# LIFE ON THE EDGE: NEW TOOLS TO TRACK FOREST-LEMUR INTERACTIONS

**Gemma Baker**<sup>1</sup>, Lydia Greene<sup>2,3</sup>, Kweku Afrifa<sup>4</sup>, Caitlin Allen<sup>1</sup>, James Bendle<sup>1</sup>, Sarah Greene<sup>1</sup>

<sup>1</sup>School of Geography, Earth and Environmental Sciences, University of Birmingham, UK. <sup>2</sup>The Duke Lemur Center, NC, USA. <sup>3</sup>Department of Biology, Duke University, NC, USA. <sup>4</sup>BioArCh, Department of Archaeology, University of York, UK

# INTRODUCTION

- Monitoring the foraging and dietary habits of lemurs is essential to their conservation, giving insight into the effects of continuing anthropogenic disturbances on Madagascar.
- Lemurs are essential to the maintenance and health of Malagasy forests, dispersing the seeds of fruiting trees, enabling pollination, and providing fertiliser and nutrient cycling. Diets are the key to assessing these interactions in greater detail.
- Therefore, complementary approaches are needed to fill the gaps in our understanding of lemur foraging habits and the interactions with their host forests. Is ORGANIC GEOCHEMISTRY the answer?

#### **PILOT SAMPLES from captive lemurs at Duke**

Lemur Center (DLC), NC, USA:  $\rightarrow$ 3 lemur species (8 per species):



Propithecus coquerel Lemur catta Coquerel's sifaka **Ringtail lemur** FOLIVORE GENERALIST

Faecal collection and

sample freeze-drying

Varecia variegata Red ruffed lemur FRUGIVORE



**Captive Enclosures** Natural Habitat Enclosures Smaller enclosures. Only Large, forested enclosures designed available food is to replicate the wild environment provisioned by the DLC. allowing foraging alongside

### LIPID BIOMARKERS

- Biomarkers are molecules indicative of biological life, they can be linked to specific organisms and/or biological processes.
- The molecules are simple and preserved in geological sediments for millions of years without being broken down. Therefore, they **Bacterial markers** will survive transport through the gastrointestinal  $\rightarrow$ Derived from the tract of a lemur. cell membranes of

n-fatty acids

#### Leaf wax markers $\rightarrow$ Produced by all higher plants, forming a waxy cuticle

*n*-alkanes

on the leaf surface



# METHODOLOGY: ORGANIC GEOCHEMISTRY

provisioned diets



Sample grinding and total lipid extraction by

organic solvents, centrifuge and ultrasonication

# **ECOSYSTEM SERVICES FROM**

## LEMURS

 $\rightarrow$  Seed dispersal of large fruiting trees:



n-Alkanes appear to reliably distinguish the **PROPORTION OF LEAF** 

MATTER in the diet given their high concentration in leaf versus fruit

*n*-Fatty acids appear to reliably distinguish a **RELIANCE ON FRUIT** 

MATTER in the diet, given that they are in significantly higher

Bacterial hopanoids show great promise. The presence of

methyldiploptene is INDICATIVE OF ANAEROBIC METHANOTROPHS in the

gut microbiome. This could be a result of something they consume that

is more fibrous than other dietary components: potentially dead wood,

bark or pine cones etc., these are documented fallback foods in the

3-OH FAs show promise as a way to identify **GEOPHAGY**. At present,

the only known way to identify this habit is by observation. Do they

**THEREFORE: organic geochemistry has a lot of promise to reconstruct** 

dietary profiles. It is possible to distinguish feeding strategies,

A selection of fruit seeds dispersed by two species of sympatric brown lemur week а two over observation period in a pristine forest.

Separation by **column** 

chromatography



cuticles.

wild.

Analysis by GC-MS and **GC-FID** 

DISCUSSION

concentrations in the cuticles of fruits than leaves.

mmhh

# RESULTS



#### **METHYLDIPLOPTENE**

 $\rightarrow$ Found in all **folivores**, and those generalists without Natural Habitat Enclosure access.

## preferences, and individual variation. These can be monitored to alert us to changes we may not notice from observation, which will inform on their relationship with the forest.

## FUTURE DIRECTIONS

partake in geophagy to enrich their gut microbiomes?

 $\rightarrow$ Completely **absent** in the **frugivores**.



A hopanoid: methyldiploptene

3-HYDROXY FATTY ACIDS (3-OH FAs)

 $\rightarrow$  3-OH FA faecal signatures show SOME overlap with soil 3-OH FAs.

 $\rightarrow$ From paired behavioural data, individuals who were observed to consume ~5% of soil in the diet fall into the overlapping section.

 $\rightarrow$ Expand dataset, including samples with paired direct dietary observation and DNA gut microbiome data, to further distil specific individual, seasonal/temporal, and habitat-specific dietary patterns from faecal biomarkers.

 $\rightarrow$  3-OH FA analysis of soil from DLC to compare to faecal 3-OH FAs, as signatures are highly regional. The identified trend may become stronger.

 $\rightarrow$ Compare faecal profiles to profiles of the original foodstuffs.

→ Apply compound-specific stable isotope analysis to infer trophic position  $(\delta^{15}N)$ , foraging patterns  $(\delta D)$  and crop raiding habits  $(\delta^{13}C)$ .

 $\rightarrow$ **EVENTUALLY**, apply and compare these datasets to wild populations.

#### References

Curtis, D.J. 2003. Diet and nutrition in wild mongoose lemurs (Eulemur mongoz) and their implications for the evolution of female dominance and small group size in lemurs. American Journal of Primatology, 124(3), 234-247. Fogel, A.T. 2015. The gut microbiome of wild lemurs: A comparison of sympatric Lemur catta and Propithecus verreauxi, 86, 85-95. Gaines, S.M. et al. 2008. Echoes of Life: What Fossil Molecules Reveal about Earth History. Oxford University Press: Oxford. Gill, F.L. et al. 2012. Lipid Analysis of Vertebrate Coprolites. Vertebrate Coprolites, 57, 93-98. Greene, L.K. et al. 2022. Forest access restores foraging and ranging behaviour in captive sifakas. Zoo Biology, 42(2), 209-222. Lara, I. et al. 2015. A focus on the biosynthesis and composition of cuticle in fruits. Journal of Agricultural and Food Chemistry, 63(16), 4005-4019. Nakamura, N. et al. 2011. Analysis of the hydrogenotrophic microbiota of wild and captive black howler monkeys (Alouatta pigra) in Palenque National Park, Mexico. American Journal of Primatology, 73(9), 909-919. Pearson, A. 2014. 12.11 Lipidomics for Geochemistry. Treatise on geochemistry, 291-336. Schwitzer, C. et al. 2014. Averting Lemur Extinctions amid Madagascar's Political Crisis. Science, 343, 842-843. Semel, B.P. et al. 2019. Assessing the function of geophagy in a Malagasy rain forest lemur. Biotropica, 51(5), 769-780. Suh, M.C. et al. 2005. Cuticular lipid composotion, surface structure and gene expression in Arabidopsis stem epidermis. Plant Physiology, 139(4), 1649-1665. Wang, C. et al. 2016. Impacts of pH and tem[erature on soil bacterial 3-hydroxy fatty acids: nent of novel terrestrial proxies. Organic Geochemistry, 94, 21-31. Wang, C. et al. 2021. Global calibration of novel 3-hydroxy fatty acid based temperature and pH proxies. Geochimica et Cosmochimica Acta, 302, 101-119.

