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Sex difference in mouth coloration and begging calls of barn swallow nestlings

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In diverse animal taxa, offspring produce signals that serve the function of soliciting parents to provision care. In birds, such 'begging' signals may consist of vocal and visual displays that influence parental decisions on allocation of critical food resources among the progeny. According to sex allocation theory, parents may gain fitness benefits from favouring offspring of either sex, depending on ecological conditions or parental quality. However, adaptive allocation of care can occur only if parents can identify the sex of their offspring, which, however, appear to be sexually monomorphic in most bird species. Begging displays may be a vehicle of such sex-specific signals. However, only very few studies have investigated sex differences in begging calls and none has analysed sex differences in a main component of begging displays of passerine birds, that is, mouth coloration. In the present study, we show that male nestling barn swallows, *Hirundo rustica*, have more brightly coloured mouths than their female broodmates early in the nestling period. Sex differences in mouth coloration disappear later in the nestling period when, however, differences in begging calls develop, confirming the results of a previous study. Thus, begging displays carry sex-specific components whose nature (visual or acoustic) changes during offspring ontogeny and might mediate the differential access of male and female offspring to parental care observed in this species, particularly under adverse rearing conditions.

Keywords: barn swallow; begging calls; begging display; *Hirundo rustica*; nestling mouth coloration; parent–offspring communication; sex allocation; sexual dimorphism

Animals have evolved behavioural and morphological characters in the young which currently serve the function of soliciting parents to provide care and/or enhancing the chances of prevailing over siblings in competition for limited parental resources (Clutton-Brock 1991; Mock & Parker 1997; Wright & Leonard 2002). In birds, parent–offspring communication and sibling competition are mediated by both visual and acoustic signals including posturing, showing a brightly coloured mouth while gaping, producing loud stereotyped vocalizations, displaying ornamental plumes and scrambling to gain additional depreciable care from parents, typically food (e.g. Kilner 2002).

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The evolution of 'begging' signals (sensu Kilner & Johnstone 1997) has been interpreted under diverse perspectives. Begging signals have been envisaged as reliable indicators of offspring quality that have been selected by parental preference for provisioning offspring with higher reproductive value (Godfray 1991, 1995a, b; Kilner & Johnstone 1997). An alternative perspective is that begging signals are mechanisms that offspring adopt to obtain a greater share of care by manipulating parental decisions to their own advantage (Macnair & Parker 1978, 1979; Parker & Macnair 1978, 1979; Parker et al. 2002). Begging signals have also been interpreted as means to allow easier localization of the offspring mouth by the attending parent, thus enhancing the efficiency of food provisioning behaviour particularly in hole-nesting species (Ficken 1965; Kilner & Davies 1998; Kilner 1999; Heeb et al. 2003; Hunt et al. 2003).

A rapidly expanding field of interest in evolutionary biology is the study of sex allocation of parental care (Fisher 1930; Trivers & Willard 1973; Charnov 1982; see also Hardy

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2002). To adaptively modulate their postnatal investment in sons and daughters, parents are expected to have evolved the capability of exploiting sex-related offspring signals. Thus, the investigation of sex differences in characters that may allow parents to discriminate between the sexes at early life stages is pivotal for sex allocation theory.

Sexual differentiation can begin early in life (see Balthazart & Adkins-Regan 2002; McCarthy & Konkle 2005). Depending on the ontogenetic trajectories, sex-related differences in phenotype may arise before offspring attain independence. Sex differences in begging signals may occur if sex-linked genes responsible for sexual differences start to be expressed or if the environment (e.g. early maternal effects such as sex hormone concentrations in the egg) to which sons and daughters are exposed differs early in life when the offspring are still attended by their parents. Some bird species (e.g. Falconiformes) show marked sexual dimorphism during the nestling stage mainly in the form of sex-related body size variation (e.g. Bortolotti 1986; Dijkstra et al. 1998). However, the chicks of most bird species appear to be monomorphic in size as well as other morphological characters at least to the naked human eye (Cramp 1994).

Very little is known about the existence of sex differences in begging signals of birds. In a study of the western bluebird, *Sialia mexicana*, no sexual dimorphism was found in sonagraphic features of begging display (Monk & Koenig 1997). In a previous study of the barn swallow, *Hirundo rustica* (Saino et al. 2003b), we found that begging calls of males differed from those of females at age 16 days but not at 12 days. However, we could find no study reporting on sex differences in mouth coloration among the nestlings of any bird species. Several correlational and experimental studies have shown that parental behaviour is influenced by the expression of such signals (e.g. Saino et al. 2000b, 2003a). For instance, mouth coloration of barn swallow nestlings has been shown to be a condition-dependent component of begging display (Saino et al. 2000a, b; de Ayala et al. 2007), which affects parental behaviour (Saino et al. 2000b; de Ayala et al. 2007).

In the present study our main aim was to test for the first time whether the coloration of nestling mouth measured at two ages (6 and 12 days after hatching, i.e. at approximately one-third or two-thirds of the nestling period) differed between male and female offspring. In addition, we conducted a confirmatory study to test for consistency of sex differences in features of begging calls which were documented earlier in the same population of barn swallows (Saino et al. 2003b).

METHODS

Field Methods and Sex Determination

We studied nestling mouth coloration in four colonies (=farms) and begging calls in eight colonies east of Milano (northern Italy), during spring 2005 and 2006. The study was conducted under permission of the local administration (Regione Lombardia). In both years, we inspected the nests every day according to standardized protocols to record adult breeding activities and hatching dates. We individually marked the nestlings with colour rings at day 6 after hatching, measured body mass (to the nearest 0.1 g; TANITA1479V9) and tarsus length (approximation 0.01 mm) at the age of 6 and 12 days and collected a blood sample in capillary tubes by puncturing the brachial vein for molecular sex determination after polymerase chain reaction amplification of the sex-specific avian CHD-1 gene, following the procedure originally devised by Griffiths et al. (1998) and reported in Saino et al. (2002). Control samples from individuals of known sex were always included in the sexing protocol. The sexing procedure was successfully validated by sexing 20 adults of known sex and resexing 20 putative males and 20 putative females that had been already sexed in another lab. Nestlings were all handled in a similar way for a few minutes. We took half of the brood at a time so that the nest was never left empty.

Recordings of Mouth Coloration

The coloration of the flanges and the palate was measured by means of a spectrometer powered by a deuterium–tungsten halogen light source (Avantes AvaSpec 2048). The reflectance (%) of the mouth was computed relative to that of a standard white tablet (WS-2). The reflection probe was positioned inside a matt black plastic tube. The end of the tube was cut at a 45°, so that when the probe was applied to the mouth specular reflection was prevented. The illuminated field was about 7 mm^2. Each reading consisted of an average of 15 scans and each mouth region (flange and palate) was measured twice. The two measures of the flanges of each side were taken close to the commissure of the mandibles. The measures of the palate were taken at the posterior end of the *Rostrum maxillare* between the two controlateral *Ruga palatina lateralis*. The standard white was checked before every chick was measured to verify 100% reflectance and the spectrometer was recalibrated before every brood was measured.

Reflectance data were summarized according to the procedure suggested by Endler (1990) and Bennett et al. (1997). First, reflectance measured at 0.34-nm intervals between 300 and 700 nm were averaged within 10-nm bandwidths. Thus, reflectance information from each spectrum was reduced to 40 reflectance measurements (one for each 10-nm bandwidth between 300 and 700 nm), and mean reflectance within any given 10-nm bandwidth was then referred to the median wavelength of that band. Since two spectra were recorded for each nestling at all measurements, the data were averaged between spectra. The means of the two controlateral measures of the flanges were averaged between sides. The 40 variables consisting of reflectance measurements for each band were subjected to principal component analysis (PCA). Only the principal components (PCs) associated to an eigenvalue larger than 1 (corresponding to approximately 2–3% of the total variance) were extracted and the coordinates (scores) of individual spectra were obtained. PCA efficiently summarized the information contained in the 40 reflectance variables: more than 97% of the total variance was accounted for by the two to four principal components that were extracted. The interpretation of PCs was based on factor loadings of each reflectance variable on any given principal component.