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Recapture probability, flight morphology, and microorganisms

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Abstract

Microorganisms on and within organisms are ubiquitous and interactions with their hosts range from mutualistic over commensal, to pathogenic. We hypothesized that microorganisms might affect the ability of barn swallows *Hirundo rustica* to escape from potential predators, with positive associations between the abundance of microorganisms and escape ability implying mutualistic effects, while negative associations would imply antagonistic effects. We quantified escape behavior as the ability to avoid capture in a mist net and hence as a small number of recaptures. Because recapture probability may also depend on timing of reproduction and reproductive success, we also tested whether the association between recapture and microorganisms was mediated by an association between recapture and life history. We found intermediate to strong positive relationships between recapture probability and abundance of *Bacillus megaterium*, but not abundance of other bacteria or fungi. The abundance of *B. megaterium* was associated with an advance in laying date and an increase in reproductive success. However, these effects were independent of the number of recaptures. This interpretation is supported by the fact that there was no direct correlation between laying date and reproductive success on one hand and the number of recaptures on the other. These findings have implications not only for predator–prey interactions, but also for capture-mark-recapture analyses of vital rates such as survival and dispersal.

Key words: bacteria, barn swallow, capture-mark-recapture analyses, fungi, *Hirundo rustica*, microbiome.

The microbiome is constituted of the microorganisms on and within a host. The effects of this diversity and abundance of microorganisms range from beneficial over neutral, to highly pathogenic. A number of diseases such as ulcer, obesity, inflammatory bowel disease, diabetes, and vaginal disease among many others have been linked to the microbiome (e.g., Forsythe et al. 2010; Larsen et al. 2010; Fettweis et al. 2011; Greenblum et al. 2012; Foster and Neufeld 2013). The extent to which organisms other than humans suffer from similar effects of perturbations of their microbiome largely remains to be determined.

Microorganisms are ubiquitous in wild and domestic animals and most are mutualistic or commensal (Krieg and Holt 1984; Hubálek 2004; Benskin et al. 2009). Microorganisms have recently been hypothesized to play an important role in predator–prey interactions. For example, uropygial glands that produce antimicrobial substances are relatively larger in prey species that are more strongly preferred by their main predator, the goshawk *Accipiter gentilis* (Møller et al. 2010a). A subsequent analysis revealed that the abundance of bacteria on the feathers of individual prey was considerably larger than that of individual non-prey (Møller et al. 2012). Microorganisms partly derived from prey also seem to play a role in delayed laying of their goshawk hosts (Møller et al. 2015), perhaps because adults in poor condition start to reproduce later (Soler et al. 2015). This has consequences for hatching success (Møller et al. 2010b) and survival prospects (Møller et al. 2013; Benskin et al. 2015). These effects could be mediated through microorganisms causing disease including increased levels of fever (Møller 2010a), or they could be due to individual hosts in poor condition having higher abundance of pathogenic nicroorganisms. A 2nd possibility is that they could be acting through flight morphology, or they could

act through microorganisms disrupting flight. Microorganisms may be beneficial by improving the quality of feathers following more detrimental microorganisms that cause degradation of feathers being outcompeted (e.g. Falagas et al. 2008; Ruiz-Rodríguez et al. 2012). For example, such degradation could be controlled by uropygial secretions, or by interference competition with less pathogenic microorganisms. Alternatively, pathogenic microorganisms may have a negative impact on the physiology of their hosts thereby causing a deterioration of their flight ability and hence their ability to out-maneuver and ultimately escape predators. A final possibility is that pathogenic microorganisms may be more common in hosts with poor physiological status and hence a weak immune response (Soler et al. 2011).

Behavior is known to be associated with the microbiome. For example, the gut–brain axis provides a direct link between mental health and the microbiome (Forsythe et al. 2010; Foster and Neufeld 2013). Thus, personality but also stress and depression can be linked directly to the community of microorganisms in the gut (e.g., Sudo et al. 2004; Forsythe et al. 2010; Foster and Neufeld 2013). Here we hypothesize that such a link between behavior and the microbiome also accounts for capture probability of free-living birds. Indeed, Soler et al. (2012) have previously suggested and provided evidence for an association between microbiome and personality in birds.

The objective of this study was to test to what extent the recapture probability of individual birds depends on the abundance and diversity of microorganisms. The rationale behind this objective is that birds with more microorganisms suffer from the costs of microorganisms causing disease in their hosts. Alternatively, birds with more microorganisms may suffer from more damage to their plumage caused by feather-degrading microorganisms. Feather-degrading bacteria are ubiquitous, and they are well-known for their ability to degrade feathers in wild and domesticated birds (e.g., Kent and Burtt 2016). Even small amounts of damage may significantly increase the risk of birds falling prey to raptors (Møller and Nielsen 2017). Either of these mechanisms may impair flight and escape ability and hence increase the probability of capture and recapture by scientists.

The 2nd objective was to test whether the abundance of microorganisms that predicted recapture probability also predicted the timing of reproduction and reproductive success. We tested whether there was a link between number of recaptures and fitness components, and between number of microorganisms and fitness components. We did so by investigating a population of barn swallows *Hirundo rustica* that has been studied since 1971, and hence all individuals were of known age (Møller 1994). Here we analyzed capture probability and reproductive success in relation to the microbiome of feather nest lining. Barn swallows are migratory insectivorous semi-colonial passerines that mainly breed in the northern hemisphere, while wintering in the tropics or the southern hemisphere. They refurbish or construct a new nest each year, and this nest is provided with feathers collected in the neighborhood of the breeding site during 1–2 weeks before start of laying. The clutch of 3–7 eggs is incubated for 2 weeks by the female and provided with food by both parents for 3 weeks. We investigated the microbiome of the barn swallow by collecting feathers from nests during laying and incubation. Because feathers of the nest lining are derived from the neighborhood before or during laying and incubation, any association between barn swallows and the microbiome of the nest must arise from secondary infection of adult barn swallows from nest lining feathers. Previous studies of microorganisms in the feather lining of barn swallow nests have shown significant effects of feather composition on fitness components (Peralta-Sánchez et al. 2010, 2011, 2014).

Materials and Methods

Methods

This study was conducted in a population of barn swallows at Kraghede (57°12'N, 10°00'E), Denmark (Møller 1994). Adults were captured at least once weekly by placing mist nets across doors ensuring that the birds breeding indoors were captured when exiting barns. Capture-mark-recapture analyses have shown that more than 98% of all adults are captured or sighted based on color rings (Møller and Szép 2002). All recaptures were also recorded. Adults were provided with an individually numbered aluminum ring, a color ring and an individual pattern of color markings on the white breast feathers. These markings allowed identification of nest owners with a pair of binoculars. We also recorded a number of morphological, behavioral, and parasitological variables before releasing the bird where it was initially captured.

Nesting data

We visited nests at least weekly and recorded laying date of the 1st egg of each clutch, clutch size, and brood size at hatching and fledging. If there were fewer eggs than final clutch size at the 1st visit, laying date was simply date of visit minus number of eggs because 1 egg is laid daily. If the number of eggs at 1st visit equaled subsequent clutch size, laying date was estimated from the size of nestlings at the 1st visit when nestlings were present, using a standard growth curve that links body mass to age.

Nest feather samples

We collected a sample of feathers from the nest during egg laying or early incubation using sterile gloves, while a 2nd feather sample was collected 2 weeks later during incubation in May–June 2014. The feathers from each nest were put in a numbered zip-lock bag and placed in a refrigerator until lab analyses. The two samples were subsequently analyzed separately, and the data derived from each sample were finally combined, since there was no evidence of differences between 1st and 2nd samples.

Analyses of bacteria: bacterial isolation

In the laboratory, we took a small piece of each feather sample and put it in a falcon tube and added an equal volume to the area of the feather (1 cm^2 feather: 1 mL PBS) with sterile phosphate buffer saline (pH 7.2). After 3 shaking periods of 5 s each in vortex, we performed serial 10-fold dilutions to 10^{-4}. Free-living bacteria were washed out of the feathers and collected in the PBS solution (Saag et al. 2011), followed by serial 10-fold dilution to 10^{-4}. To quantify cultivable and feather-degrading bacteria, in duplicate we spread 100 µL of supernatant with a sterile spreader loop on two different growth media. (1) Tryptic Soy Agar (TSA) which is a rich medium on which heterotrophic bacteria can grow thus enabling assessment of total cultivable microorganism load of feathers and (2) Feather Meal Agar (FMA) containing 15 g/L feather meal, 0.5 g/L NaCl, 0.30 g/L K2HPO4, 0.40 g/L KH2PO4,15 g/L agar to quantify feather-degrading bacterial loads (Sangali and Brandelli 2000; Shawkey et al. 2003, 2009). Fungal growth was inhibited by adding cycloheximide to TSA and FMA media (Smit et al. 2001). Plates were incubated at 28 °C for 3 days in the case of TSA and for

14 days in the case of FMA. After incubation, we counted the number of colony-forming units (CFU) of each morphotype per plate by using a dissecting microscope, and we distinguished the morphotypes on the basis of colony color, shape, size, and presence or absence of glutinous aspects.

Bacterial density was calculated by multiplying the average number of CFU by the dilution factor and by the original sample volume (Peralta-Sánchez et al. 2014). Plates with each medium type, but without microbial suspension were incubated in order to detect any contamination of media (negative controls).

Bacterial identification

Bacterial identification was done by using molecular characterization with the aid of polymerase chain reaction (PCR) for further genetic analyses. The genomic DNA was extracted from each isolate by using freeze-thaw protocol: By a wooden toothpick transfer, we crush part of a bacterial colony in a 0.5-mL Eppendorf tube containing 50 µL Tris (10 µM, pH 8.0). We started freezing and thawing in liquid nitrogen for 1–2 min until completely frozen, then quickly placing the tube in a hot water bath until completely thawed. We repeated the same process for a total of 3 cycles. We made sure that we mixed the sample tube between each cycle. To ensure complete cell lysis we put the tubes in a microwave at 270 W for 5–6 s followed by 10 s of waiting, repeated for 3 times. Then we centrifuged the sample tube for 5 min at 12,000× g in a microcentrifuge. We transferred the supernatant that contains the DNA into a new clean and sterile microcentrifuge tube by using a micropipette and discarded the pellet that contains cellular debris. We stored the tubes in −20 °C for the PCR.

PCR amplification of bacterial 16SrRNA gene

We used a DNA isolated from samples as a template for PCR to amplify the bacterial 16S rRNA gene by using the reverse primer 16S rDNA-1492R and the forward primer 16S rDNA-27F. PCR was done in a total volume of 25 µL containing 16 µL ultrapure water, 4 µL 5X buffer, 0.4 µL dNTPs 10 nM, 1 µL of 10 µM 27F, 1 µl of 10 µM 1492R, 0.2 µL Go Taq DNA polymerase and 3–5 µL genomic DNA according to Barghouthi (2011).

Microorganism isolation: fungal isolation

We snipped the feather samples accurately into small pieces and cultured them directly onto Mycobiotic Agar (which is a selective medium of fungi) following standard procedure (Deshmukh 2004), moistened with 0.5 mL of sterilized PBS. The cultures were incubated at 28 °C ± 2 and checkup daily from the 3rd day for fungal growth until a period of 4 weeks. The observed developing mycotic growths were recorded under stereoscopic binocular microscope, and then we individually and directly transferred them onto Sabouraud dextrose agar (SDA) medium with chloramphenicol (50 mg/L). The resulting products were further incubated at 28 °C ± 2 for 2 weeks to obtain a pure isolate for identification purposes.

Fungal identification

Fungal identification was done by using molecular characterization with the aid of PCR for further genetic analyses. The genomic DNA was extracted from each isolate by using the PowerSoil® DNA Isolation Kit (MO BIO). DNA was eluted in a final volume of 100 µL of 10 mM Tris-HCl, pH 8.5.

Amplification of fungal 18s rDNA gene by PCR

We used a DNA isolated from samples as a template for PCR to amplify the fungi 18S rRNA gene by using the forward primer 18S rRNA-UF1 and the reverse primer 16S rDNA-R1. PCR was done in a total volume of 25 µL containing 16 µL ultrapure water, 4 µL 5X buffer, 0.4 µL dNTPs 10 nM, 1 µL of 10 µM 27F, 1 µL of 10 µM 1492R, 0.2 µL Go Taq DNA polymerase and 3–5 µL genomic DNA.

Agarose electrophoresis

We performed gel electrophoresis in 1% agarose using 0.5× TAE buffer (Tris-Acetate-EDTA) for 25 min at 100 V. The gel was then stained with Gel Red (BIOTIUM) for 30 min. To determine the presence or absence of PCR products and to quantify the size of amplified DNA fragments, we take images under UV lamp by using the photo documentation system IP-010.SD.

DNA sequencing

All the PCR products were sent to Beckman Coulter Genomics, Takeley, Essex CM22 6TA, United Kingdom for DNA Sequencing. The sequence results were processed by using the web-based blasting program, basic local alignment search tool (BLAST), at the NCBI site (http://www.ncbi.nlm.nih.gov/BLAST), and the data were compared with the NCBI/Gene-bank database.

Statistical analyses

We used JMP (SAS 2012) for statistical analyses. We report total abundance of colonies and species richness and standard errors for fungi and bacteria. We tested whether the number of recaptures followed a Poisson distribution by fitting a Poisson model to the frequency data.

We tested whether the abundance of fungi and bacteria predicted the frequency of recaptures using a generalized linear model (GLM) with binomial error distribution when the data were classified as presence (1) or absence (0) with a logit link function, with normal error distribution when the data were normally distributed with an identity link function, or with Poisson error distribution when the data were count data with a Poisson distribution and a log link function.

We estimated effect sizes by using Cohen's (1988) guidelines for the magnitude of effects being small (Pearson $r = 0.10$, explaining 1% of the variance), intermediate ($r = 0.30$, explaining 9% of the variance), or large ($r = 0.50$, explaining 25% of the variance).

Results

Communities of microorganisms

We isolated 15 fungal species from the feather lining of barn swallow nests according to identification by PCR technique (Supplementary Table S1). For bacteria, we isolated 10 species from TSA medium (Supplementary Table S2). The different bacterial and fungal taxa were widely distributed across the phylogenetic tree of bacteria and fungi (Supplementary Figures S1a, b).

The number of fungal species ranged from 1 to 9 per sample of feathers from each nest (mean 5.48 ± 0.23 SE), $n = 54$. The number of fungal colonies ranged from 20 to 135 (mean 72.09 ± 4.34 SE), $n = 54$. The most commonly isolated fungal genus was *Aspergillus* sp.

There were 10 bacterial species isolated from TSA medium. *Bacillus* spp. was the most frequently isolated species. The number of bacterial species (in TSA) ranged from 2 to 10, (mean 4.88 ± 0.25

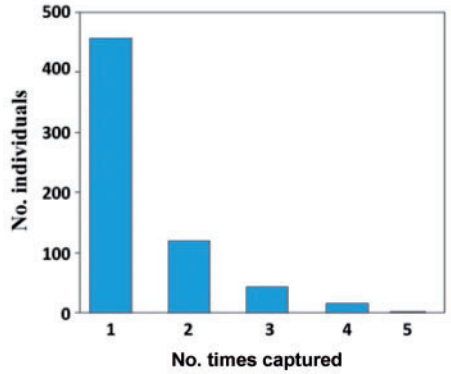

Figure 1. Frequency distribution of a number of captures of adult barn swallows.

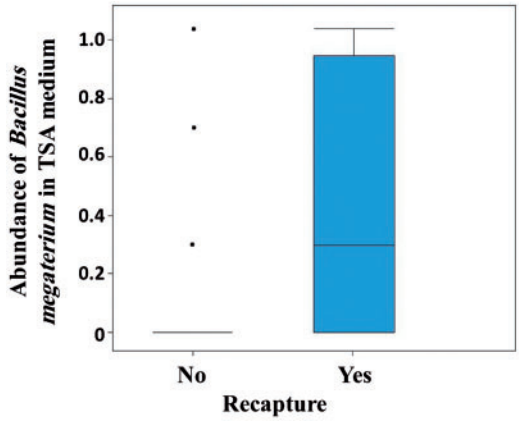

Figure 2. Box plots of the log-transformed abundance of *B. megaterium* in relation to whether adult barn swallows were recaptured or not. Box plots show means, quartiles, 5th and 95th percentiles, and extreme values.

SE), $n = 54$. The total number of bacterial colonies (in TSA) ranged from 10 to 350, (mean 70.13 ± 8.92 SE), $n = 54$.

There were 10 species of bacterial colonies in FMA medium, with *Streptomyces* spp. being the most frequently isolated species. The total number of bacterial colonies in FMA ranged from 0 to 49 (mean 19.15 ± 1.79 SE), $n = 54$. The number of bacterial species (FMA) ranged from 0 to 7 (mean 3.92 ± 0.24 SE), $n = 54$.

Microorganisms and recaptures

A total of 37.1% out of 1506 swallows were recaptured within the same breeding season. The number of recaptures ranged from 1 to 5 with a mean of 0.573 and a variance of 0.808, $n = 754$ barn swallows (Figure 1). This estimate deviated from a Poisson distribution with $\lambda = 0.573$, 95% CI = 0.521, 0.629, $\chi^2 = 67.66$, $P < 0.0001$.

A GLM with binomial error distribution and logit link function showed a significant effect of *Bacillus megaterium* with barn swallows with bacteria being more likely to be recaptured than those without ($\chi^2 = 9.753$, $P = 0.0018$; Figure 2). This model fitted the data (goodness of fit statistic $\chi^2 = 48.395$, $df = 46$, $P = 0.377$). A GLM with Poisson error distribution for count data and a log link function also showed a significant effect ($\chi^2 = 12.140$, $df = 1$, $P = 0.0005$), and this model also fitted the data (goodness of fit statistic $\chi^2 = 49.932$, $df = 46$, $P = 0.320$). Effect sizes were 0.44 and 0.50, respectively, which can be considered large effects. In contrast, there were no significant effects for any of the other bacteria or fungi on recapture probability (analyses not shown). *Bacillus megaterium* had a prevalence of 0.14 and mean 1.36 ± 0.42 SE, range 0–10.

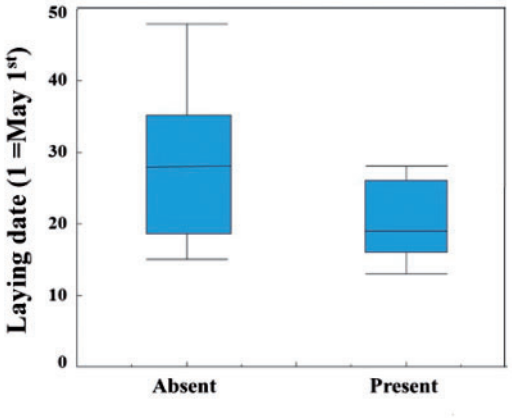

Figure 3. Box plots of laying date (1st = May 1) in relation to whether *B. megaterium* was present or absent. Box plots show means, quartiles, 5th and 95th percentiles and extreme values.

The number of recaptures was not predicted by body mass ($\chi^2 = 0.68$, $df = 1$, $P = 0.678$) or keel length ($\chi^2 = 1.16$, $df = 1$, $P = 0.282$) in a GLM with Poisson distributed number of recaptures and a log link function.

Recaptured barn swallows may more often have attended nests or laid 2nd clutches, hence causing a link between microorganisms and recapture. Barn swallow nests with 2nd clutches had more *B. megaterium* than nests with just a single clutch ($\chi^2 = 6.51$, $df = 1$, $P = 0.011$, estimate (SE) = 0.400 (0.235)). However, there was no significant association between the probability of having a 2nd clutch and the number of times barn swallows were recaptured ($\chi^2 = 1.607$, $df = 1$, $P = 0.21$).

Microorganisms and reproduction

Laying date was not predicted by the number of recaptures ($\chi^2 = 2.17$, $df = 1$, $P = 0.14$), but was predicted by the abundance of *B. megaterium* ($\chi^2 = 6.55$, $df = 1$, $P = 0.011$, estimate (SE) = −6.37 (2.40)). This effect increased when number of recaptures was deleted ($\chi^2 = 13.08$, $df = 1$, $P = 0.0003$, estimate (SE) = 23.13 (1.43)).

There was a significant negative relationship between the abundance of *B. megaterium* and laying date ($\chi^2 = 6.59$, $df = 1$, $P = 0.010$, estimate (SE) = −0.20 (0.007); Figure 3). The number of *B. megaterium* increased with the number of fledglings ($\chi^2 = 10.19$, $df = 1$, $P = 0.0014$, estimate (SE) = 0.790 (0.024); Figure 4). These findings are consistent with a trade-off between anti-bacterial defenses and investment in reproduction, or that individuals with higher reproductive success have more nest lining feathers. However, nest lining feathers appear in nests 2 weeks before start of laying, and we found no significant association between reproductive success and the number of nest lining feathers ($\chi^2 = 0.51$, $df = 1$, $P = 0.31$). There was only a weak relationship between total number of fledglings and laying date in a GLM with Poisson distribution and a log link function ($\chi^2 = 3.86$, $df = 1$, $P = 0.050$, estimate (SE) = −0.686 (0.352)).

There were no cases of nest abandonment or mortality among adult barn swallows, excluding these factors as a cause of variation in capture probability or number of recaptures.

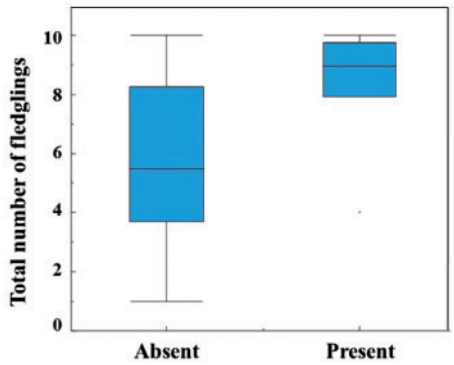

Figure 4. Box plots of the total number of fledgling in relation to whether *B. megaterium* was present or absent. Box plots show means, quartiles, 5th and 95th percentiles and extreme values.

Discussion

The main finding of this study of the diversity of microorganisms on feathers in barn swallow nests was an association between recapture probability and the abundance of the microorganism *B. megaterium*, but not any other species of microorganism. Barn swallows with more *B. megaterium* on nest lining feathers had a higher probability of being recaptured and hence falling prey to a potential predator than individual hosts with fewer microorganisms on feathers in their nests. These findings imply that the bacterial environment may play a role in predator–prey interactions (Møller et al. 2010b, 2012). We also showed that the abundance of *B. megaterium* decreased significantly with advanced laying and that the abundance of *B. megaterium* increased with increasing reproductive success measured as the number of fledglings produced during the year.

The frequency of recaptures during the same season was highly skewed with most barn swallows never being recaptured again once captured. This skew in recapture frequency is not due to lack of attempts to escape since barn swallows repeatedly fly up to the mist net and only in the very last moment turn off abruptly to avoid capture. Here we have shown that microorganisms seem to play a role in whether individuals are recaptured. Because the abundance of *B. megaterium* on feathers used as nest lining in the 1st clutch was associated with an increase in the probability of recapture, we can consider the effects reported here to be parasitic. Studies experimentally removing uropygial glands showed dramatic increases in the abundance of microorganisms on the plumage (Jacob and Ziswiler 1982). Such an increase in the abundance of microorganisms may directly impair aerodynamics since even small increases in the abundance of microorganisms on the surface of the plumage may cause turbulence. Is it feasible that recaptures directly affected the abundance of microorganisms? Perhaps both recapture and microorganisms were associated with a 3rd variable. We cannot imagine any mechanism by which such an effect could arise. However, less healthy birds may in general have higher bacterial loads and higher recapture probability. We consider this possibility unlikely given that there was no association between probability of recapture and body mass or body mass adjusted for keel length, despite this measure of body condition having been shown to repeatedly correlate with phenotypic traits in barn swallows (Møller 1994). Another possibility is that recaptured birds more often attended nests or laid 2nd clutches. Indeed, nests with 2nd clutches had more *B. megaterium* than nests with just a single clutch. However, there was no significant association between the probability of having a 2nd clutch and the number of times recaptured. This refutes these alternative explanations.

Bacillus megaterium, which accounted for an increase in recapture probability, is one of the biggest known bacteria (cell length of up to 4 μm and a diameter of 1.5 μm, i.e., it has an up to 100-times larger volume than *Escherichia coli*). It is a common soil bacterium that during the past years has become popular in biotechnology (Bunk et al. 2010). *Bacillus megaterium* strains are known to produce antimicrobial antibiotics (Malanicheva et al. 2012). *Bacillus megaterium* is also known to control microorganisms such as those involved in plant disease (Safiyazov et al. 1995). The abundance of *B. megaterium* was associated with an advance in laying date and an increase in reproductive success. Reproductive success and laying date were only weakly correlated, implying that the 2 variables were independently associated with *B. megaterium*. These effects were independent of the number of recaptures. We hypothesize that barn swallows trade early investment in reproduction against antibacterial defenses. This interpretation is supported by the fact that there was no direct correlation between laying date and reproductive success on one hand, and the number of recaptures on the other. Alternatively, barn swallows may facilitate colonization and growth of potentially beneficial bacteria in their nests.

Barn swallows prefer white feathers over other colors for lining their nests (Peralta-Sanchez et al. 2010, 2011, 2014). Feathers with white coloration have positive effects on the bacterial communities of eggshells by improving hatching success in the host (Peralta-Sanchez et al. 2010, 2011, 2014). Most bacteria living on feathers are benign and effectively prevent pathogenic bacteria from establishment and replication (e.g. Shawkey et al. 2003, 2009). We still need to assess whether that also applies to *Bacillus megaterium*, or whether the relationship between recapture probability and abundance of microorganisms is simply due to direct effects of *B. megaterium* on locomotion.

The findings reported here have potential future prospects. First, the findings suggest that barn swallows are more likely to be recaptured and, by inference, more likely to fall prey to a predator when having a larger abundance of specific microorganisms in their nests. We are unaware of any alternative explanation for the patterns described in the present paper. Obviously, this hypothesis requires a direct test by comparison of the abundance of these specific microorganisms in conspecific prey and non-prey. It also requires a direct experimental test of whether the elimination of these microorganisms reduces the probability of recapture.

Second, studies of the demography of wild organisms rely on random samples of individuals in order to obtain unbiased estimates of the demographic parameter based on capture-mark-recapture analyses (Lebreton et al. 1992). Several recent studies have demonstrated violations of this assumption (Garamszegi et al. 2009; Biro and Dingemanse 2009; Møller 2010a). For example, Garamszegi et al. (2009) showed for collared flycatchers *Ficedula albicollis* that individuals with a bold personality were over-represented, while shy individuals were less likely to be captured than expected by chance. The associations between capture probability and microorganisms that we have reported here may suggest that the microbiome plays a direct role in the estimation of demographic parameters. Indeed, Soler et al. (2012) have suggested an association between microbiome and personality in birds. We have previously shown that flight initiation distance by individual barn swallows is linked to the probability of falling prey to sparrowhawks *Accipiter nisus* (Møller 2014) and that brain size predicts capture probability (Møller 2010b). These findings and those of the present study suggest that

the microbiome of nests, probability of predation, personality and cognitive skills are all related to each other. The present study assumes that the microbiome of nest lining feathers is a proxy for the microbiome of adult feathers. This assumption is likely given that the microbiome of nest lining feathers must derive from adult barn swallows since feathers are absent from nests until at most a couple of weeks before the start of egg laying. This interpretation is supported by human studies linking personality to the gut microbiome (Forsythe et al. 2010; Foster and Neufeld 2013).

In conclusion, we have shown that the probability of recapture of adult barn swallows was significantly correlated with the abundance of a specific bacterium, *B. megaterium*. If the probability of capture reflects the probability of falling prey to the capturer, we can conclude that bacteria and hence the microbiome is involved in predator–prey interactions.

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

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