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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-Rodríguez 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).

68 However, although it is well established that preen oil changes plumage colouration, it is unknown
69 whether it does it in a way that in fact updates the message contained in the signal, so that the new
70 colouration indicates the current condition of the bearer, instead of the condition when it moulted.
71 The only evidence in favour of this hypothesis comes from the beak colour of tawny owlets (*Strix*
72 *aluco*). In these nestlings, beak brightness is influenced by preen oil, and when an infection is
73 mimicked by the inoculation of an antigen (lipopolysaccharide from the cell wall of *Escherichia*
74 *coli*, LPS), the secretion of preen oil is reduced and consequently the beak becomes lighter (Piault
75 et al. 2008). However, it is unknown whether beak colouration is used as a signal in these chicks
76 (perhaps signalling health to their parents), or the colour change detected was simply a by-product
77 of using the beak to smear the preen oil on the plumage.

78 A recent study has shown that an immune challenge reduces preen-oil production in the
79 house sparrow (*Passer domesticus*) in a body-condition-dependent fashion (Moreno-Rueda 2015),
80 similarly to tawny owlets (see Piault et al. 2008). Thus, these studies suggest that preen-oil
81 production is influenced by health status (also see Pap et al. 2013). Consequently, I hypothesise
82 that (1) the bib colour of male house sparrows may be modified by preen oil, (2) house sparrows
83 facing an immunological challenge may find their preen oil production impaired, which in turn (3)
84 will affect the colouration of their bibs, and in this way (4) bib colouration may indicate current
85 health status of males. The black bib of house sparrows intervenes in intraspecific communication
86 (Anderson 2006; Nakagawa et al. 2007), both in mate choice (Møller 1988; Griggio & Hoi 2010;
87 own unpublished data; but see Kimball 1996) and signalling dominance status (Møller 1987;
88 González et al. 2002; McGraw et al. 2003). Most studies have focussed on bib size (Nakagawa et
89 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, 37º 01′ N, 3º 37′ W) and transported to an outdoor aviary located in Moraleda de Zafayona (37º 11' N, 3º 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 m^3, 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

158 pure spectral colours are represented. Hue angle (the "colour" in common parlance) was calculated
159 as $H^* = \tan^{-1}(b^*/a^*)$ (Endler 1990).
160
161 **Statistical analyses**
162 In a first analysis, with data taken prior the inoculation of LPS and PBS, I used the *t*-test to check
163 for differences between LPS- and PBS-inoculated sparrows for body mass, wing length, body
164 condition, uropygial gland size, bib size, and bib colouration (bib lightness, saturation, and hue).
165 In addition, I examined the relationships among the different biometrical variables by running
166 Pearson correlations among bib size, bib colouration (lightness, saturation, and hue), body mass,
167 wing length, body condition, and uropygial gland size. Given that bib saturation was correlated
168 with body condition, uropygial gland size, and bib size (see Results), in order to ascertain the
169 independent relationships among these variables with bib saturation, I carried out a multiple
170 regression model (Linear Model) of type-III Sums of Squares (Quinn & Keough 2002).
171 Collinearity among continuous variables was checked by examining tolerance (Quinn & Keough
172 2002). Normality and homoscedasticity of residuals of the models were checked according to
173 Shapiro-Wilks and Levene's tests, respectively (Quinn & Keough 2002), and, when necessary, the
174 variables were log-transformed in order to improve the fit of the models. Similar multiple
175 regression models with bib lightness and hue as dependent variables were not carried out (as
176 unnecessary) because these variables did not correlate with more than one biometrical variable
177 (see below).
178 In order to confirm that the inoculation of LPS effectively produced an immune response,
179 I tested with paired *t*-test for differences in patagium swelling before and 4-hour after the
180 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 (partial $r = 0.47$, $P < 0.01$), meanwhile bib saturation remained significantly correlated with body condition (partial $r = -0.53$, $P < 0.01$), and uropygial gland size (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, ΔUGS), 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 ($r = -0.70$, $P < 0.001$).

DISCUSSION

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.

296 However, given that the bib is black, one would expect changes in the achromatic part of
297 the colouration, i.e. in the lightness. Strangely, lightness was the component of colouration least
298 affected by the experiment, uropygial gland size, or change in uropygial gland size. In this sense,
299 other studies have shown that preen oil reduces lightness (Delhey et al. 2008; Pérez-Rodríguez et
300 al. 2011). An examination of the a* (mean: 1.05; min-max: 0.40-2.24) and b* (mean: 1.56; min-
301 max: -0.18-3.56) coordinates of hue, reveals that the bib hue is brown (black is not a hue, but the
302 result of much reduced reflectance), and the use of preen oil in control birds changed the saturation
303 of that brown colour. A possibility is that the purity of colour (i.e. saturation) reflects how much
304 the bib is free of dirt, and preen oil intervenes by cleaning the bib (the highest the preening effort,
305 measured as decrease in uropygial gland size, the most saturated became the bib; see Figure 3b).
306 It is also possible that the effect of uropygial gland on bib colour is mediated by bacteria, given
307 that it has been shown that bacteria on plumage may alter feather colouration (Shawkey et al. 2007,
308 2009; Gunderson et al. 2009; Leclaire et al. 2015), and preen oil may impact on plumage bacteria
309 (Shawkey et al. 2003; Reneerskens et al. 2008; but see Czirják et al. [2013] and Giraudeau et al.
310 [2013]).

311 In addition to the results discussed above, bib colouration (hue and saturation) was
312 correlated with body condition, and, indeed, changes in bib colouration during the experiment were
313 also related to initial body condition. These findings imply that bib colouration -partially
314 dependent on preening- may indicate current physical condition. Therefore, the opportunity is
315 opened for sparrows to update information contained in bib colouration by preening. Results in
316 the experiment went in such a direction. Although the experiment failed to provoke detectable
317 changes in preen oil production or preening effort between experimental groups, it affected the
318 way in which preening affected bib colouration changes during the experiment. That is, while in

319 control birds the changes in uropygial gland size were related to changes in bib colouration, in
320 LPS-treated birds, changes in uropygial gland size were unrelated to changes in bib colouration.
321 The cause of this unexpected effect of the treatment is still unclear, but, given that preen production
322 or preening effort seemed not to be affected by the experiment, the most plausible explanation is
323 that the immune challenge affected preen oil composition. It is to say, I hypothesize that immune
324 challenged sparrows were precluded to synthetize the substances of preen oil that impact on bib
325 colouration. This hypothesis would explain why in LPS-inoculated sparrows there was no relation
326 between change in uropygial gland size and change in bib colouration, while in PBS-inoculated
327 sparrows there was.

328 In any case, it should be stressed that the results in this study suggest that (1) preen oil
329 modifies bib colouration in "healthy" house sparrows, but (2) such an effect of preen oil is
330 cancelled in immune challenged sparrows. Therefore, only healthy sparrows can modify bib
331 colouration, and, in this way, bib colouration might have the potential to signal the current health
332 of the bearer. Birds have been described to be able to signal their current health status by changing
333 colouration of bare body parts, such as the beak, which quickly changes in colour in response to
334 an immune activation (Blount et al. 2003; Faivre et al. 2003; McGraw & Ardia 2003; Alonso-
335 Alvarez et al. 2004). Also, plumage patches in some birds may vary their extension by tip abrasion
336 (e.g. the black bib of house sparrows, Møller & Erritzøe 1992). In this way, for example, pied
337 flycatchers (*Ficedula hypoleuca*) indicate current health status by varying the size of their white
338 forehead patch (Kilpimaa et al. 2004). However, condition-dependent changes in feather
339 colouration in completely moulted plumage has not been previously reported. Bear in mind that,
340 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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536 **Table 1**

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

542

	LPS (n=12)		PBS (n=9)			
	Mean	SD	Mean	SD	t_{19}	*P*
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 (cm^2)	**15.42**	**2.71**	**19.27**	**4.45**	**-2.46**	**0.024**
Initial uropygial gland size (mm^3)	0.10	0.02	0.11	0.03	-1.39	0.181
Final uropygial gland size (mm^3)	0.09	0.02	0.09	0.02	0.13	0.901
Change in uropygial gland size (mm^3)	-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

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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	$F_{1, 15}$	p	β	$F_{1, 16}$	p	β	$F_{1, 16}$	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

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564 **Figure 1**

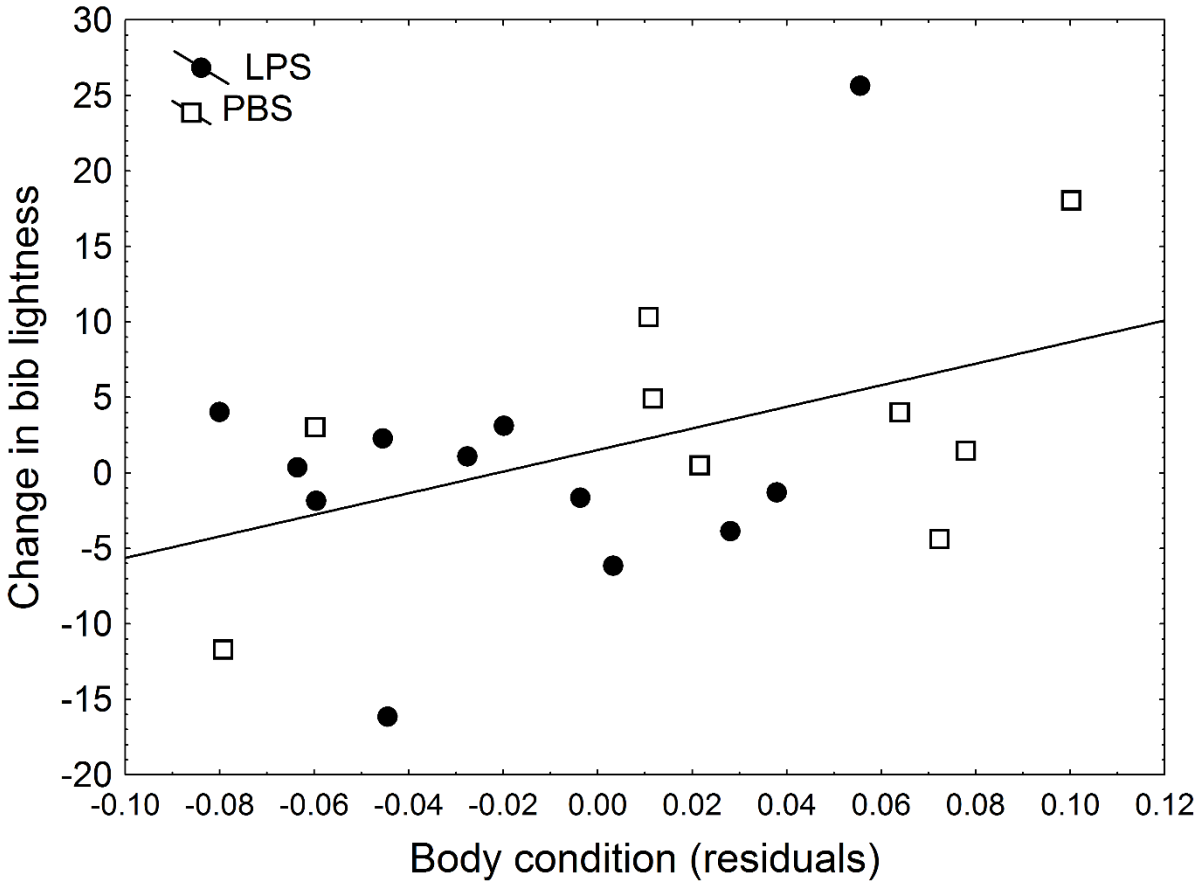
565 Relationship between body condition and (a) change in bib lightness, (b) change in bib saturation,

566 and (c) change in bib hue. Black circles are LPS-inoculated individuals, empty squares are PBS-

567 inoculated sparrows.

568

569 Figure 1a



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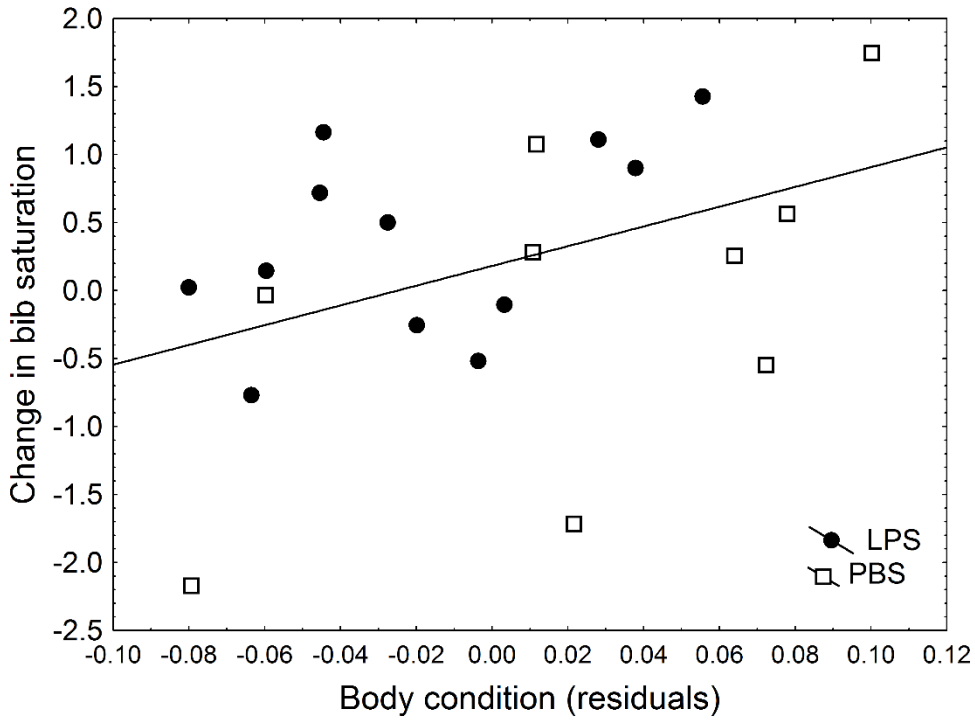
574 Figure 1b



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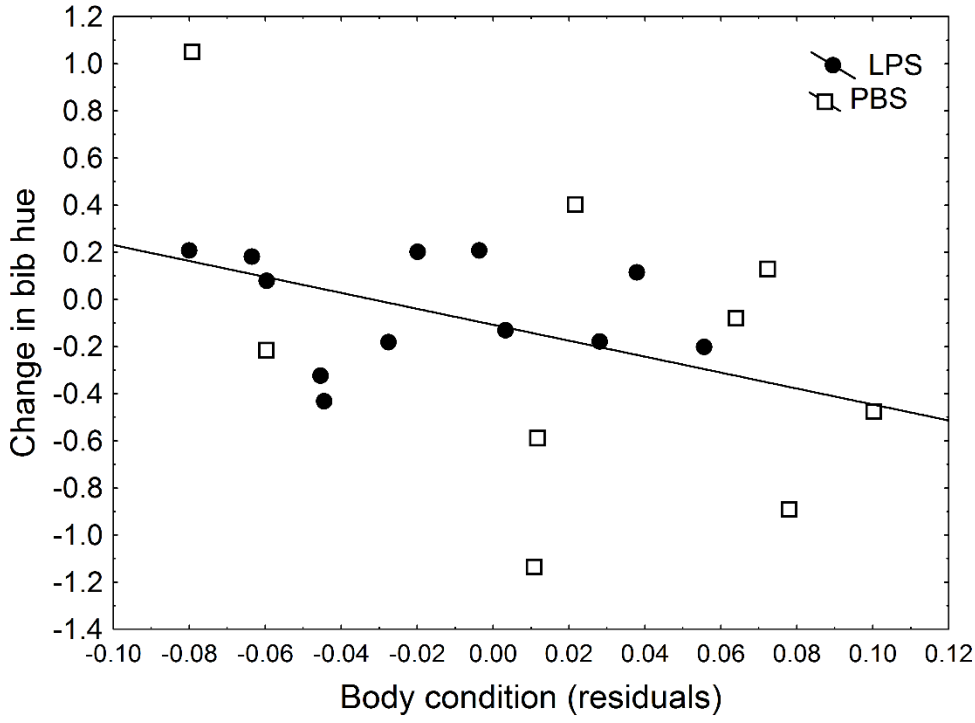
576 Figure 1c



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580 **Figure 2**

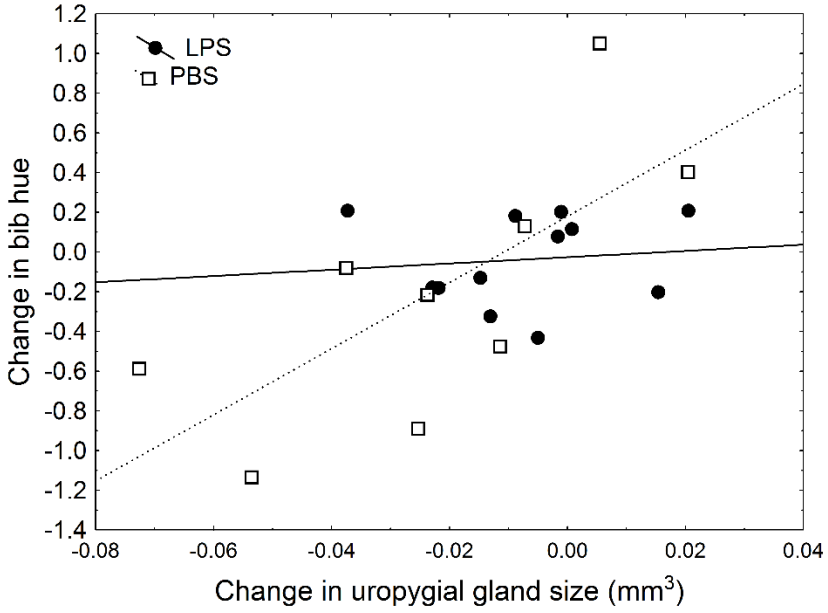
581 Relationship between change in uropygial gland size and (a) change in bib hue, and (b) change in

582 bib saturation. Black circles and solid line are LPS-inoculated individuals, empty squares and

583 dashed line are PBS-inoculated sparrows.

584

585 Figure 2a



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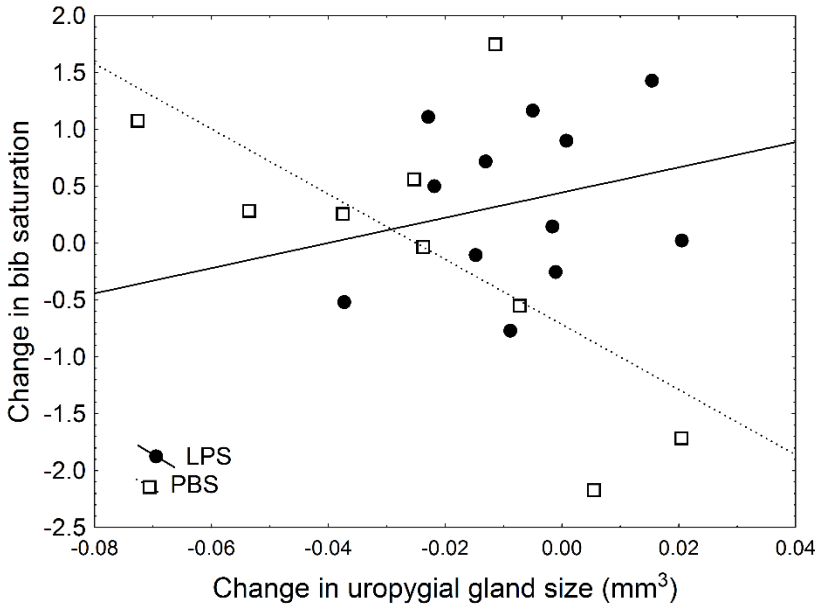
588 Figure 2b



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591 **Supplementary Material**

592

593 **Uropygial gland and bib colouration in the house sparrow**

594

595 **Gregorio Moreno-Rueda**

596

597 **Table S1**

598 Raw data of the variables measured in this study. ID indicates the identification codex for each
599 individual. Bib size is in cm^2, mass is in g, wing length in mm. UGS is uropygial gland size,
600 measured in mm^3. L*, C* and H* are referred to lightness, saturation and hue, respectively.
601

ID	Treatment	Bib size	Initial mass	Final mass	Wing length	Initial UGS	Final UGS	Initial L*	Initial C*	Initial H*	Final L*	Final C*	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	22.800	23.400	76.000	0.072	0.059	19.440	3.154	0.790	21.730	3.873	0.466

NG-O	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
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	23.446	28.800	28.100	80.500	0.129	0.103	18.650	0.966	1.457	20.110	1.529	0.566
PB-O	LPS	18.424	25.500	25.100	79.500	0.084	0.083	22.790	2.802	0.342	25.920	2.548	0.544
PW-O	PBS	15.459	26.000	26.900	82.000	0.131	0.107	15.140	1.607	1.071	18.163	1.572	0.855
PY-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
WB-WW	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
WG-O	PBS	20.455	26.900	26.300	80.000	0.113	0.133	28.290	2.996	0.354	28.780	1.280	0.758
WN-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
WR-O	LPS	17.911	24.300	24.000	80.000	0.102	0.123	14.107	2.682	0.174	18.140	2.706	0.383

608
609 **Table S2**
610 (A) Results of the lineal models examining the effect of treatment, change in body mass, change
611 in uropygial gland size (ΔUGS), body condition, and the interaction treatment×ΔUGS on colour
612 parameters (lightness, saturation, and hue) of bib. (B) Results of the lineal models examining the
613 effect of treatment, bib size, change in uropygial gland size (ΔUGS), body condition, and the
614 interaction treatment×ΔUGS on colour parameters (lightness, saturation, and hue) of bib. The
615 inclusion of change in body mass or bib size did not qualitatively change findings in Table 3 (for
616 bib lightness, the comparison should be performed with Table S3).
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	Bib lightness		Bib saturation		Bib hue	
	$F_{1, 15}$	p	$F_{1, 15}$	p	$F_{1, 15}$	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
(B) Model including bib size						
	$F_{1, 15}$	p	$F_{1, 15}$	p	$F_{1, 15}$	p
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

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621 **Table S3**
622 Results of the lineal model examining the effect of treatment, change in uropygial gland size
623 (ΔUGS), body condition and the interaction treatment×ΔUGS on bib lightness including an outlier
624 detected in Figure S1.
625

	$F_{1, 16}$	p
Treatment	5.396	0.034
Body condition	4.971	0.040
ΔUGS	1.165	0.297
Treatment*ΔUGS	5.398	0.034

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Figure 1

Correlation between change in uropygial 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.