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Differences in intestinal microbiota between avian brood parasites and their hosts

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The intestinal microbiota determines the effectiveness of digestion in vertebrates, and is influenced by the external environment (mainly the diet), gut characteristics, and phylogeny. Avian brood-parasitic nestlings of the sub-family *Cuculinae* develop in nests of phylogenetically distant passerines and can be fed with the host diet. If the shaping of bacterial communities is dominated by phylogenetic constraints, and therefore the microbiota of parasitic nestlings differs from that of host nestlings, the energy and micronutrients that parasites and hosts obtain from a similar amount of food would be different. In this case, the bacterial communities of parasitic and host nestlings would have important consequences with respect to brood parasite development. By experimentally creating mixed broods of magpies (*Pica pica*) and great spotted cuckoos (*Clamator glandarius*), we investigated their cloacal microbiota using ribosomal intergenic spacer analysis. We found significant differences in bacterial assemblages of the parasitic and host nestlings, although none of the phylotypes were specific in either great spotted cuckoos or magpies. Cuckoos presented more complex communities, which could help the brood parasitic life style and allow the digestion of food provided by different potential hosts. Moreover, the intestinal morphology is different between the two species due to phylogenetic differences in the two taxa, which would influence the dissimilar bacterial assemblages. The detected differences in microbiota of great spotted cuckoo and magpie nestlings, which might occur in other brood parasite–host systems, may imply a lower digestion efficiency in parasites. Thus, the higher level requirements of cuckoo nestlings may be explained, at least in part, by cuckoos having a suboptimal bacterial community for processing the host diet.

ADDITIONAL KEYWORDS: *Clamator glandarius* – cloacal bacteria – *Pica pica* – ribosomal intergenic spacer analysis.

INTRODUCTION

Animal growth depends on external sources of biomass that, through the digestive processes, are degraded into simple molecules for use in cellular metabolism. Apart from the variety of physical and chemical adaptations in the digestive tract involved in the digestion, there are important bacterial assemblages that are crucial for hosts, allowing the degradation and synthesis of essential nutrients, and that may also impede pathogenic bacteria colonization (Hooper *et al.*, 1998).

Bacterial communities in the intestine vary depending on environmental factors, such as diet or habitat, as well as on properties of the digestive tract, including anatomy or pH (Stevens & Hume, 1998). Most of these factors are species-specific traits and, thus, bacterial communities are relatively stable and vary between species (Dubos, 1966; Stevens & Hume,

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1998)]. Across bird species, anatomical comparisons reveal that the digestive tract is the most diverse organ system (Klasing, 1999). Moreover, the gastrointestinal microbiota has a strong phylogenetic component, as noted out by Hackstein & van Alen (1996). Thus, host phylogeny may shape the composition of optimal bacterial community for a given diet (Hackstein, Langer & Rosenberg, 1996) because of the phylogenetic constraints of gut morphology. However, there is numerous experimental evidence available concerning the effect of diet on the microbial composition of the digestive tract, allowing an optimal use of nutrients from food intake (Xu & Gordon, 2003). Therefore, bacterial communities of different species should be related to diet, but may be also phylogenetically constrained, and this may have important consequences for the evolution of digestive processes, and also affect interspecific interactions. Species whose bacterial communities are adapted to exploit the most abundant resource may have an advantage when competing for such resources versus species with a constrained microbiota. This could be particularly important for phylogenetically distant species that exploit similar resources, as is the case for nestlings of some brood parasitic species and their hosts.

Interspecific brood parasitic birds lay their eggs in nests of other species, the hosts, which incubate the eggs and feed the parasitic offspring. Most parasitic species are cuckoos (sub-family *Cuculinae*) and are phylogenetically distant from their common hosts, which are mainly Passeriformes (Davies, 2000). Moreover, although most cuckoos are insectivorous, some of them parasitize species that feed their offspring with vegetal material. This is the case for the great spotted cuckoos (*Clamator glandarius*) in Europe that mainly parasitize magpies (*Pica pica*) (Cramp, 1985). Although adult great spotted cuckoos feed exclusively on caterpillars and are specialist consumers of noxious insects that most birds avoid (Del Hoyo, Elliot & Sargatal, 1997), magpies feed their offspring with a great variety of insects, but also with vegetal materials, including grains of cereals (Martínez *et al.*, 1992).

Despite some host species feeding their foster and own nestlings with different kinds of food (Grim, 2006), the diets of parasitic and host nestlings do not differ in some other host–brood parasite systems (Brooke & Davies, 1989; Grim, 2006). When parasitized, adult magpies preferentially feed cuckoo nestlings, but the diets of cuckoo and magpie nestlings do not differ significantly (Soler *et al.*, 1995). Avian species possess a variety of adaptations for digestive processing of their diets (Duke *et al.*, 1997), including the specific anatomic plan of the digestive tract depending on the birds typical diet (Klasing, 1999), as well as their phylogenetic history. Therefore, it is likely that the gastrointestinal tracts of cuckoos and magpies differ in their anatomy. Because the morphology of the gut may plastically modify in response to changes in nutritional needs during the life cycle (Diamond, 1991), we cannot completely reject the possibility of cuckoos and magpies showing a similar gut morphology allowing the colonization of similar microbiota. A similar morphology can also be predicted as a result of evolutionary convergence, as occurs in other species with other morphological traits (Grim, 2006). In this coevolutionary scenario, the gastrointestinal microbiota of cuckoo nestlings may be adapted to exploit the magpie-nestling diet, due to the importance of the environmental component in determining cloacal bacterial communities of wild birds (Lucas & Heeb, 2005) Alternatively, gastrointestinal bacterial communities of cuckoos can be phylogenetically constrained because of differences in adult diets, and also because the two species are phylogenetically distant and thereby likely have a different digestive morphology. Thus, although cuckoo and magpie nestlings are fed with exactly the same diet, the alternative scenario predicts between-species differences in gut microbiota. This possibility may have profound consequences on the coevolutionary process in which great spotted cuckoos and magpies are involved (Soler & Soler, 2000). For example, if cuckoos are not able to digest as efficiently as their foster siblings, they would need a higher amount of food to obtain a similar quantity of energy.

In the present study, for the first time, we compare the cloacal bacterial communities of two wild species that share the same environment, and discuss possible evolutionary and ecological explanations and consequences of microbiota dissimilarities. We performed the study in a brood parasite–host system using ribosomal intergenic spacer analysis (RISA) (García-Martínez *et al.*, 1999). Moreover, we also explored the incidence of generic pathogenic bacteria in cuckoo and magpie nestlings within the same nests, which allowed us to test for interspecific differences in the structure of cloacal bacterial communities. Finally, we dissected several magpie and cuckoo nestlings of similar age to examine the morphology of their digestive tracts.

MATERIAL AND METHODS

The study area was the Hoya de Guadix (37°18′N, 3°11′W), southern Spain (1000 m a.s.l.). The vegetation is sparse, with some holm oaks (*Quercus rotundifolia*) and almond trees (*Prunus dulcis*) in which magpies nest at a high density (Soler, 1990). Parasitism of magpies by the great spotted cuckoos is quite common in the area and some evidence on an ongoing

coevolutionary process between both species has been detected in recent years (Soler & Soler, 2000).

Field work and experimental procedure

At the beginning of the breeding season of 2003, we looked for magpie nests and determined laying date, the start of incubation, and parasitism by the great spotted cuckoo. In most parasitized nests, cuckoos hatch earlier of magpie eggs (Soler, 1990). Moreover, magpies preferentially feed the larger nestling in the nest and, therefore, most magpie nestlings die by starvation in parasitized nests (Soler & Soler, 1991; Soler *et al.*, 1995). To maximize the number of nests with nestlings of the two species, soon after hatching, we manipulated magpie broods by exchanging nestlings to obtain brood sizes of two cuckoos and two magpies of the same age per experimental nest. Briefly, in nonparasitized nests, we introduced two cuckoo nestlings from other nests, matching them for age with the magpie nestlings, and removed all except two randomly selected magpie nestlings. In parasitized nests, if necessary, we introduced two magpie and one cuckoo nestling up to complete a brood of two magpie and two cuckoo nestlings of the same age. All experimental broods were formed soon after hatching, when nestlings were 1–3 days old. At this age, the original-nest effect on bacterial communities is not supposed to be a problem because the gut microbiota is strongly influenced by the nest environment (Lucas & Heeb, 2005). Cross-fostering experiments have been used to distinguish between genetic and environmental components of numerous traits (Brinkhoff *et al.*, 1999; Soler, Moreno & Potti, 2003) and, thus, this experimental approach is also useful for detecting the influence of nest of origin and nest of rearing explaining gut microbiota (Lucas & Heeb, 2005).

We formed a total of 23 experimental broods. After losses due to predation and nestling starvation, we analysed samples collected from 41 magpies and 26 great-spotted cuckoo nestlings from 27 broods. Nineteen nests were experimental broods, whereas eight were natural, nonparasitized magpie nests. From the 19 experimental nests, we collected data for the two species in 11 (16 magpies and 17 cuckoos), whereas samples from a single species (i.e. experimental nests in which we have the data only for one species) were collected in the final eight experimental nests (nine cuckoos and two magpies). In total, 18 great spotted cuckoos and 14 magpies were moved from one nest to another to create experimental broods. All nestlings were included in the analyses because, in all experimental nests, magpies and great spotted cuckoos were sharing the nests during most of the nestling period.

Bacterial sampling and laboratory analysis

Bacteria in the cloaca were sampled before fledging, when nestlings were 16–18 days old. Bacterial sampling and DNA manipulation, was carried out in exactly the same way for every individual of the two species. Bacteria were collected by injecting and repipetting 500 μL of sterile phosphate buffer (Na_2HPO_4 0.1 M and NaH_2PO_4 0.1 M, pH 7.4) in the cloaca using sterile tips and an automatic pipett. After collection, we immediately lysed the bacterial cells by adding 500 μL of lysis buffer [5% Tris HCl 50 mM, 5% sodium dodecyl sulphate, 2% ethylenediamonetetraacetic acid (EDTA) 2 mM, 3.3% NaCl 100 mM] and samples were kept in ice. Later in the laboratory, samples were stored at –20 °C until molecular analyses.

DNA was extracted from 200 μL of each sample. Samples were thermically shocked to further lyse the cells. We extracted DNA following the protocol proposed by Orsini & Romano-Spica (2001). Shortly, after adding 400 μL of a buffer pre-warmed at 65 °C (1% of 10 mM Tris HCl, 0.2% of 1 mM EDTA, 15% of 0.3 mM sodium acetate, and 1.2% of polyvinylpyrrolidone), the DNA was purified with phenol-chloroform procedure and precipitated with isopropanol overnight at –20 °C. After washing thee times with 80% ethanol, the DNA was re-suspended in TE buffer pH 8 (10 mM Tris HCl and 1 mM EDTA).

To study the diversity of the cloacal community, we used the RISA method, which amplifies the spacer region between the 16S and 23S rRNA genes in the ribosomal operon. This fragment is extremely variable in both sequence and length for the different prokaryotic species, due to the presence of several functional units within them such as tRNA genes (García-Martínez *et al.*, 1999). The primers used were S-D-Bact-1522-b-S-20 and L-D-Bact-132-1-A-18 (Ranjard, Brothier & Nazaret, 2000). The polymerase chain reaction (PCR) was performed in 50 μL, with 100 ng of DNA, 1× PCR buffer (Qiagen), 2 mM $MgCl_2$, 0.1 mg mL^{-1} BSA, 0.5 μM of each primer, 150 μM of each dNTP, and 1 U Taq polymerase (Qiagen). The amplification reaction was performed using an initial denaturation at 94 °C for 3 min, followed by 25 cycles at 94 °C for 1 min, 55 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 5 min (Ranjard *et al.*, 2000). PCR products were subsequently quantified with a fluorimeter DynaQuant (Hoefler) after staining with Hoechst Dye 1 : 10 000. To separate the PCR products (200 ng), we used a 2% Metaphor agarose (FMC Bioproducts) gel electrophoresis, during 4 h at 150 V. Each band in the gel corresponds to one operative taxonomic unit (OTU), also called phylotype, which is assumed to be one bacterial species (Atlas & Bartha, 1997).

Finally, four cuckoo and four magpie nestlings of the same age (12–14 days) and similar size, which, for other purposes, were sacrificed in 2007 and kept in alcohol (76%), were dissected, and their gastrointestinal tracts isolated for comparisons of their morphologies. Measures were taken from the gut limit under the crop until the beginning of the cloaca.

Pathogens

We checked for the presence of three generic bacterial genera considered as part of the normal gut microbiota, but that could become opportunistic pathogens. A set of 77 nestlings in total were analysed from 18 broods with the two species: 41 of them were great spotted cuckoos and 36 were magpies. The extraction method was the same as described above, and DNA from cloacal samples were checked for the presence of different DNA fragments, specific from *Salmonella* spp. (199 bp salmonella-specific fragment using primers derived from a cloned fragment of *Salmonella weltevreden* genome, Jitrapakdee *et al.*, 1995), *Campylobacter* spp. (16 rRNA region of thermotolerant *Campylobacter* species, Moreno *et al.*, 2003), and enteropathogenic *Escherichia coli* (multiplex PCR: genes for shiga toxins stx_1 and stx_2, intimine *eaeA*, and adhesine *hlyA*, generating amplification products of 180, 255, 384, and 534 bp respectively; Paton & Paton, 1998). Results from PCRs were observed in a 2% agar gel and the presence of the specific band indicates the presence of particular pathogenic bacteria.

Statistical analysis

The gels were analysed with GEL COMPARE software (Applied Maths, Kortrijk, Belgium). This program estimates degree of similarity between pairs of individuals giving rise to a similarity matrix that summarizes pairwise similarities among samples (i.e. individuals). This program uses the Dice's binary coefficient: $2a/(2a + b + c)$, where a is the number of OTUs in common for the two samples, b is the number of OTUs present only in the first sample, and c is the number of OTUs present only in the second sample. We also prepared three binary matrices for comparisons of bacterial communities: one with the two species, another one with the nest of rearing identity, and a third one with the nest of origin identity. Value 1 referred to the same and 0 referred to different values for individual within a pair (i.e. if a pair of individuals are of the same species, nest of origin, and nest of rearing, the assigned value in all three matrices would be 1).

These three matrices were correlated with that of bacterial community similarity using Mantel tests as implemented in FSTAT software (Goudet, 1995). These tests estimate the relationships between the matrices and provide partial autocorrelation coefficients and associated P-values. Statistical significances were estimated by Monte Carlo procedures after 10 000 permutations. Matrices of bacterial assemblage similarity were used as the dependent variable, whereas those of species identity, nest of rearing, and nest of origin were independent matrices (for a similar design, see Lucas *et al.*, 2005). To control for the effect of nest of origin and nest of rearing, we looked at the partial autocorrelation coefficients between bacterial community and species identity in models that include information on nest of origin and nest of rearing.

Given that the number of OTUs approximately followed a normal distribution (Shapiro–Wilk normality test: $W = 0.969$, $P > 0.9$), a t-test was performed to analyse differences in richness between the two species. Differences in prevalence of pathogenic bacteria were studied by comparing probability of infection of cuckoos and magpies within the same nest and, thus, using paired-statistical tests. We used Wilcoxon test to compare prevalence of *Campylobacter* and *Escherichia coli*. Only 6.5% of nestling demonstrated infection by *Salmonella* and, thus, we used a sign test. Analyses were performed with STATISTICA, version 6.0 (StatSoft, 2001).

RESULTS

Most of the detected bands occurred in both species, but they appeared more frequently in great spotted cuckoo samples than in those from magpie nestlings (mean of frequencies: magpies = 0.12 ± 0.14; cuckoos = 0.19 ± 0.18; paired t-test: $N = 45$, $t(1,64) = 2.55$, $P = 0.014$; Fig. 1).

Within broods variation in bacterial communities was mainly explained by species identity, either when using all the nests together (i.e. non- and experimental nests), or when using only experimental nests (Table 1). Thus, great spotted cuckoo and magpie nestlings sharing the same environment (i.e. nest) differed in their cloacal bacterial communities, as suggested by the resulting different frequencies of OTUs in each bird species (Fig. 1). In addition, after statistically controlling for species differences, we found that nest of rearing explained a significant proportion of variance of bacterial community, which indicates an important role of the environment determining the cloacal bacterial composition of both great spotted cuckoos and magpies (Table 1). This result did not vary when only experimental nests were used (Table 1).

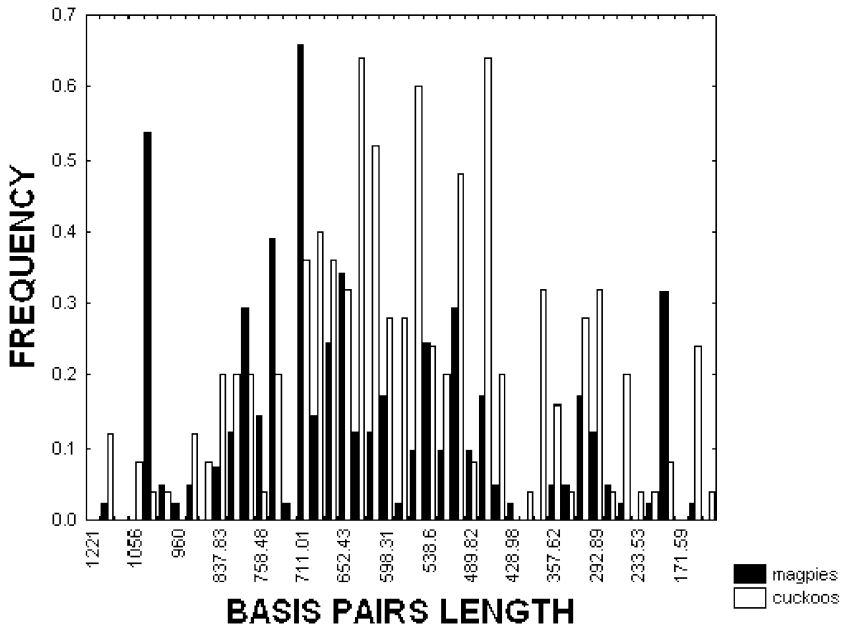

Figure 1. Prevalence of different operative taxonomic units (phylotypes) in samples of great spotted cuckoos (white, $N = 26$) and magpies (black, $N = 41$).

Table 1. Relationships between matrices of bacterial similarity and matrices of host species identity (i.e. same or different host species)

	All nests ($N = 27$)		Experimental nests ($N = 19$)	
Independent variables	*R*	*P*	*R*	*P*
Species identity	0.1643	0.0001	0.2777	0.0001
Nest of origin	0.1224	0.53	0.0205	0.0001
Nest of rearing	0.1205	0.0003	0.1245	0.0001
Species identity (controlling for nest of origin)	0.1446	0.0001	0.2936	0.0001
Species identity (controlling for nest of rearing)	0.1602	0.0001	0.2864	0.0001

Apart from the correlation coefficients and *P*-values associated with each independent variable, partial correlation coefficients for species identity, after controlling for nest of origin and nest or rearing, are shown. Sample size refers to the number of nests.

Interestingly, in spite of between-species differences in their bacterial community at the cloaca, no specific OTU for any of the two species were detected (Fig. 1). This result implies that the between-species differences in their bacterial community at the cloaca were due to great spotted cuckoos having a higher diversity of OTUs than magpie nestlings (i.e. great spotted cuckoo nestlings presented a more diverse microbiota than magpie nestlings) because the average number of OTUs per sample (±95% confidence intervals) was higher for the former ($N = 25$, 8.76 ± 3.96) than for the latter species ($N = 41$, 5.46 ± 2.61) (*t*-test: $t = -4.074$, $P = 0.00013$; Fig. 2), even after controlling for nest of rearing (GLM, nest identity as random factor and species as fixed factor; effect of species: $F_{1,28} = 15.57$, $P = 0.002$, effect of nest identity, $F_{26,28} = 1.25$, $P > 0.25$). Finally, differences in OTU richness between great spotted cuckoos and magpie nestlings did not vary in relation to nest identity since the interaction between nest and species identities was far from statistical significance ($F_{10,28} = 1.20$, $P > 0.25$).

With respect to the generalist pathogenic bacteria, except for *E. coli*, the prevalence of *Salmonella* sp. and *Campylobacter* sp. were quite low in our experimental nests. The prevalence of the three studied bacteria for magpie nestlings ($N = 36$) did not differ significantly from that estimated for great spotted

cuckoo ($N = 41$) nestlings reared in the same nest (*Salmonella*: magpies 2.70%, cuckoos 9.75%; Z = –0.7, $P = 0.8$; *Campylobacter*: magpies 8.33%, cuckoos 9.75%; Z = 0.674, $P = 0.5$; *E. coli*: magpies 47.22%, cuckoos 31.7%; Z = 1.4, $P = 0.16$).

The detected interspecific differences in bacterial communities may be explained in part by differences in the intestinal tract morphology of cuckoo and magpie nestlings (Fig. 3). Magpies, as predicted for omnivorous species (Barnes & Thomas, 1987), present a longer intestine than cuckoos (mean ± 95% confidence intervals; cuckoos: 38.50 ± 3 cm; magpies: 54.55 ± 3.87 cm; *t*-test: d.f. = 6, $P = 0.0006$), which, in spite of the low number of nestling's gut, reached statistical significance. Finally, although the intestine of the great-spotted cuckoos present two large and patent caeca sacs at the end, this is not present in magpies.

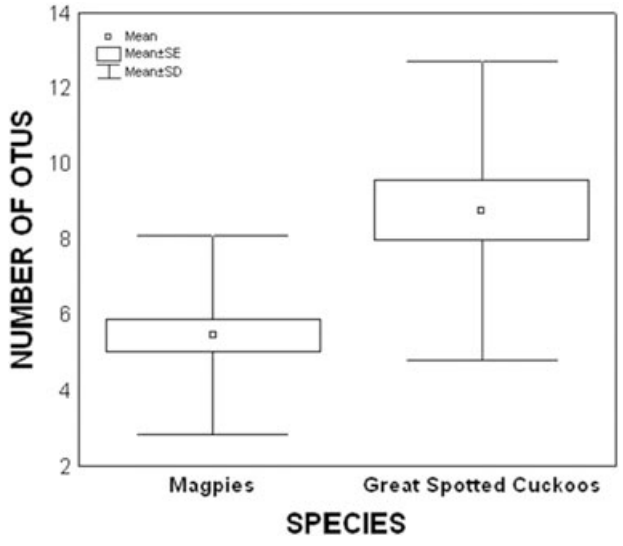

Figure 2. Number of bacterial phylotypes detected in cloacal samples of great spotted cuckoo and magpie nestlings.

DISCUSSION

We characterized the bacterial communities of cuckoo and magpie nestlings by using the RISA methodology, which identified phylotypes or OTUs in our samples allowing comparisons of bacteria communities in the digestive tracks of different animals (Lucas & Heeb, 2005). We did not detect host species-specific phylotypes but brood parasitic nestlings showed a richer microbiota than magpie nestlings and none of the detected OTUs appeared to be exclusive of great spotted cuckoo or magpie nestlings (Fig. 1). Except for two phylotypes (Fig. 1) that appeared only in 1% of the magpie samples, all bacteria phylotypes detected in magpies were also detected in cuckoos. Because great spotted cuckoo and magpie nestlings experienced the same potential colonization due, for instance, to similar diet, or to the bacterial transmission from adult hosts when feeding (Kyle & Kyle, 1993), this result is in accordance with the importance of the environmental conditions determining nestling bacterial communities (Lucas & Heeb, 2005).

Although we did not find evidence of bacteria specificity in any of the two bird species, our results showed that nestlings of great spotted cuckoos and magpies differ in their cloacal microbiota. Between species differences in microbial communities of the cloaca will reflect differences at the intestinal level (Savage, 1977; Vaahtovuo, Toivanen & Eerola, 2001), which allows the interpretation of results as differences in the bacterial community living in the digestive tract of magpies and great spotted cuckoos.

These between-species differences were not due to nest environment because, in most nests, great spotted cuckoo and magpie nestlings were reared together and analyses were corrected for differences due to nest of origin and nest of rearing. One possibility to explain the detected interspecific differences is that the digestive tracts of great spotted cuckoo and magpie nestlings differ in the chemical environment,

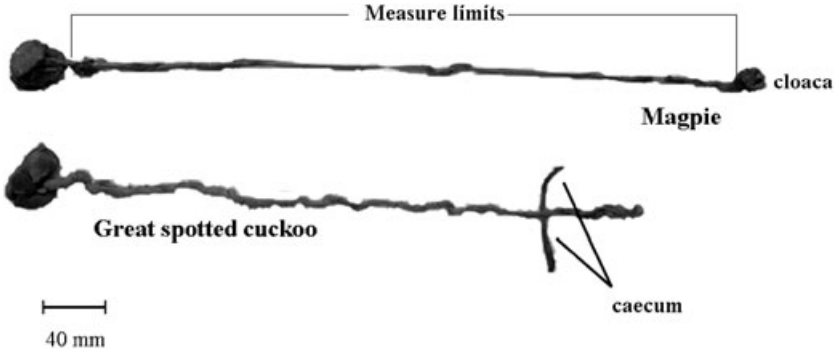

Figure 3. Intestines of a magpie (top) and a great spotted cuckoo nestling (bottom).

allowing the establishment of different microbiota. In any case, although we have no data regarding the chemical environment of the digestive tract of magpies and cuckoos, this hypothetical variation did not affect significantly the establishment of three different opportunistic pathogenic bacteria because its prevalence did not differ between great spotted cuckoo and magpie nestlings. Consequently, it is unlikely that interspecific differences in the chemical environment explained differences in the cloacal microbiota of nestlings.

Another possible explanation is related to differences in morphology of the digestive tract of cuckoos and magpies. They differ in the morphology of their digestive tracts (Fig. 3), which it is known to affect the establishment of different bacteria (Stevens & Hume, 1998; Langer, 2002). All dissected nestlings were 12–14 days old. At this age, body mass of great spotted cuckoos and magpies do not differ (Soler & Soler, 1991) and, consequently, interspecific differences in morphology of the digestive track are unlikely to be explained by differences in body size. The digestive track of cuckoos, in contrast to that of magpies, is longer and presents a well developed caecum that, at least in chicken, plays an important role in the digestion of carbohydrates by enzymes of microorganisms (Lan *et al.*, 2005). Thus, it is possible that the well developed caecum of cuckoos (Fig. 3), allows the establishment of a more diverse microbiota in great spotted cuckoos than in magpies. The caecum is also a phylogenetic character and, although it is usually small or vestigial in Passeriformes as we found in magpies (Clench & Mathias, 1995), it varies from moderate to long in the Family Cuculidae (Clench & Mathias, 1995). Those phylogenetic differences may influence the microbiota establishment.

An alternative nonfunctional explanation is that cuckoos, acquiring a greater quantity of food than their foster siblings in the nest (Soler *et al.*, 1995), would be in contact with a greater diversity of bacteria, providing greater chances of microbial colonization, and leading to cuckoos having a richer community than magpies. However, because the diet of nestlings in both species was almost the same (Soler *et al.*, 1995), this explanation is unlikely. Nevertheless, in other brood parasitic systems, when feeding parasitic nestlings, host parents decrease foraging selectivity and cuckoos increase their begging behaviour (Grim & Honza, 2006). The difference in diets between host and cuckoo nestlings may predict a richer bacterial community in cuckoos than in hosts. However, when feeding with nonsuitable food, parasitic nestlings can suffer from diarrhoea (i.e. a pathology associated with microbial disorders) and stagnate their growth (Grim, 2006), which suggest that microbiota of cuckoo nestlings is not functional for a proper digestion of unsuitable food.

Although adult magpies are omnivorous (cereals, fruits, carrion, insects, etc.), adults of great spotted cuckoos eat caterpillars almost exclusively (Cramp, 1985), and those two species are also phylogenically distant, with a different ecology and morphology, as well as environmental conditions, in their intestinal tracts. On the other hand, due to specific life-history traits, each species would present an optimal digestion of the diet as well as the establishment of the more adequate specific bacteria, as has been described previously (Langer, 2002). Therefore, it can be assumed that magpie nestlings hold a gastrointestinal microbiota allowing an optimal digestion of food carried by parents (Robbins, 1983) and, if cuckoo digestion of such diet is suboptimal, cuckoo nestlings would need more food than their foster siblings to obtain a similar amount of energy and nutrients. Therefore, the selection pressure favouring an increase on begging behaviour, and thus on feeding effort of foster parents, would be higher for cuckoos than for host nestlings. Indeed, previous studies revealed that, although body mass of fledgling cuckoos was on average lower than that of fledging magpies (Soler & Soler, 1991), cuckoo nestlings require higher amounts of food than their magpie siblings (Soler *et al.*, 1995). The higher requirements of cuckoo nestlings may be explained, at least in part, by cuckoos having a suboptimal bacterial community to process magpie diet.

However, natural selection is generally stronger in the juvenile compared with the adult phase (Endler, 1986) and, thus, adaptations allowing an optimal development of cuckoo nestlings using food items that differ from the adult diet is expected. In this scenario, a more diverse microbiota may allow a better degradation of organic matter due to niche complementarities between bacteria species (Loreau, 2001). Consequently, the detected larger bacterial diversity of cuckoo nestlings may be interpreted as a factor that favours digestion of the magpie diet. Another adaptive scenario is that the richer bacterial community detected in great spotted cuckoos would allow cuckoos to exploit other host species with different diets. In this sense, although we did not detect specific OTUs for cuckoos, it is possible that the ability of cuckoo nestlings to have a richer bacterial community in their intestinal tract allows the colonization of important digestive bacteria in different environments (i.e. nests of different species). However, studies on the digestibility of different kinds of food by great spotted cuckoos and magpies, and comparisons with other hosts species, are needed to corroborate our suggestion.

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