Fecal Composition and Its Relation to Diet

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INTRODUCTION

- Gelada monkeys (*Theropithecus gelada*) are graminivorous primates in Ethiopia's highlands. Their diet comprises grasses, as well as herbs, roots, and rarely, insects (Fashing et al., 2014).
- **Stable carbon and nitrogen isotope** ($\delta^{13}C$; $\delta^{15}N$) analysis is a widely applied, humane method for investigating the **diet** and **weaning** of live animals (Passey et al. 2004). In general, $\delta^{13}C$ reflects types of plants consumed, whereas $\delta^{15}N$ reflects trophic position, including weaning.
- It is not precisely known to what extent elemental and isotopic data from feces reflect foods **excreted,** vs. foods **used** for growth and energy.
- Independent, complementary lines of evidence into gelada diet composition that may help address this question include:

1. **Glycosyl composition analysis,** which shows types and abundances of carbohydrate residues in feces after digestion of larger molecules.
2. **Phenol sulfuric acid assays**, which measure total carbohydrate amount (Nielsen, 2010).

Figure 1. Theropithecus gelada in the Simien Mountains National Park in Ethiopia (www.simienpark.org).

Figure 2. Simien Mountains National Park, Ethiopia (www.simienpark.org).

PREVIOUS ISOTOPIC/ELEMENTAL RESEARCH

- If isotopic and elemental ratios of infant gelada feces reflect trophic position, as expected, then we would predict a **young infant** (Crymson, <1 mo) to have the **highest $\delta^{15}N$ ratio** due to suckling (high trophic position), and the **lowest carbon-to-nitrogen (CN) ratio** due to relatively fewer carbon-rich plants in the diet.
- We would expect an **older monkey** (Bucket, 19 mos) to be weaned and show **low $\delta^{15}N$** and **high CN ratios** from consumption of low trophic-level, high-carbon grasses.
- However, previous research shows a low $\delta^{15}N$ value for Crymson (**Fig 3**), contra one expectation, and a low CN ratio, consistent with the other expectation (**Fig 4**).
- The older individual, Bucket, shows an intermediate $\delta^{15}N$ value and a relatively high CN ratio (**Figs 3, 4**), consistent with expectations for a monkey being weaned.

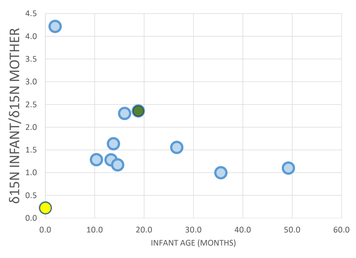


Figure 3. Each data point represents an infant's fecal $\delta^{15}N$ ratio divided by its mother's $\delta^{15}N$ ratio on paired sampling days. Crymson=yellow; Bucket=green.

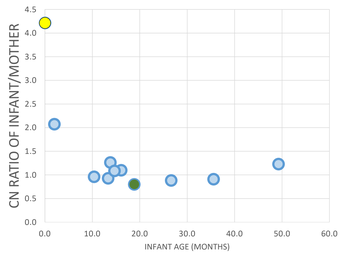


Figure 4. Each data point represents an infant's fecal CN ratio divided by its mother's CN ratio on paired sampling days. Crymson=yellow; Bucket=green.

RESEARCH QUESTIONS

- Does the low $\delta^{15}N$ value of Crymson (**Fig 3**) indicate she was weaned at <1 mo age?
- Does the intermediate $\delta^{15}N$ value of Bucket indicate he was still being weaned?
- Or, do $\delta^{15}N$ values reflect something other than weaning/diet? Stable isotope ratios also are sensitive to growth, stress, digestion, and metabolism.
- **We apply fecal carbohydrate analysis to further explore the relationship between diet, weaning, and elemental/isotopic data in the gelada.**

MATERIALS AND METHODS

- Fecal samples were collected and oven-dried in the Simien Mountains National Park in Ethiopia, 13.3064° N, 38.2641° E, in 2015-2016.
- Nine infant males and eighteen infant females aged between 0.03 and 44.38 months and their respective mothers are the subjects of this particular study.
- Samples were homogenized in a SPEX® liquid nitrogen freezer mill in the UGA Department of Anthropology Bioarcheology and Biochemistry Laboratory.
- 0.850-1.110 µg of sample were analyzed using a Costech® Elemental Analyzer coupled to a Finnigan MAT Delta Plus XL IRMS at the University of Georgia Center for Applied Isotope Studies.
- Additionally, two samples - Bucket (sampled on 2/16/16 at age 19 mos) and Crymson (sampled on 3/10/16 at age <1 mo) - were analyzed using a glycosyl composition analysis (gas chromatography and mass spectrometry) and phenol sulfuric assay using a Molecular Devices Spectra Max plate reader at the UGA Complex Carbohydrates Research Center.

CARBOHYDRATE ANALYSIS RESULTS

- **The two samples differ markedly in carbohydrate composition.** The fecal sample from older infant Bucket is **11.4%** carbohydrate compared to **61.1%** for young infant Crymson.

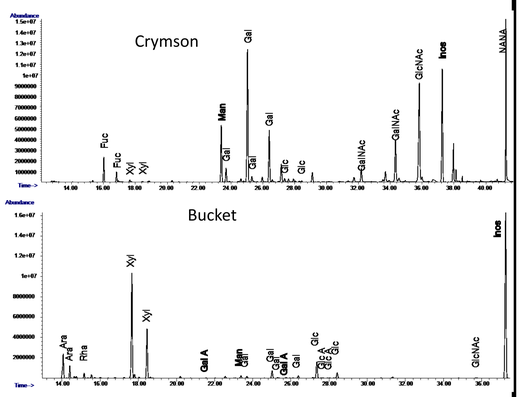


Figure 5. Glycosyl composition analysis of Crymson sample and Bucket sample: abundance of each glycosyl residue and the time when it was detected by the machine.

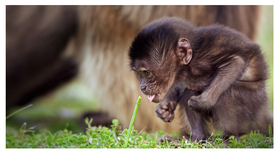
- The particular compounds also differ (Fig. 5, Table 1). The sample from Bucket contains **high amounts of arabinose and xylose**.
- The sample from **Crymson** contains **no arabinose and little xylose**, and contains relatively high amounts of **three of the five milk oligosaccharides** (Bode, 2012): fucose, galactose, and N-acetylglucosamine.

	Crymson	Bucket
Glycosyl residue:	**Mol %**	**Mol %**
Arabinose (Ara)	-	17.2
Rhamnose (Rha)	-	2.1
Xylose (Xyl)	0.5	65.6
Glucuronic Acid (GlcA)	-	0.3
Galacturonic acid (GalA)	-	0.7
Glucose (Glc)	0.8	8.9
Fucose (Fuc)	5.9	-
Mannose (Man)	6.9	0.9
Galactose (Gal)	31.1	4.0
N-Acetyl Galactosamine (GalNAc)	20.8	-
N-Acetyl Glucosamine (GlcNAc)	34.1	0.4

Table 1. Results from the glycosyl composition analysis showing carbohydrate composition of two gelada fecal samples (mole percent of each glycosyl residue out of 100).

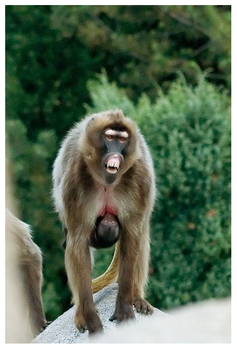
DISCUSSION AND CONCLUSIONS

- We set out to explore the relationship between three types of fecal evidence for gelada diet – isotopic, elemental, and carbohydrate.
- **Carbohydrate data suggest Crymson is almost exclusively nursed**, although minor amounts of xylose (0.5%) suggest she may be swallowing some plants already at <1 month old (see **Fig. 6**).
- **Carbohydrate data indicate Bucket's diet is almost entirely grass/plants**. The fact that Bucket's sample comprises galactose and mannose does not imply he was still nursed; mannose can be produced from glucose in the body; galactose is present in plants.
- Three lines of evidence suggest <1 mo old Crymson is suckling:
    1. Behavioral observation (Lu, pers. comm.)
    2. Fecal carbohydrate composition (this study)
    3. Fecal carbon/nitrogen elemental composition (this study)
- **In light of these carbohydrate data, we conclude that the low $\delta^{15}N$ value of Crymson is not a reflection of early weaning.** Instead, it may be related to rapid growth (Reitsema and Muir 2015). In rapidly growing infants, more nitrogen is used for tissue-building and less nitrogen is excreted. It is nitrogen excretion that causes fractionation responsible for the trophic effect.
- Complementary evidence from carbohydrate analysis of gelada feces suggests the CN ratio is a sensitive indicator of Crymson's suckling status (see also Lu et al., 2017; Reitsema et al., 2017).
- This study reveals the suckling "fingerprint" for geladas. In the future, this fingerprint could be monitored along with CN ratios to track weaning in geladas.



^Figure 6. Infant gelada exploring plant foods (Google Images)

< Figure 7. Grass-eating adult gelada (www.alltheworldsprimates.org)



>Figure 8. Nursing gelada infant (www.flickr.com, Volker Wurst)

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