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HOST MICROBE INTERACTIONS

Plumage Bacterial Assemblages in a Breeding Wild Passerine: Relationships with Ecological Factors and Body Condition

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Abstract Microorganisms have been shown to play an important role in shaping the life histories of animals, and it has recently been suggested that feather-degrading bacteria influence the trade-off between parental effort and self-preening behavior in birds. We studied a wild breeding population of great tits (*Parus major*) to explore habitat-, seasonal-, and sex-related variation in feather-degrading and free-living bacteria inhabiting the birds' yellow ventral feathers and to investigate associations with body condition. The density and species richness of bacterial assemblages was studied using flow cytometry and ribosomal intergenic spacer analysis. The density of studied bacteria declined between the nest-building period and the first brood. The number of bacterial phylotypes per bird was higher in coniferous habitat, while bacterial densities were higher in deciduous habitat. Free-living bacterial density was positively correlated with female mass; conversely, there was a negative correlation between attached bacterial density and female mass during the period of peak reproductive effort. Bacterial species richness was sex dependent, with more diverse bacterial assemblages present on males than females. Thus, this study revealed that bacterial assemblages on the feathers of breeding birds are affected both by life history and ecological factors and are related to body condition.

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Introduction

Wild animals harbor a diverse community of bacteria and fungi [reviewed in 1]. Considering the high impact of microorganisms as symbionts or parasites [2, 3], investigating microbe–host interactions may help to explain behavioral and reproductive variation within and between host populations. However, detailed research into the bacterial loads of wild animals and the influence of bacteria on hosts has been limited by the requirement for very complex methods. Recently however, molecular and microbiological techniques have developed rapidly, and avian ecologists have started to work in this field with great enthusiasm [e.g., 4–6]. Recent evidence suggests that plumage bacteria play an important role in shaping the life histories of wild birds [4, 7–9].

Birds make significant efforts to maintain plumage function and to control ectoparasite loads. Preening and other forms of grooming are critical for limiting the abundance of feather lice and other arthropods [10]. Behaviors such as anting, dusting, sunning, and the inclusion of green vegetation in nesting material may also defend against ectoparasites and bacteria [1]. Moreover, laboratory studies indicate that uropygial oil protects plumage either chemically [11] or physically [12] against damage by feather-degrading bacilli [see also 13].

Bird plumage can host various assemblages of bacteria and fungi, several of which are capable of degrading feather keratin [11, 14–16]. Several fitness consequences could result from bacterial damage to wild bird plumage. Plumage deterioration may result in decreased thermal insulation [17] and aerodynamic efficiency [18]. In the long-term, these negative effects might reduce parental survival and

reproductive success; the latter via changes in parental condition [4, 8] or indirectly through the trade-off between reproductive effort and self-preening behavior [4, 8, 19]. Furthermore, feather-degrading bacteria could also affect feather-based communication and reduce reproductive success via social dominance and mate choice [20, 21].

Feather-degrading bacteria probably associate with all orders and species of wild bird [22], and within-species, many, if not all, individuals harbor such bacteria in their plumage [9, 23]. However, to date, relatively few studies have examined the factors that shape feather-degrading bacterial assemblages on avian plumage [see 5, 8, 11, 24–26]. The variability of keratinolytic bacterial assemblages colonizing bird plumage is most likely related to avian feeding behavior [e.g., ground versus canopy birds—4] and soil characteristics [15]. As bacterial communities differ between habitats depending on soil parameters, it has been suggested that the structure of bacterial assemblages in plumage is habitat dependent, and that this might result in differences even between individuals of the same bird species [4, 24, 27]. However, to our knowledge, there is no published evidence concerning the habitat-dependent fitness consequences of feather-degrading bacteria, and only a few studies have described seasonal changes in plumage bacteria assemblages [4], e.g., during pre-breeding and breeding period [28].

The aim of our study was to explore the extent and pattern of natural variation in feather-degrading bacterial assemblages inhabiting adult great tit (*Parus major*) plumage during different breeding stages and to explore whether these patterns are related to variation in reproductive parameters. We expected that (1) bacterial load (both in terms of density and species richness) is negatively related to parental body condition and reproductive output, as suggested above, (2) bacterial load increases during the breeding season due to the seasonal increase in air temperature, increasing time exposed to contamination sources and potentially due to the cumulative effects of reproductive effort on individual condition, (3) there are habitat differences in bacterial load, with the higher loads in more heterogeneous (deciduous) habitat; and (4) there are sex differences in bacterial load, with the higher loads in the parent with the more diverse reproductive activities (females) [5].

Material and Methods

Study System

Our study was conducted near Kilingi-Nõmme (58° 7′N, 25° 5′E) in SW Estonia in 2007 and 2008. The study area covers approximately 50 km^2 and contains a mosaic of two forest types—coniferous and deciduous (see maps of the study area in [29, 30]). The main characteristics of the two habitat types are given in Table 1.

Great tits bred in wooden nest boxes with a cavity of 11×11×30 cm and an entrance diameter of 3.5–4.0 cm. Nest boxes were mounted on tree trunks at heights of 1.5–2.0 m and were arranged along linear transects, so that each transect generally consisted of 20–100 nest boxes within homogeneous (either in coniferous or deciduous) habitat. Distances between neighboring nest boxes were 50–60 m. Nest boxes were cleaned to remove old nest material before the beginning of the breeding season.

In our study area, the great tit breeding season usually starts at the end of April with a nest-building (pre-laying) period. Great tits are facultative double breeders, and in our study area, a substantial proportion of pairs (40–70%, depending on the year) breeds twice during the same season. The first breeding period lasts approximately from the end of April to the middle of June, while the second breeding period lasts approximately from the end of June to the end of July.

Collecting Basic Reproductive Parameters in the Field

All nest boxes were checked to record the laying date of the first egg, clutch size, and the hatching dates of both first and second broods. First and second broods were clearly

Table 1 Characteristics of two studied habitat types [51]

Habitat type	Deciduous	Coniferous
General description	Either isolated patches within a mostly agricultural landscape or as 250–500 m wide riparian strips along stream valleys. Mostly secondary forests	Typically managed massive stands
Soil type	Fertile moraine soils	Nutrient poor sandy or peaty soils
Age of stand	45–55 years	65–85 years
Dominant tree species	Grey alder *Alnus incana* and silver birch *Betula pendula*, rich deciduous understorey	Scots pine *Pinus sylvestris*, sometimes forming mixed stands together with Norway spruce *Picea abies* or downy birch *Betula pubescens*

distinguishable since there is no overlap between the laying dates of the first and second clutches. Second broods were confirmed by ringing data, as at least one adult from each breeding pair had already been captured in that year (see below). The number of fledglings per nest and the body parameters of fledglings and adults were also recorded. Adults were captured with automatic traps on their nests during the second half of the nestling period. In 2007, females were also captured during pre-laying stage. In total, 34 males and 127 females were trapped in 2007 and 84 males and 153 females in 2008. Males were more distrustful of the traps, compared with females; therefore, the male sample size was much lower and unequally distributed between breeding attempts. Adults were individually marked with numbered metal rings. Birds were weighed with a Pesola spring balance to a precision of 0.1 g, and tarsus measurements taken to the nearest 0.1 mm and wing length to the nearest 1 mm using digital callipers. The same parameters were measured in nestlings on day 15 post-hatch. As great tits nestlings are almost fully grown at this age [31, 32], these measurements are referred to as fledgling parameters hereafter. For fledgling parameters, brood means were used as independent data points.

Density of Free-Living and Attached Bacteria on Feathers

Adult birds were taken from nest boxes using a fresh pair of examination gloves. Within 30 s after capture, about five ventral feathers were removed from the center of the yellow chest plumage and placed into dry clean microtubes using forceps sterilized in 96% ethanol. Samples were immediately stored at 4°C and transported in a coolbox to the laboratory within a few days (maximum 7 days), where they were stored at −80°C prior to analysis. In the laboratory, 1 mL of phosphate buffered saline (PBS) solution, pH 7.2, was added to each tube, which was then vortexed for 1 min. Free-living bacteria were thus washed out from the feathers and collected in the PBS solution. To remove attached bacteria, feathers were then sonicated for 10 min in 1 mL of a solution containing 2.5% Polyethylene 6000 and 0.1% sodium deoxycholate [33]. Free-living and attached bacteria samples were stored at −80°C and afterwards counted separately. Direct counts were performed for free-living and attached bacteria using a flow cytometry machine (BD LSR II) that was calibrated to detect only bacterium-sized tagged particles. For tagging DNA-binding dye, SYBR Green was used. The number of feathers in each sample was recorded in order to calculate bacterial density per feather [as 5]. Altogether, 426 samples were analyzed. Free-living bacteria were only counted in 2007, because in 2008, many samples from first broods were destroyed due to human error. Samples of attached bacteria and species richness remained intact and suitable for analysis.

Feather-Degrading Bacterial Species Richness

Immediately after the initial feather sampling, five more ventral feathers were sampled from each adult and placed into dry clean microtubes using sterile forceps. Again, all samples were stored at 4°C while in the field. At the laboratory, sampled feathers were covered with buffer solution (1.5 mL of PBS). Feather-degrading bacterial assemblages were enriched in this buffer by incubating them for 30 days at 26°C in the dark without using a shaker [following 5]. As feathers were the only source of carbon in the enrichment media, only bacteria capable of degrading keratin were promoted. There remains the possibility that certain non-keratin-degrading species occurred at extremely high densities before enrichment of samples. In that case, the DNA of these species could have been picked up for analysis even after the enrichment procedures. However, taking into account the low mean number of phylotypes per bird (see "Results"), the probability that such species occurred in the later analyses is very low compared with that of feather-degrading species.

The ribosomal intergenic spacer analysis (RISA) method was used to analyze the structure of feather-degrading assemblages obtained in the enrichment cultures. Each RISA band is assumed to correspond to one bacterial species and will be referred to as a phylotype [following 34, 35]. Thus, the band profile reflects bacterial assemblage structure, while the number of bands corresponds to the bacterial assemblage richness [36].

In order to carry out the RISA, DNA was extracted from bacterial cultures with 200 μL extraction buffer (10 mM Tris–HCl, 1% SDS, 2 mM EDTA, 400 mM NaCl, 0.4 mg/mL proteinase K). After 5 h incubation at 37°C, 150 μL of 5 M NaCl were added, and samples were vortexed. DNA was purified two times with pure chloroform. DNA was precipitated in absolute alcohol and resuspended in 100 μL of TE buffer pH 8.0 (10 mM Tris–HCl, 1 mM EDTA).

The 16S–23S rRNA intergenic spacer was amplified using the primers S-D-Bact-1522-b-S-20 and L-D-Bact-132-a-A-18 [37]. Polymerase chain reaction was conducted in 10 μL with about 10 ng of crude DNA, 1× Taq buffer (Fermentas), 2.5 mM $MgCl_2$, 200 μM dNTPs, 5 μM of each primer, and 0.5 U of Taq polymerase (Fermentas). Amplification was performed as follows: 94°C for 3 min, 35 cycles at 94°C for 1 min, 55°C for 30 s, and 72°C for 1 min, and finally 72°C for 5 min [following 37]. Amplified products were separated using electrophoresis on a 3% agarose gel for 1 h at 140 V. Band profiles were photographed and aligned by eye (in Adobe Photoshop). Band presence was recorded for each sample. Bacterial richness was estimated for each sample as the total number of phylotypes. Altogether, 400 samples were analyzed.

Statistical Analysis

Statistical analysis was performed using Statistica 7.0 (Statsoft). Bacterial density estimates were log-transformed prior to analysis to satisfy the assumption of normality. GLMs were used to model variation in bacterial density. The following variables were included in models irrespective of their significance: the year, habitat type (deciduous or coniferous), and breeding stage (pre-laying, first brood or second brood). Other predictors (lay-date of the first egg, brood size at hatching and at pre-fledging stage, female body parameters, etc.) were removed from the initial models using a backward stepwise procedure when non-significant ($P>0.05$). In the initial models, we also included all potentially informative interactions between predictor variables. To check the validity of the final models, we also used a forward stepwise model selection approach. This approach selected the same final model as the backward stepwise approach in all cases. Effect sizes can be seen in Fig. 1 (in the case of differences between habitats and breeding stages), or standardized beta

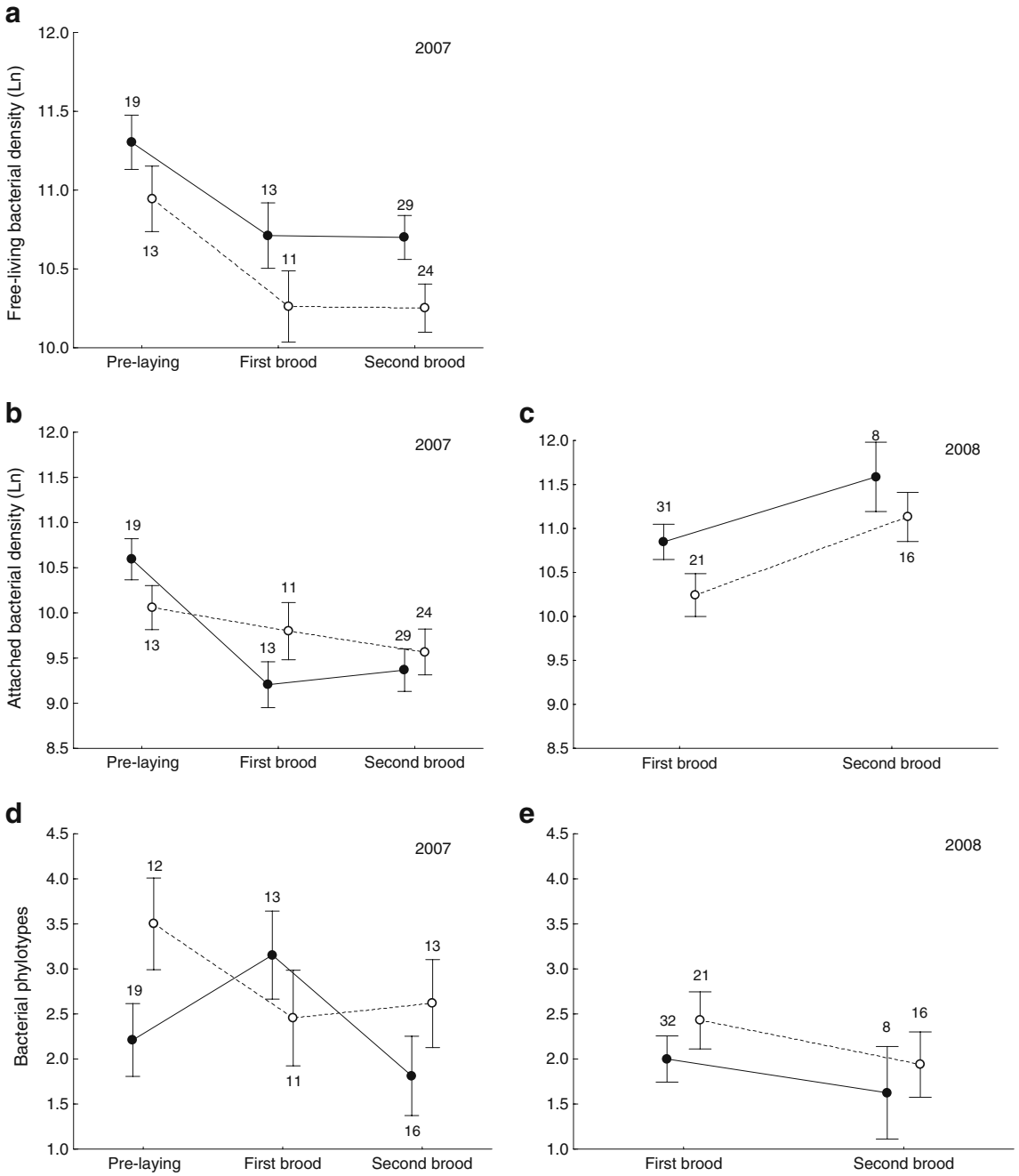

Figure 1 Bacterial densities (free-living bacteria (**a**); attached bacteria (**b**, **c**)) and species richness (**d**, **e**) in the plumage of female great tits in relation to year, habitat, and breeding stage. *Filled circles* deciduous habitat, *empty circles* coniferous habitat. *Whiskers* denote SE

(β) values are presented (in the case of significant correlative relationships).

As females were only captured during the pre-laying stage in 2007, we applied two different types of models to the female data. In the first type of model, the factor "breeding stage" contained three time points (pre-laying, first brood, and second brood), and breeding parameters were not included because no breeding data were available for the pre-laying stage. In the second type of model, the factor "breeding stage" contained only two time points (first brood and second brood), the factor "year" was included if data were available for both study years, and breeding parameters were considered as covariates. As males were not captured at the pre-laying stage, the first type of model was only applied in the case of females.

When sex differences between parents were examined, the factor "sex" was also retained in the final model irrespective of its significance. In cases where the same individual was sampled repeatedly during different breeding stages, it was represented in the data at only one randomly chosen time-point in order to avoid pseudo-replication. This was not the case in the repeated measures GLM, which was used for (1) investigating intra-individual changes between the nest-building and first-brood stage (breeding stage as a repeated measure); and (2) pairwise analysis of sex differences within breeding pairs (parental sex as a repeated measure). Intra-individual changes were not studied between the first and second broods because of very small number of such individuals captured. All significance levels apply to two-tailed tests.

Results

Free-living and attached bacterial densities on feathers were positively correlated with each other ($F_{1,106}$=5.76, P=0.018, β=0.22, corrected for sex, habitat and breeding stage, measured in 2007 only). Feather-degrading bacterial species richness was not correlated with free-living ($F_{1,78}$=1.28, P=0.26, corrected for sex, habitat and breeding stage, measured in 2007 only) or attached bacterial densities ($F_{1,214}$=0.4, P=0.5, corrected for year, sex, habitat, and breeding stage).

In males, neither bacterial density nor species richness was significantly correlated with any of the studied variables (breeding stage, habitat type, breeding parameters). Therefore, all the analyses below apply to females only, except where the two sexes were compared with each other.

Free-Living Bacterial Densities

Individual female great tits supported between 6.37×10^3 and 8.55×10^5 free-living bacteria per feather (median=4.22×10^4). The density of free-living bacteria depended on breeding stage and was significantly higher in individuals breeding in deciduous habitat compared with those in coniferous habitat (Fig. 1a; breeding stage, $F_{2,105}$=8.33, P=0.001, habitat, $F_{1,105}$=8.71, P=0.004). Tukey HSD post-hoc test revealed that the density of free-living bacteria was higher during the nest-building period than during either the first-brood or second-brood stage (pre-laying vs. first brood, P=0.005; pre-laying vs. second brood, P=0.001) but did not differ between first and second broods (P=0.99). However, this effect was not apparent in repeated measures analysis of the same individuals when sampled at nest-building and first-brood stages (T=0.29, P=0.8, N=15). Bacterial density at the nest-building stage did not differ between those females that were later observed breeding and those that were not (T=0.8, P=0.5, N=15 for both groups). During the first and second breeding stages, a positive relationship between free-living bacterial density and female mass was found (Table 2; β=0.28). No sex differences within breeding pairs were revealed (N=31 pairs; sex, F=0.3, P=0.6; sex×habitat, F=0.2, P=0.65; sex×breeding stage, F=2.19, P=0.15, investigated in 2007 only), while bacterial densities were highly significantly correlated between pair members (F=20.1, P<0.0001, β=0.58, corrected for breeding stage). None of the studied reproductive parameters were significantly related to free-living bacterial densities.

Table 2 The effects of year (density of free-living bacteria was measured only in 2007), habitat type, breeding stage (first or second broods) and body mass on bacterial densities (separately for free-living and attached bacteria) and species richness (number of feather-degrading bacterial phylotypes) in the feathers of female great tits

	DF	F	P
Density (free-living)			
Habitat type	1	5.30	0.025
Breeding stage	1	0.3	0.6
Female mass	1	5.05	0.028
Error	55		
Density (attached)			
Year	1	48.8	<0.0001
Habitat type	1	2.19	0.14
Breeding stage	1	2.90	0.037[a]
Female mass	1	0.89	0.35
Breeding stage×female mass	1	5.46	0.021
Error	125		
Species richness			
Year	1	2.89	0.09
Habitat type	1	0.4	0.5
Breeding stage	1	2.58	0.11
Error	118		

[a] Main effects were calculated without the higher-order interaction

Attached Bacterial Densities

Individual great tits supported between 1.37×10^2 and 1.26×10^6 attached bacteria per feather (median=2.89×10^4). The density of attached bacteria during the breeding period was higher in 2008 than in 2007 (Table 2, Fig. 1b, c). It was also higher during the nest-building stage than during either the first-brood or second-brood stages (Fig. 1b; breeding stage, $F_{2,105}$=8.25, P=0.001, habitat, $F_{1,105}$=0.4, P=0.5; Tukey HSD test: pre-laying vs. first brood, P=0.005, pre-laying vs. second brood, P=0.001). The decline from the nest-building stage to the first brood was confirmed by repeated measures analysis of the same individuals (T=2.61, P=0.020, N=15 pairs). This model, which was based on only 1 year (2007), did not show a significant difference between the two breeding attempts (Tukey HSD test, P=0.98; see also Fig. 1b). However, when both study years were considered in the model, an increase from the first-brood stage to the second-brood stage became significant (Table 2). During the nest-building stage, females in deciduous habitat supported a significantly higher density of attached bacteria than those in coniferous habitat (Fig. 1b; $F_{1,52}$=4.23, P=0.045). Bacterial density during the nest-building stage did not differ significantly between those females that were later found breeding and those that were not (F=1.0, P=0.3, N=15 for both groups). The model with the first and second broods combined included a significant interaction between breeding stage and female mass (Table 2). Further analysis revealed that the density of attached bacteria was negatively related to female mass during the first breeding stage (year, $F_{1,68}$=21.1, P<0.001, habitat, $F_{1,68}$=2.60, P=0.11, female mass, $F_{1,68}$=6.59, P=0.012, β=−0.32, female tarsus, $F_{1,68}$=3.85, P=0.053, β=0.27). Bacterial density was not sex dependent within breeding pairs (N=105 pairs; sex, F=2.66, P=0.10; sex×habitat, F=0.9, P=0.3; sex×breeding stage, F=0.2, P=0.7, sex×year, F=0.1, P=0.8), whereas bacterial densities were highly significantly correlated between pair members (F=10.3, P=0.002, β=0.29, corrected for year and breeding stage). None of the studied reproductive parameters were significantly related to attached bacterial densities.

Species Richness of Feather-Degrading Bacterial Assemblages

Altogether, 18 feather-degrading bacterial phylotypes were recorded on 290 birds studied during 2007 and 2008. The mean number of phylotypes per bird was 2.3±1.6 (SD), and no differences between breeding stages were revealed (Fig. 1d; breeding stage, $F_{2,78}$=1.26, P=0.29, habitat, $F_{1,78}$=1.68, P=0.20, breeding stage×habitat interaction n.s.). However, there was a weak and non-significant tendency for the number of phylotypes to decrease between the nest-building and first-brood stages within the same individuals (repeated measures analysis; N=16 females; T=1.71, P=0.11). A habitat difference in the number of phylotypes was only apparent during the nest-building stage (Fig. 1d; individuals carried fewer phylotypes in deciduous than in coniferous habitat, $F_{1,51}$=5.17, P=0.027). The models with the first and second broods combined revealed no effects of any predictors on the number of phylotypes (Table 2). However, the number of phylotypes was dependent on adult sex, such that males supported on average more phylotypes than females (Fig. 2; repeated measures analysis; N=103 pairs; sex, F=6.8, P=0.007; sex×breeding stage, F=4.65, P=0.033, sex×year, F=0.8, P=0.4). The significant sex×breeding stage interaction term indicated that the sex difference was significant during the second broods (Tukey HSD test, P=0.047), but not in the case of first broods (P=0.63). The number of phylotypes was not correlated between pair members (N=103 pairs, F=0.1, P=0.8, corrected for year and breeding stage). None of the studied reproductive parameters were significantly related to the number of phylotypes per bird.

Discussion

There are two distinguishable ecological types of bacteria in bird plumages: free-living and attached bacteria. Studies of bacterial communities in soil, water, and sediment have demonstrated that free-living bacteria are usually more labile, while attachment provides a more stable environment and protection against grazing, chemical antibiotics, or physical forces [see 33, 38, 39 for references]. However, methods used for estimating bacterial community diversity are fairly coarse (and therefore easily applicable and cheap) and do not allow ecological characteristics (free-living or attached) to be assigned to particular bacterial phylotypes. Also, due to the specific limitations of RISA analysis, a

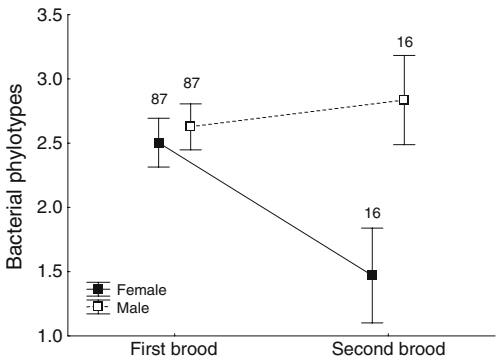

Figure 2 Feather-degrading bacterial species richness in the plumage of female and male great tits during the first and second brood periods. *Whiskers* denote SE

certain portion of true species richness might be overlooked. However, general estimates of diversity should still be reliable [36].

Bacteria, Female Condition, and Breeding Parameters

Free-living bacterial density was positively correlated with female mass; conversely, there was a negative correlation between attached bacterial density and female mass during the first broods. The latter finding provides support for the idea that attached bacteria have a harmful effect on their hosts. Attached bacteria are less sensitive to changes in host behavior than free-living bacteria, and they develop stable interactions among microbiota due to biofilm formation (see above). Attached bacteria may thus degrade feathers, reduce thermal insulation of plumage, and cause thermoregulatory stress in birds [40]. These changes together with reduced flight efficiency can negatively affect individual nutritional condition, resulting in decreased body mass, especially at the time of peak reproductive effort when there is little time for self-preening.

On the other hand, the positive correlation between free-living bacteria and female mass may indicate that free-living bacteria are "fellow travellers" rather than pests. Female breeders with high body mass are usually high-quality individuals [e.g., 41] that probably spend more time and energy searching for calcium-rich snail shells for their eggs and food for their nestlings [42] when compared with poor-quality individuals. Hence, such females are presumably more frequently in contact with possible sources of microorganisms. However, the exact mechanisms shaping the relationship between a bird's condition and its associated bacterial assemblage remain poorly understood, and experimental investigation is certainly needed.

Unlike a recent experimental study with starlings [5], we found no evidence that individuals with larger broods support more bacteria in their feathers than those with smaller broods. In our study, a correlative approach was used, and this may explain the discrepancy. Given that parents adjust their brood size according to their own reproductive potential and the availability of resources [e.g. 43], it is likely that higher quality individuals also invest relatively more into reproduction without incurring any negative effects on their self-maintenance activities. While it is possible that such a trade-off between reproductive effort and feather preening/sanitation would be revealed in exceptionally bad years, experimental manipulations of brood size or parental provisioning abilities might be more effective tools in this respect.

Seasonal and Annual Changes

Females supported significantly more bacteria (both attached and free-living) in their plumage during the nest-building period than during the first and second broods. We initially assumed (see "Introduction") that the warm and humid environment of the nest and reduced preening effort due to the increased need to devote time to feeding offspring would cause a seasonal increase in bacterial abundance on feathers. However, the pre-laying peak in bacterial density may be related to nest-building behavior that increases contact with the soil. While great tits are mainly canopy foragers during the nestling period, they spend considerable time on the ground during the nest-building phase to collect moss, dry grass, hair, wool, etc. [44], and it has been shown that transmission of bacteria is enhanced near the ground [4]. Secondly, great tits in this region roost in cavities (nest boxes in our study area) during the winter. It is possible that damp nest boxes containing old nest material harbor a diverse bacterial community and leave tits with high levels of infestation in spring. Bacterial abundance in the plumage may decline in subsequent breeding stages due to regular preening activities and reduced contact with the soil.

An alternative explanation for our result could be that the birds with a high bacteria load on their feathers at the pre-breeding phase did not start breeding at all. However, we found no difference in bacterial densities during the pre-laying period between those females that were later found breeding and those that were not. Moreover, the density of attached bacteria declined within the same individuals between the nest-building and first-brood stages. Hence, we consider this explanation unlikely.

The increase in attached bacterial load between the first and second breeding attempts can be explained by the extended exposure of plumage to various kinds of parasites, while favorable climatic conditions prevailing in midsummer during the second broods might also enhance bacterial growth and density. Moreover, bacterial density may increase during the season as a result of the cumulative negative effects of reproductive effort on individual condition and preening activities during multiple breeding attempts.

The density of attached bacteria was higher in 2008 than in 2007. The most plausible reason for this was the remarkably higher mean ambient temperature and total precipitation in the early spring of 2008 (according to the Estonian Meteorological and Hydrological Institute), which probably favored bacterial growth [4, 23, 24].

Habitat Differences

We also revealed habitat-related differences in bacterial density and species richness. These variables showed contrasting patterns of habitat-dependent variation: while the number of phylotypes per bird was higher in coniferous habitat, bacterial densities were higher in deciduous habitat.

A negative relationship between species diversity and abundance variables has previously been described for plant communities at relatively high productivity levels [45]. In bacterial communities, such a relationship can most plausibly be explained by interspecific antagonism and dominance. For example, many bacterial species produce antibacterial chemicals that suppress the growth of other bacteria [46, 47]. When fast-breeding (or fast-growing) generalist species (dominants) are present in a community, they might depress overall bacterial diversity [47–49]. Although deciduous forests are generally much more diverse habitats than managed conifers and contain more diverse microhabitats in which birds might become infected with bacteria, rapid infestation with dominant bacteria may inhibit colonization by other bacterial species.

To our knowledge, no published studies have examined habitat-related variation in plumage bacterial assemblages in the same study area, although differences could be expected. Our results indicate that the structure of bacterial communities may vary significantly between habitats even at small geographical scales. In this context, it is noteworthy that the physiological condition of breeding great tits was found to be worse in deciduous forests compared with coniferous forests of our study area [50–52]. Hence, we cannot rule out the possibility that the relatively high bacterial load associated with deciduous habitat may represent one of the factors contributing to the habitat difference in adult condition.

Sex Differences

Comparing the different parents within individual breeding pairs showed that bacterial density was correlated within-pair, but females tended on average to support fewer bacterial phylotypes than males. As within-pair differences in parental physical activity are expected to be smaller than differences between pairs [53], one would also expect within-pair bacterial densities to be correlated with each other [see also 5], especially considering the expected trade-off between parental provisioning effort and self-preening behavior. An alternative explanation could be that breeding partners share the same breeding territory, are thus exposed to similar bacterial assemblages, and may even infect each other with bacteria via their common nest and brood. However, in this case, it is hard to explain why partners support significantly different numbers of phylotypes.

On the other hand, it seems counterintuitive that males support more bacterial phylotypes than females because females are associated with higher and more diverse physical activity during different stages of reproduction, and they also roost in the nest box (males roost in the tree canopy). One possible explanation may be that if, for the reasons listed above, females come into greater contact with soil and bacteria than males do, and the dominant bacterial species may suppress bacterial diversity in their plumage to a greater extent. Secondly, given that on average, male parents invest less in their broods than females do [53, 54], they can devote more time to self-preening. It has been shown that preen waxes inhibit the growth and density of dominant bacteria but probably do not reduce the species diversity of the microbial assemblages on feathers [11]. It is also noteworthy that the difference between sexes in the mean species richness of bacteria was larger during second broods than during first broods. This was presumably related either to the higher ambient temperature during the second broods, which is more suitable for bacterial development, or with the increased time available for preening in males due to the relatively small size of second broods. However, it remains unclear why a similar sex×breeding stage interaction was not revealed in bacterial density.

Synopsis

This study revealed that bacterial assemblages on the feathers of breeding birds are affected both by avian life history and ecological factors. A negative relationship was found between attached bacterial density and parental condition. Habitat choice and the stage of the breeding season influence both feather-bacterial densities and species richness. Although there is some evidence that feather-degrading bacteria might be actively degrading feathers on living birds [9, 20, 21], further studies should consider whether preening behavior is causally related to feather-degrading bacterial composition.

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