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Sociality and the Microbiome: Gut Microbial Convergence with Infant Presence in the Black-and-White Colobus (*Colobus vellerosus*)

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Abstract

While previous studies have demonstrated that social behavior plays an important role in gut microbial variation, there is limited understanding of how changes in social cohesion affect the gut microbiome. This study provides a comprehensive examination of this longitudinal relationship in a population of black-and-white colobus monkey (*Colobus vellerosus*) at the Boabeng-Fiema Monkey Sanctuary (BFMS) in Ghana. Adult female *C. vellerosus* display increases in social interaction after the birth of an infant, indicating a social shift which I utilized to explore the association between changes in social cohesion and the gut microbiome. I used previously collected field data (2018–2020) across four social groups, resulting in 218 total fecal samples and a mean of 17.2 hours of behavioral data per female. These data sets were employed to characterize microbiomes using 16S rRNA sequencing and quantify changes in social cohesion via social network analysis. Infant presence was significantly associated with gut microbial similarity (PERMANOVA: $p<0.01$), and for three of the social groups, gut microbiomes became more similar after infant birth (GLMM: $p<0.036$). Social network analysis did not reveal significant changes in social cohesion with infant presence, indicating that other changes in social interactions not included in this analysis may explain this pattern. Future work would aim to evaluate the basis for differences in gut microbial variation between social groups and explore the presence of grooming with an infant present. Investigating the relationship between social interactions and microbial variation ultimately contributes to our understanding of the factors influencing the assembly, composition, and diversity of the gut microbiome.

1. Introduction

1.1. The Gut Microbiome and the Host

The gut microbiome consists of the community of microbes inhabiting the gastrointestinal tract of a host organism. In vertebrates, while the gut is initially colonized at birth and perhaps in utero, the microbiome exhibits considerable compositional variation throughout an individual's lifetime. These fluctuations have a range of implications on host physiological development and function. Gut microbial composition is essential for nutrient uptake and the prevention of pathogenic invasion (Suzuki et al., 2017), immune system development (Hooper et al., 2012), and the development and function of the brain and associated behavior via the gut-brain axis (Jena et al., 2020). Imbalances in the gut microbiome, known as dysbiosis, may lead to disruptions in these processes and cause negative consequences for the host. For example, studies have found evidence of associations between dysbiosis and obesity (Amabebe et al., 2020),

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depression (Kelly et al., 2016; Radjabzadeh et al., 2022), anxiety (Clapp et al., 2017) autism-like symptoms (Hsiao et al., 2013), and diabetes (Li et al., 2020). The extensive consequences of dysbiosis have caused the gut microbiome to attract considerable attention in clinical research as a system which has important implications for human health. Research in this area seeks to develop methods which could utilize gut microbes for medical applications concerning preemptive and ad hoc therapeutics for disorders correlated with dysbiosis. While our understanding of the relationship between host function and the gut microbiome continues to advance, we still lack a basic understanding of which factors act to shape the host microbiome and cause natural variation.

1.2. Social Factors Shaping the Gut Microbiome

Prior research has described factors at both the host and environmental level which have some influence on gut microbiome variation, including host diet, genetics, and social environment (Archie & Theis, 2011). The influences of host diet and genetics on gut microbial composition have been studied extensively. However, less work has been done to investigate the aspects of the host's social environment which influence the gut microbiome. As clinical intervention continues to develop at the forefront of microbiome research, understanding these social factors that contribute to microbial variation has valuable implications for the host which could ultimately inform approaches to shape a healthier gut microbiome. Exploring the social transmission of gut microbes may also help explain the evolution of sociality, as the sharing of microbes has been found to confer benefits for group members such as increased pathogen resistance and host immunity (Abt & Pamer, 2014; Ezenwa et al., 2016; Koch & Schmid-Hempel, 2011; Lombardo, 2008).

Some evidence of microbial transmission through social mechanisms has been explored in human subjects; studies have found cases of socially mediated microbial transmission through evaluations of cohabitation in adulthood (Gacesa et al., 2022; Lax et al., 2014; Song et al., 2013; Valles-Colomer et al., 2023) and infant adoption (Tavalire et al., 2021). While human studies provide valuable evidence of socially mediated microbial transmission, detailed characterization of human social behavior can be difficult due to the complexity of human spatial movement and social interactions on a day-to-day basis, which present a number of confounding environmental factors. Non-human primates present an ideal alternative study system for questions related to sociality and the gut microbiome due to their highly studied nature, the ability to collect detailed behavioral, dietary, and biological relatedness data, and their behavioral and phylogenetic similarities to humans. Studies in this area have worked to isolate and evaluate the relationship between host social environment and the non-human primate gut microbial composition in several different species.

In one of the earlier papers on this subject, Tung et al. (2015) evaluated the importance of social group membership and social networks on the structuring of the gut microbiome in a wild population of baboons. Excluding kinship, shared diet, and shared environment in their evaluation, the study found social grooming networks to be predictive of gut microbial similarity; rates of interaction between individuals directly related to compositional variation in the gut microbiome. Other studies have found similar evidence of socially mediated gut microbial transmission in this population of baboons (Grieneisen et al., 2017) as well as other non-human primate species including Verreaux's sifaka (Perofsky et al., 2017), black howler monkeys (Amato et al., 2017), and ring-tailed lemurs (Bennett et al., 2016).

While these studies provide a better understanding of the social factors that serve as mechanisms for microbial transmission at a specific cross-section in time, the rapidly changing nature of the gut microbiome implores

future work in this area to focus on more comprehensive longitudinal surveys with daily to weekly sampling of individuals spanning multiple years (Björk et al., 2019). There are several recent studies which have taken this approach in non-human primates and have begun to disentangle the factors which may contribute to inter- and intra-individual gut microbial variation. Analyses of well documented populations of chimpanzees over eight years (Moeller et al., 2016) and wild baboons over 13 years (Ren et al., 2016) revealed microbial variation correlating with both host-specific (diet, age, social behavior) and environmental factors (season, annual rainfall). In Verreaux's sifaka, environmental factors were found to define the population-level gut microbial signature, while patterns of host social interactions facilitated the persistence and variation of gut microbial communities over time within groups (Perofsky et al., 2021; Rudolph et al., 2022). In red-bellied lemurs, patterns of social contact (group membership and position within the social network) predicted gut microbial composition (Raulo et al., 2018), and distinct gut microbial profiles were detected in two resultant groups of black-and-white colobus monkeys less than nine months after a new social group split off from the main group (Goodfellow et al., 2019). This research project aims to add to this growing body of work using a combination of fine-grained data on primate social behavior and deep longitudinal sampling of individual gut microbial compositions within social groups.

With a longitudinal approach in mind, I was specifically interested in evaluating how changes in social cohesion influence gut microbial variation. In the context of my project, social cohesion refers to the average physical proximities between members of a social group. To my knowledge, there is only one paper that has directly evaluated this mechanism. The 2019 study examined human cohabitation and closeness in relationships, taking siblings and married couples (all in late adulthood) as their study subjects. As in previous studies, they found that individuals cohabitating with a spouse or partner had more similar gut microbiomes than unmarried, non-cohabitating individuals. Most importantly for the purposes of my project, the authors found that that spouses and siblings that rated themselves as having relatively "close" relationships had more similar gut microbiomes than pairs which did not rate themselves as having "close" relationships (Dill-McFarland et al., 2019). My research builds on these results by tracking expected changes in social cohesion and gut microbial variation over time in a well-documented non-human primate population.

1.3. Research Objectives and Hypotheses

To explore my research question, I focused on a population of black-and-white colobus monkeys (*Colobus vellerosus*) at the Boabeng-Fiema Monkey Sanctuary (BFMS) in central Ghana. The population of colobus monkeys at this site has been studied since 2000, leading to a detailed record of their behavior and group compositions. There are approximately 28 social groups in the area composed of uni- or multi-male/multi-female social groups with sizes ranging from 9–38 individuals (Kankam & Sicotte, 2013; Wong & Sicotte, 2006). Recent research on the BFMS black-and-white colobus population compared diet, relatedness, and the one-meter proximity network to determine which factor was the best predictor of differences in the gut microbiome across eight social groups. The study found that models of social connectedness in the one-meter proximity network best predicted variation in the gut microbiome composition between individuals (Wikberg et al., 2020). As in other non-human primate species, these results support the concept of social interaction as a factor mediating gut microbial transmission. The study also demonstrated that microbial transmission can occur in species with low rates of social interaction (grooming, time in close proximity) relative to other gregarious primate species (Teichroeb et al., 2003) and that proximity

networks can be sufficient for predicting microbial transmission.

The subfamily of monkeys to which black-and-white colobus belong (subfamily Colobinae, or colobine monkeys) are known to exhibit relatively high levels of allomothering behavior, described as an individual's attraction to and handling of another's infant (Bădescu et al., 2015; McKenna, 1979). In order to gain access to a young infant, females spend increased amounts of time grooming the mother, and thus overall grooming rates increase and individuals spend more time in close proximity when an infant younger than three months old is present in a group (Wikberg et al., 2015). Thus, based on previous research that showed 1) social interactions affect gut microbial variation, and 2) social interactions change in the presence of an infant, I used black-and-white colobus monkeys as a model to evaluate the following question, objectives, and hypotheses:

- Question*:* How is gut microbial similarity influenced by the presence of infants within social groups of adult female black-and-white colobus monkeys?
- Objective 1: Compare gut microbial similarities of adult females during time periods with and without a young infant (under three months old) present.
- Objective 2: Evaluate changes in social cohesion during time periods with and without a young infant present.
- Hypothesis 1: Female group members will have more similar gut microbiome compositions when a young infant is present in their social group than they will in the absence of a young infant.
- Hypothesis 2: Adult female group members will display higher levels of social cohesion when a young infant is present in a social group than they will in the absence of a young infant.

I used detailed demographic, behavioral, and microbial sampling from four social groups of black-and-white colobus monkeys at BFMS to quantify the longitudinal relationship between social environment and gut microbial variation. I first tested whether periods with and without young infants present correlated with variation in the gut microbial compositions of adult females in each social group. I then evaluated the type of variation that was occurring. If it was in line with my hypothesis, I expected to see adult female gut microbiomes becoming more similar to each other when a young infant was present. Next, I used social network analysis based on one-meter proximity networks to evaluate changes in social cohesion with a young infant present. Here, I hypothesized that changes in social cohesion (proximity) based on allocare behavior could be a factor contributing to the microbial variation I tested for in the first part of my analysis. This research is novel and significant because it utilizes a longitudinal approach to known social shifts surrounding infant care and employs a fine-grained data set with well-coupled behavioral and microbial sample components. The results of this study expand our understanding of the effects of changes in the social environment on the compositional variation of the gut microbiome on defined temporal scales.

2. Methods

2.1. Fieldwork and Labwork

University of Oregon graduate student Diana Christie conducted the fieldwork and labwork portions of this research. This included behavioral data collection and fecal sample collection from the study population (*Colobus vellerosus*; Boabeng-Fiema Monkey Sanctuary; Ghana). These methods have been described elsewhere (e.g., Goodfellow et al. 2019; Wikberg et al. 2020), but they are briefly documented here to provide context for the downstream data processing and analyses that I conducted.

Christie and her field assistants focused on four social groups for behavioral data collection (Redtail/RT, Wawa/WW, Winter/WT, and Splinter/SP), each containing habituated and identified individuals. Behavioral data were collected from all adult females within the four groups between 2018 and 2020, yielding two consecutive dry seasons of data. Samples were collected in the dry season (~December to April) to avoid the effects of seasonal variability on gut microbial composition (Gomez et al., 2015; Springer et al., 2017). Behavioral data were collected via continuous focal sampling, which involves tracking one individual at a time and recording frequency, duration, and type of behavior exhibited by the focal subject. This method was used to record behaviors during 10-minute intervals for all adult females in each social group. Social and feeding behaviors were recorded continuously. During a focal, point samples were also taken every two and a half minutes identifying all individuals within zero, one, three, and five meters of the focal subject. Behavioral data collection yielded a total of 240.84 hours of focal samples (mean 17.2 hours per female SD +/- 3.96).

Christie and her field assistants collected fecal samples during the same periods of time they were collecting behavioral data. Multiple samples were systematically collected from each focal subject to be used for gut microbial composition characterization via 16S rRNA sequencing. Fecal samples were collected monthly for adult females. After an identified individual defecated, 1–2 g of feces were collected using gloves and sterile collection sticks and dissolved in 4 ml of RNAlater®. The samples were stored in a freezer on site before being shipped to the Ting Lab at University of Oregon for storage at –20 °C. For the purposes of this study, these samples were used to represent the gut microbiome. However, there may be differences between the microbial composition of the samples and the true microbial communities of the host gut microbiome. Therefore, although it is more accurate to say that the samples characterized the hindgut or fecal microbiome, they were used in this context to evaluate socially mediated transmission of gut microbes between individuals.

Christie extracted DNA from each fecal sample using the Qiagen PowerFecal Pro kit, and DNA extracts were quantified on a Qubit Fluorometer. The V4 hypervariable region of the 16S rRNA gene was targeted for sequencing, as this region is useful for identifying taxa at the level of genus or species (Bukin et al., 2019). Library preparation followed protocols described in Goodfellow et al. (2019), and sequencing was conducted on a 300 base pair paired-end run on the Illumina MiSeq platform. Demultiplexing was completed by the core, matching each sample name with its appropriate set of sequenced rRNA reads. These steps produced fastq format files for each sample containing all reads for that specific sample.

2.2. Data Processing

2.2.1. Behavioral Data

The raw behavioral data were encoded in CSV files. I processed these using a combination of Microsoft Excel, Microsoft Command Prompt (CMD), and R (R Core Team, 2021). I first manually cleaned the data in Excel; any cells that were flagged for missing information were corrected and additional information was added where necessary. I then ran each sheet of focal data through a series of Command Prompt checks which involved ensuring files were in CSV format, removing Excel-formatted files, eliminating any spaces in file names or quotation marks in cells, then checking for missed corrections from the manual cleaning. Any missed corrections were subsequently fixed again in Excel, and the processing steps above were repeated. Incorrect ethogram codes were also located using an R script which would return a file with problem focal sheets. These codes were

corrected, and the script was rerun to ensure all inaccuracies were accounted for before the data were formatted for use in R. The overall results of the behavioral data processing included monthly pairwise social matrices for all adult females, and presence/absence of infants under three months. If infants were present, the number of infants under three months was also included.

2.2.2. Sequence Data

Data processing of 16S sequence reads was carried out on the University of Oregon's high performance computing cluster, Talapas, using the bioinformatics processing pipeline QIIME2 (Bolyen et al., 2019). I began by creating a bash script which would allow me to run slurm jobs as I worked through the pipeline. I used the DADA2 (Callahan et al., 2016) plugin for the next series of steps in processing. As each sample was read in both the forward and reverse direction during sequencing, I chose parameters to allow for the appropriate level of overlap between the reads before they were realigned, a process known as denoising. Too much overlap causes the program to throw out more reads as it detects a higher number of unmatched bases, but too little overlap runs the risk of incorrect matches between reads. The values I ended up choosing were determined using a combination of quality score plots generated in the demultiplexing summary and through trial and error. My parameters specified a total length of 274 base pairs (~20 base pair overlap), resulting in an average of 88.3% of reads successfully merged per sample. Once the sequences were aligned, the final step in processing was choosing appropriate sampling depth parameters, a process that results in the removal of samples with relatively low numbers of reads to maintain a robust data set for analysis. I conducted taxonomic classification using the SILVA database (Quast et al., 2013). The outcomes of microbial sample processing included an ASV table, a phylogenetic tree, and taxonomies.

2.2.3. Metadata File Creation

Using demographic and sample data, I produced a metadata file in CSV format which contained information related to each fecal sample. This metadata file was used for much of the initial processing and in QIIME2 as well as other downstream analyses. Pertinent metadata information for each sample included collection month, field season, fecal time point (a period of days within a field season where fecal samples were collected for all adult females in a social group), infant presence, and number of infants present. Infant presence was calculated by referring to a demography data sheet which lists all birth and death/disappearance dates for infants in each of the four social groups. Based on work in mice where microbial variation was tracked after cohousing (Caruso et al., 2019), I estimated the length of time for the gut microbiome to show significant levels of compositional change in an individual to be three days after the birth of an infant, with the assumption of social changes immediately after birth. After this window of time was determined, I reviewed the infant presence periods I created and found no instances of samples collected within three days of an infant birth or an infant death/disappearance and thus no samples needed to be removed from my data set.

2.2.4. Generating a Distance Matrix

I used the qiime2R package (Bisanz, 2018) to import my data from the QIIME2 pipeline into R. With the features table, phylogenetic tree, and taxonomy table from QIIME2 and the metadata file, I used the phyloseq package to create a phyloseq object, a way for microbial information to be stored, manipulated, and analyzed in R (McMurdie and Holmes, 2013). The data were then filtered to remove any samples with fewer than 5000 reads for quality control, removing nine samples out of the original 218. As there was a chance some taxa would have only appeared in those samples that were removed, I included a

command to remove empty spaces in the taxonomy table. I also included a command to remove sequence reads that mapped to chloroplasts or mitochondria instead of the ASVs I was interested in analyzing, as DNA derived from these organelles also contains the 16S gene and can represent a source of contamination.

The next step in preparing the data for statistical analysis was creating a distance matrix of beta diversity indices using Aitchison distance. Beta diversity refers to the compositional dissimilarity between microbial communities. Evaluating levels of similarity between samples allows for the partitioning of how various factors may influence the compositional similarity of the gut microbiome between individuals. I first centered log-ratio (CLR) transformed the data, converting the values from total counts to the dominance for each taxon relative to the mean of all taxa (Gloor et al., 2017). Next, the distance matrix was generated with these data using the Euclidean method. These two steps generate an Aitcheson distance matrix. This distance is widely employed for microbiome work, as it better accounts for the compositional nature of microbial data and avoids compositionality bias (Quinn et al., 2018). The distance matrix itself gives dyadic measurements of microbial similarity between all individuals in which the numerical measures in this case are based on the Aitchison beta diversity metric.

2.3. Statistical Analysis

2.3.1. Preliminary Analysis

Before beginning my analyses, I used a microbiome analytics tutorial to obtain preliminary statistics for my data set and to develop microbiome analysis skills in R using real data. I calculated and plotted the relative abundance of the phyla and observed richness using the phyloseq (McMuride and Holmes, 2013) and ggplot (Wickham, 2016) packages in R. For beta diversity analysis, I generated an Aitchison distance principal coordinate analysis using the packages microbiome (Lahti and Shetty, 2017) and vegan (Oksanen et al., 2022).

2.3.2. PERMANOVA: Testing for the Presence of Changes in Microbial Similarity

A permutational multivariate analysis of variance (PERMANOVA) identifies the effects of various factors on microbial variation and their interactions with each other via a permutational ANOVA of a distance matrix (Anderson, 2001). Broadly, the test asks if microbial variability is greater between groups or within groups for a given sample, displaying significance if variability is greater between groups. I ran a specialty version of a PERMANOVA called an adonis using the adonis2 function of the R package vegan. For the purposes of my study, I tested for differences in gut microbial similarity between time periods with and without a young infant present while controlling for other factors shown to have significant effects on gut microbial variation. While collection year and field season were both included in the metadata sheet, I chose to exclude collection year as a factor, as it is similar to field season, and field season better describes temporal changes between collection periods.

In setting up the command, each factor could be incorporated in an additive fashion; or, if I wanted to test for interactions between factors, it could be incorporated in a multiplicative fashion with another factor. Preliminary analysis involved running the PERMANOVA with different variations of factor interactions, which revealed significant interactions between social group and infant status, and between field season and collection month. Therefore, the final formula structure I used included a multiplicative interaction between social group and infant status, a multiplicative interaction between field season and collection month, and individual ID as an additive factor to account for repeat sampling among individuals.

2.3.3. Generalized Linear Mixed Model (GLMM): Evaluating Changes in Distance to Centroid

In the second part of my microbial analysis, I specifically wanted to test if changes in beta diversity (microbial similarity) with the presence of an infant were being driven by individuals in a group becoming *more similar* to one another in gut microbial composition. To do this, I modeled the effect of infant presence on distance to centroid (DTC), which measures how dispersed members of a group are from a central location. Because, in this case, dispersion of points correlates with the level of microbial similarity between samples, the measure of distance to centroid can be used to compare microbial similarity between groups (see Figure 1). I used phyloseq to subset the data by fecal time point and create Aitchison distance (beta diversity) matrices, then used the usedist package (Bittinger, 2020) to generate distance to centroid measurements for each fecal time point.

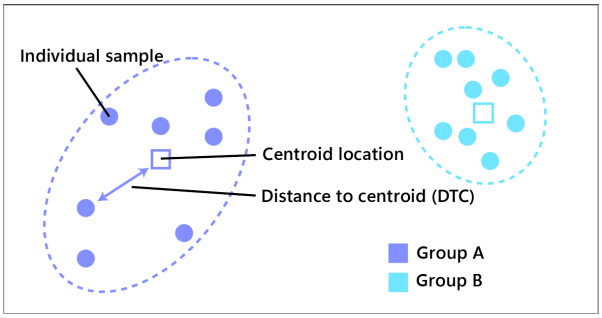


Figure 1. Conceptual figure of distance to centroid. Distance to centroid measures how dispersed all members of a group are in relation to a central point (centroid location). In this case, individual samples refer to the microbial samples, and the distance to centroid was calculated based on Aitchison distance metrics (Quinn et al., 2018).

The most appropriate way to handle repeat sampling in my data was to utilize a mixed effects model to account for random effects in addition to fixed effects. A Shapiro test for normality revealed the data were non-normal and thus unfit for a linear mixed effects model. Therefore, the glmmTMB package (Brooks et al., 2017) was used to run a generalized linear mixed model (GLMM) as it does not rely on normality as an assumption. To improve the fit of the GLMM, the outcome variable (DTC) was also log transformed. The best factor interactions for the gamma fit test were determined using AIC-based model selection (models with lowest AIC values were chosen) and the drop function in R. The selected model structure included the log link model of the GLMM with infant status as a fixed effect and group, ID, and collection month as random effects. Field season was excluded as a factor, as it could not converge as a fixed or random effect. The model was compared to the null model which fitted the data by only the random effects without infant status. The effects of the model were plotted using the effects package in R (Fox and Weisberg, 2019; Fox, 2003) to visualize the interactions between the DTC and infant status.

Because the social group Winter (WT) showed divergent patterns in the results of the GLMM (see Results), it was eventually removed from the data set, and model selection and drop were used to determine the best factor interactions. Without WT, this was a log link model of the GLMM with infant status as a fixed effect and ID and collection month as additive random effects. This model was also compared to the null model, and the effects of the model were plotted again using the effects package in R.

2.3.4. Social Networks and Centralization: Evaluating Changes in Social Cohesion

Social cohesion in this study refers to the average level of physical proximities between all adult female members in a social group. Higher social cohesion, for example, would correlate with individuals spending more time in close proximity. In order to explore changes in social cohesion associated with infant presence, I used social network analysis, a method of calculating standardized sociability measures that allows for evaluation of relationships within social groups (de Lima & Ferreira, 2021). This approach generates social networks in which points (nodes) represent individuals and the lines between

points (edges) represent the social interactions of those individuals.

For each social group, the time periods where infants were present and absent were determined, and the data were subset by infant status (young infant present = Y or N). This resulted in nine social networks being generated; there were two "Y" and one "N" infant status time periods for each social group. All networks were constructed using continuous approaches to within one meter from the focal follows collected by Christie during field sampling. The social group Redtail (RT) was excluded from this analysis because it did not have a minimum of one "Y" and one "N" infant status period with sufficiently dense behavioral data. Each distance matrix was loaded into R studio and converted to an undirected weighted edgelist using igraph (Csardi and Nepusz, 2006). Exploratory modularity analysis was run for each matrix using igraph, applying an optimization algorithm which identified groups of strongly connected individuals ("communities") in the network, differentiating each via color overlays on the social networks (Brandes et al., 2008). igraph was also used to run a statistical analysis of network-level metrics for the centralization of the social groups, which included evaluation of degree, closeness, betweenness, and eigenvector centralization values, each measuring different aspects of social structure within a group. Given the relatively small size and well-connected nature of the social groups, I chose to focus on eigenvector values for my statistical analysis, as they consider both number and strength of connections, capturing the greatest amount of variation in my data (Hanneman and Riddle, 2005).

To evaluate the relationship between infant status and the social cohesion (eigenvector centralization) of adult females in a social group, I used a nested ANOVA in R. For my data sheet, I included the eigenvector values in a table along with IDs for each social network (SPY1, SPN1, etc.), social group (SP, WW, WT), and infant status (Y/N). To test for a significant difference in social cohesion with or without a young infant present across all social groups, I ran the nested ANOVA with social group nested within infant status.

I also ran the same set of tests with a different social network metric called mean network strength. While eigenvector centralization describes the extent of cohesion around particular focal individuals in a group, mean strength more generally describes how connected all individuals are to each other, which is similar to the method used in Wikberg et al. (2015). To calculate this metric, I found the average value for each social network matrix, making sure to have each dyad represented one time. I ran the same nested ANOVA discussed above using this second network metric. I chose to run tests using both types of network metrics because they measure social structure in slightly different ways, and significant results for either one would provide insight into how social cohesion might vary with the presence of an infant.

3. RESULTS

3.1 Preliminary Analysis

After filtering, there were 209 total samples with an average of 79254 reads per sample. There were 30 phyla and 3828 taxa represented in the data set. As expected, observed amplicon sequence variants (ASV) correlated with total read count. From a visual overview, the principal coordinate analysis (PCoA) displayed subtle differences in gut microbial clustering and dispersion between the four social groups (Figure 2).

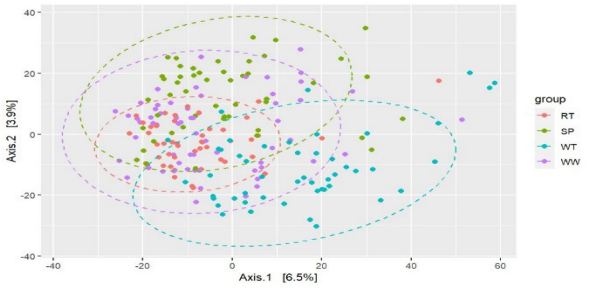


Figure 2. Principal coordinate analysis. Ordination plot generated from the identified principal coordinates of my data set. The four social groups displayed differences in clustering and dispersion based on Aitchison distance metrics.

3.2. PERMANOVA

All factors of interest showed significant effects on beta diversity (microbial similarity); collection month (R2=0.04456, p<0.001) and field season (R2=0.03502, p<0.001) explained a moderate amount of variation in beta diversity and had a significant interaction (R2=0.01995, p<0.001). After controlling for all other variables, infant presence had a small but significant effect on gut microbial variation (R2=0.007131, p<0.001). Social group and infant presence also had a significant interaction after controlling for other variables (R2=0.01907, p<0.001). See Table 1 for a full summary of PERMANOVA results.

Table 1. Summary of PERMANOVA results. All factors of interest showed significant effects on beta diversity. Infant presence exerted a small but significant effect on beta diversity (R2=0.007131, p<0.001). Social group and infant presence also showed a significant interaction (R2=0.01907, p<0.001).

	Df	SumOfSqs	R2	F	Pr(>F)
group	3	79437.033	0.0825626	7.688397	0.001
inf.pres	1	6861.465	0.0071314	1.992282	0.001
field.season	4	33692.438	0.0350181	2.445719	0.001
coll.month	7	42871.560	0.0445584	1.778301	0.001
id	22	196921.537	0.2046697	2.598990	0.001
group:inf.pres	3	18344.420	0.0190662	1.775484	0.001
field.season:coll.month	4	19194.826	0.0199501	1.393344	0.001
Residual	164	564819.659	0.5870434	NA	NA
Total	208	962142.938	1.0000000	NA	NA

3.3. Generalized Linear Mixed Model

Across all four social groups, infant presence was not found to have a significant influence on distance to centroid and the null model was selected over the full model (Table 2). However, visually there was a slight decrease in DTC when a young infant was present (Figure 3). The social group WT stood out as being significantly different than the other three social groups in the GLMM (group [WT]: p<0.021, Table 2).

Table 2. Generalized linear mixed model for distance to centroid across all social groups. Infant status did not show a significant effect on distance to centroid across all social groups (p<0.319). The social group Winter (WT) was significantly different than the other three social groups (p<0.021).

	log_centroid_distance			
Predictors	*Estimates*	*std. Error*	*CI*	*p*
(Intercept)	4.02	0.02	3.98 – 4.06	**<0.001**
young infant present [Y]	1.00	0.00	0.99 – 1.00	0.319
group [SP]	0.99	0.01	0.98 – 1.01	0.395
group [WT]	0.98	0.01	0.97 – 1.00	**0.021**
group [WW]	1.01	0.01	1.00 – 1.02	0.227
Random Effects				
σ^2	0.00			
$\tau_{00\ id}$	0.00			
$\tau_{00\ coll.month}$	0.00			
ICC	0.14			
N_{id}	26			
$N_{coll.month}$	9			
Observations	208			
Marginal R^2 / Conditional R^2	0.115 / 0.242			

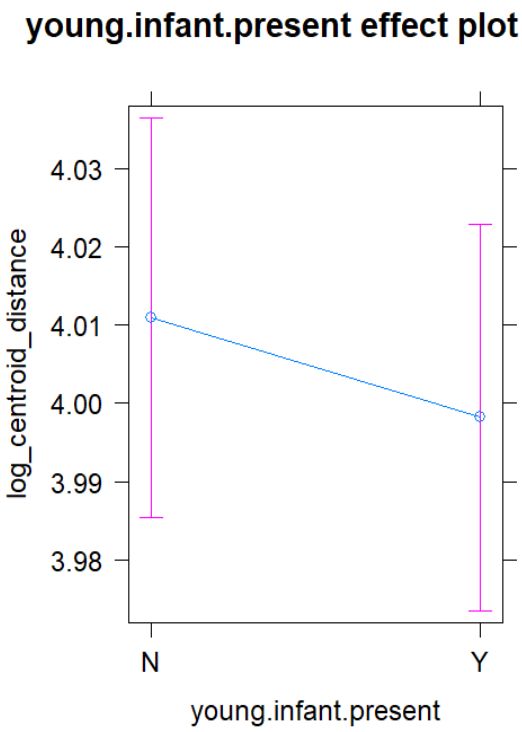


Figure 3. Effects plot of infant presence on distance to centroid across all social groups. The plotted effects of the GLMM showed a decrease in distance to centroid with a young infant present, however as seen in the GLMM results, this trend did not rise to the level of significance with all four social groups (p<0.319).

The effects plot revealed that WT also had lower distances to centroid across time points and showed a different pattern of directional differences in distance to centroid in response to infant presence (Figure 4). Based on these results, WT was removed from the data set, and the tests were rerun. The results of this second test without WT (Table 3) showed significant results for infant presence affecting distance to centroid across all remaining groups ($p< 0.036$) and the full model was selected over the null. The plotted effects again showed a decrease in distance to centroid when a young infant was present (Figure 5).

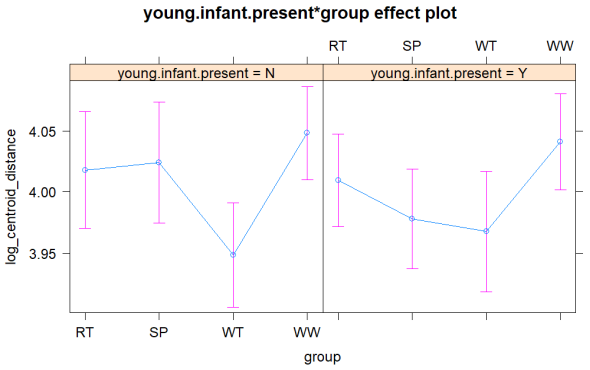


Figure 4. Effects plot of infant status on distance to centroid partitioned by social group. A visualization of the interaction between infant status and distance to centroid by social group showed that WT had an overall lower distance to centroid across time periods and showed a different pattern of directional changes in distance to centroid with a young infant present.

Table 3. Generalized linear mixed model for distance to centroid without WT. With the social group WT removed, infant presence showed a significant effect on distance to centroid for the remaining three groups in the GLMM (young infant present [Y]: $p<0.036$).

	log_centroid_distance			
Predictors	*Estimates*	*std. Error*	*CI*	*p*
(Intercept)	4.04	0.02	4.00 – 4.07	**<0.001**
young infant present [Y]	0.99	0.00	0.99 – 1.00	**0.036**
Random Effects				
σ^2	0.00			
$\tau_{00\ id}$	0.00			
$\tau_{00\ coll.month}$	0.00			
ICC	0.28			
N_{id}	21			
$N_{coll.month}$	9			
Observations	160			
Marginal R^2 / Conditional R^2	0.023 / 0.299			

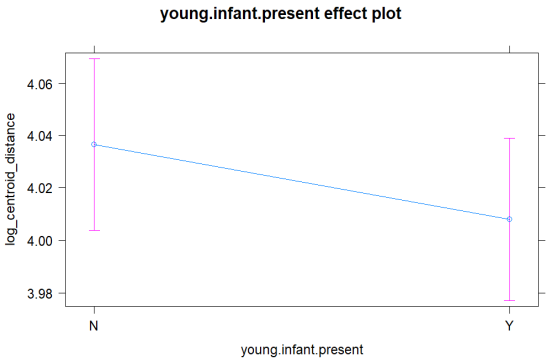
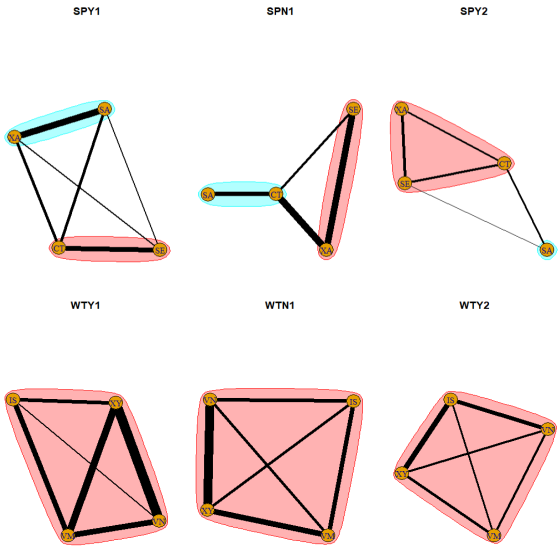


Figure 5. Effects plot of infant status on distance to centroid without WT. With WT removed from the data set, the other three social groups showed a decrease in distance to centroid with a young infant present (young.infant.present = Y).

3.4. Social Networks and Centralization

There was not a significant difference in social cohesion between time periods with or without a young infant present across all social groups based on either metric I used for my analysis (eigenvector centralization, $p<0.152$; mean strength, $p<0.496$). Based on a visual overview, I did see structural differences with and without a young infant present in the weighted edge list visualizations; the social groups SP and WW changed in some way between time periods whereas WT does not show such distinct changes. WT also lacked the sub-structuring seen in the other social groups, visualized through differences in the color overlays (Figure 6).



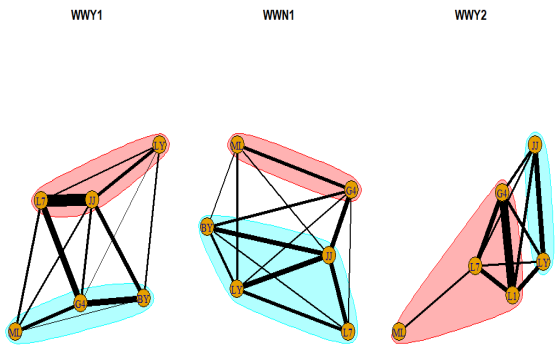


Figure 6. Weighted and undirected social networks with modularity for SP, WT, and WW. Distance matrices constructed from one-meter approaches were used to create undirected weighted edge lists with modularity analysis overlays. The edge weights denote the connection strength between individuals, and the color overlays distinguish different modules ("communities") within the network. The name of each set of matrices identifies the group (SP, WT, WW) and infant presence (Y, N). The numbers denote the time periods, differentiating between the two infant presence = Y periods, and the letters in the nodes are individuals in the group. The social group RT was excluded from the analysis as it did not have sufficient behavioral data. WT having a single-color overlay for all individuals (no sub-structuring) suggested that all members in the group were closely connected.

4. Discussion

4.1. Infant Presence Influences Gut Microbial Similarity in Adult Females

Through my first objective, I aimed to test whether changes occurred in gut microbial composition in adult female black-and-white colobus monkeys between time periods with and without a young infant present. Using a PERMANOVA, I found significant evidence of changes in gut microbial similarity between time periods while controlling for confounding variables (collection month, field season, ID). While this analysis did reveal that the presence of an infant was having a small but significant effect on gut microbial similarity, the test was quite broad and did not indicate what change was occurring. The significant interaction between social group and infant presence in the PERMANOVA also suggested that in some way, infant status was differentially affecting gut microbial similarity between social groups.

4.2. Gut Microbiomes Become More Similar with Infant Presence in Most Study Groups

While the PERMANOVA suggested that changes in microbial similarity were indeed present across groups, I was specifically interested in testing whether the gut microbiomes of adult females in social groups became more similar in the presence of a young infant. I hypothesized that the gut microbiomes of adult females would become more similar in the presence of a young infant. Across all groups, I did not see a significant interaction between infant presence and distance to centroid in the GLMM results, suggesting that infant presence was not increasing gut microbial similarity across all social groups. However, as shown in the PERMANOVA results (Table 1), it is possible that the presence of infants affected the social groups in different ways, supporting an inquiry into how certain social groups influenced the GLMM result. The GLMM results by group (Table 2) and the effects plot for the interaction between infant presence and distance to centroid (Figure 4) showed that the social group Winter (WT) was different than the other three social groups. Because of this trend, I hypothesized that WT could be disproportionately influencing the results of the GLMM, so I removed it and ran the test again. Once WT was removed, there was a significant effect of infant presence on gut microbial similarity for the remaining three social groups; the trends of WT seemed to be masking the significant results of the other groups (Table 3). The plotted effects of the model excluding WT showed a decrease in distance to centroid with a young infant present (Figure 5), suggesting that in social groups with a young infant, the overall gut microbial compositions of the female members became more similar to each other, thus supporting Hypothesis 1. It is important to interpret the results of the DTC analysis with caution; while I found evidence for

an increase in gut microbial similarity with infant presence, this effect may be group- or context-dependent, as it arose in only three out of four groups and thus requires further exploration at a group level.

4.3. No Correlation Between Infant Presence and Social Cohesion between Groups

After finding evidence of variation in gut microbial similarity following changes in infant status, my second objective aimed to explore if this variation was in fact a result of changes in social cohesion. This idea was based on a previously documented increase in grooming with a young infant present among black-and-white colobus monkeys (Wikberg et al., 2015), and the fact that social proximity was found to be the best predictor of gut microbial similarity within the same study population (Wikberg et al., 2020). I hypothesized that when a young infant was present in a group, there would be an increase in social proximity among adult females. This could in turn facilitate increased gut microbial transmission, leading to the increase in gut microbial similarity I observed in the first part of my analysis.

I did not find a significant difference in social proximity between time periods with and without a young infant present for any of the social groups, which suggests that infant presence did not exert a significant influence on social network cohesion. This result failed to support Hypothesis 2. However, it is possible that using a one-meter proximity network was too broad of a method to evaluate changes in social cohesion; allomothering has nuances that may not have been detected within the parameters of my analyses. Research on a semi-free-ranging group of capuchin monkeys found that lactating females (having recently given birth) did have a larger number of grooming partners, but this did not result in significant changes in social proximity (de Lima and Ferreira, 2021). This finding suggests that although Wikberg et al. (2015) did note increases in grooming behavior with a young infant present, this does not necessarily translate to an increase in social proximity. Female black-and-white colobus monkeys are also more likely to participate in allomothering behavior with the infants of their maternal kin (Bădescu et al., 2015). These same kin members have been found to generally spend more time in close proximity within social groups independent of allomothering behavior (Wikberg et al., 2014). If the prospective allomothers and the mother were already spending time in close proximity, when an infant was born there may not have been a change in the amount of one-meter approaches between these individuals (tested in my analysis) but rather a change in the amount of direct contact via females grooming the mother to gain access to the infant as well as females grooming the infant itself (Bădescu et al., 2015; McKenna, 1979). As in other primate species, grooming on its own is likely a mediator for transmission of gut microbes in this study population with the most direct mechanism being anogenital grooming (Tung et al., 2015), however any direct contact between the mouth and hands of one individual and the fur of another could provide a means of transmission. Adult-female-to-adult-female grooming could lead to direct transmission of gut microbes. Similarly, an infant could act as a microbial reservoir and allow for an indirect source of microbial transmission between handlers.

While my statistical analyses did not detect significant changes in social cohesion, the social networks generated in igraph did show notable changes in edge weight (connection strength) and modularity (sub-structuring) between time periods. These results further indicate that my evaluation of changes in social cohesion may not have been granular enough to capture changes occurring across time periods, suggesting the need for an investigation of other measures of social cohesion within this study population. As in the microbial analysis, WT also stood out from

the other social groups, in this case because it did not show changes in sub-structuring between time periods. This is consistent with the fact that WT had the most similar gut microbial compositions (lowest DTC) across time periods, and again demonstrates the need to further investigate differences between social groups.

4.4. Study Group Winter (WT) Is an Outlier

My results for both the microbial and social network analyses suggest that WT may have experienced very different dynamics than the other three social groups during the sampling periods. At this time, it is unclear what the cause of this difference is, but it could have been due to sampling bias and/or biological differences. I checked for any variance in sampling that could have given rise to the differences seen for WT. There were no significant differences in time between sample collection or the number of samples for each group. I did find that for both the number of days and the number of samples collected with a young infant present, there was unevenness across social groups, but the GLMM model fit I used for my DTC analysis has been shown to be relatively robust to uneven sampling (Pinheiro, 2014). The trends seen for the social group WT fail to support Hypothesis 1, which again predicted an increase in gut microbial similarity among adult females with an infant present.

It is possible that there were behavioral differences between WT and the other groups that gave rise to the differences seen in my analyses. Higher overall affiliation rates within the group could drive gut microbiome homogenization, resulting in the lower overall DTC values observed in WT and reducing the likelihood of a significant change in social structure or DTC with a young infant present. Females tend to exhibit increased rates of affiliation and grooming in periods of high stress (Cheney & Seyfarth, 2009; Engh et al., 2006; Rodrigues, 2013). If there was a threat present during time periods of data collection, such as an alpha male takeover or male immigration, the females in WT may have already been spending time in close proximity. Kinship between females has also been shown to predict rates of grooming and affiliation in other primate species (Sueur et al., 2011; Tinsley Johnson et al., 2014), and it is possible that if the females in WT had a higher degree of relatedness, then they may have already been participating in higher rates of grooming and affiliation. Conversely, allomothering behavior tends to happen among related females in this population (Bădescu et al. 2015), so if WT females had a lower degree of relatedness, perhaps they displayed less allomothering behaviors, thus explaining why the presence of an infant did not have much of an effect on gut microbial similarity.

5. Future Directions

The results of this study offer promising insights. However, further research needs to be conducted to fully explore the nuances of my study population in relation to my research question. While there were significant changes in gut microbial similarity between time periods, the change was not consistent and requires further analysis at the group level. One future direction would be evaluating WT for any biological differences compared to the other groups including kin composition or instances of high stress during the sampling period. As adult females in WT showed more similar gut microbial compositions overall (GLMM effects plot) and did not show changes in sub-structuring within their group (social network visualizations) between time periods with and without a young infant present, directly testing overall rates of affiliative behavior may be another way to help explain the differences seen in WT.

Another future direction for this work would be to evaluate social changes within groups when a young infant is present using metrics beyond one-meter approaches—mainly grooming and

infant handling. For example, it could be beneficial to create and analyze grooming rates and networks as opposed to proximity networks for each social group or weight the proximity networks with grooming rates. Future directions could also include more complex statistical approaches for determining small-scale changes in social networks. Understanding the source of microbial variation seen in my data, even if it is not the same mechanism I initially proposed, would still offer insight into the role of the social environment in shaping the composition of the gut microbiome on a temporal scale.

6. Conclusions

The gut microbiome has gained considerable attention as a system which has important implications for many aspects of host health and function. Current research has focused on investigating how gut microbial variation affects host systems. However, it is of equal importance to understand what causes gut microbial variation in the first place. This study aims to provide a more comprehensive longitudinal evaluation of how changes in social environment influence gut microbial similarity using known social changes among black-and-white colobus monkeys after the birth of an infant. I found evidence of increases in gut microbial similarity during time periods with an infant present. However, the effect was small and seems to be context- or group-dependent, motivating further investigation into the compositional and behavioral differences between social groups. This trend was also not found to be associated with any change in social proximity, and further research is required to investigate more fine-grained behavioral changes that may lead to this variation. While the means of microbial transmission was not fully revealed, this work provides insight into the temporal nature of microbial variation and builds on an understanding of how social context may influence compositional variation of the gut microbiome over time. Given the important role of the microbiome in host physiology, establishing a comprehensive understanding of the factors contributing to natural inter- and intra- individual variation could ultimately inform strategies to investigate, maintain, and shape a healthier gut microbiome.

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