestlings (via yolk and/or food). Both experimental manipulations of dietary carotenoids (e.g., Loiseau et al. 2008) and the positive relationship between mass gain and colour development (this paper) suggest that post-hatching intake is important for colour expression; in great tits, yolk carotenoids explained only a small amount of variation in nestling plumage colouration (Isaksson et al. 2006). The extent to which information regarding carotenoid intake might guide adaptive parental decisions depends on the relationship between carotenoid-richness and offspring value; whether such a relationship can drive the evolution of colouration depends on how much information contained in colours would be otherwise unavailable to parents.

A relationship between carotenoids and offspring quality is fairly well-supported. Correlative studies have documented positive relationships between carotenoid-based colouration and condition proxies (e.g., Johnsen et al. 2003, Loiseau et al. 2008, Dugas & McGraw *unpublished data*), and experimental diet supplementations have indicated positive growth and health effects of carotenoids, albeit more strongly pre-hatching (e.g., Saino et al. 2003b, McGraw, Adkins-Regan & Parker 2005, Ewen et al. 2009) than post-hatching (e.g., Fenoglio, Cucco & Malacarne 2002; see Biard et al. 2006, Fitze & Tschirren 2006, Loiseau et al. 2008 for null results). Whether brood-level colour variation offers parents information otherwise unavailable has received less attention, although it is plausible that some information would be accessible to parents only through offspring phenotype. For example, parents may not have direct knowledge of the carotenoid content of food, or the extent to which dietary carotenoids are biologically available to nestling assimilation

physiology. In the house sparrow population studied here, male parents respond to direct manipulation of offspring food intake (via experimental supplementation; Mock et al. 2005), indicating that brood-level phenotype can indeed influence provisioning decisions. Offspring ornaments influenced primarily by one parent (e.g., via maternal yolk provisioning or paternal food deliveries; Fitze et al. 2003a,b, Isaksson et al. 2006) may be of particular value to the other parent (see parallel argument for sexually-selected egg colouration: Moreno & Osorno 2003).

Among-brood differences in carotenoid-based colouration could also be explained by mechanisms other than carotenoid intake. Variation explained by dyad might reflect similarities among parents themselves if, for example, timing of breeding were linked to parental quality (Verhulst, van Balen & Tinbergen 1995, Hatch & Westneat 2007). Plausible nest-of-rearing effects might also include ecto- or endo-parasites, both of which have been shown to influence the expression of carotenoid-based colouration in birds (Brawner, Hill & Sunderman 2000, Tschirren, Fitze & Richner 2003). Nest-of-origin effects may reflect genetic or developmental differences that affect the uptake of dietary carotenoids. An experimental result showing that carotenoid supplementation affects the mean intensity of colouration but does not reduce variability suggests that carotenoid intake alone may not explain among-brood variation in carotenoid-based colouration in natural populations (e.g., Hadfield & Owens 2006). Genetic/developmental mechanisms and endoparasite infection seem especially likely to be otherwise cryptic to parents, although they may of course be accessible via cues such as body size as well.

The ability of colour to integrate past provisioning behaviour at the individual

level could allow parents either to correct previous deviations from within-brood parity or to exaggerate them. A high level of brood reduction in tandem with a tendency to preferentially feed larger or yellower nestlings (Mock et al. 2009, Dugas 2009) suggests that house sparrow parents pursue the latter strategy; patterns of mass gain in this study confirm these earlier findings. Thus, if the mechanisms underlying nest-of-origin effects (e.g., genetic or maternal effects) also generate within-brood variation, even small early colour differences, amplified by parental preferences, might lead to colour hierarchies. However, chicks with more intense day 3 colours tended to gain colour less rapidly than paler individuals, suggesting that lavish parental provisioning reduces within-brood differences and, therefore, colour-mediated competition (Bonabeau, Deneubourg & Theraulaz 1998). Still unclear is whether parents attend to signals of offspring quality (e.g., mouth colouration), other traits (e.g., body mass), or both. Colour can influence parents even when no mass differences exist (Dugas 2009), although more work is needed to assess how this trait interacts with others in shaping parental allocation patterns.

Correlative studies (e.g., de Ayala et al. 2007, Dugas & McGraw *unpublished data*) and experimental manipulations of offspring condition (e.g. immune challenges: Saino et al. 2000, 2003 or corticosterone injections: Loiseau et al. 2008) suggest that mouth colouration can signal variation among individual nestlings within broods, but do not explain the among-brood variation present in natural populations. This study reveals that most brood-level variation is explained by contemporaneous breeding and rearing conditions. Thus, among-brood colour differences may capture less about intrinsic qualities of the offspring themselves (e.g., genetic differences) than about the

circumstances in which offspring are being reared. Because the environment shapes the relationship between parental investment and payoffs in terms of offspring fitness, such information may be valuable to parents, and so might contribute to the evolution of ornamentation.

Acknowledgements

Leslyn Dillow assisted in the field, and Lauren Nichols prepared spectrometer files for analysis. Brian Davis, Jon Shik, Don Shepard, Doug Mock, P.L. Schwagmeyer, Ingo Schlupp, Rosemary Knapp and Jorge Mendoza made helpful comments on this manuscript. Funding was provided by a National Science Foundation Doctoral Dissertation Improvement Grant (# 0909882) and a G.M. Sutton Scholarship in Ornithology. All methods were approved by the University of Oklahoma IACUC (RM6-012ext) and all required permits were granted by US Fish & Wildlife and the Oklahoma Department of Wildlife Conservation.

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Tables

Table 1 Results of linear mixed models assessing the sources of variation in nestling house sparrow mouth colouration at days 3 and 6 post-hatching and the percent (%) explained by each. For brightness and relative UV intensity, chroma (a proxy for carotenoid-content) was included as a covariate to better estimate the pigment-free features of tissue reflectance.

	Random Effects					Fixed Effects					
	Estimate	SE	%	G^2	*P*		*F*•ratio	d.f.*	*P*	β	SE
Day 3											
Chroma											
dyad	0.00096	0.00042	46	10.0	0.00	mass	49.7	1,158	<0.0001	0.0060	0.001
natal(dyad)	0.00021	0.00010	10	5.4	0.02						
rearing(dyad)	0.00047	0.00018	22	13.6	<0.001						
natal*rearing(dyad)	0		0	0.0	0.99						
residual	0.00046	0.00006	22								
Brightness											
dyad	0.00146	0.00052	49	20.2	<0.001	mass	34.9	1,164	<0.0001	0.0079	0.001
natal(dyad)	0		0	0.0	0.99	chroma	100.5	1,131	<0.0001	-0.9851	0.098
rearing(dyad)	0.00004	0.00013	1	0.2	0.90						
natal*rearing(dyad	0		0	0.0	0.99						
residual	0.00147	0.00018	49								
Relative UV intensity											
dyad	0.00149	0.00088	23	4.2	0.04	mass	0.7	1,167	0.393	-0.0019	0.002
natal(dyad)	0.00047	0.00043	7	1.6	0.20	chroma	70.3	1,133	<0.0001	-1.3549	0.162
rearing(dyad)	0.00082	0.00051	13	3.4	0.07						
natal*rearing(dyad)	0		0	0.0	0.99						
residual	0.00361	0.00047	57								

Day 6												
Chroma												
	dyad	0.00152	0.00071	48	8.7	0.02	mass	24.70	1,134	<.0001	0.0033	0.001
	natal(dyad)	0.00024	0.00014	8	3.9	0.04						
	rearing(dyad)	0.00080	0.00030	26	14.7	0.00						
	natal*rearing(dyad)	0		0	0.0							
	residual	0.00058	0.00009	19								
Brightness												
	dyad	0.00061	0.00026	39	10.2	0.01	mass	27.84	1,141	<.0001	0.0035	0.001
	natal(dyad)	0.00002	0.00011	1	0.0	0.42	chroma	203.88	1,118	<.0001	-0.9786	0.069
	rearing(dyad)	0		0	0.0	0.99						
	natal*rearing(dyad	0.00011	0.00013	7	0.6	0.20						
	residual	0.00080	0.00012	52								
Relative UV intensity												
	dyad	0.00061	0.00049	16	1.8	0.11	mass	1.05	1,140	0.307	-0.0012	0.0011
	natal(dyad)	0		0	0.0	0.99	chroma	188.87	1,90.8	<.0001	-1.5649	0.1139
	rearing(dyad)	0.00059	0.00047	16	2.0	0.11						
	natal*rearing(dyad	0.00035	0.00043	9	0.7	0.21						
	residual	0.00221	0.00037	59	5.98							

* Df estimated with Satterthwaite's approximation.

Table 2 Relationship between features of nestling house sparrow flange colouration and tarsus length at days 3 and 6 post-hatching. Tarsus was entered as a fixed effect in a linear mixed model model in which dyad, nest-of-origin within dyad, nest-of-rearing within dyad and nest-of-origin by nest-of-rearing within dyad were included as random effects. For brightness and relative UV intensity, chroma was included as a covariate to better estimate the pigment-free features of tissue reflectance. Results for random effects and the fixed effect of chroma in the analysis of brightness and relative UV intensity (not shown) were similar to those presented in Table 1.

Colour parameter	day 3					day 6				
	F•ratio	d.f.*	*P*	β	SE	*F*•ratio	d.f.	*P*	β	SE
Chroma	63.0	1,153	<0.0001	0.0126	0.0016	19.6	1,131	<0.0001	0.0073	0.0017
Brightness	38.7	1,173	<0.0001	0.0165	0.0026	41.3	1,131	<0.0001	0.0105	0.0016
Relative UV intensity	0.1	1,170	0.774	0.0013	0.0044	0.4	1,151	0.525	-0.0019	0.0029

* Df estimated with Satterthwaite's approximation.

Table 3 Changes in the colouration of nestling house sparrow flanges between days 3 and 6 post-hatching assessed with repeated-measures linear mixed models in which age was included as a fixed factor and dyad, nest-of-origin within dyad, nest-of-rearing within dyad and nest-of-origin by nest-of-rearing within dyad were included as random effects. The estimated effect of day 3 and its SE is presented; positive estimates indicate that the value of a colour parameter decreased from day 3 to 6, while negative values indicate that it increased.

				effect of day 3	
	F•ratio	d.f.*	*P*	β	SE
Chroma	142.9	1,99	<.0001	-0.0508	0.0043
Brightness	1.9	1,134	0.1731	-0.0088	0.0064
Relative UV intensity	9.5	1,146	0.0025	0.0304	0.0099

* Df estimated with Satterthwaite's approximation.

Table 4 Results of a linear mixed model assessing the relationship between the change in chroma, brightness and relative UV intensity of nestling house sparrow flanges from day 3 to 6 post-hatching and change in mass. The day 3 value of colour is included as a fixed effect. For brightness and relative UV intensity, the change in chroma is also included as a covariate to control for the negative effect of carotenoid deposition on these two colour parameters.

	Random effects						Fixed effects				
	Estimate	SE	%	G^2	*P*		*F*•ratio	d.f.*	*P*	β	SE
Chroma increase											
dyad	0.00060	0.00049	32	2.2	0.15	mass change	6.1	1,96.5	0.015	0.0002	0.0001
natal (dyad)	0.00013	0.00011	7	1.3	0.30	day 3 chroma	11.4	1,94.3	0.001	-0.3467	0.1027
rearing (dyad)	0.00062	0.00032	33	7.7	0.01						
natal x rearing (dyad)	0		0	0.0	0.99						
residual	0.00054	0.00010	29								
Brightness increase											
dyad	0.00032	0.00025	26	2.2	0.15	mass change	9.0	1,73.9	0.004	0.0003	0.0001
natal (dyad)	0.00020	0.00018	16	1.7	0.20	day 3 brightness	85.1	1,81.3	<.0001	-0.4912	0.0533
rearing (dyad)	0		0	0.0	0.99	chroma change	193.0	1,84.4	<.0001	-1.2851	0.0925
natal x rearing (dyad)	0.00014	0.00013	11	1.7	0.20						
residual	0.00058	0.00011	46								
Relative UV increase											
dyad	0.00196	0.00136	33	3.8	0.06	mass change	<0.1	1,52.7	0.987	0.0000	0.0002
natal (dyad)	0.00078	0.00070	13	1.6	0.20	day 3 UV	57.4	1,81.3	<.0001	-0.5799	0.0765
rearing (dyad)	0.00016	0.00054	3	0.1	0.99	chroma change	38.0	1,37.97	<.0001	-1.1570	0.1878
natal x rearing (dyad)	0.00012	0.00076	2	0.1	0.99						
residual	0.00285	0.00061	49								

*Df estimated with Satterthwaite's approximation

Chapter III

House sparrow parents preferentially feed nestlings with mouth colours that appear carotenoid-rich

(formatted for *Animal Behaviour*)

Abstract

When the potential contribution that offspring can make to parental fitness varies, parents benefit by diverting resources towards offspring offering the highest returns on investment. In birds, nestling begging has been hypothesized to allow parental assessment of the relative contribution that each offspring can make to parental fitness. While begging is often interpreted as a signal of need, studies of nestling morphology, specifically carotenoid-based mouth colours, suggest that these traits signal not the need, but the quality of nestlings. Here, I manipulated flange colour in a free-living population of house sparrows with experimental paints that did not feature a UV reflectance peak, but otherwise approximated carotenoid-based colours within the range of natural variation. I presented parents with one nestling that appeared to have the carotenoid-rich rictal flanges associated with high condition, and one that appeared to have the carotenoid-poor flange colour associated with poor condition. Parents delivered more food items to the nestling that appeared to have carotenoid-rich flanges, a pattern driven by a strong female bias and a similar, but non-significant, trend in males. This study demonstrates that parents attend to these visual signals, rather than correlated features of nestling physiology associated with carotenoids. Broadly, this result suggests that at least some components of begging are used by parents to bias resource allocation towards their most promising, rather than most needy, offspring.

1. Introduction

Parents divide investment between both concurrent and successive offspring (Trivers 1974; Stearns 1992). When equally related to all their offspring, parents are assumed to benefit from equal distribution of investment (Trivers 1974). If, however, possible recipients of parental investment differ in the fitness returns they represent, parental fitness is maximized not by equal allocation, but by preferential allocation to offspring that offer the highest marginal fitness return on investment (Temme 1986; Haig 1990; Godfray 1991, 1995; Mock & Parker 1997).

Offspring fitness is generally assumed to be a decelerating cumulative gain function of parental investment, where a unit of investment often generates a greater marginal benefit when directed to an individual in relatively low condition (Godfray 1991, 1995). While offspring are expected to demand more than an even share of investment (Trivers 1974), inclusive fitness costs limit the selfishness of offspring (Parker et al. 1989). Both parents and offspring, then, should benefit from a signalling system through which offspring reveal some, but not all, information about the contribution that a unit of parental investment will make to their eventual fitness (Trivers 1974; Godfray 1991, 1995).

In birds, a suite of offspring traits collectively known as begging is hypothesized to serve such a signalling role, providing parents with information about the return offspring might offer on investment (Wright & Leonard 2002). Experimentally, short-term food deprivation has been used to simulate chicks of lower fitness value, and the predictions of the Godfray (1991) model seem to have been borne out: hungry nestlings beg more and receive more food (e.g. Kilner 1995, 1997;

Cotton et al. 1996, reviewed in Kilner & Johnstone 1997). Short-term periods of food deprivation probably do influence nestling fitness; all else being equal, a nestling with a full stomach is presumably at least slightly more likely to eventually breed than a nestling with an empty stomach. Under natural conditions, however, all else is unlikely to be equal; between-chick differences due to intermittent food deprivation probably do not encompass the entire range of between-chick variation in fitness value that parents encounter (Godfray 1991; Kilner & Johnstone 1997), and thus seem unlikely to account for the entirety of the information coded in parent-offspring signalling.

Offspring might differ with respect to the rate at which they convert parental investment into fitness (i.e. the slope of the function). For example, food delivered to an offspring that must share nutrients with parasites would contribute less to parental fitness than the same food delivered to a parasite-free sibling (Brown & Brown 1986; Simon et al. 2003; Fitze et al. 2004). Offspring might also differ in the maximum fitness they could attain (i.e. the asymptote of the function). For example, the young of an attractive mate might have higher potential fitness than those of an unattractive mate, and parents may be selected to increase investment under such circumstances (Burley 1986, 1988). Given that return on investment is influenced by many factors other than current hunger, components of offspring-generated signals and parental response may also reflect these among-offspring differences. Parents that can accurately discriminate high-yield from low-yield investments should have higher reproductive success than parents less able to make such distinctions, and high-yield offspring should benefit from revealing themselves as promising investments

whenever the benefits of such signalling exceed total costs.

In birds, morphological traits are good candidates for mediating such parental preferences (Saino et al. 2000; Jourdie et al. 2004; Bize et al. 2006), especially mouth parts, which nestlings automatically present to parents when begging. Fleshy rictal flanges border the mouth of young nestlings and regress as fledging approaches (Clark 1969). Carotenoid-based coloration of flanges is suggested by their reflectance profiles (Hunt et al. 2003) as well as the positive effect of dietary carotenoid supplements on colour (Saino et al. 2000, Ewen et al. 2008, Loiseau et al. 2008, Thorogood et al. 2008). Biochemical extraction has also confirmed the presence of carotenoids in house sparrow flange tissue (Dugas and McGraw *unpublished data)*. The intensity of these carotenoid-based colours is positively associated with body mass in nestling house sparrows (*Passer domesticus*; Loiseau et al. 2008; Dugas and Rosenthal 2010), immune response in nestling barn swallows (*Hirundo rustica*; Saino et al. 2000, 2003) and tarsus length in nestling stitchbirds (*Notiomystis cincta*; Ewen et al. 2008). This relationship is similar to the well-established condition-dependence of carotenoid-based ornaments that mediate adult mate choice (e.g. Hill 2002, 2006).

Here, I test the hypothesis that parents use these carotenoid-based colours to make resource allocation decisions. I manipulated the flange colours of nestling house sparrows and allowed parents to choose between nestlings with rictal flange colours that appeared carotenoid-rich versus carotenoid-poor.

2. Methods

Study animals were drawn from a free-living nest box population in Norman,

OK, USA (see Schwagmeyer et al. 2002 for details). Nests were monitored regularly to determine day of hatching (day 0). Only broods of >2 chicks were used in the experiment, and nestlings were 3–7 days old (mean ± SD=5.5 ± 1.0) when experiments were performed. At most nests (16/21 included in the final analysis), chicks were 5 or 6 days old during experimental trials. The masses of experimental chicks at the other 5 nests fell within the range of day 5 and 6 nestlings. Because parental control of resource allocation, required for parental choice, is likely maximized early in the nestling period (Royle et al. 2002), I conducted trials (within logistical constraints) at the youngest age at which flange painting could be reliably executed. On the day of testing, all chicks in the brood were weighed to the nearest 0.01g on an electronic balance. The two chicks most similar in mass were removed briefly (10-15 min) to a nearby location hidden from the parents' view for experimental manipulation, and the rest of the brood remained in the nest.

The two removed chicks were randomly assigned to one of two flange paint treatments; one approximated a carotenoid-rich colour, hereafter "yellow", and the other a carotenoid-poor colour, hereafter "pale" (see below for details about paints). The mass of chicks painted yellow (14.4 ± 3.6g) and those painted pale (14.6 ± 3.9g) did not differ significantly (Paired *t* test: t_{20} = -0.708, P =0.487). Paints were applied to the flanges using a small brush, and mouths were exposed briefly to moving air from a small, DC-powered hair dryer to ensure that paints dried fully. To facilitate individual identification on black and white video recordings, experimental chicks were also marked with small dots of white paint, either on the centre or right side of the head (markings were randomly matched with flange colour treatment).

Experimental chicks were then returned to the nest, and all other chicks in the brood removed for the duration of the trial. These nonparticipating chicks were kept warm and fed with commercial nestling food (KayTee Extract) while away from their nest. Trials ran for 90 min unless there were constraints imposed by weather or logistics (trial length = 97 ±18 min). At the conclusion of each trial, experimental nestlings were examined to ensure that paints still fully covered the flange. Paints were then removed (dried paints were easily peeled off) from the flanges of experimental nestlings, and all chicks were returned to the nest.

Parental allocation of food was recorded with a small overhead video camera placed inside the nest box with output recorded on a small digital video recorder hidden near the nest box (see Pierce & Pobprasert 2007 for description of similar technology). Parents were habituated to the camera with a black wooden dummy installed at least one day prior to the experimental trial.

To assess parental choice between the yellow and pale colour treatments, I first limited analysis to parental visits during which both chicks begged (defined as gaping prior to parental food allocation). When assessing allocation by males and females separately, I included only parents that delivered >1 food item during the 90 minute trial. I did, however, include single feeding events by one parent in the analysis of total (male + female) allocation and the calculation of overall provisioning rates. In some nests, biparental attendance (defined as the appearance, with or without food, of a male and female adult on the recording) could not be confirmed during the trial, and so a smaller sample that only included nests in which both parents appeared was also analyzed. In addition to these comparisons of parental choice, I compared total

allocation (male + female) during the entire trial, including parental visits during which only one chick begged.

Parental food deliveries could be unambiguously assigned to one chick in 97% of 285 feeding visits; the remaining events were excluded from analysis. Both chicks begged during 80% of these 277 feeding events. I used Sign tests to compare parental provisioning (total feeds, male feeds and female feeds) to the two chicks. All analyses were done with SPSS version 12.0, and post-hoc power analyses were done with G*Power 3.0.10 (Faul et al. 2007). The University of Oklahoma IACUC approved all protocols (RM6-012), and the Oklahoma Wildlife Conservation Department granted the necessary permit.

Reflectance of experimental paints

Because carotenoids create colours by absorbing short-wavelength light (i.e. greens and blue: Fox & Vevers 1960; Andersson & Prager 2006), the relative carotenoid-content of tissues can be estimated by using the relative reflectances at long and short wavelengths (Montgomerie 2006). One such colour measurement, chroma (sensu Endler 1990), has been shown through pigment extraction to predict the carotenoid content of feathers (Saks et al. 2003). Chroma is calculated as:

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

where R, Y, G and B equal the proportion of total reflectance from red (625–699nm), yellow (550–624nm), green (475–549nm) and blue (400–474nm) regions of the spectrum respectively. To create a template for experimental paints, I measured the reflectance of house sparrow flanges using a USB4000 spectrometer (Ocean Optics,

Dunedin, FL, USA). The reflectance of flange tissue at each wavelength was recorded as the percent of light reflected, relative to a white standard (WS-1) of broad-spectrum light produced by a deuterium-tungsten halogen lamp (DT-MINI-2-GS). As realistic models for flange colours, I used the mean reflectance of the 20 most and 20 least chromatic flanges of 184 measured (Fig. 1).

To manipulate flange colours, I tried a wide variety of painting techniques to find a colourant that would: 1) attach reliably to flanges, 2) entirely mask natural flange colour, and 3) accurately recreate the natural flange colours (i.e. match the models). Acrylic paint was the only method that satisfied the first two criteria, and after a broad survey of available colours, satisfied the third well. A high-carotenoid flange was best approximated by one paint (Yellow, Delta Ceramics, Delta Technical Coatings Inc, Whittier, CA, USA) and a pale flange by mixing this paint with another (Cream (11023), Anita's All Purpose Acrylic, SYNTA, Inc., Decatur, GA, USA) in a 1:10 ratio. These experimental paints approximated the chroma values of natural house sparrow flange colours. The yellow paint had a chroma value of 0.39 and the pale paint had a chroma value of 0.18. These chroma values were well within the range of natural flanges (chicks aged 2 to 9 days; range = 0.10–0.43, mean=0.24±0.05, chicks aged 3-7 days (when parents were tested in this study); range = 0.13–0.38, mean= 0.24±0.05).

Although these paints approximated the carotenoid-based coloration of nestlings, the paints did differ from natural colours in two potentially important ways. First, natural flange coloration is characterised by a reflectance peak in the UV-A wavelengths (320-400nm), whereas both paints reflected flatly in the UV (Fig. 1). UV

reflectance of flanges has been shown to influence parents of some species (de Ayala et al. 2007), although parents of other taxa appear indifferent to this colour component (Jourdie et al. 2004). Because the availability of UV light is very low in cavity nests (Hunt et al. 2003), these signals may not be very accessible to house sparrow parents. In any case, because neither paint treatment reflected UV light strongly, this difference was unlikely to drive any parental responses to the two colours. Second, paints also reflected slightly more total light than natural flanges (Fig. 1). The yellow and pale paints reflected 46% and 67% of light (relative to a white standard) respectively, while natural flange brightness ranged from 26% to 61% (mean= 42±7%). Because the absolute brightness of signals is sensitive to changes in ambient light levels (light levels change over orders of magnitude during the day: Endler 1993), the brightness of these experimental colours is still well within the range parents are likely to experience during the day.

3. Results

At least one parent delivered >1 food item when both chicks begged at 21 of 23 nests tested. In these 21 nests, males provisioned at 17 with an average of 4.9 ± 5.7 items per hour, and females provisioned at 18 nests with an average of 4.9 ± 2.7 food items per hour. Males met the more restrictive requirement of delivering >1 food item when both chicks begged at 14 of these nests, and females at 16. Biparental attendance was not confirmed during the trial in four of these 21 nests (in one nest, only the male attended, and in three, only the female attended). Of the 17 trials for which biparental care was confirmed, females delivered >1 food item when both chicks begged in 15

nests and males in 13.

Parents provisioned chicks painted yellow with more food items than chicks painted pale (yellow preferred in 18 of 21 trials, with 2 ties; Sign test: $P<0.001$; Fig. 2). This effect was driven by a significant preference by females (yellow preferred in 12 of 16 trials, with 2 ties; Sign test: $P=0.013$) and a non-significant trend in males (yellow preferred in 9 of 14 trials, with 3 ties; Sign test: $P = 0.143$). When only one chick begged, food was always delivered to the chick begging. There was no difference between treatments with respect to food obtained via solo begging in the 10 nests in which this occurred (yellow fed more often in 4 of 10 nests with 3 ties; Sign test: $P = 1.000$). When these feedings during which only one chick begged were included in the analysis, the yellow chick was fed more often in 16 of 21 nests, with no ties (Sign test: $P = 0.027$; Fig. 2). Considering only nests in which biparental care could be confirmed, the yellow chick was fed more often when both chicks begged (yellow preferred in 15 of 17 trials with 0 ties, Sign test: $P =0.002$), although this difference was not significant for females (yellow preferred in 10 of 15 trials with 1 tie, Sign test: $P =0.180$) or males (yellow preferred in 8 of 13 trials with 2 ties, Sign test: $P =0.227$) separately.

4. Discussion

Parent house sparrows allocated more food to begging chicks with mouth parts manipulated to appear carotenoid-rich. While this pattern was not significant for males alone, males did allocate more food to the yellow chick in the majority of broods, and this study lacked the statistical power to test the male effect ($1-\beta = 0.35$) or sex

differences (1-β = 0.09) fully. Carotenoid-rich colours are associated with high condition in a wide variety of animals (Olsen & Owens 1998; von Schantz et al. 1998; Møller et al. 2000), including nestling birds (Saino et al. 2000, 2003; Ewen et al. 2008) generally and house sparrow nestlings specifically (Loiseau et al. 2008). In this study, chicks were matched for mass and assigned to treatments randomly, so only the visual signal was manipulated. Thus, these results demonstrate that parents must attend to these visual signals rather than other, more cryptic, correlated changes in nestling physiology associated with carotenoids (von Schantz et al. 1998; Møller et al. 2000) or carotenoid-mediated changes in chick behaviour (Helfenstein et al. 2008).

Although nestlings with flange colours that appeared carotenoid-rich enjoyed a competitive advantage, they did not monopolize parental feedings, even when both chicks were begging (Fig. 2). So while it is clear that flange colour can influence resource allocation, the magnitude of this effect during an entire breeding cycle remains unclear. In particular, nestling signals like mouth colour probably become less important later in the nesting cycle (e.g. Kilner 1997), when control of resource allocation may shift towards offspring (Royle et al. 2002).

A general pattern of parental response to nestling morphology, including carotenoid-based mouth colours, suggests that offspring expressing traits associated with high condition are often rewarded with increased parental investment at both the between- and within-brood level. Parent stitchbirds provision carotenoid-supplemented broods (which have yellower mouth parts, see also Thorogood et al. 2008) at a higher rate than control broods (Ewen et al. 2008), although house sparrow parents do not (Loiseau et al. 2008). Skin colours are, in some species, associated with

nestling condition (Jourdie et al. 2004; Bize et al. 2006; Soler et al. 2007), and experimental manipulations have demonstrated that parents attend to this visual signal and favour nestlings within a brood that appear in good condition (Jourdie et al. 2004). The redness of nestling barn swallow palate colours (scored with photo editing software) is positively associated with several fitness correlates (Saino et al. 2000, 2003), and parents prefer nestlings with mouth parts manipulated to appear more red (Saino et al. 2000). It is not clear how such manipulation influences the reflectance of barn swallow mouths, shown elsewhere (with reflectance spectrometry) to be yellow in coloration (Hunt et al. 2003; de Ayala et al. 2007), but this result was also interpreted as a parental response to a condition-dependent visual signal (Saino et al. 2000). Although carotenoid-based plumage colour has the potential to inform parents about nestling condition in great tits (Tschirren et al. 2003), variation in this trait has no influence on parental allocation (Tschirren et al. 2005). Parents can also express preferences conditionally. House sparrow parents preferred chicks with naturally (vs. experimentally) yellower flange colours, but only when chicks were injected with corticosterone (a treatment designed to simulate stress; Loiseau et al. 2008). Parent Alpine swifts (*Apus melba*) and European starlings (*Sturnus vulgaris*) preferred nestlings with experimentally manipulated skin colours indicating good condition, but only late in the breeding season (Bize et al. 2006).

However, parents also respond to offspring morphology that is not condition-dependent, suggesting that adaptive allocation of limited resources is not the only explanation for parental attendance to nestling morphology. For example, parent barn swallows respond to manipulations of flange tissue that reduce UV reflectance, a

colour parameter associated with condition when expressed in nestling skin in several other species (Jourdie et al. 2004; Soler et al. 2006, Bize et al. 2007), but not condition-dependent in barn swallow flange tissue (de Ayala et al. 2007). If UV reflectance contributes positively to the visual conspicuousness of nestlings (Hunt et al. 2003), barn swallow parents could be responding to this feature of mouths. In the current study, the pale paint reflected more total light than the yellow paint (Fig. 1), and so was probably more conspicuous (flange-nest and flange-palate contrast) to parents (Osorio et al. 1999; Hunt et al. 2003; Avilés et al. 2008). Parental preferences for yellow were, then, particularly unlikely to result from a parental response to conspicuousness. Parental preferences have also been hypothesized to result from sensory biases, specifically for red mouths (Kilner 1999). This hypothesis is supported by the finding that experimental reddening of naturally yellow nestling palates sometimes results in increased parental allocation (Götmark & Ahlström 1997; Heeb et al. 2003; but see Noble et al. 1999), but this manipulation probably also influences the visual conspicuousness (flange-palate contrast) of nestlings (Götmark & Ahlström 1997; Heeb et al. 2003) and so is difficult to interpret.

While some components of the begging signal may provide parents with information about the hunger level of individual offspring (e.g. Kilner 1997), the expression of carotenoid-based mouth colours and other condition-dependent nestling traits is consistent with an alternative possibility that components of begging reveal the long-term quality of nestlings. Under a variety of realistic circumstances (e.g. low resource availability), the fitness interests of various family members are probably not maximised by equal allocation of resources to all brood members (Mock 1987; Davis

et al.1999; Mock & Parker 1997). In some families, sibling competition can be primarily responsible for the division of parental investment and determining the recipient of reduced care (Mock & Parker 1997). However, when parents maintain control of allocation, it is their behaviour that must determine the division of resources, and when skewed distribution is adaptive, parents should benefit by attending to offspring-generated traits that identify the most appropriate candidate for reduced investment (i.e. the individual with the worst future prospects). It is perhaps not surprising then that offspring have evolved condition-dependent ornamental traits and that parents have evolved responses to them.

Although the evolution of begging signals is constrained by the relatedness of senders and receivers (Godfray 1991,1995), offspring still compete for parental investment (Trivers 1974). Because morphological traits are associated with fitness prospects of individual nestlings, and because such traits are often amenable to experimental manipulation (e.g. Lyon et al. 1994), these traits offer excellent opportunities to test the hypothesis that some components of offspring signalling have coevolved with parental responses that favour offspring in high, rather than low, condition.

5. Acknowledgements

Jennifer Place provided assistance in the field and helped with scoring videos. Doug Mock, P.L. Schwagmeyer, Rosemary Knapp, Ingo Schlupp, Jorge Mendoza, Don Shepard, Rose Thorogood, Peter Dunn, one anonymous reviewer and the ZEEB Graduate Review provided helpful comments that greatly improved the quality of this

manuscript. This project was funded by a George Miksch Sutton Scholarship in Ornithology and I was supported by an Alumni Fellowship in Zoology from the University of Oklahoma.

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7. Figures and figure captions

Figure 1: Mean flange reflectance from chicks with high and low chroma values (a) were used as models on which to base experimental paints (b). Carotenoid content of tissue is estimated by comparing reflectance at short wavelengths (400-549nm) and long (550-700nm) wavelengths; tissues with higher carotenoid content have a greater relative reflectance of long wavelength light.

Figure 2: Proportion of parental feeds allocated to nestling house sparrows with flanges painted yellow (vs. pale) when both chicks begged (black bars) and when all feeds, including those during which only one chick begged, were considered (grey bars) in 21 nests. A dotted line shows the null expectation of equal allocation.

Figure 1

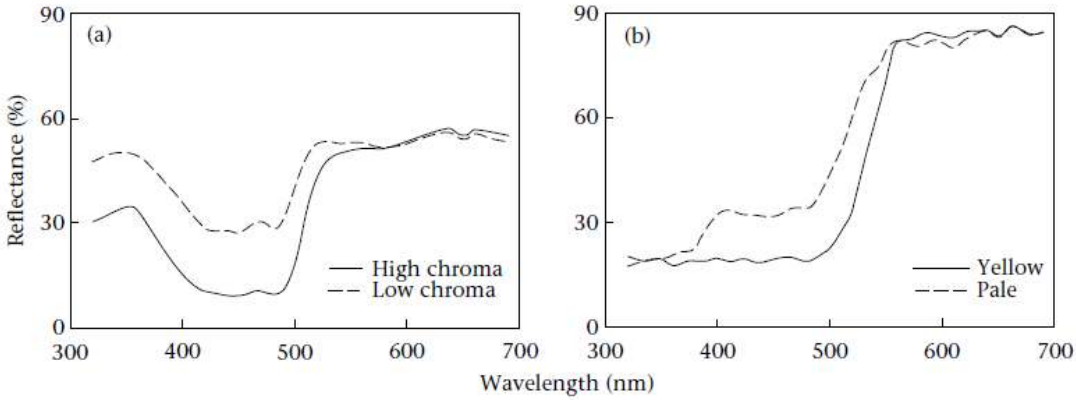
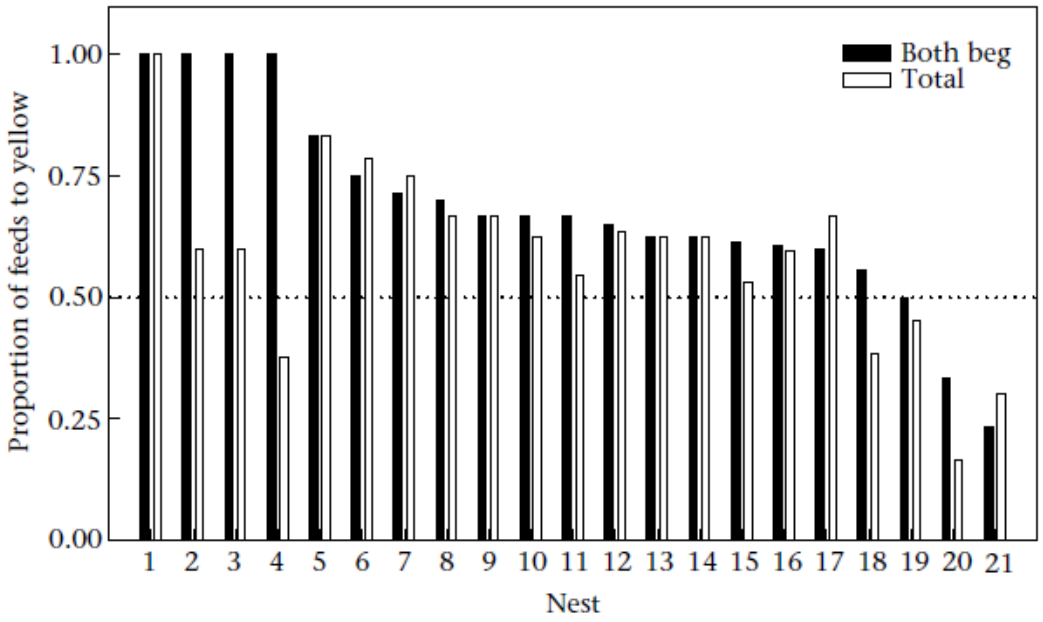

(a)
(b)
Reflectance (%)
90
60
30
0
300
400
500
600
700
Wavelength (nm)
High chroma
Low chroma
Yellow
Pale

Figure 2


Both beg
Total
Proportion of feeds to yellow
1.00
0.75
0.50
0.25
0.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Nest

Chapter IV

Carotenoid-rich mouth colors influence the conspicuousness of nestling birds

(formatted for *Behavioral Ecology and Sociobiology*)

Abstract

When allocating investment among offspring, parents might maximize their fitness by biasing investment towards offspring with the best direct fitness prospects. The observed preferences of avian parents for carotenoid-rich mouth colors that advertise good condition has been interpreted as support for this hypothesis. However, because these condition-dependent visual signals might also make offspring more visually conspicuous, active parental preferences for carotenoid-rich traits are difficult to distinguish from passive responses to differences in detectability among offspring. Here, we used a visual model to examine how mouth colors influence the visual conspicuousness of nestling house sparrows (*Passer domesticus*) to parents under a suite of realistic ambient light conditions. We found little evidence that mouths rich in carotenoids provided more conspicuous targets to parents than mouths poor in carotenoids. While other features of mouth color may have evolved to increase conspicuousness, our results suggest that carotenoid-based coloration is not a product of detectability pressures, and rather may serve as a signal of nestling quality.

Keywords: begging, carotenoids, detectability, mouth color, visual signaling, *Passer domesticus*

1. Introduction

Offspring influence the allocation of limited parental care. This influence may be the product of competition between siblings (Kacelnik et al. 1995; Parker et al. 2002) or may be exerted when parents seemingly control how resources are distributed (Lyon et al. 1994; Kilner 1995). To the extent that parents actively bias distribution, they are expected to do so non-randomly with respect to variation in the fitness prospects of individual offspring (i.e. the return on investment that each offspring offers; Trivers 1974; Godfray 1991, 1995). For example, vocalizations (e.g. Price and Ydenberg 1995), stereotyped postures (e.g. Smith and Montgomerie 1991) and specialized morphologies (e.g. Kilner 1997; Saino et al. 2000; Jourdie et al. 2004) expressed during begging displays by avian nestlings all vary with offspring state (i.e. hunger and/or condition) and all influence the allocation of parental care (references above).

While positive parental responses to signals of hunger (Mondloch 1995; Kilner 1995, 1997) suggest that fair allocation is the parents' goal, parental response to variation in offspring condition paints a very different picture. In many nestling birds, the mouth is decorated with carotenoid-based colors (Hunt et al. 2003; Loiseau et al. 2008, Thorogood et al. 2008) that vary reliably with standard measures of condition (body mass in the house sparrow (*Passer domesticus*): Loiseau et al. 2008, immune response in the barn swallow (*Hirundo rustica*): Saino et al. 2000, 2003, tarsus length in the hihi (*Notiomystis cincta*): Ewen et al. 2008), mirroring the more familiar condition-dependence of carotenoid-based sexual ornaments expressed by adults (e.g. Hill 2002). When parents respond to variation in carotenoid-based mouth colors, they

do so by delivering more food to mouths that are, or are manipulated to appear, carotenoid-rich (Saino et al. 2000; Loiseau et al. 2008; Ewen et al. 2008; Dugas 2009), paralleling the common female mating preference for carotenoid-rich male ornaments in a wide variety of taxa (e.g. Houde 1997; Hill 2002).

A critical question is whether parents actively favor offspring in good condition or whether allocation differences are merely a function of variation in nestling detectability (Royle et al. 2002; Galván et al. 2008). In the latter case, the evolution of nestling mouth colors might have been driven, largely or exclusively, by properties of the parental visual system, as might proximate parental responses to variation in this trait. The perception of color signals is restricted by ambient light conditions as well as the visual system of the receiver (Endler 1993a), and variation in ambient light can drive the evolution of visual signals (e.g. Marchetti 1993; Stuart-Fox 2007). Because many birds nest in relatively dim locations (e.g. dense vegetation, cavities: Avilés et al. 2008), mouth parts may have evolved simply to increase the conspicuousness of nestlings, facilitating efficient food transfer from parent to offspring (Ingram 1920; Kilner and Davies 1998; Avilés et al. 2008). Consistent with this detectability hypothesis, comparative evidence suggests that evolution of the nestling mouth has been influenced by nest lighting environment (Ficken 1965; Kilner and Davies 1998; Kilner 1999; Hunt et al. 2003; Avilés et al. 2008). Experimental manipulation also suggests that parental responses to nestling mouth colors are driven by an interaction between colors and ambient light: under low-light conditions only, artificially reducing mouth-color contrast in nestling great tits (*Parus major*) reduced

nestling mass gain when parents provisioned (Heeb et al. 2003; but see Götmark and Ahlström 1997).

Parental responses to carotenoid-rich mouths could indicate that parents use this condition-dependent trait to direct resources to especially promising offspring, but any such insights are limited by our lack of knowledge about how carotenoids influence visual conspicuousness. Here, we used a visual modeling approach (Gomez and Théry 2007) to determine how carotenoid-based flange color of nestling house sparrows, a condition-dependent trait (Loiseau et al. 2008, this paper) to which parents respond positively (Loiseau et al. 2008; Dugas 2009), influences the conspicuousness of the flanges of nestling house sparrows (*Passer domesticus*). If carotenoid-rich mouths are more detectable than carotenoid-poor mouths, an active parental preference is indistinguishable from an essentially passive response to the most conspicuous visual target. If, however, carotenoid-rich mouths are not more detectable, parental response can be interpreted as the result of parental favoritism.

2. Materials and Methods

Study animals were drawn from a free-living population of house sparrows occupying nest boxes in Norman, Oklahoma, USA (for details see Schwagmeyer et al. 2002) from May to July 2006. Clutches were monitored regularly to establish day of hatching (day 0). On days 2, 3 and 5, chicks were removed from nests briefly (10 minutes) and taken to a nearby car, hidden from the view of parents, where they were weighed to the nearest 0.01g on an electronic balance and assessed for mouth part coloration. On the first day of measurement, marker was applied to the legs of chicks

to identify individuals on subsequent days. We sampled entire broods, but to avoid potential desertion by parents, at least one nestling remained in the nest at all times.

House sparrows are typically cared for in the nest for 14 days post-hatching (Anderson 2006). Because parental attendance to visual signals requires parental control of allocation, we focused on younger ages, when parental control is likely maximized (Royle et al. 2002; Dugas 2009). Although we would have liked to sample chicks even earlier in the nestling period, handling and color sampling was limited by the small size of young nestlings; we sampled day 2 chicks only after considerable experience with the larger day 3 nestlings.

Measuring reflectance

In house sparrows, gaping nestlings display a red palate bordered by clearly delineated yellow flanges (Fig. 1) colored by carotenoids (Loiseau et al. 2008; Dugas and McGraw *unpublished data*). We measured the reflectance of these two regions using a USB4000 spectrometer (Ocean Optics, Dunedin, FL, USA). Tissue was illuminated by light produced by a deuterium-tungsten halogen lamp (Ocean Optics DT-MINI-2-GS) and spectrometer output was recorded using SpectraSuite software (Ocean Optics). Reflectance is quantified as the percentage of light that tissue reflects at each wavelength relative to a uniformly reflective white standard (WS-1). To control ambient light, which might interfere with accurate color measurements, the color of nestling mouth parts was measured inside a portable "dark box" constructed with wood and dark cloth. The nestling's mouth was gently held open, and the reflectance probe was placed at a 90 degree angle to the tissue (Andersson and Prager 2006). Four reflectance measurements were taken from both the palate and the rictal

flanges, and medians for each tissue were used for further analysis. White standards were re-sampled every four measurements (e.g. between flange and palate measurements) and dark standards, which calibrate the spectrometer to background noise (e.g. that generated by heat), were taken between each brood.

Because visual conspicuousness is defined, in part, by the contrast between the flange and the nest, we also sampled reflectance from nesting material. Older nestlings compact and soil nesting material as they approach fledging, and so we collected 10 nests in which eggs were laid but did not hatch and sampled their color in the lab. Ten reflectance measurements were taken from each nest (evenly spaced in the nest cup), and a mean of these 100 measurements was used to represent the average nesting material background.

Measuring ambient light

House sparrows occupy a wide variety of nest sites including dirt burrows, free-standing nests within tree branches, and natural and artificial cavities; in all, light illuminates nestlings through a narrow opening (Anderson 2006). We measured ambient light in an empty nest box (boxes have a 2.5 cm round opening that is 12.5 cm above the 11 x 11 cm floor) with a USB 4000 spectrometer and a 600nm UV/VIS irradiance probe fitted with a cosine-corrector (CC3-UV) and calibrated with an LS-1 Cal lamp (Ocean Optics). The irradiance probe passed through a small hole in the bottom of the nest box, and was fixed into position. Dark cloth was used to prevent light from entering the box from below.

To model signals under realistic lighting conditions, we sampled light in the center of the box at two vertical positions (7 and 9 cm above the floor) based on likely

positions of chicks within the nest. We estimated these as modal nest height (distance from the wooden floor to bottom of the nest cup, 2cm) + mean body length (mean ± 1 SD; day 2 chicks, 5.2 ± 0.6 cm; day 5 chicks, 7.1± 0.7 cm). Ambient light was measured at three times of day: 20 minutes after sunrise, 1 hr after sunrise and at noon (times are hereafter referred to as dawn, morning and noon). In this population, feeding rates are highest in the two hours after sunrise (Schwagmeyer and Mock 1997). The nest box was rotated so that all measurements were taken with the box facing each cardinal direction. To approximate the lighting conditions parents experienced before entering a box, we also sampled ambient light directly above the box at each measurement time. The habitat near nest boxes is homogenous and almost entirely open (free from trees or other obstructions which would change the composition of irradiance spectra: Endler 1993a), so this irradiance is a good estimate of light conditions experienced by parents prior to feeding bouts.

Quantifying color

To estimate the carotenoid content of colors non-lethally, we calculated the chroma, or saturation, of the reflectance curve (sensu Endler 1990). Carotenoids produce colors rich in long wavelengths (e.g. yellow, orange, red) via absorption of short-wavelength light (e.g. blue and green; Fox and Vevers 1960; Andersson and Prager 2006). Comparison of reflection at short and long wavelengths is, therefore, a commonly used proxy for the carotenoid concentration of tissues (see Montgomerie 2006 for review). We chose chroma (Endler 1990) to serve a carotenoid proxy because it has been empirically demonstrated to predict the carotenoid content of tissues (Saks

et al. 2003) including the flange tissue of nestling house sparrows (Dugas and McGraw *unpublished data*).

Quantifying contrast.

To capture the conspicuousness of nestling flanges, we determined their contrast with both the nesting material and the palate, using the model of Gomez and Théry (2007) to calculate chromatic and achromatic contrast. We began by computing quantal catch:

$$Q_i = \int_{300}^{700} R(\lambda) I(\lambda) S_i(\lambda) \mathrm{d}\lambda$$

where λ is the wavelength in nanometers, R is reflectance, I is spectral irradiance, and S_i refers to the spectral sensitivity of each of the $i = 4$ cone classes in house sparrows (Chen and Goldsmith 1986). Quantal catch was computed for reflectance functions R of each nestling flange color, for the corresponding palate color, and for average nest reflectance, using the 24 measured irradiance functions I: ambient light measurements taken at two positions within the box at all four cardinal directions at three times of day.

We then corrected quantal catch to take into account receptor saturation and model color constancy (Gomez and Théry 2007):

$$q_i = \frac{Q_i}{Q_i + Q_i^B}$$

For Q^B, we used irradiance measurements taken directly above the nest box (parent eyes were adapted to current outside lighting conditions, i.e. dawn, morning and noon). Our logic for this was that parents entering the box for short periods are likely

to remain light-adapted to outdoor conditions between entering the nest cavity and feeding nestlings (see Reynolds et al. 2009 for review; for a sample of 11 house sparrow pairs, the modal time that parents spent in the box before delivering food averaged 4.5 ± 2 sec, D.W. Mock *unpublished data*). Corrected q_i were normalized to relative excitations after Gomez and Théry (2007), producing a three-dimensional, tetrahedral color spaces defined by the maximal responses of each cone class. Chromatic contrast between two color patches (flange vs. palate, or flange vs. nesting material) was defined as the Euclidean distance between them. Achromatic contrast was defined as the square root of the squared difference in the summed response of double cones (modeled by combining medium and long wavelength absorbance spectra) to each color patch (Gomez and Théry 2007).

Data analysis.

With ambient light measurements taken at two positions within the box at all four cardinal directions at three times of day, there were 24 potential variables that captured the chromatic and achromatic contrast of each flange against both the nesting material and the palate. For each of four contrast elements (achromatic and chromatic contrast for flange vs. nest and flange vs. palate), contrast scores were entered into a principal components analysis (PCA) to reduce the number of potential dependent variables for later analysis.

To confirm a relationship between flange color and mass in this population (such a relationship was previously reported in a French population of house sparrows; Loiseau et al. 2008), we used a general linear model (GLM) with mass entered as the

dependent variable, brood as a random factor, and flange chroma as a covariate (sensu de Ayala et al. 2007). Because some chicks were measured at more than one age, each age group (days 2: 22 chicks from 6 broods, 3: 65 chicks from 19 broods , and 5: 34 chicks from 11 broods) was considered separately. To examine the relationship between nestling age and color, we used a repeated measures analysis of variance with chicks measured at all three ages (14 chicks from 4 broods) and on larger subsets of chicks measured on two of the three days. Brood of origin was included as a between-subjects factor.

We then examined the relationship between the chroma of each flange and the associated contrast PC scores using correlations (N=121). First, because we were interested only in how carotenoid content influenced detectability, and to maximize the range of colors included, we considered each individual flange measurement as an independent data point. However, because it is at least possible that this relationship could be influenced by other features of reflectance (e.g. brightness) which could be associated with brood of origin or individual identity, we also analyzed a reduced data set in which we included only one nestling per brood and each brood only once (final N=23). When broods were measured more than once, we excluded brood samples so that the distribution of ages would be as even as possible, and chose one chick per brood randomly (final N at day 2=6, day 3=10 and day 5=7). Alpha was set at 0.05 throughout.

3. Results

Flange colors and nestling mass

Chroma was positively associated with nestling mass on day 2 and 3, but not on day 5 (see Table 1). Average chroma increased with age (repeated-measures $F_{2,20}$=4.11, p=0.032). Analysis of chicks measured on two of the three days revealed a similar pattern; chroma increased from day 2 to 3 (repeated-measures $F_{1,11}$=7.31, p=0.021) and from day 3 to 5 (repeated-measures $F_{1,20}$=8.89, p=0.007). Although we considered a relatively narrow age range here, variation in flanges of these day 2-5 nestlings (range=0.10-0.35, mean±SD=0.237±0.05) actually exceeded the variation in a sample of day 3-9 nestlings in 2008 (range=0.13-0.32, mean±SD= 0.238±0.04).

Principal components analysis of contrast scores

Both achromatic and chromatic flange-palate contrast were explained by single principal components (PCs) with eigenvalues greater than one (28.83 for achromatic, 23.52 for chromatic), explaining 99% and 98% of variance respectively. Both were characterized by highly positive loadings under all conditions. Flange-nest achromatic contrast had two PCs extracted, explaining 99% of the variance together (PC1: 67%, eigenvalue=16.10; PC2: 32%, eigenvalue=7.74). Flange-nest-achromatic PC1 had highly positive loadings for all morning and noon lighting conditions, while PC2 had highly positive loadings from only dawn conditions. Chromatic flange-nest contrast was described by two PCs, together explaining 95% of the variance (PC1: 89%, eigenvalue=21.34; PC2: 6%, eigenvalue=1.55). PC1 had highly positive loadings at the high position at dawn and noon, but highly negative loadings at the low position at dawn and under morning conditions. PC2 had positive loadings at the low position at dawn, facing east at morning, and west at noon. Details of PC loadings are presented in the appendix.

Flange colors and contrast

Achromatic Contrast

Flange chroma was negatively associated with contrast between the flange and nest under morning and noon light conditions (flange-nest PC1), but was not associated with flange-nest contrast at dawn (flange-nest PC2), or contrast between the flange and palate (flange-palate PC; Table 2).

Chromatic Contrast

Flange chroma was negatively associated with the first flange-nest PC score, meaning that a high-chroma (carotenoid-rich) flange had higher chromatic contrast with nesting material in the morning and at the low position at dawn, but lower contrast at the high position at dawn and at noon. Flange chroma was also negatively associated with the second flange-nest PC score, which had positive loadings from the low position at dawn and facing east in the morning and west at noon (Table 2). Flange chroma was, however, positively associated with chromatic contrast between the flange and palate (flange-palate PC; see Table 2). While the relationships between chroma and the second flange-nest PC score and the flange-palate PC score were not significant in the reduced data set, the patterns were in similar directions as in the full data set (Table 2).

4. Discussion

Flange chroma, a proxy for carotenoid content, was positively associated with nestling mass (a reliable predictor of recruitment in this population: Schwagmeyer and Mock 2008) and age. In the achromatic channel, the only effect of flange chroma was

a slight reduction in the contrast between the flange and nesting material. Flange chroma positively influenced chromatic contrast between the flange and palate, but the effect of chroma on chromatic flange-nest contrast was inconsistent across lighting conditions. Overall, we found little support for the idea that carotenoid-rich flanges would be more visually detectable to parents than carotenoid-poor flanges. This result suggests parental preferences for carotenoid-rich mouth parts (Saino et al. 2000; Loiseau et al. 2008) can be safely interpreted as parental choices rather than essentially passive responses to detectability.

While birds use both achromatic and chromatic contrast to detect objects (Osorio et al. 1999; Schaefer et al. 2006), both experimental and comparative evidence suggest that the achromatic channel is more likely to mediate parental location of nestling mouths. Experimental tests suggest that birds rely principally on achromatic contrast for the detection of movement, edges and patterns (Osorio et al. 1999; Jones and Osorio 2004), needed for the task facing provisioning parents: detecting the flanges bordering a moving mouth and placing food at its center. The general signal design of mouth colors also suggests that high achromatic contrast is a conserved feature of the mouth: flanges and palates contrast greatly in brightness, even when they have similarly-shaped reflectance curves (i.e. are the same color: Hunt et al. 2003). Differences between cavity and open nesters also support the achromatic detectability hypothesis: flanges of cavity nesters are brighter (Hunt et al. 2003; Avilés et al. 2008) and less densely colored (closer to white) than those of open nesters (Kilner and Davies 1998; very dense carotenoids should also decrease chromatic contrast, see Andersson and Prager 2006). We found the effect of carotenoid-based

colors on achromatic contrast to be negligible or negative, suggesting that carotenoids are unlikely to influence parental allocation by making chicks more visually detectable (Figure 1).

A reduction in achromatic contrast is an unavoidable consequence of carotenoid deposition. Carotenoids create colors by subtracting short-wavelength light (Shawkey and Hill 2003; Andersson and Prager 2006), and so the deposition of these pigments in the flanges decreases the brightness of these structures. Because flanges are brighter than both surfaces with which they are juxtaposed, the palate and nesting material (Hunt et al. 2003; Avilés et al. 2008), subtractive carotenoid-based colors necessarily lowers the achromatic conspicuousness of the flange. When animals are illuminated by light relatively rich in medium-wavelength light (e.g. below green leaves), the negative effect of carotenoids on overall brightness will be greater than when light is rich in long wavelengths (Endler 1993). Of course, the absolute effect of pigment deposition on signal conspicuousness will depend on the absolute light levels at the nest (Avilés et al. 2008). In cavity nests, nestling behavior (e.g. stretching the neck upwards, begging at the cavity portal) can offer nestlings, especially older ones, some control over their signaling environment and this will be an important factor to consider in future behavioral studies.

Yellows more often color the mouths of cavity nesters than the orange or red carotenoids (Ficken 1965, but see Kilner and Davies 1998) that might more accurately signal quality (Hill 1996) or exploit parental sensory biases (Kilner 1999), but would impose an even higher detectability cost (Andersson 2000). While the detectability hypothesis can explain interspecific patterns of mouth colors, it does not explain the

presence of carotenoid-based colors in nestling mouths. Rather, our results indicate that carotenoids are signal components that come at the cost of impaired detectability (see also Andersson 2000), and suggest that nestlings may have faced competing pressures to produce a trait that is detectable and reflects quality. The yellow (i.e. not orange or red) colors of cavity nesters (Ficken 1965; Kilner 1999) could, then, be interpreted not as an adaptation that increases detectability *per se*, but rather the result of constraints imposed by detectability requirements.

While achromatic contrast is probably used for detecting the mouth, Osorio et al. (1999) suggest that the chromatic channel might be better suited to encode information about color differences between individuals. Color parameters associated with high carotenoid content enhanced chromatic contrast between the flange and nest only under a few lighting conditions (and lowered contrast in most conditions), but increased chromatic contrast between the flange and palate under all light conditions. This contrast between the flange and palate (both intrinsic to the chick) might be the most reliable component with which to assess quality. While birds display preferences for colors mediated by factors other than chromatic contrast (Ham and Osorio 2007), this effect of carotenoids is intriguing and supports the hypothesis that these colors have evolved in a signaling context. A context-dependent response to skin color in parent birds (Bize et al. 2006) also strongly suggests that parental preferences for visual signals can be mediated by active choice rather than a passive response to signal intensity.

The field of sensory ecology has traditionally focused on overall detectability as an important determinant of behavioral preference (e.g. Ryan and Keddy-Hector

1992; Endler 1993b; Cummings 2007), but recent theoretical (Arnqvist 2006) and empirical (Macias García and Ramirez 2005; Wong and Rosenthal 2006) evidence suggests that sensory detectability can be decoupled from signal attractiveness. Future research could benefit from a nuanced approach that considers the varied effects of pigment-based coloration on surface reflectance and their effects on receiver behavior.

5. Acknowledgements

We thank Jennifer Place for assistance with field work and Doug Mock and P.L. Schwagmeyer for generously providing access to birds. The Institutional Animal Care and Use Committee of the University of Oklahoma approved all protocols (R06-012). Research was funded by a George Miksch Sutton Scholarship in Ornithology to MD, who was also supported by an Alumni Fellowship and Adams Scholarship in Zoology from the University of Oklahoma. Doug Mock, P.L. Schwagmeyer, Rosemary Knapp, Ingo Schlupp, Anne Magurran, Marty Leonard, Tatiana Czeschlik several anonymous reviewers and the ZEEB Graduate Review all provided helpful critiques of manuscript drafts.

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7. Tables and Table Legends

TABLE 1:

Age	Mass				
	Num. *df*	Den. *df*	*F* ratio	*p* value	beta (SE)
2					
Chroma	1	15	12.11	0.003	26.40 (7.59)
Brood	5	15	2.61	0.069	
3					
Chroma	1	45	5.58	0.023	27.36 (11.59)
Brood	18	45	1.63	0.094	
5					
Chroma	1	22	1.81	0.192	NS
Brood	10	22	0.74	0.683	

Table 1: Results of GLMs assessing the relationship between flange chroma and mass at three ages (2, 3 and 5 days post-hatching). Mass was entered as the dependent variable, chroma as a covariate, and brood ID as a random factor.

TABLE 2:

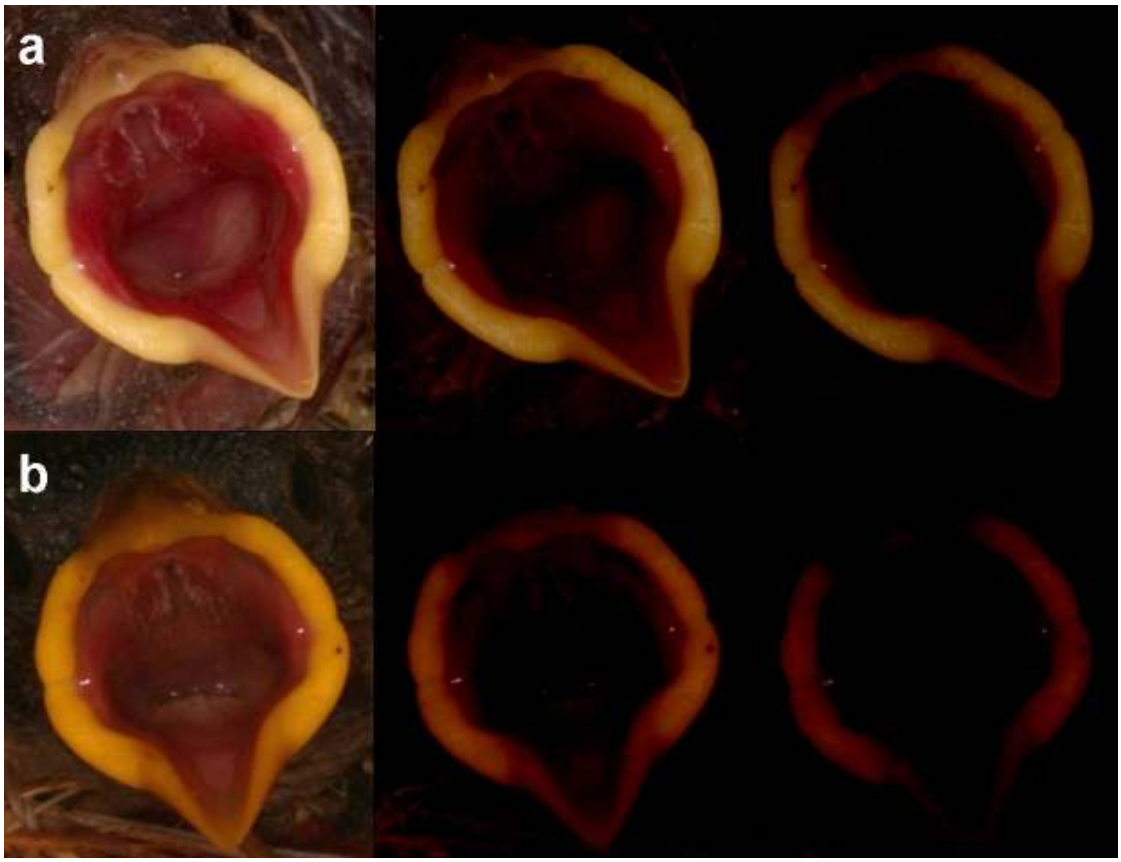
	Chroma			
	All nestlings (N=121)		One nestling per brood (N=23)	
	R	*p* value	*R*	*p* value
Achromatic				
Flange-Nest PC1	-0.237	0.009	-0.635	0.001
Flange-Nest PC2	0.141	0.124	0.188	0.390
Flange-Palate PC	-0.023	0.798	-0.381	0.073
Chromatic				
Flange-Nest PC1	-0.439	<0.001	-0.752	<0.001
Flange-Nest PC2	-0.565	<0.001	-0.381	0.054
Flange-Palate PC	0.303	0.001	0.369	0.083

Table 2: Correlations between flange chroma and achromatic and chromatic contrast PC scores. We included all nestling mouths in one analysis, and in a second, used a reduced data set with only one chick per brood.

8. Figures and legends

Figure 1: Images of (a) carotenoid-poor and (b) carotenoid-rich house sparrow nestling mouths manipulated to illustrate how ambient light influences achromatic detectability. Mouths were photographed digitally under identical lighting conditions with identical camera settings. The brightness (total red/green/blue value) of both photos was then reduced incrementally (moving left to right) using ImageJ (Abramoff et al. 2004) to illustrate for human vision the effect of low ambient light on the conspicuousness of the two mouths.

Figure 1



Appendix

Loadings of contrast values under each ambient light condition on principal component scores representing achromatic (A) and chromatic (B) contrast of the flange against the palate and nesting material.

(A)

TIME	DIRECTION	POSITION	FLANGE-PALATE	FLANGE-NEST PC1	FLANGE-NEST PC2
DAWN	EAST	HIGH	0.999	0.188	0.980
DAWN	EAST	LOW	0.987	0.207	0.966
DAWN	NORTH	HIGH	0.993	0.179	0.976
DAWN	NORTH	LOW	0.996	0.201	0.976
DAWN	SOUTH	HIGH	0.994	0.180	0.976
DAWN	SOUTH	LOW	0.991	0.204	0.971
DAWN	WEST	HIGH	0.990	0.176	0.973
DAWN	WEST	LOW	0.995	0.202	0.975
MORNING	EAST	HIGH	1.000	0.995	-0.096
MORNING	EAST	LOW	0.997	0.993	-0.084
MORNING	NORTH	HIGH	0.995	0.990	-0.105
MORNING	NORTH	LOW	0.999	0.995	-0.089
MORNING	SOUTH	HIGH	0.998	0.993	-0.101
MORNING	SOUTH	LOW	0.996	0.993	-0.084
MORNING	WEST	HIGH	0.990	0.985	-0.110
MORNING	WEST	LOW	0.999	0.995	-0.089
NOON	EAST	HIGH	0.999	0.995	-0.098
NOON	EAST	LOW	0.999	0.995	-0.088
NOON	NORTH	HIGH	1.000	0.995	-0.096
NOON	NORTH	LOW	0.999	0.995	-0.089
NOON	SOUTH	HIGH	0.998	0.994	-0.100
NOON	SOUTH	LOW	0.999	0.995	-0.088
NOON	WEST	HIGH	0.999	0.994	-0.099
NOON	WEST	LOW	0.999	0.995	-0.090

(B)

TIME	DIRECTION	POSITION	FLANGE-PALATE	FLANGE-NEST PC1	FLANGE-NEST PC2
DAWN	EAST	HIGH	0.995	0.993	0.058
DAWN	EAST	LOW	0.955	-0.867	0.407
DAWN	NORTH	HIGH	0.990	0.986	0.100
DAWN	NORTH	LOW	0.974	-0.933	0.285
DAWN	SOUTH	HIGH	0.993	0.990	0.080
DAWN	SOUTH	LOW	0.963	-0.914	0.319
DAWN	WEST	HIGH	0.991	0.986	0.094
DAWN	WEST	LOW	0.971	-0.924	0.301
MORNING	EAST	HIGH	0.995	0.576	0.558
MORNING	EAST	LOW	0.996	0.945	0.255
MORNING	NORTH	HIGH	0.995	-0.980	0.109
MORNING	NORTH	LOW	0.997	-0.981	0.165
MORNING	SOUTH	HIGH	0.996	-0.935	0.285
MORNING	SOUTH	LOW	0.992	-0.953	0.267
MORNING	WEST	HIGH	0.993	-0.977	0.043
MORNING	WEST	LOW	0.998	-0.985	0.145
NOON	EAST	HIGH	0.992	0.979	0.110
NOON	EAST	LOW	0.999	0.993	0.110
NOON	NORTH	HIGH	0.993	0.985	0.099
NOON	NORTH	LOW	0.999	0.985	0.169
NOON	SOUTH	HIGH	0.989	0.967	0.130
NOON	SOUTH	LOW	1.000	0.995	0.098
NOON	WEST	HIGH	0.992	0.978	0.129
NOON	WEST	LOW	1.000	0.707	0.623