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ORIGINAL PAPER

Preen gland removal increases plumage bacterial load but not that of feather-degrading bacteria

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Abstract The preen gland is a holocrine sebaceous gland of the avian integument which produces an oily secretion that is spread on the plumage during preening. It has been suggested that birds may defend themselves against feather-degrading bacteria (FDB) and other potential pathogens using preen gland secretions. However, besides some in vitro studies, the in vivo bacterial inhibitory effects of the preen oil on the abundance of feather-associated bacterial species has not yet been studied in passerines. Here we tested the effect of gland removal on the abundance of FDB and other-cultivable bacterial loads (OCB) of male house sparrows (*Passer domesticus*). Our results did not support earlier results on in vitro antibacterial activity of preen oil against FDB since the absence of the preen gland did not significantly affect their loads related to the control birds. In contrast, we found that preen gland removal led to higher loads of OCB. This result suggests that the antimicrobial spectrum of the preen oil is broader than previously thought and that, by reducing the overall feather bacterial loads, the preen gland could help birds to protect themselves against a variety of potentially harmful bacteria.

Keywords Bacterial abundance · Experimental gland removal · Feather-degrading bacteria · In vivo antibacterial effect · Other-cultivable bacteria · Preen gland

Introduction

The ecology and evolution of bird–bacteria interactions have received burgeoning attention during the last decade,

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mainly because birds carry a wide variety of emerging bacterial pathogens (reviewed by Hubálek 2004), and this microbial community may have adverse fitness consequences for their avian hosts (Moreno et al. 2003). Most of the studies have focused primarily on the avian cloacal (e.g., Lucas and Heeb 2005; Ruiz-Rodríguez et al. 2009a, b; White et al. 2010) and eggshell microflora (e.g., Cook et al. 2005; Shawkey et al. 2009a; Peralta-Sánchez et al. 2010; Ruiz-De-Castañeda et al. 2011; Soler et al. 2011, 2012), while research on feather-associated bacteria was mostly neglected (reviewed by Gunderson 2008), although maintaining feathers in good condition is essential for important functions like flight performance, insulation and signalling.

Avian plumage harbours rich communities of bacteria (Shawkey et al. 2005; Bisson et al. 2007, 2009), some of which degrade feathers (henceforth feather-degrading bacteria [FDB]). FDB are polyphyletic, and have been shown to be present in a wide array of bird species (Burtt and Ichida 1999; Whitaker et al. 2005). They are distinguished from other bacteria by their capacity to degrade β-keratin (Burtt and Ichida 1999; Lucas et al. 2003; but see Cristol et al. 2005), which is the principal building block of feathers, and these bacteria may impose substantial selection pressures on host plumage (Shawkey et al. 2007; Gunderson et al. 2009). FDB could compromise lifetime fitness through potentially playing a role in sexual selection as well (Shawkey et al. 2009b), as they were shown to affect the ultraviolet chroma and brightness of plumage signals (Shawkey et al. 2007; Gunderson et al. 2009). To preclude or reduce the negative effects of FDB, it has been postulated that birds have evolved several defence mechanisms they can use against these microorganisms (Clayton 1999; Gunderson 2008). Besides modifying the melanin content of the feathers and using different body maintenance behaviours (e.g., sunning, bathing, anointing; reviewed by Gunderson 2008), birds can use another antibacterial mechanism when coating their plumage with preen gland secretions during preening. These secretions could play a central role as a defence mechanism (Gunderson 2008; Møller et al. 2009).

Multiple functions have been attributed to preen oil, a topic that has been debated intensively in the ornithological literature. Preen oil can act as a water repellent (Jacob and Ziswiler 1982; Giraudeau et al. 2010a), reduce the risk of predation (Reneerkens et al. 2005; Møller et al. 2010), enhance plumage appearance (Piersma et al. 1999), protect feathers from mechanical fatigue (Moreno-Rueda 2011), serve as chemosignal in avian intra- and inter-specific communication (Leclaire et al. 2011; Whittaker et al. 2011; Campagna et al. 2012), regulate the number of ectosymbionts (Galván et al. 2008; Moreno-Rueda 2010; but see Pap et al. 2010a) and the loads of microorganisms (FDB and dermatophytes; Jacob et al. 1997; Shawkey et al. 2003; Soler et al. 2008; Reneerkens et al. 2008; Møller et al. 2009). It has been shown that the experimental removal or blockage of the preen gland deteriorates plumage condition in rock pigeons (*Columba livia*; Moyer et al. 2003) and mallards (*Anas platyrhynchos*; Giraudeau et al. 2010a). These earlier studies have hypothesized that birds maintain the physical integrity of feathers by inhibiting the proliferation and activity of FDB with preen oils coated onto their feathers (Moyer et al. 2003; Giraudeau et al. 2010a). In this perspective, recent studies have demonstrated the in vitro antibacterial effects of preen oil of different bird species against Gram-positive FDB (Shawkey et al. 2003; Soler et al. 2008; Reneerkens et al. 2008). However, Muza et al. (2000) stressed that patterns occurring in vitro may not be same as those taking place in vivo. A recent study by Giraudeau et al. (2013) showed that mallards deprived access to the preen gland had no significant effect on feather bacterial loads. These contrasting results and caveats prompted us to test experimentally the antibacterial effect of preen oil under in vivo conditions in a passerine species.

Here, we describe a test for the in vivo antibacterial properties of the preen oil by surgical removal of preen glands in house sparrows. We examined the effect of presence or absence of preen gland on the abundance of FDB and other-cultivable bacteria (OCB) that inhabit the hosts' plumage. Provided that preen oil may have antibacterial properties against FDB (as shown by the in vitro studies of Shawkey et al. 2003; Soler et al. 2008), we predicted higher FDB loads in birds with removed preen gland as compared with sham-operated conspecifics. We also expect a treatment effect on OCB loads, which could be either an increase or a decrease depending on the nature of the interactions (synergism or antagonism, respectively) between various bacterial species that do not grow independently from each other (Møller et al. 2009; Czirják et al. 2010) within feather associated bacterial communities. Currently, there is scarce knowledge about the interactions among complex bacterial communities on feathers and this precludes us to make more specific predictions.

Materials and methods

General methods and aviary conditions

Adult male house sparrows ($n=14$) were caught with mist nets (Ecotone, Poland) on the 3rd November 2007 from a cattle farm situated near Cluj Napoca (46°46′N, 23°33′E), Transylvania, central Romania. After capture, birds were transported into a large outdoor aviary [5×4×2.5 m (length × width × height)] situated at the Campus of the Babeş-Bolyai University, Cluj Napoca. Sparrows were fed ad libitum with a mixture of seeds and supplemented with extra protein source (grated boiled eggs and mealworms). To

increase the comfort of the birds, we provided shelters (small bushes), perches and nest boxes inside the aviary, while sand and fresh water was made available ad libitum throughout the experiment. For details on aviary conditions, see Pap et al. (2010b).

Surgery protocol

After 4 days of habituation to confinement, 7 birds were randomly assigned to the glandectomized group (i.e., surgical removal of the gland) while the other 7 served as controls (sham-operated; see below). Birds in the two groups did not differ significantly either in body mass ($F_{1,12}$=2.75, P=0.12) or tarsus length ($F_{1,12}$=0.60, P=0.45). Birds were anaesthetized using ketamine–xylazine combination (Pap et al. 2010a), and then an incision was made adjacent to the gland for birds in sham-operated group. The preen gland from birds assigned to the glandectomized group was excised using the method described by Mureşan et al. (2009) and Pap et al. (2010a). Skin closure was performed with absorbable surgical thread (Dexon™ S, Covidien, USA) using a "U" suture and before closure the wound was treated with an antibiotic powder (Baneocin, Biochemie Austria GMBH). After surgery, we covered the scar with an aluminum spray (Aluminium spray; CP-Pharma, Hungary) to prevent birds from pulling out their suture. The anaesthesia, wound closure and wound treatment procedures were similarly applied in both groups. Post-operation, all birds were moved in individual cages and received antibiotics orally (Enrofloxacin, 2 ml sol. 10 % l^{-1} drinking water; Krka, Slovenia) for 10 days consecutively. Following recovery (decided by visual inspection of wound and condition), birds were moved back to the same large outdoor aviary. Samples for microbiological analyses were collected twice from each bird: 4 days after the birds were re-introduced into the aviary (i.e., 2 weeks after surgery, hereafter T1) and 1 month later (hereafter T2), at the end of the experiment.

Due to the invasive nature of the experimental procedure, we used as small of a sample size (n=7 per group) necessary to detect an effect (Moyer et al. 2003 used the same sample size to test for the effect of preen gland removal on ectoparasites). During the experiment one glandectomized bird died shortly after the first microbiological sampling due to unknown reasons. At the end of the study, the birds were released in good condition to their population of origin (Pap et al. 2010a). The experimental procedures were approved by the Romanian Academy of Sciences (under license #2257).

Feather sampling and microbiological analyses

Bird handlers washed their hands with ethanol and waited until it evaporated before touching the birds for microbiological sampling to avoid exogenous contaminations. Immediately after capture, feathers from predefined regions (chest, tail and head) were removed with sterile tweezers and placed in separate sterile Eppendorf tubes for each body region before storage at −20 °C. Storage conditions and duration can affect bacterial viability and diversity (Achá et al. 2005). Our samples were stored at −20 °C without any cryoprotectant, and due to this limitation the abundances of the different bacterial groups measured are probably underestimated. However, since all samples were stored under the same condition, we do not expect that this led to a methodological bias.

Microbiological analyses were performed under sterile conditions separately for each body region, using the method described by Czirják et al. (2010). Briefly, after obtaining a bacterial solution with both free-living and attached microorganisms, 100 μl of these suspensions was spread in duplicate on two growth media. To assess the total cultivable bacterial load of the feathers, we used Tryptic Soy Agar (#22091, Fluka), a rich medium on which both heterotrophic bacteria and fungi grow, and added 0.1 mg l^{-1} cycloheximide (#01810, Fluka) to inhibit fungal growth (TSAcy). To assess the FDB load, we used Feather Meal Agar containing 15 g l^{-1} hydrolyzed feather meal (Saria Industries Bretagne S.A.S, France), 0.5 g l^{-1} NaCl, 0.30 g l^{-1} K_2HPO_4, 0.40 g l^{-1} KH_2PO_4, 15 g l^{-1} agar and 0.1 mg l^{-1} cycloheximide (FMAcy; Gunderson, personal communication). Due to the chemical and thermo-treatment of the feathers, in hydrolyzed feather meal the β-keratin is broken to smaller peptide units, which might have affected our results. However, a pilot study on a subset of samples (n=14) revealed significant correlation between the abundances of FDB measured on FMAcy with artisanal and commercial, hydrolyzed feather meal (Pearson's product moment correlation, r=0.65, P=0.01).

Due to the environmental origin of the feather-associated bacteria (Burtt and Ichida 1999; Lucas et al. 2003), plate cultures were incubated at 25 °C for 3 and 14 days in the case of TSAcy and FMAcy, respectively. Most bacteria grow faster on TSAcy compared with the FMAcy, and as shown by previous studies shorter incubation times on TSAcy are required to assess the total cultivable bacterial load of the feathers. After 3 days of incubation, the colonies started to merge on TSAcy, while 2 weeks of incubation were needed to clearly distinguish the single colonies on FMAcy (Shawkey et al. 2009b; Czirják et al. 2010). The number of visible colony-forming units (CFU) on each plate was counted, averaged between duplicates and microbial concentrations were expressed as CFU mg^{-1} feathers for each medium and for each body region. The abundance of OCB was estimated as the total abundance of cultivable bacteria minus FDB abundance (i.e., TSAcy minus FMAcy; Møller et al. 2009; Czirják et al. 2010). The bacterial counts for different body regions were added together for each media type as estimates of total FDB and OCB load

on each individual (Shawkey et al. 2009b; Czirják et al. 2010).

Statistical analyses

We obtained data for 14 and 13 individuals during the first and second sampling, respectively. All analyses were carried out using general linear models as implemented in R statistical environment version 2.11.1 (R Development Core Team 2010). The four dependent variables (FDB and OCB loads at first sampling time and the difference in FDB and OCB loads expressed as T2 minus T1 values, hereafter labelled with Δ) were analyzed separately. The FDB load at first sampling was $\log_{10}$-transformed to meet the normality assumption of parametric tests. The dependent variables had a normal distribution of error (Shapiro–Wilk test, all $W>0.91$, all $P>0.19$) and variances were homogenous (Bartlett test, all $K^2<2.49$, all $P>0.12$). Treatment was included as fixed factor and mass and tarsus length were entered as covariates in a full factorial design. We reached minimal models by backward stepwise removal of non-significant terms. Mean ± SE values are shown throughout.

Results

The abundance of feather-associated bacteria in male house sparrows was highly variable (Table 1). At T1, none of the bacterial groups differed significantly between the two experimental groups (general linear model, FDB: $F_{1,12}=0.97$, $P=0.34$; OCB: $F_{1,12}=1.85$, $P=0.20$), and there was a significant positive correlation between FDB and OCB (Pearson's product moment correlation: $r=0.64$, $P=0.01$, $n=14$).

The ΔFDB loads were not significantly different between birds in the sham-operated and glandectomized groups ($F_{1,11}=0.07$, $P=0.80$; Fig. 1a). In contrast, ΔOCB was significantly affected by the gland removal, as bacterial loads in the glandectomized group showed a significantly greater increase relative to the sham-operated group ($F_{1,11}=5.30$, $P=0.04$; Fig. 1b). The correlation between FDB and OCB was not significant at T2 ($r=0.34$, $P=0.25$, $n=13$).

Table 1 Summary statistics of the abundance (CFU mg^{-1} feather) of two groups of bacteria isolated from feathers of adult male house sparrows

Bacterial group	Mean	SE	Median	Range
T1				
FDB	101.69	24.33	74.11	18.57–311.17
OCB	2,447.79	455.42	2,240.24	397.00–5,990.57
T2				
FDB	133.35	18.71	135.72	21.56–254.77
OCB	3,504.61	484.97	3,664.50	991.40–7,541.20

Feather-degrading bacteria (FDB) and other-cultivable bacterial (OCB) loads were measured twice, at the start of the experiment (T1, $n=14$) and 1 month later (T2, $n=13$, see Materials and methods for details)

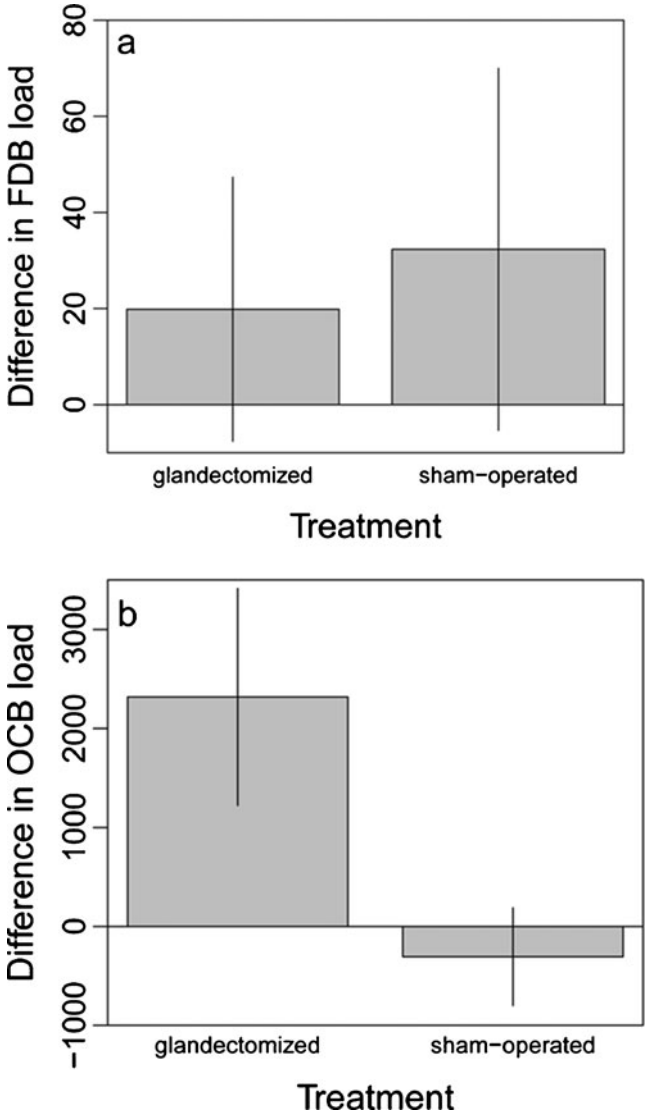

Fig. 1 Changes (post-treatment minus pre-treatment) in feather-associated bacterial loads (CFU mg^{-1} feather) of captive male house sparrows in presence (sham-operated group) or absence (glandectomized group) of preen glands. Mean load±SE of **a** feather-degrading bacteria (*FDB*) and **b** other-cultivable bacteria (*OCB*) are shown

Discussion

We found that preen gland removal in male house sparrows had no significant effect on the changes of FDB loads whereas it significantly affected the changes in the abundance of OCB. Interestingly, our results contrast with previous in vitro experiments which showed antibacterial effects of the preen oil against Gram-positive FDB (Shawkey et al. 2003; Soler et al. 2008; Reneerkens et al.

2008). We emphasize that our study is one of the first to test the antibacterial properties of the preen oil in vivo. Our results also provide evidence that preen gland secretions reduce the increase in the loads of OCB, thus possibly reducing the densities of potentially harmful bacteria. In an earlier experiment with mallards we have shown that having access to preen glands or not did not affect the loads of FDB or of total cultivable bacteria of plumage (Giraudeau et al. 2013).

It should be noted that previous in vitro tests have used a limited number of isolated bacterial strains, mainly *Bacillus licheniformis*, to test the potential effects of preen gland secretions (Shawkey et al. 2003; Soler et al. 2008; Reneerkens et al. 2008). In contrast, in our experiment, the differences between the absence or presence of preen gland secretions were tested on the bacterial abundances naturally present on the feathers, in which case, susceptibility to preen gland secretions takes interactions among bacterial strains into account. Our results suggest that FDB were better able to survive the chemical defences of hosts since the presence or absence of preen gland secretions did not affect their abundance. In contrast, OCB increased in densities more successfully when hosts were deprived of gland secretions, indicating that their proliferation is likely controlled by preen oil. In support of this hypothesis, it is worth noting that at the end of the experiment birds without preen glands tended to have greater overall bacterial loads on their plumage than sham-operated birds ($P=0.09$). Future examinations of bacterial community structure and composition could help elucidate the susceptibility of specific bacterial strains to preen gland secretions and the potential interactions between selection pressures exerted by preen gland secretions, FDB and other feather-dwelling microorganisms (Soler et al. 2012).

Results from our in vivo study raise questions concerning the mechanisms potentially responsible for the discrepancies with the earlier in vitro studies. First, differences could have emerged owing to methodological issues, as we tested the antibacterial activity of the preen oil in vivo on overall bacterial abundances, whereas previous results were obtained in vitro effects against specific isolated strains or a model feather-degrading bacterial species, *B. licheniformis*. Furthermore, in vitro studies used different techniques (the "disc diffusion test" in Shawkey et al. 2003; the "spot-on plate test" in Soler et al. 2008). Thus, it is likely that in vitro studies have overestimated the antibacterial capacity of the preen oil when testing the minimum inhibitory and bactericidal concentrations out of the natural context of feathers. This statement is reinforced by results of the in vivo experiment on mallards, where blocking access to preen gland did not affect the loads of FDB (Giraudeau et al. 2013). To date, the only study which tested the in vitro antibacterial capacity of preen oil applied by the birds on their feathers suggested that the main function of preen oil is to form a physical barrier that prevents FDB colonization on plumage (Reneerkens et al. 2008). This hypothesis could explain the observations that experimental removal or blockage of the preen gland is associated with a deterioration of plumage condition (Moyer et al. 2003; Giraudeau et al. 2010a), without changing the abundances of FDB (Giraudeau et al. 2013).

We did not find a significant effect of gland removal on increases of FDB loads, although they were expected based on published in vitro effects. It is possible that our captive birds faced different time and energy constraints than free-living house sparrows. To compensate the lack of access to gland secretions, our captive birds could have spent more time performing self-maintenance behaviours, such as water- and dust-bathing or sunning (Saranathan and Burtt 2007; Gunderson 2008; Clayton et al. 2010). A study on captive Northern cardinals (*Cardinalis cardinalis*) showed that the birds spent a substantial amount of time sunning or water bathing and that FDB did not degrade their plumage (Cristol et al. 2005). In our aviaries, the sparrows had ample time and opportunities to water-, dust- and sun-bath. However, we think that if these behavioural defences have general bactericidal effects, they should have affected FDB and OCB in similar way. Differences between the two bacteria subgroups in terms of resistance against preen oil, host colonization strategy and competitive ability clearly deserves further scrutiny to elucidate the differential effect of gland secretions.

An alternative hypothesis could be that preen gland removal affected the behaviour and the physiology of the birds, which led to our non-significant difference in FDB loads after preen gland removal. However, it has been previously shown that preen gland removal or blockage did not affect the behaviour in rock pigeons (Moyer et al. 2003), house finches (*Carpodacus mexicanus*; López-Rull et al. 2010) or mallards (Giraudeau et al. 2010b). Moreover, the surgical extirpation of the preen gland of rock pigeons did not affect serum lipids, cholesterol or calcium metabolism (Montalti et al. 2006). Thus, our results were probably not due to a change in the birds' behaviour or physiology.

Besides FDB, the avian plumage harbours numerous other bacterial species (Shawkey et al. 2005; Bisson et al. 2007, 2009), including potential pathogens such as *Pseudomonas* or *Salmonella* spp. (Mallinson et al. 1989; Shawkey et al. 2005). In fowl, Bandyopadhyay and Bhattacharyya (1996) suggested that preen gland secretions were responsible for controlling bacterial growth and community composition on the skin and in the green wood hoopoes (*Phoeniculus purpureus*) it has been demonstrated that the preen oil is effective against a wide range of bacterial pathogens (Burger et al. 2004). In contrast to these results, in mallards preen oil does not have a significant effect on OCB loads (Giraudeau et al., unpublished data). As

found by Burger et al. (2004), our findings emphasize the negative effect of preen gland on the abundance of other-cultivable bacteria and indicate that the antibacterial spectrum of preen oil is wider than previously thought. The chemical composition of the preen oil of different bird species is highly variable (Jacob and Ziswiler 1982), which could explain the differences in the antibacterial spectrum. Further in vivo and in vitro comparative studies could determine whether the chemical composition or other underlying mechanism of the antibacterial properties can explains the differences found between studies (Giraudeau et al. 2013).

It has been shown that both the quantity (Pap et al. 2010a) and the chemical composition (Reneerkens et al. 2005, 2008; Soini et al. 2007; Martín-Vivaldi et al. 2009) of the preen oil changes seasonally. Since our study was carried out in the non-breeding, winter period, it would be important to examine the generality of our results by testing in different seasons and by extending to the whole bacterial communities using culture-independent techniques (Shawkey et al. 2005; Mennerat et al. 2009). Identifying the bacteria that are specifically affected by preen oils would open promising avenues towards a better understanding of the antibacterial function of preen glands and their secretions in different bird species.

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References

Achá SJ, Kühn I, Mbazima G, Colque-Navarro P, Möllby R (2005) Changes of viability and composition of the *Escherichia coli* flora in faecal samples during long time storage. J Microbiol Meth 63:229–238

Bandyopadhyay A, Bhattacharyya SP (1996) Influence of fowl uropygial gland and its secretory lipid components on the growth of skin surface bacteria of fowl. Indian J Exp Biol 34:48–52

Bisson I-A, Marra PP, Burtt EH, Sikaroodi M, Gillevet PM (2007) A molecular comparison of plumage and soil bacteria across biogeographic, ecological, and taxonomic scales. Microb Ecol 54:65–81

Bisson I-A, Marra PP, Burtt EH, Sikaroodi M, Gillevet PM (2009) Variation in plumage microbiota depends on season and migration. Microb Ecol 58:212–220

Burger BV, Reiter B, Borzyk O, du Plessis MA (2004) Avian exocrine secretions: I. Chemical characterization of the volatile fraction of the uropygial secretion of the green woodhoopoe, *Phoeniculus purpureus*. J Chem Ecol 30:1603–1611

Burtt EH Jr, Ichida JM (1999) Occurrence of feather-degrading bacilli in the plumage of birds. Auk 116:364–372

Campagna S, Mardon J, Celerier A, Bonadonna F (2012) Potential semiochemical molecules from birds: a practical and comprehensive compilation of the last 20 years studies. Chem Senses 37:3–25

Clayton DH (1999) Feather-busting bacteria. Auk 116:302–304

Clayton DH, Koop JAH, Harbison CW, Moyer BR, Bush SE (2010) How birds combat ectoparasites. Open Ornithol J 3:41–71

Cook MI, Beissinger SR, Toranzos GA, Arendt WJ (2005) Incubation reduces microbial growth on eggshells and the opportunity for trans-shell infection. Ecol Lett 8:532–537

Cristol DA, Armstrong JL, Whitaker JM, Forsyth MH (2005) Feather-degrading bacteria do not affect feathers on captive birds. Auk 122:222–230

Czirják GÁ, Møller AP, Mousseau TA, Heeb P (2010) Microorganisms associated with feathers of barn swallows in radioactively contaminated areas around Chernobyl. Microb Ecol 60:373–380

Galván I, Barba E, Piculo R, Cantó JL, Cortés V, Monrós JS, Atiénzar F, Proctor H (2008) Feather mites and birds: an interaction mediated by uropygial gland size? J Evol Biol 21:133–144

Giraudeau M, Duval C, Guillon N, Bretagnolle V, Gutierrez C, Heeb P (2010a) Effects of access to preen gland secretions on mallard plumage. Naturwissenschaften 97:577–581

Giraudeau M, Czirják GÁ, Duval C, Bretagnolle V, Eraud C, McGraw KJ, Heeb P (2010b) Effect of restricted preen-gland access on maternal self maintenance and reproductive investment in mallards. PLoS One 5:e13555

Giraudeau M, Czirják GÁ, Duval C, Bretagnolle V, Gutierrez C, Guillon N, Heeb P (2013) Effect of preen oil on plumage bacteria: an experimental test with the mallard. Behav Process in press

Gunderson AR (2008) Feather-degrading bacteria: a new frontier in avian and host–parasite research? Auk 125:972–979

Gunderson AR, Forsyth MH, Swaddle JP (2009) Evidence that plumage bacteria influence feather coloration and body condition of eastern bluebirds *Sialia sialis*. J Avian Biol 40:440–447

Hubálek Z (2004) An annotated checklist of pathogenic microorganisms associated with migratory birds. J Wildl Dis 40:639–659

Jacob J, Ziswiler V (1982) The uropygial gland. In: Farner DS, King JR, Parkes KC (eds) Avian biology, vol 6. Academic Press, New York, pp 199–324

Jacob J, Eigener U, Hoppe U (1997) The structure of preen gland waxes from pelecaniform birds containing 3,7-dimethyloctan-1-ol: an active ingredient against dermatophytes. Z Naturforsch 52:114–123

Leclaire S, Merkling T, Raynaud C, Giacinti G, Bessière J-M, Hatch SA, Danchin É (2011) An individual and a sex odor signature in kittiwakes? Study of the semiochemical composition of preen secretion and preen down feathers. Naturwissenschaften 98:615–624

López-Rull I, Pagán I, Garcia CM (2010) Cosmetic enhancement of signal coloration: experimental evidence in the house finch. Behav Ecol 21:781–787

Lucas FS, Heeb P (2005) Environmental factors shape cloacal bacteria assemblages in great tit *Parus major* and blue tit *P. caeruleus* nestlings. J Avian Biol 36:510–516

Lucas FS, Broennimann O, Febbraro I, Heeb P (2003) High diversity among feather-degrading bacteria from a dry meadow soil. Microb Ecol 45:282–290

Mallinson ET, Tate CR, Miller RG, Bennett B, Russek-Cohen E (1989) Monitoring poultry farms for *Salmonella* by drag-swab sampling and antigen-capture immunoassay. Avian Dis 33:684–690

Martín-Vivaldi M, Ruiz-Rodríguez M, Soler JJ, Peralta-Sánchez JM, Méndez M, Valdivia E, Martín-Platero AM, Martínez-Bueno M (2009) Seasonal, sexual and developmental differences in hoopoe *Upupa epops* preen gland morphology and secretions: evidence for a role of bacteria. J Avian Biol 40:191–205

Mennerat A, Mirleau P, Blondel J, Perret P, Lambrechts MM, Heeb P (2009) Aromatic plants in nests of the blue tit *Cyanistes caeruleus* protect chicks from bacteria. Oecologia 161:849–855

Møller AP, Czirják GÁ, Heeb P (2009) Feather micro-organisms and uropygial antimicrobial defences in a colonial passerine bird. Funct Ecol 23:1097–1102

Møller AP, Erritzøe J, Nielsen JT (2010) Predators and microorganisms of prey: goshawks prefer prey with small uropygial glands. Funct Ecol 24:608–613

Montalti D, Gutiérrez AM, Reboredo GR, Salibián A (2006) Removal of the uropygial gland does not affect serum lipids, cholesterol and calcium levels in the rock pigeon *Columba livia*. Acta Biol Hung 57:295–300

Moreno J, Briones V, Merino S, Ballesteros C, Sanz JJ, Tomás G (2003) Beneficial effects of cloacal bacteria on growth and fledging size in nestling Pied Flycatchers (*Ficedula hypoleuca*) in Spain. Auk 120:784–790

Moreno-Rueda G (2010) Uropygial gland size correlates with feather holes, body condition and wingbar size in the house sparrow *Passer domesticus*. J Avian Biol 41:229–236

Moreno-Rueda G (2011) House sparrows *Passer domesticus* with larger uropygial glands show reduced feather wear. Ibis 153:195–198

Moyer BR, Rock AN, Clayton DH (2003) Experimental test of the importance of preen oil in Rock Doves (*Columba livia*). Auk 120:490–496

Mureşan C, Pap PL, Czirják GÁ, Bolfa P (2009) Excision of the uropygial gland in the house sparrow. Lucr Şt Med Vet Timişoara XLII:111–114

Muza MM, Burtt EH Jr, Ichida JM (2000) Distribution of bacteria on feathers of some Eastern North American birds. Wilson Bull 112:432–435

Pap PL, Vágási CI, Osváth G, Mureşan C, Barta Z (2010a) Seasonality in the uropygial gland size and feather mite abundance in house sparrows: natural covariation and an experiment. J Avian Biol 41:653–661

Pap PL, Czirják GÁ, Vágási CI, Barta Z, Hasselquist D (2010b) Sexual dimorphism in immune function changes during the annual cycle in the house sparrows. Naturwissenschaften 97:891–901

Peralta-Sánchez JM, Møller AP, Martín-Platero AM, Soler JJ (2010) Number and colour composition of nest lining feathers predict eggshell bacterial community in barn swallow nests: an experimental study. Funct Ecol 24:426–433

Piersma T, Dekker M, Sinninghe Damsté JS (1999) An avian equivalent of make-up? Ecol Lett 2:201–203

R Development Core Team (2010) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, http://www.R-project.org

Reneerkens J, Piersma T, Sinninghe Damsté JS (2005) Switch to diester preen waxes may reduce avian nest predation by mammalian predators using olfactory cues. J Exp Biol 208:4199–4202

Reneerkens J, Versteegh MA, Schneider AM, Piersma T, Burtt EH Jr (2008) Seasonally changing preen-wax composition: Red Knots' (*Calidris canutus*) flexible defense against feather-degrading bacteria? Auk 125:285–290

Ruiz-De-Castañeda R, Vela AI, González-Braojos S, Briones V, Moreno J (2011) Drying eggs to inhibit bacteria: incubation during laying in a cavity nesting passerine. Behav Process 88:142–148

Ruiz-Rodríguez M, Lucas FS, Heeb P, Soler JJ (2009a) Differences in intestinal microbiota between avian brood parasites and their hosts. Biol J Linn Soc 96:406–414

Ruiz-Rodríguez M, Soler JJ, Lucas FS, Heeb P, Palacios MS, Martín-Gálvez D, de Neve L, Pérez-Contreras T, Martínez JG, Soler M (2009b) Bacterial diversity at the cloaca relates to an immune response in magpie *Pica pica* and to body condition of great spotted cuckoo *Clamator glandarius* nestlings. J Avian Biol 40:42–48

Saranathan V, Burtt EH Jr (2007) Sunlight on feathers inhibits feather-degrading bacteria. Wilson J Ornithol 119:239–245

Shawkey MD, Mills KL, Dale C, Hill GE (2005) Microbial diversity of wild bird feathers revealed through culture-based and culture-independent techniques. Microb Ecol 50:40–47

Shawkey MD, Pillai SR, Hill GE (2003) Chemical warfare? Effects of uropygial oil on feather-degrading bacteria. J Avian Biol 34:345–349

Shawkey MD, Pillai SR, Hill GE, Siefferman LM, Roberts SR (2007) Bacteria as an agent for change in structural plumage color: correlational and experimental evidence. Am Nat 169:S112–S121

Shawkey MD, Firestone MK, Brodie EL, Beissinger SR (2009a) Avian incubation inhibits growth and diversification of bacterial assemblages on eggs. PLoS One 4:e4522

Shawkey MD, Pillai SR, Hill GE (2009b) Do feather-degrading bacteria affect sexually selected plumage color? Naturwissenschaften 96:123–128

Soini HA, Schrock SE, Bruce KE, Wiesler D, Ketterson ED, Novotny MV (2007) Seasonal variation in volatile compound profiles of preen gland secretions of the dark-eyed junco (*Junco hyemalis*). J Chem Ecol 33:183–198

Soler JJ, Martín-Vivaldi M, Ruiz-Rodríguez M, Valdivia E, Martín-Platero AM, Martínez-Bueno M, Peralta-Sánchez JM, Méndez M (2008) Symbiotic association between hoopoes and antibiotic-producing bacteria that live in their uropygial gland. Funct Ecol 22:864–871

Soler JJ, Peralta-Sánchez JM, Flensted-Jensen E, Martín-Platero AM, Møller AP (2011) Innate humoural immunity is related to eggshell bacterial load of European birds: a comparative analysis. Naturwissenschaften 98:807–813

Soler JJ, Peralta-Sánchez JM, Martín-Platero AM, Martín-Vivaldi M, Martínez-Bueno M, Møller AP (2012) The evolution of size of the uropygial gland: mutualistic feather mites and uropygial secretion reduce bacterial loads of eggshells and hatching failures of European birds. J Evol Biol 25:1179–1191

Whitaker JM, Cristol DA, Forsyth MH (2005) Prevalence and genetic diversity of *Bacillus licheniformis* in avian plumage. J Field Ornithol 76:264–270

Whittaker DJ, Richmond KM, Miller AK, Kiley R, Bergeon Burns C, Atwell JW, Ketterson ED (2011) Intraspecific preen oil odor preferences in dark-eyed juncos (*Junco hyemalis*). Behav Ecol 22:1256–1263

White J, Mirleau P, Danchin E, Mulard H, Hatch SA, Heeb P, Wagner RH (2010) Sexually transmitted bacteria affect female cloacal assemblages in a wild bird. Ecol Lett 13:1515–1524