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Limited microbiome differences in captive and semi-wild primate populations consuming similar diets

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Abstract

Gut microbial communities are shaped by a myriad of extrinsic factors, including diet and the environment. Although distinct human populations consistently exhibit different gut microbiome compositions, variation in diet and environmental factors are almost always coupled, making it difficult to disentangle their relative contributions to shaping the gut microbiota. Data from discrete animal populations with similar diets can help reduce confounds. Here, we assessed the gut microbiota of free-ranging and captive rhesus macaques with at least 80% diet similarity to test the hypothesis that hosts in difference environments will have different gut microbiomes despite a shared diet. Although we found that location was a significant predictor of gut microbial composition, the magnitude of observed differences was relatively small. These patterns suggest that a shared diet may limit the typical influence of environmental microbial exposure on the gut microbiota.

Keywords: captivity, diet, microbiome, primate

Introduction

Environmental factors can affect the microbiota by dictating the extent to which microbes can be transmitted between individuals and between individuals and their environments (Stamper et al. 2016, Tasnim et al. 2017, Parajuli et al. 2018, Manus et al. 2020). These factors include exposure from social networks, the built environment, xenobiotics, and outdoor green space (Maurice et al. 2013, Lax et al. 2014, Mills et al. 2017, Sarkar et al. 2020). Accordingly, we commonly see differences in microbiome composition and structure in both human and animal populations living in different locations. For example, humans living in more urban, industrialized settings have distinct gut microbiomes compared to humans living in more rural, nonindustrialized settings (De Filippo et al. 2010, Obregon-Tito et al. 2015). Similarly, wild and captive

conspecific mammals have different microbial signatures (Clayton et al. 2016, Gibson et al. 2019). However, large diet shifts are often associated with processes such as industrialization in humans (Jew et al. 2009, Mancabelli et al. 2017) and captivity in animals (Gibson et al. 2019, Van Leeuwen et al. 2020). As such, observational studies of human and animal populations involve natural confounds of diet, geography, and environment (Yatsunenka et al. 2012, Obregon-Tito et al. 2015, Van Leeuwen et al. 2020), often even when a single population is targeted (Urlacher et al. 2016, Gurven et al. 2017). Further, human intervention studies typically do not alter diets or environments for more than a few weeks or months, making it difficult to assess long-term impacts (Wu et al. 2011, David et al. 2014). Finally, controlled studies of laboratory animal models involve settings with reduced opportunities for mi-

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crobial transmission due to high sanitation and altered social contact, limiting applicability to free-ranging populations (Clayton et al. 2016).

To better understand the potential factors underlying microbiome differences in conspecific hosts sampled in distinct locations, it is crucial to measure microbial differences in populations with similar diets but distinct environments. Natural experiments in which populations of wild animals have been exposed to human-influenced diets, similar to those of captive populations, offer this approach. These populations are currently understudied but can provide insight into whether the effect of host population or location persists despite a similar diet.

Here, we use data from free-ranging and captive rhesus macaques (*Macaca mulatta*) occupying distinct environments in Puerto Rico with at least 80% diet similarity to explore the extent to which microbiome structure varies with location despite a shared diet. Rhesus macaques were introduced to Cayo Santiago (CS), a small uninhabited island off the coast of Puerto Rico, in the early 20th century, resulting in a free-ranging, semiwild population that exists to this day and has been extensively studied (Kessler and Rawlins 2016). Between 1974 and 1984, a subset of macaques from CS were transferred to the Sabana Seca (SS) Field Station on the mainland, where they are maintained in a captive research environment. Because the population on CS has outgrown the naturally available resources on the island, the macaques are provided with commercial monkey chow, i.e. delivered by boat daily. Therefore, in addition to originating from the same founder population, the macaques at SS and at CS have the same core diet. However, macaques at SS inhabit a built environment with reduced outdoor exposure and social contact as well as increased sanitation and medical intervention. Populations of wild primates that are provisioned with humanized food—much of which is low fiber high fat commercial chow—while also living in natural social groups and environments are rare, making this a unique system for exploring these microbial dynamics. We hypothesized that despite similarities in diet, we would observe microbiome differences in macaques at each location. Specifically, we predicted that the macaques from CS would have increased microbial diversity compared to those from SS since their environments are more conducive to microbial dispersal. We also expected SS macaques would have decreased relative abundances of microbial taxa that have been associated with environmental exposure in previous studies of wild cercopithecines [e.g. specific strains of Actinobacteria, Firmicutes, and Proteobacteria (Grieneisen et al. 2019)] compared to the CS macaques.

Methods

Study site

CS is a free-ranging semiwild population of rhesus macaques (*M. mulatta*) inhabiting the island of CS off the coast of Puerto Rico. CS rhesus macaques are provisioned daily with water and commercial monkey chow (8773 Teklad NIB Primate Diet Modified). Additionally, CS macaques have no predators and limited home ranges (Maestriperi and Hoffman 2012), which may lead to longer lifespans and less group dispersal as well as less energy put toward vigilance and more time for social interactions compared to completely wild macaques. Nevertheless, CS individuals occupy an otherwise wild environment where they inhabit cliffs, forests, thickets, and scrub areas, are exposed to other animals such as birds and lizards and engage in behaviors such as geophagy. Additionally, CS monkeys also inadvertently consume seaweed and

other debris when they forage. Medical intervention, and associated exposure to pharmaceuticals and antimicrobials, are rare.

SS is a captive research population of rhesus macaques on mainland Puerto Rico. At SS, macaques consume a diet made up almost entirely of the same commercial monkey chow provided at CS, and are housed in enclosures of different sizes—all of which are smaller than the average group home range on CS. Some individuals live in groups of about 15 individuals in indoor-outdoor corrals with concrete tiled floors. Others are housed indoors in groups of 1–4 in enclosures with metal floors. SPF (specific pathogen free) macaques are housed in these types of indoor enclosures as well. All of our samples were collected from macaques in indoor enclosures. All enclosures lack air conditioning and utilize natural light, and even indoor enclosures are housed in “outdoor rooms” that allow animals to see the outdoors. All enclosures have access to a well-water system so the animals can have water *ad libitum*. Enclosures are cleaned daily; disinfection is performed every 2 weeks. SS individuals have continuous veterinary care, including biannual tuberculosis testing, biannual deworming with Ivermectin, and rabies vaccination.

Dietary data collection

At CS, feeding ecology data was collected in 2010 and 2012 using 10-minute continuous focal animal samples (Altmann 1974). These data were collected from individuals in the two social groups from which most of our individuals were sampled. Individuals were sampled randomly once a day, where activity of focal subjects was recorded as one of four mutually exclusive states: feeding, resting, traveling, or grooming. Doing so, allowed us to compute the duration of time a subject spend in any given state during the sample. When subjects were feeding, we recorded whether they were consuming monkey chow or naturally available vegetation. We collected behavioral data between 07:30 a.m. and 14:00 p.m. and data collection was stratified to ensure equal sampling of individuals throughout the day and over the course of the year. We collected a total of 4819 focals for a total of 803.2 h of observation. During all recorded feeding events (i.e. when a focal animal was feeding on either chow or plants) CS monkeys consumed 81% (+/– 12.7%) chow and 19% (+/– 12.7%) plants (i.e. tree leaves, grass, and flowers; Table S1, Supporting Information). Consumption of chow vs. plants did not vary across seasons. At SS, macaques eat once a day between 9:00 and 11:00 a.m. and are provisioned with the same chow as the CS macaques. They generally do not have access to natural vegetation but are supplemented with fruit (2–3 times a week) and seeds (1–2 times a week). Therefore, chow does not make up 100% of their diet. However, because most of the provisioned food is consumed and the enrichment foods are a very small proportion of the monkeys' diet (SS staff, personal communication), we are confident that chow makes up more of the SS diet than the CS diet. As a result, we estimate that the diets of the two populations is at least 80% similar. The monkeys at both CS and SS are fed the dry, pelleted 8773 Teklad NIB Primate Diet Modified, which is made up of 20% protein, 5% fat, and 10% fiber. Ingredients are listed in Table S2 (Supporting Information).

Sample collection

In 2010, there were 2295 macaques at SS (969 conventional and 1326 SPF) and 1211 macaques at CS. We collected data from two groups at CS ($N = 32$) and opportunistically collected samples from individuals at SS ($N = 34$) across rainy and dry seasons. Feces uncontaminated with urine, water, or another animal's feces

Table 1. Sample demographics.

Location	Total N	N adults (4.08–21 years)	N Juveniles (0.58–3.33 years)	Year	Season
CS	32	19	13	See below	Rainy, dry
		0	7	2009	
		0	6	2010	
		19	0	2012	
SS	34	15	19	See below	Rainy, dry
		7	8	2009	
		8	11		
				2010	

were collected noninvasively and immediately after defecation in 2009–2010 in both conventional and SPF indoor enclosures from adults and juveniles at SS and from juveniles at CS. All fecal samples were linked to an animal with a confirmed identity. To supplement our dataset, we also integrated noninvasively collected samples from adults at CS that were collected 24 months later (2012). A summary of sample demographics can be found in Table 1. Samples were collected in both rainy and dry seasons across all years. We detected no marked interannual patterns in microbiota composition (2009 vs. 2010 vs. 2012, $P > .05$) and no differences between conventional and SPF colonies at SS (PERMANOVA, $P > .05$; distinct microbial taxa listed in Table S3, Supporting Information), allowing us to combine all samples in the same analysis. Samples from SS were frozen immediately while samples from CS were stored in a cooler on ice packs for a period of approximately 2–7 h until they could be transferred to a -20°C freezer. Samples were maintained at either -20 or -80°C until processing. All research procedures were approved by the Caribbean Primate Research Center in Puerto Rico and the University of Colorado, Boulder.

Microbiota analyses

We assessed gut microbiota taxonomic composition in adult and juvenile macaques from both populations ($N = 66$) using 16S rRNA gene amplicon sequencing. DNA was extracted from the fecal samples using the MOBio PowerSoil Kit. The V4 region of the 16S rRNA gene was amplified using the Earth Microbiome Project protocol (Thompson et al. 2017) and the 515Fa/806 primer set (Caporaso et al. 2010). Extraction and PCR negative controls were both included in the sequencing run. We barcoded and pooled all amplicons in equimolar concentrations for sequencing on an Illumina MiSeq V2 platform at the University of Colorado, Boulder.

Forward single-end sequences were demultiplexed and processed using QIIME2 version 2020.2 (Bolyen et al. 2019). The dada2 algorithm was used to trim sequences and cluster amplicon sequence variants (ASVs). The removal of chloroplast and mitochondria sequences as well as chimeric sequences resulted in a total of 1 542 122 reads with an average of 23 265 reads per sample, and taxonomy was assigned using the GreenGenes 13.8 reference database. All samples were rarefied to 10 000 reads per sample based on alpha rarefaction curves (Figure S1, Supporting Information). A total of three samples were rarefied out, so subsequent microbiota and statistical analyses were conducted on a dataset of 63 individuals. Alpha and beta diversity metrics were calculated in QIIME2, where alpha diversity metrics included Faith's phylogenetic distance, Shannon diversity index, and bacterial richness, and beta diversity metrics included unweighted and

weighted UniFrac and Bray–Curtis distances. We also calculated core microbiotas for adults and juveniles at 96% (what microbial taxa 96% of individuals shared) and 100% (what microbial taxa 100% of individuals shared).

Statistical analyses

All statistical analyses were performed in R (version 4.1.2; Bunn and Korpela 2013) on the filtered relative abundance table at the microbial ASV taxonomic level, with P -value cutoffs at 0.05. To identify predictors of microbial community composition, we utilized permutational analyses of variance (PERMANOVA) on the unweighted and weighted UniFrac distance matrices using the *adonis* function (Oksanen and Simpson 2009) in the *vegan* package in R. The model was structured with dependent variables: location (CS vs. SS), age group (adults vs. juveniles), season, and sex ($N = 63$). Collection year was initially included in the models but was not significant; as such, we did not include it in reported models. We used beta dispersion tests to evaluate variation in the magnitude of interindividual differences between locations and age groups (*betadisp* in *vegan*; $N = 63$; Anderson 2006). We also used the *nlme* package in R (PINHEIRO and J. 2012) to run a linear regression to examine the effects of the fixed variables location, age, sex, and season on alpha diversity indices ($N = 63$). Additionally, we used analysis of composition of microbiomes with bias correction (ANCOM-BC) with a cut-off of log fold changes above two or below two to estimate differential abundance of gut microbes at the genus level between locations and age groups. Finally, we visualized UniFrac distances and alpha diversity metrics by constructing nonmetric multidimensional scaling (NMDS) and violin plots, respectively, using the *ggplot2* package (Wickham 2015).

Results

Location (PERMANOVA, unweighted UniFrac: $F_{1,62} = 3.57$, $R^2 = 0.054$, P -value $< .001$; weighted UniFrac: $F_{1,62} = 6.78$, $R^2 = 0.097$, P -value $< .001$) was the most significant predictor of overall gut microbial community composition, followed by age group (adults vs. juveniles; unweighted UniFrac: $F_{1,62} = 1.48$, $R^2 = 0.022$, P -value = .025; Fig. 1) and season (unweighted UniFrac: $F_{2,62} = 1.43$, $R^2 = 0.043$, P -value = .007). Sex was not a significant predictor of microbial composition within and between locations. Yet, it must be noted that each of these variables explained less than 10% of microbial variation. Linear mixed effects models demonstrated that gut microbial diversity did not differ across locations, age groups, sexes or season. When examining which taxa characterized each location, we found that the abundances of only 22 out of 284 microbial genera (7%) were significantly different (ANCOM-BC, q -value < 0.01) between CS and SS (Table 2). We found that no individual taxon exhibited significantly different relative abundances across age groups between both locations or within SS.

Because the microbiome is shaped early in life by environmental exposures, we wanted to compare juveniles and adults across locations to see if patterns differed by age. We found no effect of age on microbial diversity across or within populations. While age was a predictor of overall gut microbial community composition, the effect of age was no longer significant within each location. Across both populations, beta dispersion tests showed differences between age groups for unweighted UniFrac distances (F-model = 15.339, P -value $< .001$). Within each population, dispersion patterns also differed significantly between adults and juveniles (CS unweighted UniFrac, F-model = 22.702, P -value $< .001$; SS unweighted UniFrac, F-model = 24.956, P -value $< .001$; SS weighted

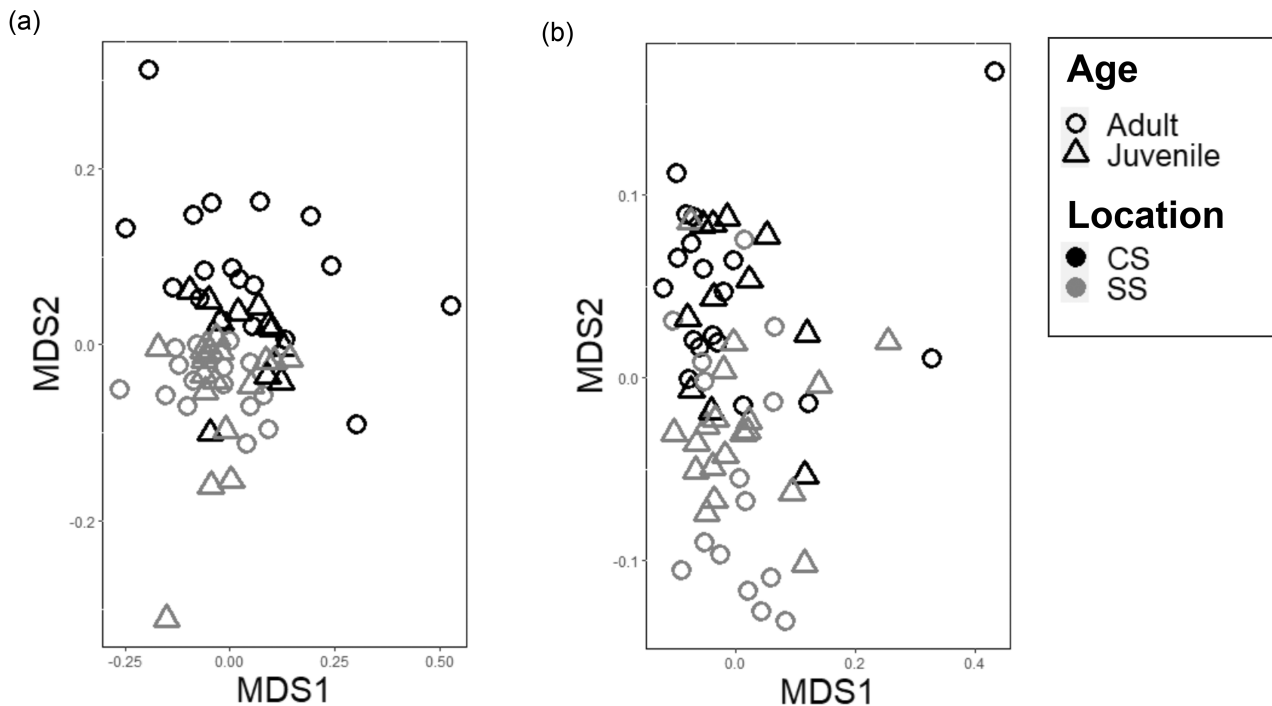


Figure 1. NMDS plots (unweighted and weighted UniFrac distances) to assess differences in gut microbial composition in adult and juvenile macaques sampled at CS (semiwild) and SS (captive).

Table 2. Genera which were significantly different between locations.

Taxa	CS Average relative abundance (%) +/-SD)	SS Average relative abundance (%) +/-SD)
Prevotella	0.084 (0.012)	0.966 (0.016)
Eubacterium	0.054 (0.001)	0.026 (0.0003)
Mogibacterium	0.028 (0.001)	0.005 (0.0002)
Phascolarctobacterium	0.020 (0.0005)	0.011 (0.0005)
Sarcina	12.67 (0.124)	7.908 (0.059)
Bulleidia	0.272 (0.012)	0.122 (0.006)
Bacteroides	0.982 (0.001)	0.766 (0.007)
Oscillospira	0.584 (0.003)	0.211 (0.004)
Dialister	0.757 (0.014)	0.294 (0.012)
Prevotellaceae	0.196 (0.0001)	0.028 (0.0004)
Methanobrevibacter	3.562 (0.035)	2.216 (0.049)
Coprococcus	0.034 (0.001)	0.009 (0.0003)
Erysipelotrichaceae	0.179 (0.001)	0.153 (0.002)
Ruminococcaceae	0 (0)	0.001 (4.38E-05)
Faecalibacterium	1.223 (0.016)	0.845 (0.010)
Streptococcus	0.233 (0.004)	0.860 (0.005)
Catenibacterium	0.446 (0.005)	0.991 (0.012)
Methanosphaera	0.003 (0.0001)	0.038 (0.0007)
Lactobacillus	0.01 (0.0002)	0.196 (0.002)
Rickettsiales	0.004 (0.0001)	0.013 (0.0001)
Treponema	0.016 (0.0003)	0.758 (0.012)
Prevotella spp.	0.021 (0.004)	0.328 (0.0004)

UniFrac, F-model = 5.306, P-value = .01). ANCOM-BC showed that the abundances of four out of 87 microbial genera specific to CS, including those from the families Erysipelotrichaceae, Ruminococcaceae, Bacteroidaceae, and Lactobacillaceae, significantly differed between adults and juveniles (Table 3).

Table 3. Microbial families and genera which were significantly different between ages within CS.

Taxa	Adults Average relative abundance (%) +/-SD)	Juveniles Average relative abundance (%) +/-SD)
Erysipelotrichaceae	0.043 (0.079)	0.141 (0.013)
Gemmiger	0.708 (0.254)	0.316 (0.042)
Bacteroides	0.214 (0.226)	0.163 (0.254)
Lactobacillus	0.138 (0.208)	0.259 (0.498)

Discussion

This study used captive and semiwild macaques in Puerto Rico with at least 80% diet similarity to examine if microbial differences could be detected across locations despite a shared diet. Although we found that location (captive vs. semiwild), age, and season were significant predictors of gut microbial composition, the magnitude of observed differences was relatively small. These patterns suggest that diet and its effects on microbial communities may shape the gut microbiota to a greater extent than do other environmental differences associated with location. Moving forward, studies should quantitatively assess the impact of diets alongside other factors to determine the relative influence of dietary and environmental factors on the gut microbiota.

Location impacts macaque microbial community composition

We found small, significant differences in CS and SS macaque gut microbial community composition. It is possible that these differences are a result of the slight dietary divergence between populations. Although monkey chow comprised the majority of the diet

at both locations, CS macaques were observed to dedicate 19% of their feeding time to natural vegetation on the island. Additionally, CS macaques sometimes eat or chew on debris that washes up on the beach, including seaweed and plastic, and inadvertently consume small amounts of sea water. Studies of captive primates suggest that dietary supplementation with natural browse from multiple plant species can influence the composition and diversity of the gut microbiota (Greene et al. 2018, 2020). We do not have individual-level data describing the types of plants CS macaques use to supplement their diets, or the nutritional content of those plants. However, when CS macaques supplementally feed, they undoubtedly draw from a higher diversity of plant food items with higher fiber content than SS macaques do, who feed on domesticated fruits and seeds that are provided a few times per week. These differences could lead to the observed microbiome differences.

Furthermore, although we do not have quantitative environmental data, it is likely that some of the observed microbial differences stem from the differences in the macaques' physical environments and associated microbial exposures. As the physical environment has been shown to transmit and potentially select for certain environmental bacteria (Liu et al. 2021, Li et al. 2016, Bornbush et al. 2022), it can serve as a strong influence on gut microbiota composition. Indeed, in wild baboons, soil and other geological properties predicted gut microbiota composition across sites (Grieneisen et al. 2019). This is likely a result of baboons' terrestrial lifestyle increasing exposure to soil microbial communities. Like baboons, macaques are terrestrial primates. Therefore, it is possible that differential exposure to soil at CS and SS leads to differences in gut microbial community composition, especially because the CS macaques engage in geophagy (Mahaney et al. 1995). Indeed, between CS and SS, we observed differences in the relative abundances of microbial taxa such as *Paraprevotellaceae* and *Mogibacterium*, whose relative abundances in baboon guts were previously associated with variation in soil properties (Grieneisen et al. 2019). Since diet does not change across seasons at both sites, the seasonal differences in microbiome structure that we detected in our models are likely driven by temporal variation in other factors such as these environmental exposures.

Macaque social environments may also contribute to the patterns in our data. SS macaques sampled for this study were housed in indoor enclosures with limited contact with other macaques, while CS macaques live in large social groups and frequently contact other macaques and animals (e.g. they share their habitat with iguanas). Direct and indirect social contact among hosts sharing the same environment shapes pathways of microbial transmission, and variation in these pathways can lead to differences in the gut microbiota both within and between groups of hosts (Tung et al. 2015, Burns et al. 2017, Raulo et al. 2018).

Finally, differences in prophylactic medical treatment could result in different microbial communities across locations. Unlike at CS, antibiotics such as Tylosin, Enrofloxacin, and Trimethoprim-sulfa are administered as needed for diarrhea at SS, and individuals are treated annually with antiparasitics such as Ivermectin. Antibiotics have been shown to affect host physiology long-term as they deplete microbial members, leaving increased niche and nutrient availability within the gut ecosystem (Francino 2016). In particular, Tylosin exposure results in reduced microbial diversity and reduced relative abundances of *Fusobacteraceae* and *Veillonellaceae* in dogs (Manchester et al. 2019), while humans exposed to Enrofloxacin exhibit reduced *Bacteroidetes* and *Proteobacteria* relative abundances (Kim et al. 2012). Similarly, helminth and parasite prevalence has been associated with variation in the gut mi-

crobiota (Berrilli et al. 2012, Kuthyar et al. 2021, Martínez-Mota et al. 2021). For example, *Prevotellaceae*, *Paraprevotellaceae*, and *Faecalibacterium* relative abundances have previously been positively correlated with increased helminth abundance (Lee et al. 2014, Ramanan et al. 2016, Martínez-Mota et al. 2021). Patterns such as reduced relative abundances of *Bacteroides* and unknown *Paraprevotellaceae* in SS individuals could be linked to antibiotics and antiparasitics, but more targeted studies are necessary to address this question.

Shared diet may limit differences in free-ranging and captive macaque microbiotas

Overall, our data indicate that gut microbiota differences between CS and SS macaques are relatively limited. Despite inhabiting disparate social and physical environments, less than 10% of the variation in the microbiota composition data was explained by location. In contrast, studies of macaques in wild and semiwild environments generally report much higher variation in microbiota composition. These include studies of captive and wild long-tailed macaques in Thailand (*M. fascicularis*, 53% of variation explained by location; Sawaswong et al. 2021), wild, provisioned, and captive Tibetan macaques in China (*M. thibetana*, mycobiome data, 23%–39%; Sun et al. 2021), and Japanese macaques in Japan (*M. fuscata*, 28%–32%; Lee et al. 2019). Similarly, in contrast to most studies of wild and captive primates (Frankel et al. 2019, Hale et al. 2019, Lee et al. 2019), including macaques, microbial diversity did not differ significantly between sites in our study, and only 7% of microbial genera exhibited differences in relative abundances between sites.

Because the diets of the two macaque populations in our study were both composed primarily of low-fiber monkey chow, with much greater qualitative differences in the physical and social environments, the microbiome similarities we detected suggest that a high fat, low fiber humanized diet shapes the macaque gut microbiota to a greater extent than do other environmental factors. Low-fiber diets have previously been shown to lead to marked differences in the microbiome over multiple time scales in experimental lab studies in which the environment is held constant. (David et al. 2014, Sonnenburg et al. 2016). However, the current study represents one of the first instances of two populations in distinct environmental conditions consuming such a similar diet. While we cannot completely disentangle diet and genetics in this study since SS and CS macaques share genetic origins, previous studies consistently demonstrate that diet and other environmental factors have a stronger effect on intrahost species microbial community structure than does genetics (Carmody et al. 2015, Rothschild et al. 2018). In fact, a study of captive and wild vervets with shared genetic origins but distinct diets and environmental exposures reported marked differences in gut microbiome composition, indicating that shared genetic variation is not sufficient to override the influence of other factors on microbial community structure (Amato et al. 2015). Therefore, we are confident that the limited genetic variation that exists is not significantly influencing the microbiotas between the two macaque populations in our study.

Moving forward, it will be important to determine whether there is a threshold of diet alteration necessary to shift the gut microbiota. Given that there is interindividual variation in the amount of monkey chow individuals consume on CS, this population can continue to be leveraged to explore these questions. Similarly, with the addition of quantitative data describing macaque physical and social environments, we can more robustly test the relative importance of individual host factors in shaping the gut

microbiota. For example, at SS, social groups are kept in different enclosures, which may exhibit microhabitat differences in environmental microbial exposure. Varying social group sizes in outdoor and indoor corals may also allow different levels of microbial transmission between conspecifics (Tung et al. 2015). Careful measurements of these factors at the individual level in each population as well as experimental manipulation will provide additional insight into the dynamics driving urban-industrialized microbiota phenotypes.

Potential effects of microbiota differences on health

While the microbiota differences that we detected across locations were relatively limited, they may still have important health impacts. For example, there may be health outcomes relevant to the Old Friends hypothesis (Rook 2009), which argues that exposure to a diverse array of environmental microbes is necessary for training the immune system in early life and that limited exposure to these microbes can result in impaired immune development and function. Because the built environment and periodic medical intervention at SS alters macaque exposure to environmental microbes and influences which taxa can stably establish, early life immune priming may be altered in this population, leading to potential downstream health implications. In addition to immune training, other aspects of physiology and development may be impacted as a result of disrupted microbial exposure in early life. Integrating detailed health and microbiome data collected from individuals longitudinally will allow us to test these relationships and identify potential thresholds of microbial divergence necessary to affect health outcomes.

Conclusions

Our data comparing captive and semiwild macaques consuming similar diets allowed us to explore the extent to which diet can limit the impact of differential environmental exposure on gut microbiota structure. While we found evidence that variation in host environments is associated with differences in gut microbial community composition, the magnitude of these differences suggest that a shared diet plays a more important role in shaping the gut microbiota. Future studies should further quantify environmental differences across sites and consider experimentally varying access to chow on CS. By capitalizing on human-influenced wild animal populations such as the one here, we can continue to disentangle the multitude of covariates associated with diet and isolate mechanisms through which the microbiota can be altered. Further, these data contribute to the overall literature on the primate gut microbiota and importantly provide insight on how provisioning captive animals with humanized chow may impact the gut microbiota, which could then impact animal health. Understanding these connections may improve health outcomes for both free-ranging and captive primate populations.

Supplementary data

Supplementary data are available at [FEMSEC](#) online.

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