Behavioural Processes 92 (2013) 1–5

Contents lists available at SciVerse ScienceDirect

Behavioural Processes

journal homepage: www.elsevier.com/locate/behavproc

Effect of preen oil on plumage bacteria: An experimental test with the mallard

M. Giraudeau[a,*], G.Á. Czirják[a,b,1], C. Duval[a,2], V. Bretagnolle[c], C. Gutierrez[d], N. Guillon[c], P. Heeb[a]

[a] Laboratoire Évolution et Diversité Biologique (EDB), UMR 5174 Centre National de la Recherche Scientifique (CNRS), Université Paul Sabatier (UPS), Ecole Nationale de Formation Agronomique (ENFA), 118 Route de Narbonne, F-31062 Toulouse, France
[b] Department of Infectious Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Mănăștur Street 3-5, Ro-400372 Cluj-Napoca, Romania
[c] Centre d'Etudes Biologiques de Chizé, CNRS UPR 1934, F-79360 Beauvoir-sur-Niort, France
[d] Laboratoire de Microbiologie et Génétique Moléculaire (LMGM), UMR 5100 CNRS-UPS, F-31000 Toulouse, France

ARTICLE INFO

Article history:
Received 15 February 2012
Received in revised form 1 August 2012
Accepted 1 August 2012

Keywords:
Anas platyrhynchos
Feather-degrading bacteria
Preen oil
Microbial load

ABSTRACT

Feathers are essential for avian thermoregulation, communication or flight and a reduction of plumage condition may alter these functions and reduce individual fitness. Recently, descriptive studies provided evidence that birds carry feather-degrading bacteria on their plumage that have the ability to degrade feathers rapidly under laboratory conditions. If such bacteria reduce avian fitness, natural selection should favour the evolution of anti-bacterial defences to limit the effects of these detrimental microorganisms. Preening behaviour and associated preen gland secretions have been proposed as the main factor used by birds to limit feather-degrading bacterial growth and some recent *in vitro* studies provided evidence that uropygial oil inhibited the growth of some keratinolytic strains in passerines. However, preen oil antimicrobial properties remained to be experimentally tested *in vivo*.

We conducted an experiment with mallards (*Anas platyrhynchos*) onto which we fixed a removable mechanism that blocked access to the uropygial gland in a first group of mallards, whilst birds in a second group had the same removable mechanism but access to their gland and a third group of birds had normal access to their gland. We found no significant effect of our treatment on total and feather-degrading bacterial loads. Three hypotheses may explain the discrepancy between our results and previous *in vitro* studies. First, *in vitro* studies may have over-estimated the bactericidal properties of the preen oil. Second, preen gland deprivation may have affected only a small portion of the feather-degrading bacterial community. Third, ducks and passerine oils might have different bactericidal properties.

1. Introduction

Feathers are characteristic of birds and are key to their thermoregulation (Stettenheim, 2000), communication (Andersson, 1994; Shuster and Wade, 2003), and flight (Rayner, 1988). Thus, a reduction of plumage conditions may alter these functions and reduce individual fitness (Booth et al., 1993; Swaddle and Witter, 1997; Ferns and Lang, 2003; Williams and Swaddle, 2003). Evidence has recently been provided that birds carry bacteria on their feathers and that some of them are keratinolytic and can rapidly degrade feathers under laboratory conditions (Burtt and Ichida, 1999; Goldstein et al., 2004; Lucas et al., 2005). Feather-degrading bacteria are a group of bacteria able to decompose β-keratin, a protein which represents more than 90% of feather mass (Onifade et al., 1998; Lucas et al., 2003; Gunderson, 2008). Given the functional importance of maintaining feather quality, a number of anti-bacterial defences are expected to have evolved in birds (Clayton, 1999; Gunderson, 2008).

Anti-bacterial mechanisms, as recently summarized by Gunderson (2008), could belong to four types: feather structure and colour (protective value of particular pigments like melanin against keratinolytic bacteria), sunbathing (sunlight reducing the number of viable keratinolytic bacteria, Saranathan and Burtt, 2007), moult (moult may reduce plumage bacterial loads, Burtt and Ichida, 1999, but see Giraudeau et al., 2010a) and preening behaviours (Clayton, 1999; Shawkey et al., 2003; Grande et al., 2004; Gunderson, 2008). Preening behaviours may act against feather-degrading bacteria either by the action of anti-bacterial properties of the uropygial oil compounds (Shawkey et al., 2003; Jacob et al., 1997), by forming a physical barrier between feather-degrading bacteria and feather surface (Reneerkens et al., 2008) or by the presence of antimicrobial substances within the uropygial gland secreted by symbiotic bacteria (Martin-Platero et al., 2006; Soler et al., 2008; Ruíz Rodríguez et al., 2009). In

* Corresponding author. Present address: School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA.
E-mail address: giraudeau.mathieu@gmail.com (M. Giraudeau).
[1] Present address: Wildlife Diseases Research Group, Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Strasse 17, 10315 Berlin, Germany.
[2] Present address: Centre for Ornithology, School of Biosciences, Birmingham University, Birmingham, UK.

0376-6357/$ – see front matter.
http://dx.doi.org/10.1016/j.beproc.2012.08.001

different studies, experimental surgical removal of preen glands (rock doves, *Columba livia*, Moyer et al., 2003) or temporary blockage of preen gland access (mallard, *Anas platyrhynchos*, Giraudeau et al., 2010b) led to degradation of plumage, consistent with the hypothesis of preen oil acting against keratinolytic bacteria. In addition, *in vitro* studies on House Finch (*Carpodacus mexicanus*) showed that preen oil inhibits the growth of several species of feather-degrading bacteria (Shawkey et al., 2003). Preen oil of Green Wood Hoopoe (*Phoeniculus purpureus*; Burger et al., 2004) and Red Knot (*Calidris canutus*; Reneerkens et al., 2008) inhibit the growth of bacteria such as *Bacillus licheniformis*. However, these studies were carried out *in vitro* and may have overestimated the *in vivo* anti-bacterial properties of preen oil. In addition, these studies tested the potential antibacterial properties of the preen oil on a single species of keratinolytic bacteria. Since the community of feather-degrading bacteria consists of a diverse assemblage of bacterial species, these studies may not be conclusive.

Here, we experimentally quantified the *in vivo* effect of preen oil on both total and feather-degrading bacterial loads. Using a novel design, we temporarily blocked access to the uropygial gland secretions with a removable mechanism on captive mallards (*A. platyrhynchos*) and we compared bacterial loads between feathers of birds with, or without, access to the preen gland after six months of treatment. In accordance with *in vitro* studies (Shawkey et al., 2003; Burger et al., 2004), we predicted that birds without preen gland access would have a higher feather-degrading bacterial loads on their plumage after six months of experiment compared to control birds. Moreover, as keratinolytic bacteria represent an important part of total cultivable bacteria (Shawkey et al., 2008; Czirják et al., 2010), we expected a higher total cultivable bacterial load on plumage of experimental birds compared to control ones.

2. Methods

Experiments were carried out between October 2007 and April 2008 at the Centre d'Etudes Biologiques de Chizé (CEBC) in western France using a semi-captive population of adult mallards (*A. platyrhynchos*) descended from individuals caught in the wild. The birds (3–5 years old, $N = 57$) were held in a large open enclosure (1 ha with a pool of 25 m^2) with free access to water and food *ad libitum*. Housing conditions and experiment were carried out in compliance with European legal recruitment and national permissions (European convention, ETS123).

2.1. Experimental design

We randomly assigned and fitted birds in a group with APO (anti-preen-oil mechanism, Giraudeau et al., 2010b,c; $N = 13$ males and 12 females). APO was designed to prevent bill-uropygial gland contact and the spread of the preen gland secretions on the feathers. The device consisted of a rubber tube of 1 cm in diameter and 2.5 cm in height, glued to the feathers (with loctite super glue-3) and skin around the small feathered nipple of the uropygial gland. We reinforced this structure with a flexible plastic square (pierced in the middle at the tube level) glued to the rubber tube and set around the uropygial gland (see details in Giraudeau et al., 2010b,c). As a control, a second group of birds ($N = 6$ males and 7 females) was fitted with CPO (control preen oil mechanism) which was identical to the APO, except the fact that the rubber ring was not present; only the flexible plastic square was used. Thus, bird equipped with the CPO had access to their preen gland through the hole pierced in the middle of the flexible plastic square. Finally, a third group of birds was kept with no device at all ($N = 11$ males and 8 females). Ducks were visually observed with binoculars at least twice a week to check that the APO and CPO remained attached. At the end of the experiment the devices were removed by gently pulling the plastic square. We detected no obvious signs of stress or unusual behaviour after fitting the birds with the systems (M. Giraudeau, pers. observations). Moreover, in preliminary studies, we did not find any significant effect of APO on preening, courtship, bathing and foraging time (Giraudeau et al., 2010b,c). Thus, we believe that our treatments did not lead to a change of bird behaviour during the experiment.

There was no significant differences of body condition and size between birds in the different treatment groups at the start of the experiment (all $P > 0.3$). In addition, changes in body condition during the experiment were not significantly influenced by the treatments ($P > 0.3$).

2.2. Bacterial sampling

To quantify plumage bacterial loads, we followed microbiological and sampling methods already published by our group and others (Møller et al., 2009; Czirják et al., 2010; Giraudeau et al., 2010a). Two feathers from a standardized position on the back (3 cm under the base of the neck) were collected at the beginning and at the end (6 months later) of the experiment and placed in a sterile 1.5 ml plastic tube. Birds did not molt during the experiment. Previous work by our group indicates that sampling two feathers from each bird at each time period provides suitable data to investigate temporal variations of feather bacterial loads (Giraudeau et al., 2010a). In addition, previous studies revealed significant correlations between back, wing-flash and head samples obtained from the same individuals, indicating that within individuals, bacterial loads on back feathers are indicative of bacteria intensity in other plumage areas in mallard (Giraudeau et al., 2010a); as it was already shown for passerine species (Shawkey et al., 2008; Gunderson et al., 2009). Tubes were subsequently kept in a dark envelope at −20 °C until microbiological analyses. We used only the 2 cm at the extremity of the feathers because preliminary tests showed that it was a quantity sufficient to obtain a bacterial suspension countable on TSA media (between 30 and 200 CFU per plates). In addition, Muza et al. (2000) showed that most of the bacteria in the plumage are on the distal half of the feathers. In order to obtain both the free-living and attached micro-organisms (Lucas et al., 2005), feather tips were sonicated three times for 15 min (5 min each with 5 min pause between each) in 0.8 ml of sterile physiological (0.90%, w/v) saline solution. After sonication the samples were vortexed for 20 s and the bacterial suspensions were transferred in a sterile 1.5 ml plastic tube. One half ml of sterile physiological saline was added to the tube with the feather tips and vortexed again for 20 s. The supernatant was transferred to the same sterile eppendorf, obtaining a ∼1.3 ml solution. Afterwards, the feathers were dried for 24 h at 70 °C and weighed to the nearest 1 μg on an analytical balance (AT1 Comparator, Mettler Toledo).

2.3. Microbiological analyses

To quantify cultivable and feather-degrading bacterial loads, we used two different growth media. Firstly, tryptic soy agar (TSA, #22091, Fluka) a rich medium on which heterotrophic bacteria grow, thus enabling assessment of total cultivable micro-organism load of the feathers. Secondly, we quantified feather-degrading bacterial loads using feather meal agar (FMA) containing 15 $g\,l^{-1}$ feather meal, 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 (Sangali and Brandelli, 2000; Shawkey et al., 2003, 2007). We inhibited fungal growth by adding 100 mg mL^{-1} of cycloheximide to the TSA and FMA media (Smit et al., 2001).

To measure bacterial counts, we spread 100 μl of each microbial suspension on 3 plates of the different growth media and the plates were incubated at 25 °C, for 3 days in the case of TSA, and for 14 days

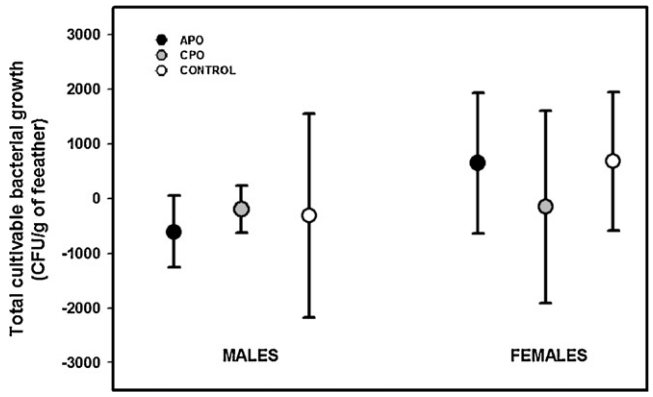

Fig. 1. Changes of cultivable bacterial abundance on back feathers of mallards during a six-month interval. Treatments consisted of anti-preen-oil mechanism (APO), control preen-oil mechanism (CPO) and control birds (for details see Section 2). Mean (±SD).

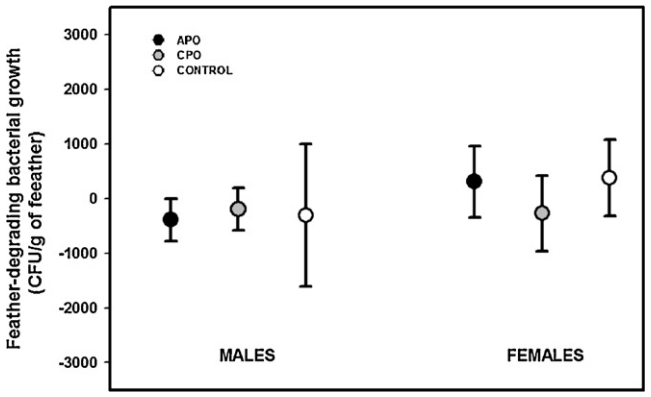

Fig. 2. Changes of feather-degrading bacterial abundance on back feathers of mallards during a six-month intervals (see legend in Fig. 1 for a description of treatments). Mean (±SD).

in the case of FMA (Shawkey et al., 2003, 2007; Møller et al., 2009). Three plates with each media type but without microbial suspension were also incubated in order to detect any contamination of media. After incubation, the number of visible colony forming units (CFU) on each plate was counted. Counts were then corrected by the initial volume of the suspension and by feather mass. The microbial concentration for each medium type was expressed as CFU per mg of feather (CFU mg^{-1}). We used 2 cm-feather tips and only one dilution because preliminary tests showed that the counts remained between 0 and 200 CFU per plate (M. Giraudeau, unpublished data). All counts were performed blindly by one of us (MG).

2.4. Statistical analyses

We calculated a difference of the bacterial counts between the end and the beginning of the experiment ($bacteria_{end} - bacteria_{beginning}$). This difference was considered as an index of the change in bacterial loads during the experiment. Since our data were not normally distributed we used non-parametric tests to examine the effects of sex and treatments on bacterial counts at the beginning of the experiment and on the change of bacterial loads during the experiment. Statistical analyses were realized with STATISTICA 6.0 software.

3. Results

We found a high repeatability for cells counts of the three plates of each media (repeatability: 0.97, Lessells and Boag, 1987), indicating that our method of bacteria quantification is highly repeatable.

Before birds were fitted with APO or CPO, cultivable and feather-degrading bacteria loads were not significantly different for the three groups of birds (TSA: $H_{2,54} = 1.3$, $P = 0.53$; FMA: $H_{2,54} = 4.6$, $P = 0.1$). However, the two sexes differed in their total and feather-degrading bacterial load (TSA: $H_{2,54} = 6.5$, $P = 0.01$; FMA: $H_{2,54} = 5.1$, $P = 0.02$); males had more bacteria on their plumage than females.

Changes between initial and final sampling of both total bacterial load and feather-degrading bacterial load were not significantly affected by our treatments in both sexes (males: TSA: $H_{2,27} = 0.74$, $P = 0.69$; FMA: $H_{2,27} = 1.05$, $P = 0.59$; females: TSA: $H_{2,24} = 5.2$, $P = 0.08$; FMA: $H_{2,24} = 4.2$, $P = 0.12$). Whether mallards were able, or not, to use their uropygial oil during six months did not impact the bacterial loads on feathers (see Figs. 1 and 2).

4. Discussion

Uropygial oil has been proposed as a first line of defence against feather-degrading bacteria (Jacob et al., 1997; Clayton, 1999; Shawkey et al., 2003; Gunderson, 2008), a hypothesis supported by *in vitro* studies (Shawkey et al., 2003; Burger et al., 2004; Reneerkens et al., 2008). Preen oil may limit feather-degrading bacteria growth through its bactericidal properties (Shawkey et al., 2003) and/or by forming a physical barrier that prevented the enzymes secreted by the bacilli from reaching the feather keratin (Reneerkens et al., 2008). However, to the best of our knowledge, no *in vivo* experimental test had ever been performed to confirm this hypothesis, and our study is the first longitudinal experiment that monitors total cultivatable and feather-degrading bacteria loads with or without preen gland access.

In a previous experiment (Giraudeau et al., 2010b), we showed a decrease of plumage condition for mallards equipped with an APO compared to those equipped with CPO, demonstrating the efficacy of our APO mechanism to prevent the spreading of preen oil on plumage. In addition, these results showed that preen oil helps to maintain feathers in good condition. One possible explanation for these earlier effects could have been that preen oil limited the growth of feather-degrading bacteria. We tested this hypothesis in the present study but unexpectedly, did not detect any significant differences of plumage bacterial loads (total cultivable and feather-degrading bacteria) on the plumage of birds with or without access to their preen gland. Indeed, being able or not to use uropygial oil during six months did not have any significant impact on feather's bacterial loads (Figs. 1 and 2).

Three hypotheses may explain the discrepancy between our results and previous *in vitro* studies which clearly showed that uropygial oil can inhibit the action of feather-degrading bacteria (Shawkey et al., 2003; Burger et al., 2004; Reneerkens et al., 2008). First, *in vitro* studies may have over-estimated preen oil effect on microbial isolates by applying a large quantity of oil which may not reliably reflect the natural amounts spread by birds on their feathers (Shawkey et al., 2003; Burger et al., 2004). However, this criticism does not apply to Reneerkens et al. (2008) where the amount of oil on each feather was applied by the birds themselves. Future *in vitro* experiments need to address this issue.

Second, preen oil could have bactericidal effect on some bacterial strains but not all. Most studies have focused on the genus *Bacillus*, and more specifically on *B. licheniformis* (Shawkey et al., 2003; Burger et al., 2004; Reneerkens et al., 2008) but several other species of feather-degrading bacteria occur on plumage (Burtt and Ichida, 1999; Shawkey et al., 2003, 2007, 2008; Burger et al., 2004; Reneerkens et al., 2008; Bisson et al., 2009). Absence of preen oil

on plumage may have affected only a small portion of the feathers-degrading bacterial community which may explain why we did not detect any effect of preen gland access deprivation on feather-degrading cultivable bacteria. Further studies focusing on the effect of preen gland of bacterial community assemblages and identification of resistant and susceptible species, could bring support for this hypothesis.

A third possibility might be that ducks and passerine oils have different bactericidal properties. This last hypothesis is probable as Sweeney et al. (2004) showed significant variation in feathers waxes already within families of passerines. There is clearly a need for assessing microbial community structure and composition in more details, using newly available techniques (reviewed in Head et al., 1998; Kirk et al., 2004; Dorigo et al., 2005; Sessitsch et al., 2006; Bisson et al., 2007; Gunderson, 2008). However, future studies will have to take into account the fact that molecular techniques may also identify DNA of non-living microorganisms which are not playing an active role on the plumage.

Shawkey et al. (2005) found that *Bacillus* spp. are dominant in the plumage of house finch using culture-dependent methods but molecular phylogenetic methods revealed that *Pseudomonas* spp. dominate the plumage bacterial community in the same individuals. Their results suggest that culture-dependent methods isolate a portion of the bacterial community present in the plumage. Thus, future studies should repeat our experiment using culture-independent methods.

In our experiment, we found a significant difference of feather bacterial loads between the two sexes. The most probable explanation for this difference could be a temporal variation of preen oil composition between the sexes. Indeed, female mallards, but not males, show qualitative shifts from mono- to diester preen waxes during courtship and incubation (Kolattukudy et al., 1985) which are within the sampling periods of our experiment. Reneerkens et al. (2008) studied the efficacy of both mono- and diester preen oils of red knots (*C. canutus*) to limit the action of *B. licheniformis* and found that both were effective at reducing the rate of feather degradation. However, more work is needed to investigate the potential differences of mono and diester preen oil bactericidal effects on the entire community of feather-degrading bacteria.

To conclude, this study provides the first *in vivo* experiment about the interaction between preen oil and plumage-associated bacteria, showing an absence of bactericidal effect of preen oil against total cultivable and feather-degrading bacteria in mallards. An examination of the potential bactericidal effect of preen oil at the level of the bacterial community could constitute a promising future way of the research.

Acknowledgements

We are grateful to Nadine Guillon, Leopold Denoufoux and Matthew D. Shawkey for their help. This project was partly supported by a French research grant from the ANR to Philipp Heeb (ANR-05, NT05-3_42075) and the "Laboratoire d'Excellence" TULIP (ANR-10-LABX-41). MG was supported by a PhD scholarship of the French Government and a Fyssen grant and GAC by an EGIDE grant from French Ministry of Foreign Affairs.

References

Andersson, M., 1994. Sexual Selection. Princeton University Press, New Jersey.
Bisson, I.A., Marra, P.P., Burtt Jr., E.H., Sikaroodi, M., Gillevet, P.M., 2007. A molecular comparison of plumage and soil bacteria across biogeographic, ecological, and taxonomic scales. Microb. Ecol. 54, 65–81.
Bisson, I.A., Marra, P.P., Burtt Jr., E.H., Sikaroodi, M., Gillevet, P.M., 2009. Variation in plumage microbiota depends on season and migration. Microb. Ecol. 58, 212–220.
Booth, D.T., Clayton, D.H., Block, B.A., 1993. Experimental demonstration of the energetic cost of parasitism in free-ranging hosts. Proc. R. Soc. B: Biol. Sci. 253, 125–129.
Burger, B.V., Reiter, B., Borzyk, O., Du Plessis, M.A., 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 Jr., E.H., Ichida, J.M., 1999. Occurrence of feather degrading bacilli in the plumage of birds. Auk 116, 364–372.
Clayton, D.H., 1999. Feather busting bacteria. Auk 116, 302–304.
Czirják, G.Á., Møller, A.P., Mousseau, T.A., Heeb, P., 2010. Microorganisms associated with feathers of barn swallows in radioactively contaminated areas around Chernobyl. Microb. Ecol. 60, 373–380.
Dorigo, U., Volatier, L., Humbert, J.F., 2005. Molecular approaches to the assessment of biodiversity in aquatic microbial communities. Water Res. 39, 2207–2218.
Ferns, P.N., Lang, A., 2003. The value of immaculate mates: relationships between plumage quality and breeding success in shelducks. Ethology 109, 521–532.
Giraudeau, M., Czirják, G.Á., Duval, C., Guillon, N., Gutierrez, C., Bretagnolle, V., Heeb, P., 2010a. No detected effect of moult on feather bacterial loads in mallards. J. Avian Biol. 41, 678–680.
Giraudeau, M., Duval, C., Guillon, N., Bretagnolle, V., Gutierrez, C., Heeb, P., 2010b. Effects of access to preen gland secretions on mallard plumage. Naturwissenchaften 97, 577–581.
Giraudeau, M., Czirják, G.Á., Duval, C., Bretagnolle, V., Eraud, C., McGraw, K.J., Heeb, P., 2010c. Effect of restricted preen-gland access on maternal self maintenance and reproductive investment in mallards. PLoS ONE 5, 13555.
Goldstein, G., Flory, K.R., Browne, B.A., Majid, S., Ichida, J.M., Burtt Jr., E.H., 2004. Bacterial degradation of black and white feathers. Auk 121, 656–659.
Grande, J.M., Negro, J.J., Torres, M.J., 2004. The evolution of bird plumage colouration: a role for feather-degrading bacteria? Ardeola 51, 375–383.
Gunderson, A.R., 2008. Feather-degrading bacteria: a new frontier in avian and host-parasite research. Auk 125, 1–8.
Gunderson, A.R., Forsyth, M.H., Swaddle, J.P., 2009. Evidence that plumage bacteria influence feather coloration and body condition of eastern bluebirds, *Sialia sialis*. J. Avian Biol. 40, 440–447.
Head, I.M., Saunders, J.R., Pickup, R.W., 1998. Microbial evolution, diversity, and ecology: a decade of ribosomal RNA analysis of uncultivated microorganisms. Microb. Ecol. 35, 1–21.
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. C 52, 114–123.
Kirk, J.L., Beaudette, L.A., Hart, M., Moutoglis, P., Klironomos, J.N., Lee, H., Trevors, J.T., 2004. Methods of studying soil microbial diversity. J. Microbiol. Methods 58, 169–188.
Kolattukudy, P.E., Bohnet, S., Rogers, L., 1985. Diesters of 3-hydroxy fatty acids produced by the uropygial glands of female mallards uniquely during the mating season. J. Lipid Res. 28, 582–588.
Lessells, C.M., Boag, P.T., 1987. Unrepeatable repeatabilities: a common mistake. Auk 104, 116–121.
Lucas, F.S., Broennimann, O., Febbraro, I., Heeb, P., 2003. High diversity among feather-degrading bacteria from a dry meadow soil. Microb. Ecol. 45, 282–290.
Lucas, F.S., Moureau, B., Jourdie, V., Heeb, P., 2005. Brood size modifications affect plumage bacterial assemblages of European starlings. Mol. Ecol. 14, 639–646.
Martin-Platero, A.M., Valdivia, E., Ruiz-Rodriguez, M., Soler, J.J., Martin-Vivaldi, M., Maqueda, M., Martinez-Bueno, M., 2006. Characterization of antimicrobial substances produced by *Enterococcus faecalis* MRR 10-3, isolated from the uropygial gland of the hoopoe (*Upupa epops*). Appl. Environ. Microbiol. 72, 4246–4249.
Møller, A.P., Czirják, G.Á., Heeb, P., 2009. Feather micro-organisms and uropygial anti-microbial defenses in a colonial passerine bird. Funct. Ecol. 23, 1097–1102.
Moyer, B.R., Rock, A.N., Clayton, D.H., 2003. An experimental test of the importance of preen oil in rock doves (*Columba livia*). Auk 120, 490–496.
Muza, M.M., Burtt Jr., E.H., Ichida, J.M., 2000. Distribution of bacteria on feathers of some Eastern North American birds. Wilson Bull. 112, 432–435.
Onifade, A.A., Al-Sane, N.A., Al-Musallam, A.A., Al-Zarban, S., 1998. A review: potentials for biotechnological applications of keratin-degrading microorganisms and their enzymes for nutritional improvement of feathers and other keratins as livestock feed resources. Bioresour. Technol. 66, 1–11.
Rayner, J.M.V., 1988. Form and function in avian flight. In: Johnston, R.F. (Ed.), Current Ornithology, vol. 5. Plenum Press, New York, pp. 1–66.
Reneerkens, J., Versteegh, M.A., Schneider, A.M., Piersma, T., Burtt Jr., E.H., 2008. Seasonally changing preen-wax composition: red knots' (*Calidris canutus*) flexible defense against feather-degrading bacteria? Auk 125, 285–290.
Ruíz Rodríguez, M., Valdivia, E., Soler, J.J., Martín Vivaldi, M., Martín Platero, A.M., Martínez Bueno, M., 2009. Symbiotic bacteria living in the hoopoe's uropygial gland prevent feather degradation. J. Exp. Biol. 212, 3621–3626.
Sangali, S., Brandelli, A., 2000. Feather keratin hydrolysis by a *Vibrio* sp. strain kr2. J. Appl. Microbiol. 89, 735–743.
Saranathan, V., Burtt Jr., E.H., 2007. Sunlight on feathers inhibits feather-degrading bacteria. Wilson J. Ornithol. 119, 239–245.
Sessitsch, A., Hackl, E., Wenzl, P., Kilian, A., Kostic, T., Stralis-Pavese, N., Tankouo Sandjong, B., Bodrossy, L., 2006. Diagnostic microbial microarrays in soil ecology. New Phytol. 171, 719–736.
Shawkey, M.D., Pillai, S.R., Hill, G.E., 2003. Chemical warfare? Effects of uropygial oil on feather-degrading bacteria. J. Avian Biol. 34, 345–349.
Shawkey, M.D., Mills, K.L., Dale, C., Hill, G.E., 2005. Microbial diversity of wild bird feathers revealed through culture-based and culture-independent techniques. Microb. Ecol. 50, 40–47.

Shawkey, M.D., Pillai, S.R., Hill, G.E., Siefferman, L.M., Roberts, S.R., 2007. Bacteria as an agent for change in structural plumage color: correlational and experimental evidence. Am. Nat. 169, S112–S121.
Shawkey, M.D., Pillai, S.R., Hill, G.E., 2008. Do feather-degrading bacteria affect sexually selected plumage color? Naturwissenschaften 96, 123–128.
Shuster, S.M., Wade, M.J., 2003. Mating Systems and Strategies. Princeton University Press, Princeton, NJ.
Smit, E., Leeflang, P., Gommans, S., van den Broek, J., van Mil, S., Wernars, K., 2001. Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. Appl. Environ. Microbiol. 67, 2284–2291.
Soler, J.J., Martín-Vivaldi, M., Ruiz Rodriguez, M., Valdivia, E., Martin Platero, A.M., Martinez Bueno, M., Peralta Sanchez, J.M., Mendez, M., 2008. Symbiotic association between hoopoes and antibiotic-producing bacteria that live in their uropigial gland. Funct. Ecol. 22, 864–871.
Stettenheim, P.R., 2000. The integumentary morphology of modern birds—an overview. Am. Zool. 40, 461–477.
Swaddle, J.P., Witter, M.S., 1997. The effects of moult on the flight performance, body mass, and behavior of European Starlings (*Sturnus vulgaris*): an experimental approach. Can. J. Zool. 75, 1135–1146.
Sweeney, R.J., Lovette, I.J., Harvey, E.L., 2004. Evolutionary variation in feather waxes of passerine birds. Auk 121, 435–445.
Williams, E.V., Swaddle, J.P., 2003. Moult, flight performance and wingbeat kinematics during take-off in European Starlings *Sturnus vulgaris*. J. Avian Biol. 34, 371–378.