

Condition-dependent effects of corticosterone on a carotenoid-based begging signal in house sparrows

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Abstract

Begging is a complex display involving a variety of different visual and auditory signals. Parents are thought to use these signals to adjust their investment in food provisioning. The mechanisms that ensure the honesty of begging displays as indicators of need have been recently investigated. It has been shown that levels of corticosterone (Cort), the hormone released during the stress response, increase during food shortage and are associated with an increased begging rate. In a recent study in house sparrows, although exogenous Cort increased begging rate, parents did not accordingly adjust their provisioning rate. Here, we tested the hypothesis that Cort might affect the expression of other components of begging displays, such as flange color (a carotenoid-based trait). We experimentally increased levels of circulating Cort and investigated the effects of the treatment on (1) the flange coloration of the nestlings, (2) the behavioral response and (3) the parental allocation of food and (4) nestling condition and cell-mediated immune response. We found that Cort affected flange coloration in a condition-dependent way. Cort-injected nestlings had less yellow flanges than controls only when in poor body condition. Parental feeding rate was also affected by the Cort treatment in interaction with flange color. Feeding rate of Cort-injected nestlings was negatively and significantly correlated with flange color (nestlings with yellower flanges receiving more food), whereas feeding rate and flange color were not correlated in control chicks. We also found that nestlings injected with Cort showed a weaker immune response than controls. These results suggest that, indeed, Cort has the potential to affect multiple components of the begging display. As Cort levels naturally raise during fasting, parents have to take into account these multiple components to take a decision as to optimally share their investment among competing nestlings.

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Introduction

Nestling begging displays have attracted considerable attention because these signals are thought to have evolved to resolve the conflict between parents and offspring (Godfray, 1991, 1995; Johnstone, 1999; Johnstone and Godfray, 2002). In altricial birds, nestlings are selected to demand (i) more resources than parents are selected to provide, and (ii) a larger

share of parental investment than their siblings (Trivers, 1974; Parker and MacNair, 1979). Nestling begging displays are based on a complex series of behavioral, acoustic and visual traits (e.g., intensity of begging, body posture, vocal display, mouth coloration). These different traits may have multiple functions and deliver information about nestling quality to parents as to influence their feeding decisions (Johnstone, 1996; Partan and Marler, 2005).

The nestling period is critical in altricial birds since it may affect the entire life history trajectory (Lindström, 1999; Verhulst et al., 2006; Alonso-Alvarez et al., 2006). During this critical life stage, nestlings face a multitude of environmental stressors that can last from a few hours (e.g., cold stress, fasting) to nearly the

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entire rearing period (e.g., sibling competition, parasites). Glucocorticoids participate to the control of the organism's response to stress and the whole body homeostasis. For example, fasting increases the production of corticosterone (Cort), which in turn, stimulates foraging and locomotor activities (Wingfield et al., 1990; Breuner et al., 1998; Angelier et al., 2007), increases food intake (Astheimer et al., 1992; Wingfield and Silverin, 1986; Koch et al., 2002) or enhances plasma glucose levels (Norris, 1997; Remage-Healey and Romero, 2001). These responses all serve to cope with periods of food restriction.

Corticosterone has already been reported to affect begging behavior in black-legged kittiwake chicks (*Rissa tridactyla*). An experimental elevation of baseline Cort was found to increase begging (Kitaysky et al., 2001) and as expected, parents adjusted their food provisioning to the level of begging and fed Cort-treated chicks more than controls (Kitaysky et al., 2001). More recently, injections of Cort were found to produce a similar effect in nestlings of House sparrows (*Passer domesticus*). Cort-injected chicks enhanced their begging intensity. Surprisingly and contrary to the prediction, parents did not respond by increasing food supply, suggesting that high begging levels may be seen by the parents as an indication of irreversibly poor condition or they may adjust provisioning rates according to one or several other signals, which may be modified by Cort (Loiseau et al., 2007). Corticosterone has indeed multiple physiological effects on the regulation of metabolism, the immune system and the antioxidant function (Sapolsky et al., 2000; Barriga et al., 2002; Lin et al., 2007; Roberts et al., 2007). For instance, an experimental study in broiler chickens highlighted the effect of short-term Cort administration on oxidative damage (Lin et al., 2004). Corticosterone administration decreased lipid peroxidation and significantly increased non-enzymatic antioxidants to prevent the development of oxidative injury (Lin et al., 2004). Among antioxidants, there are several molecules of particular interest, such as vitamins E and C, glutathione, uric acid, flavonoids and carotenoids. Among these antioxidants, evolutionary ecologists have mostly focused on carotenoids because, while playing an important role in immunoregulation and immunostimulation (Chew and Park, 2004), they also play a role in the coloration of skin, teguments and feathers in several vertebrates (Goodwin, 1986). For instance, nestlings of many bird species have yellow to red mouth and flange color and the hue of these colored traits is due to carotenoids (Ficken, 1965). Two hypotheses non-mutually exclusive have been proposed to explain the evolution of bright mouth color in altricial birds. The first one suggests that a high contrast between gapes and flanges increases nestling detectability in dark nests (Heeb et al., 2003; Kilner and Davies, 1998); the second hypothesis states that parents may use changes in mouth coloration, depending on the condition of the nestling, as a signal of offspring condition, need or immunocompetence (Kilner, 1997; Saino et al., 2000, 2003). Thus condition-dependent mouth coloration could reveal different components of nestling condition on which parents would base their feeding decisions.

In the light of the previous results on the effects of experimental Cort treatment on begging and parental provisioning rate in house sparrows (Loiseau et al., 2007), we wished to test the potential effect of Cort on flange coloration and the

allocation of carotenoids between the signaling function and the immune system. We experimentally increased the level of circulating Cort in nestlings in order to mimic a food shortage, in a repeated acute way, and we assessed the effect of the treatment on (i) the behavioral response of nestlings, (ii) the nestling color flanges, (iii) the parental allocation of food and (iv) the nestlings growth rate and immune response. We predict that if begging reflects offspring need, parents should adjust their rate of provisioning according to nestling begging rate and/or the color of flanges. We also predict that Cort-treated nestlings should have a weaker immune response than controls. In the same population, in an independent group of nests, we also experimentally supplemented the diet of nestlings with carotenoids. Here, we predicted that carotenoid-supplemented nestlings should have yellower flanges, should be more fed than control nestlings and should have a better immune response than controls.

Materials and methods

The study was carried out in Spring 2005 in a house sparrow population breeding in nest boxes at the Centre d'Etude Biologique de Chizé, France (46°09'N, 0°24'W, Chastel et al., 2003). The authors attest to possess the legal authorized use of wild animals "Certificat d'autorisation d'expérimenter sur animaux vivants N°79-2" delivered to O. Chastel by "Services Vétérinaires des Deux Sèvres".

Sixty-seven broods were studied from April 15 to July 22, 2005. All nests were checked regularly before and during egg laying to determine the date of clutch initiation, clutch size and the hatching date. Five days after hatching, all nestlings were ringed with a numbered metal ring. Nestling body mass (± 0.1 g) was measured daily from day 5 to day 10, whereas tarsus length was measured on day 5 and 10.

Broods were alternatively (based on laying date) assigned to one of the two experimental treatments: Cort injections and carotenoid supplementation. Thus, in half of the broods ($n=32$ broods and 127 nestlings), nestling Cort levels were experimentally increased by daily subcutaneous injections in the inner part of the leg from day 5 to day 8 of age. Within each brood, half of the nestlings were injected with Cort and the other half were used as controls. Nestlings were alternatively assigned to one of the two treatments (the first nestling taken out of the nest injected with Cort, the second with oil and so on). In broods with an odd number of chicks, one nestling was randomly allocated to one of the two treatments. Cort-treated nestlings received 20 μg of Cort, dissolved in 20 μl of peanut oil, at day 5 and 6 and 25 μg of Cort at day 7 and 8 [to adjust the doses (1.33 mg Cort/kg) to the body mass of older nestlings]. These doses are half of those used in a previous study (Loiseau et al., 2007) to avoid supraphysiological effects, and mimic an acute stress as natural as possible. Control nestlings received a daily injection of 20 μl of peanut oil only. Nestlings were always injected in the morning (between 8:00 and 11:00 am).

A validation of the circulating corticosterone concentrations due to the injections was done in a previous study (Loiseau et al., 2007) where the dose used was 2.67 mg Cort/kg (Remage-Healey and Romero, 2001). To measure changes in corticosterone levels (induced by Cort administration), blood samples were taken before injection, 1 h and 2 h after the corticosterone injection. We found a significant difference in raise of corticosterone after 1 h between corticosterone-injected nestlings and control nestlings. Actually, one potential problem with this dose is that corticosterone injections could produce a supraphysiological raise in corticosterone levels. Thus, we decided to use the half of the dose (i.e., 1.33 mg Cort/kg). The validation of this new dose has also been done in Remage-Healey and Romero (2001), with a significant increase of corticosterone levels.

The other half of broods ($n=32$ broods and 136 nestlings) was assigned to the carotenoid treatment. To deliver carotenoids to the nestlings, we fed them with carotenoid-injected mealworms. Each nestling was fed with a worm, daily. Mealworms were injected with 40 μl of a solution containing 420 μg of lutein and 21 μg of zeaxanthin (Kemin France SRL, Nantes, France). This dose was based on a previous study where great tit nestlings were supplemented with

950 µg of lutein per day (Fitze et al., 2003). We decided to divide this dose by two because it was approximately 10 times higher than the natural daily lutein intake. We injected the solution into mealworms with a syringe Myjector (0.3 ml–29 G). The needle was inserted ventrally, into the posterior abdomen, between two segments. If the fluid leaked from the mealworm after the injection, it was not used. Control worms were injected with the same volume (40 µl) of PBS. Mealworm size and mass were 27.49 ± 1.44 mm (mean \pm SE) and 0.159 ± 0.016 g (mean \pm SE), respectively. As for the Cort treatment, half of the nestlings within a brood were fed with carotenoid-injected worms and the other half were fed with PBS-injected worms, to be used as controls.

Nestling and parent behavior were recorded using an infrared video camera (28 × 28 × 30 mm) fixed on the roof of the nest box. Nest boxes were recorded, in the afternoon, for a period of 4 h, when chicks were 8 days old (i.e., after the 4th day of treatment). All nestlings were marked individually on their head with small dye spots. Parents usually resumed normal provisioning about 15 min after the installation of the camera. Videotapes were screened to extract several variables: begging intensity, begging rate and feeding rate. Begging intensity was defined as the time spent with the mouth open. Begging rate was expressed as the number of begs per chick per hour. We distinguished primary begging (occurring when parents arrived at the nest) and secondary begging (occurring between feeding visits in the absence of the parents). Finally, we defined the feeding rate as the number of feeds per chick per hour.

At day 5 (before treatment) and day 8 (after recording), the flanges of each nestling were photographed in a standardized photographic chamber. A digital camera (Nikon Coolpix 4500, 100 ISO, day light color balance, 1/500 s shutter speed, F9.1 aperture, maximal optical zooming, 20 cm fixed focusing, color space RGB) was put on a 30 × 30 × 30 cm box with a hole the size of the lens on the top. Light was provided by an electronic flash (Nikon SB 20, manually set to its minimal power) fixed on an opening on the side opposite to the experimenter. To ensure a uniform illumination of the head of the birds and to minimize shining spots on the bills, no direct light came from the flash unit to the bird. Instead, the heads of the birds only received the light reflected by the white walls of the chamber. Landmarks in the box allowed a precise, repeatable positioning of the birds. A grey plastic board (18% reflection) fixed to the bottom of the chamber was including in every shot in order to control for any color drift between photos. We acknowledge that the range of our color measurements is less extended than the colors perceived by the birds. They possess biologically functional receptors for UV light (Cuthill et al., 2000) to which our equipment was insensitive. As noted by Bennett et al. (1994), “for heuristic purposes, it may be useful to express color patterns in subjective terms that humans can readily understand”. We assume that differences perceived by the digital camera correlate with differences visible to birds.

Analysis of the photographs was done with the software Adobe PhotoShop (version 8.0). We measured the Hue (H), the Saturation (S) and the Brightness (B) of nestling flanges for day 5 and 8. We also measured the H–S–B values of the grey plastic board (to assess variation between shots), and used them to correct the birds measures: the residuals of the regression between H–S–B values of the flange coloration and those of the grey plastic board in a principal component analysis as an overall measure of the flange coloration. In order to estimate for each bird the color variation between days 5 and 8, we subtracted the H, S and B values of the eighth day to the values of the fifth day. We then performed a principal component analysis of the three differences to obtain one overall estimate of the flange coloration. We used the first axis of the principal component analysis (60% of total variance explained for Cort treatment; 52% of total variance explained for carotenoid treatment) to estimate the effect of treatment on flange color variation.

We assessed the chick cell-mediated immune response when nestlings were 9 days old. Nestlings were subcutaneously injected with 0.025 mg of phytohemagglutinin (PHA) dissolved in 0.04 ml of phosphate buffered saline (PBS), in the right wing patagium (Bonneaud et al., 2003). We quantified the immune response by subtracting the thickness of the right wing patagium prior to injection from the thickness of the same wing 24 h post-injection (with a thickness gauge ± 0.01 mm); a strong immune response was indicated by a large swelling (Goto et al., 1978). The PHA assay is a reliable indicator of *in vivo* cellular immunity (Goto et al., 1978; McCorkle et al., 1980) and it is used commonly to assess cell-mediated immune response in ecological immunology studies (Lee et al., 2005; Martin et al., 2004). Here, we would test if a repeated Cort administration could involve long-term effects on nestling capacity to respond to an immune challenge. Indeed, the immunosuppressive and anti-

inflammatory actions of glucocorticoids have been recognized to affect cytokines action and others mediators that promote immune and inflammatory reactions (Sapolsky et al., 2000). It is well known that corticosterone administration has a direct effect on immune system (in the few hours following injection) but also could show important impacts in the few days after repeat administration (e.g., regression of the primary lymphoid organs). In addition, we tested effects of the carotenoids supplementation on nestling capacity to respond to PHA. Blood samples were also taken to assess if PHA response could be associated to the plasma carotenoid concentration.

Carotenoids assays

At 9 days old, blood samples were collected (~50 µl) to assess the amount of plasma carotenoids. The analyses were carried out at the Laboratoire de Parasitologie Evolutive following Alonso-Alvarez et al. (2004). Twenty microliters of plasma were diluted in 180 mL of absolute ethanol. The dilution was mixed in a vortex, and the flocculent protein was precipitated by centrifuging the sample at 1500 × g for 10 min. We examined the supernatant in a spectrophotometer and determined the optical density of the carotenoid peak at 450 nm. Carotenoid concentration was determined from a standard curve of lutein. All samples from the same individual were analyzed in the same plate.

Statistical analyses

We used mixed linear models with fixed and random effects (Proc Mixed, SAS, 1999). Given that nestlings share genes and the environment (the nest), they cannot be considered as independent observations from a statistical point of view. To take into account this non-independence, nest identity was always included in the models as a random factor. The Cort and carotenoid treatments were included as fixed factors and several covariates (brood size, hatching date, body mass) were also added. Repeated measurements models were used when variables were measured several times for the same individual, such as body mass and tarsus length. The assumptions underlying the use of the linear model were systematically checked and the \log_{10} -transformation was applied when necessary.

Results

Corticosterone treatment

Effect of exogenous Cort on flange color

At day 5, before the beginning of the Cort injections, nestling flange coloration was positively correlated with body mass

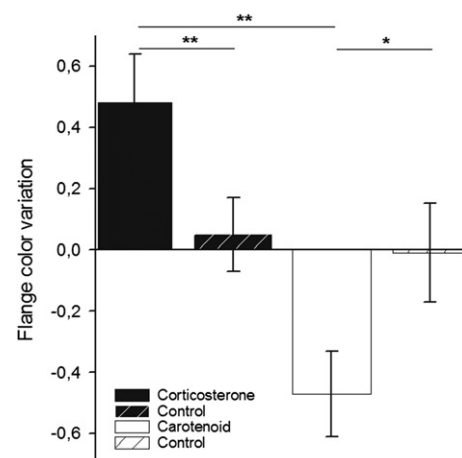


Fig. 1. Effect of corticosterone and carotenoids on flange color variation (difference in flange color between day 8 and day 5). Positive values indicate less yellow flanges and negative values indicate yellower flanges. One asterisk: significant difference ($P < 0.05$), two asterisks: significant differences ($P < 0.01$).

($F_{1,99}=4.81, P=0.03$) and negatively with hatching date ($F_{1,99}=26.74, P<0.0001$).

Corticosterone administration affected the change in flange coloration with Cort-injected chicks having less yellow flanges (Fig. 1). However, the strength of the effect also depended on the body mass of the nestlings, as shown by the interaction between treatment and body mass (Table 1; Fig. 2). Variation in flange color from day 5 to day 8 was negatively correlated with body mass in Cort-treated nestlings (low values indicate yellower flanges) ($F_{1,34}=9.85, P=0.003$), whereas the correlation was non-significant for control chicks ($F_{1,27}=0.18, P=0.67$). Difference in flange coloration between control and Cort-chicks was statistically non-significant for nestlings with large body mass (when body mass was larger than 21.5 g), presumably experiencing good body condition ($F_{1,26}=2.84, P=0.10$; Fig. 2).

Effect of administration of exogenous Cort on nestling begging behavior

Primary begging rate was not affected by Cort treatment ($F_{1,74}=0.89, P=0.35$) but was significantly and positively correlated with feeding rate ($F_{1,74}=12.59, P=0.0007$). Secondary begging rate was affected by the Cort treatment in interaction with body mass ($F_{1,72}=8.00, P=0.006$; Table 2). Control chicks begged significantly more than Cort-treated nestlings when body mass was larger than 20 grams ($F_{1,38}=10.07, P=0.003$).

Behavioral response of parents

The provisioning rate of Cort-injected nestlings did not differ from the one of control chicks ($F_{1,71}=0.19, P=0.66$). However, including begging rate and flange color variation into the model revealed that provisioning rate was affected by the interaction between flange color variation and Cort treatment (Table 3; Fig. 3). Among Cort-treated nestlings, less colored nestlings were less fed than chicks with yellower flanges ($F_{1,25}=7.46, P=0.011$), whereas for control chicks there was no correlation between changes in flange color and feeding rate ($F_{1,20}=0.10, P=0.75$; Fig. 3).

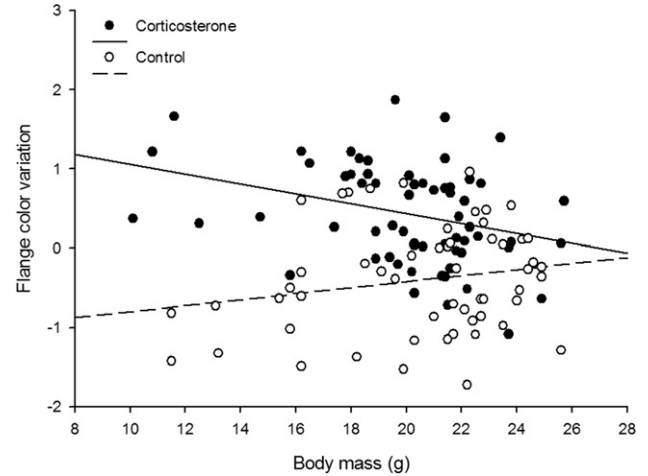


Fig. 2. Correlation between flange color variation (difference in flange color between day 8 and day 5) and body mass (g) at day 8 for Cort-injected and control chicks. Positive values indicate less yellow flanges and negative values indicate yellower flanges.

Effects of exogenous Cort on nestling growth, immune response, plasma carotenoids and return rate the next year

We used a repeated measurement model to assess the effect of daily injections of Cort on body mass and tarsus length. The treatment had no effect on body mass gain ($F_{1,506}=1.17, P=0.28$). Body mass was negatively correlated with flange color at the age of 8 days ($F_{1,93}=10.05, P=0.002$). At day 10, nestling body mass was positively correlated with plasma carotenoids ($F_{1,83}=21.98, P<0.0001$; Fig. 4a) and the immune response ($F_{1,83}=20.79, P<0.0001$). As for body mass, tarsus growth was not affected by the Cort treatment ($F_{1,506}=1.17, P=0.28$), but was negatively correlated with hatching date ($F_{1,136}=4.46, P=0.034$).

Cort-injected nestlings exhibited a weaker response to the PHA challenge than control nestlings ($F_{1,85}=16.10, P=0.0001$; Fig. 5).

Finally, at day 10, plasma carotenoids were not explained by the treatment ($F_{1,87}=0.09, P=0.76$) but were negatively correlated with flange coloration ($F_{1,87}=4.96, P=0.028$), brood

Table 1
Generalized linear mixed model exploring the effect of the hormonal treatment (Cort vs. oil) on flange color

Fixed effect	Flange color variation		
	$F_{1,86}$	Estimate (SE)	P
Treatment	13.24	4.665 (1.282)	0.0005
Body mass	0.79	0.059 (0.048)	0.3757
Hatching date	0.50	8.02E-8 (0.00)	0.4810
Brood size	0.23	0.064 (0.135)	0.6341
Body mass* Treatment	8.69	-0.183 (0.062)	0.0041
Random effect	Z	P	
Nest	2.41	0.0079	

The dependent variable was the difference between flange color at day 8 and day 5. The model included the treatment as a fixed factor, body mass, brood size and hatching date as covariates, as well as the interactions between the treatment and the covariates. The nest was included as a random factor.

Table 2
Generalized linear mixed model exploring the effect of the hormonal treatment (Cort vs. oil) on secondary begging rate

Fixed effect	Secondary begging rate		
	$F_{1,72}$	Estimate (SE)	P
Treatment	7.32	7.320 (0.008)	0.0085
Feeding rate	12.00	12.00 (0.001)	0.0009
Body mass	0.19	0.190 (0.665)	0.6654
Brood size	0.50	-0.047 (0.067)	0.4823
Flange color variation	2.44	0.033 (0.021)	0.1225
Hatching date	0.58	-4.51E-8 (0.00)	0.4499
Body mass* Treatment	8.00	8.000 (0.006)	0.0061
Random effect	Z	P	
Nest	3.16	0.0008	

The model included the treatment as a fixed factor, flange color, feeding rate, body mass, brood size and hatching date as covariates, as well as the interactions between the treatment and the covariates. The nest was included as a random factor.

Table 3
Generalized linear mixed model exploring the effect of the hormonal treatment (Cort vs. oil) on feeding rate

Fixed effect	Feeding rate		
	$F_{1,71}$	Estimate (SE)	P
Treatment	1.07	0.035 (0.034)	0.3047
Flange color variation	3.30	0.010 (0.022)	0.0735
Begging rate	46.98	0.784 (0.114)	<0.0001
Body mass	6.93	0.019 (0.007)	0.0104
Brood size	4.86	-0.075 (0.034)	0.0307
Hatching date	0.14	-1.19E-8 (0.00)	0.7129
Flange color* Treatment	8.05	-0.084 (0.029)	0.0059
Random effect	Z	P	
Nest	2.92	0.0018	

The model included the treatment as a fixed factor, flange color, begging rate, body mass, brood size and hatching date as covariates, as well as the interactions between the treatment and the covariates. The nest was included as a random factor.

size ($F_{1,87}=4.86$, $P=0.03$) and hatching date ($F_{1,87}=38.76$, $P<0.0001$).

We checked whether Cort nestlings were less able to acquire a breeding site (a nest-box in the following spring). The proportion of individuals that acquired a nest-box in 2006 did not differ between the two treatments (Cort 10/72, 13.9%; control 11/67, 16.4%; generalized linear model: $\chi^2_1=0.21$, $P=0.65$).

Carotenoid treatment

Effects of carotenoid supplementation on flange coloration and the behavioral response of parents

At day 5, before the beginning of the carotenoid treatment, nestling flange coloration was negatively correlated with hatching date ($F_{1,105}=6.31$, $P=0.013$). The carotenoid supplementation had a significant effect on flange color variation, with carotenoid-supplemented nestlings having yellower flanges than controls

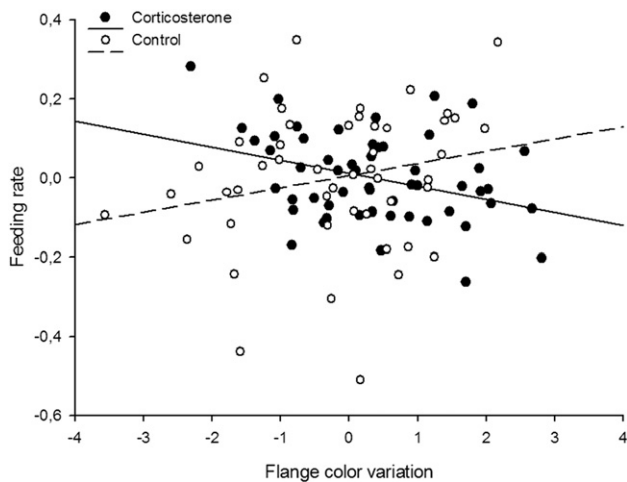


Fig. 3. Correlation between feeding rate (\log_{10} -number of feedings per nestling per hour) and flange color variation (difference in flange color between day 8 and day 5) for Cort-injected and control chicks. Positive values indicate less yellow flanges and negative values indicate yellower flanges.

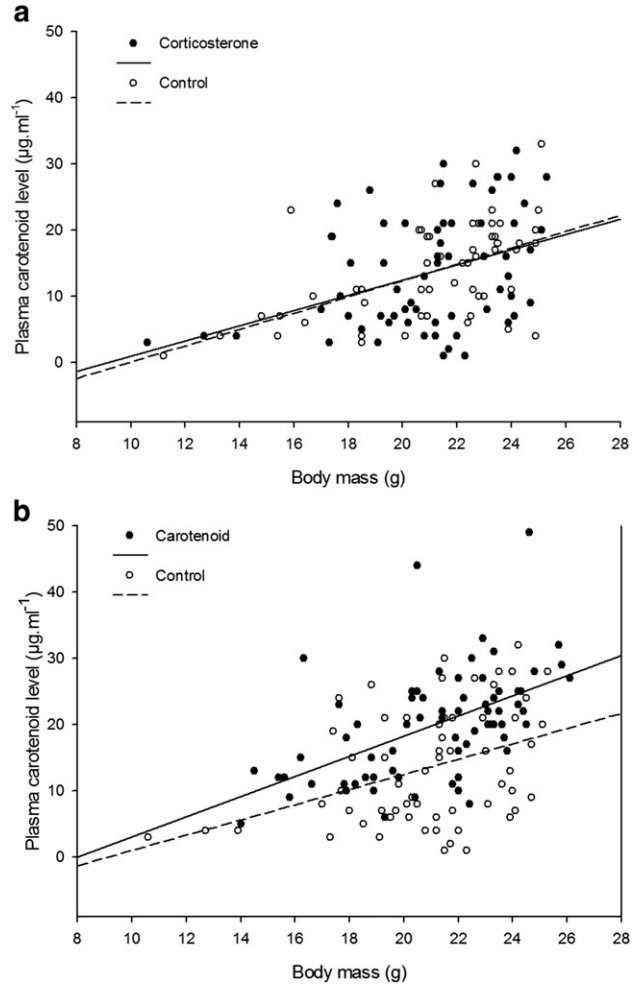


Fig. 4. Positive correlation between plasma carotenoid levels ($\mu\text{g ml}^{-1}$) and body mass (g) at day 10 for (a) corticosterone and (b) carotenoid treatments.

($F_{1,104}=6.16$, $P=0.015$; Fig. 1). However, the provisioning rate of carotenoid supplemented nestlings did not differ from the provisioning rate of control chicks ($F_{1,55}=0.51$, $P=0.48$).

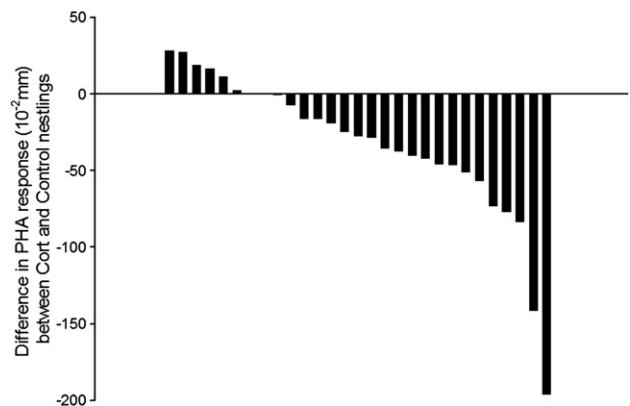


Fig. 5. Difference in immune response against a PHA challenge between Cort and control nestlings. Positive values indicate a stronger immune response in Cort nestlings and negative values indicate a stronger immune response in control nestlings. Each bar represents a nest.

Effects of carotenoid supplementation on nestling growth, immune response, plasma carotenoids and return rate the next year

A repeated measurements model showed that daily carotenoid supplementation had no effect on body mass gain ($F_{1,133}=0.08$, $P=0.77$), but body mass decreased with hatching date ($F_{1,133}=15.71$, $P=0.0001$). As for body mass, tarsus growth was not affected by the carotenoid treatment ($F_{1,128}=0.03$, $P=0.87$).

The immune response did not differ between carotenoid supplemented and control chicks ($F_{1,97}=3.53$, $P=0.63$), whereas, as expected, plasma carotenoids were higher in supplemented chicks compared to controls ($F_{1,96}=33.00$, $P<0.0001$; Fig. 4b). Plasma carotenoids were also positively correlated with body mass ($F_{1,54}=14.16$, $P=0.0003$; Fig. 4b) and negatively with hatching date ($F_{1,54}=44.07$, $P<0.0001$).

The proportion of individuals that acquired a nest-box in 2006 did not differ between the two treatments (carotenoid supplemented 17/72, 23.6%; controls 13/64, 20.3%; generalized linear model: $\chi^2_1=0.22$, $P=0.64$). Finally, we tested if there was a difference in the likelihood to acquire a breeding site between carotenoid supplemented and Cort-injected chicks in 2006, and found that the two groups had similar probabilities to breed in a nest box the following year (generalized linear model: $\chi^2_1=2.09$, $P=0.15$).

Discussion

As predicted, exogenous administration of Cort had a substantial effect on nestling flange coloration. Cort-injected nestlings exhibited a less yellow flange color than controls depending on their body mass. We also found that Cort-treated nestlings begged less than controls in the absence of parents when in good body condition. Parents seemed to adjust somehow their provisioning in response to the treatment since Cort-injected nestlings were fed significantly less than control chicks when flanges were pale. On the contrary, Cort-treated chicks with yellower flanges were fed more than controls. Overall these findings suggest that Cort may affect both behavioral and color displays used during parent–offspring communication, in a condition-dependent way. Thus, if hunger means producing more Cort, and Cort modulates the begging signal, but with its potential costs (on T cell-mediated immune response and body condition), this would ensure the honesty of nestling begging as a signal of condition and thus of need.

The effect of experimental treatments on begging displays

Begging is a complex display involving a variety of different visual and auditory components (Leonard et al., 2003; Kilner, 2002). Nestlings adopt multiple displays to advertise their condition and need. Several studies have investigated the role of hormones (testosterone and corticosterone) in the modulation of begging behaviors (Rubolini et al., 2005; Groothuis and Ros, 2005; Goodship and Buchanan, 2006; Kitaysky et al., 2001; Loiseau et al., 2007). However, to our knowledge, no study had addressed the potential effect of corticosterone on the

expression of a colored trait used during parent–offspring communication.

In this study, we showed that manipulation of Cort levels affected both behavioral and color-based signals, and these effects depended on the body mass of nestlings. Nestlings in best body condition, that undergo repeated acute stress, begged less than controls in the absence of parents. The false alarms characteristics (secondary begging) have been investigated in several studies (Price and Ydenberg, 1995; Leonard and Horn, 2001; Budden and Wright, 2001; Leonard et al., 2005). Hunger decreases the threshold needed for a chick to respond to an external stimulus, increasing thus the likelihood that it will respond to the parental stimulus. Alternatively, begging in the absence of parents can be seen as a long-distance signal that can be perceived by parents outside the nest and further stimulate parents to search food. Whatever the benefits, begging also has costs both in terms of energy expenditure and the likelihood to attract predators to the nest (Briskie et al., 1999; Dor et al., 2007). This result suggests that the Cort treatment may modulate the nestlings' decision to beg depending on their physiological state.

The effect of Cort was not restricted to begging behavior. In agreement with our prediction, we also found that Cort modified the expression of a colored signal. Nestlings with a poor body condition were not able to maintain an intense yellow flange color when facing an acute stress (Cort injections). Because flange color depends on carotenoids, we might speculate that Cort made carotenoids less available for the expression of the signal. There are two possible, non-exclusive, pathways, based on the physiological role played by carotenoids, that could account for the effect of Cort on flange color. First, since Cort induces an immunosuppression, carotenoids might be adaptively diverted to the immune function to buffer the immunosuppressive effect of Cort. Second, since chronic stress can induce oxidative damage (Lin et al., 2004), carotenoids might be preferentially allocated to scavenge reactive oxygen species. In both cases, Cort-injections would have altered the optimal allocation rule of carotenoids between the signaling, the immune and the scavenging function.

In addition, we suggest that carotenoid-based coloration may depend on the nestling capacity to absorb and transport carotenoids in tissues by proteins and lipids (e.g., triglycerides; McGraw and Parker, 2005; Fitze et al., 2007). Indeed, because plasma triglyceride levels may decrease in response to stress (Remage-Healey and Romero, 2001), we assumed that corticosterone modulate carotenoid coloration via lipoprotein-mediated mechanism.

Effects of experimental treatments on parent behavior

Exogenous administration of Cort had an effect on nestling food provisioning and this effect depends on the flange color. We found that feeding rate was negatively correlated with flange color in Cort-injected nestlings, whereas no relationship was found in control chicks. This result might appear counterintuitive, since we expected flange color to affect feeding rate in both groups. Previous work has shown that gape and flange

color is effectively used as a signal in the communication between parents and offspring where nestlings reveal their body condition and immunocompetence (Saino et al., 2000, 2003; De Ayala et al., 2007). However, also already mentioned above, begging display is based on multiple signals and it is possible that the relative information provided by each of these signals changes when nestlings face a chronic stress. Cort-injected nestlings with pale flanges were probably seen by parents as offspring providing consistent information on poor quality and low survival prospects. We therefore suggest that by manipulating Cort levels we indeed altered the consistency of the information provided by begging signals.

The supplementation of nestlings with carotenoids, although producing the expected effect on flange color, did not affect the feeding rate of carotenoid-supplemented chicks. This result is in contrast with previous work that showed a parental preference for more intensely colored nestlings in a few species (Götmark and Ahlström, 1997; Kilner, 1997; Kilner and Davies, 1998). We do not know why, in our study, parents did not respond to carotenoid treatment. One possible reason could be that the carotenoid-induced change in flange color was not enough to produce a substantial variation in the parental perception of nestling quality within the brood. Indeed, control nestlings maintained their “natural” flange color and this might have masked any potential effect of carotenoid supplementation on parental feeding decision.

To conclude, we suggest that Cort treatment, mimicking a repeated food shortage, may modulate the expression of multiple begging signals: flange color and begging rate. In accordance with the ‘multiple redundant signal’ hypothesis (Johnstone, 1995, 1996), parents may use the information gathered from two or more signal components to gain a better estimate of chick’s condition and optimally share resources among nestlings.

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References

- Angelier, F., Shaffer, S.A., Weimerskirch, H., Trouve, C., Chastel, O., 2007. Corticosterone and foraging behavior in a pelagic seabird. *Physiol. Biochem. Zool.* 80, 283–292.
- Alonso-Alvarez, C., Bertrand, S., Devevey, G., Gaillard, M., Prost, J., Faivre, B., Sorci, G., 2004. An experimental test of the dose-dependent effect of carotenoids and immune activation on sexual signals and antioxidant activity. *Am. Nat.* 164, 651–659.
- Alonso-Alvarez, C., Bertrand, S., Devevey, G., Prost, J., Faivre, B., Chastel, O., Sorci, G., 2006. An experimental manipulation of life-history trajectories and resistance to oxidative stress. *Evolution* 60, 1913–1924.
- Astheimer, L.B., Buttemer, W.A., Wingfield, J.C., 1992. Interactions of corticosterone with feeding, activity and metabolism in passerine birds. *Ornis Scand.* 23, 355–365.
- Barriga, C., Marchena, J.M., Lea, R.W., Harvey, S., Rodriguez, A.B., 2002. Effect of stress and dexamethasone treatment on circadian rhythms of melatonin and corticosterone in ring dove (*Streptopelia risoria*). *Mol. Cell. Biochem.* 232, 27–31.
- Bennett, A.T.D., Cuthill, I.C., Norris, K.J., 1994. Sexual selection and the mismeasure of color. *Am. Nat.* 144, 848–860.
- Bonneaud, C., Mazuc, J., Gonzalez, G., Haussy, C., Chastel, O., Faivre, B., Sorci, G., 2003. Assessing the cost of mounting an immune response. *Am. Nat.* 161, 367–379.
- Breuner, C.W., Greenberg, A.L., Wingfield, J.C., 1998. Noninvasive corticosterone treatment rapidly increases activity in Gambel’s whitecrowned sparrows (*Zonotrichia leucophrys gambelii*). *Gen. Comp. Endocrinol.* 111, 386–394.
- Briskie, J.V., Martin, P.R., Martin, T.E., 1999. Nest predation and the evolution of nestling begging calls. *Proc. R. Soc. Lond., B* 266, 2153–2159.
- Budden, A.E., Wright, J., 2001. Falling on deaf ears: the adaptive significance of begging in the absence of a parent. *Behav. Ecol. Sociobiol.* 49, 474–481.
- Chastel, O., Lacroix, A., Kersten, M., 2003. Pre-breeding energy requirements: thyroid hormone, metabolism and the timing of reproduction in house sparrows *Passer domesticus*. *J. Avian Biol.* 34, 298–306.
- Chew, B.P., Park, J.S., 2004. Carotenoid action on the immune response. *J. Nutr.* 134, 257S–261S.
- Cuthill, I.C., Partridge, J.C., Bennett, A.T.D., Church, S.C., Hart, N.S., Hunt, S., 2000. Ultraviolet vision in birds. *Adv. Study Behav.* 29, 159–214.
- De Ayala, R.M., Saino, N., Moller, A.P., Anselmi, C., 2007. Mouth coloration of nestlings covaries with offspring quality and influences parental feeding behavior. *Behav. Ecol.* 18, 526–534.
- Dor, R., Keddar, H., Winkler, D.W., Lotem, A., 2007. Begging in the absence of parents: a “quick on the trigger” strategy to minimize costly misses. *Behav. Ecol.* 18, 97–102.
- Ficken, M.S., 1965. Mouth colour of nestling passerines and its use in taxonomy. *Wilson Bull.* 77, 71–75.
- Fitze, P.S., Tschirren, B., Richner, H., 2003. Carotenoid-based colour expression is determined early in nestling life. *Oecologia* 137, 148–152.
- Fitze, P.S., Tschirren, B., Gasparini, J., Richner, H., 2007. Carotenoid-based plumage colors and immune function: is there a trade-off for rare carotenoids? *Am. Nat.* 169, S137–S144.
- Godfray, H.C.J., 1991. Signalling of need by offspring to their parents. *Nature* 352, 328–330.
- Godfray, H.C.J., 1995. Evolutionary theory of parent–offspring conflict. *Nature* 376, 133–138.
- Goodship, N.M., Buchanan, K.L., 2006. Nestling testosterone is associated with begging behaviour and fledging success in the pied flycatcher, *Ficedula hypoleuca*. *Proc. R. Soc. Lond., B Biol. Sci.* 273, 71–76.
- Goodwin, T.W., 1986. Metabolism, nutrition, and function of carotenoids. *Annu. Rev. Nutr.* 6, 273–297.
- Goto, N., Kodama, H., Okada, K., Fujimoto, Y., 1978. Suppression of phytohemagglutinin skin response in thymectomised chickens. *Poult. Sci.* 57, 246–250.
- Götmark, F., Ahlström, M., 1997. Parental preference for red mouth of chicks in a songbird. *Proc. R. Soc. Lond., B* 264, 959–962.
- Groothuis, T.G.G., Ros, A.F.H., 2005. The hormonal control of begging and early aggressive behavior: experiments in black-headed gull chicks. *Horm. Behav.* 48, 207–215.
- Heeb, P., Schwander, T., Faoro, S., 2003. Nestling detectability affects parental feeding preferences in a cavity-nesting bird. *Anim. Behav.* 66, 637–642.
- Johnstone, R.A., 1995. Honest advertisement of multiple qualities using multiple signals. *J. Theor. Biol.* 177, 87–94.
- Johnstone, R.A., 1996. Multiple displays in animal communication: ‘backup signals’ and ‘multiple messages’. *Phil. Trans. R. Soc. L. B.* 351, 329–338.
- Johnstone, R.A., 1999. Signaling of need, sibling competition, and the cost of honesty. *PNAS* 96, 12644–12649.
- Johnstone, R.A., Godfray, H.C.J., 2002. Models of begging as a signal of need. In: Wright, J., Leonard, M. (Eds.), *The Evolution of Begging: Competition, Cooperation, and Communication*. Kluwer, Dordrecht, pp. 1–20.

- Kilner, R.M., 1997. Mouth colour is a reliable signal of need in begging canary nestlings. *Proc. R. Soc. Lond., B Biol. Sci.* 264, 963–968.
- Kilner, R.M., 2002. The evolution of complex begging displays. In: Wright, J., Leonard, M.L. (Eds.), *The Evolution of Begging*. Kluwer Academic Publishers, Netherlands, pp. 87–106.
- Kilner, R.M., Davies, N.B., 1998. Nestling mouth colour: ecological correlates of a begging signal. *Anim. Behav.* 56, 705–712.
- Kitaysky, A.S., Wingfield, J.C., Piatt, J.F., 2001. Corticosterone facilitates begging and affects resource allocation in the black-legged kittiwake. *Behav. Ecol.* 12, 619–625.
- Koch, K.A., Wingfield, J.C., Buntin, J.D., 2002. Glucocorticoids and parental hyperphagia in ring doves (*Streptopelia risoria*). *Horm. Behav.* 4, 9–21.
- Lee, K.A., Martin, L.B., Wikelski, M.C., 2005. Responding to inflammatory challenges is less costly for a successful avian invader, the house sparrow (*Passer domesticus*), than its less-invasive congener. *Oecologia* 145, 244–251.
- Leonard, M.L., Horn, A.G., 2001. Begging in the absence of parents by nestling tree swallows. *Behav. Ecol.* 12, 501–504.
- Leonard, M.L., Horn, A.G., Parks, E., 2003. The role of posturing and calling in the begging display of nestling birds. *Behav. Ecol. Sociobiol.* 54, 188–193.
- Leonard, M.L., Horn, A.G., Mukhida, A., 2005. False alarms and begging in nestling birds. *Anim. Behav.* 69, 701–708.
- Lindström, J., 1999. Early development and fitness in birds and mammals. *Trends Ecol. Evol.* 14, 343–348.
- Lin, H., Decuyper, E., Buyse, J., 2004. Oxidative stress induced by corticosterone administration in broiler chickens (*Gallus gallus domesticus*): 2. Short-term effect. *Comp. Biochem. Physiol.* 139, 745–751.
- Lin, H., Sui, S.J., Jiao, H.C., Jiang, K.J., Zhao, J.P., Dong, H., 2007. Effects of diet and stress mimicked by corticosterone administration on early postmortem muscle metabolism of broiler chickens. *Poult. Sci.* 86, 545–554.
- Loiseau, C., Sorci, G., Dano, S., Chastel, O., 2007. Effects of experimental increase of corticosterone levels on begging behavior, immunity and parental provisioning rate in house sparrows. *Gen. Comp. Endocrinol.* doi:10.1016/j.ygcen.2007.03.004.
- Martin, L.B., Pless, M., Svoboda, J., Wikelski, M., 2004. Immune activity in temperate and tropical house sparrows: a common-garden experiment. *Ecology* 8, 2323–2331.
- McCorkle, F., Olah, I., Glick, B., 1980. Morphology of the phytohemagglutinin-induced cell response in the chickens wattle. *Poult. Sci.* 59, 616–623.
- McGraw, K.J., Parker, R.S., 2005. A novel lipoprotein-mediated mechanism controlling sexual attractiveness in a colourful songbird. *Physiol. Behav.* 87, 103–108.
- Norris, D.O., 1997. Secretion and action of glucocorticoids. *Vertebrate Endocrinology*. Academic Press, Boston, pp. 308–311.
- Parker, G.A., MacNair, M.R., 1979. Models of parent–offspring conflict: I. Monogamy. *Anim. Behav.* 26, 97–110.
- Partan, S.R., Marler, P., 2005. Issues in the classification of multimodal communication signals. *Am. Nat.* 166, 231–245.
- Price, K., Ydenberg, R., 1995. Begging and provisioning in broods of asynchronously-hatched yellow-headed blackbird nestlings. *Behav. Ecol. Sociobiol.* 37, 201–208.
- Ramage-Healey, L., Romero, M., 2001. Corticosterone and insulin interact to regulate glucose and triglyceride levels during stress in a bird. *Am. J. Physiol., Regul. Integr. Comp. Physiol.* 281, R994–R1003.
- Roberts, M.L., Buchanan, K.L., Hasselquist, D., Evans, M.R., 2007. Effects of testosterone and corticosterone on immunocompetence in the zebra finch. *Horm. Behav.* 51, 126–134.
- Rubolini, D., Romano, M., Boncoraglio, G., Ferrari, R.P., Martinelli, R., Galeotti, P., Fasola, M., Saino, N., 2005. Effects of elevated egg corticosterone levels on behavior, growth, and immunity of yellow-legged gull (*Larus michahellis*) chicks. *Horm. Behav.* 47, 592–605.
- Saino, N., Ninni, P., Calza, S., Martinelli, R., DeBernardi, F., Møller, A.P., 2000. Better red than dead: carotenoid-based mouth coloration reveals infection in barn swallow nestlings. *Proc. R. Soc. Lond., B* 267, 57–61.
- Saino, N., Ambrosini, R., Martinelli, R., Ninni, P., Møller, A.P., 2003. Gape coloration reliably reflects immunocompetence of barn swallow (*Hirundo rustica*) nestlings. *Behav. Ecol.* 14, 16–22.
- Sapolsky, R.M., Romero, M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Rev.* 21, 55–89.
- Sas, 1999. *SAS User's Guide*. SAS Institute, Cary, North, Carolina, USA. (Version 8 edition).
- Trivers, R., 1974. Parent–offspring conflict. *Am. Zool.* 14, 249–294.
- Verhulst, S., Holveck, M.J., Riebel, K., 2006. Long-term effects of manipulated natal brood size on metabolic rate in zebra finches. *Biol. Lett.* 2, 478–480.
- Wingfield, J.C., Silverin, B., 1986. Effects of corticosterone on territorial behavior of free-living male Song sparrows *Melospiza melodia*. *Horm. Behav.* 20, 405–417.
- Wingfield, J.C., Schwabl, H., Mattocks, J.P.W., 1990. Endocrine mechanisms of migration. In: Gwinner, E. (Ed.), *Bird Migration: Physiology and Ecophysiology*. Springer-Verlag, New York, pp. 232–256.