

**Supplementary Information for Uno et al., High-resolution stable isotope profiles of modern elephant (*Loxodonta africana*) tusk dentin and tail hair from Kenya: implications for identifying seasonal variability in climate, ecology, and diet in ancient proboscideans**

<b>Background and Methods.....</b>	<b>1</b>
<i>Site Background.....</i>	<i>1</i>
<i>Observational, Meteorological, and Remote Sensing Data .....</i>	<i>1</i>
<i>Sample preparation of ivory for histological analysis.....</i>	<i>2</i>
<i>Sample preparation for stable isotope analysis .....</i>	<i>3</i>
<i>Stable isotope analysis.....</i>	<i>4</i>
<i>Diet to enrichment and percent C4 calculations .....</i>	<i>5</i>
<i>Tusk and tail hair growth rates .....</i>	<i>6</i>
<b>Supplementary Discussion.....</b>	<b>8</b>
<i>SNR plant isotopes.....</i>	<i>8</i>
<i>Carbon isotopes in hair and tusk dentin.....</i>	<i>9</i>
<b>References .....</b>	<b>10</b>

## Background and Methods

### *Site Background*

The Samburu National Reserve (SNR) and Buffalo Springs National Reserve (BSNR), located at 0.5° N, 37.5° E, range in elevation from 800-1200 m, cover ~330 km<sup>2</sup> in the Samburu-Laikipia region, and provide a safe haven for the largest population of elephants living outside of protected areas in Kenya (Wittemyer et al., 2013). The primary source of water in the Reserves is the semi-permanent Ewaso Ng'iro River and its tributaries; the vegetation communities vary from *Acacia* (*Vachellia spp.*) and doum palm-dominated forests along the riparian corridors to *Vachellia-Commiphora* semiarid bushlands and *Acacia* wooded grasslands in the drier regions (Cerling et al., 2009; Wittemyer, 2001).

### *Observational, Meteorological, and Remote Sensing Data*

A suite of observational, meteorological, and remote sensing data provide the framework for behavioral, life history, climatic, and location information necessary to interpret the stable isotope profiles from the tusk and tail hairs. The use of GPS collars to track the location and movement of elephants provides much greater information than traditional radio collar methods (Douglas-Hamilton, 1998; Douglas-Hamilton et al., 2005). R37 was fitted with a GPS collar that recorded her location at 1 to 3 hour intervals for a nearly continuous period from 2001 through September 2006. From November of 1997 to July 1999, daily observational transects were conducted, primarily along water courses in and near the Reserves, identifying 767 individual elephants in the area (Wittemyer, 2001), and observational transects continue to date with the support of Save the Elephants staff.

Field observational data for R37 include known calving events on July 8, 1999 and April 27, 2004, inferred calving events (based on the age of existing calves) in the fall of 1996 and in 1992, and approximately 100 feeding observations of the Swahilis family between July 2001 and October 2002. Using data from GPS collars and field observations,

Wittemyer and Getz (2007) determined the rank of 20 family unit matriarchs in and around the Reserves; R37's rank of 13th classified her as mid-low in the social hierarchy and as a result, she had a larger home range than higher ranking matriarchs (549 km<sup>2</sup>, LoCoH method); spent less time in protected areas (i.e., the Reserves) than individuals with higher social rank; and had no core area in the Reserves as defined by the area in which 50 % of GPS points lie (Wittemyer et al., 2007).

Daily precipitation records spanning 1957 through 2006 come from measurements made at Archer's Post, located near the east end of the Reserves (Figure 1). The biannual wet seasons are associated with the migration of the Tropical Rain Belt. The majority of annual precipitation occurs during these seasons, referred to as the "long rains" (March-May) and the "short rains" (October-December). From 2000 to 2005, the average rainfall in the Reserves during the "long rains" and the "short rains" was 143 mm (max.: 259 mm; min.: 63 mm) and 193 mm (max.: 356, min.: 55 mm), respectively (Cerling et al., 2009).

Normalized Difference Vegetation Index (NDVI) is a satellite measurement that can be used as a proxy for net primary productivity (NPP), where

$$\text{NDVI} = (\text{NIR} - \text{RED}) / (\text{NIR} + \text{RED}), \quad (1)$$

and NIR and RED are the amounts of near-infrared and red light, respectively, reflected by the vegetation as measured by the satellite sensors (Pettorelli et al., 2005). NDVI works as a proxy for NPP, measured on a scale from -1 to +1, because chlorophyll absorbs RED and mesophyll scatters NIR. An NDVI value <0 indicates an absence of vegetation. We use NDVI data from two different satellites in this study. NDVI data from 1982 through 1987 come from the National Oceanic and Atmospheric Administration Advanced Very High Resolution Radiometer (AVHRR) satellite and has a spatial resolution of 8-16 km, with data collection taking place on the 1st, 11th, and 21st day of each month. NDVI data spanning 2000 to 2006 come from the Satellite Pour l'Observation de la Terre (SPOT). SPOT has spatial resolution of several meters and a semimonthly temporal resolution (Pettorelli et al., 2005). NDVI is summed over an area defined by a polygon that circumscribes nearly all GPS and observational location data for elephants using the Reserves (Figure 1).

### ***Sample preparation of ivory for histological analysis***

To develop spatially accurate sampling plans of tusk dentin and to conduct histological analysis for determining tusk growth rate, one longitudinal surface of each tusk dentin slab was polished and thin sections were made from the transverse surface of the proximal end of every other slab from the pulp cavity margin to the tip of the tusk.

For each thin section, the proximal ~5 mm was transversely cut, polished to ~1000 grit on one side, epoxied to a standard petrographic slide (27 x 46 mm), and cut to a thickness of *ca.* 250 microns with a Buehler® Isomet low speed saw. Tusk dentin thin sections were polished to a thickness of 120 ± 30 microns using successively finer grits of emery paper

ranging from 600 to 1200 grit, followed by 1.0 to 0.3 micron Buehler® aluminum oxide grit on a high-speed polisher. Longitudinal surfaces of tusk dentin slabs were polished by hand using successively finer grits of emery paper ranging from 320 to 1200 grit followed by high-speed polish with 1.0 to 0.3 micron Buehler® aluminum oxide grit.

Photomicrographs of the tusk dentin thin sections were taken using a Canon® Powershot A640 digital camera coupled to a petrographic microscope with an Alexis Scientific Clearshot Series 600 digital camera adapter. The camera shutter was controlled via computer using Canon® software. Images were taken at approximately 35x magnification under transmitted plane polarized light. A photomicrograph of a stage micrometer with 10 µm graduations was included with each sequence of thin section photomicrographs for scale. Approximately 20 to 30 photomicrographs were taken to cover a vertical transect of a tusk dentin thin section. Individual photomicrographs were stitched together in Adobe® Photoshop to produce a composite image from the pulp cavity margin or approximate structural axis of the tusk to the outer edge (Figure 3B).

Polished tusk dentin slabs were scanned in color at 9600 dpi using an Epson® 4490 Photo flatbed scanner (Figure 3A). Images were converted to gray scale and levels were adjusted in Photoshop to enhance growth increments for measurement of thicknesses.

### ***Sample preparation for stable isotope analysis***

Tusk dentin. Tusk dentin was sampled for stable isotope analyses by serial sampling parallel to second-order growth increments in tusk dentin slabs using a Micromill (Merchantek Micromill, now Electro Scientific Industries of Portland, Oregon, USA). The Micromill is equipped with a computer-controlled x-y-z stage with (~10-20) micron scale accuracy, a vertically mounted variable-speed (1200-35000 RPM) drill, and a 40x binocular microscope with a camera that is connected to the computer. A 1-mm carbide endmill bit was used for all milling (Brasseler # H21.11.010). A total of 639 samples were milled from three tusk dentin slabs (R37-DEN-1169, -1053, and -412). Tusk dentin slabs were individually mounted to the Micromill stage using hot-melt glue with the polished longitudinal surface facing up and approximately parallel to the stage. Second-order growth increments are visible under 40x magnification of the binocular microscope (Figure 3B), and these were used as guides to map out mill paths (scans) on the tusk dentin surface. Using the Scan Tool in the Micromill software, a series of “master” scan lines were drawn along a growth increment every ~1500 µm from the initial starting point towards the CDJ. Next, a series of mill paths were interpolated at ~100 µm intervals between master scan lines using the Interpolate Tool. Mill-path lengths were approximately 4.5 cm (min: 4.2 cm; max.: 4.6 cm). The average milling depth was 1000 µm (min.: 500 µm; max.: 1120 µm). Optimum milling depth is limited by the curvature of the growth increments. For example, near the pulp cavity horn where curvature is high, a relatively shallow (~500 µm) milling depth is required to prevent significant time averaging resulting from milling through multiple growth increments. Average sample yields (for a 100 µm width, 1000 µm depth, and a 4.5 cm length mill

path) were 5.3 mg (min <1; max ~10 mg) of dentin powder. Sample powders were collected onto weighing paper and placed into 0.65 ml centrifuge tubes.

Tail Hair. Tail hair was wiped with acetone to remove adhering contaminants (e.g., dust, oils, and dung). Elephant tail hair is quite thick, often on the order of 1-3 mm in diameter, and thus can be serially sampled for isotope analysis without cutting the hair into segments. Each hair was marked at 5 mm increments from the proximal (root) to the distal end and subsequently sampled at the midpoint between marked intervals (e.g., at 2.5, 7.5, and 12.5 mm). Approximately 500 µg was removed with a razor blade and transferred into a tin capsule for analyses. Wittemyer et al. (2009) determined an average tail hair growth rate of  $0.81 \pm 0.11$  mm/day for female elephants (n=38), and thus a 5 mm sampling interval represents approximately six days.

### ***Stable isotope analysis***

The carbonate component of hydroxylapatite in tusk dentin was analyzed for carbon and oxygen stable isotope ratios, reported as  $\delta$ -values relative to the Vienna Pee Dee Belemnite (VPDB) standard using permil (‰) notation where

$$\delta^{13}\text{C} \text{ } (\delta^{18}\text{O}) = (R_{\text{sample}} / R_{\text{standard}} - 1) \quad (2)$$

and  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the  $^{13}\text{C}/^{12}\text{C}$  ( $^{18}\text{O}/^{16}\text{O}$ ) ratios in the sample and in the standard, respectively, and the  $\delta^{13}\text{C}$  ( $\delta^{18}\text{O}$ ) value of VPDB is defined as 0 ‰.

Serially sampled tusk dentin powders were reacted with a 30 % hydrogen peroxide solution in 0.65 ml centrifuge tubes for 30 minutes, rinsed with DI water and centrifuged three times, and dried overnight at 60° C. Pretreatment results in a loss of *ca.* 40 % of initial sample mass. A subset of samples initially milled at 500 µm depths did not produce enough powdered dentin to be pretreated. That is, given the loss of powder during pretreatment, there would not have been enough powder for isotopic analyses, and therefore, the samples were analyzed untreated. Relatively low CO<sub>2</sub> yields were observed in the untreated (micromilled) powders during isotopic analysis that were attributed to low wettability during acid digestion. Dentin powder obtained from the same tusk slabs with a hand-held drill (Dremel) produced CO<sub>2</sub> yields more consistent with, but slightly lower than enamel. Thus, when sample mass was sufficient, pretreatment with H<sub>2</sub>O<sub>2</sub> was used to remove the organics. This resulted in improved wettability of dentin powder during acid digestion that led to increased CO<sub>2</sub> yields.

Dentin powder was digested in 100 % H<sub>3</sub>PO<sub>4</sub> (phosphoric acid) using a Finnigan CarboFlo coupled to the dual inlet on a Finnigan MAT 252 Isotope Ratio Mass Spectrometer (IRMS) at SIRFER. The CarboFlo system is a hybrid positive pressure/vacuum system with a common acid bath (CAB). Approximately 800 to 1000 µg of tusk dentin was weighed out into a silver capsule, which was used as a precautionary measure to oxidize any SO<sub>2</sub> produced during digestion in phosphoric acid.

Silver capsules containing powdered dentin were dropped from an autosampler carousel into the vigorously stirred CAB and reacted for 20 minutes at 90° C. A microcapillary tube submerged in the acid bath flushed it with 20 ml/minute of He and a 40 ml/minute He stream flushed the autosampler. The combined He streams swept the liberated H<sub>2</sub>O and CO<sub>2</sub> through a dry ice/ethanol trap to remove water and then through a liquid nitrogen (LN) trap to collect the CO<sub>2</sub>. Following the reaction period, the LN trap was isolated from positive pressure and evacuated with a rotary vacuum pump to ~10<sup>-3</sup> Torr. The LN trap was removed and the CO<sub>2</sub> was cryogenically transferred to a microvolume held at -170°C. Once transferred to the microvolume, the CO<sub>2</sub> was analyzed through the dual inlet system on the IRMS. A single point calibration of limestone internal standard reference material (SRM), UU Carrara ( $\delta^{13}\text{C} = +2.05\text{‰}$ ;  $\delta^{18}\text{O} = -1.85\text{‰}$ ), was used to calibrate to the VPDB scale. Internal SRMs of tusk dentin (R37-DEN:  $\delta^{13}\text{C} = -11.58\text{‰}$ ;  $\delta^{18}\text{O} = -1.84\text{‰}$ ) and modern enamel (MCM:  $\delta^{13}\text{C} = -13.44\text{‰}$ ;  $\delta^{18}\text{O} = -11.29\text{‰}$ ) were used for data correction and had average standard deviations of ~0.1 ‰ for  $\delta^{13}\text{C}$  and ~0.2 ‰ for  $\delta^{18}\text{O}$  across all analytical runs. The  $\delta^{18}\text{O}$  values of tusk dentin were calculated using a temperature-dependent acid fractionation factor of 1.00819 for calcite based on equation 2 in Passey et al. (2007).

Tail hair segments were analyzed for stable carbon and nitrogen isotope ratios, reported as  $\delta$ -values relative to the VPDB standard for carbon and atmospheric nitrogen (AIR) standard for nitrogen as above in Eq. 2. Hair samples were combusted in a Costech 4010 Elemental Analyzer at 1650° C and inlet to a Finnigan MAT 252 IRMS via continuous flow at the University of Utah's Stable Isotope Ratio Facility for Environmental Research (SIRFER). A single point calibration was used to calibrate to the VPDB and AIR reference scales. An internal standard reference material, SIRFER yeast ( $\delta^{13}\text{C} = -20.02\text{‰}$ ;  $\delta^{15}\text{N} = -1.24\text{‰}$ ), was used to correct sample values using linear or single value offset corrections. The mean standard deviation of SIRFER yeast pooled from all runs for the five tail hairs is 0.15 ‰ for  $\delta^{13}\text{C}$  and 0.13 ‰ for  $\delta^{15}\text{N}$ .

### ***Diet to enrichment and percent C4 calculations***

To calculate the %C4 in diet using  $\delta^{13}\text{C}_{\text{dentin}}$ ,  $\delta^{13}\text{C}$  data of plants ( $\delta^{13}\text{C}_{\text{plant}}$ ), and an enrichment factor between diet and bioapatite ( $\epsilon^*_{\text{diet-apatite}}$ ) must be determined. We use an enrichment factor of + 14.1 ‰ for  $\epsilon^*_{\text{diet-apatite}}$  based the value determined for large ungulates by Cerling and Harris (1999). The body mass dependent equations of Tejada-Lara et al. (2018) for a ~2100 kg elephant return a similar value of + 14.3 ‰. To calculate  $\delta^{13}\text{C}_{\text{diet}}$  from  $\delta^{13}\text{C}_{\text{hair}}$ , we use a  $\epsilon^*_{\text{diet-hair}}$  value of 3.1 ‰.

The  $\delta^{13}\text{C}$  values for C<sub>3</sub> and C<sub>4</sub> plants come from a large data set (n = 265) of plants collected in the Reserves between October 2004 and May 2006 during both wet and dry seasons (Cerling et al., 2009). The average  $\delta^{13}\text{C}$  values from this data set are  $-27.4 \pm 1.0\text{‰}$  for C<sub>3</sub> plants ( $\delta^{13}\text{C}_{\text{C3}}$ ) and  $-13.4 \pm 1.0\text{‰}$  for C<sub>4</sub> plants ( $\delta^{13}\text{C}_{\text{C4}}$ ). We use a two-end member mixing model to calculate % C<sub>4</sub>, where

$$\%C4 = (\delta^{13}C_{\text{diet}} - \delta^{13}C_{C3}) / (\delta^{13}C_{C4} - \delta^{13}C_{C3}) \times 100 \quad (3)$$

For tail hair  $\delta^{13}C$  ( $\delta^{13}C_{\text{hair}}$ ) values, we take an additional step toward reconstructing diet using the reaction progress variable (RPV) model, originally applied by Ayliffe et al. (2004) to horse tail hair and described in greater detail by Cerling et al. (2007). The RPV allows us to reconstruct instantaneous  $\delta^{13}C_{\text{diet}}$  and the  $\%C4$  vegetation in diet. The RPV model considers turnover time of dietary carbon using three pools that have different fractional contributions to hair. Pool turnover times are described by first-order rate constants, expressed as half-lives ranging from several to ~150 days.

### ***Tusk and tail hair growth rates***

The growth rate of serially sampled tissues must be known or determined in order to compare the stable isotope data to environmental or meteorological time series data such as NDVI or rainfall. A commonly used method for determining the growth rate of hair is visual matching (VM), whereby isotope profiles from serially sampled hairs ( $n \geq 2$ ) collected from the same individual are compared (Wittemyer et al., 2009). The growth rate of hair appears to be constant (West et al., 2004), but different hairs from the same individual may have different growth rates.

The growth rate of tusks can be determined using  $^{14}C$  bomb-curve dating (Uno et al., 2013) or by histological methods whereby growth increments with a known periodicity are counted (e.g., Fisher, 1996, 2008; Fisher and Fox, 2003). Tusk growth rates are not likely constant throughout the life of an elephant (Fisher, 2001). Fisher and Fox (2003) have shown mammoths and mastodons exhibit intra-annual and long-term variation in tusk growth rates, and it follows that their extant relatives probably do, too.

We use the VM technique described by Wittemyer et al. (2009) to determine growth rates of individual hairs of R37. To determine a growth rate, we first calculate the time between two collection dates of tail hairs that have overlapping isotope profiles. We then select one or more match points between the profiles, such as a peak in the  $\delta^{13}C$  profile, and align the overlapping profiles in Microsoft Excel by adjusting the relative position of the two profiles along a length scale (e.g., mm). Next, we calculate the distance from the proximal end of the more recent hair to the proximal end of the other hair. Finally, we divide this distance by the time between collection dates to get a growth rate for the more recent hair. To determine the growth rate of the second hair, the growth rate of that hair can be adjusted so that the differences in multiple match points between the two hairs are minimized. This can also be done mathematically in several ways, but Wittemyer et al. (2009) find no difference between methods. The VM technique can be applied to various metabolically inert tissue types, including elephant tusks. For tusks, visual matching was used to determine a linear growth rate that best matched  $\delta^{13}C$  peaks to NDVI peaks.

Bomb-curve  $^{14}\text{C}$  dating is another method for determining the growth rate of a metabolically inert tissue (Uno et al., 2013). Tissues that have been accurately dated include enamel, tusk dentin, hair, and horn. However, Uno et al. (2013) show elephant tusks yielded more accurate growth rates than tail hair. This is in part because tusks often represent decades of time, whereas tail hairs generally represent about one year's time. Tail hairs dated were also collected relatively recently (1998 to 2006), when the slope of the bomb-curve is shallower and resolving ages less than a year or two apart becomes challenging. Using the bomb-curve  $^{14}\text{C}$  dating technique, a linear growth rate of  $4.13 \pm 0.39$  cm/yr along the tusk axis was determined for the R37 tusk (Uno et al., 2013). Although tusk growth rate appears to be linear over the last 30 years of life based on bomb-curve  $^{14}\text{C}$  dating methods, the sampling resolution is too coarse to detect intra-annual variation. As for changes in long-term growth rates, we would expect the greatest variation to occur from the juvenile stage through early adulthood (~25 years of age), when overall growth rates are highest (Laws, 1966). This ontogenetic period is not present in the tusk of R37.

The large sample mass and sampling geometry required for determining growth rates by bomb-curve  $^{14}\text{C}$  measurement yields an axial growth rate, and converting this to a radial growth rate (normal to the pulp cavity appositional surface) for comparison to the VM and histologically determined radial growth rates is a complicated task. It requires that the angle between the tusk axis and the pulp cavity be known, and this can only be done if the tusk axis is present or can be reasonably approximated. It was only possible to do this on sample 1053 (Uno et al., 2013).

The third method to measure tusk growth rate is histological analysis of periodic growth increments, which provides evidence of intra-annual variations in growth rate. Growth rate was measured radially from the CDJ to the tusk axis. Using thin sections made from tusk dentin slabs 412, 1053, and 1169, we measured the thickness of (approximately weekly) second-order growth increments normal to the pulp cavity surface, or the appositional surface. Increment thicknesses were measured using an ImageJ plug-in called IncMeas v1.2, written by Adam Rountrey. We smoothed the increment thickness data with a 10-point running mean.

For direct comparison to the VM method, we limit growth increment thickness data to the intervals that were serially sampled, rather than the entire transverse section of the slabs. We do this because in addition to intra-annual variation in radial growth rate, there is also variation in increment thickness along the pulp cavity from the horn of the pulp cavity to the pulp cavity margin. That is, there is not a uniformly thick layer of dentin deposited along the pulp cavity each day (or week). A transect normal to the pulp cavity from the tusk axis to the CDJ is spatially equivalent to moving from the horn to the margin of the pulp cavity (Figure 2). Incremental thickness is thinner at the horn and the margin of the pulp cavity; therefore, sampling near the CDJ and near the intersection of a growth increment with the tusk axis, whose spatial equivalents are the pulp cavity margin and horn, respectively, was avoided when possible. One exception to this is in tusk dentin slab 412. Due to geometric constraints imposed by preparation of adjacent slabs, slab 412

had to be sampled within 1.6 mm of the CDJ, and within 5.4 mm of the intersection of growth increments with the tusk axis.

**Tuning growth rates.** As a final step, we tune the stable isotope data based on the growth rates determined by methods described in main text Section 3.4 and by using the well-established relationship between  $\delta^{13}\text{C}$  and NDVI. In East Africa, NDVI is primarily controlled by rainfall: within days after the seasonal rains begin or any other significant rainfall event ( $\sim >30$  mm), NDVI begins to increase as grasses sprout and to a lesser extent, shrubs and trees increase photosynthetic activity above dry season baseline (Cerling et al., 2004; Cerling et al., 2009; Cerling et al., 2006; Wittemyer et al., 2009). Cerling et al. (2009) report peak NDVI occurs approximately 20 days after peak rainfall, and that the  $\delta^{13}\text{C}$  peak in tail hair, which is a result of increasing  $\text{C}_4$  vegetation in diet, occurs approximately 16 days after the NDVI peak. We use the software program AnalySeries (v 2.0.4.2), to tune  $\delta^{13}\text{C}$  peaks to NDVI peaks. This involves aligning the  $\delta^{13}\text{C}$  peaks with NDVI peaks by increasing or decreasing the tissue growth rate between two match points. After tuning, we shift each isotopic time series by +16 days to account for the lag between  $\delta^{13}\text{C}$  and NDVI peaks based on the lag calculated by Cerling et al. (2009).

## Supplementary Discussion

### *SNR plant isotopes*

Plant surveys and observational data from SNR show nearly all  $\text{C}_4$  vegetation in the area is grass. The only exception is the salt bush, *Salsola dendroides*, which elephants are known to include in their diet. Therefore, we attribute the increase in  $\delta^{13}\text{C}_{\text{dentin}}$  values primarily to a dietary switch to more grazing of  $\text{C}_4$  grasses. The  $\delta^{13}\text{C}_{\text{dentin}}$  values show grazing peaks approximately five weeks after the initial rains of a wet season and two weeks after peak NDVI. Codron et al. (2012) show similar results for  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  in tusk bioapatite from elephants in South Africa, where tusks were sampled every  $\sim 6$  months. However, the lower sampling resolution and the lack of corresponding NDVI or rainfall limits the interpretation of those longer records.

Previously published isotopic data from plant samples collected in SNR and BSNR from October 2004 to July 2005 and in May 2006 provide an important framework for interpreting the hair data (Cerling et al., 2009). Nearly 200 samples were collected during the former interval, which was drier than usual. About 70 samples come from the latter interval, but these were collected towards the end of the long rains. We use the same end-member values for  $\text{C}_3$  and  $\text{C}_4$  plants discussed for RPV model inputs. The plant  $\delta^{15}\text{N}$  values ( $\delta^{15}\text{N}_{\text{plant}}$ ) range from about 0 ‰ to + 12 ‰, with a majority of values lying between + 7 and + 12 ‰. Notable exceptions include a few  $\text{C}_4$  grasses (*Andropogon* sp. and *Cynodon dactylon*) that had average  $\delta^{15}\text{N}_{\text{plant}}$  values of + 4.7 and + 3.1 ‰, respectively, and the  $\text{C}_3$  shrub *Indigofera*, which showed values ranging from + 0.4 to + 7.7 ‰. Values near 0 ‰ are indicative of nitrogen fixation, and this was primarily observed in *Indigofera schimperi* from non-riparian zones.



### ***Carbon isotopes in hair and tusk dentin***

Tusk and hair carbon isotope records show good agreement through time. Where we have synchronous records (2000-2006), a linear regression yields the relationship  $\delta^{13}\text{C}_{\text{diet-dentin}} = 0.64 * \delta^{13}\text{C}_{\text{diet-hair}} - 8.75$  ( $R^2 = 0.49$ ).

This section reviews processes, both biological and analytical, that may contribute to the mismatch between  $\delta^{13}\text{C}_{\text{diet}}$  calculated from the RPV model results and dentin. Although the hair and dentin profile data match well, when the RPV model is applied to the hair, the resulting dietary range (0 to 68 %C4 in diet) is much higher than that observed in the dentin data (7 to 39 %C4), suggesting that the dietary record of the tusk profile is damped by physical sampling or analysis of dentin, or by biological processes that have not been fully accounted for.

The first factor that may contribute to the lower range in the  $\delta^{13}\text{C}_{\text{dentin}}$  profile could be a result of time averaging through tissue formation, a physiological process. While the composition and formation of dentin differs from that of enamel, it may have a short maturation process whereby the initial and final densities of dentin differ. If this is the case, the maturation process is likely to be much less protracted and severe in tusk dentin than it is for enamel which is on the order of years (Uno et al., 2020). Data that support (but do not confirm) a maturation process in tusk dentin include preliminary Fourier Transform Infrared (FTIR) spectroscopy data from a transect along the thin section for slab 1169. The data show increasing relative intensity in the phosphate peak and decreasing relative intensity in the primary amide peak, a proxy for protein content, moving from the pulp cavity toward the CDJ (Uno, 2012).

The second is that dentin from different time periods may have been mixed by inadvertently milling across second-order features. This represents a source of physical mixing that results in time averaging. During serial sampling, the milling edge was checked to ensure that it was parallel to growth increments every 10 samples (~1 mm). On several occasions, corrections of 50 to 100  $\mu\text{m}$  were made to realign millpaths to be parallel with the second-order growth increments. A combination of physical and physiological time-averaging is also plausible.

A third factor, albeit secondary to the previous two mentioned, could be a damping of the range in  $\delta^{13}\text{C}_{\text{dentin}}$  during analysis on the IRMS. There are two important differences observed between enamel bioapatite and tusk dentin bioapatite. First, the  $\text{CO}_2$  yields from  $\text{H}_2\text{O}_2$ -treated tusk bioapatite, measured as a voltage per unit mass of sample ( $\text{mV}/\mu\text{g}$ ), were lower than for enamel bioapatite from R37 enamel by about 30%. This was compensated for by analyzing greater amounts of sample, but in some cases, where sample mass was limited, voltages were slightly lower (~1000 to 1300 mV) than for normal carbonate samples ( $\geq 1500$  mV). All samples included in analyses were within the range of linearity on the IRMS (1000 to 8000 mV). The second difference is that tusk bioapatite also took longer to digest in the CAB, which imparts a memory effect on subsequent samples. The memory effect would act to dampen the  $\delta^{13}\text{C}_{\text{dentin}}$  values

because when samples are run in sequence, the contribution of more negative  $\delta^{13}\text{C}$  from the sample just before the  $\delta^{13}\text{C}_{\text{dentin}}$  maximum would cause the maximum value to be more negative. To address the issue of a memory effect, the reaction time in the CAB was increased from 10 to 30 minutes, which reduced the memory effect to ~70 to 200 mV. In the case of low-voltage samples (~1000 mV) this would impart a 7 to 20 % memory effect, but for a majority of samples, the memory effect was smaller, on the order of 2 to 4 %.

Finally, differences in the protein content of  $\text{C}_3$  vs.  $\text{C}_4$  vegetation will result in different  $\delta^{13}\text{C}$  values for hair and tusk bioapatite. The observed lower amplitudes in  $\delta^{13}\text{C}_{\text{dentin}}$  could be explained if  $\text{C}_4$  vegetation has higher protein content than  $\text{C}_3$  vegetation. This would result in a greater proportional contribution to the  $\delta^{13}\text{C}_{\text{hair}}$  than to the  $\delta^{13}\text{C}_{\text{dentin}}$ , and could produce the higher amplitude observed in the  $\delta^{13}\text{C}_{\text{hair}}$  profile. Weight percent nitrogen (%N) data from Samburu plants show the opposite; the average %N in  $\text{C}_3$  vegetation is higher ( $3.3 \pm 1.0$  %,  $n = 139$ ) than in  $\text{C}_4$  grasses ( $1.6 \pm 0.8$  %,  $n = 53$ ).

Given the four possibilities discussed above, the most likely causes of the lower amplitude in the  $\delta^{13}\text{C}_{\text{dentin}}$  profile are due to dentin maturation, which we could not assess in this study, and time averaging introduced through milling. Both should be evaluated in future work on paired dentin-hair isotope profiles. There may also be other factors that have not been identified that result in the lower than expected ranges in the  $\delta^{13}\text{C}_{\text{dentin}}$  profile.

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### **List of Supplementary Tables**

Table S1. Second-order increment measurements for growth rate determinations in tusk dentin slabs.

Table S2. Stable carbon and oxygen isotope profile data (n=597) from R37 tusk dentin with tuned and untuned dates

Table S3. Rainfall data from Archer's Post for the periods represented by tusk dentin and tail hair isotope profiles.

Table S4. NDVI data from Samburu National Reserve for the periods represented by tusk dentin and tail hair isotope profiles.

Table S5. Correlation coefficients of tusk isotope ( $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ ) values and climatic variables (rainfall and NDVI). For  $R^2 > 0.10$ , the highest or two highest values are in bold.

Table S6. Stable carbon and nitrogen isotope profile data (n=272) from tail hair with tuned and untuned dates