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RESEARCH ARTICLE

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Divergence in gut microbial communities mirrors a social group fission event in a black-and-white colobus monkey (*Colobus vellerosus*)

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Host behavior and social factors have increasingly been implicated in structuring the composition of gut microbial communities. In social animals, distinct microbial communities characterize different social groups across a variety of taxa, although little longitudinal research has been conducted that demonstrates how this divergence occurs. Our study addresses this question by characterizing the gut microbial composition of an African Old World monkey, the black-and-white colobus (*Colobus vellerosus*), before and after a social group fission event. Gut microbial taxonomic composition of these monkeys was profiled using the V-4 hypervariable region of the bacterial 16S ribosomal RNA gene, and pairwise-relatedness values were calculated for all individuals using 17 short tandem repeat loci and partial pedigree information. The two social groups in this study were found to harbor distinct microbial signatures after the fission event from which they emerged, while these communities were not divergent in the same individuals before this event. Three genera were found to differ in abundance between the two new social groups: *Parabacteroides*, *Coprococcus*, and *Porphyromonadaceae*. Additionally, although this fission happened partially along lines of relatedness, relatedness did not structure the differences that we found. Taken together, this study suggests that distinct gut microbial profiles can emerge in social groups in <1 year and recommends further work into more finely mapping the timescales, causes, and potentially adaptive effects of this recurring trend toward distinct group microbial signatures.

KEYWORDS
group fission, gut microbiome, social groups, sociality

1 | INTRODUCTION

The mammalian gut harbors a dynamic microbial community which contributes to host physiology, metabolism, and defense (Barbáchano et al., 2017; Cho & Blaser, 2012; Huttenhower et al., 2012). This community both shapes host phenotypes and is shaped by host characteristics that can include phylogeny, genetic variation, environment, and spatial distribution (Amato, 2013; Amato et al., 2016; Barelli et al., 2015; Leamy et al., 2014; Wu et al., 2011). Moreover, behavior and social context can contribute to gut microbiome

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composition, and diet choice, habitat use, mate choice, and social networks have all been shown to modulate gut diversity (Archie & Tung, 2015; Ezenwa, Gerardo, Inouye, Medina, & Xavier, 2012). In fact, the social transmission of beneficial microbes has been cited as one of the benefits associated with group living (Lombardo, 2008), and it has been suggested that more similar gut communities between hosts may confer similar "ecosystem services" to their hosts (Costello, Stagaman, Dethlefsen, Bohannan, & Relman, 2012). Given that conspecifics in the same social group likely encounter highly comparable ecological challenges, the social transmission, and ultimate convergence of a group's gut microbiota into the consortia that provides the most ideal ecosystem services for that particular group's set of demands could prove to be of evolutionary benefit.

Distinguishing the complex and intertwined forces that shape this dynamic community, however, is difficult. Studies of wild populations can help to address this difficulty, providing insight into the forces at play in natural communities as well as how they change over time (Amato, 2013). In particular, studies of wild primates and other highly social animals allow us to answer important questions about how social forces shape these changes in some of our own closest living relatives. For example, in a number of wild primate populations, more closely associated individuals have more homogenous gut microbiome compositions (Amato et al., 2017; Moeller et al., 2016; Perofsky, Lewis, Abondano, Di Fiore, & Meyers, 2017) and distinct gut microbiota characterize different social groups in the same population (Bennett et al., 2016; Degnan et al., 2012; McCord et al., 2014; Springer et al., 2017; Tung et al., 2015). Taken together, these findings highlight the importance of social context in gut microbiome composition. Furthermore, immigrant males which have resided in a new social group for a longer period of time have more similar gut microbiota to the resident males of that group, suggesting that the convergence of group member microbiota may occur over a span of months to years (Grieneisen, Livermore, Alberts, Tung, & Archie, 2017). Because dietary shifts typically alter the composition of microbial communities over a shorter time scale of days to weeks, this finding suggests that distinct group communities are not solely the result of changes in diet (Bonte et al., 2012; Turnbaugh et al., 2009; Williams, Hornig, Parekh, & Lipkin, 2012). However, further studies are needed to more thoroughly examine the time scales over which these convergences occur in natural communities.

Group fission events provide an ideal natural system for interrogating such questions. These are a means of group proliferation in social animals that occur when the costs of living in a certain group have grown to outweigh the benefits (Sueur & Maire, 2014). When this happens, one or more social groups will break off from the original group, oftentimes splitting along lines of relatedness (Snyder-Mackler, Alberts & Bergman, 2014; Widdig et al., 2006). This type of event provides us the opportunity for unique insight into the physiological and behavioral changes in individuals following such an event as well as the time scales over which they occur. For example, fission events and variations in group size have enabled insight into the effects of social context on grooming networks, fertility, and cortisol levels in primates (Dunbar, MacCarron, & Robertson, 2018; Henzi, Lycett, & Weingrill, 1997; Markham, Gesquiere, Alberts, & Altmann, 2015). Here, we report on the gut microbiome compositions of a group of ursine colobus or white-thighed black-and-white colobus (*C. vellerosus*) before, and less than a year after, a fission event. The aim of this study was to examine the plasticity of the gut microbiome shortly following a fission event as a way of gaining insight into the time scale over which microbiomes diverge into the distinct microbiomes that have been shown to characterize different social groups.

2 | METHODS

2.1 | Study system

The Boabeng-Fiema Monkey Sanctuary (BFMS) is a 1.92 km^2 dry semideciduous forest (Hall & Swaine, 1981) located in central Ghana (7°43′N and 1°42′W). Ursine colobus or white-thighed black-and-white colobus (*C. vellerosus*) is one of two diurnal primate species that resides at BFMS (Saj, Teichroeb, & Sicotte, 2005). This is an arboreal, folivorous monkey (Saj & Sicotte 2007a, 2007b) that lives in uni-male or multi-male multi-female groups of 9–38 animals (Kankam & Sicotte, 2013; Wong & Sicotte, 2006). Dispersal is male-biased in this species (Teichroeb, Wikberg, & Sicotte, 2011), although females do show facultative dispersal (Sicotte et al., 2017; Teichroeb et al., 2011; Teichroeb, Wikberg, & Sicotte, 2009; Wikberg, Sicotte, Campos, & Ting, 2012). Female social networks are affected by the presence of infants, kinship, and immigration status, but not by dominance rank (Wikberg, Teichroeb, Bădescu, & Sicotte, 2013; Wikberg et al., 2014a, 2014b; Wikberg, Ting, & Sicotte, 2015). Several groups of *C. vellerosus* have been followed systematically since 2000 for behavioral, demographic, and ecological data (e.g., Bădescu, Sicotte, Ting, & Wikberg, 2015; Teichroeb & Sicotte, 2012). Fecal samples were collected on a regular basis from each focal female in our study groups between 2006 and 2009 (e.g., Wikberg et al., 2015). This study follows the fission of one social group into two daughter groups (named NP and DA) over the course of 1 year (2006–2007).

2.2 | Ethical note

This study was approved by the University of Calgary's Animal Care Committee, and conducted with permission from the Ghana Wildlife Division and the management committee at BFMS. This study adhered to the American Society of Primatologsts' Principles for the Ethical Treatment of Non-Human Primates.

2.3 | Sample collection and short tandem repeat (STR) genotyping

All fecal samples from the study period were collected using masks, fresh gloves, sterile sticks, and sterile tubes to minimize contamination. One to two grams of feces were mixed with 6 μl of RNAlater® (Thermo Fisher Scientific, Waltham, MA) immediately upon collection and stored at −20°C in the field. After shipment to the Ting lab,

samples were again stored at −20°C until DNA extraction. DNA was extracted from two or more samples of each individual using a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) with a slightly modified manufacturer protocol (Wikberg et al., 2012), and negative controls were processed with each round of extraction. Seventeen STR loci were amplified using Qiagen's multiplex polymerase chain reaction (PCR) kit (Qiagen, Hilden, Germany) with a modified protocol and analyzed on an ABI 3730 DNA analyzer (following Wikberg et al., 2012). We determined how many replicates were needed to confirm homozygote genotypes based on real-time PCR DNA quantification (Morin, Chambers, Boesch, & Vigilant, 2001). Two replicates were used to confirm heterozygote genotypes.

2.4 | Gut microbial profiling

We collected metagenomic data from matched genotyped samples from female members of the original social group collected from June to August, 2006 ($n = 12$ samples) and from matched genotyped samples from the same female individuals residing in the two daughter groups from July to August, 2007 (NP with $n = 6$ samples; DA with $n = 6$ samples). DNA was extracted again as above and quantified using a Qubit dsDNA BR Assay Kit protocol using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific). Samples containing at least 1.0 ng/μl were chosen for preparation and sequencing of the V-4 hypervariable region of the bacterial 16S ribosomal RNA gene in the Genomics and Cell Characterization Core Facility at the University of Oregon. Two hundred nanograms of DNA diluted in 10 μl of H_2O were PCR amplified using barcoded Illumina 515F and 806R primers. Targets were amplified in reactions of 1 μl DNA, 1.25 μl of 10 μM primer mix, 10.25 μl H_2O, and 12.5 μl NEB Q5 hot start 2× Master Mix. The thermal cycling profile was as follows: initial denaturing at 98°C for 0:30, 20–30 cycles of 98°C for 0:10, 61°C for 0:20, and 72°C for 0:20, and a final extension of 72°C for 2:00. PCR products were cleaned using Ampure XP beads (Beckman Coulter, Brea, CA), quantified and normalized. Barcoded amplicons were pooled and pair-end sequenced with 150 base pair reads on a partial medium output run on the Illumina NextSeq platform (Illumina, San Diego, CA). Sequences were then demultiplexed and denoised using DADA2 (Bolger, Lohse, & Usadel, 2014). Taxonomic units were assigned using the Qiime2 pipeline. An Operational Taxonomic Unit (OTU) table was generated for samples rarefied to an even sampling depth of 46,040 reads per sample, retaining 1,104,960 sequences for 24 samples. Negative controls were processed at both the extraction and library preparation (PCR) stages, and they were sequenced and carried through the data processing pipelines. No evidence of contamination was found via fluorometry or gel electrophoresis during laboratory work, nor was there any evidence of contaminating sequences in the Illumina reads for the negative controls.

2.5 | Data analyses

Unless otherwise noted, all subsequent statistical analyses were run in R (R Core Team, 2018). To test whether average gut microbial composition differed by social group, samples were analyzed using four groups based on social group at the time of sample collection: original group which became NP after the fission, original group which became DA after the fission, DA, and NP. Beta diversity was calculated for the samples as Bray–Curtis dissimilarity using the *phyloseq* package for R (McMurdie & Holmes, 2013). This metric was selected over metrics accounting for evolutionary relatedness as it represents a quantitative measure of community dissimilarity based on relative abundance without adjusting for the phylogenetic proximity of OTUs, thereby allowing insight into the structure without accounting for relatedness among microbial taxa. We also ran a PERMANOVA using the *adonis* function in the *vegan* package, with social group (prefission DA, prefission NP, postfission DA, postfission NP), age class (subadult and adult), collection site (mature forest and woodland), and reproductive status of the individual (cycling, noncycling, pregnant, and lactating) as predictors in the model. Linear effect size analysis (LefSe) was run for the groups before and after the fission event at a KW α value of 0.01 and with a Linear Discriminant Analysis (LDA) score of 3.0 (Segata et al., 2011).

To assess whether the fission occurred along lines of host relatedness, we calculated relatedness following Wikberg et al. (2012). A Kruskal–Wallis test was run using metrics of average group relatedness for the three social groups (original group, DA, NP). Finally, we determined whether host relatedness explained differences in gut microbial beta diversity seen between groups. We investigated the correlation between the Bray–Curtis dissimilarity matrix and the host relatedness matrix for each group using Mantel tests, and Spearman rank correlation statistics were computed with 999 permutations.

3 | RESULTS

In the summer of 2006, one of our study groups (28 individuals) showed elevated levels of female aggression. Subgroups started to range 50 m apart for periods of time, although the subgroups always convened during the day (Wikberg, unpublished data). *C. vellerosus* typically exhibit a smaller group spread, and 50 m is used to define a between-group encounter in this species (Sicotte & Macintosh, 2004). The home range of the original group spanned approximately 0.20 km^2 through both woodland and mature forest. By May of 2007, this group had fissioned into two daughter groups: NP (10 individuals) and DA (18 individuals; Table 1). These two groups both remained on the original home range, splitting the range after the fission (Figure 1). Both daughter groups ranged in subsets of the original range that included both woodland and mature forest, but DA ranged 0.15 km^2, moreso in woodland areas, while NP's new range was 0.054 km^2, moreso in mature forest after the fission event.

The original group was broken down into two groups for the sake of analysis—those female individuals which eventually split into the DA group and those females that split into the NP group. Average Bray–Curtis dissimilarity was 0.527 ($SD \pm 0.046$) for the two groups before the fission event, while it was 0.538 ($SD \pm 0.048$) for the two social

TABLE 1 Composition of the different social groups before and after the fission event

	Males			Females			Total
	Adults + subadults	Juveniles	Infants	Adults + subadults	Juveniles	Infants	
Prefission	9	0	2	12	4	1	28
Postfission DA	5	2	1	7	3	0	18
Postfission NP	1	0	2	6	0	1	10

Although this study only examined the gut microbial compositions of females, males are included in this table for insight into group composition overall.

groups after the fission event. In the PERMANOVA, age class ($p = 0.378$, $df = 1$, $F = 1.028$), collection site ($p = 0.482$, $df = 2$, $F = 0.992$), and reproductive status ($p = 0.790$, $df = 2$, $F = 0.860$) were not significant predictors of Bray–Curtis dissimilarity, but social group was a significant predictor ($p = 0.001$, $df = 3$, $F = 4.416$). Pairwise comparisons indicated that Bray–Curtis dissimilarity between the two social groups was not significant before the fission ($p = 0.085$, $n = 12$, pseudo $F = 1.23$), while differences after the fission were significant ($p = 0.02$, $n = 12$, pseudo $F = 1.62$). Significant differences were also found between the 2006 and 2007 sampling periods for all groups sampled ($p < 0.01$, $n = 24$, pseudo $F = 2.19$). Group membership explained 7.0% of the variation in gut microbiome diversity, while the largest predictor of variation was sampling year (2006 vs. 2007), explaining 31.4% of the variation in diversity (Figure 2).

The average pairwise-relatedness among females in the social group before the fission was 0.153, while it was 0.232 in the postfission NP group and 0.125 in the postfission DA group (Figure 3). This average pairwise-relatedness increased by 0.079 in the NP social group from the original DA group, suggesting that this fission event may have happened partially along lines of female relatedness, with a group of more closely related female individuals splitting off to form the new NP group. However, the average pairwise-relatedness decreased by 0.028 between the prefission group and the new DA group. The pairwise-relatedness was not statistically different between any of the groups ($p = 0.103$, $df = 2$, $\chi^2 = 4.5518$). The Mantel test comparing Bray–Curtis dissimilarity and relatedness showed no statistically significant correlation between these variables ($r < 0.01$, $p = 0.494$).

On the whole, individual gut samples were vastly dominated by Firmicutes (57–78%) and Bacteriodes (2–13%). The next most prevalent phyla across samples were Tenericutes (3–10%) and Verucomicrobia (<1–16%). LefSe found no differences between the groups before the fission and three genera to differ between the two new social groups after the fission: *Parabacteroides*, *Coprococcus*, and *Porphyromonadaceae* (Figure 4).

4 | DISCUSSION

Distinct gut microbiota characterize different social groups across a wide range of taxa (Amato et al., 2017; Bennett et al., 2016; Degnan et al., 2012; Tung et al., 2015). Examining the process and the time scale over which these divergences occur is important to understand the influence that social context can exert on gut microbiome assembly. Some recent research has focused on the time scale across which an individual's microbiome converges with that of a new social

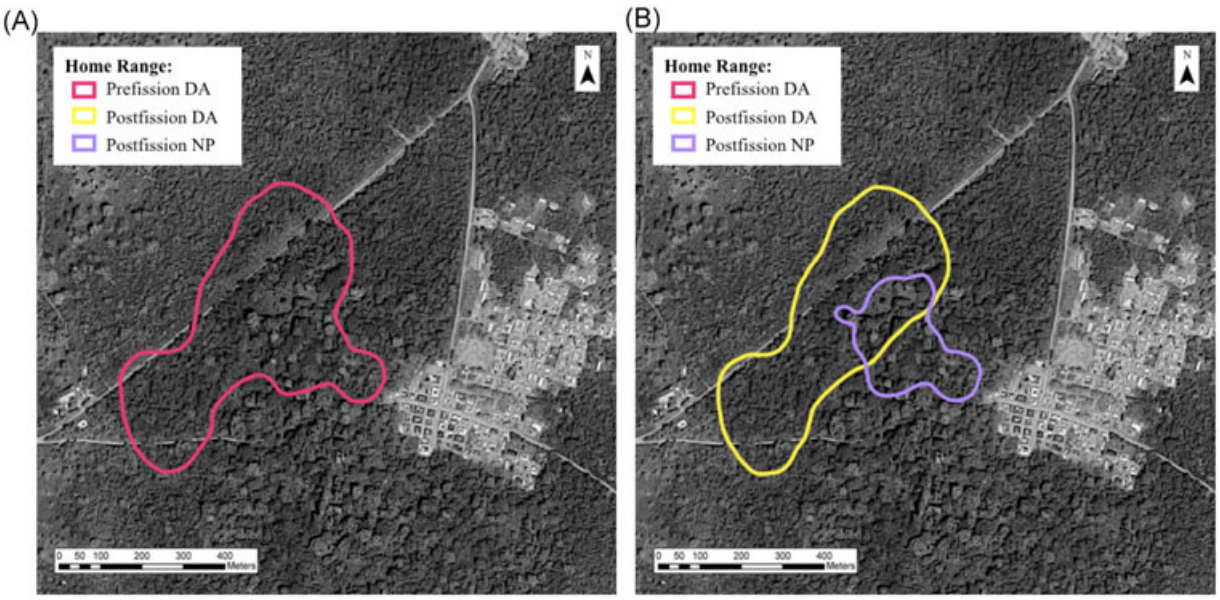

FIGURE 1 Home range distributions of the DA and NP groups before and after the fission event. (A) The original group maintained a large home range in the summer of 2006. (B) The two product groups split the original home range by the summer of 2007

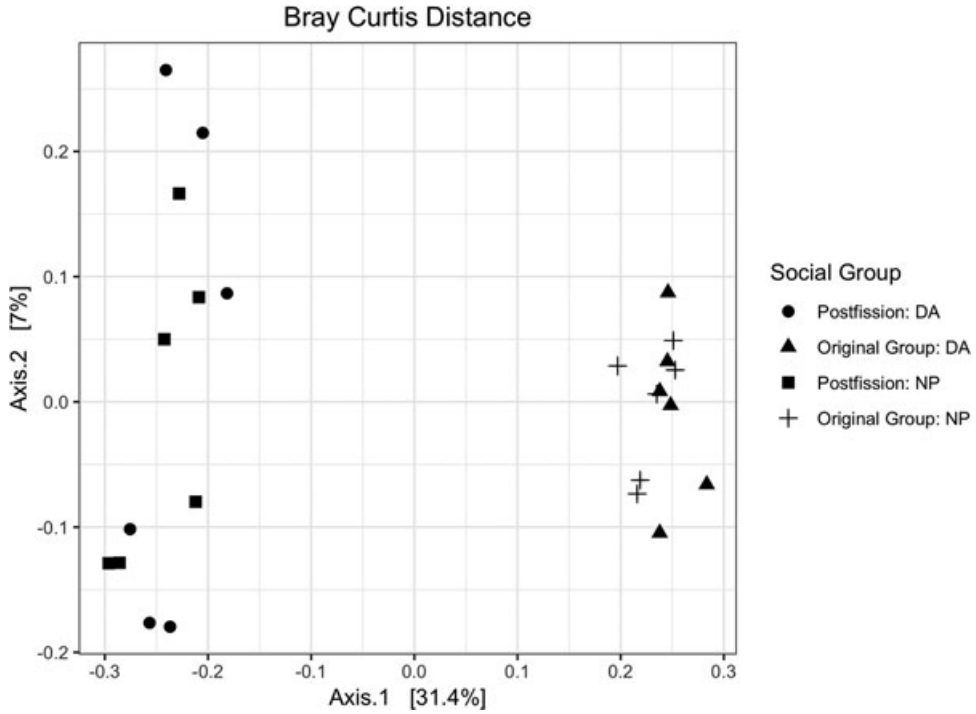

FIGURE 2 Postfission group membership predicts Bray–Curtis dissimilarity of the NP and DA groups. No significant difference in gut microbial diversity was observed before the fission event ($p = 0.085$, $n = 12$, pseudo $F = 1.23$), while <9 months after the fission event these groups showed unique microbial signatures ($p = 0.02$, $n = 12$, pseudo $F = 1.62$). Significant differences were found in gut microbial diversity between all groups across the years sampled ($p < 0.01$, $n = 24$, pseudo $F = 2.19$)

group. Grieneisen et al. (2017) found that the longer an immigrant male baboon resided in a new social group, the more closely his core and noncore microbiomes resembled those of the adult members of that group, suggesting that the process of group convergence takes place over a span of months to years. Amaral et al. (2017) also found that, when newly weaned infants joined new social groups, their gut microbiomes converged to resemble their new groups within two weeks. Our study focuses on the time scale across which social groups diverge from one another. We found distinct gut microbial signatures to characterize two daughter groups of colobus <9 months after the fission event that resulted in these groups. Before this fission, the same individuals did not harbor distinct microbial communities, although the difference between them did approach significance. This trend may be due to sampling the original group at a timepoint during the initial stages of the fission event. Taken together, this finding both indicates that distinct gut microbial profiles can emerge in two new social groups in <9 months and suggests that the process of group-specific microbial divergence may begin before the establishment of those groups. Additional time-points leading up to and following a fission event are needed to more finely map the timescale across which these communities diverge and to better understand the mechanisms driving divergence.

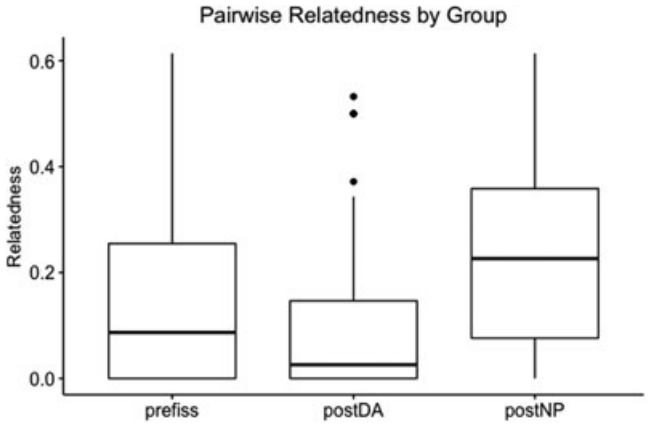

FIGURE 3 Pairwise-relatedness as calculated using 17 STR loci are represented by social group. On average, pairwise-relatedness increased by 0.079 in the NP social group from the original group. However, the pairwise-relatedness between groups was not statistically different ($p = 0.103$, $df = 2$, $\chi^2 = 4.55$). STR: short tandem repeat

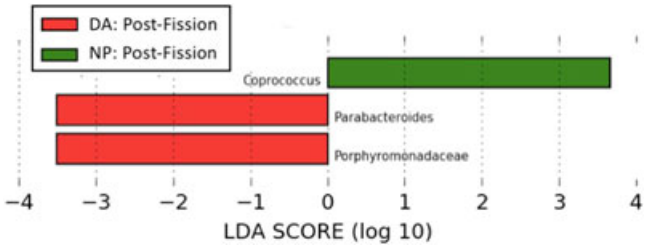

FIGURE 4 LDA scores for taxa differing significantly between product group. Three genera were found in different relative abundance between these two groups. Linear effect size analysis (LefSe) was run for the groups at a KW alpha value of 0.01 and an LDA score of 3.0

Hosts can gain microbes through changes in social context, such as alterations in direct and indirect interactions with conspecifics that provide access to different microbes, potentially affecting gut microbiome composition (Lombardo, 2008). In social animals, changes in group composition, size, and social networks could all contribute to this type of shift. In this study, group composition of females before and after the fission remained similar overall (Table 1); thus the number and age structure of females in each fission product are unlikely to be driving our results. There were, however, other changes in social context in DA social group during the postfission field season, including two males immigrating to DA group and one infant dying. Further investigation into how changes in social environment and social stress affect the gut microbiome are required to determine how these events may have influenced the observed gut microbiome divergence.

Other factors potentially contributing to the observed microbial shifts are diet and/or ranging patterns. While diet has been suggested as a primary driver in structuring the gut microbiome (Amato et al., 2014; Hale et al., 2018; Muegge et al., 2011), diet has not been found to explain differences in gut microbial beta diversity between individuals and groups in our population (Wikberg, Christie, Campos, Sicotte, & Ting, 2017; Wikberg unpublished data). Alternatively, despite all females in NP and DA group using a distinct part of their group's home range as well as a large overlap zone between the two groups (Figure 1) and collection site being a nonsignificant predictor variable for gut microbial dissimilarity, we cannot rule out the effects of habitat use on the observed shifts. While product groups ranged in subsets of the original range that included both woodland and mature forest, DA ranged primarily in woodland areas after the fission while NP's new range tended toward the mature forest. Because even small changes in environment can expose animals to new reservoirs of environmentally derived microbes, it is possible that the divergence observed between the two product groups is in part driven by spatial distribution and habitat use.

As has been reported in other folivorous species, individual gut samples were vastly dominated by Firmicutes and Bacteriodes with low but consistent proportions of Tenericutes and Verucomicrobia (Amato et al., 2016; Yildirim et al., 2010). LefSe revealed three genera to differ between the two product groups of this study. The new DA group was found to have relatively more *Porphyromonadaceae* and *Parabacteriodes* than the NP group after the fission, while the genus *Coprococcus* was found to be at greater prevalence in the NP group than the new DA group. The *Coprococcus* genus is in the order Clostridiales, which can assist in the degradation of plant material and is likely to reflect the folivorous diet of these animals (Barelli et al., 2015). It has been found at differential abundances in different social groups of baboons, suggesting that this might be a genus with a strong propensity for social transmission (Grieneisen et al., 2017). This genus is also commonly used to gauge individual gut health, and decreased levels of *Coprococcus* have been shown to accompany a stress response (Derrien, Johan, & Vileg, 2015), which could be related to the changes in social context and/or increased ranging in woodland habitat seen in the DA social group. Because quadrats characterized as "woodland" at BFMS have been previously found to have fewer large trees, less species diversity, and a lower basal area of colobus food trees than those in the interior of the forest (Teichroeb & Sicotte, 2018), it is possible that the DA group's increased ranging in this type of habitat may partially account for the elevated levels of *Coprococcus* observed in this group. While our analyses showed that collection site (woodland forest vs. mature forest) was not found to be a significant predictor of variation in this study, more detailed study on the effects of ranging patterns and habitat use are required.

This fission event resulted in an increase in average pairwise-relatedness for the NP and a decrease for the DA group, although there were no significant differences in mean relatedness between the original group and the postfission groups. This is a common phenomenon in animals that disperse by group fission, and an increase in relatedness in fission product groups has been demonstrated widely across primate species (Snyder-Mackler et al., 2014; Widdig et al., 2006). Although host genetic variation can play a significant role in shaping the diversity of the gut microbiome (Goodrich et al., 2014), previous studies have found little evidence for a strong role of host genetics in structuring the microbial communities of wild primates (Amato et al., 2017; Degnan et al., 2012; Spor, Koren, & Ley, 2011). In this particular data set, no correlation existed between beta diversity and relatedness. Taken together, our results suggest that even though NP group contained some close female kin dyads, relatedness did not play a significant role in structuring the differences in beta diversity seen between the two groups.

Finally, the largest proportion of variation between groups in this study was explained by year, rather than group membership. Previous studies have found temporal variation in the gut microbiomes of other folivorous primates to change in response to seasonal changes in food availability (Amato et al., 2015; Springer et al., 2017), which is consistent with past observations in this study population (Wikberg et al., 2016, Wikberg unpublished data). However, because longitudinally collected samples in this study were all from the wet season, the observed differences would need to be explained by some aspect of interannual variation in food availability during the same season. Further work is needed to clarify this possibility, including more sampling points between years and seasons as well as detailed data on changes in diet and food availability through time.

Overall, we used a longitudinal approach that provides a new perspective into how social groups acquire distinct gut microbial communities and the time period over which these divergent communities establish. This has significant consequences for understanding the role of social context in shaping the unique microbial signatures associated with distinct social groups across a wide variety of taxa. Further work is recommended into more finely mapping the timescales and factors that result in this divergence, especially within the context of the potentially adaptive effects of this recurrent, social-context dependent trend.

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