

## Seasonal Frugivory Modulates the Gut Microbiome of Captive Dwarf Lemurs (*Cheirogaleus medius*) During Fattening

Arielle Y. Kim<sup>1</sup> · Lydia K. Greene<sup>1,2</sup> · Anne D. Yoder<sup>1</sup> · Marina B. Blanco<sup>1,2</sup>

Received: 27 August 2024 / Accepted: 3 December 2024 / Published online: 26 December 2024 © The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2024

The evolutionary history of lemurs is tied to the seasonal, unpredictable, and erratic weather events that characterize Madagascar (Dewar & Richard, 2007), and different lineages evolved distinct ecological strategies to cope with energetic constraints. Dwarf lemurs (*Cheirogaleus* spp.) turned to obligate hibernation to conserve energy during lean seasons. Rather than foraging, they rely on deposited fat reserves. Dwarf lemurs fatten ahead of hibernation during the late rainy season by selecting ripe fruits (Fietz & Daussman, 2006). Fruit sugars, primarily fructose, are endogenously converted to lipids that are predominantly stored in the animals' tails (Fietz & Daussman, 2006; Blanco *et al.*, 2022). During fattening, dwarf lemurs can double their body mass in just a few weeks.

This seasonal feast-fast cycle has implications for the gut microbiome. In Madagascar, the dwarf lemur gut microbiome shows circannual variation linked to the hibernation cycle (Greene *et al.*, 2022). During the fattening season, community diversity is low and the *Mycoplasma* genus blooms. Both features likely relate to



Badge earned for open practices: Open Data Badge. Experiment materials and data are available in the repository at https://www.ncbi.nlm.nih.gov/sra/PRJNA1189778.

Handling Editor: Joanna M. Setchell

Arielle Kim and Lydia K. Greene co-first authors.

Lydia K. Greene lydiakgreene@gmail.com

- <sup>1</sup> Department of Biology, Duke University, Durham, NC, USA
- <sup>2</sup> Duke Lemur Center, Duke University, Durham, NC, USA

seasonal frugivory, with fruit-rich diets promoting microbes that scavenge simple fruit sugars and fibers and outcompete generalists. The bloom in *Mycoplasma* is intriguing, as close relatives of this taxon are linked to adiposity in laboratory mice (Turnbaugh *et al.*, 2008). Nevertheless, we lack resolution to determine how the gut microbiome shifts across fattening, because sampling is currently limited to a single timepoint within this season (Greene *et al.*, 2022).

We introduced seasonal dietary regimens in captive fat-tailed dwarf lemurs (*Cheirogaleus medius*) at the Duke Lemur Center (DLC) in Durham, NC (Fig. 1a) and examined consequences to gut microbiome structure. During the fattening season, we switched lemurs from a generalized diet to a frugivorous one that mimicked seasonally foraged fare in Madagascar (Blanco *et al.*, 2022). If seasonal frugivory consistently modulates gut microbiome features in dwarf lemurs, we expect captive individuals to exhibit decreased microbiome diversity and concurrent blooms in *Mycoplasma* after fattening.

We studied 14 dwarf lemurs (4 females; 10 males; 0.5–14 years old) that were singly or socially housed in temperature-controlled rooms on a shifting NC-like photoperiod during the fattening season (mid-August – October) in 2020. During the active season (March – mid-August), we provided lemurs a generalized diet of fruit-and-vegetable mix, primate chow, and mealworms. During fattening (mid-August – November), we provided them with a frugivorous diet of fresh and dried fruit, honey, and limited chow. The diets were calorically equivalent (40 kcal), but the frugivorous diet contained double the sugar and half the fat and protein (Blanco *et al.*, 2022). Between August and October, dwarf lemurs gained significant body mass (Wilcoxon signed-rank test: W = 105, n = 28, p < 0.001; Fig. 1b), and tail girth (Wilcoxon signed-rank test: W = 105, n = 28, p < 0.001; Fig. 1c).

We sampled lemurs from August 17–21 and switched diets the following day. We sampled lemurs again between October 15–22. From anesthetized lemurs, we inserted a sterile swab into the rectum, rotated for 10 s, snipped tips into tubes, and stored them at -80 °C. This study was approved by Duke University's IACUC (Protocol A213-20-11).

We extracted gDNA from swabs, sequenced the 16S rRNA gene at Argonne National Laboratory (Lemont, IL), and processed data using QIIME 2 (version 2022.8) (Greene *et al.*, 2022). After quality filtering, we computed alpha diversity, namely Observed Features, the Shannon index, and Faith's Phylogenetic Diversity and performed paired Wilcoxon signed-rank tests to determine differences between sampling timepoints. We identified microbial taxa by comparisons to the SILVA 138 database and used MaAsLin2 to determine differences in microbial abundance between sampling timepoints. For methodological details and references, see the supplementary material.

After 2 months of consuming the frugivorous diet, the dwarf lemurs' gut microbiomes showed a non-significant reduction in Observed Features (Wilcoxon signedrank test: W = -61, n = 28, p = 0.06; Fig. 1d), a significant reduction in the Shannon index (Wilcoxon signed-rank test: W = -85, n = 28, p = 0.005; Fig. 1e), and no significant difference in Phylogenetic Diversity (Wilcoxon signed-rank test: W = -33, n = 28, p = 0.33; Fig. 1f).

The dwarf lemurs' gut microbiomes were dominated by well-known bacterial phyla and genera (Fig. 1g). Six genera accounted for >5% of the gut microbiome



**Fig. 1** Dwarf lemur traits and gut microbiome features in response to seasonal frugivory. (**a**) Dwarf lemur at the Duke Lemur Center and patterns of host (**b**) body mass and (**c**) tail girth between sampling timepoints. Also shown are metrics of gut microbiome alpha diversity, namely (**d**) Observed Features, (**e**) Shannon index, and (**f**) Faith's Phylogenetic Diversity (PD) between sampling timepoints. Microbiome composition is visualized as (**g**) stacked bar charts of the taxa that accounted for >1% of the microbiome, on average, within one sampling timepoint, with colors reflecting phyla and shades reflecting distinct genera. Others, in white, includes the summation of all taxa that failed to reach the 1% cutoff. The major microbial genera, i.e., those that account for >5% of the gut microbiome on average within one sampling timepoint, are depicted in boxplots, namely (**h**) *Bacteroides*, (**i**) *Cerasicoccus*, (**j**) *Campylobacter*, (**k**) *Mycoplasma*, (**l**) *Escherichia-Shigella*, and (**m**) *Prevotella*. Across visualizations, the generalized diet is denoted by white circles; the frugivorous diet by black circles. ns p > 0.10; <sup>§</sup> $p \le 0.10$ ; <sup>\*\*</sup> $p \le 0.05$ ; <sup>\*\*\*</sup> $p \le 0.001$ .

when averaged across conspecifics within timepoints; all but one was differentially abundant between timepoints. *Bacteroides* (MaAsLin2: Coefficient = -0.82, n = 28, p = 0.011; Fig. 1h) and *Cerasicoccus* (MaAsLin2: Coefficient = -1.98, n = 28, p = 0.015; Fig. 1i) were more abundant before the diet change. *Campylobacter* (MaAsLin2: Coefficient = 1.39, n = 28, p = 0.028; Fig. 1j), *Mycoplasma* (MaAsLin2: Coefficient = 4.32, n = 28, p = 0.003; Fig. 1k), and *Escherichia-Shigella* (MaAsLin2: Coefficient = 2.83, n = 28, p < 0.001; Fig. 1l) were more abundant after the diet change. We found no difference in *Prevotella* abundance between timepoints

(MaAsLin2: Coefficient = 1.2, n = 28, p = 0.20; Fig. 1m). For full results of differential abundance testing, see the supplementary material.

We find that seasonal frugivory modulates host metabolism (Blanco *et al.*, 2022) and gut microbiome structure during the fattening season in dwarf lemurs. Frugivorous diets, consumed for 2 months prior to hibernation, enabled hosts to gain weight and fat reserves. Frugivorous diets also led to a reduction in microbiome diversity and increased *Mycoplasma*, results that match patterns from wild dwarf lemurs during fattening in Madagascar (Greene *et al.*, 2022). Despite the small scope of our study, we highlight that captive dwarf lemurs can be good proxies of their wild kin.

Whether the lemurs' gut microbes respond to host dietary intakes or facilitate fattening, as is seen in laboratory mice (Turnbaugh *et al.*, 2008), remains to be tested. If true, the gut microbiome would help lemurs prepare for hibernation, as it does in bears (Sommer *et al.*, 2016). Future studies could examine gut microbiome function, while measuring concurrent changes to dwarf lemur physiology and transcriptomes across species and conditions. This approach could pinpoint ubiquitous host-microbiome patterns inherent to the feast-fast cycle and perhaps to the evolution of primate hibernation.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10764-024-00477-9.

Acknowledgments The authors thank Laura Ellsaesser, Cathy Williams, Megan Davison, Cat Ostrowski, and Kay Welser for facilitating sample logistics. We thank Sarah Owens and Stephanie Greenwald from Argonne National Lab for sequencing data. Funding was provided by the NSF (DBI PRFB 1906416, to LKG), a Duke Microbiome Center Development Grant (to ADY), and DLC discretionary funds (to MBB). This is Duke Lemur Center publication #1601.

Author Contributions LKG and MBB incepted and designed the study, collected samples, and performed laboratory work in collaboration with ADY. AK and LKG performed bioinformatics and statistics and, with MBB, wrote the manuscript. All authors contributed to the final version.

Data Availability Raw sequence data are available online (NCBI SRA PRJNA1189778).

## Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

**Inclusion & Diversity Statement** One or more of the authors self-identifies as an underrepresented ethnic minority. One or more of the authors self-identifies as a member of the LGBTQ+ community.

## References

- Blanco, M. B., Greene, L. K., Ellsaesser, L. N., Schopler, B., Davison, M., Ostrowski, C., Klopfer, P. H., Fietz, J., & Ehmke, E. E. (2022). Of fruits and fats: High-sugar diets restore fatty acid profiles in the white adipose tissue of captive dwarf lemurs. *Proceedings of the Royal Society B*, 289, 1977.
- Dewar, R. E., & Richard, A. F. (2007). Evolution in the hypervariable environment of Madagascar. Proceedings of the National Academy of Sciences, 104, 13723–13727.
- Fietz, J., & Dausmann, K. H. (2006). Big is beautiful: Fat storage and hibernation as a strategy to cope with marked seasonality in the fat-tailed dwarf lemur (*Cheirogaleus medius*). In L. Gould & M. L. Sauther (Eds.), *Lemurs: Ecology and Adaptation* (pp. 97–110). Springer.

- Greene, L. K., Andriambeloson, J.-B., Rasoanavio, H. A., Yoder, A. D., & Blanco, M. B. (2022). Variation in gut microbiome structure across the annual hibernation cycle in a wild primate. *FEMS Microbiology Ecology*, 98, fiac070.
- Sommer, F., Ståhlman, M., Ilkayeva, O., Arnemo, J. M., Kindberg, J., Josefsson, J., Newgard, C. B., Fröbert, O., & Bäckhed, F. (2016). The gut microbiota modulates energy metabolism in the hibernating brown bear Ursus arctos. Cell Reports, 14, P1655-1661.
- Turnbaugh, P. J., Bäckhed, F., Fulton, L., & Gordon, J. I. (2008). Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe*, 3, 213–223.