PROCEEDINGS B

royalsocietypublishing.org/journal/rspb

Research

Cite this article: Díaz-Lora S, Pérez-Contreras T, Azcárate-García M, Peralta-Sánchez JM, Martínez-Bueno M, José Soler J, Martín-Vivaldi M. 2021 Cosmetic coloration of cross-fostered eggs affects paternal investment in the hoopoe (*Upupa epops*). *Proc. R. Soc. B* **288**: 20203174. https://doi.org/10.1098/rspb.2020.3174

Received: 22 December 2020
Accepted: 6 April 2021

Subject Category:
Behaviour

Subject Areas:
behaviour, evolution, microbiology

Keywords:
eggshell colour, female signalling, male investment, symbiotic bacteria, *Upupa epops*, uropygial secretion

Author for correspondence:
Silvia Díaz-Lora
e-mail: silviadiazlora@ugr.es

Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.5401651.

THE ROYAL SOCIETY PUBLISHING

Cosmetic coloration of cross-fostered eggs affects paternal investment in the hoopoe (*Upupa epops*)

Silvia Díaz-Lora[1], Tomás Pérez-Contreras[1,3], Manuel Azcárate-García[4], Juan Manuel Peralta-Sánchez[2], Manuel Martínez-Bueno[2,3], Juan José Soler[3,4] and Manuel Martín-Vivaldi[1,3]

[1]Departamento de Zoología, Facultad de Ciencias, [2]Departamento de Microbiología, Facultad de Ciencias, and [3]Unidad asociada: Coevolución: cucos, hospedadores y bacterias simbiontes, Universidad de Granada (UGR), Granada, Spain
[4]Departamento de Ecología Funcional y Evolutiva, Estación Experimental de Zonas Áridas (EEZA-CSIC), Almería, Spain

SD-L, 0000-0001-8008-2752; TP-C, 0000-0002-2271-1706; MA-G, 0000-0002-6421-4572; JMP-S, 0000-0003-4648-7988; MM-B, 0000-0002-1488-5642; JJS, 0000-0003-2990-1489; MM-V, 0000-0002-5432-425X

The signalling hypothesis suggests that avian eggshell coloration is a sexually selected female signal advertising her quality to its male partner, thereby stimulating his provisioning rate. This hypothesis has been tested for structural eggshell pigments, but not for cosmetic colorations, such as that produced by the uropygial secretion on eggshells. During the breeding season, female hoopoes (*Upupa epops*) host in their uropygial glands symbiotic bacteria. Females actively smear the eggshells with their secretion, protecting embryos from pathogenic trans-shell infections and changing eggshell coloration. Because the colour of the secretions is related to their antimicrobial potential, cosmetic eggshell coloration may act as a cue or even as a post-mating sexually selected signal if it affects male provisioning rates. To experimentally test this hypothesis, we cross-fostered already-smeared clutches between hoopoe nests, and quantified male feeding behaviour to females before and after the experiment. This approach allows disentanglement of the effects of female quality and of egg coloration on male investment. In accordance with the hypothesis, males adjusted their provisioning rate to the eggshell cosmetic coloration. This is, to our knowledge, the first experimental demonstration that egg colour stained with uropygial secretion could act as a post-mating sexual signal of female quality to males.

1. Introduction

Individual quality can be reflected by phenotypic traits that influence mate choice preferences [1,2] or differential reproductive investment after mating [3,4], being, therefore, sexually selected. Most studies have mainly explored these sexually selected traits in males [5,6], while the role of sexual selection explaining the evolution of females traits have only been recently considered [7,8]. In particular, female post-mating sexual signals causing differential investment by males, are less known, despite the fact they may be responsible for an important component of female reproductive success in species with paternal care [5,9].

An example of the post-mating sexual signal of a female reflecting her quality is avian eggshell coloration [9–19]. Intra- and interspecific variation in eggshell colour have intrigued researchers for a long time. Several adaptive and non-adaptive explanations have been proposed [15,20–25], although not all of them have received a similar level of support [25–27]. The possibility that eggshell coloration is sexually selected (SSEC hypothesis) [15] implies two assumptions: (i) females signal their quality (and hence the potential quality of their descent) to the male partner by means of egg coloration; and (ii) male partners adjust their reproductive investment (for example, their

provisioning rate to the female and nestlings) to eggshell coloration. Several studies have found an association between eggshell coloration and the quality of females in some bird species (e.g. [18,19,28,29]). As for the second assumption, a relationship between parental investment and eggshell coloration has been found through correlational [30,31] and experimental studies [9,13,14,32,33]. However, other studies failed to find such a relationship [18,34–38]. These mixed results make necessary further experimental studies to explore such association, measuring parental investment during the incubation period. This approach will ensure that changes in male investment are caused by eggshell colour rather than by confounding variables associated for instance with nestling phenotype (reviewed in [39]).

The SSEC hypothesis has been mainly focused on pigment-based coloration owing to antioxidant and other physiological properties of biliverdin and protoporphyrin [14,16,30,32,36,40,41], the responsible pigments of the eggshell coloration. However, other external sources of pigments are involved in eggshell coloration. This is, for instance, the case of the eggshell spots caused by the activity of some ectoparasites [42–45]. Deliberate application of cosmetic substances, such as the uropygial secretion, could also affect eggshell coloration, and may function as a cue or signal of female characteristics that would influence male parental investment [46]. The effect of the cosmetic use of uropygial secretion on avian coloration has mainly been investigated for feathers [47–51] and in scenarios of pre-mating sexual selection [48,52,53]. However, it may also function on eggs as post-mating sexual signals, and correlative results suggest that it may be the case in hoopoes eggshell cosmetic colours [31].

Female hoopoes actively cover their eggs with the uropygial secretion taken with the beak, which causes a colour change of the eggshell, from bluish to greenish-grey [46]. The coloration of the uropygial secretion is caused partially by the bacteria living within the uropygial gland of females [40,54–56]. When the female stains the eggshell with the secretion, she transfers these bacteria to the egg surface [57], protecting embryos from pathogenic infections and increasing hatching success [57]. Interestingly, previous studies have demonstrated an association between coloration, the density of bacterial symbionts, and antimicrobial properties of the uropygial secretion of hoopoe females [31,46,58]. In all these studies, the main colour parameter of secretions related to bacterial presence, abundance or antimicrobial activity is saturation. Secretions with higher colour saturation are related to a lower antimicrobial capacity of the secretion [46]. Similarly, eggshells (coated with secretion by females) with higher colour saturation are related to a lower bacterial load of the uropygial secretion [31]. Therefore, more saturated eggshell colours could indicate less antimicrobial capacity of the secretion. Consequently, the cosmetic coloration of the eggshells of hoopoes could inform of the antimicrobial properties of the symbiotic bacterial community hosted within the female's uropygial gland. Thus, differential reproductive investment of males in nests whose eggshell coloration indicates a high antimicrobial capacity of the mate, would be of selective advantage [59]. Therefore, sexual selection could, at least partially, drive the evolution of this cosmetic coloration [46]. Benefits for males differentially investing in reproduction based on egg colour would include higher hatching success [57], and adaptive microbiota that their offspring would acquire from mothers [60–62]. Although correlative evidence support this claim [31], egg colour might covariate with several characteristics including female and male parental quality [28] and, thus, an experimental approach is necessary to discern causes and consequences of this detected association. Unlike most studies of SSEC hypothesis, we focus on male provisioning rate to the female during the incubation period, instead of chick provisioning.

Here, we cross-fostered hoopoe clutches already coated by the secretion of their mothers. We monitored male feeding behaviour to incubating females before (i.e. with their own eggs) and after the experiment (i.e. with experimental cross-fostered eggs). We predicted that, within nests, differences in male provisioning rate should be related to differences in cosmetic coloration between original and cross-fostered clutches. Specifically, the male provisioning rate should increase when the colour saturation of the cross-fostered clutch decreases.

2. Material and methods

(a) Study species

The hoopoe is a cavity nester that readily nests in nest-boxes and is distributed throughout Europe, Africa and Asia. Females lay one or two clutches, typically of six to eight eggs from February to July [63]. They start to incubate with the first or second egg, generating an asynchronous hatching and, therefore, a considerable size hierarchy within the brood [64]. The uropygial gland of incubating females increases in size, producing a greater amount of secretion compared with non-breeding females [58], allowing them to cover the egg surface of the entire clutch [46]. Moreover, unlike other birds, the external structure of hoopoe eggshells is full of small crypts helping in the retention of the coloured secretion [57]. Hoopoes start to paint eggshells with secretion with the first or second egg laid. Few days after the end of laying, the cosmetic coloration is uniform for the entire clutch. Only the female incubates and the male feeds her while she is in the nest (from the start of incubation until the first hatched nestling is around 8 days old). This allows males to see the eggs while provisioning females at the nest entrance. That is because females of hoopoes are small relative to the nest-box size, and they always need to move off the eggs to reach the male's food delivery (see video recording in the electronic supplementary material, S1). Moreover, females go out from the nest several times a day during incubation. The male always tries to feed the female in the nest-box and, when she is not there, males look inside the nest many times, being able to see the eggs (see video recordings in the electronic supplementary material, S1–S3). In addition, hoopoes perform a striking courtship behaviour in which males do not give the prey to female the first time of offering, but they repetitively move the head back and forth at the nest entrance, while females stand up uncovering the clutch, allowing the male to have more time to see the eggs (see video recording in the electronic supplementary material, S3).

Both sexes care for offspring, providing them food until fledglings abandon the nest at 24–30 days old [65]. Within our study population, there is variation in the colour of the uropygial secretions and eggshells among individuals [46] (see the coloration of each egg in our sample in the electronic supplementary material, S5).

(b) Study area and general procedures

The fieldwork was carried out during the 2016 breeding season at the Hoya de Guadix (37°C, 18′ N, 11′ W), Granada (southern Spain) in a hoopoe population studied over the last 25 years.

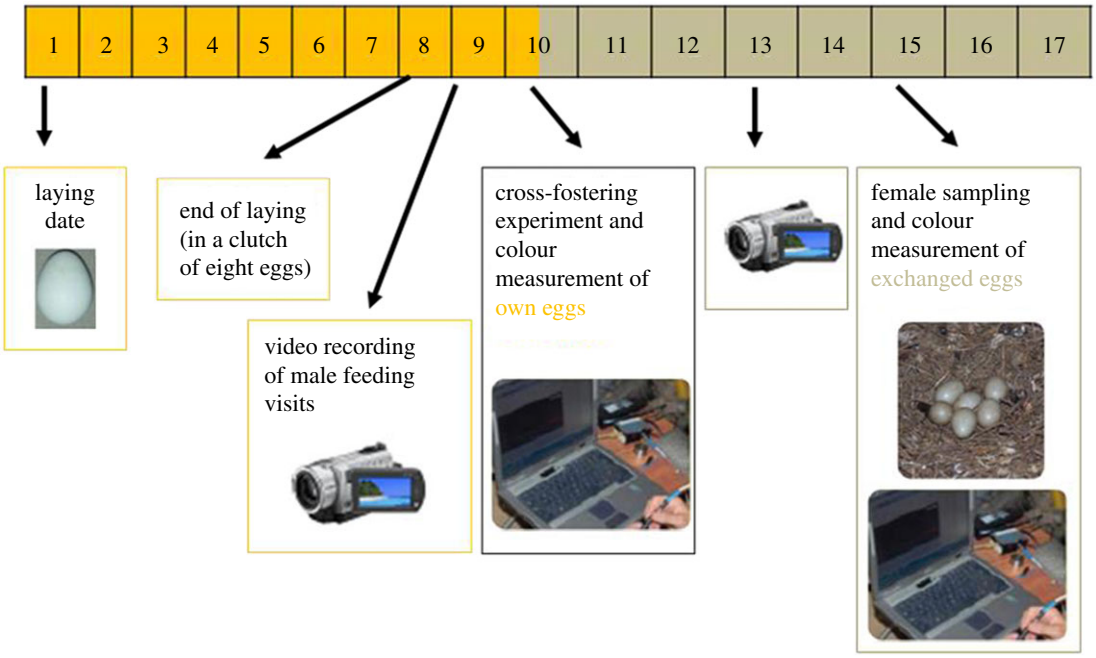

Figure 1. Schematic of the experimental design in a nest with eight eggs. Orange boxes (from 1 to 10) are days when the nest contained the female's own eggs, and grey ones (from 10 to 17) the days with exchanged eggs belonging to another female. (Online version in colour.)

They breed in cork nest-boxes situated in trees (dimensions: 24 cm (bottom-to-hole height), 35 × 18 × 21 cm (internal height × width × depth) and 5.5 cm (entrance diameter)), and in natural cavities.

Nest-boxes were visited every 5 days from early March to the end of July. Laying date was the day when the female laid her first egg, assuming that one egg was laid daily [64]. The hatching date was the day on which the first nestling hatched. Females were captured by hand inside the nest twice, during the cross-fostering experiment for marking (see below), and 15 days after the laying date for sampling. We measured tarsus length with a calliper (accuracy 1 mm) and body mass with a hanging scale (Pesola 0–100 g, accuracy 1 g). The body condition of the females was estimated as residuals of body mass on tarsus—length^3 [66–68]. Individuals were also ringed with numbered aluminium rings (Spanish Institute for Nature Conservation, ICONA) and with unique colour ring combinations. Disposable latex gloves previously cleaned with 96% ethanol were used to prevent cross-contamination among nests and among individuals.

(c) Experimental design

Eggs were cross-fostered between pairs of nests with a maximum laying date difference of 3 days. After laying completion, and at least 2 days after laying of the sixth egg, the six eggs that were completely covered with coloured secretion at this stage (i.e. those that female had laid first) were exchanged between two synchronous nests. For clutches larger than six eggs, the remaining non-cross-fostered eggs were removed and artificially incubated at 37.5°C in an incubator (Covatutto 24 Eco, Novital) in the laboratory. As a result, the cross-fostering experiment implied that clutch sizes were reduced in 47 nests, were equal in 13 nests and increased in one nest. In donor nests, the removed six eggs were temporarily replaced with artificial plastic eggs (cleaned with 96% ethanol to avoid cross-nests contamination), therefore, avoiding a drastic reduction in clutch size that could have influenced nest desertion. The plastic and the extra eggs were removed few minutes later, when the six experimental eggs collected from the other nest were introduced into the nest-box. Cross-fostering lasted less than 30 min and exchanged eggs were transported in a portable incubator. Hatchlings from surplus eggs were placed in a brood of its original nest or, in the case of predation, on nests of similar phenology. In the meantime, they were fed by hand with previously frozen fly larvae (asticot).

(d) Paternal investment

Males feeding visits to incubating females were video-recorded twice: 0–5 days after laying the last egg, with the original clutch, and 3 days after the cross-fostering experiment, with the exchanged-experimental eggs (figure 1). For video recording, digital video cameras (Sony Handycam DCR SR190 and DCR-SR55 models) were placed several metres away from the nest, camouflaged among stones, vegetation or trunks. Each recording registered periods of approximately 3 h starting around 16.00. However, there was variation in the time lasting until the behaviour of males and females normalized (from 65 to 120 min), and in the duration of the video (e.g. in five nests, owing to technical problems with batteries, only time from 31 to 57 min could be analysed after the behaviour was normalized). Parents were identified by their colour-ring combinations and/or other characteristics of their plumage or body. Male provisioning rate was calculated as the number of prey carried per hour (feeding rate) multiplied by the average relative size of all the prey carried. Relative prey size was estimated on an ordinal scale from 1 to 3 (1 when the length of the prey was less than a quarter of the beak, 2 when it was between a quarter and a half and 3 when it was bigger than a half the beak) [65]. In some feeding attempts (three samplings of three different males), the prey was not visible, and the average prey size of all studied males was considered to estimate conservatively the amount of food carried. A total of 122 videos, from 61 nests, including 41 first clutches, eight replacement clutches, 11 second clutches and one third clutch, were analysed through VLC Media Player (v. 2.2.6).

(e) Egg colour and biometric measurements

Eggshell coloration was measured twice; the day of the cross-fostering, and after several days of incubation in the foster nest (days 10 and 15 in the example of figure 1). These two measures allow estimation of differences between original and experimental eggs and between experimental eggs before and after the experiment.

Eggshell coloration was measured with an Ocean Optics S2000 spectrometer connected to a deuterium-halogen light (D2-W, Mini). A black bag wrapping the tip of the optical probe and the egg was used to standardize ambient light conditions. All measures were performed as quickly as possible, close to the nest-box and blocking the entrance to prevent nest desertion by parents. Females were captured within the nest-box, kept inside a bag during egg measurements and placed back in the nest after returning the eggs.

The spectrometer was calibrated using a standard white and black reference before measuring each clutch. Reflectance spectra at 10 nm intervals from 300 to 700 nm were obtained for the six experimental eggs of each clutch. Eggshell colour was measured on five equidistant points along the long egg axis. Prior to all analysis, reflectance curves were corrected for noise using triangular smoothing and negative values were set to zero [69].

Estimated colour variables took into account animal vision [70,71] by considering the visual parameters of a physiological model for a tetrachromatic violet vision as implemented in Avicol V.6 software [69]. Specifically, an Endler & Mielke [72] model was used with the correction for dark colours and the non-logarithmic transformation of the photoreceptor response values [73]. The model was run considering ambient light conditions inside a nest-box, and since no information is available on the violet spectral sensitivity of the hoopoe [74], that of peafowls (*Pavo cristatus*) was used [75], the best representative for violet sensitive birds for which enough data are available [76]. Three colour variables were estimated with the spectra information: Theta, Phi (both with spherical coordinates) and Chroma (r). Theta measures an angle (between −180° and 180°) in the red-green-blue plane [72]. Phi measures an angle (between −90° and 90°) in the ultraviolet/violet sensitive (UV/V) range with maximum stimulation of the violet cone at 90°. Both measures inform on hue, while r values inform on colour saturation, i.e. the distance to the centre of the tetrahedron [73,77]. Maximum potential chroma (r_{max}) depends on hue values since the colour space is not a sphere. For this reason, achieved Chroma (rA, hereafter saturation), computed as $rA = r/r_{max}$ was used in our analyses [73]. Theta values for our samples were all located in the region between the red (Theta = −30) and green (Theta = 90) vertices of the tetrahedron (range: from −14.74 to 85.04). Because Phi always takes values between −90 and 90 (range from −86.86 to −49.62 in our sample), we can use these two variables in linear scales, given that increasing values always imply changes of hue in the same direction.

To verify that eggshell coloration was more variable among than within nests, repeatability was calculated for each egg (among the five measures per egg), and among the eggs within the same clutch. To this aim, we used 26 randomly chosen nests. Eggshell coloration among the five measures per egg (general linear model (GLM): Theta: $r = 0.61$, $F_{203,806} = 8.96$, $p < 0.0001$; Phi: $r = 0.71$, $F_{203,816} = 13.5$, $p < 0.0001$; rA: $r = 0.67$, $F_{203,816} = 11.35$, $p < 0.0001$) and among the eggs of each nest (GLM: Theta: $r = 0.83$, $F_{25,754} = 31.40$, $p < 0.0001$; Phi: $r = 0.60$, $F_{25,754} = 10.1$, $p < 0.0001$, rA: $r = 0.88$, $F_{25,754} = 45.12$, $p < 0.0001$) was repeatable and, thus, average colour values of clutches were used in subsequent analyses.

Egg length and breadth were measured using a calliper (accuracy 0.01 mm) and egg volume was estimated from Hoyt's formula (size = 0.51 × length × width^2; [78]). Egg volume was more variable among than within nests (GLM: $r = 0.58$, $F_{51,260} = 9.41$, $p < 0.001$) and, thus, average values were used in the analyses.

(f) Statistical analysis

A best-subset general regression model (GRM) was used to verify that the original eggshell coloration was the best predictor of egg coloration after some days in nests of adoptive females. One nest was excluded from this analysis because it was considered an outlier that influenced these results (see analysis of Mahalanobis distances below).

The breeding attempt was considered as a continuous variable indicating increasing levels of breeding effort already performed by pairs in the season. A value of 1 was assigned to first clutches, 1.5 to replacement clutches (when the previous clutch of the female was not successful), 2 to second clutches (i.e. after successfully reared a first brood), 2.5 to replacement of second clutches and so on. A nest that followed two replacement clutches, and a first successful clutch was evaluated as 1.75. The breeding attempt did not influence eggshell coloration (GLM: Theta: $F_{1,59} = 0.55$, $p = 0.462$; Phi: $F_{1,59} = 0.84$, $p = 0.363$; rA: $F_{1,59} = 0.01$, $p = 0.933$).

To test the effect of the experiment on the provisioning rate of males, we analysed the relationship between differences in the colour of eggs (values of the three variables obtained in the physiological model) of the exchanged clutches, and differences in the amount of food carried per hour by males. These differences were calculated as values after the interchange minus those estimated before the interchange. We controlled for other variables that could explain inter-individual differences in feeding effort in this phase such as laying date, breeding attempt, clutch size, female body condition and differences in egg sizes caused by the exchange. The expected associations were tested by means of a GRM selecting the best subset of predictors by mean of Mallow's CP [79], equivalent to Akaike's information criterion [80]. The existence of outliers in the dependent and independent variables was explored by estimating Mahalanobis distances (i.e. plotting standard residuals against deleted residuals). The analysis showed the existence of one possible outlier, but its exclusion did not qualitatively affect the results, and was maintained in the analyses.

The residuals of all statistical models followed a normal distribution (Kolmogorov–Smirnov, $p > 0.20$). All statistical tests were performed with Statistica 7 software [81].

3. Results

The variables retained in the best model explaining eggshell colorations after 4–6 days in the adoptive nest were those describing coloration of the same eggs before the cross-fostering experiment (best subset GRM1: initial Theta value: $F_{1,58} = 0.76$, $p = 0.385$, adjusted $R^2 = -0.004$; GRM2: initial Phi value: $F_{1,58} = 17.88$, $p < 0.001$, adjusted $R^2 = 0.22$; GRM3: initial saturation value: $F_{1,58} = 9.76$, $p = 0.003$, adjusted $R^2 = 0.13$).

The change in provisioning rates of males before and after the experimental exchange of clutches was significantly related to colour differences between original and experimental eggs (table 1). Moreover, when eggshell colour did not vary, male feeding effort did not change either (see non-significant intercept, table 1). An increase in egg colour saturation (rA2–rA1 positive) produces a reduction of the male provisioning rate (figure 2*a*). In the case of Phi, the detected negative relationship implies a reduction in provisioning rates when the amount of violet in egg colour increases (figure 2*b*). In addition to colour variables, only the difference in egg volume entered in the best model, with a non-significant negative trend (figure 2*c* and table 1). In the nests in which the experiment reduced egg volume, males tended (non-significantly) to increase feeding effort to females when incubating experimental eggs (see all models in the electronic supplementary material, S4).

4. Discussion

We have found experimental support to the prediction that male provisioning rates to incubating female hoopoes

Table 1. General regression model exploring the association between changes (second minus first measures) in male provisioning rates and changes in eggshell coloration caused by the cross-fostering of clutches between pairs of hoopoe nests. (The best subset of predictors was selected by the mean of Mallow's CP. The numbers after variable names refer to values of the original (1) or adopted (2) clutches. Statistically significant values are in italics. Whole model statistics: $F_{3,57} = 3.65$, $p = 0.018$, $R^2 = 0.161$.)

predictor		in best subset	*T*	*p*	*β*	s.e.
intercept			0.59	0.5578		
eggshell colour changes:	Theta 2–Theta 1	pooled				
	Phi 2–Phi 1	retained	2.72	*0.0085*	−0.36	0.13
	rA2–rA1	retained	2.11	*0.0396*	−0.28	0.13
other predictors:	laying date	pooled				
	breeding attempt	pooled				
	clutch size	pooled				
	egg volume 2–1	retained	1.72	0.0907	−0.21	0.12
	fem. body cond.	pooled				

depended on the coloration of the eggshells. This coloration is owing to the presence of symbiotic bacteria in the uropygial secretion of breeding females, which actively use it to smear and colour their eggs. The colour of uropygial secretion reflects its antimicrobial properties [46,57] and, thus, our results suggest that hoopoe eggshell coloration might be a post-mating sexually selected indicator of female quality in terms of antimicrobial capabilities of their secretion.

Male provisioning rate was affected by two specific eggshell colour variables: saturation (rA) and hue (Phi, the violet colour variable), in both cases with a negative relationship. Because saturation of eggshell colour is negatively related to the bacterial load of the uropygial secretion [31], our results show that males feed females more frequently when the saturation of the cosmetic eggshell coloration indicates a higher load of symbiotic bacteria in the uropygial secretion. Moreover, uropygial secretions of less saturated coloration have higher antimicrobial capacity [46], probably mediated by symbiotic antimicrobial producer bacteria [40,46,54,55]. Therefore, our results suggest that eggshell colour saturation is a cue or signal that males use to evaluate the antimicrobial capacity of female secretion.

As for changes in Phi, the negative relationship found with changes in male provisioning rate after controlling for rA suggests that this component of cosmetic eggshell colour is also affecting male behaviour, despite not being known which particular information it may convey. To further investigate how the colour variables vary with the presence of the secretion, it would be interesting to investigate eggshell coloration before and after being covered by the uropygial secretion.

Besides egg coloration, the final model also retained egg size. A non-significant reduction in provisioning rates was associated with an increase in egg size of experimental clutches. However, if egg size reflects female and/or embryo quality, the sign of the association is contrary to that expected by SSEC. This negative differential investment could be the result of compensating the lower potential prospects of offspring ('the compensation hypothesis', [82]); a possibility that should be further tested. Further investigations on the effects of egg size on male provisioning rate are, however, necessary to look for possible adaptive value of such association.

Some authors have argued that the dim environment of hole nests [83] prevents the evolution of visual signals such egg coloration within those environments ([84], but see [85]). However, there is enough behavioural evidence to conclude that birds have impressive colour discrimination and scotopic visual abilities, being also able to discriminate in dim light conditions [86], and inside a nest cavity [84,85,87,88]. Thus, it is likely that sexual signals in general, and those of egg coloration in particular, function within hole-nest environments [84,87]. In the present study, we have analysed the eggshell coloration of hoopoes taking into account the stimuli of the avian cones inside a nest-hole, and find out the expected associations, which, therefore, support that, in spite of related dim light conditions, such traits function in hoopoes.

Our results are consistent with the assumption of the SSEC hypothesis that relates male investment with eggshell coloration [15]. Moreover, although we have not explored costs associated with the signal, the use of uropygial secretion to colour the eggs is probably costly. Uropygial secretions might be a limited resource of females with associated costs of production, and colouring the eggs is a time-consuming activity. In addition, eggshell coloration is probably an honest signal that cannot be falsified and would accurately indicate characteristics of the secretion and the female. These bacteria determine secretion colour [40,58], but depend on females characteristics, such as those influencing the immune system or amount and quality of the resources that bacteria use to grow [60,62,89]. Thus, females cannot falsify the signal. Finally, uropygial secretion protects embryos from pathogenic infections increasing hatching success [57], and thus, the colour of the eggshells would predict hatching success; a prediction of the SSEC hypothesis to be experimentally tested in the future.

Therefore, although further studies are necessary to elucidate costs associated with the use of uropygial secretion to colour the eggs, or to point out mechanisms assuring honest signalling, our findings support the SSEC hypothesis. Interestingly, although this hypothesis was proposed to explain intrinsic eggshell pigmentation, our results support an extension of the SSEC hypothesis to cosmetic eggshell coloration [46]. However, to ensure that secretion colour is not only a cue that hoopoe males follow to adjust reproductive investment, but a trait that evolves as consequence of sexual

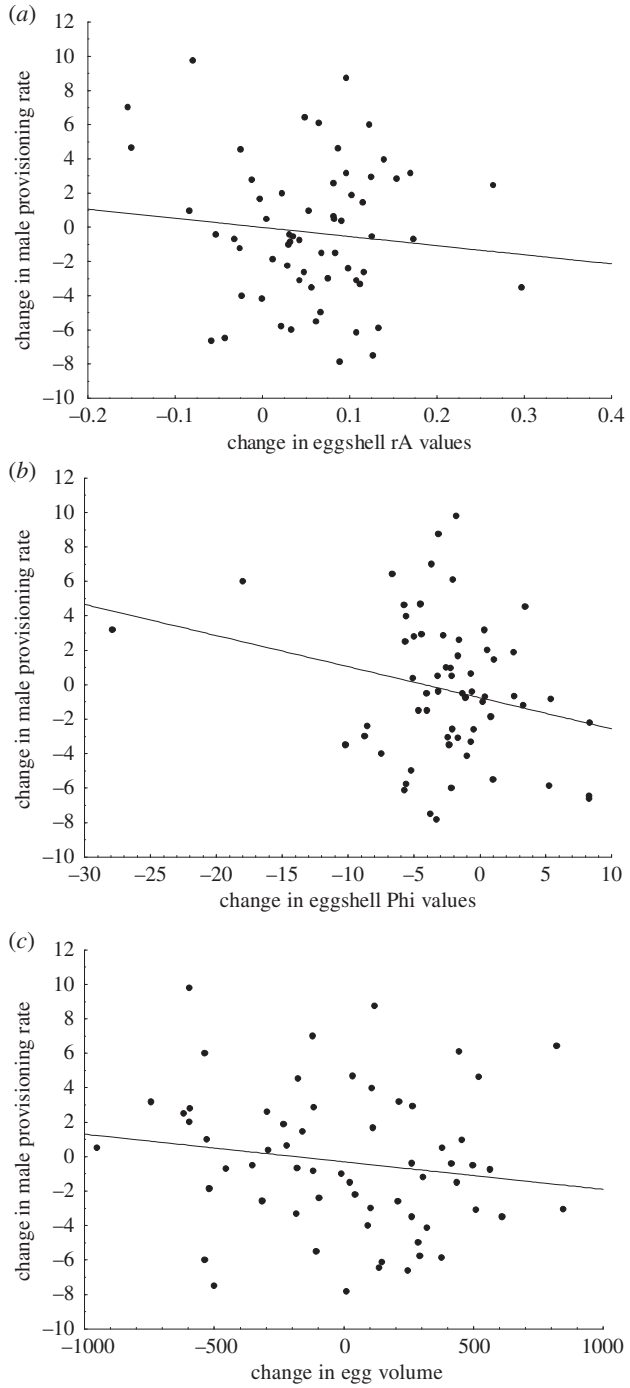

Figure 2. Relationship between changes in male provisioning rates (male provisioning rate 2–1) and those of the changes in: (*a*) saturation eggshell colour values (rA2–rA1); (*b*) Phi values of the eggshells (Phi 2–Phi 1); and (*c*) egg volume (egg volume 2–egg volume 1).

selection (i.e. post-mating sexual signal), it is necessary to explore whether the colour of uropygial secretion has evolved, at least partially, to attract males, even though coloration was mediated by hosted symbiotic bacteria.

Uropygial secretions of birds, in general, have antimicrobial substances [90] that may reach eggshells during incubation because of the contact with belly feathers previously stained with it. In addition, the uropygial secretion modifies the coloration of different parts of the body surfaces of birds, such as feathers and bills [47–51,91]. Therefore, the possibility that secretions change the colour of the eggshells [46], reflecting antimicrobial capacity mediated by cosmetic uropygial secretions, may also be applied to other bird species. It might be worth studying the possibility that eggshell cosmetic coloration owing to the use of uropygial secretion has a possible sexually selected component in species other than hoopoes.

The uropygial secretion deposited on the eggshells by females will change not only eggshell coloration, but also the odour of the clutch. Thus, males might use one or both characteristics to adjust their feeding effort. We have previously shown that when bacteria are eliminated from uropygial glands with antibiotics, secretions become red, and, simultaneously, the amount of associated chemical volatiles was drastically reduced [55,58]. Our experiment does not allow ruling out the possibility that males cue on eggshell odour. However, given that their own females are continuously producing a great amount of secretion daily, these fresh secretions probably obscure the odour of dry secretions coming from foster eggs. Thus, even though we cannot completely discard that other properties of the secretion covering eggs are the signals detected by males, the most probable explanation for our results is that males detected the change in egg colour. It is possible that the relative portion of variance unexplained by the model was owing to the limitation of our estimates of male provisioning rate (low number of feedings per hour in hoopoes during incubation), which would add to error variance that our experiment could not explain. However, despite this limitation, the colour variables are associated with male provisioning rates in the expected direction, which makes our inferences more robust.

The use of cosmetics has been reported for a wide variety of animals, including several species of fishes, mammals and birds (reviewed in [48]). For instance, similarly to hoopoes, it has been shown how tropical reef fishes secrete biochemical compounds with antibiotics into the epithelial mucus [92,93]. These compounds lack UV reflectance, and their coloration may signal characteristics of the mucus, but also individual quality in terms of the capacity for obtaining food sources rich in UV-blockers [94]. In mammals, the red kangaroo (*Megaleia rufa*) and the grey possum (*Trichosurus vulpecula*) have coloured patches in the pelage owing to the secretion of integumentary glands [95], being a sexually dimorphic character due to cosmetic coloration. Two studies in birds have shown how the plumage colour change owing to deliberate staining of the cosmetic (by using iron-red soils or uropygial secretion) may help individuals to communicate their quality [50,53] in scenarios of social communication, including mate choice [50]. Differing from these and some other examples of cosmetic colorations, the eggshell coloration of hoopoes are showing not only the physiological or cultural abilities of individuals, but also the properties of their bacterial symbionts. As far as we know, hoopoes painting their eggs with their own uropygial secretion is the first example of animals using cosmetic coloration that can show characteristics of their antimicrobial producing bacterial symbionts. Our experimental results suggest that this behaviour is probably maintained and selected by differential feeding investment of males in a typically post-mating sexually selected process [3,4].

Ethics. The study was conducted according to relevant Spanish national (Decreto 142/2013, 1 de octubre) and regional guidelines. The protocols adhered to the ASAB/ABS Guidelines for the Use of Animals in Research and it was approved by the ethics committee of the University of Granada (Comité de Ética en Experimentación Animal, CEEA, ref. 785). All necessary permits for hoopoe's manipulations were provided by Consejería de Medio Ambiente de la Junta de Andalucía, Spain (ref: SGYB/FOA/AFR/CFS and SGMN/GyB/JMIF). Our study area is not protected but privately owned, and the owners allowed us to work in

their properties. The time spent in each hoopoe nest was the minimum necessary for the experiment.

Data accessibility. Data used in this paper are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.9s4mw6mfg [96].

Authors' contributions. M.M.-V. and J.J.S. conceived the ideas and designed methodology; S.D.-L., M.A.-G. and J.M.P.-S. collected the data; S.D.-L., T.P.-C. and M.M.-V. analysed the data; contributed reagents/materials/analysis tools: M.M.-V., M.M.-B. and J.J.S.; S.D.-L. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication. All authors agree to be held accountable for the work performed therein.

Competing interests. We declare we have no competing interests.

Funding. Spanish Ministerio de Economía y Competitividad, Ministerio de Ciencia, Innovación y Universidades, and European (FEDER) funds: BES-2014-069116, CGL2013-48193-C3-1-P, CGL2013-48193-C3-2-P CGL2017-83103-P.

Acknowledgements. We thank Natalia Juárez García-Pelayo for assistance in fieldwork and Estefanía López Hernández for assistance in laboratory work. We benefited from comments by two referees (Daniel Hanley and an anonymous referee) that improved the quality of the manuscript. The research group also benefits from facilities, including an apartment, provided by the city authorities of Guadix, where a small laboratory to quickly process the samples were installed.

References

1. Andersson M. 1994 *Sexual selection*. Princeton, NJ: Princeton University Press.
2. Andersson M, Simmons LW. 2006 Sexual selection and mate choice. *Trends Ecol. Evol.* **21**, 296–302. (doi:10.1016/j.tree.2006.03.015)
3. Burley N. 1988 The differential allocation hypothesis. An experimental test. *Am. Nat.* **132**, 611–628. (doi:10.1086/284877)
4. Sheldon BC. 2000 Differential allocation: tests, mechanisms and implications. *Trends Ecol. Evol.* **15**, 397–402. (doi:10.1016/S0169-5347(00)01953-4)
5. Lyu N, Servedio MR, Lloyd H, Sun Y. 2017 The evolution of postpairing male mate choice. *Evolution* **71**, 1465–1477. (doi:10.1111/evo.13241)
6. Edward DA, Chapman T. 2011 The evolution and significance of male mate choice. *Trends Ecol. Evol.* **26**, 647–654. (doi:10.1016/j.tree.2011.07.012)
7. Fitzpatrick CL, Servedio MR. 2018 The evolution of male mate choice and female ornamentation: a review of mathematical models. *Curr. Zool.* **64**, 323–333. (doi:10.1093/cz/zoy029)
8. Soler JJ, Morales J, Cuervo JJ, Moreno J. 2019 Conspicuousness of passerine females is associated with the nest-building behaviour of males. *Biol. J. Linn. Soc.* **126**, 824–835. (doi:10.1093/biolinnean/blz015)
9. Soler JJ, Navarro C, Pérez-Contreras T, Avilés JM, Cuervo JJ. 2008 Sexually selected egg coloration in spotless starlings. *Am. Nat.* **171**, 183–194. (doi:10.1086/524958)
10. Soler JJ, Ruiz-Castellano C, Figuerola J, Martínez-de la Puente J, Ruiz-Rodríguez M, Tomás G. 2018 Egg colouration predicts brood size, telomere length and body condition of spotless starling fledglings. *J. Avian Biol.* **49**, 1–12. (doi:10.1111/jav.01686)
11. Hargitai R, Boross N, Nyiri Z, Eke Z. 2018 Effects of food limitation on the intensity of blue-green and brown eggshell coloration: an experimental study with the canary. *J. Avian Biol.* **49**, e01486. (doi:10.1111/jav.01486)
12. Moreno J, Morales J, Lobato E, Merino S, Tomás G, Martínez-De La Puente J. 2005 Evidence for the signaling function of egg color in the pied flycatcher *Ficedula hypoleuca*. *Behav. Ecol.* **16**, 931–937. (doi:10.1093/beheco/ari072)
13. Soler JJ, Moreno J, Aviles JM, Møller AP. 2005 Blue and green egg-color intensity is associated with parental effort and mating system in passerines: support for the sexual selection hypothesis. *Evolution* **59**, 636–644. (doi:10.1111/j.0014-3820.2005.tb01022.x)
14. Moreno J, Morales J, Lobato E, Merino S, Tomás G, Martínez-De La Puente J. 2006 More colourful eggs induce a higher relative paternal investment in the pied flycatcher *Ficedula hypoleuca*: a cross-fostering experiment. *J. Avian Biol.* **37**, 555–560. (doi:10.1111/j.2006.0908-8857.03915.x)
15. Moreno J, Osorno JL. 2003 Avian egg colour and sexual selection: does eggshell pigmentation reflect female condition and genetic quality? *Ecol. Lett.* **6**, 803–806. (doi:10.1046/j.1461-0248.2003.00505.x)
16. Moreno J, Osorno JL, Morales J, Merino S, Tomás G. 2004 Egg colouration and male parental effort in the pied flycatcher *Ficedula hypoleuca*. *J. Avian Biol.* **35**, 300–304. (doi:10.1111/j.0908-8857.2004.03407.x)
17. Giordano M, Costantini D, Pick JL, Tschirren B. 2015 Female oxidative status, egg antioxidant protection and eggshell pigmentation: a supplemental feeding experiment in great tits. *Behav. Ecol. Sociobiol.* **69**, 777–785. (doi:10.1007/s00265-015-1893-1)
18. Krist M, Grim T. 2007 Are blue eggs a sexually selected signal of female collared flycatchers? A cross-fostering experiment. *Behav. Ecol. Sociobiol.* **61**, 863–876. (doi:10.1007/s00265-006-0315-9)
19. Siefferman L, Navara KJ, Hill GE. 2006 Egg coloration is correlated with female condition in eastern bluebirds (*Sialia sialis*). *Behav. Ecol. Sociobiol.* **59**, 651–656. (doi:10.1007/s00265-005-0092-x)
20. Lahti DC. 2008 Population differentiation and rapid evolution of egg color in accordance with solar radiation. *Auk* **125**, 796–802. (doi:10.1525/auk.2008.07033)
21. Hanley D, Doucet SM, Dearborn DC. 2010 A blackmail hypothesis for the evolution of conspicuous egg coloration in birds. *Auk* **127**, 453–459. (doi:10.1525/auk.2009.09090)
22. Swynnerton CFM. 1916 On the coloration of the mouths and eggs of birds. II. On the coloration of eggs. *Ibis* (*Lond. 1859*) **4**, 529–606. (doi:10.1111/j.1474-919X.1916.tb07950.x)
23. Wallace A. 1889 *Darwinism: an exposition of the theory of natural selection, with some of its applications*, 2nd edn. New York, NY: MacMillan.
24. Gaston AJ, De Forest LN, Noble DG. 1993 Egg recognition and egg stealing in murres (*Uria spp.*). *Anim. Behav.* **45**, 301–306. (doi:10.1006/anbe.1993.1034)
25. Underwood T, Sealy S. 2002 Adaptive significance of egg coloration. In *Avian incubation: behaviour, environment, and evolution* (ed. DC Deeming), pp. 280–298. New York, NY: Oxford University Press.
26. Kilner RM. 2006 The evolution of egg colour and patterning in birds. *Biol. Rev. Camb. Philos. Soc.* **81**, 383–406. (doi:10.1017/S1464793106007044)
27. Hanley D, Cassey P, Doucet SM. 2013 Parents, predators, parasites, and the evolution of eggshell colour in open nesting birds. *Evol. Ecol.* **27**, 593–617. (doi:10.1007/s10682-012-9619-6)
28. Moreno J, Lobato E, Morales J, Merino S, Tomás G, Martínez-De La Puente J, Sanz JJ, Mateo R, Soler JJ. 2006 Experimental evidence that egg color indicates female condition at laying in a songbird. *Behav. Ecol.* **17**, 651–655. (doi:10.1093/beheco/ark014)
29. Holveck MJ, Guerreiro R, Perret P, Doutrelant C, Grégoire A. 2019 Eggshell coloration indicates female condition during egg-laying: a field experiment in blue tits. *Biol. J. Linn. Soc.* **128**, 181–200. (doi:10.1093/biolinnean/blz082)
30. Hanley D, Heiber G, Dearborn DC. 2008 Testing an assumption of the sexual-signaling hypothesis: does blue-green egg color reflect maternal antioxidant capacity? *Condor* **110**, 767–771. (doi:10.1525/cond.2008.8634)
31. Díaz-Lora S, Pérez-Contreras T, Azcárate-García M, Martínez-Bueno M, Soler JJ, Martín-Vivaldi M. 2020 Hoopoe (*Upupa epops*) male feeding effort is related to female cosmetic egg colouration. *J. Avian Biol.* **51**, e02433. (doi:10.1111/jav.02433)
32. English PA, Montgomerie R. 2011 Robin's egg blue: does egg color influence male parental care? *Behav. Ecol. Sociobiol.* **65**, 1029–1036. (doi:10.1007/s00265-010-1107-9)
33. Poláček M, Griggio M, Mikšík I, Bartíková M, Eckenfellner M, Hoi H. 2017 Eggshell coloration and its importance in postmating sexual selection. *Ecol. Evol.* **7**, 941–949. (doi:10.1002/ece3.2664)

34. Honza M, Požgayová M, Procházka P, Cherry MI. 2011 Blue-green eggshell coloration is not a sexually selected signal of female quality in an open-nesting polygynous passerine. *Naturwissenschaften* **98**, 493–499. (doi:10.1007/s00114-011-0790-3)
35. Johnsen A, Vesterkjær K, Slagsvold T. 2011 Do male pied flycatchers (*Ficedula hypoleuca*) adjust their feeding effort according to egg colour? *Ethology* **117**, 309–317. (doi:10.1111/j.1439-0310.2011.01876.x)
36. Stoddard MC, Fayet AL, Kilner RM, Hinde CA. 2012 Egg speckling patterns do not advertise offspring quality or influence male provisioning in great tits. *PLoS ONE* **7**, e40211. (doi:10.1371/journal.pone.0040211)
37. Bulla M, Šálek M, Gosler AG. 2012 Eggshell spotting does not predict male incubation but marks thinner areas of a shorebird's shells. *Auk* **129**, 26–35. (doi:10.1525/auk.2012.11090)
38. Fronstin RB, Doucet SM, Christians JK. 2016 Haematocrit, eggshell colouration and sexual signaling in the European starling (*Sturnus vulgaris*). *BMC Ecol.* **16**, 31. (doi:10.1186/s12898-016-0084-x)
39. Riehl C. 2011 Paternal investment and the 'Sexually Selected Hypothesis' for the evolution of eggshell coloration: revisiting the assumptions. *Auk* **128**, 175–179. (doi:10.1525/auk.2011.10171)
40. Soler JJ, Martín-Vivaldi M, Ruiz-Rodríguez M, Valdivia E, Martín-Platero AM, Martínez-Bueno M, Peralta-Sánchez JM, Méndez M. 2008 Symbiotic association between hoopoes and antibiotic-producing bacteria that live in their uropygial gland. *Funct. Ecol.* **22**, 864–871. (doi:10.1111/j.1365-2435.2008.01448.x)
41. Kaur H, Hughes MN, Green CJ, Naughton P, Foresti R, Motterlini R. 2003 Interaction of bilirubin and biliverdin with reactive nitrogen species. *FEBS Lett.* **543**, 113–119. (doi:10.1016/S0014-5793(03)00420-4)
42. López-Rull I, Gil M, Gil D. 2007 Spots in starling *Sturnus* unicolor eggs are good indicators of ectoparasite load by *Carnus hemapterus* (Diptera: Carnidae). *Ardeola* **54**, 131–134.
43. Avilés JM, Pérez-Contreras T, Navarro C, Soler JJ. 2009 Male spotless starlings adjust feeding effort based on egg spots revealing ectoparasite load. *Anim. Behav.* **78**, 993–999. (doi:10.1016/j.anbehav.2009.07.020)
44. Tomás G, Martín-Gálvez D, Ruiz-Castellano C, Ruiz-Rodríguez M, Peralta-Sánchez JM, Martín-Vivaldi M, Soler JJ. 2018 Ectoparasite activity during incubation increases microbial growth on avian eggs. *Microb. Ecol.* **76**, 555–564. (doi:10.1007/s00248-017-1140-6)
45. Azcárate-García M, Díaz-Lora S, Tomás G, Soler JJ. 2020 Spotless starlings prefer spotless eggs: conspecific brood parasites cue on eggshell spottiness to avoid ectoparasites. *Anim. Behav.* **166**, 33–39. (doi:10.1016/j.anbehav.2020.05.017)
46. Soler JJ, Martín-Vivaldi M, Peralta-Sánchez JM, Arco L, Juárez-García-Pelayo N. 2014 Hoopoes color their eggs with antimicrobial uropygial secretions. *Naturwissenschaften* **101**, 697–705. (doi:10.1007/s00114-014-1201-3)
47. Montgomerie R. 2006 Cosmetic and adventitious colors. In *Bird colouration I. Mechanisms and measurements* (eds E Hill, K McGraw), pp. 399–427. Cambridge, MA: Harvard University Press.
48. Delhey K, Peters A, Kempenaers B. 2007 Cosmetic coloration in birds: occurrence, function, and evolution. *Am. Nat.* **169**, S145–S158. (doi:10.1086/510095)
49. Piault R, Gasparini J, Bize P, Paulet M, McGraw KJ, Roulin A. 2008 Experimental support for the makeup hypothesis in nestling tawny owls (*Strix aluco*). *Behav. Ecol.* **19**, 703–709. (doi:10.1093/beheco/arm152)
50. Amat JA, Rendón MA, Garrido-Fernández J, Garrido A, Rendón-martos M, Pérez-gálvez A. 2011 Greater flamingos *Phoenicopterus roseus* use uropygial secretions as make-up. *Behav. Ecol.* **65**, 665–673. (doi:10.1007/s00265-010-1068-z)
51. Pérez-Rodríguez L, Mougeot F, Bortolotti GR. 2011 The effects of preen oils and soiling on the UV – visible reflectance of carotenoid-pigmented feathers. *Behav. Ecol. Sociobiol.* **65**, 1425–1435. (doi:10.1007/s00265-011-1153-y)
52. López-Rull I, Pagán I, Macías Garcia C. 2010 Cosmetic enhancement of signal coloration: experimental evidence in the house finch. *Behav. Ecol.* **21**, 781–787. (doi:10.1093/beheco/arq053)
53. Negro JJ, Margalida A, Hiraldo F, Heredia R. 1999 The function of the cosmetic coloration of bearded vultures: when art imitates life. *Anim. Behav.* **S8**, 14–17. (doi:10.1006/anbe.1999.1251)
54. Martín-Platero AM, Valdivia E, Ruíz-Rodríguez M, Soler JJ, Martín-Vivaldi M, Maqueda M, Martínez-Bueno M. 2006 Characterization of antimicrobial substances produced by *Enterococcus faecalis MRR 10-3*, isolated from the uropygial gland of the hoopoe (*Upupa epops*). *Appl. Environ. Microbiol.* **72**, 4245–4249. (doi:10.1128/AEM.02940-05)
55. Martín-Vivaldi M, Peña A, Peralta-Sánchez JM, Sánchez L, Ananou S, Ruiz-Rodríguez M, Soler JJ. 2010 Antimicrobial chemicals in hoopoe preen secretions are produced by symbiotic bacteria. *Proc. R. Soc. B* **277**, 123–130. (doi:10.1098/rspb.2009.1377)
56. Ruiz-Rodríguez M, Martínez-Bueno M, Martín-Vivaldi M, Valdivia E, Soler JJ. 2013 Bacteriocins with a broader antimicrobial spectrum prevail in enterococcal symbionts isolated from the hoopoe's uropygial gland. *FEMS Microbiol. Ecol.* **85**, 495–502. (doi:10.1111/1574-6941.12138)
57. Martín-Vivaldi M, Soler JJ, Peralta-Sánchez JM, Arco L, Martín-Platero AM, Martínez-Bueno M, Ruiz-Rodríguez M, Valdivia E. 2014 Special structures of hoopoe eggshells enhance the adhesion of symbiont-carrying uropygial secretion that increase hatching success. *J. Anim. Ecol.* **83**, 1289–1301. (doi:10.1111/1365-2656.12243)
58. Martín-Vivaldi M, Ruiz-Rodríguez M, Soler JJ, Peralta-Sánchez JM, Méndez M, Valdivia E, Martín-Platero AM, Martínez-Bueno M. 2009 Seasonal, sexual and developmental differences in hoopoe *Upupa epops* preen gland morphology and secretions: evidence for a role of bacteria. *J. Avian Biol.* **40**, 191–205. (doi:10.1111/j.1600-048X.2009.04393.x)
59. Haaland TR, Wright J, Kuijper B, Ratikainen II. 2017 Differential allocation revisited: when should mate quality affect parental investment? *Am. Nat.* **190**, 534–546. (doi:10.1086/693484)
60. Martín-Vivaldi M, Soler JJ, Martínez-García Á, Arco L, Juárez-García-Pelayo N, Ruiz-Rodríguez M, Martínez-Bueno M. 2018 Acquisition of uropygial gland microbiome by hoopoe nestlings. *Microb. Ecol.* **76**, 285–297. (doi:10.1007/s00248-017-1125-5)
61. Martínez-García Á, Martín-Vivaldi M, Ruiz-Rodríguez M, Martínez-Bueno M, Arco L, Rodríguez-Ruano SM, Peralta-Sánchez JM, Soler JJ. 2016 The microbiome of the uropygial secretion in hoopoes is shaped along the nesting phase. *Microb. Ecol.* **72**, 252–261. (doi:10.1007/s00248-016-0765-1)
62. Ruiz-Rodríguez M, Soler JJ, Martín-Vivaldi M, Martín-Platero AM, Méndez M, Peralta-Sánchez JM, Ananou S, Valdivia E, Martínez-Bueno M. 2014 Environmental factors shape the community of symbionts in the hoopoe uropygial gland more than genetic factors. *Appl. Environ. Microbiol.* **80**, 6714–6723. (doi:10.1128/AEM.02242-14)
63. Martín-Vivaldi M, Palomino JJ, Soler M, Soler JJ. 1999 Determinants of reproductive success in the hoopoe *Upupa epops*, a hole-nesting non-passerine bird with asynchronous hatching. *Bird Study* **46**, 205–216. (doi:10.1080/00063659909461132)
64. Cramp S. 1998 *The complete birds of the western Palearctic on CD-ROM*. Optimedia. Oxford, UK: Oxford University Press.
65. Martín-Vivaldi M, Doña J, Romero Masegosa J, Soto Cárdenas M. 2014 Abubilla - *Upupa epops* Linnaeus, 1758. *En Encicl. Virtual los Vertebr. Españoles. Salvador. A., Morales, M. B. (Eds.). Mus. Nac. Ciencias Nat. Madrid*. See http//www.vertebradosibericos.org/.
66. Senar JC, Pascual J. 1997 Tarsus lenght predictor body size. *Ardea* **85**, 269–274.
67. Labocha MK, Hayes JP. 2012 Morphometric indices of body condition in birds: a review. *J. Ornithol.* **153**, 1–22. (doi:10.1007/s10336-011-0706-1)
68. Peig J, Green AJ. 2010 The paradigm of body condition: a critical reappraisal of current methods based on mass and length. *Funct. Ecol.* **24**, 1323–1332. (doi:10.1111/j.1365-2435.2010.01751.x)
69. Gómez D. 2006 AVICOL a program to analyse spectrometric data. Last update october 2011. Free executable available at http://sites.google.com/site/avicolprogram/ or from the author at dodogomez@yahoo.fr.
70. Renoult JP, Kelber A, Schaefer HM. 2017 Colour spaces in ecology and evolutionary biology. *Biol. Rev.* **92**, 292–315. (doi:10.1111/brv.12230)
71. Endler JA. 1990 On the measurement and classification of color in studies of animal color patterns. *Biol. J. Linn. Soc.* **41**, 315–352. (doi:10.1111/j.1095-8312.1990.tb00839.x)

72. Endler JA, Mielke PW. 2005 Comparing color patterns as birds see them. *Biol. J. Linn. Soc.* **86**, 405–431. (doi:10.1111/j.1095-8312.2005.00540.x)
73. Stoddard MC, Prum RO. 2008 Evolution of avian plumage color in a tetrahedral colour space: a phylogenetic analysis of New World buntings. *Am. Nat.* **171**, 755–776. (doi:10.1086/587526)
74. Ödeen A, Håstad O. 2003 Complex distribution of avian color vision systems revealed by sequencing the SWS1 opsin from total DNA. *Mol. Biol. Evol.* **20**, 855–861. (doi:10.1093/molbev/msg108)
75. Hart NS. 2002 Vision in the peafowl (Aves: *Pavo cristatus*). *J. Exp. Biol.* **205**, 3925–3935.
76. Hastad O, Victorsson J, Odeen A. 2005 Differences in color vision make passerines less conspicuous in the eyes of their predators. *Proc. Natl Acad. Sci. USA* **102**, 6391–6394. (doi:10.1073/pnas.0409228102)
77. Saino N *et al.* 2013 Viability is associated with melanin-based coloration in the barn swallow (*Hirundo rustica*). *PLoS ONE* **8**, e60426. (doi:10.1371/journal.pone.0060426)
78. Hoyt DF. 1979 Practical methods of estimating volume and fresh weight of bird eggs. *Auk* **96**, 73–77.
79. Mallows CL. 1973 Some comments on Cp. *Technometrics* **15**, 661–675. (doi:10.1080/00401706.1973.10489103)
80. Boisbunon A, Canu S, Fourdrinier D, Strawderman W, Wells MT. 2013 AIC, Cp and estimators of loss for elliptically symmetric distributions. *arXiv*: 1308.2766.
81. Statsoft I. 2006 STATISTICA (data analysis software system), version 7. See www.statsoft.com.
82. Bluhm CK, Gowaty PA. 2004 Reproductive compensation for offspring viability deficits by female mallards, *Anas platyrhynchos*. *Anim. Behav.* **68**, 985–992. (doi:10.1016/j.anbehav.2004.01.012)
83. Wesolowski T, Maziarz M. 2012 Dark tree cavities – a challenge for hole nesting birds? *J. Avian Biol.* **43**, 454–460. (doi:10.1111/j.1600-048X.2012.05704.x)
84. Holveck MJ, Doutrelant C, Guerreiro R, Perret P, Gomez D, Grégoire A. 2010 Can eggs in a cavity be a female secondary sexual signal? Male nest visits and modelling of egg visual discrimination in blue tits. *Biol. Lett.* **6**, 453–457. (doi:10.1098/rsbl.2009.1044)
85. Avilés JM, Vikan JR, Fossøy F, Antonov A, Moksnes A, Røskaft E, Stokke BG. 2010 Avian colour perception predicts behavioural responses to experimental brood parasitism in chaffinches. *J. Evol. Biol.* **23**, 293–301. (doi:10.1111/j.1420-9101.2009.01898.x)
86. Olsson P, Lind O, Kelber A. 2015 Bird colour vision: behavioural thresholds reveal receptor noise. *J. Exp. Biol.* **218**, 184–193. (doi:10.1242/jeb.111187)
87. Avilés JM, Soler JJ, Hart NS. 2011 Sexual selection based on egg colour: physiological models and egg discrimination experiments in a cavity-nesting bird. *Behav. Ecol. Sociobiol.* **65**, 1721–1730. (doi:10.1007/s00265-011-1180-8)
88. Avilés JM, Soler JJ. 2009 Nestling colouration is adjusted to parent visual performance in altricial birds. *J. Evol. Biol.* **22**, 376–386. (doi:10.1111/j.1420-9101.2008.01655.x)
89. Rodríguez-Ruano SM, Martín-Vivaldi M, Martín-Platero AM, López-López JP, Peralta-Sánchez JM, Ruiz-Rodríguez M, Soler JJ, Valdivia E, Martínez-Bueno M. 2015 The hoopoe's uropygial gland hosts a bacterial community influenced by the living conditions of the bird. *PLoS ONE* **10**, e1369734. (doi:10.1371/journal.pone.0139734)
90. Jacob J, Ziswiler V. 1982 The uropygial gland. In *Avian biology, vol. VI* (eds DS Farner, JR King, KC Parkes), pp. 359–362. London, UK: Academic Press.
91. Kemp AC. 2001 Family Bucerotidae (hornbills). In *Handbook of the birds of the world, Piciformes to bucerotiformes, vol. 6* (eds J Del Hoyo, A Elliott, J Sargatal), pp. 436–523. Barcelona, Spain: Lynx Edicions.
92. Shephard KL. 1994 Functions for fish mucus. *Rev. Fish Biol. Fish.* **4**, 401–429. (doi:10.1007/BF00042888)
93. Videler H, Geertjes GJ, Videler JJ. 1999 Biochemical characteristics and antibiotic properties of the mucous envelope of the queen parrotfish. *J. Fish Biol.* **54**, 1124–1127. (doi:10.1006/jfbi.1999.0935)
94. Zamzow JP, Losey GS. 2002 Ultraviolet radiation absorbance by coral reef fish mucus: photo-protection and visual communication. *Environ. Biol. Fishes* **63**, 41–47. (doi:10.1023/A:1013846816869)
95. Nicholls EM, Rienits KG. 1971 Tryptophan derivatives and pigment in the hair of some Australian marsupials. *Int. J. Biochem.* **2**, 593–603. (doi:10.1016/0020-711X(71)90031-0)
96. Díaz-Lora S, Pérez-Contreras T, Azcárate-García M, Peralta-Sánchez JM, Martínez-Bueno M, José Soler J, Martín-Vivaldi M. 2021 Data from: Cosmetic coloration of cross-fostered eggs affects paternal investment in the hoopoe (*Upupa epops*). Dryad Digital Repository. (https://doi.org/10.5061/dryad.9s4mw6mfg)