

## RESEARCH ARTICLE

Environmental Variables Across *Pan troglodytes* Study Sites Correspond With the Carbon, but Not the Nitrogen, Stable Isotope Ratios of Chimpanzee Hair

MARGARET J. SCHOENINGER\*, CORINNA A. MOST, JIM J. MOORE, AND ANDREW D. SOMERVILLE  
 Department of Anthropology, University of California, San Diego, La Jolla, California

Diet influences the stable isotope ratios of carbon and nitrogen ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values) in animal tissue; but here we explore the influences of particular aspects of the local environment on those values in chimpanzees (*Pan troglodytes*). In this article we present new  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in Gombe chimpanzees using hairs collected from night nests in 1989. Then, we explore the influence of environmental factors by comparing our Gombe data to those from eight additional *Pan* study sites with previously published stable isotope data. We compare chimpanzee  $\delta^{13}\text{C}_{\text{hair}}$  and  $\delta^{15}\text{N}_{\text{hair}}$  values to specific characteristics of local site ecology (biome and ecoregion) and to local Mean Annual Precipitation (MAP) to test hypotheses based on known effects of these variables on the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in plant tissues. The comparison shows that hair from chimpanzees living in savanna sites with lower MAP have higher  $\delta^{13}\text{C}_{\text{hair}}$  values than do chimpanzees living in woodland and forested sites with higher MAP. These results demonstrate the potential of using  $\delta^{13}\text{C}$  values in primate tissue to indicate aspects of their local ecology in cases where the ecology is uncertain, such as samples collected early in the last century and in fossil hominins. In contrast to expectations, however, chimpanzee  $\delta^{15}\text{N}_{\text{hair}}$  values from some savanna sites with lower MAP are lower, not higher, than those living in more forested areas with higher MAP. It is likely that diet selectivity by chimpanzees affects  $\delta^{15}\text{N}_{\text{hair}}$  values to a greater extent than does the influence of precipitation on plants. *Am. J. Primatol.* 78:1055–1069, 2016. © 2015 Wiley Periodicals, Inc

**Key words:** stable isotopes; carbon and nitrogen; chimpanzee diet and ecology

## INTRODUCTION

Chimpanzees (*Pan troglodytes*) often serve as referential models in human evolution studies or as parts of conceptual models describing species close to the last common ancestor of the human and ape lineages [see Moore, 1996]. Today, chimpanzees are found in a range of ecosystems, from closed canopy rainforests [e.g., Boesch & Boesch-Achermann, 2000] to closed woodlands [Goodall, 1986; Nishida & Uehara, 1983a] to more open “savanna” sites consisting of grasslands and variable amounts of closed and/or open woodland [McGrew et al., 1981; Moore, 1992; Ogawa et al., 2007]. Multiple lines of evidence suggest that the habitats of our early relatives, that is the australopithecines and early *Homo* sp. varied as well. For example, fossil fauna and the stable carbon isotope ratios ( $\delta^{13}\text{C}$  values in per mil, ‰, notation) of fossil soil organics and pedogenic carbonates from several sites suggest that these early hominins inhabited woodland, bush savanna, and more open savanna sites, or mosaic ecological regions where plant communities varied over space within a single site [Cerling et al., 2011; Kingston, 2007; Reed, 1997; Wynn, 2000].

Observations of chimpanzee feeding behavior and the composition of their fecal material indicate that diets consist largely of fruit and leaves in all habitats [Basabose, 2002; Newton-Fisher, 1999; Nishida, et al., 1983; Nishida & Uehara, 1983b; Potts et al., 2011; Watts et al., 2012; Wrangham, 1977], and although ingested plant foods vary across seasons and habitats, the focus remains on fruit and leaves with little ingestion of high fiber foods [Macho & Lee-Thorp, 2014; Oelze et al., 2014; Tutin & Fernandez, 1993; Wrangham et al., 1998]. The sites with published feeding information on chimpanzee diets are few [Wrangham, 1977], and those with long-

After this article was originally published there was an error found in the label for the second column in Table II, the correct label is “ $\delta^{15}\text{N}(\text{‰})$ ”.

\*Correspondence to: Margaret J. Schoeninger, Anthropology Department, University of California-San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0532. E-mail: mjschoen@ucsd.edu

Received 26 April 2015; revised 8 October 2015; revision accepted 18 October 2015

DOI: 10.1002/ajp.22496  
 Published online 29 October 2015 in Wiley Online Library (wileyonlinelibrary.com).

term records [Boesch & Boesch-Achermann, 2000; Goodall, 1986] are biased towards woodland and forest regions [Collins & McGrew, 1988; McGrew et al., 1988]. However, published  $\delta^{13}\text{C}$  values in chimpanzee tissues (hair, bone) from more open sites also show no indication of high fiber  $\text{C}_4/\text{CAM}$  foods such as grasses, sedges, and succulents [Schoeninger et al., 1999, 2006a]. In contrast, fossil hominin tooth enamel  $\delta^{13}\text{C}$  data appear to indicate that the hominins ate some amounts of  $\text{C}_4/\text{CAM}$  foods [Cerling et al., 2013; Sponheimer & Lee-Thorp, 1999; Sponheimer et al., 2005, 2013; Wynn et al., 2013] with intra-annual dietary variation in some species [Sponheimer et al., 2006b]. Indeed, some researchers suggest that the ingestion of  $\text{C}_4$  foods was fundamental to the separation of the ape and human lineages [Codron et al., 2008].

In interpreting  $\delta^{13}\text{C}$  data in extant and fossil animals, however, commonly overlooked are the influences of the local ecology or ecosystem type (subsequently referred to more specifically as biome and ecoregion following The Nature Conservancy nomenclature) and Mean Annual Precipitation (MAP). These variables can affect the rate of local photosynthetic activity and the isotopic composition of the carbon dioxide available to plants [Farquhar et al., 1982, 1989; O'Leary, 1981], which consequently could affect the  $\delta^{13}\text{C}$  values in primate plant foods. The resultant variation in plant food  $\delta^{13}\text{C}$  values could affect the  $\delta^{13}\text{C}$  values in the animals that feed on them. Most stable isotope studies on nonhuman primates and on fossil hominins focus on diet, which is estimated based on the animal's position along a straight mixing line drawn between the worldwide average  $\delta^{13}\text{C}$  values for  $\text{C}_3$  plants and  $\text{C}_4$  plants [commonly taken from O'Leary, 1981, 1988]. Yet, small early studies on New World and Old World primates, including chimpanzees, showed a correlation between aspects of local ecology and the  $\delta^{13}\text{C}$  values in primate tissues, which showed a range of almost 4‰ [Schoeninger et al., 1997, 1999]. Using the average  $\delta^{13}\text{C}$  values for  $\text{C}_3$  and  $\text{C}_4$  plants to estimate the percent  $\text{C}_3$  plants in the diets of the primates with the highest  $\delta^{13}\text{C}$  values would have suggested that they fed on some amount of  $\text{C}_4$  plants, which observational data disputes.

Studies of living and recent primates (including humans) also interpret tissue nitrogen stable isotope ratios ( $\delta^{15}\text{N}$  values, ‰ notation) in terms of diet [for example: Fahy et al., 2013; Oelze et al., 2014; Richards et al., 2001, 2008; Schoeninger et al., 1999]. Yet, we have evidence in support of an early proposal [Heaton et al., 1986, 1987] that nitrogen stable isotope ratios in plants can be influenced by aridity. Analyses of multiple precipitation gradients from across the globe find that plant  $\delta^{15}\text{N}$  values exhibit significant negative correlations with MAP [Amundson et al., 2003; Aranibar et al., 2004; Austin

& Vitousek, 1998; Craine et al., 2009; Martinelli et al., 1999; Schulze et al., 1998]. Animals incorporate  $\delta^{15}\text{N}$  values of consumed plants in their bodily tissues, and several studies have demonstrated significant correlations between bone collagen  $\delta^{15}\text{N}$  values and MAP [Ambrose, 1991; Hartman, 2011; Johnson et al., 1998; Murphy & Bowman, 2009; Pate & Anson, 2008] and/or temperature [Stevens et al., 2006] in a variety of animals although not in primates. Such relationships do not manifest in all species. Studies on North American jackrabbits, for example, found no correlations between bone collagen  $\delta^{15}\text{N}$  values and precipitation or temperature [Ugan & Coltrain, 2011].

In the present study, we investigate whether there is a correlation between several environmental indicators, on the one hand, and the  $\delta^{13}\text{C}$  values and  $\delta^{15}\text{N}$  values in chimpanzee hair on the other. More specifically, we test the hypothesis that chimpanzee hair stable isotope ratios correlate with ecoregion, plant biome and precipitation. To do this, we analyze samples from one site (i.e., Gombe, Tanzania) for which there were no published stable isotope data, and compare the results with published data from other chimpanzee research sites across Africa. A negative correlation between the  $\delta^{13}\text{C}$  values in chimpanzees and their local MAP would support previous arguments against using single  $\delta^{13}\text{C}$  values as the end member of  $\text{C}_3$  plants in diet estimates for extant primates and in fossil hominins. Instead, as presented most clearly by Murphy & Bowman [2009] the full range of  $\delta^{13}\text{C}$  values of  $\text{C}_3$  plants must be considered. If there is a negative correlation between MAP and chimpanzee  $\delta^{15}\text{N}$  values, then some measure of aridity must be considered when interpreting the  $\delta^{15}\text{N}$  values in living and recent primates (including humans). Also, if supported, these broader comparisons should improve future modeling of chimpanzee habitats from animal samples that were collected without specific ecological information. For earlier material such as fossil hominins, such results of the comparison would indicate that tooth enamel  $\delta^{13}\text{C}$  values should be interpreted with attention to habitat ecology in addition to diet.

Over the past several decades, analyses of carbon and nitrogen stable isotope ratios have become routine in studies of animal diet [Koch, 2007; Sandberg et al., 2012]. The approach is based on the premise that animal stable isotope ratios vary in direct relation with the stable isotope ratios in the animal's diet [DeNiro & Epstein 1978, 1981], which in the case of primates is largely plant based. In those cases where we lack the ideal of baseline data [Crowley, 2012; Warinner et al., 2013], we base expectations on well-established principles of stable isotope biogeochemistry [Fry, 2006; Hoefs, 2009; Lajtha & Marshall, 1994; Rundel et al., 1989]. This latter approach is the one employed in the present study.

The primary source of variation in  $\delta^{13}\text{C}_{\text{plant}}$  values is the plant's method of photosynthesis [Smith & Epstein, 1971], but we now know that several variables other than the photosynthetic pathway affect the final  $\delta^{13}\text{C}_{\text{plant}}$  value [Marshall et al., 2007]. The following discussion focuses largely on  $\text{C}_3$  plants because these variables affect  $\text{C}_3$  plants to a far greater extent than they do  $\text{C}_4$  plants [Marino & McElroy, 1991] and, as discussed below, living primates largely feed on  $\text{C}_3$  plants. The most commonly cited average for  $\text{C}_3$  plants worldwide is  $-27\text{‰}$ ; but the values show a range from  $-37\text{‰}$  to  $-20\text{‰}$  [O'Leary, 1988]. Specific values result from a balance between the photosynthetic rate and the  $\delta^{13}\text{C}$  value of the carbon dioxide ( $\text{CO}_2$ ) available to the plant [Farquhar et al., 1989] both of which are affected by available water, altitude, temperature, plant phylogeny, canopy cover, and leaf type [Farquhar et al., 1982; Marshall et al., 2007]. Global distributions of  $\delta^{13}\text{C}_{\text{plant}}$  values, however, demonstrate that of all these variables MAP has the strongest influence on  $\text{C}_3$  plants [Diefendorf et al., 2010; Kohn, 2010]. A compilation of over 1,300  $\text{C}_3$  plant samples from 570 individual sites that span a large range of MAP, Mean Annual Temperature (MAT), altitude, and latitude show a monotonic decrease between  $\delta^{13}\text{C}_{\text{plant}}$  values and increasing MAP with far lower effects of MAT, altitude, latitude, and other variables than expected [Kohn, 2010]. The highest  $\delta^{13}\text{C}_{\text{plant}}$  values come from scrub bushlands like those in Israel [ $-22\text{‰}$  see Hartman & Danin, 2010] and extremely arid environments (e.g., the Atacama Desert) [up to  $-20\text{‰}$  see Kohn, 2010] while those below  $-31.5\text{‰}$  come from tropical closed canopy forests that result from the uptake of recycled  $^{13}\text{C}$ -depleted  $\text{CO}_2$  released from soil respiration as well as lower light levels [Bonafini et al., 2013; van der Merwe & Medina, 1991; Vogel, 1978; Yakir & Israeli, 1995]. In addition to the distribution of  $\delta^{13}\text{C}_{\text{plant}}$  values, overall plant biomass varies linearly with precipitation globally [Kohn, 2010]. Usually there is a greater amount of canopy cover with lower  $\delta^{13}\text{C}_{\text{plant}}$  values in regions with higher MAP although there are regions where local temperature and specific soil types can affect these relationships [Michaletz et al., 2014].

In contrast to  $\text{C}_3$  plants the range in  $\text{C}_4$  plants (arid adapted grasses and a few species within the sedge family) is much smaller, approximately  $4\text{‰}$ , with a range from  $-15\text{‰}$  to  $-11\text{‰}$  and a mean  $\delta^{13}\text{C}_{\text{plant}}$  value of  $-13\text{‰}$  (O'Leary, 1988). Crassulacean Acid Metabolism plants (CAM), which are also arid adapted plants [Ehleringer, 1978; Stowe & Teeri, 1978; Tieszen et al., 1979] include cacti and succulents with  $\delta^{13}\text{C}$  values that can overlap with  $\text{C}_4$  plants [Smith & Epstein, 1971].

The habitats of most primates are characterized as having predominantly  $\text{C}_3$  plant species [Cerling et al., 2004] and their diets reflect this bias. Thus far, there is little evidence that either  $\text{C}_4$  or CAM plants

are significant foods for living nonhuman primates [Crowley et al., 2010; Sandberg et al., 2012; Schoeninger, 2014]. While macaques observed raiding maize fields on a seasonal basis had somewhat elevated  $\delta^{13}\text{C}_{\text{hair}}$  values, their bone collagen indicated a pure  $\text{C}_3$  diet [O'Regan et al., 2008]. Although baboon fecal samples recovered beneath a succulent plant (i.e., CAM) in a marginal environment had  $\delta^{13}\text{C}$  values in the  $\text{C}_4$  range, baboon tooth samples from the same area indicated a complete  $\text{C}_3$  diet [Codron et al., 2006]. Even geladas, which feed extensively on grass live in regions of high altitude that are relatively cool with relatively high rainfall where most plant species, including grasses, are  $\text{C}_3$  [Fashing et al., 2014].

Because primates feed so extensively on  $\text{C}_3$  plants, it was unsurprising that primate  $\delta^{13}\text{C}$  values varied with canopy cover (which, in turn, varies with MAP) rather than dietary specifics [Schoeninger et al., 1999]. Still, recent work demonstrates that some  $\text{C}_3$  plant foods can have strikingly different  $\delta^{13}\text{C}$  values than other foods within the same ecoregion [representative examples included in: Cerling et al., 2004; Codron et al., 2005; Oelze et al., 2014]. For example, some fruits within the Ituri Forest had carbon stable isotope ratios that were much higher (e.g.,  $-30\text{‰}$  to  $-31\text{‰}$ ) than those in leaves taken from the canopy floor ( $-37\text{‰}$ ) [Cerling et al., 2003]. Yet, within the canopy (i.e., the main area of feeding for primates) both leaves and fruits had values that were  $5\text{‰}$  higher than leaves and fruits from the subcanopy. Significantly, there was no association between primate  $\delta^{13}\text{C}$  values and the differential mix of fruit and leaves among sympatric folivorous, frugivorous, and omnivorous primate species; all species had  $\delta^{13}\text{C}$  values within  $1\text{‰}$  of each other [Cerling et al., 2004].

Some, although not all, fruits eaten by both chimpanzees and gorillas at Loango, Gabon had  $\delta^{13}\text{C}$  values that are about  $1\text{‰}$  higher, on average, than leaves, and the chimpanzees there had higher  $\delta^{13}\text{C}_{\text{hair}}$  values than did the gorillas [Oelze et al., 2014]. Because approximately 60–75% of the carbon used in synthesizing tissue proteins comes from dietary protein [Fernandez in press; Froehle et al., 2010] Oelze et al. [2014] concluded that the higher  $\delta^{13}\text{C}$  values in chimpanzees reflected their higher fruit intake relative to gorilla, and that fruit provided significant amounts of protein to the chimpanzees. However, fruits normally consist of carbohydrates with little to no protein [Fleagle, 2013; Murray et al., 2001] and the C:N ratios in fruits at Loango [Oelze et al., 2014] are extremely high, which is consistent with general expectations for fruit. To be certain of the situation with regards to protein at Loango, nutritional analyses must be done on the various plant foods before it can be concluded whether or not the fruits provide chimpanzee dietary protein. On the other hand, 25–40% of the carbon in collagen (and hair) comes from nutritional sources other

than protein, and fruit carbohydrate (simple sugar and nonstructural carbohydrate) is the most likely source. Therefore, the higher  $\delta^{13}\text{C}_{\text{hair}}$  values in chimpanzees versus gorillas could still be a result of fruit intake even though it is not the protein fraction of the diet that is the source. Alternatively, perhaps the situation at Loango is similar to that at Tai where chimpanzees have a similar focus on fruit. Researchers there concluded that the fruit diet at Tai provided overall protein intake lower than recommended by the National Research Council resulting in diets that could be protein deficient [N'guessan et al., 2009].

The variation in nitrogen stable isotope ratios ( $^{15}\text{N}/^{14}\text{N}$  represented by  $\delta^{15}\text{N}$  values) within the biosphere differs from that of carbon stable isotope values. The variation in plant  $\delta^{15}\text{N}$  values is determined by the  $\delta^{15}\text{N}$  value of the nitrogen used by plants, which can be atmospheric ( $\text{N}_2$ ) via bacterial nodules on plant roots or, more commonly, soil nitrogen [Shearer & Kohl, 1994]. Animal  $\delta^{15}\text{N}$  values are determined by  $\delta^{15}\text{N}$  values in their foods [DeNiro & Epstein, 1981], which as mentioned previously are mostly plant based. In general, herbivores overall have  $\delta^{15}\text{N}$  values in their tissues that are approximately 3‰ higher than the plants on which they feed, and carnivores are approximately 3‰ higher than the animals on which they feed [Minagawa & Wada, 1984; Schoeninger & DeNiro, 1984; Schoeninger, 1985] although there can be quite a bit of variation around these values [Koch, 2007]. Individual chimpanzees observed eating animal material had higher  $\delta^{15}\text{N}_{\text{hair}}$  values than did their more herbivorous companions [Fahy et al., 2013], the results of a similar study on bonobos were less clear [Oelze et al., 2011] and in both cases, the overall effect was quite small.

Global soil  $\delta^{15}\text{N}$  values vary between  $-2.0\%$  and  $10.3\%$  [Amundson et al., 2003] depending on the amount of biomass degradation, the parent material, precipitation, salinity, and various processes such as leaching, ammonia volatilization, and denitrification [Heaton, 1987; Shearer & Kohl, 1994]. But, based on the available data thus far African soil  $\delta^{15}\text{N}$  values vary by less than 3‰, on average, and within the region where the chimpanzee sites are located, the soil  $\delta^{15}\text{N}$  values vary by less than 2‰ (9–10.3‰) [Amundson et al., 2003]. In sum, the range of baseline  $\delta^{15}\text{N}$  values across the chimpanzee sites is, on average, much lower than global distributions and nitrogen cycling processes would suggest.

Plant  $\delta^{15}\text{N}$  values are normally lower than soil and across Africa they can be 2–5‰ lower [Amundson et al., 2003], which increases the expected range of  $\delta^{15}\text{N}$  values within plants. Several studies, based on work begun in the early part of the last century, demonstrate that the greatest effects on plant  $\delta^{15}\text{N}$  values are MAP and MAT [Amundson et al., 2003; Austin & Vitousek, 1998; Handley et al., 1999; Martinelli et al., 1999]. In general, systems that are

wet and cool have lower  $\delta^{15}\text{N}$  values [Evans, 2001] compared to those systems that are open, warm and dry [Amundson et al., 2003; Austin & Vitousek, 1998; Shearer & Kohl, 1986]. Some studies suggest that in temperate latitudes with marked seasonal variation in MAT, the animals most affected are ones that must drink water [Cormie & Schwarcz, 1994, 1996]. Water independent species, such as jackrabbits in North America and northern Mexico, can show no correlation between the  $\delta^{15}\text{N}$  values of bone collagen and MAP [Ugan & Coltrain, 2011], or show significant positive correlation with MAT rather than with MAP (Somerville, in preparation). MAT is expected to have the smaller effect across the chimpanzee sites because of the relatively narrow range of MAT across within the tropical latitudes inhabited by chimpanzees. Additionally, although altitude can vary within sites, the magnitude is small relative to that observed affecting soil  $\delta^{15}\text{N}$  values [Amundson et al., 2003] and chimpanzees feeding is not limited to a single altitude.

Analyses from precipitation gradients across Australia, Africa, and in Israel find that  $\delta^{15}\text{N}$  values in plants and animals consistently show higher  $\delta^{15}\text{N}$  values in environments with lower water availability indices or with lower MAP, than in wetter zones [Ambrose, 1991; Grocke et al., 1997; Hartman, 2011; Heaton et al., 1986; Murphy & Bowman, 2006, 2009; Pate & Anson, 2008; Sealy et al., 1987]. This pattern occurs because water is a limiting nutrient in dryer systems resulting in a relative excess of soil nitrogen, which "... leaves the system more readily through fractionating pathways" [Murphy & Bowman, 2009:1046] that enrich the remaining soil nitrogen in  $^{15}\text{N}$ . The relationship, however, is not linear in either plants or in animals. Studies on Australian macropods [Murphy & Bowman, 2006; Pate & Anson, 2008] and eutherian mammals in Africa [Heaton et al., 1986] found the largest increases in bone  $\delta^{15}\text{N}$  values in regions with less than 400 MAP with smaller increases in regions with >400 and <1,000 MAP and little to no effect above 1,000 MAP. There is a marked range of MAP across the chimpanzee sites that could affect chimpanzee hair  $\delta^{15}\text{N}$  values.

## METHODS

Our study analyzed samples from Gombe National Park in western Tanzania, which has been the location of Jane Goodall and colleagues' ongoing research since 1960 [see Goodall, 1986; Wilson, 2012 for overviews]. It covers an area of about 35.4 km<sup>2</sup>, and is a mosaic of evergreen forest, open woodland, and grassland [Clutton-Brock & Gillett, 1979; Goodall, 1986; Wilson, 2012]. The dry season lasts from May to October [Wallis, 1995], average yearly rainfall has been variously reported from 750 to 1250 mm [Teleki et al.,

1976] to 1546 [Clutton-Brock & Gillett, 1979]. Our study included samples from the Kasekela community [Goodall, 1986], which is located centrally in the park.

Stable isotope analysis was performed on hair from 13 known individuals (see Table I) collected in 1989 from night nests by J.J.M. and collaborators as part of another project. The hair collection involved no invasive techniques nor did it involve any animals directly; that is these were naturally shed hairs. The project received approval from IACUC and followed the American Society of Primatologists' principles for the ethical treatment of nonhuman primates. The Tanzanian Commission on Science and Technology (COSTECH) permitted data collection on the Gombe chimpanzee community.

In addition to the new data in our study, we compiled published data from eight other study sites to evaluate the association between habitat characteristics and rainfall levels (MAP, an indirect proxy for vegetation) with carbon and nitrogen stable isotope ratios across regions and researchers (Table II). These sites include Ishasha, Zaire and Ugalla, Tanzania [Schoeninger et al., 1999]; Fongoli, Senegal [Sponheimer et al., 2006a]; Kibale, Uganda [Carter, 2001]; a restricted area within central Cameroon [Macho & Lee-Thorp, 2014]; Tai, Cote d'Ivoire [Fahy et al., 2013]; Ganta, Liberia [Smith et al., 2010]; and Loango, Gabon [Oelze et al., 2014] (Fig. 1).

When the original data were derived from bone collagen samples [e.g., Smith et al., 2010 from Ganta], we converted the values to  $\delta^{13}\text{C}_{\text{hair}}$  values. In doing so, we chose not to follow Fahy et al. [2013] in using the value of 2‰ because the source they cite is a paper on nitrogen stable isotopes [DeNiro & Epstein, 1981], and the earlier paper on carbon [DeNiro & Epstein, 1978] reported on chitin and bone collagen, but not keratin. Instead, we applied the conversion (−1.4‰

for  $\delta^{13}\text{C}$  values and −0.86‰ for  $\delta^{15}\text{N}$  values) following O'Connell et al. [2001] even though the data are limited to modern British people and the  $\Delta^{13}\text{C}$  values show a large amount of variation (i.e., S.D. = 0.45 for  $\delta^{13}\text{C}$  values and 0.17 for  $\delta^{15}\text{N}$  values). We chose not to follow Crowley et al. [2010] in using apparent enrichment values ( $\epsilon^*$ ) because while they are necessary when comparing materials with vastly different  $^{13}\text{C}/^{12}\text{C}$  ratios relative to the PDB or SMOW standards (e.g., geochemical versus biological materials) [Hoefs, 2009] we compare only biological materials (e.g., hair versus bone collagen) that are relatively similar to one another. In addition, the majority of ecological and biological papers use delta values. The reader can convert the  $\delta^{13}\text{C}_{\text{hair}}$  values to apparent enrichment values using the following equations: (1)  $\epsilon^* = (\alpha - 1)$  and (2)  $\alpha_{\text{collagen-keratin}} = (1,000 + \delta^{13}\text{C}_{\text{collagen}}) / (1,000 + \delta^{13}\text{C}_{\text{hair}})$  where  $\epsilon^*$  is the apparent enrichment value and  $\alpha$  is the apparent fractionation factor [see Crowley et al., 2010 for apparent enrichment and alpha values]. In any case, within this study any errors resulting from these choices will be constants (with the exception of the Ganta data) and will not affect the assessments of relationships between the variables.

In addition, museum samples (Ganta and Cameroon) collected in the last century had to be corrected as adjustment for the lower  $\delta^{13}\text{C}$  values in today's atmospheric  $\text{CO}_2$ . We chose not to follow [Kohn, 2010] who applied a correction of 0.023‰/year to plant samples collected within the last 10–15 years because the museum samples were collected in the early 1940's. The massive changes in the  $\delta^{13}\text{C}$  values of atmospheric carbon dioxide (Keeling Curve at Scripps Institution of Oceanography, <https://scripps.ucsd.edu>) have occurred since 1960 so atmospheric  $\delta^{13}\text{C}$  values in the years of museum collecting would be virtually identical to 1960. For the Ganta and Cameroon specimens, we subtracted an additional 1.1‰ from the bone collagen  $\delta^{13}\text{C}$  values [following Smith et al., 2010] who relied on Hoppe's estimate from prehistoric bison tooth enamel samples standardized against ice core data [Hoppe et al., 2006]. This figure is almost identical to the 1.2‰ used by Crowley [Crowley et al., 2011a] who relied on Chamberlain's estimate from prehistoric condors that was also standardized against ice core data [Chamberlain et al., 2005].

The different Pan sites were characterized using The Nature Conservancy's (TNC) online spatial dataset for the terrestrial ecoregions and biomes of the world (see <http://maps.tnc.org/>). Categories of the TNC system were designated through collaborations with the World Wildlife Fund and over 1,000 scholars from diverse fields, including ecologists, biogeographers, taxonomists, and conservationists. The categories provide highly accurate spatial categorization of different

**TABLE I. Isotope Results From Gombe Chimpanzee (*P. troglodytes*) Hair**

Spec #	Name	Sex	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C:N
MS-6174	Evered	M	−23.6	3.2	3.7
MS-6175	Fifi	F	−23.1	3.1	3.7
MS-6177	Faustino	M	−22.7	3.4	3.7
MS-6179	Frodo	M	−22.7	3.8	3.4
MS-6181	Gimble	M	−23.1	4.0	3.5
MS-6182	Goblin	M	−23.5	3.6	3.5
MS-6186	Prof	M	−22.8	3.0	3.6
MS-6190	Pax	M	−23.0	3.9	3.5
MS-6191	Sandi	F	−23.7	3.1	3.6
MS-6192	Spindle	M	−23.2	3.6	3.7
MS-6193	Sparrow	F	−23.2	3.8	3.5
MS-6194	Tubi	M	−22.7	3.4	3.6
MS-6197	Wilkie	M	−23.0	3.4	3.7

TABLE II. Summary Statistics of Stable Isotope Values From Chimpanzee Sites

Site	Biome <sup>a</sup>	Ecoregion	MAP (mm)	$\delta^{13}\text{C}$ (‰)			$\delta^{15}\text{N}$ (‰)		
				<i>n</i>	Mean	SD	<i>n</i>	Mean	SD
Ishasha <sup>b</sup>	TSGSS	Sahelian Acacia Savanna	750	8	-23.1	0.2	7	5.9	0.8
Fongoli <sup>c</sup>	TSGSS	West Sudanian Savanna	950	36	-22.2	0.4	36	2.9	0.3
Ugalla <sup>b</sup>	TSGSS	Central Zambebian Miombo Woodlands	1050	12	-22.0	0.3	8	2.3	0.8
Gombe <sup>d</sup>	TSGSS	Central Zambebian Miombo Woodlands	1250	13	-23.1	0.3	13	3.5	0.3
Kibale <sup>e</sup>	TSMBF	Albertine Rift Montane Forest	1500	15	-23.5	0.5	15	7.0	0.8
Cameroon <sup>f</sup>	TSMBF	Northwestern Congolian Lowland Forests	1700	39	-24.9	0.9	39	9.1	1.7
Tai <sup>g</sup>	TSMBF	Western Guinean Lowland Forest	1800	52	-24.9	0.5	52	7.4	0.9
Ganta <sup>h</sup>	TSMBF	Western Guinean Lowland Forest	1956	37	-24.6	0.5	21	6.2	0.7
Loango <sup>i</sup>	TSMBF	Atlantic Equatorial Coastal Forest	2215	14	-24.6	0.5	14	4.8	0.4

<sup>a</sup>Biome types. TSGSS, Tropical and subtropical savannas, grasslands, and shrublands; TSMBF, Tropical and subtropical moist broadleaf forests. Both designations follow the nature conservancy nomenclature.

<sup>b</sup>Schoeninger et al. [1999].

<sup>c</sup>Sponheimer et al. [2006].

<sup>d</sup>This study.

<sup>e</sup>Carter [2001].

<sup>f</sup>Macho and Lee-Thorp [2014].

<sup>g</sup>Fahy et al. [2013].

<sup>h</sup>Smith et al. [2010] with MAP data from Harley [1939].

<sup>i</sup>Oelze et al. [2014].

environmental regions [Olson et al., 2001]. The freely available georeferenced maps provide a standard reference for scholars around the globe. All chimpanzee sites included in the present meta-study fall within two broad biomes: Tropical and Subtropical Moist Broadleaf Forests (TSMBF) and Tropical and Subtropical Savannas, Grasslands, and Shrublands (TSGSS). Within these categories, seven separate terrestrial ecoregions are represented and are identified in Table II.

Rainfall figures (MAP) in the literature can vary greatly as a function of sampling period and methodological issues (as noted above for Gombe). We use the values reported for individual sites by the authors of the papers reporting the stable isotope data. In the case of Gombe, we chose the high end of the range reported by Teleki and colleagues [750–1250 mm; Teleki et al., 1976], which covered a 10-year period, because the value presented by Clutton-Brock & Gillett [1979;

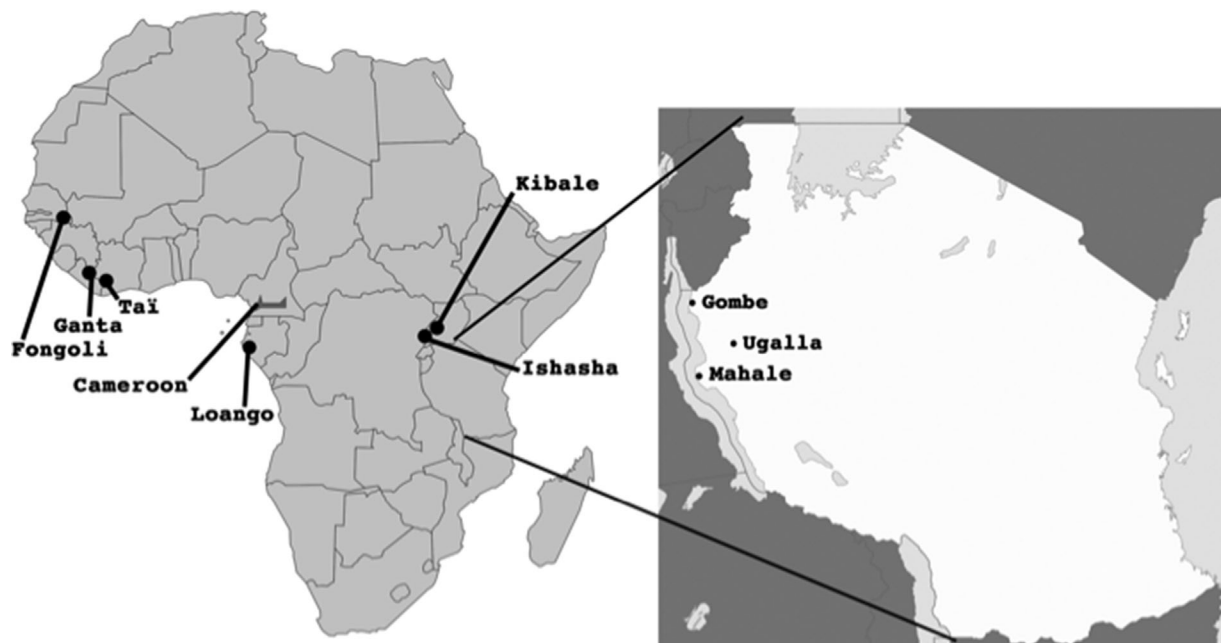


Fig. 1. Map of sites used in the present study.

1546 mm] fell so far outside the 10-year report. For Ganta, we follow Smith et al. [2010] who base their estimate on a 6 year average of values recorded between 1927 and 1936 [Harley, 1939].

To test for differences between males and females at Gombe, we used the non-parametric Mann–Whitney U test. Comparisons of chimpanzee  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  hair values between the two biomes were made with independent samples *t*-tests. Simple linear regressions determined the relationship between MAP and stable isotope ratios. A one-way analysis of variance (ANOVA) and Tukey's post hoc tests of significance assessed the differences in stable isotope ratios between the individual sites. All statistical analyses were conducted with SPSS v. 22.

All hair samples were washed in sequential ultra-sonic baths of double-distilled de-ionized water and acetone to remove environmental contaminants and extraneous body oils. Hairs were dried at 50°C overnight in a laboratory oven. To homogenize bulk samples, hairs were finely cut with a stainless steel scalpel into small (~1 mm) pieces and mixed. Between 0.5 and 1.0 mg were added directly to tin capsules for combustion. Samples were analyzed in an automated fashion on a Thermo-Finnigan Delta XP Plus, Conflow and Costect EA in the Analytical Laboratory at Scripps Institute for Oceanography (SIO). Data are expressed using the standard formula  $\delta = ((R_{\text{sample}}/R_{\text{standard}}) - 1) \times 1,000$  where  $R = {}^{13}\text{C}/{}^{12}\text{C}$  or  ${}^{15}\text{N}/{}^{14}\text{N}$ . The carbon sample was standardized relative to the Pee Dee Belemnite standard (PDB) while the nitrogen sample was standardized relative to the ambient inhalable reservoir (AIR). Repeated analysis of an

internal laboratory standard over the last 5 years indicates a precision of 0.12‰ and 0.15‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively. All hair samples had C:N ratios falling within the acceptable range of 2.9–3.8 [O'Connell et al., 2001].

## RESULTS

There are no statistically significant differences in hair stable isotope values between males and females within the Kasekela community at Gombe ( $\delta^{13}\text{C}_{\text{hair}}$  females = -23.2‰ and males = -23.0‰;  $P = 0.573$ ,  $\delta^{15}\text{N}_{\text{hair}}$  females = 3.3‰ and males; = 3.6‰  $P = 0.371$ ). We note that the sample size for females is small ( $N = 3$  versus 10 males), and more samples are needed to be sure of the apparent lack of difference. Based on the lack of significant differences, however, we combined the samples when comparing with the published data from other sites.

When the Gombe isotope data are combined with data from the other *Pan* sites (Fig. 2), some patterns emerge. Highly significant differences exist between the individual chimpanzee sites in both carbon ( $P < 0.001$ ; Table III) and nitrogen ( $P < 0.001$ ; Table IV) stable isotope values. There is a statistically significant negative regression equation ( $r^2 = 0.5579$ ,  $df = 285.3$ ,  $P < 0.001$ ) between MAP and  $\delta^{13}\text{C}_{\text{hair}}$  values at the various study sites (Fig. 2a) although two sites fall off the line. Ishasha, which has the lowest MAP value, falls below the line and Ugalla with 250 mm more MAP per year than Ishasha falls above the line. In addition, most of the Cameroon samples fall below the line and there are two outliers. The simple

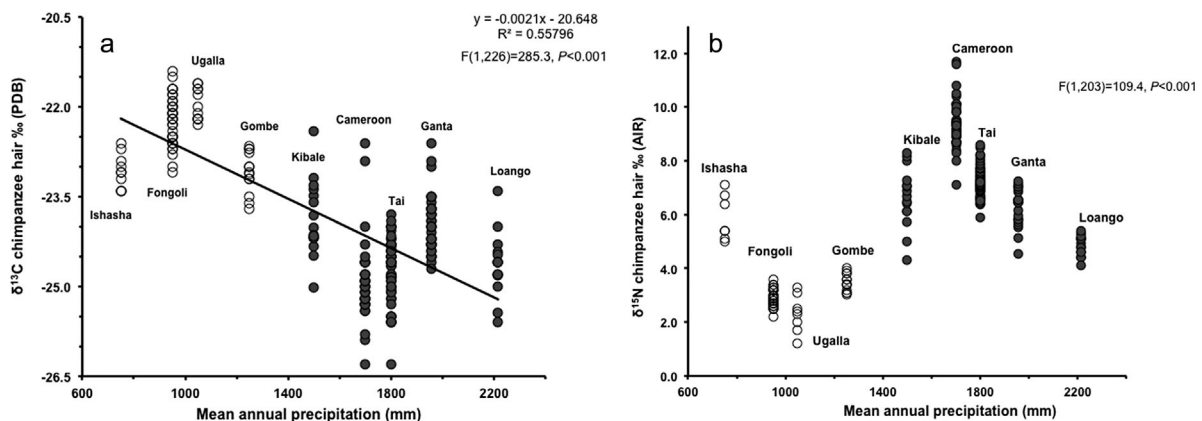


Fig. 2. Carbon ( $\delta^{13}\text{C}_{\text{hair}}$  values) and nitrogen ( $\delta^{15}\text{N}_{\text{hair}}$  values) stable isotope ratios in chimpanzee hair plotted against Mean Annual Precipitation (MAP). The data come from Gombe (this study) and from eight other, previously published chimpanzee sites. The open symbols indicate Tropical and Subtropical Savanna, Grassland, and Shrubland Biome (TSGSS) sites; the closed symbols indicate Tropical and Subtropical Moist Broadleaf Forest (TSMBF) Biome sites. (a) The  $\delta^{13}\text{C}_{\text{hair}}$  values show a statistically significant negative correlation with MAP as expected based on the worldwide distribution of  $\text{C}_3$  plant  $\delta^{13}\text{C}$  values. The higher  $\delta^{13}\text{C}_{\text{hair}}$  values come from sites within TSGSS Biome sites, and within this Biome the Savanna Ecoregion sites have higher MAP values than those from Woodland Ecoregions. The lower  $\delta^{13}\text{C}_{\text{hair}}$  values come from TSMBF Biome sites and within this Biome those with higher  $\delta^{13}\text{C}_{\text{hair}}$  values come from Forest Ecoregions with lower MAP than those with higher precipitation levels. (b) The  $\delta^{15}\text{N}_{\text{hair}}$  values show no clear pattern in the plot against MAP although there is a statistically significant positive relationship, which is opposite to expectations based solely on the ecological variation in soil, plant, and faunal  $\delta^{15}\text{N}$  values from across the globe. The values probably reflect an as yet unidentified differential in diet selectivity between the two types of Biomes (see text for discussion).

TABLE III. Pairwise Comparisons of  $\delta^{13}\text{C}_{\text{hair}}$  Values Between Chimpanzee Site Locations

	Fongoli	Ugalla	Gombe	Kibale	Cameroon	Tai	Ganta	Loango
Ishasha	<b>0.00</b> **	<b>0.00</b> **	1.00	0.01	*	<b>0.00</b> **	<b>0.00</b> **	<b>0.00</b> **
Fongoli		<b>0.00</b> **	0.86	<b>0.00</b> **	<b>0.00</b> **	<b>0.00</b> **	<b>0.00</b> **	<b>0.00</b> **
Ugalla			<b>0.00</b> **	<b>0.00</b> **	<b>0.00</b> **	<b>0.00</b> **	<b>0.00</b> **	<b>0.00</b> **
Gombe				0.01	*	<b>0.00</b> **	<b>0.00</b> **	<b>0.00</b> **
Kibale						<b>0.00</b> **	0.99	<b>0.00</b> **
Cameroon						0.63	<b>0.00</b> **	0.63
Tai							<b>0.00</b> **	1.00
Ganta								<b>0.00</b> **

Bold values indicate statistical significance between the sites compared.

\*\* $P < 0.05$ .

\* $P < 0.001$ .

linear regression assessing the relationship between  $\delta^{15}\text{N}_{\text{hair}}$  and MAP found a significant positive regression equation ( $r^2 = 0.35012$ ,  $df = 109$ ,  $P < 0.001$ ). But the relationship is not entirely clear because MAP explains only 35% of the variation in  $\delta^{15}\text{N}_{\text{hair}}$  values. For that reason, we have plotted the data without a regression line (Fig. 2b).

As seen in the plot of  $\delta^{15}\text{N}_{\text{hair}}$  against  $\delta^{13}\text{C}_{\text{hair}}$  values (Fig. 3), most of the samples from the Tropical and Subtropical Moist Broadleaf Forests (TSMBF) biomes (Kibale, Cameroon, Tai, Ganta, and Loango) exhibit  $\delta^{13}\text{C}_{\text{hair}}$  values between  $-24.0\%$  and  $-26\%$  whether they are from Montane or Lowland Forest Ecoregions. In contrast, most of the samples from the Tropical and Subtropical Savannas, Grasslands, and Shrublands (TSGSS) biomes (Ishasha, Fongoli, Ugalla, and Gombe) have  $\delta^{13}\text{C}_{\text{hair}}$  values between  $-23.0\%$  and  $-21.5\%$  whether they are Savanna or Miombo Woodland ecoregions. There is some overlap of samples from Gombe, Fongoli, and Ishasha (TSGSS biomes) with those from Ganta and Kibale (TSMBF biomes). Independent sample  $t$ -tests found a highly significant difference in  $\delta^{13}\text{C}_{\text{hair}}$  hair values between TSGSS ( $N = 71$ , Mean =  $-22.5\%$ , SD = 0.57) and TSMBF ( $N = 157$ , Mean =  $-24.6\%$ , SD = 0.7) biomes;  $t(226) = 21.6$ ,  $P < 0.001$ . The majority of the  $\delta^{15}\text{N}_{\text{hair}}$

values from the TSMBF biomes fall between 5‰ and 10‰, and those from TSGSS biomes fall between 2‰ and 4‰. Ishasha is an exception: its  $\delta^{15}\text{N}_{\text{hair}}$  values fall well within the values for the TSMBF biomes even though it is located within a Savanna ecoregion of the TSGSS Biome. Even so, independent sample  $t$ -tests found a highly significant difference in  $\delta^{15}\text{N}_{\text{hair}}$  values between TSGSS ( $N = 64$ , Mean = 3.3‰, SD = 1.1) and TSMBF ( $N = 141$ , Mean = 7.5‰, SD = 1.6) biomes;  $t(173.75) = -18.73$ ,  $P < 0.01$ .

## DISCUSSION

The correlation between MAP and  $\delta^{13}\text{C}_{\text{hair}}$  values follows the predicted pattern of higher  $\delta^{13}\text{C}$  values in animal tissues from drier regions with some exceptions although the chimpanzee  $\delta^{13}\text{C}_{\text{hair}}$  values at Ishasha are, on average, almost 1‰ lower than expected. These chimpanzees live in the gallery forest that lines a perennially flowing river with their night nests situated within the gallery forest and most feeding takes places within this more humid region [Sept, 1992]. In contrast, the chimpanzees from Ugalla have  $\delta^{13}\text{C}_{\text{hair}}$  values that are higher than expected based on reported MAP (Fig. 2a) although they are well within expected values for eating a complete  $\text{C}_3$  diet. Ugalla has leguminous trees that form a continuous but thin

TABLE IV. Pairwise Comparisons of  $\delta^{15}\text{N}_{\text{hair}}$  Values Between Chimpanzee Site Locations

	Fongoli	Ugalla	Gombe	Kibale	Cameroon	Tai	Ganta	Loango
Ishasha	<b>0.00</b> **	<b>0.00</b> **	<b>0.00</b> **	0.233	<b>0.00</b> **	<b>0.00</b> **	0.92	0.03 *
Fongoli		0.41	0.14	<b>0.00</b> **	<b>0.00</b> **	<b>0.00</b> **	<b>0.00</b> **	<b>0.00</b> **
Ugalla			<b>0.00</b> **	<b>0.00</b> **	<b>0.00</b> **	<b>0.00</b> **	<b>0.00</b> **	<b>0.00</b> **
Gombe				<b>0.00</b> **	<b>0.00</b> **	<b>0.00</b> **	<b>0.00</b> **	<b>0.00</b> **
Kibale					<b>0.00</b> **	0.09	0.76	<b>0.00</b> **
Cameroon						<b>0.00</b> **	<b>0.00</b> **	<b>0.00</b> **
Tai							<b>0.00</b> **	<b>0.00</b> **
Ganta								<b>0.00</b> **

Bold values indicate statistical significance between the sites compared.

\*\* $P < 0.05$ .

\* $P < 0.001$ .



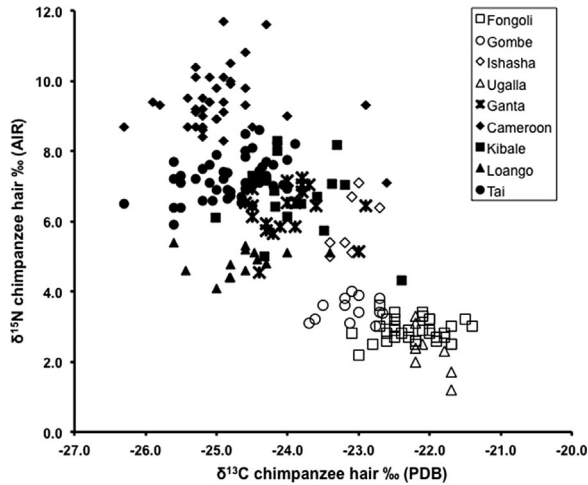


Fig. 3. Plot of the  $\delta^{13}\text{C}_{\text{hair}}$  and  $\delta^{15}\text{N}_{\text{hair}}$  values from Tropical and Subtropical Savanna, Grassland, and Shrubland Biome (TSGSS represented by the open symbols) and Tropical and Subtropical Moist Broadleaf Forest (TSMBF represented by the closed symbols) Biomes. The TSGSS Biome sites are significantly higher in  $\delta^{13}\text{C}_{\text{hair}}$  values and lower in  $\delta^{15}\text{N}_{\text{hair}}$  values. Ishasha, in contrast to the other TSGSS Biome sites, has  $\delta^{15}\text{N}_{\text{hair}}$  values that fall within the distribution of TSMBF sites. These chimpanzees feed in the gallery forest that lines the ever-flowing river and may have access to different foods than those living in the other TSGSS Biome sites.

canopy, which allows enough light for  $\text{C}_4$  grasses in the understory (the botanical identifier for a TSGSS biome). It is possible that Ugalla is a case where the MAP is an unreliable indicator of canopy cover or that the lacey leaves of leguminous trees have relatively high  $\delta^{13}\text{C}$  values. A large project is collecting vegetation samples at present, and the stable isotope analyses of the samples should further our understanding of this site [Piel et al. unpublished data].

The average values for the rest of the sites fall on or close to the regression line, although there is a lot of scatter in individual  $\delta^{13}\text{C}_{\text{hair}}$  values around the line. Some of this scatter is probably the result of chimpanzee dietary selectivity. For example, although the average  $\delta^{13}\text{C}_{\text{hair}}$  value at Loango falls on the regression line, many individuals fall above the line, which may reflect the elevated  $\delta^{13}\text{C}$  values in some fruits eaten by chimpanzees as discussed by Oelze et al. [2014]. To evaluate the possibility that the  $\delta^{13}\text{C}_{\text{hair}}$  values reflect fruit-eating rather than MAP, we compiled published data on percent of feeding time spent on fruit, leaves, flowers, bark, stem/pith, seeds, and other (Table V). Such data are not available for the majority of the sites included in the present study and we decided that there was not a way to compare feeding information taken from fecal material directly with percent of feeding time so we list those sites as not having available feeding data and report only on sites with observations of feeding. Semliki, Uganda [Hunt & McGrew, 2002], in a TSGSS biome with a permanently flowing

river, shows the lowest amount of feeding time spent on fruit (39%) of all the sites for which we have data. Fongoli, Senegal [Pruetz, 2006] another TSGSS biome site reports 62.5% feeding time devoted to fruit based on a limited sample size. We include three different dietary estimates for Gombe, Tanzania (also a TSGSS biome), all of which cite Wrangham [1977]. Two of these report amounts of feeding time spent on fruit (59.4% and 63%) that are relatively similar to each other [Newton-Fisher, 1999; Watts et al., 2012] and to Fongoli; but the third [Morgan & Sanz, 2006] reports a much lower amount of time (43%). Based on personal observations, JMM thinks that the lower value is unlikely; but we include it because it is often cited.

For the TSMBF biome sites, Kanyawara [Wrangham et al., 1996] and Ngogo [Watts et al., 2012] at Kibale, Uganda (a TSMBF biome) are similar to each other (>70% of feeding time spent on fruit), and also Tai, Cote d'Ivoire (77%) [based on Doran, 1997; Watts et al., 2012]. But, Budongo, Uganda, another TSMBF biome reports 64.5% of feeding time spent on fruit [Newton-Fisher, 1999], which is similar to that reported for Fongoli and Gombe (TSGSS biome sites). Overall the wetter sites (Kibale and Tai; but not Budongo) report greater percent feeding time focused on fruit and have lower  $\delta^{13}\text{C}_{\text{hair}}$  values than do the dryer sites (Fongoli and Gombe) suggesting a negative correlation between fruit eating and  $\delta^{13}\text{C}_{\text{hair}}$  value whereas the higher  $\delta^{13}\text{C}$  values in some fruits at Loango suggest that a higher fruit intake would associate with higher, not lower,  $\delta^{13}\text{C}_{\text{hair}}$  values [Oelze et al., 2014]. In other words, the relationship (if there is one) is the opposite of that expected based on assumptions that fruit  $\delta^{13}\text{C}$  values would be higher than leaf  $\delta^{13}\text{C}$  values. Our results, however, compare individuals from multiple populations over a large geographic scale, and do not necessarily negate isotopic patterning across different foods within single sites where data are available on the  $\delta^{13}\text{C}$  values of individual foods. But, they serve to underscore the possibility of error if using high  $\delta^{13}\text{C}$  values in primate tissues to indicate fruit-eating.

Therefore at this time, we can say only that the  $\delta^{13}\text{C}_{\text{hair}}$  values correlate with MAP as expected based on a worldwide survey of  $\text{C}_3$  plants [Kohn, 2010], and not with fruit-eating. The average  $\delta^{13}\text{C}_{\text{hair}}$  values demonstrate a range in  $\delta^{13}\text{C}_{\text{hair}}$  values over 4‰ where all of the animals eat  $\text{C}_3$  foods. Therefore, the general pattern strongly supports the need to take aspects of habitat ecology (biome and ecoregion) into consideration when interpreting the  $\delta^{13}\text{C}$  values in tissues from extant primates and also fossil primates, including hominins.

In a simple example, an early paper on  $\delta^{13}\text{C}_{\text{hair}}$  values in tooth enamel of *Australopithecus africanus*

TABLE V. Percent of Time Feeding

Site	Fruit (%)	Leaves (%)	Flowers (%)	Bark (%)	Other (%)	Stem/pith (%)	Seeds (%)	Total
Fongoli <sup>a</sup>	62.5	16	11	2.5		3		95
Gombe <sup>b</sup>	43	27	10	0	7	8	7	102
Gombe <sup>c</sup>	63	19						82
Gombe <sup>d</sup>	59.4	21.2			19.4			100
Kibale- Kanyawara <sup>e</sup>	79	2.6			16.9			98.5
Kibale-Ngogo <sup>f</sup>	70.7	19.6	2.5			2.2	4	99
Tai <sup>g</sup>	77	17.5			1		4	99.5
Semliki <sup>h</sup>	39	30	3	12				84
Budongo <sup>i</sup>	64.5	19.7	8.8			3.2		96.2
Not available								
Cameroon <sup>j</sup>								
Ganta <sup>j</sup>								
Loango <sup>k</sup>								
Ugalla <sup>j</sup>								
Ishasha <sup>j</sup>								

<sup>a</sup>Pruetz [2006].<sup>b</sup>Morgan and Sanz [2006] citing Wrangham [1977].<sup>c</sup>Newton-Fisher [1999] citing Wrangham [1977].<sup>d</sup>Watts [2012] citing Wrangham [1977].<sup>e</sup>Wrangham et al. [1996].<sup>f</sup>Watts [2012].<sup>g</sup>Based on Doran [1997] and Watts [2012].<sup>h</sup>Hunt and McGrew [2002].<sup>i</sup>Newton-Fisher [1999].<sup>j</sup>No collection or no systematic collection.<sup>k</sup>Fecal prevalence, Oelze et al [2014].

reports an average value of  $-8.2\%$ , concluding “this early hominid...ate large quantities of carbon-13 enriched foods...” [Sponheimer & Lee-Thorp, 1999:368]. Corrected for differences in atmospheric carbon dioxide  $\delta^{13}\text{C}$  values ( $-1.5\%$ ) and the offset between diet and tooth enamel  $\delta^{13}\text{C}$  values (approximately  $10\%$  for a species with a simple gastrointestinal tract) yields a diet value of  $-19.7\%$ . This value is approximately  $2\%$  higher than that expected for  $\text{C}_3$  plants in an open, but not desert, environment [Hartman & Danin, 2010]. Diagenetic alteration of approximately  $1\%$  [following Lee-Thorp, 2000] is expected to enrich the tooth enamel in  $^{13}\text{C}$  for a  $\text{C}_3$  feeder [see Schoeninger et al., 2003]. Subtracting the diagenetic alteration of  $1\%$ , lowers the estimated diet  $\delta^{13}\text{C}$  value to  $-20.7\%$  or approximately  $1\%$  higher than that expected for a  $\text{C}_3$ -feeding early hominin feeding in the dryer portion of a Tropical and Subtropical Savanna, Grassland, and Shrubland biome and indicates a diet around  $5\%$   $\text{C}_4$  foods. Using the lowest *Australopithecus africanus* value reported by Sponheimer & Lee-Thorp [1999], that is  $-11.3\%$ , the estimated diet value ( $-22.8\%$ ) falls within the range for  $\text{C}_3$  plants in this biome. Using the highest value  $-5.6\%$  corrected in the same manner as presented above, gives a diet estimate of  $-17.1\%$ , which would include upwards to  $25\%$   $\text{C}_4$  foods. If, on the other hand, *Australopithecus africanus* had a more complex gastrointestinal tract as suggested by the Expensive Tissue Hypothesis [Aiello & Wheeler, 1995]. A diet to enamel offset of

$14\%$  as reported for modern hindgut and foregut fermenters [Passey et al., 2005] results in a diet estimate of  $-21.1\%$  or  $5\%$   $\text{C}_4$  plants in the diet. We do not purport to know the specifics of the digestive tract in *Australopithecus africanus*; but this simple exercise suggests that the overall calculation of diet in fossil species is more complicated than is often presented.

In contrast, the  $\delta^{15}\text{N}_{\text{hair}}$  values do not follow the predicted pattern of higher values in regions of lower rainfall. In fact, our regression analysis found a weak, but significant, correlation of increasing  $\delta^{15}\text{N}_{\text{hair}}$  values with higher levels of MAP, which is the exact opposite of the expected relationship. The overall scatter indicates that although the relationship between  $\delta^{15}\text{N}_{\text{hair}}$  values and MAP is statistically significant, it is probably meaningless biologically. More likely, chimpanzee  $\delta^{15}\text{N}_{\text{hair}}$  values reflect diet selectivity within individual sites as suggested previously [Fahy et al., 2013; Oelze et al., 2014; Schoeninger et al., 1999] rather than any direct effect of MAP. Some unexpected and interesting relationships appear in the data, however. With one exception (Ishasha), the chimpanzees from the TSGSS biomes have  $\delta^{15}\text{N}_{\text{hair}}$  values are significantly lower than those from the TSMBF biomes. Again, we considered the possibility that the explanation might be different amounts of fruit eating in TSGSS and TSMBF biomes; but we lack a clear pattern of association of fruit-eating with specific biomes. Another possibility is that the chimpanzees in savanna and woodland ecoregions

may be eating more flowers, pods, and leaves of leguminous trees that are engaged in  $N_2$ -fixation. Such a relationship was shown for mantled howler monkeys (*Alouatta palliata*) in a similar environment [Schoeninger et al., 1997] and in sportive lemurs (*Lepilemur leucopus*) [Schoeninger et al., 1998]. The consumption of leguminous species by chimpanzees is suggested by fecal data from Ugalla [see Schoeninger et al., 1999 for original references] and by species lists noting that leguminous plants are eaten throughout the year at Fongoli [Pruetz, 2006]. We look forward to more detailed publications including dietary information at TSGSS sites [e.g., Bogart & Pruetz, 2011; Hernandez-Aguilar et al., 2007; Stewart & Piel, 2014; Webster et al., 2014] and also truly comparative data from TSMBF sites.

It is unlikely that the variation in  $\delta^{15}N_{\text{hair}}$  values is due to differences in soil baseline  $\delta^{15}N$  values. The difference between the average of the four sites within TSGSS biomes (3.3‰) and the five sites within the TSMBF biomes (7.5‰) is 4.2‰. If Ishasha is dropped because it is a riverine environment (2.9‰ average of Fongoli, Ugalla, and Gombe) the average difference between biome types is 4.6‰. In contrast, the anticipated variations in soil values in this region of Africa are on the order of 2‰ [Amundson et al., 2003]. We look forward to additional studies on  $\delta^{15}N$  values in plants and soils from across Africa since the expected relationship between MAP and  $\delta^{15}N_{\text{hair}}$  values is unsupported in our study. The data thus far do not support the use of nitrogen stable isotope data in habitat reconstructions; but could be extremely useful in identifying specific aspects of diet choice in chimpanzees from different sites.

In summation, we show that the carbon stable isotope data in chimpanzees correlate with environmental variation in ecology (forest vs savanna) and with Mean Annual Precipitation (MAP) across most of the nine chimpanzee habitats for which we have stable isotope data, and can serve as indirect estimators of those habitats. The only exception is the chimpanzee habitat within a riverine gallery forest that lies within a larger region of low rainfall. Where similar regions occur within the fossil record (e.g., the Turkana basin in northern Kenya at 3.9 my with sites along a perennially flowing Omo River), regional habitat reconstructions [Cerling et al., 2011] may not capture the local areas in which fossil hominins lived and fed. For example, if the  $\delta^{13}C$  values in tooth enamel of fossil hominin material collected along a perennial river are at the lower end of the expected range for eating  $C_3$  foods, it would suggest that they are feeding and sleeping in a forest even though the overall ecoregion might look to be a grassland or savanna based on associated faunal species or soil  $\delta^{13}C$  values. Those with values that are high for eating  $C_3$  foods such as *Australopithecus anamensis* [Cerling et al., 2013] could be feeding away from the forest on  $C_3$

foods rather than including small amounts of  $C_4$  foods in their diet as is often suggested [Codron et al., 2008; Sponheimer & Lee-Thorp, 1999].

Most importantly, researchers can use stable isotope data to address scales of space and time that are impractical by direct observation over the course of long periods of time and between distant ecological regions. The stable isotope values can, in turn, provide the basis for modeling aspects of the habitats of animals whose behavior is unobservable such as mouse lemurs [e.g., Crowley et al., 2011b] or chimpanzees where the specimens were collected without habitat information. Indeed, we demonstrate that we must consider that the carbon stable isotope values of extinct hominins and other nonhuman primate species contain more information than a diet positioned somewhere along a  $C_3$  to  $C_4$  continuum.

## ACKNOWLEDGMENTS

We thank the editors of this volume for the opportunity to participate in a session at the American Association of Physical Anthropologists and to submit a manuscript as well as the efforts of two reviewers whose comments greatly improved the article. We also thank the funding sources, which supported this project and the ones in which the hairs were collected (The Regents of the University of California to MJS and a CARTA Fellowship to CAM). Dr. Bruce Deck oversaw the stable isotope analyses at the Analytical Facility of The Scripps Institution of Oceanography at UCSD, and Kristen Snodgrass assisted with sample preparation. This research compiles with all policies of ethical research and treatment of non-human primates and adheres to the American Society of Primatologists (ASP) Principles for the Ethical Treatment of Non-Human Primates, with all protocols of the Institutional Animal Care Committee at UCSD, and with all legal requirements of the host country, that is Tanzania.

## REFERENCES

- Aiello LC, Wheeler P. 1995. The expensive-tissue hypothesis: the brain and the digestive system in human and primate evolution. *Current Anthropology* 36:199–221.
- Ambrose SH. 1991. Effects of diet, climate and physiology on nitrogen isotope abundances in terrestrial foodwebs. *Journal of Archaeological Science* 18:293–317.
- Amundson R, Austin AT, Schuur EAG, et al. 2003. Global patterns of the isotopic composition of soil and plant nitrogen. *Global Biogeochemical Cycles* 17:1031–1042.
- Aranibar JN, Otter L, Macko SA, et al. 2004. Nitrogen cycling in the soil-plant system along a precipitation gradient in the Kalahari sands. *Global Change Biology* 10:359–373.
- Austin AT, Vitousek PM. 1998. Nutrient dynamics on a precipitation gradient in Hawaii. *Oecologia* 113:519–529.
- Basabose A. 2002. Diet composition of chimpanzees inhabiting the montane forest of Kahuzi, Democratic Republic of Congo. *American Journal of Primatology* 58:1–21.

- Boesch C, Boesch-Achermann H. 2000. The chimpanzees of the Tai Forest. Oxford: Oxford University Press. p 326.
- Bogart S, Pruett JD. 2011. Insectivory of savanna chimpanzees (*Pan troglodytes* verus) at Fongoli, Senegal. *American Journal of Physical Anthropology* 145:11–20.
- Bonafini M, Pellegrini M, Ditchfield P, Pollard AM. 2013. Investigation of the 'canopy effect' in the isotope ecology of temperate woodlands. *Journal of Archaeological Science* 40:3926–3935.
- Carter ML. 2001. Sensitivity of stable isotopes ( $^{13}\text{C}$ ,  $^{15}\text{N}$ , and  $^{18}\text{O}$ ) in bone to dietary specialization and niche separation among sympatric primates in Kibale National Park, Uganda [Doctoral dissertation]. Chicago: University of Chicago. p 267.
- Cerling TE, Harris J, Passey B. 2003. Diets of East African bovidae based on stable isotope analyses. *Journal of Mammalogy* 84:456–470.
- Cerling TE, Hart JA, Hart TB. 2004. Stable isotope ecology in the Ituri Forest. *Oecologia* 138:5–12.
- Cerling TE, Levin NE, Passey BH. 2011. Stable isotope ecology in the Omo-Turkana Basin. *Evolutionary Anthropology* 20:228–237.
- Cerling TE, Manthi FK, Mbua EN, et al. 2013. Stable isotope-based diet reconstructions of Turkana Basin hominins. *Proceedings of the National Academy of Sciences* 110:10501–10506.
- Chamberlain CP, Waldbauer JR, Fox-Dobbs K, et al. 2005. Pleistocene to recent dietary shifts in California condors. *Proceedings of the National Academy of Sciences* 102:16707–16711.
- Clutton-Brock TH, Gillett J. 1979. A survey of forest composition in the Gombe National Park, Tanzania. *African Journal of Ecology* 17:131–158.
- Codron D, Lee-Thorp JA, Sponheimer M, de Ruiter D, Codron J. 2006. Inter- and intrahabitat dietary variability of chacma baboons (*Papio ursinus*) in South African savannas based on fecal  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and %N. *American Journal of Physical Anthropology* 129:204–214.
- Codron D, Lee-Thorp JA, Sponheimer M, de Ruiter D, Codron J. 2008. What insights can baboon feeding ecology provide for early hominin niche differentiation? *International Journal of Primatology* 29:757–772.
- Codron J, Codron D, Lee-Thorp JA, et al. 2005. Taxonomic, anatomical, and spatio-temporal variations in the stable carbon and nitrogen isotopic compositions of plants from an African savanna. *Journal of Archaeological Science* 32:1767–1772.
- Collins DA, McGrew WC. 1988. Habitats of three groups of chimpanzees (*Pan troglodytes*) in western Tanzania compared. *Journal of Human Evolution* 17:553–574.
- Cormie AB, Schwarcz HB. 1996. Effects of climate on deer bone  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ : lack of precipitation effects on  $\delta^{15}\text{N}$  for animals consuming low amounts of C4 plants. *Geochimica et Cosmochimica Acta* 60:4161–4166.
- Cormie AB, Schwarcz HP. 1994. Stable isotopes of nitrogen and carbon of North American white-tailed deer and implications for paleodietary and other food web studies. *Paleogeography, Paleoclimatology, Paleoecology* 107:227–241.
- Craine JM, Elmore AJ, Aida MPM, et al. 2009. Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New Phytologist* 183:980–992.
- Crowley BE. 2012. Stable isotope techniques and applications for primatologists. *International Journal of Primatology* 33:673–701.
- Crowley BE, Carter ML, Karpanty SM, et al. 2010. Stable carbon and nitrogen isotope enrichment in primate tissues. *Oecologia* 164:611–626.
- Crowley BE, Godfrey LR, Irwin MT. 2011a. A glance to the past: subfossils, stable isotopes, seed dispersal, and lemur species loss in Southern Madagascar. *American Journal of Primatology* 73:25–37.
- Crowley BE, Thoren S, Rasoazanabary E, et al. 2011b. Explaining geographical variation in the isotope composition of mouse lemurs (*Microcebus*). *Journal of Biogeography* 38:2106–2121.
- DeNiro MJ, Epstein S. 1978. Influence of diet on distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* 42:495–506.
- DeNiro MJ, Epstein S. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* 45:341–351.
- Diefendorf AF, Mueller KE, Wing SL, Koch PL, Freeman KH. 2010. Global patterns in leaf  $^{13}\text{C}$  discrimination and implications for studies of past and future climate. *Proceedings of the National Academy of Sciences of the United States* 107:5738–5742.
- Doran D. 1997. Influence of seasonality on activity patterns, feeding behavior, ranging, and grouping patterns in Tai chimpanzees. *International Journal of Primatology* 18:183–206.
- Ehleringer JR. 1978. Implications of quantum yield differences on the distributions of C3 and C4 grasses. *Oecologia* 31:255–267.
- Evans RD. 2001. Physiological mechanisms influencing plant nitrogen isotope composition. *Trends in Plant Science* 6:121–126.
- Fahy G, Richards MR, Riedel J, Hublin JJ, Boesch C. 2013. Stable isotope evidence of meat eating and hunting specialization in adult male chimpanzees. *Proceedings of the National Academy of Sciences* 110:5829–5833.
- Farquhar GD, Ehleringer JR, Hubick KT. 1989. Carbon isotope discrimination and photosynthesis. *Annual Reviews of Plant Physiology and Plant Molecular Biology* 40:503–537.
- Farquhar GD, O'Leary MH, Berry JA. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology* 9:121–137.
- Fashing PJ, Nguyen N, Venkataraman VV, Kerby JT. 2014. Gelada feeding ecology in an intact ecosystem at Guassa, Ethiopia: variability over time and implications for theropit and hominin dietary evolution. *American Journal of Physical Anthropology* 155:1–16.
- Fernandez R. in press Quantitative diet reconstruction of a Neolithic population using a Bayesian Mixing model (FRUITS): the case study of Ostorf (Germany). *American Journal of Physical Anthropology*.
- Fleagle JG. 2013. *Primate adaptation and evolution*. Oxford: Elsevier. p 441.
- Froehle AW, Kellner CM, Schoeninger MJ. 2010. Effect of diet and protein source on carbon stable isotope ratios in collagen: follow up to Warinner and Tuross. *Journal of Archaeological Science* 37:2662–2670.
- Fry B. 2006. *Stable isotope ecology*. New York: Springer. p 308.
- Goodall J. 1986. *The chimpanzees of gombe: patterns of behavior*. Cambridge, MA: Harvard University Press. p 673.
- Grocke DR, Bocherens H, Mariotti A. 1997. Annual rainfall and nitrogen-isotope correlation in macropod collagen: application as a palaeoprecipitation indicator. *Earth and Planetary Science Letters* 153:279–285.
- Handley LL, Austin AT, Robinson D, et al. 1999. The  $^{15}\text{N}$  natural abundance ( $\delta^{15}\text{N}$ ) of ecosystem samples reflects measures of water availability. *Australian Journal of Plant Physiology* 1999:26.
- Harley GW. 1939. Roads and trails in Liberia. *Geographical Review* 29.
- Hartman G. 2011. Are elevated  $\delta^{15}\text{N}$  values in herbivores in hot and arid environments caused by diet or animal physiology? *Functional Ecology* 25:122–131.

- Hartman G, Danin A. 2010. Isotopic values of plants in relation to water availability in the Eastern Mediterranean region. *Oecologia* 162:837–852.
- Heaton THE. 1987. The  $^{15}\text{N}/^{14}\text{N}$  ratios of plants in South Africa and Namibia: relationship to climate and coastal/saline environments. *Oecologia* 74:236–246.
- Heaton THE, Vogel JC, von la Chevallerie G, Gollett G. 1986. Climatic influence on the isotopic composition of bone nitrogen. *Nature* 322:822–823.
- Hernandez-Aguilar R, Moore J, Pickering TR. 2007. Savanna chimpanzees use tools to harvest the underground storage organs of plants. *Proceedings of the National Academy of Sciences of the United States* 104:19210–19213.
- Hoefs J. 2009. *Stable isotope geochemistry*. New York: Springer-Verlag. p 201.
- Hoppe KA, Paytan A, Chamberlain CP. 2006. Reconstructing grassland vegetation and paleotemperatures using carbon isotope ratios of bison tooth enamel. *Geology* 34:649–652.
- Hunt KD, McGrew WC. 2002. Chimpanzees in the dry habitats of assirik, senegal and semliki wildlife reserve, uganda. In: Boesch CGH, LFM, editors. *Behavioural diversity in chimpanzees and bonobos*. Cambridge: Cambridge University Press. p 35–51.
- Johnson BJ, Fogel ML, Miller GH. 1998. Stable isotopes in modern ostrich eggshell: a calibration for paleoenvironmental applications in semi-arid regions of southern Africa. *Geochimica et Cosmochimica Acta* 62:2451–2461.
- Kingston JD. 2007. Shifting adaptive landscapes: progress and challenges in reconstructing early hominid environments. *American Journal of Physical Anthropology* 134:20–58.
- Koch PL. 2007. Isotopic study of the biology of modern and fossil vertebrates. In: Mitchener R, Lajtha K, editors. *Stable isotopes in ecology and environmental science*. USA: Wiley-Blackwell. p 99–154.
- Kohn MJ. 2010. Carbon isotope compositions of terrestrial C3 plants as indicators of (paleo) ecology and (paleo) climate. *Proceedings of the National Academy of Sciences* 107:19691–19695.
- Lajtha K, Marshall JD. 1994. Sources of variation in the stable isotopic composition of plants. In: Lajtha K, Michener RH, editors. *Stable isotopes in ecology and environmental science*. Berlin: Blackwell Scientific Publications. p 1–21.
- Lee-Thorp JA. 2000. Preservation of biogenic carbon isotopic signals in Plio-Pleistocene bone and tooth mineral. In: Ambrose SH, Katzenberg MA, editors. *Biogeochemical approaches to paleodietary analysis*. New York: Kluwer Academic/Plenum Publishers. p 89–115.
- Macho GA, Lee-Thorp JA. 2014. Niche partitioning in sympatric Gorilla and Pan from Cameroon: implications for life history strategies and for reconstructing the evolution of hominin life history. *PLoS ONE* 9:1–17.
- Marino BD, McElroy MB. 1991. Isotopic composition of atmospheric  $\text{CO}_2$  inferred from carbon in  $\text{C}_4$  plant cellulose. *Nature* 349:127–131.
- Marshall JD, Brooks JR, Lajtha K. 2007. Sources of variation in the stable isotopic composition of plants. In: Michener R, Lajtha K, editors. *Stable isotopes in ecology and environmental science*. 2 ed. USA: Wiley-Blackwell. p 22–60.
- Martinelli LA, Piccolo MC, Townsend AR, et al. 1999. Nitrogen stable isotopic composition of leaves and soil: tropical versus temperate forests. *Biogeochemistry* 46:45–65.
- McGrew WC, Baldwin PJ, Tutin CEG. 1981. Chimpanzees in a hot, dry and open habitat: Mt. Assirik, Senegal, West Africa. *Journal of Human Evolution* 10:227–244.
- McGrew WC, Baldwin PJ, Tutin CEG. 1988. Diet of wild chimpanzees (*Pan troglodytes verus*) at Mt. Assirik, Senegal: I. composition. *American Journal of Primatology* 16:213–226.
- Michaletz ST, Cheng D, Kerkhoff AJ, Enquist BJ. 2014. Convergence of terrestrial plant production across global climate gradients. *Nature* 512:39–43.
- Minagawa M, Wada E. 1984. Stepwise enrichment of  $^{15}\text{N}$  along food chains: further evidence and the relation between  $\delta^{15}\text{N}$  and animal age. *Geochimica Cosmochimica Acta* 48:1135–1140.
- Moore J. 1992. “Savanna” chimpanzees. In: Nishida T, McGrew WC, Marler P, Pickford M, de Waal FBM, editors. *Topics in primatology, Vol I: human origins*. Tokyo: University of Tokyo Press. p 99–118.
- Moore J. 1996. Savanna chimpanzees, referential models and the last common ancestor. In: McGrew WC, Marchant L, Nishida T, editors. *Great ape societies*. Cambridge: Cambridge University Press. p 275–292.
- Morgan D, Sanz C. 2006. Chimpanzee feeding ecology and comparisons with sympatric gorillas in the Goulougo Triangle, Republic of Congo. In: Hohmann G, Robbins MM, Boesch C, editors. *Feeding ecology in apes and other primates: ecological, physical and behavioral aspects*. Cambridge, England: Cambridge University Press. p 97–122.
- Murphy BP, Bowman D. 2006. Kangaroo metabolism does not cause the relationship between bone collagen  $\delta^{15}\text{N}$  and water availability. *Functional Ecology* 20:1062–1069.
- Murphy BP, Bowman DMJS. 2009. The carbon and nitrogen isotope composition of Australian grasses in relation to climate. *Functional Ecology* 23:1040–1049.
- Murray SS, Schoeninger MJ, Bunn HT, Pickering TR, Marlett JA. 2001. Nutritional composition of some wild plant foods and honey used by Hadza foragers of northern Tanzania. *Journal of Food Composition and Analysis* 14:3–13.
- N’guessan AK, Ortmann S, Boesch C. 2009. Daily energy balance and protein gain among Pan troglodytes verus in the Tai National Park, Côte d’Ivoire. *International Journal of Primatology* 30:481–496.
- Newton-Fisher N. 1999. The diet of chimpanzees in the Budongo Forest Reserve, Uganda. *African Journal of Ecology* 37:344–354.
- Nishida T, Uehara S. 1983a. Natural Diet of chimpanzees (*Pan troglodytes schweinfurthii*): long-term record from the Mahale Mountains Tanzania. *African Studies Monograph* 3:109–130.
- Nishida T, Uehara S. 1983b. Natural diet of chimpanzees (*Pan troglodytes schweinfurthii*): long-term record from the Mahale Mountains, Tanzania. *African Study Monographs* 3:109–130.
- Nishida T, Wrangham RW, Goodall J, Uehara S. 1983. Local differences in plant-feeding habits of chimpanzees between the Mahale Mountains and Gombe National Park, Tanzania. *Journal of Human Evolution* 12:467–480.
- O’Connell TC, Hedges REM, Healey MA, Simpson A. 2001. Isotopic comparison of hair, nail and bone: modern analyses. *Journal of Archaeological Science* 28:1247–1255.
- O’Leary MH. 1981. Carbon isotope fractionation in plants. *Phytochemistry* 20:553–567.
- O’Leary MH. 1988. Carbon isotopes in photosynthesis. *BioScience* 38:328–336.
- O’Regan H, Chenery C, Lamb A, et al. 2008. Modern macaque dietary heterogeneity assessed using stable isotope analysis of hair and bone. *Journal of Human Evolution* 55:617–626.
- Oelze V, Fuller B, Richards M, et al. 2011. Exploring the contribution and significance of animal protein in the diet of bonobos by stable isotope ratio analysis of hair. *Proceedings of the National Academy of Sciences* 108:9792–9797.
- Oelze V, Head J, Robbins MM, Richards MP, Boesch C. 2014. Niche differentiation and dietary seasonality among sympatric gorillas and chimpanzees in Loango National Park (Gabon) revealed by stable isotope analysis. *Journal of Human Evolution* 66:95–106.
- Ogawa J, Idani J, Moore J, Hernandez-Aguilar. 2007. Sleeping patterns and nest distribution of chimpanzees in the savanna woodland, Ugalla, Tanzania. *International Journal of Primatology* 28:1397–1412.

- Olson DM, Dinerstein E, Wikramanayake ED, et al. 2001. Terrestrial ecoregions of the world: a new map of life on earth: a new global map of terrestrial ecoregions provides an innovative tool for conserving biodiversity. *BioScience* 51:933–938.
- Passey BH, Robinson TF, Ayliffe LK, et al. 2005. Carbon isotope fractionation between diet, breath CO<sub>2</sub>, and bioapatite in different mammals. *Journal of Archaeological Science* 32:1459–1470.
- Pate F, Anson T. 2008. Stable nitrogen isotope values in arid-land Kangaroos correlated with mean annual rainfall: potential as a palaeoclimatic indicator. *International Journal of Osteoarchaeology* 18:317–326.
- Potts K, Watts D, Wrangham RW. 2011. Comparative feeding ecology of two communities of chimpanzees (*Pan troglodytes*) in Kibale National Park, Uganda. *International Journal of Primatology* 32:669–690.
- Pruetz JD. 2006. Feeding ecology of savanna chimpanzees (*Pan troglodytes verus*) at Fongoli, Senegal. In: Hohmann G, editor. *Feeding ecology in apes and other primates: ecological, physical and behavioural aspects*. Cambridge: Cambridge University Press. p 161–182.
- Reed KE. 1997. Early hominid evolution and ecological change through the African Plio-Pleistocene. *Journal of Human Evolution* 32:289–322.
- Richards MP, Pettitt PB, Stiner MC, Trinkaus E. 2001. Stable isotope evidence for increasing dietary breadth in the European mid-Upper Paleolithic. *Proceedings of the National Academy of Sciences* 98:6528–6532.
- Richards MP, Taylor G, Steele T, et al. 2008. Isotopic dietary analysis of a Neanderthal and associated fauna from the site of Jonzac (Charente-Maritime), France. *Journal of Human Evolution* 55:179–185.
- Rundel PW, Ehleringer JR, Nagy KA, editors. 1989. *Stable isotopes in ecological research*. 1st ed. New York: Springer-Verlag. p 525.
- Sandberg PA, Loudon JE, Sponheimer M. 2012. Stable isotope analysis in primatology: a critical review. *American Journal of Primatology* 47:969–989.
- Schoeninger MJ. 1985. Trophic level effects on <sup>15</sup>N/<sup>14</sup>N and <sup>13</sup>C/<sup>12</sup>C ratios in bone collagen and strontium levels in bone mineral. *Journal of Human Evolution* 14:515–525.
- Schoeninger MJ. 2014. Stable isotopes and the evolution of human diet. *Annual Review of Anthropology* 43:413–430.
- Schoeninger MJ, DeNiro MJ. 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. *Geochimica et Cosmochimica Acta* 48:625–639.
- Schoeninger MJ, Hallin K, Reeser H, Valley JW, Fournelle J. 2003. Isotopic alteration of mammalian tooth enamel. *International Journal of Osteoarchaeology* 13:11–19.
- Schoeninger MJ, Iwaniec UT, Glander KE. 1997. Stable isotope ratios monitor diet and habitat use in New World Monkeys. *American Journal of Physical Anthropology* 103:69–83.
- Schoeninger MJ, Iwaniec UT, Nash LT. 1998. Ecological attributes recorded in stable isotope ratios of arboreal prosimian hair. *Oecologia* 113:222–230.
- Schoeninger MJ, Moore J, Sept JM. 1999. Subsistence strategies of two “savanna” chimpanzee populations: the stable isotope evidence. *American Journal of Primatology* 49:297–314.
- Schulze E-D, Williams RJ, Farquhar GD, et al. 1998. Carbon and nitrogen isotope discrimination and nitrogen nutrition of trees along a rainfall gradient in northern Australia. *Australian Journal of Plant Physiology* 25:413–425.
- Sealy JC, van der Merwe NJ, Lee Thorp JA, Lanham JL. 1987. Nitrogen isotopic ecology in southern Africa: implications for environmental and dietary tracing. *Geochimica et Cosmochimica Acta* 51:2707–2717.
- Sept JM. 1992. Was there no place like home? *Current Anthropology* 33:187–207.
- Shearer G, Kohl D. 1986. N<sub>2</sub>-Fixation in field settings: estimations Based on natural <sup>15</sup>N abundance. *Functional Plant Biology* 13:699–756.
- Shearer G, Kohl DH. 1994. Information derived from variation in the natural abundance of <sup>15</sup>N in complex biological systems. In: Bunzel E, Saunders WHJ, editors. *Isotopes in organic chemistry: heavy atom isotope effects*. Amsterdam: Elsevier Science Pub. p 191–237.
- Smith BN, Epstein S. 1971. Two categories of <sup>13</sup>C/<sup>12</sup>C ratios for higher plants. *Plant Physiol* 47:380–384.
- Smith C, Morgan M, Pilbeam D. 2010. Isotopic ecology and dietary profiles of liberian chimpanzees. *Journal of Human Evolution* 58:43–55.
- Sponheimer M, Alemseged Z, Cerling TE, et al. 2013. Isotopic evidence of early hominin diets. *Proceedings of the National Academy of Sciences* 110:10513–10518.
- Sponheimer M, Lee-Thorp J, de Ruiter D, et al. 2005. Hominins, sedges, and termites: new carbon isotope data from Sterkfontein valley and Kruger National Park. *Journal of Human Evolution* 48:301–312.
- Sponheimer M, Lee-Thorp JA. 1999. Isotopic evidence for the diet of an early hominid, *Australopithecus africanus*. *Science* 283:368–370.
- Sponheimer M, Loudon JE, Codron D, et al. 2006. Do “savanna” chimpanzees consume C<sub>4</sub> resources? *Journal of Human Evolution* 51:128–133.
- Sponheimer M, Passey BH, de Ruiter D, Guatelli-Steinberg D, Cerling TE, Lee-Thorp JA. 2006. Isotopic evidence for dietary variability in the early hominin *Paranthropus robustus*. *Science* 314:980–982.
- Stevens RE, Lister AM, Hedges REM. 2006. Predicting diet, trophic level and palaeology from bone stable isotope analysis: a comparative study of five red deer populations. *Oecologia* 149:12–21.
- Stewart F, Piel A. 2014. Termite fishing by wild chimpanzees: new data from Ugalla, western Tanzania. *Primates* 55:35–40.
- Stowe LG, Teeri JA. 1978. The geographic distribution of C<sub>4</sub> Species of the dicyledonae in relation to climate. *The American Naturalist* 112:609–623.
- Teleki G, Hunt E, Jr., Pflifferling J. 1976. Demographic observations (1963–1973) on the chimpanzees of Gombe National Park, Tanzania. *Journal of Human Evolution* 71:20–27.
- Tieszen LL, Senyimba MM, Imbamba SK, Troughton JH. 1979. The distribution of C<sub>3</sub> and C<sub>4</sub> grasses and carbon isotope discrimination along an altitudinal and moisture gradient in Kenya. *Oecologia* 37:337–350.
- Tutin C, Fernandez M. 1993. Composition of the diet of chimpanzees and comparisons with that of sympatric lowland gorillas in the Lope Reserve, Gabon. *American Journal of Primatology* 30:195–211.
- Ugan A, Coltrain J. 2011. Variation in stable nitrogen values in black-tailed jackrabbits (*Lepus californicus*) in relation to small-scale differences in climate, soil and topography. *Journal of Archaeological Science* 38:1417–1429.
- van der Merwe NJ, Medina E. 1991. The canopy effect, carbon isotope ratios and foodwebs in Amazonia. *Journal of Archaeological Science* 18:249–259.
- Vogel JC. 1978. Recycling of carbon in a forest environment. *Oecologia Plantarum* 13:89–94.
- Wallis J. 1995. Seasonal influence on reproduction in chimpanzees of Gombe National Park. *International Journal of Primatology* 16:435–451.
- Warinner C, Garcia NR, Tuross N. 2013. Maize, beans and the floral isotopic diversity of highland Oaxaca, Mexico. *Journal of Archaeological Science* 40:868–873.
- Watts D, Potts K, Lwanga J, Mitani J. 2012. Diet of chimpanzees (*Pan troglodytes schweinfurthii*) at Ngogo, Kibale National Park, Uganda. *American Journal of Primatology* 144:130–144.

- Webster T, McGrew WC, Marchant L, Payne C, Hunt K. 2014. Selective insectivory at Toro-Semliki, Uganda: comparative analyses suggest no 'savanna' chimpanzee pattern. *Journal of Human Evolution* 71:20–27.
- Wilson M. 2012. Long-term studies of the Gombe chimpanzees. In: Kappeler P, Watts D, editors. *Long-term field studies of primates*. New York: Springer. p 357–384.
- Wrangham RW. 1977. Feeding behaviour of chimpanzees in Gombe National Park, Tanzania. In: Clutton-Brock TH, editor. *Primate ecology: studies of feeding and ranging behaviours in lemurs, monkeys and apes*. London: Academic Press. p 504–538.
- Wrangham RW, Chapman CA, Clark-Arcadi AP, Isabirye-Basuta G. 1996. Social ecology of Kanyawara chimpanzees: implications for understanding the costs of great ape groups. In: McGrew WC, Marchant L, Nishida T, editors. *Great ape societies*. Cambridge: Cambridge University Press. p 45–57.
- Wrangham RW, Conklin-Brittain NL, Hunt KD. 1998. Dietary response of chimpanzees and cercopithecines to seasonal variation in fruit abundance. I. Antifeedants. *International Journal of Primatology* 19:949–970.
- Wynn JG. 2000. Paleosols, stable carbon isotopes, and paleoenvironmental interpretation of Kanapoi, Northern Kenya. *Journal of Human Evolution* 39:411–432.
- Wynn JG, Sponheimer M, Kimbel WH, et al. 2013. Diet of *Australopithecus afarensis* from the Pliocene Hadar Formation, Ethiopia. *Proceedings of the National Academy of Sciences* 110:10495–10500.
- Yakir D, Israeli Y. 1995. Reduced solar irradiance effects on net primary productivity (NPP) and the  $d^{13}C$  and  $d^{18}O$  values in plantations of *Musa* sp., *Musaceae*. *Geochimica et Cosmochimica Acta* 59:2149–2151.