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Stable isotope turnover and variability in tail hairs of captive and free-ranging African elephants (*Loxodonta africana*) reveal dietary niche differences within populations

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Abstract: Many herbivore species expand their dietary niche breadths by switching from browse-rich diets in dry seasons to grass-rich diets in rainy seasons, in response to phenological changes in plant availability and quality. We analyzed stable isotope series along tail hairs of captive and free-ranging African elephant (*Loxodonta africana* (Blumenbach, 1797)) to compare patterns of seasonal dietary variability across individuals. Results from elephants translocated from the wild into captivity, where their diets are semicontrolled, revealed tail hair growth rates of ~ 0.34 mm/day, on average, and relatively rapid isotope turnover through the transition from wild into captivity. Sampling hairs at 10 mm increments thus archives dietary chronologies at a resolution suitable for tracking diet switches at seasonal, and even subseasonal, scales. Hairs of free-ranging elephants showed extensive carbon isotopic variability within individuals, consistent with seasonal switches between C_3 -browsing and C_4 -grazing. Similarly extensive, but asynchronous, shifts in nitrogen isotope ratios were also observed, suggesting an influence of factors other than seasonality. Across individuals, switching patterns differed across habitats, and across age classes, with older, larger animals including increasing amounts of C_3 browse into their diets. These results demonstrate how stable isotope approaches characterize complex patterns of resource use in wildlife populations.

Key words: African elephant, C_3 browse, C_4 grass, diet switching, Kruger Park, *Loxodonta africana*, seasonality, weaning.

Résumé : De nombreuses espèces herbivores élargissent leur niche alimentaire en passant d'un régime riche en brout durant les périodes sèches à un régime riche en graminées durant les périodes pluvieuses, en réponse à des changements phénologiques à la disponibilité et la qualité des végétaux. Nous avons analysé des séries d'isotopes stables le long de poils de queue d'éléphants d'Afrique (*Loxodonta africana* (Blumenbach, 1797)) vivant en captivité et en liberté afin de comparer les motifs de variation saisonnière de l'alimentation d'un individu à l'autre. Les résultats pour des éléphants initialement en liberté placés par la suite en captivité dans des conditions où leur alimentation est partiellement contrôlée, ont révélé des taux de croissance moyens des poils de queue de $\sim 0,34$ mm/jour et une modification assez rapide de la composition isotopique associée à la transition de l'état libre à l'état captif. Ainsi, l'analyse isotopique des poils à des intervalles de 10 mm permet l'archivage des chronologies d'alimentation à une résolution permettant de cerner les changements d'alimentation à l'échelle saisonnière, voire plus courte. Les poils d'éléphants en liberté présentaient une grande variabilité individuelle des isotopes de carbone, ce qui appuie la thèse de passages saisonniers d'une alimentation reposant sur le broutage de plantes C_3 à une alimentation reposant sur le pâturage de plantes C_4 . Des modifications tout aussi importantes, bien que non synchrones, des rapports d'isotopes d'azote ont également été observées, ce qui laisse croire à l'influence de facteurs autres que la saisonnalité. Pour l'ensemble des individus, les motifs de changement d'alimentation variaient selon l'habitat et la classe d'âge, l'alimentation des animaux plus vieux et plus grands comprenant des proportions croissantes de brout de plantes C_3 . Ces résultats démontrent l'utilité des isotopes stables pour caractériser les motifs complexes d'utilisation des ressources par des populations d'animaux sauvages. [Traduit par la Rédaction]

Mots-clés : éléphant d'Afrique, brout de plantes C_3 , graminées C_4 , changement d'alimentation, parc Kruger, *Loxodonta africana*, saisonnalité, sevrage.

Introduction

Mammal herbivores use food resources that are unevenly distributed across space and through time (Senft et al. 1987; du Toit 2003). Many species have therefore evolved flexible foraging behaviours, in particular broadening their niches by vary-

ing diets in response to phenological changes in availability and quality of different plant food groups (du Toit 2003; Owen-Smith 2008). Seasonal diet switching is most obvious in intermediate-feeding taxa—species that forage successfully on both browse (woody and herbaceous dicots) and grass. Al-

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though intermediate-feeding occurs in relatively few taxa in African savannas (compared with assemblages in temperate habitats: Hofmann 1989; du Toit 2003), species like the African elephant (*Loxodonta africana* (Blumenbach, 1797)) and the impala (*Aepyceros melampus* (Lichtenstein, 1812)) are well known to vary the browse and grass composition of their diets through the seasonal cycle, in some cases switching from pure browsing during dry seasons to pure grazing during rainy seasons when fresh grass becomes available in abundance (Owen-Smith 1988; Codron et al. 2006a; 2011b; Kos et al. 2012).

Seasonal switches between browsing and grazing occur because of trade-offs between the relative costs and benefits of foraging on either plant group. During the rainy season, new grass growth offers the benefit of a continuously distributed resource—ensuring a high encounter rate and minimizing search time—with high concentrations of digestible fibre and nutrients like protein in the leaves (Owen-Smith 1997; O'Connor et al. 2007). Browsing is often more costly, because these plants have less digestible (more lignified) cell walls and many also produce high concentrations of secondary compounds with antifeedant properties (Meissner et al. 1999; Hummel et al. 2006; Codron et al. 2007). With the cessation of rains, grasses die back, becoming more lignified, and many of them transport nutrients to roots for winter storage, where they are inaccessible to most large herbivores (Meissner et al. 1999). During dry seasons, browse plants (especially trees) become relatively more profitable because while they are generally more sparsely distributed across the landscape and may have elevated concentrations of phenols (see Owen-Smith and Cooper 1989), their leaves retain higher concentrations of soluble nutrients than grass at that time.

These drivers of switching become more complex over a variety of intraspecific scales. For instance, the degree of seasonal switching is not constant across populations; niche breadths appear to become limited in habitats with low browse species diversity, or where high grass biomass is maintained year-round (Jachmann and Bell 1985; Owen-Smith 1988; Codron et al. 2006a, 2006b, 2011b). Intrinsic constraints, primarily changes in nutritional requirements and accessibility of resources across body size classes, also influence levels of dietary variability, especially for large taxa like elephants in which size varies considerably with age and (or) is strongly dimorphic across the sexes (Shannon et al. 2006; Woolley et al. 2011). In many animal populations, diets also differ between individuals of the same age or sex class (Bolnick et al. 2003, 2007), and although such individual niche segregation throughout the entire seasonal cycle is unlikely for mammal herbivores (Codron et al. 2012), the phenomenon nevertheless presents a potential source of variability contributing to the dietary niche breadths of whole populations.

Among free-ranging populations, diet switches and niche breadths of individuals are readily documented by stable isotope profiles of incrementally growing, inert tissues such as teeth, hooves, whiskers, and tail hairs (Cerling et al. 2006, 2009; Chereil et al. 2009; Newsome et al. 2009; Codron et al. 2012). This is because the isotope composition of an animal's diet is reflected by the isotope composition of its body tissues; metabolically inert tissues, in which no turnover takes place after deposition of the material, archive this information in chronological increments along the growth axis. Provided an animal's foods differ in isotope composition, diet switches can be recorded over a variety of temporal scales, consistent with the rate of tissue growth (Tieszen et al. 1983). However, for many species, the rate of tissue growth and the rate at which new isotopic information is incorporated (or earlier information “decays”) are uncertain. Experiments with captive animals on controlled diet regimes have revealed that isotopic turnover through a diet switch is a time-dependent process, occurring along an exponential gradient which only asymptotes once the specific tissue's nutrient pool has equilibrated with the new diet (Ayliffe et al. 2004; Cerling et al. 2007; Martínez del

Rio and Anderson-Sprecher 2008). Because of this, tracking of isotopic diet switches along growth axes of incremental tissues is attenuated, i.e., the full extent of a switch may not be fully expressed in a time series derived from tissues of animals not yet in equilibrium with the new diet. A number of turnover models have been forwarded to enable researchers to account for such attenuation effects, but there is as yet no consensus regarding the choice of model and the parameter values to use for different species in various contexts (see e.g., Carleton et al. 2008; Wittemyer et al. 2009).

Herbivores living in subtropical African savannas regularly forage on foods differing markedly in isotope composition. Here there exists a bimodal distribution of $^{13}\text{C}/^{12}\text{C}$ ratios among terrestrial plants, differentiating C_3 (^{13}C -depleted) from C_4 (^{13}C -enriched) biomass, meaning that $^{13}\text{C}/^{12}\text{C}$ ratios of herbivore tissues reflect relative proportions of browse (C_3 dicots) and grass (C_4) consumed (Vogel 1978; Tieszen et al. 1979; Lee-Thorp and van der Merwe 1987; Cerling and Harris 1999). Herbivore $^{15}\text{N}/^{14}\text{N}$ ratios are also related to nitrogen isotope compositions of the diet; for instance, higher ^{15}N abundances are often associated with plants (and hence animals) in arid, open habitats (e.g., Muzuka 1999; Codron et al. 2005; Murphy and Bowman 2006). However, factors including environmental stress, ecophysiological stress, and diets comprising high protein quality also contribute to elevated ^{15}N abundances in mammal herbivores (Ambrose 1991; Sponheimer et al. 2003; Robbins et al. 2005). In this regard, hormone analysis is known to be a precise and widely accepted tool for monitoring responses to perceived environmental, physiological, or psychological stressors (Ganswindt et al. 2012). On that note, the noninvasive assessment of glucocorticoid metabolite concentrations has been shown to be a useful approach to provide information on the level of stress experienced by elephants (Ganswindt et al. 2010b).

Our aim was to investigate isotopic evidence for differences in switching and dietary variability between elephants from different habitats and of different age classes in a South African savanna, the Kruger National Park, using serial analysis of tail hairs. As an initial step, we estimated rates of growth and isotope turnover in elephant tail hairs using results from elephants translocated from the wild into captivity where they are fed semicontrolled diets. These results allow us to parameterize isotope mixing models and so validate diet interpretations of free-ranging individuals by accounting for isotope turnover, as well as to compare results of different modeling approaches. Additionally, assessed fecal glucocorticoid metabolite levels in captive elephants kept at the same facility also allows us to determine whether these individuals generally perceived stressors that may influence overall ^{15}N abundances. Using this knowledge to better interpret tail hair data from free-ranging animals from Kruger Park, we investigated differences in the magnitude of temporal diet switches between individuals from different habitats and different age classes. In particular, we hypothesized that (i) isotopic niche breadths would be narrower in northern than southern Kruger Park habitats due to less seasonal switching arising from the low tree species diversity in the former landscapes (Codron et al. 2011b), and (ii) isotopic niche breadths would broaden with animal age as larger individuals tolerate and access a wider variety of food items (Woolley et al. 2011).

Materials and methods

Captive elephant tail hairs

For the isotope turnover experiment, we procured tail hairs from seven captive elephants (one hair per animal) that had been translocated from private nature reserves in the Limpopo Province, South Africa, to the Elephants for Africa Forever (EFAF) Mooketsi Sanctuary, ~50 km from the town of Tzaneen. One female, Andile, was kept at the EFAF Elephant Whispers Sanctuary in Hazyview, Mpumalanga Province (Table 1). Three individuals

Table 1. Length, time intervals, and estimated growth rates of captive African elephant (*Loxodonta africana*) tail hairs sampled for this study.

Individual (sex, age at capture)	Hair length (mm)	Hair length (mm) in captivity	Growth rate (mm/day)	Total days in sample	Growth phases in isotope profiles									
					Free-range			Captivity (phase 1)			Captivity (phase 2)			
					n	mm	Days	n	mm	Days	n	mm	Days	
Individuals from Louis Trichardt (captured: 8 March 2007; hair sampled after 496 days on 17 July 2008)														
Andile (F, 11)	267	165	0.33	803	10	100	301	34	100	301	23	67	201	
Chova (M, 11)	239	182	0.37	651	10	56	153	41	104	283	26	79	215	
Moya (M, 10)	195	184	0.37	526	7	10	27	23	115	310	26	70	189	
Individuals from Messina (captured: 6 March 2008; hair sampled after 132 days on 16 July 2008)														
Shan (F, 8)	239	39	0.30	809	20	200	677				31	39	132	
Chishuru (M, 11)	227	47	0.36	638	17	170	477				40	57	160	
Musina (F, 7)	191	28	0.21	900	18	162	764				30	29	137	
Nuanedi (F, 8)	224	22	0.17	1344	31	202	1212				23	22	132	

Note: Hair growth rates estimated as hair length (mm) in captivity/days in captivity. Total days represented are for the entire hair sequence, estimated as growth rate \times hair length.

were translocated in March 2007 from a private reserve near the town of Louis Trichardt, and the other four in March 2008 from a reserve in the Messina region (Table 1). All animals were between 7 and 11 years old at the time of capture and appeared to be in good body condition in captivity (information provided by EFAF staff). However, at the time of capture, Musina appeared to have stunted molar development and soft, flaky ivory, indicative of nutritional stress, and Nuanedi had one septic tusk that required surgery in captivity (R. Hensman, personal communication, 2009). Captive elephants were fed a diet comprising a mixture of C_4 grass ($\delta^{13}C = -12.4\% \pm 0.26\%$ (mean \pm SD), $n = 2$), pellets (consisting mainly of C_4 products including local grasses ($\sim 58.8\%$), hominy chop (19.6%), molasses (15.7%), and horse meal (4.9%); $\delta^{13}C = -13.4\% \pm 0.35\%$, $n = 15$), and freshly cut C_3 browse ($\delta^{13}C = -30.2\% \pm 1.2\%$, $n = 19$), and they were also allowed to roam freely in the surrounding reserve for some part of the day. Given that free-ranging elephant populations in the region have largely C_3 -based diets ($\sim 60\%$, on average; Codron et al. 2011b), we expected that tail hairs of captive animals would reflect a shift towards the ^{13}C -enriched values of their predominantly C_4 diets in captivity. $\delta^{15}N$ values of supplemental feeds (pellets = $3.9\% \pm 0.22\%$ and C_4 grass = $3.0\% \pm 2.48\%$) were higher than those of naturally-occurring forage ($0.7\% \pm 1.51\%$), thus we also expected a shift towards higher $\delta^{15}N$ values in captivity. Water was provided ad libitum.

Hairs were sampled in July 2008, 496 days after capture for the group from Louis Trichardt and 132 days after capture for the group from Messina (Table 1). To ensure that isotope series along hair strands represented growth in free-ranging (older growth at the distal end) and captive (newer growth near the root) conditions, we sampled the longest hairs possible from each individual, including the hair follicle. Hair lengths ranged from 191 to 267 mm; based on published tail hair growth rates for African elephant (Cerling et al. 2006, 2009), we expected these lengths would archive an isotopic chronology extending to the period before capture in most individuals.

Each hair was cleaned with acetone and then sectioned at 10 mm intervals from the root towards the tip using a scalpel. Between 0.45 and 0.6 mg of material was removed from within the centre of each increment, ensuring that a distance of precisely 10 mm was maintained between subsamples. Later on, the hair section(s) representing the transition from free-ranging to captivity were resampled at 1 mm increments to obtain a higher temporal resolution needed for modeling isotope turnover. Subsamples were weighed individually into tin cups and loaded in an automated Elemental Analyzer (Carlo Erba, Milan) where they were combusted and the resultant CO_2 and N_2 gases were introduced to a Delta XP mass spectrometer (Finnigan, Bremen, Germany) via a continuous flow inlet system. $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios are presented in conven-

tional delta (δ) notation, in units permil (‰), relative to the Vienna PeeDee Belemnite (VPDB) and atmospheric N_2 standards, respectively. Standard deviations of repeated measures of laboratory standards (Merck Gel, Valine, and chocolate powder) were less than 0.1‰ and 0.3‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. The C/N ratio of all subsamples was within the expected range for biological proteins, i.e., 2.7–3.6 (DeNiro 1985).

Fecal glucocorticoid metabolite levels in captive elephants

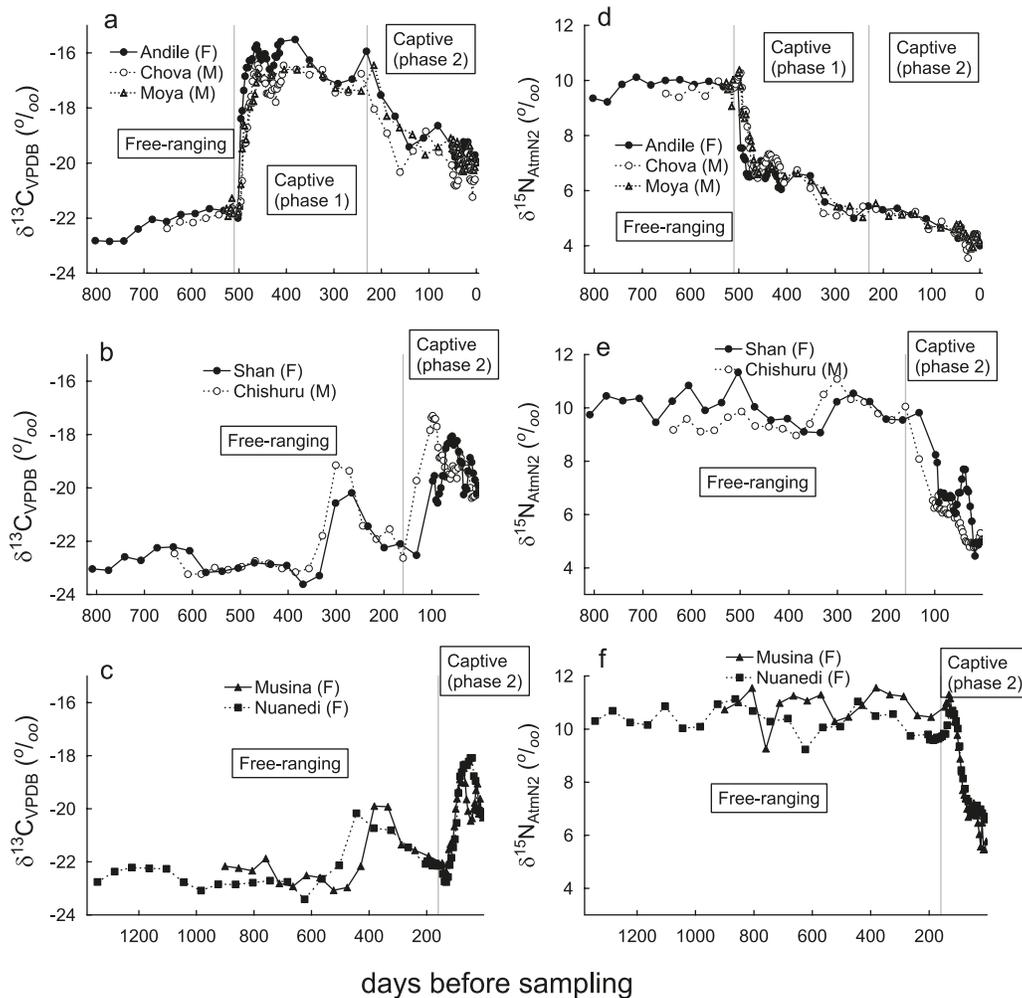
To be able to explain potential differences in $^{15}N/^{14}N$ ratios between captive and free-ranging elephants, baseline fecal glucocorticoid metabolite (FGM) levels were assessed in captive elephants as a stress-related indicator for the potential impact of environmental and ecophysiological factors.

A total of 213 individually allocated fecal samples from 12 different elephants housed at EFAF facilities were collected between May–September 2006 and March–May 2007. In addition, the three individuals (Andile, Chova, and Moya) that arrived at EFAF Mooketsi Sanctuary in March 2007 were monitored for the first 4 weeks after arrival to assess the effect of translocation. Fecal material was collected according to Ganswindt et al. (2010b). Collected samples were lyophilized, pulverized, and sieved to remove undigested fibrous material. Approximately 0.05 g of the fecal powder was then extracted with 80% ethanol in water (3 mL) (Ganswindt et al. 2010b). The resulting extracts were measured for immunoreactive FGM using an enzyme immunoassay detecting FGMs with a 5β - 3α -ol-11-one structure (Möstl et al. 2002), which have been shown to reliably reflect adrenocortical function in African elephants (Ganswindt et al. 2003; Viljoen et al. 2008). Cross-reactivities of the antibody used are described in Möstl et al. (2002). Assay procedures followed the protocol published by Ganswindt et al. (2002). Serial dilutions of extracted fecal samples gave displacement curves that were parallel to the respective standard curve. Sensitivity (90% binding) of the assay was 3 pg/well. Intra- and inter-assay coefficients of variation, determined by repeated measurements of high and low value quality controls, ranged between 4.2% and 16.9%.

Free-ranging elephant hairs

Tail hairs of free-ranging elephants were provided by the Game Capture Unit of Kruger National Park. Kruger Park is a semi-arid savanna reserve, $\sim 2\,000\,000$ ha in size, situated in the northeast of South Africa. The reserve lies in the summer rainfall zone of South Africa, characterized by warm, wet seasons between October and March and dry seasons between April and September. Mean annual rainfall is ~ 500 mm but varies geographically within the Park. Habitats are widely heterogeneous, linked with changes in climate, geology, and topography (Venter et al.

Figs. 1a–1f. Tail hair stable isotope profiles for seven individual African elephants (*Loxodonta africana*) translocated from free-ranging to captive conditions. Distinctions between free-ranging and captive phases are clearly evident in the data, entailing a sharp shift to higher $\delta^{13}\text{C}$ values (consistent with the C_4 composition of pelleted feeds) and lower $\delta^{15}\text{N}$ values. Time (days) on the x axis are estimated based on the length of the hair (mm) and known number of days in captivity.



2003); for our study, we differentiate simply between the mopane (*Colophospermum mopane*) dominated northern habitats and the *Acacia–Combretum* savanna woodlands of the south, based on fundamental differences in elephant diets and diet breadths (see above and Codron et al. 2011b).

We used data from hairs collected from 19 individuals within Kruger Park (one hair per individual), obtained during routine veterinary operations that include immobilization and tissue sampling for cold storage. Hairs ranged from 80 to 290 mm in length and were sampled at 10 mm increments from the root to the tip (data presented in supplementary material of Codron et al. 2012). Proveniences, including date of capture, locality, sex, and age group, were provided by the Game Capture Unit. Age categories provided were estimates based on animal size: calf is an individual still suckling; juvenile is a weaned individual; subadult is an animal approaching adult size but not yet reproductively active; adult is a cow that has had a calf or a bull that is able to compete for females (P. Buss, personal communication, 2005). In total the sample comprised 8 individuals from the northern part of Kruger Park (one male adult, two male and two female subadults, one juvenile male, and one male and one female calf) and 11 individuals from the southern part (three female adults, five female subadults, one female juvenile, and two male calves).

Analysis of isotope series

Visual inspection of captive elephant tail hair series revealed a sharp transition from wild to captive diet regimes (Figs. 1a–1f), from where we could estimate the growth rate of each hair (mm/day) because the exact date of capture and sampling was known (Table 1). However, only the three individuals that had been translocated in 2007 (Andile, Chova, and Moya) showed signs of reaching isotopic equilibrium with the new diet. The four individuals translocated in 2008 probably had not been in captivity long enough to attain this equilibrium, and hence had to be excluded from models of isotope turnover. Furthermore, a shift in captive diet conditions following a poor harvest of local grass in the wet season of 2008 (R. Hensman, personal communication, 2009) was also evident in the series of even Andile, Chova, and Moya (note especially the reduction in $\delta^{13}\text{C}$ values in Fig. 1a to levels similar to captive diets of the other four individuals shown in Figs. 1b and 1c). Although $\delta^{15}\text{N}$ values continued to shift in the same direction even after the second switch in captivity (Fig. 1d), this portion of the sequence could not be modeled for either isotope given the overall change in diet regime. Considering only the portion representing the initial transition period (labeled Captive (phase 1) in Figs. 1a and 1d and Table 1; Captive (phase 2) in Figs. 1a–1f and Table 1) represents the shorter period in captivity after conditions

had changed), we fitted a single-pool turnover model to the data for Andile, Chova, and Moya to estimate the rate constant (λ) for isotopic turnover in these individuals:

$$[1] \quad \delta X_t = \delta X_{eq} - (\delta X_{eq} - \delta X_0)e^{-\lambda t}$$

where δX_t is the isotopic value of a unique subsample representing a given point in time (t , in days), δX_0 is the isotope value at the start of the transition period, and δX_{eq} is the model-derived isotope value of the sample when the animal is at equilibrium with the diet. Model fitting was carried out using the least squares nonlinear regression module of STATISTICA version 8.0 (Statsoft Inc. 2007).

Hairs from Kruger Park animals were used to compare diets and dietary variability across animals from different habitats and of different age classes (males are poorly represented in our sample, and hence sex-based differences are omitted from our analyses). Comparisons of longitudinal series across individuals are problematic because of autocorrelations, and many of our series are too short to account statistically for this phenomenon. Therefore, our analysis is based on comparisons of metrics including the median, range, minimum, and maximum δ value of each individual. We assume that the range of δ values along a hair strand represents the individual's isotopic niche breadth; however, for conservatism we also compared ranges between the 25th and the 75th percentiles, as well as between the 10th and the 90th percentiles, and indicate cases in which patterns differed from comparisons of the range. Habitat effects were tested using Mann–Whitney U tests (Statsoft Inc. 2007) to compare data for individuals from the northern Kruger Park with individuals from the south. Age effects were tested using Spearman's rank correlations (for each habitat independently), ranking age classes from 1 to 4, where 1 indicates a calf and 4 indicates an adult.

To account for attenuation in the data for free-ranging elephants, we estimated a "corrected" δ value for each subsample, rewriting eq. 1 as (and see, e.g., Cerling et al. 2006, 2009)

$$[2] \quad \delta X_{eq} = \frac{\delta X_t - \delta X_{t-1}e^{-\lambda t}}{1 - e^{-\lambda t}}$$

where δX_{eq} is the estimated value if attenuation had not occurred and δX_{t-1} is the initial value (equivalent to δX_0 in eq. 1). For λ , we used the mean value derived from our analysis of captive elephant hairs. The adjusted series were then submitted to the same analytical comparisons as described above.

For comparability with previous studies of elephant tail hair chronologies (Cerling et al. 2006, 2009), we also employed a three-pool turnover model to correct Kruger Park tail hair series, initially parameterized for horses (Ayliffe et al. 2004):

$$[3] \quad \delta X_{eq} = \frac{\delta X_t - f_1\delta X_{t-1}e^{-\lambda_1 t} + f_2\delta X_{t-1}e^{-\lambda_2 t} + f_3\delta X_{t-1}e^{-\lambda_3 t}}{1 - f_1\delta X_{t-1}e^{-\lambda_1 t} + f_2\delta X_{t-1}e^{-\lambda_2 t} + f_3\delta X_{t-1}e^{-\lambda_3 t}}$$

where f_1 , f_2 , and f_3 are the fractional contributions of each isotope pool represented by three independent rate constants (λ_1 , λ_2 , and λ_3). Results of the three approaches (raw data, single-pool adjusted, and three-pool adjusted) were compared using repeated measures (RM) ANOVAs, which included the individual as a between-subjects factor to ensure correct error terms were being compared (Statsoft Inc. 2007).

Finally, to enhance interpretation of $\delta^{13}\text{C}$ series, we converted these data to estimates of % C_4 grass intake using a linear mixing model:

$$[4] \quad \% \text{C}_4 \text{ in diet} = \frac{\delta^{13}\text{C}_{\text{hair}} - \Delta_{\text{HD}} - \delta^{13}\text{C}_{\text{C}_3 \text{ plants}}}{\delta^{13}\text{C}_{\text{C}_4 \text{ plants}} - \delta^{13}\text{C}_{\text{C}_3 \text{ plants}}}$$

where Δ_{HD} is the diet–hair isotope spacing, or discrimination, assumed to be 3.1‰ for large herbivorous mammals (Cerling and Harris 1999). Mean $\delta^{13}\text{C}$ values of C_3 and C_4 vegetation for northern and southern Kruger Park habitats are from Codron et al. (2005).

Results

Captive elephants

As anticipated, a switch from free-ranging to captive conditions entailed a sharp rise in $\delta^{13}\text{C}$ values, consistent with the high C_4 composition of captive diets (Figs. 1a–1c). Over this period, however, hair $\delta^{15}\text{N}$ values declined, opposite to what was expected based on comparisons of supplemental and natural forages (Figs. 1d–1f). Some individuals also showed a smaller $\delta^{13}\text{C}$ peak shortly before captivity (Figs. 1b, 1c)—but no corresponding shift in $\delta^{15}\text{N}$ —likely reflecting elevated levels of C_4 grazing among free-ranging animals in the wet season just prior to capture.

Estimated hair growth rates varied from 0.30 to 0.37 mm/day across individuals (0.34 ± 0.03 mm, mean \pm SD), except for the two individuals that initially suffered from nutritional or physical stress, or from pathology, which showed much slower growth (0.21 and 0.17 mm/day in Musina and Nuanedi, respectively; Table 1). The mean value of 0.34 mm/day suggests a 250 mm elephant hair represents approximately 2 years of growth, and if subsampled at 10 mm increments (as in the case of our Kruger Park hair samples) records shifts at approximately monthly intervals, on average.

Nonlinear regression models fitting eq. 1 to the Captive (phase 1) portion of hairs from Andile, Chova, and Moya were significant in all cases ($r^2 = 0.88$ – 0.92 for $\delta^{13}\text{C}$ and 0.68 – 0.92 for $\delta^{15}\text{N}$, $p < 0.0001$; Table 2). Turnover rate constants (λ) derived from these models varied from 0.0722 to 0.1445 (0.1007 ± 0.0385 , mean \pm SD) for $\delta^{13}\text{C}$ and from 0.0370 to 0.1353 (0.0738 ± 0.0536) for $\delta^{15}\text{N}$, across individuals, implying half-lives ($\ln(2)/\lambda$) of 4.8–9.6 days for the former (7.5 ± 2.46) and 5.1–8.7 days (12.6 ± 6.9) for the latter isotope, respectively.

The assessment of FGM concentrations in elephants housed at EFAF facilities revealed hormone levels comparable with those from elephants in the wild (Student's t test, $t_{[12,58]} = 396$, $p = 0.646$; data from Ganswindt et al. 2005b, 2010a, 2010b). The five animals (25–39 samples/animal) monitored in 2006 had individual mean FGM baseline values between 0.38 and 0.80 $\mu\text{g/g}$ dry mass (DM) (mean 0.58 $\mu\text{g/g}$ DM), and the seven elephants (9 samples/animal) monitored in 2007 showed individual mean FGM levels between 0.48 and 0.69 $\mu\text{g/g}$ DM (mean 0.59 $\mu\text{g/g}$ DM). During the first month after arrival, Andile, Chova, and Moya (5 samples/animal) showed FGM levels between 0.86 and 2.24 $\mu\text{g/g}$ DM (individual mean values of 1.45, 1.31, and 1.80 $\mu\text{g/g}$ DM, respectively), which corresponds to an elevation of 47%–285% when compared with the overall mean FGM baseline value of the 12 abovementioned elephants (0.58 $\mu\text{g/g}$ DM). The revealed hormone values for Andile, Chova, and Moya indicate that translocation and the subsequent adaptation to a new environment resulted in an increase in FGM concentrations similar to those observed for elephants translocated within Kruger Park (Viljoen et al. 2008).

Free-ranging individuals from Kruger Park: variability and attenuation effects

Average (median) $\delta^{13}\text{C}$ values of tail hairs from Kruger Park elephants were similar across individuals (medians ranged from -21.9% to -18.8%). However, substantial variations were recorded within individuals, with differences of 3.3‰ to 7.9‰ along the length of the hair of any one individual (Fig. 2). Median $\delta^{15}\text{N}$ values

Table 2. Single-pool isotope turnover models fitted to captive African elephant (*Loxodonta africana*) tail hair data for the first phase of captivity (see Figs. 1a–1f).

Individual (sex)	df	F	R ²	p	δX_{eq} (–95% to +95% CI)	λ (–95% to +95% CI)	$t_{1/2}$
$\delta^{13}C$							
Andile (F)	3, 31	19 221.280	0.8849	0.0000	–16.21 (–16.37 to –16.05)	0.1445 (0.1090 to 0.1801)	4.80
Chova (M)	3, 38	23 817.710	0.8930	0.0000	–16.99 (–17.17 to –16.81)	0.0855 (0.0652 to 0.1058)	8.11
Moya (M)	3, 20	15 636.970	0.9241	0.0000	–16.85 (–17.11 to –16.59)	0.0722 (0.0507 to 0.0938)	9.60
$\delta^{15}N$							
Andile (F)	3, 31	2 213.602	0.6750	0.0000	6.41 (6.22 to 6.60)	0.1353 (0.0719 to 0.1986)	5.12
Chova (M)	3, 38	2 194.814	0.7823	0.0000	6.38 (6.07 to 6.69)	0.0492 (0.0302 to 0.0682)	14.07
Moya (M)	3, 20	2 284.360	0.9203	0.0000	5.85 (5.52 to 6.17)	0.0370 (0.0258 to 0.0482)	18.70

Note: δX_{eq} is the asymptotic δ value expected after complete turnover. $\Delta\delta X$ is the total change (%) of the diet switch. λ is the turnover rate constant. $t_{1/2}$ is the isotopic half-life, i.e., $\ln(2)/\lambda$.

differed more notably across individuals (medians ranged from 4.9‰ to 11.0‰). There were also large shifts in $\delta^{15}N$ values along some hairs (of up to 6.6‰), although some individuals varied by much less (by as little as 0.8‰). Shifts in $\delta^{13}C$ values along individual hair strands most likely reflect a seasonal pattern, with peaks indicating switches to increased C_4 grazing during the rainy season. There was no consistent correlation between $\delta^{13}C$ and $\delta^{15}N$ values, thus at this stage the shifts in $\delta^{15}N$ values cannot be interpreted in terms of seasonality.

Adjustments of observed $\delta^{13}C$ and $\delta^{15}N$ values for attenuation during isotope turnover had only minor effects on the overall results, but did influence results of isotope mixing models to some extent. For example, while corrections using both a single-pool turnover model (parameterized from results of this study) and a three-pool turnover model (parameterized for horses in Ayliffe et al. 2004) visually increased the size of isotopic shifts observed—consistent with predicted effects of attenuation (an example from one individual is shown in Fig. 3a), there was no significant difference in mean $\delta^{13}C$ values derived from raw data or either turnover model (RM ANOVA, $F_{[2,530]} = 0.420$, $p = 0.657$). Despite this, there were some instances in which substantial differences did arise between the three data series: whereas the single-pool model never differed from observed values by >1.0‰, the three-pool model sometimes differed from observed values by as much as 3.5‰, usually in a positive direction indicating that when turnover effects are operating, tail hair series may underrepresent the C_4 component of elephant diets. Actually, significant differences did arise between the three series of estimated % C_4 intake (RM ANOVA, $F_{[2,530]} = 7.791$, $p < 0.001$); a Bonferroni post hoc test revealed the difference was only between the three-pool model compared with the other two series ($p < 0.05$ in both cases). The mean difference in estimated % C_4 intake was less than 1.5%, but the significance of the effect arises because there were individual points along the series where the three-pool turnover model predicted diets comprising as much as 30% more C_4 compared with the other two series. In other words, whereas raw data and the single-pool model predicted mean diets comprising from 0% to ~50% C_4 grass, the three-pool model suggested that diets comprising as much as ~75% C_4 grass are evident in some phases of the series.

Adjustments of observed $\delta^{15}N$ values for attenuation also had no significant effect (RM ANOVA, $F_{[2,530]} = 1.204$, $p = 0.301$). Nonetheless, there were again individual observations in which dampening of $\delta^{15}N$ switches along hair strands were evident (of up to 1‰ for the single-pool model and 4.5‰ for the three-pool model).

Free-ranging Individuals from Kruger Park: habitat- and age-related differences

Given that turnover adjustments did have some influence on diet reconstructions, albeit small on average, we focused the remainder of our analyses on results of those models rather than the observed data. However, it must be noted that calibration of tail hair data from Kruger Park elephants using our growth

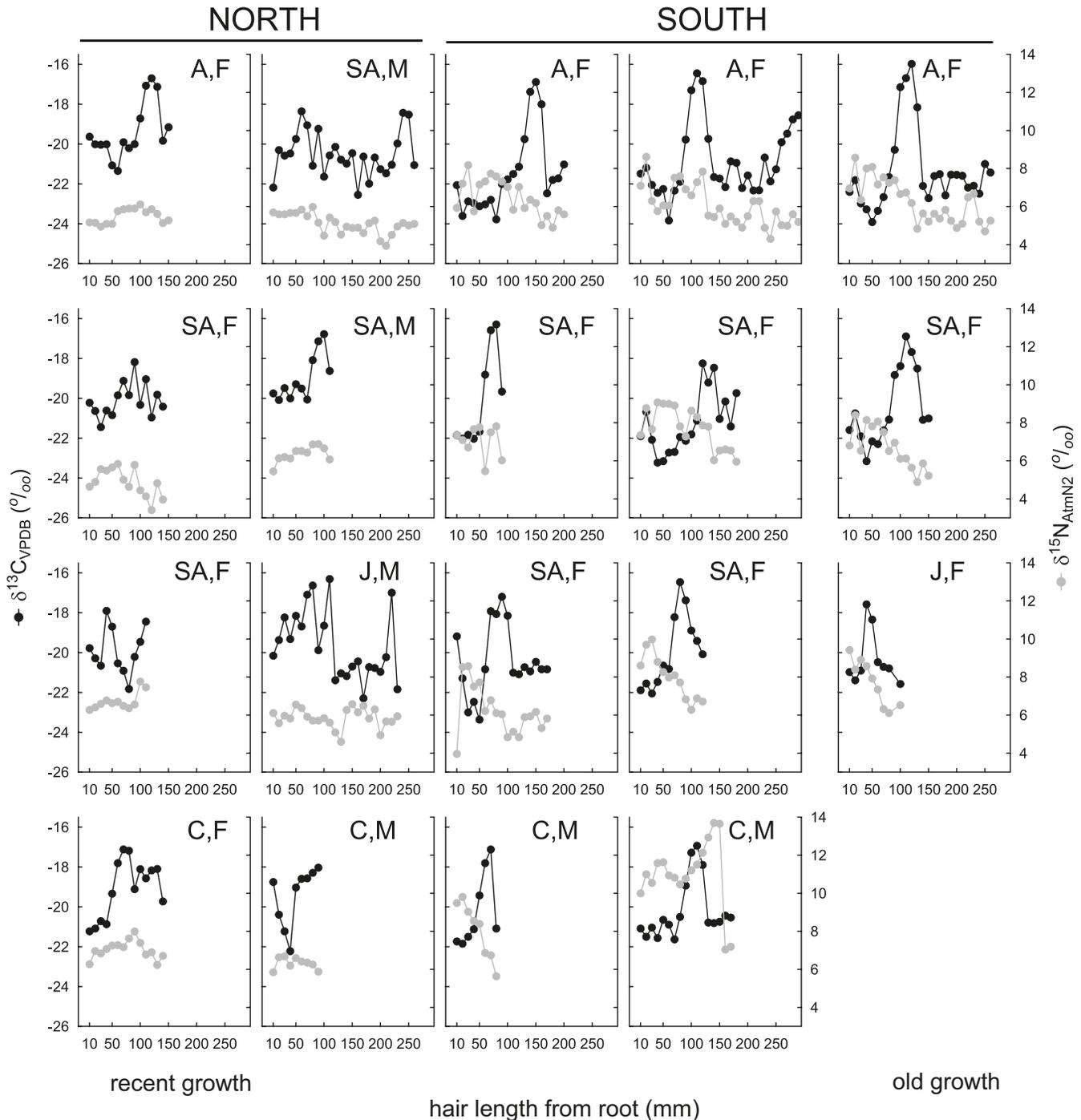
rates—and those of Cerling et al. (2006, 2009)—did not reveal synchrony in the timing of isotopic shifts across individuals, implying there was significant variation in growth rates among the free-ranging individuals. Thus, hypotheses tested below focus on differences in isotopic variability across regions and across age classes, but not on the timing of switches.

Median $\delta^{13}C$ values of hairs from the northern Kruger Park was higher than in the south, implying significantly more C_4 grass consumption by the elephants in the north (~27% compared with ~16% C_4 intake, on average, respectively; Mann–Whitney U test, $U_{[8,11]} = 1.0$, $p < 0.001$; Fig. 4a). Also, the range of values observed along hair strands was significantly lower for individuals from the north (shifts of ~40% and ~30% C_4 intake, respectively; Mann–Whitney U test, $U_{[8,11]} = 10.0$, $p = 0.005$; Fig. 4b). Comparisons of maximum and minimum values across individuals revealed that the differences in diet ranges do not arise because of shifts in maximum C_4 grass intake (Mann–Whitney U test, $U_{[8,11]} = 29.0$, $p = 0.216$; Fig. 4c), but because the minimum C_4 grass intake is lower in the south than the north (Mann–Whitney U test, $U_{[8,11]} = 20.0$, $p = 0.046$; Fig. 4d).

Age-related shifts in $\delta^{13}C$ values and in estimated % C_4 grass intake occurred among southern but not northern Kruger Park elephants (probably because of the lower levels of variability in the latter). Among individuals from the south, median $\delta^{13}C$ values decreased from calf to adult, implying a tendency towards lower % C_4 intake with age (from >20% to <10%; $R_S = -0.729$, $p = 0.011$; Fig. 4a). This change coincided with an increased $\delta^{13}C$ variability with age (variations in dietary C_3/C_4 composition were in the range of ~30% for calves and ~50% for adults; $R_S = 0.753$, $p = 0.008$; Fig. 4b), which occurred due to a decrease in minimum but not maximum (Fig. 4c) C_4 grass intake with age ($R_S = -0.921$, $p < 0.001$; Fig. 4d). Similar results for north–south differences in diet and for age effects were found for series corrected using the three-pool turnover model, except that these revealed no significant shift in median % C_4 intake with age ($R_S = -0.454$, $p = 0.161$), again suggesting that the observed data and even the single-pool model had underrepresented the upper levels of C_4 grass in the diet in some cases. The decrease in estimated % C_4 intake with age among southern elephants nevertheless persisted in these series ($R_S = -0.789$, $p = 0.004$).

For the $\delta^{15}N$ series, elephants from the south had higher median values (7.5‰ compared with 6.0‰ for the north; Mann–Whitney U test, $U_{[8,11]} = 18.0$, $p = 0.032$; Fig. 4e), and consistent with the results for $\delta^{13}C$, more variable values (ranges averaged 4.2‰ compared with 1.8‰ for the north; Mann–Whitney U test, $U_{[8,11]} = 0.0$, $p < 0.001$; Fig. 4f). However, in this case the difference occurred because of higher maxima for the south (Mann–Whitney U test, $U_{[8,11]} = 1.0$, $p < 0.001$; Fig. 4g), whereas minimum $\delta^{15}N$ values were similar for both regions (Mann–Whitney U test, $U_{[8,11]} = 37.0$, $p = 0.563$; Fig. 4h). An effect of age class on $\delta^{15}N$ values was also recorded for elephants from the south (but not from the north), with median, maximum, and minimum $\delta^{15}N$ values all declining

Fig. 2. Tail hair stable isotope profiles for 19 individual African elephants (*Loxodonta africana*) from Kruger National Park, South Africa. The two left-hand columns represent individuals from the northern Kruger Park, whereas the right-hand columns represent the south. Age classes and sex: A, adult; SA, subadult; J, juvenile; C, calf; F, female; M, male.



with age (respectively, $R_s = -0.864$, $p < 0.001$; $R_s = -0.599$, $p = 0.042$; and $R_s = -0.758$, $p = 0.007$; Figs. 4e, 4g, and 4h). Because both the maxima and the minima shifted in the same way with age, variability in $\delta^{15}\text{N}$ did not differ across age classes ($R_s = 0.169$, $p = 0.619$; Fig. 4f).

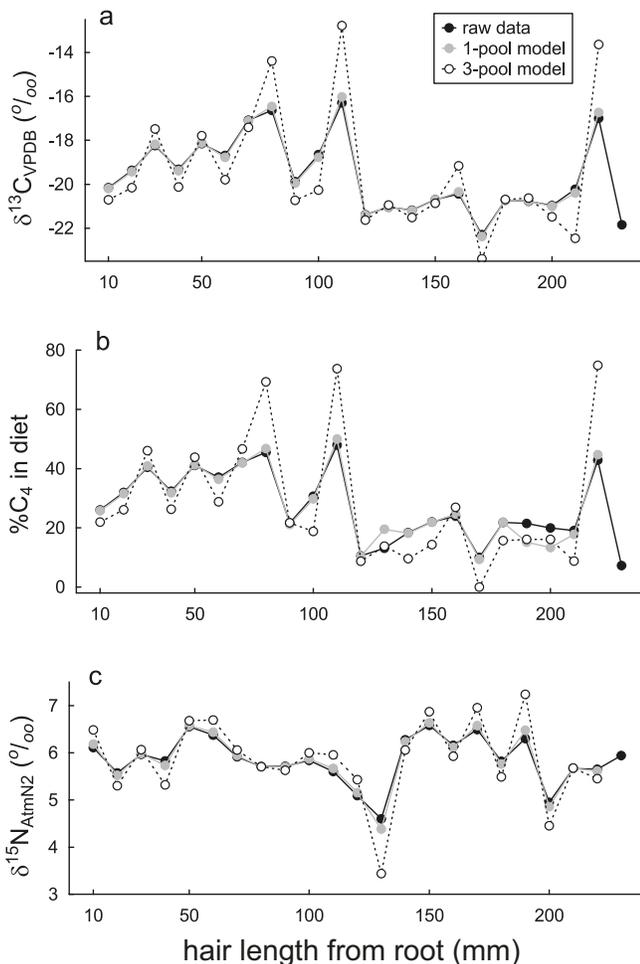
Discussion

Stable isotope series along tail hairs of elephants from Kruger Park revealed habitat-, season-, and age-related diet switches within individuals. The temporal scale recorded by our sampling

protocol was validated using tail hairs of elephants fed semicon-trolled diets in captivity, including a diet shift from free-range to captive conditions.

Data from captive elephants are limited because their diets were not homogenous, introducing uncertainty in estimates of δX_{eq} , which in turn can lead to upwards or downwards bias in estimates of the turnover rate constant λ (Martínez del Río et al. 2009; Wolf et al. 2009). Because of uncertainty in estimates of δX_{eq} , we were also unable to fit multiple turnover pools to our model, which often provide more realistic λ values (Carleton

Figs. 3a–3c. Comparison of observed stable isotope series in tail hair of African elephants (*Loxodonta africana*) with adjusted series based on a single-pool and a three-pool turnover model, respectively. This example is for one individual from Kruger Park; the same comparisons were made for all individuals included in this study. Note that because of attenuation during tissue isotope turnover, the magnitude of diet switches are slightly dampened without data adjustments.



et al. 2008). Thus, parameters estimated from our experiment are probably not generally applicable, although they do compare favorably with rate constants derived from experiments using other large herbivores (Ayliffe et al. 2004) and were therefore deemed suitable for our purposes.

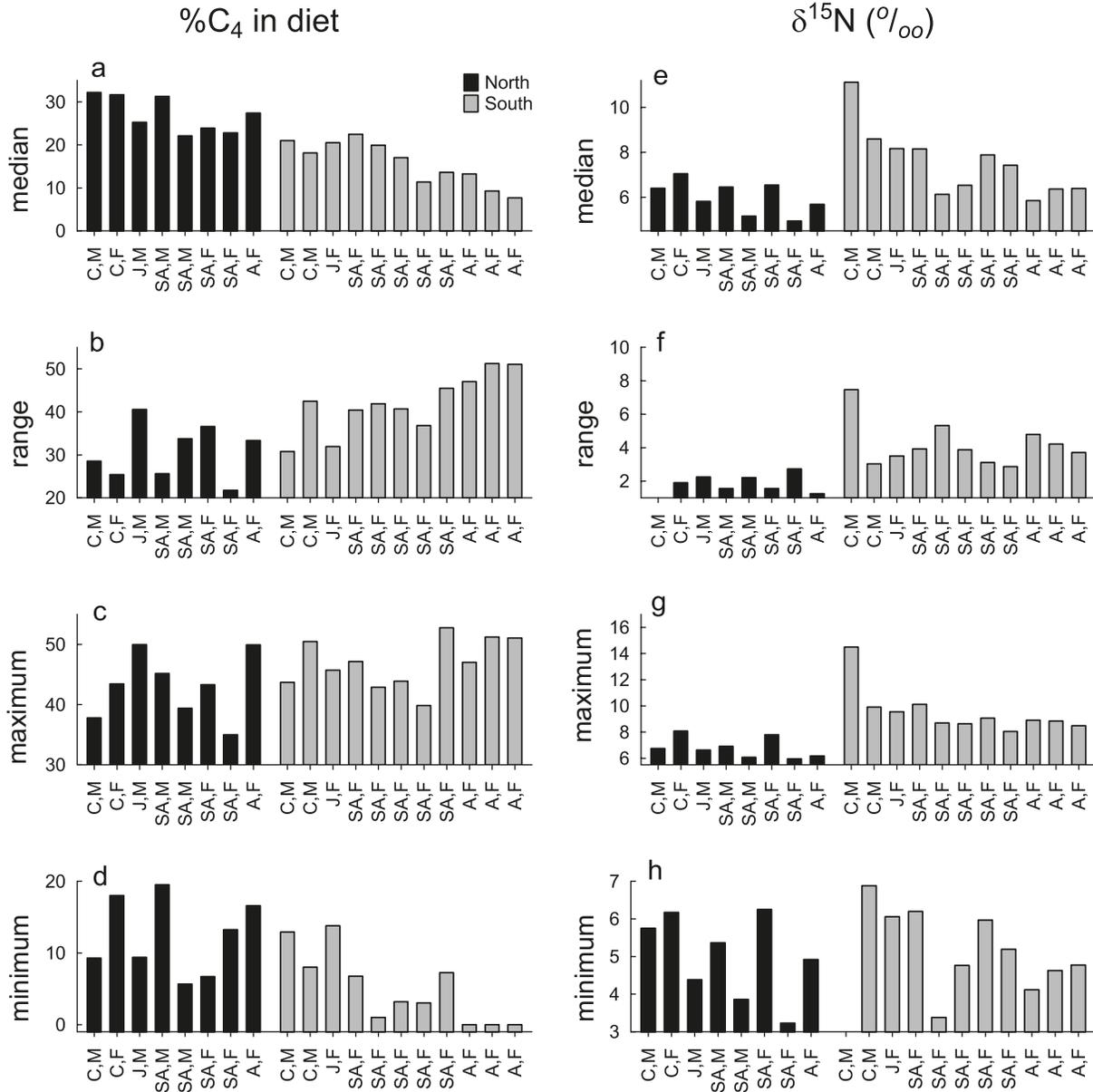
Adjustment of data series from free-ranging elephant tail hairs using our parameters derived from a single-pool model did not differ, on average, from series adjusted using a three-pool turnover model parameterized from a longer-term controlled feeding experiment of horses (Ayliffe et al. 2004). In some cases, diet switches to increased C_4 grass intake seemed to be underrepresented based on our parameters, and more so in the raw data. Actually, estimates of $\%C_4$ intake in elephants based on isotope turnover and mixing models are known to be sensitive to small changes in parameter values (Wittemyer et al. 2009); consistent with our results is that, due to model sensitivity, estimates of C_4 intake are often underrepresented in proteinaceous tissues like hair (Codron et al. 2011a). These observations highlight the need for more accurate information about isotope turnover rates in subject taxa.

Growth rates derived from captive elephants in this study were approximately 0.34 mm/day. However, when assuming a fixed

growth rate, we were unable to detect synchrony in isotopic trajectories of free-ranging elephant hairs. Understanding sources of variation in hair growth rates is thus a limiting factor for isotopic tracking of diet histories. Some sources of variation evident in our data are worth discussing. First, the figure derived here is about half of that recorded for East African elephants (Cerling et al. 2006, 2009). The latter results indicate sex-based differences, with growth rates of males being slower than for females (approximately 0.55 and 0.81 mm/day, respectively). In our study, hair growth rates for males were faster (0.36–0.37 mm/day) than for females (0.30–0.33 mm/day), but unfortunately sample sizes were too small ($n = 2$ and $n = 3$, respectively) to test this hypothesis statistically. The difference in growth rate between southern and East African elephants also suggests that geography is a contributing factor, but whether this is environmentally or genotypically induced is unclear. The fact that the elephants used in our study were relatively young (11 years or less) is unlikely to account for the geographic difference because one would expect faster growth in young growing animals. Stress and (or) pathology may also contribute to variation in hair growth rates, as evinced in our data for the two elephants that were in poor condition when they were captured (and subsequently treated in captivity).

At this stage we therefore cannot be certain about the rate of hair growth of each free-ranging individual, but it is clear from results of captive elephants that tail hair series sampled at 10 mm increments archived diet records at finer-than-seasonal scales. Among free-ranging individuals from Kruger Park, $\delta^{13}C$ shifts were evident in all cases, corresponding to expectations for gradual increases in C_4 grass intake through the wet season and gradual increases in C_3 browse intake in the dry season (Owen-Smith 1988; O'Connor et al. 2007; Codron et al. 2011b). Temporal diet switching was of such a magnitude that the isotopic niche breadths of individuals overlapped substantially, supporting the idea that while in many smaller taxa individuals may have specialized niches within their populations (Bolnick et al. 2003, 2007), large herbivores like the elephant do not use this foraging strategy (Codron et al. 2012). Nevertheless, our results do show that dietary niches of herbivores can differ across habitats and that individuals of different demographic units may use different components of the resource. In elephants, there appears to be a broadening of dietary niche breadths with age, which is expected because larger herbivores can tolerate lower quality food items and can therefore use a larger proportion of the total resource (Van Soest 1994; Clauss et al. 2003), and (or) because larger size facilitates access to food items, in particularly browse, that may be inaccessible for smaller individuals. Actually, our data indicate that the minimum $\delta^{13}C$ value (maximum level of C_3 biomass intake) along an elephant tail hair series decreases with age, whereas the maximum $\delta^{13}C$ value (maximum level of C_4 biomass intake) is independent of age. This finding shows that rather than expanding the C_4 component of the dietary niche, the rate of browsing increases with age, which probably occurs because as elephants become larger they can access food resources higher up in the canopy, and using their trunks have a longer vertical feeding envelope. Previously, Sukumar et al. (1987) reported lower $\delta^{13}C$ values in adult compared with juvenile Asian elephants (*Elephas maximus* L., 1758), which is consistent with our results. An observational study in another South African savanna also showed that adult females fed from higher up in the tree canopy when foraging in proximity to juveniles, but whether this behaviour is altruistic or due to intraspecific competition is not yet resolved (Woolley et al. 2011). Nonetheless, the latter observation may explain why we only found differences between age classes among southern Kruger Park elephants, as these specimens all come from a single herd. Hairs from the north represent individuals from multiple herds, and it may be that age-related dietary differences only arise when individuals within a herd are interacting with each other.

Figs. 4a–4h. Comparison of Kruger Park African elephant (*Loxodonta africana*) diet metrics showing differences between the northern and southern Kruger Park habitats, as well as age-related patterns (data adjusted using a single-pool isotope turnover model). Because the distribution is biased towards females, sex-related comparisons are excluded, but individuals' sexes are marked in the plots. Age classes and sex: A, adult; SA, subadult; J, juvenile; C, calf; F, female; M, male.



The general habitat effect on isotope niches of elephants is that individuals from the southern Kruger Park had broader isotope niche breadths than those from the north, which occurred because the δ¹³C minima recorded for the south were lower than those for the north, with the net effect that elephants from the south consumed more C₃ browse, on average. This concurs with previous results from faeces (Codron et al. 2011b) and from ivory (Codron et al. 2012) that were previously interpreted to be the result of the homogeneous woody vegetation characterizing northern Kruger Park habitats. Based on our tail hair data, elephant δ¹⁵N niches were also broader in the south than in the north and some of the variation in the south was linked with age-related decreases. Reduced δ¹⁵N values from calf to juvenile stages are consistent with expected trophic shifts between suckling and nonsuckling individuals (see e.g., Balasse et al. 2001; Polischuk et al. 2001).

Although patterns in δ¹⁵N series agree with the hypothesis of overall greater variations in behaviour among southern Kruger Park elephants, they do not necessarily indicate differences in diet composition. For one, there is no consistent difference in δ¹⁵N values of vegetation from northern and southern Kruger Park (Codron et al. 2005). Similarly, tail hair δ¹⁵N values of captive elephants did not reflect the diet shift: values decreased following the switch from wild into captivity, despite that the bulk of their diet in captivity had a higher mean δ¹⁵N value than most of the natural vegetation in the area. One factor that could contribute to low consumer δ¹⁵N values is if the diet comprises relatively low-quality proteins (i.e., a small proportion of dietary proteins are actually retained and digested; Robbins et al. 2005). Such nutritional stress is, however, not expected for animals in captivity, where animals were fed dietary supplements and where they were observed to gain mass consistently (R. personal communication,

2009). Furthermore, the revealed fecal glucocorticoid metabolite (FGM) levels for elephants at EFAF indicate that these animals are not differently affected by potential stress-related environmental and ecophysiological factors than their free-ranging counterparts. Elephants at EFAF had FGM levels comparable with those from elephants sampled in zoological institutions located in Europe and the United States (Ganswindt et al. 2005a), as well as from animals monitored in East and South Africa (Ganswindt et al. 2005b, 2010a). Actually, the low $\delta^{15}\text{N}$ values of elephants at EFAF may indicate relatively low levels of nutritional stress because when nutrients are limiting, animals rely more heavily on catabolically derived proteins, effectively synthesizing body proteins at higher trophic levels and resulting in higher $\delta^{15}\text{N}$ values (Hobson et al. 1993). The reverse of this trend could explain the relatively high $\delta^{15}\text{N}$ values of free-ranging elephants in this study and free-ranging elephants may have experienced diets of lower biological value, if for example tannins in their natural forages were implicated in precipitating proteins through the alimentary canal.

Studies of diet patterns among wildlife have focused largely on mean values, or seasonal mean values, of individuals and populations. Stable isotope series from incremental tissues like hair record diet histories within individuals, which provide new insights into dietary variability within individuals. These new insights are making important contributions to our understanding of how populations of different species are structured by their unique foraging strategies (Bolnick et al. 2007; Araújo et al. 2011). Such knowledge can make it possible to manage discrete units of populations in different ways, according to the habitat and dietary requirements of each. In high-impact species like elephants, such functionally distinct units may indicate that each has a different degree and type of impact on vegetational landscapes. Future studies focusing explicitly on how different management policies (which treat either the whole population or each age class within it as the ecological unit) would, or do, influence elephant populations and the impacts they have on habitats are a potentially important next step towards understanding biodiversity consequences of growing elephant populations.

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