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Article

Plumage brightness and uropygial gland secretions in barn swallows

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Abstract

The uropygial gland has been hypothesized to play a role in sexual signaling through a "make-up" function derived from the effects of secretions from the gland on the appearance of the plumage and bare parts of the body. Here we show that plumage brightness of dorsal feathers of individual barn swallows *Hirundo rustica* was greater in mated than in unmated individuals. In addition, plumage brightness increased with colony size. Furthermore, plumage brightness was positively correlated with the amount of wax in the uropygial gland, negatively correlated with time of sampling of uropygial wax (perhaps because more wax is present early in the morning after an entire night of wax production without any preening), and negatively correlated with the number of chewing lice that degrade the plumage. Experimentally preventing barn swallows from access to the uropygial gland reduced plumage brightness, showing a causal link between secretions from the uropygial gland and plumage brightness. These findings provide evidence consistent with a role of uropygial secretions in signaling plumage brightness.

Key words: brightness, coloration, preening wax, sexual selection, uropygial gland

Many species of animals change their exterior phenotype by using bodily or extra-bodily substances (reviews in Berthold 1967; Grammer et al. 2003; Montgomerie 2006; Delhey et al. 2007). The function of such use of substances for skin, plumage or pelage can broadly be divided into signaling or mating advantages and advantages in terms of antimicrobial defenses although these explanations may not be mutually exclusive. The first hypothesis suggests that the use of substances that are applied to the body surface increases the attractiveness of an individual and thereby improves its mating success or its success with respect to other recipients of the visual signal. Several recent studies have suggested that secretions from the uropygial gland (an exocrine gland in birds that produces complex biochemicals that are smeared with the beak across the plumage) may act as a cosmetic that increases plumage brightness and hence improves sexual attractiveness (e.g., Andersson and Amundsen 1996; Blanco et al. 1999; Figuerola and Senar 2005; Galván and Sanz 2006). López-Rull et al. (2010) and Pérez-Rodríguez et al. (2011) showed evidence of a change in reflectance of plumage in response to application of wax. Several studies have suggested that soiling of the plumage may affect ultraviolet reflectance (Pérez-Rodríguez et al. 2011; Surmacki 2011) by preventing or reducing reflectance from the surface of feathers that otherwise are covered by uropygial secretions. For example, Negro et al. (1999) suggested that the application of red ferro-oxide to the plumage of bearded vultures *Gypaetus barbatus* was due to its attractiveness, although a functional hypothesis suggesting an oxidative effect is just as compatible with available data (Arlettaz et al. 2002). Recently, Hirao et al. (2009) showed that cockerels *Gallus domesticus* copulated less frequently with uropygial glandectomized chickens than with

sham-operated females. They also showed that olfactory bulbectomized cocks copulated equally often with sham-operated and uropygial glandectomized chickens, while sham-operated males copulated less often with uropygial glandectomized chickens than sham-operated females. These findings may reflect a role of the uropygial gland in sexual selection.

The alternative functional hypothesis is that the application of such external substances to skin or plumage is for antimicrobial use (Moreno-Rueda 2018). Secretions of the exocrine uropygial gland are complex, and interspecifically highly diverse chemical compounds with known anti-microbial effects on bacteria and fungi (review in Jacob and Ziswiler 1982). The extreme diversity of these biochemical compounds, as least compared to most other biochemicals, and the fact that they have a strong phylogenetic signal, imply that they have been subject to intense selection, presumably caused by coevolutionary interactions between hosts and parasites. Uropygial gland secretions have a negative effect on bacterial and fungal infections of feathers and the skin (Jacob and Ziswiler 1982; Bandyopadhyay and Bhattacharyya 1996, 1999; Shawkey et al. 2003; Møller et al. 2009). Comparative studies have shown that there are significant viability effects associated with large uropygial glands (Møller et al. 2010a, 2010b), and that large uropygial glands are associated with greater abundance of feather mites and a higher diversity of chewing lice in species of birds (Galván et al. 2008; Møller et al. 2010a). Two publications by Galván et al. (2008; Galván and Sanz 2006) related uropygial secretions to mite abundance. Pap et al. (2010), Meléndez et al. (2014), and Møller et al. (2010a, 2010b), however, did not find relationships between mite abundance and uropygial gland size. Therefore, this relationship is at best weak. Reneerkens et al. (2005) showed that many ground-nesting shorebirds and ducks (hence two independent evolutionary events) have evolved diester uropygial gland secretions that are less volatile and potentially less easy to detect by predators than monesters (at least for an olfactory-based searching dog). Furthermore, the composition of these secretions changes over the annual cycle from monester during the non-breeding season to diester during the breeding season (Reneerkens et al. 2002, 2005). A function of secretions from the uropygial gland in predator deterrence (Steyn 1999) or olfactory crypsis (Reneerkens et al. 2005) can be accommodated into an antimicrobial context. Some studies have shown that the size of the uropygial gland is related to the expression of secondary sexual characters (Galván and Sanz 2006; Moreno-Rueda 2010), while other secondary sexual characters have shown no such relationship between the size of the gland and the expression of secondary sexual characters (Galván and Sanz 2006; Møller et al. 2009; Moreno-Rueda 2010). For nestling tawny owls *Strix aluco* that uropygial gland secretions reduced rather than increased brightness of beak color, and that production of wax from the uropygial gland is impaired by a stimulation of the immune system (see also Moreno-Rueda 2015). Again, these results may suggest a role in crypsis rather than make-up, and the effect of immune stimulation on production of wax from the uropygial gland could reflect either a sexual function with increased brightness being associated with uropygial gland secretions or an antimicrobial function with brighter coloration being reflecting immunocompetence.

The objectives of this article were to test the hypothesis that the uropygial gland plays a role in plumage brightness. The barn swallow *Hirundo rustica* was used as a model system because individuals are easy to capture, follow and experimentally manipulate. We focused on the brightness of the dark color of the back, which has been shown to be highly repeatable within years (Garamszegi et al. 2006). Surprisingly, plumage brightness has been the focus of much less research than other components of coloration, which makes it a natural target of further study (Romano et al. 2017a, 2010b). First, we tested the prediction that plumage brightness of male barn swallows is greater in mated than in unmated males. While there is an excess of males in the population, females always find a mate (Møller 1994). The blue dorsal plumage of male barn swallows is known to be 8–9% brighter than that of females, implying sexual dichromatism and hence a benefit in terms of sexual selection (Perrier et al. 2002). Second, we tested if plumage brightness is related to degree of sociality as reflected by colony size. Barn swallows breeding in large colonies have more plumage-degrading bacteria than barn swallows from small colonies (Møller et al. 2009), but it remains unknown whether colonial barn swallows produce more wax and hence have brighter plumage than solitarily breeding conspecifics. We tested whether that was the case in the present study. Third, we tested if plumage brightness increases when an individual produces more wax. Fourth, we tested if plumage brightness depends on the time of collection of the feather sample. When a plumage sample was collected early in the morning, such a sample would have been preened with larger amounts of wax that had accumulated during the night and deposited on feathers during the early morning peak in preening activity (Møller 1991b). This implies a direct effect of secretions from the uropygial gland on plumage brightness. Fifth, we tested if barn swallows have brighter plumage when having few chewing lice that are known to degrade the plumage (Møller 1991a, 1994; Vas et al. 2008). Some previous studies have also suggested that chewing lice may be affected by uropygial secretions (Møller et al. 2009; see also Moreno-Rueda 2010). Finally, we test if experimentally preventing access to the uropygial gland reduced plumage brightness, as would be expected if secretions from the uropygial gland were the cause of plumage brightness.

Materials and Methods

Uropygial glands and wax from glands

Barn swallows were captured at Kraghede, Denmark (57°12'N, 10°00'E) during May–August 2008 and again in June 2012 in a study population that has been followed since 1971 (Møller 1994). There were no survivors between 2008 and 2012 so there was no pseudo-replication. All barn swallows were provided by a numbered ring and color rings for individual identification and a number of different measurements were taken, parasite abundance recorded and blood samples extracted.

APM measured the length, width and height of the uropygial gland with a digital caliper with a precision of 0.01 mm. Subsequently volume of the uropygial gland was calculated from these three linear measurements (Møller et al. 2009 for methods). This measure of external gland size is strongly positively correlated with the mass of the gland when removed from the body in different species of birds (Møller et al. 2010a). The estimated size of the gland is highly repeatable on different days (see Møller et al. 2009 for data).

Subsequently, APM extracted secretions from the uropygial gland by gently touching it repeatedly with a 5 μl micro-capillary tube until secretions had ceased to emerge. The amount in the micro-capillary

tube was subsequently measured with a digital caliper to the nearest 0.001 μl. Seven barn swallows captured in the morning on two different days showed a high degree of repeatability in amount of uropygial wax of 0.88 ($SE = 0.13$) (based on ANOVA with individual identity as classification variable, Møller et al. 2009).

Colony size

We estimated colony size as the number of pairs breeding in a given site throughout the breeding season because individuals hardly ever move from one breeding site to another once they have chosen a breeding site (Møller 1994). Only 3 out of more than 4,000 individuals ever moved site and then always to the nearest neighboring site.

Mating success and tail length

APM estimated mating success from the presence of a male and a female within each of the small breeding territories at any time during the breeding season (see Møller 1994 for details). Thus, males remained unmated throughout the entire breeding season as judged by their high singing activity and their continuous attempts to attract females (Møller 1985). Only males were unmated. APM measured the length of the tail to the nearest mm using a ruler with a precision of 1 mm (see Møller 1994 for details).

Plumage brightness

APM collected a sample of 5–10 feathers from the centre of the blue back, the red throat, and the white belly of each individual barn swallow before storing these feathers in zip-lock bags and in complete darkness until color measurements were made in October–December 2010. This should prevent fading, although the fact that all individuals were measured simultaneously should prevent any bias. FMG overlapped five blue back feathers from each sample, simulating their natural position on the bird, on a receptacle made from black matte cardboard. Spectral reflectance data, relative to a white reflectance standard, were obtained from these samples ($N = 228$) using an Avantes AvaSpec-2048 spectrometer provided with a deuterium-halogen light source (Avantes, Eerbek, Netherlands).

The end of the reflectance probe was fitted with a black plastic cylinder that helped to block the ambient light and to keep a standardized distance to the feathers (approximately 2 mm). Three replicate readings were taken from each sample, holding the probe at 90°. The spectrometer was recalibrated after measuring each individual. Data on plumage brightness were computed using Avasoft 7.1, obtaining reflectance values from 300 to 700 nm, averaged every 10 nm. Brightness was calculated as the total amount of light reflected by the feathers, by summing up the total reflectance obtained along the wavelength range 300–700 nm (Endler 1990). FMG made all color measurements blindly with respect to information on uropygial wax or other variables, preventing any bias in measurements. Repeatability of brightness for a small sample of nine individuals sampled twice was $F = 86.77$, $df = 8, 9$, $r^2 = 0.98$, $P < 0.0001$, $R = 0.98$ ($SE = 0.02$)).

Chewing lice and feather mites

Upon capture APM counted the number of holes in the feathers of wings and tail of adult barn swallows, which were presumably made by the chewing louse *Brueelia* sp. (Møller 1991a; Vas et al. 2008), although other causes (e.g., feather-degrading bacteria, see Fülöp et al. 2016) have been proposed (review in Vágási 2014). These holes are clearly visible when the feathers are held against a light source. The abundance of holes is highly repeatable not only on different sampling days within a season, but also among seasons (Møller 1991a, 1994). In an experiment conducted in June 2015 at Kraghede, Denmark, with a tail feather of a barn swallow placed in a petri dish in the dark at 38°C with 0, 5, or 25 chewing lice, there were no additional holes in feathers in the five dishes without chewing lice after 2 weeks, there was an increase by 1.4 holes ($SE = 0.51$) in five dishes with five lice, and there was an increase by 5.4 holes ($SE = 0.75$) in five dishes with 25 lice. These differences were significant in a GLM with a Poisson distribution and a log link function ($\chi^2 = 40.13$, $df = 2$, $P < 0.0001$).

Table 1. Plumage brightness of adult barn swallows in relation to amount of uropygial wax, abundance of chewing lice and time during the day when the wax sample was collected

Variable	Sum of squares	*df*	*F*	*P*	Estimate (*SE*)	Effect size
Uropygial wax	17,208.28	1	8.41	0.0041	47.62 (16.42)	0.19
Time of sampling	33,413.84	1	16.32	<0.0001	−0.041 (0.010)	0.26
Chewing lice	16,049.39	1	7.84	0.0056	−16.803 (6.001)	0.19
Tail length	11,370.55	1	5.55	0.019	0.945 (0.401)	0.16
Sex	13,622.72	1	6.65	0.011	12.024 (4.661)	0.17
Error	446,297.73	218				

The model had the statistics $F = 8.25$, $df = 5, 218$, $r^2 = 0.14$, $P < 0.0001$. Effect size was estimated as pearson's product-moment correlation coefficient.

Experimental manipulation of access to the uropygial gland

APM developed a small plastic container to place over the uropygial gland as previously done to prevent sperm transfer from the cloaca (Michl et al. 2008). During early June 2012, the small 10 mm diameter containers were either placed over the uropygial gland (treatment) or just above the uropygial gland (controls) of males using a string attached with glue to the container. APM captured 26 birds and assigned these randomly to the treatment or the control group. The birds were recaptured 10 days later, and all but one still had the small container attached to the body, as at the start of the experiment, showing that the treatment was effective. The single individual without a container was eliminated from the analyses. Feathers were removed from the back at recapture as described above and plumage brightness was subsequently measured.

Statistical analyses

We $\log_{10}$-transformed the abundance of parasites (by adding a constant of one), gland size, volume of secretions from the uropygial gland, and colony size. Brightness fulfilled the criterion for homoscedasticity (Shapiron–Wilk W-test, $W < 0.98$, $P > 0.13$).

We compared plumage brightness, parasite abundance, gland size, and amount of secretions between sexes and between mated and unmated individuals, respectively, using JMP version 10 (SAS 2012). Only males were unmated so mating status and sex could not be included in the same model. Likewise, we related these variables to colony size by using mean values per colony.

We developed best-fit generalized linear models by reducing full models (including the volume of uropygial secretions, body mass, time of sampling, the number of chewing lice, the number of feather mites, tail length, and tarsus length) until the final model only contained factors with an associated $P < 0.10$. These models are reported in the 'Results' section or in Table 1. To assess possible problems of collinearity, we calculated variance inflation factors that in all cases were less than 3, which is much less than the commonly accepted

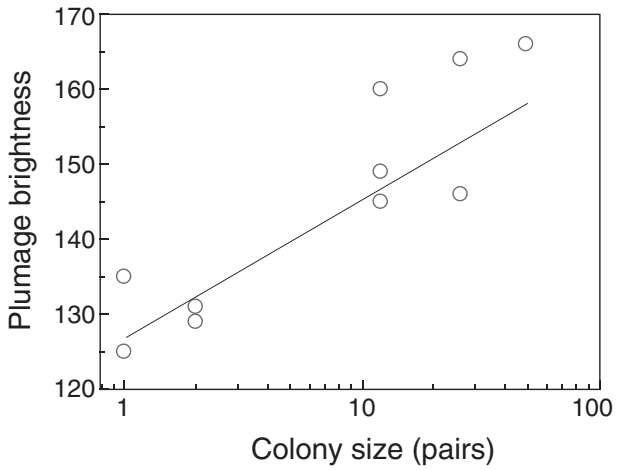

Figure 1. Mean plumage brightness of dorsal feathers for barn swallows in relation to colony size.

levels for significant collinearity of 5–10 (McClave and Sincich 2003). We tested for effects of the experiment on amount of secretion and plumage brightness using experimental treatment as a factor. Effect size was estimated as Pearson's product–moment correlation coefficient is a standardized measure of the magnitude of effects r (Rosenthal 1994). We assessed relationships based on effect sizes according to the criteria listed by Cohen (1988) for small (Pearson $r = 0.10$, explaining 1% of the variance), intermediate (Pearson $r = 0.30$; 9% of the variance), or large effects (Pearson $r = 0.50$; 25% of the variance). All values reported are means (SE).

Results

Plumage brightness, mating status, and colony size

We recorded plumage brightness of the blue feathers from the back of adult male and female barn swallows. Plumage brightness was on average 157 ($SE = 3$), range 59–303, $N = 228$. A total of 114 mated male barn swallows had brighter plumage than the five unmated swallows ($F = 10.75$, $df = 1$, $P = 0.001$). Plumage brightness for mated males was 155 (5), $N = 114$, compared to 94 (18), $N = 6$ unmated males. This effect of plumage brightness was independent of tail length (partial effect of brightness on mating success after inclusion of tail length as an additional predictor of mating success ($F = 4.36$, $df = 1$, $P = 0.008$). Brightness was significantly larger in males than in females ($F = 8.24$, $df = 1$, 224, $P = 0.0045$; males: 143 (6), females: 172 (6)), and increased with tail length ($F = 7.20$, $df = 1$, 224, $P = 0.0078$, slope (SE) = 1.14 (0.43)].

Mean plumage brightness of males and females combined increased with colony size [Figure 1; linear regression based on mean brightness for ten colonies: $F = 32.01$, $df = 1$, 8, $r^2 = 0.80$, $P = 0.0005$, slope (ES) = 21.22 (3.75)]. Thus, plumage brightness was greater in larger colonies.

The amount of uropygial wax was slightly larger in females than in males [$F = 4.57$, $df = 1$, 223, $r^2 = 0.02$, $P = 0.03$; males: 1.96 (0.02), females: 2.01 (0.02)], while there was no significant difference in gland size between males and females ($F = 0.68$, $df = 1$, 223, $r^2 = 0.003$, $P = 0.41$). The amount of uropygial wax was not related to colony size ($F = 0.10$, $df = 1$, 223, $r^2 = 0.0005$, $P = 0.75$), nor was the abundance of chewing lice ($F = 2.47$, $df = 1$, 223, $r^2 = 0.01$, $P = 0.12$). The relationship beween the size of the uropygial gland and colony size was not significant either ($F = 1.05$, $df = 1$, 223, $r^2 = 0.0005$, $P = 0.31$).

Plumage brightness, amount of wax in uropygial glands and parasites

Plumage brightness was predicted by five factors that explained 14% of the variance (Table 1). Brightness increased with the amount of uropygial wax, with an intermediate effect size (Figure 2A). When plumage was sampled earlier in the morning, plumage brightness was greater, with an intermediate effect size (Figure 2B). Plumage brightness increased with tail length (Table 1). Plumage brightness was higher in males than in females (Table 1). Finally, barn swallows with more chewing lice had less bright plumage coloration, with an intermediate effect size (Figure 2C). In contrast, there was no significant effect of feather mites on plumage brightness ($F = 0.23$, $df = 1$, 225, $P = 0.63$).

Experimental manipulation of uropygial glands and plumage brightness

Experimental birds had significantly more wax in their uropygial glands than controls ($F = 8.66$, $df = 1$, 23, $r^2 = 0.27$, $P = 0.0073$; experimental: 2.05 (0.03), control: 1.84 (0.07)). Plumage brightness was lower in the group of experimental birds compared to the controls (Figure 3; $F = 8.40$, $df = 1$, 23, $r^2 = 0.27$, $P = 0.0079$).

Discussion

To summarize, plumage brightness of adult barn swallows differed significantly between mated and unmated individuals and increased with colony size. Brightness was greater in individuals that had more uropygial wax, when feathers were sampled earlier in the morning and when there were few chewing lice. Experimentally preventing birds from access to the uropygial gland caused a decrease in plumage brightness. This finding is consistent with another study showing that changes in uropygial gland size are correlated with changes in bib coloration in house sparrows *Passer domesticus* (Moreno-Rueda 2016). These findings match with key predictions of the make-up hypothesis, that is, that uropygial wax or other substances should enhance the brightness of plumage or naked body parts (Andersson and Amundsen 1996; Blanco et al. 1999; Piersma et al. 1999; Figuerola and Senar 2005; Galván and Sanz 2006; Montgomerie 2006; Delhey et al. 2007), with this increased brightness providing a mating advantage.

West-Eberhard (1983) has suggested that many characters have evolved as a means of facilitating social competition. Plumage brightness increased strongly and significantly with degree of sociality as reflected by colony size in the barn swallow. This correlation may arise as a consequence of (1) differential recruitment of individuals with particularly bright plumage to large colonies, (2) larger uropygial glands and hence more secretions in birds from larger colonies, or (3) a difference in abundance of microorganisms among barn swallows breeding in colonies of different sizes. Møller et al. (2009) have previously reported that barn swallows breeding in large colonies have significantly more feather degrading bacteria than conspecifics breeding solitarily or in small colonies. We found a significant increase in the amount of secretions and size of uropygial glands among barn swallows from colonies of different sizes (Møller et al. 2009). We were unable to discriminate among these hypotheses with available data.

We found evidence consistent with microbes and ectoparasites affecting plumage brightness. Barn swallows with small uropygial glands have previously been shown to have more feather degrading bacteria (Møller et al. 2009) and hence less bright plumage than

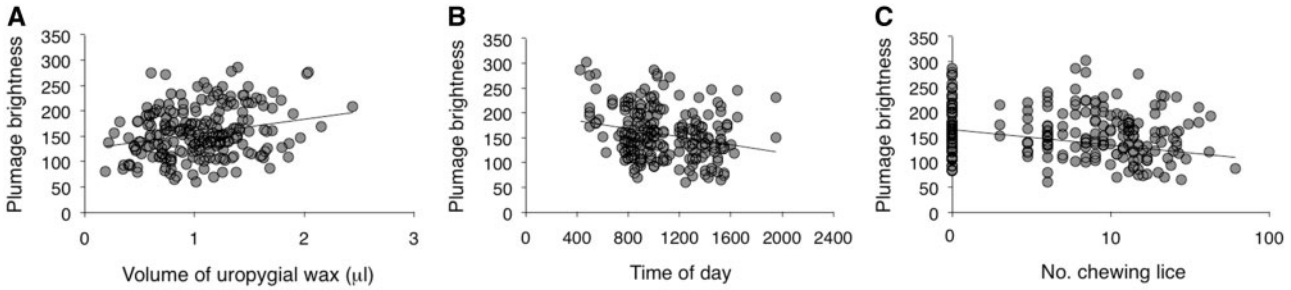

Figure 2. Plumage brightness of dorsal feathers for individual barn swallows in relation to (A) amount of uropygial wax (μl), (B) time of day [ranging from 0 (0) to 24 (2400)] when wax was sampled, and (C) number of chewing lice in adult barn swallows. The lines are the regression lines.

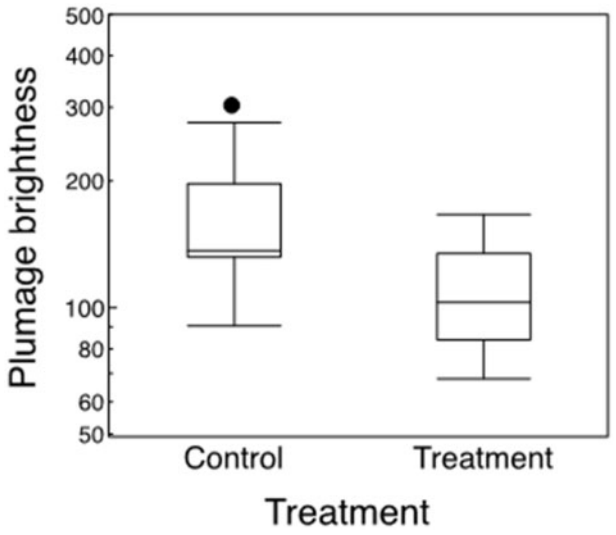

Figure 3. Boxplots of plumage brightness of dorsal feathers for individual barn swallows in relation to experimentally manipulated access to the uropygial gland. The plot shows median, quartiles, 5- and 95-percentiles and an extreme value.

barn swallows with large glands. Furthermore, females have more bacteria than males and hence differ in plumage brightness. Finally, barn swallows living in larger colonies have more feather-degrading bacteria than solitarily breeding individuals, thus differing in plumage brightness (Møller et al. 2009). We hypothesize that barn swallows with small uropygial glands have more feather degrading bacteria than conspecifics with large glands if secretions have antimicrobial properties. A second hypothesis is that secretions directly increase plumage brightness, although that is inconsistent with secretions directly reducing the abundance of feather degrading bacteria as already shown in a previous study (Møller et al. 2009). Furthermore, bird species with larger uropygial glands for their body size have a greater diversity of chewing lice (Møller et al. 2009; see also Moreno-Rueda 2010). Galván et al. (2008) showed a greater diversity of feather mites in species of birds with relatively larger uropygial glands, although Møller et al. (2009) could not replicate this result for a larger sample of species. Here we have shown that plumage brightness of barn swallows decreases with the abundance of chewing lice, but not with the abundance of feather mites. This difference between parasite taxa is as expected, because chewing lice cause damage to the microstructure of the plumage (Møller 1991a; Vas et al. 2008), while that is not the case for feather mites (Galván et al. 2008).

In conclusion, the plumage of barn swallows was brighter in mated than in unmated individuals, in accordance to the hypothesis that plumage brightness is a sexually selected trait. Individuals with brighter plumage bred in larger colonies showing an association between plumage brightness and sociality. Plumage brightness increased with the amount of uropygial wax and the time when feathers were sampled, and with a decrease in the abundance of chewing lice that damage feathers through feeding. An experimental reduction in access to uropygial wax reduced plumage brightness providing direct evidence for a causal link between plumage brightness and uropygial wax.

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