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Chemical regulation of body feather microbiota in a wild bird

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Abstract

The microbiota has a broad range of impacts on host physiology and behaviour, pointing out the need to improve our comprehension of the drivers of host–microbiota composition. Of particular interest is whether the microbiota is acquired passively, or whether and to what extent hosts themselves shape the acquisition and maintenance of their microbiota. In birds, the uropygial gland produces oily secretions used to coat feathers that have been suggested to act as an antimicrobial defence mechanism regulating body feather microbiota. However, our comprehension of this process is still limited. In this study, we for the first time coupled high-throughput sequencing of the microbiota of both body feathers and the direct environment (i.e., the nest) in great tits with chemical analyses of the composition of uropygial gland secretions to examine whether host chemicals have either specific effects on some bacteria or nonspecific broad-spectrum effects on the body feather microbiota. Using a network approach investigating the patterns of co-occurrence or co-exclusions between chemicals and bacteria within the body feather microbiota, we found no evidence for specific promicrobial or antimicrobial effects of uropygial gland chemicals. However, we found that one group of chemicals was negatively correlated to bacterial richness on body feathers, and a higher production of these chemicals was associated with a poorer body feather bacterial richness compared to the nest microbiota. Our study provides evidence that chemicals produced by the host might function as a nonspecific broad-spectrum antimicrobial defence mechanism limiting colonization and/or maintenance of bacteria on body feathers, providing new insight about the drivers of the host's microbiota composition in wild organisms.

KEYWORDS
host–microbiota interactions, microbiome, *Parus major*, Preen gland, skin microbiota

1 | INTRODUCTION

The microbiota, that is, the diverse microbial communities inhabiting eukaryotic hosts, provides multiple essential functions for the host, from digestion and nutrient synthesis to protection against pathogens and can even mediate behaviour (Evans, Buchanan, Griffith, Klasing, & Addison, 2017; Ezenwa, Gerardo, Inouye, Medina, & Xavier, 2012; Fukuda et al., 2011; Jacob, Immer, et al., 2014; Jacob et al., 2015; Roggenbuck et al., 2014; Yatsunenko et al., 2012). It is also, however, associated with various diseases, such as infections, obesity or diabetes (Evans et al., 2017; Ley et al., 2005; Turnbaugh & Gordon, 2009; Wen et al., 2008), making our understanding of the drivers of microbiota composition of general importance. Numerous studies have documented the extensive variability in microbiota composition in animal hosts, both among individuals and among body parts of a given individual (Ding & Schloss, 2014; Edwards

et al., 2015; Roggenbuck et al., 2014; Spor, Koren, & Ley, 2011). This variability can be attributed to factors such as host diet and genotype (Benson et al., 2010; Spor et al., 2011), with a significant proportion of the microbiota being acquired from the environment or diet and depend on the microbial colonization history of the host (Benson et al., 2010; Morgan, Segata, & Huttenhower, 2013; Roggenbuck et al., 2014; Tannock, 2007). However, it remains unclear to what extent and by which mechanisms a host can control the acquisition and maintenance of its microbiota.

The microbiota of host body surfaces is particularly important as such surfaces are the first point of contact between the host and the environmental microbial pool, acting as an interface between the host and its environment (Kulkarni & Heeb, 2007; Schommer & Gallo, 2013). Unsurprisingly, body surfaces are home to abundant and diverse microbial communities (Schommer & Gallo, 2013) and are suggested to play significant roles in host health (Fredricks, Jacob et al., 2015; Muletz, Myers, Domangue, Herrick, & Harris, 2012; Schommer & Gallo, 2013). Skin microbiota disturbance (i.e., dysbiosis), for example, has been linked with various diseases (Benskin, Wilson, Jones, & Hartley, 2009; Fredricks, 2001; Hubálek, 2004; Schommer & Gallo, 2013). Some studies have suggested interactive effects of host traits and environmental factors in driving skin microbiota (reviewed in Schommer & Gallo, 2013). However, the factors responsible for microbiota richness and composition on a host's body surfaces remain poorly understood (Schommer & Gallo, 2013), and still biased towards humans or laboratory animals. Given the relevance of the microbiota for the ecology and evolutionary biology of hosts (Bestion et al., 2017; Evans et al., 2017; Ezenwa et al., 2012; Jacob et al., 2015; Leclaire, Jacob, Greene, Dubay, & Drea, 2017; McFall-Ngai et al., 2013), our comprehension of the drivers of microbiota composition must extend to nonmodel wild organisms to improve our understanding of the occurrence and mechanisms underlying such processes.

Almost all bird species possess an external gland, the uropygial gland, which produces oily secretions used to coat feathers. Well known for their waterproofing properties (Jacob & Ziswiler, 1982) and their role in plumage signalling and communication (Lopez-Rull, Pagan, & Macias Garcia, 2010; Piault et al., 2008; Piersma, Dekker, & Sinninghe Damsté, 1999), these secretions have also been suggested to function as an antimicrobial defence mechanism used to regulate bacterial communities on feathers (Czirjak et al., 2013; Fülöp, Czirják, Pap, & Vágási, 2016; Jacob, Immer, et al., 2014; Leclaire, Pierret, Chatelain, & Gasparini, 2014; Martín-Vivaldi et al., 2009; Møller, Czirjak, & Heeb, 2009; Shawkey, Pillai, & Hill, 2003). Accordingly, by experimentally manipulating great tit (*Parus major*) microbiota, Jacob, Immer, et al. (2014) demonstrated that individuals modify the quantity and chemical composition of uropygial secretions produced depending on their exposure to bacteria. Similarly, captive feral pigeons (*Columba livia*) increase the quantity of secretions produced and time spent preening when exposed to experimentally increased feather bacterial loads (Leclaire et al., 2014). Furthermore, house finch (*Carpodacus mexicanus*) secretions have been found to inhibit the in vitro growth of several isolated bacterial strains (Shawkey et al., 2003), but evidence for such inhibition effects on feather bacteria is mixed and mostly limited to in vitro studies (Czirjak et al., 2013; Giraudeau et al., 2013).

Given their suspected antimicrobial activity, secretions are expected to contain antimicrobial peptides, acids or alcohols, either directly produced by the birds or indirectly through a symbiosis with specific bacteria living in the gland (Martin-Vivaldi et al., 2010; Soler, Martín-Vivaldi, Peralta-Sánchez, & Ruiz-Rodríguez, 2010; Soler et al., 2008). However, most bird species produce secretions that are mainly composed of esters (Jacob & Ziswiler, 1982; Leclaire et al., 2011, 2012; Reneerkens, Piersma, & Sinninghe Damste, 2002; Whittaker et al., 2010). As with all lipids, esters are energy stores that might be used by some microorganisms for growth (Ara et al., 2006; Shelley, Hurly, & Nichols, 1953). Therefore, coating feathers with uropygial secretions could favour rather than inhibit the colonization and maintenance of some microorganisms on feathers. Such a promicrobial effect could help saturate the microbial niche on feathers by favouring commensal or mutualistic microorganisms and result in a limited colonization or reduced activity of pathogenic microorganisms, or instead have negative consequences for the hosts if pathogenic bacteria exploit these esters to colonize and grow (Currie, Scott, Summerbell, & Malloch, 1999; Davis et al., 2007; Jacob, Immer, et al., 2014; Soler et al., 2010). Alternatively, these oily secretions might have nonspecific broad-spectrum effects, acting as a physical barrier limiting colonization ability of any bacteria on feathers. Because body feathers are potentially exposed to a large diversity of environmental bacteria, selection might indeed favour the evolution of nonspecific antimicrobial activities. Altogether, these studies suggest that uropygial secretions potentially function to control colonization of feathers by environmental microorganisms (Czirjak et al., 2013; Fülöp et al., 2016; Jacob, Immer, et al., 2014; Martín-Vivaldi et al., 2009; Møller et al., 2009; Shawkey et al., 2003). However, our comprehension of the mechanisms behind this regulation remains in its infancy.

Here, we aimed at advancing our understanding of the mechanisms underlying body surface microbiota regulation by investigating the relationships between host chemical production and their associated microbiota in a wild bird (great tits; *P. major*), following previous work experimentally demonstrating that several phenotypic and fitness traits depend on the microbiota (Jacob, Immer, et al., 2014; Jacob et al., 2015). To do so, we coupled high-throughput sequencing of body feather and environmental (i.e., nest) bacterial communities with chemical analyses of the composition of uropygial gland secretions. We first compared the composition of body feather and nest bacterial communities. Birds indeed spend a significant amount of time in their nests during breeding. Bacteria from the nests thus constitute an important source of bacterial colonization of bird feathers (Jacob, Immer, et al., 2014). We then investigated the relationships between chemical compounds produced by the host uropygial gland and feather bacterial community composition and examined two, nonexclusive, hypotheses. First, gland chemicals might have specific effects on certain bacterial taxa, either favouring commensals or hindering pathogens. Under this hypothesis, we expected significant co-occurrence or co-exclusions between

chemicals and certain bacteria within the body feather microbiota. Second, uropygial gland chemicals might have nonspecific broad-spectrum effects, affecting the colonization or maintenance of any bacteria on feathers. For this second hypothesis, we tested for correlations between chemicals and the richness and composition of body feather bacterial communities.

We further investigated whether these chemicals could be involved in the acquisition of microbes from the environment by testing whether chemicals correlate with the differences in bacterial richness and composition between body feathers and the nest. Finally, males and females usually differ in their microbiota: female great tits spend more time in the nest, host higher bacterial loads and richness compared to males (Jacob, Immer, et al., 2014), and show higher investment in gland secretions (Jacob, Immer, et al., 2014). Here, we thus investigated whether the sexes differ in their body feather bacterial communities, composition of gland secretions and in their chemical–microbiota relationships.

2 | METHODS

2.1 | Studied populations

The study was performed during the 2013 reproductive period on two great tit populations breeding in woodcrete nest boxes in south-west France (Lauragais: 43°39′N, 1°54′E; Moulis: 42°58′N, 1°05′E). Nest boxes were checked at least twice a week from mid-March to detect the onset of nest construction, then everyday from its completion, allowing accurate determination of lay date. Adults were captured on the nest between 8 and 13 days after hatching and were measured (wing to the nearest mm, tarsus to the nearest 0.1 mm and mass to the nearest 0.05 g) under permits to A.S. Chaine from the French bird ringing office (CRBPO; no. 13619) and animal care permits from the state of Ariège (Préfecture de l'Ariège, Protection des Populations, no. A09-4) and the Région Midi-Pyrenées (DIREN, no. 2012-07).

2.2 | Microbiota sampling and analysis

Nest bacterial communities were sampled at day 3 posthatching using sterilized tweezers. Following Jacob, Immer, et al. (2014), samples were taken from a standardized position in the centre of the nest cup. Each sample was placed in a separate sterile Eppendorf tube filled with 1 ml phosphate buffer saline (PBS) and stored at −20°C until laboratory analyses. During adult trapping in the nests, feather bacterial communities were sampled by collecting ~20 ventral feathers from each individual at a standardized position close to the left leg (Jacob, Immer, et al., 2014). As with nest samples, feathers from each bird were placed in separate 1 ml PBS and stored at −20°C. All sampling and manipulations were made after systematically washing hands and materials with 70% ethanol to avoid cross-contaminations.

Nest and feather samples were sonicated and vortexed to detach microorganisms from nest material and feathers (Czirják, Møller, Mousseau, & Heeb, 2010; Jacob, Immer, et al., 2014; Møller et al., 2009). Bacterial DNA was then extracted using the Promega extraction protocol (Promega, Fitchburg, WI, USA; for details see Carriconde et al., 2008). PCRs were performed in 30 μl volumes containing 3 μl of 1/10 diluted DNA extract, 1U of AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA, USA), 2.5 mM of $MgCl_2$, 1× of Taq buffer, 0.2 mM of each dNTP and 4 ng of bovine serum albumin (Promega, Fitchburg, WI, USA). PCR conditions consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation (95°C for 30 s), annealing (57°C for 30 s) and elongation (72°C for 30 s). The universal primer pair used specifically amplifies the v5–6 region (ca 295 bp length) of the bacterial 16S rRNA gene (BACTB-F: GGATTAGATACCCTGGTAGT; and BACTB-R: CACGACACGAGCTGACG; Fliegerova et al., 2014). To discriminate samples after sequencing, both forward and reverse primers were labelled at the 5′ end with a combination of two different 8-bp tags. PCR products were purified using the QIAquick PCR purification Kit (Qiagen GmbH, Hilden, Germany) and pooled. Amplicon multiplex was prepared with the METAfast method and sequenced with an Illumina MiSeq platform using the 2 × 250 bp protocol (Fasteris SA, Plan-les-Ouates, Switzerland). PCR blank controls were included in the sequenced multiplex to detect and withdraw potential reagent contaminants.

The sequence reads were analysed as recommended by Taberlet et al. (2012) with some adjustments using the OBITools package (Boyer et al., 2015). Briefly, after paired-end reads assembly, reads were assigned to their respective samples (respectively, 0 and 2 mismatches allowed on tag and primer sites). Reads with low assembly scores or containing ambiguous bases (i.e., "N") were excluded. Strictly identical reads were dereplicated, and singletons (i.e., one single occurrence over the entire data set) were removed. Potential PCR/sequencing errors were detected and removed using the OBICLEAN algorithm (Boyer et al., 2015). The remaining sequences were then clustered into OTUs (operational taxonomic units) based on their similarity using the SUMACLUST algorithm (Kopylova et al., 2016; Mercier, Boyer, Bonin, & Coissac, 2013), with a 97% similarity threshold. Next, we removed all OTUs with a total read abundance <10 reads or detected in only one sample (Taberlet et al., 2012; Zinger et al., 2016). Finally, we used the PCR blank controls to remove contaminant OTUs from the data set (241 low-abundance OTUs removed out of 6,413 OTUs). The most abundant sequences of each OTU were then taxonomically assigned using the RDPII classifier (Wang, Garrity, Tiedje, & Cole, 2007) with the RDPII database release 11 (May 2015; Tables S2 and S3). We here considered a taxonomic assignment as reliable when its probability (provided at each taxonomic level) was >0.8. A total of 65 samples from adult great tits (43 females and 22 males) and 48 samples from nests have been sequenced in this study.

2.3 | Gland chemical sampling and analysis

Uropygial gland secretions were sampled during adult trapping by draining the gland papilla with a glass capillary. Filled capillaries were then immediately transferred in 2-ml sealed glass vials and stored at −20°C until extraction of organic compounds within 6 months.

Following previously developed procedures (Jacob, Immer, et al., 2014; Leclaire et al., 2011, 2017; Martin-Vivaldi et al., 2010), samples were diluted in 500 μl hexane, evaporated and then diluted in 150 μl of dichloromethane and vortexed for 1 min to extract organic chemical compounds. Analyses were performed on a mass spectrometer quadrupole detector (ISQ QD) coupled to a Trace 1300 gas chromatography (Thermo Fisher Scientific Inc) with a capillary column (Restek RTX-5MS 30 m × 0.25 mm, 0.25 μm film thickness, 5% diphenyl and 95% dimethylpolysiloxane) and a splitless injector (300 °C). Ionization was performed by electron impact (70 eV, source temperature 250 °C). Helium was the carrier gas (1.2 ml/min). The oven temperature was initiated at 50 °C for 1 min. After the injection of the sample (1 μl), the oven was programmed to increase 10 °C/min to 240 °C, then at 5 °C/min to 300 °C and held for 40 min. The scan range of the mass spectrometer was 60 to 500 m/z. Blanks were regularly interspersed throughout the sample analyses. To generate composition matrices, resulting profiles were analysed using the *runGC* function (*metaMS* R-package; Wehrens, Weingart, & Mattivi, 2014), with 43 samples analysed manually and blindly using Xcalibur software to verify the resulting composition matrix. As we cannot standardize the quantity of secretions sampled by the GC-MS, we used a matrix of intra-individual relative quantity of compounds in all analyses. A standard mixture of alkanes from n-C12 to n-C60 (Supelco, Sigma-Aldrich, 0.01% w/w for each component) was used as a reference for computing Kovats retention indices (KI). Then, compound identification was performed based on mass spectral fragmentation patterns and comparison with the NIST mass spectral library (Table S4).

2.4 | Statistical analyses

All analyses were performed using R software (version 3.2.2; R Core Team, 2017). We first used linear mixed models to compare bacterial and chemical richness between host sexes, along phenotypic traits (tarsus length, body mass, scaled mass index; Peig & Green, 2009) and with lay date and populations defined as fixed effects, and with nest identity as a random factor (*lme*, *nlme* R-package). Then to investigate the role of uropygial gland chemicals for body feather microbiota, we constructed an association network (*igraph R*-package). This approach consists of exploring the connections between items (i.e., proteins, individuals, species) by drawing the association structure of these items based on their presence/absence or abundances in a series of samples (Barberán, Bates, Casamayor, & Fierer, 2012; Faust & Raes, 2012; Faust et al., 2012). This approach is now often used in microbiota research and helps investigate the potential interactions between microbial taxa from large microbial data sets generated by high-throughput sequencing (Barberán et al., 2012; Faust & Raes, 2012; Faust et al., 2012). Here, we constructed a network comprising both bacterial OTUs and feather chemical compounds to explore the potential interactions between chemicals produced by the uropygial gland and the bacteria present on bird body feathers. Following previous key studies using this approach, pairwise associations were inferred from Spearman's rank correlations between bacteria and chemical relative abundances. We excluded correlation coefficients with absolute values lower than 0.6 as these are often reported to be unreliable (Barberán et al., 2012). Correlations were obtained from bacteria and chemical relative abundances across samples, which were expressed as the per cent abundance of OTUs/chemicals in each sample.

Chemicals from the uropygial gland might act individually or synergistically on the microbiota. To assess this potential synergistic effect, we constructed a second network comprising only gland chemicals to define groups of chemicals co-occurring in the samples based on relative abundances. Modules of chemical compounds were obtained with the walktrap algorithm, a community detection algorithm that allows definition of densely connected subgraphs through random walks (*igraph* R-package; Pons & Latapy, 2006), with Spearman correlations >0.6 (Barberán et al., 2012). Negative correlations between chemical relative abundances were considered equivalent to no correlation in this analysis, allowing us to split chemicals that negatively co-occur in different modules. Because our aim was to test for correlations between these groups of chemicals and the richness and composition of the bacterial communities (see below), we discarded chemical modules present in less than 30% of samples, as these may lead to spurious associations (Faust et al., 2012).

To investigate whether uropygial gland chemicals have broad-spectrum effects on body feather bacterial communities, we tested for correlations between the modules of uropygial gland chemicals and body feather bacterial richness (i.e., number of OTUs), with modules of chemicals calculated as the summed relative abundance of all chemical compounds included in a given module. We used linear mixed models followed by a backward selection procedure. We defined log-transformed bacterial richness (i.e., the number of OTUs in a sample) as the dependent variable, modules of chemicals as explanatory variables, and included host phenotype (i.e., body mass and tarsus length), sex, lay date and population to control for host and environmental particularities. Nest identity was defined as a random factor (*lme*, *nlme* R-package). Models were checked for normality and homoscedasticity of residuals. We additionally performed these analyses using the Scaled Mass Index as a measure of bird phenotype (Peig & Green, 2009).

We then investigated whether host chemicals might act as a filter between environmental bacterial communities and body feathers. To do so, we computed the difference between bacterial richness in nests and body feathers as $Bacterial\ richness_{nest} - Bacterial\ richness_{feather}$, expressed in number of OTUs. The values of this metric hence increase when bacterial richness in the nests increase compared to the feathers, meaning a reduced proportion of environmental bacterial colonizing body feathers. We used this difference as a dependent variable in linear mixed models, with modules of chemicals as explanatory variables, host phenotype, sex, lay date and population as covariates/cofactors, and nest identity as a random factor. Models were checked for normality and homoscedasticity of residuals. Finally, to test for general differences in bacterial community composition between body feather and nests, and for effects of

chemicals on body feather bacterial community composition, we used PERMANOVA (i.e., nonparametric multivariate analysis of variance; *adonis*, *vegan* R-package) using relative abundance-based Bray–Curtis dissimilarity and 999 permutations, with nests as a *strata* argument within which to constrain permutations.

3 | RESULTS

3.1 | Body feather and environmental microbiota

Females hosted higher bacterial richness on their body feathers than males (females: mean ± *SE* = 262.02 ± 18.72 OTUs; males: 184.73 ± 19.01; estimate ± *SE* = 0.32 ± 0.11; $t_{1,65}$ = 2.94; p = .009), and both sexes were home to lower bacterial richness compared to the nest microbiota (333.60 ± 23.22 OTUs in nests; estimate ± *SE* = −0.34 ± 0.08; $t_{1,113}$ = 4.20; p < .001). We found no significant correlation between bird phenotype and body feather bacterial richness (tarsus length: estimate ± *SE* = 15.89 ± 23.85; $t_{1,65}$ = 0.67; p = .52; body mass: estimate ± *SE* = −2.05 ± 12.86; $t_{1,65}$ = −0.16; p = .88; scaled mass index: estimate ± *SE* = −3.96 ± 11.44; $t_{1,65}$ = −0.35; p = .74). As expected, as females spend more time in nests than males, the difference in bacterial richness between nests and body feathers was higher in males (sex × location interaction: estimate ± *SE* = 0.35 ± 0.12; $t_{1,111}$ = 2.89; p = .005; females: estimate ± *SE* = −0.23 ± 0.09; $t_{1,43}$ = 2.59; p = .013; males: estimate ± *SE* = −0.58 ± 0.10; $t_{1,22}$ = 6.06; p < .001).

Bacterial richness in the nests significantly increased with lay date (estimate ± *SE* = 8.48 ± 3.7; $t_{1,65}$ = 2.29; p = .026) and differed between populations (estimate ± *SE* = 200.80 ± 43.30; $t_{1,65}$ = 4.64; p < .001), but appeared unaffected by bird phenotype (tarsus length: estimate ± *SE* = 16.34 ± 31.13; $t_{1,65}$ = 0.53; p = .60; body mass: estimate ± *SE* = −16.47 ± 19.89; $t_{1,65}$ = −0.83; p = .41; scaled mass index: estimate ± *SE* = −1.56 ± 8.17; $t_{1,65}$ = −0.19; p = .85).

Male and female body feather microbiota composition differed from nest microbiota (Figure 1; $F_{1,112}$ = 13.20; p = .001), and body feather microbiota composition differed between sexes ($F_{1,64}$ = 2.32; p = .006). This difference in composition between nest and body feathers differed between sexes slightly but not significantly ($F_{1,112}$ = 1.32; p = .080). Both nest and body feather microbiota were mainly composed of Actinobacteria, Proteobacteria, Bacteroidetes, Firmicutes and unidentified OTUs (Figure 2), but body feathers host more γ-Proteobacteria and unidentified Proteobacteria than nests, and fewer Actinobacteria and unidentified OTUs (Figure 2).

3.2 | Chemical composition of the uropygial gland secretions

Our analyses revealed the presence of 121 chemical compounds in great tit uropygial gland secretions, with individuals showing a mean chemical richness of 34.95 ± 0.68 compounds. There was no significant difference in chemical richness (i.e., the number of chemical compounds in a sample) between sexes (estimate ± *SE* = 1.92 ± 1.63; $t_{1,65}$ = 1.18; p = .25) nor by phenotypic traits (tarsus length: estimate ± *SE* = −0.89 ± 1.48; $t_{1,65}$ = −0.60; p = .56; body mass: estimate ± *SE* = 0.26 ± 0.80; $t_{1,65}$ = 0.32; p = .75; scaled mass index: estimate ± *SE* = 0.007 ± 0.023; $t_{1,65}$ = 0.29; p = .77), but chemical richness significantly differ between populations (5.00 ± 1.79; $t_{1,65}$ = 2.79; p = .008). Among these chemicals, 76.99 ± 1.51% (mean ± *SE*) were esters, 3.16 ± 0.52% were acids, 0.46 ± 0.12% were alcohols, and 19.37 ± 1.52 could not be identified. Females showed a higher proportion of acids than males (estimate ± *SE* = 0.03 ± 0.01; $t_{1,65}$ = 3.16; p = .005; p > .05 for all other types of compounds).

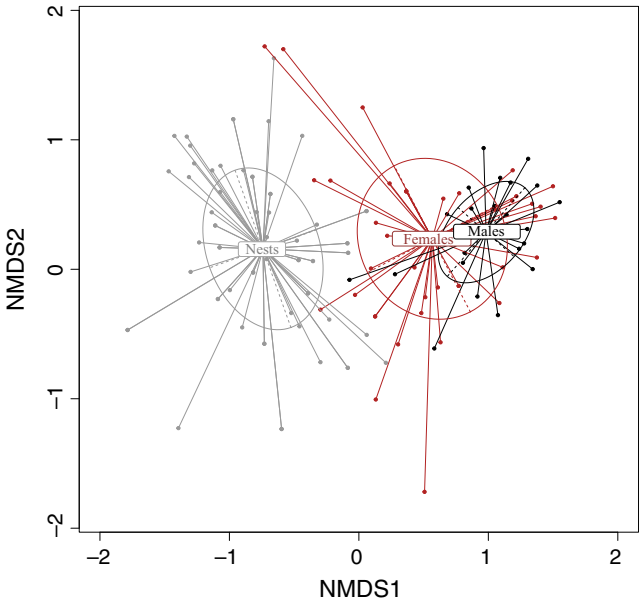

FIGURE 1 Male and female body feather microbiota composition differ from nest microbiota (NMDS analysis using relative abundance-based Bray–Curtis dissimilarity; stress value 0.18; PERMANOVA: $F_{1,112}$ = 13.20; p = .001). Circles represent scatter diagrams (*s.class* function, *ade4* R-package; inertia ellipse size coefficient = 1) [Colour figure can be viewed at wileyonlinelibrary.com]

3.3 | Specific effects of chemicals?

We used a network approach (Barberán et al., 2012; Faust & Raes, 2012; Faust et al., 2012) to explore specific relationships between chemical compounds and feather bacteria. Drawing a network including Spearman rho correlation coefficients of absolute values higher than .6, we found no evidence for specific relationships between gland chemicals and specific bacteria (Figure 3). First, we did not find any negative correlations between pairs of chemicals and bacteria. Second, only some chemical compounds co-occurred with bacteria, but they were among the rarest compounds in the data set (Figure 3). These results suggest that great tits do not produce antimicrobial compounds with specific effects on certain bacteria, either favouring commensals or hindering pathogens in their uropygial secretions.

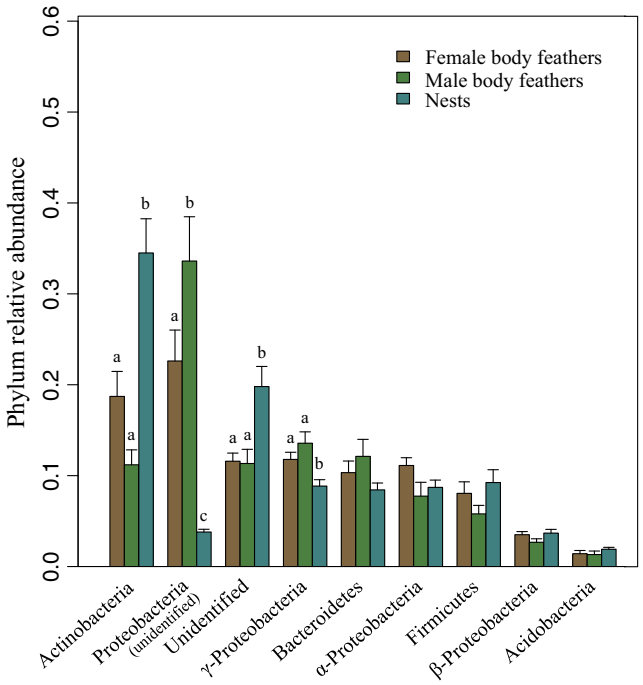

FIGURE 2 Bacterial composition of nests and body feather microbiota. Nest and body feather microbiota are mainly composed of Actinobacteria, Proteobacteria, Bacteroidetes, Firmicutes and unidentified operational taxonomic units (OTUs), but body feathers host more γ-Proteobacteria and unidentified Proteobacteria and less Actinobacteria and unidentified OTUs than the nests. Mean ± *SE* of the relative abundance of the major phyla is shown. Proteobacteria are split in subphyla, and unidentified OTUs are included as a separate group. Letters indicate when phylum relative abundance significantly differs between categories (linear mixed models with nest as random factor and Bonferroni multiple testing correction of *p* values) [Colour figure can be viewed at wileyonlinelibrary.com]

3.4 | Broad-spectrum effects of chemicals?

Using a network approach to summarize the chemical compounds into modules of chemicals that co-occur in the samples, we retained 10 modules with >30% prevalence in the data set (Figure S1). Testing for correlations between these groups of compounds and body feather microbiota, we found that one group was negatively correlated to bacterial richness (estimate ± *SE* = −0.87 ± 0.25; $t_{1,65} = -3.55$; $p = .003$; Figure 4). We did not find a significant interaction between this group of compounds and sex on feather bacterial richness ($t_{2,65} = -0.32$; $p = .75$), but male secretions contained significantly more of these compounds than female secretions relative to other compounds (estimate ± *SE* = 0.23 ± 0.05; $t_{1,65} = 4.67$; $p < .001$). Moreover, this negative correlation occurred in both studied populations, and was stronger in the population where body feather bacterial richness was higher (module × area interaction: $F_{2,65} = 9.24$; $p = .008$; Lauragais: estimate ± *SE* = −436.08 ± 103.85; $t_{1,26} = -4.20$; $p = .004$; Moulis: estimate ± *SE* = −123.23 ± 46.06; $t_{1,39} = -2.68$; $p = .025$).

This module contained 18 chemicals (16 esters, 1 acids and 1 unidentified compounds; *highlighted in grey in* Figure S1; Table S4), and post hoc analyses reveal that it was negatively correlated with OTU richness of each major bacterial phylum (linear mixed model: module × phylum interaction: $F_{8,585} = 1.59$; $p = .12$). We further tested whether the negative relationship with these chemicals came from an indirect effect of host condition, as condition might itself affect both feather microbiota and investment in gland compounds. We did not find significant effects of phenotypic traits on the relative abundance of these chemicals (linear mixed model: tarsus length: $t_{1,65} = 2.61$; $p = .13$; body mass: $t_{1,65} = 1.98$; $p = .18$; scaled mass index: $t_{1,65} = 0.29$; $p = .77$), nor differences between populations ($t_{1,65} = 0.53$; $p = .60$). Finally, chemical modules did not significantly correlate with body feather microbiota composition (PERMANOVA; all $p > .1$).

3.5 | Gland chemicals and the relationship between feather and environmental microbiota

We then tested whether uropygial gland secretions are involved in the acquisition of bacteria from nest microbiota. First, we found that the relative abundance of the module of chemicals linked to lower body feather bacterial richness was also positively correlated with the difference between nest and body feather bacterial richness (estimate ± *SE* = 289.55 ± 75.91; $t_{1,65} = -3.81$; $p = .001$; Figure 5; no significant effect for other modules, all $p > .05$). This module was negatively correlated to OTU richness in each major bacterial phylum (Table S1), suggesting a potential broad-spectrum effect as found for body feather microbiota. This means that the more birds invested in the production of these chemical compounds, the lower was the bacterial richness on feathers was compared to their nests (Figure 5). This correlation appeared stronger in the population where body feather bacterial richness was higher (module × area interaction: $F_{2,65} = 8.25$; $p = .011$; Lauragais: estimate ± *SE* = 531.67 ± 144.73; $t_{1,26} = 3.67$; $p = .008$; Moulis: estimate ± *SE* = 122.89 ± 64.37; $t_{1,39} = 1.91$; $p = .089$), despite no significant difference between populations in investment in this module (see above). Finally, we found no significant correlation between chemical modules and the difference of microbiota composition between nests and feathers (abundance-based Bray–Curtis dissimilarity; all $p > .10$). Importantly, bacterial richness in the nests did not significantly correlate with the relative abundance of gland chemical modules (all $p > .1$), suggesting that these chemicals play a role in determining body feather microbiota with no further direct or indirect effect on environmental microbiota.

4 | DISCUSSION

In this study, we coupled high-throughput sequencing of both host body surface and nest bacterial communities with chemical analyses of the composition of great tit uropygial gland secretions to examine the role host chemicals might play in the regulation of feather microbiota. In this species, the time spent nest building, incubating and nestling rearing exposes adults to the dense and diverse nest

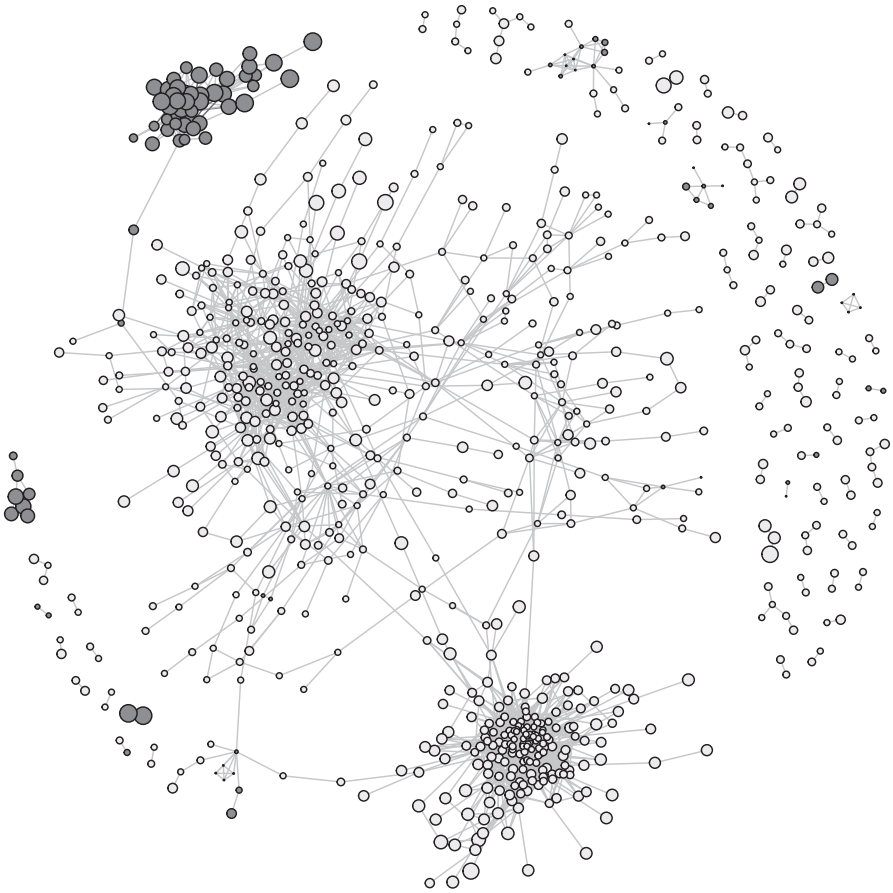

FIGURE 3 Co-occurrence network of uropygial gland chemicals (*dark grey*) and feather bacterial operational taxonomic units (OTUs) (*light grey*). Connections represent Spearman correlations >|.6|. Strong positive co-occurrences appear in grey, negative co-occurrences in red (only in the upper left, between chemical compounds). Network graph was built using Fruchterman–Reingold layout (igraph R-package; iterations = 500, maximum change = number of OTUs/chemicals, cooling exponent = 3; Barberán et al., 2012). Node size is proportional to bacteria or chemical prevalence in the data set

microbiota (Jacob, Immer, et al., 2014; Kilgas, Saag, Mägi, Tilgar, & Mänd, 2012; Saag, Tilgar, Mänd, Kilgas, & Mägi, 2011), which in turn is expected to colonize body feathers and potentially affect health and reproduction (Jacob et al., 2015). Indeed, experimental modifications of the nest microbiota have been shown to lead to changes of feather microbiota (Jacob, Immer, et al., 2014), and had significant consequences for host phenotype and fitness (Jacob et al., 2015). Coating feathers with secretions from the uropygial gland might allow a bird to control colonization of body feathers by environmental microorganisms to some degree (Czirjak et al., 2013; Fülöp et al., 2016; Jacob, Immer, et al., 2014; Møller et al., 2009; Ruiz-Rodriguez, Valdivia, et al., 2009; Shawkey et al., 2003).

Oily uropygial secretions could contain antimicrobial chemicals inhibiting the growth of specific microorganisms, or promicrobial compounds favouring some commensal or mutualistic bacteria (Møller et al., 2009; Shawkey et al., 2003; Soler et al., 2010). However, we found no evidence for specific effects of uropygial gland chemicals favouring commensals or hindering pathogens. First, we did not find any significant negative correlations between pairs of chemicals and bacteria (Figure 3), suggesting that great tits do not produce narrow-spectrum antimicrobial compounds in their uropygial secretions. Second, only some chemical compounds co-occurred with bacteria. These were among the rarest compounds in the secretions (Figure 3) and were in turn correlated with rare and relatively isolated bacteria in the network (Figure 3). We further cannot rule out that these correlations only appeared by chance given the large number of correlations considered in network approaches (Barberán et al., 2012; Faust & Raes, 2012; Faust et al., 2012). The presence of specific bacteria on the hosts might also induce the production of these compounds by the birds, or could be the result of the degradation of more complex chemical compounds into subproducts by these bacteria, which would explain these rare co-occurrences (Jacob, Immer, et al., 2014).

Chemicals coated on feathers might otherwise have broad-spectrum effects, either by favouring the maintenance of a diverse microbiota or on the contrary by acting as a physical barrier limiting colonization ability of any bacteria on feathers. Indeed, because body feathers are potentially exposed to a large diversity of environmental bacteria, selection might indeed favour the evolution of nonspecific antimicrobial actions. In this species for instance, a recent experimental study showed that the overall bacterial load in nests, but not microbiota composition, affects parental investment, costs of reproduction and nestling condition (Jacob et al., 2015). After summarizing the chemical community data into modules of chemicals that co-occur in the samples, we found that one module of chemicals was negatively correlated to bacterial richness on body feathers (Figure 4). Furthermore, the more birds invested in these chemicals, the poorer body feather bacterial richness was compared to the nest microbiota (Figure 5), a relationship observed within all major bacterial phyla. Our results suggest that these chemical compounds

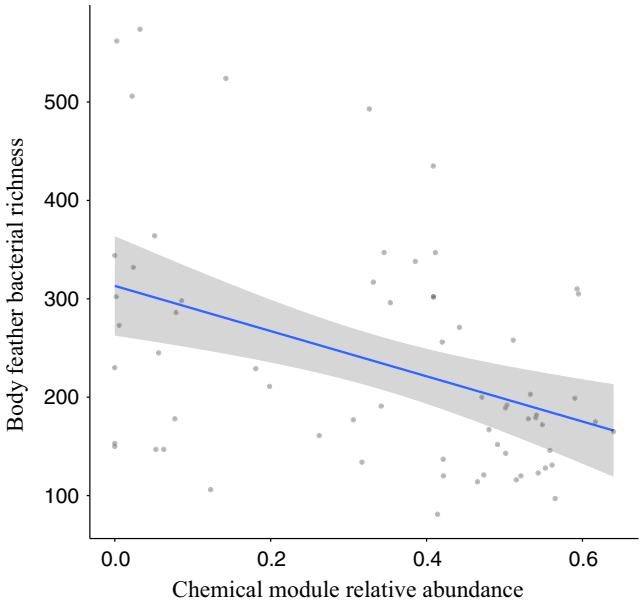

FIGURE 4 Bacterial richness on great tit body feathers is negatively correlated to a module of chemical compounds produced by the uropygial gland. The line shows predicted values from a linear mixed model including module abundance as explanatory variable and nest identity as a random effect; the grey area represents the standard error of model predictions [Colour figure can be viewed at wileyonlinelibrary.com]

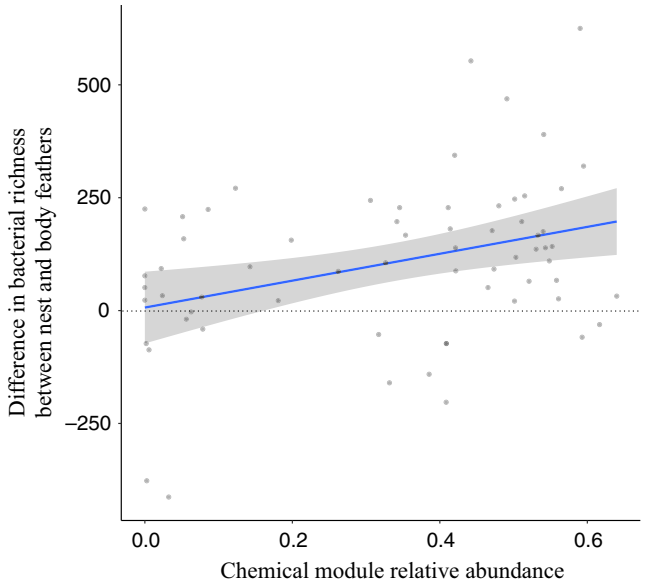

FIGURE 5 The difference in bacterial richness between nest and body feathers increases with the abundance of a module of chemical compounds produced by the uropygial gland. The line shows predicted values from a linear mixed model including cocktail abundance as explanatory variable and nest identity as a random effect; the grey area represents the standard error of model predictions. The dashed line represents no difference between nest and feathers in bacterial richness, and feathers host fewer bacteria than nests when points are above this line [Colour figure can be viewed at wileyonlinelibrary.com]

produced by the birds might function as a nonspecific broad-spectrum antimicrobial defence mechanism limiting colonization and/or maintenance of bacteria on body feathers (Czirjak et al., 2013; Fülöp et al., 2016; Jacob, Colmas, Parthuisot, & Heeb, 2014; Jacob, Immer, et al., 2014; Møller et al., 2009; Ruiz-Rodriguez, Valdivia, et al., 2009; Shawkey et al., 2003).

Among the 18 chemicals included in the antimicrobial module, 14 were esters, 2 were acids and 2 remained unidentified. Esters are oily substances that are unlikely to have a direct antimicrobial effect, but could form an oily physical barrier limiting colonization of bacteria from the environment. Alternatively, the presence of acidic compounds in the uropygial gland secretions might explain the broad-spectrum antimicrobial action observed. In humans, differences between men and women in skin acidity have been suggested to be a potential explanation for the higher skin microbiota diversity on women (Fredricks, 2001; Giacomoni, Mammone, & Teri, 2009; Schommer & Gallo, 2013). Furthermore, men have higher sebum production than women, a substance that consists mostly of acids and esters (Giacomoni et al., 2009). Here, we found that female great tits showed a higher proportion of acidic compounds in their uropygial gland secretions compared to males in addition to a previously found higher production of secretions (Jacob, Immer, et al., 2014). However, the module of chemicals we identified to be negatively correlated with body feather bacterial richness represented a larger fraction of secreted chemicals in males than in females. Such sex-specific investment in coating body feathers with gland secretions might thus explain the observed bacterial richness differences between males and females. Sexes indeed significantly differed in the richness and composition of their microbiota. Furthermore, males and females usually differ in their investment in reproduction, a physiologically demanding activity that can increase oxidative stress and is associated with increased susceptibility to infection by parasites (Metcalfe & Monaghan, 2013; Monaghan, Metcalfe, & Torres, 2009; Sheldon & Verhulst, 1996). Interestingly, such differences in reproductive strategies and associated costs might in part result from sex differences in microbiota richness and composition. For instance, the microbiota has been found responsible for a significant part of the oxidative costs of reproduction in this species, and sexes differ in their investment in antimicrobial defence mechanisms (Jacob, Immer, et al., 2014; Jacob, et al., 2015). Deciphering the relative role of the microbiota in sex-specific reproductive investment might thus help explain the evolution of sexual dimorphism and sex-specific reproductive strategies.

So far we have assumed that uropygial secretions influence the microbiota, but an alternative hypothesis is that changes in environmental or feather microbiota resulted in modification of the chemical composition of uropygial secretions produced (Jacob, Immer, et al., 2014). However, we found no significant correlation between uropygial gland chemicals and the nest microbiota as would be expected under this hypothesis. Although experimentally investigating how these gland chemicals affect the colonization and maintenance of feather microbiota is an important next step, our results strongly

suggest that great tit preening behaviour plays a role in the colonization of feathers by environmental microorganisms.

Interestingly, our study revealed that both body feather bacterial richness and uropygial gland chemical richness differ between the two populations we studied. Furthermore, the relationship between increased investment in specific chemical modules and lower body feather bacterial richness compared to the nest was stronger in the population where bacterial richness was the highest. Empirical studies have provided evidence for variability of microbiota composition along environmental gradients, such as for instance along temperature, altitude, vegetation diversity and cover (Delgado-Baquerizo et al., 2018; Fierer, 2017; Thompson et al., 2017), and in response to climate warming (Bestion et al., 2017). When facing such differences in microbial exposure, hosts are expected to adjust their investment in antimicrobial defences, as experimentally demonstrated in this species (Jacob, Immer, et al., 2014). Contrasts between populations in uropygial gland chemicals might thus be one way in which hosts deal with different microbial environments. Although our work was limited to two populations, this result points out the potential importance of breeding habitat characteristics in shaping the microbiota and host–microbiota interactions (Lucas & Heeb, 2005; Ruiz-Rodriguez, Lucas, Heeb, & Soler, 2009), and urges the need for experimental approaches to shed light on how hosts and their microbiota will respond to environmental changes (Bestion et al., 2017; Jacob et al., 2015).

Developing our knowledge of the drivers of a host's microbiota composition is of major importance for the comprehension of host–microbe interactions and their consequences for the hosts' ecology and evolution (Benson et al., 2010; Ezenwa et al., 2012; Fukuda et al., 2011; Spor et al., 2011; Sullam et al., 2012; Yatsunenko et al., 2012). Here, we found no evidence for specific effects of chemical compounds on body feather microbiota, but rather that chemicals produced by the host uropygial gland might function as a broad-spectrum antimicrobial defence mechanism limiting colonization of feathers by environmental bacteria. Given the known consequences (positive or negative) of the microbiota for the host (Ezenwa et al., 2012; Fukuda et al., 2011; Gilbert, Sapp, & Tauber, 2012; Yatsunenko et al., 2012), including in this species (Jacob et al., 2015), further studies should investigate the detailed mechanisms underlying this defence mechanism, its potential plasticity (Jacob, Immer, et al., 2014; Whittaker et al., 2011) and costs and benefits of such a broad-spectrum microbial regulation.

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DATA AVAILABILITY

The data supporting the findings of this study are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.k36t400.

AUTHOR CONTRIBUTIONS

S.J., L.S., A.S.C. and P.H. defined the research project and set up the sampling protocol. L.S., A.S.C., A.R. and P.H. performed the fieldwork and sampling. S.J. and L.Z. performed molecular and bioinformatics analyses. S.J., C.D. and L.B. performed the chemical analyses. S.J. analysed the data, with the help of L.Z. and L.B. S.J. wrote the first draft of the manuscript, and all authors contributed to manuscript editing.

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REFERENCES

Ara, K., Hama, M., Akiba, S., Koike, K., Okisaka, K., Hagura, T., … Tomita, F. (2006). Foot odor due to microbial metabolism and its control. *Canadian Journal of Microbiology*, *52*, 357–364. https://doi.org/10.1139/w05-130

Barberán, A., Bates, S. T., Casamayor, E. O., & Fierer, N. (2012). Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME Journal*, *6*, 343–351. https://doi.org/10.1038/ismej.2011.119

Benskin, C. M. H., Wilson, K., Jones, K., & Hartley, I. R. (2009). Bacterial pathogens in wild birds: A review of the frequency and effects of infection. *Biological Reviews*, *84*, 349–373. https://doi.org/10.1111/j.1469-185X.2008.00076.x

Benson, A. K., Kelly, S. A., Legge, R., Ma, F., Low, S. J., Kim, J., … Pomp, D. (2010). Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. *Proceedings of the National Academy of Sciences of the United States of America*, *107*, 18933–18938. https://doi.org/10.1073/pnas.1007028107

Bestion, E., Jacob, S., Zinger, L., Di Gesu, L., Richard, M., White, J., & Cote, J. (2017). Climate warming reduces gut microbiota diversity in a vertebrate ectotherm. *Nature Ecology & Evolution*, *1*, 0161. https://doi.org/10.1038/s41559-017-0161

Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., & Coissac, E. (2015). Obitools: A unix-inspired software package for DNA metabarcoding. *Molecular Ecology Resources*, *16*, 176–182.

Carriconde, F., Gardes, M., Jargeat, P., Heilmann-Clausen, J., Mouhamadou, B., & Gryta, H. (2008). Population evidence of cryptic species and geographical structure in the cosmopolitan ectomycorrhizal fungus, *Tricholoma scalpturatum*. *Microbial Ecology*, *56*, 513–524. https://doi.org/10.1007/s00248-008-9370-2

Currie, C., Scott, J., Summerbell, R., & Malloch, D. (1999). Fungus-growing ants use antibiotic-producing bacteria to control garden parasites. *Nature*, *398*, 701–704. https://doi.org/10.1038/19519

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. *Microbial Ecology*, *60*, 373–380. https://doi.org/10.1007/s00248-010-9716-4

Czirjak, G., Pap, P., Vagasi, C., Giraudeau, M., Mureçan, C., Mirleau, P., & Heeb, P. (2013). Preen gland removal increases plumage bacterial load but not that of feather-degrading bacteria. *Naturwissenschaften*, *100*, 145–151. https://doi.org/10.1007/s00114-012-1005-2

Davis, M. E., Brown, D. C., Baker, A., Bos, K., Dirain, M. S., Halbrook, E., … Rehberger, T. (2007). Effect of direct-fed microbial and antibiotic supplementation on gastrointestinal microflora, mucin histochemical characterization, and immune populations of weanling pigs. *Livestock Science*, *108*, 249–253. https://doi.org/10.1016/j.livsci.2007.01.063

Delgado-Baquerizo, M., Oliverio, A. M., Brewer, T. E., Benavent-González, A., Eldridge, D. J., Bardgett, R. D., … Fierer, N. (2018). A global atlas of the dominant bacteria found in soil. *Science*, *359*, 320–325. https://doi.org/10.1126/science.aap9516

Ding, T., & Schloss, P. D. (2014). Dynamics and associations of microbial community types across the human body. *Nature*, *509*, 357–360. https://doi.org/10.1038/nature13178

Edwards, J., Johnson, C., Santos-Medellín, C., Lurie, E., Podishetty, N. K., Bhatnagar, S., … Sundaresan, V. (2015). Structure, variation, and assembly of the root-associated microbiomes of rice. *Proceedings of the National Academy of Sciences of the United States of America*, *112*, E911–E920. https://doi.org/10.1073/pnas.1414592112

Evans, J. K., Buchanan, K. L., Griffith, S. C., Klasing, K. C., & Addison, B. (2017). Ecoimmunology and microbial ecology: Contributions to avian behavior, physiology, and life history. *Hormones and Behavior*, *88*, 112–121. https://doi.org/10.1016/j.yhbeh.2016.12.003

Ezenwa, V. O., Gerardo, N. M., Inouye, D. W., Medina, M., & Xavier, J. B. (2012). Animal behavior and the microbiome. *Science*, *338*, 198–199. https://doi.org/10.1126/science.1227412

Faust, K., & Raes, J. (2012). Microbial interactions: From networks to models. *Nature Reviews Microbiology*, *10*, 538–550. https://doi.org/10.1038/nrmicro2832

Faust, K., Sathirapongsasuti, J. F., Izard, J., Segata, N., Gevers, D., Raes, J., & Huttenhower, C. (2012). Microbial co-occurrence relationships in the human microbiome. *PLoS Computational Biology*, *8*, e1002606. https://doi.org/10.1371/journal.pcbi.1002606

Fierer, N. (2017). Embracing the unknown: Disentangling the complexities of the soil microbiome. *Nature Reviews Microbiology*, *15*, 579–590. https://doi.org/10.1038/nrmicro.2017.87

Fliegerova, K., Tapio, I., Bonin, A., Mrazek, J., Callegari, M. L., Bani, P., … Wallace, R. J. (2014). Effect of DNA extraction and sample preservation method on rumen bacterial population. *Anaerobe*, *29*, 80–84. https://doi.org/10.1016/j.anaerobe.2013.09.015

Fredricks, D. N. (2001). Microbial ecology of human skin in health and disease. *Journal of Investigative Dermatology Symposium Proceedings*, *6*, 167–169. https://doi.org/10.1046/j.0022-202x.2001.00039.x

Fukuda, S., Toh, H., Hase, K., Oshima, K., Nakanishi, Y., Yoshimura, K., … Ohno, H. (2011). Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature*, *469*, 543–547. https://doi.org/10.1038/nature09646

Fülöp, A., Czirják, G. Á., Pap, P. L., & Vágási, C. I. (2016). Feather-degrading bacteria, uropygial gland size and feather quality in House Sparrows *Passer domesticus*. *Ibis*, *158*, 362–370. https://doi.org/10.1111/ibi.12342

Giacomoni, P. U., Mammone, T., & Teri, M. (2009). Gender-linked differences in human skin. *Journal of Dermatological Science*, *55*, 144–149. https://doi.org/10.1016/j.jdermsci.2009.06.001

Gilbert, S. F., Sapp, J., & Tauber, A. I. (2012). A symbiotic view of life: We have never been individuals. *The Quarterly Review of Biology*, *87*, 325–341. https://doi.org/10.1086/668166

Giraudeau, M., Czirják, G. Á., Duval, C., Bretagnolle, V., Gutierrez, C., Guillon, N., & Heeb, P. (2013). Effect of preen oil on plumage bacteria: An experimental test with the mallard. *Behavioural Processes*, *92*, 1–5. https://doi.org/10.1016/j.beproc.2012.08.001

Hubálek, Z. (2004). An annotated checklist of pathogenic microorganisms associated with migratory birds. *Journal of Wildlife Diseases*, *40*, 639–659. https://doi.org/10.7589/0090-3558-40.4.639

Jacob, S., Colmas, L., Parthuisot, N., & Heeb, P. (2014). Do feather-degrading bacteria actually degrade feather colour? No significant effects of plumage microbiome modifications on feather colouration in wild great tits. *Naturwissenschaften*, *101*, 929–938. https://doi.org/10.1007/s00114-014-1234-7

Jacob, S., Immer, A., Leclaire, S., Parthuisot, N., Ducamp, C., Espinasse, G., & Heeb, P. (2014). Uropygial gland size and composition varies according to experimentally modified microbiome in Great tits. *BMC Evolutionary Biology*, *14*, 134. https://doi.org/10.1186/1471-2148-14-134

Jacob, S., Parthuisot, N., Vallat, A., Ramon-Portugal, F., Helfenstein, F., & Heeb, P. (2015). Microbiome affects egg carotenoid investment, nestling development and adult oxidative costs of reproduction in Great tits. *Functional Ecology*, *29*, 1048–1058. https://doi.org/10.1111/1365-2435.12404

Jacob, J., & Ziswiler, V. (1982). The uropygial gland. In D. S. Farner, J. R. King & K. C. Parkes (Eds.), *Avian biology* (pp. 199–324). London, UK: Academic Press Inc.

Kilgas, P., Saag, P., Mägi, M., Tilgar, V., & Mänd, R. (2012). Plumage bacterial load increases during nest-building in a passerine bird. *Journal of Ornithology*, *153*, 833–838. https://doi.org/10.1007/s10336-011-0801-3

Kopylova, E., Navas-Molina, J. A., Mercier, C., Xu, Z. Z., Mahé, F., He, Y., … Knight, R. (2016). Open-source sequence clustering methods improve the state of the art. *mSystems*, *1*, e00003–e00015.

Kulkarni, S., & Heeb, P. (2007). Social and sexual behaviours aid transmission of bacteria in birds. *Behavioural Processes*, *74*, 88–92. https://doi.org/10.1016/j.beproc.2006.10.005

Leclaire, S., Jacob, S., Greene, L. K., Dubay, G. R., & Drea, C. M. (2017). Social odours covary with bacterial community in the anal secretions of wild meerkats. *Scientific Reports*, *7*, 3240. https://doi.org/10.1038/s41598-017-03356-x

Leclaire, S., Merkling, T., Raynaud, C., Giacinti, G., Bessière, J.-M., Hatch, S. A., & Danchin, E. (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. https://doi.org/10.1007/s00114-011-0809-9

Leclaire, S., Merkling, T., Raynaud, C., Mulard, H., Bessiere, J.-M., Lhuillier, E., … Danchin, E. (2012). Semiochemical compounds of preen secretion reflect genetic make-up in a seabird species. *Proceedings of the Royal Society B-Biological Sciences*, *279*, 1185–1193. https://doi.org/10.1098/rspb.2011.1611

Leclaire, S., Pierret, P., Chatelain, M., & Gasparini, J. (2014). Feather bacteria affects plumage condition, iridescent color and investment in preening in pigeons. *Behavioral Ecology*, *25*, 1192–1198. https://doi.org/10.1093/beheco/aru109

Ley, R. E., Bäckhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., & Gordon, J. I. (2005). Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 11070–11075. https://doi.org/10.1073/pnas.0504978102

Lopez-Rull, I., Pagan, I., & Macias Garcia, C. (2010). Cosmetic enhancement of signal coloration: Experimental evidence in the house finch. *Behavioral Ecology*, *21*, 781–787. https://doi.org/10.1093/beheco/arq053

Lucas, F. S., & Heeb, P. (2005). Environmental factors shape cloacal bacterial assemblages in great tit *Parus major* and blue tit *P. caeruleus* nestlings. *Journal of Avian Biology*, *36*, 510–516. https://doi.org/10.1111/j.0908-8857.2005.03479.x

Martin-Vivaldi, M., Pena, A., Peralta-Sanchez, J. M., Sanchez, L., Ananou, S., Ruiz-Rodriguez, M., & Soler, J. J. (2010). Antimicrobial chemicals in hoopoe preen secretions are produced by symbiotic bacteria. *Proceedings of the Royal Society B-Biological Sciences*, *277*, 123–130. https://doi.org/10.1098/rspb.2009.1377

Martín-Vivaldi, M., Ruiz-Rodríguez, M., José Soler, J., Manuel Peralta-Sánchez, J., Méndez, M., Valdivia, E., … Martínez-Bueno, M. (2009). Seasonal, sexual and developmental differences in hoopoe *Upupa epops* preen gland morphology and secretions: Evidence for a role of bacteria. *Journal of Avian Biology*, *40*, 191–205. https://doi.org/10.1111/j.1600-048X.2009.04393.x

McFall-Ngai, M., Hadfield, M. G., Bosch, T. C., Carey, H. V., Domazet-Lošo, T., Douglas, A. E., … Wernegreen, J. J. (2013). Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 3229–3236. https://doi.org/10.1073/pnas.1218525110

Mercier, C., Boyer, F., Bonin, A., & Coissac, E. (2013). *Sumatra and sumaclust: Fast and exact comparison and clustering of sequences*. Programs Abstr. SeqBio 2013 Workshop, pp. 27–29.

Metcalfe, N. B., & Monaghan, P. (2013). Does reproduction cause oxidative stress? An open question. *Trends in Ecology & Evolution*, *28*, 347–350. https://doi.org/10.1016/j.tree.2013.01.015

Møller, A. P., Czirjak, G. Ã., & Heeb, P. (2009). Feather micro-organisms and uropygial antimicrobial defences in a colonial passerine bird: Ecology of feather micro-organisms. *Functional Ecology*, *23*, 1097–1102. https://doi.org/10.1111/j.1365-2435.2009.01594.x

Monaghan, P., Metcalfe, N. B., & Torres, R. (2009). Oxidative stress as a mediator of life history trade-offs: Mechanisms, measurements and interpretation. *Ecology Letters*, *12*, 75–92. https://doi.org/10.1111/j.1461-0248.2008.01258.x

Morgan, X. C., Segata, N., & Huttenhower, C. (2013). Biodiversity and functional genomics in the human microbiome. *Trends in Genetics*, *29*, 51–58. https://doi.org/10.1016/j.tig.2012.09.005

Muletz, C. R., Myers, J. M., Domangue, R. J., Herrick, J. B., & Harris, R. N. (2012). Soil bioaugmentation with amphibian cutaneous bacteria protects amphibian hosts from infection by *Batrachochytrium dendrobatidis*. *Biological Conservation*, *152*, 119–126. https://doi.org/10.1016/j.biocon.2012.03.022

Peig, J., & Green, A. J. (2009). New perspectives for estimating body condition from mass/length data: The scaled mass index as an alternative method. *Oikos*, *118*, 1883–1891. https://doi.org/10.1111/j.1600-0706.2009.17643.x

Piault, R., Gasparini, J., Bize, P., Paulet, M., McGraw, K. J., & Roulin, A. (2008). Experimental support for the makeup hypothesis in nestling tawny owls (*Strix aluco*). *Behavioral Ecology*, *19*, 703–709. https://doi.org/10.1093/beheco/arm152

Piersma, T., Dekker, M., & Sinninghe Damsté, J. S. (1999). An avian equivalent of make-up? *Ecology Letters*, *2*, 201–203. https://doi.org/10.1046/j.1461-0248.1999.00078.x

Pons, P., & Latapy, M. (2006). Computing communities in large networks using random walks. *Journal of Graph Algorithms and Applications*, *10*, 191–218. https://doi.org/10.7155/jgaa.00124

Reneerkens, J., Piersma, T., & Sinninghe Damste, J. S. (2002). Sandpipers (Scolopacidae) switch from monoester to diester preen waxes during courtship and incubation, but why? *Proceedings of the Royal Society B-Biological Sciences*, *269*, 2135–2139. https://doi.org/10.1098/rspb.2002.2132

R Core Team (2017). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Roggenbuck, M., Bærholm Schnell, I., Blom, N., Bælum, J., Bertelsen, M. F., Pontén, T. S., … Hansen, L. H. (2014). The microbiome of New World vultures. *Nature Communications*, *5*, 5498. https://doi.org/10.1038/ncomms6498

Ruiz-Rodriguez, M., Lucas, F., Heeb, P., & Soler, J. (2009). Differences in intestinal microbiota between avian brood parasites and their hosts. *Biological Journal of the Linnean Society*, *96*, 406–414. https://doi.org/10.1111/j.1095-8312.2008.01127.x

Ruiz-Rodriguez, M., Valdivia, E., Soler, J. J., Martin-Vivaldi, M., Martin-Platero, A. M., & Martinez-Bueno, M. (2009). Symbiotic bacteria living in the hoopoe's uropygial gland prevent feather degradation. *Journal of Experimental Biology*, *212*, 3621–3626. https://doi.org/10.1242/jeb.031336

Saag, P., Tilgar, V., Mänd, R., Kilgas, P., & Mägi, M. (2011). Plumage bacterial assemblages in a breeding wild passerine: Relationships with ecological factors and body condition. *Microbial Ecology*, *61*, 740–749. https://doi.org/10.1007/s00248-010-9789-0

Schommer, N. N., & Gallo, R. L. (2013). Structure and function of the human skin microbiome. *Trends in Microbiology*, *21*, 660–668. https://doi.org/10.1016/j.tim.2013.10.001

Shawkey, M. D., Pillai, S. R., & Hill, G. E. (2003). Chemical warfare? Effects of uropygial oil on feather-degrading bacteria. *Journal of Avian Biology*, *34*, 345–349. https://doi.org/10.1111/j.0908-8857.2003.03193.x

Sheldon, B. C., & Verhulst, S. (1996). Ecological immunology: Costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution*, *11*, 317–321. https://doi.org/10.1016/0169-5347(96)10039-2

Shelley, W., Hurly, H., & Nichols, A. (1953). Axillary odor: Experimental study of the role of bacteria, apocrine sweat, and deodorants. *Archives of Dermatology*, *68*, 430.

Soler, J. J., Martín-Vivaldi, M., Peralta-Sánchez, J. M., & Ruiz-Rodríguez, M. (2010). Antibiotic-producing bacteria as a possible defence of birds against pathogenic microorganisms. *The Open Ornithology Journal*, *3*, 93–100. https://doi.org/10.2174/1874453201003010093

Soler, J. J., Martín-Vivaldi, M., Ruiz-Rodríguez, M., Valdivia, E., Martín-Platero, A. M., Martínez-Bueno, M., … Méndez, M. (2008). Symbiotic association between hoopoes and antibiotic-producing bacteria that live in their uropygial gland. *Functional Ecology*, *22*, 864–871. https://doi.org/10.1111/j.1365-2435.2008.01448.x

Spor, A., Koren, O., & Ley, R. (2011). Unravelling the effects of the environment and host genotype on the gut microbiome. *Nature Reviews Microbiology*, *9*, 279–290. https://doi.org/10.1038/nrmicro2540

Sullam, K., Essinger, S., Lozupone, C., O'Connor, M., Rosen, G., Knight, R., … Russell, J. A. (2012). Environmental and ecological factors that shape the gut bacterial communities of fish: A meta-analyses. *Molecular Ecology*, *21*, 3363–3378. https://doi.org/10.1111/j.1365-294X.2012.05552.x

Taberlet, P., Prud'Homme, S. M., Campione, E., Roy, J., Miquel, C., Shehzad, W., … Coissac, E. (2012). Soil sampling and isolation of extracellular DNA from large amount of starting material suitable for metabarcoding studies. *Molecular Ecology*, *21*, 1816–1820. https://doi.org/10.1111/j.1365-294X.2011.05317.x

Tannock, G. W. (2007). What immunologists should know about bacterial communities of the human bowel. *Seminars in Immunology*, *19*, 94–105. https://doi.org/10.1016/j.smim.2006.09.001

Thompson, L. R., Sanders, J. G., McDonald, D., Amir, A., Ladau, J., Locey, K. J., … Earth Microbiome Project Consortium. (2017). A communal catalogue reveals Earth's multiscale microbial diversity. *Nature*, *551*, 457–463.

Turnbaugh, P. J., & Gordon, J. I. (2009). The core gut microbiome, energy balance and obesity: The core gut microbiome, energy balance and obesity. *Journal of Physiology*, *587*, 4153–4158. https://doi.org/10.1113/jphysiol.2009.174136

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environment Microbiology*, *73*, 5261–5267. https://doi.org/10.1128/AEM.00062-07

Wehrens, R., Weingart, G., & Mattivi, F. (2014). metaMS: An open-source pipeline for GC–MS-based untargeted metabolomics. *Journal of Chromatography B*, *966*, 109–116. https://doi.org/10.1016/j.jchromb.2014.02.051

Wen, L., Ley, R. E., Volchkov, P. Y., Stranges, P. B., Avanesyan, L., Stonebraker, A. C., … Chervonsky, A. V. (2008). Innate immunity and intestinal microbiota in the development of Type 1 diabetes. *Nature*, *455*, 1109–1113. https://doi.org/10.1038/nature07336

Whittaker, D. J., Soini, H. A., Atwell, J. W., Hollars, C., Novotny, M. V., & Ketterson, E. D. (2010). Songbird chemosignals: Volatile compounds in preen gland secretions vary among individuals, sexes, and populations. *Behavioral Ecology*, *21*, 608–614. https://doi.org/10.1093/beheco/arq033

Whittaker, D. J., Soini, H. A., Gerlach, N. M., Posto, A. L., Novotny, M. V., & Ketterson, E. D. (2011). Role of testosterone in stimulating seasonal changes in a potential avian chemosignal. *Journal of Chemical Ecology*, *37*, 1349–1357. https://doi.org/10.1007/s10886-011-0050-1

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., … Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. *Nature*, *486*, 222–227.

Zinger, L., Chave, J., Coissac, E., Iribar, A., Louisanna, E., Manzi, S., … Taberlet, P. (2016). Extracellular DNA extraction is a fast, cheap and reliable alternative for multi-taxa surveys based on soil DNA. *Soil Biology & Biochemistry*, *96*, 16–19. https://doi.org/10.1016/j.soilbio.2016.01.008

SUPPORTING INFORMATION

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