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## ANIMAL HOST-MICROBE INTERACTIONS

## Social behaviour and gut microbiota in red-bellied lemurs (*Eulemur rubriventer*): In search of the role of immunity in the evolution of sociality

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## Abstract

- Vertebrate gut microbiota form a key component of immunity and a dynamic link between an individual and the ecosystem. Microbiota might play a role in social systems as well, because microbes are transmitted during social contact and can affect host behaviour.
- 2. Combining methods from behavioural and molecular research, we describe the relationship between social dynamics and gut microbiota of a group-living cooperative species of primate, the red-bellied lemur (*Eulemur rubriventer*). Specifically, we ask whether patterns of social contact (group membership, group size, position in social network, individual sociality) are associated with patterns of gut microbial composition (diversity and similarity) between individuals and across time.
- 3. Red-bellied lemurs were found to have gut microbiota with slight temporal fluctuations and strong social group-specific composition. Contrary to expectations, individual sociality was negatively associated with gut microbial diversity. However, position within the social network predicted gut microbial composition.
- 4. These results emphasize the role of the social environment in determining the microbiota of adult animals. Since social transmission of gut microbiota has the potential to enhance immunity, microbiota might have played an escalating role in the evolution of sociality.

### KEYWORDS

cooperation, immunity, lemur, microbial transmission, microbiota, social network, sociality

#### Dedication

Authors want to dedicate this article to the memory of Prof. Ilkka Hanski, who was both an important collaborator and a great inspirer of the underlying ideas of this research. His influential work on metapopulation theory was the unequivocal basis of our ideas of mammalian social networks as a metapopulation of bacterial communities. We regret that he passed away during the writing of the manuscript but believe that in the diverse metapopulation of scientists affected by his legacy, his ideas will continue to flourish and grow in enriching harmony of isolation and contact.

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## 1 | INTRODUCTION

The vertebrate gut microbiota is of growing scientific interest, because of the emerging understanding of its role as a functional link between the host physiology and the surrounding ecosystem. As such, an animal's gut microbiome is increasingly seen as an important and plastic part of its phenotype, in that it is crucial for immune system development (Hooper, Littman, & Macpherson, 2012; Kato, Kawamoto, Maruya, & Fagarasan, 2014), digestion (Flint & Bayer, 2008; Mackie, 2000; Turnbaugh et al., 2006), behaviour (Archie & Theis, 2011; Bravo et al., 2011; Cryan & Dinan, 2012; Ezenwa, Gerardo, Inouye, Medina, & Xavier, 2012; Montiel-Castro, González-Cervantes, Bravo-Ruiseco, & Pacheco-López, 2013; Sharon et al., 2010) and fitness (Rosengaus, Zecher, Schultheis, Brucker, & Bordenstein, 2011; Ruokolainen, Ikonen, Makkonen, & Hanski, 2016; Shin et al., 2011). The association between social behaviour and a host's microbiota is of particular interest, since this relationship is most likely bidirectional: social contact transmits microbes that can in turn modify social behaviour (Dinan & Cryan, 2012; Sharon et al., 2010). For instance, social grooming resulted in greater similarity of gut microbiota in baboons (Tung et al., 2015). On the other hand, through the vagus nerve of the microbiotagut-brain axis (Bravo et al., 2011; Montiel-Castro et al., 2013), the microbiota is known to affect the hormonal stress response system with downstream effects on behaviour (Crumeyrolle-Arias et al., 2014; Sudo et al., 2004).

Recently, these ideas of interaction between gut microbiota and behaviour have evoked novel research on the proximate patterns of microbial transmission due to social contact (Amato et al., 2017; Kort et al., 2014; Moeller et al., 2016; Tung et al., 2015). Many social behaviours, such as grooming, huddling or mating include physical contact and can function as potential pathways for microbial transmission. For example, Kulkarni and Heeb (2007) showed that experimentally induced bacteria were transmitted across a group of zebra finches (Taeniopygia guttata) via preening and sexual behaviours in less than a day. Accordingly, patterns of parasite transmission can reflect the structure of the host social network (Drewe, 2009; Godfrey, Moore, Nelson, & Bull, 2010; Griffin & Nunn, 2012; MacIntosh et al., 2012; Rimbach et al., 2015; Zohdy, Kemp, Durden, Wright, & Jernvall, 2012). However, while most work to date has focused on the relationships between social contact and immunologically challenging pathogen transmission (Alexander, 1974; Altizer et al., 2003; Moller, Dufva, & Allander, 1993; Turnbull et al., 2011), social contact can also enhance transmission of microbes that benefit immunity (Archie & Theis, 2011; Gilbert, 2015; Lombardo, 2008; Troyer, 1984). Many authors have even suggested that pro-social and affiliative contact behaviours such as grooming, licking or kissing might have partly evolved to serve this beneficial microbial transmission (Ezenwa et al., 2012; Lombardo, 2008; Montiel-Castro et al., 2013; Troyer, 1984).

Social transmission of mutualistic microbes affects two important aspects of the host's microbiota: (1) within-host microbial diversity (alpha diversity), and (2) between-host similarity of microbial communities. First, within-host gut microbial diversity (alpha diversity) can be enhanced by frequent social transmission of microbes among multiple hosts. For example, frequent social interactions enhanced gut microbial richness in chimpanzees (Pan troglodytes) relative to time periods when they were less sociable (Moeller et al., 2016). Diverse microbiota has been long suggested to be a requisite for a resilient immunity (Blaser & Falkow, 2009; Hooper et al., 2012; Keeney & Finlay, 2011; Lozupone, Stombaugh, Gordon, Jansson, & Knight, 2012), in the same way biodiversity makes macroecosystems more resilient to change (Gunderson, 2000; Levine & D'Antonio, 1999). For example, gut microbial diversity was found to protect desert locusts against pathogen invasion (Schistocerca gregaria, Dillon, Vennard, Buckling, & Charnley, 2005). Secondly, when microbes are transmitted through affiliative behaviours in a social network, individuals most closely socially linked can be expected to share more similar microbial communities. Recent work has found that frequent intimate kissing enhances mutual transmission of mouth microbiota in humans (Kort et al., 2014), and social proximity can predict gut microbial composition in howler monkeys (Alouatta pigra, Amato et al., 2017) and in baboons (Papio cynocephalus) regardless of shared environment, diet or relatedness (Tung et al., 2015).

Although host stress physiology (Bailey et al., 2011; Stothart et al., 2016), diet (Doré & Blottiere, 2015; Turnbaugh et al., 2009) and other environmental aspects (Benson et al., 2010; Friswell et al., 2010) are known to affect gut microbial composition, the role of social transmission cannot be ignored. Here, we explore the transmission dynamics of gut microbiota within a host social network of red-bellied lemurs. In addition to exploring these interactions on this population, our aim is to propose new avenues and testable hypotheses for future research on interactions of social dynamics and microbiota in wild populations.

The red-bellied lemur (*Eulemur rubriventer*) is native to Madagascar and lives in groups comprising an adult male, adult female and their offspring of different ages (Overdorff, 1996; Overdorff & Tecot, 2006; Tecot, 2008; Tecot, Baden, Romine, & Kamilar, 2013; Tecot, Singletary, & Eadie, 2016). They are a good species for studying the social transmission of microbiota because (1) groups are relatively stable; (2) they have fixed territories (consistent space use throughout the year, see Tecot, Singletary, & Eadie 2016) with little overlap and almost no contact with other groups (Overdorff & Tecot, 2006); (3) all group members participate in social interactions, such as grooming, caring for young or huddling, but vary in how much they socialize and with whom (Tecot, Singletary, & Eadie 2016); and (4) they exhibit strong seasonality in reproduction, diet and behaviour (Tecot, 2008, 2010), making it possible to determine seasonal effects on gut microbiota.

By using social network analysis, we examine inter-individual differences in host social behaviour and how these relate to gut microbial composition. Our overall hypothesis is that patterns of social contact are associated with patterns of gut microbial composition between individuals and across time. Specifically, we ask (1) whether the nature of this social association (indicated by group membership and position in social network) is positively correlated with gut microbial similarity, and (2) whether the amount of close social interaction (indicated by group size and individual sociality) is positively correlated with gut microbial alpha diversity. To gain a more comprehensive view of the role of social lifestyle in shaping the gut microbiota, we explore the temporal dynamics of the host microbiota and social behaviour, and further identify what taxa best describe major trends.

### 2 | MATERIALS AND METHODS

### 2.1 | Behavioural data collection

Data were collected from family groups of red-bellied lemurs on the Vatoharanana trail system in Ranomafana National Park. Madagascar. between August 2013 and February 2014. This time of the year is when infants were born (this study; Tecot, 2010) and fruit availability was generally low (Tecot, 2008). Behavioural data were collected from 28 individually identifiable adult and subadult focal individuals in eight groups on a rotating basis: each group was followed during full-day observation periods, rotating between the groups daily (mean interval 10.7 days, ± SD 8.5 days). During each follow we noted group demography, including group size (see Data S1) and composition (age, sex, identity). Age was classified as "adult" or "juvenile" based on appearance (body size), and known family composition and births. We used scan sampling and instantaneous recording (Altmann, 1974) to collect data on behavioural states at 5-min intervals. When recording social behaviours (mutual grooming, huddling), the partner's identity was also recorded. Inter-observer reliability among the field team (N = 4)was tested repeatedly until all observers were within 95% agreement (Gwet, 2008). Only the groups with more than two individuals and more than 40 hr of behavioural data were used to investigate questions related to social behaviour (total 19 individuals from 5 groups).

### 2.2 | Faecal sample collection

During behavioural observations, faecal samples were collected from all focal individuals within the group and ad libitum from additional identified groups when encountered (N = 36 individuals). Faecal samples were collected immediately upon defecation (N = 110) following (Amato et al., 2013), and placed into Eppendorf tubes filled with RNAlater. At least one sample per individual was collected during Season 1 (September–October). We later sampled 13 of the same individuals in Season 2 (November–January) to allow for seasonal comparisons. To increase sample size and analyse demographic correlates such as age, sex, temporal trends and group size, additional samples were opportunistically collected whenever encountering individuals from non-focal groups (in total, three extra groups were sampled).

## 2.3 | Indices of social interaction and social network analysis

Because most social contact in this species comprises social grooming and huddling (resting or sleeping in close physical contact; S. Tecot, unpubl.), two different indices for individual sociality (SI) were constructed: (1) SI<sub>Groom</sub>, calculated as the proportion of time an individual engaged in social grooming behaviours relative to its total observation time; and (2)  $SI_{Huddle}$ , calculated as the proportion of time an individual spent huddling with others relative to its total time spent resting.

In addition, pairwise association indices were constructed from the behavioural data using a Simple Ratio Index method for social network analyses (Cairns & Schwager, 1987; Ginsberg & Young, 1992; Whitehead, 2008). Simple Ratio Index is defined as:

$$I = \frac{X}{[X + y_{AB} + yA + yB]},$$

where X = the number of sampling periods in which individuals A and B were observed associated,  $y_{AB}$  is the number of sampling periods in which A and B were observed but not associated,  $y_A$  = the number of sampling periods in which only A was observed, and  $y_B$  = the number of sampling periods in which only B was observed. Paralleling the sociality indices described above, we constructed two association indices to characterize how social contact was distributed between individuals: (1) Al<sub>Groom</sub>, indicating the time each pair spent grooming each other relative to the total observed grooming time in the group, and (2) Al<sub>Huddle</sub>, indicating the time each pair spent huddling together relative to the total time these individuals were observed huddling with someone.

Because infants were born into groups during the study period, likely affecting the physiological states and patterns of social behaviour within groups (Tecot, 2008, 2013; Tecot & Baden, in press), both indices were constructed separately for two time periods: (1) Season 1: before infants were born (September–October in 2013), and (2) Season 2: after infants were born (November 2013–January 2014). Infant birth was the most evident change between seasons, although weather and environmental parameters were also changing gradually across the study period, making season 2 also higher in overall rainfall and temperature.

### 2.4 | Gut microbial DNA analysis

Samples were stored in RNAlater for 1–6 months at –20°C until they could be transported (at ambient temperature) to the United States for further storage (6 months at ambient temperature, to avoid re-freezing that can degenerate DNA) and processing. RNAlater is known to be a powerful preserving medium for DNA, even in room temperature (Song et al., 2016), and in any case, all samples were stored in room temperature for the same length of time. DNA analyses were done in the Knight Laboratory at the University of Colorado according to the Earth Microbiome Project protocols (EMP; see http://www.earthmi crobiome.org/emp-standard-protocols/16s/). DNA extraction was done using MO Bio PowerSoil kits. PCR was run with primers targeted at the V4 region of the 16S rRNA gene (EMP amplification primers 515F/806R, according to Caporaso et al., 2012). The resulting amplicons were sequenced using Illumina MiSeq V2 platform (150 bp length, using EMP Sequencing primers, according to Caporaso et al., 2012).

Due to short read length, we were unable to join many of the forward and backward reads. Thus, only forward reads were used in Operational Taxonomic Unit (OTU) clustering, according to widely used methods (see, for example, Amato et al., 2016). It is important to note that, because there is very little overlap between reads, base calls would have been made from a single read even if all read pairs could have been joined. We determined that there was no systematic bias in OTU clustering between the forward and backward read sequences. Forward reads were used because there is more variation in this part of the gene region. Clustering sequences into OTUs was done de novo with UPARSE pipeline (with 97% similarity threshold), with a quality filtering using maximum expected error  $\leq 2$ . Further preprocessing was done with R, using the PHYLOSEQ package (McMurdie & Holmes, 2013). Preprocessing of data included simultaneously removing non-bacterial taxa (chloroplasts and mitochondria) and bacteria that were likely not part of the gut community (Cyanobacteria and phytopathogenic Xanthomonadales, for a table of removed OTUs, see Data S2). Also, samples with fewer than 5,100 reads were excluded from further analysis. This threshold was chosen because library size had no effect on Shannon diversity estimates for samples above this threshold (see Data S3). Subsequently, sequence data (OTU table) were not rarefied because rarefying discards usable data (see McMurdie & Holmes, 2014) and is not necessary if weighted metrics are used (see Haegeman et al., 2013). Accordingly, sequence data were used only as relative abundances of each taxon per sample. Samples in the processed data had a mean library size of 11,742 reads (standard deviation 3,758, range 5,626-22,617). The bacterial content in faecal material is so high that soil contamination is likely to play a minimal role in analysed microbial community composition. To ensure that taxa were not contaminants from faecal contact with the soil, the distribution of abundances was examined. and rare taxa with a maximum relative abundance below  $10^{-4}$  in any of the samples were dropped. This was done after removing the nongut microbial taxa.

### 2.5 | Statistical analyses

Statistical analyses and plots were done using R (R Core Team 2014; packages: PHYLOSEQ, McMurdie & Holmes, 2013; VEGAN, Oksanen et al., 2014; GUNIFRAC, Chen, 2012; LABDSV, Roberts, 2013; GGPLOT2; Wickham, 2009; RANDOMFOREST, Liaw & Wiener, 2002). Microbial communities in samples were described with Shannon diversity indices and the Bray-Curtis dissimilarity index (Legendre & Legendre, 2012). All indices were based on relative abundance, as indices based on presence/ absence are suspected to bias the impact of rare species (Haegeman et al., 2013). Because monthly change in microbiota was found to be small ( $R^2$  = .04, p < .01), data from roughly 2 months before and after infants were born in focal groups were treated as temporally uncontrolled time points (Season 1 and Season 2). Under this assumption, since the data were unbalanced, the data were divided into three different subsets (see Data S4):  $S_0$  = total data (*n* = 98; 6 months, Sep-Feb) used to explore the effects of demographic variables (group identity, group size, sex, age, pregnancy) on microbial diversity, and to compare overall spatial vs. temporal variation in microbial composition (similarity);  $S_1$  (n = 28): one sample per individual, all collected during Season 1, used to explore the effects of demographic factors on overall composition of microbiota (similarity);  $S_2$  (n = 22): two samples per individual, Seasons 1 and 2, both with corresponding behavioural data, used to explore the effects of time, sociality and position in social network on microbiota.

To test for associations between group membership or other demographic factors (age, sex, month, reproductive state) and gut microbial similarity, we used permutational multivariate regression on microbial distance matrices (PERMANOVA: adonis function in the VEGAN package in R) created from  $S_0$  and  $S_1$  datasets. In addition,  $S_1$  microbial data were clustered using K-means partitioning (Legendre & Legendre, 2012). The optimal number of K-means clusters was found using the 'cascadeKM' function in VEGAN package (Oksanen et al., 2014), using default options. PERMANOVA was used to test whether clusters differed significantly from each other. While the optimal number of clusters was found to be three, the data were also partitioned into eight clusters, motivated by the clustering of eight family groups in PCoA ordination (using Bray-Curtis dissimilarity of S<sub>1</sub> data). Correlations between gut microbial similarity (Bray-Curtis dissimilarity) and social association within group (matrix of association indices, S2 data) were tested with Mantel tests of matrix correlation (Legendre & Legendre, 2012).

To test whether *gut microbial alpha diversity* differed among levels of sociality (via sociality indices), group sizes or time points, we used generalized estimation equation (GEE), controlling for other between-individual variation. Lastly, *identity of taxa* that best characterize any given trend was determined with random forest analysis (Breiman, 2001), using Mean Decrease Accuracy corrected with standard deviation.

## 3 | RESULTS

## 3.1 | Species-specific trends in composition and diversity of gut microbiota

The gut microbiota of red-bellied lemurs was dominated by the phyla *Bacterioidetes*, *Proteobacteria* and *Firmicutes* (Figure 1). Unknown taxa



**FIGURE 1** Gut microbial composition of the eight study groups, represented by relative abundances of different Phyla. White area covers unknown taxa

represent a large part of gut microbiota (Figure 1). Individual-level variation was an order of magnitude greater than temporal variation: looking at the total data ( $S_0$ ), differences between individuals explained 44% of variation, while monthly change accounted for 4% of the remaining variation ( $R^2 = .44$  and  $R^2 = .04$ , respectively, p < .01; for tabled bioinformatics results for all PERMANOVA tests and GEE models, see Data S5). Sex had no significant effect on gut microbiota, but pregnant female microbiota differed significantly from others ( $R^2 = .1$ , p = .03,  $S_1$  data). Individual age had no detectable effect on overall gut microbial composition in the total data but was a significant predictor of gut microbial alpha diversity in a GEE model of a smaller subset of data ( $S_2$ ).

## 3.2 | Effects of group membership and social association on gut microbial similarity

Group identity was the most important measured factor explaining variation in gut microbial profiles. Using PERMANOVA on similarity matrices on the whole  $S_0$  data, none of the individual variation was independent from the Family Group effect on microbial composition (p = .43 after controlling for Month and Family Group). Using  $S_1$  data (one sample per individual) and controlling for time, age and sex, up to 28% of the variation could be explained by group membership ( $R^2 = .28$ , p < .01). As a further validation of the importance of social groups, K-means partitioning of the  $S_1$  data into eight groups (the number of family groups in the dataset) corresponded closely with actual group membership ( $\chi^2 = 130$ ,  $p = 3.38e^{-12}$ ). Figure 2 shows PCoA ordination of samples, clustering together according to family group membership and further forming three superclusters for unknown reason.

The pronounced effect of family group identity on the similarity of gut microbiota was not due to differences in alpha diversity



**FIGURE 2** Lemur family groups have distinct gut microbiota. PCoA orientation shows how S1 data cluster according to eight family groups represented by colour and connected with lines (clusters differ from each other,  $R^2 = .08$ , p = .014 and match family groups p < .01) and form three bigger clusters for unknown reason ( $R^2 = .24$ , p = .001)

between groups ( $S_{0,}$ , GEE model, controlling for time, sex, age, pregnancy, p = .13). Rather, between-group differences in microbiota were best characterized by differential relative abundances (indicated by 10 OTUs with the highest random forest Mean Decrease Accuracy, Data S6) of microbial taxa belonging to classes *Bacteroidia*, *Clostridia* and *Betaproteobacteria*.

Pairwise association indices (AI<sub>Groom</sub> and AI<sub>Huddle</sub>) were both negatively correlated with microbiota dissimilarity (and thus positively correlated with similarity) during Season 2 (both,  $R^2 = .7$ , p < .01), but not during Season 1 (AI<sub>Huddle</sub>, p = .4; AI<sub>Groom</sub>, p = .6). In both behaviours, individuals showed clear patterns of social preference (aggregation of partners was non-random): AI<sub>Groom</sub> values ranged from 0.1 to 0.6, AI<sub>Huddle</sub> values ranged from 0.1 to 0.5.

# 3.3 | Effects of group size and individual sociality on gut microbial alpha diversity

Group size was not correlated with gut microbial alpha diversity. Individual sociality was negatively associated with microbial diversity:  $SI_{Groom}$  was negatively correlated with gut microbial alpha diversity between individuals ( $S_2$  data, controlling for time, p < .01), and a similar correlation was apparent between time points (population-wide decrease in diversity was associated with simultaneous increase in  $SI_{Groom}$ ) (Figure 3a). The individual-level correlation between alpha diversity and sociality was strongest during Season 1 (Figure 3b).  $SI_{Huddle}$  had a similar time-dependent association with alpha diversity as  $SI_{Groom}$ , although it was not significant (Figure 3c).

### 3.4 | Temporal trends in microbiota

Microbial composition itself was also subject to a small seasonal change ( $S_0$  data, monthly change  $R^2 = .04$ , p < .01), driven by a sharp population-wide change in diversity during the time when infants were born (Figure 4a). This seasonal change was associated with decreasing diversity ( $R^2 = -.2$ , p = .01), which was in turn associated with increasing relative abundance of *Gammaproteobacteria*, paralleled by a reduction in the relative abundance of *Clostridia*. Furthermore, individual variation in gut microbial composition was higher during Season 2 both within groups and at the population level (Figure 4b, see also Data S7). While the gut microbiota of pregnant females differed slightly from that of other group members, all individuals experienced similar changes in microbial composition across seasons and after infant birth.

## 4 | DISCUSSION

#### 4.1 | Species-specific trends in gut microbiota

This study shows that social environment is an important modulator of microbiota in red-bellied lemurs. Our findings add to the increasing evidence of group-specific microbiota in highly social species (Bennett et al., 2016; Degnan et al., 2012; Gomez et al., 2015; Leclaire, Nielsen, & Drea, 2014; Song et al., 2013; Theis, Schmidt,



**FIGURE 3** Individual sociality indices are negatively associated with gut microbial alpha diversity. (a) Within the whole population, decreasing Groom indices (SI<sub>Groom</sub>, black-lined circles) are associated with increasing gut microbial alpha diversity (blue filled dots) in time. (b) Among individuals, SIGroom, is negatively correlated with gut microbial alpha diversity in both Seasons. (c) SI<sub>Huddle</sub> is negatively correlated with gut microbial alpha diversity only in Season 1. For summary of temporal changes of mean group-wise microbial dissimilarity and Huddle index, see Data S7

& Holekamp, 2012; Tung et al., 2015) and support the view that individual-level social relationships are associated with microbial similarity (Amato et al., 2017; Kort et al., 2014; Moeller et al., 2016; Tung et al., 2015). Interestingly, we found no correlations between age or sex and overall gut microbial composition. This is remarkable, because individual endocrinology, which varies with age and sex, is commonly thought to be associated with gut microbiota (Markle et al., 2013; Stothart et al., 2016). However, our measures of age were categories of juvenile or adult and more refined estimates of age may yield different results.

## 4.2 | Social association and gut microbial similarity

While genetic factors and diet are likely to strongly affect the composition of microbiota (Benson et al., 2010; Doré & Blottiere, 2015; Goodrich et al., 2014; Khachatryan et al., 2008; Lanyon et al., 2007; Turnbaugh et al., 2009), it is unlikely that these factors explain the majority of observed differences in microbial profiles between family groups. Firstly, as frugivores with a temporally changing diet (Overdorff, 1993; Tecot, 2008), one would expect temporal changes in gut microbiota to be apparent. However, during the course of this study, gradual seasonal changes in the environment and diet were observed (A. Raulo, personal communication), but temporal variation in gut microbial composition remained small compared to the differences observed across groups. This finding suggests that environmental fluctuations had only a relatively small effect on the gut microbiota. However, diet likely does play a role of some kind in both the observed temporal changes as well as group differences, and this is currently being analysed with data from the same population. Secondly, groups had some territory overlap and were observed feeding in the same trees on different occasions, but clustering patterns of microbiota did not follow their general geographical distribution (A. Raulo, personal communication). Finally, within each red-bellied lemur family group, the gut microbial composition of the breeding pair (presumably not closely related) differed from each other as much as from their presumed offspring (closely related), suggesting that genetic relatedness may have little effect on microbial similarity within groups, although this needs to be formally tested. We are currently exploring this relationship with genetic kinship data on the population level (Diakiw, 2017; L.O. Diakiw, T. Tecot & A. Baden, in prep.). Thus, it is





most likely that group-specific gut microbiota in red-bellied lemurs is largely due to shared social contact, reflecting the social life of this species, with high within-group cohesion and extremely low interaction between members of different groups (Overdorff & Tecot, 2006; Tecot, Singletary, & Eadie 2016).

Shared microbiota are likely to play several important roles in the social context, for example, by affecting social recognition and bonding through synthesizing pheromones crucial for mammalian group-specific scent marks (Archie & Theis, 2011; Douglas & Dobson, 2013; Theis et al., 2012, 2013). More importantly, shared microbiota can be seen as a mechanism of immunity synchronization in a small group. Individuals become accustomed to their gut microbes and indeed the same gut bacteria might be mutualistic or pathogenic depending on the individual or situation (Backhed et al., 2012; Barribeau, Villinger, & Waldman, 2012; Feng & Elson, 2011; Stilling, Bordenstein, Dinan, & Cryan, 2014). Sharing microbiota ensures that all group members are accustomed to similar bacterial communities and will not infect each other with potential pathogens. Concurrently, following the metapopulation theory (Levin, 1974; Wilson, 1992), distributing common microbial allies within a social network enables a more diverse and resilient microbiota to be present within a social group, increasing the group's potential to adapt to changing environments. Extending microbiota beyond an individual evokes interesting evolutionary consequences, and implications for future experimental confirmation. For example, not all socially transmittable microbes are beneficial and an interesting future field of research lies in recognizing situations where an individual can gain beneficial microbial transmission while avoiding pathogenic transmission (Amato, 2016). However, distinguishing pathogenic (or potentially pathogenic) microbes from beneficial ones can be tricky, especially in an endemic species with a large portion of gut microbes unclassified, like in this study. More detailed research, even strain-specific analyses, are needed to separate immunologically challenging and beneficial taxa. When we have an idea of the highresolution taxonomic diversity of microbiota, we can start to map the extent to which individuals choose their social company with respect to current immunological challenges, for example, by avoiding conspecifics with dissimilar microbiota and preferring the company of those with more similar microbiota (see Barribeau et al., 2012; Sharon et al., 2010). Social preference for similar microbiota could lead to social discrimination patterns between individuals with a different initial genetic basis for immunity (e.g. MHC genotype). This can conflict with adaptive mating strategies (e.g. mating with a dissimilar MHC genotype: Hamilton & Zuk, 1982; Apanius, Penn, Slev, Ruff, & Potts, 1997), inducing a fluctuating trade-off between current immunological benefits and the immunological quality of offspring.

Given that the family groups with offspring were very small (three to six individuals) and generally all individuals interacted with each other, it is remarkable that even within-group patterns of association were related to gut microbial similarity, although only during Season 2. Thus, some members of the group were more tightly bonded, spending more time grooming and huddling with each other, and this was reflected in their gut microbial similarity. This result is interesting, because if social contact can function as a transmission route for microbes (Kort et al., 2014; Kulkarni & Heeb, 2007; Moeller et al., 2016; Tung et al., 2015), these microbes can carry information of social contact as well.

## 4.3 | Amount of social contact and gut microbial alpha diversity

Sharing microbiota might be an underestimated force behind groupwide immunity. However, contrary to our expectations we found generally no support for the common idea that increasing social contact would increase gut microbial alpha diversity. Specifically, we found no correlation between group size and alpha diversity in this species, possibly due to small variation between group sizes (N = 2-6 individuals). Furthermore, although social association influenced microbial

transmission, individual sociality (i.e. SI<sub>Groom</sub>) was negatively correlated with gut microbial alpha diversity. There are several plausible explanations for this. First, sharing microbiota within social groups could lead to an enrichment of certain bacteria present in the community resulting in decreasing diversity estimates in weighted diversity indices, such as the Shannon index. This might be especially pronounced if high values of individual sociality indices are due to intense interaction with just one other individual instead of many. Another likely reason for the observed pattern is that both lower alpha diversity and intense social behaviour are caused by a third factor, such as stress. Stress is known to increase affiliative behaviour in primates (Aureli, Cords, & Van Schaik, 2002; Engh et al., 2006) and reduce gut microbial diversity (Bailey et al., 2011; Stothart et al., 2016). In accordance, preliminary analyses on samples from the same population have shown that individual faecal cortisol levels are correlated with microbial composition in our study population (S. Tecot, A. Baden, unpubl.; see Data S8), although more data are needed to explore whether cortisol levels are associated with overall diversity or other aspects of gut microbiota. Taken together, understanding how taxonomic subsets with different transmission dynamics construct the composition of microbiota calls for more research, which should take into account the effects of both transmission (which microbes can enter the gut) as well as host physiology, genotype and co-infection dynamics (who can establish a population in the gut).

## 4.4 | Temporal dynamics in microbiota, and social behaviour

Interestingly, individual sociality (SI<sub>Groom</sub>) was correlated with gut microbial alpha diversity more strongly during Season 1 (*before* infants were born) yet social association within the group (AI<sub>Groom</sub> and AI<sub>Huddle</sub>) predicted gut microbial similarity only during Season 2 (*after* infants were born). These trends are best explained by pervasive larger scale temporal patterns in microbiota and behaviour. Importantly, in the transition between Seasons 1 and 2, individuals tended to become less similar in microbial composition (variation in similarity and diversity increase) as well as more distant in their social relationships (mean AI<sub>Groom</sub> was 0.4 before and 0.28 after infant birth). Thus, the delicate trend of social association predicting microbial similarity becomes visible only when individual variation is high enough. On the other hand, the trend of individual sociality correlating with microbial diversity is lost to the overall decreasing diversity during Season 2.

A major part of the gradual change in gut microbiota is likely caused by a shift in diet (Doré & Blottiere, 2015; Turnbaugh et al., 2009). However, there was a clear population level shift towards lower microbial diversity and similarity at the time when infants were born to groups. While coinciding dietary changes might play a role here as well, an alternative explanation is offered by the major changes in hormonal profiles associated with infant birth. In our study population, just before infant birth hormonal profiles are also most similar between individuals (Tecot, 2008; S. Tecot et al., in prep, see also Data S8) and sociality indices are highest, reflecting the system of allomaternal care in the species (Tecot, Baden, Romine, & Kamilar, 2012; Tecot et al., 2013; Tecot, & Baden, in press), where all group members reflect the hormonal status of the pregnant female (in red-bellied lemurs: Tecot, 2008; Tecot, Singletary, & Eadie 2016; in other species with allomaternal care: Storey, Walsh, Quinton, & Wynne-Edwards, 2000; Nunes, Fite, Patera, & French, 2001; Ziegler, Washabaugh, & Snowdon, 2004). Thus, synchronized microbiota prior to infant birth might be a result of increased transmission with higher sociality indices, or alternatively, a result of synchronized hormonal profiles. The population-level shift in gut microbiota prior to infant birth was associated with increasing abundance of Proteobacteria and decreasing Firmicutes. In humans, both of these trends in gut microbial composition are linked to third trimester changes in pregnant women's microbiota (Koren et al., 2012), that are likely under hormonal control. This pattern has been suggested to represent an adaptive adjustment of the microbiota to enhance beneficial maternal transmission to offspring and reduce the risk of pathogen infection from the mother to the infant (Koren et al., 2012).

# 5 | CONCLUSIONS AND FUTURE DIRECTIONS

Along with the effects of diet, physiology, environment, and genes described elsewhere, habitual social contact seems to be important in determining aspects of an individual's microbiota. In addition to early maternal exposure, it appears that social environment may continue to modify an individual's microbial community. In turn, microbes might modify social behaviour through their effects on the central nervous system (microbe-gut-brain axis) and hormones. The emerging understanding of these reciprocal interactions between the host and the mutualist microbiota can inspire novel perspectives on the evolution of social systems.

However, to understand the role of microbiota in the evolution and ecology of host social systems, one needs to consider a range of processes occurring at scales both within and among hosts. We hope that future research will be conducted on (1) the interplay between individual genotype/physiology and transmission dynamics in affecting the composition and function of microbiota; (2) combined effects of dietary differences and population's social and spatial structure on gut microbial composition; (3) the effects of different social systems (different patterns of connectedness) on the microbiota and immune function of the species; and (4) the identity of the specific subset of microbiota that relies largely on social transmission. Socially transmittable microbiota might be an important synchronizing force in a tightly bonded social group. Synchronizing immunity, endocrine profiles and subsequent behavioural responsiveness allows more cohesive sociality and effective cooperation. In the evolution of cooperative groups, individuals increasingly work "as one." Thus, when the functional unit of the species shifts from individuals towards groups, immunological, physiological and behavioural synchrony become more adaptive. The evolution of vertebrate social systems has been long seen through trading aspects of individual fitness. However, in the world of holobionts (Zilber-Rosenberg & Rosenberg, 2008), units of hosts and their

microbiota, there are no simple alliances: evolving social systems are restructuring ecosystems by affecting patterns of connectedness between hosts, and thus also microbial meta-populations. These connections in turn create whole new niches for microbial life.

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### AUTHORS' CONTRIBUTIONS

A.R., A.L., S.R.T. and A.L.B. conceived the ideas and designed methodology regarding the data collection and behavioural analyses; R.K., K.A., R.S., K.E.N. and B.W. designed and carried out the microbial analyses; A.R. and A.L. collected the data; A.R. and L.R. analysed the data; A.R. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

#### DATA ACCESSIBILITY

The raw data can be openly accessed at European Nucleotide Archive http://www.ebi.ac.uk/ena/data/view/PRJEB22571.

The metadata for demographic information and sociality indices are available as online Supporting Information.

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#### REFERENCES

- Alexander, R. D. (1974). The evolution of social behavior. Annual Review of Ecology and Systematics, 5, 325–383.
- Altizer, S., Nunn, C. L., Thrall, P. H., Gittleman, J. L., Antonovics, J., Cunningham, A. A., ... Pulliam, J. R. C. (2003). Social organization and parasite risk in mammals: Integrating theory and empirical studies. *Annual Review of Ecology Evolution and Systematics*, 34, 517–547.

- Altmann, J. (1974). Observational study of behavior: Sampling methods. *Behaviour*, 49, 227–267.
- Amato, K. R. (2016). Incorporating the gut microbiota into models of human and non-human primate ecology and evolution. *American Journal of Physical Anthropology*, 159, 196–215.
- Amato, K. R., Martinez-Mota, R., Righini, N., Raguet-Schofield, M., Corcione, F. P., Marini, E., ... Leigh, S. R. (2016). Phylogenetic and ecological factors impact the gut microbiota of two Neotropical primate species. *Oecologia*, 180, 717–733.
- Amato, K. R., Van Belle, S., Di Fiore, A., Estrada, A., Stumpf, R., White, B., ... Leigh, S. R. (2017). Patterns in gut microbiota similarity associated with degree of sociality among sex classes of a neotropical primate. *Microbial Ecology*, 74, 250–258.
- Amato, K. R., Yeoman, C. J., Kent, A., Righini, N., Carbonero, F., Estrada, A., ... Gillis, M. (2013). Habitat degradation impacts black howler monkey (*Alouatta pigra*) gastrointestinal microbiomes. *The ISME Journal*, 7, 1344–1353.
- Apanius, V., Penn, D., Slev, P. R., Ruff, L. R., & Potts, W. K. (1997). The nature of selection on the major histocompatibility complex. *Critical Reviews in Immunology*, 17, 179–224.
- Archie, E. A., & Theis, K. R. (2011). Animal behaviour meets microbial ecology. Animal Behaviour, 82, 425–436.
- Aureli, F., Cords, M., & Van Schaik, C. P. (2002). Conflict resolution following aggression in gregarious animals: A predictive framework. *Animal Behaviour*, 64, 325–343.
- Backhed, F., Fraser, C. M., Ringel, Y., Sanders, M. E., Sartor, R. B., Sherman, P. M., ... Finlay, B. B. (2012). Defining a healthy human gut microbiome: Current concepts, future directions, and clinical applications. *Cell Host* & *Microbe*, 12, 611–622.
- Bailey, M. T., Dowd, S. E., Galley, J. D., Hufnagle, A. R., Allen, R. G., & Lyte, M. (2011). Exposure to a social stressor alters the structure of the intestinal microbiota: Implications for stressor-induced immunomodulation. *Brain Behavior and Immunity*, 25, 397–407.
- Barribeau, S. M., Villinger, J., & Waldman, B. (2012). Ecological immunogenetics of life-history traits in a model amphibian. *Biology Letters*, 8, 405–407.
- Bennett, G., Malone, M., Sauther, M. L., Cuozzo, F. P., White, B., Nelson, K. E., ... Amato, K. R. (2016). Host age, social group, and habitat type influence the gut microbiota of wild ring-tailed lemurs (*Lemur catta*). *American Journal of Primatology*, 78, 883–892.
- Benson, A. K., Kelly, S. A., Legge, R., Ma, F. R., Low, S. J., Kim, J., ... Pomp, D. (2010). Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 18933–18938.
- Blaser, M. J., & Falkow, S. (2009). What are the consequences of the disappearing human microbiota? *Nature Reviews Microbiology*, 7, 887–894.
- Bravo, J. A., Forsythe, P., Chew, M. V., Escaravage, E., Savignac, H. M., Dinan, T. G., ... Cryan, J. F. (2011). Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proceedings of the National Academy of Sciences of the United States of America, 108, 16050–16055.
- Breiman, L. (2001). Random forests. Machine Learning, 45, 5-32.
- Cairns, S. J., & Schwager, S. J. (1987). A comparison of association indices. Animal Behaviour, 35, 1454–1469.
- Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., ... Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal*, 6, 1621–1624.
- Chen, J. (2012). GUniFrac: Generalized UniFrac distances. R package version 1.0. Retrieved from http://CRAN.R-project.org/package=GUni Frac
- Crumeyrolle-Arias, M., Jaglin, M., Bruneau, A., Vancassel, S., Cardona, A., Dauge, V., ... Rabot, S. (2014). Absence of the gut microbiota enhances

anxiety-like behavior and neuroendocrine response to acute stress in rats. *Psychoneuroendocrinology*, 42, 207–217.

- Cryan, J. F., & Dinan, T. G. (2012). Mind-altering microorganisms: The impact of the gut microbiota on brain and behaviour. *Nature Reviews Neuroscience*, 13, 701–712.
- Degnan, P. H., Pusey, A. E., Lonsdorf, E. V., Goodall, J., Wroblewski, E. E., Wilson, M. L., ... Ochman, H. (2012). Factors associated with the diversification of the gut microbial communities within chimpanzees from Gombe National Park. Proceedings of the National Academy of Sciences of the United States of America, 109, 13034–13039.
- Diakiw, L. (2017). Determinants of the adult microbiome: Kinship, dispersal, and social relationships. MA thesis, University of Arizona, Tucson, AZ.
- Dillon, R. J., Vennard, C. T., Buckling, A., & Charnley, A. K. (2005). Diversity of locust gut bacteria protects against pathogen invasion. *Ecology Letters*, 8, 1291–1298.
- Dinan, T. G., & Cryan, J. F. (2012). Regulation of the stress response by the gut microbiota: Implications for psychoneuroendocrinology. *Psychoneuroendocrinology*, 37, 1369–1378.
- Doré, J., & Blottiere, H. (2015). The influence of diet on the gut microbiota and its consequences for health. *Current Opinion in Biotechnology*, 32, 195–199.
- Douglas, A. E., & Dobson, A. J. (2013). New synthesis: Animal communication mediated by microbes: Fact or fantasy? *Journal of Chemical Ecology*, 39, 1149.
- Drewe, J. A. (2009). Who infects whom? Social networks and tuberculosis transmission in wild meerkats. Proceedings of the Royal Society B-Biological Sciences, 277, 633–642.
- Engh, A. L., Beehner, J. C., Bergman, T. J., Whitten, P. L., Hoffmeier, R. R., Seyfarth, R. M., & Cheney, D. L. (2006). Behavioural and hormonal responses to predation in female chacma baboons (*Papio hamadryas* ursinus). Proceedings of the Royal Society B-Biological Sciences, 273, 707-712.
- Ezenwa, V. O., Gerardo, N. M., Inouye, D. W., Medina, M., & Xavier, J. B. (2012). Animal behavior and the microbiome. *Science*, 338, 198–199.
- Feng, T., & Elson, C. O. (2011). Adaptive immunity in the host-microbiota dialog. *Mucosal Immunology*, 4, 15–21.
- Flint, H. J., & Bayer, E. A. (2008). Plant cell wall breakdown by anaerobic microorganisms from the mammalian digestive tract. *Incredible Anaerobes: From Physiology to Genomics to Fuels*, 1125, 280–288.
- Friswell, M. K., Gika, H., Stratford, I. J., Theodoridis, G., Telfer, B., Wilson, I. D., & McBain, A. J. (2010). Site and strain-specific variation in gut microbiota profiles and metabolism in experimental mice. *PLoS ONE*, 5, e8584.
- Gilbert, J. A. (2015). Social behavior and the microbiome. Elife, 4, e07322.
- Ginsberg, J. R., & Young, T. P. (1992). Measuring association between individuals or groups in behavioural studies. *Animal Behaviour*, 44, 377–379.
- Godfrey, S. S., Moore, J. A., Nelson, N. J., & Bull, C. M. (2010). Social network structure and parasite infection patterns in a territorial reptile, the tuatara (Sphenodon punctatus). International Journal for Parasitology, 40, 1575–1585.
- Gomez, A., Petrzelkova, K., Yeoman, C. J., Vlckova, K., Mrazek, J., Koppova, I., ... Leigh, S. R. (2015). Gut microbiome composition and metabolomic profiles of wild western lowland gorillas (*Gorilla gorilla gorilla*) reflect host ecology. *Molecular Ecology*, 24, 2551–2565.
- Goodrich, J. K., Waters, J. L., Poole, A. C., Sutter, J. L., Koren, O., Blekhman, R., ... Ley, R. E. (2014). Human genetics shape the gut microbiome. *Cell*, 159, 789–799.
- Griffin, R. H., & Nunn, C. L. (2012). Community structure and the spread of infectious disease in primate social networks. *Evolutionary Ecology*, 26, 779–800.
- Gunderson, L. H. (2000). Ecological resilience In theory and application. Annual Review of Ecology and Systematics, 31, 425–439.
- Gwet, K. L. (2008). Computing inter-rater reliability and its variance in the presence of high agreement. British Journal of Mathematical & Statistical Psychology, 61, 29–48.

- Haegeman, B., Hamelin, J., Moriarty, J., Neal, P., Dushoff, J., & Weitz, J. S. (2013). Robust estimation of microbial diversity in theory and in practice. *ISME Journal*, 7, 1092–1101.
- Hamilton, W. D., & Zuk, M. (1982). Heritable true fitness and bright birds A role for parasites. *Science*, *218*, 384–387.
- Hooper, L. V., Littman, D. R., & Macpherson, A. J. (2012). Interactions between the microbiota and the immune system. *Science*, 336, 1268–1273.
- Kato, L. M., Kawamoto, S., Maruya, M., & Fagarasan, S. (2014). The role of the adaptive immune system in regulation of gut microbiota. *Immunological Reviews*, 260, 67–75.
- Keeney, K. M., & Finlay, B. B. (2011). Enteric pathogen exploitation of the microbiota-generated nutrient environment of the gut. *Current Opinion in Microbiology*, 14, 92–98.
- Khachatryan, Z. A., Ktsoyan, Z. A., Manukyan, G. P., Kelly, D., Ghazaryan, K. A., & Aminov, R. I. (2008). Predominant role of host genetics in controlling the composition of gut microbiota. *PLoS ONE*, 3, e3064.
- Koren, O., Goodrich, J. K., Cullender, T. C., Spor, A., Laitinen, K., Backhed, H. K., ... Ley, R. E. (2012). Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell*, 150, 470–480.
- Kort, R., Caspers, M., van de Graaf, A., van Egmond, W., Keijser, B., & Roeselers, G. (2014). Shaping the oral microbiota through intimate kissing. *Microbiome*, 2, 41.
- Kulkarni, S., & Heeb, P. (2007). Social and sexual behaviours aid transmission of bacteria in birds. *Behavioural Processes*, 74, 88–92.
- Lanyon, C. V., Rushton, S. P., O'Donnell, A. G., Goodfellow, M., Ward, A. C., Petrie, M., ... Penn, D. J. (2007). Murine scent mark microbial communities are genetically determined. *FEMS Microbiology Ecology*, 59, 576–583.
- Leclaire, S., Nielsen, J. F., & Drea, C. M. (2014). Bacterial communities in meerkat anal scent secretions vary with host sex, age, and group membership. *Behavioral Ecology*, 25, 996–1004.
- Legendre, P., & Legendre, L. F. (2012). *Numerical ecology*. Amsterdam, the Netherlands: Elsevier.
- Levin, S. A. (1974). Dispersion and population interactions. *The American Naturalist*, 108, 207–228.
- Levine, J. M., & D'Antonio, C. M. (1999). Elton revisited: A review of evidence linking diversity and invasibility. Oikos, 87, 15–26.
- Liaw, A., & Wiener, M. (2002). Classification and regression by randomForest. R News, 2, 18–22.
- Lombardo, M. P. (2008). Access to mutualistic endosymbiotic microbes: An underappreciated benefit of group living. *Behavioral Ecology and Sociobiology*, 62, 479–497.
- Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K., & Knight, R. (2012). Diversity, stability and resilience of the human gut microbiota. *Nature*, 489, 220–230.
- MacIntosh, A., Jacobs, A., Garcia, C., Shimizu, K., Mouri, K., Huffman, M. A., & Hernandez, A. D. (2012). Monkeys in the middle: Parasite transmission through the social network of a wild primate. *PLoS ONE*, 7, e51144.
- Mackie, R. I. (2000). Mutualistic fermentative digestion in the gastrointestinal tract: Diversity and evolution. *American Zoologist*, 40, 1112.
- Markle, J. G., Frank, D. N., Mortin-Toth, S., Robertson, C. E., Feazel, L. M., Rolle-Kampczyk, U., ... Danska, J. S. (2013). Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. *Science*, 339, 1084–1088.
- McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R Package for reproducible interactive analysis and graphics of microbiome census data. *PLoS* ONE, 8, e61217.
- McMurdie, P. J., & Holmes, S. (2014). Waste not, want not: why rarefying microbiome data is inadmissible. *PLoS computational biology*, 10, e1003531.
- Moeller, A. H., Foerster, S., Wilson, M. L., Pusey, A. E., Hahn, B. H., & Ochman, H. (2016). Social behavior shapes the chimpanzee panmicrobiome. *Science Advances*, 2, e1500997.
- Moller, A. P., Dufva, R., & Allander, K. (1993). Parasites and the evolution of host social-behavior. Advances in the Study of Behavior, 22, 65–102.

- Montiel-Castro, A. J., González-Cervantes, R. M., Bravo-Ruiseco, G., & Pacheco-López, G. (2013). The microbiota-gut-brain axis: Neurobehavioral correlates, health and sociality. Frontiers in Integrative Neuroscience, 7, 63–78.
- Nunes, S., Fite, J. E., Patera, K. J., & French, J. A. (2001). Interactions among paternal behavior, steroid hormones, and parental experience in male marmosets (*Callithrix kuhlii*). *Hormones and Behavior*, 39, 70–82.
- Oksanen, J., Blanchet, G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., ... Wagner, H. (2014). vegan: Community Ecology Package. R package version 2.2-0. Retrieved from http://CRAN.R-project.org/ package=vegan
- Overdorff, D. J. (1993). Similarities, differences, and seasonal patterns in the diets of Eulemur rubriventer and Eulemur fulvus rufus in the Ranomafana National Park, Madagascar. International Journal of Primatology, 14, 721–753.
- Overdorff, D. J. (1996). Ecological correlates to activity and habitat use of two prosimian primates: Eulemur rubriventer and Eulemur fulvus rufus in Madagascar. American Journal of Primatology, 40, 327–342.
- Overdorff, D. J., & Tecot, S. (2006). Social pair-bonding and resource defense in wild red-bellied lemurs (*Eulemur rubriventer*). In L. Gould, & M. L. Sauther (Eds.), *Lemurs: Ecology and adaptation* (pp. 235–254). New York, NY: Springer.
- R Core Team. (2014). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org/
- Rimbach, R., Bisanzio, D., Galvis, N., Link, A., Di Fiore, A., & Gillespie, T. R. (2015). Brown spider monkeys (*Ateles hybridus*): A model for differentiating the role of social networks and physical contact on parasite transmission dynamics. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 370, 20140110.
- Roberts, D. W. (2013). labdsv: Ordination and multivariate analysis for ecology. R package version 1.6-1. Retrieved from http://CRAN.R-project. org/package=labdsv
- Rosengaus, R. B., Zecher, C. N., Schultheis, K. F., Brucker, R. M., & Bordenstein, S. R. (2011). Disruption of the termite gut microbiota and its prolonged consequences for fitness. *Applied and Environmental Microbiology*, 77, 4303–4312.
- Ruokolainen, L., Ikonen, S., Makkonen, H., & Hanski, I. (2016). Larval growth rate is associated with the composition of the gut microbiota in the Glanville fritillary butterfly. *Oecologia*, 181, 895–903.
- Sharon, G., Segal, D., Ringo, J. M., Hefetz, A., Zilber-Rosenberg, I., & Rosenberg, E. (2010). Commensal bacteria play a role in mating preference of Drosophila melanogaster. Proceedings of the National Academy of Sciences of the United States of America, 107, 20051–20056.
- Shin, S. C., Kim, S. H., You, H., Kim, B., Kim, A. C., Lee, K. A., ... Lee, W. J. (2011). Drosophila microbiome modulates host developmental and metabolic homeostasis via insulin signaling. *Science*, 334, 670–674.
- Song, S. J., Amir, A., Metcalf, J. L., Amato, K. R., Xu, Z. Z., Humphrey, G., & Knight, R. (2016). Preservation methods differ in fecal microbiome stability, affecting suitability for field studies. *MSystems*, 1, e00021–16.
- Song, S. J., Lauber, C., Costello, E. K., Lozupone, C. A., Humphrey, G., Berg-Lyons, D., ... Fierer, N. (2013). Cohabiting family members share microbiota with one another and with their dogs. *Elife*, 2, e00458.
- Stilling, R. M., Bordenstein, S. R., Dinan, T. G., & Cryan, J. F. (2014). Friends with social benefits: Host-microbe interactions as a driver of brain evolution and development? Frontiers in Cellular and Infection Microbiology, 4, 147.
- Storey, A. E., Walsh, C. J., Quinton, R. L., & Wynne-Edwards, K. E. (2000). Hormonal correlates of paternal responsiveness in new and expectant fathers. *Evolution and Human Behavior*, 21, 79–95.
- Stothart, M. R., Bobbie, C. B., Schulte-Hostedde, A. I., Boonstra, R., Palme, R., Mykytczuk, N., & Newman, A. E. (2016). Stress and the microbiome: Linking glucocorticoids to bacterial community dynamics in wild red squirrels. *Biology Letters*, 12, 20150875.
- Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X. N., ... Koga, Y. (2004). Postnatal microbial colonization programs the

hypothalamic-pituitary-adrenal system for stress response in mice. *Journal of Physiology-London*, 558, 263–275.

- Tecot, S. (2008). Seasonality and predictability: The hormonal and behavioral responses of the red-bellied lemur, Eulemur rubriventer, in southeastern Madagascar. Dissertation, The University of Texas at Austin, Austin, TX.
- Tecot, S. R. (2010). It's all in the timing: Birth seasonality and infant survival in Eulemur rubriventer. International Journal of Primatology, 31, 715–735.
- Tecot, S. R. (2013). Variable energetic strategies in disturbed and undisturbed rain forests: *Eulemur rubriventer* fecal cortisol levels in south-eastern Madagascar. In J. Masters, M. Gamba, & F. Genin (Eds.), *Leaping ahead* (pp. 185–195). New York, NY: Springer.
- Tecot, S. R., & Baden, A. L. (In Press). Profiling caregivers: Hormonal variation underlying allomaternal care in wild red-bellied lemurs. *Physiology* and Behavior.
- Tecot, S., Baden, A., Romine, N., & Kamilar, J. (2012). Infant parking and nesting, not allomaternal care, influence Malagasy primate life histories. *Behavioral Ecology and Sociobiology*, 66, 1375–1386.
- Tecot, S., Baden, A., Romine, N., & Kamilar, J. (2013). Reproductive strategies and infant care in Malagasy primates. In K. B. H. Clancy, K. Hinde, & J. N. Rutherford (Eds.), Building babies: Primate development in proximate and ultimate perspective (Developments in primatology: Progress and prospects) (pp. 321–359). New York, NY: Springer.
- Tecot, S., Singletary, B., & Eadie, E. (2016). Why "monogamy" isn't good enough. American Journal of Primatology, 78, 340–354.
- Theis, K. R., Schmidt, T. M., & Holekamp, K. E. (2012). Evidence for a bacterial mechanism for group-specific social odors among hyenas. *Scientific Reports*, 2, 615.
- Tecot, S., Singletary, B., & Eadie, E. (2016). Why "monogamy" isn't good enough. American Journal of Primatology, 78, 340–354.
- Theis, K. R., Venkataraman, A., Dycus, J. A., Koonter, K. D., Schmitt-Matzen, E. N., Wagner, A. P., ... Schmidt, T. M. (2013). Symbiotic bacteria appear to mediate hyena social odors. *Proceedings of the National Academy* of Sciences of the United States of America, 110, 19832–19837.
- Troyer, K. (1984). Microbes, herbivory and the evolution of social-behavior. Journal of Theoretical Biology, 106, 157–169.
- Tung, J., Barreiro, L. B., Burns, M. B., Grenier, J. C., Lynch, J., Grieneisen, L. E., ... Archie, E. A. (2015). Social networks predict gut microbiome composition in wild baboons. *Elife*, 4, e05224.
- Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, 444, 1027–1031.

- Turnbaugh, P. J., Ridaura, V. K., Faith, J. J., Rey, F. E., Knight, R., & Gordon, J. I. (2009). The Effect of diet on the human gut microbiome: A Metagenomic analysis in humanized gnotobiotic mice. *Science Translational Medicine*, 1, 6ra14.
- Turnbull, C., Hoggard, S., Gillings, M., Palmer, C., Stow, A., Beattie, D., ... Beattie, A. (2011). Antimicrobial strength increases with group size: Implications for social evolution. *Biology Letters*, 7, 249–252.
- Whitehead, H. (2008). Analyzing animal societies: Quantitative methods for vertebrate social analysis. Chicago, IL: University of Chicago Press.
- Wickham, H. (2009). ggplot2 elegant graphics for data analysis introduction. Berlin, Germany: Springer Science and Business Media.
- Wilson, D. S. (1992). Complex interactions in metacommunities, with implications for biodiversity and higher levels of selection. *Ecology*, 73, 1984–2000.
- Ziegler, T. E., Washabaugh, K. F., & Snowdon, C. T. (2004). Responsiveness of expectant male cotton-top tamarins, *Saguinus oedipus*, to mate's pregnancy. *Hormones and Behavior*, 45, 84–92.
- Zilber-Rosenberg, I., & Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: The hologenome theory of evolution. *FEMS Microbiology Reviews*, 32, 723–735.
- Zohdy, S., Kemp, A. D., Durden, L. A., Wright, P. C., & Jernvall, J. (2012). Mapping the social network: Tracking lice in a wild primate population (*Microcebus rufus*) to infer social contacts and vector potential. *Integrative and Comparative Biology*, 52, E200.

### SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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