Captivity humanizes the primate microbiome

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The primate gastrointestinal tract is home to trillions of bacteria, whose composition is associated with numerous metabolic, autoimmune, and infectious human diseases. Although there is increasing evidence that modern and Westernized societies are associated with dramatic loss of natural human gut microbiome diversity, the causes and consequences of such loss are challenging to study. Here we use nonhuman primates (NHPs) as a model system for studying the effects of emigration and lifestyle disruption on the human gut microbiome. Using 16S rRNA gene sequencing in two model NHP species, we show that although different primate species have distinctive signature microbiota in the wild, in captivity they lose their native microbes and become colonized with Prevotella and Bacteroides, the dominant genera in the modern human gut microbiome. We confirm that captive individuals from eight other NHP species in a different zoo show the same pattern of convergence, and that semicaptive primates housed in a sanctuary represent an intermediate microbiome state between wild and captive. Using deep shotgun sequencing, chemical dietary analysis, and chloroplast relative abundance, we show that decreasing dietary fiber and plant content are associated with the captive primate microbiome. Finally, in a meta-analysis including published human data, we show that captivity has a parallel effect on the NHP gut microbiome to that of Westernization in humans. These results demonstrate that captivity and lifestyle disruption cause primates to lose native microbiota and converge along an axis toward the modern human microbiome.

Human microbiome | primate microbiome | dietary fiber | dysbiosis | microbial ecology

Humans with metabolic disorders, autoimmune and inflammatory conditions affecting the intestines, colorectal cancer, and infectious diseases often exhibit dysbiosis, a state of microbial imbalance (1). Petersen and Round described three categories of dysbiosis: loss of beneficial microbial organisms, expansion of pathobionts or potentially harmful microorganisms, and loss of overall microbial diversity, all of which can occur simultaneously (2). In addition to the evident effect of lifestyle on the pathophysiology of many diseases (3), there is mounting evidence that an intimate interplay exists between the gut microbiota and the development of diseases, including obesity (4–6), Crohn’s disease and ulcerative colitis (7), diabetes (8–10), nonalcoholic fatty liver disease (11), Kwashiorkor (12), and many others. Thus, understanding how lifestyle affects the development and maintenance of the gut microbiome is an important health issue.

Significance

Trillions of bacteria live in the primate gut, contributing to metabolism, immune system development, and pathogen resistance. Perturbations to these bacteria are associated with metabolic and autoimmune human diseases that are prevalent in Westernized societies. Herein, we measured gut microbial communities and diet in multiple primate species living in the wild, in a sanctuary, and in full captivity. We found that captivity and loss of dietary fiber in nonhuman primates are associated with loss of native gut microbiota and convergence toward the modern human microbiome, suggesting that parallel processes may be driving recent loss of core microbial biodiversity in humans.
the United States (i.e., Western) and in developing nations (i.e., non-Western). Both red-shanked doucs and mantled howling monkeys are folivorous NHPs, consuming a diet that is nutritionally poor and difficult to digest compared with diets consumed by nonfolivores. These species are rarely housed in captivity, in part because of the challenge of replicating their wild diets. Our ability to sample from captive, semicaptive, and wild populations from the same species gave us a unique opportunity to study the relationship between lifestyle and disturbance of the native gut microbiota in primates.

Results
Captivity Reproducibly Alters the Primate Microbiome. We performed amplicon sequencing of the V4 region of the 16S rRNA gene on fecal samples collected from captive and wild red-shanked doucs ($n = 93$) and captive and wild mantled howling monkeys ($n = 56$). We obtained samples from wild doucs in Vietnam and from captive doucs in two zoos in different continents (Southeast Asia, United States). Captive and wild howler samples were collected in Costa Rica. We first examined microbiome composition differences according to captivity status. To determine whether significant differences in gut microbiomes were present between captive and wild populations, we calculated unweighted UniFrac distances between all samples (22). This distance metric has been effective previously for distinguishing both highly divergent and subtly divergent microbial ecosystems (23). Examination of a principal coordinates analysis plot revealed that although the gut microbiomes of wild NHP populations (doucs and howlers) are highly divergent, captivity causes them to converge toward the same composition (Fig. 1 and SI Appendix, Fig. S1). This is true despite the highly distinct diet, gut physiology, and geographical location of the two species, and is true across three independent zoos in three countries. We use unweighted UniFrac distances throughout our analysis, as they provide much better clustering of our experimental data by population than weighted UniFrac or Bray Curtis distances (SI Appendix, Fig. S2). This indicates that the clustering is likely driven by presence or absence of key taxa in different populations, rather than by shifts in the ratios of dominant members of the microbiota.

To confirm this pattern of microbiome convergence, we collected samples from an additional captive population comprising 33 individuals from eight different species, housed at a single zoological institution in the United States ($n = 33$), different from the US institution housing the sampled doucs. We found that the novel captive population had experienced a similar trend of convergence toward the same captive microbiome state (Fig. 1). To further test the hypothesis that this convergence toward the captive microbiome was a result of the major disruptions to diet and lifestyle associated with captivity, we obtained microbiome samples from 18 individual doucs housed at a primate sanctuary in Vietnam. These “semicaptive” animals are exposed to a less severe form of captivity than the fully captive animals. They are provided with diets consisting mostly of local plants obtained daily from the jungle by caregivers, but these plants do not represent the full diversity of plants the animals would normally eat in the wild, the animals are kept in large caged enclosures, and the animals also have increased exposure to humans relative to wild animals. However, they are not given antibiotics or other medicines and are not fed the manufactured or refined diets typical of zoos. One of the distinguishing features of our analysis of these animals is that they belong to the same species (red-shanked douc) as the wild and captive individuals to which we are comparing them. Notably, we found these animals to have an intermediate level of disruption to the gut microbiota, falling approximately in between the wild doucs and the captive doucs in our distance-based analysis (Fig. 1), suggesting that the level of severity of dietary and lifestyle disruption is associated with the level of disruption to the native gut microbiota. Analysis-of-similarities (ANOSIM) statistical analysis confirmed that NHP gut microbial communities grouped by captivity status (ANOSIM $R = 0.69$; $P = 0.001$), and a random-forest classifier obtained 100% cross-validation accuracy at discriminating wild douc, wild howler, semicaptive, and captive groups, with the sole exception of the recently captured howler monkey, which classified with the wild howlers (Fig. 2 and SI Appendix, Figs. S3 and S4). These analyses confirmed that the wild primate populations had unique signature microbiota that were lost in captivity. An additional meta-analysis including data previously published by Muegge et al. also confirmed that captive primates lose their native microbial biodiversity and converge toward a similar perturbed state (SI Appendix, Fig. S5) (17).

The Captive Primate Microbiome Is Characterized by Loss of Diversity. To investigate gut microbial diversity, including previously unknown taxa, we performed open-reference operational taxonomic units (OTUs) picking on the NHP samples. We tested for significant differences in within-individual biodiversity between primes in different living conditions. Adding to previously published results describing decreased NHP diversity in captivity (24), we found that gut microbial diversity decreased in accordance with the severity of captivity, with the highest alpha diversity observed in NHPs living under the most natural conditions (i.e., wild), the lowest alpha diversity seen in the NHPs living under the most unnatural conditions (i.e., captive), and an intermediate number of OTUs seen in the semicaptive individuals ($t$ test wild vs.
Fig. 3. Captivity reduces native primate microbiota. (A) Bar plot of mean and spread of gut microbial biodiversity, as measured by the number of species-like OTUs in the gut microbiome, of wild, semicaptive, and captive douc populations of NHPs, using the Chao1 estimator of total OTU richness. This indicates a significant loss of biodiversity from wild populations to semicaptive populations, and again from semicaptive to captive. Error bars indicate SD, and asterisks denote significance at *P < 0.05, **P < 0.01, and ***P < 0.001. (B) Standard box plot of microbiome variation (unweighted UniFrac distance) explained by different experimental factors, showing that captivity in general is associated with a greater change in microbiome state than variation in host species, zoological institution, or individual.

Factors Influencing Captive Primate Microbiome Convergence. Several major lifestyle changes known to influence the gut microbiome are associated both with captivity in NHPs and with modernization in humans. These changes include dietary shifts, disease, changes in geography, and exposure to modern medicine and hygiene, including antibiotics. We performed several analyses to determine whether these factors, or host genetics, are associated with convergence toward humans and loss of microbiome diversity in captive primates.

First, we examined the relative effects of geography and location on microbiome variation. Including the additional 33 samples from the second US zoo, our samples span four different captive facilities in three different countries, all demonstrating the same trend of convergence toward the modern human microbiome in captive primates. We found that, although there is a strong effect of geography and location on the microbiota of the captive individuals, with interzoo microbiome distances being significantly greater than intrazoo microbiome distances, there is an even greater difference between captive and wild douc microbiomes (Fig. 3B) (permutation test on unweighted UniFrac distances; P < 0.001). We also note that the two captive douc populations are in very divergent geographical locations (United States and Southeast Asia), yet their microbiomes are more similar to each other than to the wild doucs. We confirmed in a meta-analysis, including samples from additional species in additional zoos (17), that although captive animals do cluster together with animals from the same zoo, all zoo animals tend to be more similar to each other, even between zoos, than they are to the wild animals (SI Appendix, Fig. S5). In addition, we note that although the captive and wild howler populations have approximately the same geographical location, we observed significant microbiome perturbations in the captive howlers. Thus, we find that geography and location alone are not sufficient to explain the observed changes in captive NHP microbiomes.

We also considered the effects of host genetics on the microbiome as a potential confounder. However, an important additional component of our study design is that we have controlled for host genetics at the species level by obtaining samples from wild and captive individuals within the same species, replicated across two different species (howler and douc). Although this does not control for within-species individual genetic variation that may cause dysbiosis (14), microbiome variation between captive individuals of the same species is smaller than variation between captive and wild individuals from the same species (Fig. 3B). Interestingly, we do find a correlation between host phylogeny and host microbiome variation within a given zooological institution (permutation test, P < 0.0001) (SI Appendix, Fig. S7), as noted previously in wild NHPs (25). However, this association is potentially confounded by diet and is less strong than the associations of microbiome state with zoological institution and captivity in general. Thus, we find that host genetics, although likely affecting the primate microbiome, has a much smaller effect on the microbiome than does captivity.

We followed several lines of study to examine the effects of dietary changes on the captive primate microbiome and found evidence that dietary perturbation is likely a primary driver of captive primate microbiome composition. Recent studies in humans and mice have supported the hypothesis that loss of natural dietary fiber causes a loss of native microbial diversity and a shift toward the modern Westernized human microbiome (26, 27). To quantify dietary perturbation in a subpopulation of wild, captive, and semicaptive doucs, we collected samples of typical plants and other dietary components for chemical
analysis. We tracked wild doucs over the course of ~9 mo and observed them feeding on 57 different plant species, whereas the semicaptive doucs were offered 43 different plant species over the course of 1 y. In contrast to the high dietary diversity (i.e., number of plant species) consumed by the wild and semicaptive doucs, the captive doucs were fed far fewer plant species, and thus consumed a much less diverse diet. Specifically, the Southeast Asia zoo doucs were offered ~15 plant species and the US zoo doucs were offered only one plant species over the course of 1 year. We measured crude protein, crude fat, soluble sugars, acid detergent fiber, and neutral detergent fiber, in addition to several minerals, in the typical diets of each of these populations (wild, semicaptive, captive in Asia, captive in the United States). For neutral detergent fiber content in the wild and semicaptive douc diets, we used previously measured values from Ulibarri (28) and Otto (29), respectively (SI Appendix, Table S1). Although we do not have specific dietary chemical analysis for each individual animal in these populations, dietary content tends to be homogeneous within these captive and wild primate groups (see SI Appendix for detailed discussion of captive primate diet homogeneity). We then tested the hypothesis that decreased total neutral detergent fiber is associated with loss of native microbiota in captive primates and convergence away from the wild douc microbiome (ANOVA, \( P = 1.4 \times 10^{-7} \)). We found that populations consuming high-fiber diets had microbiomes more similar to those of wild doucs, and populations consuming low-fiber diets had microbiomes more similar to those of modern humans (Fig. 4A). Functional pathways predicted using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) (30) indicated that wild NHP (notably douc) microbiomes possessed increased metabolism of pyruvate, butanoate, glycerophospholipid, and propanoate (random forests feature importance score > 0.01), consistent with increased plant fiber degradation (SI Appendix, Fig. S8). However, these predicted functional profiles should be regarded as suggestive only because of the lack of accounting for unknown species and functional variability within OTU clusters.

We also estimated total raw plant dietary content for all NHPs and human subjects, using chloroplast sequences observed in the 16S amplicon sequencing data. We found that chloroplast content was substantial in wild populations (~3% of all amplicon sequences, on average), but that chloroplast content decreased in semicaptive and captive animals and was nearly completely absent from the human samples and US-based captive primate samples (Fig. 4B). Finally, we performed deep shotgun metagenomic sequencing (28.2 ± 6.6 million sequences per sample) on a subset of 30 captive and wild howlers and doucs and aligned the sequences to known plant reference genomes at 97% identity. Although the existing plant reference genomes were not the same species as those being consumed by the wild primates, we nonetheless identified a large proportion of fecal DNA sequences in the wild primates that were homologous to regions of known cultivated plant genomes, ranging up to ~40% of all observed sequences. In contrast, we observed almost no plant DNA in the captive samples, with the one exception of an individual howler monkey that had been rescued for electrical burn treatment within the last 48 h who had levels similar to those of the wild animals (Mann–Whitney U test, \( P < 0.0001 \)) (Fig. 4C).

This same animal was also an outlier in the principal coordinates analysis, clustering with the wild howler monkeys (Fig. 1). Plant DNA in captive NHP microbiomes also represented lower dietary plant alpha diversity than in wild NHP microbiomes (Mann–Whitney U test of Shannon index, \( P < 0.0001 \)) (SI Appendix, Fig. S9).

We considered whether transition to captivity can produce major microbiome perturbations in an adult individual. We obtained birth location records for the semicaptive douc and tested the hypothesis that decreased total neutral detergent fiber content (black); same, but showing mean ecological similarity to humans (blue). Doucs consuming more fiber more closely resemble wild doucs in their microbiome; doucs consuming less fiber more closely resemble humans. (B) The same samples plotted in Fig. 1, colored by host species. Also shown is the primary axis of correlation of chloroplast relative abundance, a proxy measurement for dietary raw plant content, with sample positions. A smoothed local regression curve for chloroplast ratio (i.e., relative abundance) along the primary axis of variation shows decreasing raw plant consumption from wild, to semicaptive, to captive primates, with almost no raw plant consumption in the humans or US captive primates. (C) Fraction of whole-genome shotgun data aligning at 97% identity to known plant genomes for 14 captive individuals (9 douc, 5 howler) and 16 wild individuals (8 douc, 8 howler). Wild individuals have a high fraction of plant DNA in their stool; captive individuals have almost none, with the exception of a single outlier individual who was recently rescued from the wild for treatment of electrical burns (also an outlier in Fig. 1).
individuals and determined that approximately half of them were born in the wild (eight of 18). This provided us with an opportunity to test the hypothesis that transition to captivity has caused the observed disruption to the natural microbiota in the adult individuals. If the within-individual transition from wild to semicaptive caused only part of the observed disruption to the gut microbiota, then we would expect the wild-born sanctuary-housed doucs to resemble the wild doucs more closely than do those born in the sanctuary. However, we found that wild-born doucs were no closer to wild doucs than were the captive-born doucs (unweighted UniFrac permutation test, \( P = 0.663 \)) (Fig. 1). This supports the hypothesis that transition to captivity can cause the observed microbiome disruption in these animals and indicates that a substantial part of the captivity-related microbiome perturbation can be acquired within an individual’s lifetime. This may have implications in the study of migration-related dysbiosis in humans.

We examined medical records for differences in antibiotic use and disease between individuals that could be affecting or affected by the microbiome. Sadly, five of the nine captive douc individuals we sampled died of gastrointestinal-related diseases including wasting and gastroenteritis within the following year. We tested whether individuals who died had microbiomes more divergent from the wild douc microbiome than their captive counterparts within the same zoo. We observed a positive trend, but there were only five deceased and four living individuals, and the trend was not significant (\( t \) test, \( P = 0.35 \)) (SI Appendix, Fig. S10). This suggests there may be an association between the captive primate microbiome and disease, but the findings are not conclusive and require larger sample size for proper testing.

Finally, we determined that 11 of the 33 animals sampled from the second US zoo had never been prescribed antibiotics in their lives, based on their complete medical records. In contrast, the remaining 22 individuals had received an average of 4.6 ± 4.0 courses throughout life. We used this information to test whether the microbiomes of the 22 individuals that had received antibiotics more closely resembled modern human microbiomes, but they did not (\( t \) test, \( P = 0.5507 \)), nor were they lower in diversity or less similar to wild primate microbiomes (SI Appendix, Fig. S11). This finding suggests that antibiotics are not a strong driver of the convergence of the captive primate microbiome and disease, but the findings are not conclusive and require larger sample size for proper testing.

Crevatin in Primates Partially Parallels Modernization in Humans.

Using standardized methodologies across studies (i.e., sequencing of the V4 region), we were able to conduct a meta-analysis combining NHP populations with previously published samples from adult humans living in both Westernized (United States, \( n = 129 \)) and non-Westernized (Malawi and Venezuela, \( n = 21, 34 \), respectively) countries (23). This analysis demonstrated that the axis of convergence seen in Fig. 1 continues from wild to captive NHPs, and then toward non-Westernized humans, and finally to Westernized humans, suggesting that a similar loss of signature biodiversity seen in captive NHPs has taken place as humans adapted to modern society. Interestingly, we observed higher relative abundances in captive primates of \textit{Bacteroides} and \textit{Prevotella}, the two dominant human gut microbiome genera compared with in wild and semicaptive NHPs (Fig. 5). In addition, a higher relative abundance of \textit{Bacteroides} was seen in Westernized humans compared with non-Westernized humans. The high relative abundance of \textit{Bacteroides} seen in both captive NHPs and Westernized humans is suggestive that captivity moves captive NHPs in the same direction along the \textit{Bacteroides} gradient as does Westernization in humans.

Discussion

Our analysis of species-matched wild, semicaptive, and captive individuals in two different species (red-shanked douc and mantled howler monkey) demonstrates that NHPs lose substantial portions of their signature microbiota in captivity and that they become colonized by human-associated gut bacterial genera \textit{Bacteroides} and \textit{Prevotella}. Species in both \textit{Bacteroides} and \textit{Prevotella} are capable of polysaccharide degradation, suggesting their emergence may be associated with a shift in diversity or types of dietary polysaccharides, rather than with overall loss of dietary fiber. In an additional captive NHP population, represented by 33 individuals across eight species at an independent zoo, we found that captive rearing caused these individuals to converge toward the same perturbed microbiome state as the captive douces and howlers. Thus, we found that multiple populations of captive primates converged along an axis leading away from the wild primate microbiome state and toward the modern human microbiome state.

It is important to note that we do not know whether the microbiome perturbations we observe contribute to captive primate disease or are merely a consequence of gastrointestinal disease caused by other factors. However, perturbation of the gut microbiome has known causal and predictive roles in human gastrointestinal diseases, particularly in predisposing individuals to infection (31–33). This suggests there may be a broader role for maintaining keystone gut microbial genetic and functional diversity in the conservation of NHPs, although further research is needed to determine which microbiome perturbations represent dysbiosis, and which are merely benign or even beneficial adaptations to changes in lifestyle and diet. Previous studies have shown that changes in diet are directly associated with shifts in gut microbial community structure (18, 20, 34). By leveraging our study design and zoological medical records, we were able to rule out geography, host genetics, antibiotics exposure, and birth in captivity as the primary determinants of the captive primate microbiome. In contrast, chemical dietary analysis, deep shotgun sequencing, and tracking of fecal chloroplast ratios pointed to loss of dietary plant fiber as a primary driver of captive primate microbiome perturbation.

Recent studies have shown that modern humans have lost a substantial portion of their natural microbial diversity (34–36). Given the massive loss of gut microbiome diversity in captive primates in this study, captive NHPs may provide an informative model for understanding the effects of modernization and mass human migration on the development of human diseases linked to diet and the microbiome, such as obesity and diabetes. Our meta-analysis including Westernized and non-Westernized human microbiomes suggests that loss of dietary fiber may be
Supplemental Text and Methods

Random forests classifier and cross-validation estimation of accuracy
Using the random forests classifier, we determined the most discriminative genus-level taxa between wild, semi-captive, and captive NHPs. We then assessed the accuracy of the classifier using 10-fold cross-validation. In other words, we trained the classifier on 90% of the samples, and then used the discovered signatures to predict which populations the remaining 10% of samples belonged, and then repeated the process 10 times. This analysis revealed that individual primate populations have such distinct signature microorganisms that they can be identified from their microbiota with an estimated 99.6% accuracy (Figure 2). From this analysis we identified the bacterial genera most important for distinguishing the populations (random forests feature importance score ≥ 0.01). We found that wild NHPs possessed higher relative abundances of a variety of microbes, including Collinsella, Tannerella, Oscillospira, Coprococcus, etc., while captive NHPs possessed higher relative abundances of Bacteroides, Prevotella, Parabacteroides, Treponema, etc. Compared to wild and captive NHPs, our population of semi-captive NHPs possessed higher relative abundances of a variety of microbes, including Akkermansia, Turicibacter, Methylobacterium, and other taxa.

Importance of concordant data generation methods for meta-analysis
A major challenge in performing the meta-analyses combining microbiome data from multiple sources is the presence of batch effects due to study bias from different processing methods. To extract meaningful patterns from our comparison of NHP and human microbiome data required joint analysis of multiple wild populations of species, together with previously published human data. Previous work has characterized gut microbiome variation in a number of specific groups of primate species, such as chimpanzees, African apes, and baboons (1, 2). However, these published data are generated using varied approaches to sample storage, DNA extraction, amplification, and DNA sequencing, impeding efforts toward large-scale meta-analyses. Quantitation of microbiome data requires application of consistent, standardized methods to avoid batch effects. In this study, all NHP fecal samples were obtained by our group using the same protocols, were processed using comparable methods, and were sequenced at the same sequencing facility using the same method (i.e., EMP method; V4 region) as published human data (3), which resulted in wild, semi-captive, and captive NHP microbiome samples that were amenable to meta-analysis.

Detailed discussion of captive primate diet homogeneity
Captive NHP diets are dramatically different from those of wild or even semi-wild NHPs. Diets fed to captive NHPs are typically generalized and rarely species-specific. NHP species are regularly categorized as folivores, frugivores, and omnivores based on the dietary niche they occupy. In captive settings, this results in NHPs being given very similar, if not identical diets (4). However, even if wild primates do belong in similar feeding guilds, different feeding ecologies, morphologic and physiologic adaptations, and habitats all contribute to varied nutrient intake in the wild (5), typically rich in plant fibers. In contrast, the recommended diet of most captive NHPs is based primarily on corn and soy. It is high in fat (5%) and protein (23%) while low in fiber (14%) (6) when compared to the diet of wild leaf-eating NHPs that has approximately 0% fat, 10-13% protein and 23-54% fiber (7). Unlike corn and soy, tree leaves contain plant secondary compounds (such as alkaloids, phenolics, and cyanide) in addition to nutrients (8–12). For example the Golden Bamboo lemur consumes four times the human-lethal dose of cyanide every day (13), indicating highly specialized digestive capability across different primates. Thus, loss of dietary fiber content and fiber diversity in captive NHPs is a likely contributor to their concomitant loss of gut health and microbial diversity.

Detailed discussion of emergence of Bacteroides
One of the marked effects of captivity on the gut microbiome of NHPs, as well as Westernization on the gut microbiome of humans, is an increase in relative abundance of Bacteroides. Using both wild ape and human microbiomes, Moeller et al. (2014) determined that Bacteroides has increased in relative abundance in humans living in the USA greater than fivefold since their divergence from other human populations. The bacterial genus Bacteroides has a known positive association with the consumption of a diet rich in animal fat and protein (1, 14), which are major components of a
Western diet. A Western diet is considered to be a diet high in fat and animal protein (e.g., red meat), high in sugar, and low in plant-based fiber (15–17). Previous studies examining the relationship between dietary patterns and dysbiosis suggest a strong association between Western lifestyle, notably diet, and a dysbiotic gut microbiome (14, 15, 17), as the Western diet is evolutionarily discordant from the diet of ancestral humans (15, 18). Taken together, the relative abundance of *Bacteroides* in the gut appears to be strongly regulated by dietary intake.

**Breakdown of Douc and Howler sample population**

Doucs: Fecal samples (n = 111) were collected from captive (n = 27 samples, 9 individuals), semi-captive (n = 18 samples, 18 individuals), and wild (n = 66 samples from 7 known individuals and 39 unknown individuals) red-shanked doucs (*Pygathrix nemaeus*) between 2012-2013. One captive population was located at the Philadelphia Zoo in the USA while another was located at the Singapore Zoo in SE Asia. Doucs housed at the Endangered Primate Rescue Center in Ninh Binh, Vietnam served as the semi-captive population. Doucs inhabiting Son Tra Nature Reserve, Da Nang, Vietnam (16°06’—16°09’N, 108°13’—108°21’E) served as the wild population in this comparative study (19).

Howlers: Fecal samples (n = 56) were collected from captive (n = 5 samples, 5 individuals) and wild (n = 51 samples, 28 known individuals, 17 unknown individuals) mantled howling monkeys (*Alouatta palliata*) between July-August 2010. Howlers inhabiting the forests of Hacienda La Pacifica, which is a privately owned cattle/tilapia farm of approximately 2,000 hectares located at the base of the Cordillera de Tilaran in the Province of Guanacaste, Costa Rica (latitude 10°28’N, longitude 85°07’W), served as the wild subjects (20, 21). Howlers housed at Las Pumas Rescue Center, which is located within Hacienda La Pacifica, served as the captive subjects.

The remaining eight NHP species sampled consisted of captive individuals housed at the Como Zoo in Saint Paul, MN (Supplemental Table 2).
Figure S1. Primate microbiome clustering by captivity, location, and species does not depend on inclusion of USA zoo #2 samples. Principal coordinates plot of unweighted UniFrac distances between all primate samples shown in Figure 1, excluding the 33 samples from the second USA zoo. Similar to the plot in Figure 1, this plot shows separation of the wild doucs and howlers, and convergence of the douc and howler microbiomes toward the same state in captivity. Principal coordinates analysis was performed only on the subset of samples to demonstrate that the observed clustering was not driven by the second USA zoo samples.
Figure S2. Captive primate dysbiosis converges toward the modern human microbiome. Principal coordinates plot of (a) weighted UniFrac and (b) Bray-Curtis distances between all samples, showing ecological distance between gut microbial communities in wild, semi-captive (from a sanctuary), and captive nonhuman primates, as well as non-westernized humans, and humans living in the USA (i.e., Westernized). Unweighted UniFrac (Figure 1) provided much stronger clustering of our experimental data by population than weighted UniFrac or Bray Curtis distances, indicating that the clustering is likely driven by presence or absence of key taxa in different populations, rather than by shifts in the ratios of dominant members of the microbiota. These distances based on relative abundance show captive primates overlapping more with the non-westernized modern humans.
Figure S3. Heatmap of most predictive taxa discriminating gut microbiomes of two wild primate species. The transformed relative abundance of the 20 most strongly predictive bacterial genera for discriminating between two species of primates, the red-shanked douc and the mantled howling monkey, as determined by the random forests classifier. This heatmap shows very strong signature species for each of these groups.
Figure S4. Captivity reduces native primate microbiota. Standard box plot of microbiome variation (unweighted UniFrac distance) explained by different experimental factors, showing that captivity in general is associated with a greater change in microbiome state than variation in host species, zoological institution, or individual. (c) Stacked bar plot of relative abundance of the 20 most abundant genera across all wild and captive douc and howler individuals. Bars above zero correspond to genera more prevalent in wild primates; bars below zero correspond to genera more common in captive primates.
Figure S5. Captivity causes nonhuman primate microbiomes to converge toward the same compositional state. Principal coordinates plot of genus-level unweighted UniFrac distances showing ecological distance between gut microbial communities in wild, semi-captive (from a sanctuary), and captive nonhuman primates plotted by population location (left panel) and captivity status (right panel). Novel NHP microbiomes (doucs, howlers, and Como Zoo population) are based on V4 16S sequences (R1 only), and previously published NHP microbiomes (Saint Louis Zoo and Namibia_Wild) by Muegge et al. (2011) are based on V2 16S sequences. Although in wild populations the douc and howler microbiomes are highly distinctive, captivity causes them to converge toward the same composition. Semi-captive doucs (green) fall in between wild and captive doucs along the same axis of convergence. The inclusion of additional captive nonhuman primate populations, represented by 14 distinctive primate species (Como Zoo and Saint Louis Zoo), further highlights the convergence that occurs when primates are kept in captivity. We note that although location is an important driver of microbiome variation (left panel), the effect of zoo location is smaller than the overall effect of captivity. This is also shown in Figure 3b.
Figure S6. Rarefaction curves for different primate groups. (left) Chao-1 estimator as a measure of alpha diversity; (right) Observed number of OTUs as a measure of alpha diversity. Red: wild; blue: semi-captive; orange: captive. We also compared diversity between groups of different captivity status using data at the full rarefaction depth (14100 sequences/sample). Dropping singleton OTUs present in only one sample, the wild NHPs (2544.3 ± 390.9 OTUs) harbored the highest number of OTUs (i.e., greatest diversity), followed by the semi-captive NHPs (2141.4 ± 293.0 OTUs), and captive NHPs (1967.9 ± 538.2 OTUs). By this metric, wild NHPs had significantly higher diversity than captive or semi-captive ($t$-test $p = 2.1 \times 10^{-21}, 1.9 \times 10^{-5}$, respectively), and captive had higher diversity than semi-captive ($t$-test $p = 0.040$). We repeated this analysis with the Chao1 estimator of the true number of OTUs (i.e., species richness) in our samples. Using the Chao1 estimator differences were significant between all three populations ($t$-test $p = 4.6 \times 10^{-39}, 7.3 \times 10^{-8}, 0.0099$ for wild vs. captive, wild vs. semi-captive, and captive vs. semi-captive, respectively) (see Figure 3a).
Figure S7. Association of microbiome and host phylogeny. (a) Host phylogeny from Perelman et al. (22) for the 10 unique primate species sampled in this study. (b) Microbiome phylogeny of all captive, semi-captive, and wild individuals according to unweighted UniFrac distance, using the Nei-Saitou neighbor-joining method (23). This shows major clustering by zoological institution location, and minor nested clustering by host species. (c) Host phylogeny for subset of species present in USA Zoo #2. (d) Microbiome phylogeny as in (b) for samples from USA Zoo #2. This shows some concordance with (c) but concordance is hard to assess due to multiple individuals sampled per species. (e) Microbiome phylogeny as in (d) but depicting a phylogeny built from the average distances between species, averaging over individuals, for comparison with (c). (f) Direct comparison of 8-species host phylogeny (c) with 8-species microbiome-based phylogeny (e), showing significant concordance between the two phylogenies ($p < 0.0001$, permutation test of cophenetic distance (23) when permuting species labels).
Figure S8. Heatmap of KEGG level 2 metabolic pathways that discriminate the four major populations. Only pathways with random forests feature importance > 0.01 are shown. Wild NHPs (notably doucs) possessed higher relative abundances of a variety of pathways, including pyruvate metabolism, butanoate metabolism, glycerophospholipid metabolism, and propanoate metabolism (random forests feature importance score > 0.01), consistent with increased plant fiber degradation.
Figure S9. Dietary plant diversity and the non-human primate microbiome. Estimated dietary plant diversity (Shannon index) in captive and wild doucs and howlers based on whole-genome shotgun data aligning at 97% identity to known plant genomes. These data include 14 captive individuals (9 douc, 5 howler) and 16 wild individuals (8 douc, 8 howler). Wild individuals have higher dietary plant diversity based on plant DNA in their stool (Mann-Whitney U test, p < 0.001), despite the fact that whole genome reference databases only contain a small number of cultivated plant genomes.
Figure S10. Severity of captive douc dysbiosis and risk of mortality. Beeswarm plot with median and upper/lower quartiles of average unweighted UniFrac distance from each captive douc to all wild douc individuals, stratified by survival status at 1 year post-sampling. Distances are normalized to the mean within each captive population in order to adjust for microbiome variation associated with sampling location. Individuals who died tended to have microbiomes more divergent from the wild douc microbiome than their captive counterparts within the same zoo, but there were only 5 deceased and 4 living individuals and the trend was not significant (t-test, p = 0.35).
Figure S11. Antibiotic exposure and captive primate microbiome dysbiosis. (a) Beeswarm plot of mean ecological similarity of individual captive primates to all modern humans, stratified by antibiotic exposure (USA zoo #2 samples only). This shows that antibiotic exposure in captive primates was not associated with having a more human-like microbiome. 11 individual animals sampled from the second USA zoo had never taken antibiotics, while the remaining 22 individuals had received an average of 4.6 +/- 4.0 courses throughout life. (b) as in (a) but showing unweighted UniFrac distance of the individuals in the left panel to wild primates. (c) Shannon diversity of 33 captive individuals in the USA Zoo #2, 11 of whom had never had antibiotics. There is no statistical difference between the two groups, indicating that lifetime exposure to antibiotics is not related to captive primate microbiome diversity. These results demonstrate that lifetime exposure to antibiotics is not likely to be causing captive primate microbiome diversity.
Figure S12. **Sequencing quality scores in forward and reverse reads.** Forward-read quality scores (a) and Reverse-read quality scores (b) plotted against nucleotide position. Forward-read score lower standard deviation drops consistently below q=25 at approximately 185 bases; reverse-read score lower standard deviation drops consistently below q=25 at approximately 100 bases.
## Supplemental Tables

### Supplemental Table 1. Nutrient content from dry matter aggregate dietary material for each population.

<table>
<thead>
<tr>
<th>Diet component</th>
<th>Wild</th>
<th>EPRC</th>
<th>Southeast Asia Zoo(^1)</th>
<th>USA Zoo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein (%)</td>
<td>9.46</td>
<td>16.52</td>
<td>13.37</td>
<td>16.7</td>
</tr>
<tr>
<td>Crude Fat (%)</td>
<td>*</td>
<td>3.23</td>
<td>3.12</td>
<td>3.71</td>
</tr>
<tr>
<td>Soluble Sugars (%)</td>
<td>2.7</td>
<td>2.28</td>
<td>*</td>
<td>7.9</td>
</tr>
<tr>
<td>Acid detergent Fiber (%)</td>
<td>46.76(^2)</td>
<td>23.2(^3)</td>
<td>23.07</td>
<td>8.65</td>
</tr>
<tr>
<td>Neutral detergent Fiber (%)</td>
<td>53.67(^2)</td>
<td>35.6(^3)</td>
<td>31.97</td>
<td>12.64</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>0.49</td>
<td>1.05</td>
<td>0.22</td>
<td>0.72</td>
</tr>
<tr>
<td>Potassium (%)</td>
<td>0.96</td>
<td>0.76</td>
<td>0.21</td>
<td>0.29</td>
</tr>
<tr>
<td>Sodium (%)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.24</td>
<td>0.27</td>
</tr>
<tr>
<td>Zinc (mg/kg)</td>
<td>19.4</td>
<td>10.16</td>
<td>8.25</td>
<td>26.3</td>
</tr>
<tr>
<td>Iron (mg/kg)</td>
<td>26.5</td>
<td>33.73</td>
<td>20.74</td>
<td>64.33</td>
</tr>
</tbody>
</table>

\(^1\)Southeast Asian zoo diet also included a vitamin and mineral supplement which was not included in the analysis.

\(^2,3\)Values marked with (\(^2\)) are from Ulibarri (2013) and those marked with (\(^3\)) are from Otto (2005) as NDF and ADF were not available from the laboratory analyses for these diets. Data from Ulibarri (2013) were weighted according to feeding season to represent the proportion of plants part selected.

*Not detected.
Supplemental Table 2. *Primate species and associated lifestyles included in this study.*

<table>
<thead>
<tr>
<th>NHP species common name</th>
<th>NHP species scientific name</th>
<th>Lifestyle</th>
<th>Provenance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red-shanked douc</td>
<td><em>Pygathrix nemaeus</em></td>
<td>Wild</td>
<td>Son Tra Nature Reserve (Da Nang, Vietnam)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Semi-captive</td>
<td>Endangered Primate Rescue Center (Ninh Binh, Vietnam)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Captive</td>
<td>Singapore Zoo (Singapore, Singapore)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Captive</td>
<td>Philadelphia Zoo (Philadelphia, PA, USA)</td>
</tr>
<tr>
<td>Mantled howling monkey</td>
<td><em>Alouatta palliata</em></td>
<td>Wild</td>
<td>Hacienda La Pacifica (Guanacaste, Costa Rica)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Captive</td>
<td>Las Pumas Rescue Center (Guanacaste, Costa Rica)</td>
</tr>
<tr>
<td>Western lowland gorilla</td>
<td><em>Gorilla gorilla gorilla</em></td>
<td>Captive</td>
<td>Como Zoo (Saint Paul, MN, USA)</td>
</tr>
<tr>
<td>Sumatran orangutan</td>
<td><em>Pongo abelii</em></td>
<td>Captive</td>
<td>Como Zoo (Saint Paul, MN, USA)</td>
</tr>
<tr>
<td>De Brazza's monkey</td>
<td><em>Cercopithecus neglectus</em></td>
<td>Captive</td>
<td>Como Zoo (Saint Paul, MN, USA)</td>
</tr>
<tr>
<td>Black-handed spider monkey</td>
<td><em>Ateles geoffroyi</em></td>
<td>Captive</td>
<td>Como Zoo (Saint Paul, MN, USA)</td>
</tr>
<tr>
<td>White-faced saki</td>
<td><em>Pithecia pithecia</em></td>
<td>Captive</td>
<td>Como Zoo (Saint Paul, MN, USA)</td>
</tr>
<tr>
<td>Blue-eyed black lemur</td>
<td><em>Eulemur macaco flavifrons</em></td>
<td>Captive</td>
<td>Como Zoo (Saint Paul, MN, USA)</td>
</tr>
<tr>
<td>Emperor tamarin</td>
<td><em>Saguinus imperator subgrisescens</em></td>
<td>Captive</td>
<td>Como Zoo (Saint Paul, MN, USA)</td>
</tr>
<tr>
<td>Geoffroy's tamarin</td>
<td><em>Saguinus geoffroyi</em></td>
<td>Captive</td>
<td>Como Zoo (Saint Paul, MN, USA)</td>
</tr>
</tbody>
</table>
References


