



Behavioral Ecology (2014), 00(00), 1–9. doi:10.1093/beheco/aru074

## Original Article

# Bacterial communities in meerkat anal scent secretions vary with host sex, age, and group membership

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Received 17 February 2014; revised 31 March 2014; accepted 2 April 2014.

The contribution of bacterial fermentation to the production of vertebrate scent signals has long been suspected, but there is still relatively little information about the factors driving variation in microbial composition in animal scent secretions. Our study subject, the meerkat (*Suricata suricatta*), is a social mongoose that lives in territorial, family groups and relies heavily on scent for social communication. Unusually in mammalian research, extensive life-history data exist for multiple groups inhabiting the same ecosystem, allowing for a study of both individual variation and group differences in the host's microbial communities. Using a culture-independent sampling technique, we explored the relationship between a signaler's sex, age/dominance, genotype or group membership, and the microbiota of its anal scent secretions. We found differences in the microbiota of males and females, but only after the animals had reached sexual maturity. Although bacterial communities in meerkat scent secretions were not more similar between kin than between nonkin, they were more similar between members of the same group than between members of different groups. Collectively, these results are consistent with a potential role for reproductive hormones in determining a host's bacterial assemblages, as well as an influence of sociality (such as intragroup allo-marking behavior) and/or microhabitat in the acquisition of bacterial assemblages. This study provides a key starting point for understanding the role of microbes in the variation of scent composition in mammals.

**Key words:** bacteria, meerkat, microbiota, odor, scent.

## INTRODUCTION

Bacteria are diverse, enormously versatile, and ubiquitous (Singleton 2004). As fundamental associates of animal bodies, they are essential to biochemical processes within their host, so their presence can ultimately influence animal behavior (Sharon et al. 2010; Archie and Theis 2011; Ezenwa et al. 2012; McFall-Ngai et al. 2013). Beyond the behavioral effects associated with bacterial infections (reviewed in Hart 1988), however, we understand little about bacteria's more routine contribution to host behavior and communication. Recently, in fruit flies (*Drosophila melanogaster*),

resident bacteria have been shown to influence their host's reproductive behavior, probably through their effect on olfactory cues associated with mate choice (Sharon et al. 2010). Here, we explore variation in host bacterial communities to better understand the role bacteria may play in olfactory communication. Because bacteria are found in animal glandular secretions (Albone et al. 1974; Soler et al. 2008; Theis et al. 2013), their influence on their host's scent cues has long been suggested (Gorman et al. 1974; Albone 1984), but this purported relationship has only recently received renewed attention owing to the advent of new technologies (Archie and Theis 2011; Douglas and Dobson 2013).

According to the fermentation hypothesis (Albone et al. 1974; Gorman 1976), bacterial communities can influence their host's

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scent via the direct production of odorants or the metabolization of organic compounds produced endogenously by the host (Gorman et al. 1974; Albone 1984; reviewed in Müller-Schwarze 2006 and Archie and Theis 2011). Experimental evidence from the Indian mongoose, *Herpestes aurofunicatus*, for example, shows that bacteria isolated from the host's anal pocket and cultured in vitro produce the same volatile fatty acids as those present in the host's anal scent secretions (Gorman et al. 1974). Moreover, in vivo production of these volatile fatty acids can be inhibited by treating the host's anal pocket with penicillin (Gorman et al. 1974). More recently, in members of Hyaenidae, the bacterial communities deriving from the host's anal scent secretions were found to be dominated by fermentative bacteria (Theis et al. 2013). The composition of these bacterial communities covaried with the composition of the volatile compounds in the scent secretions, again suggesting that symbiotic bacteria may underlie odorant composition (Theis et al. 2013).

As bacterial communities can be shaped by several factors, including their host's genotype, physiology, diet, and social relationships (Voigt et al. 2005; Lanyon et al. 2007; White et al. 2010; Theis et al. 2012; van Dongen et al. 2013), differences in the microbiota of scent secretions between individuals are likely to influence the scent cues underlying individual, kin, or group recognition (Albone et al. 1974; Gorman 1976; Archie and Theis 2011). For instance, in humans, armpit odor is generated by bacterial metabolization of odorless compounds secreted in sweat (reviewed in James et al. 2013). As armpit bacterial communities differ among individuals (Leyden et al. 1981; Xu et al. 2007), bacteria have been suggested to be at the origin of individual scent signatures (reviewed in Archie and Theis 2011 and James et al. 2013). Likewise, because closely interacting individuals may readily cross contaminate one another (Boulay et al. 2000; Safi and Kerth 2003) and mothers may transmit bacteria to their offspring during the early stage of life (Davies 1971; Mändar and Mikelsaar 1996, reviewed in Funkhouser and Bordenstein 2013), bacteria may also be at the origin of group or kin scent signatures (Theis et al. 2012). Clearly, more studies examining patterns in the microbiota of animal scent secretions are needed to shed additional light on the potential role of bacteria in driving scent signal variation.

Here, we investigated if bacterial communities present in the scent secretions of meerkats, *Suricata suricatta*, which are cooperatively

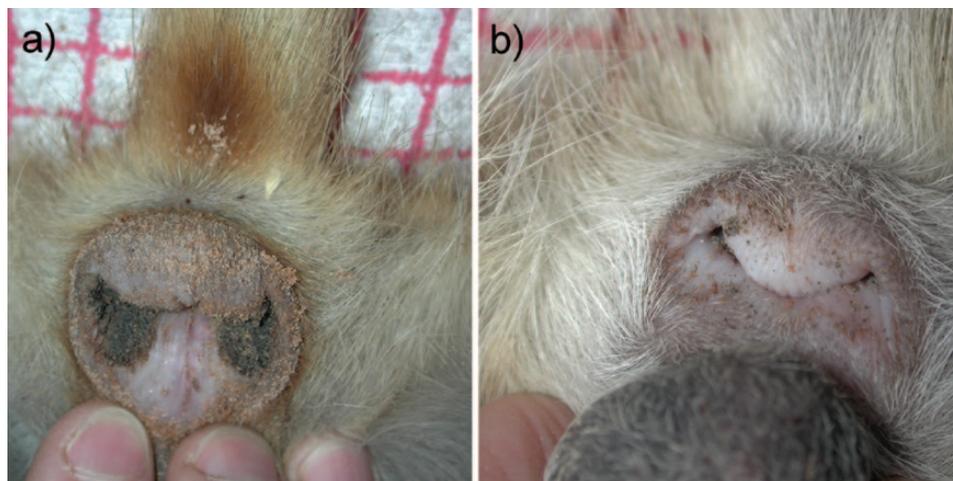
breeding mongooses, varied with individual and group factors. We studied meerkats from a wild population living in the Kalahari because an unusually rich and complete life-history data set exists for multiple groups inhabiting the same ecosystem. Meerkats occur in territorial groups of 2–50 individuals, generally consisting of a dominant breeding pair and various subordinates of both sexes that assist in raising the dominant pair's offspring (Clutton-Brock et al. 1998; Doolan and Macdonald 1999). Dominance within each sex is usually acquired by the oldest subordinate, so age is often highly correlated with social status (Clutton-Brock et al. 1999b). Both sexes possess an anal pouch (Figure 1) that is extruded during scent marking, a behavior that functions to maintain group territories (Jordan 2007). Meerkats often investigate the anogenital region of conspecifics. They can discriminate sex, social status, kinship, and group membership based on odor alone (Gsell 2002; Mares et al. 2011; Leclaire et al. 2013), suggesting an important role for scent in social communication. Using a culture-independent molecular technique, we tested if the bacterial communities in meerkat anal gland secretions varied with sex, age or social status, kinship, group membership, and geographic distance between groups.

## MATERIALS AND METHODS

### Study site and subjects

We conducted this study on 25 males and 25 females of a wild meerkat population, comprising 11 neighboring groups, at the Kalahari Meerkat Project (KMP) in South Africa. The KMP is part of the Kuruman River Reserve (26°58'S, 21°49'E), which is situated on ranch land in the southern Kalahari. Details about this site have been published previously (Clutton-Brock et al. 1999a). Maturity in meerkats occurs at 7–11 months. By age and social status, our subjects included 8 pups (< 3 month old), 29 mature subordinates (range: 7–47 months), and 13 dominant adults (range: 15–94 months; mean age:  $62 \pm 7$  months).

All of the meerkats at the KMP are habituated to close observation by humans. Individuals are implanted with subcutaneous transponder chips and are recognizable in the field by unique dye marks applied to the fur of awake animals (Jordan et al. 2007). At least 1 animal per group is fitted with a radio collar (Sirtrack,



**Figure 1**

Photographs of the anal pouches of a dominant (a) and subordinate (b) male meerkat. Note the accumulation of secretion in the dominant individual that, presumably via scent marking, appears to have stained the base of his tail.

Havelock North, New Zealand) to facilitate locating groups. Each focal group is visited approximately once every 3 days to record all key life-history events, including group movements (see below) and changes in dominance status or group composition.

### Scent secretion and sand sampling

We collected scent samples in November 2011 by rubbing sterile cotton swabs (Copan sterile plain swabs; Copan Italia, Brescia, Italy) against the interior wall of the anal pouch of awake, freely behaving meerkats (hereafter, we refer to these samples as deriving from the “anal pouch”). In our study population, most meerkats are sufficiently well habituated that secretion samples can be collected directly from their anal pouch while meerkats are resting near their sleeping burrows. This was our preferred method of collection as it allowed us, relatively noninvasively, to sample a large number of animals. Our 50 focal subjects were chosen randomly from among the most habituated meerkats. Individuals from the same group were not all sampled on the same date. We also collected 3 control samples (or blanks) by opening the swab tubes for few seconds in the field.

To ensure that bacteria collected from the anal pouch, as described previously, were mainly representative of the bacteria associated with anal gland secretions, more so than of bacteria associated with other substances (e.g., deriving from feces or the environment), we collected pure anal gland secretions from 10 adult meerkats ( $n = 2$  females and 8 males) that were captured and anaesthetized during the course of other studies. For these latter collections (hereafter referred to as deriving from the “anal gland”), we partially everted the anal pouch, gently pressed the anal gland, and collected the “pure” exudate in sterile 1.5-mL tubes. In addition, we collected 31 sand samples from the 11 territories of our focal subjects, including from currently used burrows ( $n = 8$  samples), from burrows used in the recent past ( $n = 14$  samples), and from several random locations within the territories ( $n = 9$  samples). We collected sand with a sterile scoop and placed the sample in a sterile 2-mL tube.

We stored all of our samples at  $-20^{\circ}\text{C}$  at the KMP until transport from the field to the laboratory in a cooler filled with ice packs. The samples arrived at the laboratory refrigerated and were then kept frozen at  $-20^{\circ}\text{C}$  until analysis.

All protocols were approved by Duke University’s Institutional Animal Care and Use Committee (protocol registry numbers: A171-09-06 and A143-12-05) and by the University of Pretoria’s Animal Use and Care Committee (ethical approval number: EC074-11, to C.M.D.).

### Kinship data for estimating pairwise genetic distances between individuals

We obtained kinship data, as expressed by pairwise coefficients of relatedness, from pedigree relationships. Full details on DNA extraction, genotyping, and pedigree methods are described in Nielsen et al. (2012). In brief, DNA was extracted from tail tip samples and genotyped at up to 18 variable microsatellite loci. A combination of genetic data and behavioral records was used to infer parentage for the whole population from the KMP’s inception in 1993 until late 2011. Based on the pedigree relationships, a matrix of pairwise coefficients of relatedness (i.e., the percentage of genes shared by common descent) was calculated for the whole population.

### Locational data for estimating pairwise geographical distances between groups

We obtained data on group location during group visits in September–November 2011, using handheld global positioning

system units (Garmin International Inc., Olathe, KS; for details see Jordan et al. 2007). We used these data to estimate group home range, calculated using the 95%-fixed kernel method and reference bandwidth value for smoothing (Worton 1995). We calculated the geographical distance between 2 group’s home ranges as the distance between their centroids. We estimated home ranges and pairwise geographic distances using adehabitatHR package in R statistical software (R Development Core Team 2010). Global positioning system coordinates were recorded for each sand sample, which allowed us to determine pairwise geographic distances between sand samples.

### Molecular analyses of microbiota

After allowing the samples to thaw to room temperature, we added 400  $\mu\text{L}$  of sterile water into the collection tube, which we vortexed for 1 min. We then transferred the mixture, including the swab tip (which we cut using sterilized scissors), into a 1.5-mL vial. After 10 min of centrifugation at 13 200 rpm, we removed the swab tips from the vials and then extracted DNA from each sample using a WIZARD Genomic DNA Purification Kit (Promega, Lyon, France). We resuspended final pellets in 20  $\mu\text{L}$  of sterile water. We extracted DNA from the sand samples using this same protocol. We extracted DNA from the 10 pure secretion samples using the Qiagen DNeasy® Blood and Tissue Kit and the standard protocol designed for the purification of total DNA from Gram-positive bacteria (Qiagen, Venlo, Netherlands; July 2006).

To characterize the bacterial communities present in each sample, we performed automated ribosomal intergenic spacer analyses (ARISA; Ranjard et al. 2000b). This DNA fingerprinting method is based on the amplification of the internal transcribed spacer (ITS) region lying between the 16S and 23S ribosomal RNA genes in the ribosomal operon. The ITS region is extremely variable, in both sequence and length, for different bacterial species. Therefore, the DNA amplification profile obtained with ARISA allows straightforward estimation of bacterial diversity, avoiding biases inherent in classical culture-based techniques (Ranjard et al. 2000b).

We amplified the ITS using the FAM (6-carboxyfluorescein)-labeled primer S-D-Bact-1522-b-S-20 (5’-[6FAM] TGCGGCTGG ATCCCTCCTT-3’) and the unlabeled primer L-D-Bact-132-a-A-18 (5’-CCGGGTTTCCCCATTCGG-3’; Ranjard et al. 2000a). We performed the PCR amplification in 10- $\mu\text{L}$  mixtures containing 200- $\mu\text{M}$  deoxynucleotide triphosphates, 0.20  $\mu\text{M}$  of each primer, 0.13 units of GoTaq DNA polymerase, 1 $\times$  PCR buffer (Promega, Fitchburg, WI), and 1- $\mu\text{L}$  DNA extract, using the following protocol: initial denaturation at  $95^{\circ}\text{C}$  for 3 min, 40 cycles consisting of denaturation at  $95^{\circ}\text{C}$  for 30 s, annealing at  $55^{\circ}\text{C}$  for 1 min, elongation at  $72^{\circ}\text{C}$  for 1 min, and a final elongation at  $72^{\circ}\text{C}$  for 10 min.

We then mixed 1  $\mu\text{L}$  of the PCR products with 8.6  $\mu\text{L}$  of highly deionized formamide and 0.4  $\mu\text{L}$  of Genescan 1200 LIZ size standard (Applied Biosystems, Foster City, CA). The mixtures were denatured at  $95^{\circ}\text{C}$  for 5 min before separation with a 48-capillary 3730 DNA Analyzer (Applied Biosystems) using POP-7 polymer and the manufacturer’s default electrophoresis run settings. Data analysis and genotyping were performed with GeneMapper software (Applied Biosystems).

For each sample, the sequencer produced an ARISA profile in which each peak corresponds to 1 phylotype or operational taxonomic unit (OTU). In the various samples, the sequencer detected ITS fragments ranging in size from 255 to 1055 base pairs. We used

Jaccard distance based on presence/absence of OTUs to estimate dissimilarity in microbiota composition for each dyad of samples.

### Statistical analyses

To determine if the composition of bacterial communities present in the meerkat's anal pouch depended on the animal's sex, age/social class (i.e., pups, mature subordinates, and dominant adults) or any interaction between these factors, we used a PERMANOVA with 5000 permutations (i.e. nonparametric multivariate analysis of variance, Adonis function, VEGAN package in R; Oksanen et al. 2007), based on Jaccard distance for OTU presence/absence data. PERMANOVA allows distance-based tests of significance for comparing a priori groupings as in a classical partitioning. Within adult meerkats, age was highly related to social status (Anova:  $F_{1,40} = 55.69$ ,  $P < 0.0001$ ). Therefore, to determine if bacterial communities in adults were related to age, while partialling out any effect of social status (and vice versa), we used PERMANOVAs with type I sum of squares and the covariate entered first in the model formula. To determine if richness of bacterial communities (i.e., number of OTUs per sample) was associated with sex, age/social class, or their interaction, we used linear mixed models with group identity as a random effect.

Because dyads of individuals from the same group were genetically more related to one another than were dyads of individuals from different groups (permutation  $t$ -test:  $t_{944} = 32.36$ ,  $P = 0.0002$ ), we needed to consider the effect on bacterial communities of kinship when testing the effect of group membership (and vice versa). We thus used partial mantel tests with 5000 permutations, in which pairwise Jaccard distance between bacterial communities was the dependant variable, group membership (scored as 0 for individuals of the same group and 1 for individuals of different groups; mantel tests being able to handle binary matrices, Borcard and Legendre 2012) or coefficient of relatedness was the fixed effect, and coefficient of relatedness or group distance respectively was a covariate.

To determine if the composition of bacterial communities were more similar between groups whose territories were closer to one another, geographically, we compared a matrix of geographic distances between groups to a matrix of bacterial distances between groups using Mantel tests with 5000 permutations. The bacterial distance between 2 groups was calculated as the mean distance of all possible dyads consisting of 1 member of group 1 and 1 member of group 2.

To determine if bacterial communities in sand varied among sample type (i.e., currently used burrows, burrows used in the recent past, and random locations within the territories), we used a PERMANOVA based on Jaccard distances. To determine if bacterial communities in sand varied with geographic factors, we compared a matrix of geographic distances to a matrix of bacterial distances using Mantel tests with 5000 permutations. Lastly, using Mantel test, we determined if sand samples collected within the same meerkats' territory were more similar to each other than to sand samples collected in different territories, by correlating a matrix of bacterial distances to a matrix of territory locations (0 for sand samples collected within the same meerkats' territory and 1 for sand samples collected from different meerkat territories).

We graphically represented similarities between individuals using nonmetric multidimensional scaling (NMDS) based on the Jaccard distance matrix and assessed the reliability of the NMDS representation using stress values. Stress  $< 0.2$  is usually considered to be acceptable. We performed all statistical tests using R statistical software (R Development Core Team 2010). Values are expressed as mean  $\pm$  standard error throughout.

## RESULTS

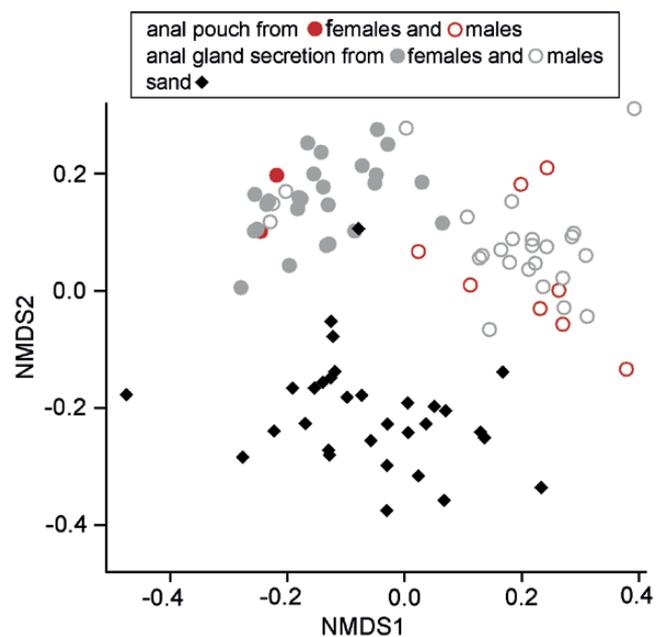
### Comparison of the bacterial communities in anal pouch, anal gland, and sand samples

The total number of OTUs detected in samples derived from the anal pouch ( $n = 50$  samples), from pure secretions of the anal gland ( $n = 10$  samples), and from sand ( $n = 31$  samples) were 251 OTUs, 122 OTUs, and 120 OTUs, respectively. By individual sample, they averaged  $26 \pm 1$  OTUs per sample,  $25 \pm 2$  OTUs per sample, and  $17 \pm 1$  OTUs per sample, respectively. Blank samples ( $n = 3$ ) averaged  $3 \pm 1$  OTUs per sample. The OTUs found in blank samples were excluded from the anal pouch and pure secretion profiles.

The composition of bacterial communities differed significantly among sample types (i.e., anal pouch, pure secretions, and sand;  $F_{2,88} = 3.59$ ,  $P < 0.0001$ ; Figure 2). Although bacterial communities present in the anal pouch and in pure anal gland secretion were extracted with different DNA extraction protocols, they did not differ reliably from each other ( $F_{1,58} = 1.42$ ,  $P = 0.053$ ), however, they were strongly and significantly different from the bacterial communities in sand (sand vs. anal pouch:  $F_{1,79} = 5.70$ ,  $P < 0.0001$  and sand vs. pure anal secretion:  $F_{1,39} = 2.58$ ,  $P < 0.0001$ ; Figure 2).

### Sand samples and geographic factors

The composition of bacterial communities in sand did not differ among sample types (i.e., currently used burrows, burrows used in the recent past, and random locations within the territories;  $F_{2,28} = 1.02$ ,  $P = 0.37$ ). In addition, bacterial community composition was not correlated to territory location. That is, bacterial communities in sand collected from the same group's territory were not more similar than bacterial communities in sand collected from different territories (Mantel test:  $r = 0.04$ ,  $P = 0.15$ ). Lastly, dissimilarity in bacterial communities in sand did not correlate with geographic distances between sand samples (Mantel test:  $r = -0.12$ ,



**Figure 2** NMDS analysis based on Jaccard distances in bacterial communities, showing separation of samples by sample type (anal pouch, anal gland secretion, and sand) and sex (3D stress: 0.18).

$P = 0.10$ ). Hereafter, our results are derived from analyses of the anal pouch secretions alone.

### Sex and age/status differences in anal pouch microbiota

The composition of bacterial communities in anal pouch samples related to the interaction between sex and age/social class ( $F_{2,44} = 3.68, P = 0.0018$ , Figure 3). Differences between the sexes were observed in subordinate adults and dominants ( $F_{1,27} = 6.12, P < 0.001$  and  $F_{1,11} = 2.02, P = 0.0012$ ), but not in pups ( $F_{1,6} = 1.08, P = 0.36$ ). Male pups had bacterial communities that differed from those of adult subordinate and dominant males ( $F_{1,18} = 3.15, P < 0.001$  and  $F_{1,7} = 2.13, P = 0.009$ ). Within adult males, bacterial communities varied with social status ( $F_{1,18} = 1.48, P = 0.020$ ; Figures 1 and 3), but not with age ( $F_{1,18} = 1.16, P = 0.21$ , Figure 4). In contrast, within adult females, bacterial communities varied with age ( $F_{1,18} = 1.47, P = 0.025$  Figure 4), but not with social status ( $F_{1,18} = 0.87, P = 0.73$ ). Richness of the bacterial community in the anal pouch related to the interaction between sex and age ( $F_{1,44} = 6.50, P = 0.01$ ). Richness increased with age in females, ( $F_{1,19} = 11.95, P = 0.002$ ), but did not vary with age in males ( $F_{1,19} = 11.95, P = 0.39$ ).

### Genetic and group differences in anal pouch microbiota

Bacterial community composition was correlated to meerkat group membership (Mantel test:  $r = 0.16, P = 0.0004$ ; Figure 5), that is, individuals belonging to the same group had more similar bacterial communities than did individuals belonging to different groups. When controlling for group membership, distance in bacterial communities did not correlate with genetic distance (Mantel test:  $r = -0.03, P = 0.30$ ). Individuals whose group territories were nearer to one another were more genetically related (Mantel test:  $r = 0.32, P = 0.02$ ), but they did not have more similar bacterial

communities than did individuals from groups whose territories were farther apart (Mantel test:  $r = 0.07, P = 0.37$ ).

## DISCUSSION

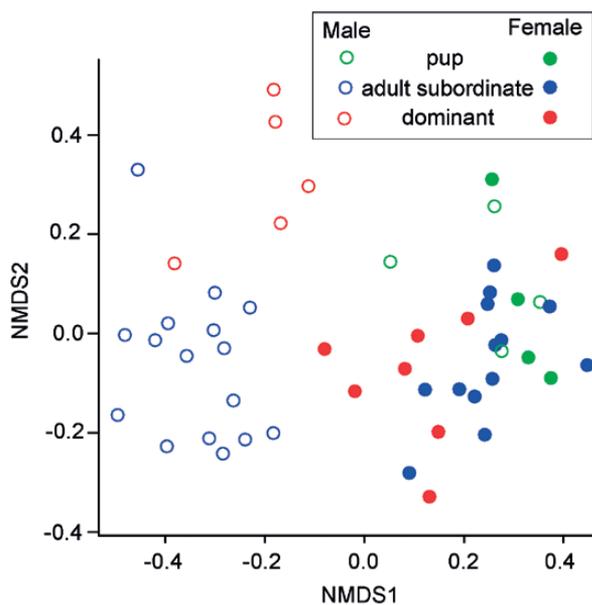
Using a culture-independent technique, we explored the relationship between various demographic, social, or ecological variables and the microbiota in meerkat anal pouches or its scent secretions. Most notably, we found similarities in the microbiota deriving from individuals of the same sex, age or dominance class, and social group. The latter finding, which is comparable to a group “signature” (Theis et al. 2012), could not be readily explained by kinship or geographical location, at least as represented by the bacteria in sand from within the animals’ territories. Although we cannot rule out potential effects of shared microhabitat on group similarities, our data are consistent with a social mechanism of bacterial transfer.

Because meerkats mark their territories by dragging their extruded anal pouch against various substrates in their environment, bacterial communities present in their anal pouch could represent a mixture of bacteria deriving from anal gland secretions, excrement, and environmental substances. Nevertheless, the structure of bacterial communities deriving from the anal pouch was comparable to that deriving from pure glandular secretions, both of which differed from the structure of bacterial communities in sand. Based on these comparisons, we suggest that our primary sampling technique adequately captured bacteria associated with the host animal. The socio-demographic patterns we describe in meerkat microbiota, therefore, likely reflect host traits more so than environmental markers.

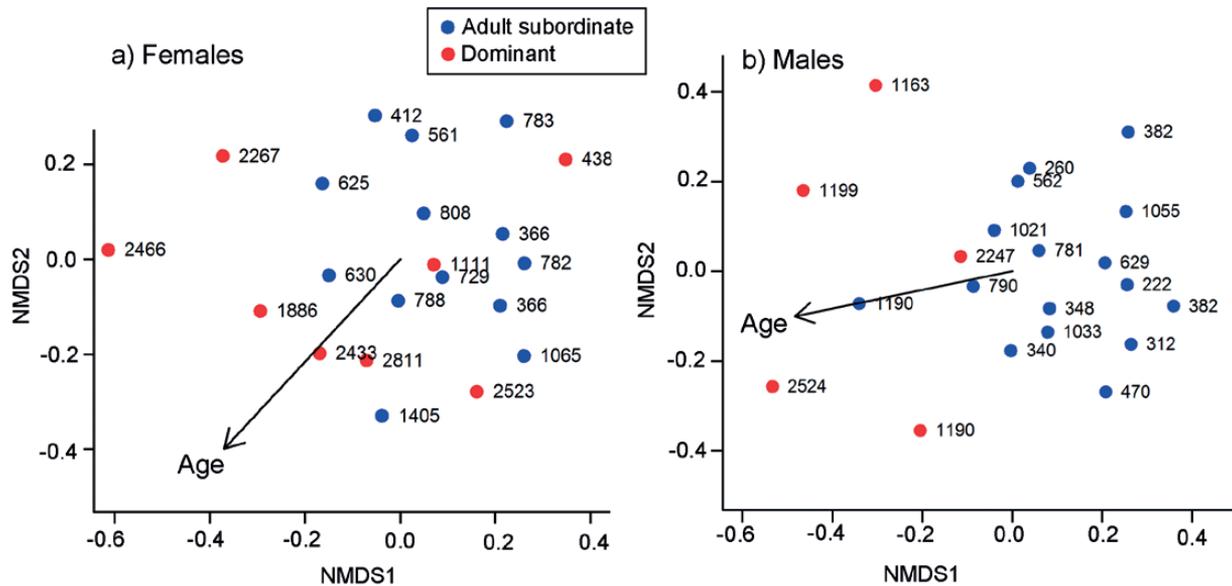
Host sex differences in microbiota were evident only after the meerkats reached adulthood, which suggests a role for host maturation or reproductive hormones in tailoring bacterial communities, either through selective acquisition or loss. Sex differences in the integument microbiota of adults have been shown in various species spanning multiple taxonomic groups, including humans (Fierer et al. 2008), greater sac-winged bats, *Saccopteryx bilineata* (Voigt et al. 2005), white-tailed deer, *Odocoileus virginianus* (Alexy et al. 2003), and great tits, *Parus major* (Saag et al. 2011). As is typical of vertebrate reproductive development, sexual maturity in meerkats is associated with sex-specific hormonal changes (Moss et al. 2001) that might directly affect microbiota (Styrt and Sugarman 1991; Komukai et al. 1999; Freestone et al. 2008) or the microenvironment in which they flourish.

Sex differences in microbiota also may be related to sex differences in host behavior. For instance, sex differences in the microbiota of greater sac-winged bats may owe to the male-specific behavior of cleaning wing sacs and filling them with secretions transferred from the genital region (Voigt and von Helversen 1999; Voigt et al. 2005), and in humans may owe to the higher frequency of washing by women than men (Fierer et al. 2008). In meerkats, adult males, unlike adult females, regularly prospect outside their territory (Young et al. 2007), while nevertheless maintaining their group membership. Prospecting males may thus experience different microhabitats, including varying soils (beyond sand), vegetation, livestock, or water sources, as well as different conspecifics. In the process, they may acquire bacterial assemblages that differ from those of more sedentary females and their young.

Dominant and subordinate males also had different microbiota, despite the fact that there is no major difference in their reproductive hormone profiles (Carlson et al. 2004). They do, however, differ

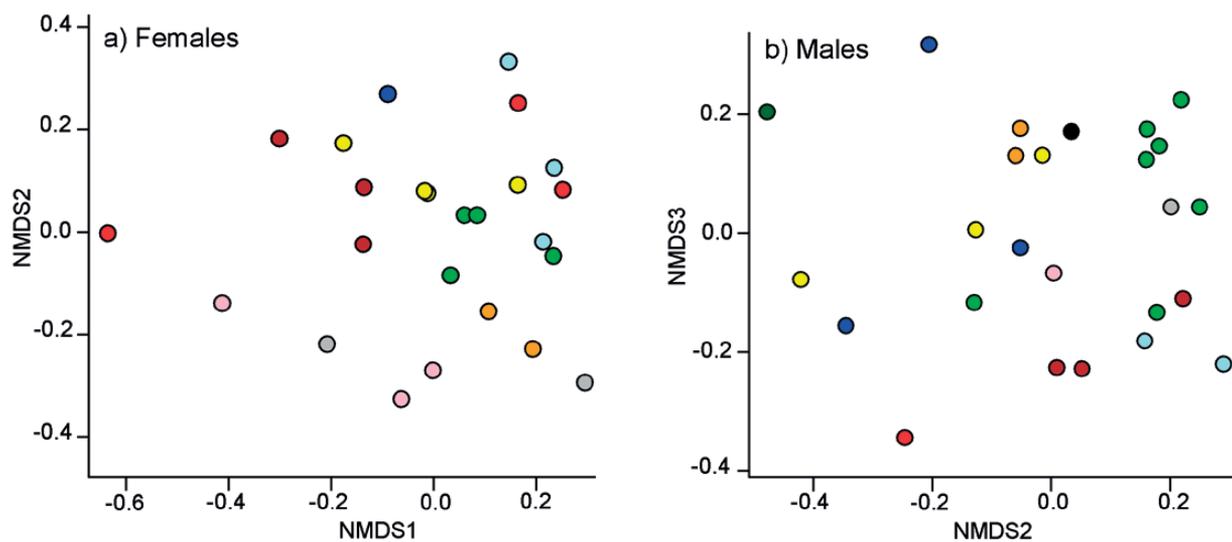


**Figure 3** NMDS analysis based on Jaccard distances in bacterial communities, showing separation between meerkats by their sex and age/social class (3D stress: 0.15).



**Figure 4**

NMDS analysis based on Jaccard distances in bacterial communities in (a) adult females (2D stress: 0.20) and (b) adult males (2D stress: 0.19), showing meerkat age (in days) and separation of meerkats by their social class. We fitted age vectors onto the NMDS plots using the envfit function in the package VEGAN.



**Figure 5**

NMDS analysis based on Jaccard distances in bacterial communities in (a) females (3D stress: 0.14) and (b) males (3D stress: 0.14), showing separation of host individuals by group membership. Each color represents a different group.

significantly in their social roles within their group, with dominant males monopolizing reproduction and subordinate males helping to rear offspring (Clutton-Brock et al. 1998; Griffin et al. 2003). Various neuroendocrine or physiological differences may underlie different social roles (e.g., Shively and Kaplan 1984; Creel 2001), some of which may affect bacterial assemblages in ways we have yet to recognize. Differences in the microbiota of dominant and subordinate males may also be related to differences in behavior. Subordinate males engage in extraterritorial prospecting forays more frequently (Young et al. 2007), and scent mark less often than do dominant males (Drea CM, personal observations). In addition, dominant males produce more anal gland secretion than do subordinate males (Leclaire S, personal observations). Differences in the

production of secretion may affect the glandular environment in a manner that influences the establishment and growth of bacterial assemblages. Notably, both sweat secreted by humans and preen oil secreted by hoopoes, *Upupa epops*, contain antimicrobial peptides (Schitteck et al. 2001; Martín-Platero et al. 2006). If the same were true of meerkat anal gland secretions, the enlarged or more productive glands of dominant males may contain greater quantities of antimicrobial substances that could help explain rank-related differences in male bacterial assemblages.

Unlike bacterial communities in adult males, bacterial communities in adult females did not vary with social status. Instead, they varied with female age, suggesting that bacterial assemblages may be influenced by the physiological changes associated with aging. In humans,

the microbiota associated with skin are known to change throughout life (Somerville 1969; Oh et al. 2012), which may be related to changes with age in sebum production, skin surface pH (Luebberding et al. 2013), or skin immune components (Sunderkötter et al. 1997). In female meerkats, anal gland mass increases with age (Lynch 1980), which may influence the establishment and growth of bacterial assemblages.

The composition of the microbiota present in meerkat anal pouches was more similar within groups than between groups—a finding that is comparable to prior reports in other species. Notably, in spotted hyenas, *Crocuta crocuta*, the microbiota present in anal gland secretions are more similar within clans than between clans (Theis et al. 2012) and, in great tits, the bacterial density present on feathers are more similar within mated pairs than between pairs (Saag et al. 2011). As a territorial and cooperatively breeding species, meerkats from the same group share the same socio-ecological environment. Beyond experiencing the close quarters of subterranean burrows, meerkats engage in social interaction, such as allogrooming, allonursing, and babysitting, that increase the likelihood of bacterial transmission between members of the same group. Indeed, tuberculosis, for example, can rapidly spread through a meerkat group via social interactions (see Drewe 2010). Moreover, meerkats likely expose one another, specifically, to the bacterial assemblages in their anal pouches via over-marking previously deposited scent, marking conspecifics, or scent rubbing (see Drea et al. 2002).

Social transmission of bacteria better accounts for our findings than does either genetic inheritance or microhabitat exposure. Notably, we did not find any correlation between kinship and the microbiota inhabiting the host's anal pouch. Moreover, members of neighboring groups were both more closely related and more likely to share similar habitats, given partial overlap in their territories (Jordan et al. 2007), but the geographical distance between groups failed to predict the composition of host bacterial communities. Although dismissing a role for host genetics in the bacterial assemblages of meerkats might require comparisons across more distantly related animals than are available at the KMP (see Nielsen et al. 2012), broader comparisons would likely introduce other variables that would confound interpretation. Moreover, studies on the control of host genetics over the composition of host microbiota have yielded inconsistent patterns across species (Spor et al. 2011). For instance, in mice, the microbiota in the gut or scent marks are influenced by genetic background and genotype of the major histocompatibility complex (Lanyon et al. 2007; Zomer et al. 2009; Benson et al. 2010). By contrast, in humans, gut microbiota is not more similar in monozygotic than in dizygotic twins and has been suggested to have low heritability (Turnbaugh et al. 2009; Yatsunen et al. 2012). The role of genetics in the variability of host microbial communities thus remains an area requiring further research.

Although bacteria in scent secretions are influenced by individual and group factors in meerkats and other animals (Theis et al. 2012; Sin et al. 2012), whether bacteria partly generate scent cues used in individual or group recognition remains unknown (Archie and Theis 2011). Although useful for investigating patterns of host variability, the culture-independent method used here does not allow the identification of bacteria phylotypes. Therefore, determining if meerkat scent gland secretions contain fermentative, odor-producing bacteria, as recently found in striped hyenas, *Hyaena hyaena*, spotted hyenas (Theis et al. 2012; Theis et al. 2013), and European badgers, *Meles meles* (Sin et al. 2012), will require implementing next-generation sequencing methods. The rapid improvements and

cost reduction of these methods promise to reveal much about the importance of bacteria in the production of social odors in animals.

## FUNDING

This study was supported by a Fondation Fyssen postdoctoral grant (to S.L.) and a research grant from the Association for the Study of Animal Behavior (to S.L.) and National Science Foundation (IOS-1021633 to C.M.D.). The kinship molecular analyses were financed by Cambridge and Zurich Universities. During the span of this study, the KMP was financed by Cambridge, Duke, and Zurich Universities.

We thank T. Clutton-Brock and M. Manser for providing access to the animals and their records at the KMP. We thank A. Szabo, L. Greene, N. Thavarajah, and J. Samson for their help in the field, and L. Raux for her help with molecular analyses. We are grateful to the Kotze family and other farmers neighboring the Kuruman River Reserve for graciously allowing us to work on their land and to the Northern Cape for permission to conduct the research.

**Handling editor:** Madeleine Beekman

## REFERENCES

- Albone ES. 1984. Microorganisms in mammalian semiochemistry. In: Albone ES, editor. Mammalian semiochemistry. New York: John Wiley & Sons. p. 135–164.
- Albone ES, Eglinton G, Walker JM, Ware GC. 1974. The anal sac secretion of the red fox (*Vulpes vulpes*); its chemistry and microbiology. A comparison with the anal sac secretion of the lion (*Panthera leo*). *Life Sci.* 14:387–400.
- Alexy KJ, Gasset JW, Osborn DA, Miller KV, Russell SM. 2003. Bacterial fauna of the tarsal tufts of white-tailed deer (*Odocoileus virginianus*). *Am Midl Nat.* 149:237–240.
- Archie EA, Theis KR. 2011. Animal behaviour meets microbial ecology. *Anim Behav.* 82:425–436.
- Benson AK, Kelly SA, Legge R, Ma F, Low SJ, Kim J, Zhang M, Oh PL, Nehrenberg D, Hua K, et al. 2010. Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. *Proc Natl Acad Sci USA.* 107:18933–18938.
- Borcard D, Legendre P. 2012. Is the Mantel correlogram powerful enough to be useful in ecological analysis? A simulation study. *Ecology.* 93:1473–1481.
- Boulay R, Hefetz A, Soroker V, Lenoir A. 2000. *Camponotus fellah* colony integration: worker individuality necessitates frequent hydrocarbon exchanges. *Anim Behav.* 59:1127–1133.
- Carlson AA, Young AJ, Russell AF, Bennett NC, McNeilly AS, Clutton-Brock T. 2004. Hormonal correlates of dominance in meerkats (*Suricata suricatta*). *Horm Behav.* 46:141–150.
- Clutton-Brock TH, Gaynor D, Kansky R, MacColl ADC, McIlrath GM, Chadwick P, Brotherton PNM, O'Riain JM, Manser M, Skinner JD. 1998. Costs of cooperative behaviour in suricates (*Suricata suricatta*). *Proc R Soc Lond B.* 265:185–190.
- Clutton-Brock TH, Gaynor D, McIlrath GM, Maccoll ADC, Kansky R, Chadwick P, Manser M, Skinner JD, Brotherton PNM. 1999a. Predation, group size and mortality in a cooperative mongoose, *Suricata suricatta*. *J Anim Ecol.* 68:672–683.
- Clutton-Brock TH, Maccoll A, Chadwick P, Gaynor D, Kansky R, Skinner JD. 1999b. Reproduction and survival of suricates (*Suricata suricatta*) in the southern Kalahari. *Afr J Ecol.* 37:69–80.
- Creel S. 2001. Social dominance and stress hormones. *Trends Ecol Evol.* 16:491–497.
- Davies PA. 1971. Bacterial infection in the fetus and newborn. *Arch Dis Child.* 46:1–27.
- Doolan S, Macdonald D. 1999. Co-operative rearing by slender-tailed meerkats (*Suricata suricatta*) in the Southern Kalahari. *Ethology.* 105:851–866.
- Douglas AE, Dobson AJ. 2013. New synthesis: animal communication mediated by microbes: fact or fantasy? *J Chem Ecol.* 39:1149.

- Drea CM, Vignieri SN, Cunningham SB, Glickman SE. 2002. Responses to olfactory stimuli in spotted hyenas (*Crocuta crocuta*): I. Investigation of environmental odors and the function of rolling. *J Comp Psychol*. 116:331–341.
- Drewe JA. 2010. Who infects whom? Social networks and tuberculosis transmission in wild meerkats. *Proc Roy Soc Lond B Bio*. 277:633–642.
- Ezenwa VO, Gerardo NM, Inouye DW, Medina M, Xavier JB. 2012. Animal behavior and the microbiome. *Science*. 338:198–199.
- Fierer N, Hamady M, Lauber CL, Knight R. 2008. The influence of sex, handedness, and washing on the diversity of hand surface bacteria. *Proc Natl Acad Sci USA*. 105:17994–17999.
- Freestone PP, Sandrini SM, Haigh RD, Lyte M. 2008. Microbial endocrinology: how stress influences susceptibility to infection. *Trends Microbiol*. 16:55–64.
- Funkhouser LJ, Bordenstein SR. 2013. Mom knows best: the universality of maternal microbial transmission. *PLoS Biol*. 11:e1001631.
- Gorman ML. 1976. A mechanism for individual recognition by odour in *Herpestes auro-punctatus* (Carnivora: Viverridae). *Anim Behav*. 24:141–145.
- Gorman ML, Nedwell DB, Smith RM. 1974. Analysis of contents of anal scent pockets of *Herpestes auro-punctatus* (Carnivora-Viverridae). *J Zool*. 172:389–399.
- Griffin AS, Pemberton JM, Brotherton PNM, McIlrath G, Gaynor D, Kansky R, O’Riain J, Clutton-Brock TH. 2003. A genetic analysis of breeding success in the cooperative meerkat (*Suricata suricatta*). *Behav Ecol*. 14:472–480.
- Gsell AC. 2002. Marking behaviour and hierarchy structures in meerkats: do marks indicate rank? Basel (Switzerland): University of Basel.
- Hart BL. 1988. Biological basis of the behavior of sick animals. *Neurosci Biobehav Rev*. 12:123–137.
- James AG, Austin CJ, Cox DS, Taylor D, Calvert R. 2013. Microbiological and biochemical origins of human axillary odour. *FEMS Microbiol Ecol*. 83:527–540.
- Jordan NR. 2007. Scent-marking investment is determined by sex and breeding status in meerkats. *Anim Behav*. 74:531–540.
- Jordan NR, Cherry MI, Manser MB. 2007. Latrine distribution and patterns of use by wild meerkats: implications for territory and mate defence. *Anim Behav*. 73:613–622.
- Komukai Y, Amai H, Goto N, Kusajima Y, Sawada T, Saito M, Takahashi KW. 1999. Sex differences in susceptibility of ICR mice to oral infection with *Corynebacterium kutscheri*. *Exp Anim*. 48:37–42.
- Lanyon CV, Rushton SP, O’donnell AG, Goodfellow M, Ward AC, Petrie M, Jensen SP, Morris Gosling L, Penn DJ. 2007. Murine scent mark microbial communities are genetically determined. *FEMS Microbiol Ecol*. 59:576–583.
- Leclaire S, Nielsen JF, Thavarajah NK, Manser M, Clutton-Brock TH. 2013. Odour-based kin discrimination in the cooperatively breeding meerkat. *Biol Lett*. 9:20121054.
- Leyden JJ, McGinley KJ, Hölzle E, Labows JN, Kligman AM. 1981. The microbiology of the human axilla and its relationship to axillary odor. *J Invest Dermatol*. 77:413–416.
- Luebberding S, Krueger N, Kerscher M. 2013. Age-related changes in skin barrier function - quantitative evaluation of 150 female subjects. *Int J Cosmet Sci*. 35:183–190.
- Lynch CD. 1980. Ecology of the suricate, *Suricata suricatta* and yellow mongoose, *Cynictis penicillata* with special reference to their reproduction. Bloemfontein (South Africa): Memoirs van die Nasionale Museum. p. 145.
- Mandar R, Mikelsaar M. 1996. Transmission of mother’s microflora to the newborn at birth. *Biol Neonate*. 69:30–35.
- Mares R, Young AJ, Levesque DL, Harrison N, Clutton-Brock TH. 2011. Responses to intruder scents in the cooperatively breeding meerkat: sex and social status differences and temporal variation. *Behav Ecol*. 22:594–600.
- Martín-Platero AM, Valdivia E, Ruiz-Rodríguez M, Soler JJ, Martín-Vivaldi M, Maqueda M, Martínez-Bueno M. 2006. Characterization of antimicrobial substances produced by *Enterococcus faecalis* MRR 10-3, isolated from the uropygial gland of the hoopoe (*Upupa epops*). *Appl Environ Microbiol*. 72:4245–4249.
- McFall-Ngai M, Hadfield MG, Bosch TC, Carey HV, Domazet-Lošo T, Douglas AE, Dubilier N, Eberl G, Fukami T, Gilbert SF. 2013. Animals in a bacterial world, a new imperative for the life sciences. *Proc Natl Acad Sci*. 110:3229–3236.
- Moss AM, Clutton-Brock TH, Monfort SL. 2001. Longitudinal gonadal steroid excretion in free-living male and female meerkats (*Suricata suricatta*). *Gen Comp Endocrinol*. 122:158–171.
- Müller-Schwarze D, editor. 2006. Chemical ecology of vertebrates. Cambridge (MA): Cambridge University Press.
- Nielsen J, English S, Goodall-Copestake W, Wang J, Walling C, Bateman AW, Flower TP, Sutcliffe RL, Samson J, Thavarajah NK, et al. 2012. Inbreeding and inbreeding depression of early life traits in a cooperative mammal. *Mol Ecol*. 21:2788–2804.
- Oh J, Conlan S, Polley EC, Segre JA, Kong HH. 2012. Shifts in human skin and nares microbiota of healthy children and adults. *Genome Med*. 4:77.
- Oksanen J, Kindt R, Legendre P, O’Hara B, Stevens MHH, Oksanen MJ, Suggests M. 2007. Vegan: Community Ecology Package. Available from: <http://CRAN.R-project.org/package=vegan>.
- R Development Core Team. 2010. R: a language and environment for statistical computing [Internet]. Vienna (Austria): R Foundation for Statistical Computing [cited 2013 July 01]. Available from: [www.R-project.org](http://www.R-project.org).
- Ranjard L, Brothier E, Nazaret S. 2000a. Sequencing bands of ribosomal intergenic spacer analysis fingerprints for characterization and microscale distribution of soil bacterium populations responding to mercury spiking. *Appl Environ Microbiol*. 66:5334–5339.
- Ranjard L, Poly F, Nazaret S. 2000b. Monitoring complex bacterial communities using culture-independent molecular techniques: application to soil environment. *Res Microbiol*. 151:167–177.
- Saag P, Tilgar V, Mänd R, Kilgas P, Mägi M. 2011. Plumage bacterial assemblages in a breeding wild passerine: relationships with ecological factors and body condition. *Microb Ecol*. 61:740–749.
- Safi K, Kerth G. 2003. Secretions of the interaural gland contain information about individuality and colony membership in the Bechstein’s bat. *Anim Behav*. 65:363–369.
- Schitteck B, Hipfel R, Sauer B, Bauer J, Kalbacher H, Stevanovic S, Schirle M, Schroeder K, Blin N, Meier F, et al. 2001. Dermcidin: a novel human antibiotic peptide secreted by sweat glands. *Nat Immunol*. 2:1133–1137.
- Sharon G, Segal D, Ringo JM, Hefetz A, Zilber-Rosenberg I, Rosenberg E. 2010. Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. *Proc Natl Acad Sci USA*. 107:20051–20056.
- Shively C, Kaplan J. 1984. Effects of social factors on adrenal weight and related physiology of *Macaca fascicularis*. *Physiol Behav*. 33:777–782.
- Sin YW, Buesching CD, Burke T, Macdonald DW. 2012. Molecular characterization of the microbial communities in the subcaudal gland secretion of the European badger (*Meles meles*). *FEMS Microbiol Ecol*. 81:648–659.
- Singleton P. 2004. Bacteria in biology, biotechnology and medicine. Chichester (UK): John Wiley & Sons.
- Soler JJ, Martín-Vivaldi M, Ruiz-Rodríguez M, Valdivia E, Martín-Platero AM, Martínez-Bueno M, Peralta-Sánchez JM, Méndez M. 2008. Symbiotic association between hoopoes and antibiotic-producing bacteria that live in their uropygial gland. *Funct Ecol*. 22:864–871.
- Somerville DA. 1969. The normal flora of the skin in different age groups. *Br J Dermatol*. 81:248–258.
- Spor A, Koren O, Ley R. 2011. Unravelling the effects of the environment and host genotype on the gut microbiome. *Nat Rev Microbiol*. 9:279–290.
- Styrt B, Sugarman B. 1991. Estrogens and infection. *Rev Infect Dis*. 13:1139–1150.
- Sunderkötter C, Kalden H, Luger TA. 1997. Aging and the skin immune system. *Arch Dermatol*. 133:1256–1262.
- Theis KR, Schmidt TM, Holekamp KE. 2012. Evidence for a bacterial mechanism for group-specific social odors among hyenas. *Sci Rep*. 2:615.
- Theis KR, Venkataraman A, Dycus JA, Koonter KD, Schmitt-Matzen EN, Wagner AP, Holekamp KE, Schmidt TM. 2013. Symbiotic bacteria appear to mediate hyena social odors. *Proc Natl Acad Sci USA*. 110:19832–19837.
- Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, et al. 2009. A core gut microbiome in obese and lean twins. *Nature*. 457:480–487.
- van Dongen WF, White J, Brandl HB, Moodley Y, Merkling T, Leclaire S, Blanchard P, Danchin E, Hatch SA, Wagner RH. 2013. Age-related differences in the cloacal microbiota of a wild bird species. *BMC Ecol*. 13:11.
- Voigt CC, Caspers B, Speck S. 2005. Bats, bacteria, and bat smell: sex-specific diversity of microbes in a sexually selected scent organ. *J Mammal*. 86:745–749.
- Voigt CC, von Helversen O. 1999. Storage and display of odour by male *Saccopteryx bilineata* (Chiroptera, Emballonuridae). *Behav Ecol Sociobiol*. 47:29–40.

- White J, Mirleau P, Danchin E, Mulard H, Hatch SA, Heeb P, Wagner RH. 2010. Sexually transmitted bacteria affect female cloacal assemblages in a wild bird. *Ecol Lett.* 13:1515–1524.
- Worton BJ. 1995. Using Monte Carlo simulation to evaluate kernel-based home range estimators. *J Wildlife Manage.* 59:794–800.
- Xu Y, Brereton RG, Trebesius K, Bergmaier I, Oberzaucher E, Grammer K, Penn DJ. 2007. A fuzzy distance metric for measuring the dissimilarity of planar chromatographic profiles with application to denaturing gradient gel electrophoresis data from human skin microbes: demonstration of an individual and gender-based fingerprint. *Analyst.* 132:638–646.
- Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP, et al. 2012. Human gut microbiome viewed across age and geography. *Nature.* 486:222–227.
- Young AJ, Spong G, Clutton-Brock T. 2007. Subordinate male meerkats prospect for extra-group paternity: alternative reproductive tactics in a cooperative mammal. *Proc R Soc Lond B.* 274:1603–1609.
- Zomer S, Dixon SJ, Xu Y, Jensen SP, Wang H, Lanyon CV, O'Donnell AG, Clare AS, Gosling LM, Penn DJ, et al. 2009. Consensus multivariate methods in gas chromatography mass spectrometry and denaturing gradient gel electrophoresis: MHC-congenic and other strains of mice can be classified according to the profiles of volatiles and microflora in their scent-marks. *Analyst.* 134:114–123.