Detecting intraannual dietary variability in wild mountain gorillas by stable isotope analysis of feces

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We use stable isotope ratios in feces of wild mountain gorillas (Gorilla beringei) to test the hypothesis that diet shifts within a single year, as measured by dry mass intake, can be recovered. Isotopic separation of staple foods indicates that intraannual changes in the isotopic composition of feces reflect shifts in diet. Fruits are isotopically distinct compared with other staple foods, and peaks in fecal δ13C values are interpreted as periods of increased fruit feeding. Bayesian mixing model results demonstrate that, although the timing of these diet shifts match observational data, the modeled increase in proportional fruit feeding does not capture the full shift. Variation in the isotopic and nutritional composition of gorilla foods is largely independent, highlighting the difficulty for estimating nutritional intake with stable isotopes. Our results demonstrate the potential value of fecal sampling for quantifying short-term, intradividual dietary variability in primates and other animals with high temporal resolution even when the diet is composed of C3 plants.

C3 photosynthesis | feeding ecology | great apes | Uganda

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Traditional approaches to reconstructing diet in living animals, such as field observation and microhistological examination of gut and fecal contents, can be difficult to apply to many known individuals over long periods of time (1, 2). Stable isotope analysis is a powerful tool for rapidly and noninvasively investigating animal ecology across various spatiotemporal scales. The stable carbon isotope composition of mammalian tissues and excreta can be used to empirically reconstruct diet, although other ecological factors such as habitat and geography may also be important (3–4). If locally available food sources are isotopically distinct, mathematical mixing models can be used to estimate the proportional contribution of multiple foods in isotopic mixtures such as animal tissues or feces (5). This technique represents an independent supplement to observation-based methods and is particularly advantageous when studying animals that are difficult to observe or feeding on items that are difficult to quantify.

African great apes are the closest extant relatives of modern humans and extinct human relatives and therefore have been used as referential models for reconstructing hominin adaptations (6). Abundant observational data demonstrate that extant African apes consume foods that vary dramatically throughout the year (7–9). It has been suggested based on intratooth isotopic variability that some early hominins also had seasonally variable diets (10). Interpreting these values requires comparisons with extant African great apes, yet whereas the isotope ecology of chimpanzees and bonobos (genus Pan) has received some attention (11–13), gorillas (genus Gorilla) are not well studied (14).

Isotope studies of diet generally rely on the divergent photosynthetic pathways of C3 trees, shrubs, and temperate grasses compared with C4 tropical grasses and sedges and the associated bimodal distribution of stable isotope ratios of carbon (δ13C) among plants in tropical regions (15, 16). However, this distinction is not useful if the dietary contribution of C4 vegetation is minimal, as documented for chimpanzees and bonobos (11–13, 17). Isotopic variability among C3 plants results from variation in canopy position and microhabitat, with lower canopy and forest floor foliage exhibiting more negative δ13C values compared with upper canopy and canopy gap leaves because of low irradiance and photosynthetic use of soil respired CO2 in the forest understory (18–23). Nonleaf C3 plant matter such as fruit sometimes exhibit more positive δ13C values than associated foliage (22–24). The stable nitrogen (δ15N) isotope ratios of vegetation integrates terrestrial nitrogen cycling and varies with temperature, precipitation, salinity, nutrient cycling, and resource availability (25). δ15N values in animal tissue integrate dietary input, trophic level, climate, and physiology (26, 27).

The time scale at which isotopes are incorporated into animal tissue depends on the timing of tissue synthesis. Bulk samples of bone, tooth enamel, and hair provide a broad measure of diet integrated over the period of formation, generally months to years (28, 29). Most previous stable isotope analyses of African great ape tissues have followed this approach (11, 12, 17, 30). Diet change within a single year can be estimated by serial microsampling tissues that form incrementally, have relatively rapid isotope turnover rates, and are resistant to isotopic exchange after formation, such as enamel or hair (31, 32). Intratooth enamel isotope measurements represent a highly time-averaged signal due to the prolonged period of mineralization during amelogenesis (32, 33). Although hair is not subject to this effect, extended formation times and a lag in equilibration of the body amino acid pool with synthesizing tissues result in signal attenuation in both tissue types (33–35). Little carbon isotopic variation throughout the year was found in an interindividual comparison of chimpanzee hair collected in different seasons (0.4‰) and intradividual serial samples of bonobo hair (1.4‰) from Senegal and the Democratic Republic of Congo (DRC), respectively (12, 13). This stability may reflect a lack of variability in the supply of protein, which is preferentially incorporated in hair keratin relative to other macronutrients (36). Greater intraannual variability in δ13C values is evident in serially sectioned hair from taxa that feed to some degree on C3 foods, such as generalist chama baboons (Papio ursinus) from South Africa (~3‰), as well as primarily browsing elephants (Loxodonta africana) from Kenya (~6‰) and forest hogs (Hylochoerus meinertzhageni) from Uganda (~3‰) (37–39).

Stable isotopes in feces, which are composed of undigested food matter, gut microbes, digestive secretions, and sloughed epithelial tissues, have been demonstrated in experimental and wild settings to reflect ingested diet in mammalian herbivores and primates (4, 40–43). Digestive processes along the mammalian gastrointestinal


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tract do not seem to consistently alter the stable isotopic composition of feces relative to diet (44, 45). Digestibility did not impact the isotopic enrichment between feces and diet in Mongolian C3/C4 mixed-feeding domestic goats (46), but δ13C values in domestic sheep feces overrepresented less digestible foods in a controlled feeding experiment (47). The relevance of this to understanding 13C-incorporation rates in feces in natural settings, when differences in digestibility are not artificially enhanced, is currently unclear. Fecal sampling provides an isotope record of diet change limited only by gut retention time, which in gorillas, as in most other primates and mammals, is a few days (48–51), and can resolve subtle dietary shifts marked by signal attenuation in other tissues (40, 52–54). Isotopic equilibration of all sources contributing to feces may take several months, however, resulting in time-averaging of short-term diet shifts (47). Monthly fecal collections of a variety of South African ungulates reveals a greater range of fecal δ13C values annually among mixed-feeders (~6‰) compared with dietary specialists (~2‰) such as browsers and grazers (54). Stable isotope analysis of monthly fecal samples from South African chacma baboons reveals a range in δ13C values similar to mixed-feeding ungulates (39), as expected given the highly flexible diets of baboons (55, 56), suggesting that stable isotope analysis of feces may be able to resolve fine-scale diet shifts in individual animals.

We present dry mass fruit intake and stable isotope data of feces from four individually recognizable wild mountain gorillas and plant foods in Bwindi Impenetrable National Park, Uganda. Observational data demonstrate that mountain gorillas (Gorilla beringei) feed primarily on herbaceous leaves, but prefer fruit when it is seasonally available, with additional tree leaves, wood, and peel also contributing to the staple diet (7, 57, 58). The feeding behavior of this group has been extensively studied, with consistent fruit-feeding peaks in February–March and June–July (7, 57–59). We use carbon isotopes in gorilla feces to estimate changes in fruit feeding over a 10-mo period with a Bayesian multiple source isotope mixing model (SIAR) (60). A Bayesian approach can be used to estimate diet composition in underdetermined systems (i.e., more diet sources than isotopes) and can directly account for uncertainty and variation in the isotopic composition and elemental concentrations of sources and consumer tissues, as well as trophic enrichment factors (60). We use dry mass fruit intake to evaluate the accuracy of mixing model results. We also examine the relationships between the isotopic and nutritional composition of staple foods (58). These data generally highlight the utility of stable isotope analysis of mammal feces for estimating variation in staple food dietary input, particularly changes in frugivory, and inform our understanding of the stable isotope ecology of hominoid primates in particular.

Results
Mean dry mass fruit consumption varied bimonthly by all age-sex classes (mixed model, F = 36.63, P < 0.0001). Fruit feeding was greater in February–March and June–July than in October–November, December–January, and April–May (multiple comparisons of least squares means, P < 0.0001; Table S1). We measured the stable isotope composition of staple foods (n = 73) collected during the period of behavioral observation from 10 species representing 99.1% of total dry mass intake (Fig. 1; Table S2). Staple diet categories included herbaceous leaves (50.9%), fruit (19.3%), tree leaves (12.0%), wood (11.7%), and peel (5.2%). We found statistically significant differences among staple food δ13C values (Kruskal-Wallis ANOVA, χ2 = 43.8856, df = 4, P < 0.0001). Fruit δ13C values were more positive than herbaceous leaves and tree leaves, and tree leaf δ13C values were more negative than herbaceous leaves and wood (Kruskal-Wallis multiple comparison post hoc test, P < 0.05). We also found statistically significant differences among staple food δ15N values (Kruskal-Wallis ANOVA, χ2 = 32.1268 df = 4, P < 0.0001). Tree leaves had more positive δ15N values than herbaceous leaves, peel, and wood, and wood had more negative δ15N values than fruit (Kruskal-Wallis multiple comparison post hoc test, P < 0.0001). Weighted staple diet δ13C and δ15N mean values were −28.4‰ and 3.2‰, respectively. The mean δ13C and δ15N values of all feces samples (121 samples from four individuals) were −28.1 ± 0.9‰ and 3.9 ± 0.5‰. The isotope enrichment ε between feces and staple diet was 0.3%ε for δ13C and 0.6%ε for δ15N. We did not detect a relationship (linear least-squares regression, P > 0.05) between staple food δ13C or δ15N values with either fiber content (neutral detergent fiber, hemicellulose, and cellulose) or nonstructural carbohydrates (see Table S2 for nutrition data). We also did not detect a relationship between staple food δ15N values and protein. Staple food δ13C values increased with decreasing protein (linear least-squares regression, F = 7.196, r2 = 0.3749, P = 0.0199).

An additional 67 samples from 41 plant species, including bark, fungus, pith, shoot, stem, and twig, collected during the period of behavioral observation were analyzed to understand the isotopic composition of the whole diet (Table S3). Including staple foods, all plants had mean δ13C and δ15N values of −28.1 ± 2.2‰ and 2.2 ± 2.7‰, respectively. Mean δ13C values of individual species ranged from −32.9‰ to −22.2‰, whereas mean δ15N values of individual species ranged from −5.3‰ to 7.4‰. The most 13C-enriched food was fungus (−22‰), although only one measurement was made, whereas tree leaves were the most negative (−30.1‰). Nonstaple food items were rarely consumed (<1% dietary intake) (61) and likely did not contribute substantially to the isotopic composition of feces. Using a separate sample of staple fruits (18 samples of three species) seeds were manually separated from pulp and skin to approximate the undigested and digested components, respectively. Stable isotope analysis revealed that seeds had a mean δ13C value (−26.9‰) indistinguishable from pulp and skin (−27.1‰) (Wilcoxon test, paired, P = 0.3832; Table S4). The mean δ15N value of fruit seeds (4.9‰) was greater than pulp and skin (4.1‰) (Student t test, paired, P = 0.007513). Greater mass of pulp and skin was associated with greater mass of seeds in Chrysophyllum (linear least-squares regression, r2 = 0.5189, P < 0.001), Maesa (r2 = 0.4142, P < 0.001), and Myriantus (r2 = 0.6198, P < 0.001; Fig. S1). Seed mass increases more slowly than pulp/skin in each species (slope of linear regression less than 1).

The mean fecal δ13C value for these gorillas was −28.1‰, and the mean range of variation was 3.7‰ (Table 1; Table S5). All four individuals exhibited a consistent peak in δ13C values during
February and March, with a second, smaller peak or series of peaks during June and July (Fig. 2; Fig. S2). Likewise, a uniform trough in δ¹³C values was evident in both January and May. The mean fecal δ¹³C value was 3.7‰ and the mean range of variation was 2.1‰ (Table 1; Table S5). Temporal variation in feces δ¹³C values across individuals was not consistent (Fig. S2). Successive pooled monthly increments were different (P < 0.001), except for November–December (ANOVA, post hoc Tukey's honestly significant difference (HSD); Fig. 3). The timing of these peaks corresponded with fruit feeding peaks revealed by observational data. The total range in proportional fruit feeding estimated by the SIAR model was 14%, whereas dry mass intake estimates revealed a range of 48% range of variation (Table S1). Months with increased fruit feeding are associated with decreased herbaceous leaf feeding, but variation in the estimated intake of other staple foods was minor, varying by ≤10% throughout the 10-mo interval (Table S6).

**Discussion**

**Gorilla Diet.** Our results demonstrate that stable isotope analysis of feces collected from wild mammals feeding on C₃ foods can recover significant variation within a single year. Carbon isotopes in the Bwindi gorilla feces reveal a pattern of intrannual variability consistent across all four individuals (Fig. 2; Fig. S2). The range in fecal δ¹³C values within individual profiles, averaging 3.7‰, is greater than large mammalian herbivore dietary specialists feeding primarily on either C₃ or C₄ foods (54) but similar to C₃/C₄ mixed-feeding baboons (39). This range in δ¹³C values substantially exceeds previously reported values for intrindividual and seasonal-scale variation in individual hominoid primates (12, 13). *Pan* is known to exhibit variable feeding behavior (7–9), and isotopic homogeneity may be an artifact of tissue choice (i.e., analyzing hair instead of feces) or minimal isotopic separation among staple plant foods (13). Isotopic separation of fruit from other staple foods (Fig. 1), lack of consistent variation in feces δ¹⁵N values, and correspondence in time with observed changes in fruit intake suggest that δ¹³C peaks in gorilla feces reflect increased frugivory (Fig. 3; Fig. S2). There is no consistent relationship between home range and fruit feeding among the Bwindi gorillas (57), suggesting this pattern of isotopic variability is not likely driven by geography. Additionally, although fruit feeding among the Bwindi gorillas may be driven by changes in fruit availability throughout the year (59), it is not related to rainfall seasonality (62). Dry mass fruit intake is similar to previous fruit feeding estimates for Bwindi gorillas, which exceeds observed fruit feeding in Virunga mountain gorillas (7, 57–59, 61). Lowland gorillas (*Gorilla gorilla*) consume fruit more regularly than mountain gorillas (2, 9). Differences in fruit intake among gorilla populations likely reflect spatiotemporal variation in the distribution of fruit species across habitats (59, 62). Chimpanzees eat fruit more consistently year round, even when fruit availability is low (7–9).

The SIAR Bayesian mixing model reveals a distinct pattern of increasing frugivory associated with δ¹³C peaks (Fig. 3). Modeled increases in frugivory are associated primarily with decreased feeding on herbaceous leaves (Table S6), which is consistent with previously reported high folivory during low frugivory months for this population (58). The model also reveals a reduced range of variation in estimated proportional fruit intake compared with dry mass estimates (Fig. 3). Dry matter diet digestibility is ∼18% lower during months of high frugivory compared with low frugivory months due to the ingestion of untransformed, largely undigested seeds (58). However, this is unlikely to bias the representation of staple fruit in feces, because the mass of seeds is positively correlated with pulp/skin (Fig. S1), and δ¹³C values of seeds and pulp/skin are indistinguishable (Table S4). Signal loss in modeled frugivory peaks is likely due in part to the rate of isotope incorporation in feces. Stable isotopes in feces can record diet shifts with a resolution of a few days, yet complete equilibration with a new diet may take up to several months (47). Although the resolution of our observational data is limited to 2-mo intervals, some δ¹³C peaks in individual fecal profiles span less than 2 mo (Fig. 2), suggesting that fecal equilibration time may exceed the duration of diet shifts in these gorillas and result in some degree of time-averaging in fecal isotope profiles. This signal loss also means that isotopes in feces may be somewhat buffered against day-to-day variability. Additionally, estimated fruit intake may have been truncated by grouping stable isotope data into monthly intervals for SIAR input. This grouping procedure was chosen as an acceptable compromise between input sample size and time resolution (*Materials and Methods*) but reduced the range in feces δ¹³C values by almost half to ∼2‰. Despite some signal loss, the timing of diet change is preserved with greater temporal resolution than observational data. Individual δ¹³C profiles reveal that, within the directly observed February–March fruit feeding peak, the maxima seems to have occurred in February for one individual (Zs) and March for the others (Fig. 2; Fig. S2). Within the observed June–July peak, δ¹³C

<table>
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<th>Individual</th>
<th>δ¹³C mean (‰)</th>
<th>δ¹³C range (‰)</th>
<th>δ¹⁵N mean (‰)</th>
<th>δ¹⁵N range (‰)</th>
<th>Fecal samples (n)</th>
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<td>2.2</td>
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<tr>
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<td>2.2</td>
<td>29</td>
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<tr>
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<td>3.4</td>
<td>4</td>
<td>2.1</td>
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<tr>
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**Fig. 2.** Stable carbon isotope (δ¹³C) profiles from feces of four gorilla individuals (Rk and Zs are silverbacks; Bn is an adult female; By is a juvenile).
maxima are less uniform (Fig. 2; Fig. S2), consistent with the greater dispersion in dry mass intake values (Table S1).

The stable carbon isotope enrichment between diet and feces (δ13C_feces - δ13C_diet) of 0.3‰ found here is more enriched than previously reported values, whereas the stable nitrogen isotope enrichment of 0.6‰ contrasts with most experimentally derived values of approximately +3.0‰ (40, 63). Whereas these diet-feces enrichments are relatively minor, particularly compared with other tissues, even small differences can impact diet estimates when foods are isotopically similar. Variability in fractionation factors highlights the need for additional experimental work particularly on primates, with direct sampling of gut microflora and differentially digestible components that control the isotopic composition of feces. Future stable isotope mixing models would be improved with the incorporation of potential interindividual differences in feces-diet spacing, which can change depending on the digestibility of foods consumed (45).

**Bwindi Plants.** The mean stable carbon isotopic composition of vegetation from Bwindi (−28.1 ± 2.2‰) is more positive than other tropical African rainforests including the Ituri Forest (DRC) and Kakamega Forest (Kenya), which both exhibit mean δ13C values <30‰ (22, 28). Greater carbon isotope fractionation and 13C depletion of forest floor and lower canopy vegetation results in δ13C values between −33‰ and −37‰. Plant δ13C values from the lowland rainforest in Salonga National Park (DRC) are more similar to Bwindi, whereas vegetation from Kibale National Park (Uganda) is more positive than Bwindi (13, 30). Kibale and Bwindi are mid- and high-altitude afro montane forests with lower mean annual precipitation, resulting in less closed canopy structure and thus reduced isotope fractionation in subcanopy plants (22, 64–67). Mean δ13C values of plants from Bwindi, Kibale and Salonga may also partially reflect sample collection bias toward primate food plants. This isotopic variability emphasizes the need to understand the isotopic composition of plants among different C3-dominated habitats, such as tropical forests, which may differ by geography or climate. The average δ15N value of Bwindi plants (2.2 ± 2.7‰) is typical of tropical forest vegetation (68). This value is more negative than the staple diet δ15N mean value because it includes additional plant parts that are more negative than leaves and fruits (Table S3). Although mean staple food and mean total diet plants have similar δ13C values (differing by <1‰), particular diet categories have distinct δ13C values that would have been masked by analyzing plants based solely on within-habitat presence or without consideration of feeding preferences. The more positive δ13C values of fruit and wood may stem from preferential respiratory release of isotopically light carbon leading to an enrichment in δ13C of the plant carbon source pool available for nonleafy biomass production (69). Wood has more negative δ15N values, which is consistent with previous studies on tropical forest ecosystems (70, 71). While represented by only a single sample, the very positive δ13C value of fungus falls within the range of previously reported saprotrophic fungi δ13C values and is more positive than cultivated representatives of the genus by only 1–2‰ (72, 73). Isotopic variability among gorilla foods suggests it may be possible to detect similar source separation among the diets of other primates and forest-dwelling mammals. Further investigation is required, however, to assess the taxonomic, physiological, and microenvironmental boundary conditions for which these patterns hold. Variation in the nutritional and isotopic composition of gorilla staple foods is largely independent. Fecal isotope values primarily record feeding behavior rather than changes in nutritional intake. The negative relationship between staple food carbon isotope values and crude protein concentrations is weak. Our results illustrate the difficulty in tracking seasonal shifts in nutrient intake with isotopes in primate tissues and excreta in C3-dominated diets.

Implications for Hominin Paleoecology. Laser ablation stable isotope analysis of South African australopith tooth enamel reveals substantial intratooth isotopic variability, with differences between about 1‰ and 7‰ (10, 74). Using growth increments (peri-kymata) on the enamel surface to estimate elapsed time, variation in δ13C values has been interpreted as seasonal dietary variability within the lifetimes of individual hominins (74). Although this might instead represent seasonal movement across differing habitats (74), a strontium isotope analysis of South African hominin tooth enamel reveals 87Sr/86Sr values characteristic of local geologic substrates among a majority of specimens (75). Strontium and carbon isotopes are both incorporated in enamel during amelogenesis; therefore, carbon isotope variation in most individuals likely represents a dietary rather than a geographic signal. The magnitude of intra-individual δ13C fluctuation in gorilla feces falls within the range of many reported hominin enamel profiles, although the maximum range among hominins exceeds that of the almost 4‰ maximum range for gorillas (10). Although fecal isotope time series closely reflects the magnitude of isotopic variation in diet, enamel profiles represent a substantially time-averaged signal due to the prolonged process of enamel mineralization, which masks isotopic variability associated with very short time scales such as days or weeks (32, 33). Thus, the isotopic record in hominin enamel represents a minimum estimate of dietary variability that may have included significant shifts from C3- to C4-dominated diets on a seasonal scale, which supports hypotheses...
suggesting fossil hominids had more variable diets than African great apes (10).

**Conclusion**

Our findings reveal that some aspects of dietary flexibility in gorillas, as observed directly and similar to known patterns in all great apes, can be measured with stable isotope analysis of feces. Stable isotopes can be used to detect shifts in feeding on staple foods within a single year even when the diet is composed of C3 foods. The isotopic separation of fruit from other Bwindi gorilla staple foods demonstrates the potential for extending this technique for detecting frugivory in other animals. Future work with Bayesian mixing models has great potential for estimating the magnitude and timing of short-term diet shifts even when foods are distributed within a relatively restricted isotopic range. Establishing an isotopic baseline comprising locally consumed foods, rather than bulk habitat averages that do not represent the staple diet or are derived from other geographic areas, is critical for making such fine-scale diet estimates. This approach should be particularly useful in situations where the time and effort demanded by continuous field observation is not desirable or possible, such as diet estimates in populations of threatened or endangered species in relation to anthropogenic habitat degradation and climate-induced shifts in resource availability.

**Materials and Methods**

Bwindi Impenetrable National Park, located in the Albertine Rift of southwestern Uganda, is composed of contiguous tropical montane rainforest characterized by an undulating topography of steep hills and valleys. It extends over an area of ~331 km² and ranges in altitude from 1,160 to 2,607 m (62). Behavioral observation and fecal and food plant sampling at the Ruhija site, inhabited by the Kyaguriri group of gorillas, was conducted from October 2009 to July 2010 (Table 3; Fig. 6). There are four sample sets of seeds and pulp from the three staple fruits included in the gorilla diet were collected in January 2012 to test whether fruit seeds and pulp had similar isotopic compositions. Additional details on the study site, behavioral observation, and sampling are provided in [MATERIALS AND METHODS](#).

Plant and fecal samples were dried and ground before analysis. 13C/12C and 15N/14N ratios of plant material (800–1,000 μg), feces (500–700 μg), and standards (400–600 μg) were combusted on a Costech 4010 Elemental Analyzer at 950 °C. The isotope ratios of resulting gases were measured on a Finnigan MAT 252 isotope ratio mass spectrometer. Values are reported using the conventional permil (‰) notation where δ^13C or δ^15N = [(Rsample/Rstandard) – 1] × 1,000, using the international isotope standards V-PDB (Vienna Pee Dee Belcliminate) for δ^13C and AIR (atmospheric air) for δ^15N. Repeated isotope analysis of laboratory standards of yeast and spinach were made to measure analytical precision, which was < 0.1% for δ^13C and < 0.2% δ^15N. Details of nutritional analysis are outlined elsewhere (58, 61). Isotope enrichment (ε*) was calculated as a function of the apparent fractionation factor (α*), where ε* = (1,000 + δ^13Cfood(plant)/δ^13C(standard)) × (1,000 + δ^15Nsolution)/δ^15N(standard)) – 1. Additional details on statistical analyses are provided in [MATERIALS AND METHODS](#).

We analyzed the stable isotope composition of foods and feces with the package Stable Isotope Analysis in R (SIAR v. 4.1.1) in R, which performs well under a variety of complex scenarios (51, 52). This mixing model is ideal for estimating mixtures (i.e., diet composition) where there are more sources than isotopes, because under such circumstances model results are undetermined. SIAR can incorporate uncertainty in the isotopic composition of sources and consumer tissues or excreta, elemental concentration of food sources, and trophic enrichment factors. Uncertainty is also incorporated in diet estimates, which are provided as posterior probability distributions of probable solutions. The model works best when each group includes multiple observations, which provides more accurate estimates of intragroup variance. Data can be grouped according to ecological relevance, such as demographic groups, sampling locations, or sampling periods. Stable isotope measurements from gorilla feces were grouped into monthly intervals to maximize intragroup sample size while maintaining temporal resolution comparable to direct observational data. SIAR is a nonhierarchical model and cannot estimate diets of individuals within a group.

Required model input includes stable isotope data for diet sources (Table S2) and consumer tissues or feces (Table S5), as well as trophic enrichment factor (TEF) data on the isotopic separation between diet sources and consumers. TEF estimates (mean and SD) from the literature can be used when diet-tissue enrichment is unknown. However, we were able to properly calculate this value, and incorporating diet-tissue enrichment uncertainty was unnecessary. An additional advantage of a Bayesian mixing model is the ability to incorporate prior probabilities, which directs the model according to previously established knowledge. Prior information on each staple diet source was included in the model, including mean annual intake (from ref. 61) and elemental concentrations (Table S2). A minor value (<1%) was added proportionately to the annual contribution of each source so that they sum to unity, as required by the model. An estimate of statistical dispersion for one diet category can also be supplied, and the known SD for annual fruit intake (7.4%) was used here. The model output includes a posterior probability distribution function of the proportional contribution of each source for each month. Correlations in this model between predicted contribution of fruit and other sources were weak (<0.46). As SIAR outputs were problematic with correlations >0.6 and posterior probability distributions were narrow, these results represent robust estimates of fruit feeding (76). Routing should not impact these results, as the model incorporates source elemental concentrations, and excreta should record bulk dietary input regardless of differences in digestibility among sources (46). Additionally, the less digestible fraction of fruit consumed by these gorillas is isotopically identical to the more digestible fraction (Results).

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16. Tieszen LL, Senyimba MM, Imbamba SK, Troughton JH (1979) The distribution of C3 and C4 vegetation and incorporating diet-tissue enrichment uncertainty was unnecessary. An additional advantage of a Bayesian mixing model is the ability to incorporate prior probabilities, which directs the model according to previously established knowledge. Prior information on each staple diet source was included in the model, including mean annual intake (from ref. 61) and elemental concentrations (Table S2). A minor value (<1%) was added proportionately to the annual contribution of each source so that they sum to unity, as required by the model. An estimate of statistical dispersion for one diet category can also be supplied, and the known SD for annual fruit intake (7.4%) was used here. The model output includes a posterior probability distribution function of the proportional contribution of each source for each month. Correlations in this model between predicted contribution of fruit and other sources were weak (<0.46). As SIAR outputs were problematic with correlations >0.6 and posterior probability distributions were narrow, these results represent robust estimates of fruit feeding (76). Routing should not impact these results, as the model incorporates source elemental concentrations, and excreta should record bulk dietary input regardless of differences in digestibility among sources (46). Additionally, the less digestible fraction of fruit consumed by these gorillas is isotopically identical to the more digestible fraction (Results).

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