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Reconstructing Diet from the Ground Up
Isotopic Dietary Ecology of Chimpanzees at Ngogo, Kibale National Park, Uganda

By

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Ph.D.

Anthropology

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Date

# Reconstructing Diet from the Ground Up
## Isotopic Dietary Ecology of Chimpanzees at Ngogo, Kibale National Park, Uganda

By

Bryce Andrew Carlson
B.S., University of Michigan, 2003

Advisor: John Kingston, Ph.D.

An abstract of
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James T. Laney School of Graduate Studies of Emory University
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Abstract

Reconstructing Diet from the Ground Up
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By Bryce Andrew Carlson

The central questions in biological anthropology relate to processes of acclimation, adaptation, and evolution that shaped and continue to shape our species in the face of an ever-changing environment. One of the most direct ways to access these questions is through dietary reconstruction. There is no more intimate relationship between an organism and its environment than through the resources it consumes. Plants and animals alike take their environment in to construct, maintain, or reproduce themselves. The geographical distribution, physical properties, and social significance of various dietary resources have played key roles in the evolution of morphology, social structure, foraging, reproductive behavior, life history, etc.

Of the numerous methods utilized for recovering our evolutionary relationships with food, dietary reconstruction via stable isotopic analyses is uniquely capable of quantifying the intake of both animal and plant based resources. To date, however, most applications remain limited in scope as a result of insufficient ecological data on stable isotopic variation and distribution within environments relevant for hominin evolution. This project seeks to lay such a framework for future analyses, to characterize the isotopic dietary ecology of a large bodied omnivorous primate in a $C_3$-dominated East African environment.

This project examines the dietary ecology of Ngogo chimpanzees (Kibale National Park, Uganda) with three interconnected analyses. The first investigates temporal variation in nutrient composition and foraging behavior. The second characterizes dietary ecology from a stable isotopic perspective and establishes an interpretive framework for previous and future dietary reconstructions from extant, as well as extinct, primates. The third analysis investigates whether foods are isotopically heterogeneous, or specifically, whether the highly digestible macronutrient fraction is isotopically equivalent to the less digestible neutral detergent fiber fraction.

These three analyses engage chimpanzee dietary ecology as a means for investigating our evolving relationships with food in the near and distant past, and represent novel contributions including (1) the report of diurnal nutrient variation associated with chimpanzee foraging behavior, (2) the isotopic characterization of a chimpanzee dietary niche within a closed canopy East African forest, and (3) the first fiber fraction isotopic analysis of the diet for any primate.

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# Chapter 1

# INTRODUCTION

A number of the central questions in biological anthropology relate to our origins as the human lineage and the course of our evolution. They relate both to variability and universals observed within our species today, and to the processes of acclimation, adaptation, and evolution that shaped and continue to shape our species in the face of an ever-changing environment.

One of the most direct ways to access these questions related to our evolution is through dietary reconstruction. There is no more intimate relationship between an organism and its environment than through the resources it consumes. Plants and animals alike take their environment in to construct, maintain, or reproduce themselves. The distribution, physical properties, and social significance of various dietary resources have played key roles in the evolution of morphology, social structure, foraging, reproductive behavior, life history, etc.

Our literature is populated with hypotheses that explain our origins and evolution in dietary terms. Examples include the Expensive-Tissue Hypothesis (Aiello and Wheeler 1995), the Grandmothering Hypothesis (O'Connell et al. 1999), the Home Base or Central Place Foraging Hypotheses (Bunn 1986; Isaac 1983), life history models (Kaplan et al. 2000), the aquatic diet constraint hypothesis (Broadhurst et al. 1998; Carlson and Kingston 2007), as well as hunting and scavenging hypotheses (Domínguez-Rodrigo 2002).

These hypotheses are constructed at the level of specific nutrients, food types, or characteristics of the whole diet as evolutionary constraints and selective pressures. A nutrient may constrain the selection of a given trait if it is limiting within the diet and essential for the development or function of that trait. Dietary components may also act as agents of adaptive change, influencing the evolution of specific anatomical, physiological, life history, and behavioral traits.

*Homo sapiens* evolved primarily within a foraging context, and later in association with agriculture and industrial food production and manufacture. Our species is still acclimating, adapting, and evolving today within a world subsisting increasingly upon the products of industrial agriculture, and the legacies of our foraging and early agricultural past remain with us (for better or worse). To understand how organisms acclimate to dietary change in the short term, adapt behaviorally and genetically over generations, and evolve as a species in the longer term, we gain much by investigating these processes as recoverable within the archeological and paleontological record.

Our species' evolutionary relationship with food has relevance for modern health and disease, digestive metabolism and physiology, disordered eating behavior, sustainable food production and consumption, and trade relationships between geopolitical bodies.

Over the past few decades several hypotheses have attempted to explain the discordance between our modern environment, diet, and patterns of chronic disease from an evolutionary perspective: Thrifty Genotype (Neel 1962; Neel et al. 1998), Thrifty Phenotype (Hales and Barker 1992; Hales and Barker 2001), Non-Thrifty Genotype (Allen and Cheer 1996), and the Thrifty Epigenotype (Stoger 2008). Each attempts to explain current patterns of morbidity and mortality in evolutionary terms, but also struggles to fully account for the heterogeneous experience of metabolic disease today.

The rising global demand for meat, and it's underlying social and cultural significance, is at least partly connected with our over 5 million year history of omnivory, over 2 million year history of butchery and the role those resources played within the context of a foraging niche.

To understand the evolutionary legacy of the perceptions of taste and the social/cultural relevance of certain foods one needs to track the history and prehistory of consumptive behavior. The generation of hypotheses relating food and evolution is important, and testing those hypotheses remains essential and difficult. We need to identify when and under what conditions particular resources were utilized, who gained access (e.g. males and/or females, adolescents and/or adults), the relative significance within the overall dietary niche (e.g. seasonal, fallback, preferred, staple), and how that significance changed over time and within different environmental conditions.

Paleoanthropologists have utilized several methodological approaches to examine consumptive behavior across the hominin lineage including stable isotopic analyses (Lee-Thorp et al. 2003), trace element analyses (Burton 1996; Burton and Wright 1995), faunal bone assemblages with morphological (Blumenschine and Pobiner 2007; Dominguez-Rodrigo 1997; Marean and Kim 1998; Monahan 1996) or distributional modification (Bunn 1991; Dominquez-Rodrigo and Pickering 2003), as well as dental functional morphology (Ungar 2004), and macro- and microwear of the teeth (Lalueza et al. 1996; Mahoney 2006; Ungar 1998). Despite research efforts over the last several decades, anthropologists today are only marginally closer to understanding *who* was eating *how much* and *when*. And while the consumption of animal flesh has received most attention, reconstructing the relative and absolute contribution of plant source foods to the ancestral hominin dietary niche remains a significant challenge.

Dietary reconstructions from stable isotopic analyses are uniquely capable of testing *how much*, and directly quantify the intake of both animal and plant based resources. Ratios of $^{13}C/^{12}C$ ($\delta^{13}C$) and $^{15}N/^{14}N$ ($\delta^{15}N$) are known to vary predictably by dietary intake (Ambrose and DeNiro 1986), geography and climate (Codron et al. 2005; Kohn 2010). Sponheimer and Lee-Thorp (1994; 1999; 2003; 2006) have contributed to our understanding of the diets of *Australopithecus africanus, Paranthropus robustus*, and early *Homo* utilizing stable isotopic analyses of $\delta^{13}C$ within fossilized tooth enamel. These authors report an isotopic signal intermediate between $C_3$ browsers and $C_4$ grazers for these South African hominin taxa, indicating some contribution to the diet of $C_4$ plant material or butchery of $C_4$ consumers (Lee-Thorp et al. 1994). This is a pattern unlike most living primates, leading to debate over the origins of such a signal (Peters and Vogel 2005). Some speculate significant contributions of underground storage organs (Yeakel et al. 2007) or termites (Peters and Vogel 2005). A more recent application to *Paranthropus boisei* in East Africa (Cerling et al. 2011; van der Merwe et al. 2008) have documented $C_4$ rich diets for *P. boisei* and been interpreted as suggestive of papyrus or sedge consumption.

While providing valuable insight into early hominin diets, these examples of stable isotopic contributions to paleodietary reconstruction reveal the interpretive limitations of current methods. Most appear limited to coarse distinctions between dietary inputs of $C_3$ vs. $C_4$ resources. And as is the case with interpretation of the australopith isotopic data, overlap between dietary components (e.g. termites, $C_4$ grasses, $C_4$ tubers, and meat from $C_4$ grazers) severely confounds interpretation (Cerling et al. 2011; Lee-Thorp et al. 2003; Peters and Vogel 2005; van der Merwe et al. 2008).

The ability to identify consumptive behavior via isotopic analyses requires underlying variability in the isotopic composition of the diet. One can only reconstruct

aspects of the dietary ecology that are isotopically distinct. Variables of interest may include the contributions of different food types (e.g. fruit, leaves, seeds, etc.), and/or how those contributions change by season or time of day. To date, most isotopic analyses have focused on the large isotopic differentiation between the consumption of plant resources utilizing the $C_3$ and $C_4$ photosynthetic pathways, which may differ by 10‰ or more. Less research, however, has examined the isotopic variability within a predominantly $C_3$ ecosystem, which dominated much of early hominin evolution. While $C_3$ and $C_4$ resources differ significantly, the range of $\delta^{13}C$ within $C_3$ environments can be just as great. Should those resources vary systematically by species, food type, season, elevation or other ecological attribute, the differential consumption of those foods, or foods with those attributes, may be reconstructed from tissue analysis of the consumer.

This project seeks to quantify the underlying isotopic variability within a rich $C_3$ ecosystem with geographic and ecological significance for the evolution of the hominin lineage. To investigate whether food type, plant species, seasonality, elevation, or height within the canopy may carry a unique isotopic signature potentially recoverable from assimilated tissue. This project additionally seeks to address whether the nutrient and non-nutrient constituents of individual foodstuffs vary isotopically. In summary, these analyses define an ecological template upon which to interpret previous and future dietary reconstructive analyses from similar environments today as well as the near and distant past.

## Site and Sample Identification

To advance methods of dietary reconstruction as outlined above, access to a rich $C_3$ environment supporting at least one species of large bodied primate was necessary. Several sites within Uganda were identified as potentially appropriate within this framework, including Kalinzu-Maramagambo Forest Reserves/Queen Elizabeth Park, Bwindi Impenetrable National Park, Kibale National Park, Budongo Forest Reserve, and Murchison Falls Park Conservation Area. Given time and funding limitations, this project focused on Kibale National Park where extant chimpanzees, baboons and several smaller species of monkey are found. Permission and support was extended from the directors of the Ngogo Chimpanzee Project (John Mitani and David Watts), and the project commenced there in November 2009 with the financial support of the National Science Foundation, the Leakey Foundation, and the administrative support of the Ugandan Wildlife Authority, Ugandan National Council for Science and Technology, and the United States Department of Agriculture.

## Ngogo, Kibale National Park, Uganda

The Ngogo chimpanzees (Kibale National Park, Uganda) live in proximity of the Western Great Rift Valley, nearly 24 km east of the Rwenzori Mountains. This area has been described as a mosaic of forest types, characterized variously as moist evergreen forest, moist montane, lowland tropical rain forest, and mixed tropical deciduous forest (Struhsaker 1975). The location between Central and East Africa has led to a $C_3$ dominated environment with a uniquely large mammalian faunal assemblage (Table 1.1).

Although humans have occupied areas of the Ngogo territory in the recent past, however, the ecology remains largely undisturbed. The territory is characterized as a

closed canopy forested environment, with open grassy patches the chimpanzees regularly cross to exploit additional resources, patrol their territorial borders, or hunt for monkeys. The Ngogo chimpanzee community is the largest observed in equatorial Africa numbering approximately 150, and has been under observation by John Mitani and David Watts (and students and colleagues) since 1995. Most males are now well habituated and females are becoming increasingly so.

## Dietary Collection

Over approximately 4 months of collection, split between one wet season and one dry season, I collected approximately 350 samples from 40 plant species, constituting 60 specific dietary items, and representative of approximately 91% of the chimpanzee dietary niche, as measured by observed feeding time (rank list compiled by David Watts, personal communication). Many of these resources are also consumed by sympatric primate species including red-tail monkeys, baboons, red- and black and white colobus, mangabey, and L'Hoest monkeys. For each food item I collected samples from at least 5 different trees or vegetation patches within the Ngogo range to account for within-species variability. Fruit is available only seasonally and so most of those samples were collected in one season or the other, but rarely both. Leaves from saplings and herbaceous vegetation, on the other hand, are consumed year round and so were sampled in both wet and dry seasons. Samples were collected in the field between approximately 7am and 2pm, and immediately cut up and dried for several hours over relatively low temperatures in a Coleman camp oven positioned over a kerosene stove.

Plant components of the Ngogo chimpanzee diet were defined broadly and collected opportunistically. A list of consumed species was created from several sources including published literature, unpublished observational data (David Watts, personal communication), the experience of local field assistants, and personal observation. Identification of each species proceeded over months of study, and assistance from local field guides and camp staff.

To look broadly at the chimpanzee dietary niche the plant sampling protocol for included approximately 91% of the diet. Chimpanzees (like many primates) consume a large number of resources within their environment. A list of species consumed over a 1-year period might include over one hundred different items, but over 60% of the diet is defined by only about 10 different species, and nearly 85% defined by approximately 20 species. In addition to coverage of the chimp dietary niche, sampling of additional plants was conducted to assess potential inter-anatomical differences both within and across species. For example, is the isotopic signal for "fruit" unique from "flowers"? Therefore, some flowers, seeds, and fruits were collected despite only rare consumption by the chimpanzees.

The collection protocol began in December 2009 following direct observation of the Ngogo chimpanzees, and continued for approximately 3 weeks. The second round of collection occurred over the course of a few weeks between June and July 2010. Fruit was selected from the ground that appeared (1) the appropriate stage of ripeness, and contained (2) no bite marks, (3) no insect damage, and (4) no mold. Leaves of saplings were sampled upon encounter from patches exploited by the chimpanzees, but not necessarily the same sapling. Leaves of mature trees were sampled from the ground and preference was given to less mature leaves as they appear most consumed by the

chimpanzees. Leaf buds were selected along with leaves of mature trees, from the ground and when available. The pith of herbaceous vegetation was sampled selectively from patches of relatively large specimens (selective criteria of the chimpanzees). The outer husk was peeled away and the fleshy inner pith sampled preferentially from the lowest sections of the plant (lowest 6-12 inches). Flowers were sampled opportunistically upon encounter following similar principles to fruit selection. Cambium, roots and dead wood were likewise sampled opportunistically.

To ensure sufficient sampling coverage, the above protocol eventually shifted from collection following direct observation of chimpanzee consumption, to searching the Ngogo range for previously identified but unsampled foods. For each food species, every effort was made to sample widely across the Ngogo range to capture potential geographic variation in nutritional or isotopic characteristics. Each sample was labeled with its identification, date, time, location collected (nearest trail intersection and GPS coordinate), as well as the relative stage of development (e.g. ripeness of fruits, or maturity of leaves).

Samples were dried within hours of collection at 150-170°F for approximately 3.5 hours or until completely desiccated within a small oven. A kerosene fueled backpacking stove was positioned under a Coleman camping oven, and samples transferred only after the stove burned cleanly and the oven reached the appropriate temperature (approximately 10-20 minutes). This was done to minimize exposure of samples to carbon contamination from kerosene soot and ensure uniform conditions for each batch of samples. Leaves typically dried in 2-3 hours while fruit often took 3-4 hours. Drying time and temperature were determined to minimally impact nutrient composition (low temperatures) in the least amount of time required to prevent molding.

## Analyses

All samples for isotopic analyses were processed in the Biogeochemical Anthropology Laboratory, Emory University. These facilities include the necessary equipment to prepare material for nutrient analysis, liquid chromatography, gas chromatography, and mass spectroscopy. For bulk and neutral detergent fiber isotopic analyses, working collaborations for use of additional facilities were established with the Analytical Chemistry Laboratory, Odum School of Ecology, University of Georgia (Thomas Maddox). For nutritional analyses and fiber extraction, a collaboration was established with Jessica Rothman and the Primate Nutritional Ecology Laboratory at Hunter College of CUNY.

## Component Chapters

The first content chapter (Chapter 2), entitled "Dietary Ecology: Diurnal nutrient cycling among young saplings of *Pterygota mildbraedii* and *Celtis africana*" examines an often under appreciated aspect of dietary ecology: time. Chimpanzees are repeatedly observed to consume sapling leaves and herbaceous vegetation (pith) preferentially in the early to late afternoon. Plants utilize photosynthesis to sequester carbon during the day in the form of sucrose, which is then mobilized at night to maintain metabolic processes in the absence of photosynthetic activity. As such, nutritional content of these young leaves was hypothesized to contain the greatest concentration of nutrients at the end of the light cycle (early evening). The most sensitive nutrient to light/dark flux should be sucrose as

that is the direct product of photosynthesis. But, I also expected lower amplitude cycling in the concentrations of protein and structural carbohydrate. While the effect size may differ by species, as the fundamentals of photosynthesis should be identical between all $C_3$ plants, I expected little to no difference between different species.

This study measured the nutrient composition of two commonly consumed sapling leaves within the chimpanzee dietary niche at three time points over the course of the day: shortly after sunrise, around midday, and again in late evening. This analysis sought to examine whether preferential consumption correlates with nutrient content, digestibility, or other attributes of dietary quality resulting from photosynthetic activity and nutrient accumulation throughout the light period.

The second content chapter (Chapter 3), entitled "Isotopic Dietary Ecology: Bulk $\delta^{13}C$ and $\delta^{15}N$ characterization of the chimpanzee dietary niche," defines a bulk isotopic template for a closed canopy $C_3$ dietary niche in eastern equatorial Africa. Previous studies have revealed significant differences in $\delta^{13}C$ attributable to height within the forest canopy (Cerling et al. 2004; Schoeninger et al. 1998). In general, greater geochemical or biological cycling of molecules within a system leads to greater isotopic fractionation and ultimately differentiation of isotopic composition. Plant resources along the ground are more depleted in $^{13}C$ as a result of utilizing highly recycled $CO_2$ available at the forest floor. Similarly, different species of plants (within a predominantly $C_3$ or $C_4$ environment) as well as the different anatomical components of a single species (ie. leaves, fruits, seeds, etc) undergo unique metabolic processes that utilize carbon and nitrogenous compounds that have been more or less recycled and should therefore appear isotopically distinct. Yet, to date, no such differences have been found (Codron et al. 2005).

In this study, over 300 samples were collected between both dry and wet seasons representing over 40 taxa and 60 distinct food items. Seasonality, elevation, food type, species, height within the canopy, and location within community range were all assessed as independent variables within a model to characterize bulk $\delta^{13}C$ and $\delta^{15}N$ variability. Following previous studies on $C_3$ isotopic ecology (Kohn 2010), the values of $\delta^{13}C$ are likely to vary significantly, with $\delta^{15}N$ less so. The central questions within this chapter are which variables are most and least responsible for this variability and to what extent the differences observed can be utilized to reconstruct (or control for) those attributes within the organism's consumptive history.

The third and final content chapter (Chapter 4), entitled "Compound Specific Isotopic Dietary Ecology: Neutral detergent fiber $\delta^{13}C$ within the chimpanzee dietary niche," examines the isotopic fractionation between the highly digestible macronutrients (non-structural carbohydrates, proteins and lipids) and less digestible fiber fractions (structural carbohydrates). Feeding trials from a number of mammalian species have revealed variable, but similar, isotopic fractionation from the diet to tissue. Such fractionation may occur at the level of digestion, through metabolic processing, or assimilation within the tissue. Assessing the isotopic composition of digestible nutrients $\delta^{13}C$ (e.g. from non-structural carbohydrate, lipids and protein) relative to less digestible non-nutrient $\delta^{13}C$ (e.g. fiber fractions) is an essential first step in uncovering the mechanisms of diet-to-tissue fractionation, and the variability in that process may allow further refinement of isotopic analyses for uncovering metabolic physiology and pathology.

Neutral detergent fibers (NDF) were extracted from 23 of the most common foods in the Ngogo chimpanzee dietary niche and analyzed via isotope ratio mass spectroscopy for $\delta^{13}C$. Neutral detergent fiber fractions were then compared to the bulk isotopic signature from each sample to assess whether the less digestible component of each food differs significantly from the more digestible components. Fractionation from bulk to NDF was examined using pairwise comparison and ANOVA to determine whether food type, species, or other ecological attributes may variously affect the fractionation.

As all nutrients are constructed from distinct metabolic processes, and each reaction therein is an opportunity for isotopic fractionation, I expect to find some level of fractionation isotopic distinction between the bulk sample and NDF fraction. The nature of this fractionation and the variability between species or food types will go far to advance our understanding of digestive fractionation and dietary reconstruction via stable isotopic methods.

Finally, the chapter entitled "Discussion and future direction", examines the significance of each chapter in advancing the dietary reconstruction of modern primates and extinct hominins via stable isotopic methods. Therein, the results from each chapter are addressed as well as the potential for compound specific isotopic analyses, in particular, to allow the reconstruction of individual food items and life history variables. The future of this emerging field is rich, and the final chapter of this dissertation concludes by outlining a research program capable of reaching that potential.

Table 1.1

Large mammalian fauna of Kibale National Park, Uganda

| Non-primate taxa | Primate taxa |
|---|---|
| Elephant | Red colobus |
| Bushbuck | Black & white colobus |
| Red duiker | Blue monkey |
| Blue duiker | Redtail monkey |
| Giant forest hog | L'Hoesti monkey |
| Buffalo | Grey cheaked mangabey |
| | Baboon |
| | Chimpanzee |

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# Chapter 2

# DIETARY ECOLOGY: DIURNAL NUTRIENT CYCLING AMONG YOUNG SAPLINGS OF *Pterygota mildbraedii* AND *Celtis africana*

## Introduction

Primatologists have often observed and reported diurnal variation in the consumptive behavior of wild chimpanzees, with ripe fruit most commonly consumed in the morning and early afternoon and young leaves in the afternoon or early evening. Plant biologists have also recognized that the processes of photosynthesis, translocation and storage result in nutrient cycling associated with light and dark periods (Hodges 1967; Kokubun and Shimada 1994; Kramer and Kozlowski 1979).

The primary end products of photosynthesis are sucrose and starch. These carbohydrates are synthesized, pooled and transported as sucrose, and variably stored as starch or structural carbohydrates such as cellulose and lignin (Bazzaz 1997). As products of photosynthesis, the concentration of carbohydrates within the leaves and phloem sap fluctuate diurnally in association with light intensity (Huber 1981).

The principal source of nitrogen for protein synthesis in forest trees is derived from soil microorganisms and absorbed through the root system in the form of ammonium and nitrate. The translocation of nitrogen from roots to leaves occurs within the xylem sap and is dependant upon leaf transpiration rate. Nitrate concentration within xylem sap has been shown to reach a peak around midday (Siebrecht et al. 2003),

and suggests a mechanism by which protein concentration within the leaves may also vary over the course of the light cycle.

Despite this evidence suggesting diurnal nutrient cycling of both carbon and nitrogenous compounds, little work to date has specifically addressed this source of nutrient variation within the context of primate dietary ecology. Frugivorous primates often make use of color, texture and taste to determine ripeness of fruits and preferentially consume those resources when the relative quality and digestibility is high (ie. ripe). These relationships have been reported for various resources over the course of days and weeks, as foods mature or ripen. However, those mechanisms that allow primates to sense nutrient quality over the course of a plants development (e.g. maturing leaves and ripening fruits), should operate on a much shorter temporal scale as well.

Two commonly consumed species of young sapling leaves within the Ngogo chimpanzee dietary niche (*Pterygota mildbraedii* and *Celtis africana)* show preferential utilization in the afternoon and early evening. If nutrient content increases throughout the day as a result of accumulated photosynthetic products and/or translocated nitrogenous compounds to the leaves, as plant biologists have recognized, the utilization of these two species of saplings would occur when they are of a higher nutrient quality and/or digestibility.

This analysis sought to examine whether this preferential consumption (over the course of 24 hours) would correlate with nutrient content, digestibility, or other attributes of dietary quality resulting from photosynthetic activity and nutrient accumulation throughout the light period.

## Methods

Young leaves were collected from five saplings of *Pterygota mildbraedii* and *Celtis africana* at dawn, midday, and dusk towards the end of the dry season in late-July and early August. The distribution of these trees within the dietary niche affects how they are consumed and therefore how they were sampled for this study.

*Pterygota mildbraedii* often begins its life cycle in patches of thin low growing saplings with few branches and very large leaves (up to 30x18cm across). Any given sapling contains only a handful of leaves of the appropriate stage of maturity (young) for chimpanzee consumption, and thus a feeding bout often includes leaves from multiple neighboring saplings. As such, *Pterygota* leaves for this analysis were similarly collected. On July 21st 2011, young leaves were collected from five individual saplings between 8:15a and 8:40a, again between 1:15p and 1:25p, and finally from 6:20p and 6:30p. Samples were collected from the same patch at each time point, but no two samples originated from the same sapling.

The *Pterygota* patch was found near C/7 of the Ngogo trail system within a habitat best characterized as closed canopy with a relatively open understory, and moderate light passing to the forest floor. The sky was overcast from sunrise until approximately 10:30a, partly cloudy from 10:30-12:30p, with light rain from 12:30-2:00p and overcast skies remaining for the rest of the afternoon and evening.

*Celtis africana,* unlike *Pterygota*, is less clustered in distribution. Saplings often grow solitarily with many thin branches and many small leaves (from 2x1cm to 4x7cm across). Their distribution and prolific production of leaves result in chimpanzees foraging on a single sapling before moving on to another dietary resource or activity. Therefore, for this study, five different *Celtis* saplings were identified, flagged, and sampled at each of the

three time points: 7:30am, 1:00p, and 6:30p. Each *Celtis* sapling was found along the Grassland Trail between 5.5 (just east) and 6.5 (just north), and collected on a sunny August 5th 2011. This area is characterized as forest margin with a relatively low canopy, thick undergrowth, and patchy sunlight exposure on the forest floor. Leaves from each sapling were selected across all branches, so each sample (morning, midday, evening) would have similarly aged and positioned leaves. There was also a selection bias at each time point for younger and smaller leaves in accordance with chimpanzee preference.

Both *Celtis* and *Pterygota* samples were immediately returned to camp and oven dried at 150° F for approximately 200 minutes. To control for cross contamination, samples were separated within the drying oven by an aluminum foil partition, transported one by one and labeled accordingly at each stage of the process. Following drying, each sample was transferred to a 1-quart Ziploc freezer bag.

Nutrient analyses proceeded in collaboration with the Primate Nutritional Ecology Laboratory (Jessica Rothman) at Hunter College of the City University of New York (see Appendix for analytical protocol). Sugars analyses followed previously published methods by Hall et al (Hall et al. 1999). Neutral detergent fibers (NDF), acid detergent fibers (ADF), acid detergent lignin (ADL), crude protein, and simple sugars were selected for investigation as they constitute most of the dry mass within leaves and their hypothesized relationship with photosynthetic processes and nutrient accumulation (e.g. simple sugars) was clear. Lipids were excluded from this analysis as their composition within leaf structures is often less than 1% of total dry weight (Kramer and Kozlowski 1979), and sufficient sample was unavailable.

## Results

Each species exhibited diurnal variation in some nutritional constituents and not others (Table 2.1). Given that each displayed different patterns of variation, they are reported separately below.

*Pterygota mildbraedii*

Five samples of leaves were collected from a single patch of *Pterygota mildbraedii* samplings at three time points over the course of the day for a total of 15 samples (Table 2.1). Mean values of all three fiber fractions (NDF, ADF, and ADL) decreased over time (Figures 2.1, 2.2 and 2.3). Neutral detergent fiber remained constant through midday before decreasing in the early evening, whereas ADF and ADL decreased linearly with increasing length of the photoperiod. The relationship between these three fiber fractions and time of day was both strongly correlated and statistically significant (Spearmans r = -0.6992, -0.8315, and -0.812 for NDF, ADF, and ADL at $p=0.0071$, $<0.0001$, and 0.0003 respectively). These shifts are likely dietarily significant for browsers and omnivorous primates alike as the decreasing concentrations of NDF, ADF and ADL represent shifts between 10 and 33% over the course of the light cycle towards increased digestibility.

In contrast to mean fiber fraction concentrations, crude protein and sugars were not significantly correlated with time of day in *Pterygota* (Table 2.1). While crude protein remained stable, variance decreased by nearly three-fold throughout the day. Given the small sample size, this relationship may well be an artifact of sampling.

*Celtis africana*

Five *Celtis africana* saplings were repeat-sampled at each of three time points throughout the day, for a total of 15 samples. Unlike *Pterygota*, mean concentrations of ADF and ADL did not significantly change across time period collected. After controlling for inter-sapling variability via repeat-measures one-way ANOVA, time of day resulted in a small but statistically significant effect for NDF but not for ADF or ADL (Table 2.1).

Mean sugars concentration did show a significant correlation with time of day and cumulative exposure to light, with late afternoon concentrations nearly double that in the early morning (Table 2.1, Figure 2.4). The correlation between concentration and time of sampling was statistically significant ($p=0.0160$) and after controlling for inter-sapling variability nearly so ($p=0.0737$).

## Discussion

The nutritional composition of both sapling species increased in quality or digestibility throughout the day. In *Celtis africana,* sugars became increasingly concentrated, while most fiber fractions and total protein remained constant (increasing quality). Alternatively, the concentration of all fiber fractions (NDF, ADF, and ADL) within the sapling leaves of *Pterygota mildbraedii* decreased throughout the day, while sugars and protein remained constant (increasing digestibility). Quality and digestibility are both preferable dietary attributes resulting in greater nutritional or energetic return. That both saplings exhibited increasing quality or digestibility, consistent with preferential

consumption, conforms to the initial hypothesis and previous literature. Not anticipated, however, was that each would vary nutritionally in different ways.

While all plants synthesize sucrose within their leaves from water and carbon dioxide, the storage and ultimate transport of that carbon occurs differently across plant taxa. Some species pool carbon as sucrose within the leaves and transport it directly for utilization by other parts of the plant. Others store sucrose within the leaves as starch (Huber 1989) prior to future utilization. These variable mechanisms may explain the difference observed in sugar accumulation over time between these two species. If *Celtis* partitions it's photosynthate differentially toward sucrose accumulation, while *Pterygota* does not, we might expect the pattern observed here with respect to diurnal accumulation in sucrose.

However, the decreasing fiber concentrations within leaves of *Pterygota* throughout the day may not be explained by differential partitioning. If total structural carbohydrate (fiber fractions) remains constant throughout the photoperiod, but sugars or starch increase, the percentage of mass taken up by structural carbohydrate should decrease throughout the day. That structural carbohydrate fractions do not decrease throughout the day in *Celtis* is curious given increases in sugars. There may be a compensatory decrease in some other unmeasured component, or the total structural carbohydrate may be increasing throughout the photoperiod (i.e. leaf growth).

These results were significant and interesting, but much work remains to elucidate how the nutritive profile of these dietary resources changes throughout the day. To examine the effects noted above for sugars, starch and structural carbohydrate, future studies must take care to analyze the contributions of carbohydrate fractions as well as other nutritive and non-nutritive components (ash, lipids, etc.). The sample size of this

study was fairly small, and as evidenced by the sugars concentrations within *Celtis* leaves, there may be important effects requiring greater sample size to reach statistical significance.

In addition to sapling leaves, future work should include a greater number of dietary items (food types and species) collected across different lighting conditions (e.g. sunny vs. overcast, canopy filtering, etc), soil types, and degree of water stress. Among species of herbaceous vegetation, for example, the pith consists of both xylem and phloem channels containing the sugars and other nutrients fixed in the leaves or the roots and shuttled throughout the plant. One might expect significant diurnal variation within these resources as nutrient concentrations likely represent the current metabolic activity of the plant, which is strongly affected by photosynthetic activity and transpiration from the leaves.

As resource sinks, fruits, seeds and underground storage organs may show some diurnal variation. However, given the quantity of nutrients already stored relative to those accumulated over the course of any given day, such diurnal variation may be insignificant or below physiological detection for the consuming omnivorous primate.

This study is one of the first to show the nutritional profile of resources within a chimpanzee dietary niche vary diurnally, and that time of consumption is correlated with greater quality (*Celtis africana*) and digestibility (*Pterygota mildbraedii*). As frugivorous primates are able to track fruit quality using visual cues (e.g. color, size), so too perhaps are they able to track the quality of other resources using taste or other senses as well. There are a number of other factors that contribute to resource utilization aside from nutrient content or energetic return. For highly social species, intra-group dynamics much also be considered.

Among the Ngogo chimpanzees, much time in the early morning through midday hours are spent in large fruit trees. Preferential consumption of fruit in the early hours of the day may be most significantly related to energetic return or other nutritional priorities. But the fruit trees also allow for group feeding and therein the fusion of individual chimpanzee groups and the re-establishment of social bonds. Sapling consumption, alternatively, occurs mostly in the afternoon when individuals come out of the trees and move about or in small groups. Both social dynamics and nutritional characteristics may help explain the differential timing of resource consumption (fruit in morning, saplings in the afternoon), so future work must include observation and analysis of both dynamics to tease association from causation.

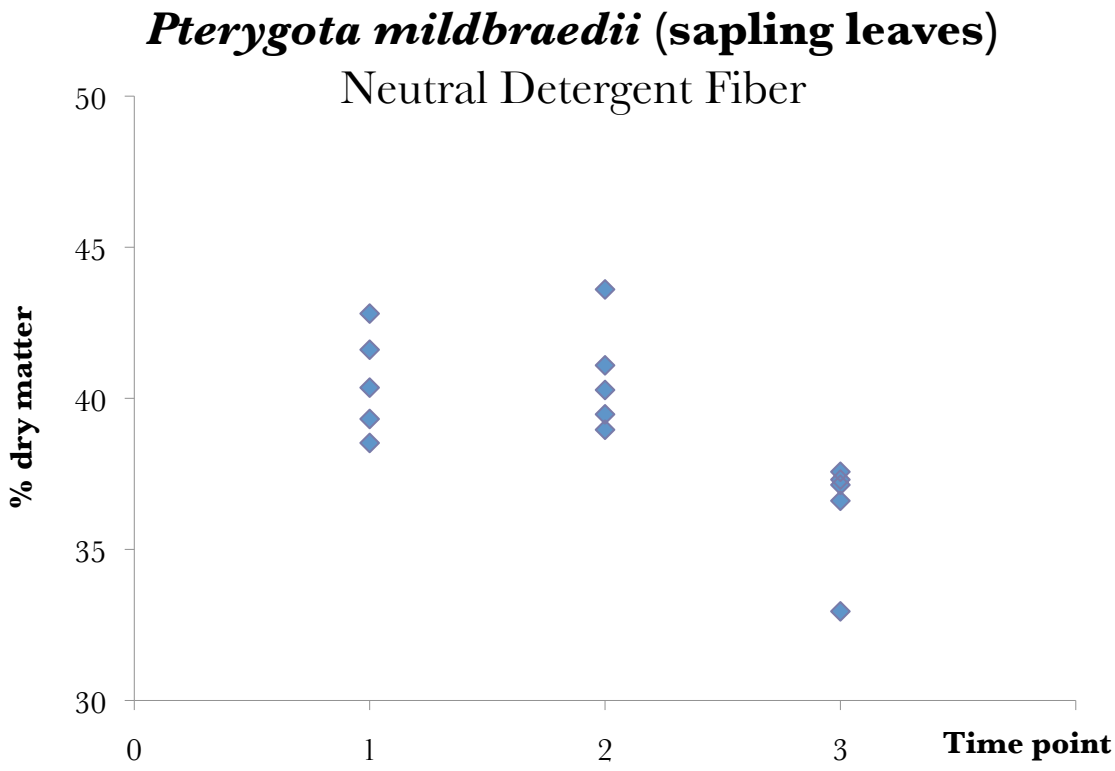
## Conclusion

Sapling leaves represent one dietary resource for Ngogo chimpanzees preferentially consumed between midday and early evening. Research on plant physiology also indicates nutrient accumulation occurs within leaves in the form of starch and sugars throughout the light cycle.

Two species of sapling leaves commonly consumed within the Ngogo chimpanzee diet were studied here and shown to exhibit diurnal variation in quality and digestibility. *Celtis africana* sapling leaves became increasingly concentrated in sugars throughout the day, while protein and the fiber fractions ADF (acid detergent fiber) and ADL (acid detergent lignin) remained constant. Conversely, the sapling leaves of *Pterygota mildbraedii* became decreasing concentrated in the fiber fractions NDF, ADF, and ADL, while protein and sugar concentrations remained constant.

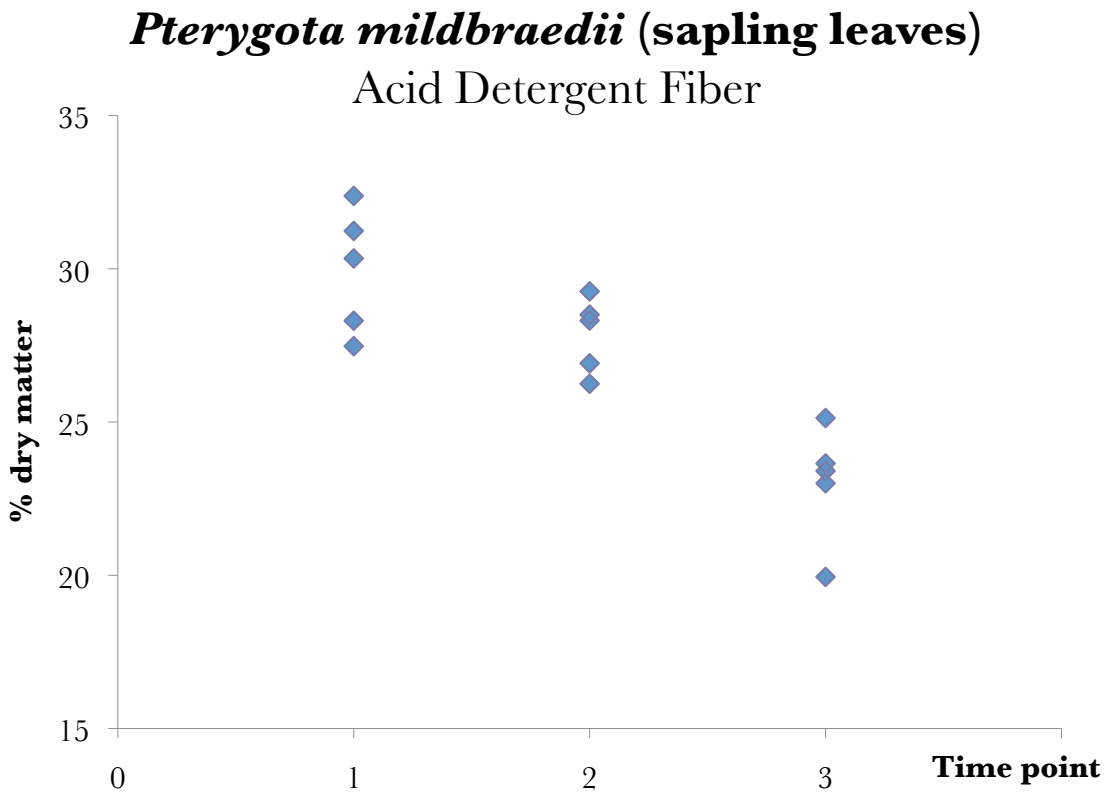
Diurnal variation in nutritional value, especially associated with variation in consumption, indicates that consumptive behavior may consciously or unconsciously track nutritive value, even within a given resource. Many factors contribute to consumptive behavior, including as ripeness of fruit, the age of leaves, energetic and social costs. As optimal foraging models become increasingly complex with the addition of numerous relevant energetic and social variables, so too might time of resource acquisition be another important addition.

Figure 2.1



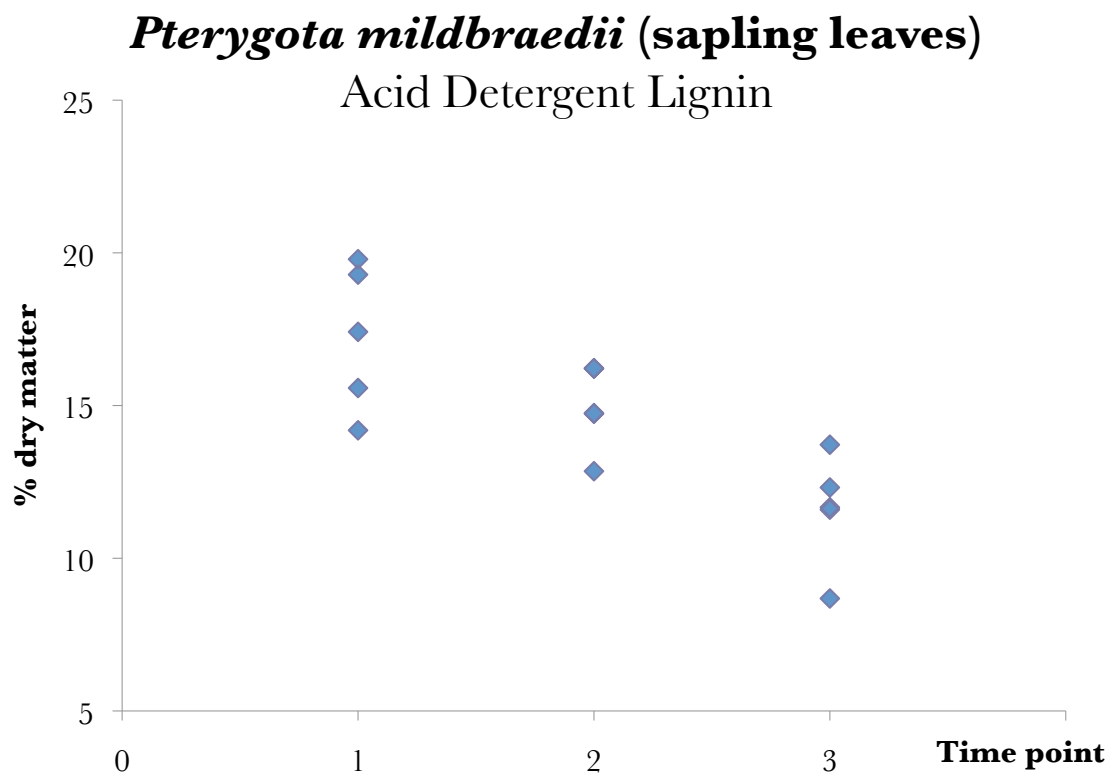

Concentration of neutral detergent fiber (NDF) by time of day for sapling leaves of *Pterygota mildbraedii*. Time point 1 corresponds to approximately 8:30a, time point 2 corresponds to around 1:15p and time point 3 corresponds to approximately 6:30p. Each point on the graphic represents the % dry matter of NDF for one sample at that time point.

Figure 2.2



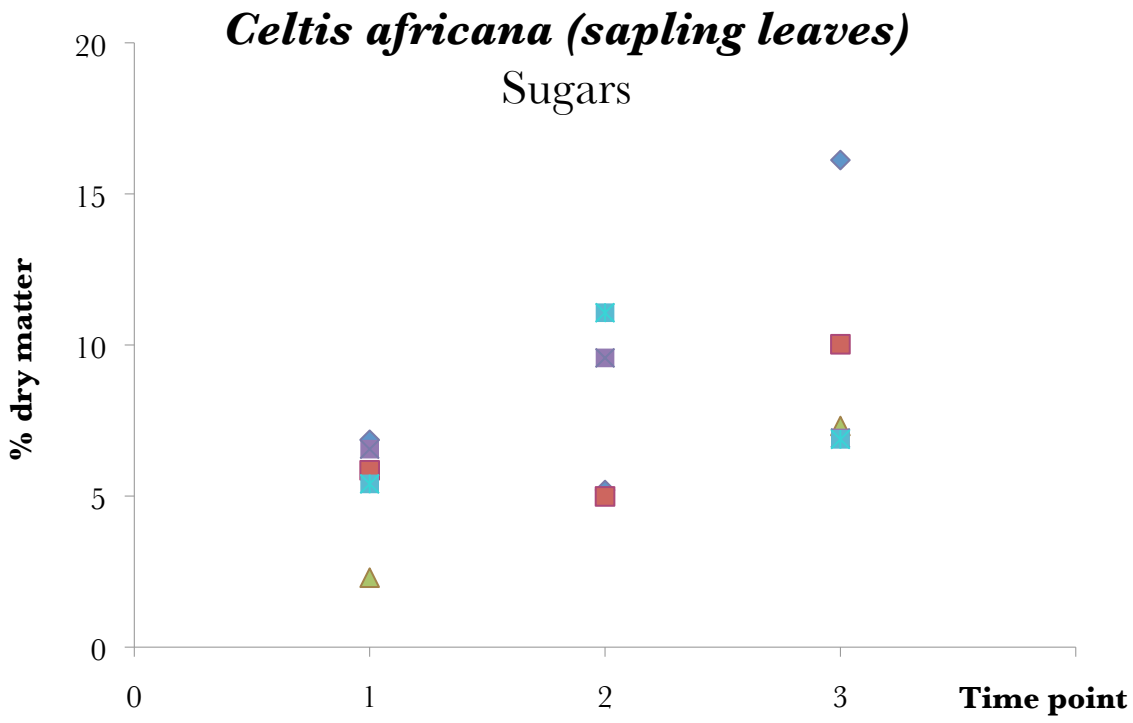

Concentration of acid detergent fiber (ADF) by time of day for sapling leaves of *Pterygota mildbraedii*. Time point 1 corresponds to approximately 8:30a, time point 2 corresponds to around 1:15p and time point 3 corresponds to approximately 6:30p. Each point on the graphic represents the % dry matter of ADF for one sample at that time point.

Figure 2.3




Concentration of acid detergent lignin (ADL) by time of day for sapling leaves of *Pterygota mildbraedii*. Time point 1 corresponds to approximately 8:30a, time point 2 corresponds to around 1:15p and time point 3 corresponds to approximately 6:30p. Each point on the graphic represents the % dry matter of ADL for one sample at that time point.

Figure 2.4




Concentration of sugars by time of day for sapling leaves of *Celtis africana*. Time point 1 corresponds to approximately 7:30a, time point 2 corresponds to around 1:00p and time point 3 corresponds to approximately 6:00p. Each point on the graphic represents the % dry matter of sugars for one sample at that time point. Five sapling trees were sampled serially throughout the day and each tree is identified by color/symbol above.

Table 2.1

Time of day effect on several nutrients in young saplings leaves of *Pterygota mildbraedii*

| Time collected | *n* | NDF | ADF | ADL | Protein | Sugars |
|---|---|---|---|---|---|---|
| 8:30 | 5 | 40.5 ± 1.7 | 29.9 ± 2.0 | 17.3 ± 2.4 | 26.2 ± 2.8 | 4.7 ± 3.1 |
| 13:30 | 5 | 40.7 ± 1.8 | 27.8 ± 1.2 | 15.0 ± 1.4 | 26.1 ± 2.1 | 4.5 ± 2.2 |
| 18:30 | 5 | 36.3 ± 1.9 | 23.0 ± 1.9 | 11.6 ± 1.8 | 27.0 ± 1.0 | 4.3 ± 4.2 |
| *Spearman's r* | | **-0.6992** | **-0.8315** | **-0.8126** | 0.1890 | -0.0567 |
| *p-value (Spearman's r)* | | **0.0037** | **0.0001** | **0.0002** | 0.5000 | 0.8409 |
| *p-value (ANOVA)* | | **0.0072** | **< 0.0001** | **0.0003** | 0.5263 | 0.8358 |

NDF=neutral detergent figer, ADF=acid detergent fiber, ADL=acid detergent lignin
Each nutrient value expressed as mean % of dry matter ± 1 standard deviation
Statistical analyses utilzed One-Way ANOVA to calculate variance explained by time of day

Table 2.2

Time of day effect on several nutrients in young saplings leaves of *Celtis africana*

| Time collected | *n* | NDF | ADF | ADL | Protein | Sugars |
|---|---|---|---|---|---|---|
| 7:30 | 5 | 31.3 ± 1.3 | 17.1 ± 1.3 | 6.1 ± 0.9 | 25.9 ± 3.2 | 5.4 ± 1.8 |
| 13:00 | 5 | 30.5 ± 0.7 | 16.5 ± 0.5 | 6.0 ± 0.5 | 25.1 ± 3.5 | 7.7 ± 3.1 |
| 18:30 | 5 | 30.2 ± 2.2 | 16.8 ± 2.1 | 6.5 ± 1.7 | 25.2 ± 3.5 | 9.5 ± 3.9 |
| *Spearman's r* | | -0.2268 | -0.0756 | 0.1323 | -0.0756 | **0.6290** |
| *p-value (Spearman's r)* | | 0.4163 | 0.7889 | 0.6384 | 0.7889 | **0.0160** |
| *p-value (ANOVA)* | | **0.0463** | 0.6466 | 0.5266 | 0.1194 | 0.0737 |

NDF=neutral detergent figer, ADF=acid detergent fiber, ADL=acid detergent lignin
Each nutrient value expressed as mean % of dry matter ± 1 standard deviation
Each of 5 *Celtis africana* saplings were repeat sampled at 7:30a, 1:00p, and 6:30p.
Statistical analyses utlized a One-Way Repeated Measures ANOVA to calculate variance explained by time of day while controlling for repeat sapling of each tree.

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SAS CODE

```
/*ISOTOPIC DIETARY ECOLOGY:
/*DIURNAL NUTRIENT CYCLING AMONG YOUNG SAPLINGS OF
/*Pterygota mildbraedii AND Celtis africana

/*DISSERTATION RESEARCH
/*BRYCE CARLSON*/

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        RUN;

PROC CONTENTS DATA=TOD;
        RUN;

PROC SORT DATA=TOD; BY time;
PROC FREQ DATA=TOD;
        TABLES species;
        BY time;
        RUN;

PROC MEANS DATA=TOD;
        VAR NDF ADF ADL Protein Sugars;
        BY time;
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    RUN;

PROC MEANS DATA=TOD;
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    BY time;
    WHERE species="Ca";
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    VAR NDF ADF ADL Protein Sugars;
    WITH time;
    WHERE species="PTm";
    RUN;

PROC CORR DATA=TOD SPEARMAN;
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    WITH time;
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    RUN;

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```

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    RUN;


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    CLASS tree;
    MODEL NDF ADF ADL Protein Sugars = time tree;
    WHERE species="Ca";
    RUN;
```

# TECHNIQUE FOR FINDING NEUTRAL DETERGENT FIBER

Reprinted from the Primate Nutritional Ecology Laboratory Manual

at Hunter College of CUNY

by Jessica Rothman

**Definition:**

This method determines Neutral Detergent Fiber, which is the residue remaining after digesting in a detergent solution. The fiber residues are predominantly hemicelluloses, cellulose, and lignin.

*** Before Starting- You MUST Do a Dry Matter at the Same Time You Prepare Samples for NDF ***

**Apparatus:**

1. Analytical Balance

2. Oven—capable of maintaining a temperature of 105±2°C.

3. Digestion instrument—(ANKOM 200 fiber analyzer).

4. Filter bags (F57, ANKOM Technology).

5. Weighing paper, brushes, and scoops for handling the sample.

5. Heat sealer—sufficient for sealing the filter bags closed to ensure complete closure

6. Desiccator—glass vessel and lid with vacuum grease and desiccant inside that enables the removal of moisture in the air around the filter bags.

7. Industrial Strength Sharpie—solvent and acid resistant ink

8. Data Sheet

**Reagents:**

1. Neutral Detergent Solution: to make 4 L of ND Solution, add 2L of deionized water to a large plastic beaker and start stirring on a stir-plate. Then add:

120.0g- Sodium Lauryl Sulfate

74.44g- Ethylenediamine-tetraacetic disodium salt, dehydrate

27.24g- Sodium borate

18.24g- Sodium phosphate dibasic, anhydrous

Pipet into the solution 40 ml of Triethylene Glycol and finish by adding 2.0L of deionized water.

2. Alpha-amylase, heat stable bacterial alpha amylase (ANKOM Technology)

**Procedure:**

1. Use a solvent resistant marker to label the filter bags. Record the weight of filter bag (W1). Any mislabeled bags should be saved and reused.

*Note*—Do not pre-dry filter bags; any moisture will be accounted for by the blank bag correction.

2. Weigh 0.45-0.55 g of prepared sample onto weighing paper and record the sample weight (W2). Using the weigh paper as a funnel, pour your weighed sample into the filter

bag, using a brush to ensure all weighed sample is transferred to the filter bag. Avoid placing the sample on the upper 4 mm of the bag.

3. Using a heat sealer (set to 4.5), completely seal the upper edge of the filter bag within 4 mm of the top.

*Note*—Use sufficient heat to completely seal the filter bag (the red light should come on, and go off) and allow enough cool time (2 sec) before removing the bag from the heat sealer.

Spread the sample uniformly inside the filter bag by shaking and flicking the bag to eliminate clumping.

4. Weigh one blank (empty) bag and include in run to determine blank bag correction (C1, see Number Note 1).

5. Place a maximum of 24 bags into the Bag Suspender. All nine trays should be used regardless of the number of bags being processed. Place three bags per tray and then stack trays on center post with each level rotated 120 degrees (the notches should be lined up over the ridges along the bag suspender edge). Your control bag should be included at the middle of the Bag Suspender. Insert the Bag Suspender with bags into the fiber analyzer vessel and place the weight on top to keep it submerged. *Note*—Prior to inserting the Bag Suspender, if the vessel temperature is warm from a previous run, add cold water and exhaust.

6. When processing 24 sample bags, add 1900-2000mL of ambient ND solution to the fiber analyzer vessel. If processing less than 20 bags, add 100 ml/bag of ND solution (use minimum of 1500 mL to ensure Bag Suspender is covered). Add 4.0 mL of alpha-amylase to the solution in the vessel.

7. Set timer for 75 min and check that the thermostat (green numbers) is set to 100$^{oC}$, turn Agitate ON and confirm agitation, close the lid tightly and turn Heat ON. Start the timer. Check back frequently to ensure that as the solution heats, none is leaking from around the top of the vessel. Also, check that the temperature in the vessel (red numbers) has reached 100$^{oC}$.

8. With 15 minutes to go in extraction, prepare the two water boiling kettles with deionized water for rinsing and set kettles to boil.

9. At the end of extraction, turn Heat and Agitate off. Open the drain valve (slowly at first) and exhaust hot solution before opening lid. The waste from this assay can be collected in large, plastic beakers and disposed of down the sink. *Note*—The solution in the vessel is under pressure. The exhaust valve needs to be opened to release the pressure and solution prior to opening the lid.

10. After the solution has been exhausted, close the exhaust valve and open the lid. Add 1900mL of (70-90°C) rinse water and 4.0 mL of alpha-amylase to the first and second rinses. Turn Agitate on and rinse for 5 min. Repeat hot water rinses a total of three times.

11. When the rinsing process is complete remove the samples. Gently press out excess water from bags. Place bags in a 400 mL beaker, with a 250ml beaker stacked inside to hold samples submerged and add enough histological grade acetone to cover bags and soak for 3-5 min. Remove bags from acetone and place on a baking sheet to air-dry.

12. Completely dry the samples in oven at 105±2°C for 30 min. Desiccate samples for 30 minutes to cool before weighing samples. *Note*—Do not place bags in the oven until acetone has completely evaporated.

**Calculations:**

% NDF (as-received basis) = ((W3 - (W1 x C1))/$W_2$) x 100

Where: W1 = Bag weight

W2 = Sample weight

W3 = Dried weight of bag with fiber after extraction process

C1 = Blank bag correction (final ovendried weight divided by the original blank bag weight)

**Notes:**

*Caution*

Powdered chemicals will irritate the mucous membranes. A dust mask and gloves should be worn when handling this chemical. Acetone is extremely flammable. Avoid static electricity and use a fume hood when handling.

**Numbered notes:**

1. A running average blank bag correction factor (C1) should be used in the calculation of fiber. The inclusion of a blank bag in each run is mainly used as an indicator of particle loss. A C1 larger than 1.0000 indicates that sample particles were lost from filter bags and deposited on the blank bag. Any fiber particle loss from the filter bags will generate erroneous results. If particle loss is observed then grinding method needs to be evaluated.

**Citation, CBE Style:**

Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74(10): 3583-3597.

# TECHNIQUE FOR FINDING ACID DETERGENT FIBER

Reprinted from the Primate Nutritional Ecology Laboratory Manual

at Hunter College of CUNY

by Jessica Rothman

**Definition:**

This method determines Acid Detergent Fiber, which is the residue remaining after digesting with H2SO4 and HTAB. The fiber residues are predominantly cellulose and lignin.

**Apparatus:**

1. Analytical Balance

2. Oven—capable of maintaining a temperature of 105±2°C.

3. Digestion instrument—(ANKOM 200 fiber analyzer).

4. Filter bags (F57, ANKOM Technology).

5. Weighing paper, brushes, and scoops for handling the sample.

5. Heat sealer—sufficient for sealing the filter bags closed to ensure complete closure

6. Desiccator—glass vessel and lid with vacuum grease and desiccant inside that enables the removal of moisture in the air around the filter bags.

7. Industrial Strength Sharpie—solvent and acid resistant ink

8. Data Sheet

**Reagents:**

1. Acid Detergent Solution: To make 2.0L of acid detergent solution: Combine chemicals in a large plastic beaker using a stir plate. Chemicals should ONLY be combined IN THIS ORDER!
1800ml deionized water, 200ml 10N Sulfuric acid solution, and 40g Hexadecyltrimethyl Ammonium Bromide (HTAB).

**Procedure:**

***If continuing from NDF, start at step 5 with your dried and weighed sample bags***

1. 1. Use a solvent resistant marker to label the filter bags. Record the weight of filter bag (W1). Any mislabeled bags should be saved and reused.
*Note*—Do not pre-dry filter bags; any moisture will be accounted for by the blank bag correction.

2. Weigh 0.45-0.55 g of prepared sample onto weighing paper and record the sample weight (W2). Using the weigh paper as a funnel, pour your weighed sample into the filter bag, using a brush to ensure all weighed sample is transferred to the filter bag. Avoid placing the sample on the upper 4 mm of the bag.

3. Using a heat sealer (set to 4.5), completely seal the upper edge of the filter bag within 4 mm of the top.

*Note*—Use sufficient heat to completely seal the filter bag (the red light should come on, and go off) and allow enough cool time (2 sec) before removing the bag from the heat sealer.

Spread the sample uniformly inside the filter bag by shaking and flicking the bag to eliminate clumping.

4. Weigh one blank (empty) bag and include in run to determine blank bag correction (C1, see Number Note 1).

*****5. Place a maximum of 24 bags into the Bag Suspender. All nine trays should be used regardless of the number of bags being processed. Place three bags per tray and then stack trays on center post with each level rotated 120 degrees (the notches should be lined up over the ridges along the bag suspender edge). Your control bag should be included at the middle of the Bag Suspender. Insert the Bag Suspender with bags into the fiber analyzer vessel and place the weight on top to keep it submerged. *Note*—Prior to inserting the Bag Suspender, if the vessel temperature is warm from a previous run, add cold water and exhaust.

6. When processing 24 sample bags, add 1900-2000mL of ambient temperature AD solution to the fiber analyzer vessel. If processing less than 20 bags, add 100 ml/bag of AD solution (use minimum of 1500 mL to ensure Bag Suspender is covered).

7. Set timer for 60 min and check that the thermostat (green numbers) is set to 100$^{oC}$, turn Agitate ON and confirm agitation, close the lid tightly and turn Heat ON. Start the timer. Check back frequently to ensure that as the solution heats, none is leaking from around the top of the vessel. Also, check that the temperature in the vessel (red numbers) has reached 100$^{oC}$.

8. At end of extraction, turn the Timer, Heat and Agitate off. Open the drain valve (slowly at first) and exhaust hot solution before opening lid. Waste from the assay should flow into the large sulfuric acid waste containers to the right of the machine. *Note*—The solution in the vessel is under pressure. The exhaust valve needs to be opened to release the pressure and solution prior to opening the lid.

9. After the solution has been exhausted, close the exhaust valve and open the lid. Remove the bag suspender and samples. Place sample bags into a large plastic beaker and rinse under hot water in the sink. Rinse the bag suspender thoroughly under hot water as well. Rinse until bubbles stop forming, rinse bubbles from the sink. Continue to vigorously rinse the samples for 15-20 minutes.

10. When the rinsing process is complete remove the samples. Gently press out excess water from bags. Place bags in a 400 mL beaker, with a 250ml beaker stacked inside to hold samples submerged add enough histological grade acetone to cover bags and soak for 3-5 min. Remove bags from acetone and place on a baking sheet to air-dry.

11. Completely dry the samples in oven at 105±2°C for 30 min. Desiccate samples for 30 minutes to cool before weighing samples. *Note*—Do not place bags in the oven until acetone has completely evaporated.

**Calculations:**

% ADF (as-received basis) = ((W3 - (W1 x C1)) /W2) x 100

Where: W1 = Bag weight

W2 = Sample weight

W3 = Dried weight of bag with fiber after extraction process

C1 = Blank bag correction factor (final oven-dried weight divided by original weight)

**Notes:**

*Caution*

Sulfuric acid is a strong acid and will cause severe burns. Protective clothing should be worn when working with this acid. Always add acid to water and not the reverse. HTAB will irritate the mucous membranes. A dust mask and gloves should be worn when handling this chemical. Acetone is extremely flammable. Avoid static electricity and use a fume hood when handling.

**Numbered Notes:**

1. A running average blank bag correction factor (C1) should be used in the calculation of fiber. The inclusion of a blank bag in each run is mainly used as an indicator of particle loss. A C1 larger than 1.0000 indicates that sample particles were lost from filter bags and deposited on the blank bag. Any fiber particle loss from the filter bags will generate erroneous results. If particle loss is observed then grinding method needs to be evaluated.

**Citation, CBE Style:**

Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74(10): 3583-3597.

# PROCEDURE FOR DETERMINING ACID DETERGENT LIGNIN

Reprinted from the Primate Nutritional Ecology Laboratory Manual

at Hunter College of CUNY

by Jessica Rothman

## Reagents:

Sulfuric acid (72% by weight)

Sodium bicarbonate (Arm & Hammer baking soda) in aqueous solution

## Apparatus:

1. Analytical Balance—capable of weighing down to 0.1 mg.
2. Oven—capable of maintaining a temperature of 105±2°C.
3. Weighing paper, brushes, and scoops for handling the sample.
4. ANKOM F57 Filter bags
5. Impulse bag sealer
6. Desiccator
7. 3- 400 and 2- 600 ml glass beakers
8. Spatula for turning samples
9. Glass baking dish
10. Data sheet

## Procedure:

***If beginning procedure after ADF, skip to step 6***

1. Use a solvent resistant marker to label the filter bags. Record the weight of filter bag (W1). Any mislabeled bags should be saved and reused.

*Note*—Do not pre-dry filter bags; any moisture will be accounted for by the blank bag correction.

2. Weigh 0.45-0.55g of prepared sample onto weighing paper and record the sample weight (W2). Using the weighing paper as a funnel, pour your weighed sample into the filter bag, using a brush to ensure all weighed sample is transferred to the filter bag. Avoid placing the sample on the upper 4 mm of the bag.

3. Using a heat sealer (set to 4.5), completely seal the upper edge of the filter bag within 4 mm of the top.

*Note*—Use sufficient heat to completely seal the filter bag (the red light should come on, and go off) and allow enough cool time (2 sec) before removing the bag from the heat sealer. Spread the sample uniformly inside the filter bag by shaking and flicking the bag to eliminate clumping.

4. Weigh one blank bag and include in run to determine blank bag correction (C1, see Number Note 1).

5. Perform ADF determination (see Technique for ADF)

*****6. After samples are dried and weighed from ADF determination, stack A and B samples separately. Place control bag in the middle of the stack. Place sample bags into the larger of the 2 glass beakers.

7. Place several scoops of baking soda into the glass baking dish, and add enough water to create a shallow aqueous solution. Similarly, add a couple scoops of baking soda to one of the 400ml beakers and fill with water to create an aqueous solution. This is to help catch and neutralize any acid spills.

8. Place the sample beakers into the glass baking dish. Add a sufficient quantity of 72% sulfuric acid to cover the filter bags. Using a spatula, turn over the stack of filter bags to ensure all samples are well coated in acid.

9. Place the 400ml beaker inside the 600ml beaker to keep bags submerged. "Bouce" the 400ml beaker up and down on the stack of fiber bags (30x) to agitate the stack. The stack of filter bags should be turned over with the spatula and agitated every 30 minutes for a total of 3 hours.

10. After 3 hours pour off the sulfuric acid into the appropriate waste container and place fiber bags in a large plastic beaker. Vigorously rinse the bags in the sink under hot water until all the acid is removed and water is a neutral pH. Lay samples out on a cookie sheet and let air dry.

11. Place air dry samples in the drying oven (105$^{oC}$) for 30 minutes. Transfer the dry samples to the desiccator and let cool to room temperature (30 minutes). Weigh the cool and dry samples and record the weight on the data sheet.

**Calculation:**

ADL (as received) = ((W3-(W1*C1)) /W2)*100

Where:

W1= bag weight

W2= sample weight

W3= post ADL dry weight

C1- blank bag correction (final oven dried weight/original blank bag weight)

**Citation, CBE Style:**

Van Soest PJ. 1963. The use of detergents in the analysis of fibrous feeds. II. A rapid method for determination of fiber and lignin. J Assoc Offic Agr Chem 46: 829-835

# DETERMINATION OF CRUDE PROTEIN BY COMPLETE COMBUSTION

Reprinted from the Primate Nutritional Ecology Laboratory Manual

at Hunter College of CUNY

by Jessica Rothman

## Apparatus:

1. Analytical Balance
2. Foil protein sample bags
3. Black protein bag stand
4. Standard sample- Orchard Leaves
5. Colored 5 x 16 well rack to store and keep samples labeled
5. Scoop and brushes for handling sample
6. Data Sheet for N analysis

## Procedure:

1: Place the black protein bag stand on the balance and place an empty foil protein bag onto the stand so it forms a small cup, then zero out the weight

2. Weigh out 0.10g of sample directly into the foil cup and record the weight (to 4 decimals) on the weight sheet

3. To close the bag, pinch the four corners of the foil pouch together. Tightly twist the bag into a teardrop shape. Be careful to not rip the bag

4. Place the sample bag in the colored rack and record the numbered well that the bag was placed in.

5. Weigh out 0.10g of orchard leaves as a standard sample every 10 samples. "Blanks" are an open air blank ran through the nitrogen analyzer, and DO NOT need a foil sample bag.

**Using the Nitrogen Analyzer:**

1. The lab tech can help you turn on and prepare the N analyzer for use on your samples

2. To load samples, place the samples in an open carousel position. Record this position number in the third column of the weight sheet. Sample carousel rotates in the direction shown by the arrow on top.

3. On the computer, enter your sample name and sample weight in the appropriate columns. Every 10 samples run 1 blank sample (that is 1 empty carousel position) and 1 orchard leaf control. This pattern is also illustrated on the protein sample data sheet.

4. Before you finish running your samples alert the lab tech so that they can be ready to shut the nitrogen analyzer down as soon as it is finished.

*Remember, gases are expensive so try to run samples en mass*

**Citation, CBE Style:**

[AOAC] Assoc of Official Analytical Chem. 1995. Official methods of analysis 990.03. 16th ed. Arlington (VA).

# ACID DETERGENT INSOLUBLE NITROGEN

Reprinted from the Primate Nutritional Ecology Laboratory Manual

at Hunter College of CUNY

by Jessica Rothman

1. Follow the procedure for preparing and analyzing samples as described for Acid Detergent Fiber.

2. After samples have been run through ADF procedure and a final dry weight has been recorded, cut the top of the fiber bag to remove the sample inside. Use a spatula to break apart the AD residue in the fiber bag. Use this residue as your sample to bag as described for Crude Protein Analysis.

**Calculation:**

ADIN (DM basis) =( $\%N_s$ x %ADF(DM basis))/100

$\%N_s$ = percent of nitrogen in ADF residue

%ADF(DM basis) = percent ADF calculated using ADF procedure

**Citation, CBE Style:**

Licitra G, Hernandez TM, Van Soest PJ. 1996. Standardization of procedures for nitrogen fraction of ruminant feeds. Anim Feed Sci Tech 57 (4) 347-358.

Rothman JM, Chapman CA, Pell AN. 2008. Fiber-bound nitrogen in gorilla diets: implications for estimating dietary protein intake of primates. Am J Primatology 70: 690-694.

## NEUTRAL DETERGENT INSOLUBLE NITROGEN

Reprinted from the Primate Nutritional Ecology Laboratory Manual

at Hunter College of CUNY

by Jessica Rothman

1. Follow the procedure for preparing and analyzing samples as described for Neutral Detergent Fiber.

2. After samples have been run through NDF procedure and a final dry weight has been recorded, cut the top of the fiber bag to remove the sample inside. Use a spatula to break apart the ND residue in the fiber bag. Use this residue as your sample to bag as described for Crude Protein Analysis.

**Calculation:**

ADIN (DM basis) =( $\%N_s$ x %ADF(DM basis))/100

$\%N_s$ = percent of nitrogen in ADF residue

%ADF(DM basis) = percent ADF calculated using ADF procedure

**Citation, CBE Style:**

Licitra G, Hernandez TM, Van Soest PJ. 1996. Standardization of procedures for nitrogen fraction of ruminant feeds. Anim Feed Sci Tech 57 (4) 347-358.

# Chapter 3

# ISOTOPIC DIETARY ECOLOGY: BULK $\delta^{13}C$ AND $\delta^{15}N$ CHARACTERIZATION OF THE CHIMPANZEE DIETARY NICHE AT NGOGO, KIBALE NATIONAL PARK, UGANDA

## Introduction

An element's isotopic composition can provide a means of reconstructing it's ecological cycling through biotic and abiotic processes (Kohn 2010; Schoeller 1999). The isotopic composition of an organism's tissue results from dietary intake followed by differential routing of the component elements during digestion and absorption, synthetic and other metabolic processes, and deposition within the tissue. Each of these component processes modifies the dietary isotopic signature in measurable ways, allowing stable isotopic analysis of individual tissues to reconstruct dietary and metabolic history (O'Brien et al. 2002; Petzke et al. 2006; Petzke and Lemke 2009).

Stable isotope ratios can track consumptive behavior as well as attributes of the local environment. The $\delta^{13}C$ of leaves vary significantly according to mode of photosynthesis ($C_3$, $C_4$, or CAM), as well as position within the forest canopy (Cerling et al. 2004; Schoeninger et al. 1998) and mean annual precipitation (Kohn 2010). Analysis of $\delta^{15}N$ among individual animals shows a strong association with trophic level (Fizet et al. 1995; Hedges and Reynard 2007; Petzke et al. 2005b; Schwarcz and Schoeninger 1991), and among plants we see unique and characteristic values for nitrogen-fixing plants (e.g. legumes) relative to non-fixers.

Within a paleodietary context, Sponheimer and Lee-Thorpe have contributed much to our understanding of *Australopithecus* and *Paranthropus* diet via analyses of the $\delta^{13}C$ within fossilized tooth enamel (Lee-Thorp et al. 2010; Sponheimer et al. 2005; Sponheimer and Lee-Thorp 1999; Sponheimer and Lee-Thorp 2003; Sponheimer et al. 2006). Both hominin taxa exhibit isotopic ratios intermediate between $C_3$ browsing and $C_4$ grazing species, indicating some contribution to the diet of $C_4$ plant material or animals who themselves were $C_4$ consumers (Lee-Thorp et al. 1994; Sponheimer et al. 2007). This is a pattern unlike most extant primates that have been analyzed isotopically, leading to debate over the consumptive behaviors likely to have produced such a signal (Peters and Vogel 2005; Sponheimer and Lee-Thorp 2003; Yeakel et al. 2007).

Plio-Pleistocene and archaeological isotopic analyses require understanding how stable isotopes are cycled through ecosystems. Ecological surveys of the isotopic composition and distribution of East African flora and fauna are essential for reconstructing Plio-Pleistocene consumption via stable isotopic analyses. Such analyses provide a modern template, or proxy, from which to interpret the analysis of hominin remains.

Despite several studies reporting significant isotopic variability attributable to canopy height and mean annual precipitation, previous ecological surveys have reported little or no isotopic variation across a $C_3$ dominated landscape (Codron et al. 2005). This survey collected a large number of samples across a South African savanna and therefore wasn't representative of the closed-canopy $C_3$ dominated environments that contain today's great apes and likely our earliest hominin ancestors as well. The presence or absence of isotopic variability across an actual or hypothetical dietary niche carries incredible significance for dietary reconstruction. If little variability exists within $C_3$ or $C_4$

dominated landscapes, dietary ecologists are left unable to model dietary consumption except as a combination of $C_3$ and $C_4$ resources. If, however, isotopic variation is found to exist with $C_3$ dominated environments, and that variation may be attributed to ecologically meaningful variables, such is the foundation for reconstituting dietary ecology from the distance of weeks up to millions of years.

The canopy effect has previously been shown to result in isotopic differentiation by consumption at different heights in the forest canopy. Within a chimpanzee dietary niche, such a differentiation may correlate with access to preferred foods (e.g. canopy fruit as opposed to less preferred ground based sapling leaves and pith). Applied to chimpanzee dietary ecology over the course of a month or year, longitudinal sampling of hair may allow tracking seasonal access to fruit and fallback foods via the impact of the canopy effect on driving tissue level isotopic variation.

Within the context of hominin evolution, should the canopy effect remain a consistent element within all $C_3$ environments (especially those across East Africa with relevance for hominin evolution), the isotopic differentiation between canopy and ground resources may allow access to the origins and subsequent evolution of terrestriality. However, to date, no work has systematically examined isotopic dietary ecology within an East African $C_3$ dominated environment.

In general, greater geochemical or biological cycling of molecules within a system leads to greater isotopic fractionation and ultimately differentiation of isotopic composition. The canopy effect results from plant resources along the ground utilizing highly recycled $CO_2$ available at the forest floor, the recycling of which results in a relative depletion of $^{13}C$. Similarly, different species of plants as well as the different anatomical components of a single species (ie. leaves, fruits, seeds, etc) undergo unique

metabolic processes that should result in greater or lesser fractionation of atmospheric carbon and nitrogen. As a result, leaves may contain an isotopic signature distinguishable from fruit, which may be distinguishable from seeds, etc. Additionally, some plant resources may contain such unique metabolic physiologies that all anatomical components are enriched or depleted in $^{13}C$ or $^{15}N$ relative to the rest of the dietary niche. If such is the case, these resources may prove recoverable utilizing bulk isotopic analysis, even from within a $C_3$ dominated dietary environment.

This project seeks to establish an isotopic dietary template with specific relevance for hominin evolution. To that end, over the course of 12 weeks of sampling spanning wet and dry seasons, approximately 350 plant samples were collected representing approximately 60 food items within the chimpanzee niche, 40 individual taxa, 10 food types, and approximately 90% of the chimpanzee dietary niche at Ngogo, Kibale National Park, Uganda (Table 3.1). By characterizing the stable isotopic (C and N) variation and distribution within the dietary niche of a large-bodied omnivorous primate, this study takes one small step toward the development of a comprehensive frame of reference for reconstructing the consumptive behavior of our hominin ancestors.

## Methods

Plant components of the Ngogo chimpanzee diet were defined broadly and collected opportunistically. A list of consumed plant species was generated from several sources including published literature, unpublished observational data (personal communication: David Watts), experience of local field assistants, and personal observation. Identification of each species proceeded over several months of study during

and between field season, and benefited from assistance of local field guides and camp staff.

Collection began in late November 2009 and continued for approximately 3 weeks at trees following direct observation of the Ngogo chimpanzees. Sampling strategy included:

- *Fruit* was selected from the ground that appeared (1) the appropriate stage of ripeness, and contained (2) no bite marks, (3) no insect damage, and (4) no mold.
- *Leaves of saplings* were sampled upon encounter from patches exploited by the chimpanzees, but not necessarily the same sapling.
- *Leaves of mature trees* were sampled from the ground and preference was given to less mature leaves as they appear most consumed by the chimpanzees.
- *Leaf buds* were selected along with leaves of mature trees, from the ground and when available.
- The *pith of herbaceous vegetation* was sampled selectively from patches of relatively large specimens (selective criteria of the chimpanzees). The outer husk was peeled away and the fleshy inner pith sampled preferentially from the lowest sections of the plant (lowest 6-12 inches).
- *Flowers* were sampled opportunistically upon encounter following similar principles to fruit selection.
- *Cambium, roots* and *dead wood* were likewise sampled opportunistically.

To ensure the broadest sampling coverage of the chimpanzee dietary niche, the above protocol eventually shifted from collection associated with direct observation of consumption to searching the Ngogo range for species known to be preferred by

chimpanzees. For each food species, every effort was made to sample widely across the Ngogo range to capture potential geographic and seasonal variation in nutritional or isotopic characteristics. Each sample was labeled with its identification, date, time, location collected (nearest trail intersection and GPS coordinate), as well as the relative stage of development (e.g. ripeness of fruits, or maturity of leaves). This sampling strategy was followed the last 1-2 weeks in December 2009, and again over approximately 5 weeks between June and July 2010.

Samples were dried within hours of collection at 150-170°F for approximately 3.5 hours or until completely desiccated within a small kerosene backpacking stove/oven. The backpacking stove was positioned immediately under a Coleman backpacking oven, and samples transferred only after the stove burned cleanly and the oven reached the proper temperature (approximately 10-20 minutes). This was done to minimize exposure of samples to carbon contamination from kerosene soot and ensure uniform conditions for each batch of samples. Leaves typically dried in 2-3 hours while fruit often took 3-4 hours. Drying time and temperature were determined to minimally impact nutrient composition (low temperatures) in the least amount of time required to prevent molding.

Approximately 5 mg of each sample were then frozen with liquid nitrogen and ground to a fine powder with mortar and pestle. Following grinding, all samples were vacuum dried to ensure complete dehydration. Between 1 and 2 mg of each powder were then transferred to individual tin capsules for isotopic analysis.

Bulk isotopic analyses were conducted in three batches between December 2010 and February 2011 within the Analytical Chemistry lab at the University of Georgia, Odum School of Ecology in collaboration with Tom Maddox.

# Results

The bulk $\delta^{13}C$ and $\delta^{15}N$ values of plant tissues from Ngogo were highly variable across taxa (Table 3.2), with $\delta^{13}C$ values ranging from -35.6 to -21.8‰ and $\delta^{15}N$ ranging from -0.41 to 9.44‰. Average $\delta^{13}C$ and $\delta^{15}N$, however, fell within that expected from previously published literature on $C_3$ and $C_4$ plants (Kohn 2010). The $\delta^{13}C$ of *Monodora myristica* and *Morus lactea* sapling leaves were most negative (-32.7±1.4 and -33.7±1.0‰ respectively). Leaf buds and flowers of *Morus lactea* (-24.2±0.2 and -24.5±0.8‰ respectively) and flowers of *Pterygota mildbraedii* (-24.4±1.0‰) tended to be least negative among $C_3$ plants. The mean $\delta^{13}C$ for $C_3$ plants was -27.7±2.6‰ (relative to PDB). Nitrogen isotopic values, likewise, ranged significantly from 1.4±2.3‰ (pith of *Acanthus sp.*) to a maximum of 6.9±0.8‰ (fruit and seeds of *Celtis durandii*), with an average $\delta^{15}N$ of 4.5±1.8‰.

Most plants within the Ngogo chimpanzee dietary niche utilize the $C_3$ photosynthetic pathway (large cluster in Figure 3.1). Only one plant species (*Cyperus papyrus*) utilizes the $C_4$ pathway, and it correspondingly carried unique $\delta^{13}C$ and $\delta^{15}N$ values of -10.6±0.5‰ and 1.5±0.4‰ respectively (see Table 3.2).

Given the large range of carbon and nitrogen values among dietary resources, multivariate ANOVA was utilized to tease apart the anatomical, photosynthetic, seasonal, and other environmental sources of $\delta^{13}C$ and $\delta^{15}N$ variability. A simple model including photosynthetic pathway alone ($C_3$ vs. $C_4$ resources) accounted for approximately 45% of the variance in $\delta^{13}C$ (R-square = 0.451). This indicates that approximately 55% of the

variance within this sample could be attributed to factors other than $C_3$ v. $C_4$ photosynthesis.

To examine the contributions of other environmental, anatomical, or taxonomic variables, a multivariate ANOVA model was developed. The multivariate model was built stepwise and included the following variables: food type (plant anatomy), season (dry vs. wet), photosynthetic pathway of the plant species, height within the canopy (dichotomous: canopy vs. ground), and elevation (see Table 3.5). The full model, including all of the above variables, produced an R-square of 0.782 indicating these parameters accounted for an additional 35% of the sample variance. However, a reduced model, including only those variables with statistically significant estimates (photosynthetic pathway, food type, and elevation) produced an R-square nearly equal to the full model (R-square=0.776).

The best ANOVA model is one that includes only variables whose contributions significantly increase the total variance explained by the model. One means of testing for significant variables is to create one model with independent variables of interest included, and then examining the effect and statistical significance of each within the model. The other process is to build a number of different models one variable at a time while examining the explanatory power of the model and the statistical significance of each variable of interest within the model. Combining these two processes, the variation in bulk $\delta^{13}C$ within this sample was best explained by variability attributable to (1) photosynthetic pathway, (2) food type, and (3) elevation. These two processes of model building also revealed that while canopy height was a significant contributor to sample variance, it was largely controlled for by food type (which explained more variability than canopy height alone). Given that pith and sapling leaves were the only ground resources,

and that no food type contained both ground and canopy resources, once food type was entered in the model there was little remaining variability to be explained by the addition of the "canopy" variable. So, even though both variables were statistically significant within the full model, adding canopy to a model that already contained "food type" only explained an additional 0.3% of the total variance (Table 3.5).

The variance of $\delta^{15}N$, however, was less well defined by those same variables. A multivariate ANOVA model was developed following the same process to examine the contributions of each variable individually and in unison. As with $\delta^{13}C$, the relationship between elevation, food type, and photosynthetic pathway upon $\delta^{15}N$ were statistically significant, whereas season collected, and canopy height were not (Table 3.6). However, the full and reduced models were only able to explain approximately 25% of the total variance in $\delta^{15}N$ (R-square of 0.256 and 0.249 respectively), indicating approximately 75% of the sample's variance attributable to other (undefined) factors.

A more explicit discussion of the three principal sources of isotopic variability follows:

*Variability attributable to anatomy (food type)*

As a dietary class, leaves of saplings and pith were the most $^{13}C$ depleted of all classes at -31.1±2.4 and -30.5±2.1‰ respectively (Table 3.3). Pairwise comparisons within ANOVA testing the null hypthesis that means for each food type were equal (e.g. $\mu_{ls}=\mu_{fl}$) showed these two dietary items (sapling leaves and pith) significantly different from all others ($p<0.0001$, 2-tailed, unequal variances), but not different from each other ($p=0.768$, 2-tailed, unequal variances). Conversely, fruits, seeds, fruits with seeds, tree

leaves, leaf buds, cambium, and roots were isotopically indistinguishable in both carbon and nitrogen. Some statistical differences were apparent (e.g between flowers and fruits, fruits/seeds, and leaves), but there is sufficient overlap to confound reconstructions of their individual contributions to tissue level $\delta^{13}C$ or $\delta^{15}N$ values.

The unique signatures of pith and sapling leaves are appear upon comparison with other food types (Figure 3.2). The separation between food types is also observable within individual species (eg. Figure 3.3). This differentiation is in part a result of the canopy effect (with ground based sapling leaves and pith significantly depleted in $^{13}C$), but also independent of it. Within the ANOVA model, food type accounted for more total variability than canopy alone, and as can be seen in Figure 3.4, the distribution of $\delta^{13}C$ between canopy resources (ie. flowers, fruit, etc.) appears to shift slightly between food types.

Any isotopic separation between food classes appears largely defined by $\delta^{13}C$. While some differences in $\delta^{15}N$ between food types were statistically significant (e.g. tree leaves from fruit, sapling leaves, pith, and roots), considerable overlap existed between most resources.

*Variability attributable to canopy*

$\delta^{13}C$ has previously been reported to vary within the canopy as a result of carbon dioxide recycling at the forest floor (Cerling et al. 2004; Schoeninger et al. 1998). To test this relationship within the Ngogo chimpanzee dietary niche, resources were classified as "canopy" or "ground" resources. Canopy resources were defined as those originating from the canopy (e.g. flowers, fruits, tree leaves, leaf buds, etc). Ground resources were

defined as those originating from the ground (e.g. sapling leaves and pith). Fruit and flowers collected from the ground, therefore, were defined as canopy resources.

Canopy samples averaged -26.6±1.7‰ while ground samples averaged -30.7±2.3‰. This difference was statistically significant ($p<0.0001$), and a similar magnitude (4.1‰ between canopy and ground resources) to that previously reported by Cerling et al (2004). Within the ANOVA model, canopy position was also statistically significant ($p=0.0149$) after controlling for photosynthetic pathway, food type, season, and elevation.

*Variability attributable to elevation*

Elevation was also a significant grouping variable for bulk $\delta^{13}C$ and $\delta^{15}N$ ($p=0.0122$ and 0.0003 respectively). The effect of elevation change within this sample amounted to a 2.13‰ enrichment in $^{13}C$ and 2.96‰ depletion in $^{15}N$ for every 1000 feet of gain (Tables 3.5 and 3.6 respectively).

## Discussion

*Carbon*

Previous isotopic work at Kibale National Park examined the isotopic signature of of chimpanzee tissues (collagen, apatite, and hair), but not the underlying dietary signal providing the molecular framework for that tissue (Carter 2001). Carter reported for the Ngogo chimpanzees $\delta^{13}C_{collagen}$ -21.7‰, $\delta^{13}C_{apatite}$ -15.1‰, and $\delta^{13}C_{hair}$ -21.8.1‰ (Carter

2001). Average fractionation from tissue to diet suggested a dietary $\delta^{13}C$ average between -28 and -24‰ (Smith et al. 2010). This would suggest that the individuals sampled did not consume a great quantity of ground resources (i.e. pith and sapling leaves). Such consumption would have resulted in more depleted hair, collagen, and apatite values than measured by Carter (2001).

This is born out by long-term observations on the Ngogo feeding ecology. Of the top twenty consumed food items, ranked by observed feeding time, eighteen are ripe fruits and only two sapling or mature leaves (David Watts, personal communication). The average $\delta^{13}C$ of ripe fruit at Ngogo is -26.6‰, well within the predicted range from previously analyzed Ngogo chimpanzee tissue (Carter 2001).

Pith and sapling leaves, two major fallback resources utilized by the Ngogo chimpanzees carried $\delta^{13}C$ significantly more depleted than any other food type within the dietary niche. Each was statistically different from all other food types, but not different from the other. This is most likely a result of the canopy effect, whereby biogenic carbon dioxide is recycled at the forest floor and results in the accumulation of carbon dioxide more depleted in $^{13}C$ than at the canopy top.

Within the Ngogo community, fruits are available seasonally with alternating periods of relative abundance and scarcity. Herbaceous vegetation (pith) and sapling leaves, however, are available in relative abundance year round. Correspondingly, during periods of relative fruit scarcity the chimpanzees appear to spend more time consuming sapling leaves and pith.

Isotopic analyses are capable of tracking the consumption of items which may be differentiated isotopically. That pith and sapling leaves are uniquely and especially depleted in $^{13}C$ within the Ngogo dietary niche allows one to track their consumption.

This is especially valuable given the significance of these resources within the niche as fallback foods. Within a habituated community and long term repeated sapling of individual chimpanzees, one may utilize this isotopic characteristic to track access to preferred resources. Serially sampling strands of hair might also prove capable of elucidating cycles of fruit abundance and scarcity. As ripe fruit becomes increasingly available and consumed within the niche, hair synthesized during that period will incorporate amino acids less depleted in $^{13}C$. Conversely, during periods of relative fruit scarcity, consumption of pith and sapling leaves will increase and hair synthesized during this period will incorporate amino acids more depleted in $^{13}C$.

As pith and sapling consumption appear to be the most significant dietary factors capable of depleting tissue $\delta^{13}C$, by sampling a single individual over time one may track their consumption by associating depleted tissue $\delta^{13}C$ with increased consumption of pith and/or sapling leaves. Individual activity patterns or pathology could also influence fractionation, resulting in what might appear as a dietary shift. Therefore, future work must couple behavioral observation with dietary and tissue sampling to further elucidate the various contributions to tissue level variance in $\delta^{13}C$ as well as $\delta^{15}N$.

*Nitrogen*

In contrast to bulk $\delta^{13}C$, the variation in $\delta^{15}N$ across the Ngogo chimpanzee dietary niche is more ambiguous with respect to how it might be utilized to reconstruct consumptive behavior. None of the environmental variables included within the ANOVA models accounted for much of the sample variance in nitrogen isotopes. The full and reduced models, each accounted for only about 25% of the total variance (Table

3.6). In contrast, the best-fit model for $\delta^{13}C$ accounted for over 75% of the total variance (Table 3.5).

To utilize any isotope in the reconstruction of diet, one must find and take advantage of variation between dietary items of interest. This data showed that no food groups (Figure 3.5), individual species, or most other attributes of dietary ecology (incl. canopy height and seasonality) could be meaningfully differentiated by $\delta^{15}N$ signature. The one exception was the effect of elevation, where ANOVA estimated $\delta^{15}N$ to decrease by 2.96‰ for every 1000 feet of elevation gained.

Previous literature has indicated that nitrogen fixing species contain $\delta^{15}N$ values close to 0 (close to atmospheric ratios) whereas non-fixing species are relatively enriched in $^{15}N$ (Kurdali and Al-Shamma'a 2009). Nitrogen fixing status was not assessed in this study, and given 75% of the $\delta^{15}N$ variance remains unresolved, utilization of $N_2$-fixing rhizomes may be one of the key environmental variables necessary to make sense of the nitrogen data.

*General (carbon and nitrogen)*

The effect of canopy position on $\delta^{13}C$ has previously been reported. Within closed canopy forested environments, carbon dioxide is increasingly recycled at the forest floor by resident microbiota. The result is a gradation of highly depleted $^{13}CO_2$ at the forest floor to carbon dioxide far less depleted at the canopy crown (Cerling et al. 2004). Schoeninger and colleagues reported that this effect may be utilized to differentiate between feeding strategies within the canopy, as different primates utilized resources at different heights with corresponding depletion in $^{13}C$ (Schoeninger et al. 1998). Later,

Carter noted this effect may be utilized to investigate changing arboreality among Miocene apes and the early hominins (Carter 2001).

The canopy effect on carbon observed within the Ngogo chimpanzee niche, 4.1‰, was highly significant and consistent with previous findings lending further support to its potential utility in extant primate dietary ecology and the evolution of extinct primate terrestriality.

For extant chimpanzees, canopy and ground based resources are essentially synonymous with preferred and fallback foods. Fruits, matured within the canopy and consumed therein or after falling to the ground are seasonally available highly preferred over other food groups within the chimpanzee niche. Of the top 20 food items consumed by the Ngogo chimpanzees, 17 are fruits. Across the Ngogo range, fruit availability is variable and loosely associated with wet and dry seasons. As sapling leaves and pith are nearly ubiquitous across the Ngogo chimpanzee range, their consumption is often inversely proportional to that of ripe fruit. So, during periods of decreased fruit availability, consumption of sapling leaves and pith (ground based resources) increase.

Given that season differences in isotopic signature of any given resource were non-significant, longitudinal repeat sampling from individual chimpanzees (eg. hair) could reveal variation in consumption of the fallback foods. Increased consumption of the ground based sapling leaves and/or pith would lead to depletion in the whole body pool of $^{13}C$ and all tissues synthesized from it. Alternatively, increased consumption of canopy matured fruit would lead to a less depleted pool of $^{13}C$ and the tissues synthesized at that time would likewise exhibit higher values of $\delta^{13}C$.

By tracking consumption with personal observation and concurrently sampling hair, one may begin to define several variables related to isotopic dietary ecology here-to-

fore unknown: (a) time between initial consumption and the representation of that isotopic signature within the chimpanzees tissue; (b) time horizon represented by a single strand of chimpanzee hair; (c) the magnitude of dietary shift necessary to produce a recoverable or identifiable isotopic shift within the tissues.

Once the above relationships are defined within a habituated population, the isotopic differentiation between canopy and ground resources may be utilized to study the consumptive behavior of unhabituated groups of primates as well. By sampling the resources utilized over the course of a year, one may track access by sampling the hair left behind in night nests or after grooming sessions on the ground. Among unhabituated groups, the ability to recover meaningful dietary information in the absence of observation is critical and a tremendous resource for both science and conservation.

The application of the canopy effect for studying the origins and evolution of hominin terrestriality has been previously expressed, but this study presents a framework previously unavailable. This is the first time that canopy height has been assessed alongside a number of other ecological and dietary variables of interest, and been shown one of the most significant drivers of $\delta^{13}C$ variation within a $C_3$ dominated environment. Our earliest hominin ancestors likely lived in a similarly closed-canopy, $C_3$ dominated environment within East Africa. Current technology allows sampling apatite from modern or fossilized tooth enamel on a scale that minimizes destruction and thereby allows the recovery of biogenic carbon amenable for isotopic analysis. The differentiation between canopy and ground based resources should be identifiable from such sampling, and after elucidating the magnitude of dietary change required to produce a recoverable/identifiable signal would allow the investigation of such consumption at the origins of habitual hominin terrestriality.

However, following an increasing adoption of ground based foraging is a presumed increase in consumption of $C_4$ resources or animals who themselves are $C_4$ resource consumers (ie. grazers). Parsing the contributions of ground based $C_3$ resources (more negative $\delta^{13}C$) from ground based $C_4$ resources (less negative $\delta^{13}C$) remains a challenge and requires further investigation of the isotopic and nutritional contributions of animal source foods within extant primate groups (e.g. chimpanzees and baboons). Extracting biogenic nitrogen from fossilized material may dramatically expand the ability to differentiate between the consumption of paleodietary components, but as yet has not been explored within an anthropological context.

Perhaps most interesting among environmental variables examined herein is the statistically significant attribution of elevation change for both $\delta^{13}C$ and $\delta^{15}N$. This affect may be related to or directly controlled by changing partial pressures of carbon dioxide and atmospheric nitrogen at increasing elevation. Alternatively, this affect may result from microclines within the Ngogo range. At Ngogo, the relief ranges from approximately 1200 to 1500m, with no more than 300m (or approximately 1000 ft.) between the lowest and highest points of the foraging range. The lowest elevations are largely characterized by streambeds, and swamp ecosystems. The highest elevations, conversely, are associated with forest margin habitats, and open patches of grassland. Therefore, the affect of elevation within this sample may simply track ecological factors attributed to the local topography and not remain consistent across study sites.

If this effect is found consistently across study sites, it may provide a critical alternative explanation for what has previously been interpreted as a $C_4$ shift in consumption among australopiths, *Paranthropus* and early *Homo*. Extrapolated to a 2000 ft. elevation gain, the dietary $\delta^{13}C$ and $\delta^{15}N$ would shift +4.36 and -5.84‰ respectively.

Therefore, the +4‰ shift in $\delta^{13}C$ observed for all South African hominin may be explained if these individuals were foraging approximately 650 m. higher than the associated fauna. This may not be very likely. However, it may be one alternative way of achieving the same tissue level isotopic signature.

The ultimate goal for isotopic dietary reconstruction is to differentiate between individual resources. At the level of bulk carbon and nitrogen, specific differentiation within a closed canopy $C_3$ dietary niche remains problematic. Figure 3.7 depicts the individual carbon and nitrogen isotopic signatures for ten common foods within the Ngogo dietary niche. To differentiate their contributions to tissue isotopic composition, one must identify an isotopic domain where there is substantial separation between dietary signatures. Unfortunately, there is no systematic separation in $\delta^{13}C$ or $\delta^{15}N$ among these resources.

Mixing models developed to back calculate a range of potential dietary contributions to tissue isotopes often take advantage of differentiation between two or three stable isotopes and then are only capable of teasing apart the relative contributions of only a few resources, typically 2-3 (Phillips and Gregg 2001; Phillips and Gregg 2003). This presents a significant challenge for reconstructing the diet of eclectic omnivorous species such as the Ngogo chimpanzees, which have been observed to utilize over 100 different dietary items from nearly as many different plant species.

Nutrient specific isotopic analyses are an emerging methodology with the potential for providing a solution to the problem of equifinality associated with bulk isotopic analyses. Nutrient specific isotopic analyses recognize that foods and tissues are built from individual building blocks, all of which have the potential to carry unique isotopic signatures characteristic of their unique biochemical origins and history. Hair,

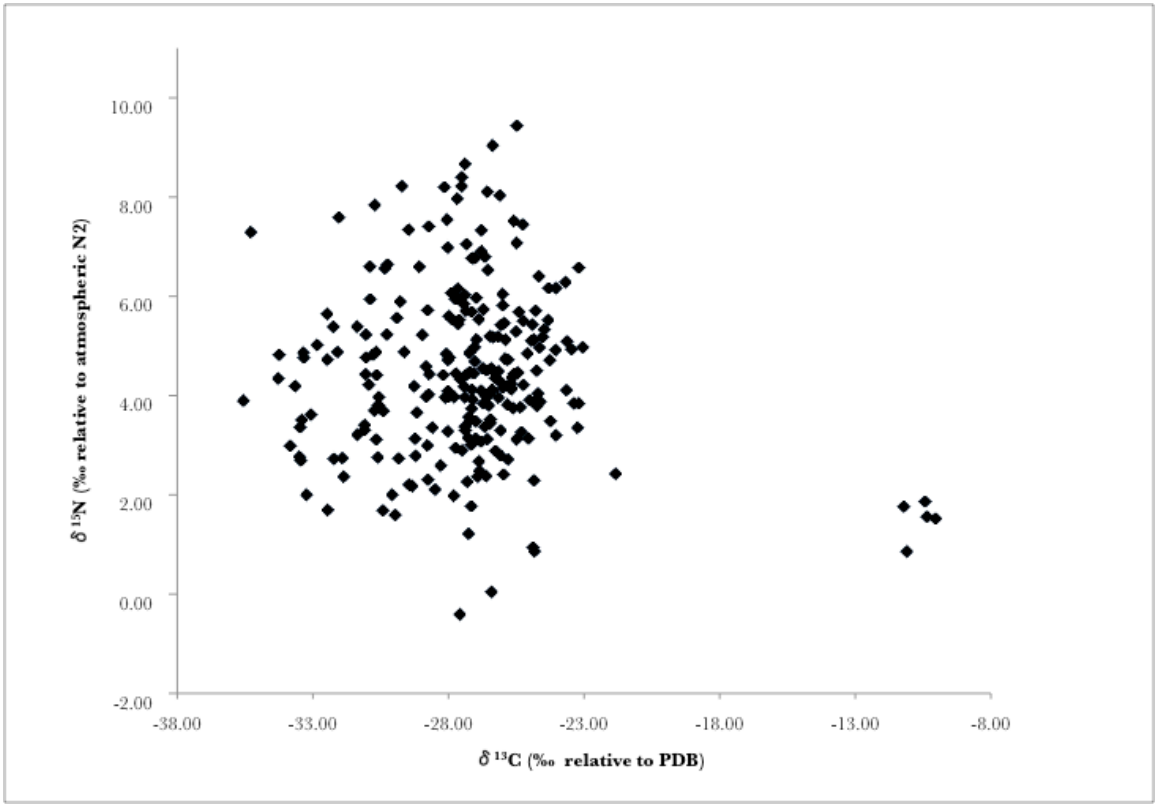
bone collagen, and enamelins and amelogenins within tooth enamel are all made of individual amino acids, which have been incorporated directly from the diet (essential amino acids) or synthesized from other dietary precursors. By analyzing each amino acid separately, one is able to capture and reconstruct their unique histories (O'Brien et al. 2005; Petzke et al. 2005a). This is an exciting direction for future research and one that could significantly alter the landscape of ecological and dietary reconstruction.

## Conclusion

Dietary reconstruction via stable isotopic analyses requires an environmental or dietary template to interpret the signature obtained from the organism. These results suggest that apart from carbon fractionation in $C_3$ and $C_4$ photosynthetic plants, there may be a number of additional variables responsible for driving variability among and between plant species utilizing the same photosynthetic pathway. Isotopic variability within the Ngogo chimpanzee dietary niche was most significantly attributable to photosynthetic system ($C_3$ or $C_4$), plant anatomy (i.e. food type), and elevation.

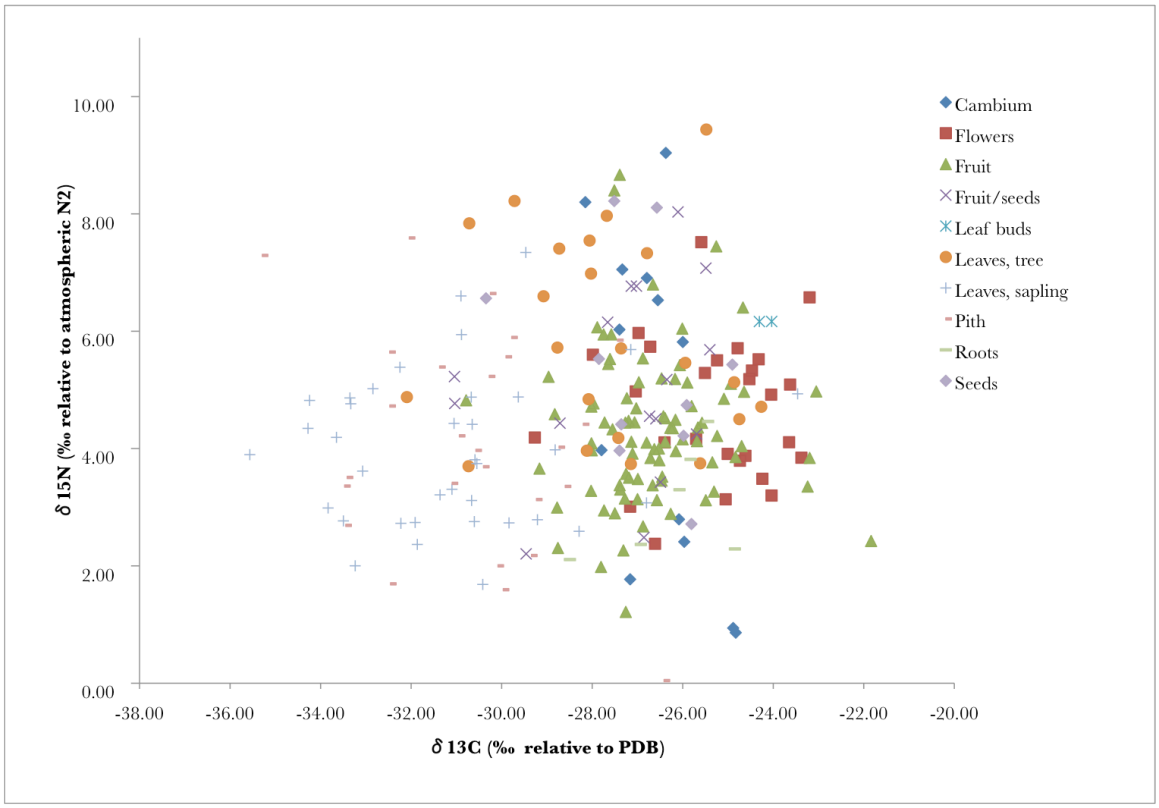
The $\delta^{13}C$ and $\delta^{15}N$ values within this $C_3$ dominated ecosystem were highly variable, with $\delta^{13}C$ ranging nearly 15‰ among $C_3$ resources from most to least depleted and $\delta^{15}N$ ranging approximately 10‰ from most to least enriched. A considerable amount of the variability in $\delta^{13}C$ could be attributed to photosynthetic pathway, food type, and elevation. However, these same variables could only account for 25% of the variability among $\delta^{15}N$. Acknowledging and characterizing this environmental variability is a critical step for the application of stable isotopic methods to extant primate populations, archaeological populations, and hominin remains alike.

Figure 3.1



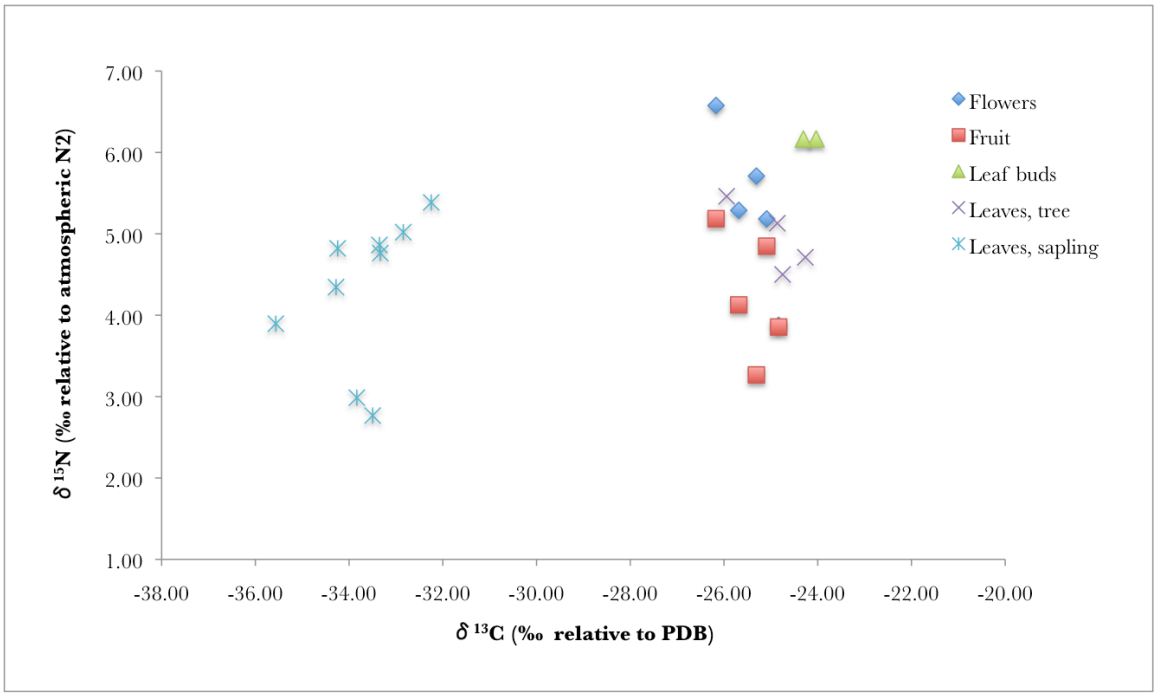

Bulk isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of the Ngogo chimpanzee dietary niche. Cluster to the left are all $C_3$ plants, whereas the small cluster to the right represents the small contribution of $C_4$ resources (the pith from papyrus).

Figure 3.2



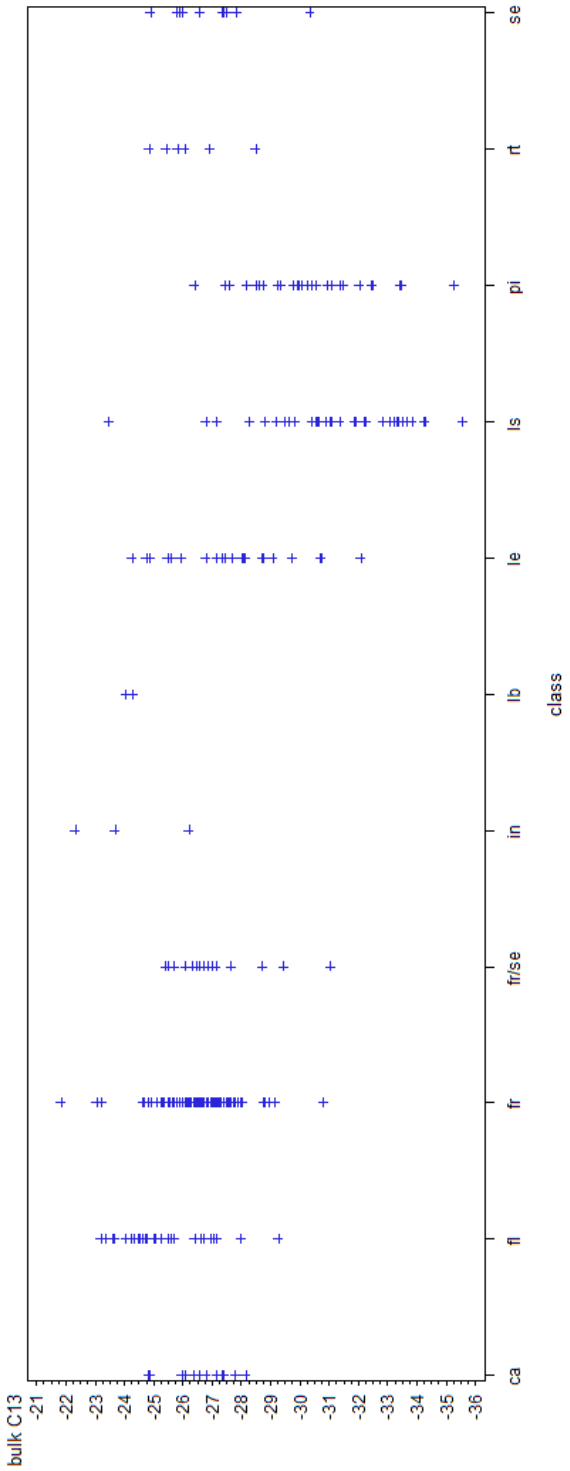

Bulk isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of the Ngogo chimpanzee dietary niche by food type. Clustering of sapling leaves and pith are observed to the left (more depleted), while flowers and fruit appear clustered to the right (less depleted).

Figure 3.3



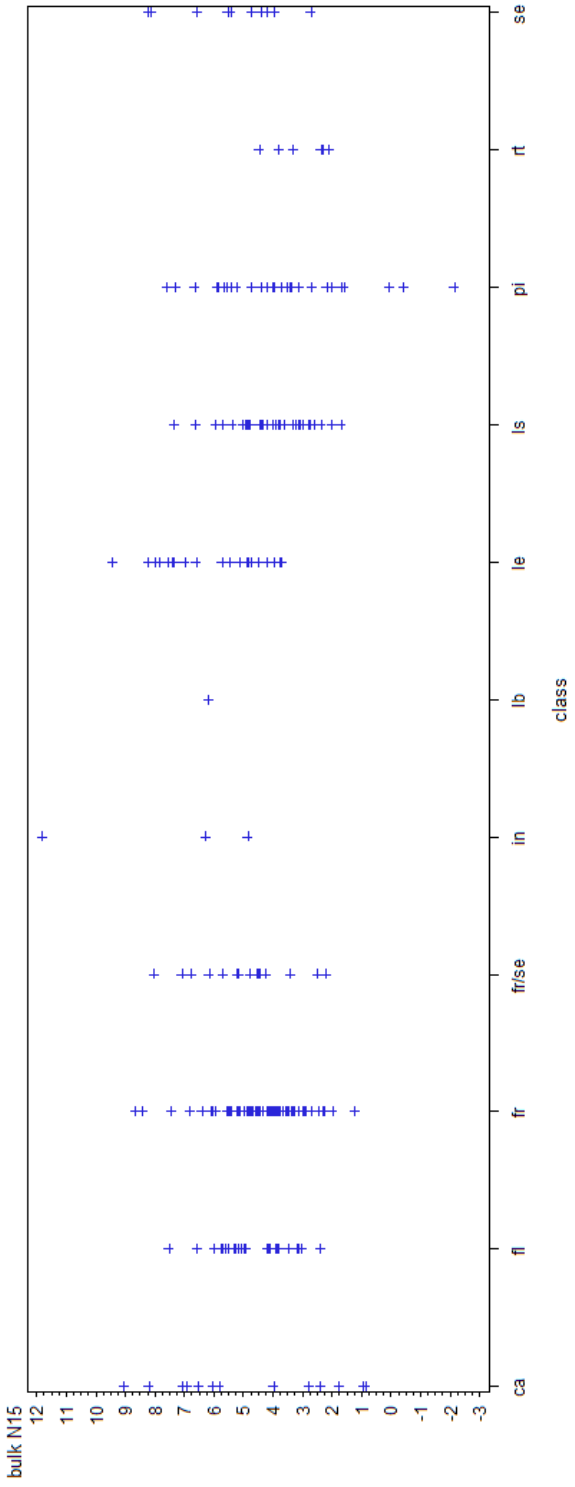

Bulk isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of *Morus lactea* by anatomical component (food type). Clear differentiation of the sapling leaves is observed to the left (more depleted). Other food types are clumped by $\delta^{13}C$ but may be differentiated by $\delta^{15}N$, with fruit least enriched and flowers and leaf buds most enriched in $^{15}N$.

Figure 3.4



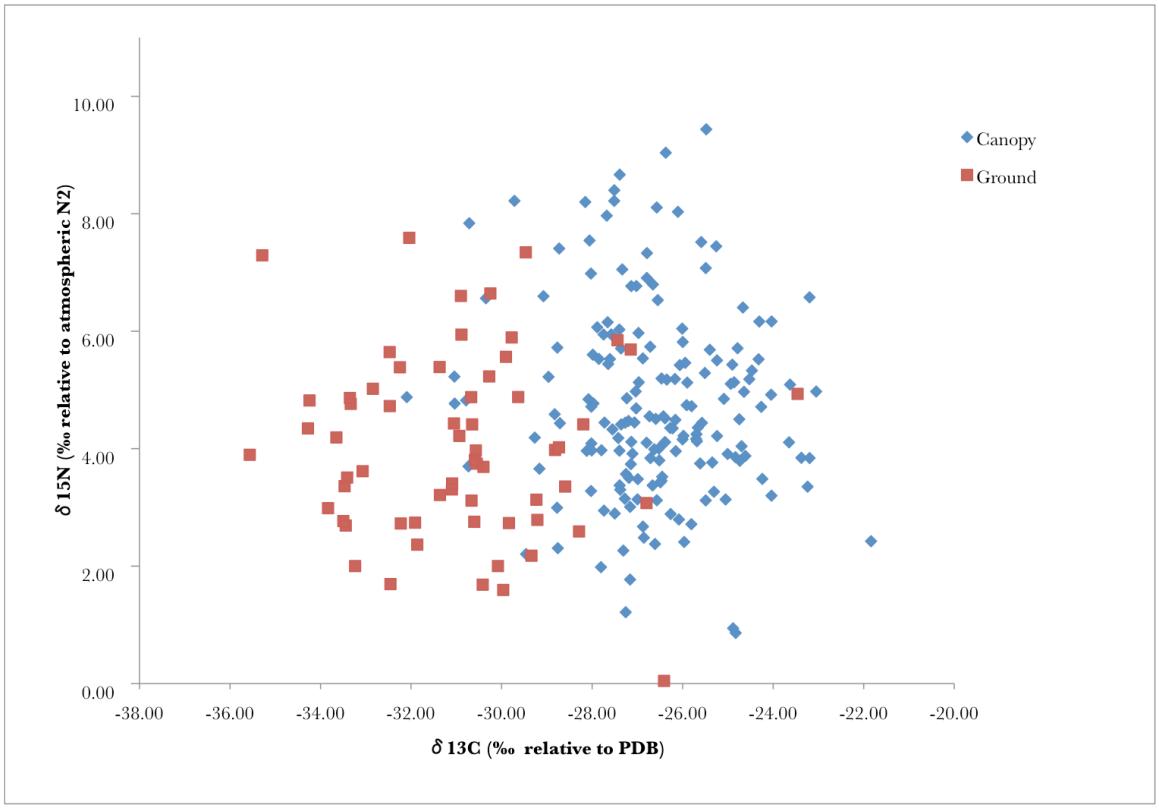

Differentiation in $\delta^{13}C$ by food type. Ca=cambium, fl=flower, fr=fruit, fr/se=fruit/seed, in=insect, lb=leaf buds, le=leaves of tree, ls=leaves of sapling, pi=pith, rt=root, se=seed.

Figure 3.5



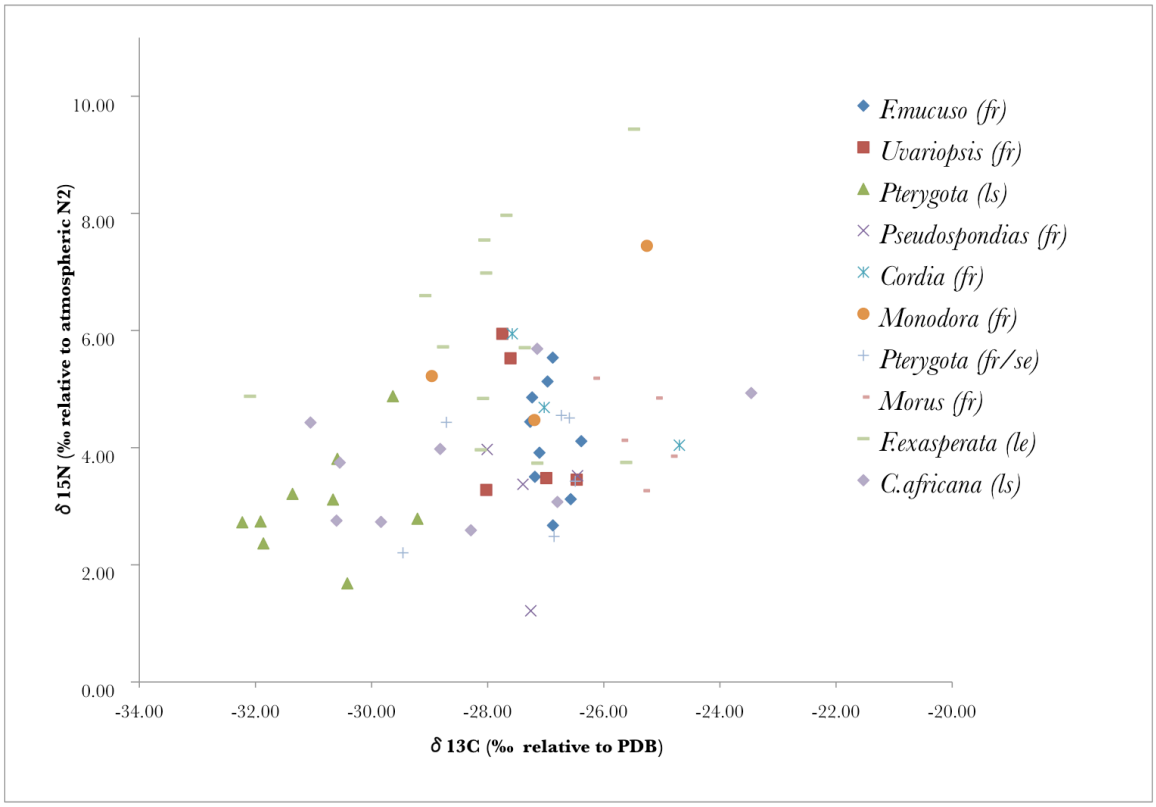

Differentiation in $\delta^{15}N$ by food type. Ca=cambium, fl=flower, fr=fruit, fr/se=fruit/seed, in=insect, lb=leaf buds, le=leaves of tree, ls=leaves of sapling, pi=pith, rt=root, se=seed.

Figure 3.6




Bulk isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of the Ngogo chimpanzee dietary niche: canopy v. ground. Differentiation can clearly be seen between canopy resources (on average) to the right and ground resources (on average) to the left.

Figure 3.7




Bulk isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) from 10 of the most commonly consumed foods within the Ngogo chimpanzee dietary niche. Given substantial overlap between individual resources, dietary reconstruction of individual species or resources using bulk isotopic analyses remains a challenge.

# Table 3.1

Sampled plant taxa by food type

| | Number of samples collected per food type | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Taxa | Fr | Se | Fr+se | Ls | Le | Lb | Pi | Fl | Ca | Rt | Dw | Total |
| *Acanthus sp.* | | | | | | | 7 | | | | | 7 |
| *Afromomum sp.* | | | | | | | 19 | | | | | 19 |
| *Aningeria altissima* | 5 | 5 | | | | | | | | | | 10 |
| *Antiaris toxicaria* | | | | | | | | 2 | | | | 2 |
| *Celtis africana* | | | | 9 | | | | | | | | 9 |
| *Celtis durandii* | | | 6 | | | | | | | | | 6 |
| *Celtis mildbraedii* | | | | 3 | 1 | | | | | | | 4 |
| *Chaetacme aristata* | | | | | 5 | | | | 8 | | | 13 |
| *Chrysophyllum albidum* | 9 | 9 | | | | | | | | | | 18 |
| *Cola gigantea* | 3 | 3 | | | | | | | | | | 6 |
| *Cordia millenii* | 3 | 3 | | | | | | 2 | | | | 8 |
| *Cyperus papyrus* | | | | | | | 5 | | | | | 5 |
| *Ficus brachylepis* | | | 5 | | | | | | | | | 5 |
| *Ficus capensis* | | | 2 | | | | | | | | | 2 |
| *Ficus cyathistipula* | | | 5 | | | | | | | | | 5 |
| *Ficus dawei* | | | 3 | | | | | | | | | 3 |
| *Ficus exasperata* | | | 7 | | 12 | | | | | | | 19 |
| *Ficus mucuso* | | | 9 | | | | | | | | | 9 |
| *Ficus natalensis* | | | 6 | | | | | | | | | 6 |
| *Ficus stipulifera* | | | 1 | | | | | | | | | 1 |
| *Ficus thonningii* | | | 2 | | | | | | | | | 2 |
| *Ficus vallis-choudea* | | | 1 | | | | | | | | | 1 |
| *Marantochloa sp.* | | | | | | | 5 | | | | | 5 |
| *Mimusops bagshawei* | | | 6 | | | | | | | | | 6 |
| *Monodora myristica* | 3 | 3 | 5 | 6 | | | | 10 | | | | 27 |
| *Morus lactea* | | | 5 | 9 | 4 | 2 | | 5 | | | | 25 |
| *Neoboutonia macrocalyx* | 5 | 5 | | | | | | | | 5 | 2 | 17 |
| *Piper sp.* | | | | | | | 9 | | | | | 9 |
| *Premna angolensis* | | | | | | | | | 5 | | | 5 |
| *Pseudospondias microcarpa* | 4 | 4 | | | | | | | | | | 8 |
| *Pterygota mildbraedii* | | | 6 | 9 | | | | 10 | 5 | | | 30 |
| *Tabernamontana holstii* | | | 5 | | | | | | | | | 5 |
| *Treculia africana* | 4 | 4 | | | | | | | | | | 8 |
| *Uvariopsis congensis* | 7 | 7 | | | | | | | | | | 14 |
| *Warburgia ugandensis* | | | 7 | | | | | | | | | 7 |
| Unidentified | | | 1 | | | | | 1 | | | 2 | 4 |
| Unidentified fig | | | 2 | | | | | | | | | 2 |
| Unidentified fern | | | | 1 | | | | | | | | 1 |
| Total samples | 43 | 43 | 84 | 37 | 22 | 2 | 45 | 30 | 18 | 5 | 4 | 333 |
| Total taxa represented | 9 | 9 | 19 | 6 | 4 | 1 | 5 | 6 | 3 | 1 | 2 | - |

KEY: Fr=fruit, Se=seeds, Fr/se=fruit with seeds, Ls=leaves of saping, Le=leaves of mature tree , Lb=leaf buds, Pi=pith, Fl=flower, Ca=cambium, Rt=roots, Dw=dead wood

# Table 3.2

Anatomical differences in the carbon and nitrogen isotopic composition by taxon

| | | | Plant Part | | Species average | |
|---|---|---|---|---|---|---|
| Taxon | Plant Part | *n* | $\delta^{13}C$ (‰) | $\delta^{15}N$ (‰) | $\delta^{13}C$ (‰) | $\delta^{15}N$ (‰) |
| *Acanthus sp.* | Pith | 7 | -29.4 ± 2.3 | 1.4 ± 2.3 | -29.4 ± 2.3 | 1.4 ± 2.3 |
| *Afromomum sp.* | Pith | 8 | -29.5 ± 1.8 | 3.9 ± 1.7 | -29.5 ± 1.8 | 3.9 ± 1.7 |
| *Aningeria altissima* | Fruit | 5 | -25.7 ± 1.5 | 4.8 ± 0.3 | -25.7 ± 1.5 | 4.8 ± 0.3 |
| *Celtis africana* | Leaves, sapling | 9 | -29.1 ± 1.6 | 3.8 ± 1.1 | -29.1 ± 1.6 | 3.8 ± 1.1 |
| *Celtis durandii* | Fruit+seeds | 5 | -26.2 ±0.8 | 6.9 ± 0.8 | -26.2 ±0.8 | 6.9 ± 0.8 |
| *Celtis mildbraedii* | Leaves, sapling | 3 | -30.3 ± 0.8 | 6.1 ± 1.2 | -30.2 ± 0.7 | 6.6 ± 1.5 |
| | Leaves, tree | 1 | -29.7 | 8.2 | | |
| *Chaetacme aristata* | Leaves, tree | 5 | -28.9 ± 1.8 | 6.1 ± 2.0 | -27.8 ± 1.5 | 6.5 ± 1.7 |
| | Cambium | 8 | -27.1 ± 0.7 | 6.7 ± 1.5 | | |
| *Chrysophyllum albidum* | Fruit | 9 | -27.3 ± 1.0 | 4.7 ± 1.7 | -27.3 ± 1.0 | 4.7 ± 1.7 |
| *Cordia millenii* | Fruit | 3 | -26.4 ± 1.5 | 4.9 ± 1.0 | -26.2 ± 1.3 | 4.0 ± 1.4 |
| | Flowers | 2 | -25.8 ± 1.1 | 2.8 ± 0.5 | | |
| *Cyperus papyrus* | Pith | 5 | -10.6 ± 0.5 | 1.5 ± 0.4 | -10.6 ± 0.5 | 1.5 ± 0.4 |
| *Ficus brachylepis* | Fig | 5 | -28.3 ± 1.6 | 4.3 ±1.4 | -28.3 ± 1.6 | 4.3 ±1.4 |
| *Ficus dawei* | Fig | 3 | -26.0 ± 0.9 | 4.0 ± 0.8 | -26.0 ± 0.9 | 4.0 ± 0.8 |
| *Ficus exasperata* | Fig | 7 | -26.3 ± 1.4 | 4.9 ± 1.0 | -27.3 ± 1.8 | 5.5 ± 1.6 |
| | Leaves, tree | 12 | -28.0 ± 1.7 | 5.9 ± 1.8 | | |
| *Ficus mucuso* | Fig | 9 | -26.9 ± 0.3 | 4.1 ± 0.9 | -26.9 ± 0.3 | 4.1 ± 0.9 |
| *Ficus natalensis* | Fig | 6 | -26.9 ± 0.8 | 3.8 ± 0.7 | -26.9 ± 0.8 | 3.8 ± 0.7 |
| *Marantochloa sp.* | Pith | 5 | -32.3 ± 1.7 | 5.5 ± 1.6 | -32.3 ± 1.7 | 5.5 ± 1.6 |
| *Mimusops bagshawei* | Fruit+seeds | 6 | -26.7 ± 0.9 | 3.7 ± 0.9 | -26.7 ± 0.9 | 3.7 ± 0.9 |
| *Monodora myristica* | Fruit | 3 | -27.1 ± 1.9 | 5.7 ± 1.5 | -28.4 ± 2.7 | 5.1 ± 1.4 |
| | Seeds | 3 | -28.6 ± 1.5 | 6.8 ± 1.4 | | |
| | Fruit+seeds | 5 | -28.4 ± 2.5 | 5.1 ± 0.7 | | |
| | Leaves, sapling | 6 | -32.1 ± 1.4 | 4.0 ± 1.5 | | |
| | Flowers | 10 | -26.6 ± 1.4 | 5.0 ± 1.3 | | |
| *Morus lactea* | Fruit+seeds | 5 | -25.4 ± 0.5 | 4.3 ± 0.8 | -28.0 ± 4.4 | 4.8 ± 1.0 |
| | Leaves, tree | 4 | -25.0 ± 0.7 | 5.0 ± 0.4 | | |
| | Leaves, sapling | 9 | -33.7 ± 1.0 | 4.2 ± 0.9 | | |
| | Leaf buds | 2 | -24.2 ± 0.2 | 6.2 ± 0.0 | | |
| | Flowers | 5 | -24.5 ± 0.8 | 5.9 ± 0.9 | | |
| *Neoboutonia macrocalyx* | Fruits | 5 | -27.6 ± 0.8 | 4.9 ± 2.7 | -26.6 ± 1.2 | 4.2 ± 2.0 |
| | Seeds | 5 | -26.1 ± 0.9 | 5.0 ± 2.0 | | |
| | Roots | 5 | -26.3 ± 1.3 | 3.0 ± 1.0 | | |
| *Piper sp.* | Pith | 9 | -31.3 ± 1.4 | 4.5 ± 1.3 | -31.3 ± 1.4 | 4.5 ± 1.3 |
| *Pseudospondias microcarpa* | Fruit | 4 | -27.3 ± 0.6 | 3.0 ± 1.2 | -27.3 ± 0.6 | 3.0 ± 1.2 |
| *Pterygota mildbraedii* | Fruit+seeds | 6 | -27.5 ± 1.3 | 3.6 ± 1.1 | -27.2 ± 2.9 | 3.4 ± 1.3 |
| | Leaves, sapling | 9 | -30.9 ± 1.0 | 3.0 ± 0.9 | | |
| | Flowers | 10 | -24.4 ± 1.0 | 1.8 ± 0.9 | | |
| | Cambium | 5 | -25.8 ± 1.0 | 1.8 ± 0.9 | | |
| *Treculia africana* | Fruit | 4 | -26.0 ± 1.4 | 3.8 ± 1.0 | -26.2 ± 1.2 | 3.9 ± 0.8 |
| | Seeds | 2 | -26.7 ± 1.0 | 4.3 ± 0.1 | | |
| *Uvariopsis congensis* | Fruit | 5 | -27.4 ± 0.6 | 4.3 ± 1.3 | | |
| *Warburgia ugandensis* | Fruit+seeds | 6 | -24.6 ± 2.2 | 4.0 ± 1.0 | -24.6 ± 2.2 | 4.0 ± 1.0 |
| Total | | 250 | | | | |

Table 3.3

Plant part differences in carbon and nitrogen isotopic composition

| Photosynth. | Plant Part | *n* | $\delta^{13}C$ (‰) | $\delta^{15}N$ (‰) |
|---|---|---|---|---|
| **C3** | **All** | **246** | **-27.7 ± 2.6** | **4.5 ± 1.8** |
| | Leaves, sapling | 36 | -31.1 ± 2.4 | 4.0 ± 1.3 |
| | Pith | 29 | -30.5 ± 2.1 | 4.1 ± 2.9 |
| | Leaves, tree | 22 | -27.7 ± 2.1 | 5.9 ± 1.7 |
| | Fruit+seeds | 16 | -27.4 ± 1.8 | 5.1 ± 1.6 |
| | Seeds | 10 | -27.0 ± 1.5 | 5.4 ± 1.8 |
| | Fruit | 85 | -26.6 ± 1.4 | 4.3 ± 1.3 |
| | Cambium | 13 | -26.6 ± 1.0 | 4.8 ± 2.8 |
| | Roots | 6 | -26.3 ± 1.3 | 3.1 ± 1.0 |
| | Flowers | 27 | -25.3 ± 1.5 | 4.7 ± 1.2 |
| | Leaf buds | 2 | -24.2 ± 0.2 | 6.2 ± 0.1 |
| **C4** | **All** | **5** | **-10.6 ± 0.5** | **1.5 ± 0.4** |
| | Pith | 5 | -10.6 ± 0.5 | 1.5 ± 0.4 |

Table 3.4

Canopy effect among C3 plants

| | *n* | $\delta^{13}C$ (‰) | $\delta^{15}N$ (‰) |
|---|---|---|---|
| Canopy | 157 | -26.6 ± 1.7 | 4.7 ± 1.6 |
| Ground | 70 | -30.7 ± 2.3 | 4.0 ± 1.9 |
| *p-value* | | *p < 0.0001* | *p=0.0091* |

*Student's TTEST for differences between canopy and sub-canopy
*1-tailed, unequal variances

Table 3.5

ANOVA - Sources of variation for bulk $\delta^{13}C$ (‰ relative to PDB)

| *Model* | *R-square* |
|---|---|
| C3/C4 | 0.451 |
| Canopy | 0.138 |
| Food type | 0.235 |
| C3/C4 + Food type | 0.768 |
| C3/C4 + Food type + Season | 0.769 |
| C3/C4 + Food type + Canopy | 0.771 |
| C3/C4 + Food type + Elevation | 0.776 |
| Full[1] | 0.782 |

| *Variable in full model*[1] | *F-value*[3] | *P-value*[4] |
|---|---|---|
| C3/C4 | 516.03 | < 0.0001 |
| Food Type | 3.98 | < 0.0001 |
| Elevation | 9.18 | 0.0027 |
| Canopy | 6.02 | 0.0149 |
| Season | 0.34 | 0.5594 |

| *Effect in reduced model*[2] | *Estimate*[5] | *P-value* |
|---|---|---|
| C4 (papyrus) | 19.4 | < 0.0001 |
| Food type = cambium | -0.215 | 0.779 |
| Food type = flower | 1.49 | 0.0232 |
| Food type = fruit | 0.0361 | 0.951 |
| Food type = fruit/seed | -0.678 | 0.335 |
| Food type = leaf bud | 2.50 | 0.0624 |
| Food type = mature leaf | -1.27 | 0.0668 |
| Food type = sapling leaf | -4.40 | < 0.0001 |
| Food type = pith | -3.64 | < 0.0001 |
| Food type = roots | 0.589 | 0.508 |
| Food type = seeds | 0.000 | . |
| Elevation (change in ‰ per 1000 ft gain) | 2.18 | 0.0122 |

[1] Full model = C3/C4 + Food type + Elevation + Season + Canopy

[2] Reduced model = C3/C4 + Food type + Elevation

[3] The F-value in ANOVA is a measure of variance between groups relative to within groups. The greater the F-value, the more between group variance relative to within group variance.

[4] P-value is the probability of obtaining a greater F-value by chance

[5] The Estimate represents the effect of each variable upon bulk $\delta^{13}C$ controlling for all other variables in the model

Table 3.6

ANOVA - Sources of variation for bulk $\delta^{15}N$ (‰ relative to atmospheric N2)

| *Model* | *R-square* |
|---|---|
| C3/C4 | 0.0546 |
| Canopy | 0.0474 |
| Food type | 0.197 |
| C3/C4 + Food type | 0.223 |
| C3/C4 + Food type + Season | 0.225 |
| C3/C4 + Food type + Canopy | 0.223 |
| C3/C4 + Food type + Elevation | 0.249 |
| Full[1] | 0.256 |

| *Variable in full model*[1] | *F-value*[3] | *P-value*[4] |
|---|---|---|
| C3/C4 | 3.93 | 0.0487 |
| Food Type | 5.26 | < 0.0001 |
| Elevation | 14.9 | 0.0001 |
| Canopy | 1.67 | 0.197 |
| Season | 0.74 | 0.391 |

| *Effect in reduced model*[2] | *Estimate*[5] | *P-value* |
|---|---|---|
| C4 (papyrus) | -2.12 | 0.0350 |
| Food type = cambium | 0.229 | 0.741 |
| Food type = flower | -0.252 | 0.671 |
| Food type = fruit | -0.705 | 0.185 |
| Food type = fruit/seed | 0.0803 | 0.900 |
| Food type = leaf bud | 1.16 | 0.336 |
| Food type = mature leaf | 1.21 | 0.0533 |
| Food type = sapling leaf | -1.14 | 0.0448 |
| Food type = pith | -1.47 | 0.012 |
| Food type = roots | -2.19 | 0.007 |
| Food type = seeds | 0.000 | . |
| Elevation (change in ‰ per 1000 ft gain) | -2.92 | 0.0003 |

[1] Full model = C3/C4 + Food type + Elevation + Season + Canopy

[2] Reduced model = C3/C4 + Food type + Elevation

[3] The F-value in ANOVA is a measure of variance between groups relative to within groups. The greater the F-value, the more between group variance relative to within group variance.

[4] P-value is the probability of obtaining a greater F-value by chance

[5] The Estimate represents the effect of each variable upon bulk $\delta^{15}N$ controlling for all other variables in the model

ANOVA: Least Squares Means for Effect of Food Type

Pr > |t| for $H_0$: LSMean(i)=LSMean(j)

Dependant Variable: Bulk $\delta^{13}C$ (‰ relative to PDB), Model=Reduced[1]

| i/j | ca | fl | fr | fr/se | lb | le | ls | pi | rt | se |
|---|---|---|---|---|---|---|---|---|---|---|
| ca | | 0.1303 | 1.0000 | 0.9995 | 0.5496 | 0.7664 | **<0.0001** | **<0.0001** | 0.9956 | 1.0000 |
| fl | 0.1303 | | **0.0096** | **0.0041** | 0.9985 | **<0.0001** | **<0.0001** | **<0.0001** | 0.9786 | 0.4025 |
| fr | 1.0000 | **0.0096** | | 0.8828 | 0.5965 | 0.0713 | **<0.0001** | **<0.0001** | 0.9990 | 1.0000 |
| fr/se | 0.9995 | **0.0041** | 0.8828 | | 0.2919 | 0.9903 | **<0.0001** | **<0.0001** | 0.8765 | 0.9938 |
| lb | 0.5496 | 0.9985 | 0.5965 | 0.2919 | | 0.0951 | **<0.0001** | **<0.0001** | 0.9376 | 0.6875 |
| le | 0.7664 | **<0.0001** | 0.0713 | 0.9903 | 0.0951 | | **<0.0001** | **0.0003** | 0.3960 | 0.7080 |
| ls | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | | 0.7677 | **<0.0001** | **<0.0001** |
| pi | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **0.0003** | 0.7677 | | **<0.0001** | **<0.0001** |
| rt | 0.9956 | 0.9786 | 0.9990 | 0.8765 | 0.9376 | 0.3960 | **<0.0001** | **<0.0001** | | 0.9997 |
| se | 1.0000 | 0.4025 | 1.0000 | 0.9938 | 0.6875 | 0.7080 | **<0.0001** | **<0.0001** | 0.9997 | |

[1] ANOVA model: Bulk $\delta^{13}C$ = C3/C4 + food type + elevation

Table 3.7

Table 8

ANOVA: Least Squares Means for Effect of Food Type

Pr > |t| for $H_0$: LSMean(i)=LSMean(j)

Dependant Variable: Bulk $\delta^{15}N$ (‰ relative to atmospheric N2), Model=Reduced[1]

| i/j | ca | fl | fr | fr/se | lb | le | ls | pi | rt | se |
|---|---|---|---|---|---|---|---|---|---|---|
| ca | | 0.9969 | 0.6394 | 1.0000 | 0.9987 | 0.7332 | 0.2140 | 0.0683 | 0.0727 | 1.0000 |
| fl | 0.9969 | | 0.9582 | 0.9997 | 0.9650 | 0.0555 | 0.4737 | 0.1428 | 0.1687 | 1.0000 |
| fr | 0.6394 | 0.9582 | | 0.7049 | 0.8054 | **<0.0001** | 0.9307 | 0.4648 | 0.4282 | 0.9462 |
| fr/se | 1.0000 | 0.9997 | 0.7049 | | 0.9953 | 0.4743 | 0.2244 | 0.0585 | 0.0780 | 1.0000 |
| lb | 0.9987 | 0.9650 | 0.8054 | 0.9953 | | 1.0000 | 0.5725 | 0.3882 | 0.2062 | 0.9939 |
| le | 0.7332 | 0.0555 | **<0.0001** | 0.4743 | 1.0000 | | **<0.0001** | **<0.0001** | **0.0002** | 0.6401 |
| ls | 0.2140 | 0.4737 | 0.9307 | 0.2244 | 0.5725 | **<0.0001** | | 0.9983 | 0.8821 | 0.5878 |
| pi | 0.0683 | 0.1428 | 0.4648 | 0.0585 | 0.3882 | **<0.0001** | 0.9983 | | 0.9902 | 0.2572 |
| rt | 0.0727 | 0.1687 | 0.4282 | 0.0780 | 0.2062 | **0.0002** | 0.8821 | 0.9902 | | 0.1711 |
| se | 1.0000 | 1.0000 | 0.9462 | 1.0000 | 0.9939 | 0.6401 | 0.5878 | 0.2572 | 0.1711 | |

[1] ANOVA model: Bulk $\delta^{15}N$ = C3/C4 + food type + elevation

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# SAS CODE

```
/*ISOTOPIC DIETARY ECOLOGY:
/*BULK d13C and d15N CHARACTERIZATION OF THE CHIMPANZEE
/*DIETARY NICHE AT NGOGO, KIBALE NATIONAL PARK, UGANDA

/*DISSERTATION RESEARCH
/*BRYCE CARLSON*/

PROC IMPORT DATAFILE="Y:\Bryce's
        Documents\Emory\Research\DISSERTATION\DATA\bulk.27mar11.xlsx"
        OUT=a replace; sheet="Full.SASinput";
        RUN;

PROC CONTENTS DATA=a;
        RUN;

DATA bulk; SET a;
        IF class="ls" THEN sapling=1;
                ELSE IF class~="ls" THEN sapling=0;
        IF class="pi" THEN pith=1;
                ELSE IF class~="pi" THEN pith=0;
        IF class="le" THEN treeleaf=1;
                ELSE IF class~="le" THEN treeleaf=0;
```

```
IF class="fr/se" THEN fruitseed=1;
        ELSE IF class~="fr/se" THEN fruitseed=0;
IF class="se" THEN seed=1;
        ELSE IF class~="se" THEN seed=0;
IF class="fr" THEN fruit=1;
        ELSE IF class~="fr" THEN fruit=0;
IF class="ca" THEN cambium=1;
        ELSE IF class~="ca" THEN cambium=0;
IF class="rt" THEN root=1;
        ELSE IF class~="rt" THEN root=0;
IF class="fl" THEN flower=1;
        ELSE IF class~="fl" THEN flower=0;
IF class="lb" THEN leafbud=1;
        ELSE IF class~="lb" THEN leafbud=0;
IF class="in" THEN insect=1;
        ELSE IF class~="in" THEN insect=0;

IF season="dry" THEN dryseason=1;
        ELSE IF season~="dry" THEN dryseason=0;
IF season="wet" THEN wetseason=1;
        ELSE IF season~="wet" THEN wetseason=0;

IF species="PTm" THEN pterygota=1;
```

```
        ELSE IF species~="PTm" THEN pterygota=0;
IF species="COm" THEN cordia=1;
        ELSE IF species~="COm" THEN cordia=0;
IF species="Ml" THEN morus=1;
        ELSE IF species~="Ml" THEN morus=0;
IF species="Mm" THEN monodora=1;
        ELSE IF species~="Mm" THEN monodora=0;
IF species="Fe" THEN exasperata=1;
        ELSE IF species~="Fe" THEN exasperata=0;
IF species="Nm" THEN neoboutonia=1;
        ELSE IF species~="Nm" THEN neoboutonia=0;
IF species="CHAa" THEN chaetacme=1;
        ELSE IF species~="CHAa" THEN chaetacme=0;
IF species="PAP" THEN papyrus=1;
        ELSE IF species~="PAP" THEN papyrus=0;
IF species="AC" THEN acanthus=1;
        ELSE IF species~="AC" THEN acanthus=0;
IF species="AF2" THEN aframomum=1;
        ELSE IF species~="AF2" THEN aframomum=0;
IF species="PI" THEN piper=1;
        ELSE IF species~="PI" THEN piper=0;
IF species="MA" THEN marantochloa=1;
        ELSE IF species~="MA" THEN marantochloa=0;
```

```
IF species="CEa" THEN celtisaf=1;
        ELSE IF species~="CEa" THEN celtisaf=0;
IF species="Cm" THEN celtismi=1;
        ELSE IF species~="Cm" THEN celtismi=0;
IF species="Fm" THEN mucuso=1;
        ELSE IF species~="Fm" THEN mucuso=0;
IF species="Uc" THEN uvariopsis=1;
        ELSE IF species~="Uc" THEN uvariopsis=0;
IF species="PSm" THEN pseudospondias=1;
        ELSE IF species~="PSm" THEN pseudospondias=0;
IF species="Aa" THEN aningeria=1;
        ELSE IF species~="Aa" THEN aningeria=0;
IF species="Mb" THEN mimusops=1;
        ELSE IF species~="Mb" THEN mimusops=0;
IF species="Ta" THEN treculia=1;
        ELSE IF species~="Ta" THEN treculia=0;
IF species="Fd" THEN dawei=1;
        ELSE IF species~="Fd" THEN dawei=0;
IF species="CHRa" THEN chrysophyllum=1;
        ELSE IF species~="CHRa" THEN chrysophyllum=0;
IF species="Fn" THEN natalensis=1;
        ELSE IF species~="Fn" THEN natalensis=0;
IF species="Cd" THEN celtisdu=1;
```

```
        ELSE IF species~="Cd" THEN celtisdu=0;
IF species="Fb" THEN brachylepis=1;
        ELSE IF species~="Fb" THEN brachylepis=0;
IF species="Wu" THEN warburgia=1;
        ELSE IF species~="Wu" THEN warburgia=0;
IF species="Termite" THEN termite=1;
        ELSE IF species~="Termite" THEN termite=0;
IF species="Ant" THEN ant=1;
        ELSE IF species~="Ant" THEN ant=0;

IF canopy="S" THEN ground=1;
        ELSE IF canopy~="S" THEN ground=0;

IF species="PAP" THEN C4=1;
        ELSE IF species~="PAP" THEN C4=0;

carbon=bulk_C13; LABEL carbon="d13C (per mil relative to PDB)";
nitrogen=bulk_N15; LABEL nitrogen="d15N (per mil relative to atmospheric
        N2)";
RUN;

/*checking that new variables were coded and labeled correctly*/
PROC CORR DATA=bulk;
```

```
    VAR bulk_C13 bulk_N15;
    WITH carbon nitrogen;
    RUN;

PROC FREQ DATA=bulk;
    TABLES class*fruitseed;
    TABLES class*treeleaf;
    TABLES class*sapling;
    TABLES class*pith;
    TABLES class*fruit;
    TABLES class*seed;
    TABLES class*root;
    TABLES class*cambium;
    TABLES class*insect;
    TABLES class*flower;
    TABLES class*leafbud;
    TABLES dryseason;
    TABLES C4;
    RUN;

PROC FREQ DATA=bulk;
    TABLES C4;
    RUN;
```

```
/*Mean carbon and nitrogen isotopic values by species and food type*/

PROC SORT DATA=bulk; BY species;

PROC MEANS DATA=bulk;

        VAR carbon nitrogen;

        BY species;

        RUN;


PROC SORT DATA=bulk; BY class;

PROC MEANS DATA=bulk;

        VAR carbon nitrogen;

        BY class;

        WHERE C4=0;

        RUN;


/*Plotting carbon and nitrogen isotopic values by food type*/

PROC GPLOT DATA=bulk;

        PLOT class*carbon;

        RUN;


PROC GPLOT DATA=bulk;

        PLOT class*nitrogen;

        RUN;


/*ANOVA for d13C*/
```

```
/*Model building, one variable at a time*/

PROC GLM DATA=bulk;
        CLASS C4;
        MODEL carbon=C4 / SOLUTION;
        RUN;


PROC GLM DATA=bulk;
        CLASS ground;
        MODEL carbon=ground / SOLUTION;
        RUN;


PROC GLM DATA=bulk;
        CLASS class;
        MODEL carbon=class / SOLUTION;
        RUN;


PROC GLM DATA=bulk;
        CLASS class;
        MODEL carbon=class C4 / SOLUTION;
        RUN;


PROC GLM DATA=bulk;
        CLASS class season;
        MODEL carbon=class C4 season / SOLUTION;
```

```
        RUN;


PROC GLM DATA=bulk;
        CLASS class;
        MODEL carbon=class C4 ground / SOLUTION;
        RUN;


PROC GLM DATA=bulk;
        CLASS class;
        MODEL carbon=class C4 elevation / SOLUTION;
        RUN;


PROC GLM DATA=bulk;
        CLASS class season;
        MODEL carbon=class C4 season ground elevation/ SOLUTION;
        RUN;


/*Full statistics on the reduced d13C model*/
PROC GLM DATA=bulk;
        CLASS class;
        MODEL carbon=class C4 elevation / SOLUTION;
        LSMEANS class / PDIFF ADJUST=tukey;
        RUN;
```

```
/*ANOVA for d15N*/

/*Model building, one variable at a time*/

PROC GLM DATA=bulk;
        CLASS C4;
        MODEL nitrogen=C4 / SOLUTION;
        RUN;


PROC GLM DATA=bulk;
        CLASS ground;
        MODEL nitrogen=ground / SOLUTION;
        RUN;


PROC GLM DATA=bulk;
        CLASS class;
        MODEL nitrogen=class / SOLUTION;
        RUN;


PROC GLM DATA=bulk;
        CLASS class;
        MODEL nitrogen=class C4 / SOLUTION;
        RUN;


PROC GLM DATA=bulk;
        CLASS class season;
```

```
        MODEL nitrogen=class C4 season / SOLUTION;
        RUN;

PROC GLM DATA=bulk;
        CLASS class;
        MODEL nitrogen=class C4 ground / SOLUTION;
        RUN;

PROC GLM DATA=bulk;
        CLASS class;
        MODEL nitrogen=class C4 elevation / SOLUTION;
        RUN;

PROC GLM DATA=bulk;
        CLASS class season;
        MODEL nitrogen=class C4 season ground elevation/ SOLUTION;
        RUN;

/*Full statistics on the reduced model*/
PROC GLM DATA=bulk;
        CLASS class;
        MODEL nitrogen=class C4 elevation / SOLUTION;
        LSMEANS class / PDIFF ADJUST=tukey;
        RUN;
```

Chapter 4

# COMPOUND SPECIFIC ISOTOPIC DIETARY ECOLOGY: NEUTRAL DETERGENT FIBER $\delta^{13}C$ WITHIN THE CHIMPANZEE DIETARY NICHE AT NGOGO, KIBALE NATIONAL PARK, UGANDA

## Introduction

Stable isotopic analyses are uniquely capable of quantifying the intake of both animal and plant based resources from an individual's hair, bone or teeth. Ratios of $^{13}C/^{12}C$ ($\delta^{13}C$) and $^{15}N/^{14}N$ ($\delta^{15}N$) are known to vary predictably by dietary intake (Ambrose and DeNiro 1986), geography and climate (Codron et al. 2005; Kohn 2010). Based on this framework, Sponheimer and Lee-Thorp (1994; 1999; 2003; 2006), van der Merwe et al (2008) and Cerling et al (2011) have contributed to our understanding of the diets of *Australopithecus africanus, Paranthropus robustus, P. bosei* and early *Homo* utilizing stable isotopic analyses of $\delta^{13}C$ from fossilized tooth enamel.

While providing valuable insight into early hominin diets, current isotopic methods for paleodietary reconstruction remain limited in application. The interpretation of bulk analyses from tooth enamel is currently limited to coarse distinctions between dietary inputs of $C_3$ vs. $C_4$ resources, or variability within $C_3$ plant ecosystems. And as is the case with interpretation of the australopith, *Paranthropus* and early *Homo* isotopic data, overlap between dietary components (e.g. termites, $C_4$ grasses, $C_4$ tubers, and meat from $C_4$ grazers) severely confounds interpretation.

To reconstruct dietary intake from modern or fossilized tissue, three pieces of information are necessary: (1) a piece of tissue, (2) a template of potential dietary items and their isotopic composition, and (3) a fractionation factor that represents the average offset between diet and tissue for the species whose diet is being reconstructed. Among chimpanzees, for example, bone collagen has been reported 5‰ less depleted in $^{13}C$ than the diet ($\delta^{13}C_{coll} = \delta^{13}C_{diet} + 5‰$), bone apatite 10-13‰ less depleted than the diet, and hair only 2-3‰ less depleted in $^{13}C$ (Smith et al. 2010). With these offset values, dietary ecologists calculate the presumed isotopic composition of the diet by analyzing the isotopic composition of the organism's tissue.

Numerous studies have suggested this offset is consistent enough for use in isotopic dietary reconstruction (Ambrose 1993; Bocherens and Drucker 2003; Cerling and Harris 1999; Cerling et al. 2004; DeNiro and Epstein 1978; Kellner and Schoeninger 2007; Krueger and Sullivan 1984; Lee-Thorp et al. 1989; Passey et al. 2005; Sponheimer et al. 2003; Tieszen and Fagre 1993). However, the origins and variability of this fractionation for humans and large bodied hominins remain largely unknown.

The example of chimpanzee offsets above were used for one particular study, but controversy exists over the most appropriate offset for a given tissue and species of mammal. This controversy is only heightened with respect to the interpretation of fossil enamel isotopic analyses. There are still many unknowns with respect to the origins and variability of diet to tissue isotopic fractionation. One such unknown is the extent to which nutrient relative to non-nutrient constituents within the diet may be isotopically heterogeneous. The total pool of carbon and nitrogen within any given food source originates from a number of nutritive and non-nutritive constituent components, some of which pass through the gastrointestinal tract unabsorbed and unassimilated into tissue.

To effectively utilize bulk isotopic analyses (which are technically simpler and more economic to conduct), the isotopic signature of the whole plant must be identical to, or systematically fractionated from, the constituents ultimately absorbed and assimilated from the digestive tract. If the less-digestible fiber fractions are significantly fractionated from the bulk signature, reconstructing the ingested signal requires accounting for such fractionation.

To examine the potential fractionation of the fibrous non-nutrient components, stable isotopic analyses were conducted on the bulk and neutral detergent fiber fractions of commonly consumed foods within the Ngogo chimpanzee dietary niche. Neutral detergent fiber (NDF) represents a class of structural carbohydrates integral to plant cell walls and includes cellulose, hemicellulose and lignin. A small fraction of NDF constituents are partially digested within the large intestine by symbiotic bacteria. However, the NDF largely represent dietary non-nutrients that traverse the alimentary tract undigested. Therefore, these analyses sought to compare the less-digestable NDF fraction to the bulk signature of several of the most commonly consumed items within the chimpanzee niche.

## Methods

Plant components of the Ngogo chimpanzee diet were defined broadly and collected opportunistically. A list of consumed species was created from several sources including published literature, unpublished observational data (personal communication: David Watts), experience of local field assistants, and personal observation.

The collection protocol was conducted throughout December 2009 and continued in June and July 2010. Fruit was selected from the ground that appeared (1) the appropriate stage of ripeness, and contained (2) no bite marks, (3) no insect damage, and (4) no mold. Leaves of saplings were sampled upon encounter from patches exploited by the chimpanzees, but not necessarily the same sapling. Leaves of mature trees were sampled from the ground and preference was given to less mature leaves as they appear most consumed by the chimpanzees. Leaf buds were selected along with leaves of mature trees, from the ground and when available. The pith of herbaceous vegetation was sampled selectively from patches of relatively large specimens (selective criteria of the chimpanzees). The outer husk was peeled away and the fleshy inner pith sampled preferentially from the lowest sections of the plant (lowest 6-12 inches). Flowers were sampled opportunistically upon encounter following similar principles to fruit selection. Cambium, roots and dead wood were likewise sampled opportunistically.

For each food species, every effort was made to sample widely across the Ngogo range to capture potential geographic variation in nutritional or isotopic characteristics. Each sample was labeled with its identification, date, time, location collected (nearest trail intersection and GPS coordinate), as well as the relative stage of development (e.g. ripeness of fruits, or maturity of leaves).

Samples were dried within hours of collection at 150-170°F for approximately 3.5 hours or until completely desiccated within a small oven. A kerosene fueled backpacking stove was positioned under a Coleman oven, and samples transferred to the oven only after the stove burned cleanly and the oven reached the appropriate temperature (approximately 10-20 minutes). This was done to minimize exposure of samples to carbon contamination from kerosene soot and ensure uniform conditions for each batch

of samples. Leaves typically dried in 2-3 hours while fruit often took 3-4 hours. Drying time and temperature were determined to minimally impact nutrient composition (low temperatures) and dry in the least amount of time required to prevent molding.

For bulk and neutral detergent fiber analyses, approximately 100 samples were selected to capture the top 20 foods within the Ngogo chimpanzee dietary niche (David Watts, personal communication) in triplicate or greater. Each sample was analyzed in bulk and again following NDF extraction. Extraction methods followed those of Van Soest et al (Van Soest et al. 1991)(see appendix). The extracted NDF was then weighed and transferred to tin capsules for IRMS. The isotopic analyses were conducted in collaboration with Thomas Maddox in the Analytical Chemistry Laboratory of the Odum School of Ecology, University of Georgia.

Statistical analyses proceeded using SAS 9.2 (see appendix for SAS code). Fractionation was determined as the difference between $\delta^{13}C_{bulk}$ and $\delta^{13}C_{NDF}$ for each sample. Paired-sample TTEST and ANOVA were used to test fractionation in $\delta^{13}C$ between bulk and NDF samples as well as assess whether fractionation varied by food type or species.

## Results

Bulk $\delta^{13}C$ of plants collected at Ngogo ranged from -23.2 to -35.6‰, with a mean of -28.0 ± 2.4‰ (Table 4.4). These samples, representing 23 of the most commonly consumed plants within the chimpanzee dietary niche at Ngogo, were a subset of total collection analyzed (n=246, $\delta^{13}C_{bulk}$ -27.7±2.6‰).

Fractionation between bulk and neutral detergent fibers (NDF) was 0.11±1.66 (mean±SD) with the NDF fraction slightly more enriched in $^{13}C$ on average than bulk samples (Table 4.1). This difference, however, was not statistically significant (p=0.52), and outside the range of analytical error.

Analysis of variance (ANOVA) revealed variability in fractionation by food type (plant anatomy) and species (Table 4.2). A reduced ANOVA model including only food type revealed that it could account for only 20% of total sample variance (R-square=0.2008, p-value=0.0022). This relationship is appears to be driven by the fruit/seed component with an NDF fraction significantly more enriched than bulk (2.0±3.2‰)(Table 4.3). The bulk to NDF isotopic offset for all other food types was non-significant.

The significance of food type for explaining variance in $\delta^{13}C$ fractionation disappeared when controlling for species. In the full ANOVA model, which includes food type, species, position within the canopy, and elevation, only plant species remains statistically significant for explaining variability in $\delta^{13}C$ fractionation (Table 4.2). Among plant species, the bulk-NDF fractionation for all but one (*Pterygota mildbraedii*) was also statistically non-significant.

The NDF fraction of *Pterygota* fruit/seeds and sapling leaves was significantly more enriched in $^{13}C$ than bulk (3.4±4.0‰ and 2.7±1.0‰ respectively)(Figure 4.2). The unique isotopic fractionation of NDF in *Pterygota* explains the early significance of fruit/seed fractionation in the ANOVA model that only included food type as an explanatory variable as well as the significance of species within the full model. Aside from the unique offset of *Pterygota*, canopy height and elevation also proved insignificant contributions to NDF fractionation (Table 4.2).

# Discussion

Analysis of variance (ANOVA) revealed that despite an overall non-significant fractionation of $\delta^{13}C_{NDF}$ from $\delta^{13}C_{bulk}$, one species (*Pterygota mildbraedii*) stood apart with an enrichment in the NDF fraction of about 3‰ relative to the bulk sample (Table 4.4).

Plants store photosynthetically produced sucrose within the leaves as either starch or sucrose. In a previous analysis among two species of leaves commonly consumed by chimpanzees (see Chapter 2), it appeared that *Pterygota* was a starch (rather than sucrose) accumulator. If *Pterygota* goes through the additional physiological step of binding sucrose units into starch before mobilizing the sucrose for utilization throughout the rest of the plant, such processing may result in increased fractionation. Sucrose molecules with a greater number of heavier $^{13}C$ molecules would be discriminated against in sequestration as starch. This would create a pool of starch within the leaves relatively depleted in $^{13}C$, while the sucrose destined for structural carbohydrate (various fiber fractions) becomes relatively enriched in $^{13}C$. This could explain the depleted $\delta^{13}C_{bulk}$ values of *Pterygota* relative to the less depleted $\delta^{13}C_{NDF}$. The unique fractionation seen in *Pterygota* would result from a fractionation effect at the level of starch synthesis, and should appear in all starch accumulating species.

It seems odd, however, if *Pterygota* were the only such species among the 20 sampled for this analysis that sequestered sucrose as starch within the plant leaves. Further analysis is required to determine the fractionation of $^{13}C$ among other nutrient pools within the plant tissue. Non-structural carbohydrates and lipids represent the other major pools of carbon within the plant's tissue, and the analysis of these highly digestible

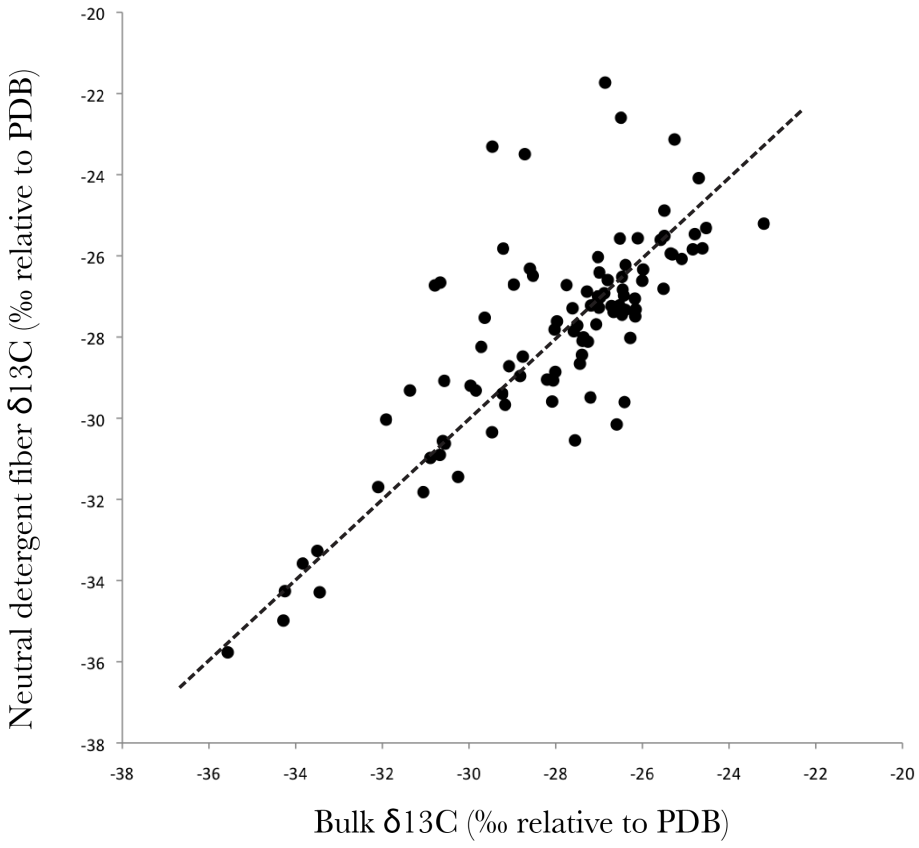
macronutrients would lend insight into the various mechanisms that lead to plant isotopic variability and the contributions of each nutrient pool to the organism's own tissues.

Most of the Ngogo plant samples, unlike *Pterygota*, exhibited no significant difference in the $\delta^{13}C$ from whole sample and that from the NDF fraction. The bulk samples consist of non-structural carbohydrates, protein, lipids, and neutral detergent fibers. The fact that the NDF fraction remains isotopically equivalent to the bulk sample indicates that the highly digestible fraction of macronutrients (as a whole) is also isotopically equivalent to the bulk isotopic signal. Individual differences may, and likely do, exist between individual macronutrients, but on average the isotopic signature from that fraction remains indistinct from the structural carbohydrates.

## Conclusion

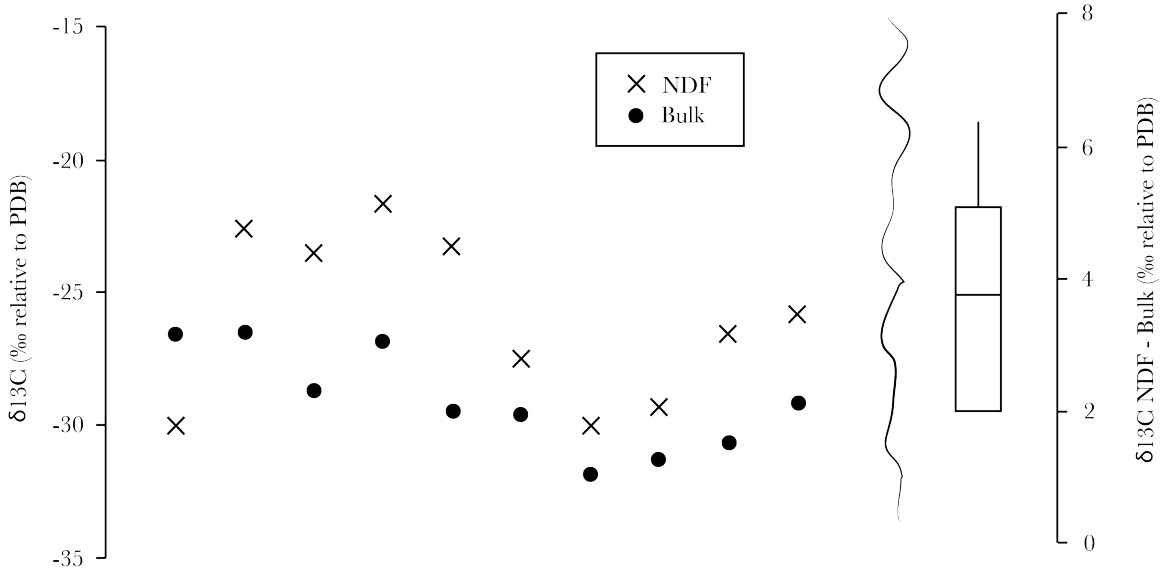
This analysis suggests that among the various sites of isotopic fractionation between consumption and tissue assimilation, the differential digestion of fiber fractions and macronutrients is likely not a significant contributor. If there is no significant isotopic difference between the digestible and indigestible components of most foods within the chimpanzee dietary niche, then fractionation from diet ($\delta^{13}C_{bulk}$) to tissue ($\delta^{13}C_{tissue}$) must result from metabolic processing, routing and biosynthesis. While much work remains, if fractionation can be defined as the result of specific metabolic processes, then variability in diet to tissue fractionation may open the door for isotopic analyses to reconstruct metabolic activity as well as diet.

Figure 4.1




Bulk versus Neutral Detergent Fiber $\delta^{13}C$ (‰ relative to PDB). Regression line represents the $H_0$: $\delta^{13}C_{bulk} = \delta^{13}C_{NDF}$. Each point represents one sample. Most samples roughly conform the null hypothesis that $\delta^{13}C_{bulk} = \delta^{13}C_{NDF}$.

Figure 4.2




*Pterygota mildbraedii* fractionation between $\delta^{13}C_{bulk}$ and $\delta^{13}C_{NDF}$. *Pterygota* was the only species that exhibited significant fractionation from bulk to NDF $\delta^{13}C$. The left side of this figure displays each set of data points for Pterygota, with NDF consistently less depleted than bulk. On the right is a box and whiskers plot of the fractionation from bulk to NDF $\delta^{13}C$ with mean approximately 3‰ and median nearly 4‰.

Table 4.1

Bulk and neutral detergent fiber (NDF) isotopic composition

| | *n* | $\delta^{13}C$ (‰) |
|---|---|---|
| Bulk sample | 97 | -28.0 ± 2.4 |
| NDF | 97 | -27.9 ± 2.6 |
| Fractionation (NDF-Bulk) | 97 | 0.1 ± 1.7 |
| *p-value* | | *0.5156* |

Table 4.2

ANOVA - Sources of variation for NDF $\delta^{13}C$ (‰ relative to PDB)

| *Model* | *R-square* | *P-value*[4] |
|---|---|---|
| Elevation | 0.0019 | 0.6750 |
| Season | 0.0048 | 0.4987 |
| Canopy | 0.0101 | 0.3271 |
| **Food type** | **0.2008** | **0.0022** |
| **Species** | **0.4288** | **0.0003** |
| **Food type +Species** | **0.4554** | **0.0009** |
| **Full**[1] | **0.4669** | **0.0017** |

| *Variable in full model*[1] | *F-value*[3] | *P-value*[4] |
|---|---|---|
| Food Type | 0.99 | 0.4022 |
| **Species** | **1.89** | **0.0330** |
| Elevation | 1.53 | 0.2208 |
| Season | 0.05 | 0.8323 |

| *Effect in "Species" model*[2] | *Estimate*[5] | *P-value* |
|---|---|---|
| *Acanthus sp.* | 0.2307 | 0.7951 |
| *Afromomum sp.* | 0.7840 | 0.3785 |
| *Aningeria altissima* | 1.3687 | 0.1489 |
| *Celtis africana* | 0.5021 | 0.5722 |
| *Celtis durandii* | 0.0590 | 0.9501 |
| *Celtis mildbraedii* | 0.3487 | 0.7114 |
| *Chrysophyllum albidum* | 0.8510 | 0.3675 |
| *Cordia millenii* | 0.2966 | 0.7724 |
| *Ficus brachylepis* | -0.1532 | 0.8708 |
| *Ficus dawei* | 0.4333 | 0.6727 |
| *Ficus exasperata* | 0.8584 | 0.3634 |
| *Ficus mucuso* | 0.4086 | 0.6646 |
| *Ficus natalensis* | 0.8416 | 0.3447 |
| *Mimusops bagshawei* | 1.0086 | 0.2580 |
| *Monodora myristica* | -0.2793 | 0.7854 |
| *Morus lactea* | 1.1277 | 0.1260 |
| *Pseudospondias microcarpa* | 1.2001 | 0.2050 |
| ***Pterygota mildbraedii*** | **-2.6082** | **0.0011** |
| *Treculia africana* | 0.9201 | 0.4344 |
| *Uvariopsis congensis* | ref | . |

[1] Full model: NDF $\delta^{13}C$ = Food type + Species + Elevation + Season + Canopy

[2] Species model NDF $\delta^{13}C$ = Species

[3] The F-value in ANOVA is a measure of variance between groups relative to within groups. The greater the F-value, the more between group variance relative to within group variance.

[4] P-value is the probability of obtaining a greater F-value by chance

[5] The Estimate represents the effect of each species upon NDF $\delta^{13}C$ controlling for all other variables in the model

Table 4.3

Bulk and neutral detergent fiber isotopic composition by food type

| | *n* | Bulk $\delta^{13}C$ (‰) | NDF $\delta^{13}C$ (‰) | NDF - Bulk |
|---|---|---|---|---|
| Flowers | 5 | -24.5 ± 0.8 | -25.7 ± 0.7 | -1.2 ± 0.5 |
| Fruit | 48 | -26.9 ± 1.2 | -27.1 ± 1.3 | -0.2 ± 1.1 |
| Fruit/seed | 9 | -27.1 ± 1.2 | -25.1 ± 2.6 | 2.0 ± 3.2 |
| Leaves, sapling | 18 | -31.4 ± 2.0 | -30.8 ± 2.8 | 0.6 ± 1.4 |
| Leaves, tree | 5 | -29.4 ± 1.7 | -29.5 ± 1.3 | -0.1 ± 1.2 |
| Pith | 10 | -29.3 ± 2.0 | -29.4 ± 2.3 | -0.1 ± 1.7 |
| Seed | 2 | -26.7 ± 1.0 | -27.2 ± 1.2 | -0.5 ± 0.2 |
| **All** | **97** | **-28.0 ± 2.4** | **-27.9 ± 2.6** | **0.1 ± 1.7** |

Table 4.4

Bulk and neutral detergent fiber isotopic composition by food item (species and type)

| | *n* | Bulk $\delta^{13}C$ (‰) | NDF $\delta^{13}C$ (‰) | NDF - Bulk |
|---|---|---|---|---|
| *Acanthus sp.* (pith) | 5 | -28.9 ± 1.6 | -28.8 ± 1.3 | 0.2 ± 2.1 |
| *Afromomum sp.* (pith) | 5 | -29.6 ± 2.4 | -30.0 ± 3.0 | -0.4 ± 1.5 |
| *Aningeria altissima* (fruit) | 4 | -26.4 ± 0.1 | -27.3 ± 0.2 | -1.0 ± 0.3 |
| *Celtis africana* (leaves, sapling) | 5 | -30.2 ± 0.9 | -30.3 ± 1.1 | -0.1 ± 0.5 |
| *Celtis durandii* (fruit/seed) | 4 | -26.4 ± 0.8 | -26.1 ± 0.8 | 0.4 ± 0.5 |
| *Celtis mildbraedii* (leaves, sapling) | 3 | -30.3 ± 0.8 | -30.7 ± 0.3 | -0.4 ± 0.4 |
| *Celtis mildbraedii* (leaves, tree) | 1 | -29.7 | -28.2 | 1.5 |
| *Chrysophyllum albidum* (fruit) | 4 | -27.5 ± 1.2 | -27.9 ± 1.3 | -0.4 ± 0.4 |
| *Cordia millenii* (fruit) | 3 | -26.4 ± 1.5 | -26.3 ± .0 | 0.1 ± 0.5 |
| *Ficus brachylepis* (fruit) | 4 | -28.4 ± 1.8 | -27.8 ± 2.2 | 0.6 ± 2.9 |
| *Ficus dawei* (fruit) | 3 | -26.0 ± 0.9 | -26.1 ± 1.5 | 0.0 ± 0.6 |
| *Ficus exasperata* (leaves, tree) | 4 | -29.3 ± 1.9 | -29.8 ± 1.3 | -0.4 ± 1.0 |
| *Ficus mucuso* (fruit) | 4 | -26.9 ± 0.3 | -26.9 ± 0.4 | 0.0 ± 0.1 |
| *Ficus natalensis* (fruit) | 5 | -26.7 ± 0.7 | -27.2 ± 0.4 | -0.4 ± 0.6 |
| *Mimusops bagshawei* (fruit) | 5 | -26.6 ± 1.0 | -27.2 ± 0.8 | -0.6 ± 0.8 |
| *Monodora myristica* (fruit) | 3 | -27.1 ± 1.9 | -26.4 ± 3.2 | 0.7 ± 2.6 |
| *Morus lactea* (fruit) | 4 | -25.4 ± 0.6 | -26.2 ± 0.6 | -0.9 ± 0.2 |
| *Morus lactea* (flowers) | 5 | -24.5 ± 0.8 | -25.7 ± 0.7 | -1.2 ± 0.5 |
| *Morus lactea* (leaves, sapling) | 5 | -34.3 ± 0.8 | -34.4 ± 1.0 | -0.1 ± 0.4 |
| *Pseudospondias microcarpa* (fruit) | 4 | -27.3 ± 0.6 | -28.1 ± 0.9 | -0.8 ± 0.3 |
| *Pterygota mildbraedii* (fruit/seed) | 5 | -27.6 ± 1.4 | -24.3 ± 3.4 | 3.4 ± 4.0 |
| *Pterygota mildbraedii* (leaves, sapling) | 5 | -30.6 ± 1.1 | -27.9 ± 1.8 | 2.7 ± 1.0 |
| *Treculia africana* (seed) | 2 | -26.7 ± 1.0 | -27.2 ± 1.2 | -0.5 ± 0.2 |
| *Uvariopsis congensis* (fruit) | 5 | -27.4 ± 0.6 | -27.0 ± 0.6 | 0.4 ± 0.4 |
| **All** | **97** | **-28.0 ± 2.4** | **-27.9 ± 2.6** | **0.1 ± 1.7** |

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SAS CODE

```
/*NUTRIENT SPECIFIC ISOTOPIC ECOLOGY:
/*NEUTRAL DETERGENT FIBER d13C WITHIN THE CHIMPANZEE
/*DIETARY NICHE AT NGOGO, KIBALE NATIONAL PARK, UGANDA

/*DISSERTATION RESEARCH
/*BRYCE CARLSON*/

PROC IMPORT DATAFILE="Y:\Bryce's
        Documents\Emory\Research\DISSERTATION\DATA\bulk.24may11.xlsx"
        OUT=a replace; sheet="Full.SASinput";
        RUN;

PROC CONTENTS DATA=a;
        RUN;

DATA fiber; SET a;
        IF class="ls" THEN sapling=1;
                ELSE IF class~="ls" THEN sapling=0;
        IF class="pi" THEN pith=1;
                ELSE IF class~="pi" THEN pith=0;
        IF class="le" THEN treeleaf=1;
                ELSE IF class~="le" THEN treeleaf=0;
```

```
IF class="fr/se" THEN fruitseed=1;
        ELSE IF class~="fr/se" THEN fruitseed=0;
IF class="se" THEN seed=1;
        ELSE IF class~="se" THEN seed=0;
IF class="fr" THEN fruit=1;
        ELSE IF class~="fr" THEN fruit=0;
IF class="ca" THEN cambium=1;
        ELSE IF class~="ca" THEN cambium=0;
IF class="rt" THEN root=1;
        ELSE IF class~="rt" THEN root=0;
IF class="fl" THEN flower=1;
        ELSE IF class~="fl" THEN flower=0;
IF class="lb" THEN leafbud=1;
        ELSE IF class~="lb" THEN leafbud=0;
IF class="in" THEN insect=1;
        ELSE IF class~="in" THEN insect=0;


IF species="PTm" THEN pterygota=1;
        ELSE IF species~="PTm" THEN pterygota=0;
IF species="COm" THEN cordia=1;
        ELSE IF species~="COm" THEN cordia=0;
IF species="Ml" THEN morus=1;
        ELSE IF species~="Ml" THEN morus=0;
```

```
IF species="Mm" THEN monodora=1;
        ELSE IF species~="Mm" THEN monodora=0;
IF species="Fe" THEN exasperata=1;
        ELSE IF species~="Fe" THEN exasperata=0;
IF species="Nm" THEN neoboutonia=1;
        ELSE IF species~="Nm" THEN neoboutonia=0;
IF species="CHAa" THEN chaetacme=1;
        ELSE IF species~="CHAa" THEN chaetacme=0;
IF species="PAP" THEN papyrus=1;
        ELSE IF species~="PAP" THEN papyrus=0;
IF species="AC" THEN acanthus=1;
        ELSE IF species~="AC" THEN acanthus=0;
IF species="AF2" THEN aframomum=1;
        ELSE IF species~="AF2" THEN aframomum=0;
IF species="PI" THEN piper=1;
        ELSE IF species~="PI" THEN piper=0;
IF species="MA" THEN marantochloa=1;
        ELSE IF species~="MA" THEN marantochloa=0;
IF species="CEa" THEN celtisaf=1;
        ELSE IF species~="CEa" THEN celtisaf=0;
IF species="Cm" THEN celtismi=1;
        ELSE IF species~="Cm" THEN celtismi=0;
IF species="Fm" THEN mucuso=1;
```

```
        ELSE IF species~="Fm" THEN mucuso=0;
IF species="Uc" THEN uvariopsis=1;
        ELSE IF species~="Uc" THEN uvariopsis=0;
IF species="PSm" THEN pseudospondias=1;
        ELSE IF species~="PSm" THEN pseudospondias=0;
IF species="Aa" THEN aningeria=1;
        ELSE IF species~="Aa" THEN aningeria=0;
IF species="Mb" THEN mimusops=1;
        ELSE IF species~="Mb" THEN mimusops=0;
IF species="Ta" THEN treculia=1;
        ELSE IF species~="Ta" THEN treculia=0;
IF species="Fd" THEN dawei=1;
        ELSE IF species~="Fd" THEN dawei=0;
IF species="CHRa" THEN chrysophyllum=1;
        ELSE IF species~="CHRa" THEN chrysophyllum=0;
IF species="Fn" THEN natalensis=1;
        ELSE IF species~="Fn" THEN natalensis=0;
IF species="Cd" THEN celtisdu=1;
        ELSE IF species~="Cd" THEN celtisdu=0;
IF species="Fb" THEN brachylepis=1;
        ELSE IF species~="Fb" THEN brachylepis=0;
IF species="Wu" THEN warburgia=1;
        ELSE IF species~="Wu" THEN warburgia=0;
```

```
IF species="Termite" THEN termite=1;
        ELSE IF species~="Termite" THEN termite=0;
IF species="Ant" THEN ant=1;
        ELSE IF species~="Ant" THEN ant=0;

IF canopy="S" THEN ground=1;
        ELSE IF canopy~="S" THEN ground=0;

IF species="PAP" THEN C4=1;
        ELSE IF species~="PAP" THEN C4=0;

bulkC=bulk_C13;
        LABEL bulkC="Bulk d13C (per mil relative to PDB)";

fiberC=fiber_C13;
        LABEL fiberC="Neutral Detergent Fiber d13C (per mil relative to PDB)";

fract=bulkC-fiberC;
        LABEL fract="Fractionation between Bulk and NDF d13C";
RUN;

/*checking that new variables were coded and labled correctly*/
PROC CONTENTS DATA=fiber;
        RUN;
```

```
PROC CORR DATA=fiber;
        VAR bulk_C13 fiber_C13;
        WITH bulkC fiberC;
        RUN;

/*reviewing summary statistics and distribution*/
/*pair=1 where the sample was analyzed for both bulk and NDF d13C*/
PROC UNIVARIATE DATA=fiber;
        VAR bulkC fiberC fract;
        WHERE pair=1;
        ID sampleID;
        HISTOGRAM bulkC fiberC fract;
        RUN;

PROC SORT DATA=fiber; BY class;
PROC MEANS DATA=fiber;
        VAR bulkC fiberC fract;
        BY class;
        WHERE pair=1;
        RUN;

PROC SORT DATA=fiber; BY species;
PROC MEANS DATA=fiber;
```

```
        VAR bulkC fiberC fract;
        BY species class;
        WHERE pair=1;
        RUN;

/*ANOVA for d13C fractionation*/
PROC GLM DATA=fiber;
        CLASS class species season;
        MODEL fract=class species elevation season ground / SOLUTION;
        RUN;
        QUIT;

PROC GLM DATA=fiber;
        MODEL fract=ground / SOLUTION;
        RUN;

PROC GLM DATA=fiber;
        MODEL fract=elevation / SOLUTION;
        RUN;

PROC GLM DATA=fiber;
        CLASS species;
        MODEL fract=species / SOLUTION;
        RUN;
```

```
PROC GLM DATA=fiber;
        CLASS class;
        MODEL fract=class / SOLUTION;
        RUN;

PROC GLM DATA=fiber;
        CLASS season;
        MODEL fract=season / SOLUTION;
        RUN;

PROC GLM DATA=fiber;
        CLASS class species;
        MODEL fract=class species / SOLUTION;
        RUN;

PROC GLM DATA=fiber;
        CLASS species;
        MODEL fract=species / SOLUTION;
        LSMEANS species / PDIFF ADJUST=tukey;
        RUN;
        QUIT;

/*creating boxplots of Pterygota fract vs. other fract*/
```

```
PROC SORT DATA=fiber; BY pterygota;

PROC UNIVARIATE PLOT DATA=fiber;

        BY pterygota;

        VAR fract;

        WHERE pair=1;

        RUN;
```

# TECHNIQUE FOR FINDING NEUTRAL DETERGENT FIBER

Reprinted from the Primate Nutritional Ecology Laboratory Manual

at Hunter College of CUNY

by Jessica Rothman

**Definition:**

This method determines Neutral Detergent Fiber, which is the residue remaining after digesting in a detergent solution. The fiber residues are predominantly hemicelluloses, cellulose, and lignin.

*** Before Starting- You MUST Do a Dry Matter at the Same Time You Prepare Samples for NDF ***

**Apparatus:**

1. Analytical Balance

2. Oven—capable of maintaining a temperature of 105±2°C.

3. Digestion instrument—(ANKOM 200 fiber analyzer).

4. Filter bags (F57, ANKOM Technology).

5. Weighing paper, brushes, and scoops for handling the sample.

5. Heat sealer—sufficient for sealing the filter bags closed to ensure complete closure

6. Desiccator—glass vessel and lid with vacuum grease and desiccant inside that enables the removal of moisture in the air around the filter bags.

7. Industrial Strength Sharpie—solvent and acid resistant ink

8. Data Sheet

**Reagents:**

1. Neutral Detergent Solution: to make 4 L of ND Solution, add 2L of deionized water to a large plastic beaker and start stirring on a stir-plate. Then add:

120.0g- Sodium Lauryl Sulfate

74.44g- Ethylenediamine-tetraacetic disodium salt, dehydrate

27.24g- Sodium borate

18.24g- Sodium phosphate dibasic, anhydrous

Pipet into the solution 40 ml of Triethylene Glycol and finish by adding 2.0L of deionized water.

2. Alpha-amylase, heat stable bacterial alpha amylase (ANKOM Technology)

**Procedure:**

1. Use a solvent resistant marker to label the filter bags. Record the weight of filter bag (W1). Any mislabeled bags should be saved and reused.

*Note*—Do not pre-dry filter bags; any moisture will be accounted for by the blank bag correction.

2. Weigh 0.45-0.55 g of prepared sample onto weighing paper and record the sample weight (W2). Using the weigh paper as a funnel, pour your weighed sample into the filter

bag, using a brush to ensure all weighed sample is transferred to the filter bag. Avoid placing the sample on the upper 4 mm of the bag.

3. Using a heat sealer (set to 4.5), completely seal the upper edge of the filter bag within 4 mm of the top.

*Note*—Use sufficient heat to completely seal the filter bag (the red light should come on, and go off) and allow enough cool time (2 sec) before removing the bag from the heat sealer.

Spread the sample uniformly inside the filter bag by shaking and flicking the bag to eliminate clumping.

4. Weigh one blank (empty) bag and include in run to determine blank bag correction (C1, see Number Note 1).

5. Place a maximum of 24 bags into the Bag Suspender. All nine trays should be used regardless of the number of bags being processed. Place three bags per tray and then stack trays on center post with each level rotated 120 degrees (the notches should be lined up over the ridges along the bag suspender edge). Your control bag should be included at the middle of the Bag Suspender. Insert the Bag Suspender with bags into the fiber analyzer vessel and place the weight on top to keep it submerged. *Note*—Prior to inserting the Bag Suspender, if the vessel temperature is warm from a previous run, add cold water and exhaust.

6. When processing 24 sample bags, add 1900-2000mL of ambient ND solution to the fiber analyzer vessel. If processing less than 20 bags, add 100 ml/bag of ND solution (use minimum of 1500 mL to ensure Bag Suspender is covered). Add 4.0 mL of alpha-amylase to the solution in the vessel.

7. Set timer for 75 min and check that the thermostat (green numbers) is set to 100$^{oC}$, turn Agitate ON and confirm agitation, close the lid tightly and turn Heat ON. Start the timer. Check back frequently to ensure that as the solution heats, none is leaking from around the top of the vessel. Also, check that the temperature in the vessel (red numbers) has reached 100$^{oC}$.

8. With 15 minutes to go in extraction, prepare the two water boiling kettles with deionized water for rinsing and set kettles to boil.

9. At the end of extraction, turn Heat and Agitate off. Open the drain valve (slowly at first) and exhaust hot solution before opening lid. The waste from this assay can be collected in large, plastic beakers and disposed of down the sink. *Note*—The solution in the vessel is under pressure. The exhaust valve needs to be opened to release the pressure and solution prior to opening the lid.

10. After the solution has been exhausted, close the exhaust valve and open the lid. Add 1900mL of (70-90°C) rinse water and 4.0 mL of alpha-amylase to the first and second rinses. Turn Agitate on and rinse for 5 min. Repeat hot water rinses a total of three times.

11. When the rinsing process is complete remove the samples. Gently press out excess water from bags. Place bags in a 400 mL beaker, with a 250ml beaker stacked inside to hold samples submerged and add enough histological grade acetone to cover bags and soak for 3-5 min. Remove bags from acetone and place on a baking sheet to air-dry.

12. Completely dry the samples in oven at 105±2°C for 30 min. Desiccate samples for 30 minutes to cool before weighing samples. *Note*—Do not place bags in the oven until acetone has completely evaporated.

**Calculations:**

% NDF (as-received basis) = ((W3 - (W1 x C1))/$W_2$) x 100

Where: W1 = Bag weight

W2 = Sample weight

W3 = Dried weight of bag with fiber after extraction process

C1 = Blank bag correction (final ovendried weight divided by the original blank bag weight)

**Notes:**

*Caution*

Powdered chemicals will irritate the mucous membranes. A dust mask and gloves should be worn when handling this chemical. Acetone is extremely flammable. Avoid static electricity and use a fume hood when handling.

**Numbered notes:**

1. A running average blank bag correction factor (C1) should be used in the calculation of fiber. The inclusion of a blank bag in each run is mainly used as an indicator of particle loss. A C1 larger than 1.0000 indicates that sample particles were lost from filter bags and deposited on the blank bag. Any fiber particle loss from the filter bags will generate erroneous results. If particle loss is observed then grinding method needs to be evaluated.

**Citation, CBE Style:**

Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74(10): 3583-3597.

# Chapter 5

# DISCUSSION AND FUTURE DIRECTION

This project examines dietary ecology among wild East African chimpanzees from three separate, yet interconnected, perspectives. The first examined temporal variability in nutrient composition and whether chimpanzee foraging behavior correlated with periods of relative quality or digestibility. The second perspective characterized the dietary ecology in terms accessible in the absence of observation, namely stable isotopic composition and variability at the level of whole foods. The third, investigated whether foods were isotopically heterogeneous, or specifically, whether the neutral detergent fiber component could be distinguished isotopically from the bulk signal.

These three perspectives engage chimpanzee dietary ecology at increasingly specific levels with correspondingly increasing applicability and significance for the study of dietary ecology among extant primates, to paleodietary reconstruction of our hominin ancestors, and the potential for studying metabolic activity and life history of extant and extinct organisms via compound specific stable isotopic analyses.

## Nutrient cycling and consumptive behavior

Foraging models have become increasingly complex over the past couple decades. Early models focused on maximization of energetic intake were complemented by evidence that some organisms instead minimize foraging time rather than maximize intake (Bergman et al. 2001). Satisficing models of foraging behavior, as opposed to

optimizing (or maximizing), were first introduced in the mid-1950s (Ward 1992), but have gained increased attention over the last couple decades (Carmel and Ben-Haim 2005; Ward 1992). These models proposed that organisms seek not to optimize caloric or nutrient intake, but rather minimize the time necessary to acquire sufficient calories and nutrients within the context of other priorities (e.g. social).

Both optimizing and satisficing models of foraging behavior typically consider foods as nutritionally static with respect to time. Seasonal considerations have been included as they relate to food availability, but rarely have foods been considered to vary nutritionally over the course of a 24-hour period.

Human consumption is highly temporal. Most cultures consume distinct types of foods in the morning, at midday, and in the early evening, as well as distinct types of foods in one season or another according to availability or preference. These practices are highly culturally constructed, and perhaps for this reason investigations have largely missed the temporal component to the foraging behavior of wild primates.

In this study, two items within the Ngogo chimpanzee dietary niche, which are consumed preferentially in the afternoon and early evening, were found to contain a higher quality nutrient profile at the time of consumption. The significance of this finding is two-fold. Reported here for the first time within the primate literature, the nutritional properties of individual dietary resources are not static, but rather variable in association with time of day. Therefore, this study inserts a new and important temporal component into the study of primate dietary ecology. When an animal consumes a given resource may be just as important as what is being consumed.

Secondly, the two dietary resources studies here are preferentially consumed at the time of day when nutrient quality is greatest. This suggests that chimpanzee feeding behavior may track nutrient quality and digestibility.

Temporal consumption at the level of a 24-hour cycle may prove inaccessible to reconstruct isotopically from fossil remains. However, as the two other chapters of this project suggest, there may be a number of other characteristics of dietary ecology that are via bulk and compound specific isotopic analyses.

## Sourcing bulk isotopic variability within a $C_3$ ecosystem

The isotopic differentiation between plants utilizing the $C_3$ and $C_4$ photosynthetic pathways has long been recognized and used as a cornerstone for dietary reconstruction via isotopic analyses. However, perhaps less appreciated, is the large range of carbon isotopic values within a predominantly $C_3$ ecosystem and dietary niche. At Ngogo, $\delta^{13}C$ values within the chimpanzee dietary niche ranged from -35.6 to -21.8‰, or a span of more than 13‰. In previous studies, this variability is typically reported as a single mean ± standard deviation and otherwise glossed over. Yet, this project revealed significant clustering within the $C_3$ environment attributable to food type (plant anatomy), height within the canopy, and elevation.

Pith and sapling leaves, two major fallback resources utilized by the Ngogo chimpanzees carried $\delta^{13}C$ significantly more depleted than any other food type within the dietary niche. This is most likely a result of the canopy effect, whereby biogenic carbon dioxide is recycled at the forest floor and results in the accumulation of carbon dioxide more depleted in $^{13}C$ than at the canopy top or the atmospheric average.

Within the Ngogo community, fruits are available seasonally with alternating periods of relative abundance and scarcity. Herbaceous vegetation (pith) and sapling leaves, however, are available in relative abundance year round. Correspondingly, during periods of relative fruit scarcity the chimpanzees appear to spend more time consuming sapling leaves and pith.

Within a habituated community and long term repeated sapling of individual chimpanzees, one may utilize this isotopic characteristic to track access to preferred resources. Serially sampling strands of hair might also prove capable of elucidating cycles of fruit abundance and scarcity. As ripe fruit becomes increasingly available and consumed within the niche, hair synthesized during that period will incorporate amino acids less depleted in $^{13}C$. Conversely, during periods of relative fruit scarcity, consumption of pith and sapling leaves will increase and hair synthesized during this period will incorporate amino acids more depleted in $^{13}C$.

Individual activity patterns or pathology could also influence fractionation, resulting in what looks like a dietary shift. Therefore, future work most couple behavioral observation with dietary and tissue sampling to further elucidate the various contributions to bulk level tissue variance in $\delta^{13}C$ as well as $\delta^{15}N$. However, given such large fractionation between terrestrial and canopy resources, this relationship could also be utilized to study the origins of terrestriality (including bipedality) among our earliest hominin ancestors (Carter 2001).

The effect of elevation change on both $\delta^{13}C$ and $\delta^{15}N$ is interesting. One hypothesis for such an effect is the changing partial pressures of carbon dioxide and atmospheric nitrogen at increasing elevation. Alternatively, this affect may simply result from microclines within the Ngogo range. At Ngogo, the relief is relatively minor with no

more than 300m between the lowest and highest points of the foraging range. The lowest elevations are largely characterized as valley bottoms, streambeds, and swampland. The highest elevations, conversely, are associated with hilltops, forest margins, and open patches of grassland. Therefore, the affect of elevation within this sample may simply track levels of water stress or other ecological factors attributed to the ecological morphology.

If this effect is found consistently across study sites, it may provide one alternative explanation for what has previously been interpreted as a $C_4$ shift in consumption among australopiths, *Paranthropus* and early genus *Homo*.

## Bulk and fiber fraction isotopic equivalence

Numerous studies have assumed a consistent fractionation from diet to tissue (Ambrose 1993; Bocherens and Drucker 2003; Cerling and Harris 1999; Cerling et al. 2004; DeNiro and Epstein 1978; Kellner and Schoeninger 2007; Krueger and Sullivan 1984; Lee-Thorp et al. 1989; Passey et al. 2005; Sponheimer et al. 2003; Tieszen and Fagre 1993). Consistent fractionation has been a necessary assumption for dietary reconstructive analyses. However, the origin and variability of that fractionation for humans and large bodied hominids remain undefined. By defining the origins and variability in isotopic composition and fractionation, one may reconstruct dietary consumption more specifically, and reveal underlying metabolic status or pathology.

Each tissue (e.g. collagen, apatite, hair) exhibits a different level of fractionation from the dietary value (Ambrose 1993). This suggests that differential metabolic routing, or processing, contributes to tissue level fractionation. Some have utilized the consistent

fractionation from diet to hair, and the deviation therefrom, to track protein stress and diagnose disordered eating behavior (Petzke et al. 2010). However, several other sites of fractionation are also possible including (a) fractionation between nutrient and non-nutrient fractions of the diet, and (b) fractionation between individual nutrients that may then contribute differentially toward tissue biosynthesis.

The results of this study (see chapter entitled "Neutral detergent fiber $\delta^{13}C$ within the chimpanzee dietary niche") showed that for most species there is no significant fractionation between $\delta^{13}C_{bulk}$ and $\delta^{13}C_{NDF}$. If the neutral detergent fiber (NDF) fraction of foods is isotopically equivalent to the whole food, this suggests that the fraction digested and absorbed is also isotopically equivalent to the whole food.

This is an encouraging result for dietary reconstructive analyses for a few reasons. (1) To define the list of potential dietary inputs one needs to record the isotopic signal of the component that is actually absorbed and ultimately incorporated into recoverable tissues. Bulk isotopic analyses are relatively quick and inexpensive to run as compared to a digestive analysis where the nutrient fractions are removed and analyzed independent from the fiber fraction. The digestive step and compound specific analyses increase the time required by 10-fold (or more), and the cost per sample by nearly 2-fold. (2) If the NDF fraction is isotopically equivalent to the bulk sample, this may open the door for isotopic analyses of fecal remains as representative of dietary inputs. For species where tissue samples are inaccessible or in short supply, fecal analyses may be more feasible. Feces include undigested material as well as bacterial cells, epithelial cells, toxins released by the body, and metabolic byproducts of the intestinal bacterial community. Many of these constituents may be separated without difficulty in the field or in the lab, and thus isotopic analysis of the undigested material may provide a reliable signature of the dietary

inputs. (3) These results shed light on the sources of tissue level fractionation and isotopic variability within the diet. The isotopic composition of different tissues is highly variable, and the molecular constituents of each tissue exhibit sometimes very different isotopic signatures (Petzke and Lemke 2009). Food is heterogeneous, comprising a number of nutrient and non-nutrient constituents. Likewise, our digestive physiology and the metabolic processes responsible for tissue level fractionation are variable. However, to date, dietary reconstruction via stable isotopic methods have treated both food and metabolic physiology as homogeneous and glossed over nutrient and tissue level variability in fractionation.

To advance isotopic analyses of past diet the variability within foods and between tissues should be explored and defined. The discovery that NDF and bulk isotopic signatures are equivalent is one small step toward characterizing the origins of diet to tissue fractionation and variability. If NDF fractions are isotopically equivalent to the whole plant, then tissue level variation must originate at inter-nutrient variability or differential physiological processing.

## Future direction: Compound specific isotopic analyses

Compound specific isotopic analyses (CSIA) make use of variability in the stable isotopic compositions of individual nutrients and their differential routing within the organism. To date, such analyses have targeted individual amino acids (McCullagh et al. 2006; Metges and Petzke 1997; Petzke et al. 2005b), fatty acids (Stott et al. 1997), other lipids (Evershed et al. 1995; Stott et al. 1999) and waxes (Evershed et al. 1994). And now,

this study is the first to examine the isotopic composition of neutral detergent fiber fractions relative to whole food.

The stable isotopic composition of any individual nutrient may vary widely from the bulk, or average, dietary value. Individual nutrients may be tracked from ingestion through absorption and metabolism to resynthesis and deposition within the tissues. Their dietary origins may be elucidated if one can retrace the metabolic fractionation and routing under defined environmental conditions. Essential nutrients, by way of being essential, must enter the diet in the active or essential form. Within hard tissues, these nutrients represent a direct and relatively unambiguous isotopic signal from the diet, because the body is incapable of *de novo* synthesis. The $\delta^{13}C$ of essential fatty acids in bone (Stott et al. 1997) and essential amino acids in blood plasma (Metges and Petzke 1997), for example, have been shown to mirror the dietary proportions.

Tracing amino acid isotopic variability from consumption to tissue synthesis offers several advantages over bulk protein analyses or compound specific methods with lipids. While inter- and intra-amino acid isotopic variability within the dietary environment is largely unknown, tissue level variation between amino acids has been shown considerable (Petzke et al. 2005b). Since half of all amino acids are dietarily essential, much of this variability is likely rooted in the diet. And given that dietary resources contain unique and definable concentrations of the essential amino acids, back calculation of specific dietary consumption may be possible at a level of detail impossible with bulk analyses alone.

Between dietary ingestion and tissue deposition, fractionation of amino acids depends on compound specific pathways universal to all humans and mammals (McLarney, Pellett et al. 1996; Baker 2005). Those pathways are defined and influenced by a number of life history and environmental conditions (e.g. pregnancy/lactation,

activity level, infection, etc.). Therefore, utilizing amino acids to trace isotopic variation from ingestion through metabolic processing to deposition within the tissue may additionally allow reconstruction of lived experience as well as diet.

*Growth and development*

Throughout infancy, childhood, and adolescence, demand for protein is increased (Matthews 1999; McLarney et al. 1996). Increased protein is essential not only for the structural development of muscle, bone, and other tissues, but for gene transcription and translation that serves to facilitate and direct growth and development in conjunction with environmental stimuli. The pattern of amino acid requirements appears to differ both in absolute quantity and relative proportions between infancy and adulthood (McLarney et al. 1996). Protein concentrations within breastmilk have evolved to meet these physiological demands, but upon weaning, foods sufficiently rich in calories and other essential nutrients (including protein) are essential to maintain normal growth and development.

Enamelins and amelogenins are the scaffolding proteins responsible for directing tooth enamel synthesis (Glimcher et al. 1990; Passey and Cerling 2002). As they are not remodeled over the life course, the $\delta^{15}N$ and $\delta^{13}C$ of these proteins are representative of early life intake and metabolism during enamelogenesis. Therefore, considerations of protein metabolism during this period are critically important when interpreting isotopic signals taken from enamel proteins. If the protein concentration within weaning foods was consistent with the adult dietary pattern, one would expect the weaning infant to be more protein stressed than the adult unless adult intake was sufficiently greater than required.

This may translate to non-essential amino acids more closely resembling a "whole diet" isotopic signal as *de novo* synthesis will likely increase. However, if protein intake is sufficient or in excess during infancy and childhood, *de novo* synthesis from dietary carbohydrate, fats or essential amino acids may likely be reduced, and the $\delta^{15}N$ and $\delta^{13}C$ more directly reflective of their specific origins within the diet.

*Pregnancy, lactation, and sex*

Pregnancy and lactation represent another obvious life history condition with an increased demand for dietary protein. Both pre- and postnataly, protein demands are increased to facilitate structural growth as well as signalling proteins involved in directing and scaffolding growth and development. Pregnancy and lactation may lead to protein stress effects similar to that during growth and development. If protein intake is only marginally capable of sustaining the pregnancy and periods of lactation, non-essential amino acids may more broadly reflect carbohydrate and fatty acid intake. Or, if maternal protein intake is insufficient, tissue level isotopic composition should reflect increased protein stress represented by enrichment in $\delta^{15}N$.

Lamont and colleagues have conducted a series of studies that show males, but not females, upregulate the oxidation of leucine during exercise (Lamont et al. 2001; Lamont et al. 2003). What may appear a gender difference, may result from lean muscle mass and mitochondrial density. The enzyme responsible for leucine metabolism (branded-chain ketoacid dehydrogenase) is upregulated in line with mitochondrial density and muscle mass (Rennie and Tipton 2000). As men, on average, have greater lean muscle mass than females this sex difference may be confounded by muscle mass. While

leucine metabolism may differ for more highly active (with a training effect of increased mitochondrial density), or muscular individuals, as an essential amino acid this may not result in an isotopic fractionation effect. However, the impact of leucine metabolism may have downstream effects on the pool of non-essential amino acids that do prove identifiable within the tissues.

*Infection, injury, and pathology*

Infection and injury have a direct impact on protein balance and therefore on nutritive demands. Immunologic response to infection include synthesis of T lymphocytes, macrophages, granulocytes, antibodies, complement-derived peptides, and/or cytokines (Wolf and Keusch 1999). Depending on the protein-energy status of the individual, the stress of infection may result in a major drain on the internal amino acid pool and affect normal amino acid dynamics. Infection and injury also lead to a hypercatabolic state, where muscle tissue is rapidly broken down to provide sufficient amino acid substrate for defense and repair respectively (Smith and Lowry 1999).

Individuals experiencing a hypercatabolic state during a significant period of tissue synthesis (childhood for tooth enamelins, over the life course for collagen, and within the last few months before death for hair keratin) should exhibit serious protein stress effects. It is within these individuals where the differences between the essential and non-essential amino acid $\delta^{15}N$ and $\delta^{13}C$ should prove greatest. Those amino acids synthesized from glycogenic precursors (incl. alanine, serine, and glycine) should most closely resemble the carbohydrate and fatty acid dietary signal. Those non-essential amino acids synthesized directly from essential amino acids (incl. tyrosine from

phenylalanine, and cysteine from methionine), should resemble the dietary carbohydrates or fats to the extent that carbon and nitrogen are introduced in the synthetic pathway (eg. significant for cysteine, but minimal for tyrosine)(Matthews 1999; Stipanuk 2000).

*Energy status*

Energy intake and the contribution of dietary protein to caloric requirements also affects protein metabolism. Given dietary intake above metabolic need, carbohydrates and fatty acids may be stored as glycogen (in muscle and the liver) and triacylglycerols (TAG, in adipose tissue) respectively. There is no mammalian mechanism for long term amino acid storage, and therefore the balance between anabolic and catabolic states depends on the concentration of essential amino acids within the diet: (1) if caloric needs are met, but protein intake is below the requirement, protein is degraded from muscle tissue to resupply the amino acid pool; (2) if caloric needs are met and protein intake is above that essential for normal function, then the excess amino acids are oxidized for energy while glucose and fatty acids are preferentially spared as glycogen and TAG; and (3) if caloric needs are not met, protein from muscle tissue may be degraded, the amino groups from the amino acids removed and the carbon skeleton oxidized (Coomes 2002; Matthews 1999).

The metabolic phases of food deprivation between meals have been defined into three distinct periods (Wang et al. 2006), with each period defined by a unique pattern of energy production and protein metabolism. In the first phase immediately following absorption of the meal, metabolism is mostly fueled by glycogenolysis and secondarily through lipolysis (the breakdown of glucose and fatty acid stores respectively). The second

phase is initiated as the liver's supply of glycogen is depleted and the body emphasizes oxidation of fatty acids stored in adipose tissue for energy production. At the same time, protein is increasingly broken down from muscle tissue to synthesize glucose. This phase is often referred to as adapted starvation. Once adipose stores of fatty acids are depleted, the third phase begins, and muscle catabolism begins in earnest. This phase cannot be sustained for long before function fails and the organism dies (Wang et al. 2006).

Depending on the feeding strategy of the individual, tissue fractionation of amino acids may be influenced by significant turnover. If food is readily available within the niche, the individual need not spend significant time in phase II summarized above. The longer an individual spends in phase II or III, the more tissue protein is catabolized and re-synthesized after the next feeding. Under such conditions, all amino acids may show increased fractionation resulting from repeated turnover. Populations experiencing greater food insecurity should face more extended and repeated fasting between meals and therefore exhibit greater fractionation of tissue amino acid $\delta^{15}N$ and $\delta^{13}C$.

## Research program

Stable isotopic means of dietary reconstruction are ripe for enrichment. Advances in ecology (O'Brien et al. 2005) and clinical medicine (Petzke et al. 2005a; Petzke et al. 2006; Petzke et al. 2010) are showing that stable isotopic analyses are a widely applicable and powerful tool. To realize this potential within paleoanthropology, primatology, and archaeology, four stages of research and development are necessary.

*Examine underlying nutritional and isotopic ecology.*

Different plants have unique isotopic signatures resulting from unique biochemistries. It remains to be determined whether niche differentiation between sympatric species may be characterized isotopically at a large or fine scale. Do canopy folivores look different from terrestrial omnivores? That is a fairly large dietary distinction with little or no overlap in resources consumed, and evidence from this work as well as previous studies suggests should be possible to differentiate isotopically given the effect of canopy height on carbon fractionation (Cerling et al. 2004; Schoeninger et al. 1998). However, it is currently unclear whether stable isotopic methods might prove capable of differentiating between the diets of two large bodied sympatric species with much overlap (e.g. chimpanzee and gorilla or baboon). It is also unclear how stable isotopic methods might differentiate between variable consumptive patterns within a single species.

Can isotopic differences be identified between classes of food types that are nutritionally meaningful (fruit and leaves, termites and papyrus, etc)? This needs to be conducted within a number of different and relevant contexts. Do the resources within a closed canopy forested environment isotopically resemble those at forest margins? Bulk isotopic analyses may not be sensitive enough to trace differential consumption on a specific level, which is where nutrient specific analyses may prove especially valuable.

In addition, to utilize nutrient specific analyses, though, requires knowledge of how these nutrients are distributed across the dietary niche – taxonomically, spatially, temporally, etc. This is especially true of the essential nutrients (e.g. essential amino acids and fatty acids), and to date little has been done to understand their availability within most primate dietary niches (or human forager diets for that matter).

*Database of interpretive templates*

Utilizing bulk and nutrient specific isotopic analyses, with an understanding of how nutrients and isotopes are distributed within a single dietary niche or ecological context, the next step is to understand how that variability may or may not shift within different niche or ecological contexts. For example, can stable isotopic analyses differentiate between the dietary niche of chimpanzees at Ngogo (forest/woodland) and those at Tai, or Mt. Assirik (closed canopy lowland forests)?

The potential to reconstruct hominin diet using stable isotopic methods requires an appropriate template, or backdrop, from which to interpret the isotopic signatures of fossil hominin material. To reconstruct the dietary ecology from a forest margin site, it is most appropriate to utilize an isotopic template that captures the isotopic variability from a similar modern site. Sites of particular interest for hominin evolution might be closed canopy sites within Eastern and Central Africa, forest margin, lacustrine, and dry savannah to name a few. Our closest living hominid ancestors, chimpanzees, can be found in each of these habitat types. But, it has been suggested that extant baboons may represent a better model for early hominin niche expansion (Codron et al. 2008). Therefore, isotopic templates incorporating the nutrient and isotopic variability within baboon dietary niches across these habitat types may also prove interesting and valuable.

*Build mixing models capable of incorporating bulk and nutrient specific data.*

To reconstruct diet using stable isotopes with the highest resolution, one must understand how the dietary signal is modified through processes of digestion and metabolism to ultimately result in the signal deposited within the tissues. By coupling field observation of dietary consumption with the subsequent isotopic analyses of the dietary and synthesized tissues (e.g. hair), one may quantify precisely how the dietary signal is fractionated within the organism differentially by age, sex, activity, etc. Mixing models must then be developed that incorporate the large amounts of data generated from nutrient and bulk isotopic analyses to identify the relative contributions of dietary intake and tissue assimilation. Once developed within a single population, the models may be expanded to test applicability in other populations and species.

Essential amino acids and essential fatty acids represent a direct signal from the diet. If certain essential nutrients are known to be limited to certain resources, nutrient specific analyses can be used to differentiate between the contributions of those resources to the diet. Dietary reconstruction from nutrient specific data may not need to incorporate the signal from all nutrients simultaneously, but rather certain nutrients may prove useful independent from all others in distinguishing between the consumption of certain resources. For example, the isotopic signature ($\delta^{13}C$ and/or $\delta^{15}N$) of one particular amino acid may prove sufficient for reconstructing the relative contributions of resources X and Y. At the same time, we may discover that the isotopic signature of docohexaenoic acid (DHA) is sufficient for reconstructing the contribution of aquatic resources to the diet (fish, shellfish). In this hypothetical example, combining the isotopic analyses of that single amino acid and DHA might allow for differentiating the relative contributions of resources X, Y, and aquatic source foods to the diet and assimilation within the tissue.

*Application to Archaeological and Paleontological material via biogenic protein*

Ultimately, reconstructing the diet of any organism requires the extraction of unaltered biogenic material from preserved soft tissue, bone or teeth. Within fossilized material, the most reliable biogenic signal is sequestered within the apatite of tooth enamel. Enamel apatite contains biogenic carbon allowing for bulk $\delta^{13}C$ of very old materials. However, accessing the more specific dietary information available through nutrient specific analyses requires the extraction of biogenic protein or fatty acids. To date, this work has been limited, primarily because beyond approximately 20 kya the biogenic protein and fatty acids within bone at the time of death become significantly contaminated with exchange from the burial environment. The densely crystalline structure of enamel, however, is relatively resistant to biogenic degredation over time. To apply the refined reconstruction that is likely possible through amino acid isotopic analyses will require the extraction of uncontaminated protein from within the enamel itself. Tooth enamel is synthesized in infancy and adolescence around a protein scaffolding which remains largely intact within the mineral structure over millions of years (Glimcher et al. 1990). That protein scaffolding, however, represents approximately 1% of the mature tooth, and the technology capable of extracting and analyzing that this small component remains undeveloped. (Porto et al. 2006).

## Broader significance

Darwinian fitness is heavily dependent upon an organisms' ability to procure dietary resources sufficient for energetic and essential nutrient requirements. Several dietary trends have been hypothesized in the adaptive radiation of Plio-Pleistocene hominins. A highly specialized niche that includes the consumption of tough seeds or more marginal resources like papyrus has been widely hypothesized for *Paranthropus*, as has a gradual increase in animal butchery and likely consumption among late australopiths and early genus *Homo*. Contribution of these food groups to the diet of early hominins is therefore of central interest in paleoanthropology.

Quantifying the relative contribution of plants and animals to the diet of extinct hominins remains difficult. This project provides a conceptual framework for such applications and improves our understanding of isotopic variability within a contemporary East African $C_3$ ecosystem. Several components of this study contribute valuable insight into the application of isotopic ecology and dietary reconstruction for studying evolution of the human lineage, including:

(1) examination of isotopic variation within a $C_3$ ecosystem

(2) investigation of diurnal nutrient cycling in association with consumptive behavior

(3) characterization of isotopic variability within a modern chimpanzee dietary niche

(4) creation of an isotopic template for a closed canopy East African forest with results of significance for studying the origins of terrestriality

(5) a novel application of compound specific isotopic analyses to characterize the isotopic contributions of less-digestible fiber fractions relative to the whole food

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