Mouth coloration of nestlings covaries with offspring quality and influences parental feeding behavior

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Altricial nestlings compete with their nest mates for resources delivered by parents. Parents may allocate food to nestlings based on reproductive value of offspring. To test the hypothesis that mouth coloration acts as a signal of nestling condition in the barn swallow *Hirundo rustica*, we investigated whether gape coloration is correlated with offspring quality and age. We also examined the role of ultraviolet (UV) flange coloration in parental allocation in a manipulative experiment. Mouth coloration changed with age, probably due to accumulation of dietary carotenoids in the tissue and an increase in the number of collagen layers. Highly UV and redder palates and brighter flanges were associated with longer tarsi and greater body mass at day 6 and with feather growth at day 12 posthatching. Although we did not find evidence that UV coloration of flanges is associated with nestling quality, parents preferentially fed young whose flanges reflected higher UV light, compared with experimentally UV-filtered nestlings. These results support the hypothesis that mouth coloration is a reliable signal of nestling condition. In addition, they show that UV flange coloration influences parental decisions regarding food allocation. *Key words:* food allocation, honest signaling, nestling mouth coloration, parent–offspring communication, parental care, UV coloration. *[Behav Ecol 18:526–534 (2007)]*

In passerine birds, nestlings entirely depend on food brought to the nest by parents. Individual offspring are selected to beg for a greater food share than that equivalent to an even allocation, and this can result in competition among siblings for access to the limited resources delivered by parents (Trivers 1974; Parker et al. 1989; Godfray 1991; reviewed in Mock and Parker 1997). Parents may have the opportunity to invest their resources differentially among offspring and therefore decide which offspring to feed preferentially (Godfray 1995). Because parents are selected to provide care in relation to the reproductive value of offspring (Clutton-Brock 1991), they should be able to assess nestling condition, which would involve a mechanism enforcing honesty on offspring signals (Saino et al. 2003).

The study of parent–offspring conflict has long focused on begging behavior (Parker et al. 1989; Godfray 1991; Whittingham et al. 2003; Royle et al. 2004). However, in addition to postures and acoustic performance, visual cues also play a role in begging and may influence the decisions of parents regarding which chick to feed (Rydén and Bengtsson 1980; Kilner 1995). Previous studies have shown that gape color influences feeding patterns with parents feeding nestlings with a brighter red gape more often (Götmark and Ahlström 1997; Kilner 1997; Kilner and Davies 1998; Saino, Ninni, Calza, et al. 2000; Heeb et al. 2003). These studies, however, have focused on human color perception and thus have ignored the potential role of ultraviolet (UV) wavelengths in parent–offspring interactions.

Passerine birds are sensitive to UV wavelengths (320–400 nm; Bennett et al. 1994; Bowmaker et al. 1997; Hart et al. 1998; Cuthill, Partridge, Bennett, Church, et al. 2000). Although UV signals have been shown to play an important role in the color communication systems of birds (reviewed in Cuthill, Partridge, Bennett, Church, et al. 2000; Cuthill, Partridge, and Bennett 2000), most attention to avian UV vision has focused on its function in mate choice (Bennett et al. 1994; Hunt et al. 1999; Cuthill, Partridge, Bennett, Church, et al. 2000; Pearn et al. 2001; reviewed in Hill 2006). Recently, the mouth and surrounding flanges of nestlings of several bird species (Hunt et al. 2003) and skin of nestling starlings (*Sturnus vulgaris*) (Jourdie et al. 2004) have been shown to reflect maximally in the UV range. These findings suggest that UV reflectance in nestlings may play a role in parent–offspring communication and influence parental decisions about food allocation.

A characteristic feature of nestling altricial birds is the gape showing distinctive wide rictal flanges, suggesting that they might function as a highly visible food target that influences parental feeding choices (Clark 1995). Gape coloration as an honest signal entails a correlation with the bearer's quality. However, convincing evidence that UV reflectance of nestling mouthparts functions as a signal requires experimental manipulation of UV coloration and further measurement of parental response.

In the present study, we assess the potential of mouth coloration as an indicator of nestling quality and investigate the role of the UV wave band in offspring–parent communication in the barn swallow (*Hirundo rustica*). First, we assess the relationship between coloration and phenotypic traits of nestlings, including body mass, tarsus length, feather growth, and T-cell–mediated immune response with the prediction that nestlings with a higher degree of reflectance are of superior

phenotypic quality. Second, we experimentally manipulated UV reflectance of the rictal flanges of barn swallow nestlings and monitored the feeding rates to offspring while predicting that this treatment would reduce parental allocation of food.

METHODS

The barn swallow is an insectivorous passerine that breeds in farm buildings. This species is semicolonial and socially monogamous, and both parents care for the young. Clutches of 2–7 eggs are incubated by the female alone in European populations (Møller 1994). Nestling mortality is typically very low, and last laid eggs in a clutch are relatively large, suggesting that this species does not adopt a brood reduction strategy based on hatch order, which largely reflects laying order (Saino et al. 2004; Ferrari et al. 2006). We studied 4 colonies of barn swallows breeding near Milano (northern Italy) during April–July 2005. Nests were inspected daily to record breeding events and hatching date. Nestlings of this species have palates that not only appear yellow to orange but also have a reflectance peak in the UV region of the spectrum (Figure 1). They also have large rictal flanges that appear pale yellow to almost white to humans and have a striking reflectance peak in the UV region (Figure 1).

General methods

In the correlational study we 1) monitored changes in color parameters of nestling gapes with age and 2) investigated the relationship between gape coloration, nestling morphology, and immunity.

Each bird was individually marked the first time it was handled. On days 6 and 12 posthatching, we measured chick mass with an electronic balance to the nearest 0.1 g (TANITA 1479V) and measured both tarsi using a digital caliper (approximation 0.01 mm). On days 12 and 17, we measured the length of the left and right eighth primaries and outermost tail feathers with a ruler (approximation 0.5 mm). On day 12, T-cell–mediated immune response was measured according to a standard in vivo test (Lochmiller et al. 1993; Saino et al. 1997). The thickness of both wing webs was first measured using a pressure-sensitive micrometer (approximation 0.01 mm). The web of the left wing was then injected with 0.05 ml of phytohemagglutinin (PHA) in phosphate-buffered saline (PBS) in a concentration of 0.5 mg PHA/0.1 ml PBS, whereas the right wing web was injected with the same amount of PBS only to serve as a control. After 24 h, the thickness of both wing webs at the inoculation sites was remeasured. The difference between the change in thickness of the left (PHA) and the right (PBS) wing web was used as an index of T-cell–mediated immune response. Greater wing web swelling indicates a stronger cell-mediated immune response and, hence, presumably better condition (Cichon and Dubiec 2005; Moreno et al. 2005).

We measured the coloration of the mouth (flanges and palate) of nestlings with an Avantes DH-2000 spectrometer. Illumination was provided by a combined deuterium–tungsten halogen light source. The percentage reflectance of the samples was relative to a standard white tablet (WS-2). The reflection probe was mounted inside a matt black plastic tube to prevent any stray light from entering. The end was cut at a 45° angle in order to hold illumination at 45° to prevent specular reflection. The illuminated field was an ellipse of about 7 mm^2, and each reading was an average of 15 scans. For every chick, each gape region (flange and palate) was measured twice. The spectrometer was calibrated against the white standard before every brood was measured, and the white was periodically checked to verify 100% of reflectance.

To analyze reflectance spectra, we adopted the approach suggested by Endler (1990) and Bennett et al. (1997) consist-
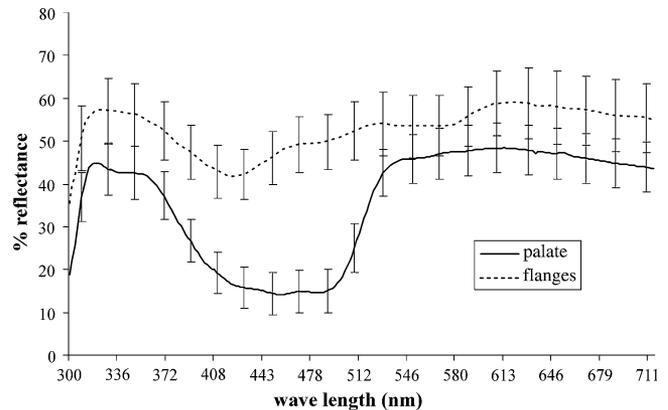
Figure 1
Reflectance curves for gape coloration of nestling barn swallows. Notice the striking peak in the 300 to 400 nm range (UV range of the spectrum) for both palate and flanges. The depression in the range of 400–550 nm of the palate's spectrum (dull line) reveals the peak of absorbance of carotenoids.

ing of summarizing the information of reflectance recorded at different wavelengths (λ) between 300 and 700 nm by applying principal component analysis (PCA). The 1165 data points from each spectrum (at 0.34–nm intervals) were reduced to the medians of computed mean reflectance on all 10-nm bandwidths or, depending on the analyses, for the mean reflectance of the medians computed over the 2 measurements recorded for each nestling and gape region (flange or palate) at each age. Each interval will hereafter be indicated by its median λ (e.g., mean reflectance between 380 and 390 nm will be associated with $\lambda = 385$). The resulting 40 variables of mean reflectances (color variables) were subjected to PCA. PCA is a multivariate statistical method that allows to summarize the information contained in several, potentially intercorrelated variables, in ideally few orthogonal variables (the principal components), which account for most of the variance in the original data set. Scores (i.e., coordinates) of individual spectra were obtained for each principal component that was associated with an eigenvalue larger than 1.0. This threshold resulted in the extraction of principal components that accounted for more than 3% of the variance in all cases. As observed in similar studies adopting the same approach to color quantification, 95% or more of the variance in reflectance data was accounted for by the first 3 principal components, suggesting that loss of information due to reduction of dimensionality was very small.

Repeatability of reflectance profiles and variation with age were tested using spectra recorded on a sample of 26 nestlings from 6 different broods at age 4, 6, 8, 10, 12, 14, and 17 days. The spectra recorded at all ages on the flanges or the palate were subjected to PCA. One-way analyses of variance (ANOVAs) of PCA scores showed highly significant effects of individual for both the flanges and the palate (see Results).

Variation in PCA scores from the analyses of flange or palate spectra on all ages pooled (see above) in relation to age was analyzed by general linear mixed models (GLMMs) with a repeated-measures design where brood to which nestlings belonged was included as a random effect factor and age was entered as a 7-levels factor. For the purpose of this analysis, mean PCA scores of the 2 spectral measurements belonging to the same individual were used.

We investigated the pattern of covariation between gape coloration (flange and palate) and nestling morphology or immunity at age 6 ($n = 143$ nestlings in 33 broods) and 12 days ($n = 142$ nestlings in 33 broods; 1 nestling had died), when all

nestlings in our sample were measured. We performed a GLMM where morphological and immunity traits were entered as the dependent variables, brood was included as a random effect, and PCA scores as covariates. We considered nestling morphological traits as dependent variables because we wanted to test whether gape coloration can signal nestling phenotypic values of traits that may influence their fitness. From this perspective, gape coloration variables were considered predictors of nestling morphology and immune response. We assume that higher phenotypic values (i.e., longer tarsi, larger body mass, higher immune response, and longer feathers) are associated with higher quality chicks. Principal components of gape coloration were computed for the flanges and the palate, and for age 6 and 12 days separately using the maximum sample of spectra available, by averaging the reflectances for each median (see above) between the 2 spectral measurements from each nestling. The final models were obtained by a step-down procedure where the term with the largest P value was excluded at each step.

Experimental manipulation of UV coloration

This experiment was designed to test whether parent barn swallows differentially allocate food to nestlings whose UV coloration in flanges had been manipulated. We used different chicks than those used in the correlational study. We experimentally manipulated UV reflectance by applying UV filter in a dry oily vehicle and only the vehicle as a control. The UV filter treatment consisted of a specially designed suspension that chemically reduces UV reflectance within the 300- to 400-nm wavelength band. The active UV filter ingredient was Uvinul A plus (diethylamino hydroxybenzoyl hexyl benzoate) (5%), and the vehicle was composed by cyclopentasiloxane (55%), diisopropyl adipate (15%), C_{12-15} alkyl benzoate (20%), and ethylhexyl ethylhexanoate (10%). We assessed the effects of treatments by comparing total reflectance before treatments with relative reflectance after the application of the UV filter treatment or the control solutions. We performed paired t-tests of treated surfaces before and after the application of the corresponding treatment. The control solution moderately diminished UV reflectance to 82.5% ± 17% of the reflectance before the application of the control treatment ($t = 2.73$, degrees of freedom [df] = 6, and $P = 0.034$). The UV filter considerably reduced the UV range of the spectra, to 33% ± 25.3% of the reflectance before the application ($t = 7.00$, df = 6, and $P = 0.0004$). The reflectance in the non-UV range of the spectrum was not significantly affected by any of the treatments (all $t < 1.60$ and $P > 0.13$). Therefore, the effects of UV filter and control solution above 400 nm were equivalent. We compared reflectance of the UV-filtered and control surfaces using a t-test for independent data. UV reflectance (300–400 nm) after the application of the UV filter was 29.7% (±26%) compared with the control treatment ($t = 4.39$, df = 11, and $P = 0.001$).

On day 12, we measured the mass of all young. We randomly chose 2 chicks and assigned them to 1 of the 2 treatments, respectively. We spread the superior and inferior sides of the flanges of the 2 experimental chicks with a small amount of either the UV filter or the control with a paintbrush and returned the young to the nest. During the observation period, we temporarily removed the remaining nestlings from the broods. The nestlings that were removed were kept safe and warm.

All observations were taken from underneath a concealed covering, which was placed the day before to allow parents to habituate to the device. We recognized the chicks by their position in the nest (left or right), which was randomly assigned, and put a divisional septum that prevented them from swapping positions. For 40 dyads of chicks, we recorded 20 feeding events per dyad and counted how many times each chick was fed and the total time elapsed per trial. For 25 nests, we did not allow nestlings to feed for 1 h prior the experiment, whereas for 15 nests offspring were not deprived of food prior to the trail. One hour of food deprivation is commonly encountered by barn swallow nestlings under natural conditions when weather is bad (Turner and Rose 1989). We expected that the effect of the UV filter treatment varied with the level of hunger of the chicks, being more pronounced among food-deprived chicks. Nevertheless, our main aim was to test the effect of UV reflectance manipulation on parental feeding decision. Because parents take more time in completing the 20 feeding events with satiated chicks, we decided to continue the experiment only on food-deprived chicks to guarantee accomplishing the field schedule.

The effects of treatment and food deprivation on feeding rates to individual nestlings were analyzed by t-tests for paired data (effect of treatment) or by 2-sample t-tests for independent data (effect of food deprivation; see below). The size of the effects of treatments on feeding rates between the 2 deprivation regimens was tested using a z test following Wolf (1986).

RESULTS

Repeatability of reflectance spectra and variation in coloration with age

Repeatability of reflectance measures for each gape region (flange or palate) was tested by applying PCA to mean reflectance data computed for each 10-nm interval on 2 spectral measurements recorded for 26 nestlings at each of 7 different ages (see Methods).

PCA on palate reflectance led to the extraction of 3 principal components, which overall accounted for 98.5% of the variance in reflectance data (PC1: 82.0%; PC2: 12.6%; and PC3: 3.9%). In all the PCAs run with our raw data, PC1 invariably described mean reflectance, namely "brightness," which is typical for spectra data because this often forms the majority of between-spectra variation (see Cuthill et al. 1999). Subsequent PCs captured variation in spectral shape. The loadings of mean reflectance at all wavelengths (λ) on PC1 were positive, whereas those on PC2 decreased with increasing λ and became negative for λ greater than 515 nm. Thus, PC2 for palate spectra represents variation in the relative amounts of short wavelengths, with high and positive values being related to UV wavelengths. Loadings on PC3 increased with increasing λ and became positive for λ greater than 415 nm. The patterns of coefficients of PC3 suggest that it accounts for medium to long wavelength, implying that high values are indicative of "yellow to red" hue. The 2 PCs extracted on flange spectra accounted for 98.0% of the total variance (PC1: 94.7% and PC2: 3.3%). Similarly to the analyses of palate spectra, reflectance at all λ had positive loadings on PC1. The loadings on PC2 decreased from low to high λ values, becoming negative for λ greater than 445 nm. Thus, PC2 represents the relative amounts of "UV" hue in flanges.

One-way ANOVAs with nestling as a factor on PC1–PC3 scores of individual spectra from the palate showed significant variation among nestlings at all 7 ages ($4.10 < F_{25,26} < 50.10$, P always < 0.001, and $0.61 < R^2 < 0.96$). A highly significant among-individual variation existed for flange PC1 scores at all ages ($3.30 < F_{25,26} < 8.00$, P always < 0.005, and $0.53 < R^2 < 0.77$), with the only exception of age 14, when among-individual variation was marginally significant ($F_{25,26} = 2.19$, $P = 0.026$, and $R^2 = 0.37$). A significant among-nestling variation in PC2 scores also existed for flange coloration at all ages ($2.80 < F_{25,26} < 18.10$, P always < 0.005, and $0.48 < R^2 < 0.89$).
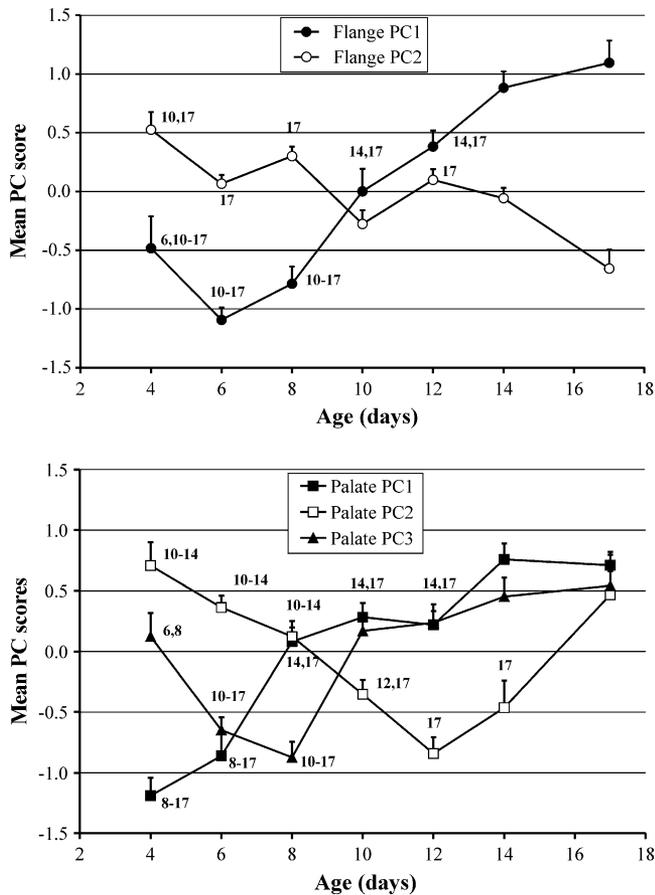
Figure 2
Mean values of scores of reflectance spectra on the principal components extracted for flange and palate coloration at different ages. The numbers in the body of the panels indicate significant differences for Bonferroni post hoc tests from GLMM presented in the Results between mean PCA scores at the focal age with respect to the age indicated. For brevity, only differences between each age and later ages are indicated. Thus, for example, mean palate PC2 at age 12 days differed significantly from those at ages 4, 6, 8, 10, and 17 days, but not from those at age 14 days. Sample size is 26 nestlings for each age and gape region.

Results qualitatively similar to those presented above were obtained when PCAs were run on reflectance data for each separate gape region per age set of data. In fact, 1-way ANOVAs showed that variation between the 2 spectral measurements from the same individual was highly significantly ($P < 0.005$) smaller than variation among individuals at all ages. Therefore, these results show that spectral color measurements were reliable measures of "true" gape coloration at the selected regions and that chicks differed in gape coloration at any given age during the nestling period.

In the analyses of PC scores in relation to age, brood was included as a random factor (see Methods). Age had a significant effect on flange PC1 and PC2 scores (PC1: $F_{6,170} = 62.28$, $P < 0.0001$; and PC2: $F_{6,170} = 5.52$, $P < 0.0001$). PC1 scores first declined between age 4 and 6 days and then monotonically increased with age (Figure 2). Overall, PC2 scores declined with age, but significant differences between pairs of ages were almost exclusively found between age 17 and earlier ages (Figure 2). When the analysis on flange PC2 was rerun while including age as a continuous covariate, rather than as a factor, a highly significant negative relationship was found

($F_{1,175} = 21.50$, $P < 0.0001$, and coefficient [standard error] $= -0.069$ [0.015]).

Age also influenced scores of individual nestlings on palate PCs (PC1: $F_{6,170} = 41.83$, $P < 0.0001$; PC2: $F_{6,170} = 13.33$, $P < 0.0001$; and PC3: $F_{6,170} = 12.55$, $P < 0.0001$). PC1 scores increased with age, PC2 scores declined till age 12 and increased at later ages, whereas PC3 showed a complex pattern of variation whereby scores at age 6 and 8 were similar and significantly smaller than those at age 4 days or 10 days or older.

Gape coloration and nestling morphology and immunity

Age 6
PCA extracted 1 factor (variance explained: 95.7%) from the flange and 3 factors (total variance: 98.8%) from the palate spectra data recorded at age 6 days (Table 1). The loadings of reflectance averaged over 10-nm λ intervals were positive and high (>0.85) for both flange and palate PC1. As stated formerly, PC1 always indicates variation in brightness (the same applies for age 12). The unsigned loadings on palate PC2 were generally low except for λs between 400 and 510 nm (Figure 3). Thus, PC2 for palate denotes variation in the relative amounts of short to medium wavelengths in relation to both UV and long wavelengths. Finally, the loadings on palate PC3 declined almost monotonically from the UV region of the spectrum (Figure 3), thus reflecting variation in the amount of UV hue.

In ANOVAs with brood as a random effect where scores of each PC were included separately, body mass and tarsus length were significantly predicted by PC2 of the palate (details not shown). ANOVAs where scores on all PCs were entered simultaneously confirmed the highly significant effects of palate PC2 on body mass and tarsus length, and also disclosed significant effects of PC1 of both gape regions (Table 1). The significant effects of flange PC1 on body mass and palate PC2 on body mass and tarsus length were confirmed after step-down selection of independent predictors (see Methods).

Age 12
At age 12, PCA extracted 2 factors from flange spectra (total variance: 95.9%) and 3 factors from palate spectra (total variance: 96.9%; Table 2). For both gape regions, the loadings of reflectance for each 10-nm interval on PC1 were large (>0.65) and positive. Loadings on flange PC2 were large and positive for low λs (320–450 nm) and then declined to large negative values for high wavelengths, whereas loadings on palate PC2 increased to a high plateau between 380 and 520 nm and then steeply declined to large negative values for λ > 540 (see Figure 3). Flange PC2 described variation in UV- to short-wavelength reflectance. It was positively related to "UV blue" and negatively related to "red" wavelengths. Likewise at age 6, palate PC2 explained variation in short to medium wavelengths ("blue-green" hue) and was negatively related to "yellow-red" hue. Loadings on palate PC3 had relatively low unsigned values except for a negative peak in the UV region. Hence, PC3 accounted for variation in the amount of UV hue but in the opposite direction as at age 6 days.

Separate ANOVAs of body mass and tarsus length at day 12 where brood was included as a random effect and PCs were included as covariates one at a time showed no relationship with flange PC1 or PC2 nor with palate PC1–PC3 (details not shown). Analyses of covariance (ANCOVA) where all PCs were included simultaneously confirmed that body mass and tarsus length did not significantly covary with gape color components, and the same result was obtained after the step-down procedure of selection of nonsignificant predictors (Table 2).

ANCOVA of feather characters showed a significant relationship of outermost rectrix length with flange PC1 and PC2 and with palate PC2 and PC3. In addition, we observed

Table 1

GLMM ANOVAs of body mass and tarsus length of nestlings at age 6 where brood was included as a random factor

	Initial model					Step-down model				
	Num. (df)	Den. (df)	F	P	Coeff. (SE)	Num. (df)	Den. (df)	F	P	Coeff. (SE)
Body mass										
Flange PC1 (95.7%)	1	137	7.54	0.0067	0.83 (0.30)	1	88.2	6.09	0.016	0.59 (0.24)
Palate PC1 (88.4%)	1	88.2	2.22	0.140	−0.52 (0.35)					
Palate PC2 (6.7%)	1	95.8	20.69	<0.0001	−1.02 (0.22)	1	117	19.60	<0.0001	−0.95 (0.22)
Palate PC3 (3.6%)	1	126	0.06	0.813	0.05 (0.20)					
Tarsus length										
Flange PC1	1	138	5.01	0.027	0.20 (0.09)					
Palate PC1	1	87.4	3.51	0.064	−0.18 (0.10)					
Palate PC2	1	85.9	13.26	0.0005	−0.23 (0.06)	1	126	9.06	0.0032	−0.16 (0.05)
Palate PC3	1	121	0.69	0.409	0.05 (0.06)					

Initial models included the scores of flange and palate reflectance spectra on factors extracted by PCA and associated with eigenvalues >1. Step-down models were obtained by excluding from the model at each step the covariate that had the smallest statistical effect on the response variable under scrutiny, until the model included on significant terms. SE, standard error.

significant covariation of wing feather length with flange PC2 and with palate PC2 and PC3. ANOVAs where all PCs were included as covariates showed a significant relationship between outermost tail feather length and flange PC1, and palate PC2 and PC3 (Table 2), whereas wing feather length significantly covaried with palate PC2 and PC3 (Table 2). The results of these analyses on feather characters were confirmed by step-down covariance analyses (Table 2).

Finally, cell-mediated immunity, reflecting a major component of acquired immunity, was not significantly predicted by any color component and this was confirmed by ANCOVA where all PCs were included simultaneously (Table 2).
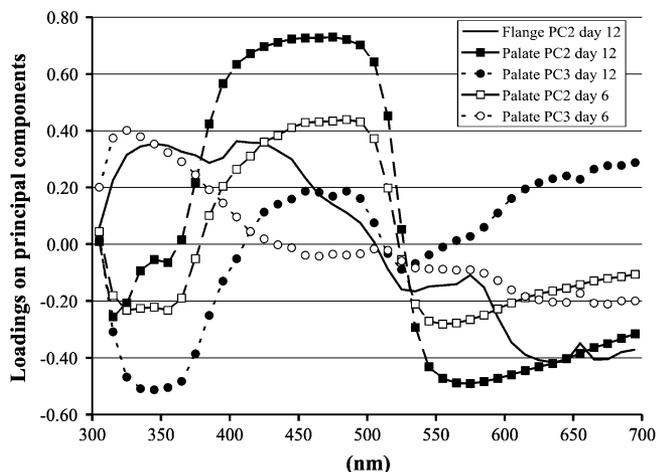
Figure 3
Loadings of reflectances at each 10-nm λ interval on the principal components extracted from flange and palate spectra recorded at age 6 and 12 days on nestlings that were included in the analyses of morphology and immunity in relation to gape coloration. Loadings on PC1s are not shown because they were large and positive at all λs. Flange PC2 at day 12 represents the relative amounts of UV hue. Loadings on palate PC2 at day 12 present high values between 380 and 520 nm. Palate PC2 at day 6 shows relative higher values between 400 and 510 nm, denoting variation of these intermediate wavelengths in relation to both UV and long wavelengths. Palate PC3 at days 6 and 12 show changes almost monotonically, reflecting variation of UV hue, but in the opposite direction.

Manipulation of UV reflectance

Parent barn swallows preferentially allocated food to nestlings with the control treatment compared with UV-manipulated chicks, regardless of their hunger state. For non–food-deprived dyads of chicks, parents fed control nestlings at significantly higher rates than UV-manipulated nestlings ($t = 3.54$, df = 14, $P = 0.003$, and $n = 15$ pairs of nestlings; Figure 4). Similarly, UV-manipulated nestlings received fewer feedings per unit time than control nestlings in the food-deprived dyads ($t = 5.15$, df = 24, and $P < 0.001$, based on 25 pairs of nestlings; Figure 4). The size of the effects of treatment on feeding rates did not differ between food deprivation regimens ($z = 0.70$ and $P = 0.24$; Wolf 1986).

Cumulative feeding rates to food-deprived dyads were significantly larger than feeding rates to non–food-deprived dyads ($t = 2.64$, df = 38, and $P = 0.012$).

DISCUSSION

The conspicuous mouths of nearly all altricial birds are a key signal in parent–offspring communication (Kilner and Johnstone 1997; Kilner and Davies 1998; Kilner 1999; Saino and Møller 2002). Most studies have focused on coloration that is visible to humans (Götmark and Ahlström 1997; Kilner 1997; Kilner and Davies 1998; Saino, Ninni, Calza, et al. 2000; Heeb et al. 2003; Saino et al. 2003), and, consequently, the function of UV coloration in begging displays is virtually unknown. In a manipulative experiment in which the UV reflectance of both skin and flanges was reduced, Jourdie et al. (2004) found that reducing UV reflectance of skin, but not flanges, influenced mass gain of offspring. Flanges are an exclusive feature in young birds, suggesting that they function to stimulate parental feeding. For instance, Schuetz (2005) found that common waxbill (*Estrilda astrild*) nestlings whose flanges were colored black experienced reduced growth compared with controls, presumably due to differences in provisioning among young. It has been proposed that flanges can act as a beacon around nestling mouths to make chicks detectable (Ingram 1920) serving as a target for food delivery (Pycraft 1907), particularly in dark nests (Ficken 1965; Kilner and Davies 1998). Barn swallows nest in relatively dark places (Møller 1983). Nestlings in darker nests improve their conspicuousness through the relative color and size of the flange that borders their mouths (Kilner and Davies 1998). Thus, differences in food allocation could be due to differences in

Table 2

GLMM ANOVAs of body mass, tarsus length, feather characters and an index of acquired immune response of nestlings at age 12 where brood was included as a random factor

	Initial model					Step-down model				
	Num. (df)	Den. (df)	F	P	Coeff. (SE)	Num. (df)	Den. (df)	F	P	Coeff. (SE)
Body mass										
Flange PC1 (87.6%)	1	134	1.13	0.289	0.16 (0.15)					
Flange PC2 (8.4%)	1	114	2.17	0.144	−0.28 (0.19)					
Palate PC1 (67.8%)	1	123	0.67	0.415	−0.14 (0.17)					
Palate PC2 (23.1%)	1	92.1	0.01	0.928	−0.02 (0.17)					
Palate PC3 (6.0%)	1	132	0.15	0.699	0.06 (0.16)					
Tarsus length										
Flange PC1	1	135	0.14	0.707	−0.01 (0.04)					
Flange PC2	1	85.8	0.04	0.847	−0.01 (0.04)					
Palate PC1	1	108	1.79	0.184	−0.05 (0.04)					
Palate PC2	1	71.4	0.02	0.896	−0.01 (0.04)					
Palate PC3	1	121	1.36	0.246	−0.05 (0.04)					
Outermost rectix length										
Flange PC1	1	136	5.18	0.024	0.63 (0.28)	1	135	4.16	0.043	0.52 (0.25)
Flange PC2	1	90.4	3.23	0.076	−0.60 (0.33)					
Palate PC1	1	110	0.01	0.954	0.02 (0.30)					
Palate PC2	1	71.8	7.86	0.007	−0.90 (0.32)	1	87.1	9.15	0.003	−0.92 (0.31)
Palate PC3	1	124	6.43	0.013	0.74 (0.29)	1	138	12.55	0.0005	0.87 (0.25)
Eighth primary length										
Flange PC1	1	134	0.42	0.517	0.27 (0.42)					
Flange PC2	1	70.9	2.89	0.093	−0.82 (0.48)					
Palate PC1	1	98	0.01	0.954	0.03 (0.44)					
Palate PC2	1	57.7	4.41	0.040	−0.95 (0.45)	1	67.5	5.08	0.028	−0.99 (0.44)
Palate PC3	1	113	3.57	0.062	0.82 (0.43)	1	129	8.36	0.0045	1.07 (0.37)
Wing web swelling response										
Flange PC1	1	132	0.16	0.687	−0.03 (0.06)					
Flange PC2	1	70.8	0.92	0.340	−0.07 (0.07)					
Palate PC1	1	98.8	1.72	0.193	0.08 (0.06)					
Palate PC2	1	59.4	0.22	0.642	0.03 (0.06)					
Palate PC3	1	111	0.32	0.571	0.04 (0.06)					

Initial models included the scores of flange and palate reflectance spectra on factors extracted by PCA and associated with eigenvalues >1. Step-down models were obtained by excluding from the model at each step the covariate that had the smallest statistical effect on the response variable under scrutiny, until the model included on significant terms. SE, standard error.

chick detectability to parents. Hunt et al. (2003) suggested that UV reflectance increases the contrast against the nest background, which has the lowest reflectance in shorter wavelengths. In addition to detectability, parents could preferentially feed young with the greatest contrast between the color of the gape and the surrounding flanges (Kilner and Davies 1998).

Integumentary coloration is produced by pigments and by the interaction of light with biological surfaces (i.e., structural coloration; Prum and Torres 2003). It is very likely that the characteristic hue of rictal flanges (Figure 1) in nestlings is due to structural colors produced by collagen arrays (Prum R, personal communication, see also Prum, Torres, Kovach, et al. 1999; Prum and Torres 2003; Prum 2006). Based on the spectra of mouth coloration that we obtained, we hypothesize that flange coloration is structural, and both structural and pigmentary coloration appear to occur in nestlings' palate. PCA for palate separated into 2 variables (PC2 and PC3) the coloration in the UV range of the spectrum from coloration at longer wavelengths. This implies that these qualities are independent (orthogonal by definition), which upholds the distinct nature of structural coloration from pigment-based coloration (physiology, development, and/or selection pressures). Blue, violet, and UV hues are typically structural colors (Auber 1957; Dyck 1976) that result from tissue nanostruc-

ture that coherently scatter light (Prum, Torres, Kovach, et al. 1999). Gape coloration in nestling birds is believed to be partly based on carotenoids which produce yellow to red hues (Ficken 1965; Goodwin 1984). The palate of barn swallow nestlings has a reflectance spectrum with a broad plateau in reflectance across all longer wavelengths (550–750 nm) (Figure 1), which is typical of carotenoid pigments (Prum and Torres 2003). The depression in the range of 400–550 nm of the spectrum (Figure 1) reveals the peak of absorbance of carotenoids (Ke et al. 1970; Frank 1993), whereas blood flow would result in a different spectral shape. In contrast, integumentary structural colors produce a definite reflectance peak (Prum, Torres, Kovach, et al. 1999; Prum and Torres 2003), as is the case for the UV range for both nestlings' palate and flanges (Figure 1).

Gape coloration may reveal offspring need, condition, and immunocompetence as suggested in other studies (Kilner 1997; Saino, Ninni, Incagli, et al. 2000; Saino et al. 2003). In barn swallows, redder gape coloration is positively associated with the immunological competence of nestlings (Saino, Ninni, Calza, et al. 2000). Hence, carotenoid-pigmented mouths signal individual quality (Saino, Ninni, Calza, et al. 2000; Saino et al. 2003). The costs of acquisition, processing, and maintenance of carotenoids and their use in other physiological processes like immunity and antioxidant activity support their function as
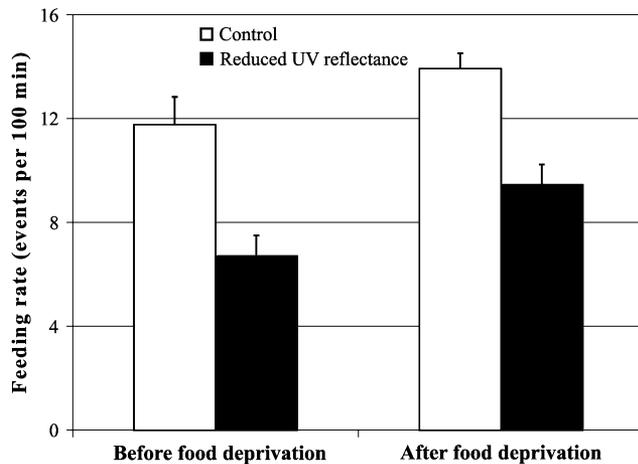
Figure 4
Feeding rates (feedings per 100 min + standard error) to non–food-deprived nestlings and starving nestlings according to experimental manipulation of rictal flange UV coloration. Feeding rates to starving chicks were significantly larger than feeding rates to non–food-deprived nestlings. The size of the effects of the UV manipulation treatments did not differ between food deprivation regimens. Asterisks denote significant effects of UV manipulation on parental feeding preference.

honest signals of individual quality (Olson and Owens 1998; Møller et al. 2000; Hill 2002). More recently, structural-based coloration has been shown to be condition dependent in a number of studies (Keyser and Hill 2000; McGraw et al. 2002; Siefferman and Hill 2005a, 2005b, 2005c; Siefferman et al. 2005), thus revealing their potential as honest signals. Although these studies have assessed plumage coloration, the tidiness with which the collagen structure is assembled might also depend on the bearer's quality because structural colors require extraordinarily precise bioengineering with costs of accurately producing them (Prum 2006) making them necessarily honest (Iwasa et al. 1991). Therefore, flange coloration might signal chick condition. Chicks in better condition must be able to produce highly ordered nanostructures that coherently scatter light that is associated with UV hue and brightness (Prum et al. 1998, 2003; Prum, Torres, Kovach, et al. 1999; Prum, Torres, Williamson, and Dyck 1999; Shawkey et al. 2003).

Gape coloration of barn swallow nestlings changes with age. Overall brightness (PC1), for both flanges and palate, increases as chicks age (Figure 2). Conversely, UV hue (PC2) tends to decrease with age, except for the palate in the last stage of the nestling period (Figure 2), when they are near fledging age. This progression in mouth coloration could be due to an increase in the number of collagen layers in the gape with age (Prum 2006). Variation in overall thickness or number of nanostructures could affect brightness (total reflectance) or chroma (saturation or purity of hue) of a structural color (Prum 2006). Palate PC3 appears to be related to carotenoid-based coloration, as it describes medium- to long-wavelength hues. Taken as a whole, palate PC3 increases as chicks develop. The tone tends to be dominated by the UV component when chicks are younger, but the palate tends to become "redder" with age. This component of gape coloration could reflect the accumulation of carotenoids from the diet in the gape tissue (Goodwin 1984). Gape coloration of nestlings is associated with other phenotypic traits such as body mass, skeletal size, and feather growth (see Tables 1–2). Palate PC2 for ages 6 and 12 days describes the extent of short to medium wavelengths (Figure 3). Large values in PC2 are thus related to high amounts of blue-green hue

(absorbance peak of carotenoids). When depression in the "blue-green" range of the spectrum is greater (lower values in PC2) a more saturated "UV red" hue is produced (see Figure 1). A highly saturated UV red hue is associated with high phenotypic values in chicks. Thus, PC2 is strongly correlated with body mass and tarsus length 6 days posthatching and feather growth 12 days posthatching (Tables 1–2).

As explained previously, this feature appears to be linked to concentration of carotenoids in the palate in addition to structural properties of the tissue. This is further corroborated by the positive correlation of palate PC3 scores at age 12 with feather growth (both the outermost rectrix and eighth primary remige) (Table 2). Flange PC1 accounted for brightness and was positively correlated with body mass at age 6 and outermost rectrix length at age 12, suggesting that nestling barn swallows that experience enhanced growth also display redder palates and more reflective flanges. Flange PC2 did not predict any aspect of nestling condition. Thus, apparently, UV coloration in flanges does not reveal condition. PCA scores predicted different phenotypic traits associated with chick quality at days 6 and 12 posthatching. PCA scores predicted body mass and tarsus length in 6-day-old chicks, when they experience maximal growth rates. Chicks at age 12 have attained their peak mass before mass recession starts, and skeletal growth is completed. Therefore, body mass and tarsus length at this age may not be a good indicator of nestling condition and growth, while feather growth continues until fledging, constituting a key trait of chick quality.

Food deprivation increased the frequency of visits by parents. Hungry nestlings honestly signal need by increasing the intensity of begging displays such as postures and vocalizations (Saino, Ninni, Incagli, et al. 2000). Although we expected a differential effect of UV manipulation with the hunger level of chicks, the size of the effects of the treatments were similar between the 2 food deprivation regimens. Thus, parents adjust the feeding rates according to the whole-brood begging intensity, but make similar decisions when it comes to feed the competing nestlings in the nest.

In conclusion, this study reveals that gape coloration is influenced by nestling condition and nestling maturity. Although we failed to find a relationship between UV coloration in flanges (PC2) and nestling quality, parents preferentially provided food to nestlings whose flanges exhibited greater UV reflectance. This finding upholds the idea that flanges have a decisive role in parental feeding choices (Clark 1995) by improving nestling detectability (Pycraft 1907; Kilner and Davies 1998; Heeb et al. 2003), increasing contrast with the background and color of the gape (Kilner and Davies 1998), and/or signaling nestling condition. Mouth coloration is probably the result of both structural and pigmentary phenomena and is associated with other nestling phenotypic traits, which in turn provide support for the hypothesis of honest signaling. Nevertheless, the function of rictal flanges might be mainly providing conspicuousness to nestlings in dark nests, although this interpretation is not exclusive to a signaling role, due to the potential costs of production of structural coloration (Keyser and Hill 2000; McGraw et al. 2002; Siefferman and Hill 2005a, 2005b, 2005c; Siefferman et al. 2005). There is still the intriguing possibility that flanges and palate coloration have an intricate role in parent–offspring conflict. If flanges only provide detectability, offspring could control food allocation and parents passively feed the competing offspring presenting the largest overall stimulus (Royle et al. 2004). On the other hand, parents might control food allocation by actively choosing between competing signalers as revealed by the honest signal of carotenoid-based coloration of the palate (Godfray 1991, 1995). Future research should address the ultimate role of different types of coloration in parent–offspring

communication as well as the characterization of mouth composition of nestlings as a color-producing signal.

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