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: microbiome, primate, diet, captivity

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, Haiser and Turnbaugh 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, non-industrialized settings (Obregon-Tito *et al*. 2015; De Filipo *et al*. 2010). Similarly, wild and captive conspecific mammals have different microbial

signatures (Gibson *et al*. 2019; Clayton *et al*. 2016). However, large diet shifts are often associated with processes such as industrialization in humans (Jew, AbuMweis and Jones 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 (Yatsunenko *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 microbial 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, a small uninhabited island off the coast of Puerto Rico, in the early 20th century, resulting in a free-ranging, semi-wild population that exists to this day and has been extensively studied (Kessler and Rawlins 2016). Between 1974 and 1984, a subset of macaques from Cayo Santiago were transferred to the Sabana Seca Field Station on the mainland, where they are maintained in a captive research environment. Because the population on Cayo Santiago (CS) has outgrown the naturally available

resources on the island, the macaques are provided with commercial monkey chow that is delivered by boat daily. Therefore, in addition to originating from the same founder population, the macaques at Sabana Seca (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

Cayo Santiago (CS) is a free-ranging semi-wild population of rhesus macaques (*Macaca mulatta*) inhabiting the island of Cayo Santiago 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 (Maestripieri 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.

Sabana Seca (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 two weeks. SS individuals have continuous veterinary care, including bi-annual tuberculosis testing, bi-annual 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, travelling 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 and 14:00 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 4,819 focals for a total of 803.2 hours 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) (*Supplementary Table 1*). Consumption of chow vs. plants did not vary across seasons. At SS, macaques eat once a day

between 9:00 to 11:00 AM 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 *Supplementary Table 2*.

Sample Collection

In 2010, there were 2,295 macaques at SS (969 conventional and 1,326 SPF) and 1,211 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 were collected non-invasively 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 non-invasively 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 inter-annual patterns in microbiota composition (2009 vs 2010 vs 2012, $p > 0.05$) and no differences between conventional and SPF colonies at SS (PERMANOVA, $p > 0.05$; distinct microbial taxa listed in *Supplementary Table 3*), 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 hours until they could be transferred to a -20 degree C freezer. Samples were maintained at either -20 degrees C or -80 degrees 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 ribosomal RNA 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, Colorado.

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 (*Supplementary Figure 1*). 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 inter-individual 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 non-metric 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 < 0.001; weighted UniFrac: $F_{1,62}$=6.78, R^2=0.097, p-value < 0.001) 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=0.025) (**Figure 1**) and season (unweighted UniFrac: $F_{2,62}$= 1.43, R^2=0.043, p-value=0.007). 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 < 0.001). Within each population, dispersion patterns also differed significantly between adults and juveniles (CS unweighted UniFrac, F-model=22.702, p-value < 0.001; SS unweighted UniFrac, F-model=24.956, p-value < 0.001; SS weighted UniFrac, F-model = 5.306, p-value=0.01). 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 Lactobacilliceae, significantly differed between adults and juveniles (**Table 3)**.

Discussion

This study used captive and semi-wild 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. semi-wild), 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.* 2020; Bornbush *et al.* 2022; Li *et al.* 2016), 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 anti-parasitics 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 Veilloneaceae 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 microbiota (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 anti-parasitics, 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 semi-wild 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 intra-host 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 inter-individual 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 semi-wild 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.

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References

Altmann J. Observational Study of Behavior: Sampling Methods. Behaviour 1974;49:227-266

Amato KR, Yeoman CJ, Cerda G *et al.* Variable responses of human and non-human primate gut microbiomes to a Western diet. *Microbiome* 2015, DOI: 10.1186/s40168-015-0120-7.

Anderson MJ. Distance-based tests for homogeneity of multivariate dispersions. *Biometrics* 2006;62:245–53.

Berrilli F, Di Cave D, Cavallero S *et al.* Interactions between parasites and microbial communities in the human gut. *Front Cell Infect Microbiol* 2012;2:141.

Bolyen E, Rideout JR, Dillon MR *et al.* Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* 2019;37:852–7.

Bornbush S, Greene LK, Rahobilalaina S *et al.* Gut microbiota of ring-tailed lemurs (*Lemur catta*) vary across natural and captive populations and correlate with environmental microbiota. *Animal Microb* 2022;4:29.

Bunn A, Korpela M. Crossdating in dplR. 2013, DOI: 10.1016/j.dendro.2008.01.002.

Burns AR, Miller E, Agarwal M *et al.* Interhost dispersal alters microbiome assembly and can overwhelm host innate immunity in an experimental zebrafish model. *Proc Natl Acad Sci U S A* 2017, DOI: 10.1073/pnas.1702511114.

Caporaso JG, Kuczynski J, Stombaugh J *et al.* QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 2010;7:335–6.

Carmody RN, Gerber GK, Luevano JM *et al.* Diet Dominates Host Genotype in Shaping the Murine Gut Microbiota. *Cell Host Microbe* 2015;17:72–84.

Clayton JB, Vangay P, Huang H *et al.* Captivity humanizes the primate microbiome. *Proc Natl Acad Sci* 2016;113:10376–81.

David LA, Maurice CF, Carmody RN *et al.* Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014, DOI: 10.1038/nature12820.

De Filippo C, Cavalieri D, Di Paolo M *et al.* Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *PNAS* 2010; 107:14691-14696.

Francino MP. Antibiotics and the human gut microbiome: Dysbioses and accumulation of resistances. *Front Microbiol* 2016, DOI: 10.3389/fmicb.2015.01543.

Frankel JS, Mallott EK, Hopper LM *et al.* The effect of captivity on the primate gut microbiome varies with host dietary niche. *Am J Primatol* 2019;81:e23061.

Gibson KM, Nguyen BN, Neumann LM *et al.* Gut microbiome differences between wild and captive black rhinoceros - implications for rhino health. *Sci Rep 2019*;9:7570

Greene LK, McKenney EA, O'Connell TM *et al.* The critical role of dietary foliage in maintaining the gut microbiome and metabolome of folivorous sifakas. *Sci Rep 2018*;8:1–13.

Greene LK, Williams C V., Junge RE *et al.* A role for gut microbiota in host niche differentiation. *ISME J 2020 147* 2020;14:1675–87.

Grieneisen LE, Charpentier MJE, Alberts SC *et al.* Genes, geology and germs: Gut microbiota across a primate hybrid zone are explained by site soil properties, not host species. *Proc R Soc B Biol Sci* 2019;286, DOI: 10.1098/rspb.2019.0431.

Gurven M, Stieglitz J, Trumble B *et al.* The Tsimane Health and Life History Project: Integrating anthropology and biomedicine. *Evol Anthropol Issues News Rev* 2017;26:54–73.

Hale VL, Tan CL, Niu K *et al.* Gut microbiota in wild and captive Guizhou snub-nosed monkeys, Rhinopithecus brelichi. *Am J Primatol* 2019;81, DOI: 10.1002/AJP.22989.

Jew S, AbuMweis SS, Jones PJH. Evolution of the Human Diet: Linking Our Ancestral Diet to Modern Functional Foods as a Means of Chronic Disease Prevention. *J Med Food* 2009;12:925–34.

Kessler MJ, Rawlins RG. A 75-year pictorial history of the Cayo Santiago rhesus monkey colony. *Am J Primatol* 2016;78:6–43.

Kim BS, Kim JN, Yoon SH *et al.* Impact of enrofloxacin on the human intestinal microbiota revealed by comparative molecular analysis. *Anaerobe* 2012;18:310–20.

Kuthyar S, Kowalewski MM, Roellig DM *et al.* Effects of anthropogenic habitat disturbance and Giardia duodenalis infection on a sentinel species' gut bacteria. *Ecol Evol* 2021;11:45–57.

Lax S, Smith DP, Hampton-Marcell J *et al.* Longitudinal analysis of microbial interaction between humans and the indoor environment. *Science* 2014;345:1048–52.

Lee SC, Tang MS, Lim YAL *et al.* Helminth Colonization Is Associated with Increased Diversity of the Gut Microbiota. Davies SJ (ed.). *PLoS Negl Trop Dis* 2014;8:e2880.

Lee W, Hayakawa T, Kiyono M *et al.* Gut microbiota composition of Japanese macaques associates with extent of human encroachment. *Am J Primatol* 2019;81:e23072.

Li H, Li T, Yao M *et al.* Pika gut may select for rare but diverse environmental bacteria. *Front Microbiol* 2016; 7:1269.

Liu W, Sun Z, Ma C *et al.* Exposure to soil environments during earlier life stages is distinguishable in the gut microbiome of adult mice. Gut Microbes 2021;13:1830699.

Maestripieri D, Hoffman CL. Behavior and social dynamics of rhesus macaques on Cayo Santiago. *Bones, Genetics, and Behavior of Rhesus Macaques: Macaca Mulatta of Cayo Santiago and Beyond*. Springer New York, 2012, 247–62.

Mahaney WC, Stambolic A, Knezevich M *et al.* Geophagy amongst rhesus macaques on Cayo Santiago, Puerto Rico. *Primates* 1995;36:323–33.

Mancabelli L, Milani C, Lugli GA *et al.* Meta-analysis of the human gut microbiome from urbanized and pre-agricultural populations: The urbanization/industrialization of humans and gut microbiomes. *Environ Microbiol* 2017;19:1379–90.

Manchester AC, Webb CB, Blake AB *et al.* Long-term impact of tylosin on fecal microbiota and fecal bile acids of healthy dogs. *J Vet Intern Med* 2019;33:2605–17.

Manus MB, Kuthyar S, Perroni-Marañón AG *et al.* Infant Skin Bacterial Communities Vary by Skin Site and Infant Age across Populations in Mexico and the United States. *mSystems* 2020, DOI: 10.1128/msystems.00834-20.

Martínez-Mota R, Righini N, Mallott EK *et al.* The relationship between pinworm (Trypanoxyuris) infection and gut bacteria in wild black howler monkeys (Alouatta pigra). *Am J Primatol* 2021:e23330.

Maurice CF, Haiser HJ, Turnbaugh PJ. Xenobiotics Shape the Physiology and Gene Expression of the Active Human Gut Microbiome. *Cell* 2013;152:39–50.

Mills JG, Weinstein P, Gellie NJC *et al.* Urban habitat restoration provides a human health benefit through microbiome rewilding: the Microbiome Rewilding Hypothesis. *Restor Ecol* 2017;25:866–72.

Obregon-Tito AJ, Tito R, Metcalf J *et al.* Subsistence strategies in traditional societies distinguish gut microbiomes. *Nat Commun* 2015.

Oksanen J, Simpson GL. *The Vegan Package*., 2009.

Parajuli A, Grönroos M, Siter N *et al.* Urbanization reduces transfer of diverse environmental microbiota indoors. *Front Microbiol* 2018, DOI: 10.3389/fmicb.2018.00084.

Pinheiro, J. nlme : linear and nonlinear mixed-effects models. R package version 3.1-103. *Httpcranr-Proj* 2012.

Ramanan D, Bowcutt R, Lee SC *et al.* Helminth infection promotes colonization resistance via type 2 immunity. *Science* 2016;352:608–12.

Raulo A, Ruokolainen L, Lane A *et al.* Social behaviour and gut microbiota in red-bellied lemurs (Eulemur rubriventer): In search of the role of immunity in the evolution of sociality. *J Anim Ecol* 2018, DOI: 10.1111/1365-2656.12781.

Rook GAW ed. *The Hygiene Hypothesis and Darwinian Medicine*. Basel: Birkhäuser Basel, 2009.

Rothschild D, Weissbrod O, Barkan E *et al.* Environment dominates over host genetics in shaping human gut microbiota. *Nature* 2018;555:210–5.

Sarkar A, Harty S, Johnson KVA *et al.* Microbial transmission in animal social networks and the social microbiome. *Nat Ecol Evol* 2020;4:1020–35.

Sawaswong V, Praianantathavorn K, Chanchaem P *et al.* Comparative analysis of oral-gut microbiota between captive and wild long-tailed macaque in Thailand. *Sci Rep 2021 111* 2021;11:1–13.

Sonnenburg ED, Smits SA, Tikhonov M *et al.* Diet-induced extinctions in the gut microbiota compound over generations. *Nature* 2016;529:212–5.

Stamper CE, Hoisington AJ, Gomez OM *et al.* The Microbiome of the Built Environment and Human Behavior: Implications for Emotional Health and Well-Being in Postmodern Western Societies. *International Review of Neurobiology*. 2016.

Sun B, Xia Y, Garber PA *et al.* Captivity Is Associated With Gut Mycobiome Composition in Tibetan Macaques (Macaca thibetana). *Front Microbiol* 2021;12:665853.

Tasnim N, Abulizi N, Pither J *et al.* Linking the gut microbial ecosystem with the environment: Does gut health depend on where we live? *Front Microbiol* 2017, DOI: 10.3389/fmicb.2017.01935.

Thompson LR, Sanders JG, McDonald D *et al.* A communal catalogue reveals Earth's multiscale microbial diversity. *Nature* 2017;551:457–63.

Tung J, Barreiro LB, Burns MB *et al.* Social networks predict gut microbiome composition in wild baboons. *eLife* 2015, DOI: 10.7554/eLife.05224.

Urlacher SS, Liebert MA, Snodgrass JJ *et al.* Heterogeneous effects of market integration on sub-adult body size and nutritional status among the Shuar of Amazonian Ecuador. *https://doi.org/101080/0301446020161192219* 2016;43:316–29.70

Van Leeuwen P, Mykytczuk N, Mastromonaco GF *et al.* Effects of captivity, diet, and relocation on the gut bacterial communities of white-footed mice. *Ecol. Evol. 2020;*10:4677-4690.

Wickham MH. *Package "ggplot2" Type Package Title An Implementation of the Grammar of Graphics*., 2015.

Wu GD, Chen J, Hoffmann C *et al.* Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes. *Science* 2011;334:105–8.

Yatsunenko T, Rey FE, Manary MJ *et al.* Human gut microbiome viewed across age and geography. *Nature* 2012, DOI: 10.1038/nature11053.

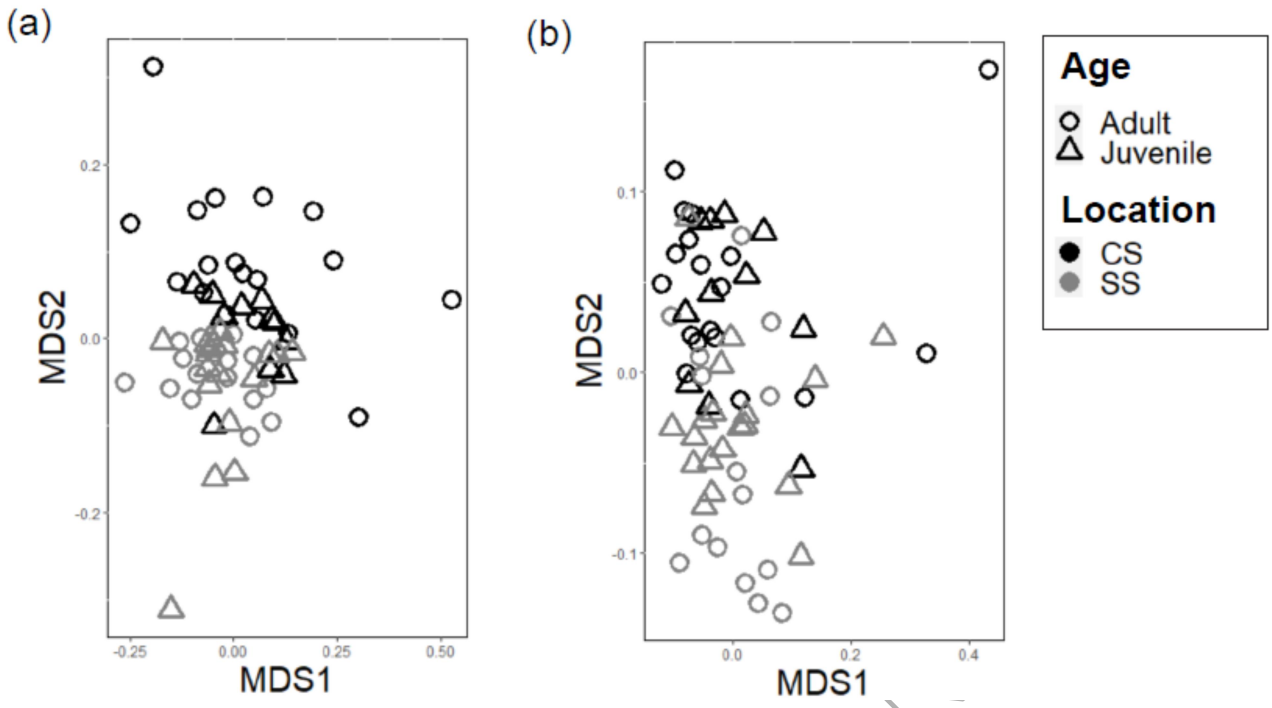

Figure 1. Non-metric Multi-dimensional Scaling plots (unweighted and weighted UniFrac distances) to assess differences in gut microbial composition in adult and juvenile macaques sampled at Cayo Santiago (semi-wild) and Sabana Seca (captive).

Table 1, Sample demographics

Location	Total N	N Adults (4.08-21 yrs)	N Juveniles (0.58-3.33 yrs)	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	

Table 2, Genera which were significantly different between locations.

	CS	SS
Taxa	Average Relative Abundance (% +/- SD)	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 (0.00001)
Treponema	0.016 (0.0003)	0.758 (0.012)
Prevotella spp.	0.021 (0.004)	0.328 (0.0004)

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

	Adults	**Juveniles**
Taxa	Average Relative Abundance (% +/- SD)	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)