RESEARCH ARTICLE

Revised: 20 December 2018

Stable isotope variation in savanna chimpanzees (*Pan troglodytes verus*) indicate avoidance of energetic challenges through dietary compensation at the limits of the range

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Funding information

Krekeler Foundation; Iowa State University; Max Planck Society Innovation Fund; Max-Planck-Gesellschaft; National Geographic Society

Abstract

Objectives: Food scarcity is proposed to be a limitation to chimpanzees at the limits of their range; however, such a constraint has never been investigated in this context. We investigated patterns of δ^{13} C and δ^{15} N variation along a latitudinal gradient at the northwestern West African chimpanzee (*Pan troglodytes verus*) range limit with the expectation that isotope ratios of chimpanzees at the range limit will indicate different dietary strategies or higher physiological constraints than chimpanzees further from the edge.

Materials and methods: We measured δ^{13} C and δ^{15} N values in hair (*n* = 81) and plant food (*n* = 342) samples from five chimpanzee communities located along a latitudinal gradient in Southeastern Senegal.

Results: We found clear grouping patterns in hair $\delta^{13}C$ and $\delta^{15}N$ in the four southern sites compared to the northernmost site. Environmental baseline samples collected from these sites revealed overall higher plant $\delta^{15}N$ values at the northernmost site, but similar $\delta^{13}C$ values across sites. By accounting for environmental baseline, $\Delta^{13}C$ and $\Delta^{15}N$ values were clustered for all five sites relative to total *Pan* variation, but indicated a ${}^{13}C$ -enriched diet at the range limit.

Discussion: Clustering in Δ^{13} C and Δ^{15} N values supports that strategic shifting between preferred and fallback foods is a likely ubiquitous but necessary strategy employed by these chimpanzees to cope with their environment, potentially allowing chimpanzees at their limits to avoid periods of starvation. These results also underline the necessity of accounting for local isotopic baseline differences during inter-site comparison.

KEYWORDS

carbon, niche, nitrogen, δ^{13} C, δ^{15} N

1 | INTRODUCTION

Carbon and nitrogen isotope values (δ^{13} C and δ^{15} N) are advantageous tools used in disciplines like paleoanthropology and ecology due to their ability to inform us on the diet and habitat of an individual. Fundamental to the use of stable isotopes as an indicator of diet is the idiom "you are what you eat" (Kohn, 1999), in that an individual's isotopic composition is a reflection of the isotopic ratio of the food items it consumes. These isotopes provide semi-quantified information on the diet of the individuals from which the tissues are sampled (Schwarcz & Schoeninger, 1991), and can be used, for example, to differentiate between photosynthetic pathways in plant-based diets (DeNiro & Epstein, 1978), or used as indicators of trophic level (for example as between plant- and animal-based diets: Minagawa & Wada, 1984). As a result, these isotopes have served as the basis upon which studies of dietary composition, change, and niche partitioning have been undertaken in a wide range of taxa (e.g., Balasse, Bocherens, Mariotti, & Ambrose, 2001; Caraveo-Patino, Hobson, & Soto, 2007; Codron, Lee-Thorp, Sponheimer, de Ruiter, & Codron, 2006; Codron, Lee-Thorp, Sponheimer, De Ruiter, & Codron, 2008; Harrison et al., 2007; Koch et al., 1995; Lee, Schell, McDonald, & Richardson, 2005; Zazzo et al., 2007). American Journal of PHYSICAL ANTHROPOLOGY

Because tissues used for stable isotope analyses (e.g., hair, teeth, and bone) reliably record the nature of isotopic intake, isotope ecology has recently gained popularity in primatological research (Crowley, Rasoazanabary, & Godfrey, 2014; Oelze et al., 2016; Sandberg, Loudon, & Sponheimer, 2012; Schoeninger, 2009; Schoeninger, Iwaniec, & Nash, 1998). Furthermore, a particular advantage of using δ^{13} C and δ^{15} N as dietary indicators is the possibility to incorporate otherwise unobtainable data on groups unhabituated to researcher presence in the face of rapid species decline (e.g., Kühl et al., 2017).

Pan species are frequently used as referential models for hominin ecology and dietary reconstruction; therefore these species have likewise represented a particular focus within primate isotopic research (e.g., Carter, 2001; Fahy, Boesch, Hublin, & Richards, 2015; Fahy, Richards, Riedel, Hublin, & Boesch, 2013; Macho, 2016; Macho & Lee-Thorp. 2014: Oelze et al., 2016: Oelze, Head, Robbins, Richards, & Boesch, 2014; Schoeninger, Moore, & Sept, 1999; Schoeninger, Most, Moore, & Somerville, 2016; Smith, Morgan, & Pilbeam, 2010; Sponheimer et al., 2006; van Casteren et al., 2018). As a result, Pan isotope ecology has developed into a growing discipline in which both broad- and fine-scale dietary variation within and between individuals have been detected noninvasively. For example, Fahy et al. (2013) demonstrated that interindividual differences in hunting prowess among wild chimpanzees is detectable using $\delta^{15}N$ values on the basis that better hunters are likely to have a higher proportion of meat in their diet and therefore have higher $\delta^{15}N$ values than less successful hunters. Additionally, researchers have begun to investigate the use of nitrogen isotopes for detecting periods of nutritional stress in multiple taxa (e.g., Kempster et al., 2007; McCue & Pollock, 2008), more recently extending to great apes (Deschner et al., 2012; Vogel et al., 2011). Such patterns are built upon the tenet that increases in the metabolization of animal body tissue (whether extrinsic via meat consumption or intrinsic via fasting or starvation) effect observable δ^{15} N increases in the tissues of the consumer (Fuller et al., 2005).

However, in larger-scale comparisons of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values across multiple ecological conditions potential conclusions become less straightforward. Initial studies attempted to evaluate aspects of primate diet and ecology using tissue samples alone, yet the recognition of the variability of tropical ecosystems led researchers to embrace the incorporation of environmental context into cross-site comparisons (Crowley et al., 2014; Oelze et al., 2011; Oelze et al., 2016). For example, one prominent contextual measure of isotope system variability was mean annual precipitation (MAP). However, attempts to explain isotopic variation between chimpanzee sites using MAP have resulted in contradictory findings, as significant empirical support for this relationship exists for δ^{13} C values, but widespread consensus is lacking for δ^{15} N (Loudon, Sandberg, Wrangham, Fahey, & Sponheimer, 2016; Oelze et al., 2016; Schoeninger et al., 2016). Although attempts at explaining climatic contributions aids in understanding broad-scale patterns of variation in Pan isotopes, the relevance of the ecological context in isotopic comparisons across varied habitats necessitated the establishment of environmental isotopic baselines before fine-scale variation could be evaluated. Isotopic variability of Pan food items (Carlson & Crowley, 2016; Carlson & Kingston, 2014; Loudon et al., 2016) and the incorporation of these items into body tissues (Tsutaya, Fujimori, Hayashi, Yoneda, & Miyabe-Nishiwaki, 2017) has recently received greater focus in the literature. Still, scant research (Oelze et al., 2016) has directly incorporated these baselines when drawing inter-site comparisons, which instead use the offset between baseline plant isotope values and body tissue isotope values (known as Δ^{13} C and Δ^{15} N) as the unit of comparison, thereby allowing a stronger basis upon which primate interactions with their environment can be evaluated across sites.

Within Pan isotope ecology, particular focus has been placed on chimpanzees in more open mosaic habitats due to their use as referential models for human evolution. To date, data from a growing number of sites have been published (Ugalla, Ishasha: Schoeninger et al., 1999, Gombe: Schoeninger et al., 2016, Fongoli: Sponheimer et al., 2006, Kayan: Oelze et al., 2016, Issa: van Casteren et al., 2018), however each employ starkly different sampling approaches, and the majority of these studies lack baseline environmental data. Here we describe patterns of isotopic variation in chimpanzees inhabiting a savanna environment along a latitudinal gradient in Senegal, at the northwestern reaches of chimpanzee distribution in Africa. The climate of southeastern Senegal is characterized by strong seasonality in rainfall and temperatures which consistently exceed 40 °C, comprising exceptional conditions expected to be particularly taxing to chimpanzees (Pruetz, 2007; Pruetz & Bertolani, 2009; Wessling, Kühl, Mundry, Deschner, & Pruetz, 2018).

This region likewise represents the northern margins of the West African chimpanzee (Pan troglodytes verus) range. Multiple hypotheses have been proposed to explain the potential drivers of the chimpanzee range margin in this region (Kortlandt, 1983; McGrew, Baldwin, & Tutin, 1981), although food scarcity has been the predominant (albeit untested) hypothesis as limiting chimpanzee biogeographic distribution here (Kortlandt, 1983; Lindshield, 2014). Previous work at Fongoli, a habituated chimpanzee research site approximately 65 km south of the previous IUCN estimated range limit (Humle et al., 2008), has demonstrated that chimpanzees in this region employ a range of compensatory behaviors to cope with this environment (coprophagy: Bertolani & Pruetz, 2011; tool-assisted hunting: Pruetz & Bertolani, 2007, Pruetz et al., 2015; pool use: Pruetz & Bertolani, 2009; cave use: Pruetz, 2007), and appear to be physiologically challenged due to the extremity of this environment to varying degrees (Wessling, Kühl, et al., 2018).

Although for Fongoli chimpanzees challenges of dehydration and thermoregulation appear more stressful than maintaining adequate energetic status (Wessling, Kühl, et al., 2018), the relative importance of these challenges may not remain identical for chimpanzees who survive at the very edge of the chimpanzee range. An important question remains as to what drives chimpanzee occurrence in an extreme savanna habitat, and ultimately, what is most limiting to chimpanzees inhabiting the very edges of their tolerable habitat? If environmental conditions are assumed to be less suitable to a species the closer to the edge of their range (Hutchinson, 1961), then we should expect that biomarkers of the physiological challenges faced in this habitat become more pronounced toward the range margin. Equally, if chimpanzees at the range edge encounter increasing environmental constraint, we should expect them also to develop adaptations for overcoming these constraints as they are able. For example,

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individuals may increasingly rely upon the consumption of fallback foods, or less-preferred food items eaten when preferable items become scarce (Marshall & Wrangham, 2007), such as termites and nonreproductive plant organs (Boesch & Boesch-Achermann, 2000; Bogart & Pruetz, 2011; Goodall, 1986; Head, Boesch, Makaga, & Robbins, 2011; Morgan & Sanz, 2006; Piel et al., 2017; Pruetz, 2006; Tutin, Ham, White, & Harrison, 1997; Wrangham et al., 1991).

Here, we describe patterns of isotopic variation in chimpanzees at the edges of the chimpanzee range limit. We expected that if maintaining adequate nutritional and dietary requirements is increasingly challenging with decreasing distance to the chimpanzee biogeographical range edge, then we should predict fundamental differences in isotopic ratios among groups at the range edge according to their location. Alternatively, if chimpanzees are modifying their diet to accommodate potential environmental inadequacies, dietary variation should be visible in stable isotopic variation among these groups. Specifically, we predict that (a) if chimpanzees are more likely to encounter periods of starvation closer to the range margins, that chimpanzee tissues will be $\delta^{15}N$ enriched at the edge of the range, and (b) that if food scarcity is a limitation to chimpanzee distribution, then chimpanzee δ^{13} C and δ^{15} N isotopic profiles will indicate heavier reliance upon less-preferred food items or fallback foods. Contingent upon the investigation of these predictions is the hypothesis that isotopic baseline signatures remain largely identical across Senegal, and that dietary differences or physiological responses to food scarcity will be the sole origin of chimpanzee isotope variation.

2 | METHODS

We collected plant and chimpanzee hair samples along a latitudinal gradient at the edges of the West African chimpanzee range margin in southeastern Senegal (Figure 1). These five sites included Fongoli (FSCP: 12°40'N, 12°13'W; southern-most site), a research chimpanzee community habituated since 2005, Kayan (12°12'W 13°12'N), one of the research sites surveyed by the Pan African Programme (Oelze et al., 2016), two unhabituated chimpanzee sites located between Fongoli and Kayan (Kanoumering: 12°6'W 12°47'N, Makhana: 12°7'W 13°5'N) as well an additional site (Hérémakhono) located beyond the previously estimated IUCN range limit (12°0'W 13°26'N; Humle et al., 2008). Surveying at and beyond the previously estimated IUCN West African chimpanzee range limit indicated that if Hérémakhono does not represent the very northern-most vestige where chimpanzees can be found, it is likely very near to it (Wessling, unpublished data). In addition, initial comparison of chimpanzee densities across these sites suggests densities are far lower at Hérémakhono than estimated at the four other sites (Wessling, unpublished data).

This region of Senegal belongs to the Sudano-Guniean vegetation belt (Ba et al., 1997) and can be described as a savanna-woodland mosaic comprising gallery forest, woodland, and grassland. The climate is highly seasonal, and is particularly extreme in comparison to habitats typical for the chimpanzee across its biogeographical range (Wessling et al., 2018). The region undergoes a significant annual dry season (October–April) during which little to no rain falls, and temperatures reach 46 °C (Wessling, Kühl, et al., 2018). Mean annual rainfall for the region is 945 mm (FSCP, unpublished data), the majority of which falls during the short wet season (June-August).

2.1 | Isotope analyses

We collected chimpanzee hair samples between April 2012 and February 2014 from vacated chimpanzee night nests whenever encountered and accessible. Samples (n = 81) were selected for analysis based on sample quality (weight, length, and root present, fresh nest, temporal resolution) of the sample, and were prepared following the protocol outlined in Oelze et al. (2016). Hair samples ranged from 2 to 9 cm in maximum length (mean \pm SD: 6.4 \pm 1.5 cm), and were aligned at the root bulb and sectioned into 2.5 mm, 5 mm, 10 mm, or 1.5 cm segments, as segment weight allowed (min. target weight: 0.2 mg). These sections were then transferred into tin capsules for analysis at the Max Planck Institute for Evolutionary Anthropology (MPIEVA) in Leipzig, Germany on a Flash EA 2112 (Thermo-Finnigan[®], Bremen, Germany) coupled to a DeltaXP mass spectrometer (Thermo-Finnigan[®]) or on a EuroEA3000 (EuroVector SpA[®], Milan, Italy) coupled to a MAT 253 IRMS (Thermo-Finnigan[®]) at the German Research Center for Environmental Health, Institute for Groundwater Ecology, Neuherberg, Germany,

We sampled plant items from each of the aforementioned sites. This sample set was composed of known or potential food items for the Fongoli community (Pruetz, 2006; FSCP unpublished data). We followed a similar plant sampling approach at the unhabituated chimpanzee sites for which no feeding data were available. Plant samples were collected opportunistically during an initial pilot study (April–June 2012) as well as systematically throughout the study period (January 2013–February 2014). Sampling occurred quarterly at three sites (Hérémakhono, Kanoumering, and Makhana), whereas sampling was continuous at the two sites (Fongoli and Kayan) with continuous research presence.

To control for potential canopy effects in plant samples (Cerling, Hart, & Hart, 2004; Schoeninger et al., 1998; Van der Merwe & Medina, 1991), we specifically targeted our sampling to the bottom of the arboreal canopy, as low canopy samples have been suggested to be sufficiently representative of δ^{13} C levels in arboreal primate diet food items (Blumenthal, Rothman, Chritz, & Cerling, 2016; Krigbaum, Berger, Daegling, & McGraw, 2013; Nelson, 2013). Rather than restrict our analyses to fruit items as has previously been recommended (Crowley, 2012; Oelze et al., 2016), we included plant parts from all potential Fongoli food species so as to encompass the wider range of potential environmental isotopic contributions, but targeted known Fongoli dietary items when available. If we were unable to collect food items during the phenological stage at which they are eaten at Fongoli (e.g., new leaves or ripe fruit) we collected a sample from the species during another phenological stage (i.e., mature leaves or unripe fruit), or in few cases, another plant organ altogether (e.g., mature leaves instead of ripe fruit). As we were unable to directly estimate dietary isotopic inputs, this sampling protocol provided us with an environmental (not dietary) baseline, representative of potential (not actual) dietary availability at each site. Kayan hair and fruit sample isotope analyses have previously been published (Oelze et al., 2016), although published results were averages of duplicate measurements;



FIGURE 1 Location of the five chimpanzee research sites relative to the *Pan troglodytes verus* IUCN range limit (green area, Humle et al., 2008) within southeast Senegal

instead, here we included original single measurements in our models, and supplemented this dataset with previously unpublished nonfruit plant samples from Kayan. In few cases (n = 5), individual plants in our dataset which had been sampled multiple times over the course of a year were included in the dataset as single plant items.

Plant samples were dried using silica gel and sun exposure, and stored dry until shipped for analysis. Prior to analysis, we dried samples in an oven at 50 °C for a minimum of 12 hr, homogenized them using a ball mill, after which they were redried at 50 °C for a minimum of 12 hr, and weighed into tin capsules for measurement. Plant samples were measured by three different groups: at (a) MPIEVA (see above), in (b) Leipzig, Germany on a Flash HT Plus (Thermo Scientific[®], Waltham) coupled to a MAT 253 IRMS (Thermo-Finnigan[®]) in collaboration with IsoDetect GmbH, or (c) on a Flash2000 Elemental Analyzer, coupled with a Conflo IV and a Delta V Advantage Isotope Ratio Mass Spectrometer (Thermo Fisher, Dreieich, Germany) at the University of Leipzig. All samples from Kayan were run in Neuherberg, Germany (see above) via partnership with IsoDetect GmbH, therefore we group these samples within the IsoDetect GmbH analyses (see below).

We expressed the stable isotope ratios of carbon and nitrogen as $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios using the delta (δ) notation in parts per thousand or permil (‰). This is relative to the international standard materials Vienna PeeDee Belemite (vPDB) and atmospheric N2. We included repetitive measurements in each run of CH7, CH6, N1, N2, USGS 25, USGS 40, and USGS 41 and internal-laboratory standard materials to calculate analytical error; these results suggested analytical error to be <0.3 ‰ (2 σ) for both δ^{13} C and δ^{15} N. In general, we aimed to analyze samples in duplicate (at minimum) and across multiple laboratories whenever possible. In practice, we analyzed an average of 2.4 ± 2.4 δ^{15} N (mean ± SD, range: 1–24) and 2.6 ± 2.5 δ^{13} C (range: 1-32) runs per sample, with only 54 and 14 sample results originating from single analyses for $\delta^{15}N$ and $\delta^{13}C$ isotopes, respectively. We observed interlaboratory analytical differences and therefore accounted for this discrepancy with the use of an additional term in our models (see below).

To ensure analytical quality, we excluded all plant analyses for which sample amplitudes were less than one-third or three times the average amplitude of run standards for carbon (n = 112) and nitrogen (n = 409) isotope analyses of plant samples, and four samples for hair carbon isotope analyses. Additionally, as an extra precaution to avoid potential biases in site averages due to sampling of extreme plant items (e.g., C₄ grasses), plant sample isotope values were excluded if they exceeded two standard deviations from the overall mean of the dataset ($n_{carbon} = 80$, $n_{nitrogen} = 25$). We excluded all hair results (n = 4) with atomic C:N ratios outside the 2.6–3.8 range originally proposed by O'Connell, Hedges, Healey, and Simpson (2001). Three additional hair δ^{13} C results were excluded as they were extreme outliers (<–28‰) unlikely to be true measurements.

In total, we successfully analyzed 764 segments from 81 hair samples for δ^{13} C and δ^{15} N. For plant samples, we analyzed 621 runs of 265 plant samples from 53 species representing 116 unique plant parts for δ^{15} N, and 864 runs of 340 samples from 54 species representing 120 unique plant parts for δ^{13} C. This included 42 food items for which identical plant parts were collected from the same species in at least two sites.

2.2 | Statistical analyses

To test for potential inter-site differences in baseline plant sample isotopic values, we fitted linear mixed models (LMM) with a Gaussian error function using the "Ime4" package (Baayen, 2008) in R (version 3.4.0; R Core Team, 2017) for $\delta^{15}N$ and $\delta^{13}C$ values separately. Each individual plant sample analysis was included in the model as a single data point, and controlled for 'sample ID' as a random effect. We used the factor 'site' as our single test predictor, and included 'lab' as a fixed effect to control for potential interlaboratory differences. To account for potential seasonal variation in plant isotopic ratios, we included a generic seasonal term (sine and cosine of Julian date upon which each sample was collected: Stolwijk, Straatman, & Zielhuis, 1999) as a control predictor. For samples in which only the collection month was noted (n = 54), we assigned these samples to the monthly midpoint (15th). Lastly, as different plant tissues and species may differ in their isotope ratios, we controlled for plant species and plant part as random effects, with consideration of plant part maturity as potentially isotopically distinct (per Blumenthal et al., 2016; Schielzeth & Forstmeier, 2009). Due to difficulty of in-field identification, we grouped two Lannea species (L. microcarpa and L. acida), as well as several Ficus species into a single level for each genus (with the exception of the following species which were treated separately: sur, ingens, cordata, sycomorus, trichopoda, umbellata, asperfolia, abutilifolia, vallis-choudae). To keep type I error rate at the nominal 5%, we included random slopes (Barr, Levy, Scheepers, & Tily, 2013; Schielzeth & Forstmeier, 2009) for season within species and plant part as well as site within species. We used a likelihood ratio test to compare the fit of these full models to their respective null models, lacking only the test predictor (Forstmeier & Schielzeth, 2011).

We visually inspected qq-plots and residuals plotted against fitted values and verified that the assumptions of normally distributed and homogeneous residuals were met in each model. These plots revealed relatively wide (i.e., long-tailed) distributions of model residuals, suggesting our models were possibly conservative in their ability to identify significant effects of our test predictor. Further, there was a slight positive correlation between model residuals and fitted values, which is likely due to a large number of random effect levels occurring relatively rarely in the dataset and leading to more prominent "shrinkage" (Baayen, 2008). We evaluated model stability by excluding levels of the random effects one at a time and comparing the estimates derived from these datasets with those derived for the full dataset. This revealed no influential random effects. We verified our models did not suffer from collinearity among predictors by determining Variance Inflation Factors (VIF; Field, 2005) for a standard linear model excluding all random effects (maximum VIF: 1.07).

We chose not to test for statistical differences between sites for chimpanzee hair sample analyses due to our inability to account for potential pseudo-replication arising from multiple sampling of the same individuals in the unhabituated sites (Mundry & Oelze, 2016). Instead, we describe broad-scale comparison among these groups using only site averages and δ^{13} C and δ^{15} N ranges. We did, however, calculate site averages using an LMM for each site and isotope which controlled for the random effect of "sample ID". Lastly, we calculated isotopic fractionation (Δ) following Oelze et al. (2016) to control for plant baseline effects and to allow for inter-site dietary comparison. In brief, isotope fractionation values, or Δ^{13} C and Δ^{15} N, are calculated as the difference between average site hair isotope values and average baseline plant values (using intercept and estimates per site in our plant models). All research was noninvasive and was approved by the Max Planck Society.

3 | RESULTS

Our model reported no significant differences in plant δ^{13} C ratios among the five sites (full-null model comparison: χ^2 = 3.986, *df* = 4, *p* = .408), indicating Hérémakhono (as the reference category) was not significantly higher in plant δ^{13} C values than the four southern **TABLE 1** Model results for the effect of 'site' on δ^{13} C (a) and δ^{15} N (b) ratios in plant samples

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(a) δ ¹³ C Term	Estimate ± SE	p-value
(Intercept)	-28.88 ± 0.42	-
Site (Fongoli) ^a	0.19 ± 0.30	
Site (Kanoumering) ^a	-0.24 ± 0.39	0.377
Site (Kayan) ^a	-0.28 ± 0.28	
Site (Makhana) ^a	0.13 ± 0.35	
Sine (Julian date)	0.36 ± 0.20	-
Cosine (Julian date)	-0.26 ± 0.23	-
Lab (MPI) ^b	0.10 ± 0.07	-
Lab (UNI) ^b	-0.69 ± 0.10	-
(b) δ^{15} N Term	Estimate ± SE	p-value
(b) δ ¹⁵ N Term (Intercept)	Estimate ± SE 3.55 ± 0.37	p-value -
(b) $\delta^{15}N$ Term (Intercept) Site (Fongoli) ^a	Estimate ± SE 3.55 ± 0.37 -1.37 ± 0.33	p-value -
 (b) δ¹⁵N Term (Intercept) Site (Fongoli)^a Site (Kanoumering)^a 	Estimate ± SE 3.55 ± 0.37 -1.37 ± 0.33 -1.62 ± 0.36	<i>p</i> -value - <0.001
 (b) δ¹⁵N Term (Intercept) Site (Fongoli)^a Site (Kanoumering)^a Site (Kayan)^a 	Estimate ± SE 3.55 ± 0.37 -1.37 ± 0.33 -1.62 ± 0.36 -1.62 ± 0.39	p-value - <0.001
 (b) δ¹⁵N Term (Intercept) Site (Fongoli)^a Site (Kanoumering)^a Site (Kayan)^a Site (Makhana)^a 	Estimate ± SE 3.55 ± 0.37 -1.37 ± 0.33 -1.62 ± 0.36 -1.62 ± 0.39 -1.10 ± 0.40	<i>p</i> -value - <0.001
 (b) δ¹⁵N Term (Intercept) Site (Fongoli)^a Site (Kanoumering)^a Site (Kayan)^a Site (Makhana)^a Sine (Julian date) 	Estimate \pm SE 3.55 ± 0.37 -1.37 ± 0.33 -1.62 ± 0.36 -1.62 ± 0.39 -1.10 ± 0.40 -0.12 ± 0.12	p-value - <0.001
 (b) δ¹⁵N Term (Intercept) Site (Fongoli)^a Site (Kanoumering)^a Site (Kayan)^a Site (Makhana)^a Sine (Julian date) Cosine (Julian date) 	Estimate \pm SE 3.55 \pm 0.37 -1.37 \pm 0.33 -1.62 \pm 0.36 -1.62 \pm 0.39 -1.10 \pm 0.40 -0.12 \pm 0.12 0.13 \pm 0.17	p-value - <0.001
 (b) δ¹⁵N Term (Intercept) Site (Fongoli)^a Site (Kanoumering)^a Site (Kayan)^a Site (Makhana)^a Sine (Julian date) Cosine (Julian date) Lab (MPI)^b 	Estimate \pm SE 3.55 \pm 0.37 -1.37 \pm 0.33 -1.62 \pm 0.36 -1.62 \pm 0.39 -1.10 \pm 0.40 -0.12 \pm 0.12 0.13 \pm 0.17 -0.13 \pm 0.14	<pre>p-value</pre>

^a Estimate refers to the comparison with Hérémakhono as the reference category.

^b Estimate refers to the comparison with IsoDetect as the reference category.

sites (Fongoli, Kanoumering, Makhana, Kayan; Table 1(a)) when controlling for potential biases among the sites. $\delta^{15}N$ significantly differed among the five sites ($\chi^2 = 23.637$, df = 4, p < .001), with plant $\delta^{15}N$ values of Hérémakhono baseline plant samples significantly higher than in the four other sites (Table 1(b)). Model-based site $\delta^{13}C$ plant averages ranged between -28.7 and -29.2% across the five sites, whereas the four southern-most sites averaged between 0.9% and 1.6% lower than Hérémakhono (3.5%) in $\delta^{15}N$ plant values (Table 2).

We also found clear differences between the southern sites and Hérémakhono in mean chimpanzee hair isotope values (Table 3; Figure 2). Average δ^{13} C and δ^{15} N values for the southern communities ranged from –23.1 to –22.6‰ and 3.2 to 3.6‰, respectively, whereas Hérémakhono samples averaged at least 0.9‰ higher in δ^{13} C and 1.0‰ higher in δ^{15} N than even their most similar counterparts. The southern sites exhibited very minor overlap with the range of isotopes observed at Hérémakhono. In general, intra-site variation in hair δ^{13} C and δ^{15} N values was greater than inter-site average variation, with intra-site variation ranging from 1.2‰ to 2.6‰ in δ^{13} C and 1.1‰ to 2.4‰ in δ^{15} N among the five sites, with Fongoli exhibiting the highest variation in both isotopes while likewise being the most heavily sampled site by at least twofold.

By accounting for inter-site variation in baseline plant samples at each site, Δ^{13} C and Δ^{15} N values were tightly clustered for all five sites relative to total *Pan* variation (Figure 3), averaging 6.3% for Δ^{13} C (range: 5.8% to 7.2%) and 1.2% in Δ^{15} N (range: 1.0% to 1.6%) across sites. Maximum difference between Δ values across sites were

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TABLE 2 Plant baseline δ^{13} C and δ^{15} N values from five sites in Senegal, ordered in table from south (top) to north (bottom), and their descriptive statistics for 340 (δ^{13} C) and 265 (δ^{15} N) samples

Site	n _{13C}	n _{15N}	$\delta^{13}C^{a}$ (model-based; x ± SE)	$\delta^{13}C^{b}$ (x ± SE)	δ ¹³ C minimum, maximum	$\delta^{15} N^a$ (model-based; x ± SE)	δ^{15} N ^b (x ± SE)	δ ¹⁵ N minimum, maximum
Fongoli	154	122	-28.7	-28.6 ± 0.4	-32.4, -24.0	2.2	2.0 ± 0.2	-3.0, 7.2
Kanoumering	38	35	-29.1	-28.4 ± 0.4	-32.1, -24.6	1.9	1.9 ± 0.3	-1.6, 7.3
Makhana	46	33	-28.7	-28.3 ± 0.4	-32.3, -24.6	2.4	2.1 ± 0.3	-1.8, 6.4
Kayan	69	51	-29.2	-28.8 ± 0.3	-32.3, -24.6	1.9	1.9 ± 0.7	-1.7, 5.3
Hérémakhono	35	26	-28.9	-28.8 ± 0.3	-32.3, -24.9	3.5	3.2 ± 0.4	-0.9, 7.2

^a Model-based averages derived from intercept and estimates of respective LMMs containing the full dataset.

^b Controlling for species, plant part, sample, season, and lab and the random slopes of season within plant part and species, within each site.

1.4‰ and 0.6‰ for Δ^{13} C and Δ^{15} N respectively, with highest Δ values at Hérémakhono (Δ^{13} C) and Kanoumering (Δ^{15} N).

Lastly, Figure 4 demonstrates the variability of different plant food items within our dataset, across all species and sites, and relative to the modeled averages of each plant part controlling for sampling biases. Broadly, $\delta^{15}N$ demonstrated little variation among plant parts with the exception of bark which was ¹⁵N depleted (although the model failed to account for this variability). Conversely, $\delta^{13}C$ was relatively more variable across plant parts, with mature leaves, new leaves, and bark averaging lower than ripe and unripe fruit. Flowers and pith averaged higher than fruit in $\delta^{13}C$, but these differences were not reflected in the random intercepts for these parts. Nonetheless, all plant parts with the exception of bark (sampled from only two species) varied across the range of values observed for $\delta^{13}C$ and $\delta^{13}N$ plant analyses, indicating strong interspecific variability in these isotope systems.

4 | DISCUSSION

Altogether, isotope ratios for Fongoli samples were similar to previously published results on the same population (Loudon et al., 2016; Sponheimer et al., 2006), both in hair and plant datasets, despite differences between datasets in analyses, sample size, and dietary coverage. Our larger plant sample set indicated a wider range of potential isotopic δ^{13} C and δ^{15} N inputs than previously published estimates lacking information on the nature of the plant samples (Loudon et al., 2016), and approach ranges similar to that seen in wetter habitats (Carlson & Crowley, 2016; Carlson & Kingston, 2014; Oelze et al., 2016). Nonetheless, inter-study similarity in Fongoli hair isotope averages indicated interannual dietary fidelity at Fongoli.

Generally, we found that chimpanzees at all five sites exhibited relatively high Δ^{13} C and low Δ^{15} N values in comparison to chimpanzees from other habitat types. Our results support that low $\delta^{15}N$ values in this region are due in part to equally low environmental baseline levels (Sandberg et al., 2012), and contradict assertions that dry African sites reveal δ^{15} N enriched values (Heaton, Vogel, von La Chevallerie, & Collett, 1986). In addition, $\Delta^{15}N$ values for all sites averaged lower than would be expected for a trophic level increase between a consumer and its diet (~3%; Minagawa & Wada, 1984). Low $\Delta^{15}N$ values in Senegal have previously been suggested either to be due to relatively low meat consumption, or to result from the regular consumption of ¹⁵N depleted Macrotermes termites (n = 4, mean 0.2‰ δ^{15} N, Oelze, unpublished data; Oelze et al., 2016, Sandberg et al., 2012). Mound-building termites are abundant (Bogart & Pruetz, 2011) and ubiquitous in the study region (Wessling, personal observation), and are consumed at higher rates at Fongoli than other chimpanzee sites (Bogart & Pruetz, 2011). Termite remains in Kayan fecal samples (Oelze et al., 2016) indicate termite consumption across multiple sites in Senegal, thereby supporting ¹⁵N-depleted termite consumption as a potential cause for low $\Delta^{15}N$ across our sites. Additionally, Fongoli chimpanzees appear to consume meat less frequently, with only 99 hunts observed over 9 years (Pruetz et al., 2015), relative to other chimpanzee communities such as Taï where hunts are observed on average every 4 days (Samuni et al., 2018), or Ngogo with an average 73 hunts per year (Watts & Mitani, 2002). If low hunting rates is a region-wide pattern, comparatively infrequent meat consumption may also explain lower Δ^{15} N values in our samples relative to sites like Ngogo and Taï. Nonetheless, direct behavioral comparison is needed to confirm potential drivers of low Δ^{15} N in Senegalese chimpanzee hair samples.

As for chimpanzee isotopic variation across our five sites, we found strong similarities in δ^{13} C and δ^{15} N hair sample averages and significant overlap in the ranges of these isotope ratios among the

TABLE 3 Descriptive statistics for 81 chimpanzee hair sample δ^{13} C and δ^{15} N values from five sites located along a latitudinal gradient in Senegal, ordered in table from south (top) to north (bottom), as well as site mean Δ^{13} C and Δ^{15} N in comparison with plant baselines per site

Site	n _{samples}	n _{segments}	δ^{13} C ^a (mean ± SE)	δ^{13} C minimum, maximum	δ^{15} N ^a (mean ± SE)	δ^{15} N minimum, maximum	$\Delta^{13}\text{C}^{\text{b}}$	$\Delta^{15} N^{b}$
Fongoli	37	378	-22. 6 ± 0.1	-23.5, -20.9	3.2 ± 0.1	1.9, 4.3	6.1	1.0
Kanoumering	16	145	-22.8 ± 0.1	-23.7, -22.3	3.5 ± 0.1	2.7, 4.8	6.3	1.6
Makhana	11	110	-22.9 ± 0.1	-23.5, -22.1	3.6 ± 0.0	3.0, 4.1	5.8	1.2
Kayan	10	89	-23.1 ± 0.1	-23.9, -22.3	3.2 ± 0.1	2.4, 4.2	6.1	1.3
Hérémakhono	7	43	-21.7 ± 0.1	-22.2, -21.0	4.6 ± 0.1	3.8, 5.3	7.2	1.1

^a Controlling for hair sample.

^b Delta values calculated using model-based plant estimates and site hair averages.



FIGURE 2 (a) δ^{13} C and δ^{15} N hair sample averages for five locations along a north-south gradient in Senegal, with ranges of all hair segments for each site. Sample means (points) and site ranges (polygons) are depicted for Fongoli (red), Makhana (yellow), Kanoumering (blue), Kayan (green), and Hérémakhono (purple). (b) Hair sample averages controlled for site plant sample baselines (Δ^{13} C and Δ^{15} N)

four southern communities. However, chimpanzees from our northernmost site, Hérémakhono, demonstrated δ^{13} C and δ^{15} N values around 1‰ higher than their southern counterparts. In absence of corresponding environmental baseline data, these results might have been suggestive of significant differences among the communities either in dietary composition, energetic status, or both.

However, calculations of Δ^{13} C and Δ^{15} N allow us to control for plant baselines and turn our attention to the dietary behavior or other potential physiologically-based influence on isotopic variation that we sought to assess in this study. By accounting for the observation that Hérémakhono plant samples averaged 1.4‰ in δ^{15} N higher than the southern groups, we identified that $\Delta^{15}N$ values were functionally identical across sites with a maximum 0.6‰ variation for $\Delta^{15}N$ among the site averages. Therefore, regardless of potential sources of variation in δ^{15} N values of environmental baselines, the scale of differences in plant baselines among these sites accounted for the majority of isotopic differences in δ^{15} N values in our hair samples. The low degree of Δ^{15} N variation between sites is contradictory to our predictions that chimpanzees at Hérémakhono experience more pronounced periods of nutritional stress than their counterparts. However, as plant $\delta^{13}\text{C}$ values were statistically indistinguishable across sites, the mean Hérémakhono Δ^{13} C value (7.0%) remained higher than its most similar counterpart by 0.9‰, twice the maximum difference in averages among the four southern sites (0.4‰). Taken together, as δ^{13} C differences between chimpanzees from different sites are not explained by differences in environmental baseline, dietary rather than direct environmental isotopic differences must account for differences in Δ^{13} C, with the diet of Hérémakhono chimpanzees proving to be relatively ¹³C enriched.

Without direct behavioral evidence, identification of dietary explanations for ¹³C enrichment at Hérémakhono remains speculative, although a few possible candidates emerge from our data. Within our sample set, the most likely candidate explaining ¹³C enrichment could be a greater reliance on flowers at Hérémakhono than at other sites, as flowers were the only plant food type which averaged higher in δ^{13} C across species than ripe fruits (although all random intercepts in this model fell below the ripe fruit average). It is also possible that specific ¹³C enriched food items within this dataset (e.g., *Afzelia* or *Parkia* fruits)

could also be candidates for overall dietary ¹³C enrichment, but that this was not a consistent pattern among plant parts and species.

Another common explanation for ¹³C enrichment is the consumption of C_4 plants. Fongoli chimpanzees have been observed to infrequently consume C_4 plants like grasses (Pruetz, 2006), therefore it could also be possible that greater consumption of C_4 grasses may explain ¹³C enrichment at Hérémakhono. Furthermore, typical C_4 crop species like corn and sugarcane are grown within the study region, although there have been no reports of crop raiding of these species from chimpanzees at any of our sites. Yet, chimpanzees at Hérémakhono have been exclusively reported to annually crop-raid mango trees located within corn fields in the months of May and June (Wessling, pers. observation). Although we do not have isotopic data



FIGURE 3 Average Δ^{13} C and Δ^{15} N values per *Pan* research site; size of points denotes relative sizes of hair datasets (range: 6–36). Δ values are calculated based on the following hair and plant datasets: Oelze et al., 2016; Loudon et al., 2016; van Casteren et al., 2018; this study

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FIGURE 4 Plant part variation in δ^{13} C (top) and δ^{15} N (bottom) values. Shown are medians (thick horizontal lines; "MED"), quartiles (boxes), percentiles (vertical lines; 2.5 and 97.5%), and random effect intercepts (red diamond; "REI") of each plant part (left to right: ripe fruit, unripe fruit, mature leaves, new leaves, bark, flower, and pith)

from mango fruits, it is likely that these fruits are ¹³C enriched if the fields containing them are irrigated (Wallace et al., 2013), or are sunnier than locations where wild plant foods were sampled (e.g., France, 1996).

Regardless, isotope systems are highly complex and sources of variation are multiplicitous. Therefore, conclusions in wild great ape isotope ecology should remain relatively tentative until supported with behavioral consensus and more detailed physiological studies. Although consistent patterns among plant parts and species have been described, the complexities of identifying origins of isotopic variation in *Pan* diets should promote researchers to be cautious in their interpretations and coarse in their conclusions.

Previous comparison across habitats has established that food availability in savanna environments is scarcer than more forested habitats (Wessling, Deschner, et al., 2018). It may therefore be possible that food scarcity is a real threat to chimpanzees at the edge of their range, but that they are able to employ flexible strategies to counteract this threat or dampen its effects below a measureable threshold (Kempster et al., 2007). Data from the only site for which feeding strategy is known, the habituated community of Fongoli, suggests that although savanna chimpanzees are ripe-fruit specialists, they depend to a greater extent on fallback foods when preferred ripe fruit items are not available (Bogart & Pruetz, 2011; Pruetz, 2006). As the four unhabituated chimpanzee communities demonstrated similar Δ^{15} N ratios to Fongoli, it is likely this is a ubiquitous but necessary strategy employed by these chimpanzees to cope with a seasonal and challenging environment.

However, we did not observe a measureable difference in $\Delta^{15}N$ values for Hérémakhono chimpanzees, which suggests that these chimpanzees also do not endure periods of starvation or energetic stress to greater degrees than the southern communities. Such a strategy would only lead to low inter-site variation in diet where dietary pressures are equal in their magnitude. However, if floristic diversity and/or density are reduced at Hérémakhono compared to the southern sites, it may be possible that Hérémakhono chimpanzees are more reliant upon fallback foods like flowers, grasses, or cultivated plants to compensate for potential dietary shortcomings relative to their southern counterparts. Such a strategy may explain why we observed clear differences in Hérémakhono chimpanzee Δ^{13} C values, but not in Δ^{15} N values. Yet, although laboratory experiments on bonobos (Deschner et al., 2012) and other taxa (McCue & Pollock, 2008) have demonstrated ¹⁵N enrichment due to nutritional stress, it may be possible that energetic deficits in situ are not easily identifiable using isotopic data. In this vein, Vogel et al. (2011) failed to find a $\delta^{15}N$ effect of nutritional scarcity in wild orangutans, even when other markers of energetic deficit were observed. Therefore, we offer this conclusion with caution, and acknowledge the need of further investigation into potential ecological differences and their potential physiological consequences among these sites.

Nonetheless, inter-site differences in average $\delta^{13}C$ and $\delta^{15}N$ values were smaller than intra-site variation among hair segments, and total observed $\delta^{13}C$ and $\delta^{15}N$ intra-site variation across all five communities are comparable to the range of variation in other chimpanzee communities across Africa (Oelze et al., 2016). It is notable, however, that δ^{13} C and δ^{15} N hair ranges for these sites varied considerably and extended across the range of observed variation for Pan sites (e.g., Oelze et al., 2016), suggesting the isotopic composition of chimpanzee diets in these areas varies substantially within the yearfurther conforming to behavioral observation that the Fongoli diet flexibly responds to availability of various food items, and relies more heavily upon fallback food items when necessary. Such relatively tight grouping in Δ^{13} C and Δ^{15} N site averages may therefore suggest that chimpanzees at all sites have developed similar dietary adaptations for coping with reduced food availability when encountered, and that this strategy extends across the range of food scarcity encountered in our study area, albeit to varying degrees.

It is worth noting that the variability in site ranges suggests that the extent of intra-annual dietary variation could also vary from site to site, although such inference should remain cautious as isotopic ranges per site appeared to increase with increasing sample size. Further investigations into intra-annual variation in isotopic values and small-scale ecological variation will illuminate the flexibility afforded to individuals living in relatively food-scarce environments (Wessling, Deschner, et al., 2018).

Lastly, this research underlines the necessity of accounting for potential baseline differences in isotope ecology of a species when engaging in inter-site comparisons. Multiple authors (e.g., Carlson & Crowley, 2016; Oelze et al., 2016) have previously proposed this approach, although conclusions continue to be drawn from wild ape isotope analyses without incorporation of environmental baselines (e.g., Loudon et al., 2016; Schoeninger et al., 2016). Our research highlights that such baselines are necessary not only across varied habitats, but also within habitats which might appear superficially similar. In absence of environmental baselines, our understanding and interpretation of isotopic variation in our data would have led to inaccurate conclusions. For example, the lack of plant baseline data in our research would have potentially led us to conclude that enrichment of δ^{15} N levels in Hérémakhono chimpanzees potentially stems from greater periods of starvation due to food scarcity at the extreme edge of chimpanzee distribution relative to their southern counterparts. Given that Hérémakhono chimpanzees occur at low densities (Wessling, unpublished data) and are likely inhabiting the extreme edges of tolerable chimpanzee habitat, such a conclusion from our data may not have been implausible.

We argue further that accounting for environmental baselines in inter-site comparison will likewise remain ineffective if potential sources of isotopic variation within unbalanced plant baseline datasets are ignored. Typically, baseline values are calculated on a site-by-site basis as the average of all plant samples analyzed at each site, and usually sample sets are intentionally limited to certain components of the diet (e.g., ripe fruit: Loudon et al., 2016; Oelze et al., 2016). However, by expanding the dataset to encompass the wider range of the chimpanzee diet, while accounting for the effects of plant species, plant part, and season across the entire comparative dataset, we were able to distinguish patterns of differentiation in environmental baselines across our dataset which likewise would have been overlooked had we not benefited from the explanatory power of mixed models. Again, plant baseline averages appeared to be nearly identical in δ^{13} C values across sites when averages were created site-by-site: instead controlling for plant species and plant part in the entire dataset helped distinguish inter-site differences otherwise masked by biases in sampling across sites. In consideration of these methodological effects, these results therefore underline that future primate isotopic research should be as comprehensive in scope as feasibly possible and unwaveringly careful in interpretation. The implications of our methods likewise extend beyond nonhuman primates to humans and other taxa, and highlight that caution is needed in interpretation of all comparative isotopic datasets when environmental baselines cannot be established.

5 | CONCLUSION

Our results show that Hérémakhono, likely the northern-most chimpanzee community on the continent and at the physical edge of the West African chimpanzee range, had distinctly higher δ^{13} C and δ^{15} N values relative to their counterparts further south in Senegal. However, baseline plant δ^{15} N values also were significantly higher than those from the four southern sites, thereby suggesting nitrogen fractionation was similar for chimpanzees across all five Senegalese sites. These results support two main conclusions. First, that Hérémakhono chimpanzees are likely addressing potential dietary constraints using ¹³C enriched food items at the range edge as they have a measurably δ^{13} C enriched diet compared to the four southern sites. In doing so, it appears that they are able to avoid potentially higher risks of starvation relative to their southern counterparts to below a measureable threshold using dietary flexibility. Our second conclusion is methodological; due to the numerous and varied ways in which local environments can impact an individual's isotopic profile, accounting for baseline environmental profile while concurrently accounting for sources of bias within each baseline is compulsory when comparing geographically distinct groups. This remains pertinent even when absolute distances between comparative groups may be small (in this case <100 km), or when habitats would appear to be superficially similar. By coupling environmental baseline data with our interpretation of hair isotopic variation, we demonstrate dietary similarities across five chimpanzee communities living at the limits of their biogeographical range.

ACKNOWLEDGMENTS

We thank the Republic of Senegal and the Département des Eaux et Forêts, et de Chasses for their permission to conduct this work. For their assistance in coordination of the Pan African Programme we thank Mimi Arandjelovic, Paula Dieguez, and Claudia Herf. For assistance in the field we thank Jacques Tamba Keita, Kaly Bindia, Madi Keita, Dondo Kante, and Michel Sadiakho. For their assistance with analyses we thank Michael Richards, Annabelle Reiner, Ulrike Wacker, Sven Steinbrenner, and the staff at IsoDetect GmbH, as well as Klervia Jaouen for her invaluable support in the process. We thank Colleen Stephens for her assistance with figure development. Sincere thanks to Liran Samuni, Christophe Boesch, Roger Mundry, and two anonymous reviewers for their helpful comments on earlier versions of this manuscript. This research was funded by the Max Planck Society, the National Geographic Society, Iowa State University, the Max Planck Society Innovation Fund, and the Krekeler Foundation.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Wessling EG, Oelze VM, Eshuis H, Pruetz JD, Kühl HS. Stable isotope variation in savanna chimpanzees (*Pan troglodytes verus*) indicate avoidance of energetic challenges through dietary compensation at the limits of the range. *Am J Phys Anthropol.* 2019;168:665–675. <u>https://doi.</u> org/10.1002/ajpa.23782