Comparative Isotope Ecology of African Great Apes

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Abstract

The isotope ecology of great apes is a useful reference for palaeodietary reconstructions in fossil hominins. As extant apes live in C_3-dominated habitats, variation in isotope signatures is assumed to be low compared to hominoids exploiting C_4-plant resources. However, isotopic differences between sites and between and within individuals were poorly understood due to the lack of vegetation baseline data. In this comparative study we included all species of free-ranging African great apes (Pan troglodytes, Pan paniscus, Gorilla sp.). First, we explore differences in isotope baselines across different habitats and whether isotopic signatures in apes can be related to feeding niches (faunivory and folivory). Secondly, we illustrate how stable isotopic variations within African ape populations compare to other extant and extinct primates and discuss possible implications for dietary flexibility. Using 701 carbon and nitrogen isotope data points resulting from 148 sectioned hair samples and an additional collection of 189 fruit samples we compare six different great ape sites. We investigate the relationship between vegetation baselines and climatic variables, and subsequently correct great ape isotope data to a standardized plant baseline from the respective sites. We gained temporal isotopic profiles of individual animals by sectioning hair along its growth trajectory. Isotopic signatures of great apes differed between sites, mainly as vegetation isotope baselines were correlated with site-specific climatic conditions. We show that controlling for plant isotopic characteristics at a given site is essential for faunal data interpretation. While accounting for plant baseline effects, we found distinct isotopic profiles for each great ape population. Based on evidence from habituated groups and sympatric great ape species these differences could possibly be related to faunivory and folivory. Dietary flexibility in apes varied, but temporal variation was overall lower than in fossil hominins and extant baboons, shifting from C_3 to C_4-resources, providing new perspectives on comparisons between extinct and extant primates.

Introduction

The feeding ecology of different primate species is a valuable reference for reconstructing the ecological niche of extinct hominoids. In particular, great apes reveal a diversity of behavioral traits and ecological adaptations which provide a unique framework for ecological reconstructions in our earliest ancestors in Pleistocene Africa (e.g. Stanford, 2006). For fossil hominins, various lines of evidence (e.g. skeletal and dental morphology, dental wear and plant residuals in calculus) can provide information on feeding behavior (e.g. summarized by Ungar and Sponheimer, 2011). Recently, stable isotope analysis in both extant and extinct primates is increasingly utilized to link past and present feeding ecology (Ungar and Sponheimer, 2011; Antón et al., 2014).

However, our current knowledge on the feeding ecology diversity of wild apes may be biased by the small number of communities which are subject to long-term research. Increasing evidence from previously unstudied wild great ape populations suggests general classifications may to be over-simplified. Each unstudied population can be expected to preserve its own unique behavioral or even cultural traits (Whiten et al., 1999). At the same time ape populations are declining at high rates and have already gone extinct in many regions (Junker et al., 2012; Tranquilli et al., 2012). Hence, there is a need to develop new techniques and strategies to compare the ecology of extant great apes across populations, habitats and species in a standardized, yet timely manner.

Isotope analysis is a well-established tool in the study of wildlife ecology (Wolf et al., 2009) and in paleoanthropology (Ungar and Sponheimer, 2011; Sponheimer et al., 2013). The stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are increasingly utilized biochemical markers to reconstruct the feeding ecology of primates, particularly in populations which cannot be directly observed (Schoeninger et al., 1998; Schoeninger, 2009). The differentiation into separate or overlapping ecological niches can be assessed in sympatric primate species

(Schoeninger et al., 1998; Macho and Lee-Thorp, 2014; Oelze et al., 2014). Stable isotope analysis in hair keratin is a particularly useful approach when investigating temporal and inter- and intra-individual dietary variation in great apes (Oelze et al., 2011, 2014; Fahy et al., 2013). The key advantages are that a hair strand records the dietary signature of a single individual over a long time period (several subsequent months) and hair isotopic data obtained from different individuals and species can be compared (Schwertl et al., 2005; Cerling et al., 2009; Oelze et al., 2011, 2014). In consumers, $\delta^{13}C$ values are mainly associated with the photosynthetic pathway of food plants (C_3, CAM or C_4) (DeNiro and Epstein, 1978). In forested habitats, the predominant habitat of great apes, the so called 'canopy effect' is thought to result in vertical variation in plant $\delta^{13}C$ values (van der Merwe and Medina,1991; Graham et al., 2014). Due to the isotopic fractionation with each step in the food chain, $\delta^{15}N$ values are commonly related to the trophic level of an animal, e.g. if it can be considered herbivore, omnivore or carnivore (Minagawa and Wada, 1984). Stable isotope ecology is based on the principle "you are what you eat" as the isotopic characteristics of the main food components are incorporated into consumers' body tissue with a predictable enrichment (indicated by Δ) caused by isotopic fractionation (DeNiro and Epstein, 1978, 1981; Minagawa and Wada, 1984; Kohn, 1999).

The first studies on great apes used bulk hair samples from unhabituated apes and did not control for local baseline isotopic values in the environment as no plant samples were used for analysis (Schoeninger et al., 1999; Sponheimer et al., 2006a). Cross-site comparisons of these data are hindered as isotopic baseline values are known to differ between regions and habitats due to biotic and abiotic factors in the soil and plants (Dawson et al., 2002; Casey and Post, 2011). Thus, conclusions drawn on primate dietary behavior without controlling for isotopic baseline effects may be misleading. For example, the low $\delta^{15}N$ values in 'savanna chimpanzees' have been interpreted as legume consumption which would cause depleted

$\delta^{15}N$ values (Schoeninger et al., 1999; Sponheimer et al., 2006a). This explanation was suggested as open dry habitats are thought to commonly reveal rather high $\delta^{15}N$ values (Heaton et al., 1986). However, no vegetation samples were used to validate this assumption. We seek to show in this study that controlling for ecological isotopic characteristics at a given site is essential for data interpretation. A good example of this need is the high $\delta^{15}N$ values reported for wild bonobos from Salonga National Park, which may have been interpreted as intensive faunivory if local plant baseline values had not been considered. However, these high $\delta^{15}N$ values in bonobos were best explained by an overall ^{15}N-enriched ecosystem which was supported by the data from other sympatric herbivores (Oelze et al., 2011).

Recently, two separate attempts to compare chimpanzee stable isotope characteristics across several sites came to strikingly contradicting results. As neither study could account for plant baselines they found different patterns between climate and chimpanzee hair isotope data (Schoeninger et al., 2016, Loudon et al., in press). Here, we propose to systematically correct great ape hair isotope values by a standardized local plant sample set when examining inter-site isotopic variation. While $\delta^{13}C$ values in plants are mainly determined by plant physiology, forest cover (UV-radiation and air ventilation), water availability and altitude (Tieszen, 1991; Dawson et al., 2002), $\delta^{15}N$ values mainly vary with the nitrate content of the local geological substrate and its assimilation by micro flora and fauna (Robinson et al., 1998; Martinelli et al., 1999). Thus, every location should have a unique plant isotope baseline. Recently, Crowley and colleagues (Crowley et al., 2014) suggested calculation of a mean isotope ratio of a standardized vegetation sample (e.g. only C_3, non-leguminous plants) for each site and subsequent discussion of the deviation measured in the primate body tissue signatures from this plant baseline. This deviation from food source to consumer tissue is referred to as isotopic discrimination or fractionation (Δ) and describes the trophic

enrichment from diet to tissue. In this study we follow this suggested approach by systematically using plant baseline data in our comparison of hair isotope data from great apes.

In a recent review on primate isotope studies Sandberg and colleagues (2012) noted that the inter-individual variation of $\delta^{13}C$ and $\delta^{15}N$ values in bonobos and chimpanzees is low. Further they state: "A significant characteristic of the chimpanzee stable isotope data is the virtual lack of variability within populations and between those living in similar environments, particularly for carbon" (Sandberg et al., 2012, p. 980). We would argue previous analyses of bulk hair samples likely muted the isotopic variation within the growth trajectory of a given hair strand, as bulk samples represent an average of the variation within a hair sample. Moreover, most great apes feed predominantly on C_3-plant based resources. Thus, the overall isotopic variation should be within the C_3-plant range (Carlson and Kingston, 2014) and will not be as distinctive as in primates switching between C_3 and C_4-resources such as in some baboon populations (e.g. Codron et al., 2008 and references therein). Nevertheless, isotopic variation will be detectable and indicative of differences in feeding behavior if the data can be related to a sample of local food plants (Oelze et al., 2014). For example, in an isotope study using local plant samples and sequential sections of hair samples of sympatric lowland gorillas and central chimpanzees from Gabon, Oelze and colleagues (2014) showed that there was significant intra-annual variation in hair $\delta^{13}C$ in gorillas as they seasonally shifted their dietary proportions of low canopy foliage and fruit. At the same site, temporal variation in $\delta^{15}N$ values was highly significant in chimpanzees and ranged over ~1.5 ‰, suggested to be a result of utilizing different fruit species (Oelze et al., 2014). These nuanced temporal patterns would not have been detected in bulk samples of ape hair. In western chimpanzees from Taï National Park, Ivory Coast, intra-individual variation was detectable even in such bulk hair samples. Here, male chimpanzees had significantly

higher mean $\delta^{15}N$ values than females as they consumed meat more frequently. Males observed to be successful hunters revealed the highest $\delta^{15}N$ values in the community (Fahy et al., 2013).

Here, we present the most comprehensive $\delta^{13}C$ and $\delta^{15}N$ dataset measured to date in non-human primates. It includes all species of African apes living in different habitats ranging from evergreen forests to mosaic landscapes to savanna woodlands (see Figure 1 and Table 1). Samples were obtained from six field sites in six African countries, including three subspecies of chimpanzees (Pan troglodytes troglodytes, Pan troglodytes verus, Pan troglodytes schweinfurthii), western lowland gorillas (Gorilla gorilla gorilla), mountain gorillas (Gorilla beringei beringei) and bonobos (Pan paniscus). This dataset is represented by 701 hair keratin isotope data from 148 hair samples and an additional collection of 189 fruit samples.

[Figure 1 and Table 1 here]

Our first main objective is to examine how stable isotope signatures of extant great apes compare to each other across their range in tropical Africa. Our hypothesis is that stable isotope signatures of different great ape populations are explained by a) site-specific environmental conditions (plant baseline) and b) differences in feeding behavior, particularly by differing levels of faunivory and folivory. Our second objective is to compare the temporal isotopic profiles among African great apes and to data from fossil hominins. We assume that great apes will vary in their temporal variability in both isotope systems but given their dependence on C_3-food resources we assume extant apes will reveal less variation in $\delta^{13}C$ than the two species of fossil hominins for which temporal resolution $\delta^{13}C$ data is available.

To assess the impact of environmental baseline effects on local isotope signatures we compare a standardized plant sample comprising fruits from each site with regards to climatic parameters. To directly compare the isotope signatures of the different ape populations we correct the respective hair $\delta^{13}C$ and $\delta^{15}N$ values to this local fruit baseline. We compare the resulting $\Delta^{13}C$ and $\Delta^{15}N$ values between species and sites and seek to draw inferences on feeding niches particularly with regard to folivory and faunivory. Finally, we discuss temporal isotopic variation between sites and within the different sections of individual hair samples alongside the only published primate isotope data with temporal resolution, available for extant baboons and fossil hominins from South and East Africa to illustrate the differences between different ape populations and the isotope characteristics of extinct hominoids.

Material and methods

Sample and data collection

We collected great ape hair samples and a selection of fruit species potentially consumed by great apes at the respective sites (Table SI 1 and Table SI 2). Great ape hair samples were collected non-invasively from nests at six different African study sites. Partially, hair and plant isotope data from the sites Salonga, Loango and Taï have been published previously (Oelze et al., 2011, 2014). Please see Table 1 and the supplementary information (SI) for environmental details about the respective sites as well as on sample collection and export. We included potential plant food samples from each site. Identifying plants consumed by unhabituated apes is difficult and limited to indirect evidence, such as feeding signs and fecal analyses. We sampled vegetation at each site, mostly based on presence in dung or feeding signs or observational data (such as for Loango, Taï and Salonga, see SI for further details), yet we do not claim that all plants sampled are essential to the diet of these primate

populations. However, we are confident that the sampled items are representative for the isotopic characteristics in potential primate food plants at each site and thus valid as plant baselines. We limited the vegetation sample to non-leguminous C_3-plants as recommended by Crowley and colleagues (2014) to produce standardized vegetation baseline data sets for all sites. Further, we limited the sample to fruits to avoid sample $\delta^{13}C$ biases resulting from different representation of photosynthetic (leaves) versus non-photosynthetic plant parts (Cernusak et al., 2009) or low versus high canopy plant parts (Medina and Minchin, 1980). Leaves are staple foods for apes, but substantial differences in $\delta^{13}C$ can easily be introduced by sampling from different canopy layers by different field teams. Also leaf age has an effect on $\delta^{13}C$ values of foliage (Blumenthal et al., 2016). We did not exclude several genera of the family Euphorbicaceae and Vitaceae as their $\delta^{13}C$ values did not indicate the utilization of CAM photosynthesis (Table SI 1).

In order to relate vegetation baselines to climatic parameters, precipitation in mm and mean temperature were measured at each site on a daily basis or the respective information was extracted from the literature. The supplementary information (SI) provides further details on each site and the cited literature.

Isotope analysis

Hair samples were washed in a chloroform/methanol solution (2:1 v/v) in a rotator over night to remove lipids and external contaminants (O'Connell and Hedges, 1999). Dried hair strands were sorted for hairs with roots in the telogen stage (pale yellowish root bulbs) under a microscope to limit analysis to only those hairs in the same phase of the growth cycle (Williams et al., 2011). Also all short, fractured and thin hairs were excluded to avoid hair of infant individuals (Oelze et al., 2011; Oelze, 2016). As a result each individual hair sample typically consisted of 12 hairs of 6-7 cm length with equal thickness and color. These hairs

were then aligned at their roots and sectioned with a scalpel into 5 or 10 mm sections as weight allowed. Finally, each hair section (≥0.2 mg) was transferred into tin capsules for isotopic measurement. All plant items were dried in an oven at 50 °C for several days, homogenized with a pestle and mortar and weighed into tin capsules (~2 mg) for duplicate isotopic measurement. All measurements were performed parallel to IAEA standards well as several internal standard materials (see below) in a Flash EA 2112 (Thermo-Finnigan®, Bremen, Germany) coupled to a DeltaXP mass spectrometer (Thermo-Finnigan®, Bremen, Germany) at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, or in a EuroEA3000 (EuroVector SpA®, Milan, Italy) coupled to a MAT 253 IRMS (Thermo-Finnigan®, Bremen, Germany) at the German Research Center for Environmental Health, Institute for Groundwater Ecology, Neuherberg, Germany. The stable isotope ratios of carbon and nitrogen are expressed as the ratio of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios using the delta (δ) notation in parts per thousand or permil (‰) relative to the international standard materials Vienna PeeDee Belemite (vPDB) and atmospheric N_2. Analytical error calculated from repetitive measurements of international (CH7, CH6, N1, N2, USGS 25, USGS 40 and USGS 41) and lab-internal standard materials (caffeine, methionine) included in each run is less than 0.2 ‰ (2σ) for $\delta^{13}C$ and $\delta^{15}N$. Intra-laboratory differences were tested using the internal standards material methionine in each run and reproducibility was better than 0.2 % (2σ) for $\delta^{13}C$ and $\delta^{15}N$. To assure analytical quality we excluded all hair isotope data with atomic C:N ratios outside the acceptable 2.6 to 3.8 range (O'Connell et al., 2001).

Fossil hominin and primate isotope data from the literature

Although bulk $\delta^{13}C$ data is available from many fossil hominin taxa (summarized in Ungar and Sponheimer, 2011), sequential $\delta^{13}C$ data obtained from laser ablation isotope ratio mass spectrometry in dental enamel is only available from Paranthropus robustus (Sponheimer et

al., 2006b) and Australopithecus africanus (Lee-Thorp et al., 2010). Here, we investigate how temporal isotopic variation in these fossil species relates to isotopic variation in the African great ape populations we present in this study. In this comparison we also include the only other sequential primate isotope dataset available, published by Codron and coworkers (2008) on free ranging chacma baboons (Papio ursinus).

The laser ablation isotope data from fossil teeth were converted to hair keratin values for comparison with great ape hair. Differences between isotope ratios of different body tissues are well described but also seem to vary slightly between species of the same order (Crowley et al., 2010). We are aware of slight inaccuracies in absolute values for each conversion step but we here aim to grossly compare the overall isotopic patterns relative to modern apes. We converted the original enamel laser ablation $\delta^{13}C$ values a) to bulk enamel values by +0.5 ‰ (Henry et al., 2012), b) from carbonate to collagen 6.1 ‰ (±0.2 ‰) and c) from collagen to hair keratin by +0.63 ‰ (±0.4 ‰) after (Crowley et al., 2010) and corrected for fossil fuel effects by subtracting 1.2 ‰ (Friedli et al., 1986). Finally, we used the software WebPlotDigitizer (Version 3.6, Rohatgi 2015) to extract data from figures presenting sequential $\delta^{13}C$ values in hair samples of nine free ranging chacma baboons (Papio ursinus) from the literature (Codron et al., 2008).

Statistical analyses

To analyse and compare the isotopic patterns in plants and within and between great ape populations we conducted several statistical analyses in R (version 3.1.0, R Development Core Team, 2014). We mostly used general linear mixed-effects models (GLMM) with Gaussian error distribution using the R-package 'lme4' (Bates et al., 2014). We tested the effect of climate on vegetation baseline samples, the differences between males and female Pan to assess sex differences in $\Delta^{13}C$ and $\Delta^{15}N$ values, isotopic differences between

sympatric Pan and Gorilla, and finally we also tested the differences in $\Delta^{13}C$ and $\Delta^{15}N$ values between different chimpanzee populations across Africa (see Table SI 3 for a summary of all the statistical models).

Analyses of vegetation baselines

We analysed the plant data separatley from the hair dataset to investigate site-specific patterns. We tested the relationship between fruit stable isotope ratios and rainfall (see Table 2). As temperature and rainfall were correlated (Spearman correlation, $r = 0.54$, $n = 187$, $p < 0.0001$) we tested only the effect of mean annual precipitation on plant isotope values. To account for the fact that we repeatedly sampled the same fruit species across sites (five out of total of 189 samples) we additionally ran two GLMMs on the responses $\delta^{13}C$ (model 1A) and$\delta^{15}N$ (model 1B), including the random effect of species (and thus controlling for repeated observations of the same plant species) and the fixed effect of precipitation. We gained p-values by conducting a likelihood ratio test comparing the full model with a null model excluding the fixed effect of precipitation and only including the random effect of species. Here and in the following analyses we also present effect sizes (R^2) for all fixed effects as a whole which represent the variance explained by them (Nakagawa and Schielzeth, 2013). We conducted model diagnostics by visually inspecting the normality and homogeneity of the residuals in a histogram, a qq-plot and residuals plotted against fitted values and found no issues with these assumptions. To assess model stability we re-ran each model on reduced datasets excluding levels of random effects, one at a time, compared the results of these models with the full model and found no evidence for influential fruit species.

Calculation and comparison *of* $\Delta^{13}C$ and $\Delta^{15}N$

The isotopic fractionation (Δ) between diet and body tissue such as hair keratin can be expected to range between 2 and 3 ‰, although experimental fractionation values are not available for large bodied primates such as great apes. In a controlled feeding experiment with captive vervet monkeys (Chlorocebus aethiops sabaeus), Macharia and colleagues (2014) measured $\Delta^{13}C$ and $\Delta^{15}N$ values of 2.8 ‰ and 2.9 ‰, respectively in hair samples of males and lower $\Delta^{13}C$ and $\Delta^{15}N$ of 2.4 ‰ and 2.2 ‰ in hair of lactating females. In a previous study on wild bonobos $\Delta^{13}C$ and $\Delta^{15}N$ around 3 ‰ were reported for both sexes (Oelze et al., 2011). Here, we used the $\Delta^{13}C$ and $\Delta^{15}N$ values calculated for Taï chimpanzees in comparison with those calculated for the different unhabituated chimpanzee communities. Long-term observations on the Taï population confirmed the sex-differentiated pattern in $\delta^{15}N$ to be related to higher meat consumption frequencies in males (Fahy et al., 2013). Moreover, the Taï $\delta^{13}C$ values clearly represent a frugivorous closed canopy forest dwelling chimpanzee population (Boesch and Boesch-Achermann, 2000). When interpreting the $\Delta^{13}C$ values of unhabituated chimpanzees, we additionally related these data to those of gorillas which are generally regarded as more folivorous than chimpanzees (e.g., Head et al., 2011 and references therein). A more folivorous diet in gorillas is also supported by isotopic evidence (Macho and Lee-Thorp, 2014; Oelze et al., 2014).

As a crude approximation of the isotopic fractionation (Δ) between local plant isotope ratios and the respective great ape hair isotope ratios we subtracted the local mean fruit isotope ratio from each hair isotope value, both in $\delta^{13}C$ and $\delta^{15}N$. We gained mean $\Delta^{13}C_{hair\text{-}fruit}$ ($\Delta^{13}C$ in the following) and $\Delta^{15}N_{hair\text{-}fruit}$ values ($\Delta^{15}N$ in the following) for the different ape species represented at Loango and Bwindi and also separately for the males and females at Salonga and Taï (see Figure 2). Differences in $\Delta^{13}C$ and $\Delta^{15}N$ between sites and sympatric ape species should therefore represent dietary differences rather than baseline conditions in each locality.

Feeding niches of sympatric gorillas and chimpanzees

To compare how sympatric apes differed in their $\Delta^{13}C$ and $\Delta^{15}N$ values, we conducted two GLMMs, each for Loango (model 2 A and B) and Bwindi (model 3A and B), on the responses $\delta^{15}N$ and $\delta^{13}C$ with species as a main fixed effect and hair sample and 'info' (coding for additional information on individual or nest group if available, see Table SI 2) as random effects. For each model we gained the p-value for the effect of species using a likelihood ratio test comparing the full model with a null-model only comprising the random effects. Model diagnostics were conducted as outlined above for the plant baseline model and suggested no violations with the model assumptions or model instability.

Feeding niches of Pan across sites

To compare the $\Delta^{13}C$ and $\Delta^{15}N$ values of the different chimpanzee populations in this study, we used the isotope dataset of the well-studied Taï chimpanzees as a reference to aid the interpretation of results. Particularly, we used Taï males as the reference category (dummy coding the factor 'site') in the model to which the $\Delta^{15}N$ values of the other chimpanzee sites could be compared as a previous study (Fahy et al., 2013) revealed significant differences between the sexes in hair $\delta^{15}N$ values due to the higher meat eating frequencies in males. We tested the sex differences in Taï chimpanzees again in a GLMM controlling for potential pseudoreplication effects of individual and repeated sampling of hair per individual by including individual and hair ID as random effects. We ran two GLMMs on the responses $\delta^{13}C$ (model 4A) and $\delta^{15}N$ (model 4B) with the fixed effect of sex and the random effect of individual and hair sample and gained p-values by conducting a likelihood ratio test with a null model only including the random effects. Model diagnostics were conducted as outlined above.

We tested the differences between all adult Salonga bonobos and Taï chimpanzee males and females separately from other Pan populations as we could fully control for individual (and hair sample) by including individual (and sample ID) as random effect at these two sites and gain robust results. A previous analysis of the bonobo dataset revealed no differences in male and female stable isotope ratios and the authors concluded overall levels of meat consumption to be low (Oelze et al., 2011). If this is correct bonobo $\Delta^{15}N$ values should be similar to Taï females. To test the differences in the response $\Delta^{15}N$ we ran two GLMMs separately for bonobos and Taï males (model 5A) and for bonobos and Taï females (model 5B). We checked model assumptions and stability in both models as outlined above and detected no issues with these diagnostic parameters.

As Taï males were significantly higher in $\delta^{15}N$ than Taï females we used the Taï males as a reference category when dummy coding the factor site (and in case of tai coding for a combination of sex and site) and fitted two models to obtain estimates and p-values for the differences between all chimpanzee sites in this study with regard to $\Delta^{13}C$ (model 6A) and $\Delta^{15}N$ (model 6B). For the $\Delta^{13}C$ model we used Taï female chimpanzees (slightly lower $\delta^{13}C$ values) as a reference category to gain the model coefficients. However, using males as the reference category revealed the same results as male and female Taï chimpanzees did not differ significantly in $\delta^{13}C$. Overall model significance was tested using likelihood ratio tests comparing the full models to the respective null models excluding the fixed effect of 'site'. We conducted model diagnostics by visually inspecting a qq-plot and a scatter plot with residuals plotted against fitted values and found no issues with the assumption of normally distributed and homogenous residuals. We assessed model stability as outlined above which confirmed the robustness of both models.

Maximum isotopic variation within sites, species and individuals

To compare the isotopic variation between sites we calculated the ranges (minimum to maximum isotope values) in $\delta^{13}C$ ($\delta^{13}C_{range}$) and $\delta^{15}N$ ($\delta^{15}N_{range}$) for each site separately for species occurring at the same site (Table 4). We also calculated these ranges per site and specimen for the fossil hominin and baboon data extracted from the literature.

Statistical limitations – dealing with potential pseudoreplication

As we included data obtained from unhabituated and thus unidentified great ape individuals, we cannot exclude erroneous p-values as the result of potentially pseudoreplicated data in our statistical analyses (Mundry and Oelze, 2016). The issue of pseudoreplication is still largely ignored in (primate) isotope ecology, although it can lead to type I and type II errors if non-independent data points (e.g., from the same individual) are treated as if they were independent, although they are not (Hurlbert, 1984).

In the unhabituated chimpanzee populations reported in this study, we cannot fully exclude that pseudoreplication may have occurred as hairs were not consistently collected from single nest groups. However, we assume unintentional re-sampling of the same individuals will be a rare case. Based on estimations reported by Mundry and Oelze (2016) there is a chance to have sampled hair from a at least one individual at least twice at Kayan (9 samples from ~ 15 individuals), Sapo (23 samples from ~ 42 individuals), and Bwindi (10 samples from ~ 40 individuals), but it is rather unlikely for Loango (9 samples from ~ 123 individuals). For the datasets from gorillas we report here, pseudoreplication is less problematic. Most of the Bwindi mountain gorilla hair samples were collected from single nest sites of habituated groups, minimizing the possibility of re-sampling of the same individual. Several gorilla hair samples from Loango had been identified from the same silverback individual (see Table SI 2), which was controlled for in a previous analyses (and also in this study). Also, excluding

data from potentially re-sampled individuals from the analysis did not suggest any erroneous influence on the overall results (Oelze et al., 2014).

We fully controlled for repeated measurements of the same hair sample by including hair sample ID as random effects in all statistical analyses. We controlled for individual in the analysis on the Taï and Salonga datasets which contained information on individual identity.

Results

Vegetation baselines

We found a significant negative relationship between annual precipitation and $\delta^{13}C$ values of fruits (model 1A: $R^2 = 0.05$, estimate= 0.002, SE= 0.00, $\chi^2 = 8.7$, df = 1, $p = 0.0031$) and a significant positive relationship between precipitation and fruit $\delta^{15}N$ values (model 1B: $R^2 = 0.17$, estimate= 0.000, SE= 0.00, $\chi^2 = 27.0$, df = 1, $p < 0.0001$). We found the lowest $\delta^{13}C$ values in evergreen/semi-deciduous forest habitats (Salonga, Taï and Sapo), intermediate values in plants from a mosaic habitat (Loango) and the highest $\delta^{13}C$ values in habitats with open forest cover, here reflected by afro-montane (Bwindi) and savanna-woodland habitat (Kayan) (Figure S1A, Table 2). We also found a climatic gradient in fruit $\delta^{15}N$ values, with the highest values in Salonga and Taï, intermediate values in Sapo, Loango and Bwindi and remarkably low $\delta^{15}N$ values in the woodland of Kayan (Figure S1B).

[Table 2 here]

Isotopic fractionation in African great apes

We calculated the isotopic fractionation (Δ) in both stable isotope systems for all ape populations in this study (Figure 2) and found the largest $\Delta^{13}C$ values in chimpanzees from Kayan and Taï and the largest $\Delta^{15}N$ values in Sapo chimpanzees. In order to be able to

compare isotopic pattern between ape populations, independently from vegetation baseline characteristics related to climate, we used $\Delta^{13}C$ and $\Delta^{15}N$ values in all subsequent analyses.

[Figure 2 here]

Niche differences between sympatric gorillas and chimpanzees

The differences between mean isotope values of sympatric gorillas and chimpanzees were significant; except for no differences in $\Delta^{15}N$ values at Bwindi (see Figure 2 and 3). As reported in a previous analysis on the same dataset from Loango (Oelze et al., 2014), the differences in $\Delta^{13}C$ between sympatric gorillas and chimpanzees were significant (model 2A, $R^2 = 0.38$, estimate= 0.705, SE= 0.128, $\chi^2 = 17.25$, df = 1, $p < 0.001$). In Bwindi differences in $\Delta^{13}C$ between chimpanzees and gorillas were significant (model 3A, $R^2 = 0.20$, estimate = 0.376, SE = 0.131, $\chi^2 = 7.54$, df = 1, p = 0.006). At both sites chimpanzee $\Delta^{13}C$ values were higher than those of gorillas. While Loango chimpanzees were significantly higher in $\Delta^{15}N$ than sympatric gorillas (model 2B, $R^2 = 0.40$, estimate = 0.789, SE = 0.235, $\chi^2 = 12.86$, df = 1, $p < 0.001$), we found no significant differences between sympatric chimpanzees and mountain gorillas in Bwindi (model 3B, $R^2 = 0.10$, estimate = -0.312, SE = 0.187, $\chi^2 = 3.30$, df = 1, p = 0.069).

Feeding niches of Pan

Our models on the Taï chimpanzee dataset confirmed the previously reported significant differences in $\delta^{15}N$ between Taï males and females (model 4B, $R^2 = 0.35$, estimate = 0.875, SE = 0.23, $\chi^2 = 10.64$, df = 1, p = 0.001), with higher $\delta^{15}N$ values in males. There were no differences in $\delta^{13}C$ between Taï males and females (model 4BA, $R^2 = 0.01$, estimate = 0.156, SE = 0.224, $\chi^2 = 0.37$, df = 1, p = 0.5) (see Figure 2 and 3).

Comparisons between Taï chimpanzee males and all adult Salonga bonobos revealed that their $\Delta^{15}N$ values did not differ (model 5A, $R^2 = 0.01$, estimate = -0.080, SE = 0.157, $\chi^2 = 0.25$, df = 1, p = 0.6), but bonobos were significantly higher in $\Delta^{15}N$ than Taï females (model 5B, $R^2 = 0.46$, estimate = 0.7867, SE = 0.112, $\chi^2 = 20.72$, df = 1, $p < 0.001$), (see Figure 3B). [Table 3 and Figure 3 here]

We tested the isotopic differences between the different chimpanzee populations while accounting for vegetation baseline effects. We used Taï males as the reference category in the linear model for $\Delta^{15}N$ for which the full-null model comparison was highly significant (model 6B, $R^2 = 0.82$, $\chi^2 = 71.5$, df = 5, $p < 0.001$). We then used Taï females as a reference category in the $\Delta^{13}C$ model which was also highly significant (model 6A, $R^2 = 0.82$, $\chi^2 = 67.2$, df = 5, $p < 0.001$). The model estimates and related p-values suggested significant differences between Taï chimpanzees and the other chimpanzee populations of our study (see Table 3 for further statistical results, Figure 3A and B).

Temporal isotopic variation in extinct and extant hominoids

By sectioning all hair samples we gained sequential isotopic profiles for all African great apes allowing for evaluating the isotopic and thus dietary flexibility of the different species and populations. We found isotopic variation within and between the different sub-species of chimpanzees (see Figure 4) as well as in gorillas and bonobos (Figure 5). However, when we compared our data with the published $\delta^{13}C$ data from chacma baboons (Figure 6), overall variation over time in great apes was relatively low. The extent of isotopic variation over time varied between populations in both isotope systems (Table 4 and Figure 7). Among the great apes in this study maximum variation in $\delta^{15}N$ was similar across sites and only Sapo chimpanzees appeared to have higher variation over time. The maximum variation in $\delta^{13}C$ differed slightly between sites and species. Lowland gorillas from Loango showed the largest

variation in $\delta^{13}C$. Also Sapo chimpanzees appeared to be flexible in the $\delta^{13}C$ component of their diet (Figure 4, Sapo B). On the other hand, the range in $\delta^{13}C$ in bonobos from Salonga was moderate and similar to Loango chimpanzees (Table 4). Mountain gorillas, which rather consistently feed on terrestrial herbs throughout the year (summarized by Doran and McNeilage, 1998), showed even lower variation.

[Figure 4, 5 and 6 here]

We gained converted mean $\delta^{13}C_{hair\ keratin}$ values of -15.5 ‰ for Australopithecus africanus and -14.1 ‰ for Paranthropus robustus, respectively, which we compared to hair isotope data from extant baboons (Figure 6). Values ranged from -11.3 ‰ to -18.8 ‰ in Au. africanus and from -10.2 ‰ to -17.2 ‰ in P. robustus (Figure 6). As this conversion was based on a range of extant primate taxa we assume they are the best fit to fossil hominin data. Alternative conversion calculations directly from dental enamel to (hoof) keratin suggested by Cerling and Harris (1999) revealed mean values of -19.8 ‰ for Au. africanus and -18.5 ‰ for P. robustus. These values are based on various ungulate species and may not be appropriate for primates, yet are interesting as the resulting hominin mean $\delta^{13}C$ values were identical to the range of mean $\delta^{13}C$ values in hair of nine baboons (-19.8 ‰ to -18.5 ‰) for which a C_4-plant diet of 21-30 % was proposed (Codron et al., 2008).
Variation in $\delta^{13}C$ varied by more than 7‰ in A. africanus and P. robustus whereas the range in $\delta^{13}C$ in great apes did not exceed 3.1‰ (Figure 7C). The extent of temporal isotopic variation of baboons fell between apes and fossil hominins.

[Figure 7 and Table 4 here]

Discussion and conclusion

This study provides insights into ape isotopic ecology on several levels. First, the isotopic signature in ape habitat across the African continent, characterized in our study by fruit baseline isotopic patterns, was clearly influenced by the local rainfall regime. Second, the variability in ape isotopic patterns was larger than previously thought; this concerns comparisons across species, habitats and populations as well as over time. Last, our comparative approach provides new opportunities for interpreting isotopic signatures in fossil hominins.

As noted above, we cannot exclude that pseudoreplication caused some overestimation of isotopic differences when comparing isotope ratios between and within sites without being able to control for individual. However, we consistently controlled for pseudoreplication whenever possible.

Site comparison using vegetation baselines - the effects of habitat and precipitation

We compared the vegetation isotope baselines from each site and observed significant gradients in $\delta^{13}C$ and $\delta^{15}N$. Depleted $\delta^{13}C$ values can be related to the so called 'canopy effect', which is commonly described to result from low CO_2 respiration due to low air ventilation in the understory of dense forests with high crowned tree cover (van der Merwe and Medina, 1991). Graham et al. (2014) could show for two tropical and temperate closed canopy forest sites that isotopic fractionation in carbon is negatively related to height and light availability but positively related to humidity. The most depleted ^{13}C values in tropical C_3 dominated forest are typically found in the forest understory where UV radiation is limited and humidity is commonly high (van der Merwe and Medina, 1991; Cerling et al., 2004; Graham et al., 2014; Oelze et al., 2014; Blumenthal et al., 2016). We found support for this

pattern from comparisons at a large spatial scale in fruits from different forest habitats with differing levels of humidity (precipitation) and forest cover (habitat type, see Table 1).

The Salonga forest, with low mean $\delta^{13}C$ values, can be considered a moist closed canopy tropical forest, similar to the Ituri forest also located in the center of the Congo Basin (Cerling et al., 2004). Also the primary forest of Taï in West Africa was similarly influenced by canopy effects in $\delta^{13}C$ suggesting that, besides precipitation, canopy cover could be a main factor determining $\delta^{13}C$ in plants.However, the Taï mean fruit $\delta^{15}N$ baseline was less ^{15}N-enriched as compared to the Congo Basin. The other extreme of the habitat spectrum is represented by open habitat and with long and harsh dry periods such as Kayan, where we found particularly low $\delta^{15}N$ and high $\delta^{13}C$ values.

Our data showed that dense and humid forest habitats in tropical Africa revealed the lowest $\delta^{13}C$ and highest $\delta^{15}N$ values whereas more open canopy and low precipitation sites revealed the highest $\delta^{13}C$ and lowest $\delta^{15}N$ signatures in plants. This pattern contradicts the global pattern found in foliar $\delta^{15}N$ values, which predicts a negative correlation with mean annual precipitation and the highest N availability in dry, warm ecosystems (Craine et al., 2009). Although Craine and coworkers (2009) sampled leaves and we sampled fruit, the isotopic differences between different anatomical parts of plants are small (Cernusak et al., 2009) and should not explain these contradicting findings. However, one may consider that in the global study design by Craine et al., (2009) tropical Africa was not included. The study of Craine et al. (2009) suggested that the association of trees with specific types of mycorrhizal fungi has an effect on $\delta^{15}N$ values in foliage, with the lowest values found in ericoid and ectomycorrhizal species when controlling for mean annual temperature. Ericoid and ectomycorrhizal fungi are particularly valuable for plants in dry habitats poor in N, such as heathlands in temperate climatic zones (Read et al., 2004). We think it is well possible that, although savanna vegetation is dominated by arbuscular mycorrhizal associations (Read et

al., 2004), ectomycorrhizal fungi may be particularly important for fruit trees growing in the savanna-woodlands of tropical Africa. Further isotope research on African savanna vegetation may further shed light on this aspect of plant isotope ecology.

In light of these contradictions in spatial plant isotope patterns we would like to emphasize the necessity to control for isotopic baselines using local vegetation sampling when analyzing faunal material. The isotope biochemistry of plants may be too complex to draw inferences based on climate variables alone (Dawson et al., 2002; Casey and Post, 2011).

Considerations of plant baseline values are essential for great ape isotope ecology. For example, we assume that previous conclusions made about Fongoli chimpanzee feeding ecology based on bulk hair isotope values (Sponheimer et al., 2006a) are possibly incorrect. The plant data we presented here from the neighboring site of Kayan, located approximately 50km northwest of Fongoli, suggest that an overall ^{15}N-depleted plant baseline was responsible for the depleted δ^{15}N values in Fongoli chimpanzee hair and not the consumption of plant items from the Leguminosae family. Additional investigations of the isotope ecology of the dry and open habitat site of Ugalla in Tanzania (Schoeninger et al., 1999) as suggested also by Sandberg and colleagues (2012) would likewise offer a better understanding of the issue.

Niche differences between sympatric gorillas and chimpanzees

We analyzed the hair samples of sympatric gorillas and chimpanzees with overlapping home ranges in a lowland mosaic habitat in Gabon (Loango) and an afromontane forest in Uganda (Bwindi). Fecal analysis at both sites suggested that chimpanzees were more frugivorous than gorillas (Stanford and Nkurunungi, 2003; Head et al., 2011). While we detected this niche differentiation in δ^{13}C, the patterns in δ^{15}N as well as the overall isotopic variation in hair were not consistent.

In Bwindi niche partitioning in $\delta^{13}C$ resembled the pattern found in Loango with the frugivorous and folivorous species differentiating particularly in $\delta^{13}C$ (see Figure 2, Bwindi and Loango). Gorillas, particularly mountain gorillas, depend heavily on low canopy vegetation (Doran and McNeilage, 1998; Robbins et al., 2006), which is commonly ^{13}C-depleted (Medina and Minchin, 1980; Carlson and Kingston, 2014; Oelze et al., 2014). Gorillas appeared to have consistently lower $\delta^{13}C$ values than sympatric chimpanzees, independent of the habitat type. This pattern had also been described for historic gorilla and chimpanzee populations from several locations in Cameroon (Macho and Lee-Thorp, 2014). Based on our knowledge on the feeding niche differentiation of sympatric gorillas and chimpanzees (e.g., Stanford, 2006; Head et al., 2011) we suggest that lower $\Delta^{13}C$ values may be associated with higher levels of folivory. On the other hand, the $\delta^{15}N$ differentiation between gorillas and chimpanzees seems more complex and appeared likely dependent on the vegetation baseline and plant species diversity at a given site. Unlike previous studies on sympatric gorillas and chimpanzees (Macho and Lee-Thorp, 2014; Oelze et al., 2014) Bwindi gorillas had almost the same mean $\delta^{15}N$ values as sympatric chimpanzees. This patternlikely resulted from the rather uniform $\delta^{15}N$ isotope baseline in this afromontane forest where fruit, herbaceous leaves and tree leaves all had rather similar mean $\delta^{15}N$ values (Blumenthal et al., 2012).

Feeding niches of *Pan* – $\delta^{15}N$ evidence for faunivory

We used the Taï chimpanzee dataset to facilitate the interpretation of patterns in $\Delta^{13}C$ and $\Delta^{15}N$ across chimpanzee populations. We propose that the correction for local vegetation baseline using the $\Delta^{13}C$ and $\Delta^{15}N$ approach allows for comparisons between sites. Chimpanzee populations with high levels of consumed vertebrate protein will reveal similar

or higher $\Delta^{15}N$ values to Taï chimpanzee males, whereas populations with low levels of faunivory will be more similar to (or even lower than) Taï chimpanzee females.

The comparison between Salonga bonobos (both sexes) and Taï female and male chimpanzees suggests that bonobos had a $\Delta^{15}N$ signature comparable to Taï males and distinct from Taï females. This conclusion is not supported by behavioral data which suggested that Taï males eat meat frequently (Fahy et al., 2013) whereas it is a much more rarely observed behavior in the bonobos from Salonga forest (Hohmann and Fruth, 1993; Surbeck et al., 2009). It also contradicts the interpretation of a previous dataset on the bonobos from Salonga forest which suggested the bonobo $\delta^{15}N$ values to not exhibit significant levels of faunivory (Oelze et al., 2011). However, the study by Oelze et al. (2011) was the first integrating environmental samples in great ape isotope ecology and there was no good comparative data basis from other habituated groups to aid data interpretation. The novel approach across sites and species we present in this study suggests that faunivory did indeed play a role in bonobo feeding behavior. Low ranking adolescent males had significantly lower $\delta^{15}N$ values than other group members (Oelze et al., 2011), which could indicate that young males were regularly excluded from preferred high trophic level food resources, such as meat. However, this is not necessarily confirmed by observational data as both sexes and different age classes have access to meat (Hohmann and Fruth, 1993; Surbeck et al., 2009). The comparison of $\Delta^{15}N$ patterns between Taï chimpanzees and bonobos is thus not fully conclusive.

The results of the chimpanzee $\Delta^{15}N$ comparison model (see Table 3 and Figure 3B) suggested that the only population with significantly higher trophic level than the faunivorous Taï males may be Sapo in neighboring Liberia with a mean $\Delta^{15}N$ of 4.1 ‰. If so, hunting frequencies in this yet largely unstudied population would be remarkably high. In a small scale survey in Sapo forest, Anderson and colleagues (1983) made the rather exceptional discovery of high

numbers of flesh and bone remains in chimpanzee feces. While this evidence is anecdotal, the $\Delta^{15}N$ pattern of Sapo chimpanzees may indeed suggest high hunting frequencies in this population or that some particularly ^{15}N-enriched plant sources played an important role in the diet, as it has been suggested for Loango chimpanzees (Oelze et al., 2014). However, the vegetation sample from Sapo was very limited (n=6) and, thus, the calculated $\Delta^{15}N$ value is certainly assiciated with large uncertainty. We conclude that hunting behavior in the chimpanzees from Sapo requires further investigation before meaningful conclusions on their meat eating frequencies can be drawn.

All other chimpanzee populations reported and discussed in this study, including Bwindi, Loango and Kayan, revealed $\Delta^{15}N$ values significantly lower than those of Taï males but comparable to Taï females (see Figure 3A and Table 3). This suggests that none of these populations was hunting at high frequencies. High altitude dwelling chimpanzees from Bwindi showed a $\Delta^{15}N$ pattern similar to Taï females. Their low $\Delta^{15}N$ (1.9 ‰) value suggests faunivory was not frequent. However, previous fecal analysis on Bwindi chimpanzees revealed that 4.3% of all dung samples contained vertebrate remains, mainly from duikers and small monkeys (Stanford and Nkurunungi, 2003). This indicated that hunting/scavenging events did indeed occur but were probably rarer than in Taï or Gombe (Wrangham and Van Zinnicq Bergmann Riss, 1990). During the sample collection period for the present study, however, no evidence of meat consumption could be recorded. None of the 100 opportunistically collected chimpanzee dung samples contained bones, skin or invertebrate remains (Pan African Programme, unpublished data).

Also for chimpanzees from the mosaic habitat in Loango, the $\Delta^{15}N$ values were low (2.1 ‰) and comparable to Taï female chimpanzees (see Figure 3A). In a previous diet study remains of vertebrates were infrequently found in dung samples from this population (Head et al.,

2011). As habituation is ongoing with the Loango Ape Project future observational data will validate or reject this hypothesis on low hunting frequencies.

Chimpanzees from Kayan showed similar evidence for rare meat consumption given their low $\Delta^{15}N$ value. During 12 subsequent months of fecal sampling at Kayan, one bone fragment (tentative identification of an immature baboon) was found in chimpanzee feces (personal observation H. Eshuis and V. Oelze) suggesting that the chimpanzees at this site did hunt and consume meat. However, even if this community showed similar dietary patterns to the nearby Fongoli community, its rate of meat consumption was still relatively low (Pruetz et al., 2015), as compared to other sites like Taï (Boesch and Boesch-Achermann, 2000; Fahy et al., 2012).

Feeding niches of *Pan* – $\delta^{13}C$ evidence for folivory

When exploring the difference in $\Delta^{13}C$ between the different chimpanzee populations we used data from the forest dwelling Taï chimpanzees as a means of comparison but additionally refered to isotopic evidence from lowland and mountain gorillas to interpret the results. Taï chimpanzees live in a forest habitat, rich in high crowned trees and high in fruit productivity. Although their diet is supplemented by foliage (mainly young leaves) and seasonally with nuts, they are predominantly frugivorous (Boesch and Boesch-Achermann, 2000). Meat eating did not seem to affect $\delta^{13}C$ values (Fahy et al., 2013). Our model revealed interesting differences between sites in their $\Delta^{13}C$ patterns. Bwindi, Sapo and Loango chimpanzees had significantly lower mean $\Delta^{13}C$ values than Taï chimpanzees (see Figure 3A and Table 3). Based on the significant isotopic differences between sympatric gorillas and chimpanzees (see above) and the fact that foliage is commonly ^{13}C-depleted compared to fruits (Cernusak et al., 2009), we suggest that low $\Delta^{13}C$ values indicate increased folivory.

Bwindi chimpanzees were significantly lower in $\Delta^{13}C$ than Taï chimpanzees (see Figure 3A, Table 3). The mean $\Delta^{13}C$ value of 2.7 ‰ suggests that, in addition to fruits, ^{13}C-depleted plant foods such as tree and herbaceous leaves could have been an important dietary source. The afromontane Bwindi forest had the highest altitude of all our study sites and, possibly, the lowest fruit diversity as tree species density and diversity decreases with increasing altitude (Goldsmith, 2003). Hence, it may not be surprising that we found isotopic indication for a possible contribution of foliage to the chimpanzees' diet. Also the difference to the $\Delta^{13}C$ value of the folivorous mountain gorillas was remarkably small (0.4 ‰). Stanford and Nkurunungi (2003) found 27% of Bwindi chimpanzee dung samples containing leaf and pith remains but emphasized that fecal analysis can underestimate the frequency of foliage. Here, stable isotope analysis of hair seemed to provide a particularly useful complementary method to detect folivory in primates.

According to our comparisons the Sapo chimpanzees' $\Delta^{13}C$ values suggest that they may also had consumed larger quantities of foliage than chimpanzees in nearby Taï forest. However, the small fruit sample from this site may have led to erroneous Δ-calculations. The $\Delta^{13}C$ value of 3.2 ‰ resembled the $\Delta^{13}C$ of lowland gorillas (Figure 3A) and may indicate an unusually high frequency of foliage and herb consumption. In contrast to the Bwindi habitat, fruit species diversity and abundance can be expected to be relatively high in a lowland forest such as Sapo forest. We thus expected to find a largely frugivorous Pan community similar to Taï. However, compared to Taï National Park, the Sapo forest seems to be more degraded by human land use and secondary forest and swamp areas are currently more common. Hence, diversity and productivity of fruit bearing trees may be depleted and Sapo chimpanzee may had fallen back on more foliage. A detailed environmental comparison using habitat and phenology data within the Pan African Programme will further shed light on this suggested scenario.

The mean $\Delta^{13}C$ value of Loango chimpanzees (3.3 ‰) were significantly lower than in Taï, suggesting their $\Delta^{13}C$ level was on average 0.7 ‰ lower than that of those of a closed forest chimpanzee population. However, it was also clearly higher than the $\Delta^{13}C$ pattern of sympatric gorillas which are more folivorous but also highly depend on ripe fruit (Head et al., 2011). We assume their level of folivory could be intermediate between lowland gorillas and closed canopy chimpanzees from Taï.

The savanna chimpanzees from Kayan had a significantly higher mean $\Delta^{13}C$ value than chimpanzees from Taï males (the mean estimate was 0.45 ‰ higher). We think these higher $\Delta^{13}C$ values are related to the consumption of plants growing outside the canopy cover of the woodland and gallery forest patches or even suggest the irregular consumption of C_4-resources. The habituated chimpanzee community from nearby Fongoli has been observed to frequently consume multiple species of termites throughout the year (Bogart and Pruetz, 2008, 2011). Based on findings of Macrotermes remains in chimpanzees feces collected at Kayan (personal observations by H. Eshuis) it seems likely that Kayan chimpanzees also foraged for termites. For southern African savanna habitats, Sponheimer and colleagues (2005) could show that some termite species indeed have mixed diets consisting of C_3 and C_4-plants. They concluded that termite feeding may explain the C_4-signal in some fossil hominin taxa (Sponheimer et al., 2005). Although no respective isotope data is yet available for termites from Senegalese chimpanzee habitats, isotope data from several Macrotermitinae species in other parts of the West African savanna suggest that termites indeed harvest C_4-plants for fungus production (Lepage et al., 1993). The consumption of C_4-resources by savanna chimpanzees has been questioned partly because previous isotope studies were uncertain about the local vegetation isotope baselines (Schoeninger et al., 1999; Sponheimer et al., 2006a). At the time of our study, more than 65% of the habitat at Fongoli consisted of wooded grassland containing C_4-plants such as elephant grass (Bogart and Pruetz, 2011)

which could be an important dietary source for termites and other chimpanzee prey species. We suggest that C_4-resources had indeed contributed to the diet of the savanna chimpanzees in eastern Senegal and caused their isotope signatures to be slightly higher than those of forest populations, even when corrected for baseline values of locally available fruit. This hypothesis will need to be thoroughly tested with a larger dataset, including more chimpanzee hair samples and different prey species, including termites.

Temporal variation in diet – isotopic evidence for dietary flexibility in hominoids

So far, only one two studies could directly compare the $\delta^{13}C$ signatures of chimpanzee dental enamel with those of fossil African hominins. Neslon (2013) presented isotope data from bulk dental enamel from chimpanzees living in a closed canopy forest habitat in Kibale National Park, Uganda. She compared these data with isotope data from bulk enamel samples from fossil hominids and found that forest chimpanzees are a good model for the fossil ape Sivapithecus, but that fossil hominins, such as Ardipithecus, must have dwelled in a more open environment. Fahy and coworkers (2015) also recently presented dental enamel and bone apatite data from forest dwelling chimpanzees in Taï National Park, Ivory Coast and described the large differences to the C_4-biased isotopic pattern found in fossil hominins. From these studies one can conclude that forest chimpanzees are indeed not a useful model for fossil hominin species adapted to savanna woodland habitats and specialized to C_4-plant resources. However, we suggest to add the aspect of temporal isotopic variation as a measure of dietary flexibility to this discussion. Also, other great ape species and populations such as savanna chimpanzees but also gorillas deserve more consideration in this context.

We presented novel temporal isotopic sequences from various African great ape habitats and compared them with dental enamel $\delta^{13}C$-sequences from early hominins. We aimed at using temporal isotopic variation within individuals as a measure for the dietary flexibility of the

different species. Unlike extant great apes most fossil hominins from East and South Africa have been found to heavily rely on C_4-plant based food sources (Ungar and Sponheimer, 2011). So far, Southern African chacma baboons seem to be the best model species for the hominin C_4-dietary pattern (Figure 6). They temporarily utilize considerable quantities of C_4-plants and this is well reflected in their low hair $\delta^{13}C$ values and in the variation in $\delta^{13}C$ over time (Sponheimer et al., 2006a; Codron et al., 2008).

Hair and enamel are incremental tissues forming over time, retaining dietary isotope signatures once secreted and matured (summarized in Humphrey et al., 2008). In large bodied hominoids, dental formation lasts several years from initiation of enamel mineralization to crown completion (Reid and Dean, 2006), whereas hair can be expected to grow at a speed of 1 cm per month (Tobin, 2005; Carlitz et al., 2014). Hence hair keratin should respond much more rapidly to temporal or seasonal dietary shifts and record them in the subsequent weeks or months. However, Figure 7 illustrates that the isotopic variation of Au. africanus and also P. robustus was higher than that found in hair of extant baboons, which also shift in their reliance on C_4-plants (Codron et al., 2008 and references therein). When comparing the ranges in $\delta^{13}C$ at the different sites of extant great apes we found that none of the African populations in this study revealed isotopic variation comparable to extinct hominins. If we fully exclude that technical constraints during laser ablations measurement may erroneously enhance the $\delta^{13}C$ variability as it has been suggested for strontium isotope analyses (Balter et al., 2012; but also see Le Roux et al., 2014) this difference seems fundamental.

The question remains whether the remarkably high variation in $\delta^{13}C$ in fossil hominins implies dramatic dietary shifts between distinct food resources and thus indicates dietary flexibility or plasticity. It is still debated which plant (or animal) food resources could potentially have been consumed by hominins (Sponheimer et al., 2005; Codron et al., 2008; Stewart, 2014). Also, would these require different foraging strategies and ranging patterns or

more adaptive mastication and digestive physiology? If hominins (or their prey) were simply shifting from C_3-grass to C_4-grass, the socioecological and also physiological implications would probably be marginal compared to temporary shifts from abundant low energy herbaceous vegetation to clumped, highly seasonal and high caloric fruits (Clutton-Brock and Harvey, 1977; Hohmann et al., 2006). Did the isotopic approach comparing extant great apes with fossil hominins reach a dead end?

We do not think so. First, our data suggest that to some extent savanna chimpanzees may be a promising model for moderate C_4-resource utilization. Isotopic evidence from several fossil hominoid taxa does not suggest a strong C_4-plant dependence, including Australopithecus sediba (Henry et al., 2012) and Ardipithecus ramidus (White et al., 2009). These species (or specimens analyzed) seem to have foraged in C_3-dominated habitats (with C_4-plants yet available), similar to landscapes inhabited by savanna chimpanzees today. We think future work on the isotope ecology of savanna chimpanzees across Africa will greatly improve our comprehension of the ecological niche of these hominins.

Second, research on fossil Miocene great ape species such as Sivapithecus (Nelson, 2007, 2013) and Gigantopithecus blacki (Nelson, 2014 and references therein) would benefit from the isotopic comparison with the various African great apes species living in different habitats as presented here. The new data available for G. blacki indicate the habitat of these large bodied fossil apes were comprised of dense canopy forest. We suggest that low $\delta^{13}C$ signatures in G. blacki are best resembled by extant gorillas, which would match the dental morphological assessments (Kupczik and Dean, 2008).

In summary, the comprehensive dataset on African great apes and their respective food plants we present here illustrates the need of vegetation baseline data to disentangle effects related to habitat and those related to feeding behavior. We show that chimpanzee groups vary in their degrees of faunivory and folivory and that the approach we suggest here is applicable to

compare species and populations across different ecosystems. Finally, temporal isotopic variation in African great apes is detectable and differed between groups. Compared to fossil and extant primate species utilizing on C_4- resources however, variation over time was low even in apes living in mosaic and woodland savanna habitats.

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