The Importance of Visual Cues for Nocturnal Species: Eagle Owl Fledglings Signal with White Mouth Feathers

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Abstract

Complex begging display by bird offspring has predominantly been investigated in diurnal species, which have conspicuous gape colours or plumage features. In nocturnal species, in contrast, such visual communication has received little attention because the assumption is that they exclusively rely on vocal communication. Here, we use a field experiment to investigate whether eagle owls, *Bubo bubo,* communicate through visual signals at night. We artificially decreased the brightness of the white feathers surrounding fledgling eagle owls' mouths during the post-fledging dependence period, and investigated the effect of this treatment by comparing the condition of these birds to that of birds who received a control treatment. Several physiological parameters considered in our analyses indicate that control owlets were in better condition than owlets with brightness-reduced mouth feathers, which suggests that they received more or better food from feeding parents who discriminated between those young. Brightness-dependent reactions of parent owls suggest that visual signalling may be more widely employed than previously thought, and studying birds at night may reveal sophisticated strategies of animal communication.

Introduction

One of the most widely investigated aspects of parental care is the begging behaviour performed by offspring before or during resource allocation by parents. Begging is an activity designed to solve family conflicts over parental feeding, and it regulates both parent-offspring conflict (Trivers 1974) and sibling competition (Mock & Parker 1997). As a general trend, the more intense the begging (and the signals associated with this behaviour), the more resources are provided by parents (Kilner & Johnstone 1997; Godfray & Johnstone 2000).

Early studies on begging assumed that offspring employ a single signal (i.e. begging calls) and most interest was directed towards young vocalizations. However, begging may involve several different signals such as posturing and plumage features (Leonard et al. 2003 and reviews in Budden & Wright 2001 and Wright & Leonard 2002). The combination of vocal and visual components of begging might provide parents with additional information on the state of the offspring and/or reflect different aspects of offspring condition (Johnstone 1996; Kilner 2002 and references therein; Leonard et al. 2003). The different elements involved in begging displays may

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interact synergistically and, in particular, visual cues may increase parental response to vocal cues (Rowe 1999). Finally, offspring detectability by parents can be enhanced by brightness contrast between the white fleshy borders of the gape and its dark surroundings (Kilner and Davis 1998; Kilner 2002; Hunt et al. 2003).

Studies on bird begging have mainly focused on diurnal species (but see for example Roulin et al. 2000; Roulin 2001) and many different hypotheses on the functions of begging have been proposed (reviewed in Wright & Leonard 2002). However, while visual communication in diurnal birds is presently a major topic in behavioural ecology, the possibility that nocturnal species could also communicate using visual signals is a largely unexploited field of investigation. Because any visual signal used in the dark should optimize the use of the scarce light available, achromatic plumage patches (i.e. pigment-free white feathers) are the best candidates for signalling at night, when contrast is more important than colour (Aragonés et al. 1999). The total amount of light reflected by a white patch may be exploited as a high-contrast signal against dark backgrounds (Endler 1993; Endler & Théry 1996). Indeed the conspicuousness of fleshy flanges bordering an offspring's gape increases with decreasing illumination in or close to the nest, providing parents with more conspicuous targets when feeding under low ambient light (Kilner and Davis 1998).

We previously showed that achromatic plumage has a signalling role in adult eagle owls *Bubo bubo* (Penteriani et al. 2006, 2007). Intriguingly, a white border of feather appears at the edges of eagle owls' mouths just before fledging (approx. 35 d of age, Fig. 1a), and it becomes considerably less apparent upon dispersal [approx. 150 d of age; Fig. 1b(A–D)], when juveniles and adults have a similar appearance. From fledging to dispersal the young continue to depend upon their parents for food. During this period, telemetry data indicate that the mean distance of owlets from the nest is 500 m and the mean distance between siblings is higher than 200 m (Penteriani et al. 2005).

In the current study, we test the hypothesis that such white feathers play a role in parent-offspring communication during the post-fledging dependence period. We hypothesize that if parent eagle owls adjust food allocation based on the visual signals of offspring, then experimentally reducing the brightness of the mouth feathers should engender poorer physical condition in the young.

Materials and Methods

Study Site and Experimental Design

Our experiment was carried out from late March to early May 2005 on 19 owlets from seven different nests located in the Sierra Norte of Seville (37°30′N, 06°03′W, SW Spain; more details in Penteriani et al. 2005).

To locate owlets after fledging, they were fitted with 30-g harness mounted backpacks (Biotrack Ltd, Wareham BH20 5AJ, Dorset, UK) at the age of 30–35 d (see Penteriani et al. 2005 for more details on radiotracking procedures). The weight of the tags corresponds to <3% of the weight of the smallest adult male (1550 g) of our eagle owl population ($x \pm SE$: 1667 ± 104.8 g, n = 9 males), and only a bit more of the weight of owlets at the stage at which we put the tag ($x \pm SE$: 1246 ± 195.5 g, n = 55). Because at this time the young are still growing, backpacks were adjusted in such a way that the Teflon ribbon could expand and allow for the increased body size. The capture of owlets was always very easy and safe because, in the age range in which we carried out the experiment, they stay motionless when humans approach. After 3 yr of continuous radiotracking of 50 eagle owls (both breeders and floaters), we never recorded a possible adverse effect on birds, several nestling marked by backpacks being now successful breeders of our population (M. M. Delgado & V. Penteriani, unpubl. data).

Nestlings were aged following Penteriani et al. (2005) and sex was determined by molecular procedures using DNA extracted from blood (Griffiths et al. 1998).

From the time that each owlet was 35 d old (when it is still growing, Penteriani et al. 2005), it was visited every 7 d during a period of 3 wk. On the first visit, owlets were randomly allocated to an experimental group: either control or brightness-reduced (10 and nine chicks respectively). Both treatments were represented within each nest (n = 7), although the number of nestlings per treatment was not always balanced ($\tilde{x}$ three chicks per nest, range 2–4). The allocation of birds to each treatment did not differ with the hatching order of chicks within the nest ($\chi^2 = 2.20$, df = 3, p = 0.532).

The total reflectance (hereafter also 'brightness') of the mouth feathers was measured just before the treatment as the sum of the reflectance spectra in the range 360–700 nm using a Minolta CM-2600d

Fig. 1: (a) The white mouth feathers of an owlet during the post-fledging period (approx. 75 d old). (b) Evolution of the white edge of the mouth of eagle owl young, from 10 d old to dispersal. (a) Ten-day-old eagle owls at nest, begging for food. At this stage, the white feathers around the mouth have still not appeared. (b) Three young of 32 (in the middle), 30 (on the right) and 28 (on the left) d old begging when female (on the right of the picture) arrives at the nest with a rabbit. At this stage (approx. a week before fledging) white feathers start to become evident, mainly in the older offspring. (c) Fledgling owl waiting for parental feeding a few hundred meters from the nest. At this stage, a large white edge surrounds the mouth. (d) Adult eagle owl. White feathers are less apparent now and a new, white patch has appeared as a large badge on the throat (see Penteriani et al. 2006).

portable spectrophotometer (Minolta Co., Ltd., Osaka, Japan) with UV (xenon flashlight source) and visible light (standard illuminant D65). Brightness before manipulation showed 23% and 77% between- and within-nest variation respectively (Fig. 2). The size of the white border did not differ between treatments within the nest (width: $F_{1,6} = 0.60$, $p = 0.467$; length $F_{1,6} = 0.56$, $p = 0.483$).

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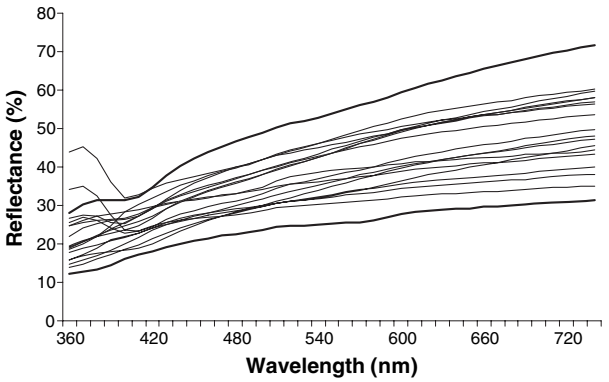

Fig. 2: Reflectance spectra of the natural (pre-treatment) variation on brightness of the owlet mouth feathers (n = 19). Bold lines represent the maximum and minimum recorded values. Brightness before manipulation showed 23% and 77% between- and within-nest variation, respectively (calculated from the sum of squares; see Materials and Methods).

Similarly, brightness did not show significant differences between groups ($F_{1,6} = 0.38$, $p = 0.559$).

To produce two different levels of reflectance, and following the same procedure than in Penteriani et al. (2007), the white feathers were smeared with differing amounts of a 40/60% (w/w) mixture of duck preen gland fat. The birds received one or two layers of the mixture (controls and treated birds respectively), which were spread with a brush on the white surface. The treatment covered the entire white surface, being therefore independent from the size of the trait. Feathers of the brightness-reduced offspring were also smeared with an UV-absorbing chemical (50/50 w/w blend of Parsol 1789 and MCX; Roche, Dubendorf, Switzerland). We used this UV filter because part of the reflectance spectrum of this patch belongs to the UV range (Penteriani et al. 2006). The manipulation produced two groups of chicks whose brightness (x ± SE: control = 1401.9 ± 120 nm, brightness-reduced = 777.3 ± 116 nm) significantly differed within the nest ($F_{1,6} = 19.13$, $p = 0.007$). The manipulation reduced the brightness of the mouth's feathers within the range of total reflectance recorded in our population (V. Penteriani & M. M. Delgado, unpubl. data). Over the 3 wk of the experiment, we measured the total reflectance of the owlet mouth feathers at each visit, to verify that the treatment was effective during the whole duration of the experiment. If necessary, we washed and reapplied both the preen gland fat and UV-blocking substances. At the time of each new treatment, we verified that the new process of washing and reapplying these oily substances did not cause substantial feather changes in reflectance (see Penteriani et al. 2007).

During this first visit, we also took both morphometric measurements and blood samples (2 ml, from the brachial vein). Morphometric measurements included length of forearm (using a digital calliper, ± 0.1 mm) and body weight (to the nearest 10 g), two of the most useful body measurements in describing growth patterns in this species (Penteriani et al. 2005). Blood samples were stored in tubes with heparin at 4°C until arrival at the laboratory, where they were centrifuged for 10 min at 1699 g and the plasma was separated and stored at −78°C.

During the second visit, T-cell-mediated immune response of fledglings was evaluated by means of phytohaemagglutinin skin tests (e.g. Alonso-Alvarez & Tella 2001). Young were injected subcutaneously in the wing web with 100 μl of 2 mg/ml PHA-P (phytohaemagglutinin from *Phaseolus vulgaris*; SIGMA, reference: L-8754) diluted in phosphate-buffered saline (PBS) after the injection site was marked. The thickness of the wing web was measured (±0.01 mm) with a spessimeter at the injection site immediately prior to and 6 h after the test. This procedure has been recently validated by Navarro et al. (2003) and minimized the handling disturbance of young. Moreover, Martin et al. (2006) have described in sparrows (*Passer domesticus*) that the highest lymphocyte response is detected at the skin site after only 6 h from the injection. T-cell immune challenge was performed simultaneously in both wings to obtain an improved measurement of spatial repeatability (i.e. Granbom et al. 2004; see also Smits et al. 2001). The swelling in the wing web of each wing was measured three times. As the repeatability of these six measurements was highly significant ($F_{18,95} = 4.50$, $p < 0.001$, $R = 0.40$; following Lessells & Boag 1987), x-values were used for subsequent statistical analyses. Swelling of the wing web (i.e. the cell mediated immune-response) was calculated as the difference in thickness of the wing web prior to and 6 h after the injection. Following Smits et al. (1999), we did not use control wings (injected with PBS).

One week after the immunity test (i.e. 2 wk after taking the first sample) young were again weighed and measured, and blood samples were taken from them for the last time. To obtain plasma, blood samples were processed and stored as above described.

We did not record any adverse effects of the chemicals that we smeared on the feathers, as well as of the repeated blood sampling or the PHA skin

test. In fact, the individuals used for experimental purpose showed both survival and dispersal patterns similar to the juveniles that we marked in both this same year and in the previous 2 yr (M. M. Delgado & V. Penteriani, unpubl. data).

Blood smears (fixed with GIEMSA method) were used to determine by microscopy (×40) the prevalence and intensity of *Leucocytozoon spp.* infestation. *Leucocytozoon* shows very high prevalence in other owl species (e.g. Korpimäki et al. 1993; Tome et al. 2005), being associated with reduced breeding output in adults (Korpimäki et al. 1993).

From plasma samples, cholesterol and uric acid concentrations were determined using a spectrophotometer (Screenpoint 2; COR SRL, Ginestra Florentina, Italy), using commercial kits (BIOLABO). All measurements were performed in duplicate in the same assay. Repeatability was very high (uric acid: $F_{11,12} = 506.2$, $p < 0.001$, $R = 0.99$; cholesterol: $F_{11,12} = 43.67$, $p < 0.001$, $R = 0.96$; Lessells & Boag 1987). Cholesterol level has been proposed as a good index of body condition in birds because it predicts variability within an individual range of body mass, that is, from the maximum to the lowest level before the death for starvation (Alonso-Álvarez et al. 2002; Alonso-Álvarez & Velando 2003). Meanwhile, uric acid has been used as body condition index in raptors (e.g. Ferrer 1990, 1992, 1993), high levels being associated with advanced level of starvation (García-Rodriguez et al. 1987; see also Alonso-Alvarez & Ferrer 2001). Moreover, as uric acid is a nitrogen waste derived from protein metabolism, high concentrations can be also indicative of high dietary protein intake in raptors (Lumeij & Remple 1991), as well as in other avian species (Okumura & Tasaki 1969; Kern et al. 2005).

Statistical Analyses

The differences between both treatments were determined by exploring within-nest variability. Analysing within-nest variability is appropriate because the hypothesis should be tested into the context where selection could be acting, thus controlling for other confounding variables (e.g. differences in food availability between nests). In this way, 'nest identity' was used as the subject of analysis. Repeated measurement mixed models were applied (PROC MIXED in SAS software; SAS Institute 2001), testing the treatment (control and brightness-reduced) as within-subject effect (REPEATED statement in PROC MIXED). Nest identity was termed as subject (SUBJECT statement in PROC MIXED). This is a conservative procedure compared to the simpler use of nest identity as a random effect (e.g. Bennington & Thayne 1994; Wolfinger & Chang 1995). Moreover, to allow balanced statistical tests, an average value was obtained when two chicks from the same experimental group were present in a same nest. The analyses were then weighted by the number of young used to obtain such x-value (one or two; WEIGHT statement in SAS). To control for initial variability, the initial values of each dependent variable were always included as a covariate. The sex of the young was also tested as fixed factor. However, the effect of the sex and its interaction with the experimental treatment were never significant (always $p > 0.15$), being therefore removed from the models. In a similar way, Julian calendar date and nestling age were tested as covariates and later removed (always $p > 0.10$). The lower degrees of freedom in some tests are explained by the lost of one blood sample due to haemolysis and by the deterioration of a blood smear during the storage (tests of plasma biochemicals: n-1; tests on parasite rates: n-2). *Leucocytozoon* prevalence was non-normally distributed, being tested by non-parametric statistics. The rest of dependent variables met the normal distribution requirement (Shapiro–Wilk tests). Data shown in results and figures are least square $x \pm SE$ from the mixed models (LSMEANS statement is SAS).

Results

Birds allocated to each treatment did not differ in body mass and forearm length on the first visit ($F_{1,6} = 0.06$, $p = 0.81$ and $F_{1,6} = 0.07$, $p = 0.67$ respectively). Similarly, the other dependent variables did not show any significant initial bias ($p > 0.10$ in each case).

Body mass and body size did not differ significantly between treatments at the end of the experiment (body mass: $F_{1,5} = 2.39$, $p = 0.183$, initial body mass as covariate: $F_{1,5} = 12.71$, $p = 0.016$; $x \pm SE$: control = 1600 ± 58, brightness reduced = 1485 ± 63; forearm length: $F_{1,5} = 3.36$, $p = 0.126$, initial length as covariate: $F_{1,5} = 10.96$, $p = 0.021$; control = 187.9 ± 2.3, brightness reduced = 184.4 ± 2.5). Moreover, size-controlled body mass at the end of the study did not differ (body mass: $F_{1,5} = 0.31$, $p = 0.604$, forearm length as covariate: $F_{1,5} = 9.42$, $p = 0.028$).

Among biochemical indices of condition, uric acid concentration was lower in individuals with experi-

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mentally-reduced brightness ($F_{1,4} = 9.48$, $p = 0.036$, initial level as covariate: $F_{1,4} = 0.06$, $p = 0.822$; Fig. 3a). Meanwhile, although cholesterol was higher in control birds, it did not differ significantly between both treatments ($F_{1,4} = 2.46$, $p = 0.192$, initial level: $F_{1,4} = 2.05$, $p = 0.225$; Fig. 3b). Both results also held when the covariate was removed (uric acid: $F_{1,5} = 10.24$, $p = 0.024$; cholesterol: $F_{1,5} = 1.33$, $p = 0.300$).

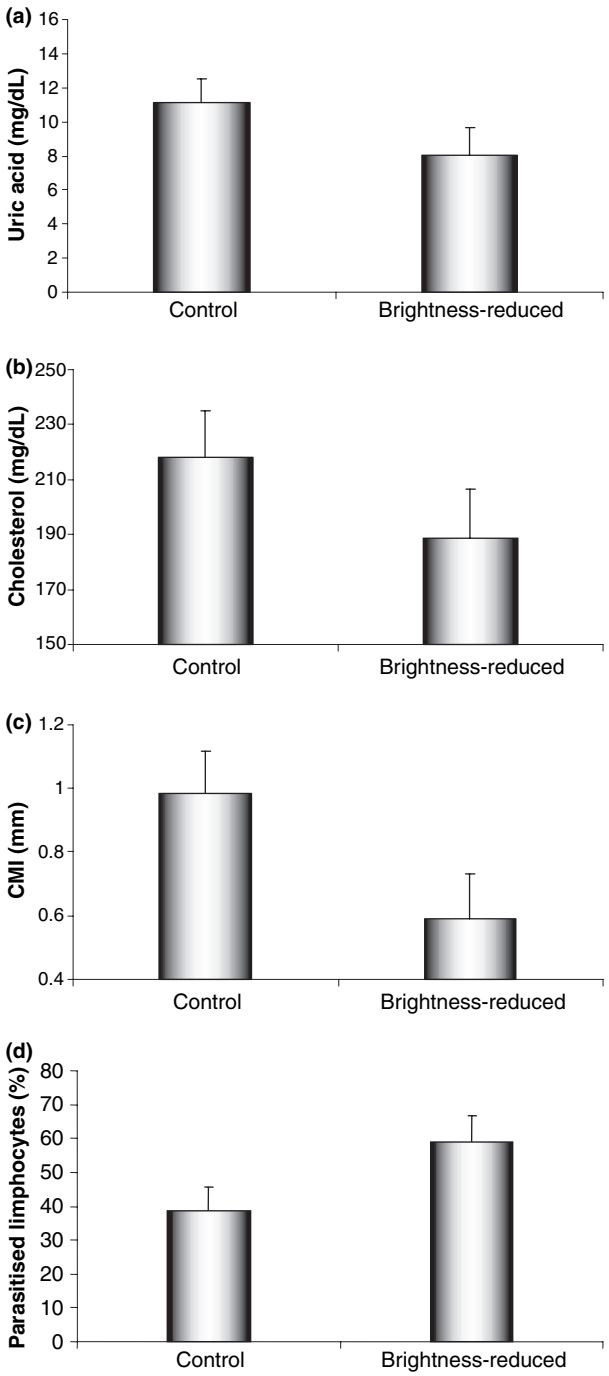

Fig. 3: Uric acid (a) and cholesterol (b) plasma levels, T-cell mediated immune response (c) and intensity of *Leucocytozoon* infestation (d) between eagle owl fledglings with control and brightness-reduced mouth feathers. Bars represent values at the end of the experiment taking into account initial variability (least squared $x \pm SE$ from the models; see Materials and Methods).

Brightness-reduced individuals showed a weaker T-cell mediated immune response ($F_{1,6} = 8.99$, $p = 0.024$; Fig. 3c). Finally, *Leucocytozoon* prevalence was very high in both groups (control: 88%; brightness reduced: 80%; $\chi^2 = 0.133$, $df = 1$, $p = 0.72$). However, the proportion of parasitized lymphocytes (i.e. intensity) was significantly higher in brightness-reduced birds ($F_{1,3} = 28.58$, $p = 0.013$; Fig. 3d). The same occurs when the initial levels are included into the model ($F_{1,2} = 36.24$, $p = 0.027$; initial level: $F_{1,2} = 1.56$, $p = 0.339$).

Discussion

Our results indicate that the white edge of the mouths of fledgling eagle owls plays a role in parent-offspring communication during feeding, as control owlets showed a better physical condition than owlets with brightness-reduced feathers. This suggests that feeding parents can actively discriminate between their young. However, we have to note that, due to the nocturnal habits of the species and the distances among young after fledging, it was impossible to directly measure the response of the receivers (e.g. to record direct evidence of active discrimination by parents).

High uric plasma levels in control birds would reflect their higher protein intake (e.g. Lumeij & Remple 1991; Kern et al. 2005). Such result would be consistent with their stronger T-cell mediated immune response. In fact, the nature of this particular immune defence is associated with the amount of dietary protein among different avian species (e.g. Lochmiller et al. 1993; Saino et al. 1997; González et al. 1999). Moreover, the absence of differences in body mass (and in size-controlled body mass) would discard the possibility that uric acid would be reflecting different levels of starvation (García-Rodriguez et al. 1987). The higher intensity of *Leucocytozoon* infestation in brightness-reduced fledglings completes this scenario. Thus, control birds would have received more/or better food, allowing to mount stronger immune responses and avoiding parasite infestations.

The fact that no significant effect on body mass/-size variability was detected supports the idea that results were mostly associated with differences in

food quality (high or low protein content). Parents would tear to pieces the prey when arriving close to the young. Owlets with high brightness feathers would first receive the food items, which probably contain a higher proportion of muscles (i.e. protein). Thus, though the total amount of food could not differ, the nutritional quality of the diet could have deeply diverged. Alternative, the lack of significant differences in body mass/size could be due to the low sample size, which would not allow rejecting the null hypothesis.

From the results as a whole we can hypothesize that: (1) parent owls differentiated between manipulated and control offspring, allocating fewer or worse resources to owlets with brightness-reduced mouth feathers. This suggests that visual cues might signal some aspect of owlet quality to parents; and (2) white mouth feathers appear to influence parents during feeding after fledging when foraging is particularly costly due to large inter-sibling distances and the wide range of movements made by fledglings in the vicinity of the nest (see Introduction).

Results from studies in diurnal species seem to support these ideas. In fact, diurnal parents have been shown to: (1) be more attentive to variation in gape morphology when foraging becomes costly (Reed & Freeman 1991); (2) allocate more food to more brightly coloured offspring (Lyon et al. 1994; Kilner 1999; Saino et al. 2000a); and (3) reduce provisioning rates of young with manipulated gape flanges (Schuetz 2005). Moreover: (4) Tshirren et al. (2005) concluded that the breast plumage coloration of nestling great tits (*Parus major*) might be important in parent-offspring interactions only during post-fledging; and (5) chicks of precocial species only remain ornamented during the time in which they depend upon their parents for food (Krebs & Putland 2004).

Several studies proved that mouth colours associated with begging signals can indicate a need for food as well as other components of the offspring's general state (i.e. condition-dependent traits) and, consequently, influence parental decisions in favour of offspring with higher reproductive potential (Kilner & Johnstone 1997; Saino et al. 2000a; Saino & Møller 2002). For example, mouth colour seems to signal health in nestling barn swallows *Hirundo rustica* (Saino et al. 2000b) and hunger in some finch species (Kilner & Davis 1998). Finally, Mennill et al. (2003) showed that an achromatic trait, the white and black plumage of the black-capped chickadee *Poecile atricapilla*, describes status-related variability and sexual dichromatism. Therefore, we can speculate that the white feathers of this owl have evolved under selective pressure on parents to maximize their fitness during parental care.

However, we cannot ignore the possibility that parental food allocation simply followed a fixed mechanism set by scramble competition (Parker et al. 2002), in which parents passively fed the offspring presenting the greatest stimulus (i.e. highest brightness).

Our results could also lend further support to the hypothesis that mouth colours of nestling also act to enhance offspring detectability (see Kilner 2002; Heeb et al. 2003; Hunt et al. 2003). In fact, the white border appears when offspring need to be fed quite far from the nest and siblings can be relatively distant from each other (Penteriani et al. 2005). In the dark, white feathers could aid parents in locating their offspring during begging. If this is the case, poor reflecting nestlings could have an increase in energy expenditure (e.g. through increased movements due to a reduction in parental feeding) leading to the observed changes between experimentally reduced brightness and control individuals.

In conclusion, the trait might have evolved under selection for both reliable signalling of offspring general state (i.e. signalling hypothesis; Godfray 1991) and/or increased conspicuousness enhancing their detectability in dark surroundings (e.g. a beacon around offspring mouths; Kilner and Davis 1998; Kilner 1999).

An alternative hypothesis on offspring plumage ornamentation suggests that it could appear as a non-selected by-product of selection on adult phenotypes (Krebs & Putland 2004). We can exclude this possibility because the white marks exhibited by owlets differ from the white badge of the adults, and a different, appealing divergence can be highlighted; both offspring and adult eagle owls show white plumage markings, but the former is a broad border around the mouth while the latter is a wide badge on the throat. This shift in visual cues may be related to a substantial modification in vocal signalling. In fact, the smaller white mark of young is associated with the 'soft' call of young (Penteriani et al. 2005), mainly used for short-range communication (e.g. with siblings and parents), whereas the bigger badge of adults (only shown during vocalizations) plays a role in long-range communication (e.g. territorial context; Penteriani et al. 2007), their call being audible up to 1–1.5 km away.

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Above all, the brightness-dependent reaction of parent owls suggests that visual signalling could represent an overlooked element in nocturnal animals' communication, which may be more widely employed than previously thought. Until now, visual communication has been considered important only for diurnal birds, but more sophisticated strategies of animal communication may still be revealed by studying birds at night. Living in the dark does not necessarily mean a blind life.

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