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ORIGINAL PAPER

Hoopoes color their eggs with antimicrobial uropygial secretions

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Abstract Uropygial gland secretions are used as cosmetics by some species of birds to color and enhance properties of feathers and teguments, which may signal individual quality. Uropygial secretions also reach eggshells during incubation and, therefore, may influence the coloration of birds' eggs, a trait that has attracted the attention of evolutionary biologists for more than one century. The color of hoopoe eggs typically changes along incubation, from bluish-gray to greenish-brown. Here, we test experimentally the hypothesis that dark uropygial secretion of females is responsible for such drastic color change. Moreover, since uropygial secretion of hoopoes has antimicrobial properties, we also explore the association between color and antimicrobial activity of the uropygial secretion of females. We found that eggs stayed bluish-gray in nests where female access to the uropygial secretion was experimentally blocked. Furthermore, experimental eggs that were maintained in incubators and manually smeared with uropygial secretion experienced similar color changes that naturally incubated eggs did, while control eggs that were not in contact with the secretions did not experience such color changes. All these results strongly support the hypothesis that female hoopoes use their uropygial gland secretion to color the eggs. Moreover, saturation of the uropygial secretion was associated with antimicrobial activity against *Bacillus licheniformis*. Given the known antimicrobial potential of uropygial secretions of birds, this finding opens the possibility that in scenarios of sexual selection, hoopoes in particular and birds in general signal antimicrobial properties of their uropygial secretion by mean of changes in egg coloration along incubation.

Keywords Antimicrobials · Bacteria · Egg color change · Cosmetics · Eggshells · Sexual signal · Uropygial gland secretion

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Introduction

Differences in egg coloration among avian species have attracted the attention of evolutionary biologists for more than a century (Underwood and Sealy 2002). Several hypotheses have been proposed in highly diverse scenarios as predation, brood-parasitism and parasitic egg rejection avoidance, sexual selection, thermal isolation, or even filtering solar radiation (Underwood and Sealy 2002; Kilner 2006). Recently, it has also been suggested that eggshell pigments may play an antimicrobial function (Ishikawa et al. 2010) preventing trans-shell infection by pathogenic microorganisms. Incubating birds may also transfer antimicrobials in the uropygial secretions to the eggshells, behavior that may also protect embryos from pathogenic infections due to the antimicrobial activity of these secretions (Cook et al. 2005; Soler et al. 2008a; Martín-Vivaldi et al. 2009). Moreover, because

uropygial secretions influence coloration of smeared tissues (see below), it may also affect color of eggshells (Martín-Vivaldi et al. 2009).

The use of uropygial secretion as cosmetic to color feathers is well known (Delhey et al. 2007; Lopez-Rull et al. 2010; Amat et al. 2011; Perez-Rodriguez et al. 2011), but its influence on egg coloration has only been suggested for European hoopoes (*Upupa epops*) (Martín-Vivaldi et al. 2009). Antimicrobial functioning of the uropygial secretion against feather-degrading bacteria has been shown for different bird species (Shawkey et al. 2003; Møller et al. 2009) including hoopoes (Soler et al. 2008a; Ruiz-Rodríguez et al. 2009). Because of the antimicrobial properties of uropygial secretion, its spreading on eggshells by hoopoe females could also function for impeding trans-shell embryo infection by microbial parasites (Martín-Vivaldi et al. 2009). Therefore, if the uropygial secretion was the responsible of the eggs color changes in hoopoes, this color could reflect the antimicrobial capacity of incubating females. Testing this hypothesis is particularly important for hoopoes since (i) most of the antimicrobial characteristics of the uropygial secretion in this species are mediated by the symbiotic bacteria growing in the uropygial gland (Soler et al. 2008a; Martín-Vivaldi et al. 2010; Ruiz-Rodriguez et al. 2013) and (ii) color changes are detected few days after laying (i.e., before finishing laying stage, the first laid eggs being easily distinguishable (browner) from the last ones (Martín-Vivaldi et al. 2009)). Finding evidences of an association between color of secretions and their antimicrobial activity would open the possibility that egg coloration inform males of characteristics of the uropygial secretion of incubating females which may affect paternal effort (feeding to nestlings and to incubating and brooding females) and the evolution of the symbiotic association.

We here experimentally explore the hypothesis that eggshell coloration of hoopoe eggs at the end of the incubation was in fact the consequence of hoopoe females spreading uropygial secretion on the eggshells. However, this striking color change may also be caused by dust from the bottom of the nest, or contact with the skin of incubating females. Trying to distinguish between these possibilities, we studied color changes along the incubation period in eggshells of (i) hoopoe eggs artificially incubated in the lab that were and were not smeared with hoopoe uropygial secretion and (ii) eggs incubated by hoopoe females whose access to their uropygial secretion was and was not experimentally blocked.

The possibility that uropygial secretion affects eggshell coloration may apply to most of bird species. Uropygial secretions when spread on feathers mainly affect the UV reflectance (Lopez-Rull et al. 2010; Perez-Rodriguez et al. 2011); one of the color traits better detected inside hole nests (dim conditions) (Avilés et al. 2006, 2008). Thus, even in hole-nesting bird species, pair members may be able to appraise characteristics of the incubating partner through the change in egg coloration due to the application of uropygial secretion. Since characteristics of uropygial secretion are likely related to phenotypic quality of birds (Delhey et al. 2007; Piault et al. 2008; Soler et al. 2012), the changes in eggshell color due to uropygial secretions may reflect such phenotypic characteristics in a scenario similar to that proposed for feather color change by mean of preening.

Secondly, we also explore the association between coloration of the uropygial secretion of the incubating females and the antimicrobial activity of their secretion against *Bacillus licheniformis*, a well-known feather-degrading bacterium. Finding evidence of such relationship would allow predictions in a hypothetical scenario of postmating sexual selection acting on egg coloration showing antimicrobial efficiency of incubating females.

Material and methods

Study populations

Field work was performed in 2008–2010 in the Hoya de Guadix (37° 18′ N, 38° 11′ W), southern Spain, where hoopoes breed in crops, forests, and gullies within nest boxes placed in trees or buildings (for a more detailed description of the study area see Martín-Vivaldi et al. 1999). The experiment to block female hoopoes from having access to the uropygial gland was performed during 2009–2010 in a captive population in installations of our research group in the Hoya de Guadix in Granada (University of Granada) and in Finca Experimental la Hoya in Almería (Estación Experimental de Zonas Áridas, CSIC) since 2006. Breeding pairs were housed in independent cages at least 3 m×2 m×2 m installed in the open, with access to soil and provided with live food (crickets, fly larvae, and meat (beef heart)) ad libitum. The treatments were balanced within each captive subpopulation. Uropygial secretions of incubating females for evaluation of antimicrobial activity were collected during 2013 in Guadix wild population.

Experimental manipulation of female access to uropygial gland secretion

Access of female hoopoes to the uropygial secretion was manipulated by using sterile cat catheters (Buster, width 1.0 mm) inserted in the opening of the papilla of the uropygial gland connected to flexible silicone tubes with 8 mm width and 70 mm length that served as a store of the secretion, by means of small sections of two tubes of intermediate widths (Fig. 1a). The flexible tube connected with the catheter was fixed to the skin with surgical glue and adhesive bandage. In experimental birds, the tube was plugged to the entrance of the gland, blocking the access of females to their uropygial gland

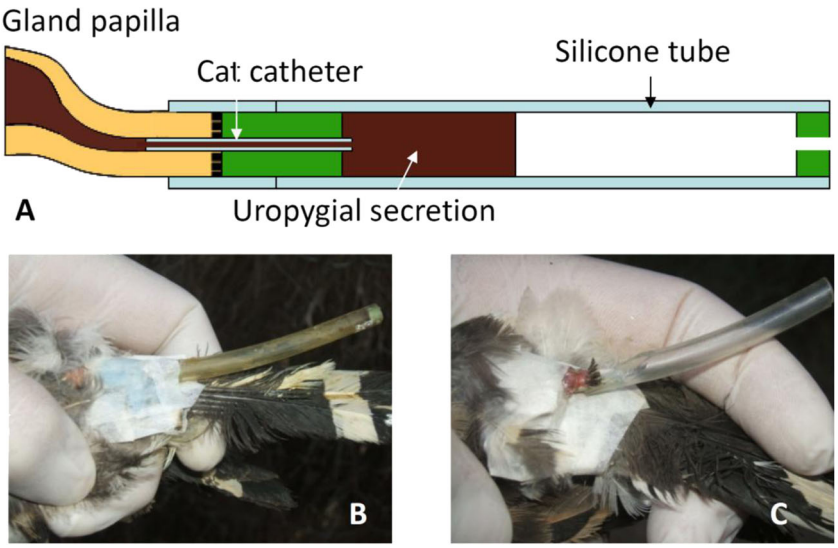

Fig. 1 **a** Scheme of the insertion of the cat catheter in the gland papilla opening that allows collecting uropygial secretion in the silicone tube while blocking the access to secretion for experimental females. Pictures **b** and **c** show the appearance of experimental and control I (tubes that did not cover gland entrance) females with flexible silicone tubes plugged to the uropygial gland

secretion, which was retained in the plastic tube (Fig. 1b). Every second day, we removed and changed the plastic tube and checked the fixation of the apparatus on the gland opening. In order to control for possible effects in females of having a plugged tube of 7 cm on their uropygial gland, a first group of control birds (control I) were provided with a similar structure of tubes. In this case, it was fixed by a plastic ring glued surrounding the entrance of the uropygial gland, but it did not cover the entrance and not block the normal access of females to secretions (Fig. 1c). A third group of breeding females (control II) was visited and handled at the same rate as those of experimental and control I groups, but no plastic structure was fixed on them.

During incubation and the first half of the nestling period, female hoopoes stay within the nest the whole day; all food that they consume is provided by the male (Martín-Vivaldi et al. 1999; Krištín 2001), and they only leave the cavity for defecation few times daily. None of the experimental females died in the course of the experiment. Moreover, the experiment did not affect the body condition of females as those that did or did not wear flexible silicon tubes, or those with and without access to uropygial secretion did not differ in body mass reduction experienced from first to second reproductive events (body mass measured at the beginning of each breeding attempt, when laying the first eggs; Martín-Vivaldi et al. (2014)). Thus, we are confident that our experimental manipulation did not negatively affect adequate nourishment and health of breeding females in our conditions of captivity.

Experimental smearing of hoopoe eggs with uropygial gland secretion

In 2008, we collected two recently laid eggs per clutch from six clutches in our captivity population and incubated them artificially in the lab (Covatutto 24 Eco, Novital) at 37.5ºC. These eggs were collected on the day of laying when more than three eggs were already in the nest. In addition, the two last laid eggs from two clutches that were abandoned during the laying period were also used for the experiment. Color of uropygial secretion of nestlings and its antimicrobial properties do not apparently differ from that of females (Martín-Vivaldi et al. unpublished data). We used secretions both from incubating females or nestlings because of the large volume of secretion needed for the experiment and because collecting uropygial secretion implies risky disturbance of incubating or brooding females. Secretion was collected with a micropipette directly from the inside of the uropygial glands after feathers around the gland were separated and washed with ethanol to avoid contamination. One egg from each clutch was daily painted for 6–7 days (mean=6.5, SD=1.2 days) with uropygial secretion, and the other was smeared with water as a control treatment to explore the effect of uropygial secretion on eggshell coloration. A clean cotton swab was moistened with a small amount (5–10 μl) of the secretion or water and used to smear them on the egg surface trying to impregnate the whole egg surface each time.

Eggshell and uropygial secretion color measurements

Egg color measurements were obtained from two randomly selected areas of each egg and consisted on reflectance at 10-nm intervals for wavelengths between 360 and 700 nm using a portable spectrophotometer (Konica Minolta Sensing (Seoul, South Korea), CM-2600d). Eggs were illuminated by a xenon light source, and the observer angle established at 10° (CIE1964). The measurements were taken relative to standard white (CM-A145, Konica Minolta Sensing) and dark references (CM-A32, Konica Minolta Sensing), which we calibrated before measurement of each clutch. Spectrophotometric data were transformed to the L*C*h color space; values that

were directly provided by the spectrophotometer. The L axis represents lightness and varied from 0 (absolute black) to 100 (absolute white); the C axis represents chroma or saturation and range from gray (C=0) to pure color of a given hue (with maximum values varying depending on hue). The h represents hue with units in the form of angle degrees ranging from 0° (red) through 90° (yellow), 180° (green), 270° (blue), and back to 0°. Preliminary analyses with the spectrometric information summarized in three PCA axes indicated that uropygial secretion does not affect UV (i.e., PC3, results not shown) of the eggshells, which support the use of LCh color space which does not include information of UV wavelengths.

Incubation length of hoopoe eggs extends to 17 days (Martín-Vivaldi et al. 1999). For the description of natural changes in hoopoe egg color as well as in the artificial incubation experiment, eggs were measured twice: on the day of laying and 15 days after the start of incubation. However, eggs were measured only at the end of the incubation in the experiment manipulating female access to uropygial secretions. Color of the uropygial secretion of incubating females (N=7) was measured with the spectrophotometer as we did for eggs, but on a piece of blotting paper after gently smearing 2 μl of the collected secretion on a 1-cm-diameter circle.

Antagonistic activity of uropygial gland secretion

Antagonistic activity of the uropygial gland secretion (UGS) of female hoopoes was estimated as the area of the inhibition halo that the secretion produces in Petri dishes inoculated with culture of *B. licheniformis* D13. This bacterium is a well-known feather-degrading bacterium that is commonly used as indicator bacterium in tests of antagonism (Martín-Platero et al. 2006; Soler et al. 2008a; Ruiz-Rodríguez et al. 2012, 2013). Interestingly, we know that antimicrobial activity of substances in the uropygial secretion of hoopoes against this strain is usually related to activity against other bacteria species including pathogens of embryos such as *Micrococcus* spp., *Escherichia coli*, and *Staphylococcus* spp. (Martín-Platero et al. 2006; Soler et al. 2008a; Ruiz-Rodríguez et al. 2012, 2013). Thus, we used activity level against *B. licheniformis* as a proxy of general antimicrobial activity of secretions. Overnight cultivation of *B. licheniformis* in 5 ml Brain Heart Infusion broth was mixed with 15 ml Brain Hearth Agar and the blend was poured into an empty plate.

In order to improve the detection of inhibition zones in the antagonistic tests by using a transparent phase, and given that both bacteriocins and other chemicals produced by symbiotic bacteria found in UGS (Martín-Platero et al. 2006; Martín-Vivaldi et al. 2010) are water soluble, UGS were centrifuged at 9,279*g* for 5 min, and the watery phase was used for the tests. We took 2–4 μl of this phase that were added directly to plates inoculated with the indicator bacteria that were cultivated at 32ºC for 24 h before estimating the area of the clearing zone as the index of antimicrobial activity. To do so, we photographed the plate with a digital camera and later estimated the number of pixels of the diameter of the growth inhibition area relative (%) to the number of pixels of the diameter of the complete plate measured in the same digital image.

Statistical analyses

The changes along incubation on hoopoe egg coloration were examined by mean of general linear models (GLM), with color scores as dependent variables, time of color measurements (i.e., laying vs. hatching) as the fixed factor, and nest identity as random factor to account for nonindependence of color values of eggs from the same nest. The effects of experimental smearing of artificially incubated eggs with water or uropygial secretion of hoopoes were analyzed by means of repeated measures MANOVA with color factors as dependent variables, and treatment (i.e., water vs. UGS), and time of measurements (i.e., before vs. after treatment) as two independent within-factors. Because our prediction was focused on differential color changes of eggs under experimental treatments, we also estimated the interaction between both within-factors.

For exploring the effect of the experiment preventing females from accessing uropygial secretions, we first tested for differences in egg coloration of females under different control treatments. Coloration of eggshells of control I and control II females did not differ significantly for any of the color factors (F<1.14, df=1,94, P>0.240) after controlling for year variation. Thus, we combined data from both kinds of controls to improve the statistical power of the analyses. The effects of the experimental treatment on eggshell coloration were analyzed using GLMs with the three different color factors as dependent variables and the experimental treatment as the fixed factor. Moreover, since we measured more than one egg per experimental clutch, clutch identity nested within experimental treatment was also included in the model as a random factor.

The association between coloration and antimicrobial activity of the uropygial secretion was explored by mean of nonparametric rank correlations.

Results

Uropygial secretion and eggshell coloration

The bluish-gray eggs of hoopoes drastically changed to dark greenish-brown color along the incubation period in natural clutches in which females smeared their uropygial secretion to eggs (MANOVA, Wilks=0.048, F=288.22, df=3,59,

$P<0.0001$; Fig. 2a). This egg color change implied a significant decrease of brightness ($F=322.44$, df=1,61, $P<0.0001$), an increase in saturation ($F=450.88$, df=1,61, $P<0.0001$), and an increase in hue ($F=349.06$, df=1,61, $P<0.0001$) along the incubation (Fig. 2b). The detected effects of incubation on eggshell coloration of hoopoes were similar to those found for artificially incubated eggs that were brushed with UGS, while those brushed with water (i.e., control treatment) did not experience color changes (Table 1, visual differences in color changes experienced by experimental and naturally incubated hoopoe eggs are shown in Fig. 3).

Preventing females from accessing the uropygial gland secretion drastically reduced color changes of eggs along incubation (blue line in Fig. 3a) in comparison to the eggs of control females (brown line in Fig. 3a). In addition, in control artificially incubated eggs (smeared with water, i.e., without contact with the UGS), the coloration did not change along the incubation (Fig. 3c). Incubated eggs from females with access to the uropygial secretion were significantly darker ($F=122.55$, df=1,68.9, $P<0.0001$), more saturated ($F=120.73$, df=1,71.7, $P<0.0001$), and of higher hue value ($F=53.57$, df=1,70.2, $P<0.0001$) than those of females under the experimental treatment (Fig. 4). Visual differences in coloration of eggshells in nests of experimental and control females are shown at the bottom of Fig. 4. These results strongly suggest a link between the changes in color and the retention of uropygial secretion by eggshells of hoopoes.

Coloration and antimicrobial activity of uropygial secretion

Average brightness, saturation, and hue values of uropygial secretion of the seven sampled females was 66.05 %

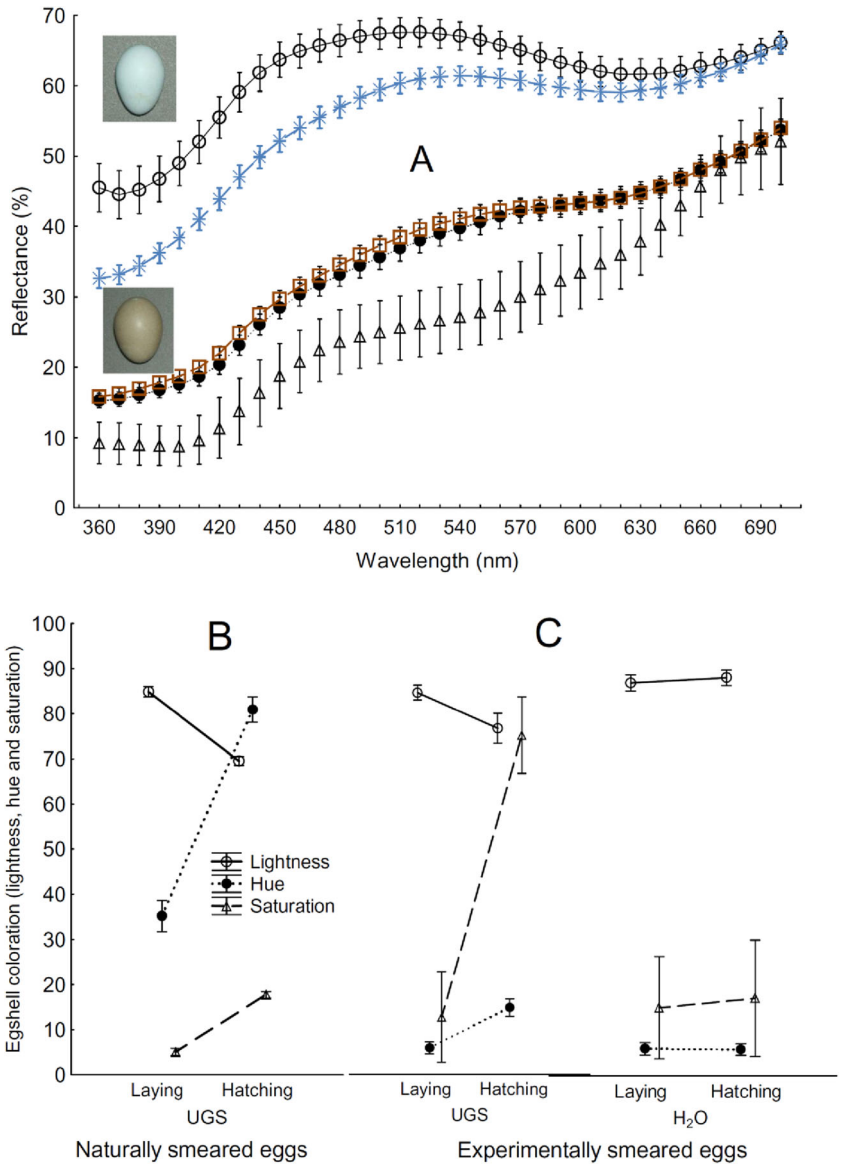

Fig. 2 Change in color that hoopoe eggs suffered from laying to hatching time. Subfigure **a** shows reflectance spectra (±95 % confidence intervals) of recently laid bluish-gray hoopoe eggs at the day of laying (*empty circles*) and that of brown eggs at the end of the incubation period (*full circles*). *Empty squares* and *blue stars* represent the color of incubated eggs of experimental females that did and did not have access to the uropygial gland secretion, respectively. Finally, *triangles* show reflectance spectra of uropygial secretion measured on a white piece of paper. Subfigures **b** and **c** show means± 95 % confidence intervals of color scores of naturally and artificially (smeared with uropygial secretion (*UGS*) or water (H_2O)) incubated hoopoe eggs respectively, at laying and close to hatching time

Table 1 Repeated measures MANOVA of color (i.e., brightness, hue, and saturation) change along the incubation experienced by eggs brushed with UGS and water (i.e., treatment effect) during the incubation. Univariate results for each of the color variable are also shown. The interaction between incubation and treatment indicates whether or not egg color change during incubation consistently varied for eggs under different experimental treatment

	MANOVA			Brightness		Hue		Saturation	
	Wilks	$F_{3,5}$	P	$F_{1,7}$	P	$F_{1,7}$	P	$F_{1,7}$	P
Treatment (1)	0.063	24.90	0.0020	28.71	0.0011	51.24	0.0001	66.19	<0.0001
Incubation (2)	0.013	130.60	<0.0001	17.11	0.0044	425.78	<0.0001	69.51	<0.0001
(1)×(2)	0.034	47.90	0.0004	32.32	0.0007	156.84	<0.0001	67.06	<0.0001

(SE=1.37), 25.68 % (SE=0.56), and 81.01° (SE=0.67), respectively. The reflectance curve of uropygial secretions was of similar shape than that of eggshells close to hatching, but with relatively lower and higher values at intermediated (500–570 nm) and high (640–700 nm) wavelengths, respectively (triangles in Fig. 2a). These differences may be due to the reflectance of the blotting paper where we spread the secretion which differs from that of the eggshell. All the female uropygial secretions tested inhibited growth of *B. licheniformis* (diameter of inhibition halo=7.02 mm, SE=0.12 mm). Size of the inhibition halo did not depend on volume of used secretion (i.e., watery phase) (Spearman rank correlation=0.347, P=0.205). The only color variable that resulted associated with antimicrobial activity of the secretion was chroma, with secretions of less saturated coloration showing higher antimicrobial activity (Spearman rank correlation=−0.893, P=0.007; Fig. 5), being the relationship with brightness and hue far from statistical significance (Spearman rank correlation<0.250, P>0.589).

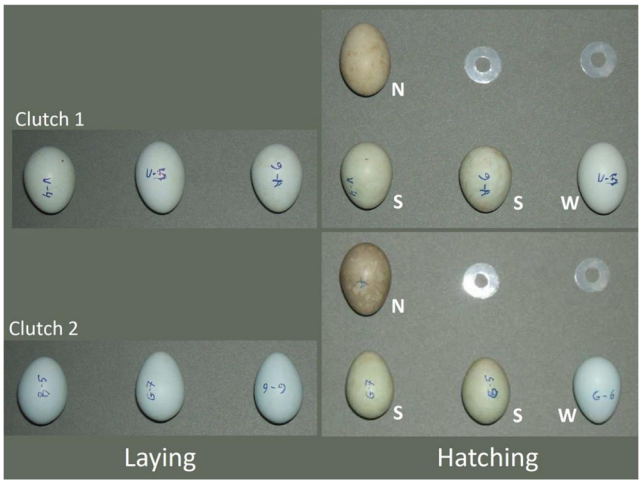

Fig. 3 Changes in the color of hoopoe eggs along the incubation period. On the *left*, pictures of three eggs from two different clutches at laying. On the *right*, we show color at the end of the incubation period of an egg of the same clutches incubated by the female in the nest (*N*), and three eggs maintained in the incubator; two of them regularly smeared with uropygial secretion (*S*) and the other one with water (*W*)

Discussion

Our main results show that recently laid bluish-gray eggs of hoopoes changed during the incubation stage to a darker and more saturated coloration of higher hue value (i.e., greenish-brown coloration). This color change was drastically reduced in nests where we blocked experimentally female access to the uropygial secretion. Furthermore, eggshells that in the lab were experimentally smeared with uropygial secretion experienced similar color changes than naturally incubated eggs did, while control eggs incubated in the lab without contact with secretion did not experience such color changes. All these results strongly support the hypothesis that uropygial secretion changes the eggshell coloration during incubation. Moreover, we detected a negative association between color saturation and antimicrobial activity of UGS of incubating females.

The uropygial secretion of incubating hoopoes is of special characteristics (i.e., antibiotic properties and brown color) that are mediated by symbiotic bacteria living within the uropygial gland (Soler et al. 2008a; Martín-Vivaldi et al. 2009, 2010; Ruiz-Rodriguez et al. 2013). The dark color of uropygial secretions from hoopoes is an indication of the presence of symbiotic bacteria within the uropygial gland (Soler et al. 2008a), and secretions from individuals with antibiotic treated uropygial glands are paler and redder than those from control individuals (Martín-Vivaldi et al. 2009). Thus, as preen secretion changes the eggshell coloration during incubation, eggshell coloration may inform males of the presence, abundance, or even particularities of the bacterial community of females affecting the color of the secretion. Symbiotic bacteria of the uropygial gland of hoopoes confer secretion with important antimicrobial properties (Martín-Platero et al. 2006; Martín-Vivaldi et al. 2010) that are likely transferred from mother to offspring (Ruiz-Rodríguez et al. 2012). Thus, we speculated with the possibility that color of the uropygial gland secretion was related to its antimicrobial properties and found support for this association. Secretions with more saturated color were those with the lower antimicrobial activity. Interestingly, secretions of hoopoes experimentally

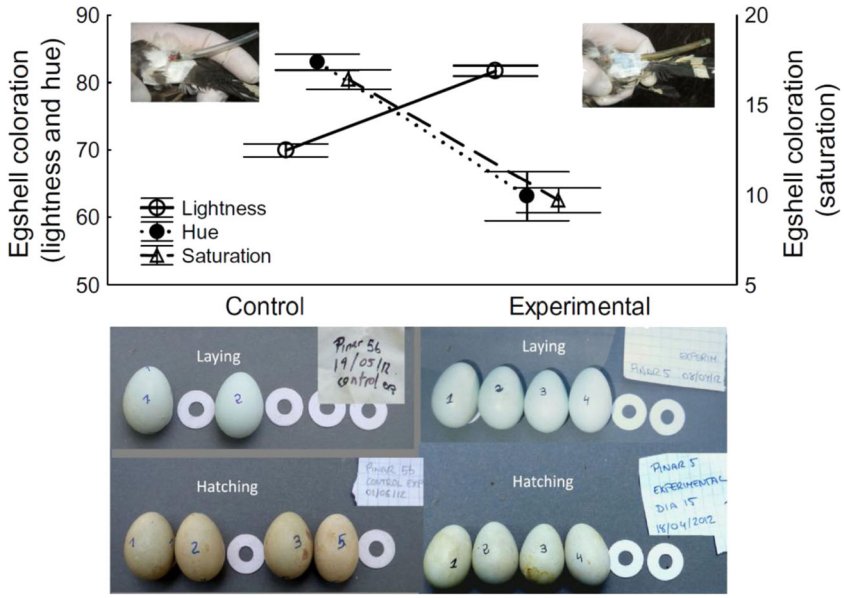

Fig 4 Means±95 % confidence intervals of color scores of naturally incubated hoopoe eggs, close to hatching time, by control (*left*) and experimental females without access to the uropygial secretion (*right*). Pictures below were taken in 2012 and show eggs of one control and one experimental female at the time of laying and hatching. Eggs in different photos were those present in the nest at the time of visits. Labels of the photo at the right-base of the figure were too separate of the eggs in the original photo and were cut and pasted within the same picture

treated with antibiotics to reduce the load of antimicrobial producing symbionts became paler in the black axis of Küppers (2002) color tables (Martín-Vivaldi et al. 2009), and this axis is strongly negatively related with chroma as estimated here (i.e., linear regression of chroma measured with the Minolta CM-2600d apparatus at 49 color squares from Küppers (2002) on their values in seven levels of blacks (40–99) and seven levels of magenta (40–99); $R=-0.99$, $P<0.0001$). Thus, secretions of less saturated chroma may be those from glands containing more symbiotic bacteria, thereby explaining their higher antimicrobial activity. Consequently, although more research is necessary to understand the relationship between antimicrobial properties and coloration of uropygial secretion of hoopoes, our results suggest that eggshell coloration of hoopoes could reflect antimicrobial quality of females.

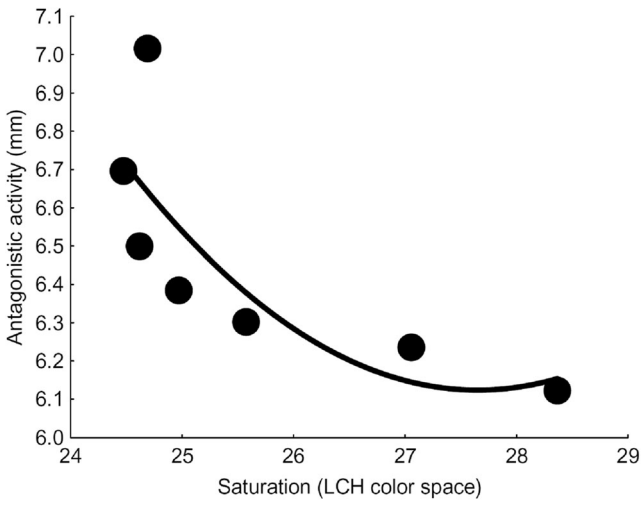

Fig. 5 Relationship between the antimicrobial activity of uropygial gland secretion of hoopoes against *Bacillus licheniformis*, measured as the diameter in millimeter of the inhibition halo, and saturation of the secretion color

Recognizing antimicrobial quality of females by evaluating egg coloration would be of selective advantage for males both if this implies direct benefits given the higher prospects of offspring protected by such secretions (Martín-Vivaldi et al. 2014) and indirect ones if beneficial bacteria would pass to the offspring through vertical transmission. In both cases, it may lead males to invest differentially in reproduction responding to a postmating sexually selected signal (Burley 1988; Soler et al. 1998; Moreno et al. 2004) as has, for instance, been demonstrated to occur in spotless starlings (*Sturnus unicolor*) (Soler et al. 2008b), pied flycatchers (*Ficedula hypoleuca*) (Moreno et al. 2008), and American robins (*Turdus migratorius*) (English and Montgomerie 2011) responding to blue-green eggshell color intensity. The link between color of the eggs and the application of uropygial secretion on them found in this manuscript, together with the detected negative association between color saturation of the uropygial secretion and the antimicrobial activity, opens the possibility of exploring the hypothesis that the cosmetic use of uropygial secretion to color the eggs by hoopoes has a sexual selection component. Hoopoes nest in dimly lit cavities where assessing color changes may be difficult due to limitation of their visual system (Wesolowski and Maziarz 2012), which some researchers have suggested may preclude a signal role of egg coloration (Reynolds et al. 2009). However, we are aware of several hole-nesting birds that are able to discriminate between visual stimuli that, according to visual color modeling, should not be possible (Holveck et al. 2010; Avilés et al. 2010, 2011). Thus, exploring the effect of egg coloration (i.e., saturation) on paternal behavior experimentally is necessary before confirming the hypothetical signaling role of uropygial secretion of hoopoes.

The hypothesis that egg colorations reflect antimicrobial capacity of incubating individuals may also apply to other species since the uropygial secretion may reach eggshells when belly feathers became in contact with eggs during incubation (Cook et al. 2005). On the one hand, uropygial secretions of birds include antimicrobial substances (Jacob and Ziswiler 1982), some of them active against feather-degrading bacteria (Ruiz-Rodríguez et al. 2009), that may reach eggshells and protect embryo from pathogenic infections (Cook et al. 2005; Soler et al. 2010, 2012; Møller et al. 2010). On the other hand, uropygial secretions influence coloration of feathers (Delhey et al. 2007; Lopez-Rull et al. 2010; Amat et al. 2011; Perez-Rodriguez et al. 2011) and bills (Piault et al. 2008) of different species of birds. Thus, it is possible that changes in eggshell coloration due to uropygial secretion drawn in information on antimicrobial characteristics of incubating individuals.

Here, we show strong evidence that hoopoes make use of uropygial secretion as a cosmetic for eggshells producing drastic changes in egg coloration. More subtle changes may also occur in some other species of birds demonstrating phenotypic characteristics of incubating individuals that may be alternative or complementary to that provided by feather coloration due to uropygial secretion. A nonfunctional alternative possibility is that the detected changes in eggshell coloration did not have a signaling function, but were simply the consequence of hoopoes smearing eggshells with antimicrobials that protect embryos from trans-shell infections. Further experimental work is needed for testing the signaling function and we hope that our results encourage such research not only in hoopoes but also in other bird species.

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Ethical standards The experiment was conducted according to relevant Spanish national guidelines (Real Decreto 1201/2005, de 10 de Octubre) and under the permission of Junta de Andalucía, Dirección General de Gestión del Medio Natural which authorized the establishment and maintenance of the captive breeding population (Resolución de 14 de Abril de 2008) and conceded the permits required to perform the present research according to Spanish regulations (Resoluciones de 14 de Abril de 2008 and 23 de Marzo de 2010).

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