

Bacteria on nestling skin in relation to growth in pied flycatchers

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Abstract The association between skin bacterial communities and nestling growth is poorly understood. We estimated the abundance of heterotrophic bacteria on skin of nestlings and their association with growth in the pied flycatcher *Ficedula hypoleuca*. At two different nestling ages, we swabbed a delimited area of the naked belly skin of nestlings and measured them. Skin bacterial loads on day 13, but not on day 7, were positively associated with brood size and with nestling wing length. Larger broods develop in less hygienic conditions, which may stimulate bacterial growth. Skin bacteria may favor wing feather growth through competition with harmful bacteria, or faster feather growth may facilitate bacterial growth through the accumulation of remains on the skin surrounding growing feathers.

Keywords Brood size · *Ficedula hypoleuca* · Nestlings · Pied flycatcher · Skin bacterial loads · Wing length

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Zusammenfassung

Das Verhältnis zwischen Hautbakterien und Wachstum bei Nestlingen des Trauerschnäppers

Die Zusammenhänge zwischen Hautbakteriengemeinschaften und dem Wachstum von Nestlingen sind bisher kaum bekannt. Wir bestimmten die Häufigkeit heterotropher Bakterien auf der Haut von Nestlingen und deren Verbindung mit dem Wachstum bei Trauerschnäppern *Ficedula hypoleuca*. Dazu wurden die Nestlinge in zwei verschiedenen Altersstadien gemessen und gleichzeitig ein Abstrich von einer definierten Stelle der nackten Bauchhaut genommen. Die Besiedlung der Haut mit Bakterien am 13., nicht jedoch am 7. Lebenstag, zeigte einen positiven Zusammenhang mit der Brutgröße sowie mit der Flügelänge der Nestlinge. Größere Bruten entwickeln sich unter weniger hygienischen Bedingungen, was das Bakterienwachstum begünstigen könnte. Hautbakterien könnten durch Konkurrenz mit schädlichen Bakterien das Wachstum der Schwungfedern begünstigen; alternativ könnte ein schnelleres Federwachstum durch die Ansammlung von Hautresten rund um wachsende Federn die Bakterienvermehrung fördern.

Introduction

Nestling development has frequently been studied without considering microbial effects (O'Connor 1984; Starck and Ricklefs 1998). The effects of skin bacteria have not been considered even though nest materials are in close contact with the bare skin of nestlings, which could favor microbial colonization. Thus, potentially pathogenic bacteria may colonize the bare skin of growing altricial nestlings and could thereby influence their growth and development by

affecting thermoregulation or degrading growing feathers (Burt and Ichida 1999; Clayton 1999; Muza et al. 2000; Ruiz-de-Castañeda et al. 2012). For example, González-Braojos et al. (2012) found that nestlings raised in old reused nests had higher bacterial loads on their belly skin than those raised in freshly built nests. Moreover, they found that bacterial loads of nests showed a negative association with nestling feather growth. This association suggests that feather-degrading bacteria (FDB) (Burt and Ichida 1999) may be able to colonize growing feathers during the nestling stage. In other studies of freshly built nests, it has been observed that skin bacteria have no effect on the weight of nestlings, although possible associations with the growth of structures such as wings or tarsi have not been addressed (Berger et al. 2003; Gwinner and Berger 2005).

The study reported in the present paper was performed to explore the changes in abundance of heterotrophic bacteria on nestling skin and their potential associations with nestling growth. We estimated heterotrophic bacterial loads on the nestling skin of pied flycatchers *Ficedula hypoleuca* at two ages. We measured mass, tarsus, and wing length as estimators of organ growth, skeletal growth, and feather growth, respectively. We hypothesized that: (1) skin bacterial loads should increase with nestling age, as Gwinner and Berger (2005) observed in starling nestlings; (2) given that skin microbial colonization is related to season (Berger et al. 2003; Gwinner and Berger 2005), bacterial loads on the skin should increase with hatching date; (3) bacterial loads should be positively related to brood size if parents have a reduced capacity to clean nests by removing fecal sacs because they need to feed many nestlings, or because nests containing large broods are warmer (Dunn 1976), which could stimulate bacterial growth; (4) if bacteria on skin affect thermoregulation or are able to invade other tissues, skin bacterial loads should be negatively associated with general nestling growth; and (5) feather development could be related to skin bacterial load if bacteria on the skin affect feather growth.

Methods

General field methods

We conducted the study during the spring of 2011 on a population of pied flycatchers breeding in artificial nestboxes in a montane forest of Pyrenean oak, *Quercus pyrenaica*, at 1,200 m.a.s.l. in Valsain, central Spain (40°54'N, 4°01'W). The pied flycatcher is a small hole-nesting passerine of European woodlands (Lundberg and Alatalo 1992). Nests were followed by performing daily visits, allowing the hatching dates and brood sizes to be recorded.

On day 7, we swabbed the naked belly skin of two randomly chosen nestlings (for more details of the bacterial sampling and laboratory work involved, see González-Braojos et al. 2012). After obtaining each sample, we ringed and weighed the nestling and measured its tarsus and wings. On day 13, the same nestlings were swabbed and measured again in the same way.

Statistical analyses

All variables were normally distributed or successfully normalized via natural logarithms. Analyses were conducted with either SAS, IBM SPSS Statistics 21 (2012), or Statistica (Statsoft). To test hypotheses (1)–(3), we used a mixed linear model (proc mixed) with repeated measures by age with skin bacterial load as the dependent variable, nest ID as the random factor, and brood size and hatching date as the covariables. To test hypotheses (4)–(5), we used mixed linear models with nestling biometry measures at different ages as the dependent variable, and skin bacterial loads at different ages, hatching date, and brood size as independent effects, including nest ID as a random factor. For growth, we used the Variance Components module in Statistica, which uses the Satterthwaite correction for the degrees of freedom while controlling for the same variables. Nonsignificant variables were removed sequentially ($p > 0.05$), obtaining a final model with only significant effects.

Results

Skin bacterial loads did not change with nestling age [day 7: 3.652 ± 0.258 (ln(CFU/cm²), mean \pm SE); day 13: 3.500 ± 0.275 (ln(CFU/cm²), mean \pm SE); $F_{1,53} = 0.41$,

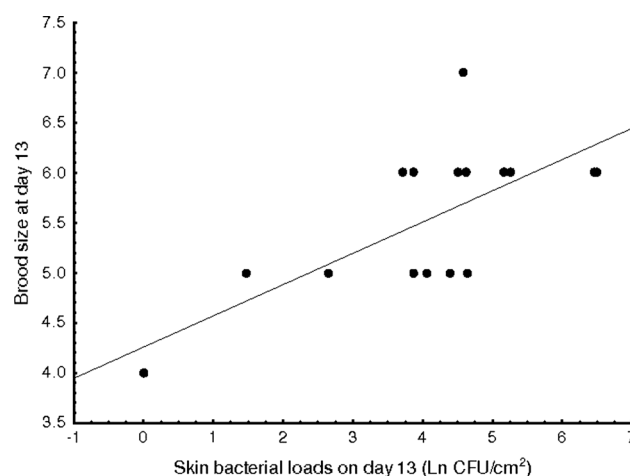


Fig. 1 Association between skin bacterial load on day 13 and brood size at the same age. We have included only a randomly selected nestling per nest

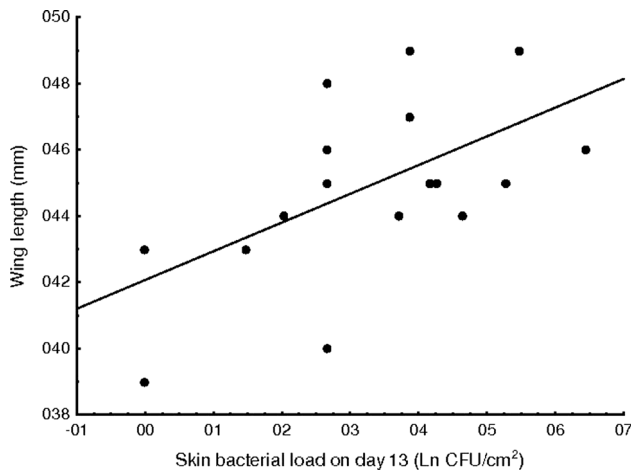


Fig. 2 Association between skin bacterial load on day 13 and wing length at the same age. We have included only a randomly selected nestling per nest

$p = 0.523$]. Bacterial loads were positively correlated with brood size ($F_{1,53} = 6.41$, $p = 0.014$, Fig. 1), but this significant correlation was due only to differences at age 13 ($R^2 = 0.006$ on day 7 and $R^2 = 0.300$ on day 13). Bacterial loads were not correlated with hatching date ($F_{1,53} = 1.62$, $p = 0.208$).

No biometric variable was significantly associated with hatching date, brood size or skin bacterial loads (all $p > 0.07$) on day 7. While there were no significant effects of mass and tarsus length on day 13 (all $p > 0.17$), wing length at that age showed a significant positive association with skin bacterial load at that age after sequential backward deletion of nonsignificant effects ($F_{1,34,49} = 4.394$, $p = 0.043$, Fig. 2). None of the biometric parameters increased significantly with age (all $p > 0.10$).

Discussion

Our results show that the bacterial load on nestling skin did not change with age between days 7 and 13. Furthermore, there was no association between skin bacterial load and hatching date. However, the results support the possibility that larger broods imply enhanced bacterial growth. Finally, we have found a positive association of skin bacteria with primary feather length. These results will be discussed below.

Unlike Gwinner and Berger (2005), who found that bacterial load increased with age (they measured bacterial loads at 1, 9, and 14 days) on the skin of starling nestlings, we did not find any increase, possibly because the bacterial community was already established on nestling skin by day 7. While Berger et al. (2003) and Gwinner and Berger (2005) found seasonal variations in skin microbiota, we did

not find any association between skin bacterial load and hatching date, probably because there were only slight variations in the hatching date in our sample (range: 8 days), in contrast to other studies, where the range of hatching dates encompassed 2 months, so seasonal changes in temperature may have affected bacterial growth.

Large broods showed higher bacterial loads on day 13. This may be explained by the higher parental provisioning intensity at the end of the nestling period, which may preclude efficient nest sanitation (Cantarero et al. 2013). Therefore, fecal sacs containing intestinal bacteria would accumulate in these nests, allowing the bacteria to colonize the skin of nestlings. In 2009, we measured the number of fecal sacs in nestboxes (nestcup and nestbox walls) in the same population when we measured the nestlings at 13 days, and found that nestboxes with larger broods contained more fecal sacs (R^2 adjusted = 0.093; $F_{1,43} = 5.552$, $p = 0.023$), providing some support for our explanation.

Only wing length on day 13 was associated with bacterial load on belly skin, i.e., nestlings with longer wings had more bacteria. The lack of association of skin bacterial load with mass or skeletal growth indicates that skin bacteria do not remove resources that are important for growth, a result also found by other authors (Berger et al. 2003; Gwinner and Berger 2005). It should be noted that those authors did not look for a relationship between skin bacteria and wing length.

A possible explanation for our results regarding wing length may be competition between bacterial strains for space and nutritive resources offered by the skin. This may contribute to the exclusion of bacteria that are harmful to the host in some host–bacteria associations (e.g., Martín-Platero et al. 2006; Soler et al. 2008; Ruiz-Rodríguez et al. 2009). As we have not studied the diversity of bacteria on skin, we cannot provide support for this hypothesis. On the other hand, FDB affect feather degradation rather than feather growth, and feather degradation may not affect wing length unless it causes feather breakage. Nests containing nestlings with more developed wing feathers may shed greater amounts of keratin, fat, and other tissues, which may favor the growth of keratinolytic and other bacteria that may access nestling belly skin. According to this possible explanation, nestlings with more developed feathers would also have more bacteria on their skin. We cannot differentiate between these two explanations for the positive association of feather development with skin bacterial load.

Summarizing, this is the first study to show the potential effects of nest hygiene on skin microbiota and the positive association of skin bacteria with nestling wing growth. More studies are needed to clarify the patterns revealed in the present study.

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