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Uropygial gland and bib colouration in the house sparrow

Gregorio Moreno-Rueda

Birds frequently signal different qualities by plumage colouration, mainly during mating. However, plumage colouration is determined during the moult, and therefore it would indicate the quality of individual birds during the moult, not its current quality. Recent studies, however, suggest that birds could modify plumage colouration by using cosmetic preen oil. In this study, I show that bib colouration is related to uropygial gland size and body condition in male house sparrows (Passer domesticus). Moreover, I conducted an experiment in which a group of sparrows were inoculated with an antigen, mimicking an illness. In control birds, short-term changes in bib colouration were related to both body condition and change in uropygial gland size. Therefore, birds that spent more preen oil (thus, reducing uropygial gland size), showed a greater colouration change. However, bib colouration did not change with use of preen oil in experimental birds inoculated with the antigen. That is, the simulated illness cancelled the effect of preen oil on bib colouration. Given that the experiment did not affect preen oil production, this finding suggests that the immune challenge provoked a change in the composition of preen oil, affecting its cosmetic properties. In short, the results of this study suggest that (1) male house sparrows produce cosmetic preen oil that alters the colouration of their bibs; (2) the more effort in preening, the more change in bib colouration; and (3) in this way, bib colouration has the potential to signal current health status, since less healthy birds showed less capacity to change bib colouration.
Uropygial gland and bib colouration in the house sparrow

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ABSTRACT

Birds frequently signal different qualities by plumage colouration, mainly during mating. However, plumage colouration is determined during the moult, and therefore it would indicate the quality of individual birds during the moult, not its current quality. Recent studies, however, suggest that birds could modify plumage colouration by using cosmetic preen oil. In this study, I show that bib colouration is related to uropygial gland size and body condition in male house sparrows (*Passer domesticus*). Moreover, I conducted an experiment in which a group of sparrows were inoculated with an antigen, mimicking an illness. In control birds, short-term changes in bib colouration were related to both body condition and change in uropygial gland size. Therefore, birds that spent more preen oil (thus, reducing uropygial gland size), showed a greater colouration change. However, bib colouration did not change with use of preen oil in experimental birds inoculated with the antigen. That is, the simulated illness cancelled the effect of preen oil on bib colouration. Given that the experiment did not affect preen oil production, this finding suggests that the immune challenge provoked a change in the composition of preen oil, affecting its cosmetic properties. In short, the results of this study suggest that (1) male house sparrows produce cosmetic preen oil that alters the colouration of their bibs; (2) the more effort in preening, the more change in bib colouration; and (3) in this way, bib colouration has the potential to signal current health status, since less healthy birds showed less capacity to change bib colouration.

Keywords: cosmetic, house sparrow, LPS, mate choice, *Passer domesticus*, sexual selection, uropygial gland.
INTRODUCTION

Animals frequently use patches of colouration in communication (Bradbury & Vehrencamp 2011). Bird plumage colouration, in particular, is a recurrent model system for the study of communication based on colouration (Hill & McGraw 2006). Plumage colouration is produced by pigments embedded in the feathers, as well as by the structure of keratin layers (Hill & McGraw 2006). For communication being useful (for the receiver), colour patches should convey some type of information about determinate quality of the bearer (Searcy & Nowicki 2005). In this sense, signals based on plumage colouration have the problem that coloured plumage patches are formed during the moult and thus presumably reflect bearer condition (for a given quality) during the moult (e.g. Vágási et al. 2010), not the current quality status. Although current bearer quality status might be correlated with that during the moult (Saks et al. 2003), this may not be necessarily true. For example, if the patch colour signals health status during the moult and the population suffers an epidemic after the moult was completed, the signal would become uninformative.

Nevertheless, plumage colouration is not invariable and indeed changes with time (e.g., Örnborg et al. 2002; Figuerola & Senar 2005; Delhey et al. 2006), primarily by physical abrasion. Moreover, plumage colouration may change from the addition of cosmetics (reviews in Montgomerie 2006b; Delhey et al. 2007). One of the main cosmetics used by birds is the secretion of uropygial gland (hereafter, preen oil), an oily secretion that birds spread on their plumage during preening (Clark 2004). Several studies show that preen oil effectively alters plumage colouration (Surmacki & Nowakowski 2007; López-Rull et al. 2010; Amat et al. 2011; Pérez-Rodriguez et al. 2011; but see Delhey et al. 2008). Indeed, it has been proposed that, by changing plumage colouration with the addition of preen oil, birds may “update” the information contained in the signal about the quality status of the sender (Montgomerie 2006b; López-Rull et al. 2010).
However, although it is well established that preen oil changes plumage colouration, it is unknown whether it does it in a way that in fact updates the message contained in the signal, so that the new colouration indicates the current condition of the bearer, instead of the condition when it moulted. The only evidence in favour of this hypothesis comes from the beak colour of tawny owlets (*Strix aluco*). In these nestlings, beak brightness is influenced by preen oil, and when an infection is mimicked by the inoculation of an antigen (lipopolysaccharide from the cell wall of *Escherichia coli*, LPS), the secretion of preen oil is reduced and consequently the beak becomes lighter (Piault et al. 2008). However, it is unknown whether beak colouration is used as a signal in these chicks (perhaps signalling health to their parents), or the colour change detected was simply a by-product of using the beak to smear the preen oil on the plumage.

A recent study has shown that an immune challenge reduces preen-oil production in the house sparrow (*Passer domesticus*) in a body-condition-dependent fashion (Moreno-Rueda 2015), similarly to tawny owlets (see Piault et al. 2008). Thus, these studies suggest that preen-oil production is influenced by health status (also see Pap et al. 2013). Consequently, I hypothesise that (1) the bib colour of male house sparrows may be modified by preen oil, (2) house sparrows facing an immunological challenge may find their preen oil production impaired, which in turn (3) will affect the colouration of their bibs, and in this way (4) bib colouration may indicate current health status of males. The black bib of house sparrows intervenes in intraspecific communication (Anderson 2006; Nakagawa et al. 2007), both in mate choice (Møller 1988; Griggio & Hoi 2010; own unpublished data; but see Kimball 1996) and signalling dominance status (Møller 1987; González et al. 2002; McGraw et al. 2003). Most studies have focussed on bib size (Nakagawa et al. 2007), but bib lightness and saturation are strongly correlated (negatively and positively,
respectively) with bib size (Václav 2006). Moreover, bib size is positively related to immunocompetence (Møller et al. 1996; González et al. 1999).

In order to test this hypothesis, I examined the relationship between bib colouration and uropygial gland size, and carried out an experiment in which a group of house sparrows were inoculated with LPS, while a second group served as control (these birds were sham-inoculated with phosphate-buffered saline, PBS). Subsequently, I examined changes in bib colouration as a consequence of the experiment.

METHODS

General procedure

The study was conducted during March 2011 with 21 adult male house sparrows captured with mist-nets on a farm in Padul (SE Spain, 37° 01’ N, 3° 37’ W) and transported to an outdoor aviary located in Moraleda de Zafayona (37° 11’ N, 3° 57’ W). No bird suffered any damage during capture, transport, maintenance in the aviary, or as a consequence of the experiment. The aviary structure followed the recommendations of the European directive as well as national legislation. Measuring about 20 m³, the aviary was built with bricks at the base (1-m height) and a complete wall, the remaining being covered with a mesh supported by a metal framework. The structure was designed to avoid injuring the birds. A roof was provided to protect birds from rainfall and direct sunlight. All birds were individually marked with colour rings, and were supplied with food (seed mixture, fruit, and different vitamins and minerals) and water with *ad libitum* access, as well as diverse perches, and trays with water and powder for bathing and dust bathing. The aviary, and especially the food and water containers, were carefully cleaned and disinfected before the capture
of the birds. Confinement lasted for a week, and when the study ended, the sparrows were released in the same place where they had been captured. The study was performed with the permission of the Andalusian government.

On 03 March 2011, 12 sparrows were subcutaneously injected in the patagium with 0.1 mg of LPS (serotype 055:B5, L-2880, Sigma Aldrich), diluted in 0.01 ml of isotonic phosphate-buffered saline. LPS acts as an antigen, provoking a humoral immune reaction that mimics an infection, and diverts energy from other functions to the immune system. Consequently, inoculating LPS usually lowers body mass in the house sparrow (Bonneaud et al. 2003; Moreno-Rueda 2011). Another 9 sparrows were injected with 0.01 ml of PBS as a sham control. To determine whether the antigen effectively stimulated the immune system, I measured the thickness of the patagium where the substances were inoculated with a pressure-sensitive micrometre (Mitutoyo; accuracy 0.01 mm). Measurements were taken before injecting the substance and four hours afterwards, when the immune response to LPS is maximal (Parmentier et al. 1998). Then, I tested whether the patagium was significantly swelled in LPS-inoculated birds, which indicates an immune response to the antigen.

I took a number of measurements just prior the experiment and just when the experiment was ended (on 10 March). Firstly, I measured the length, width, and height (from the base of the gland to the base of the papilla) of the uropygial gland (three times each) with a digital calliper (accuracy 0.01 mm), and estimated its size by multiplying the three measurements, which is a good estimator of gland volume and preen-oil production (Pap et al. 2010). The repeatability (Lessells & Boag 1987) of the uropygial gland size estimation was 0.76 ($F_{1,19} = 72.7$, $p < 0.001$). Also, body mass was measured with a digital balance (accuracy 0.1 g), and wing length was measured with a ruler (accuracy 0.5 mm). Body condition was estimated as the residuals of the regression of body
mass (log-transformed) against the wing length (log-transformed) as a measurement of skeletal body size (review in Green 2001). Furthermore, I measured bib size by means of photography on gridded paper with a digital camera (Fujifilm 10.2 megapixels; following Figuerola & Senar 2000). The camera was mounted on a tripod, consistently at the same distance from birds, which were held with the breast plumage combed in order to ensure a normal position. Afterwards, patch surface areas were measured with the program Image J (Abramoff et al. 2004). Photos were scaled using the gridded paper as reference. Then, I adjusted the area using the “colour threshold” tool, and measured the area of bib of each bird with the “analyse particles” tool.

Plus, the colouration of the bib was measured with a spectrophotometer (Minolta CM-2600d). After reference calibration of white, the spectrophotometer was placed over the bib and three beams of light were projected through a hole of 3 mm in diameter. As a result, three reflectance measurements were taken and automatically averaged (Andersson & Prager 2006). The spectrophotometer did not measure the ultraviolet spectrum, whose measurement is unnecessary given that house sparrows do not reflect ultraviolet radiation in the bib (Václav 2006). Bib colouration was measured in the L*a*b* colour-space of the Commission Internationale d’Eclairage (CIE; Montgomerie 2006a). L*a*b* is a three-dimensional rectangular colour space. L* axis represents lightness (0 is completely black, 100 is completely white); a* axis represents red-green gradient (positive values are red, negative values are green); b* axis represents blue-yellow gradient (positive values are yellow, negative values are blue). From L*a*b* values I determined the saturation, i.e., the radiance in a specific part of the spectrum in relation to the radiance from the whole visible spectrum. Saturation was calculated as $C^* = [(a*)^2 + (b*)^2]^{1/2}$, measured as the percentage distance from the centre of the colour space to its circumference, where
pure spectral colours are represented. Hue angle (the “colour” in common parlance) was calculated as $H^* = \tan^{-1}(b^*/a^*)$ (Endler 1990).

Statistical analyses

In a first analysis, with data taken prior the inoculation of LPS and PBS, I used the $t$-test to check for differences between LPS- and PBS-inoculated sparrows for body mass, wing length, body condition, uropygial gland size, bib size, and bib colouration (bib lightness, saturation, and hue). In addition, I examined the relationships among the different biometrical variables by running Pearson correlations among bib size, bib colouration (lightness, saturation, and hue), body mass, wing length, body condition, and uropygial gland size. Given that bib saturation was correlated with body condition, uropygial gland size, and bib size (see Results), in order to ascertain the independent relationships among these variables with bib saturation, I carried out a multiple regression model (Linear Model) of type-III Sums of Squares (Quinn & Keough 2002). Collinearity among continuous variables was checked by examining tolerance (Quinn & Keough 2002). Normality and homoscedasticity of residuals of the models were checked according to Shapiro-Wilks and Levene's tests, respectively (Quinn & Keough 2002), and, when necessary, the variables were log-transformed in order to improve the fit of the models. Similar multiple regression models with bib lightness and hue as dependent variables were not carried out (as unnecessary) because these variables did not correlate with more than one biometrical variable (see below).

In order to confirm that the inoculation of LPS effectively produced an immune response, I tested with paired $t$-test for differences in patagium swelling before and 4-hour after the inoculation in both LPS- and PBS-inoculated sparrows. I estimated the change in patagium
swelling (final minus initial patagium swelling) and used a $t$-test for unpaired samples to examine for differences in change in patagium swelling between treatments. Also, I used the $t$-test to check for differences between treatments in body mass, uropygial gland size, and bib colouration (lightness, saturation, and hue), measured once the experiment ended (7 days after inoculations). In addition, I estimated the change in body mass, uropygial gland size, and every component of bib colouration as final value minus initial value. Then, I tested whether the treatment differentially affected the change of these variables by using $t$-test. Paired $t$-test were also used to examine the change in uropygial gland size.

Lastly, in order to examine which variables were related to changes in bib colouration during the experiment, I carried out a matrix of correlations among the change in bib colouration variables (change in lightness, saturation, and hue) and bib size, body condition, and both the change in uropygial gland size and in body mass. I identified that changes in colouration were correlated with body condition and change in uropygial gland size (see Results). Therefore, in order to ascertain the independent effect of treatment, body condition, and change in uropygial gland size, I performed a set of Linear Models with the change in every component of bib colouration (lightness, saturation, and hue) as dependent variables, and body condition, change in uropygial gland size, treatment, and all the interactions among these variables as predictors. Non-significant interactions were removed from final models. As described above, normality and homoscedasticity of residuals of the models were checked according to Shapiro-Wilks and Levene's tests, respectively (Quinn & Keough 2002), and, when necessary, the variables were log-transformed in order to improve the fit of the models. The complete dataset is available in Table S1.
RESULTS

Descriptive relationships among variables prior the experiment

Prior to the experiment, no statistical differences were found between control (PBS-inoculated) and experimental (LPS-inoculated) house sparrows in initial body mass, wing length, body condition, uropygial gland size, and bib colouration (light, saturation, and hue) (Table 1). Despite the randomization of the treatment assignment, bib size was significantly larger in PBS- than in LPS-inoculated birds (Table 1), nevertheless, this did not seem to affect results of the experiment (see below). Bib size was not significantly correlated with any colour parameter (Table 2), although there was an almost significant trend for sparrows with larger bibs to have more saturated bibs \( (r = 0.416, P = 0.061) \). Bib saturation, moreover, was negatively correlated with mass, body condition, and uropygial gland size (Table 2). Notice that uropygial gland size was not correlated with body condition \( (r = 0.17, P = 0.45) \) or bib size \( (r = -0.10, P = 0.66) \), and body condition was not correlated with bib size \( (r = 0.18, P = 0.43) \). When body condition, uropygial gland size, and bib size were included as predictors in a multiple regression model, a significant correlation between bib size and bib saturation emerged \( (\text{partial } r = 0.47, P < 0.01) \), meanwhile bib saturation remained significantly correlated with body condition \( (\text{partial } r = -0.53, P < 0.01) \), and uropygial gland size \( (\text{partial } r = -0.40, P = 0.01) \) (multiple \( R = 0.83, F_{3, 17} = 12.46, P < 0.001 \), adjusted \( R^2 = 0.63 \), tolerance > 0.92). On the other hand, bib hue was also positively correlated with uropygial gland size, but bib lightness was not correlated with any variable (Table 2). Saturation and hue were significantly correlated among themselves, but not with lightness (Table 2).

Effects of the experiment on the measured variables
The experimental treatment had a significant effect on the house sparrows’ immune system. Sparrows in the LPS-inoculated group showed a significant patagium swelling 4 h after the inoculation (average variation in patagium thickness: 0.37 ± 0.20 mm, \( t_{11} = 6.29, P < 0.001 \)), while the control group showed no swelling (change in thickness: -0.03 ± 0.06 mm, \( t_{8} = 1.33, P = 0.22 \)).

Moreover, the change in patagium thickness was significantly greater in the experimental group than in the control group (Table 1). Sparrows treated with LPS weighed significantly less than controls 7 days after the inoculation, but without significant changes in body mass between treatments (Table 1). For all individuals considered together, the uropygial gland size decreased during the experiment (paired \( t \)-test, \( t_{20} = 2.77, P = 0.011 \)); nevertheless, the change in uropygial gland size did not differ between treatments (Table 1). Regarding bib colour, after the experiment, saturation was significantly higher in LPS-treated sparrows than in controls (Table 1), but no other effect of the treatment on bib colour was detected.

**Determinants of changes in bib colouration during the experiment**

The change in uropygial gland size correlated positively with the change in hue (\( r = 0.61, P = 0.003 \)). Body condition also was related to changes in colouration, as birds in better condition had a greater change in lightness (\( r = 0.44, P = 0.046 \)) and trended (almost significantly) to have a greater change in saturation (\( r = 0.41, P = 0.066 \)) and hue (\( r = -0.40, P = 0.073 \)) (Figure 1). Changes in body mass or bib size were not related to change in any colouration parameter (in all cases \( |r| < 0.4, P > 0.85 \)), and their inclusion in the models below did not qualitatively change the results (Table S2). No other variable correlated with changes in colouration (data not shown).

In a more detailed analysis, I examined the effect of the treatment, change in uropygial gland size (hereafter, \( \Delta \text{UGS} \)), and body condition on the change in colouration by using Linear
Models in which, therefore, the effect of each variable was controlled for the effect of the other variables introduced in the model. Change in hue was significantly determined by ΔUGS, with an almost significant effect of treatment, body condition, and the interaction treatment×ΔUGS (Table 3). More specifically, in the control group, there was a significant correlation between ΔUGS and change in hue ($r = 0.72$, $P = 0.03$), suggesting that the use of preen oil affected bib hue. By contrast, in the experimental group, such a correlation was inexistent ($r = 0.11$, $P = 0.73$; Figure 2a).

Similarly, for saturation, after the experiment was carried out, I found a significant effect of body condition, treatment, and the interaction treatment×ΔUGS on change in bib saturation (Table 3). In this model, a significant effect of the treatment emerged: in sparrows inoculated with LPS, bib saturation did not change significantly (average change 0.45, with lower and upper 95%CI limits of -0.004 and 0.91). However, in the control group, the bib became less saturated, with an average change of -0.54 (95% CI limits: -1.06 and -0.026; significantly below zero [Nakagawa & Cuthill 2007]). Moreover, there was a positive effect of body condition on change in saturation ($\beta = 0.568$). As for hue, experimental individuals showed no correlation between change in hue and ΔUGS ($r = 0.25$, $P = 0.43$), while in control birds, there was an almost significant trend for a greater decrease in uropygial gland size accompanying greater change in saturation ($r = -0.66$, $P = 0.053$; Figure 2b).

For the case of lightness, in a first analysis, I found a significant interaction between ΔUGS and treatment (Table S3), but this finding seemed to be caused by an outlier (Figure S1). When the outlier was removed from the analyses, no significant result emerged (Table 3). Lastly, the change in lightness correlated with change in saturation ($r = 0.44$, $P = 0.045$), but not with the change in hue ($r = -0.38$, $P = 0.092$). In turn, changes in saturation and hue were strongly correlated ($r = -0.70$, $P < 0.001$).
The findings in this study suggest that preen oil influences bib colouration in male house sparrows. This conclusion arises from two evidences. First, bib saturation was correlated with uropygial gland size—which is a good surrogate of preen oil production (Pap et al. 2010). Although several works have found an effect of preen oil on feather colouration (e.g. López-Rull et al. 2010; Amat et al. 2011; Pérez-Rodríguez et al. 2011), I am aware of only other work reporting a relationship between natural inter-individual variation in plumage colouration and uropygial gland size, concretely in great tits (*Parus major*), in which yellow brightness is positively correlated with uropygial gland size (Galván & Sanz 2006). In addition, in house sparrows, uropygial gland size is positively correlated with the size of the wing bar (Moreno-Rueda 2010). As a whole, these studies suggest that part of the variation in inter-individual bird plumage colouration is due to differences in the capacity of individual birds to produce preen oil.

Second, during the experiment, in control birds, changes in hue and saturation of bib colouration were correlated with changes in uropygial gland size. Changes in uropygial gland size during the experiment may be interpreted as a consequence of preen oil use in preening (i.e. preening effort). Therefore, findings in this study suggest that the more birds used oil in preening, the more they changed the colouration of their bibs. It is well established that time invested in preening influences changes in colouration in birds (Zampiga et al. 2004; Griggio et al. 2010; Leitão & Mota 2015), and, moreover, access to preen oil affects plumage colouration (López-Rull et al. 2010). Therefore, the consistent relationships between uropygial gland size and bib saturation—and the correlated changes in both variables—suggest that the (intra- and inter-individual) variation in bib saturation was related to the use of preen oil as a cosmetic.
However, given that the bib is black, one would expect changes in the achromatic part of the colouration, i.e. in the lightness. Strangely, lightness was the component of colouration least affected by the experiment, uropygial gland size, or change in uropygial gland size. In this sense, other studies have shown that preen oil reduces lightness (Delhey et al. 2008; Pérez-Rodríguez et al. 2011). An examination of the $a^*$ (mean: 1.05; min-max: 0.40-2.24) and $b^*$ (mean: 1.56; min-max: -0.18-3.56) coordinates of hue, reveals that the bib hue is brown (black is not a hue, but the result of much reduced reflectance), and the use of preen oil in control birds changed the saturation of that brown colour. A possibility is that the purity of colour (i.e. saturation) reflects how much the bib is free of dirt, and preen oil intervenes by cleaning the bib (the highest the preening effort, measured as decrease in uropygial gland size, the most saturated became the bib; see Figure 3b). It is also possible that the effect of uropygial gland on bib colour is mediated by bacteria, given that it has been shown that bacteria on plumage may alter feather colouration (Shawkey et al. 2007, 2009; Gunderson et al. 2009; Leclaire et al. 2015), and preen oil may impact on plumage bacteria (Shawkey et al. 2003; Reneerkens et al. 2008; but see Czirják et al. [2013] and Giraudeau et al. [2013]).

In addition to the results discussed above, bib colouration (hue and saturation) was correlated with body condition, and, indeed, changes in bib colouration during the experiment were also related to initial body condition. These findings imply that bib colouration -partially dependent on preening- may indicate current physical condition. Therefore, the opportunity is opened for sparrows to update information contained in bib colouration by preening. Results in the experiment went in such a direction. Although the experiment failed to provoke detectable changes in preen oil production or preening effort between experimental groups, it affected the way in which preening affected bib colouration changes during the experiment. That is, while in
control birds the changes in uropygial gland size were related to changes in bib colouration, in
LPS-treated birds, changes in uropygial gland size were unrelated to changes in bib colouration.
The cause of this unexpected effect of the treatment is still unclear, but, given that preen production
or preening effort seemed not to be affected by the experiment, the most plausible explanation is
that the immune challenge affected preen oil composition. It is to say, I hypothesize that immune
challenged sparrows were precluded to synthetize the substances of preen oil that impact on bib
colouration. This hypothesis would explain why in LPS-inoculated sparrows there was no relation
between change in uropygial gland size and change in bib colouration, while in PBS-inoculated
sparrows there was.

In any case, it should be stressed that the results in this study suggest that (1) preen oil
modifies bib colouration in “healthy” house sparrows, but (2) such an effect of preen oil is
cancelled in immune challenged sparrows. Therefore, only healthy sparrows can modify bib
colouration, and, in this way, bib colouration might have the potential to signal the current health
of the bearer. Birds have been described to be able to signal their current health status by changing
colouration of bare body parts, such as the beak, which quickly changes in colour in response to
an immune activation (Blount et al. 2003; Faivre et al. 2003; McGraw & Ardia 2003; Alonso-
Alvarez et al. 2004). Also, plumage patches in some birds may vary their extension by tip abrasion
(e.g. the black bib of house sparrows, Møller & Erritzøe 1992). In this way, for example, pied
flycatchers (Ficedula hypoleuca) indicate current health status by varying the size of their white
forehead patch (Kilpimaa et al. 2004). However, condition-dependent changes in feather
colouration in completely moulted plumage has not been previously reported. Bear in mind that,
although it is well documented that preen oil affects feather colouration (above), until now, this is
the first study showing a link between immune response, preen oil, and changes in plumage
colouration in a bird species.

A still open question, nonetheless, is why the activation of the immune system would
provoke changes in preen-oil composition or production. Preen oil is composed mainly of waxes,
and therefore highly energetic components, undoubtedly very costly to produce, as implied by
different lines of evidence. For example, food restriction in red knots (*Calidris alpina*) reduces
their capacity to produce diester waxes, which are presumably more costly to produce than
monoester waxes (Reneerkens et al. 2007). Moreover, experiments of immune activation
conducted by Piault et al. (2008) and Moreno-Rueda (2015) suggest that preen-oil production is
costly. Indeed, uropygial gland size has been found to be correlated with body condition and cell-
mediated immune response in house sparrows (Moreno-Rueda 2010). On the other hand, the
activation of the immune system implies heavy energy costs (review in Schmid-Hempel 2011; see
Martin et al. [2003] for a study in house sparrows). Therefore, it is very likely that, in LPS-
inoculated sparrows, the immune system and the uropygial gland competed for energy. Although
it seems that preen-oil production was not impaired, it is possible that preen-oil composition
changed to less energy-demanding waxes, as reported for red knots (see Reneerkens et al. 2007).

Lastly, it should be noticed that it is unknown whether the colour of the bib is used as a
signal by house sparrows. Nevertheless, it presumably is, given that the signal depends on the
eumelanin concentration (Jawor & Breitwisch 2003). If this were not so, sparrows could cheat
with a large but thinly melanised (lighter) bib, but by contrast, bib lightness is negatively correlated
with bib size (Václav 2006), suggesting that sparrows that may synthesise much eumelanin
produce larger and darker bibs than do sparrows that synthesise less eumelanin. Moreover, the
relationship found between bib saturation and body condition suggests that there is information
contained in bib colouration. However, experimental studies modifying bib colouration would be welcome to test whether bib colour acts as signal.

In conclusion, the findings in this study suggest that healthy house sparrows modify bib colouration (in particular bib saturation) by preening. However, the effect of preen oil on bib colouration is cancelled in immune challenged sparrows. In this way, the results reported here suggest how house sparrows might use preening to update the information about their health status contained in the colouration of their bibs.

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Table 1

Means (with SD) of variables measured between control (PBS-inoculated) and experimental (LPS-inoculated) sparrows, and the result of the test for the differences. Raw values were used to calculate the means, but variables were transformed when necessary for the t-test. Swelling of the patagium is related to the response to the inoculation of LPS or PBS accordingly. Significant differences appear in bold.

|                          | LPS (n=12) | PBS (n=9) | t       | P     |
|--------------------------|------------|-----------|---------|-------|
|                          | Mean       | SD        | Mean    | SD    |       |
| Initial body mass (g)    | 26.26      | 1.86      | 27.58   | 1.31  | -1.82 | 0.085 |
| Final body mass (g)      | **25.73**  | **1.71**  | **27.21** | **1.15** | **-2.25** | **0.037** |
| Change in body mass (g)  | -0.53      | 0.54      | -0.37   | 0.63  | -0.65 | 0.524 |
| Wing length (mm)         | 80.58      | 1.94      | 80.89   | 0.86  | -0.46 | 0.651 |
| Body condition           | -0.02      | 0.04      | 0.02    | 0.06  | -1.86 | 0.078 |
| Bib size (cm²)           | **15.42**  | **2.71**  | **19.27** | **4.45** | **-2.46** | **0.024** |
| Initial uropygial gland size (mm³) | 0.10 | 0.02 | 0.11 | 0.03 | -1.39 | 0.181 |
| Final uropygial gland size (mm³) | 0.09 | 0.02 | 0.09 | 0.02 | 0.13 | 0.901 |
| Change in uropygial gland size (mm³) | -0.01 | 0.02 | -0.02 | 0.03 | 1.55 | 0.138 |
| Swelling of the patagium | **0.37**   | **0.20**  | **-0.03** | **0.06** | **6.16** | **<0.001** |
| Initial bib lightness    | 21.77      | 5.16      | 19.14   | 5.72  | 1.10  | 0.284 |
| Initial bib saturation   | 2.14       | 0.74      | 1.92    | 1.01  | 0.56  | 0.584 |
| Initial bib hue          | 0.57       | 0.26      | 0.82    | 0.53  | -1.19 | 0.251 |
| Final bib lightness      | 22.23      | 7.59      | 22.05   | 6.52  | 0.06  | 0.955 |
| Final bib saturation     | **2.50**   | **0.68**  | **1.86** | **0.59** | **2.24** | **0.037** |
| Final bib hue            | 0.53       | 0.21      | 0.62    | 0.37  | -0.74 | 0.471 |
| Change in lightness      | 0.47       | 9.58      | 2.91    | 8.39  | -0.61 | 0.549 |
| Change in saturation     | 0.36       | 0.71      | -0.06   | 1.26  | 0.98  | 0.339 |
| Change in hue            | -0.04      | 0.23      | -0.20   | 0.67  | 1.22  | 0.236 |
Table 2
Matrix of correlations among the variables measured in house sparrows before the start of the experiment and bib colouration (hue, saturation, and lightness). Significant or almost significant correlations in bold. * for P < 0.05, ** for P < 0.01, and § for P = 0.061.

|                        | Bib lightness | Bib saturation | Bib hue |
|------------------------|---------------|----------------|---------|
| **Body mass**          | -0.048        | -0.618**       | 0.300   |
| **Wing length**        | 0.153         | -0.345         | 0.155   |
| **Body condition**     | -0.161        | -0.515*        | 0.257   |
| **Uropygial gland size** | -0.335     | -0.542*        | 0.453*  |
| **Bib size**           | -0.083        | 0.416§         | -0.269  |
| **Bib lightness**      | 0.326         | -0.226         |         |
| **Bib saturation**     | -0.664**      |                |         |

Table 3
Results of the lineal models examining the effect of treatment, change in uropygial gland size (ΔUGS), body condition, and the interaction treatment×ΔUGS on the change in colour parameters (lightness, saturation, and hue) of bib. Degree of freedom of error term were 15 for lightness, as an outlier (Figure S1) was removed (see Table S3 for the results including the outlier). Significant results in bold.

|                        | Change bib lightness | Change bib saturation | Change bib hue |
|------------------------|----------------------|-----------------------|----------------|
|                        | β        | F₁,₁₅   | p  | β        | F₁,₁₆   | p | β        | F₁,₁₆   | p |
| **Treatment**          | 1.880    | 0.191   |    | 7.501    | **0.015** |   | 4.043    | 0.062   |   |
| **Body condition**     | 0.33     | 1.703    | 0.212 | 0.57    | 11.530   | **0.004** | -0.35 | 3.783    | 0.070   |   |
| **ΔUGS**               | 0.01     | 0.001    | 0.977 | -0.15   | 0.740    | 0.402 | 0.42    | 4.774    | **0.044** |
| **Treatment*ΔUGS**     | 1.875    | 0.191   |    | 7.550    | **0.014** |   | 4.052    | 0.061   |   |
Figure 1

Relationship between body condition and (a) change in bib lightness, (b) change in bib saturation, and (c) change in bib hue. Black circles are LPS-inoculated individuals, empty squares are PBS-inoculated sparrows.

Figure 1a
Figure 1b

Figure 1c
Figure 2

Relationship between change in uropygial gland size and (a) change in bib hue, and (b) change in bib saturation. Black circles and solid line are LPS-inoculated individuals, empty squares and dashed line are PBS-inoculated sparrows.

Figure 2a

Figure 2b
Supplementary Material

Uropygial gland and bib colouration in the house sparrow

Gregorio Moreno-Rueda

Table S1

| ID  | Treatment | Bib size | Initial mass | Final mass | Wing length | Initial UGS | Final UGS | Initial L* | Final L* | Initial C* | Final C* | Initial H* | Final H* |
|-----|-----------|----------|--------------|------------|-------------|-------------|-----------|------------|----------|------------|----------|------------|----------|
| 1M  | LPS       | 14.391   | 29.000       | 28.400     | 84.000      | 0.091       | 0.076     | 26.450     | 2.125    | 0.432      | 20.300   | 2.021      | 0.301    |
| BG-O| PBS       | 13.716   | 27.600       | 26.500     | 81.500      | 0.148       | 0.075     | 11.440     | 0.866    | 1.024      | 16.370   | 1.942      | 0.436    |
| BN-O| LPS       | 11.437   | 25.300       | 24.200     | 81.000      | 0.076       | 0.067     | 21.260     | 2.449    | 0.540      | 21.630   | 1.679      | 0.722    |
| BO-WW| LPS      | 14.745   | 28.000       | 27.300     | 81.000      | 0.099       | 0.099     | 22.910     | 1.236    | 0.329      | 21.620   | 2.138      | 0.445    |
| BY-O| LPS       | 18.531   | 27.500       | 26.900     | 82.000      | 0.120       | 0.082     | 17.510     | 1.946    | 0.818      | 15.860   | 1.427      | 1.026    |
| GG-O| LPS       | 16.78    | 25.000       | 23.700     | 79.000      | 0.099       | 0.078     | 18.690     | 1.910    | 0.485      | 19.790   | 2.410      | 0.303    |
| GN-O| LPS       | 15.868   | 27.400       | 26.300     | 80.500      | 0.137       | 0.114     | 21.290     | 1.524    | 0.664      | 17.430   | 2.635      | 0.486    |
| GO-WW| PBS      | 19.224   | 29.100       | 27.900     | 80.000      | 0.075       | 0.064     | 17.040     | 1.408    | 0.537      | 35.080   | 3.156      | 0.060    |
| GP-O| PBS       | 15.537   | 28.400       | 28.300     | 80.500      | 0.114       | 0.076     | 16.400     | 1.998    | 0.401      | 20.420   | 2.255      | 0.320    |
| GR-O| PBS       | 15.955   | 27.900       | 27.800     | 82.000      | 0.114       | 0.060     | 15.420     | 1.046    | 1.744      | 25.750   | 1.329      | 0.609    |
| GW-WW| LPS       | 12.978   | 23.800       | 23.400     | 76.000      | 0.072       | 0.059     | 19.440     | 3.154    | 0.790      | 21.730   | 3.873      | 0.466    |
|  | PBS   | 26.834 | 28.300 | 28.200 | 80.000 | 0.089 | 0.082 | 22.770 | 2.675 | 0.482 | 18.390 | 2.125 | 0.611 |
|---|-------|--------|--------|--------|--------|-------|-------|--------|-------|-------|--------|-------|-------|
| NG-O | PBS   | 23.446 | 28.800 | 28.100 | 80.500 | 0.129 | 0.103 | 18.650 | 0.966 | 1.457 | 20.110 | 1.529 | 0.566 |
| NW-O | LPS   | 15.4   | 25.400 | 25.600 | 81.000 | 0.087 | 0.085 | 22.710 | 3.298 | 0.330 | 20.850 | 3.444 | 0.409 |
| OR-WW| PBS   | 18.424 | 25.500 | 25.100 | 79.500 | 0.084 | 0.083 | 22.790 | 2.802 | 0.342 | 25.920 | 2.548 | 0.544 |
| PB-O | LPS   | 15.459 | 26.000 | 26.900 | 82.000 | 0.131 | 0.107 | 15.140 | 1.607 | 1.071 | 18.163 | 1.572 | 0.855 |
| PW-O | LPS   | 10.535 | 26.400 | 25.700 | 82.000 | 0.101 | 0.096 | 34.760 | 1.231 | 0.976 | 18.610 | 2.396 | 0.544 |
| PY-O | LPS   | 18.032 | 28.500 | 28.100 | 81.000 | 0.078 | 0.094 | 19.270 | 1.268 | 0.908 | 44.920 | 2.695 | 0.707 |
| WB-WW| LPS   | 20.455 | 26.900 | 26.300 | 80.000 | 0.113 | 0.133 | 28.290 | 2.996 | 0.354 | 28.780 | 1.280 | 0.758 |
| WG-O | PBS   | 22.768 | 25.200 | 24.900 | 81.500 | 0.075 | 0.081 | 27.120 | 3.744 | 0.315 | 15.430 | 1.573 | 1.366 |
| WN-O | PBS   | 17.911 | 24.300 | 24.000 | 80.000 | 0.102 | 0.123 | 14.107 | 2.682 | 0.174 | 18.140 | 2.706 | 0.383 |
**Table S2**

(A) Results of the lineal models examining the effect of treatment, change in body mass, change in uropygial gland size (ΔUGS), body condition, and the interaction treatment×ΔUGS on colour parameters (lightness, saturation, and hue) of bib. (B) Results of the lineal models examining the effect of treatment, bib size, change in uropygial gland size (ΔUGS), body condition, and the interaction treatment×ΔUGS on colour parameters (lightness, saturation, and hue) of bib. The inclusion of change in body mass or bib size did not qualitatively change findings in Table 3 (for bib lightness, the comparison should be performed with Table S3).

|                  | Bib lightness | Bib saturation | Bib hue |
|------------------|---------------|----------------|---------|
|                  | F<sub>1,15</sub> | p   | F<sub>1,15</sub> | p   | F<sub>1,15</sub> | p   |
| (A) Model including change in body mass |               |     |               |     |               |     |
| Treatment        | 4.704         | 0.047 | 6.760         | 0.020 | 3.447         | 0.083 |
| Change in body mass | 0.161     | 0.694 | 0.015         | 0.903 | 0.275         | 0.607 |
| Body condition   | 4.628         | 0.048 | 9.410         | 0.008 | 3.813         | 0.070 |
| ΔUGS             | 0.909         | 0.355 | 0.708         | 0.413 | 4.823         | 0.044 |
| Treatment×ΔUGS   | 4.709         | 0.046 | 6.807         | 0.020 | 3.458         | 0.083 |

|                  | F<sub>1,15</sub> | p   | F<sub>1,15</sub> | p   | F<sub>1,15</sub> | p   |
| (B) Model including bib size |               |     |               |     |               |     |
| Treatment        | 4.554         | 0.050 | 5.019         | 0.041 | 2.586         | 0.129 |
| Bib size         | 0.001         | 0.982 | 2.599         | 0.128 | 0.997         | 0.334 |
| Body condition   | 4.115         | 0.061 | 15.216        | 0.001 | 4.717         | 0.046 |
| ΔUGS             | 0.916         | 0.354 | 0.019         | 0.891 | 2.437         | 0.139 |
| Treatment×ΔUGS   | 4.550         | 0.050 | 5.040         | 0.040 | 2.584         | 0.129 |

**Table S3**

Results of the lineal model examining the effect of treatment, change in uropygial gland size (ΔUGS), body condition and the interaction treatment×ΔUGS on bib lightness including an outlier detected in Figure S1.

|                  | F<sub>1,16</sub> | p   |
|------------------|------------------|-----|
| Treatment        | 5.396            | 0.034 |
| Body condition   | 4.971            | 0.040 |
| ΔUGS             | 1.165            | 0.297 |
| Treatment×ΔUGS   | 5.398            | 0.034 |
Figure 1
Correlation between change in urohygial gland size and change in bib lightness for control (PBS, empty squares, dashed line) and experimental (LPS, filled circles, black line). The possible outlier in the experimental group is indicated. Note that the analyses for bib lightness in Table 3 do not include that outlier.