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Vicky Oelze, Alice Percher, Gontran Nsi Akoué, Nory El Ksabi, Eric Willaume, et al.. Seasonality and interindividual variation in mandrill feeding ecology revealed by stable isotope analyses of hair and blood. American Journal of Primatology, 2020, 10.1002/ajp.23206 . hal-02998461

HAL Id: hal-02998461

<https://hal.science/hal-02998461>

Submitted on 14 Nov 2020

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Journal:	<i>American Journal of Primatology</i>
Manuscript ID	AJP-20-0069.R2
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Oelze, Vicky Percher, Alice NSI-Akoue, Gontran; University of Sciences and Technology of Masuku, Department of Biology; University of Sciences and Technology of Masuku, Department of Biolohy El Ksabi, Nory Willlaume, Eric; SODEPAL, Charpentier, Marie; ISEM UMR 5554
Indicate which taxonomic group was the subject of your study (select all that apply or type another option)::	Old World monkeys
Keywords:	carbon, nitrogen, sex differences, season

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**Seasonality and inter-individual variation in mandrill feeding ecology
revealed by stable isotope analyses of hair and blood**

Vicky M Oelze^{1,2}, Alice M Percher³, Gontran Nsi Akoué⁴, Nory El Ksabi⁵, Eric
Willaume⁵, Marie JE Charpentier^{3*}

¹ Anthropology Department, University of California Santa Cruz, Santa Cruz, USA
² Department of Primatology, Max Planck Institute for Evolutionary Anthropology,
Leipzig, Germany
³ Institut des Sciences de l'Évolution de Montpellier UMR5554, CNRS, IRD, EPHE,
Université de Montpellier, Montpellier, France
⁴ Université des Sciences et Techniques de Masuku, Franceville, Gabon
⁵ SODEPAL, Bakoumba, Gabon

Running head:
Stable isotope analyses in free ranging mandrills

***Corresponding author:**
Marie JE Charpentier
ISEM UMR5554
Place Eugène Bataillon cc065
34095 Montpellier cedex 05, France
Email: marie.charpentier@umontpellier.fr

Abstract

Mandrills are large bodied, terrestrial forest primates living in particularly large social groups of several hundred individuals. Following these groups in the wild to assess differences in diet over time as well as among individuals is demanding. We here use isotope analyses in blood and hair obtained during repeated captures of 43 identified free-ranging mandrills (*Mandrillus sphinx*) from Southern Gabon, to test how dietary variation relates to season as well as an individual's age and sex. We measured the stable carbon ($\delta^{13}\text{C}\%$) and nitrogen ($\delta^{15}\text{N}\%$) isotope ratios in 46 blood and 214 hair section samples as well as from a small selection of mandrill foods ($n=24$). We found some seasonal isotopic effects, with lower $\delta^{13}\text{C}$ values but higher $\delta^{15}\text{N}$ values observed during the highly competitive long dry season compared to the fruit-rich long rainy season. Variation in $\delta^{13}\text{C}$ was further predicted by individual age, with higher $\delta^{13}\text{C}$ values generally found in younger individuals suggesting that they may consume more high canopy fruit than older individuals, or that older individuals consume more low canopy foliage. The best predictor for $\delta^{15}\text{N}$ values was the interaction between age and sex, with mature and reproductively active males revealing the highest $\delta^{15}\text{N}$ values, despite the observation that males consume substantially less animal food items than females. We interpret high $\delta^{15}\text{N}$ values in these mature male mandrill blood and hair sections to be the result of nutritional stress associated to intense male-male competition, particularly during mating season. This is the first study showing isotopic evidence for nutritional stress in a free-ranging primate species and may spark further investigations into male mandrill diet and energy balance.

Keywords: carbon, nitrogen, sex differences, season

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Research Highlights

- Stable isotope analyses of hair and blood have the potential to provide short and long-term data on how individual diets may shift over the season and with individual traits.
- Dietary patterns differ across seasons, between sexes as well as between young and older mandrills.
- Mature and reproductively active male mandrills showed the highest nitrogen isotope values as a result of nutritional stress possibly associated to intense male-male competition in this species.
- This is the first study showing isotopic evidence for nutritional stress in a free-ranging primate species.

62 Introduction

63 Feeding ecology studies are essential to determine an individuals' nutritional and
64 energetic requirements and understand how they translate into different biological and
65 ecological characteristics of a species, such as ranging patterns and socio-sexual
66 behavior (e.g. Chapman and Chapman, 1990, Hohmann et al., 2006, Lambert and
67 Rothman, 2015; McGraw and Daegling, 2012). As with many other animal species,
68 primate sociality highly depends on how food is distributed in the environment. The
69 degree at which individuals use clumped and high energy food resources such as fruits
70 vs. much more dispersed lower energy foods such as foliage gives insights into the
71 evolution of sociality across primate species (Clutton-Brock and Janson, 2012; van
72 Schaik, 1989; Sterck et al., 1997).

73 Compared to other terrestrial large bodied primates in Africa, such as great apes and
74 baboons, mandrills (*Mandrillus sphinx*) are clearly understudied in the wild. Our
75 knowledge about mandrill feeding seasonality and competition is limited to a handful of
76 studies conducted in the 1980s (Harrison, 1988; Hoshino et al., 1984; Lahm, 1986;
77 Rogers et al., 1996) as well as much more recent work at a research site in Southern
78 Gabon, the Lékédi Park (Nsi Akoué et al., 2017). Our current understanding is that
79 mandrills are generalist feeders with a clear tendency towards frugivory (Lahm, 1986;
80 Nsi Akoué et al., 2017). During a 17-month behavioral survey, 57 mandrills from the
81 Lékédi population were observed consuming about 150 different plant species
82 (including more than 440 different items), representing 74% of their diet, along with
83 mushrooms (<1%), invertebrates (6%) and vertebrates (<1%) (Nsi Akoué et al., 2017).
84 Behavioral data additionally suggest that females consumed more faunal food items
85 than males (Nsi Akoué et al., 2017). Among the plant species frequently consumed,

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mandrills relied essentially on C₃ plants (e.g. *Xylopia aethiopica* and *Pentaclethra*
macrophylla), including bamboo, a C₃ plant within the grass family *Poaceae* (e.g. *Olyra*
latifolia, see Nsi Akoué et al., 2017).

While long-term and direct observations of feeding behavior generally constitute the
ideal method for determining dietary differences between individuals and across
seasons, this requires full habituation and long-term systematic study. In mandrills,
direct observations are partially impeded by the fairly large group sizes of several
hundred individuals (Abernethy et al., 2002; Harrison, 1988; Rogers et al., 1996). The
use of indirect methods to study primate diets are thus a tempting and cost-effective
alternative to some, clearly not all, aspects of direct observation. Fecal analysis provides
a good overview of what food items are eaten by a primate species over short to long
term periods and can be conducted even in unhabituated groups (e.g. Tutin and
Fernandez, 1993). However, while fecal analysis is strictly non-invasive and reveals
some aspects of what was ingested by the animals the previous day(s), the dietary
assessment is potentially biased towards indigestible food items such as fruit seeds,
tough foliage and insect chitin (Lahm, 1986) and the method requires repeated sampling
over large stretches of time. As an alternative approach, the stable isotopes of carbon
($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) are increasingly used to reconstruct diet and ecological
niches of wild animal species with less sampling effort (Crowley, 2012; Crowley et al.,
2015). For example, patterns of seasonal dietary overlap and dietary differentiation
found in isotope values of hair collected non-invasively from nests of central
chimpanzees (*Pan troglodytes troglodytes*) and lowland gorillas (*Gorilla gorilla gorilla*)
suggested plasticity in the feeding niches of these sympatric apes (Oelze et al., 2014),

consistent with previous results based on long-term and labor intensive fecal sample collection and analysis (Head et al., 2011).

Stable isotope analysis in samples of body tissue provides estimates of several aspects of the animals' diet in the recent or more distant past (Crowley, 2012; Crowley et al., 2015; Sandberg et al., 2012). Feces and blood are for example turned over by the body fairly rapidly and are related to the diet of the previous days or weeks (Hobson and Clark, 1992). By contrast, hair keratin has a much slower turnover rate of ~1cm per month (Tobin, 2005; Fourie et al., 2016), although hair growth rates are yet unknown for most wild primates necessitating to fall back on human hair growth rates as a proxy (Oelze, 2016). In hair, sampling along the growth trajectory from hair root to tip allows for a isotopic time sequence of several consecutive months (O'Connell and Hedges, 1999; Schwertl et al., 2003; Oelze, 2016). The use of sequential hair isotope analysis has shown to be particularly useful to investigate dietary changes over time in response to shifts in climate and food availability as well as dietary differences between individuals within the same social group (Oelze, 2016; Oelze et al., 2011, 2014, 2016a).

Some aspects of diet may be reconstructed using stable isotopes because the ratios of the stable isotopes of carbon ($^{13}\text{C}/^{12}\text{C} = \delta^{13}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N} = \delta^{15}\text{N}$) in animal bodies are sourced from the predominant foods, which can vary drastically and thus be relevant dietary markers (DeNiro and Epstein, 1978, 1981). On the one hand, the $\delta^{13}\text{C}$ values of plants differ substantially between C_3 and C_4 photosynthetic pathways (Tieszen, 1991), but also between high canopy and low canopy plant resources in forest habitats (Medina and Minchin, 1980; van der Merwe and Medina, 1989), and even between photosynthetic (e.g. foliage) and reproductive plant parts (e.g. fruits and flowers; Cernusak et al., 2009). In C_3 plant dominated tropical rainforest habitats, we

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133 can expect the majority of variation in $\delta^{13}\text{C}$ in herbivore consumers to be associated
134 with differences in the consumption of high versus low canopy food items (e.g. arboreal
135 fruit versus terrestrial herbs), as well as between reproductive and photosynthetic plant
136 parts (e.g. fruit versus leaves; Blumenthal et al., 2016; Oelze et al., 2016a; Roberts et
137 al., 2017). On the other hand, the $\delta^{15}\text{N}$ values in foods mainly relate to the trophic level
138 of a food item, with plants revealing the lowest $\delta^{15}\text{N}$ values (DeNiro and Epstein, 1981).
139 Primary consumers have significantly higher $\delta^{15}\text{N}$ values than the plants they eat,
140 whereas carnivores reveal higher $\delta^{15}\text{N}$ values than herbivores (DeNiro and Epstein,
141 1981).
142 While differences between some food categories can be striking, there are several
143 environmental factors that influence the isotopic variation within plants in a given
144 habitat, including temperature (Barnes et al., 2007), rainfall (Nardoto et al., 2008; Sealy
145 et al., 1987), altitude (Tieszen et al., 1979), soil composition (Dawson et al., 2002), as
146 well as canopy density (Medina and Minchin, 1980). One strategy to cope with these
147 environmental influences is to assess the local isotope baseline using a small yet
148 representative plant sample, ideally consisting of plant foods frequently consumed by
149 the study subjects (Oelze et al., 2011, 2016b). There is no widespread consensus on how
150 extensive this plant sample should be to represent the habitat, and some studies still do
151 not include any plant samples at all (Loudon et al., 2014; Schoeninger et al., 2015;
152 Sponheimer et al., 2006). However, while these baseline values are crucial when
153 comparing between primate groups ranging in different habitats with different
154 environmental conditions (Oelze et al., 2016b), they may be less essential to studies
155 focusing on intra-community differences as well as isotopic variation over time.

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4 156 Isotopic patterns can vary considerably between individuals and reveal information on
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10 159 in their $\delta^{15}\text{N}$ values, e.g. vertebrate meat (Fahy et al., 2013; Oelze et al., 2011, 2016a).
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12 160 Also the energetic demands of female reproduction (gestation, lactation) can affect her
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14 161 bodies nitrogen balance and thus skew the $\delta^{15}\text{N}$ values (Fuller et al., 2004; Oelze et al.,
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16 162 2016a). Further, there is good evidence that negative energy balance due to severe
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18 163 nutritional stress or fasting can result in shifts in nitrogen metabolism and thus $\delta^{15}\text{N}$
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20 164 values in primates (Deschner et al., 2012; Mekota et al., 2006) and other animals
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28 166 In this study, we evaluate to what extent stable isotope analysis can provide novel
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32 168 the Lékédi Park and surroundings, in Southern Gabon. In particular, we focus on the
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34 169 effects of shifting environmental conditions (season) and individual characteristics (age,
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36 170 sex) on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values measured in mandrill body tissue samples. We here
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38 171 use two different sample tissues with two very different turnover rates and thus
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40 172 reflecting different periods in time. Blood isotope data is considered here to reflect the
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42 173 recent dietary contributions of the past day(s) with relatively high accuracy, whereas
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44 174 hair section isotope data relates to the isotopic characteristics of foods consumed over
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46 175 the previous months, with data representing weekly or monthly intervals. We assess to
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48 176 what extent these two different sample types pick up the same isotopic patterns.

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51 177 Given the previous isotopic research in tropical forest of Gabon (Oelze et al., 2014), as
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53 178 well as previous observational data on this primate population (Nsi Akoue et al., 2017),
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55 179 we focus on the following predictions. Regarding the season of sampling, we expect
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isotopic values to vary between rainy and dry seasons, as fruit availability is commonly shifting with rainfall. Shifting proportions of foliage vs. fruit should particularly have an effect on the $\delta^{13}\text{C}$ values of mandrills, with high fruit intake periods being associated with high $\delta^{13}\text{C}$ values (Blumenthal et al., 2016; Cernusak et al., 2009; Oelze et al., 2016a; Roberts et al., 2017). Regarding individual age, we predict that the intense intra-sexual competition observed in fully adult males (Setchell et al., 2005), as compared to younger, non-reproductive males, may result in nutritional stress particularly during the mating season (long dry season) with higher $\delta^{15}\text{N}$ values in mature males. Finally, regarding individual sex, we expect to find slightly higher $\delta^{15}\text{N}$ values in all females as compared to all males as females have been shown to consume more animal food items than males (Nsi Akoué et al., 2017), which can be expected to have higher $\delta^{15}\text{N}$ values than plant foods.

Materials and Methods

Ethical statement

This study complies with ethical protocols approved by the CENAREST institution (authorization number: AR003/20/MESRSTT/CENAREST/CG/CST/CSAR). This research adhered to the legal requirements of Gabon and to the American Society of Primatologists principles for the ethical treatment of nonhuman primates.

Site and study population

The study group originates from 65 captive individuals released into the park on two occasions in 2002 and 2006 (Peignot et al., 2008). Food supplementation with bananas and home-made cakes three to five times a week was initiated with the release, then

gradually declined for a few years and totally ceased in April 2012 except during punctual trappings. The study population is monitored daily within the framework of a long-term field project (Mandrillus Project) started in early 2012. In 2018, the group was composed of ca. 180-200 habituated and individually identified mandrills, of which ~ 90% were born in the wild. We collected tissue samples from a total of 43 individuals (24 males, 19 females) aged 2.2-19.3 yrs. Data on individual age in this study are based on observed birth dates (n=12) or are based on general body conditions as well as tooth eruption and wear patterns (Galbany et al., 2014).

The study population ranges freely in the Lékédi Park and its surroundings near the village of Bakoumba. This private park covers an area of 116km² at an elevation varying from 430 to 610m. The habitat is composed of closed canopy forests and patches of savanna (Brockmeyer et al., 2015). Gabon is characterized by an equatorial climate with a long rainy season (Feb-May; in the studied site: monthly average temperature=23.9°C and total rainfall=240.5 mm; data pooled from 04/2012-12/2014), a long dry season (Jun-Sep; 22.1°C and 18.9 mm), a short rainy season (Oct-Nov; 23.3°C and 152.3 mm) and a short dry season (Dec-Jan; 23.5°C and 133.3 mm). These four climatic seasons have been shown to result in distinct diets in the studied mandrills, with no overlap in food selection between these seasons (see Figure 1 in: Nsi Akoué et al., 2017). In addition, reproduction is highly seasonal in mandrills, with most females cycling during the long dry season and giving birth during the short dry season or at the beginning of the long rainy season (MJEC, pers. obs). We therefore considered these four climatic seasons as a reliable indicator for the seasonality of mandrill behavior and ecology.

Sample collection

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227 We collected hair tufts and blood samples from mandrills during four capture events
228 between April 2012 and July 2014. During these events, mandrills were baited with
229 food (mostly bananas) and anesthetized via blowpipe intramuscular injection of a
230 ketamine-xylazine mix. After 30-40 min, individuals were awakened using atipamezole.
231 During anesthesia, we plucked hair from the mandrills' arms or legs and stored them in
232 paper envelopes that were then stored dry. We collected blood samples from the iliac
233 vein of the individuals using heparinized tubes. Blood samples were centrifuged (15
234 min at 3000 rpm) the day of collection to obtain blood samples that were stored at -
235 20°C (Beaulieu et al., 2017).

236 In 2014, we collected 24 food samples consumed by the studied mandrills, including 14
237 plant species, three mushroom and three insect species (Table S1). These items were
238 selected randomly among the hundreds of food items known to be consumed by the
239 studied population to gain a broad impression on the plant baseline values in the habitat,
240 without an expectation of grasping the entire picture of plant isotope diversity. All
241 selected plant species are frequently consumed by the studied mandrills (>0.10
242 occurrence per hour, as defined per: Nsi Akoué et al., 2017).

243 We prepared 214 hair sections from 62 hair samples. We selected 3 to 12 hairs of
244 similar length, diameter and growth stage from each hair tuft and followed a sequential
245 sectioning protocol (see for details: Oelze, 2016). Each hair sample provided up to 12
246 different sections of 1cm (3.5 on average), which we sometimes combined to reach a
247 weight suitable for a single isotopic measurement. We aliquoted 200 µL of 46 blood
248 samples and freeze-dried these samples for 24h before analyses. Plants were dried the
249 day of collection and homogenized thereafter. Insects and mushrooms were frozen at -

20°C the day of collection, freeze-dried during 24h and then homogenized using a pebble mill before analyses.

Hair sections (0.33 to 0.74 mg), blood (0.88 to 1.14 mg) and food samples (0.44 to 2.25mg) were weighed into tin capsules for isotopic measurements performed parallel to the IAEA standards CH6, CH7, N1 and N2 in a FLASH HT Plus coupled to a MAT 253 IRMS (both by Thermo Scientific, Waltham, MA, USA) at the commercial laboratory IsoDetect GmbH in Leipzig, Germany. Stable isotope ratios are expressed in permil (‰) using the delta (δ) notation and are calibrated against the international standard materials Vienna PeeDee Belemite (vPDB) for carbon and atmospheric N₂ (AIR) for nitrogen. Analytical error calculated from repetitive measurements of international and lab-internal standard materials in each run is less than 0.2‰ (2 σ) for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Stable isotope ratios for hair sections, blood samples and food items are hereafter referred to as $\delta^{13}\text{C}_{\text{hair}}$, $\delta^{13}\text{C}_{\text{blood}}$, and $\delta^{13}\text{C}_{\text{diet}}$ as well as $\delta^{15}\text{N}_{\text{hair}}$, $\delta^{15}\text{N}_{\text{blood}}$, and $\delta^{15}\text{N}_{\text{diet}}$. We removed one blood sample from the dataset because of its abnormally high value of C:N ratio (5.10 against an average value=3.56±0.08).

Estimation of diet-tissue fractionation factors

Despite the small sample set we have for mandrill plant food items, we calculated isotopic fractionation factors for both isotope systems ($\Delta^{15}\text{N}_{\text{hair-diet}}$ and $\Delta^{13}\text{C}_{\text{hair-diet}}$) between broad categories of plant foods (reproductive parts vs. non-reproductive parts; see: Table S1) and individual hair sections to obtain a broad impression of which plant food category is relevant to mandrill diet and is reflected in hair isotope values. In addition, this approach controls for baseline differences and allows to compare between populations and sites with differing environmental conditions (Crowley et al., 2013; Nakagawa et al., 2007; Oelze et al., 2016b).

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274 *Statistical analyses*

275 We conducted all statistical analyses in SAS Studio. To test for relationships between

276 isotopic values obtained from hair and blood samples representing the same individual

277 and the same time window, we used Pearson correlation tests. We used Linear Mixed

278 Models (LMM, proc GLIMMIX) and Linear Models (LM, proc GLM) with Gaussian

279 distributions to study the effects of seasonality and individual traits on both $\delta^{13}\text{C}$ and

280 $\delta^{15}\text{N}$ recorded in hair sections (LMM) and blood samples (LM). The normality of the

281 models' residuals was systematically verified by visual inspection of the residuals'

282 distribution in qq-plots and by conducting Kolmogorov-Smirnov tests. Note that in one

283 model ($\delta^{15}\text{N}_{\text{hair}}$), we removed six outlier samples to attain a Gaussian distribution of the

284 data (leverage test, proc robustreg). We used differences in least square means as post-

285 hoc analyses for pairwise comparisons of categorical variables when appropriate. In all

286 our statistical models performed, we considered all first order interactions and kept the

287 full models as final models excluding only non-significant interactions ($p>0.05$).

288 We ran four independent models on the response variables $\delta^{13}\text{C}_{\text{hair}}$, $\delta^{15}\text{N}_{\text{hair}}$, $\delta^{13}\text{C}_{\text{blood}}$

289 and $\delta^{15}\text{N}_{\text{blood}}$ to explore the effects of season, individual age and sex. We added the

290 factor hair sample as a random effect in the two models on hair isotope values to control

291 for pseudo-replication biases caused by multiple sampling of the hair tufts (Mundry and

292 Oelze, 2015). We did not consider individual as a random effect in the analyses based

293 on blood samples because only few animals out of our dataset were sampled more than

294 once ($n=10$).

295 We were unable to estimate mandrill hair growth rates, so we relied on human scalp hair

296 growth rates as a rough estimate (see discussion in: Oelze, 2016). Hence, for each hair

297 section analyzed, we calculated the corresponding time window based on a growth rate

of 1 cm of hair per 30 days (Tobin, 2005), with the root date identical to the hair collection date and the distal date corresponding to 30 days before.

In all four models we considered sex as a class variable and age as a continuous variable (calculated at the distal date for hair and at the date of collection for blood). We considered the effect of season as a class variable with two (blood) to three (hair) modalities including a long rainy season, a long dry season, and the two short seasons that we pooled together for the models based on hair because of limited sample sizes (7 samples only were collected during the small rainy season). In the models based on blood, we considered the season at the exact date of sampling while in the models based on hair, we considered the season at the distal date.

Food supplementation that occurred just before the beginning of sampling (early 2012) may have impacted isotope values in the distal sections of long mandrills' hair (the ones spanning the supplementation period that ceased in April 2012). Although supplementation only constituted a limited proportion of mandrills' diet, we conducted preliminary analyses by removing these potentially biased hair sections. We found similar results to those obtained from full datasets and therefore chose to include these samples in the final models.

Results

We obtained a total of 46 isotopic measurements for blood and 214 measurements in hair sections. The $\delta^{13}\text{C}_{\text{hair}}$ values range from -26.1‰ to -24.0‰ (mean $\pm 1\sigma$: $-25.1 \pm 0.3\text{‰}$), slightly higher than $\delta^{13}\text{C}_{\text{blood}}$ values which vary between -27.7‰ and -25.8‰ ($-26.6 \pm 0.4\text{‰}$). The $\delta^{15}\text{N}_{\text{hair}}$ values range from 4.21‰ to 7.8‰ ($6.0 \pm 0.5\text{‰}$) while

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321 $\delta^{15}\text{N}_{\text{blood}}$ values vary less and range between 5.6‰ and 7.5‰ ($6.4\pm0.3\text{‰}$). Isotopic
322 measurements retrieved from hair sections and blood samples collected from the same
323 individual on the same day ($n=37$) are significantly correlated (Figure S1). Despite the
324 small plant food dataset from the studied habitat, we can report that non-reproductive
325 plant parts ($-32.2 \pm 3.0\text{‰}$) yielded on average $\sim 2\text{‰}$ lower $\delta^{13}\text{C}$ values than reproductive
326 plant parts ($-30.3 \pm 2.6\text{‰}$). In addition, $\delta^{15}\text{N}$ values were on average higher in non-
327 reproductive plant parts ($6.0 \pm 3.8\text{‰}$) than in reproductive plant parts ($3.7\pm1.1\text{‰}$). The
328 few arthropods we analyzed (ants, butterfly, caterpillar) did not have higher $\delta^{15}\text{N}$ values
329 than plant foods (Table S1). Mean fractionation factors between mandrill hair and
330 reproductive plant parts, comprised by fruits, seeds and some flowers, resulted in mean
331 $\Delta^{15}\text{N}_{\text{hair-fruit}}$ values of 2.3‰ and mean $\Delta^{13}\text{C}_{\text{hair-fruit}}$ values of 5.2‰. The calculated mean
332 fractionation values between mandrill hair and non-reproductive plant parts (leaves)
333 were 0.0‰ for $\Delta^{15}\text{N}_{\text{hair-leaves}}$ and 7.1‰ for $\Delta^{13}\text{C}_{\text{hair-leaves}}$.

334

335 *Effect of season*

336 In the $\delta^{13}\text{C}_{\text{blood}}$ model, isotopic values were significantly higher during the rainy season
337 (see Table 1 for detailed statistical results; Figure 1A). In the $\delta^{13}\text{C}_{\text{hair}}$ model, however,
338 the factor season was interacting with the predictors sex and age (Table 1). In females,
339 the lowest $\delta^{13}\text{C}$ values of hair were found during the long rainy season, while we
340 observed exactly the reverse in males (Figure 2). Although the interaction between age
341 and season was not obvious, younger animals, especially males, tended to show higher
342 $\delta^{13}\text{C}$ values of hair (Figure 2). The $\delta^{15}\text{N}$ values of blood were significantly higher
343 during the long dry season compared to the long rainy season (Table 1; Figure 1B). By
344 contrast, the effect of seasonality was not captured by the $\delta^{15}\text{N}$ values of hair (Table 1).

345

346 *Effects of individual age and sex*

347 Individual age had a significant effect on $\delta^{13}\text{C}$ values of blood which decreased with
348 mandrill's age in male subjects (Table 1; Figure 3). Finally, we found significant
349 interactions between individual age and sex on $\delta^{15}\text{N}$ values of blood and hair: older
350 males showed higher values than younger males and this age effect was not observed in
351 females (Table 1; Figure 4A and B).

352

353 **Discussion**

354 We here report the first isotopic study conducted on free ranging mandrills in which we
355 investigated effects of season and individual traits such as age and sex on feeding
356 behavior. The limited plant isotope data set allowed a glimpse into the natural isotopic
357 variation of food items in the mandrill habitat to understand how to interpret findings of
358 lower or higher isotope values across seasons and individuals. Similar to isotopic values
359 reported in several forests of Central and West African countries (Oelze et al., 2014;
360 Oelze et al., 2016b), the low $\delta^{13}\text{C}$ values of the 14 plant species collected in the Lékédi
361 Park (mean: -31.3‰) indicate that mandrills' habitat is similarly depleted in ^{13}C as other
362 forest habitats in Gabon, such as Loango with a mean plant $\delta^{13}\text{C}$ value of -30.5‰
363 (Oelze et al., 2014), although additional plant isotope data would be desirable to
364 confirm this finding.

365 The $\delta^{13}\text{C}$ values of mandrills' hair sections ($-25.1 \pm 0.3\text{‰}$) are within the range of those
366 obtained from hair of sympatric great apes also living in Gabon, namely central
367 chimpanzees (from -25.7‰ to -23.4‰) and lowland gorillas (from -27.1‰ to -24.5‰)

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368 from Loango National Park (Oelze et al., 2014). Similar to these species, mandrills live
369 in dense rainforest habitats strongly affected by the so called canopy effect (van der
370 Merwe and Medina, 1989). As expected, mandrills yielded much lower $\delta^{13}\text{C}$ values than
371 those found in South African chacma baboons (ranging from -19.8‰ to -18.5‰) as
372 these baboons live in an open semiarid environment and ~20-30% of their diet is
373 comprised by C_4 plants (Codron et al., 2006). Mandrills showed on average higher
374 $\delta^{15}\text{N}_{\text{hair}}$ values ($6.0 \pm 0.4\text{‰}$) than chimpanzees and gorillas from Gabon (5.0‰ and 4.2‰,
375 resp.), which is well explained by the slightly lower mean $\delta^{15}\text{N}$ value in plants from
376 Loango (4.2 at Lékédi vs. 3.3‰ at Loango; Oelze et al., 2014). These similarities with
377 other species in both isotope systems would suggest that the dietary pattern observed in
378 mandrills is comparable to the dietary niche of terrestrial great apes in other parts of
379 Gabon, particularly to the higher level of frugivory as seen in chimpanzees. While our
380 food item isotope dataset was too small to conduct dietary mixing models, at least the
381 fractionation factors we calculated between different plant categories and mandrills
382 seem to support this finding, as the relationship between mandrill hair and the different
383 plant foods found a better match between reproductive plant parts (fruits, seeds and
384 flowers) than it did with foliage. $\Delta^{13}\text{C}_{\text{hair-fruit}}$ values between ~3 and 5‰ and $\Delta^{15}\text{N}_{\text{hair-fruit}}$
385 values between ~2 and 4‰ are reported for several populations of frugivorous great
386 apes in different regions of Africa (Oelze et al., 2016b). This very general observation is
387 well in line with direct observations of mandrill foraging behavior which suggests that
388 their diet consists of 70 to 90% of fruit (Nsi Akoue et al., 2017). For non-reproductive
389 plant parts (foliage) the fractionation values were either too low (0.0‰ $\Delta^{15}\text{N}_{\text{hair-leaves}}$) or
390 too high (7.1‰ $\Delta^{13}\text{C}_{\text{hair-leaves}}$) to be considered reflecting foliage as a substantial dietary
391 contribution. However, this mismatch could also be related to a data bias introduced by

using a very small and possibly not representative sample of leaves, that likely does not encompass the full isotopic variation within non-reproductive plant parts in the mandrills' diet. Particularly leaves can vary substantially in their $\delta^{13}\text{C}$ values depending on whether they are sampled on the forest floor, understory or high up in the canopy (Medina and Minchin, 1980; van der Merwe and Medina, 1991; Carlson and Kingston, 2014; Oelze et al., 2014, 2016a; Carlson and Crowley, 2016; Roberts et al., 2017), which may make comparisons of hair-leave values less reliable than calculation referring to reproductive plant parts alone (Oelze et al., 2016b).

We found some isotopic differences across seasons. We expected to find isotopic variation in response to shifts in fruit availability between the dry and rainy seasons. In Gabon, the rainy season generally corresponds to higher fruit availability (White, 1994; but see: Head et al., 2011) and high fruit intake periods should be associated with higher $\delta^{13}\text{C}$ values (Cernusak et al., 2009; Oelze et al., 2014, 2016b). As such, we found higher $\delta^{13}\text{C}$ values of blood across all studied mandrills and also higher $\delta^{13}\text{C}$ values of hair in males during the long rainy season compared to the long dry season. It remains unclear if this seasonal pattern in $\delta^{13}\text{C}$ values could be related to general seasonal changes within the $\delta^{13}\text{C}$ values of plant food categories. Previous research on primate forest plant foods did not suggest that seasonal variation is considerable (Carlson and Kingston, 2014) and we thus did not systematically collect samples from the same food plants across different seasons in this study.,

The $\delta^{13}\text{C}$ values of hair in females did not follow this seasonal pattern in $\delta^{13}\text{C}$ value, possibly reflecting some sex-related differences in physiology or seasonal feeding preferences. During the long dry season, most females are, indeed, cycling or are in early pregnancy (see Figure 1 in: Dibakou et al., 2019), and the specific nutritional

demands of maternal investment have been shown to affect the feeding behavior and $\delta^{13}\text{C}$ values of other large bodied primates (Oelze et al. 2016). The long dry season also caused higher $\delta^{15}\text{N}$ values in blood of the studied mandrills, although this season does not correspond to the highest animal's consumption in the studied population (Nsi Akoué et al., 2017). This apparent contradictory finding probably results from highly shifted $\delta^{15}\text{N}$ values in adult male mandrills (see below).

Variation in $\delta^{13}\text{C}$ values of blood was mainly predicted by age but not sex, whereas $\delta^{15}\text{N}$ variation was driven by the interaction between individual age and sex. In blood and, to a lesser extent, in male hair section samples, the $\delta^{13}\text{C}$ values were significantly higher in young individuals compared to old mandrills. Given that variation in plant $\delta^{13}\text{C}$ values is primarily driven by canopy height (lower values in understory than in canopy) and plant part (lower values in foliage than in fruit) in rainforest communities, we can interpret higher $\delta^{13}\text{C}$ values in young mandrills to be the result of consuming significantly less low canopy plant foods and/or foliage, but more fruit from higher in the canopy. Significant diet differences across age classes have been observed during the long dry season in the studied mandrills. At this time of the year, interestingly, the highest frequencies of fruit consumption was observed in juvenile males and adolescent females (see Supplementary Information in: Nsi Akoué et al., 2017). Although, we cannot comment further on this possible relationship, it seems puzzling as one may predict that older, more experienced mandrills should be able to find ripe fruit better than younger individuals. Alternatively, instead of high canopy fruit, the infrequent consumption of C_4 grasses may explain the higher $\delta^{13}\text{C}$ values in young individuals. However, we have little to no evidence that C_4 grasses possibly available in the open savanna patches are of any relevance to the mandrills feeding ecology. Finally, young

mandrills were found, among others, to consume significantly more *Ficus mucuso* but less *Aframomum alboviolaceum* than adults in the study population (Nsi Akoué et al., 2017). While fruits of the former should result in high $\delta^{13}\text{C}$ values, because they are generally found high in the canopy, the latter should result in low $\delta^{13}\text{C}$ values because it is a terrestrial herbaceous species. These observations are consistent with our current findings.

We anticipated to additionally find another effect of age on the dietary behavior of mandrills as particularly older and reproductively active males may undergo periods of nutritional stress during and around the mating season. An endocrinological study on this mandrill population suggested high fecal glucocorticoid values in old males during the mating season (long dry season) due to the influx of new competing males and intense and aggressive male-male competition in this species (Charpentier et al., 2018). This may suggest that older males undergo negative energy balance for several months a year. Our models and the $\delta^{15}\text{N}$ values obtained from both blood and hair sections appear to support this assumption, with increasing $\delta^{15}\text{N}$ values with age in males. We assume that higher $\delta^{15}\text{N}$ values in older males result from nutritional stress in response to the intense male-male competition during the mating season. Mature male mandrills fiercely fight over access to female and dedicate less time to feeding, resulting in higher stress levels and negative energy balance (Charpentier et al., 2018). Adult males indeed spend less time foraging compared to all other individuals (Nsi Akoué et al., 2017). The $\delta^{15}\text{N}$ values are known to increase in response to severe negative energy balance. When basic metabolic requirements in nitrogen (protein) are not met through diet, it has to be acquired from body own proteins through catabolism. Higher mobilization and excretion of body own nitrogen then contributes to a reduction in body mass (Deschner

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et al., 2012). The reduction of net energy intake has to be substantial in order to lead to reduction in body mass, and the condition has to be experienced for a prolonged period of time in order to affect the animals physiology, nitrogen balance and in consequence the bodies $\delta^{15}\text{N}$ values (Barboza and Parker, 2006; Hobson et al., 1993). Changes in $\delta^{15}\text{N}$ values due to nutritional stress have rarely been shown in non-human and human primates (Deschner et al., 2012; D’Ortenzio et al., 2015; Mekota et al., 2006, 2009). This is the first study reporting isotopic evidence for nutritional stress in a free-ranging primate supported by observational data, suggesting that nutritional stress experienced by reproductive male mandrills is traceable using isotope analysis and worth future more detailed investigation.

Finally, we anticipated to find slightly higher $\delta^{15}\text{N}$ values in all females as compared to all males. As the first direct observations of this population of mandrills suggested that females consumed more animal food items than males (Nsi Akoue et al., 2017). However, we found no support for this third prediction. We did not find higher $\delta^{15}\text{N}$ values in the three arthropods collected at the Lékédi Park than what we found in a variety of plant foods (Table S1). Further, females did not show higher average $\delta^{15}\text{N}$ values than males across age classes. In fact, we found the opposite: females generally showed slightly lower $\delta^{15}\text{N}$ values than males, which we think is mainly driven by the higher $\delta^{15}\text{N}$ levels in productively active and thus nutritionally stressed male mandrills.

This is the first isotopic investigation conducted in a large population of free-ranging mandrills. Our results partly confirm observational data, but also highlighted some potential future avenues for behavioral research, particularly regarding dietary differences between age classes as well as nutritional stress in reproductively active males.

Acknowledgements

We thank past and present field assistants of the Mandrillus Project for their help in data collection. We are grateful to Ilham Bentaleb for useful discussions regarding stable isotopes. Alice Baniel also helped with data analyses. Laboratory analyses were performed at the *Plateforme d'Analyses Chimiques en Ecologie* (PACE; Montpellier, France), the Max Planck Institute for Evolutionary Anthropology (Leipzig, Germany) and in the commercial laboratory IsoDetect GmbH (Leipzig, Germany). This study was funded by a grant from the Deutsche Forschungsgemeinschaft grant (DFG, KA-1082-20-1), and from INEE-CNRS (“Station d’Etudes en Ecologie Globale” and a “Laboratoire International Associé”) to MJEC. This is a Mandrillus Project publication number 23 and a ISEM 2020-171 SUD.

References

- Abernethy, K.A., White, L.J.T., and Wickings, E.J. (2002). Hordes of mandrills (*Mandrillus sphinx*): Extreme group size and seasonal male presence. *Journal of Zoology*, 258, 131–137.
- Barboza, P.S., & Parker, K.L. (2006). Body protein stores and isotopic indicators of N balance in female reindeer (*Rangifer tarandus*) during winter. *Physiological and Biochemical Zoology*, 79, 628–644.
- Barnes, C., Sweeting, C.J., Jennings, S., Barry, J.T., & Polunin, N.V.C. (2007). Effect of temperature and ration size on carbon and nitrogen stable isotope trophic fractionation. *Functional Ecology*, 21, 356–362.
- Beaulieu, M., Benoit, L., Abaga, S., Kappeler, P.M., & Charpentier, M.J.E. (2017). Mind the cell: Seasonal variation in telomere length mirrors changes in leucocyte profile. *Molecular Ecology*, 26, 5603–5613.
- Blumenthal, S.A., Rothman, J.M., Chritz, K.L., & Cerling, T.E. (2016). Stable isotopic variation in tropical forest plants for applications in primatology. *American Journal of Primatology*, 78, 1041–1054.
- Brockmeyer, T., Kappeler, P.M., Willaume, E., Benoit, L., Mboumba, S., & Charpentier, M.J.E. (2015). Social organization and space use of a wild mandrill (*Mandrillus sphinx*) group. *American Journal of Primatology*, 77, 1036–1048.
- Carlson, B.A., & Crowley, B.E. (2016). Variation in carbon isotope values among chimpanzee foods at Ngogo, Kibale National Park and Bwindi Impenetrable National Park, Uganda. *American Journal of Primatology*, 78, 1031–1040.
- Carlson, B.A., & Kingston, J.D. (2014). Chimpanzee isotopic ecology: A closed canopy C3 template for hominin dietary reconstruction. *Journal of Human Evolution*, 76, 107–115.
- Cernusak, L.A., Tcherkez, G., Keitel, C., Cornwell, W.K., Santiago, L.S., Knohl, A., ... Wright, I.J. (2009). Why are non-photosynthetic tissues generally ¹³C enriched compared with leaves in C3 plants? Review and synthesis of current hypotheses. *Functional Plant Biology*, 36, 199–213.
- Clutton-Brock, T., & Janson, C. (2012). Primate socioecology at the crossroads: Past, present, and future. *Evolutionary Anthropology Issues News and Reviews*, 21, 136–150.
- Codron, D., Lee-Thorp, J.A., Sponheimer, M., de Ruiter, D., & Codron, J. (2006). Inter- and intrahabitat dietary variability of chacma baboons (*Papio ursinus*) in South African savannas based on fecal $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, & %N. *American Journal of Physical Anthropology*, 129, 204–214.

- Codron, D., Lee-Thorp, J.A., Sponheimer, M., de Ruiter, D., & Codron, J. (2008). What insights can baboon feeding ecology provide for early hominin niche differentiation? *International Journal of Primatology*, 29, 757–772.
- Crowley, B.E. (2012). Stable isotope techniques and applications for primatologists. *International Journal of Primatology*, 33, 673–701.
- Crowley, B.E., Blanco, M.B., Arrigo-Nelson, S.J., & Irwin, M.T. (2013). Stable isotopes document resource partitioning and effects of forest disturbance on sympatric cheirogaleid lemurs. *Naturwissenschaften*, 100, 943–956.
- Crowley, B.E., Reitsema, L.J., Oelze, V.M., & Sponheimer, M. (2015). Advances in primate stable isotope ecology—Achievements and future prospects. *American Journal of Primatology*, 78, 995–1003.
- Dawson, T.E., Mambelli, S., Plamboeck, A.H., Templer, P.H., & Tu, K.P. (2002). Stable isotopes in plant ecology. *Annual Review of Ecology, Evolution, and Systematics*, 33, 507–559.
- DeNiro, M.J., & Epstein, S. (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochimica and Cosmochimica Acta*, 42, 495–506.
- DeNiro, M.J., & Epstein, S. (1981). Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica and Cosmochimica Acta*, 45, 341–351.
- Deschner, T., Fuller, B.T., Oelze, V.M., Boesch, C., Hublin, J.-J., Mundry, R., ... Hohmann, G. (2012). Identification of energy consumption and nutritional stress by isotopic and elemental analysis of urine in bonobos (*Pan paniscus*). *Rapid Communication in Mass Spectrometry*, 26, 69–77.
- D’Ortenzio, L., Brickley, M., Schwarcz, H., & Prowse, T. (2015). You are not what you eat during physiological stress: Isotopic evaluation of human hair. *American Journal of Physical Anthropology*, 157, 374–388.
- Fahy, G.E., Richards, M., Riedel, J., Hublin, J.-J., & Boesch, C. (2013). Stable isotope evidence of meat eating and hunting specialization in adult male chimpanzees. *Proceedings of the National Academy of Sciences*, 110, 5829–5833.
- Fourie, N.H., Brown, J.L., Jolly, C.J., Phillips-Conroy, J.E., Rogers, J., & Bernstein, R.M. (2016). Sources of variation in hair cortisol in wild and captive non-human primates. *Zoology*, 119, 119–125.
- Fuller, B.T., Fuller, J.L., Sage, N.E., Harris, D.A., O’Connell, T.C., & Hedges, R.E.M. (2004). Nitrogen balance and ^{15}N : Why you’re not what you eat during pregnancy. *Rapid Communication in Mass Spectrometry*, 18, 2889–2896.
- Galbany, J., Romero, A., Mayo-Alesón, M., Itsoma, F., Gamarra, B., Pérez-Pérez, A., ... Charpentier, M.J.E. (2014). Age-related tooth wear differs between forest and savanna primates. *PLoS ONE*, 9(4), e94938.

- 572 Harrison, M.J.S. (1988). The mandrill in Gabon's rain forest - Ecology, distribution and
573 status. *Oryx*, 22, 218–228.
- 574 Head, J.S., Boesch, C., Makaga, L., & Robbins, M.M. (2011). Sympatric chimpanzees
575 (*Pan troglodytes troglodytes*) and gorillas (*Gorilla gorilla gorilla*) in Loango National
576 Park, Gabon: Dietary composition, seasonality, & intersite comparisons. *International*
577 *Journal of Primatology*, 32, 755–775.
- 578 Hobson, K.A., & Clark, R.G. (1992). Assessing avian diets using stable isotopes .1.
579 Turnover of C-13 in Tissues. *Condor*, 94, 181–188.
- 580 Hobson, K.A., Alisauskas, R.T., & Clark, R.G. (1993). Stable nitrogen isotope
581 enrichment in avian tissues due to fasting and nutritional stress - Implications for
582 isotopic analyses of diet. *Condor*, 95, 388–394.
- 583 Hoshino, J., Mori, A., Kudo, H., & Kawai, M. (1984). Preliminary report on the
584 grouping of mandrills (*Mandrillus sphinx*) in Cameroon. *Primates*, 25, 295–307.
- 585 Lahm, S.A. (1986). Diet and habitat preference of *Mandrillus sphinx* in Gabon:
586 Implications of foraging strategy. *American Journal of Primatology*, 11, 9–26.
- 587 Lambert, J.E., & Rothman, J.M. (2015). Fallback foods, optimal diets, & nutritional
588 targets: Primate responses to varying food availability and quality. *Annual Review of*
589 *Anthropology*, 44, 493–512.
- 590 Loudon, J.E., Grobler, P., Sponheimer, M., Moyer, K., Lorenz, J.G., & Turner, T.R.
591 (2014). Using the stable carbon and nitrogen isotope compositions of vervet monkeys
592 (*Chlorocebus pygerythrus*) to examine questions in ethnoprimateology. *PLoS One*, 9,
593 e100758.
- 594 McGraw, W.S., & Daegling, D.J. (2012). Primate feeding and foraging: Integrating
595 studies of behavior and morphology. *Annual Review of Anthropology*, 41, 203–219.
- 596 Medina, E., & Minchin, P. (1980). Stratification of $\delta^{13}\text{C}$ values of leaves in Amazonian
597 rain forests. *Oecologia*, 45, 377–378.
- 598 Mekota, A.M., Grupe, G., Ufer, S., & Cuntz, U. (2006). Serial analysis of stable
599 nitrogen and carbon isotopes in hair: Monitoring starvation and recovery phases of
600 patients suffering from anorexia nervosa. *Rapid Communication in Mass Spectrometry*,
601 20, 1604–1610.
- 602 Mekota, A.M., Grupe, G., Ufer, S., & Cuntz, U. (2009). Identifying starvation episodes
603 using stable isotopes in hair Forensic approach on serial hair analysis. *Rechtsmedizin*,
604 19, 431–438.
- 605 van der Merwe, N.J., & Medina, E. (1989). Photosynthesis and $^{13}\text{C}/^{12}\text{C}$ ratios in
606 Amazonian rain forests. *Geochimica and Cosmochimica Acta*, 53, 1091–1094.
- 607 van der Merwe, N.J., & Medina, E. (1991). The canopy effect, carbon isotope ratios and
608 foodwebs in Amazonia. *Journal of Archaeological Science*, 18, 249–259.

- Mundry, R., & Oelze, V.M. (2015). Who is who matters - The effects of pseudoreplication in stable isotope analyses. *American Journal of Primatology*, 78, 1017-1030.
- Nakagawa, M., Hyodo, F., & Nakashizuka, T. (2007). Effect of forest use on trophic levels of small mammals: An analysis using stable isotopes. *Canadian Journal of Zoology*, 85, 472-478.
- Nardoto, G.B., Ometto, J.P.H.B., Ehleringer, J.R., Higuchi, N., Bustamante, M.M.D.C., & Martinelli, L.A. (2008). Understanding the influences of spatial patterns on N availability within the Brazilian Amazon forest. *Ecosystems*, 11, 1234-1246.
- Nsi Akoue, G., Mbading-Mbading, W., Willaume, E., Souza, A., Mbatchi, B., & Charpentier, M.J.E. (2017). Seasonal and individual predictors of diet in a free-ranging population of mandrills. *Ethology*, 123, 600-613.
- O'Connell, T.C., & Hedges, R.E. (1999). Investigations into the effect of diet on modern human hair isotopic values. *American Journal of Physical Anthropology*, 108, 409-425.
- Oelze, V.M. (2016). Reconstructing temporal variation in great ape and other primate diets: A methodological framework for isotope analyses in hair. *American Journal of Primatology*, 78, 1004-1016.
- Oelze, V.M., Fuller, B.T., Richards, M.P., Fruth, B., Surbeck, M., Hublin, J.-J., & Hohmann, G. (2011). Exploring the contribution and significance of animal protein in the diet of bonobos by stable isotope ratio analysis of hair. *Proceedings of the National Academy of Sciences*, 108, 9792-9797.
- Oelze, V.M., Head, J.S., Robbins, M.M., Richards, M., & Boesch, C. (2014). Niche differentiation and dietary seasonality among sympatric gorillas and chimpanzees in Loango National Park (Gabon) revealed by stable isotope analysis. *Journal of Human Evolution*, 66, 95-106.
- Oelze, V.M., Douglas, P.H., Stephens, C.R., Surbeck, M., Behringer, V., Richards, M.P., ... Hohmann, G. (2016a). The steady state great ape? Long term isotopic records reveal the effects of season, social rank and reproductive status on bonobo feeding behavior. *PLoS ONE*, 11, 1-17.
- Oelze, V.M., Fahy, G., Hohmann, G., Robbins, M.M., Leinert, V., Lee, K., ... Kühl, H.S. (2016b). Comparative isotope ecology of African great apes. *Journal of Human Evolution*, 101, 1-16.
- Peignot, P., Charpentier, M.J.E., Bout, N., Bourry, O., Massima, U., Dosimont, O., ... Wickings, E.J. (2008). Learning from the first release project of captive-bred mandrills *Mandrillus sphinx* in Gabon. *Oryx*, 42, 122-131.
- Roberts, P., Blumenthal, S.A., Dittus, W., Wedage, O., & Lee-Thorp, J.A. (2017). Stable carbon, oxygen, and nitrogen, isotope analysis of plants from a South Asian

- 647 tropical forest: Implications for primatology. *American Journal of Primatology*, 79,
648 e22656.
- 649 Rogers, M.E., Abernethy, K.A., Fontaine, B., Wickings, E.J., White, L.J.T., & Tutin,
650 C.E.G. (1996). Ten days in the life of a mandrill horde in the Lopé Reserve, Gabon.
651 *American Journal of Primatology*, 40, 297–313.
- 652 Sandberg, P.A., Loudon, J.E., & Sponheimer, M. (2012). Stable Isotope Analysis in
653 Primatology: A Critical Review. *American Journal of Primatology*, 74, 969–989.
- 654 van Schaik, C.P. (1989). The ecology of social relationships amongst female primates.
655 In V. Standen & Foley R.A. (Eds.), *Comparative socioecology: The behavioural*
656 *ecology of humans and other mammals* (pp. 195–218). Oxford, UK: Blackwell.
- 657 Schoeninger, M.J., Most, C.A., Moore, J.J., & Somerville, A.D. (2015). Environmental
658 variables across *Pan troglodytes* study sites correspond with the carbon, but not the
659 nitrogen, stable isotope ratios of chimpanzee hair. *American Journal of Primatology*,
660 78, 1055–1069.
- 661 Schwertl, M., Auerswald, K., & Schnyder, H. (2003). Reconstruction of the isotopic
662 history of animal diets by hair segmental analysis. *Rapid Communication in Mass*
663 *Spectrometry*, 17, 1312–1318.
- 664 Sealy, J.C., van der Merwe, N.J., Thorp, J.A.L., & Lanham, J.L. (1987). Nitrogen
665 isotopic ecology in southern Africa: Implications for environmental and dietary tracing.
666 *Geochimica and Cosmochimica Acta*, 51, 2707–2717.
- 667 Sponheimer, M., Loudon, J.E., Codron, D., Howells, M.E., Pruett, J.D., Codron, J., ...
668 Lee-Thorp, J.A. (2006). Do “savanna” chimpanzees consume C-4 resources? *Journal of*
669 *Human Evolution*, 51, 128–133.
- 670 Sterck, E.H., Watts, D.P., & Van Schaik, C.P. (1997). The evolution of female social
671 relationships in nonhuman primates. *Behavioral Ecology and Sociobiology*, 41, 291–
672 309.
- 673 Tieszen, L.L. (1991). Natural variations in the carbon isotope values of plants:
674 implications for archaeology, ecology and paleoecology. *Journal of Archaeological*
675 *Science*, 18, 227–248.
- 676 Tieszen, L.L., Senyimba, M.M., Imbamba, S.K., & Troughton, J.H. (1979). The
677 distribution of C3 and C4 grasses and carbon isotope discrimination along an altitudinal
678 and moisture gradient in Kenya. *Oecologia*, 37, 337–350.
- 679 Tobin, D.J. (2005). The biogenesis and growth of hair. In D.J. Tobin (Ed.), *Hair in*
680 *Toxicology. An Important Bio-Monitor* (pp. 3–33). Cambridge, UK: The Royal Society
681 of Chemistry.
- 682 Tutin, C.E.G., & Fernandez, M. (1993). Composition of the diet of chimpanzees and
683 comparisons with that of sympatric lowland gorillas in the Lopé reserve, Gabon.
684 *American Journal of Primatology*, 30, 195–211.

- 1
2
3
4 685 White, C.D., & Schwarcz, H.P. (1994). Temporal trends in stable isotopes for Nubian
5 686 mummy tissues. *American Journal of Physical Anthropology*, 93, 165–187.
6
7
8 687 White, L.J.T. (1994). Patterns of fruit-fall phenology in the Lopé Reserve, Gabon.
9 688 *Journal of Tropical Ecology*, 10, 313-322.
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Table 1: Results of the four Mixed Models on the responses of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in hair sections and blood samples, with the fixed effects of the season, individual age and sex.

Response	Sample sizes	Fixed effects	Statistics	p-value
$\delta^{13}\text{C}_{\text{hair}}$	214 hair sections	Season	F=3.82	0.024
	62 hair samples	Age	F=13.22	<0.001
	37 individuals	Sex	F=5.00	0.027
		Sex*Season	F=3.33	0.039
		Age*Season	F=3.06	0.050
$\delta^{13}\text{C}_{\text{blood}}$	46 blood samples	Season	F=21.52	<0.001
	36 individuals	Age	F=8.09	0.007
		Sex	F=0.73	0.400
$\delta^{15}\text{N}_{\text{hair}}$	208 hair sections	Season	F=0.93	0.400
	61 hair samples	Age	F=2.23	0.140
	37 individuals	Sex	F=4.34	0.004
		Sex*Age	F=6.51	0.012
$\delta^{15}\text{N}_{\text{blood}}$	46 blood samples	Season	F=33.55	<0.001
	36 individuals	Age	F=7	<0.001
		Sex	F=8.46	0.006
		Sex*Age	F=24.58	<0.001

691

692 **Figure captions**

693

694 **Figure 1:** Whisker boxplots with median, upper/lower quartiles and min/max ranges
695 illustrating the effect of seasonal differences in mandrill isotope values between the long
696 dry and the long rainy seasons revealed by the $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B) values of mandrill
697 blood.

698

699 **Figure 2:** Whisker boxplots with median, upper/lower quartiles and min/max ranges
700 illustrating the effect of seasonal differences in mandrill $\delta^{13}\text{C}$ values of hair by sex (A:
701 males, B: females) and age class (young <10 years, old > 10 years). Two age classes
702 were defined here for the sake of clarity, although age was considered as a continuous
703 predictor in our statistical models.

704

705 **Figure 3:** Scatter plot showing the relationship between individual age and $\delta^{13}\text{C}$ values
706 of blood.

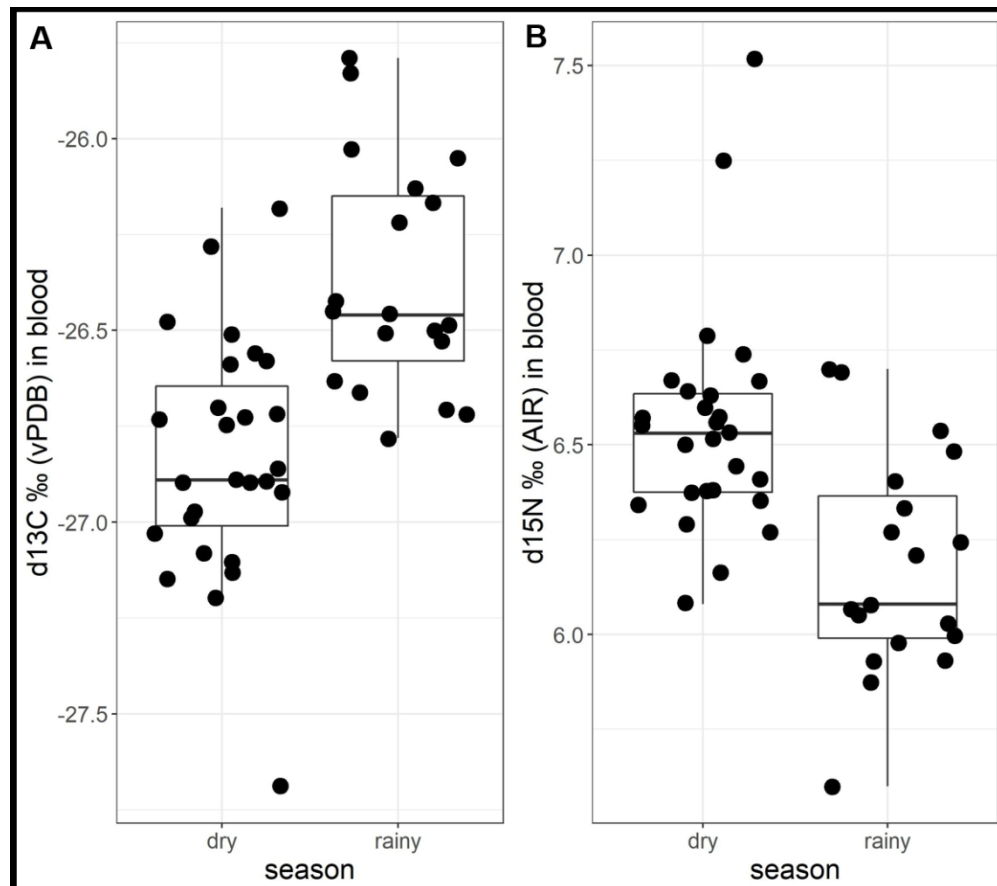
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708 **Figure 4:** Scatter plots showing the relationships between individual age in years and
709 $\delta^{15}\text{N}$ values in mandrill hair (grey symbols) and blood (black symbols) separate for
710 males (A) and females (B).

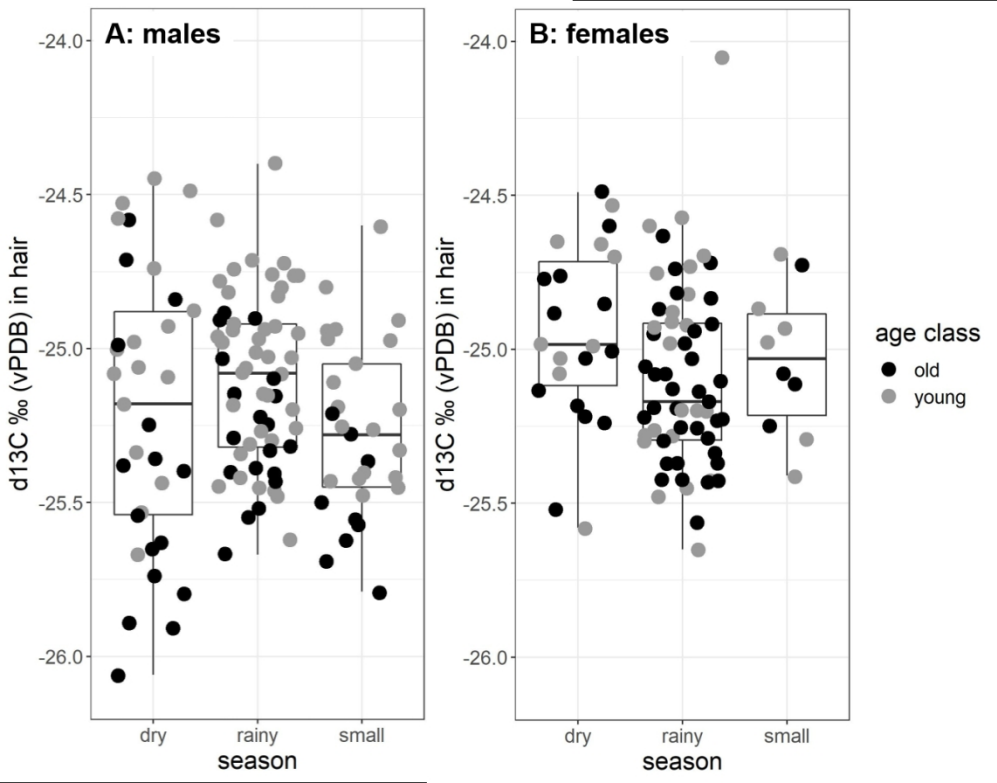
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Research Highlights

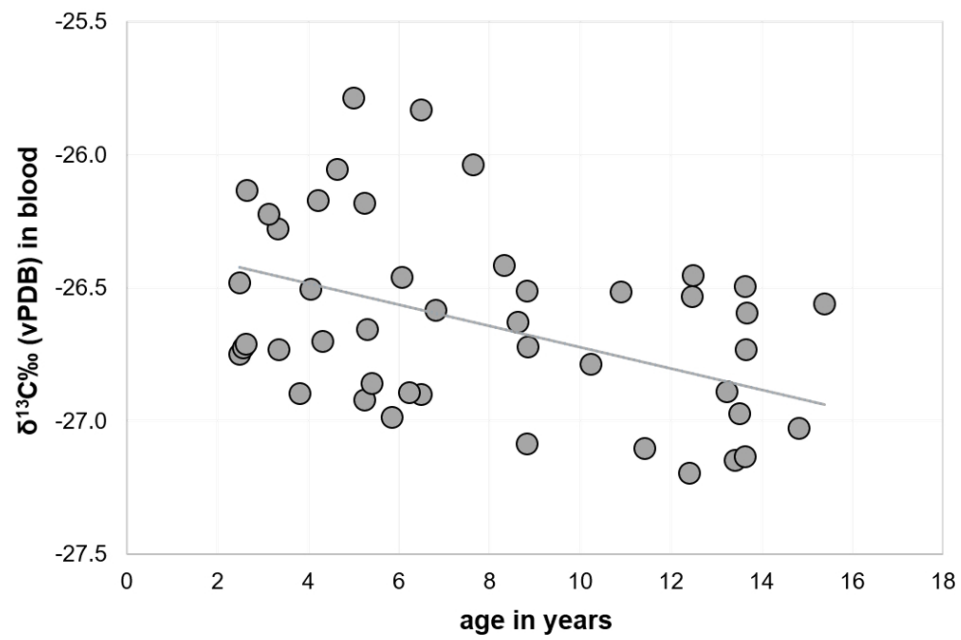
- Stable isotope analyses of hair and blood have the potential to provide short and long-term data on how individual diets may shift over the season and with individual traits.
- Dietary patterns differ across seasons, between sexes as well as between young and older mandrills.
- Mature and reproductively active male mandrills showed the highest nitrogen isotope values as a result of nutritional stress possibly associated to intense male-male competition in this species.
- This is the first study showing isotopic evidence for nutritional stress in a free-ranging primate species.



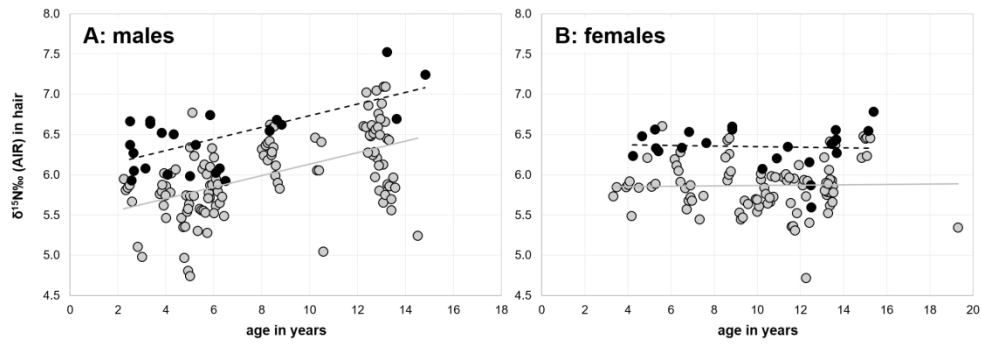
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Supplementary information:

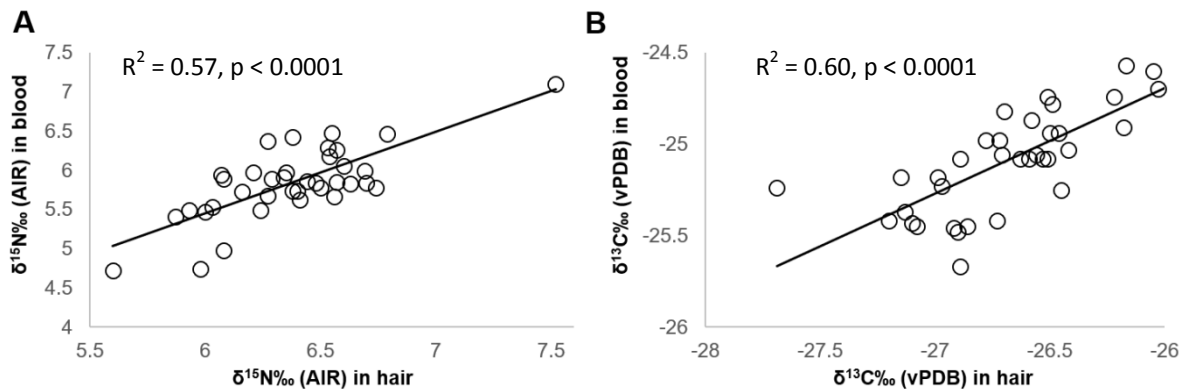


Figure S1: The $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B) values measured in hair sections and blood samples, each obtained from the same individuals calculated to be representing overlapping time windows (n=37 samples). Blood and hair isotope values are significantly correlated.

Table S1: Stable isotopic values of a small selection of mandrill food items from Lékédi park. Insects and mushrooms were not determined to species level.

Species	Food category	Plant item	$\delta^{15}\text{N}\text{‰}$ AIR	$\delta^{13}\text{C}\text{‰}$ VPDB
Ants (unidentified)	fauna	-	5.3	-25.2
Butterfly (unidentified)	fauna	-	1.5	-27.9
Caterpillar (unidentified)	fauna	-	6.7	-26.4
Mushroom 1 (unidentified)	na	-	9.0	-29.1
Mushroom 2 (unidentified)	na	-	3.0	-32.1
Mushroom 3 (unidentified)	na	-	6.8	-24.0
<i>Megaphrynium macrostachum</i>	na	leaves/fruits	4.4	-31.5
<i>Smilax anceps</i>	na	stems/seeds	2.9	-29.2
<i>Aframomum cf polyanthum</i>	na	unknown	2.3	-32.8
<i>Croton sylvaticus</i>	na	unknown	5.1	-29.7
<i>Geophila afzelii</i>	na	unknown	1.5	-34.7
<i>Renealmia macrocolea</i>	na	unknown	3.6	-33.1
<i>Uapaca guineensis</i>	na	unknown	4.0	-29.0
<i>Dialium dinklagei</i>	non-reproductive part	leaves	6.4	-32.1
<i>Lasianthus batangensis</i>	non-reproductive part	leaves	3.4	-35.0
<i>Megaphrynium macrostachum</i>	non-reproductive part	leaves	11.6	-29.9
<i>Nymphaea maculata</i>	non-reproductive part	leaves	6.6	-28.7
<i>Psychotria stenostegia</i>	non-reproductive part	stems/seeds	1.8	-35.4
<i>Cnestis corniculata</i>	reproductive part	flowers	5.4	-31.6
<i>Psychotria gillettii</i>	reproductive part	flowers	3.8	-28.9
<i>Psychotria stenostegia</i>	reproductive part	fruits	3.0	-34.0
<i>Cnestis corniculata</i>	reproductive part	seeds	2.9	-31.9
<i>Hylodendron gabunense</i>	reproductive part	seeds	4.4	-26.7
<i>Psychotria gillettii</i>	reproductive part	seeds	2.5	-28.9

^{14}N 14.00307 99.63% Stable	^{15}N 15.0001 0.37% Stable
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Photo-credit: Paul Amblard-Rambert