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HOST MICROBE INTERACTIONS

The Microbiome of the Uropygial Secretion in Hoopoes Is Shaped Along the Nesting Phase

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Abstract Microbial symbiont acquisition by hosts may determine the effectiveness of the mutualistic relationships. A mix of vertical and horizontal transmission may be advantageous for hosts by allowing plastic changes of microbial communities depending on environmental conditions. Plasticity is well known for gut microbiota but is poorly understood for other symbionts of wild animals. We here explore the importance of environmental conditions experienced by nestling hoopoes (*Upupa epops*) during the late nesting phase determining microbiota in their uropygial gland. In cross-fostering experiments of 8 days old nestlings, "sibling-sibling" and "mother-offspring" comparisons were used to explore whether the bacterial community naturally established in the uropygial gland of nestlings could change depending on experimental environmental conditions (i.e., new nest environment). We found that the final microbiome of nestlings was mainly explained by nest of origin. Moreover, cross-fostered nestlings were more similar to their siblings and mothers than to their stepsiblings and stepmothers. We also detected a significant effect of nest of rearing, suggesting that nestling hoopoes acquire most bacterial symbionts during the first days of life but that the microbiome is dynamic and can be modified along the nestling period depending on environmental conditions. Estimated effects of nest of rearing, but also most of those of nest of origin are associated to environmental characteristics of nests, which are extended phenotypes of parents. Thus, natural selection may favor the acquisition of appropriated microbial symbionts for particular environmental conditions found in nests.

Keywords Cross-fostering experiment · Horizontal transmission · Microbial symbiont · Microbial transmission · Parent-offspring comparisons · Plasticity · *Upupa epops* · Vertical transmission

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Introduction

Hosts may acquire symbionts directly by vertical transmission from parents to offspring [1, 2], or by horizontal transmission from the environment [3]. Although the vast majority of symbioses described in eukaryotes involve bacteria [1, 4], studies on mechanisms of bacterial transmission are limited to a handful of model systems [5, 6]. Horizontally transmitted bacteria are known for squids [3], tubeworms [7], and mussels [8], while mechanisms of vertical transmission have been detected for instance in ascidians [9], bryozoans [10,] and earthworms [11]. For some other model systems, microbial symbionts are acquired both vertically and horizontally, as it is the case for beneficial gastrointestinal microbiomes of animals [12] or for enterococci of the uropygial gland of hoopoes (*Upupa epops*) [13].

Modes of bacterial acquisition may determine the effectiveness of the mutualistic relationship [14]. On the one hand, fitness of vertically transmitted symbionts is

closely related to that of their hosts and, thus, enhancing reproductive success of hosts will directly benefit their own performance [14–17]. On the other hand, different symbionts may provide hosts with characteristics that are more appropriate for particular environments and/or symbiont effectiveness may vary with environmental conditions. Although vertical transmission is identified as a key route to host specialization on effective symbionts [14, 18], hosts with horizontally transmitted symbionts have the opportunity to adjust the community of symbionts to environmental characteristics [19]. In situations of variable environmental conditions with unpredictable selection pressures, a mix of vertical and horizontal transmission shaping symbiont bacterial communities might be of selective advantage for hosts because it would guarantee the simultaneous presence of potential beneficial microorganisms from different environments [5, 14, 20]. In this mixed mode of symbiont acquisition, the symbiotic community that hosts acquires from mothers and/or environment during the first days of life could change in relation to variable environmental conditions experienced later, in subsequent phases of life. Symbiotic community changes (i.e., plasticity) of hosts in relation to environmental changes are well known for gut microbiota [21, 22] but are poorly understood for other symbionts of wild animals.

We experimentally explore whether microbiome of the uropygial gland of nestling hoopoes change along the nesting period in relation to environmental conditions hosted in their uropygial gland. Symbiotic bacteria have only been detected by traditional culture methods in incubating females and nestlings [23, 24]. Nowadays, we know that males and non-reproducing females also harbor bacteria in their gland, but at very low density [25]. Interestingly, we also know that antibiotic producing enterococci are transmitted from mother hoopoes to offspring soon after hatching (vertical transmission), and that hatchlings (i.e., before uropygial gland functions) are able to incorporate new enterococci symbionts from new environments after cross-fostering experiments (horizontal transmission) [13]. The question that we try to answer here is whether the bacterial community, once it is established in the uropygial gland of nestlings (i.e., functioning), could change depending on experimental environmental conditions (i.e., new nest environment). We approached this aim by cross-fostering nestlings of intermediate age (i.e., with an established bacterial community) from different nests and characterizing bacterial communities of fledglings by mean of ARISA (automatic ribosomal intergenic spacer analysis). We first describe the bacterial communities in uropygial secretions of nestlings and females in terms of prevalence, richness, and operational taxonomic units (OTUs) composition. The relative contribution of factors acting during early (i.e., genetic plus early environment including any pre-manipulation maternal effects) and late (experimental nest environment) nesting phase were determined by (i) comparing the proportion of variance explained by nest of origin and nest of rearing, respectively. Relative contribution of early and later environments explaining microbiota of the uropygial secretion was also explored by comparing (ii) levels of similarity between siblings reared in different nests and between stepsiblings reared in the same nest, and (iii) between cross-fostered nestlings and biological and stepmothers.

Materials and Methods

Study Species, Study Area, and General Methods

The hoopoe is distributed throughout Europe, Asia, and Africa, inhabiting open woods or open areas as steppes, grasslands, pastures, semi-deserts, or crops whenever they have scattered trees, walls, or buildings providing holes for nesting and soil without tall vegetation for feeding [26–28]. Females lay one or two clutches of six to eight eggs along the breeding season, between February and July [29]. Incubation lasts 17 days and starts with the first or second egg, which results in eggs hatching asynchronously at 24 h or even greater intervals [30].

The fieldwork was performed during the breeding seasons 2010–2011 in a wild population located in the Hoya de Guadix (37°18′ N, 38°11′ W), southern Spain, where hoopoes breed in crops, forests, and gullies within nest boxes placed in trees or buildings. In 2011, hoopoes were also sampled in a captive population descendant from our wild population and breeding in captivity since 2008. The captive pairs were distributed in two different subpopulations located in south-eastern Spain, one of them in installations of the University of Granada in Hoya of Guadix (Granada), and the other one in facilities of the Estación Experimental de Zonas Áridas (CSIC) at the Finca Experimental La Hoya in Almería (36°50′ N, 2°28′ W). All females and nestlings were ringed with numbered rings and females also with color rings for individual recognition.

Nest boxes in the wild were visited twice per week, from mid-February to the end of July to record laying date, clutch size, and hatching date. Pairs of hoopoes breeding in captivity were housed in independent cages at least $3 \times 2 \times 2$ m installed in the open, scattered, and isolated to avoid interactions between pairs and ensure successful breeding. Cages had access to soil and were

provided with live food (crickets, vitamin-enriched fly larvae, and meat (beef heart)) ad libitum and were visited daily.

Experimental Design and Sampling

The cross-fostering experimental design consisted in the exchange of two experimental nestlings among pairs of nests of similar (±1 day) hatching date and similar brood size. The exchange was carried out when the oldest nestling in each nest was 8 days old (i.e., when nestlings start to produce secretion containing bacteria). Two of the four heaviest nestlings in each nest were randomly selected and exchanged with those from another nest (i.e., with the same age and similar weight). Comparisons were later performed with all nestling in the experimental nests. Nestlings were individually marked by painting their tarsus with permanent innocuous markers. Cross-fostering experiments were performed between wild nests in 2010 and in 2011 between one nest in captivity and the other in wild conditions. When this was not possible, experimental nestlings were exchanged between two captivity nests, or between two wild nests. This was done so to increase phenotypic variance among cross-fostered nests that allow a more realistic estimation of the effects of nest of origin and of nest of rearing (Falconer 1989). Transport of nestlings between nests lasted approximately 1 h and was done in a portable incubator at 37 °C to reduce stress due to temperature change.

Uropygial secretions of females were sampled before hatching date (i.e., 14 days after laying the first egg), whereas those of nestlings were sampled 10 days after nest exchange (i.e., oldest nestlings had 18 days old). Incubating females were captured within the nest box by hand, quickly sampled, and released again within the nest to reduce disturbance. For each capture, we wore new sterile latex gloves cleaned with 70 % ethanol for the whole process to limit external bacterial contamination. Before collecting samples from uropygial gland, we softly washed the circlet of feathers and surrounding skin with a cotton swab slightly soaked in ethanol to reduce the risk of contamination with external bacteria. After evaporation of the alcohol, a sterile micropipette tip (1–10 μl micropipette (Finpipette)) was introduced in the gland papilla. The papilla was pressed softly with a finger and the uropygial secretion entirely collected was transferred to a sterile microfuge tube. Afterwards, 5 μl were separated in a different sterile microfuge tube for the molecular analyses. Nestling hoopoes were sampled with identical protocol than adult females were. For further molecular analyses, all samples were individually stored in 1.2 ml sterile microfuge tubes in a portable cooler (1–3 °C) until being stored in the lab at −20 ° C the same day of sampling.

We sampled 44 nests and got information for the 44 breeding females and for 165 nestlings; 93 of them did grow in the same nests where they hatched, whereas 72 were moved to foreign nests. However, final sample sizes were reduced due to predation of wild nests or failures with ARISA. We obtained complete information of siblings that were reared in the same nests of hatching ($N=57$) or moved to another nests ($N=44$) for 28 nests. Only for 21 of these nests, we got the necessary information to compare the bacterial community of experimental nestlings with that of their foster and genetic siblings on the one hand, and with the bacterial community of their mother and stepmother on the other hand.

Laboratory Work

Bacterial genomic DNA for the uropygial secretion samples was extracted with a commercial kit (The FavorPrep™ Blood Genomic DNA Extraction Kit, Favorgen). ARISA (Fisher and Triplett 1999), which amplifies an intergenic transcribed spacer (ITS) region between the prokaryotic 16S and 23S rDNA, was used to characterize the composition of bacterial communities. This region is highly variable both in size and sequence between species and, thus, offers an appropriate taxonomic resolution for microbiota characterization (Danovaro et al. 2006). The ITS was amplified using the primer pair ITSF (5′-GTCGTAACAAGGTAGCCGTA-3′) and ITSReub (5′-GCCAAGGCATCCACC-3′) [31]. The primer ITSReub was labeled fluorescently with 6-FAM. Amplifications were performed in 50 μL reaction volumes containing ultrapure H_2O, 20 μL of 5 PRIME MasterMix (2.5×) including 1.5 mM $Mg(OAC)_2$, 200 μM dNTPs, 1.25 U Taq DNA polymerase 0.2 μM of primers, and 5 μL of diluted DNA 1:10. PCRs were carried out in Eppendorf Mastercycler Nexus. Fragments were amplified under the following conditions: initial denaturation at 94 °C 2 min, followed by 30 cycles with denaturation at 94 °C 45 s, annealing at 52 °C 45 s, and extension at 72 °C 1 min, with a final extension at 72 °C 5 min. Amplified PCR products were diluted 1:10 and denatured by heating in formamide. Fragment lengths were determined by mean of automated fluorescent capillary electrophoresis on 3130 Genetic Analyzer. Electropherogram peak values were calculated after interpolation with an internal size standard named GeneScan™ 1200 LIZ dye Size Standard (both Applied Biosystems). These analyses were performed at the Scientific Instrumentation Center of the University of Granada.

Statistical Analysis

Peak Scanner 1.0 (Applied Biosystems) was used to determine fragment length (i.e., OTUs) in terms of base pairs (bp). For binning DNA fragment lengths from different samples, we used available scripts in R-environment [http://cran.r-project.org/] at www.ecology-research.com [32] with a window size of 4 base pairs (bp) and a distance of two consecutive binning frames (i.e., shift) of 0.1. Peaks with RFI values of <0.09 % were considered as background peaks. Only fragments above a threshold of 50 fluorescence units and ranging between 100 and 1000 bp [32] were used for constructing and analyze the presence-absence matrices depicting bacterial communities.

We described the bacterial community harbored in uropygial secretion of females and nestlings with information obtained from ARISA for all individuals (44 females and 165 nestlings). To explore the differences in bacterial richness (number of OTUs per sample) between adult females and nestlings, we performed ANOVAs with one fixed factor (adult females vs. nestlings). Moreover, we explored differences in prevalence of OTUs detected in uropygial secretions of adult females and nestlings, but considering the most frequent OTUs; i.e., those that were detected in more than 30 % of females or nestlings uropygial secretion sampled. Pearson correlation coefficients were used to explore whether OTU's prevalence in females and nestling samples were related. We did this analysis with all detected OTUs and also including only those that were present in more than 30 % of females or nestlings sampled. Furthermore, we analyzed differences in the composition of bacterial communities hosted in uropygial secretions of females and nestlings by one-way PERMANOVAs analysis (Jaccard's distance), taking into consideration all females and only non-moved nestlings. Trying to reduce probability of detecting significant differences among females and nestlings due to rare OTUs, we only considered those that appeared in more than three samples of females or nestlings. We used classical multidimensional scaling analysis, principal coordinates analysis (PCoA) to graphically show variation in bacterial communities of uropygial secretions of females and nestlings. This technique represents the objects (communities) on a plot with canonical axes, where the distance between the objects shows their underlying similarity [33].

Cross-fostering experiments are a well-established approach for partitioning phenotypic variance in its genetic and environmental components in mixed statistical models that include the identity of nest of origin and rearing (nested within nest of origin) as random factors [34]. This experimental approach has been previously used to explore genetic and environmental influences determining cloacal bacterial assemblages in great tit (*Parus major*) [35] and the enterococci community of the uropigial gland of hoopoes [13]. Here, we performed cross-fostering experiments of nestlings of intermediate age to estimate the relative importance of early and late nestling periods explaining the whole microbiota of the uropygial gland of hoopoes. The effects of early nestling phase, which include genetic component and any pre-manipulation maternal and environmental effects, were estimated by the proportion of variance of microbiome composition explained by nest of origin and by mother-offspring (i.e., vertical transmission) and sibling-sibling comparisons. The effects of late nesting phase, which would only include environmental components, were estimated by the proportion of variance associated to the nest of rearing and by stepmother-offspring and sibling-stepsibling comparisons.

The similarity matrix among all bacterial communities of the sampled individuals was based in Jaccard's distance [36]. The similarity values were used as the dependent variables of PERMANOVA model using type III estimation of mean squares. This model try to explain similarity among nestlings including two random factors: nest of origin and nest of rearing (nested within nest of origin). For this model, we used only the 28 nests for which we have information for moved and non-moved nestlings from the same nest of origin. Finally, for the 21 experiments with all the information (see above), we estimated mean values of similarities among bacterial communities of experimental nestlings and those of their genetic (reared in different nests but genetically related) or foster (reared in the same environment but genetically unrelated) siblings. We estimated for the same nests mean values of similarities among bacterial communities of experimental nestlings and their genetic or foster mothers.

All multivariate analyses and figures trying to explain similarity matrices (PERMANOVAs) were performed with PRIMER v7 (PRIMER-E) software (Anderson et al. 2008). Statistical inferences (e.g., p values) of all PERMANOVAs were based on 9999 permutations. Statistical tests trying to explain variation in bacterial richness and prevalence of different bacterial strains, as well as those comparing mean values of similarities estimated for genetically related and unrelated individuals, were performed with STATISTICA 10 software [37].

Results

Description of Bacterial Communities in Uropygial Secretions: Prevalence, Richness, and Composition

We detected 143 different OTUs (length of the ITS fragment varying between 100 and 847 bp) in the bacterial community

of the uropygial secretion of female and nestling hoopoes, 141 of which were present in nestlings and 116 in females. All except two OTUs that were detected in females at very low prevalence (143 and 603 bp, 2.22 and 4.44 %, respectively) were also present in nestling samples. Prevalence of detected OTUs ranged from 0.61 % (OTU with 847 bp) to 84.44 % (OTU with 183 bp) and were similar for females and nestlings as shown by the strong positive relationships among their values (Appendix 1, $R^2 = 0.89$, $N = 143$, $t = 34.2$, $p < 0.0001$). This relationship was evident even when only considering the 28 OTUs that were present in more than 30 % of samples from females or nestlings (Fig. 1, $R^2 = 0.73$, $N = 28$, $t = 8.44$, $p < 0.0001$). Richness of bacterial community of the uropygial secretions of nestlings (mean (SE) = 22.64 (0.66)) was also similar to that of females (mean (SE) = 21.78 (1.37)) ($F = 0.34$, $df = 1, 207$, $p = 0.55$). Additionally, composition of bacterial communities of nestlings and females did not significantly differ (one-way PERMANOVA, $F = 1.53$, $df = 1, 135$, $p = 0.0572$, Fig. 2).

Effects of Early and Late Nesting Phase on the Bacterial Community

The similarity matrix among bacterial communities of the uropygial gland of experimental nestlings was significantly explained by nest of origin and nest of rearing (Table 1). The proportion of variance explained by the nest of origin was relatively larger than that explained by nest of rearing (Table 1). This result suggests that the influence of the early nesting phase (i.e., genetic factors and/or pre-manipulation maternal and environmental effects) explaining uropygial microbiome of hoopoes was relatively larger than that of late nesting period (i.e., environmental influence and maternal effects after the experiment). This inference was further confirmed by the significantly larger similarity values of comparisons of siblings reared in different nests than those for comparisons of stepsiblings reared in the same nest (GLM, $F = 19.33$, $df = 1, 20$, $p = 0.0002$; Fig. 3a). Results from comparisons of similarities between bacterial communities of cross-fostered nestlings and those of their foster and genetic mothers (Fig. 3b) were also in accordance with a relatively larger influence of the early nesting phases determining the bacterial community of the uropygial secretion of hoopoe nestlings (GLM, $F = 20.42$, $df = 1, 20$, $p = 0.0002$).

Importantly, we found statistically significant effects of nest of rearing explaining bacterial community of nestling hoopoes (Table 1), which suggest that environmental conditions experienced by hoopoes during the late nesting phase also contribute to mold microbial community.

Discussion

Our main results are twofold. The first one is that bacterial communities of the uropygial secretion of hoopoe nestlings did not differ significantly from those of their mothers. The second group of results pointed out strong pre-manipulation effects explaining the composition of bacterial communities in experimental cross-fostered nestlings. We also detected a significant effect of nest of rearing, suggesting that environmental characteristics experienced after experimental treatment contributes to

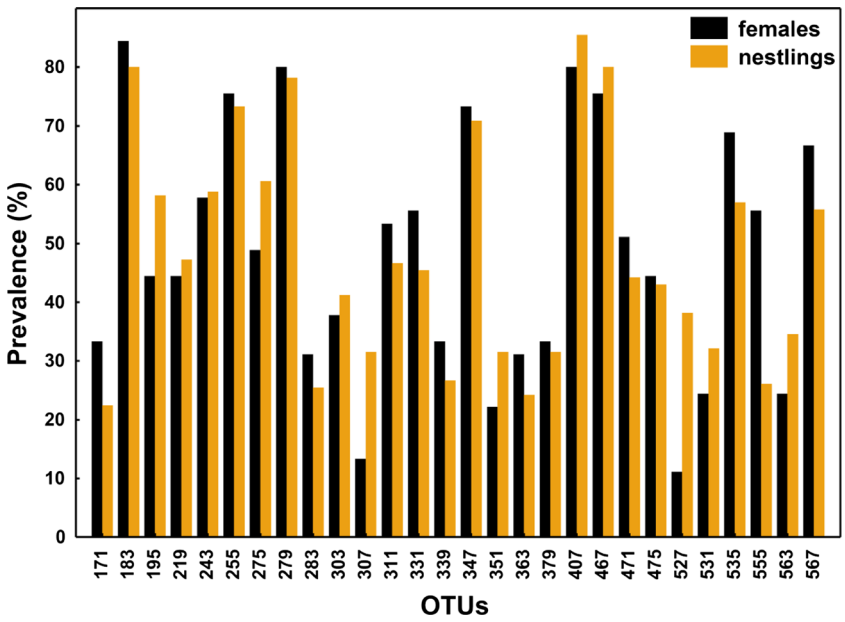

Fig. 1 Prevalence (%) of different bacterial OTUs (named by their length in terms of base pairs (bp)) found in more than 30 % of samples from uropygial glands of hoopoe nestlings ($N = 165$) and females ($N = 44$)

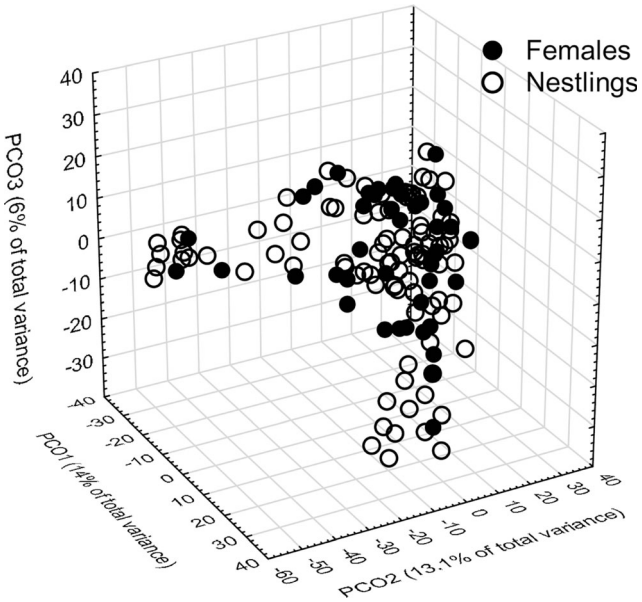

Fig. 2 Multidimensional space representation (PCoA) based on similarities of the OTUs communities harbored in uropygial secretions of hoopoe females and non-moved nestlings. The total variance explained is also shown (captured by the three axes = 33.1 %)

the microbiome of the uropygial secretion of hoopoes. Below, we discuss the importance of these findings for understanding the mechanisms of acquisition of bacterial symbionts by nestling hoopoes, and the implication for coevolutionary relationships between hoopoes and bacteria of their uropygial secretion.

Previous work has shown that the prevalence of cultivable bacterial strains (i.e., enterococci) in the uropygial secretion of females and nestlings differs [13]. These differences were at least partially due to the effect of few enterococci strains that appeared at a higher prevalence in samples of females or nestlings [13]. However, when considering the bacterial community as a whole, differences between females and nestlings did not reach statistical significance, and prevalence of different OTUs in samples from females and from nestlings correlated positively. In terms of bacterial diversity, even when considering the group of enterococci, estimates for females and nestlings did not differ significantly [13]. Thus, although prevalence of some OTUs in communities of females and nestlings may differ, the microbiome of the uropygial secretion of females and nesting hoopoes is quite similar.

Results from previous cross-fostering experiments performed by Ruiz-Rodríguez et al. [13] with recently hatched nestlings that were exchanged before they started to produce secretion (4 days old) strongly suggested that enterococci from the new environment are incorporated into the community of the uropygial secretion of nestlings, although some strains came from the uropygial secretion of mothers [13]. We here considered the whole microbiome of the uropygial gland of hoopoes, and performed the experiment with nestlings of intermediate age, once they started to produce uropygial secretion. Even with these nestlings, we also found a significant effect of nest of rearing and of nest of origin explaining variation in microbial community. Thus, the effect of nest of origin detected here also included environmental effects before the experiment and, accordingly, was stronger than that detected for enterococci community in cross-fostering experiments performed with 4-day-old nestlings. Moreover, the detected effect of nest of rearing of 8 days old cross-fostered nestlings confirms that experimental nestlings incorporate new bacteria to their uropygial microbiome once the uropygial gland is functioning. Consequently, results from these two experiments considered together suggest that nestling hoopoes acquire most bacterial symbionts during the first days of life, but that the microbiome of hoopoes is dynamic and can be modified along the nestling period depending on environmental conditions.

The effect of either nest of origin or nest of rearing included possible maternal effects that, respectively, occur before (from genetic mother) and after (from stepmother) the experimental translocation of nestlings [34, 38]. Mechanisms of vertical transmission of symbionts are by definition a maternal effect that contributes to offspring phenotype, but that is genetically determined in mothers (i.e., indirect genetic effect, [39–41]. Thus, the detected effects of nest of origin on the microbial community of nestling secretions, as well as the relatively high similarities between related nestlings, and between nestlings and mothers, may be explained by direct vertical transmission

Table 1 Results of a PERMANOVA model explaining matrices of similarity among the bacterial communities found in the uropygial secretions of hoopoe nestlings. The model includes identity of nest of origin (genetic factor) and rearing (environmental factor) nested within nest of origin. Bold *p* values are those lower than 0.05

Factors	Pseudo-F	df	*p*	Permutations	% Explained variance
(a) Nest of origin	3.56	27	>0.001	9693	37.0
(b) Nest of rearing (nested in (a))	1.28	27	0.013	9737	14.9

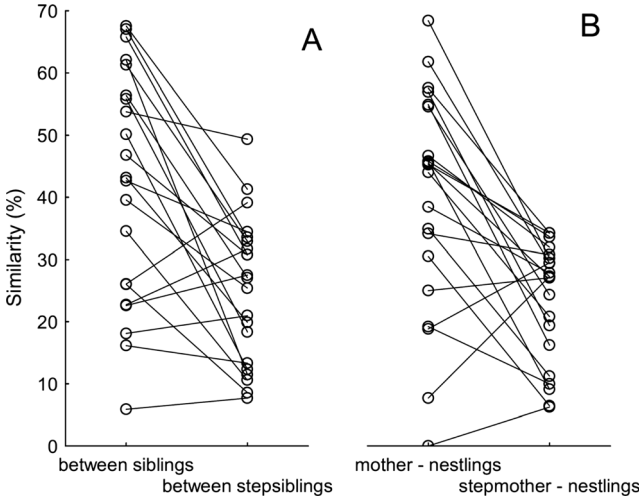

Fig. 3 Similarities in composition of bacterial communities (Jaccard's distance in percentage) among samples from cross-fostering experiments. **a** Similarity between uropygial secretions of nestlings that did not grow in their nests of origin (experimental nestlings) and genetic siblings reared in their native nests (between siblings), or foster siblings (between stepsiblings). **b** Similarities between microbiomes of the experimental moved nestlings and those of their genetic (mother–nestlings) or foster (stepmother–nestlings) mothers. *Lines* connect estimates of individuals from the same pair of cross-fostered nests

of symbionts from mother to offspring. However, previous results suggested that direct contacts with mother or nest material are not necessary for hatchling hoopoes to develop normal uropygial glands and acquire enterococci symbionts [13]. Thus, it is unlikely that the strong influence of nest of origin and the similarity among bacterial communities of related individuals detected here were exclusively explained by vertical transmission. An additional interpretation of these results is that the first microbes to colonize the hatchling glands will exclude subsequent colonists. So an empty gland acquires any microbe, but this is biased in nature because the natal nest is dominated by microbes from the mother, and later from the hatchling itself after it has been colonized. Thus, the transmission would be technically horizontal (because it has an external phase) in this scenario, but the external phase is determined by the mother similar to vertical transmission.

An alternative explanation of the strong effects of nest of origin is that related hoopoes share characteristics of their uropygial gland and/or secretion (i.e., chemical properties) that influence the composition of the bacterial community established. Bacteria from the environment that were compatible with characteristics of the uropygial gland and secretion of hoopoes would colonize hosts. Within the uropygial gland, competitive ability of different bacterial strains would depend on the particular environment (i.e., chemicals, resources, etc.) provided by hoopoes, which would determine the stabilized microbiome of the uropygial secretion [see 19]. Even if this was the explanation of the large detected microbiome similarities among relatives, the detected effects of nest of rearing suggest a plastic microbiome response to environmental changes after reaching certain stability level (i.e., uropygial gland functioning).

Nests of birds are considered as extended phenotypes of parents because nest site selection, nest building behavior, nest sanitation behavior, etc. are characters with strong genetic components [42, 43]. Thus, nests are indirect genetic (i.e., parental) effects for nestlings where natural selection would work [39]. Estimated effects of nest of rearing, but also most of those of nest of origin are likely associated to environmental characteristics of nests (included bacterial communities and characteristics of mothers). Thus, independently of the relative importance of genetic, environmental, and maternal effects, the factors determining the bacterial community of the uropygial secretion of nestling hoopoes have a considerable genetic background to be modulated by natural selection.

Detecting mechanisms explaining how beneficial microbiomes are established and maintained within their hosts is a major question in evolutionary biology [5, 44]. Here, not only we found a strong effect of nest of origin that likely included indirect genetic effects but also evidence of an influence of the environment. This effect suggests the composition of the microbial community in the uropygial secretion of hoopoes for which evidence of beneficial effects for hosts are accumulating [23, 45–47] is plastic. We expect that these results will encourage further research directed to detect factors driving phenotypic plasticity of the symbiotic microbiome of hoopoe uropygial gland, including physiological and morphological characteristics of the gland as well as characteristics of the microbiome of mothers, siblings, and nests.

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Compliance with Ethical Standards

Authorship Statement JJS and MM-V designed the study, JJS and AM-G carried out statistical analyses. AM-G together with SRR performed molecular analyses. AM-G, LA, and JMPS performed most of the field work. AM-G wrote the first version of the manuscript with substantial contribution from MRR and JJS. All authors contributed to the final version of the article.

Appendix 1

Table 2 Prevalence (%) of different bacterial OTUs (named by their length in terms of base pairs (bp)) found in all sampled uropygial glands of nestlings ($N = 165$) and females ($N = 44$). Italicized numbers show OTUs that were detected in more than 30 % of samples from females or nestlings

OTU	Females	Nestlings	OTU	Females	Nestlings	OTU	Females	Nestlings
100	11.11	9.09	295	0.00	1.21	499	2.22	5.45
103	2.22	1.82	299	22.22	13.33	503	0.00	1.21
107	0.00	0.61	*303*	37.78	41.21	507	6.67	4.85
111	6.67	10.30	*307*	13.33	31.52	511	28.89	25.45
115	4.44	1.21	*311*	53.33	46.67	515	17.78	12.73
119	0.00	9.09	315	4.44	7.27	519	17.78	14.55
123	0.00	3.64	319	28.89	28.48	523	15.56	16.97
127	15.56	16.97	323	4.44	10.91	*527*	11.11	38.18
131	13.33	27.27	327	13.33	28.48	*531*	24.44	32.12
135	11.11	15.15	*331*	55.56	45.45	*535*	68.89	56.97
139	28.89	16.36	335	11.11	24.24	539	11.11	10.91
143	2.22	0.00	*339*	33.33	26.67	543	2.22	17.58
147	13.33	22.42	343	6.67	15.15	547	0.00	2.42
151	4.44	13.33	*347*	73.33	70.91	551	6.67	6.06
155	8.89	21.82	*351*	22.22	31.52	*555*	55.56	26.06
159	2.22	7.88	355	17.78	13.33	559	15.56	8.48
163	6.67	4.24	359	2.22	2.42	*563*	24.44	34.55
167	0.00	4.24	*363*	31.11	24.24	*567*	66.67	55.76
171	33.33	2.42	367	15.56	16.97	571	8.89	12.73
179	2.22	3.03	371	0.00	1.21	575	2.22	5.45
183	84.44	80.00	375	0.00	0.61	579	11.11	4.24
187	8.89	10.91	*379*	33.33	31.52	583	17.78	18.79
191	17.78	20.61	383	0.00	0.61	587	13.33	10.91
195	44.44	58.18	387	0.00	1.21	591	2.22	2.42
199	15.56	13.94	391	4.44	5.45	595	8.89	4.85
203	4.44	1.21	395	2.22	6.06	599	6.67	4.24
207	0.00	1.21	399	11.11	6.06	603	4.44	0.00
211	2.22	3.03	403	6.67	5.45	611	6.67	4.24
215	4.44	6.67	*407*	80.00	85.45	619	4.44	2.42
219	44.44	47.27	411	11.11	13.94	639	2.22	1.21
223	4.44	3.03	415	2.22	1.21	647	8.89	4.85
227	8.89	4.85	419	8.89	18.79	651	0.00	0.61
231	15.56	10.91	423	24.44	21.82	659	2.22	4.24
235	17.78	2.42	427	13.33	20.00	667	0.00	0.61
239	11.11	10.91	431	0.00	2.42	675	0.00	0.61
243	57.78	58.79	439	8.89	8.48	679	0.00	0.61
247	15.56	6.67	451	2.22	4.85	699	2.22	9.70
251	2.22	2.42	455	4.44	1.82	703	0.00	1.82
255	75.56	73.33	459	2.22	3.03	711	2.22	2.42
259	2.22	6.06	463	4.44	3.64	715	0.00	0.61
263	20.00	18.79	*467*	75.56	80.00	719	0.00	0.61
267	0.00	1.82	*471*	51.11	44.24	731	0.00	1.21
271	15.56	18.18	*475*	44.44	43.03	755	0.00	0.61
275	48.89	60.61	479	15.56	14.55	767	4.44	2.42
279	80.00	78.18	483	2.22	1.21	775	0.00	0.61
283	31.11	25.45	487	2.22	2.42	779	0.00	0.61
287	6.67	10.91	491	24.44	17.58	847	0.00	0.61
291	0.00	6.67	495	4.44	8.48			

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