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Establishing chronologies from isotopic profiles in serially collected animal tissues: An example using tail hairs from African elephants

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ABSTRACT

While the use of stable isotopes in wildlife ecological research is growing rapidly, development of methods to establish time-specific isotope data from continuously growing animal tissues are lacking. Using serially collected tail hairs from wild African elephants (*Loxodonta africana*), we develop and compare four techniques to collate temporal isotope chronologies from metabolically inert tissues for which formation/growth overlapped in time. The influence of variation in within hair growth rates and other sources of error in the presented techniques are explored and found to be inconsequential relative to the 5-day tissue sampling interval. Using a floating point regression approach, we find a high degree of correlation between independently derived isotope profiles from the same and different individuals in the study ecosystem. Remotely sensed Normalized Difference Vegetation Index (NDVI) data is compared with the isotope derived diet chronologies from five elephants developed independently. Diet shifts from browse to grass occurring at the onset of the wet season were highly synchronized, while early dry season diets varied across individuals. These methods are applicable across a variety of keratinous tissues and even teeth and, as demonstrated by our results, can be implemented using profiles from different individuals or relating profiles to environmental variation (seasonality). As such, the presented methods allow the establishment of high resolution temporal data on diet, movement, and climatic conditions experienced by an organism in many research settings.

1. Introduction

Ratios of stable isotopes in animal tissues provide unique information on the environmental conditions experienced by organisms (West et al., 2006) and therefore new insights into animal ecology (Rubenstein and Hobson, 2004), diets of both fossil and modern animals (Hobson and Clark, 1992a,b; Bocherens et al., 1996; West et al., 2006), and climatic variability (Cerling et al., 1997; Iacumin et al., 2000). Continually growing animal tissues provide the opportunity to derive temporal records of animal diet, animal movement, and environmental variability experienced by individual animals (Dalerum and Angerbjorn, 2005). Here we present methods for establishing isotope chronologies on continually growing tissues and apply these methods to assess the relationship between seasonality and diet shifts among free ranging African elephants.

Traditional methods for estimating diet entail opportunistic point sampling of feces, stomach contents, or direct observation of feeding behavior; these methods are prone to temporal and spatial sampling error because of sample accessibility in time and space (Reynolds and Aebischer, 1991; Votier et al., 2003). Therefore, point samples will necessarily lack information in comparison to continuous records. While ratios of stable isotopes in animal tissues reflect the average diet or environmental conditions experienced by an animal during the time that tissue formed—modern or fossil (Best and Schell, 1996; West et al., 2006), continuously growing metabolically inert tissues like hair can theoretically provide such information at nearly continuous temporal resolutions (dependent on the tissue growth rate and sample mass required for analysis). In addition, collection of such tissues requires less time and is not prone to the limitations of classically employed techniques (Dalerum and Angerbjorn, 2005).

In this paper, we (1) present and compare four methods to develop time-specific isotope profiles derived from analyses of serially collected hair samples, (2) use these methods to assess potential sources of error in time-specific diet estimation caused by (a) variation in tissue growth rates over time and (b) sensitivity of elephant diet estimation to mixing pools originally parameterized on horses (West et al., 2004), and (3) provide a methodological hierarchy for chronology establishment based on the type of comparative

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information available. Finally, an example of the temporal accuracy of diet calculation is presented where we relate the timing of dietary shifts of the study elephants to seasonal ecological changes measured using rainfall and remotely sensed Normalized Difference Vegetation Index [NDVI; a metric of photosynthetic activity often employed as a surrogate for vegetative productivity (Sellers et al., 1992; Pettorelli et al., 2005)]. The role of elephants as a primary ecosystem engineer and keystone species in savanna and forest ecosystems makes understanding the interaction between elephants and the vegetative communities they inhabit essential for conservation and management activities across Africa (Laws, 1970; Dublin et al., 1990; Pringle, 2008). The analysis presented here is conducted on isotope profiles from African elephants (*Loxodonta africana*) tail hairs, though the methods presented are applicable to other taxa and tissues.

2. Methods

2.1. Isotope analysis

We collected hairs opportunistically from wild African elephants. Hairs were collected at different times across a six year period beginning in July 2000. Hair samples were exported from Kenya and imported into the USA following the Conventional on International Trade in Endangered Species (CITES) regulations. Elephant hairs were serially sectioned in 5 mm intervals before stable isotope analysis, though smaller intervals of analysis are possible (West et al., 2006). Plant samples were collected on the 1st, 11th, and 21st of each month from October 2004 through July 2005; plants included six C_3 and four C_4 species, representing both legumes and non-legumes. In addition, a single day 14-location transect of >70 plants was collected in May 2006.

$^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of elephant hair and plant material were measured on an isotope ratio mass spectrometer (Finnigan 252, Bremen, Germany) following combustion in a flow-through modified Carlo-Erba system. Values are reported using the conventional permil (‰) notation where:

$$\delta^{13}C = \left(\left(\left(^{13}C/^{12}C\right)\text{sample}/\left(^{13}C/^{12}C\right)_{\text{standard}}\right) - 1\right)*1000$$

and an analogous terminology describes $^{15}N/^{14}N$ ratios. Standards are VPDB (Vienna Pee Dee Belemnite) and AIR for $\delta^{13}C$ and $\delta^{15}N$, respectively. Uncertainties for average $\delta^{13}C$ and $\delta^{15}N$ values for plants discussed in the text are reported as the standard error. Isotope enrichment between hair and diet is approximately 3‰ for both $\delta^{13}C$ and $\delta^{15}N$ (Deniro and Epstein, 1978; Cerling and Harris, 1999; Ayliffe et al., 2004).

2.2. Study area and population

The elephants sampled in this study inhabit the region in and around the 220 km^2 Samburu and Buffalo Springs National Reserve in northern Kenya (37.5° E 0.5° N). These semi-arid parks are dominated by Acacia–Comiphora savanna and scrub bush and located along the Ewaso N'giro River, the major permanent water source in the region (Barkham and Rainy, 1976). Rainfall averages approximately 350 mm per year and occurs during biannual rainy seasons generally taking place in April/May and November/December. The elephant population using these reserves are individually identified, following well established methods (Wittemyer, 2001), allowing hair sampling from the same individual across time. We present data from analysis of 50 different tail hairs collected from 18 elephants. For a more detailed description of the study population and ecology of the study area see Wittemyer (2001).

2.3. Tissue development/growth rates

Determination of tissue growth rates enables definition of the time–length relationship of a tissue, allowing time-specific isotope chronologies to be derived. We compared four methods to establish

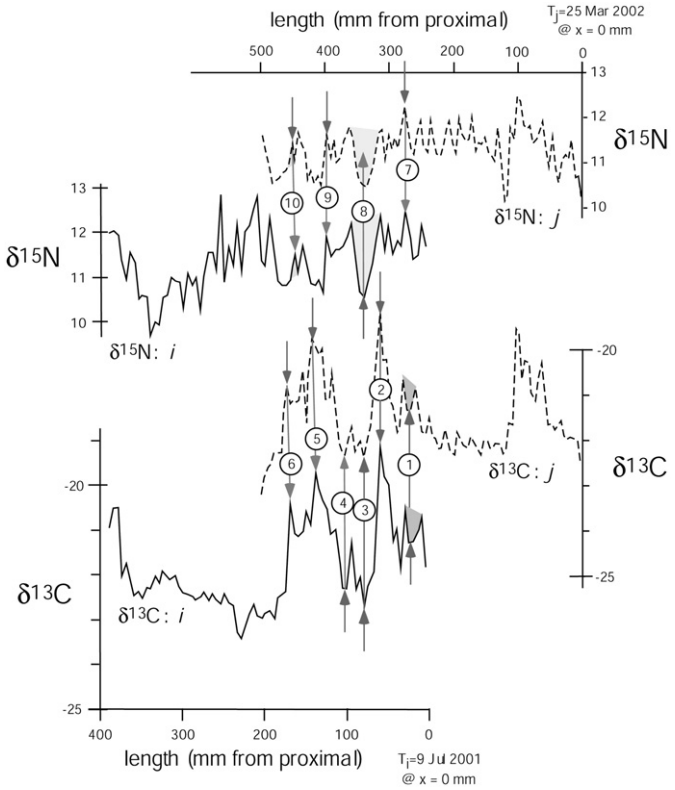

Fig. 1. The isotopic chronologies ($\delta^{13}C$ and $\delta^{15}N$) from two hairs (i, j) collected from the same individual on 9 July, 2001 (solid lines) and 25 March, 2002 (dashed lines) contain periods of identical isotopic variation. Matching points (indicated as 2–7, 9–10) or intervals (1 and 8), where isotopic profiles from the serially collected tissues mirror each other, can be used to derive time-specific growth rates of hairs and other metabolically inert tissues. Relating the difference in collection dates to differences in the lengths of tissues (top axis for hair j and bottom axis for hair i) across matching points and intervals enables accurate dating of the profiles. Secondary Y-Axis: $\delta^{15}N$ for hair collected on 25 March 2002, and $\delta^{13}C$ for hair collected on 22 March 2002. Primary Y-Axis: $\delta^{15}N$ for hair collected on 9 July 2001, and $\delta^{13}C$ for hair collected on 9 July 2001.

growth rates of tissue. We assume, for the present, that the growth rate of each individual sample is constant.

2.3.1. Algebraic solution

Consider two different hairs, i and j, collected at different dates, T_i and T_j, so that the period of time between collection dates is:

$$\Delta T_{j-i} = T_j - T_i \tag{1}$$

From visual inspection one can select points along the length of the hairs where isotope signatures appear to be identical between hairs—here we denote the length of each hairs at match point x as $H_{i(x)}$ and $H_{j(x)}$ (Fig. 1). Selection of two or more match points (x, y,…, n) allows calculation of the relative growth rates between the two hairs. For each pair of match points, $x-y$, the relative growth rate $K_{i(x-y)/j(x-y)}$ is calculated as:

$$K_{\frac{i(x-y)}{j(x-y)}} = \frac{H_{i(x)} - H_{i(y)}}{H_{j(x)} - H_{j(y)}} = \frac{G_{i(x-y)}}{G_{j(x-y)}} \tag{2}$$

where $G_{i(x-y)}$ and $G_{j(x-y)}$ are the absolute growth rates for each hair over the interval $(x-y)$. H_i at match point x is converted to the corresponding calendar date as:

$$T_{i(x)} = T_i - \frac{H_{i(x)}}{G_{i(x-y)}} \tag{3}$$

and we know that:

$$T_{i(x)} \approx T_{j(x)} \tag{4}$$

where the approximation in time is dependent on the temporal period represented by the sampling interval. So:

$$T_i - \frac{H_{i(x)}}{G_{i(x-y)}} \approx T_j - \frac{H_{j(x)}}{G_{j(x-y)}} \quad (5)$$

Assuming the absolute difference in match points is negligible, the absolute growth rate of either hair can be solved by combining Eqs. (1)–(3):

$$G_{j(x-y)} = \frac{K_{\frac{i(x-y)}{j(x-y)}} H_{j(x)} - H_{i(x)}}{K_{\frac{i(x-y)}{j(x-y)}} \Delta T_{j-i}} \quad (6)$$

and $$G_{i(x-y)} = K_{\frac{i(x-y)}{j(x-y)}} G_{j(x-y)} \quad (7)$$

The best estimate of hair growth rate is then the average of growth rates calculated across many match points, enabling calculation of the variance in estimates and confidence intervals if required.

2.3.2. Minimization solution

By using the true growth rate of hair i (G_i) to replace the interval growth rate in Eq. (3), the true date $T_{i(x)}$ at each match point x can be calculated as:

$$T_{i(x)} = T_i - \frac{H_{i(x)}}{G_i} \quad (8)$$

which, in relation to Eq. (4), means:

$$T_{j(x)} = T_j - \frac{H_{j(x)}}{G_j} \quad (9)$$

where $T_{j(x)}$ is calculated using the true growth rate of hair j (G_j). Differences in the dates of the match points x across each hair i and j is calculated as:

$$\varepsilon_x = T_{i(x)} - T_{j(x)} = \left(T_i - \frac{H_{i(x)}}{G_i}\right) - \left(T_j - \frac{H_{j(x)}}{G_j}\right) \quad (10)$$

for point x, where ε_x is derived from inaccuracies in the estimated true growth rates, G_i and G_j, in combination with slight difference in the true dates of the match points for each hair. Using multiple match points n, the sum of errors can be calculated:

$$\varepsilon_T = \sum_{x=1}^{n} \varepsilon_x \quad (11)$$

Assuming differences in the actual dates of match points are fixed (i.e. difference can not be reduced by selecting "better" match points), ε_T is dependent on the accuracy of growth rate parameters G_i and G_j. Minimization of ε_T by searching the parameter space for G_i and G_j (the minimum to maximum possible hair growth rates for the species), results in an optimized solution for the growth rates.

For individuals with more than two hairs collected on different dates, all hairs can be combined simultaneously, minimizing

$$\varepsilon_T = \sum_{w=1}^{m} \sum_{x=1}^{n} \varepsilon_x \quad (12)$$

where w is the comparison between 2 hairs and m is the number of pairs of hairs considered. To resolve the growth rates of multiple hairs simultaneously from one individual, our approach was to use a Monte Carlo simulation with random draws of growth rates until the minimum ε_T was derived. Alternatively, a minimization algorithm to search the hair growth rate parameter space could be employed, though the algorithm design need be sensitive to erroneous results from local optima solutions. Jackknife techniques were used to get estimates of variances for growth rate values (Shao and Tu, 1996).

2.3.3. Visual (spreadsheet) matching

Non numerical solutions to growth rates can be conducted through the visual alignment of the selected match points $H_{i(x)}$ and $H_{j(x)}$. Using Eq. (3) (relating hair length to time), correspondence of isotope signature from different hairs can be visually aligned on a spread sheet by increasing growth rates to stretch hair chronologies or reducing growth rates to compact chronologies. This simple method can be performed simultaneously for any number of hairs.

2.3.4. Comparison to NDVI

Previous studies have shown that elephant diet calculated using stable isotopes in hair is correlated with the intake of grass during the rainy season because of the predominance in Africa of C_4 grasses (Cerling et al., 2004, 2007). NDVI is a measure of photosynthetic activity (Sellers et al., 1992; Pettorelli et al., 2005) and so it is expected that there should be a correlation between % grass in diet and NDVI in our system. We matched peaks in $\delta^{13}C$, which is directly related to changes in the fraction of C_4 biomass in diet, with the peaks in NDVI. While matching isotopic shifts to seasonal variation is plausible in the highly seasonal savanna study ecosystem, such strong seasonal signals may not be present in all systems.

2.4. Variation in growth rates

In the previous analyses, we assume growth rates in each hair are constant, but that different hairs may have different growth rates. Variability in the growth rate of a single hair, however, is possible and would decrease the temporal accuracy of chronologies and the correlation between different hairs. The most likely time for a change in the growth rate of hair would be during periods of the heaviest metabolic stress (Robbins, 2002), which for female large mammals is likely to be the 3 month period of heavy lactation following birth (Oftedal, 1984). Births in the study population typically occur during the onset of the wet season, when high energetic expenditure associated with early lactation period can be covered by intake from high quality forage (Wittemyer et al., 2007). Of the elephants used in this study, M5 Anastasia gave birth on 10 Aug 2003 and, rather unusually, this birth occurred during the dry season when baseline metabolic stress would be relatively high on account of low quality forage. The last parturition date of M4 Cleopatra, a female from the same behaviorally characterized family as Anastasia, was 4 Dec 2002, thus metabolic costs associated with lactation were likely not a factor for her during the period of greatest constraint for Anastasia. We compare 10 $\delta^{13}C$ and 10 $\delta^{15}N$ "match points" distributed across 509 days of overlap between single hairs from both Anastasia and Cleopatra, where growth rates of hairs for the two females were established independently of each other using the minimization routine described previously. The temporal sampling interval for hairs of Anastasia and Cleopatra were 5 and 6 days, respectively. The dates of match points have uncertainty estimates based on the sampling interval of each hair, allowing calculation of the confidence interval around match point dates. We assess the proportion of match points for which differences in dates occur within and outside the temporal confidence interval, comparing the fit of Anastasia's and Cleopatra's diet chronologies before, during, and after Anastasia's metabolically intense period.

2.5. Floating mid point correlation

Because hairs grow at different rates, sectional isotope samples taken from different hairs at the same length intervals (e.g. 5 mm) do not represent the same time intervals. In order to compare chronological isotopic profiles across hairs with different growth rates, incremental data must be equated temporally between hairs. Where ~x indicates temporally nearest neighbor points in two hairs, i and j, for which the attributed date (T) of hair i occurs before the attributed date of hair j, we

calculate the temporal mid point between sequential data points from the different hairs as:

$$\Delta T_{j-1(\sim x)} = \frac{T_{j(\sim x)} - T_{i(\sim x)}}{2} \quad (13)$$

Linearly interpolated isotopic values for the mid point between temporal nearest neighbor points is calculated for hair i and j respectively as:

$$\Delta T_{j-i(\sim x)} \left[\frac{\delta^{13}C_{i(\sim x)} - \delta^{13}C_{i(\sim x+1)}}{T_{i(\sim x)} - T_{i(\sim x+1)}} \right] + \delta^{13}C_{i(\sim x)} \quad (14)$$

$$\Delta T_{j-i(\sim x)} \left[\frac{\delta^{13}C_{j(\sim x-1)} - \delta^{13}C_{j(\sim x)}}{T_{j(\sim x-1)} - T_{j(\sim x)}} \right] + \delta^{13}C_{j(\sim x)} \quad (15)$$

The degree of correlation can then be assessed between these calculated "floating" mid points across all points ($\sim x$) of each hair using standard regression techniques. Using this method, we assess the degree of correlation between isotope profiles from hairs collected on the same day from the same individual, the same individual but separated by multiple months, two different individuals from the same family group collected on different dates, and two unrelated individuals collected at different dates. The growth rates of hairs collected from the same individual are self correlated, while growth rates of hairs collected from different individuals are (*i*) derived independently (self correlated) or (*ii*) estimated by correlating isotope profiles from the different individuals to each other. For hairs from different individuals, we present correlation coefficients from both methods of analysis.

2.6. Diet model

The proportion of C_4 (grass) to C_3 (browse) comprising diet was estimated using the isotope turnover pool model of Ayliffe et al. (2004) originally parameterized from controlled experiments on horses. We use $\delta^{13}C$ end-member values of −27.5‰ and −13.5‰ for C_3 and C_4 plants, respectively, which represent average ecosystem values for the Samburu NR and nearby regions (Fig. S1). A three pool model best characterized the temporal changes in diet in controlled feeding experiments of horses (West et al., 2004; Cerling et al., 2007). Conducting controlled feeding experiments on elephants was not feasible, therefore we assessed the sensitivity of elephant diet estimates to horse derived model parameters. Proportional changes in the calculated percent of C_4 grass in diet was assessed by halving and doubling pool turnover times, fractional contribution of each pool, and the ratio between the short and long pools (holding the medium pool constant). Although ecosystem $\delta^{13}C$ end-members were derived from broad sampling of vegetation in the study system, we also assessed the sensitivity of the model to the $\delta^{13}C$ end-members. The dietary component of C_3 and C_4 vegetation is estimated using this model on isotope profiles from serially collected tail hairs of five elephants from 2001 and 2002. Variation in diet is compared with rainfall records and Normalized Difference Vegetation Index data (NDVI).

Table 1
Growth rates (±S.E.) of elephant hairs determined by three different methods described in the text.

Elephant ID	Collection date	Length (mm)	Visual (mm/day)	Algebraic (mm/day)	Minimization (mm/day)	NDVI (mm/day)
M2	7-Jul-00	115	0.73	NA	0.68 ± 0.02	NA
M5	9-Jul-01	388	0.74	0.71 ± 0.03	0.72 ± 0.01	0.70
M5	25-Mar-02	500	0.94	0.93 ± 0.02	0.93 ± 0.01	0.91
M5	10-Apr-03	550	0.92	0.93 ± 0.01	0.91 ± 0.01	0.86
M5	22-Jul-04	580	1.03	1.05 ± 0.01	1.03 ± 0.01	0.99
M4	19-Nov-03	475	0.86	0.81 ± 0.03	0.86 ± 0.01	0.80
M4	27-Jul-04	460	0.84	0.85 ± 0.02	0.84 ± 0.01	0.76
M4	27-Apr-05	415	0.89	0.89 ± 0.01	0.89 ± 0.01	0.83

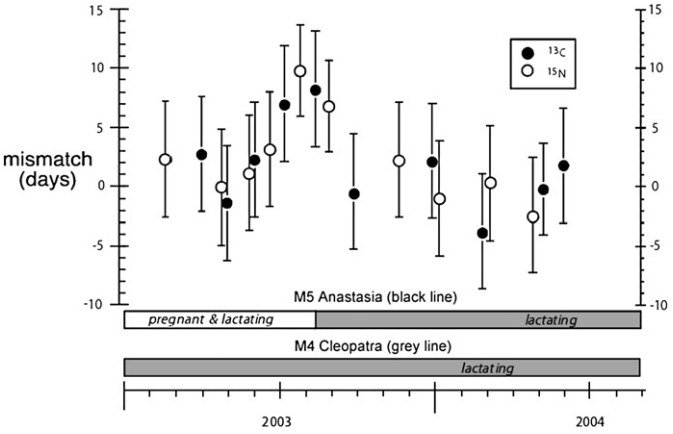

Fig. 2. Differences between match points from two individuals in the same family group, Anastasia and Cleopatra, are typically within the temporal resolution of hair sample intervals (±5.5 days). However, during Anastasia's post-partum period coinciding with the long dry season when resource quality is poor the offsets between match points were greater than the sampling error during a 2–3 month period. The reproductive state of each female is presented graphically as bars. Interestingly, this indicates that during periods of extreme metabolic stress within hair growth rates may vary slightly, though the variability in growth rate only caused an offset of <5 days greater then the sampling interval represented in each data point.

3. Results

3.1. Comparison of different growth rate calculation methods

In Table 1 we report the results from the different methods for estimating growth rates. All growth rate calculation methods were sensitive to the degree of overlap and isotopic structure for the $\delta^{13}C$ and the $\delta^{15}N$ signatures. For sample dates that differ by several months or more, providing greater overlap and number of match points, the similarity between methods was greater. Minimization of the sum of differences between match points using the Monte Carlo random draws solves for the most accurate growth rate, regardless of properties of the isotope profile overlap. The algebraic approach was the most sensitive to the number and spread of match points, becoming unreliable when the overlap was less than two months. Manual point matching in a spreadsheet application (e.g. Excel) provided comparable results to those derived from quantitative techniques, which are virtually indistinguishable (Table 1). The correlation of $\delta^{13}C$ maxima with NDVI gives growth rates that are about 6% less than the other methods (the cause of this offset is discussed below). The algebraic, minimization, and visual methods gave indistinguishable growth rates (paired *t*-test; $P>0.7$ for all comparison). The correlation to NDVI was significantly different for comparison to the other methods (paired *t*-test, $P<0.001$) for all comparisons; however, increasing the growth rate by 6% for the NDVI-derived growth rates gave growth rates indistinguishable from the other methods (paired *t*-test; $P>0.5$ for all comparisons). All techniques require identification of match points by the user and will be subject to greater error when profile isotope structure is poorly defined. Such error is, in part, a function to the inherent uncertainty imposed by the sampling interval (in our case, 5 mm), as accuracy can not be greater than the sampling interval.

3.2. Is growth rate constant?

Variation in within hair growth rates was minimal, with the range of differences in "match" dates being −4 to +9 days for the 20 match points assessed during the 509 days of overlapping data between chronologies of Cleopatra and Anastasia. Because the sampling period of each data

Table 2
Sensitivity analysis of parameters used for diet estimation.

Sensitivity	Average % change	StDev % change	Maximum % change	Diet range (%C4)
1/2 Pool length (days)	0.86	0.64	3.71	0.65–61.90
2× Pool length (days)	0.81	0.72	4.04	−2.42–69.48
1/2 Fractional contribution	2.15	1.69	9.22	3.84–56.39
1.5× Fractional contribution	4.83	4.29	26.14	−15.25–91.75
End member max + 1	(−) 1.40	0.69	4.23	0.93–61.37
End member min − 1	(+) 5.06	0.69	6.52	5.44–67.83

point is the equivalent of the average signal during approximately 5.5 days, "perfect" matches occur where differences between the dates of match points are ±5.5 days. For most of the time interval under consideration for these two females, the match points relative to each other were within the temporal sampling interval demonstrating that the time-specific isotope profiles (independently derived) of these two individuals were not distinguishable. Interestingly, the period with clear differences between the timing of match point coincides with Anastasia's parturition date during the dry season of 2003 when forage quantity and quality are low (Fig. 2). Similarly, West et al. (2004) previously found constant growth rates for tail hair form horses despite diet changes from low- to high-protein contents.

3.3. Sensitivity to diet model parameters

Prior to applying an isotope turnover pool diet model parameterized from a controlled experiment on horses to isotope profiles from elephant tail hairs, we investigated the sensitivity of model output to parameters including pool lengths, fractional contributions of pools, and isotope end members. Model outputs using elephant isotope profiles were relatively robust to variation in horse optimized model parameters (Table 2). Halving or doubling pool lengths in the model caused on average less than 1% change in dietary calculations. Doubling pool lengths lead to estimates of below 0 proportions of C_4 grass. The model was more sensitive to the fractional contributions of different pools. By halving the contribution of the shorter pools, diet estimates on average changed by slightly more than 2%. Increasing the short pool by 1.5 times elicited the greatest change, with estimates of the proportion of C_4 increasing on average by nearly 5%. Such a change, however, caused substantial below 0 estimates of the proportion of C_4 grass and, therefore, was unlikely. The model appeared to be most sensitive to changes of diet end members. In particular, an incremental decrease of 1‰ in the $\delta^{13}C$ end member on average caused an increase in the estimated proportion of C_4 grass over 5%. End members, however, for this study were well resolved

Table 3
Correlation coefficients of $\delta^{15}N$ and $\delta^{13}C$ profiles from different hairs with overlapping time intervals and $\delta^{13}C$ from hairs and NDVI from a matching period.

Category	ID Hair1	Date pulled	ID Hair2	Date pulled	Length of overlap (mm)	R^2
SameDate/ individual	S30	25-Feb-04	S30	25-Feb-04	230	0.93
SameDate/ individual	Meru35	12-Jul-01	Meru35	12-Jul-01	200	0.91
DifferentDate/ SameIndividual	M5	9-Jul-01	M5	25-Mar-02	210	0.89
DifferentDate/ FamilyRelated	M5	22-Jul-04	M4	27-Jul-04	460	0.79 (matched) 0.79 (independent)
DifferentDate/ Unrelated	M5	25-Mar-02	M31	2-Feb-02	315	0.45 (matched) 0.28 (independent)
Hair/NDVI	M5	Jan-00 to Jul-04	NDVI	Matching Period	4 1/2 years	0.33 (no lag) 0.38 (lag 10 days)

All correlations were significant with p values < 0.001.

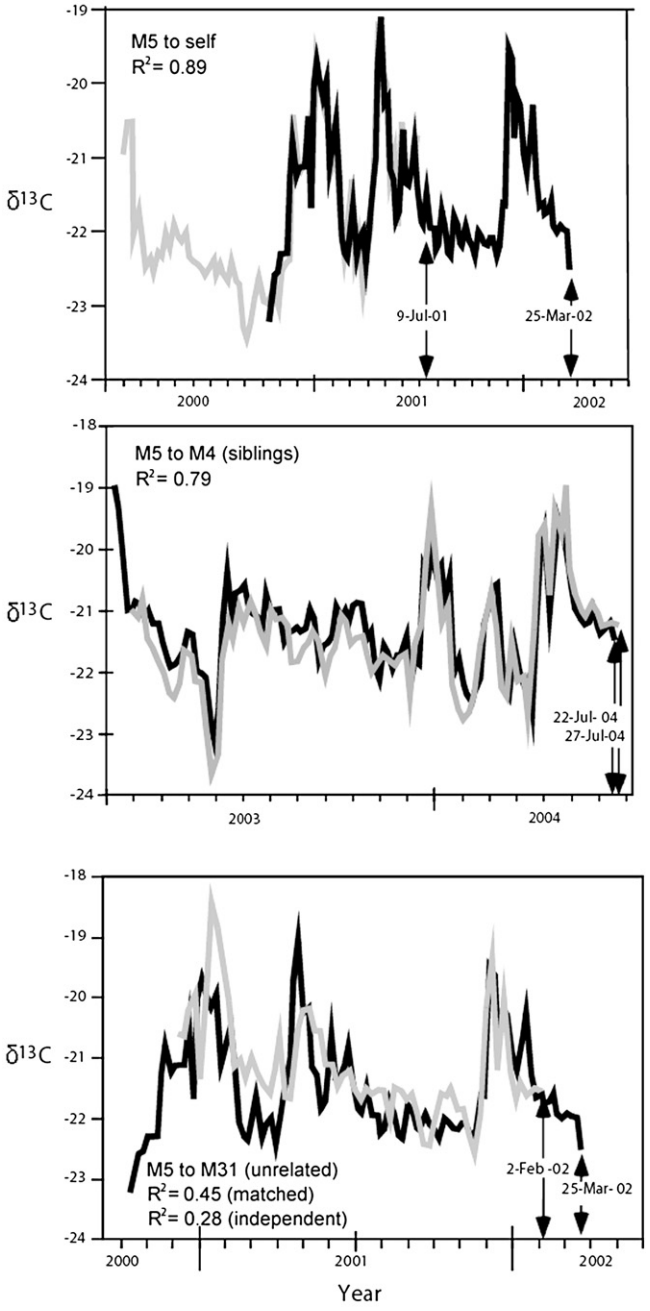

Fig. 3. $\delta^{13}C$ profiles (‰ on y-axis) from individually collected elephant tail hairs (gray and black represent chronologies from the different hairs) demonstrate intervals of strong correlation during periods where profiles overlap temporally (x-axis). Collection dates of each hair are indicated with arrows. The correlation between isotope profiles is quantified using a floating point regression technique described in the methods across the same and different individuals (Table 3). Chronologies of hairs collected from the same individual at different times (A) are strongly correlated ($R^2 = 0.89$). Hair chronologies from different individuals can be derived independently (using serially collected hairs from the same individuals) or by correlating with an established chronology from another individual. Chronologies from different individuals in the same social unit (B) demonstrate the same degree of correlation ($R^2 = 0.79$) whether derived independently or correlated to each other. Chronologies from different individuals that are not socially related (C) show less correlation ($R^2 = 0.44$), particularly when derived independently ($R^2 = 0.28$). Even independently derived chronologies, however, demonstrate similar seasonal changes.

from isotope analysis of over 300 tree and grass samples in the ecosystem (Figure S1), though use of seasonal end members would ensure greater accuracy in diet estimates. While the amplitude of model outputs varied slightly with changes to parameters, the general pattern of dietary shifting remained.

3.4. Correlation between different hairs and climatic signals

As presented in Table 3, isotope signatures were most strongly correlated between hairs collected from the same individual on the same day or for hairs collected on different dates from the same

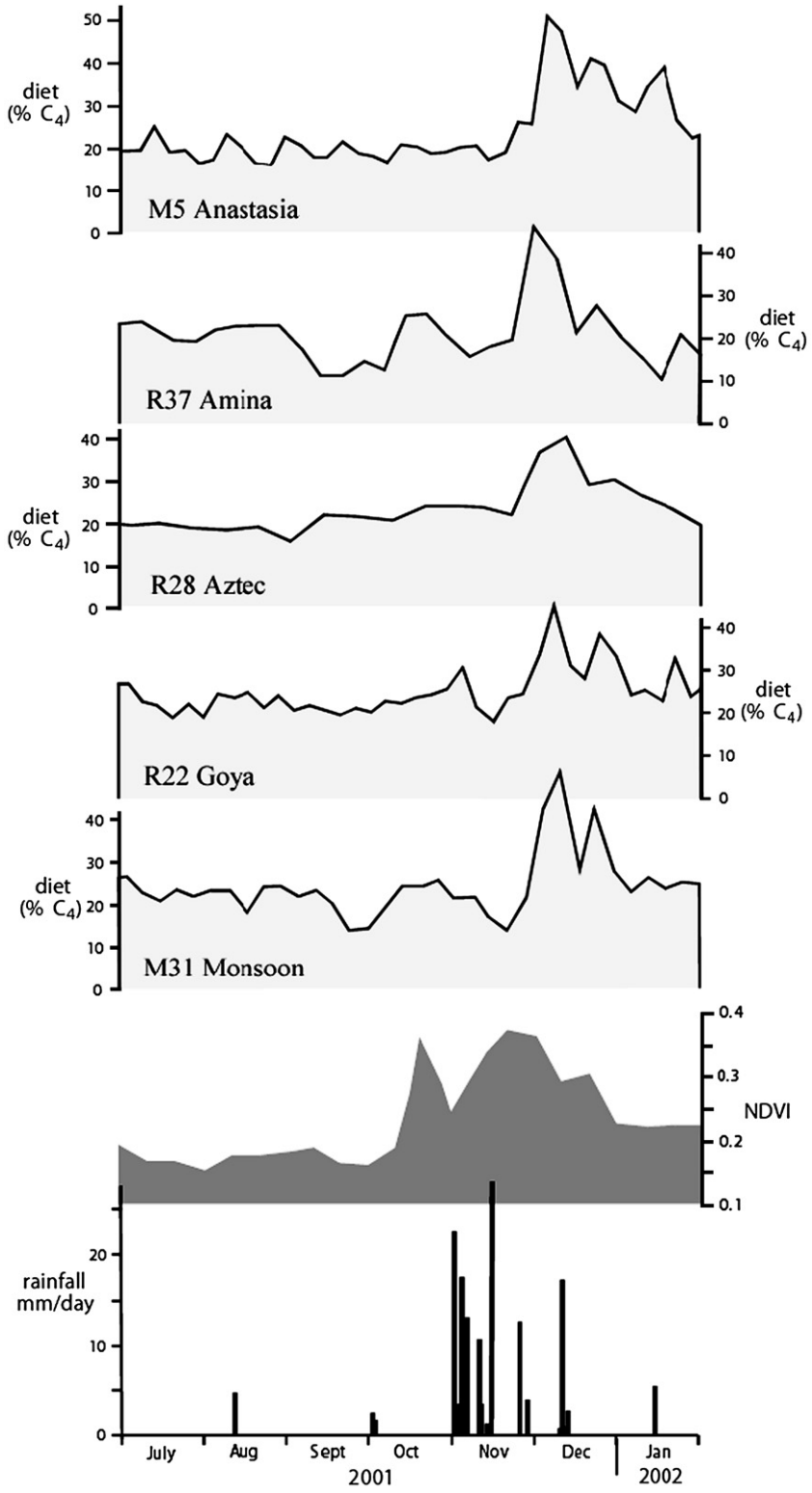

Fig. 4. Calculated equivalent C_4 percentage of diet for five elephants with independently determined chronologies for the period 1 July 2001 to 31 January 2002. (A. Anastasia. B. Amina. C. Aztec. D. Goya. E. Monsoon.). The calculated diets are compared to remotely sensed Normalized Difference Vegetation Index (NDVI) and rainfall measurements for the same period. Dietary shifts are lagged by about two weeks after seasonal vegetation productivity pulses as recorded by NDVI and four weeks after the start of the seasonal rains (F. NDVI. G. Rainfall at Archer's Post Meteorological Station).

individual. Isotope profiles from individuals within the same family demonstrated only slightly weaker correlations (Fig. 3). Even correlations between completely unrelated individuals inhabiting the same ecosystem are sufficient to derive hair growth rates. Correlations of profiles with productivity metrics like NDVI, which capture the seasonal nature of the study system, are also sufficiently strong for use in the determination of growth rates, though it is essential that use of such seasonal signals for chronology establishment is independently assessed prior to implementation in other study settings. Isotope profiles, however, demonstrate relatively consistent lags of ~6% relative to NDVI.

Differences causing decreased correlation between profiles appeared to relate primarily to small scale oscillations, as major seasonal signals were well matched across individuals. Comparison across five independently derived diet chronologies demonstrates dietary shifts among elephants are strongly related to season. During the November 2001 wet season, all five elephants demonstrated coordinated switching from a primarily C_3 browse diet to a strongly C_4 grass based diet (Fig. 4). Dietary switching lagged primary productivity pulses, as measured using remotely sensed NDVI, by about two weeks, which in turn lagged rainfall by about two weeks.

4. Discussion

By overlapping isotope chronologies from serially collected hair, we were able to calculate the growth rates of hairs using four methods (algebraic, minimization, visual, and comparison to NDVI) with a high degree of accuracy, allowing development of time-specific isotopic chronologies for wild elephants. Results were comparable across methods and not impacted by slight variation in tissue growth rates, with differences between methods or errors caused by inherent properties of hair growth generally being less than the sampling interval from which chronologies were derived. Applications in applied and theoretical ecology for time-specific isotopic data derived from continuously growing tissues abound, including in studies of temporal variation in feeding behavior (Hall-Aspland et al., 2005; Inger et al., 2006), changes in niche (Bearhop et al., 1999; Bearhop et al., 2004), indices of individual fitness characteristics (Darimont et al., 2007), and connectivity and migration between ecosystems (Best and Schell, 1996; Hobson, 2006; West et al., 2006). Furthermore, comparison between modern and fossil profiles can allow novel insight to historical climate patterns. The presented methods are applicable across a wide variety of taxa on any metabolically inert, continuously growing keratinous materials, including baleen, horn, otoliths, feathers and even teeth.

4.1. Hierarchy of methods for determining growth rates

Lacking repeated tissue samples from the same individuals decreases the ability to resolve growth rates and impacts the scale at which isotope and behavioral correlates can be analyzed, however, time-specific data can still be developed. Our analysis indicates that isotope chronologies from the same and different individuals, as well as between chronologies and seasonal ecological signals are significantly correlated (Table 3). Building on these results, we have developed a seven level framework for deriving the growth rates of

Table 4
Hierarchy of confidence levels for growth rates (1: highest; 7: lowest).

	Degree of information	Proximal end of tissue	Distal end of tissue
1	Both ends fixed	Collection date	To previous hair from same individual
2	Both ends fixed	Collection date	To older hair from related individual
3	Both ends fixed	Collection date	To older hair from unrelated individual
4	One end fixed	Collection date	To NDVI or rainfall
5	One end fixed	Collection date	Average tissue growth rate (male/female)
6	Neither end fixed	Approximate date known	Match to 1–4
7	Neither end fixed	Average tissue growth rate (male/female)	

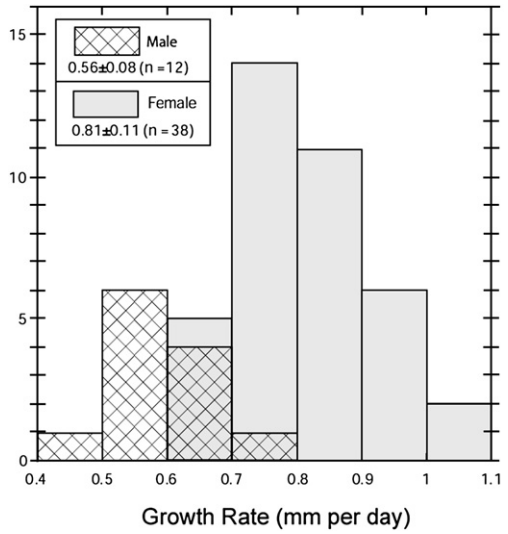

Fig. 5. The histogram of growth rates from 50 elephant tail hairs (established using the methods presented in this paper) demonstrates major differences between the growth rates across sexes. The average and standard deviation of tissue growth rates can be used to date isotopic chronologies and estimate the relative date error to sample interval ratio, information important for understanding the resolution at which chronologies are accurate.

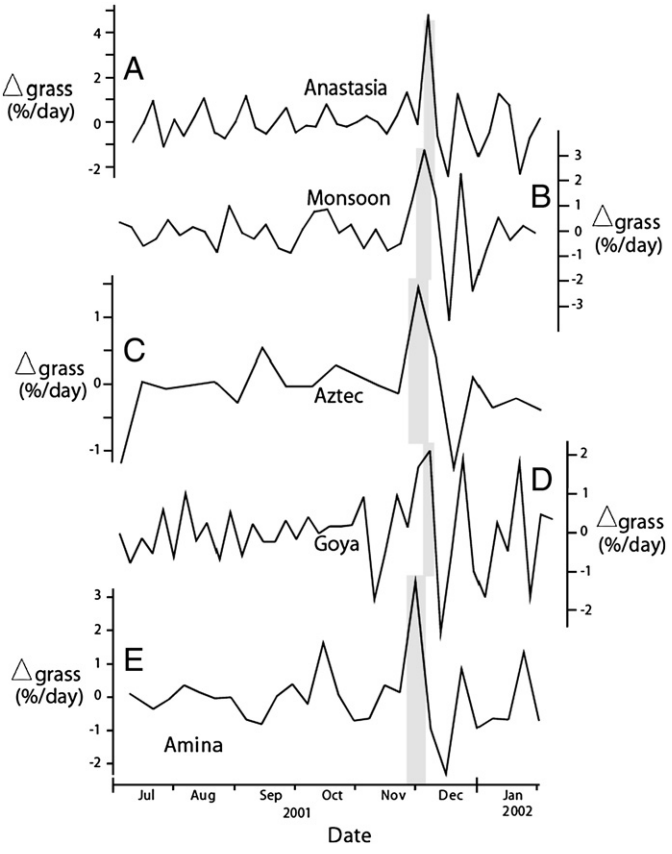

Fig. 6. The difference in calculated diets (%C_4 equivalent/day) for five elephants during one dry and one wet season demonstrate peak changes in diet occur within the same week and are related to seasonal changes. Chronologies for the five elephants were determined independently. A. Anastasia. B. Monsoon. C. Aztec. D. Goya. E. Amina.

tissues. The most accurate methods for growth rate establishment is to relate isotope chronologies to tissues samples collected from the same or different individuals from the same ecosystem (Table 4 Standards 1–3). If strong seasonal variation is known, growth rates can be derived by comparing chronologies to terrestrial seasonal indices such as rainfall, remotely sensed data like NDVI, or temperature records (Table 4 Standard 4), though ideally the use of seasonal signatures should be applied only after independent testing for seasonal dependence of isotope chronologies. Species in marine ecosystems may also display seasonal variation in their ecology that can be used for chronology establishment (Lewis et al., 2006). Where information is available on tissue growth rates, chronologies can be derived where the variance of known growth rates sets the resolution at which chronologies can be dated (Table 4 Standard 5). For instance, we have determined the growth rates of over 50 hairs from elephants in Kenya, finding that on average males have a significantly slower growth rate than females (males: 0.56 ± 0.08 mm per day; females: 0.81 ± 0.11 mm per day; (Fig. 5). Even in circumstances with minimal information (Table 4 Standards 6–7) temporal information can be applied to isotope chronologies to get an idea if the periodicity in variation relates to known phenomena.

4.2. Elephant diet shifts

We apply these methods to determine the timing of isotopic dietary signals of five elephants. Dietary experiments were not feasible for elephants, therefore we applied a three pool dietary model parameterized using controlled feeding experiments on horses as both species are hindgut fermenters utilizing cecal digestion (Robbins, 1993). We expect minor modification if these parameters are fitted experimentally for elephants. Sensitivity analysis on the impact of horse derived model parameters indicated elephant dietary estimates are most sensitive to ecosystem end-member inputs into the model (Table 2), for which we have reliable information from analysis of over 300 vegetation samples collected during both the dry and wet season. In this analysis, we use an average value for seasonal difference in end members, but an adaptive model incorporating seasonal changes in end members is likely to improve the accuracy of diet estimation by a few percentages.

Near synchronous behavior in diet shifts was found for five different elephants that were resident in the same national reserve in Kenya. Their response was to vegetative productivity, reaching maximum grass intake about 2 weeks after the NDVI/productivity peak, which in turn was delayed by about 2 weeks from the onset of the rainy season (Fig. 4). These elephants had a baseline equivalent C_4 intake of about 20% C_4 grass, which increased rapidly to about 50% for a few weeks. The major switch to grass occurs at precisely the same time (when considering the ± 5 day value of each diet record) across all five elephants (Fig. 6). The delay between rainfall and diet switching may relate to the rate of grass growth or variation in nutrient composition over the growing season (Dublin et al., 1990), and demonstrates foraging pressures on different vegetative communities vary strongly by season. Furthermore, our methods allow differences in foraging behavior to be quantified temporally, demonstrating that variation in diet across the studied individuals was greatest during the early dry season. This probably reflected differences in foraging strategies reflecting the period of greatest resource heterogeneity in the ecosystem—as a function of the water dependence of different species and the stochastic spatio-temporal nature of rainfall in savanna ecosystems. The use of such temporally accurate diet chronologies offers an important advance in the study of animal foraging behavior.

The methods we present here allow detailed assessment of the relationship between modern climate regimes and isotope chronologies derived from animal tissues. In addition to contemporary research, comparing samples analyzed from modern fauna with their ancient counterparts offers an important reference for paleoclimatology and paleoecology studies (West et al., 2006). Although rare, fossil hair is sometimes preserved in dry or permafrost environments (Macko et al., 1999), providing the opportunity to study the influence of inter-annual climate regimes in fossil species during the late Pleistocene and Holocene.

5. Conclusions

Because isotopic signals in elephant hairs demonstrated greater variation than background noise such as that introduced from laboratory influences (tissue sample resolution), isotope chronologies from continuously growing tissues could be related to time with a high degree of accuracy. Other sources of noise are inherent to isotopic data and can potentially impact interpretation of signals in tissues, such as difference in assimilation efficiencies of dietary components, metabolic routing, and differences in tissue fractionation (Gannes et al., 1997). Furthermore, an animal's physiological state may influence the incorporation of isotopes into different tissues over time. As such, it is important to relate isotope data to biological information of an organism in order to understand the contribution of environmental and physiological inputs, particularly as both are likely to be related. Such limitations are discussed in detail elsewhere (Dalerum and Angerbjorn, 2005), and highlight the importance of considering all sources of isotopic variation in interpretation of isotopic time series. In summary, isotope records in continuously growing hair offer a media whereby time-specific information on climatic and dietary conditions of an individual are recorded. Accessing this information offers an important addition to wildlife biology studies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemgeo.2008.08.010.

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