Microbes, Mothers, And Others: Allocare and Socially-Mediated Gut Microbiome

Transmission Across the *Colobus vellerosus* Lifespan

by

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DISSERTATION ABSTRACT

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Title: Microbes, Mothers, And Others: Allocare and Socially-Mediated Gut Microbiome Transmission Across the *Colobus vellerosus* Lifespan

In this dissertation, I investigate relationships between gut microbiome variation and social interactions in a natural population of black and white colobus monkey (*Colobus vellerosus*) at Boabeng-Fiema Monkey Sanctuary, Ghana. This species displays high levels of allocare, which varies across infants and increases infant contact with non-maternal adults, thus presenting an excellent opportunity to examine the role of early life social contact on the developing gut microbiome. Allocare following infant birth also changes adult social dynamics, providing a natural experiment for investigating the effects of longitudinal social change on gut microbiome variation. Thus, in studying social behavior and gut microbial variation in this species, I address gaps in knowledge related to the impact of social interactions on microbiome assembly early in life as well as how changes in social environment affect microbiome plasticity.

In Chapter I, I introduce the importance of the gut microbiome, factors shaping its variation, and *Colobus vellerosus* as a model to better understand this topic. In Chapter II, I characterize the developing colobus gut microbiome and examine how adult social partners shape it. I found that shared social group was predictive of infant-adult microbial similarity and allocare behaviors by adults likely transmitted microbes to infants. However, I was unable to pinpoint dyadic transmission of microbes between infants and adult social partners. In Chapter III, I explore the relationship between social shifts and gut microbiome plasticity. I found that grooming increased among adult females after infant birth, which coincided with an increase in adult female

gut microbial similarity. While I was unable to tie this increased microbial similarity to social relationships on very short (3-month) time scales, shorter time periods than typically used (6-month) did predict microbial similarity. In Chapter IV, I provide implications for this work, including the importance of adult social partners seeding the developing colobus gut microbiome, the underappreciated role of microbial transmission to the evolution of allocare, and how relatively short-lived changes in social relationships may cause microbial shifts in adulthood. This dissertation expands our understanding of the social factors shaping the gut microbiome, particularly in cooperatively breeding species.

This dissertation includes previously unpublished co-authored material.

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DEDICATION

To my two favorite little primates in the world, Owen and Theo.

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CHAPTER I:

INTRODUCTION

The Role of the Gut Microbiome

The animal gastro-intestinal tract is host to diverse communities of bacteria and other microbes, collectively called the gut microbiome. This complex community of microbes plays crucial roles in the development, health, and physiological function of the animal host, and thus understanding the factors shaping inter-individual variation in gut microbiomes is of great interest. The gut microbiome is essential to digestion and the absorption of nutrients, helps prevent invasion of pathogenic microbes into the body, may act as a plastic factor allowing the host to cope with changes in food availability, environmental microbes, geography, and even climate change. It has also been linked to development and modulation of the brain and typical behavioral patterns via the gut-brain axis, and aids in the development of the innate and adaptive immune systems.

The gut microbiome assists in digestion and absorption of nutrients in several ways. First, microbes residing in the gut reduce the pH of the gut lumen (Amato, 2013). This lower pH prevents the build-up of metabolic by-products that can be toxic and enables better nutrient absorption. Further, gut microbiota assist in break-down of plant structural polysaccharides (fiber) in the GI tract, reducing them to short chain fatty acids. These short chain fatty acids produced by the microbiome can supply hosts with up to 70% of their daily energy requirements, allowing hosts to derive nutrition from foods that would otherwise be indigestible (Gomez et al., 2016). In addition, gut microbiota detoxify plant toxins ingested by the host (Clayton et al., 2019; Suzuki, 2017). Finally, gut microbes may also synthesize and produce vitamins and minerals, such as folate, that are beneficial to the host (Amato, 2013; Amato et al., 2014).

Microbes residing in the gut also provide colonization resistance against enteric pathogens via competitive exclusion by depleting nutrients and resources not absorbed by the host's intestine (filling available feeding niches), and compete for receptor and binding sites on the epithelium and mucosa (filling the spatial niches available) (Bailey, 2012; Candela et al., 2012; Levine & D'Antonio, 1999; Stecher & Hardt, 2011; Suzuki, 2017). Microbes can also directly inhibit pathogen invasion chemically by releasing bacteriocins and metabolites, such as butyrate and acetate, that hinder pathogen invasion and growth (Stecher & Hardt, 2011). Thus, a complex and diverse gut microbiome is important to host health.

Further, the gut microbiome can act as a plastic factor allowing the host to compensate for environmental change (Candela et al., 2012). While certain aspects of the microbiome are thought to be relatively stable, it has also become clear that it can be extremely plastic and capable of conforming to different environmental pressures. This ability to reconfigure due to differing factors is an important function of the gut microbiome in contributing to host health and behavioral and physiological plasticity (Amato, 2016). The ways in which dietary and environmental changes can affect gut microbial communities have been well documented in humans (Amato, Jeyakumar, et al., 2019; Amato, Mallott, et al., 2019; Jha et al., 2018) and in non-human primates (Amato et al., 2014, 2015; Amato, Mallott, et al., 2019; Amato et al., 2013; Gomez et al., 2015; Gomez et al., 2016; Hale et al., 2018; Springer et al., 2017). Not only does the gut microbiome shift as a result of dietary intake, but also this shift in microbial communities can allow hosts to derive nutrition and energy from varying food sources. Indeed, humans have more plasticity in gut microbial communities in response to environmental/dietary variation than any other primate, indicating that the gut microbiome may be integral in the ability of humans to

adapt to novel environments, greater dietary diversity, climate change, and other evolutionary challenges (Amato, Jeyakumar, et al., 2019; Amato, Mallott, et al., 2019; Moran et al., 2019).

The gut microbiome also plays an important role in the development of the brain and typical behavior via the gut-brain axis. The presence of gut microbiota influences neurocognitive and behavioral development, as well as changes to brain structure and neural plasticity. Neurotransmitter development, synaptic growth, and increased anxiety behaviors have all been tied to abnormal microbiota during development in gnotobiotic hosts, highlighting the role gut microbes play in the signaling mechanisms that modulate brain development and behavior (Indrio et al. 2017; Heijtz et al. 2011; Sampson and Mazmanian 2015). Further, transplantation experiments indicate that gut microbiota can actually drive behavior in the host, as abnormal behaviors were transferred with gut microbiome transplantation (Sampson & Mazmanian, 2015).

One of the most well-studied function of the gut microbiome involves its role in the development and function of the host immune system (Bengmark, 2013; Candela et al., 2012; Hooper et al., 2012; Turnbaugh et al., 2009). Gut and mucosal colonization by microbiota early in life aids in the development and training of the immune system in a time-sensitive manner by activating specific pathways of molecular signaling (Gensollen et al. 2016; Indrio et al. 2017). Many studies in gnotobiotic animals have revealed the importance of microbiota to the proper development of lymphoid tissues, especially near the mucosal interface. Early life succession events of the gut microbiome occur concurrently with the development and expansion of the mucosal immune system, likely influenced directly and indirectly by the presence of these microbes. Neonatal T-cells learn to tolerate environmental microbes after birth, and the presence of certain microbial taxa are known to induce T-cell production (both locally and systemically), inhibit pro-inflammatory responses, and induce repair of damaged intestinal epithelium by

promoting transcription of cytoprotective genes (Bengmark, 2013; Bouskra et al., 2008; Gensollen et al., 2016; Hooper et al., 2012; Indrio et al., 2017). Gut microbiota directly stimulate T-cell proliferation via penetration of the epithelial mucosa, interaction with myeloid cells that extend through the epithelium, or production of metabolites that act on receptors expressed by host cells. Through interaction with Toll-like receptors, microbes activate signaling pathways that modulate processes including cell survival, replication, and apoptosis. These processes occur during a critical development window in early life in which differences in gut microbiota have significant effects on brain development, behavior, and immune function (Diaz Heijtz et al., 2011; Gensollen et al., 2016). Certain cellular defects associated with the intestinal immune system are irreversible if a gnotobiotic host is not conventionalized with microbes in early life, and the potentially deleterious effects of altered immune development can have lasting consequences for the host.

Sociality and the Gut Microbiome

Animal gut microbiomes can vary substantially in composition and diversity across hosts (Ley et al., 2008). In addition to a variety of other intrinsic and extrinsic factors that are known to contribute to inter-individual variation of the gut microbiome (including diet, genetics, environment), host social environment shapes the gut microbiome, and social transmission of microbes may have even played a part in the evolution of sociality (Lombardo, 2007). Socially-mediated gut microbial transmission is often achieved through physical contact between hosts or indirect contact between hosts and host waste. Many mammals, including rodents, rabbits, pigs, horses, and dogs engage in coprophagy, or the ingestion of fecal matter (Archie and Tung 2015). Eating the feces of other individuals allows for direct dispersal of microbes from one host GI tract to another's GI tract. Simple contact with feces in the environment even in the absence of

coprophagic behavior may also be enough for microbial dispersal to occur between individual hosts. Physical contact between hosts is also a clear mechanism for dispersal of microbes across individuals, and the form of this physical contact can vary depending on the host species. Grooming is employed by non-human primates to cement social bonds, reduce stress, and maintain health, and the physical contact and ingestion of detritus found in the coat of grooming partners appears to aid in microbial dispersal among baboons and other primate species, especially grooming that is focused on the ano-genital region (Tung et al. 2015; Degnan et al. 2012; Amato et al. 2017; Archie and Tung 2015).

Evidence of socially-mediated microbial transmission has been found in a wide variety of host taxa. Various types of social interactions, social behavior, and group sociality in adulthood can be highly predictive of gut microbial similarity, indicating that social contact helps shape the gut microbiome. Social group microbial "signatures" have been described in many natural populations, in which members of the same social group share more similar gut microbiomes than members of different social groups, independent of shared diet and environment (Bennett et al., 2016; Degnan et al., 2012; Grieneisen et al., 2017; Orkin et al., 2019; Perofsky et al., 2021; Raulo et al., 2018; Rudolph et al., 2022; Springer et al., 2017; Tung et al., 2015; Vernier et al., 2020; Wikberg et al., 2020). Social structuring of the gut microbiome can be seen on a more fine-grained level as well; within social groups, networks and interactions can be highly predictive of gut microbial similarity; individuals more closely associated in a social network share more similar gut microbiomes (Amato et al., 2017; Grieneisen et al., 2017; Levin et al., 2016; Moeller et al., 2016; Perofsky et al., 2017; Rose et al., 2023; Tung et al., 2015; Wikberg et al., 2020). The majority of this research has been performed in wild non-human primate populations, as non-human primate social relationships are readily quantifiable from

observational data, and non-human primates share a close phylogenetic relationship with humans. However, a small but growing body of work has confirmed these patterns in non-primates, and most utilize host spatial data (e.g. via passive integrated responder tags) to infer social relationships in place of direct behavioral observation (Antwis et al., 2018; Chiyo et al., 2014; Raulo et al., 2021). These approaches have the advantage of obtaining much larger quantities of interaction data than is achievable with observational methods but face more difficulties disentangling the effects of social interaction from shared physical environment and diet.

Although a few studies have utilized a longitudinal lens for examining the influence of social interaction on gut microbiomes, the majority of this research has relied on one or few samples per individual and thus represent snapshots in time of complex and mutable microbial communities, and less is known about how gut microbiomes fluctuate in response to social change. Many social animals exhibit social partner preference, but there are a variety of situations in which strength of social relationships or social partners change, including births, deaths, emigrations and immigrations, dominance rank changes, resource scarcity, and aging (Blaszczyk, 2017; Machanda & Rosati, 2020; Shizuka & Johnson, 2020; Teichroeb et al., 2009). Limited evidence from non-human primate studies indicates that social shifts can be reflected in microbiomes. Social group signatures appear to be continuously constructed and adapt in response to social change; e.g., recently transferred baboon males retain hallmarks of their old social group microbiomes, while immigrants who have resided in the group for a longer period of time display microbiomes that have converged with their new social group (Grieneisen et al., 2017). Distinct social group microbial signatures can also arise relatively quickly following group fission (Goodfellow et al., 2019), and social instability can be reflected by shifts in gut

microbial communities over relatively short time periods (Samartino et al., 2023). Further longitudinal studies are necessary to fully understand socially-mediated transmission dynamics and how this contributes to long-term stability and maintenance of the gut microbiome (Bjork et al., 2019; Grieneisen et al., 2023; Pinacho-Guendulain et al., 2022).

Additionally, while it is clear that host social interactions have a measurable impact on gut microbiomes during adulthood, less is known about the impact of early life social environment, during a time when gut microbial variation can have lasting consequences for health and development. There is some evidence that social environment shapes the early life gut microbiome; household composition and shared home environment impact the presence and abundance of microbial taxa in human infant guts (Azad et al., 2013; Lane et al., 2019; Tavalire et al., 2021). Further, the gut microbiomes of peer-reared laboratory primate infants display distinct compositional differences from those of maternally-reared infants, and when transitioned to peer group housing, group-specific microbial profiles emerge shortly after weaning (Amaral et al., 2014; Dettmer et al., 2019). While parental care comprises a large portion of early life interactions in young social animals, infants typically have wider social environments beyond their parents which may contribute to the assembly of their microbiomes (Pinacho-Guendulain et al., 2022). Social interactions with non-parental adults and allocare (care provided to the infant by non-parental individuals) present an excellent opportunity for examining the role of diverse early life social contact on the gut microbiome of infants (Kuthyar et al., 2019), and there is limited evidence from human research that non-parental infant care (allocare) also shapes the early life gut microbiome. The diversity and composition of a lactating mother's milk microbiome is associated with the size of the mother-infant dyad's social network and amount of care given to the infant by non-maternal caregivers, and in turn the maternal milk microbiome is

a primary mode of infant microbial acquisition (Lackey et al., 2019; McDonald & McCoy, 2019; Meehan et al., 2018). Additionally, alloparents were recently found to influence infant gut and skin bacterial diversity, which was impacted by care behaviors (e.g. alloparental co-sleeping had stronger effects on infant bacterial diversity than holding) (Manus, Sardaro, et al., 2023). Our understanding of social environmental influences during early life are limited to findings from humans and captive animals, while very little is known about these dynamics in natural populations.

Colobus vellerosus as Models

This dissertation uses the white-thighed black and white colobus monkey (*Colobus vellerosus*) as a model for understanding the influence of social interaction on gut microbiome development and plasticity. *C. vellerosus* belong to the colobine subfamily, which are united by their adaptations to a folivorous diet, including having a foregut-fermentation digestive system, an anatomical specialization that no other primates have (Matsuda et al., 2019). Black and white colobus are medium-bodied arboreal primates that live in upper forest canopies, and are characterized by black bodies and faces, white thighs, long white tails, and distinctive white ruffs around their faces (Fleagle, 1999). *C. vellerosus* have been extirpated from the majority of their previous range, are threatened by hunting and habitat destruction, and are listed as Critically Endangered and decreasing with fewer than 975 estimated mature individuals left (Goodwin et al., 2020).

As colobines, *C. vellerosus* subsist primarily on leaves (folivory), and have a variety of physiological adaptations that allow them to extract nutrients from this difficult to digest food source. Colobines have complex, multi-chambered stomachs (Matsuda et al., 2019; McKenna, 1979), very low gut pH (Beasley et al., 2015) and a very diverse and distinct gut microbial

community. Fermenting guts necessitate an alkaline chamber for fermentation of plant matter, and herbivory requires animals to rely disproportionately on microbial processes to help break down complex plant compounds, including cellulose and lignin. High stomach acidity acts as an ecological filter by preventing colonization by food-borne microbes but can also make it difficult to acquire commensal microbes. There is a relatively low risk of plant matter containing food borne pathogens, thus herbivores have much lower stomach acidity (foregut fermenters have the least acidic stomachs among birds and mammals, and colobus monkeys have the lowest pH of any primate examined) (Beasley et al., 2015), and gut microbes near the lumen further aid in lowering gut pH. The ability of gut microbiota to aid in detoxification is another a function that is integral to colobine health as many leaves contain high levels of otherwise toxic substances (Clayton et al., 2019; Suzuki, 2017). Further, the role that gut microbes play in breaking down plant polysaccharides is especially important for the nutritional status of colobines, as they derive a majority of their energy from difficult to break down leaves that would otherwise be indigestible (Gomez et al., 2016).

Colobus monkeys also display a variety of behavioral adaptations to their folivorous diet. They spend a greater amount of time feeding and resting than most other primates due to the difficulty of obtaining sufficient nutrition from this low-quality diet, and as such, colobus are known to spend less of their day engaged in social interactions than other non-folivorous primates (Christensen et al., 2023). Further, feeding competition within colobine social groups is relatively low (as leaves are relatively abundant and widely distributed, scramble competition for resources is generally higher between social groups and contest competition is lower within social groups), which allows for more relaxed/egalitarian female dominance structures and close

spatial feeding (Wikberg et al., 2014a). Additionally, colobines display unusually high levels of affiliative allocare.

Allocare is found across a wide variety of animals (including invertebrates, fish, birds, and mammals), but in primates is notably high among callitrichids, titi monkeys, owl monkeys, colobines, and humans (MacKinnon, 2011). Among primates, social group members often display a great deal of interest in infants, especially newborns, approaching to inspect, sniff, touch, and sometimes groom, hold, carry, or play with young infants (Boose et al., 2018; MacKinnon, 2011; Nicolson, 1987). Affiliative allocare behaviors can be divided into two main categories: natal attraction and infant handling (Badescu et al., 2015; Brent et al., 2008). Infant handling includes those behaviors where direct physical interaction between the allomother and infant are involved, including holding, carrying, grooming, and playing. Natal attraction involves approaching, inspecting, sniffing, and touching the infant. While many social group members may engage in natal attraction, whether that attraction progresses to actual infant handling and further allocare is determined by a number of maternal, allomaternal, and infant factors. Allocare in primates, however, very rarely involves allonursing behavior, except in unusual circumstances among a small number of primate species (black and white ruffed lemurs, ring-tailed lemurs, gray mouse lemurs, gracile capuchins, black capuchins, Bolivian squirrel monkeys, and common marmosets) and a few human populations (Hrdy, 2009; Packer et al., 1992; Ren et al., 2012).

There are a variety of ecological, physiological, and behavioral traits unique to colobines that have led to high rates of allocare, including dietary feeding adaptations, group sociality, infanticide risk, mother permissiveness, and coat coloration. The more egalitarian social structures enabled by folivory result in low female-female competition so that infants are not taken, exploited, or harmed by higher ranking females (McKenna, 1979). This dietary niche

allows for a social environment in which colobines can be relatively secure in allowing other females to handle their offspring. Additionally, a primary benefit of allocare to the mother is increased foraging efficiency, and one of the primary barriers to obtaining sufficient nutrition via a folivorous diet is foraging efficiency, thus allocare is particularly beneficial to colobines (Nicolson, 1987; Stanford, 1992; Whitten, 1982). As a result, mothers of infants who receive allocare have increased reproductive success through reduced lactation effort, accelerated weaning, shortened inter-birth intervals, and increased birth rates (Badescu et al., 2016; Hrdy, 2009; Hrdy, 1976; Maestripieri, 1994; Mitani & Watts, 1997; Nicolson, 1987; Ross & MacLarnon, 2000). Further, the relatively high risk of infanticide among many colobine species (infanticide accounted for nearly 40% of all infant mortality between 2000 and 2005 in *Colobus vellerosus* at Boabeng-Fiema Monkey Sanctuary), maternal permissiveness (colobine mothers sometimes allow their infants to be transferred within hours of birth), and bright contrasting natal coats (which act as an attractant to potential allomothers) all play a role in the high rates and particular benefits of allocare in colobines (Bădescu et al., 2016; Dolhinow & Murphy, 1982; Hrdy, 2009; Hrdy, 1976; MacKinnon, 2011; Scollay & DeBold, 1980).

Allocare is also beneficial for infants as it leads to increased post-natal growth rates and accelerated development and weaning (Mitani & Watts, 1997; Ross & MacLarnon, 2000). Faster growth rates results in increased overall body size at time of weaning, lowering the risk that juveniles will starve and reducing the risk of predation (Lee et al., 1991; Mitani & Watts, 1997). Further, reaching weaning sooner lowers an individual's overall risk of infanticide, as infanticide risk is highest among younger infants (particularly during the natal coat stage) and is non-existent after weaning (Bădescu et al., 2016).

Finally, although it is a seemingly-altruistic behavior, there are a variety of ways in which allocare may benefit the allomother as well. Allomothers may provide care for related infants to increase their own inclusive fitness, contributing to the survival of related infants and freeing up related mothers to spend more time feeding, leading to reduced weaning times and shorter inter-birth intervals (thus increasing the reproductive fitness of the related mother) (Boose et al., 2018). In a biological market, allomothers may also exchange allocare for some sort of reciprocal altruistic act or social commodity, including social alliance (in which mothers may provide future coalitionary support to allomothers), or future reciprocal allocare (in which mothers may provide future allocare to the infants of allomothers) (Boose et al., 2018; Hrdy, 1976; Stanford, 1992). Lastly, allocare may provide immature/nulliparous allomothers with experience in infant care and handling, potentially increasing their parenting skills under a relatively low-stakes situation in which mistakes will not reduce their own reproductive success (Hrdy, 1976; McKenna, 1979; Quiatt, 1979).

There are a variety of inherent risks associated with allocare as well, including higher risk of infant morbidity/mortality, and reduced foraging efficiency and social capital (for the allomother) (Maestripieri, 1994; Nicolson, 1987; Tecot & Baden, 2015). Further, allocare increases the risk of parasite and infectious disease transmission by increasing the number of individuals that an infant may have contact with during a life stage in which their immune systems are still developing and somewhat naïve. This increases the chances that an allomother may pass a disease on to the infant, which could be detrimental both to the infant's health and survival and the mother's reproductive success (Stilling et al., 2014).

Infant *C. vellerosus* are born bright white, then fade to grey and reach their characteristic black and white coloration around 3 months of age. This contrasting natal coat is thought to be

an adaptation associated with attracting allocare as protection against the high risk of infanticide found in colobines, enabling high infant visibility and increasing natal attraction by potential allomothers (Badescu et al., 2015). All adult black and white colobus females display allomothering behavior, which is particularly concentrated during this natal coat stage and affects various social behaviors, including increased adult-adult grooming (Saj & Sicotte, 2005; Wikberg et al., 2015). Potential allomothers may use grooming and other affiliative behaviors to gain infant access, and indeed grooming rates are higher in *C. vellerosus* social groups containing higher proportions of infants (Dunayer & Berman, 2018; Wikberg et al., 2015). Thus, infant birth is an expected and recurring catalyst for social change and presents a good natural experiment for examining the influence of shifting social interactions on social transmission of gut microbes.

Further, this high-allocare period overlaps with the critical window of development when gut microbiome assembly is important to long-term health (Badescu et al., 2015; Hrdy, 2009; Maestripieri, 1994; McKenna, 1979). While all black and white colobus females display allocare behaviors, rates of allocare vary according to infant, mother, and allomother characteristics, thus infants experience inter-individual variation in the allocare they receive (Badescu et al., 2015). This makes the black and white colobus an ideal natural system for assessing the contributions of early life social environment and allocare towards gut microbiome variation during development.

The *Colobus vellerosus* Gut Microbiome

Previous research has revealed that social environment plays a significant role in shaping gut microbial similarity of adult colobus at Boabeng-Fiema Monkey Sanctuary (BFMS); gut microbiomes of adult females diverged in conjunction with group fission, and new social group microbial signatures arose relatively quickly in the new daughter groups (Goodfellow et al.,

2019). Further, social network connectedness was a better predictor of gut microbial similarity than dietary similarity or relatedness, even when excluding within-group dyads (Wikberg et al., 2020). Female colobus spent 0.1% of their time grooming available social partners, and generally spend less time engaging in social contact than many other primates (Wikberg et al., 2014b). However, 1m proximity-based networks were shown to be good measures of social contact and correlated with microbial similarity, and even approaches to within 1m by individuals in different social groups was sufficient for microbial transmission. Lastly, the social transmission of black and white colobus gut microbiomes are influenced by events that shift social interactions. During a year of social instability in one group of colobus at BFMS (including alpha make takeover and subsequent infant death) only a short time scale (3-month) proximity network predicted microbial similarity among social partners while the typically employed 12-month proximity network did not, indicating that shifting social dynamics over relatively short time periods can be reflected by shifts in gut microbial communities (Samartino et al., 2023).

Boabeng-Fiema Monkey Sanctuary

Data collection for this dissertation was carried out at Boabeng-Fiema Monkey Sanctuary (BFMS) in central Ghana. BFMS is a 1.92 km^2 dry semi-deciduous forest and is the site of a community conservation effort created and maintained by the bordering villages of Boabeng and Fiema in partnership with other surrounding communities. This small area of forest contains a sacred grove important to the local traditional religion, and the conservation of the forest and wildlife at BFMS began organically as a result of these beliefs, in which the two species of monkeys in the forest (*Cercopithecus lowei* and *Colobus vellerosus*) are the sacred children of the fetish Daworo and his wife Abudwo (local spirits that offer protection, guidance, and good fortune to the villages of Boabeng and Fiema respectively). For over 150 years the monkeys and

the forest surrounding the sacred grove at BFMS were protected by local people, as hunting and killing of these monkeys was taboo. By the 1960s, the human populations of Boabeng and Fiema grew, and outside settlers who did not believe in local taboos migrated to the area, increasing timber cutting and hunting. This resulted in a steep decline of the monkey population and agricultural conversion of forest land. In response to this decline, local stakeholders led by Boabeng police officer and schoolteacher Daniel K. Akowuah formalized the protection of BFMS under the Ghanaian government in the 1970s, and reforestation efforts involving converting surrounding farmland back into buffer forest commenced (Silfee, 2022). Under Akowuah's guidance, BFMS became one of the earliest community-based conservation projects designed to prioritize local cultural values and economic needs in order to further conservation goals. Currently BFMS is a popular ecotourism site for both Ghanaian and international tourists, who learn about the history and traditions of the forest and community and the biology of both species of monkeys from local tour guides, and the sanctuary is an important source of income for many local people. BFMS has also been host to long-term primatological research on the biology and behavior of *C. vellerosus* since 2000, and thus this population is very well-described.

Dissertation Research

This dissertation utilizes social behavioral data and fecal sampling from infants, young juveniles, and adult females from 4 habituated study groups of black and white colobus monkeys at BFMS to answer questions about the influence of host social environment on gut microbiome development and plasticity. The study groups Redtail (RT), Splinter (SP), Winter (WT), and Wawa (WW) contained between 10 and 26 individuals during the study period, including between 3 and 8 adult females (see **Supplemental Table 4** for group demographics at the end of

each field season). We characterized the gut microbiomes from 312 fecal samples from 29 early

life and 26 adult females collected across a 2.5 year study period, and quantified social bonds

and diet from 449 focal hours of adult female and infant behavioral data in order to address these

questions.

While host sociality is known to shape the gut microbiome, the extent to which social

environment and allocare during early life influence the developing gut microbiome is unclear,

particularly in natural populations. Chapter II of this dissertation aimed to first characterize the

developing colobus gut microbiome, and then to elucidate how non-parental members of the

social environment shape it. We found that early life colobus gut microbiomes are shaped by

similar factors as adult colobus gut microbiomes yet remain distinct due to the selective forces of

aging and weaning. Infant colobus gut microbiomes also develop along similar trajectories

described in other mammals. Further, we found that while broad measures of social environment

were predictive of microbial similarity between infants and adults (e.g., shared social group and

maternal centrality), we were unable to pinpoint dyadic transmission of microbes between

infants and adult social partners (e.g., dyadic social connectedness did not predict microbial

similarity). Finally, we found evidence that allocare likely mediates microbial transmission, as

infants with more allomothers shared more similar microbiomes with their social group members

than infants with fewer allomothers. Taken together, results from Chapter II indicate that

developing gut microbiomes of colobus monkeys are seeded by adults in their social group, and

that microbial transmission may be an unappreciated benefit of allocare. The research in this

chapter is unpublished and includes contributions from co-authors Allyson G. King, Eva C.

Wikberg, Pascale Sicotte, Brendan Bohannan, and Nelson Ting.

Chapter III of this dissertation explores the relationship between longitudinal change in social interactions and colobus gut microbiome plasticity. By utilizing a known catalyst for social change in adult-adult social relationships (infant birth and associated allocare), this research aimed to understand how socially-mediated microbial transmission influences the maintenance and stability of the adult gut microbiome through time. We found that grooming increased among adult females during the 3-month time period after infant birth during which allocare rates are highest, and that this increase in social interaction was reflected in increased microbial similarity among adult females. However, we were unable to tie this increased microbial similarity to stronger social relationships, which we conclude is likely due to sampling constraints. Further, we determined that shorter social network time periods than typically used (6 months) predicted microbial similarity among adult females, demonstrating that tighter time frames of social interaction are employable for understanding social microbial maintenance even in lower-affiliation species. The research in this chapter is unpublished and includes contributions from co-authors Emma Freedman, Eva C. Wikberg, Pascale Sicotte, Brendan Bohannan, and Nelson Ting.

The final chapter (Chapter IV) synthesizes the results of this work and summarizes the intellectual contributions of the research described here.

CHAPTER II:

EARLY LIFE SOCIAL ENVIRONMENT AND ALLOCARE SHAPE THE

DEVELOPING COLOBUS GUT MICROBIOME

Introduction

Research in this chapter includes unpublished co-authored material. Allyson G. King

(AK), Eva C. Wikberg (EW), Pascale Sicotte (PS), Brendan Bohannan (BB), and Nelson Ting

(NT) made substantial contributions to the work in this chapter. AK assisted in fecal and

demographic data collection. EW, BB, and NT assisted in project design and development,

provided feedback on analytical design, and edited the manuscript. EW and PS provided access

to the field site and to long-term demographic data. I led project development, conducted all data

analyses, wrote the initial draft of the manuscript, and edited the manuscript.

The gut microbiome is critical to multiple aspects of host physiological function,

including digestion (Amato, 2013; Gomez et al., 2015; Gomez et al., 2016; Suzuki, 2017), the

exclusion of pathogens (Candela et al., 2012; Stecher & Hardt, 2011; Suzuki, 2017), and

development, notably of the nervous system (Diaz Heijtz et al., 2011; Indrio et al., 2017;

Sampson & Mazmanian, 2015), and the immune system (Bengmark, 2013; Candela et al., 2012;

Hooper et al., 2012; Turnbaugh et al., 2009). Among other known sources (including genetics,

environment, and diet), social environment and host social behavior is known to impact the gut

microbiome; social group microbial "signatures" are found across a wide variety of taxa (Bennett

et al., 2016; Chiyo et al., 2014; Degnan et al., 2012; Grieneisen et al., 2017; Orkin et al., 2019;

Raulo et al., 2018; Rose et al., 2023; Springer et al., 2017; Tung et al., 2015; Vernier et al., 2020;

Wikberg et al., 2020), socially interacting individuals tend to have more similar microbiomes

(Amato et al., 2017; Grieneisen et al., 2017; Levin et al., 2016; Perofsky et al., 2017; Raulo et al.,

2021; Rudolph et al., 2022; Tung et al., 2015; Wikberg et al., 2020), and grooming is known to transmit microbes among primates (Tung et al., 2015). However, less is known about the impact of social transmission on microbiome assembly early in life, a time when the gut microbiome can play an especially important role in the development of the nervous and immune systems (Diaz Heijtz et al., 2011; Gensollen et al., 2016), potentially altering host health later in life. Here, we examine the influence of early life social environment on the developing gut microbiome of a wild primate, *Colobus vellerosus*.

There is some evidence that social environment can shape the early life gut microbiome; shared home environment impacts the presence and abundance of microbial taxa in human infant guts (Azad et al., 2013; Lane et al., 2019; Tavalire et al., 2021), laboratory primate infants that are reared with their mothers displayed distinct differences in gut microbial composition from infants that were nursery reared with their peers (Dettmer et al., 2019), and group-specific microbial profiles emerge in peer group housed laboratory primates shortly after weaning (Amaral et al., 2014). While parental care (which represents the majority of young infant social interaction) and parental effects on the infant gut microbiome has been widely studied (Baniel et al., 2022; Korpela et al., 2018; Manus, Sardaro, et al., 2023; Manus, Watson, et al., 2023; Petrullo et al., 2022; Reese et al., 2021), the effects of non-parental care and interactions with non-parental adults have been largely ignored. Infants of group-living species have social interactions with a variety of non-parental conspecifics through which socially-mediated microbial transmission may occur (Pinacho-Guendulain et al., 2022), and social interactions with non-parental adults and allocare (care provided to the infant by non-parental individuals) present an excellent opportunity for examining the role of diverse early life social contact on the gut microbiome of infants (Kuthyar et al., 2019).

There is limited evidence from human research that non-parental infant care (allocare) also shapes the early life gut microbiome. The diversity and composition of a lactating mother's milk microbiome is associated with the size of the mother-infant dyad's social network and amount of care given to the infant by non-maternal caregivers (Meehan et al., 2018). Although this study did not directly examine the infant gut microbiome, the milk microbiome strongly shapes the infant gut microbiome and is likely the primary postnatal mode of microbial transmission between infant and mother (Lackey et al., 2019; McDonald & McCoy, 2019). Additionally, alloparents were recently found to influence infant gut bacterial diversity, which was impacted by care behaviors (e.g. alloparental co-sleeping had stronger effects on infant bacterial diversity than holding), although the majority of the alloparents included were young (2-5 years old) cohabitating siblings and likely do not provide the range of typical allocare behaviors (Manus, Sardaro, et al., 2023). Our understanding of social environmental influences during early life are limited to findings from humans and captive animals, while very little is known about these dynamics in natural populations. Habituated wild non-human primates provide good systems for examining the associations between social behavior and microbiomes, as social interactions and diet can be more accurately characterized than in humans and the confounding influences of captivity and laboratory model systems can be avoided (Amato, 2013; Archie & Tung, 2015; Bjork et al., 2019).

To assess the influence of early life social environment and allocare on the developing gut microbiome, we collected behavioral data and characterized the gut microbiomes of adult females, young juveniles, and infants in 4 social groups of black and white colobus (*Colobus vellerosus*) at Boabeng-Fiema Monkey Sanctuary (BFMS) in central Ghana. Previous research has revealed that social environment plays a significant role in shaping gut microbial similarity

of adult colobus at BFMS. For example, social group microbial signatures are present in adults and are continuously altered through social interactions (Goodfellow et al., 2019), and social networks predict microbial similarity even among females of different social groups (Wikberg et al., 2020). Further, as in many other members of the subfamily Colobinae, allocare behaviors are well documented in *C. vellerosus* and are particularly concentrated during the first 3 months of life, overlapping with the critical window of development where gut microbiome assembly is important to long-term health (Badescu et al., 2015; Hrdy, 2009; Maestripieri, 1994; McKenna, 1979). While all black and white colobus females display allocare behaviors, rates of allocare vary according to infant, mother, and allomother characteristics, thus infants experience inter-individual variation in the allocare they receive (Badescu et al., 2015). This makes the black and white colobus an ideal natural system for testing the effects of diverse early life social environments on gut microbiome variation.

We tested four hypotheses regarding how an infant's social environment, including allocare, influences microbiome assembly early in life. We hypothesized that infants would have higher microbiome similarity with adult females in their social group than those in other social groups (H1), consistent with the observation of social group specific microbiome signatures across a wide range of host species in adulthood (Sarkar et al., 2020). Because primate mothers are the primary source of early colonizing microbes for their infants and provide the majority of direct care and social contact in early life (even in high allocare species), we hypothesized that the gut microbiomes of maternal-offspring dyads would be more similar than non-maternal-offspring dyads (H2). We hypothesized that maternal social relationships would predict infant-adult female gut microbial similarity (H3). This hypothesis arises from the observation that mothers mediate their infants' contact with other adults, and infant social relationships often

mirror those of their mothers' (Dunayer & Berman, 2018). Finally, we hypothesized that infants with higher rates of allocare, more frequent contact with allomothers, and greater allomother diversity would display higher microbial diversity and share more microbes with their social group members (H4).

Methods

Field Site and Study Population

This study focused on a natural population of white-thighed black and white colobus monkeys (*Colobus vellerosus*). *C. vellerosus* are medium-bodied arboreal primates that live in the upper canopies of forests in West Africa, and are characterized by black bodies and faces, white thighs, long white tails, and distinctive white ruffs around their faces (Fleagle, 1999). Infant black and white colobus are born with a bright white natal coat which fades to grey and then reaches their characteristic black and white coloration around 3 months of age; this contrasting natal coat is thought to be an adaptation associated with attracting allocare as protection against the high risk of infanticide found in colobines (Badescu et al., 2015). Rates of affiliative allocare are unusually high among *C. vellerosus*, and all adult black and white colobus females display allomothering behavior, which is concentrated in the first 3 months after infant birth (during which infanticidal risk is highest) (Saj & Sicotte, 2005; Wikberg et al., 2015). Allocare among colobines includes various handling, grooming, play, and other care and contact associated behaviors, and unlike cercopithecine primates, colobine mothers can be very permissive, and have been known to allow their infants to be transferred to other females sometimes the same day it was born (Badescu et al., 2015; Dolhinow & Murphy, 1982; Scollay & DeBold, 1980) . This makes the black and white colobus an ideal natural system for testing the effects of diverse early life social environments and allocare on gut microbiome variation.

As colobines, adult colobus subsist primarily on leaves (folivory), and have a variety of physiological adaptations that allow them to extract nutrients from this difficult to digest food source. Colobines have complex, multi-chambered stomachs (Matsuda et al., 2019), very low gut pH (Beasley et al., 2015) and distinct gut microbiota that allow them to detoxify plant toxins (Clayton et al., 2019; Suzuki, 2017), break down plant structural polysaccharides (fiber) into short chain fatty acids (Gomez et al., 2016), and synthesize and produce critical vitamins and minerals (Amato, 2013; Amato et al., 2014).

Our study population resides in Boabeng-Fiema Monkey Sanctuary (BFMS) in Ghana, West Africa. BFMS is a 1.92 km^2 dry semi-deciduous forest and is the site of a community conservation effort created and maintained by the bordering villages of Boabeng and Fiema in partnership with other surrounding communities. The black and white colobus at BFMS have been studied since 2000, and thus are very well-described (Badescu et al., 2015; Badescu et al., 2016; Brent et al., 2008; Goodfellow et al., 2019; Wikberg, 2012; Wikberg et al., 2020; Wikberg et al., 2014a, 2014b; Wikberg et al., 2015). This population of *C. vellerosus* was comprised of approximately 28 social groups at the time of the study, and they reside in uni- or multi-male/multi-female groups that range in size from 9-38 individuals. Females give birth every 11-16 months and births occur year-round (Vayro et al., 2020). We focused on 4 groups of colobus that have been studied for the past 15 years: Splinter, Redtail, Winter, and Wawa. During the study period, the groups varied between 10 and 26 individuals across all age and sex classes (see **Supplemental Table 4** for full group demographics).

Two brief preliminary field seasons occurred during July – September 2017 (preliminary field season 1) and March 2018-May 2018 (preliminary field season 2) where only fecal sampling occurred. This was followed by two primary field seasons during November 2018-May

2019 (field season 1) and December 2019-March 2020 (field season 2) where we collected both behavioral data and fecal samples. Finally, we included some fecal samples from focal infants collected by colleagues between field seasons 1 and 2 during October and November 2019.

Behavioral Data Collection and Analysis

Focal data were collected from all adult females and infants across 4 social groups. Adult females were followed throughout the two primary field seasons. Infants were followed immediately after birth (or at the beginning of the field season if born beforehand and under 1 year old) until they reached 1 year of age or were weaned, whichever occurred first. During field season 2 adult females in the Redtail group were dropped from behavioral data collection for the purposes of ensuring collection of dense behavioral data on the remaining social groups. However, behavioral data were collected from all Redtail infants continuing the same methods employed in previous field seasons so they could be included in the allocare subset.

Social groups were followed from dawn at nesting site (approximately 6:30am) until 2:40pm, maintaining approximate evenness of focal behavior across all focal animals in all 4 study groups. Behavioral data were recorded during 10-minute continuous focal samples of all adult females and infants following previous procedures used by Wikberg and colleagues (Wikberg et al., 2014b). Social and feeding behaviors (including plant species and plant part) were recorded continuously. Point samples were taken every 2.5 minutes in which the state behavior of the focal animal was recorded in addition to the identity of all individuals within 0, 1, 3, and 5 meter proximities (Altmann, 1974). A detailed ethogram of all behaviors recorded during continuous focal samples can be found in Supplemental Materials (**Supplemental Table 9**).

Adult Female Behavioral Data Analysis. We utilized social network analysis of adult

female focal data to understand how the social bonds and social position of the mother influences

the gut microbiome of her offspring. Undirected adult female social networks were constructed

for primary field seasons 1 and 2 for each social group using all occurrences of 1m approaches

during focal follows. The 1m approaches matrices were weighted by the total time in view for

each dyad, such that every dyadic undirected 1m approach count was divided by the summed

total of in-view focal hours that were collected on each individual in the dyad (see **Equation 1**) .

Thus, social connectedness (within the social network) was defined as the dyadic rates of 1m

approaches per in-view hour.

$$N = \frac{M_1 A_{M2} + M_2 A_{M1}}{M_1 T + M_2 T}$$

Equation 1: Undirected dyadic social connectedness. *Social connectedness (N) is the sum of monkey 1 (M_1) approaches (A) to within 1 m of monkey 2 (M_2) and monkey 2 approaches to within 1m of monkey 1, divided by the sum of monkey 1 and monkey 2 total in view focal hours (T).*

Further social network calculations were conducting using the *igraph* package for R.

Undirected weighted 1m approach matrices were converted into adjacency matrices, and we

calculated 2 measurements of adult female centrality: strength (or weighted degree) centrality

and eigenvector centrality. Strength (or weighted degree) centrality sums all weights of all

connections for a given node, and is considered a more nuanced approach than simply using

degree centrality (which counts the number of connections any given node has), while

eigenvector centrality is based on the number of links that an individual has to other nodes and

how well connected those nodes are (Brent et al., 2011).

Infant Behavioral Data Analysis. Allocare was measured in four ways – grooming, combined natal attraction and infant handling, social connectedness, and total number of allomothers. Rates of grooming and rates of combined natal attraction and infant handling for each infant were calculated as a proportion of total dyadic in view time without directionality. Social connectedness was calculated using rates of approaches to within 1m during continuous focal follows as a proportion of dyadic time in view as described above. Total number of allomothers was calculated using the presence/absence of all social behaviors between infants and non-maternal adult females. Birth dates, maternal/offspring relationship, social group, and wean dates were determined via long-term demographic data. Wean status was determined via last observed nipple/mouth contact, which is not always an accurate representation of actual weaning, thus we created a "transition" wean status that included any samples taken within two weeks of last observed nipple/mouth contact. Behavioral data collection and cleaning resulted in a total of 375.17 adult female focal hours (mean 14.43) and 73.84 early life focal hours (mean 7.38).

Fecal Collection, Shipping, and Storage

Fecal samples were collected from all focal adult females monthly throughout primary field seasons 1 and 2, and opportunistically during preliminary field seasons 1 and 2. Further fecal samples from all focal infants were collected opportunistically throughout development. Obtaining fecal samples from infant colobus is much more difficult, thus a minimum of 1 fecal sample was obtained from each focal infant with a target of 2-5 fecal samples per individual.

Individuals were identified and followed until defecation, at which point fecal samples were collected with the use of gloves and sterile collection sticks to prevent sample

contamination. For each adult female sample, approximately 1g of feces was immediately homogenized with approximately 4mL of RNAlater in an 8mL tube and secured with parafilm to prevent leakage. As young infant colobus feces can be much smaller than adult feces, the entire sample was often collected (up to approximately 1g) and homogenized with 2-4mL of RNAlater to maintain an appropriate ratio of sample to preservation buffer. At the end of the data collection day, samples were cataloged and stored at -20°C (maximum of 8.5 hours at ambient temperatures). At the conclusion of each field season, samples were shipped at ambient temperatures back to the Ting Lab at the University of Oregon, where they were stored at -20°C until DNA extraction.

16S rRNA Gut Microbial Characterization

   Sequencing. We sequenced the V4 region of the 16S rRNA locus in 312 samples from 55 individuals (adult females: mean 8.08, SD ± 3.91, range = 1-13, early life: mean 3.52, SD ± 1.96, range = 1-9). DNA was extracted using the QIAGEN QIAamp PowerFecal Pro DNA kit (Qiagen, Hilden, Germany) according to the kit handbook protocols with the following adjustments: initial fecal sample volume consisted of 250mL of stool/RNAlater slurry, bead-beating duration was increased by 5 minutes to account for larger extraction batches, and DNA was eluted in 75uL of elution buffer. Negative controls were processed with each extraction batch, and DNA was quantified using a Qubit dsDNA Broad Range assay kit and a Qubit 2.0 Fluorometer (Thermo Fisher Scientific). All samples that contained quantifiable DNA were prepared for 16S V4 library amplification. Libraries were prepared using 515F and 806R primers containing 5' Illumina adaptor tails and dual indexing barcodes in reactions containing 12.5μL NEB Q5 hot start 2x Master Mix, 11.5μL primer mix, and 1μL DNA. Thermocycler parameters

were as follows: initial denaturing at 98º for 0:30, 24 cycles of 98º for 0:10, 61º for 0:20, and 72º

for 0:20, and a final extension at 72º for 2:00. PCR products were cleaned and pooled for

sequencing following protocols outlined in Goodfellow et al. (2019). Libraries were subject to

300 bp paired-end sequencing on the Illumina MiSeq platform and raw reads were demultiplexed

by the University of Oregon's Genome and Cell Characterization Core Facility (GC3F).

Microbial Data Processing. Demultiplexed reads were bioinformatically processed using

the Quantitative Insights Into Microbial Ecology 2 (QIIME 2) platform (Bolyen et al., 2019) and

the DADA2 plug-in (Callahan et al., 2016). 16S rRNA sequencing of the early life dataset

resulted in an average of 108,449 reads per sample. Samples were trimmed to remove low-

quality portions of reads, filtered, and aligned, yielding an average of 97,247 reads per sample.

Sequencing of the adult female dataset resulted in an average of 80,614 reads per sample.

Trimming, filtering, and aligning yielded an average of 68,936 reads per sample. ASVs were

taxonomically assigned using version 138 of the SILVA database. Rooted taxonomic trees,

taxonomic information, and ASV tables were exported to be used for statistical analysis in R.

Statistical Analyses

Rooted taxonomic trees, ASV tables, taxonomic assignments, and metadata were

imported into R using the *qiime2r* and *phyloseq* packages (McMurdie & Holmes, 2013). Data

were filtered to remove any samples with fewer than 5,000 reads and contaminants. To account

for the potentially confounding influence of repeat sampling within individuals, all analyses

include individual ID as a random effect. Further, to eliminate the influence of potential

autocorrelation among samples taken from the same individual closely in time, samples were

randomly down-sampled to a maximum of 1 sample per individual per month. Alpha and beta

diversities were calculated using the *vegan* package (Oksanen et al., 2017). Basic

characterization of gut microbial diversity through development was conducted using Linear

Mixed Effects models, PERMANOVA, and neutral assembly modelling (see Supplemental

Materials).

 We used Generalized Linear Mixed Models (GLMMs) to compare early life and adult

female gut microbiomes. Comparisons were constrained to a single sample per adult female that

was collected closest in time to the early life sample, such that repeat ID dyads could occur, but

each early life sample was compared to a single sample from a given female. Further, Aitchison

distances between early life individuals and adult females displayed marked bimodality; nursing

infants were significantly less similar to adult females than weaned juveniles, therefore age

(centered and scaled), wean status, and the interaction between age and wean status were used as

control factors in all GLMMs. GLMMs were fit using the *glmmTMB* package (Brooks et al.,

2017), the *drop1* function was used to determine final model formulas, model fit was assessed by

examining residuals, and collinearity among variables was assessed using the *performance*

package (Lüdecke et al., 2021). Variables that displayed moderate or high collinearity (VIF > 5)

were dropped from the models, except for wean status and age, which were retained because 1)

they are used as a control factor and are not the variables of interest and 2) they are both known

to highly influence gut microbial similarity between early life individuals and adults. We also fit

reduced models for all hypotheses which included all control factors and random effects but not

the primary hypothesized predictor. Finally, model selection between full and reduced models

was performed using the *model.sel* function in the *MuMIn* package, and the model with the

lowest Delta AIC value was selected as the best fit model. The following describes our approach

to each set of hypotheses tested via competing GLMMs.

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H1 Social Group Membership. We tested whether the gut microbiomes of early life individuals were more similar to those of adult females in their social groups than to those of adult females in other social groups (1385 comparisons among 555 dyads; 26 adult females and 29 early life). The identity link Gamma family full and reduced models used Aitchison distance per ID dyad as response and accounted for individual dyad and collection month as random effects, and sex dyad, age, wean status, and field season of collection as control factors. The full model included shared social group (yes/no) as predictor.

H2 Mother/Offspring. We compared beta diversity distances between early life colobus gut microbiomes and all adult females within their social group to test the hypothesis that early life colobus gut microbiomes are more similar to their mothers' than to non-maternal adult females. Comparisons were only performed within social groups to eliminate the influence of shared social group, and any offspring without mothers represented in the adult female sampling for a given time period were removed from analysis, resulting in 334 comparisons among 136 dyads (24 adult females and 27 early life). The identity link Gamma family full and reduced models used Aitchison distances per ID dyad as response and accounted for sex dyad, wean status, age, and field season of collection as control factors and collection month and ID dyad as random effects. The full model included mother/offspring pair (yes or no) as predictor.

H3 Maternal Social Relationships. To test the hypothesis that maternal social connectedness predicted similarity between offspring gut microbiomes and non-maternal adult females, we compared early life samples to non-maternal adult female samples within their social group. Maternal/offspring dyads were removed as well as any offspring of mothers without matching behavioral data, resulting in 182 comparisons among 78 dyads (20 adult females and 19 early life). The identity link Gamma family full and reduced models used

Aitchison distance per ID dyad as response and accounted for collection month and individual dyad as random effects, and social group, age, sex, wean status, and field season as control factors. The full model included mother/adult female dyad social connectedness as predictor. We also investigated the effect of maternal social bonds on early life gut microbiome variation by testing whether gut microbial similarity between offspring and social group adult females is predicted by the mother's centrality in the social network. Any offspring of mothers without matching behavioral data were removed, resulting in 229 comparisons among 100 dyads (22 adult females and 19 early life). Full and reduced log (strength centrality) and identity (eigenvector centrality) link Gamma family models used Aitchison distance per ID dyad as response and accounted for collection month and individual dyad as random effects, and social group, age, sex, and wean status as control factors (social group was removed from eigenvector models per drop1). Full models used either strength centrality or eigenvector centrality as predictor.

H4 Allocare. We investigated the effect of direct allocare on infant gut microbiome alpha and beta diversity. To test the hypothesis that amount of allocare received in early life influences infant gut microbial alpha diversity, we fit three Linear Mixed Effects (LME) models with Shannon Index as response, individual ID as random effect, and three different measures of received allocare as predictor for our allocare dataset (26 samples from 10 individuals). Measures of allocare tested include grooming, combined natal attraction and infant handling, and number of allomothers. To test whether allocare predicts gut microbial similarity (beta diversity) between adult females and early life colobus, we compared early life colobus to social group adult females from our subset of samples that contained matched infant focal data and fecal samples (127 comparisons across 42 dyads; 19 adult females and 10 early life). Full and reduced

log link Gamma family models included dyadic Aitchison distance as response and age, wean status, group, and sex as control factors and collection month and individual dyad as random effects (sex was removed from number of allomothers models per *drop1*). Full models included social connectedness (dyadic), grooming (dyadic), combined natal attraction and infant handling (dyadic), and number of allomothers as predictors.

Results

Composition of the Early Life Gut Microbiome

After quality filtering, removing contaminants, and dropping low-read samples, 102 early life and 210 adult female samples were retained across 29 early life and 26 adult female individuals. Bacterial diversity was similar across the early life sample set (5,442 unique ASVs, mean 465 +/- 109.23 SD, range 144-695; 27 phyla and 219 families), and the adult female dataset (4,119 unique ASVs, mean 535 +/- 87.89 SD, range 201-747; 29 phyla and 214 families). Nursing infant gut microbiomes were dominated by Firmicutes (75%), Bacteroidota (12%), Spirochaetota (4%), and Verrucomicrobiota (3%), while weaned juvenile gut microbiomes were dominated by similar phyla (Firmicutes 88%, Bacteroidota 4%, Spirochaetota 3%, Cyanobacteria 1%) (**Supplemental Figure 4**).

The gut microbiomes of folivorous primates during early life have not been previously described, thus we first established that early life colobus gut microbiome dynamics are similar to those documented during development of other primates (including humans) (see Supplemental Materials). Aging and development were strong predictors of microbial alpha and beta diversity; age was positively correlated with Shannon index (Linear Mixed Effects model $p<0.001$; **Figure 1a**), and significant proportions of variation in beta diversity were explained by age (13%) and weaning status (12.8%) (PERMANOVA; **Figure 1c, Figure 1d**). Further, when

compared across the colobus lifespan, infant gut microbiomes were also highly dispersed

compared to juvenile and adult female gut microbiomes, which is clearly illustrated in

ordinations (beta dispersion p<0.001; **Figure 1b**). Finally, early life gut microbiomes are

decreasingly shaped by stochastic processes (including drift) as they move from infancy through

weaning (neutral assembly modelling Root-mean-square error; **Supplemental Table 5**;

Supplemental Figure 5). This exploration also revealed that field season and month of sample

collection, sex, social group, and individual identity significantly shaped early life beta diversity,

and thus these factors were controlled for in further modeling.
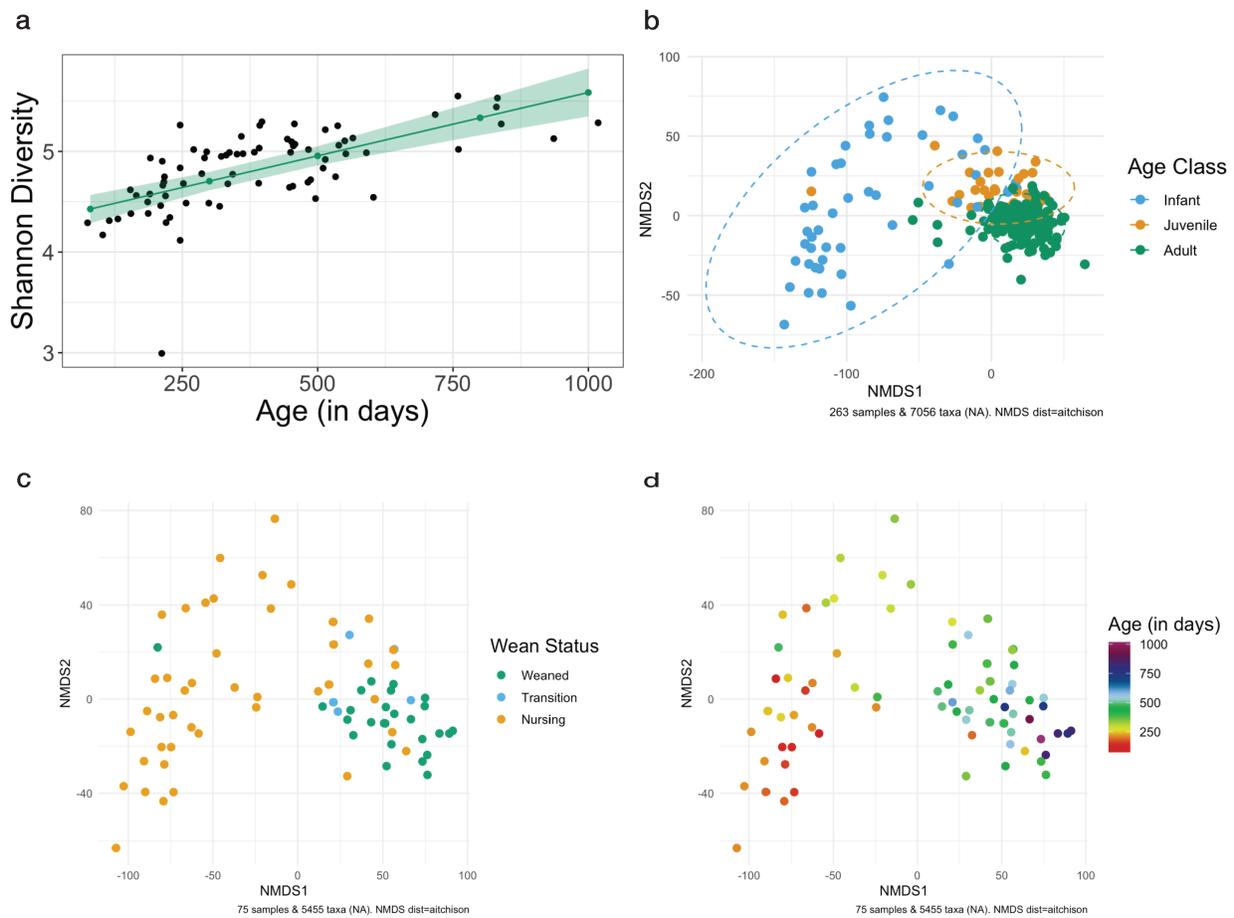
Figure 1: Gut microbiome development and variation across the lifespan. (a) Age effects on
early life colobus gut microbiome alpha diversity. The gut microbial diversity of infant and

juvenile colobus monkeys steadily increases with age (p<0.001). (b) Non-metric MultiDimensional Scaling (NMDS) ordination of all adult female, juvenile, and infant gut microbiomes (randomly down-sampled to retain a maximum of 1 sample per individual per month). Infant gut microbiomes (blue) are highly dispersed compared to other age classes, and colobus gut microbiomes converge after weaning (orange, green). (c) NMDS ordination of all infant and juvenile gut microbiomes (randomly down-sampled) colored by weaning status. Weaning status explains a large proportion of inter-individual variation in early life gut microbiomes ($R^2 = 0.128$). (d) NMDS ordination of all infant and juvenile gut microbiomes (randomly down-sampled) colored by age (in days). Age explains the largest proportion of inter-individual variation in early life gut microbiomes ($R^2 = 0.13$).

Social Environment Influences the Early Life Gut Microbiome

H1 Social Group Membership. Early life colobus gut microbiomes are more similar to adult females in their social group than adult females in other social groups. The full model containing shared social group as a predictor was selected over the reduced model, and shared social group was significant (coefficient estimate= -2.291, 95% CI: -3.826, -0.756).

H2 Mother/Offspring. Early life colobus gut microbiomes are not more similar to their mothers than to other adult females in their social group. Model selection revealed that the reduced model containing only control factors and random effects was better supported than the full model, and mother/offspring relationship was not significant (coefficient estimate = -1.257, 95% CI: -4.798, 2.234).

H3 Maternal Social Relationships. Mother social connectedness within the social network was not a predictor of offspring and non-maternal adult female gut microbial similarity, and the reduced model was selected over the full (coefficient estimate= -3.238, 95% CI: -12.829, 6.353). However, offspring with more central mothers shared more similar gut microbiomes with adult females than offspring with less central mothers; both strength centrality (coefficient estimate= -0.059, 95% CI: -0.105, -0.013) and eigenvector centrality (coefficient estimate= -

16.59, 95% CI: -28.434, -4.746) were significant predictors of offspring/adult female similarity

(**Table 1**).

Table 1: Modeling the effects of early life social environment. Competing GLMMs fixed effects, Akaike's information criterion (AIC), delta (difference in AIC between the current model and best-fit model) and Akaike weights (relative likelihood of the model). Hypothesis testing included mother/offspring similarity, shared social group, mother social connectedness, and 2 measures of mother centrality. Social group and mother centrality significantly predicted early life gut microbiomes, while mother/offspring relationship and mother social bonds did not.

Model	Model formula	AIC	Delta	Weight
Social group				
Full	Shared social group + Wean status * Age + Sex + Fieldseason	10723.10	0.00	0.96
Reduced	Wean status * Age + Sex + Fieldseason	10729.55	6.45	0.04
Mother/offspring				
Reduced	Wean status * Age + Sex + Fieldseason	2623.01	0.00	0.70
Full	Mother/offspring relationship +Wean status * Age + Sex + Fieldseason	2624.81	1.71	0.30
Mother social connectedness				
Reduced	Wean status * Age + Sex + Group + Fieldseason	1419.95	0.00	0.73
Full	Mother/adult female social connectedness + Wean status * Age + Sex + Group + Fieldseason	1421.90	1.94	0.27
Mother centrality (Strength)				
Full	Strength centrality + Wean status * Age + Sex + Group + Fieldseason	1781.12	0.00	0.88
Reduced	Wean status * Age + Sex + Group + Fieldseason	1785.10	3.99	0.12
Mother centrality (Eigenvector)				
Full	Eigenvector centrality + Wean status * Age + Sex + Group + Fieldseason	1779.97	0.00	0.87
Reduced	Wean status * Age + Sex + Group + Fieldseason	1783.73	3.76	0.13

H4 Allocare. Levels of allocare received did not predict gut microbial alpha diversity; Linear Mixed Effects models found no influence of grooming, combined natal attraction and infant handling, number of social partners, or number of allomothers on Shannon Diversity in the early life colobus gut microbiome. Dyadic allocare rates (including social connectedness, grooming, and combined natal attraction and infant handling) also did not predict gut microbial

beta diversity between early life colobus and social group adult females. In all cases, reduced

models were selected over full models, and none of the predictor variables rose to the level of

significance. However, model selection revealed that early life colobus with more allomothers

had significantly more similar gut microbiomes to adult females in their social groups than

infants with fewer allomothers (coefficient estimate= -0.034, 95% CI: -0.046, -0.022) (**Table 2**;

Figure 2).

Table 2: Modeling the effects of allocare. Competing GLMMs fixed effects, Akaike's information criterion (AIC), delta (difference in AIC between the current model and best-fit model) and Akaike weights (relative likelihood of the model). Hypothesis testing included number of allomothers, social connectedness, grooming, and combined natal attraction and infant handling. Reduced models were selected and the null hypothesis was not rejected when Delta AIC values for the reduced model was lower than for the respective full model, while full models with lower Delta AIC values were selected. When full models were selected, the null hypothesis was only rejected if the hypothesized variable was significant in the model. Number of allomothers significantly predicted early life gut microbiomes, while other measures of allocare did not.

Model	Model formula	AIC	Delta	Weight
Number of allomothers				
Full	Number of allomothers + Wean status + Age + Group	900.75	0.00	9.99
Reduced	Wean status + Age + Group	924.68	23.93	0.00
Social connectedness				
Reduced	Wean status + Age + Sex + Group	906.65	0.00	0.76
Full	Social connectedness + Wean status + Age + Sex + Group	908.90	2.25	0.24
Grooming				
Reduced	Wean status + Age + Sex + Group	906.65	0.00	0.76
Full	Grooming + Wean status + Age + Sex + Group	908.98	2.33	0.24
Natal attraction and infant handling				
Reduced	Wean status + Age + Sex + Group	906.65	0.00	0.58
Full	Natal attraction and infant handling + Wean status + Age + Sex + Group	907.27	0.62	0.42
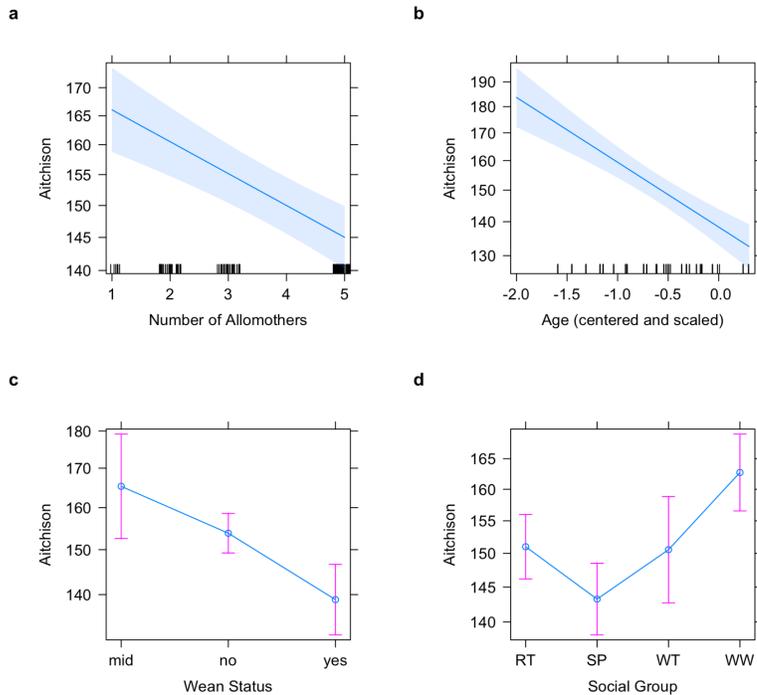
Figure 2: Effects of allomother diversity on early life gut microbiomes. Effects plots displaying the relationship between response (Aitchison distance), number of allomothers (a), and other significant control factors included in the model: (b) age centered and scaled, (c) wean status, and (d) social group. Lines show the relationships predicted by the GLMM, and shaded areas represent 95% confidence intervals. Infants with more allomothers share more similar microbiomes with adults in their social group than infants with fewer allomothers (a).

Discussion

Our understanding of how social environment shapes the gut microbiome of social animals has been largely focused on cross sectional studies of adult individuals, while less is known about these patterns in early life and through development, during which the gut microbiome can play an especially important role in the development of the nervous and immune systems. We found that nursing and development results in a drastically different early life colobus gut microbial community than that harbored by adult individuals, but infant colobus gut microbiomes are also shaped by aspects of their social environment, including by non-parental adult social partners. We suggest that microbial transmission from older individuals to infants

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may help prime developing guts for a future adult diet, easing the potentially negative health impacts of weaning (including dysbiosis, diarrhea, and enteric infections) and speeding up the ability to derive proper nutrition from a fully folivorous diet (Wei et al., 2021).

Selection and Diversity Increase Over Time in the Early Life Colobus Gut Microbiome

We found that early life colobus gut microbiomes are characterized by similar aging and development patterns found across other animals, including humans, primates, and fish (Baniel et al., 2022; Burns et al., 2016; Petrullo et al., 2022; Roswall et al., 2021) (although see Reese et al. 2021); gut microbial diversity steadily increases while inter-individual variability and volatility decreases throughout development. However, this microbial shift and selection around weaning is likely more dramatic in colobines to accommodate a folivorous diet which requires much greater microbial diversity, greater reliance on microbial processes to help break down complex plant compounds, and drastic changes in gut physiology (Amato et al., 2014; Amato et al., 2013; Beasley et al., 2015; Clayton et al., 2019; Gomez et al., 2016; Suzuki, 2017) . This pattern of microbial succession in early life is shaped not only by developmental, physiological, and dietary changes, but also via an increasingly wide social environment and new social connections (Koenig et al., 2011; Yatsunenko et al., 2012).

Early Life Gut Microbiomes are Shaped by Social Group Members

We found evidence of social acquisition of microbes throughout early life, suggesting that social environment shapes the colobus gut microbiome despite drastic developmental changes. Regardless of age group or weaning status, social group is a significant and moderate predictor of early life gut microbial similarity, akin to what has been observed during adulthood

in other social animals. Further, this pattern is visible even when comparing across age classes; infant and juvenile gut microbiomes are consistently more similar to those of adult females within their social group than to those of adult females in other social groups. Social group is likely a proxy for several conflated factors including not just social contact (direct), but also shared environment (indirect contact with shared surfaces), dietary similarity, geography, and relatedness. Establishing that this social group signature pattern is present in early life individuals is an important first step in understanding how social environment and social contact influences early life gut microbiomes. Although a few other studies have included juvenile individuals in their examinations of group-level microbial dynamics (Amato et al., 2017; Moeller et al., 2016; Perofsky et al., 2017), to our knowledge ours is the first to show that infant microbiomes display distinct characteristics across social groups. While much attention has been paid to parental (particularly maternal) effects on the infant microbiome, infants of group-living animals have complex social environments that include interactions with non-parental and non-peer social partners. These social relationships contribute to early life microbial assembly and thus should not be ignored.

Mothers and Young Offspring Do Not Share More Similar Microbiomes

Unexpectedly, early life colobus gut microbiomes are not more similar to that of their mothers, despite the role colobus mothers play as primary care providers and the central role maternal milk plays in shaping infant gut microbiomes. Mother/offspring gut microbiome similarity has been seen across a wide spectrum of host systems, including both captive and wild primates (Petrullo et al., 2022; Reese et al., 2021). Shared genetic background (which may be acting as a filter in determining what microbes can colonize and persist in the gut), shared

proximal environment, and vertical transmission (via birth, nursing, and physical contact) generally contribute to microbial similarity between mothers and their offspring (Lackey et al., 2019; Laursen, 2021).

Although it is surprising that early life colobus gut microbiomes do not share more similarity with their mothers', it is not unprecedented. A different primate study documented similar findings; the gut microbiomes of gelada mother/infant pairs are not more similar than those of random female/infant pairs (Baniel et al., 2022). Geladas (the only primate with a grass-specialized diet) are a close dietary analogue for folivorous colobines, thus the lack of similarity between mothers and their offspring may be related to the extreme dietary and physiological shifts associated with weaning to a folivorous (or in the case of geladas, graminivorous) diet. Given the drastically different demands placed on the gut microbiota of a nursing infant and an adult folivore, it is possible that microbial taxa transmitted between mothers and their infants are simply unable to colonize or persist in the other gut environment. However, this seems unlikely given the detectable microbial similarity between infants and social group adults (e.g., Hypothesis 1), which indicates that microbes transmitted from adults to infants are persisting in the infant gut. A more likely explanation, at least for the colobus, is the homogenization of microbiomes among infants and non-mothers via allocare (unlike colobus, geladas engage in lower degrees of affiliative allocare and more exploitative allocare e.g., kidnapping, as seen in most other cercopithecine primates). The high degree of allomaternal care colobus infants receive may facilitate homogenization of the gut microbiomes of infants with non-maternal group members, masking similarity between infants and their mothers.

Maternal Social Position May Facilitate Offspring Microbial Acquisition

Mothers mediate whom their infants have contact with and infant handling often mirrors maternal social relationships, thus maternal social bonds are a good approximation of infant social bonds, particularly during the high allocare period (0-3 month of age) (Dunayer & Berman, 2018). Further, the social position of the mother often predicts the rates of allocare an infant receives; higher ranking/more socially connected mothers typically have higher allocare infants (Boose et al., 2018; Hrdy, 1976). We used maternal social bonds (dyadic social connectedness within the social network) and social position (centrality within the social network) as a proxy for early life social bonds because direct focal data do not exist for all infants in our study. Social connectedness measures the strength of social bonds between 2 individuals within the social network, while social centrality quantifies the overall social position within the social network by incorporating the strength and number of social connections an individual has.

We found that infants with more central mothers had more similar gut microbiomes to adult females in their social groups than infants with less central mothers, thus maternal social position may play an important role in early life gut microbial acquisition from non-maternal social group members. This could indicate that mothers act as microbial mediators for their infants; mothers with more central social positions (more or stronger social bonds) may facilitate indirect transmission of microbes between their social partners and their offspring during affiliation. More likely (given the lack of maternal/offspring microbial similarity), maternal centrality is reflecting the influence of allocare, as infants of more central mothers are expected to receive higher degrees of allocare and likely hold more central social positions themselves. Across a wide variety of primates, having dominant or socially well-positioned mothers can

result in tangible benefits to their offspring, including inherited rank, allocare and other rearing

support, enhanced nutritional status, and lower risk of attack and infanticide (Dunayer &

Berman, 2018). We suggest that those benefits may also include microbial acquisition.

However, our analyses of social bonds (social connectedness) did not reveal a significant

impact of mother social networks on dyadic infant/adult female beta diversity. Centrality

captures how individuals fit within group-level social dynamics, and at this aggregate level, it

may be easier to detect cumulative microbial transmission. Meanwhile, social connectedness

may not result in sufficiently strong microbial transmission to detect dyadically. Epidemiological

studies of disease transmission dynamics often utilize social network centrality measures to

identify individuals who may be at greatest risk of infection, as they are most likely to be

exposed directly via close contacts, indirectly via secondary connections, and over longer periods

of contact (Christakis & Fowler, 2010). We suspect that our mixed results may be related to

these dynamics, as the strength of singular dyadic social relationships within a social network

(here social connectedness) may be a poorer measure for potential microbial transmission than a

combination of overall number of social transmission sources and strength of connections (here

centrality), particularly at the sparser resolution of behavioral sampling in our study (see also

Chapter III of this dissertation) (Raulo et al., 2021).

Allomother Diversity Impacts Infant Microbiomes

We found that infants with more allomothers share more microbes with their social group

members, pointing to a potential benefit of allocare and particularly of allo-caregiver diversity.

Dyadic allocare-associated behaviors (grooming and combined natal attraction and infant

handling) and dyadic social connectedness (between infants and adult females) were not

predictive of gut microbial similarity between infants and allomothers. However, total number of allomothers was associated with a significant increase in gut microbial similarity between infants and non-maternal adult females in their social groups, indicating that some aspect of allocare is likely mediating microbial transmission. These findings complement those of human caregiving networks; mothers of infants with larger caregiver networks/more caregivers had greater human milk microbial diversity (Meehan et al., 2018) (but see Manus, Sardaro, et al. 2023). Although we found no impact of allocare on alpha diversity, taken together with Meehan et al. (2018), our findings signify that allocare is likely a route for microbial transmission and may help seed early life microbiomes during a crucial time period where microbial colonization can have lasting consequences for infant health and immunity.

Humans and colobines are cooperatively breeding species, and thus a developing infant's immune system must contend with the increased pathogen risk inherent in exposure to a wider variety of non-maternal individuals. While the primary benefits of allocare for the infant include increased growth rates and faster development, there may be other overlooked health and wellbeing benefits (Badescu et al., 2016; Lee et al., 1991; Mitani & Watts, 1997; Ross & MacLarnon, 2000). Increased risk of pathogen/parasite transmission to relatively immunologically fragile infants is known to be a potential cost of allocare, but transmission of commensal microbiota may help mitigate that risk or even enhance health status via increased microbial diversity, playing a role in preventing pathogen invasion by filling available niches and directly inhibiting pathogen growth (Bailey, 2012), and providing functional redundancy (Tian et al., 2020). Further, acquisition of adult-associated microbiota via allocare may function to prime infant guts for weaning to a folivorous diet (with dietary-associated microbes) or prepare infant immune systems for the loss of maternal milk-derived immunologic factors (Kalbermatter et al.,

2021; Kurokawa et al., 2007). Microbial seeding could even contribute to the faster growth and development rates of allomothered infants primarily attributed to the enhanced nutritional status of their mothers and maternal milk. We suggest that socially-mediated microbial transmission may be an unappreciated benefit of allocare for cooperatively-breeding species including colobines and humans, and that social environment likely plays an important role in the assembly of the early life gut microbiome.

Limitations and Future Directions

Sample size is a major limitation of our study, as acquiring the necessary focal data and fecal samples from wild colobine populations is extremely difficult, especially from young individuals. Captivity alters the gut microbiome drastically (particularly for folivores), and thus may not be a sufficient alternative for examining natural variation and consequences of gut microbial transmission (Clayton et al., 2016; Zhou et al., 2022), and many co-operatively breeding non-human primate species can be notoriously challenging to study in the wild due to their arboreal natures. Complementary research in model experimental systems (e.g., cross-fostering and co-housing experiments) may be helpful in bridging the gap between naturalistic study of diverse early life social contact and the mechanisms and drivers of socially-mediated microbial transmission during development. Further, we can only speculate on the health impacts and long-term consequences of socially-mediated microbial transmission in early life with the data currently available. Additional longitudinal research is necessary to understand how the differences we found in microbial diversity and composition during infancy are associated with growth and development rates, weaning survival, and longevity of colobines.

CHAPTER III:

COLOBUS GUT MICROBIOME PLASTICITY AND SOCIAL DYNAMICS
FOLLOWING INFANT BIRTH

Introduction

Research in this chapter includes unpublished co-authored material. Emma Freedman
(EF), Eva C. Wikberg (EW), Pascale Sicotte (PS), Brendan Bohannan (BB), and Nelson Ting
(NT) made substantial contributions to the work in this chapter. EF assisted in data cleaning and
compilation. EW, BB, and NT assisted in project design and development and provided feedback
on analytical design. BB and NT edited the manuscript. EW and PS provided access to the field
site and to long-term demographic data. I led project development, conducted all data analyses,
wrote the initial draft of the manuscript, and edited the manuscript.

The microbial community present in the mammalian gut (aka the "gut microbiome")
plays many crucial roles in host physiology, including in development, immune function, and
digestion (Bailey, 2012; Gensollen et al., 2016; Indrio et al., 2017; Suzuki, 2017). Despite this
importance, the mammalian gut microbiome is highly variable in composition across individuals,
and understanding the source of this variation is of great interest because microbiome variation is
often associated with variation in gut microbiome function (Bengmark, 2013; Candela et al.,
2012; Falony et al., 2016; Turnbaugh et al., 2009). There is growing evidence that social
interactions can be an important driver of gut microbiome variation. Group sociality, social
interaction networks, and social behaviors (such as grooming) have been shown to be predictive
of microbial similarity in a wide variety of social animals (Amato et al., 2017; Antwis et al.,
2018; Bennett et al., 2016; Chiyo et al., 2014; Degnan et al., 2012; Grieneisen et al., 2017; Levin
et al., 2016; Moeller et al., 2016; Orkin et al., 2019; Perofsky et al., 2017; Raulo et al., 2021;

Raulo et al., 2018; Rose et al., 2023; Rudolph et al., 2022; Springer et al., 2017; Tung et al., 2015; Vernier et al., 2020; Wikberg et al., 2020). However the majority of these studies have relied on "snapshots" of microbiome composition (based on small numbers of fecal samples per individual), and while social animals exhibit social partner preference, social relationships can change over time, particularly in conjunction with significant demographic events such as infant birth (Blaszczyk, 2017; Machanda & Rosati, 2020; Shizuka & Johnson, 2020; Teichroeb et al., 2009). The goal of this study was to assess the contribution of social transmission to microbiome variation during the social shifts associated with infant birth in a social primate, the black and white colobus monkey (*Colobus vellerosus)*.

Infant birth presents a particularly good natural experiment in social change, as birth is an expected, regular occurrence, and shifts in social dynamics surrounding infant birth occur in predictable patterns, particularly in high allocare (non-parental care) species. Jockeying for access to infants among potential allomothers leads to more interaction between adult females that may not typically affiliate, while affiliation with and grooming the mother is a popular strategy for winning infant access (Dunayer & Berman, 2018; Wikberg et al., 2015). Among non-human primates, overall grooming and other affiliation rates are known to increase after infant birth; and colobine monkeys (suborder Colobinae) in particular display unusually high levels of adult female allocare following infant birth (Badescu et al., 2015; de Lima & Ferreira, 2021; Hrdy, 2009; Maestripieri, 1994; McKenna, 1979; Wikberg et al., 2015). Thus, members of this subfamily provide an excellent model for examining the influence of changing social relationships (after infant birth) on microbial transmission.

We examined social change and gut microbiome plasticity in adult females in response to infant birth in 4 social groups of a black and white colobus monkey (*Colobus vellerosus;*

hereafter black and white colobus) at Boabeng-Fiema Monkey Sanctuary in central Ghana. First, we aimed to identify and characterize affiliative changes among adult females in response to infant birth and young infant presence. We hypothesized that 1) adult female social bonds would strengthen after infant birth and during the time period when allocare is highest (infant is < 3 months old) in comparison to time periods with no young infants in the social group. Next, we characterized colobus gut microbiomes to determine whether adult female gut microbiomes change in the presence of young infants. We hypothesized that 2) adult female gut microbiomes would be more similar after infant birth and during the time period when allocare is the highest. Finally, we aimed to test whether these gut microbial shifts in the presence of young infants were driven by changes in social bonds. We hypothesized that 3) changes in social bond strength and social partner preference among adult females after infant birth would result in increased gut microbial similarity among adult female colobus.

Methods

Study Species, Study Population, and Field Site

Data were collected on a natural population of white-thighed black and white colobus, which is a species of colobine monkey endemic to Africa. *C. vellerosus* are medium-bodied arboreal primates that live in the upper canopies of forests. As colobines, *C. vellerosus* subsist primarily on leaves (folivory), and have a variety of physiological adaptations that allow them to extract nutrients from this difficult to digest food source. Colobines have complex, multi-chambered stomachs (McKenna, 1979), very low gut pH (Beasley et al., 2015) and distinct gut microbiota that allow them to detoxify plant toxins (Clayton et al., 2019; Suzuki, 2017), break down plant structural polysaccharides (fiber) into short chain fatty acids (Gomez et al., 2016), and synthesize and produce critical vitamins and minerals (Amato, 2013; Amato et al., 2014).

Further, colobines spend a greater amount of time feeding and resting than most other primates due to the difficulty of obtaining sufficient nutrition from this low-quality diet, and as such, colobines are known to be less social than other non-folivorous primates (e.g., *C. vellerosus* spend less than 1% of their time engaged in grooming, in contrast to baboons which can spend upwards of 30% of their time engaged in grooming) (Christensen et al., 2023). Thus, proximity is often used in place of grooming to quantify social bonds (described below), as grooming interactions are sparse.

Female black and white colobus give birth to white infants every 11-16 months and births occur year-round (Vayro et al., 2020). Young infant coats fade to grey and then reach their characteristic black and white coloration around 3 months of age; this contrasting natal coat is thought to be an adaptation associated with attracting allocare as protection against the high risk of infanticide found in colobines (Badescu et al., 2015). All adult black and white colobus females display allomothering behavior, which is concentrated in the first 3 months after infant birth (during which infanticidal risk is highest) and affects various social behaviors, including increased adult-adult grooming (Saj & Sicotte, 2005; Wikberg et al., 2015). High rates of allomothering and associated social shifts, as well as predictable and frequent infant birth events make this an ideal system for testing the influence of short-term social changes on gut microbiomes.

Our study population resides in Boabeng-Fiema Monkey Sanctuary (BFMS) in Ghana, West Africa. BFMS is a 1.92 km^2 dry semi-deciduous forest and is the site of a community conservation effort created and maintained by the bordering villages of Boabeng and Fiema in partnership with other surrounding communities. This population of black and white colobus is comprised of approximately 28 uni- or multi-male/multi-female social groups that range in size

from 9-38 individuals, has been studied since 2000, and thus are very well-described (Badescu et al., 2015; Badescu et al., 2016; Brent et al., 2008; Goodfellow et al., 2019; Wikberg, 2012; Wikberg et al., 2020; Wikberg et al., 2014a, 2014b; Wikberg et al., 2015). We focused on 4 groups of colobus that have been studied for the past 15 years: Splinter, Redtail, Winter, and Wawa. During the study period, the groups varied between 10 and 26 individuals across all age and sex classes (see **Supplemental Table 4** for full group demographics). Data collection was conducted across two field seasons: November 2018 - May 2019 (field season 1) and December 2019 - March 2020 (field season 2).

Fecal Sample Collection

Fecal samples were collected from each focal adult female throughout field seasons 1 and 2 approximately every 4 weeks, resulting in 163 samples from 21 individuals (mean 7.76 ± 2.21, range = 3-10) across 10 months of total data collection. Adult and sub-adult females from the same social group were sampled as closely together in time as possible, in many instances on the same day (sampling spread average: 1.33 days $\pm$ 0.20, range: 0-23 days).

Individuals were identified and followed until defecation, at which point fecal samples were collected with the use of gloves and sterile collection sticks to prevent sample contamination. For each adult female sample, approximately 1g of feces was immediately homogenized with approximately 4mL of RNAlater in an 8mL tube and secured with parafilm to prevent leakage. At the end of the data collection day, samples were cataloged and stored at -20°C (maximum of 8.5 hours at ambient temperatures). At the conclusion of each field season, samples were shipped at ambient temperatures back to the Ting Lab at the University of Oregon, where they were stored at -20°C for up to 4 years awaiting DNA extraction.

16S rRNA Gut Microbial Characterization

     Sequencing. DNA was extracted using the QIAGEN QIAamp PowerFecal Pro DNA kit (Qiagen, Hilden, Germany) according to the kit handbook protocols with the following adjustments: initial fecal sample volume consisted of 250mL of stool/RNAlater slurry, bead-beating duration was increased by 5 minutes to account for larger extraction batches, and DNA was eluted in 75uL of elution buffer. Negative controls were processed with each extraction batch, and DNA was quantified using a Qubit dsDNA Broad Range assay kit and a Qubit 2.0 Fluorometer (Thermo Fisher Scientific). All samples that contained quantifiable DNA were prepared for 16S V4 library amplification. Libraries were prepared using 515F and 806R primers containing 5' Illumina adaptor tails and dual indexing barcodes in reactions containing 12.5μL NEB Q5 hot start 2x Master Mix, 11.5μL primer mix, and 1μL DNA. Thermocycler parameters were as follows: initial denaturing at 98° for 0:30, 24 cycles of 98° for 0:10, 61° for 0:20, and 72° for 0:20, and a final extension at 72° for 2:00. PCR products were cleaned and pooled for sequencing following protocols outlined in Goodfellow et al. (Goodfellow et al., 2019). Libraries were subject to 300 bp paired-end sequencing on the Illumina MiSeq platform and raw reads were demultiplexed by the University of Oregon's Genome and Cell Characterization Core Facility (GC3F).

     Microbial data processing. Demultiplexed reads were bioinformatically processed using the Quantitative Insights Into Microbial Ecology 2 (QIIME 2) platform (Bolyen et al., 2019) and the DADA2 plug-in (Callahan et al., 2016). Sequencing resulted in an average of 87,587 reads per sample. Trimming, filtering, and aligning yielded an average of 74,368 reads per sample.

ASVs were taxonomically assigned using version 138 of the SILVA database. Rooted taxonomic trees, taxonomic information, and ASV tables were exported for statistical analysis in R.

Behavioral Data Collection and Analysis

Focal data were collected from all adult females across 4 social groups. During field season 2 adult females in the Redtail group were dropped from behavioral data collection for the purposes of ensuring collection of dense behavioral data on the remaining social groups. Social groups were followed from dawn at nesting site (approximately 6:30am) until 2:40pm, maintaining approximate evenness of focal behavior across all focal animals in all 4 study groups. Behavioral data were recorded during 10-minute continuous focal samples of all adult females and infants following previous procedures used by Wikberg and colleagues (Wikberg et al., 2014b). Social and feeding behaviors (including plant species and plant part) were recorded continuously. Point samples were taken every 2.5 minutes in which the state behavior of the focal animal was recorded in addition to the identity of all individuals within 0, 1, 3, and 5 meter proximities (Altmann, 1974). A detailed ethogram of all behaviors recorded during continuous focal samples can be found in Supplemental Materials (**Supplemental Table 9**).

Grooming is often considered the gold-standard for quantifying social relationships in primates, however the very low overall rates of grooming in colobines presents statistical problems for quantification, particularly on the short time scales we used, and thus we also incorporated proximity-based interactions to allow for more robust estimations of social bonds. Undirected adult female social networks were constructed for each social group using all occurrences of 1-meter approaches and grooming rates during focal follows during primary field seasons 1 and 2. Undirected networks do not take the actor and receiver directionality into

account, but simply quantify how many times either individual within a given dyad approached to within 1m of the other individual in the dyad. We chose undirected networks as our overall objective is to understand how social proximity and contact may transmit gut microbes, but our approach is not granular enough to understand the directionality of this transmission. The 1m approaches matrices were weighted by the total time in view for each dyad, such that every dyadic undirected 1m approach count was divided by the summed total of in-view focal hours that were collected on each individual in the dyad (see **Equation 1**). Undirected grooming rates were calculated as the number of seconds per dyadic in-view hour spent grooming. Behavioral data collection resulted in 358.59 in-view focal hours (average: 17.076 per female). Adult females spent an average of 0.34% of their time grooming each available adult female social partner ($\pm$ 0.008, range: 0-2.25%), an average of 3.8 % of their time in close proximity (1m or less) with available adult female social partners (% $\pm$ 0.04, range: 0-11.7), and on average approached within 1m of each available adult female social partner 0.33 times per hour ($\pm$ 0.037, range: 0-1.09).

Infant presence time periods were determined using demographic data of infant births and deaths/disappearances. We defined the "young infant" time period as the first 3 months of an infant's life (when allocare rates are highest) unless the infant died or disappeared before reaching 3 months old, in which case the young infant time period ended on the death/disappearance date. Fecal sampling time points within social groups were categorized as either young infant present or young infant absent. Grooming and proximity rates in the presence or absence of young infants were calculated based on time period of presence/absence, and thus were not all the same length of time, but always contained the given fecal sampling period within the time period of the network. Multiple births within a social group resulted in some

overlapping young infant present time points that may extend longer than 3 months, while infant deaths/disappearances resulted in some infant present time points shorter than 3 months. This resulted in uneven treatment groups due to a higher number of infant presence than infant absence time periods and longer infant presence than infant absence time periods (222 dyads from 26 infant present time periods and 76 dyads from 11 infant absence time periods). However, the in-view focal hours were proportionally even given the uneven dyadic totals (259.8 total in-view focal hours for infant presence time periods and 98.8 for infant absence time periods; 4.63 average in-view focal hours per female per infant presence time period, 4.02 average per female per infant absence time period). Further, the statistical approaches used are known to be robust to uneven treatment groups (Pinheiro, 2014).

Continuous feeding data including plant species and food item were used to create dietary dissimilarity matrices to characterize similarity in diet across adult females for use as a control factor in all analyses. We calculated Bray-Curtis (abundance-weighted) and Sorensen's (presence/absence) indices for dissimilarity to capture whether the abundance or simple presence of a given food item was a better predictor of microbial similarity. These indices were calculated based on combinations of unique plant species and food items based on continuous event samples of dietary intake. Dietary dissimilarity indices were aggregated to the same time frames described above for the corresponding proximity/grooming networks. Adult females ate 17 items from 41 plant species for a total of 102 unique species and item combinations. Modelling results from Sorensen's and Bray-Curtis indices for dietary dissimilarity on gut microbial similarity were similar, thus only Bray-Curtis was used in the final models as it accounts for proportion of diet and provides a finer dietary resolution than Sorensen's index.

Difference in age among adult females was calculated using demographic data and known birth dates for all but the oldest adult females in our study population; the ages of 3 geriatric adult females were estimated when research began in 2000. Female dyads were either classified as both non-mothers during fecal sample collection, one mother and one not, or both mothers. These dyadic age differences and dyadic maternal statuses were used as control factors in analyses described below.

Statistical Analyses

Aitchison distances were calculated for intra-group dyads according to fecal collection time point. Aitchison distance was used as it best accounts for the compositional nature of microbial data (Quinn et al., 2018). Rooted taxonomic trees, ASV tables, taxonomic assignments, and metadata were imported into R using the *qiime2r* and *phyloseq* packages (McMurdie & Holmes, 2013). Data were filtered to remove any samples with fewer than 5,000 reads and contaminants. To account for the potentially confounding influence of repeat sampling within individuals, all analyses include individual ID as a random effect. ASV tables were center-log transformed and Aitchison distance was calculated using the *vegan* package (Oksanen et al., 2017).

We used Generalized Linear Mixed Models (GLMMs) to assess the interactions between infant birth, adult female social bonds, and gut microbiome variation. Comparisons were constrained to within-group dyads of samples collected during the sample time point, such that samples were not compared through time (298 comparisons among 47 dyads). GLMMs were fit using the *glmmTMB* package (Brooks et al., 2017), the *drop1* function was used to determine final model formulas, model fit was assessed by examining residuals, and collinearity among

variables was assessed using the *performance* package (Lüdecke et al., 2021). We also fit

reduced models for all hypotheses which included all control factors and random effects but not

the primary hypothesized predictor(s). Finally, model selection between full and reduced models

was performed using the *model.sel* function in the *MuMIn* package, and the model with the

lowest AICdelta value was selected as the best fit model. The following describes our approach

to each hypothesis tested via competing GLMMs.

Hypothesis 1 Social Shifts After Infant Birth. We compared grooming rates and proximity

rates among adult female dyads according to the occurrence of infants in the social group to test

the hypothesis that social bond strength increases when a young infant is present in the social

group. Grooming and proximity rates were used as response variables, infant presence and age

difference were included as fixed effects, and ID dyad as a random effect. The reduced models

included all covariates except infant presence.

Hypothesis 2 Infant Presence and the Gut Microbiome. We compared gut microbial beta

diversity distances among adult female dyads according to the presence of young infants in the

social groups to test the hypothesis that adult female gut microbiomes are more similar when a

young infant is present. Aitchison distance was used as the response variable, infant presence,

maternal status, dietary dissimilarity, age difference, field season of collection, and sampling

spread (days between dyad sample collection to control for differences in within-group sampling

effort) were included as fixed effects, and ID dyad and collection month were included as

random effects (298 comparisons among 47 dyads). The reduced model included all covariates

except infant presence.

Hypothesis 3 Social Bonds Drive Gut Microbial Shifts in Infant Presence. We compared

gut microbial beta diversity distances among adult female dyads according to the presence of

infants in the social group and grooming and proximity rates to test the hypothesis that social

bonds drive microbial similarity among adult females in young infant presence. Aitchison

distance was used as the response variable, grooming, proximity, or grooming interacting with

proximity, and infant presence, maternal status, dietary dissimilarity age difference, field season

of collection, and sampling spread (days between dyad sample collection to control for

differences in within-group sampling effort) were included as fixed effects, and ID dyad and

collection month were included as random effects (298 comparisons among 47 dyads). The

reduced model included all covariates except grooming, proximity, and grooming interacting

with proximity.

Results

Hypothesis 1 Social Shifts After Infant Birth

We found that grooming rates were significantly higher (coefficient estimate = 0.498,

95% CI: 0.080, 0.916) and the full model was selected over the reduced, while proximity rates

were not (coefficient estimate = 0.010, 95% CI: -0.192, 0.212) and the reduced model was

selected over the full. Further, age difference between adult females was a strong predictor of

grooming rates and social bond strength; females closer in age spent more time grooming each

other (coefficient estimate = -0.141, 95% CI: -0.229, -0.054) and spend more time in proximity

(coefficient estimate = -0.047, 95% CI: -0.075, -0.019) than females farther apart in age

(**Supplemental Table 6**).

Hypothesis 2 Infant Presence and the Gut Microbiome

Young infant presence had a significant effect on adult female gut microbial similarity;

adult female gut microbiomes were more similar when a young infant was present in the social

group (coefficient estimate = -0.048, 95% CI: -0.077, -0.019), and the full model was selected

over the reduced. Age difference between adult females (coefficient estimate = 0.005, 95% CI:

0.004, 0.007) and field season of collection (coefficient estimate = 0.031, 95% CI: 0.003, 0.059)

were also significant predictors, while sampling spread was marginally significant (coefficient

estimate = 0.006, 95% CI: 1.50e-04, 0.011), and all other model predictors (maternal status and

diet) were not.

In order to visualize the effect of the model predictors on gut microbial similarity, we

plotted the relationship between response (Aitchison distance) and significant model predictors,

including young infant presence, age difference between adult females, field season of data

collection, and sampling spread (**Figure 3**). Adult female gut microbiomes are more similar in

the presence of young infants (**Figure 3a**), are more similar to adult females of similar ages

(**Figure 3b**), are more similar during field season 1 (**Figure 3c**), and are more similar when

collected closely in time (**Figure 3d**).
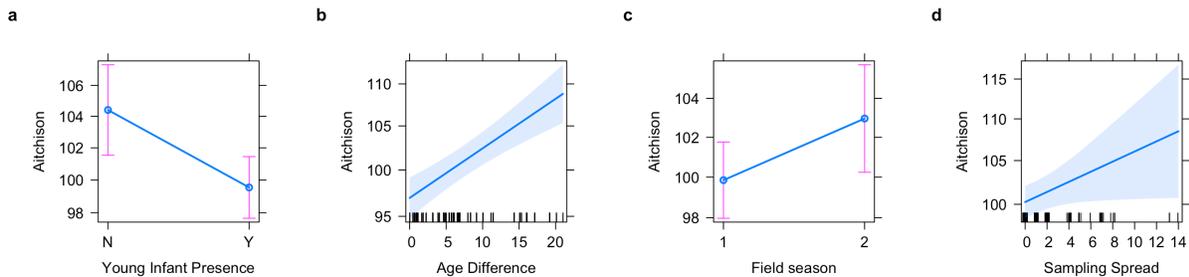
Figure 3: Effects of infant presence on adult female gut microbiomes. GLMM effects plots displaying the relationship between response (Aitchison distance) and significant model predictors, including (a) young infant presence, (b) age difference between adult females, (c) field season of data collection, and (d) sampling spread. Lines show the relationships predicted by the GLMM, and shaded areas represent 95% confidence intervals. Adult female gut microbiomes are more similar in the presence of young infants (a; Aitchison distance decreases), are more similar to adult females of similar ages (b), are more similar during field season 1 (c), and are more similar when collected closely in time (d).

Hypothesis 3 Social Bonds Drive Gut Microbial Shifts in Infant Presence

While adult female gut microbiomes are more similar in the presence of a young infant, social bonds were not significant predictors in the models (grooming: coefficient estimate = 1.2e-04, 95% CI: -3.40e-04, 5.83e-04; proximity: coefficient estimate = -0.017, 95% CI: -0.060, 0.025; proximity * grooming: coefficient estimate = 5.17e-05, 95% CI: -0.001, 0.001), and the reduced model containing only infant presence and covariates (and not grooming, proximity, or grooming interacting with proximity) was selected over the full models (**Table 3**). Age difference between adult females and field season of collection were also significant predictors in all models, sampling spread was marginally significant in the grooming model, while all other predictors (maternal status, diet, and sampling spread) were not.

Table 3: Modeling the effects of infant presence. Competing GLMMs fixed effects and response variables, Akaike's information criterion (AIC), delta (difference in AIC between the current model and best-fit model) and Akaike weights (relative likelihood of the model). Hypotheses tested include that (1) infant presence predicts grooming and proximity, that (2) infant presence predicts gut microbial similarity (Aitchison distance), and (3) social bonds drive gut microbial similarity in infant presence. Infant presence significantly predicts grooming rates, and the full model was selected over the reduced. Infant presence does not predict adult female proximities, and the reduced model was selected over the full. Infant presence predicts gut microbial similarity, and the full model was selected over the reduced. Finally, social bonds (grooming, proximity, and proximity * grooming) do not predict microbial similarity during infant presence, and the reduced model incorporating infant presence but not social bonds was selected over the full model.

Model	Model formula	AIC	Delta	Weight
Grooming in infant presence				
Full	Grooming ~ Infant presence + Age	1387.17	0.00	0.87
Reduced	Grooming ~ Age	1390.92	3.75	0.13
Proximity in infant presence				
Reduced	Proximity ~ Age	144.31	0.00	0.99
Full	Proximity ~ Infant presence + Age	155.87	11.55	0.00
Microbiomes in infant presence				
Full	Aitchison ~ Infant presence + Mother status + Diet + Age + Sample spread + Field season	2191.20	0.00	0.95
Reduced	Aitchison ~ Mother status + Diet + Age + Sample spread + Field season	2197.20	6.07	0.05
Microbiomes, infant presence, and social bonds				
Reduced	Aitchison ~ Infant presence + Mother status + Diet + Age + Sample spread + Field season	2191.20	0.00	0.52
Full	Aitchison ~ Infant presence + 1m Proximity + Mother status + Diet + Age + Sample spread + Field season	2192.70	1.54	0.24
Full	Aitchison ~ Infant presence + Grooming + Mother status + Diet + Age + Sample spread + Field season	2193.10	1.91	0.20
Full	Aitchison ~ Infant presence + 1m Proximity * Grooming + Mother status + Diet + Age + Sample spread + Field season	2196.60	5.43	0.04

Discussion

There is evidence that the gut microbiomes of social animals are continuously altered through social interactions; however, very few studies have attempted to understand how social bonds impact microbial transmission over time, particularly in the context of social change. Here, we show that shifts in social environments on relatively short time scales (3-months or less) are reflected in microbiome changes. By evaluating longitudinal changes in social relationships following infant birth, this study provides important evidence that short-lived changes in social environments are associated with changes in microbiome composition, and that the gut microbiome of social animals are plastic in response to social change, even over relatively short time periods.

Grooming Among Adult Females Increased in the Presence of an Infant

As expected based on prior research in colobines, including this study population (Wikberg et al., 2015), we found support for Hypothesis 1, as grooming rates among adult females increased significantly during young infant presence. Attempts to gain infant access increases contact among females who may not typically affiliate (including among infant handlers), increasing overall grooming rates (Badescu et al., 2015). Further, mothers often seek out more grooming opportunities to increase access to allocare, which can ultimately assist with increased foraging efficiency (Altmann & Samuels, 1992). Increased grooming can also be driven by vulnerability and serve as infanticide protection; coalitionary support from other females can shelter mothers against harassment from both males and females, and strengthening social bonds among adult females may be particularly important during a time when infants are at highest risk of infanticide (a risk that significantly decreases after an infant reaches adult coat coloration around 3 months of age) (Saj & Sicotte, 2005).

In prior research on social bonds in this study populations, 1-meter proximity networks were found to be correlated with grooming, and thus proximity was used as a proxy for social bonds in these lower-affiliation primates, allowing for more robust estimation of social interactions (Wikberg et al., 2014b). However, the grooming and proximity networks in our study did not correlate and 1-meter proximities did not increase in the presence of young infants. We suggest this may be attributable to smaller social group sizes and thus less spatial differentiation; in 2008-2009 the study groups in our population consisted of 5-11 adult and subadult females, while they only contained 4-7 adult females in 2018-2020 during our study period. We postulate that smaller social groups result in increased overall time spent in proximity amongst group members, such that social bonds are less differentiated by close proximity and

social networks are less modular (Evans et al., 2021). Small social group size likely drives increased rates of interactions among all members of the social group and may also necessitate stronger social bonds as smaller groups can be more vulnerable to territorial disputes and alpha male takeovers (Markham & Gesquiere, 2017). Indeed, affiliation rates among our study individuals display a marked increase from previous examination of our study population a decade earlier, indicating that adult females in our study population spend more time socializing than previously; grooming rates more than tripled (2008-2009: 0.1% average time grooming available female social partners; 2018-2020: 0.34% average), and time spent in close proximity increased moderately (2008-2009: 3% average time spent within 1m proximity; 2018-2020: 3.8% average time spent within 1m proximity) (Wikberg et al., 2014b). Despite these long-term changes in demography and sociality in our study population, rates of grooming were still found to increase among adult females in the presence of an infant.

Adult Female Gut Microbial Similarity Increased in the Presence of an Infant

We found support for Hypothesis 2, as the gut microbiomes of adult female colobus monkeys were more similar when a young infant (<3 months old) was present in the social group than when no young infants were present. However, we did not find support for Hypothesis 3, as modelling revealed that social bonds (grooming and proximity) were not a significant predictor of gut microbial similarity in the context of young infant presence. This raises questions regarding the mechanism by which adult female gut microbiomes are becoming more similar in the presence of infants.

Other group-level changes could promote increased microbial similarity during infant presence independently of socially-mediated transmission, such as more restricted ranging and

associated feeding changes following infant birth. However, we would expect such changes to be reflected in increased dietary similarity, which did not occur during infant presence. Another possible alternative is hormonal shifts among adult females in the group during the presence of a new infant; wild primate gut microbiome composition has been associated with hormone levels (Hickmott et al., 2022; Vlčková et al., 2018). However, a separate study would be required to test this alternative, and given the increase in grooming seen in the presence of an infant, we believe increasing microbial similarity during young infant presence is still most likely driven by social transmission and allocare.

We believe our lack of support for Hypothesis 3 is likely due to data density/infrequent affiliative events within short time-scales. Our behavioral sampling efforts are similar in density to previously published studies on colobines (Wikberg et al., 2015), but these are less dense than is typically achievable in terrestrial primates. Further, as colobines are known to display fewer affiliative interactions and spend far less time engaged in grooming than other more social animals (e.g.: baboons can spend upwards of 30% of their time engaged in grooming in contrast to less than 1% of time engaged in grooming by *C. vellerosus*), the total amount of affiliative behavior encapsulated in our shorter time scales can be quite sparse (Christensen et al., 2023). There may be a minimum threshold of behavioral data necessary to truly quantify social bonds of colobines, and the density of focal data captured in our dataset may not meet that threshold on infant presence time-scales (3 months or less).

To further explore this possibility, we performed post-hoc analyses (see Supplemental Materials for details; **Supplemental Table 7**, **Supplemental Table 8, Supplemental Figure 6**) in order to determine the minimum time-scale at which social bonds were predictive of microbial similarity in our dataset. We found that while 1-, 2-, and 3-month aggregated social networks

were not predictive of Aitchison distances among adult females, 6-month aggregate social networks were. Thus, the approximately 3-month time-scale of young infant presence may not contain sufficiently dense data to accurately quantify social bonds among our study individuals. We assert that grooming and social interactions are the most likely mechanism driving increased microbial similarity post-infant birth, and this supports our supposition that lack of behavioral data density is likely the culprit for our mixed results. It is important to note that 6-month social networks are still shorter than those typically employed by similar studies examining social network effects on the gut microbiome, which are most typically aggregated across 12 or more months. The gut microbiomes of social animals are influenced by their social environments; however, these social environments are not static, and treating them as such is inappropriate. Although there are clearly limits, especially depending on affiliation rates and data density, these results still indicate that employing shorter time-scale social networks within a longitudinal framework is feasible (Samartino et al., 2023).

Given our results, the importance of social bonding to facilitate infant allocare and better infant protection in a cooperative breeding context may have consequences for infants' microbiomes as well. We previously asserted that microbial "seeding" might be an underappreciated feature of allocare in colobines, and that mothers may act both as microbial sources and as "mediators" of interactions that result in microbial "seeding" of their infant's microbiome from other sources. If allocare is a route for microbial transmission between social group adults and infants, increasing social proximity and subsequent microbial similarity amongst allomothers and mothers may be further aiding colonization of infant guts with adult-associated microbes. Further, infant gut microbiomes are shaped by various aspects of their social environment, and both their social and microbial environment is not only continuously

constructed but also clearly influenced by adult-adult social dynamics (Lane et al., 2019; Meehan et al., 2018; Tavalire et al., 2021). Understanding the longitudinal dynamics of the gut microbiome and its plasticity in response to social change is therefore important to understanding the development of infant gut microbiomes as well.

Conclusion

We conclude that social events and subsequent changes in social environments can be used to explore the dynamics of social transmission of microbes among individuals. Our results provide a wider understanding of gut microbiome plasticity in response to social change, particularly in the context of cooperatively breeding species in which infant birth and care influences adult social relationships, and in turn adult social environments influence infant microbiome assembly. These findings support a larger body of evidence that infants can have a significant impact on social networks and interactions, and further adds that there may be microbial consequences of the social dynamics surrounding infant birth. Future research should harness other demographic events that are known to induce social change (e.g., rank changes, emigrations and immigrations, deaths, etc.) in order to understand the scale and speed of natural socially-mediated microbial transmission and how this affects the long-term maintenance and stability of gut microbiomes, particularly in species for which dense behavioral data are more readily attainable.

We also found that while very short (1-, 2-, and 3-month) time-scales may not be sufficient for examining social transmission of microbiota in lower-affiliation species, shorter time frames than typically used (6-month) are still potentially informative. We suggest that it is necessary to employ shorter social network time-scales in order to produce an accurate understanding of the ways in which social relationships continuously construct and maintain the

gut microbiomes of social animals. While the social bonds of some animals may remain relatively stable through time, a variety of demographic events and external forces can significantly alter social networks, and thus treating social networks as static structures aggregated at 12-month or longer time scales may be inappropriate. This is perhaps particularly true when considering species with less strict dominance hierarchies and social bonds that shift more often, including not only colobus monkeys, but also humans. Similarly, microbiomes are complex, dynamic communities which can remain relatively stable yet shift in expected and/or volatile ways. Deep fecal sampling through time is a major advantage of this research, and the known mutability of the microbiome highlights the need for a longitudinal lens. Future research should move away from relying on snapshots in time of this complex community.

Behavioral data density is a major limitation of our study, and while ours was equivalent to other similar research on colobines, density severely limited the usable network time-scales in this research. Although greater effort in behavioral data collection would be useful to examine whether shorter social networks in the context of short-term social change could truly be microbially predictive, our post-hoc analyses also indicate that even relatively sparse networks may be employed.

CHAPTER IV:

CONCLUSION

The gut microbiome is critical to host function, health, and wellbeing, yet it is still unclear exactly what determines natural inter-individual variation in the gut microbiome. There is growing evidence that social interaction and social environment shape the gut microbiome; research from across a wide variety of animals has shown that group-level social interactions, social relationships, and certain social behaviors are associated with microbial similarity and transmission. However, less is known about these dynamics during early life and development, and it's unclear how plastic the gut microbiome is in response to social change. This dissertation utilized focal behavioral and fecal sampling from a population of black and white colobus monkeys to answer questions about the social environmental effects and socially-mediated transmission of gut microbiota across the lifespan.

Disentangling the various factors that shape the early life gut microbiome during host development has ultimate consequences for lifelong health and wellness. In Chapter II, we characterized the early life colobus gut microbiome and examined social relationships between infant and adult colobus monkeys to elucidate the contributions of diverse social interactions on the developing gut microbiome. The early life colobus gut microbiome follows similar developmental patterns seen in other primates; diversity increases throughout the first few years of life, while inter-individual variation decreases, converging after weaning into an adult-like microbiome (Baniel et al., 2022; Petrullo et al., 2022; Roswall et al., 2021). These patterns are likely due to both developmental and behavioral changes; as the immune system develops, it more selectively filters the microbes that colonize the GI tract, while dietary, social, and

exploratory behaviors also change. The infant gut microbiome is initially colonized by maternal vaginal, milk, and skin microbiota, via birth, nursing, and contact. During aging, exposure to an increasingly wider range of environmental and dietary sources (colobus infants often mouth a variety of food long before solid food becomes a significant portion of their diet) leads to an increase in gut microbial diversity. Among colobines this increase in alpha diversity towards weaning is likely especially crucial for a successful transition to folivory; fore- and hind-gut fermenters display much greater species richness within the fermentation chambers than is seen in the intestines of non-herbivores (Suzuki, 2017). Convergence towards a widely shared gut microbial profile post-weaning and the increasing influence of selective forces also implies a gut microbial community that is functionally necessary for folivory and adult immunity. While food mouthing and environmental contact are sources for some of the microbial taxa contributing to this increased diversity and shift towards a microbial community characteristic of a folivorous diet, we postulated that colonization by some of the taxa shaping the adult microbiome may necessitate social acquisition, particularly from older social partners harboring the diverse community necessary for folivory.

Indeed, we found evidence of social structuring of the infant gut microbiome using broad measures of social interaction; early life colobus gut microbiomes are more similar to those of adult females in their social group. Further, we demonstrated that this extends to social dynamics within the social group; the microbiomes of infants of more socially central mother displayed higher similarity to their social group members than infants of less well-positioned mothers. If maternal centrality is acting as a proxy for infant centrality (infant social relationships often mirror that of their mothers), then this likely signifies that infants involved in more social interactions with more social partners are being colonized by adult female-associated microbes at

higher rates than less central infants. We also hypothesized that this could be an indicator of allocare driven microbial transmission, as mothers who are well-positioned within the social network are expected to have higher degrees of allocare aimed at their infants. Direct examination of allocare relationships between infants and non-mothers in a subset of our data confirmed that allocare is a likely route for socially-mediated microbial transmission; infants with more allomothers had more compositionally similar microbiomes to adult females in their social groups than infants with fewer allomothers.

As a seemingly-altruistic behavior, animal behaviorists have long been interested in the evolution of allocare and the selective pressures involved. Allocare is likely not truly altruistic, and the circumstances under which it may arise are often thought of as a cost/benefit trade-off. While the primary benefits of allocare for the infant include increased growth rates and faster development, there may be other overlooked health and wellbeing benefits associated with microbial colonization (Badescu et al., 2016; Lee et al., 1991; Mitani & Watts, 1997; Ross & MacLarnon, 2000). We suggest that transmission of commensal microbiota may help mitigate the risk of increased pathogen/parasite transmission associated with allocare via increased microbial diversity, preventing pathogen invasion and inhibiting pathogen growth (Bailey, 2012), and these adulthood microbes may even prepare infant immune systems for the loss of maternal milk-derived immunity (Kalbermatter et al., 2021; Kurokawa et al., 2007). Microbial transmission from older individuals to infants could also be involved in the accelerated growth and development rates associated with allocare; "seeding" the developing gut with adult-associated microbes may help prime them for a more successful weaning transition and speed up their ability to derive complete nutrition from a fully folivorous diet. Socially-mediated microbial transmission may be an unappreciated benefit of allocare for cooperatively-breeding

species, and we conclude that social environment likely plays an important role in the development of the early life colobus gut microbiome.

While social environment is an important factor in shaping the gut microbiome in adulthood, social interactions are not static and social partner preference and strength of social relationships can shift over time. Further, the gut microbiome is a complex, mutable community that is known to change in response to a variety of internal and external forces. However, our understanding of social transmission is largely based on cross-sectional studies that do not take longitudinal dynamics into account. In Chapter III we leveraged a regularly occurring, known catalyst of social change (infant birth in high-allocare colobines) to assess the contribution of social transmission to microbiome variation under shifting social dynamics. We found that increased social interactions (specifically higher grooming rates) following infant birth and during the high allocare period were reflected in greater microbial similarity among adult female social group members. While we were unable to pinpoint social transmission as the driver of this pattern, taken together these results indicate that even relatively short-lived changes in social relationships may cause microbial shifts.

Further, we demonstrated that social interaction networks aggregated over much short time periods than typically employed in similar studies (6 vs. 12 months) can sufficiently detect the contribution of social relationships in shaping gut microbiomes. Indeed, utilizing narrow social network time frames within a longitudinal framework is necessary in order to understand the ways in which social interactions contribute to microbial variation in social animals, and the known mutability of the microbiome highlights the need for a longitudinal lens.

This dissertation research also contained unexpected results: mother/offspring relationships were not microbially distinguishable from non-maternal adult/infant relationships, a

surprising result which could be attributed to the drastically different selective pressures present in a folivorous adult gut and a nursing infant gut. However, we speculate that this may in fact support our assertion that allocare mediates microbial transmission and homogenizes microbiomes between infants and adult social groups members, masking mother/offspring similarity. We believe that our other mixed findings were limited greatly by behavioral sampling density. Our inability to pinpoint social interactions as the driver of increased microbial similarity after infant birth, and the lack of predictive relationship between dyadic social connectedness and gut microbial similarity (in both Chapters II and III) was likely due to the infrequent rates at which colobines engage in grooming and an increase in overall social proximities, resulting in behavioral data that was too sparse at short time scales. Social networks aggregated across short time scales (1-, 2-, and 3-months) lacked the power to predict microbial similarity among adults, which supports this assumption. Thus, future research (particularly in species for which dense behavioral data are readily available and in complementary model system experiments) could be useful in deciphering the scale and speed of socially-mediated microbial dispersal and how this affects the long-term maintenance and stability of the gut microbiome, which we were unable to disentangle with the data available. Additionally, we could only speculate about the long-term health and fitness consequences of the early life microbial variation we described, and additional longitudinal research examining how early life microbial diversity and composition is associated with growth and development rates, weaning survival, and longevity would be invaluable.

Taken together, these results expand our understanding of the social factors shaping the gut microbiome across the lifespan, particularly in social animals in which multi-generational groups interact socially. Infants of social animals have rich social lives that extend beyond

maternal and paternal interaction, and while peer social relationships are increasingly important as infants age, non-parental adults are also important aspects of an infant's social environment. This is particularly true for infants of high allocare species. Further, the effects of these infant/adult social relationships are not unidirectional; infants impact the social lives of adults in addition to the role adults play in infant social environments, and our findings support a larger body of evidence showing that infants influence social networks and interactions among adults. In this way, one can imagine a feedback loop between infants and adults of social animals, in which infant birth and care influences adult social relationships, and in turn adult social relationships shape infant care and the early life social environment. We propose that this feedback loop is recapitulated by microbial transmission; infant birth and allocare results in increasing microbial similarity among adults, and this greater microbial similarity aids in colonizing developing infant guts via allocare.

In addition to colobines, it is notable that humans also display unusually high levels of allocare, and thus the results of this dissertation inform on our understanding of the social influences mediating human microbial variation as well, and could have important implications for the social determinants of human health. This work demonstrates the utility of natural non-human primate research, and habituated populations studied longitudinally provide particularly advantageous model systems in which natural experiments can help scientists disentangle complex social and biological dynamics.

Finally, this research contributes to a large body of work focused on the biology and behavior of *Colobus vellerosus*, a critically endangered primate at risk of extinction. This research was conducted at Boabeng-Fiema Monkey Sanctuary (BFMS) on the only known stable population of *C. vellerosus* left in the world, (approximately 400 individuals; fewer than 1000

mature individuals globally) (Goodwin et al., 2020). In characterizing the gut microbiomes of developing black and white colobus monkeys, this work provides preliminary information that future research can build upon to examine the health and wellbeing of these dwindling monkeys. Additionally, longitudinal approaches to gut microbiome research will be important for assessing the impact of habitat fragmentation and degradation on black and white colobus health. Although the BFMS core forest is protected, a portion of this population reside in surrounding fragments, and other populations of *C. vellerosus* are severely threatened by habitat loss. Research at BFMS contributes to the conservation of this species via research permit fees and through open data sharing with sanctuary management. Further, this dissertation research supported the ongoing employment of 4 Ghanaian field assistants, several of whom have been involved in colobus research for more than 15 years. In addition to supporting conservation and providing consistent benefits to human communities, long-term primatological studies are critical for examining questions related to life history, environmental variation, climate change, evolution, and population and community ecology (Chapman et al. 2017). Behavioral and biological research on the black and white colobus at BFMS has been ongoing for over 20 years, and this dissertation research contributes to this critical long-term research.

Group	Adult Males	Adult Females	Subadults and Juveniles	Infants	Total group size
December 2017					
Splinter	1	4	5	3	13
Redtail	1	7	9	6	23
Winter	2	3	6	3	14
Wawa	2	4	8	3	17
April 2018					
Splinter	1	4	5	2	12
Redtail	1	6	7	5	19
Winter	2	3	5	1	11
Wawa	3	4	8	2	17
May 2019					
Splinter	1	4	7	3	15
Redtail	1	8	8	7	24
Winter	1	4	3	2	10
Wawa	2	5	6	2	15
March 2020					
Splinter	1	4	5	5	15
Redtail	1	7	8	6	22
Winter	1	5	5	1	12
Wawa	2	6	3	3	14

Supplemental Table 4: Group demographics during data collection. Demographics of 4 study groups Splinter, Redtail, Winter, and Wawa during preliminary field seasons 1 and 2 and full field seasons 1 and 2. Demographics included were recorded at end of each field season, and group numbers occasionally change during field seasons due to births, deaths, immigration, and emigration. Thus, numbers included here are approximate and do not display the complete range of group demographics.

Establishing Early Life Gut Microbiome Dynamics

Neutral assembly modeling, alpha diversity, and beta diversity were used to characterize

the gut microbiomes of early life colobus gut microbiomes.
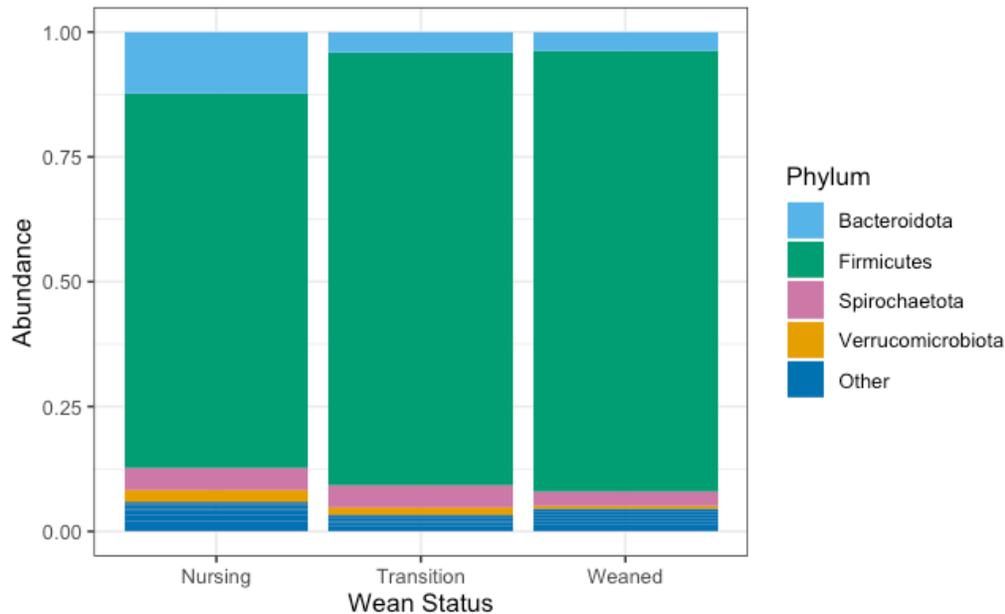
Supplemental Figure 4: Relative abundance bar plots of dominant phyla in early life. Bar plots show the top 4 phyla found in the guts according to weaning status (nursing infants, transitioning, and weaned juveniles).

Alpha Diversity

To determine which intrinsic and extrinsic factors shape early life gut microbial

diversity, we calculated Shannon's index (alpha diversity) for early life samples and used Linear

Mixed Effects models to allow for individual ID and month of collection to be used as random

effects (*lme4* package; 75 samples from 29 individuals) (Bates et al., 2015). Samples were

rarefied to an even depth for all alpha diversity analyses. Intrinsic and extrinsic variables tested

included age (measured in days), sex, social group, and social group size measured via number

of individuals.

Age during early life was positively correlated with Shannon index; older infants and young juveniles have significantly higher alpha diversity than younger infants, and this pattern is relatively linear, with alpha diversity increasing as infants age (p<0.001; **Figure 1a**). Social group was barely significant (p<0.05), while all other factors (field season and month of collection, sex, social group size) examined were not significant predictors of early life alpha diversity.

Beta Diversity

We used a specialty PERMANOVA (*adonis2* function in the *vegan* package) to evaluate the factors shaping inter-individual variation in gut microbiomes. Aitchison distance was used as a measure of beta diversity because the center log ratio transformation better accounts for the compositional nature of microbial data (Quinn et al., 2018). We used a permutational down-sampling approach; each iteration of the PERMANOVA contained a maximum of 1 random sample per individual per month (75 samples from 29 individuals), and this random down-sampling was permuted 99 times, allowing us to retain the power of the full dataset (102 samples from 29 infants) (Klinges et al., 2020). P-values were corrected using False Discovery Rate, and the mean of all corrected p-values and R^2 values was calculated to determine the results of this permutational down-sampling approach. Intrinsic and extrinsic variables tested included age, wean status, sex, individual identity, field season and month of collection, and social group. Interaction terms were included for wean status and age as well as field season and month of collection. Significant PERMANOVA results can be due to differences in centroid location, differences in dispersion around the centroid, or both. Therefore, we examined beta dispersion as heterogeneity of variances can influence PERMANOVA results (Anderson, 2017). We used the

betadisper function in the *vegan* package to assess the dispersion of all variables used in both the early life and combined dataset PERMANOVAs.

Individual identity explained the largest proportion of variation (24.8%), while a moderate amount of variation was explained by wean status (12.8%) and age (13%), and a smaller but significant proportion of variation was explained by month of collection (10%), field season of collection (6 %), social group (6.3%), and sex (1.7%). The interaction between wean status and age group was significant (6%), while the interaction between month and field season of collection was not significant and thus the interaction term was removed from the final model. Ordinations reveal how age and wean status pattern gut microbial similarity (**Figure 1c**, **Figure 1d**). Wean status ($p<0.001$), age ($p<0.01$), collection month ($p<0.01$), field season ($p<0.05$), and social group ($p<0.001$) all displayed significant differences in beta dispersion, while sex ($p>0.05$) did not.

The same permutationally down-sampled PERMANOVA approach was utilized in the combined adult female/early life dataset to examine the influence of these variables across the colobus lifespan (263 samples from 55 individuals per down-sample, retaining all 312 samples from 55 infant, juvenile, and adult individuals). Variables examined include age class, individual identity nested within social group, and field season and month of collection.

The largest portion of variation was explained by individual identity (30.88%), and small but significant portions of variation were explained by age class (2.82%), social group (2.9%), and collection month (3.83%), while field season was not a significant predictor. Beta dispersion testing revealed that infant gut microbiomes are also highly dispersed compared to juvenile and adult female gut microbiomes, which is clearly illustrated in ordinations ($p<0.001$; **Figure 1b**).

Further, collection month (p<0.01), field season (p<0.001), and social group (p<0.05) all showed significant differences in dispersion between groups.

Neutral Assembly Modeling

Neutral assembly modeling is sensitive to read depth bias, thus the infant ASV table was rarified to an even depth. Neutral assembly modeling is also sensitive to unequal treatment group size and a minimum of 20 samples per treatment group are recommended. We created the following treatment groups based on weaning status: Early nursing (samples from individuals with 180 or more days until weaning; n = 20), Late nursing (samples from individuals with 53 to 179 days to weaning, n = 20), Mid-weaning (53 days to weaning to 40 days post weaning, n = 21), and Fully weaned (samples from individuals 41 or more days post-weaning, n = 20). We used Root-mean-square error (RMSE) and the *snm.comm* function in the *iCAMP* package (Ning et al., 2020) to assess fit of the neutral assembly model and binomial model (as a null comparison) across weaning stage. Any RMSE value below 0.2 is considered a good fit, and a lower value is interpreted as a better fit.

The fit of the neutral assembly model decreases with weaning progression, indicating that early life gut microbiomes are less shaped by stochastic processes (including drift) as they move towards weaning and after they are fully weaned (RMSE = 0.164, 0.174, 0.18, and 0.22 respectively; **Supplemental Table 5**; **Supplemental Figure 5**). Throughout all 4 early life nursing and weaning stages, the neutral assembly model outperforms the binomial model (RMSE.bino = 0.54, 0.533, 0.53, 0.48 respectively), indicating that at all developmental stages, neutral assembly better explains early life colobus monkey gut microbiomes than a random sampling of the source community (binomial model) (**Supplemental Table 5**).

Supplemental Table 5: Neutral assembly model fit decreases with weaning. Full modelling output displaying decreasing fit of the neutral assembly model as early life individuals age/wean (determined via RMSE statistic. RMSE for neutral assembly model ("RMSE") and RMSE for competing binomial model ("RMSE.bino") are highlighted. Higher RMSE.bino scores in relation to RMSE scores indicate that the neutral assembly model always fits better than the binomial model regardless of wean status; ecological drift and passive dispersal better explains early life gut microbiomes than a random sampling of the source community.

Treatment.group	m	m.ci.2.5	m.ci.97.5	m.mle	maxLL	binoLL	poisLL	Rsqr	Rsqr.bino	Rsqr.pois	RMSE	RMSE.bino	RMSE.pois	AIC	BIC	AIC.bino	BIC.bino	AIC.pois	BIC.pois	N	Samples	Richness	Detect
Early nursing	0.0018263	0.0016509	0.0020133	NA	NA	959.2519	959.2374	-0.3448049	-14.111312	-14.111111	0.1623952	0.543705	0.5443669	NA	NA	1922.504	1934.375	1922.475	1934.346	23518	20	2795	4.25e-05
Late nursing	0.0028207	0.0025711	0.0030878	0.0028488	-903.1803	813.9120	813.8970	-0.1924746	-10.147804	-10.147648	0.1742873	0.528880	0.5328843	-1802.606	-1790.520	1631.824	1643.664	1631.794	1643.634	23518	20	2752	4.25e-05
Mid-weaning	0.0053231	0.0049093	0.0057649	0.0053470	-682.7387	640.3847	640.3714	0.2494837	-5.527593	-5.527501	0.1800237	0.509165	0.5309128	-1361.775	-1349.990	1284.769	1296.257	1284.743	1296.230	23518	21	2307	4.25e-05
Fully Weaned	0.0110451	0.0100624	0.0121141	0.0110497	-215.2083	599.5050	599.4912	0.2746861	-2.410334	-2.410281	0.2201604	0.473915	0.4773878	-426.167	-414.959	1203.010	1214.468	1202.982	1214.440	23518	20	2273	4.25e-05
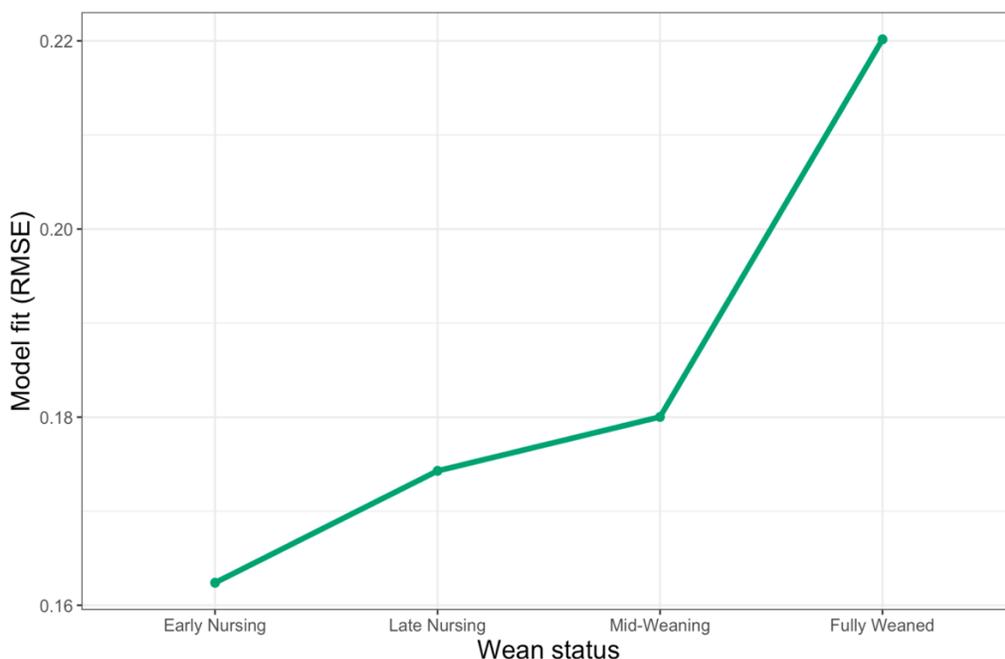
Supplemental Figure 5: Neutral assembly model fit decreases with weaning. Plot displays model fit (measured via Root Mean Square Error or RMSE) according to wean status in early life. Lower RMSE indicates better model fit; as early life colobus age and move through weaning/towards juvenilehood, the neutral assembly model fit decreases.

Adult Female Social Networks

Supplemental Table 6: Conditional model effects of infant presence and adult female age on social bonds. GLMMs fixed effects and response variables, model estimates, standard errors, z-, and p-values. Hypotheses tested include that grooming rates increase among adult females when a young infant is present in the social group, and that 1m proximity rates increase among adult female when a young infant is present, while age difference is treated as a covariate in both models. Infant presence and age significantly predict grooming rates, while only age (and not infant presence) significantly predicts adult female proximities.

Model formula	Estimate	Std. Error	Z-value	P-value
Grooming in infant presence				
(intercept)	2.13	0.41	5.21	< 0.001 ***
Grooming ~ Infant presence	0.50	0.21	2.34	0.019 *
Grooming ~ Age	-0.14	0.04	-3.17	0.002 **
Proximity in infant presence				
(intercept)	-0.80	0.15	-5.52	< 0.001 ***
Proximity ~ Infant presence	0.01	0.10	0.09	0.92
Proximity ~ Age	-0.05	0.01	-3.25	0.001 **

Post-hoc Testing

Social networks were constructed on a variety of aggregate scales to determine the minimum network size that is recapitulated in gut microbial similarity. Grooming and proximity rates were calculated separately for each time scale subset and were organized in time such that the start of a given network was approximately 2 weeks before fecal sampling of the social group occurred and ended approximately 2 weeks after the last fecal sampling for that given time period. In other words: 1-month networks were calculated from behavioral data beginning 2 weeks before the fecal sampling and ending 2 weeks after, while 2-month networks began 2

weeks before the first fecal sampling and ended 2 weeks after the second fecal sampling. It is important to caveat that our 6-month networks contain approximately 2/3 of the total data used in our 1-,2-, and 3-month networks; only data collection from field season 1 contained 6 months of continuous data, while data collection from field season 2 consisted of slightly over 3 months and thus was discarded from the 6-month analysis (see **Supplemental Table 6** for details of included data in each time scale).

Supplemental Table 7: Fecal sampling and behavioral data included in each time scale aggregate analysis. 1-, 2-, and 3-month social networks included all fecal sampling and focal behavioral data, while 6-month time scales only included data collected during field season 2.

Analysis	Total fecal samples included	Total in-view focal hours included	Total dyads	Total comparisons	Field seasons included
Social network time scales					
1-month	165	358.59	47	312	Primary 1 and 2
2-month	165	358.59	47	170	Primary 1 and 2
3-month	165	358.59	47	106	Primary 1 and 2
6-month	112 [68%]	205.5 [57%]	42 [89%]	42	Primary 1

We compared beta diversity distances among adult female dyads aggregated by 1-, 2-, 3-, and 6-month time scales to dyadic grooming and proximity rates to test the hypothesis that narrower time scale-based social bonds are predictive of microbial similarity. Only samples from primary field seasons 1 and 2 with matched focal data were included (165 samples from 21 females). Aitchison distance was used as response variable, while proximity, grooming, diet, age difference, and sampling spread were used as fixed effects for the 6-month models (ID dyad, collection month, and field season were not included as the 6-month data was aggregated across these variables) (6-month models: 42 comparison among 42 dyads). 1-, 2-, and 3-month models

included proximity, grooming, diet, age difference, sampling spread, and field season of collection as fixed effects, and ID dyad as a random effect. 1-month models included collection month as a random effect (312 comparisons among 47 dyads), 2-month models included collection month group as a random effect (170 comparisons among 47 dyads), and 3-month models included collection month group as a fixed effect (as at the 3-month timescale, there were not enough levels of month group to warrant inclusion as a random effect) (106 comparisons among 47 dyads). The reduced models for all time scales included all covariates except grooming and proximity rates.

Preliminary longitudinal testing revealed that neither grooming rates nor rates of approaches to within 1m alone significantly predicted microbial similarity among adult females, and further that grooming and proximity were not consistently correlated with each other across time scales. However, social bond strength (defined here as the interaction between grooming and 1m proximity) was predictive of gut microbial beta diversity at longer time scales. 6-month social bond strength was significantly negatively correlated with dyadically-aggregated 6-month Aitchison distances (coefficient estimate = -0.006, 95% CI: -0.010, -0.002; **Supplemental Figure 6**; **Supplemental Table 8**), and the full model was selected over the reduced model. 1-month, 2-month, and 3-month social bond strength was not significantly associated with microbial similarity, and reduced models were selected over full models (**Supplemental Table 8**).

Supplemental Table 8: Determining minimum time scales for predictive social networks. Competing GLMMs fixed effects and response variables, Akaike's information criterion (AIC), delta (difference in AIC between the current model and best-fit model) and Akaike weights (relative likelihood of the model). Time scales tested include 1-month, 2-month, 3-month, and 6-month aggregates of social behavior.

Model	Model formula	AIC	Delta	Weight
1-month networks				
Reduced	Diet + Age + Sample spread + Field season	2322.88	0.00	0.95
Full	1m Proximity * Grooming + Diet + Age + Sample spread + Field season	2328.99	6.11	0.05
2-month networks				
Reduced	Diet + Age + Sample spread + Field season	1172.54	0.00	0.91
Full	1m Proximity * Grooming + Diet + Age + Sample spread + Field season	1177.08	4.54	0.09
3-month networks				
Reduced	Diet + Age + Sample spread + Field season + Month group	673.46	0.00	0.96
Full	1m Proximity * Grooming + Diet + Age + Sample spread + Field season + Month group	679.98	6.52	0.04
6-month networks				
Full	1m Proximity * Grooming + Diet + Age	242.54	0.00	0.57
Reduced	Diet + Age	243.09	0.56	0.43
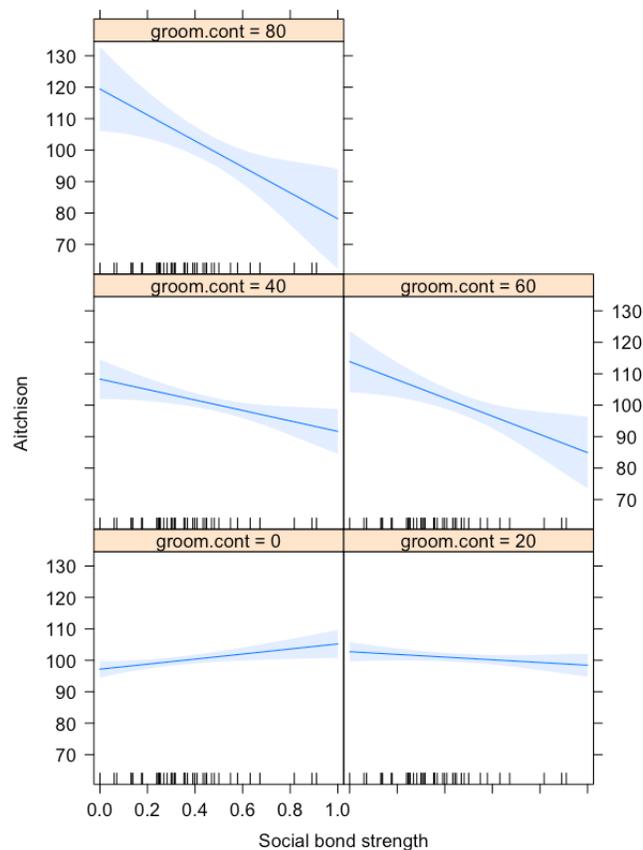
Supplemental Figure 6: Effects of 6-month aggregated social bond strength on gut microbiomes of adult females. Effects plots displaying the relationship between response

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(Aitchison distance) and social bond strength, measured via the interaction between proximity (x axes) and grooming (plot bins). Lines show the relationships predicted by the GLMM, and shaded areas represent 95% confidence intervals. Increasing line slope indicates that among dyads that groom at higher rates (i.e., grooming > 60), proximity is a good predictor of microbial similarity, and vice-versa.

Supplemental Table 9: Behavioral ethogram. Full ethogram detailing all behaviors recorded during continuous focal follows on infants and adult females.

CODE	BEHAVIOR
PROXIMITY CLASSES	
0	Body contact
1	Within 1 tail length
3	Within 3 tail lengths
5	Within 5 tail lengths
A	Approach (0, 1, 3, 5)
L	Leave (0, 1, 3, 5)
CONSPECIFICS	
XX	Unidentified/unknown
XM	Unidentified male
XF	Unidentified adult female
XJ	Unidentified juvenile
XB	Unidentified infant
NM	New male
SELF DIRECTED BEHAVIORS	
AB	Defecating
AE	Piloerect
AG	Autogroom
AH	Touch
AI	Inspect
AL	Bug slap
AP	Autoplay
AS	Scratch
AU	Urinate
SAV	Scan
AV	Vigilant
AY	Yawn
DISPLAY BEHAVIOR	
DB	Stiff leg, both legs on branch **(AD L)**
DH	Display hop **(AD L)**
DI	Stiff leg, one leg only **(AD L)**
DJ	Jump display **(AD L)**

DL	Stiff leg, one leg on branch **(AD L)**
DM	Small open mouth
DO	Open mouth
DR	Run display/run through **(AD L)**
DS	Stiff leg, two legs **(AD L)**
AFFILIATIVE BEHAVIORS	
FB	Tail grab usually by infant
FC	Friendly bite (occurs within play bout) **(modifier for * FX on ipad)**
FCH	Friendly chase **(modifier for * FX on ipad)**
FF	Play face **(modifier for * FX on ipad)**
FG E	Grooming continues at end of follow
FG S	Grooming at start of follow
FG	Start groom
FGR	Friendly grab **(modifier for * FX on ipad)**
FH	Hug
FI	Inspect
FJ	(Infant) jumps on top of someone else (often when males stiff leg)
FK	Kiss, mouth to mouth, face sniff/inspect
FL	Play present **(modifier for * FX on ipad)**
FM	Grooming open mouths, not as wide as normal open mouths
FO	Over-the-head mount **(done by infants)**
FP	Groom present
FQ	Tail hit
FS	Sniff
FT	Touch (modifier *FAC*: touch to the face)
FU	Friendly pull **(modifier for * FX on ipad)**
FV	Friendly follow **(modifier for * FX on ipad)**
* FX	Play-related behaviors (modifiers include: *FC, FCH, FF, FGR, FL , FU, FV*); not a specific behavior, lumped for ease of use in recording on ipad
* FY	Friendly grapple/wrestling/bouncing in unison
AGGRESSIVE & SUBMISSIVE (Most of these will be in the Ad lib codes)	
GA	Avoid
GB	Bite
GC	Chase
GD	Displace
GE E	Aggressive end **(ADD AT THE END OF FOCAL DISPLAYS DURING CLEANING)**
GF	Flee
GG	Fear grin
GH	Hit
GJ	Bounce
GL	Lunge

GM	Moving displace (**ADD DURING CLEANING**)
GO	Cower
GP	Pounce on
GQ	Displacement but the displayed individual stays in 1m
GV	Push, shove
GR	Grab
GS	Snap at
GT	Submissive present
GU	Pull
GW	Swipe at
GX	Contact fighting
GZ	Nose grab
INFANT-RELATED BEHAVIORS	
IBC	Infant climbs on the back of another individual
ITC	Infant climbs tail of another individual
** IC	Carried
IC F	Infant is carried at start of follow
IC E	Infant carry continues as end of follow
IDF	Infant distress face; often is accompanied by squeals, infant shows and chatters teeth, usually is clearly directed towards a specific individual
** IH	Infant held
IH F	Infant being held at start of follow
IH E	Infant hold continues at end of follow
IM	Infant exploratory movement; non-directed travel, often includes bouncing; infant stays within ½ meter radius of location
IW	Infant tries to get off ventral position
IWT	Infant waves tail
ISW	Infant swings (on tail, etc.)
IA	Infant attempts access to nipple
IAV	Infant attempts to get on ventral position/initiate hold
IN F	Nursing at start of follow
** IN	Start nursing
IN E	Nursing continues at end of focal
IO	Mother attempted to get infant off nipple
IQ	Infant Squeal: Differs from VQ (vocalize squeal) in that it is by an infant and will only be used when the infant is within 5m of the mother (Modifiers include: *W: Weak or I: Intense*)
IR	Restrained, held back, transfer resisted, infant retrieved, i.e., infant pulled to body contact
IT	Infant transfer to another individual (with receiver who is the individual infant is transferred to)
	(IA/IAV) MODIFIERS

PR	Passively rejected: Preventing access to nipple (including holding arm across nipples, pulling away or turning back on infant, lying down on branch).
AR	Aggressively rejected: Following a nursing attempt involving an overt behavior such as pushing, shoving, biting, swiping, and hitting
NR	No reaction: Mother does not respond to infant's nipple access attempts
SOCIAL FOOD-RELATED BEHAVIORS	
MA	Attempted theft of food
MC	Co-feeding (feeding in the same spot, within 1 tail length or from same cluster of leaves/food patch)
MI	Mouth food
MO	Tolerated theft
MS	Steal food
MT	Touch others food
SEXUAL BEHAVIOR	
SA	Attempted mount
SD	Dismount
SE	Sex end
SJ	Ejaculate
SH	Hip touch
SI	Inspect anogenital area
SL	Sexual slap
SM	Mount
SN	Sniff anogenital area
SP	Present
SR	Resist mount
SS	Stop thrust without dismount
ST	Mount with thrust
SW	Watches sex, individual looks at couple copulating with or without interference
VOCALIZATIONS	
VA	Click-alarm call S
VC	Click before loud call
VG	Grunt (uses modifiers G, F, IG, P, IP)
	G Grunt
	F Fast grunt
	IG Intense grunt
	P Pant grunt
	IP Intense pant grunt
VH	Cough, the vocalization, not just coughing
VK	Click when open mouth
VL	Loud call

VL D	Loud call in distance (assign group if known RT WW WT SP OD AK PN etc or U unknown)
VQ	Squeal (W weak, I intense)
VR	Fight roar
VS	Scream
VY	Yelp
VX	Unknown vocalization
FOOD ITEMS	
* IL	Ingest leaf (modifiers are the trees)
IFR	Ingest fruit (modifiers are the trees)
IFL	Ingest flower (modifiers are the trees)
ISP	Ingest seedpod (modifiers are the trees)
IOT	Ingest other **(ADD DURING CLEANING)**
	AD LIB COMMENT BELOW CODES IF NECESSARY
A	Sap
B	Bark
CC	Charcoal
D	Water, drink
E	Flower bud
F	Fruit
G	Grass
H	Pith
I	Stem of the fruit
K	Stick
L	Leaf
M	Mature leaf
O	Other
P	Seed pod
Q	Leaf bud
R	Flower
S	Seed
T	Petiole
U	Bud
V	Vine
W	Wall
X	Unknown
Y	Young leaf
TRAVEL	
* TT	Start travelling
TT S	Start travelling before start of follow
TT E	Traveling continues at end of follow

TS	Small movement
TS E	Small movement continues at end of focal
OTHER ANIMALS (Modifiers following FAV)	
OO	Observer **(Receiver 0X)**
OH	Other human besides observers **(0X)**
OM	Other monkey **(Receiver is the monkey it's looking at, followed by OM)**
ZA	Automobile (includes motorcycles, tractors, cars) **(Receiver 0X)**
ZB	Bird **(Receiver 0X)**
ZC	Branch crash **(Receiver 0X)**
ZG	Pig **(Receiver 0X)**
ZM	Mona monkey **(Receiver 0X)**
ZS	Snake **(Receiver 0X)**
ZP	Sheep **(Receiver 0X)**
ZV	Look at vocalization **(Receiver 0x)**
UNK	Unknown **(Receiver 0x)**
OTHER CODES (In focals)	
OV	Out of view
OVF	Out of view face (used only when nursing possible)
IV	In view (is NOT used for face in view)
IVF	In view face
INTERGROUP (AD L)	
IS	Intergroup start
IS S	Intergroup started before observer arrived
IE	Intergroup end
IE S	Intergroup still going on when observer leave
IL	Location of encountered group or focal group if taking other location points than those scheduled on the hour **(Ad lib notes)**
IY	Activity before, during, after intergroup **(in ad lib notes)**
CI	Comments regarding intergroups or male/female incursions/excursions **(in ad lib notes)**
COMMENTS (Put in Ad lib note section with these headings)	
C	Comments general
CB	Coat color comment
CE	Comment for data editing and analyses
CF	Comment food
CI	Intergroup
CL	Location, e.g., CL 150LOW CG1 AND CG2 or CL 150AS BETWEEN CG1 AND CG2 OR Location when tree is on the map, e.g., 150DA1 – hourly & at tree changes **(PUT IN AD LIB NOTES)**
CM	Group movement, animals relative position, leader of progression

CR	Reaction to vocalizations
CV	Description of vocalizations
CW	Wounds
CX	Comment sex/consorts
CY	Comment play/games **(ad lib with infants & males)**
SHORT-CUTS	
*	Signal to yourself for data editing. (Modifiers below)
	DEL previous line
	OT other editing issue
	RR Repeat the entry from the immediately preceding line
	ST Same time as previous state
**	Dictaphone insert **(Put into Ad Lib notes section) MODIFIER**
POINT SAMPLES CODES	
*P	Behavior
	P FDL Feed leaves
	P FDF Feed fruit
	P FDO Feed other
	P OT Other behavior
	P OV Out of view
	P RT Rest
	P SO Social
	P SS Sexual behavior
	P TT Travel
	P VV Vigilant
*PX	Proximity of all individuals in proximity. **Click this behavior for every proximity in the point sample.**
	P0 Contact
	P1 Within 1
	P3 Within 3
	P5 Within 5
	PV Ventral
	PU Unknown proximities
*PM	Proximity to mother. **Click this behavior for every point sample.**
	P0 Contact
	P1 Within 1
	P3 Within 3
	P5 Within 5
	P+ Greater than 5 away
	PN Nursing
	PV Ventral

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