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ORIGINAL ARTICLE

Distinct colour morphs in nestling European Bee-eaters *Merops apiaster*: is there an adaptive value?

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Abstract In a few bird species, dimorphism already exists in nestling and juvenile plumage coloration and these colour morphs are often attributable to different sexes. In this study we detected variation in nestling coloration among European Bee-eaters *Merops apiaster*. We identified two distinct colour morphs, namely nestlings with yellowish-brown and nestlings with green back feathers. By means of genetic methods, we determined nestling sex. It turned out that the back colour is a significant indicator for sex. Male nestlings have yellowish-brown and females green back feathers, but there are exceptions. Population sex ratio was about equal but we found sex-biased variation in several nests. Furthermore, we found evidence that colour is an indicator for condition especially in those individuals where sex and coloration do not match.

Keywords Plumage polymorphismus · Juvenile coloration · Sexual dimorphism · *Merops apiaster* · Condition dependence

Zusammenfassung Farbddimorphismus im Gefieder von Nestlingen kennt man von einigen Vogelarten. Häufig ist dieser Farbdimorphismus geschlechtsspezifisch. Wir fanden einen solchen Farbdimorphismus bei Europäischen Bienenfressern *Merops apiaster*. Nestlinge haben entweder braune oder grüne Rückenfedern. Mit Hilfe genetischer Methoden haben wir das Geschlecht bestimmt. Die Ergebnisse zeigen eine hohe aber nicht vollständige Übereinstimmung zwischen Geschlecht und Gefiederfärbung. Das Geschlechterverhältnis in unserer Population scheint ausgewogen obwohl einzelne Nester in beide Richtungen geschlechtsverschoben sein können. Unsere Ergebnisse deuten weiters darauf hin, dass Gefiederfärbung auch mit der Kondition zusammenhängt, speziell bei den Individuen bei denen Geschlecht und Färbung nicht übereinstimmen.

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Introduction

In adult birds, plumage colour polymorphism is widespread (Andersson 1994; Hill 2006). There are few species with dimorphism in nestling and juvenile plumage coloration and these morphs are often attributable to different sexes. In juvenile Bearded Reedlings (*Panurus biarmicus*), sex differences are most visible in bill colour, and in juvenile Scottish Crossbills (*Loxia scotica*), the sexes show dichromatism in contour feathers and breast coloration (Edelaar et al. 2005). Nestling dichromatism, however, does not necessarily mean sexual dimorphism as in the Mute Swan (*Cygnus olor*) (Conover et al. 2000).

Colour morphs reflecting sexual dimorphism may be important in early sex recognition as found in the Bearded Reedling (Glutz von Blotzheim and Bauer 1994) which forms pair bonds shortly after fledging and Red Crossbills

(*Loxia curvirostra*) which even reproduce successfully while still in juvenile plumage (Edelaar et al. 2005). Furthermore, assuming that production costs vary between different colours (e.g. structural-, melanin- or carotenoid-based colours), different colour morphs may reflect quality differences caused by e.g., nutrition status, infectious diseases and parasite infestations. In Spotless Starlings (*Sturnus unicolor*), it was shown that differential coloration may reflect intrinsic characteristics, such as producing an efficient immune response (Soler et al. 2007).

Variation in juvenile coloration among European Bee-eaters (*Merops apiaster*) was mentioned in Glutz von Blotzheim and Bauer (1994) and Lessells (personal communication in Kilner 2006) reported green crown coloration in female nestlings, which disappeared during pre-basic moult. In contrast, in an earlier study, we found evidence for variation in coloration of back feathers in nestling European Bee-eaters. Therefore, we wanted to investigate whether this colour variation in back feathers (1) can be attributed to distinct colour categories and result in dichromatism in nestling plumage, (2) is sex dependent, and (3) is condition dependent.

Methods

We studied nestlings from eight nests from five different colonies (colony size varied between 5 and 33 breeding pairs), all of them in man-made sandpits, usually no higher than 3–4 m, in south-western Slovakia (47°48′–47°56′N, 18°15′–18°44′E) during the breeding season of 2000. Being long-distance migrants with over-wintering areas in East and South Africa, European Bee-eaters arrive at their breeding grounds at the beginning of May and usually start digging new horizontal burrows in sandbanks (Cramp 1983). At our study sites, these burrows are about 1 m in depth. In our sample, brood size varied between three and five chicks with five being the most usual number (average ± SE, number of nestlings is 4.17 ± 0.83, $n = 8$ nests). For each nest, we determined the number of chicks surviving for 15–20 days (nestlings start to fledge at about 20 days in our population). Nest checks were conducted either by means of an endoscope or by counting the number of living or dead chicks or unhatched eggs when chicks were removed from the nest for morphological measurements, blood sampling and weighing (Hoi et al. 2002). We used a spoon tied to a pole (1.5 m in length) to remove the chicks from the nest.

For each chick, we measured wing length according to Svensson (1993) and body weight by means of an electric balance to the nearest 0.1 g. There is a marked weight recession in older chicks some days before they fledge. Since it was impossible to determine chick age accurately for most nests, and to avoid heteroscedasticity and a non-linear weight increase, we used only chicks within an approximate weight range of 47–71 g. That weight is typical for chicks between 10 and 15 days old (Belskaja 1976; Randík 1961). We calculated the relationship between wing length (as a measure of size and chick age) and body weight ($r = 0.47$, $P < 0.006$, $n = 33$). Wing length is considered to be a good predictor of nestling age in birds (Lessells and Ovenden 1989). The relationship between wing length and body mass within this age range has been shown to be highly linear (Hoi et al. 2002). Weight deviation (residual body weight) with wing length as the selection criterion (x variable) was used as a measure of chick condition. Blood samples (50–70 µl), drawn from the brachial vein, were collected for genetic sex determination.

Total genomic DNA was extracted from the blood with the DNeasy tissue kit and according to the standard procedure for animal tissues. For sex-typing, we amplified parts of the CHD gene, which is located on both the Z and the W chromosome in non-ratite birds (Dubiec and Zagalska-Neubauer 2005). The gene contains at least two introns which differ in length between the sexes. Two primer sets, P2/P8 (Griffiths et al. 1998) and 1237L/1272H (Kahn et al. 1998), binding on different sites within this DNA region, were used to approve the findings. A 2% agarose gel was used to discriminate between PCR products of homogamous individuals, with only one band, and heterogamous individuals which produce two bands of different lengths.

For colour variation, we additionally visually examined 30 fledgling European Bee-eater specimens from the Natural History Museum in Vienna. Colour measurements were taken from three adult males, three females, and three green and yellowish-brown juveniles of the museum specimens, respectively, and one individual of each category was arbitrarily chosen as an example to demonstrate the difference in colour reflectance (Fig. 2). We measured back coloration using a USB-2000 spectrometer and a DHS-2000-FHS deuterium halogen lamp, connected through a bifurcated fibre-optic probe (Ocean Optics, Eerbek, The Netherlands). To exclude disturbance by outer light sources and for keeping standardised distance and angle (90°), a black rubber cylinder was fitted on the top of the probe. Before each measurement, the spectrophotometer was recalibrated with a standard white (Ocean Optics), for calibration of black, the probe was removed from the light source and the cap of the plug closed. HUE was additionally given as a colour descriptor of reflectance spectra (e.g. Griggio et al. 2009). Measurements were taken from five standardised spots of the lower back plumage of each individual. HUE was calculated as the wavelength at peak reflectance (λ (R_{max})) for reflectance in the 500–700 ηm range.

To determine whether residual body condition explains colour, we used colour as the dependent variable (binary) and residual body mass and sex as the explanatory variables in a generalised linear model of logistic regression. Since including nest as a random effect (to avoid pseudoreplication) in a mixed model did not improve the model fit and the estimated random effect was zero, we excluded nest from the final model. Stepwise discriminant function analyses were performed to examine whether using only morphological parameters (wing, bill length and body mass) or including colour produced better results in terms of the accuracy of sex determination.

Results

In nestlings of European Bee-eaters, we found two distinct colour morphs in juvenile plumage. Colour differences are recognizable on the back feathers of the birds (Fig. 1) and can only be identified in chicks weighing about 50 g (approximately 10 days old) and more. Green nestlings have an entirely green colour on the back and the yellowish-brown morph has brown to yellowish back feathers. The distribution was clearly bimodal and there were no intermediate or ambiguous individuals. Classification by three different investigators revealed 100% concordance. Of 33 nestlings, about the same number of green ($n = 17$) and yellowish-brown ($n = 16$) nestlings occurred. Three nests (37.5%) contained only yellowish-brown, one nest (12.5%) only green and four nests (50%) contained nestlings of both morphs. Additionally, although plumage coloration became paler, differences between green and yellowish-brown fledglings can still be recognized from museum specimens in the Natural History Museum in Vienna ($n = 30$). The spectral curves show that the reflectance peaks of the backs of the yellowish-brown juvenile (HUE = 592.1), the adult male (HUE = 581.7) and the female (HUE = 595.4) are at the same wavelength, whereas the green juvenile (HUE = 575.3) is shifted towards a shorter wavelength (Fig. 2).

The sex ratio among our sample was also almost equal (18 females, 15 males). Colour morphs are non-randomly distributed between the sexes (Fig. 3; binomial test $z = -2.6$, $P = 0.009$). Overall, in 72.7% of the cases, colour is the correct predictor of sex. Therefore, colour differences alone are not sufficient to assign sex accurately. When body mass, wing and bill length and nest were included as discriminating variables, colour ($F = 8.04$, $P = 0.008$) but also body mass ($F = 9.25$, $P = 0.001$) entered the model (Wilk's lambda = 0.62, $df = 2.31$, $P = 0.001$), but according to this model only in 69.7% of the cases can the sex be correctly identified, which is no different from using colour alone. In contrast, when we used only the morphological variable, no variable entered and no significant model could be established (Wilk's lambda = 0.66, $df = 1.31$, $P = 0.2$).

The results of a GLM of a logistic regression revealed that sex is a highly significant predictor for colour ($\chi^2 = 10.06$, $P = 0.0015$) whereas condition itself is not significant ($\chi^2 = 2.48$, $P = 0.11$). We found a significant interaction term, however, between sex × condition ($\chi^2 = 4.12$, $P = 0.042$) which is indicated by the opposite effect of coloration on the condition of the sexes (Fig. 3).

Fig. 1 Shows four European Bee-eater (*Merops apiaster*) nestlings of one nest. Three nestlings with *yellowish-brown back* feathers and one with *green back* feathers (nestling on the right side). According to genetic sex determination (see "Methods"), the three *yellowish-brown* nestlings have been identified as males and the *green* nestling as a female

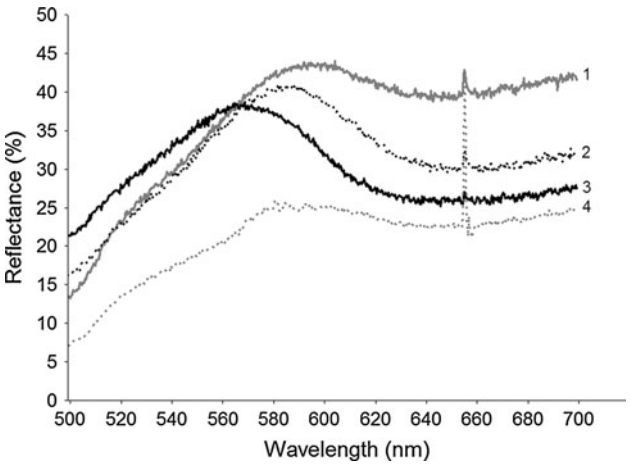

Fig. 2 Shows the spectral curves within 500–700 *η*m of the backs of *1* adult female, *2* yellowish-brown juvenile, *3* green juvenile and *4* adult male European Bee-eaters. All individuals are museum specimens from the Natural History Museum of Vienna

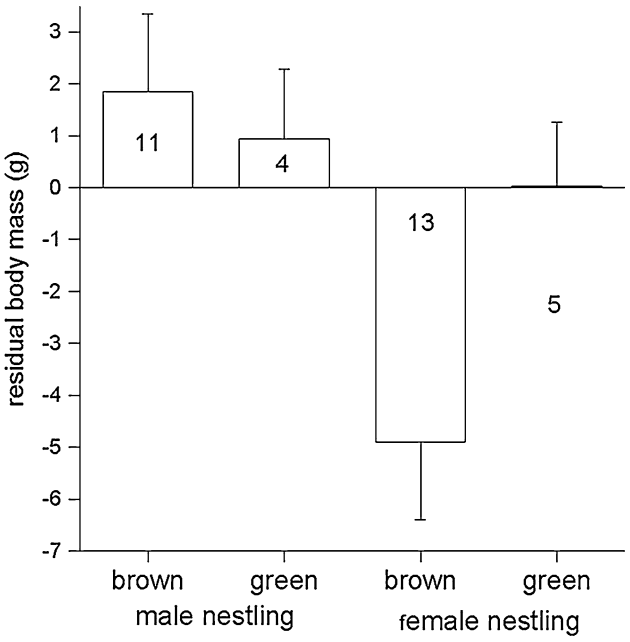

Fig. 3 Residual body mass for *yellowish-brown* and *green* male and female nestlings. Means ± SE and sample (size in *bars*) are given

Green males are lighter than yellowish-brown males, but green females are heavier than yellowish-brown females.

Discussion

Our results revealed that, when back feathers are fully developed, two distinct colour morphs can be found in the juvenile plumage of nestling European Bee-eaters. They can be unmistakably distinguished by either a greenish or a yellowish-brown coloration of the back feathers. This difference can also be seen in the reflectance curves of the green and yellow–brown juveniles (Fig. 2). As indicated by the spectrogram, the yellow–brown juvenile has a reflectance peak of back coloration on the same wavelength as an adult male or female. The green juvenile has a slightly different peak shifted towards a shorter wavelength (Fig. 2). It is interesting that a rather small shift in the peak produces an obvious visible difference in the plumage coloration. Adults in contrast seem to have a less pronounced peak and their reflectance curve does not decrease in the longer wavelengths (Fig. 2). The dichromatism in crown coloration described by Lessells (personal communication in Kilner 2006) was not obvious in our population, indicating that population differences in dichromatism may exist.

Furthermore, we found a clear tendency for these juvenile back colour morphs to be sex-specific. In European Bee-eaters, female nestlings are mostly green and male nestlings are mostly yellowish-brown. This resembles the sex differences in adults, where females differ from males in the larger area of green on the shoulder (Glutz von Blotzheim and Bauer 1994).

Differential allocation of parental investment could be a functional explanation (de Ayala et al. 2007; Tschirren et al. 2005) for sex-specific dichromatism of offspring in European Bee-eaters (Lessells 2002). Body coloration cannot be confirmed as a visual signal in cavities but juveniles are fed for about 3 weeks after fledging (Glutz von Blotzheim and Bauer 1994). Although offspring of sexually dichromatic species usually do not reveal their sex (Kilner 2006), Lessells (2002) identified conditions in which sexual advertisement could evolve, even though one sex would be penalised.

Plumage dimorphism may also play a role in the formation of early pair bonds as suggested for juvenile Bearded Reedlings (Brocchieri et al. 1992; Marin et al. 1994; Todte and Stêpniewski 2002), but in European Bee-eaters there is no clear-cut sex separation according to colour. European Bee-eaters seem to form perennial pairs (Garnett 1984) and undergo a moult during late autumn to early spring (Glutz von Blotzheim and Bauer 1994), but the time of pair formation relative to moult of the back feathers is not yet known.

Finally, the two colour morphs may have no signalling function (Johnston 1967). This is suggested by the fact that this dimorphism is not a reliable sex badge (Borras et al. 1993; Johnston 1967; Senar et al. 2002). Furthermore, sex-specific dichromatism usually increases with age (Kilner 2006). In juvenile European Bee-eaters, however, dichromatism decreases since colour differences in mature birds within wavelengths visible to humans are marginal (Glutz von Blotzheim and Bauer 1994). Whatever the function of colour may be, it can be used to sex juveniles with some confidence, in addition to the use of biometric data alone. The juvenile sex difference corresponds to the expected direction, since, like juveniles, adult females also have more green in their plumage than males, i.e. adult females show more green plumage on their lesser wing coverts than males. Also, in Lesser Grey Shrikes (*Lanius minor*), sex discrimination owing to colour variation can be improved by including biometric data (Krištín et al. 2007). Including biometric data in our nestling European Bee-eaters did not, however, improve sex discrimination (see "Results").

Further studies on the length of persistence of this dichromatism might give additional insight into terms of functional explanations. Although sample size was low, our data suggest a sex-specific difference in growth and a weak association of colour with residual body mass. Hence, it is not clear whether colour is condition dependent. Further studies are needed to evaluate the cost of producing the pigmentation used in the two colour morphs and to investigate parent–offspring as well as offspring–offspring

interactions during the early fledgling period to provide more data for evaluating possible functions of this clear-cut polymorphism. In the future, using spectrophotometry rather than colour classes would allow us to describe colour as a continuous variable and consequently could also enhance sex-specific dichromatism and colour-condition relationships (e.g. green males may be less green than females).

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