



Calcium isotopes in enamel of modern and Plio-Pleistocene East African mammals

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ABSTRACT

Calcium isotope analyses show a depletion of heavy calcium isotopes in vertebrates, compared to food sources along each trophic step. Recent studies show considerable variability of the calcium isotopic composition of bone and teeth in modern mammals, leading to inconclusive interpretations regarding the utility of Ca isotopes for trophic inference in mammal-dominated terrestrial ecosystems. Here, we analyzed modern enamel samples from the Tsavo National Park (Kenya), and fossil enamel samples dated from ca. 4 Ma to 1.6 Ma from the Turkana Basin (Kenya). We found a constancy of taxa ordering between the modern and fossil datasets, suggesting that the diagenesis of calcium isotopes is minimal in fossils. In modern herbivore samples using similar digestive physiologies, browsers are enriched in ⁴⁴Ca compared to grazers. Both grazer and browser herbivore tooth enamel is enriched in ⁴⁴Ca relative to carnivores by about +0.30‰. Used together, carbon and calcium isotope compositions may help refine the structure of the C₃ and C₄ trophic chains in the fossil record. Due to their high preservation potential, combining both carbon and calcium isotope systems represent a reliable approach to the reconstruction of the structure of past ecosystems.

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1. Introduction

Calcium (Ca) is a major element (~40% weight) in carbonate hydroxylapatite (CHA): the inorganic phase of vertebrate phosphatic tissues (i.e., bone, enamel and dentine). Ca isotope ratios, here expressed as $\delta^{44/42}\text{Ca}$ (see details thereafter), in vertebrate phosphatic tissues were first measured by means of thermal ionization mass spectrometry (Russell and Papanastassiou, 1978; Skulan et al., 1997; Skulan and DePaolo, 1999; Clementz et al., 2003). Ca stable isotope ratios have not been measured routinely by means of multi collector inductively coupled plasma mass spectrometry (MC-ICPMS) due to a major isobaric interference on ⁴⁰Ca⁺ by ⁴⁰Ar⁺, and polyatomic and doubly charged interferences on ⁴²Ca⁺, ⁴³Ca⁺ and ⁴⁴Ca⁺ beams (Wieser et al., 2004; Valdes et al., 2014; Tacail et al., 2016). Subsequent improvements of the Ca purification chemistry and in MC-ICPMS analytics further encouraged the interest of Ca isotope systematics in recent and fossil vertebrate samples with an emphasis at understanding mammal, fish and reptile biology and reconstruction of as-

sociated trophic chains (Clementz et al., 2003; Chu et al., 2006; Reynard et al., 2010, 2011, 2013; Heuser et al., 2011; Melin et al., 2014; Martin et al., 2015, 2017a, 2017b; Tacail et al., 2017a; Hassler et al., 2018).

Two direct implications of the high Ca content in CHA stimulate the interest for the analysis of Ca isotopes. The first is that minute amount of phosphatic tissue, typically 100 µg, is necessary to process the measurement of Ca isotope ratios accurately. Such a small amount of sample opens perspectives for the use of sample leftovers or the almost non-destructive sampling of precious fossils. The second is that only extreme diagenesis, with more than 80% of reworked CHA, is predicted to have an effect on the original Ca isotope composition (Martin et al., 2017a). These calculations are made using water–rock interactions and assume that secondary calcium carbonates are leached accordingly. Collagen nitrogen is rarely preserved in fossils older than the Holocene or Late Pleistocene so that its potential as a trophic indicator in the deep past is precluded. Therefore, measuring Ca isotope ratios have the potential to allow reconstructing past trophic chains in vertebrate fossils of Pleistocene age and older. So far, only trace elements, mainly the strontium–calcium and barium–calcium ratios (Balter et al., 2001; Sponheimer and Lee-Thorp, 2006) have been used to

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this end, but trace elements have the disadvantage to be potentially altered by diagenetic processes (Reynard and Balter, 2014).

Trophic level reconstruction using Ca isotopes is based on the reasoning that the whole body tissues of vertebrates are depleted in heavy Ca isotopes relative to diet. The main observation is that bone Ca is depleted in heavy isotopes by -0.54% in average (expressed as $\delta^{44/42}\text{Ca}$) when compared to dietary Ca in mammals (Skulan and DePaolo, 1999; Chu et al., 2006; Hirata et al., 2008; Tacaïl et al., 2014; Heuser et al., 2016a, 2016b). This systematic and well-conserved offset argues in favor of a shared physiological effect on Ca isotope fractionation in mammal tissues. The depletion in heavy Ca isotopes is variable among organs, but taking blood as a baseline, Tacaïl et al. (2017b), based on a compilation of available data in mammals (Skulan and DePaolo, 1999; Morgan et al., 2012; Tacaïl et al., 2014; Channon et al., 2015; and Heuser et al., 2016a) on various organisms including humans, calculated a Ca isotopic offset $\delta^{44/42}\text{Ca}$ between blood and diet of $-0.30 \pm 0.13\%$ (1SD). The observed trophic level effects in ecosystems could thus be explained by the propagation of this physiology-related isotopic fractionation from a trophic level to another.

Indeed, calcium isotope ratios were shown to decrease with trophic level position in marine ecosystems by Skulan et al. (1997) and this finding was later confirmed (Clementz et al., 2003; Martin et al., 2015, 2017b). Early work proposed a model to understand the relationship between dietary and mineralized calcium (Skulan and DePaolo, 1999) but subsequent studies raised some issues in interpreting calcium isotope values in terms of trophic fractionation, notably in terrestrial environments. Melin et al. (2014) studied calcium isotope ratios for terrestrial mammal ecosystems and concluded that while confirming the decrease in Ca isotope ratios in large carnivores, they also observed isotopic insensitivity to trophic levels between small faunivores and low trophic levels, suggesting limited applications of Ca isotopes in past ecosystems. Moreover the application of Ca isotopes for trophic level reconstruction in past continental ecosystems, including dinosaur fauna, was not conclusive (Heuser et al., 2011) although a recent study at regional scales permitted to distinguish between food sources between predatory dinosaurs (Hassler et al., 2018). Recent work offered encouraging perspectives in a Pleistocene mammalian fauna (Martin et al., 2017a) but some outliers remain difficult to interpret and may be so under the suspicion that physiological processes might be at play (Tacaïl et al., 2017a). Also, complexation of Ca with aqueous compounds (e.g. citrates, oxalates) potentially plays a role in isotopic fractionation between various plant or animal organs (Moynier and Fujii, 2017). Physiological differences have been previously discussed between fish and marine mammals (Martin et al., 2015) underlining the difficulty to interpret mammalian calcium isotope variability solely under the light of a trophic effect on fractionation processes. Importantly, a comprehensive framework of Ca isotope distribution in modern terrestrial mammals is lacking.

In an effort to fill this gap, the present work reports Ca isotope ratios of modern enamel samples from the Tsavo National Park and from Turkana Basin (Kenya) ($n = 64$), and fossil enamel samples ($n = 51$) dated from ca. 4 Ma to 1.6 Ma from the Turkana Basin (Kenya). The $^{44}\text{Ca}/^{42}\text{Ca}$ and $^{43}\text{Ca}/^{42}\text{Ca}$ isotope ratios are compared with carbon isotope ($^{13}\text{C}/^{12}\text{C}$), oxygen isotope ($^{18}\text{O}/^{16}\text{O}$), strontium–calcium (Sr/Ca), and barium–calcium (Ba/Ca) ratios.

2. Methods

2.1. Samples

Tsavo National Park is situated in southern Kenya (ca. 3.4 S, 38.6 E, 550 m elevation) and has a mean annual temperature of 25 °C and 550 mm annual rainfall (Climatological Statistics for East

Africa, 1975); it is a semi-desert bushland with riparian woodland (White, 1983). Samples of mammals were collected between 1997 and 2011 and include the long-term collections at the Tsavo Research Center near Voi; samples in this collection date back to the 1960s. Fossil samples from the Turkana Basin were collected from the National Museums of Kenya and the Turkana Basin Institute. Ages of fossils are based on the stratigraphic and geochronologic work of Brown and McDougall (2011). Both modern and fossil materials were collected as part of a paleoecology project reported earlier (Cerling et al., 2015). For all samples, powdered enamel was collected using a low-speed dental drill.

2.2. Analytical techniques

We compared samples that had undergone the standard pretreatment used in light stable isotope studies to remove organic matter and calcium carbonate (3% H_2O_2 followed by 0.1 M acetic acid as in Passey et al., 2002). Samples were analyzed for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ using digestion by 100% H_3PO_4 and analyzed on an isotope ratio mass spectrometer using the standard ‰ notation where

$$\delta^{13}\text{C}(\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1) * 1000 \quad (1)$$

where R_{sample} and R_{standard} are the $^{13}\text{C}/^{12}\text{C}$ ratios in the sample and standard, respectively. An analogous equation defines $\delta^{18}\text{O}$. The isotope standard VPDB (Vienna-PDB) is used for both carbon and oxygen isotopes.

The remaining powdered samples were treated in the clean lab at Laboratoire de Géologie de Lyon: Terre, Planètes, Environnement, ENS de Lyon, France. For each dissolved sample, a fraction was taken for concentration analyses and another fraction was kept for purification of calcium. Concentration analyses were performed by means of inductively coupled plasma mass spectrometer (ICP-MS Agilent Technologies 7500 Series) for trace elements such as Sr, Ba, U, and major elements were measured on an inductively coupled plasma atomic emission spectrometer ICP-AES (Thermo electron corporation ICAP 6000). Measurements were controlled through a set of blanks and standards such as SRM1486. Calcium was purified following the protocol described in previous work using Eichrom Sr-specific resin (Sr-spec Eichrom®) and cation-exchange resin (AG-50WX-12) with ultrapure solutions of nitric and hydrochloric acids as elution agents (see details in Tacaïl et al., 2014; Martin et al., 2015, 2017a, 2017b). The purified fraction was measured for Ca isotopes on a Thermo Neptune Plus MC-ICPMS at medium resolution in static mode. Delta values were obtained using the standard bracketing method using the ICP Ca Lyon standard issued from a Specpure calcium plasma standard solution (Alfa Aesar) (Tacaïl et al., 2014, 2016, 2017a; Martin et al., 2015, 2017a, 2017b; Hassler et al., 2018). SRM1486 was used as a secondary standard during each analytical sequence. Uncertainties are reported in Table S1 and represent 2 standard deviations of these analyses. $\delta^{44/42}\text{Ca}$ values are defined as:

$$\delta^{44/42}\text{Ca}(\text{‰}) = \left(\frac{^{44}\text{Ca}/^{42}\text{Ca}_{\text{sample}}}{^{44}\text{Ca}/^{42}\text{Ca}_{\text{ICP Ca Lyon}}} - 1 \right) * 1000 \quad (2)$$

where $\delta^{44/42}\text{Ca}$ is the normalized difference in per mil (‰) between a sample and our in-house ICP Ca Lyon standard. In this work, all measurements are expressed in $\delta^{44/42}\text{Ca}$ (Table S1) and we invite the reader to refer to supplementary material (Fig. S1, Table S2) for details regarding conversions of data from the literature. Calcium isotope values are often expressed as $\delta^{44/40}\text{Ca}$ values in the literature. As a guideline, the magnitude of variations of

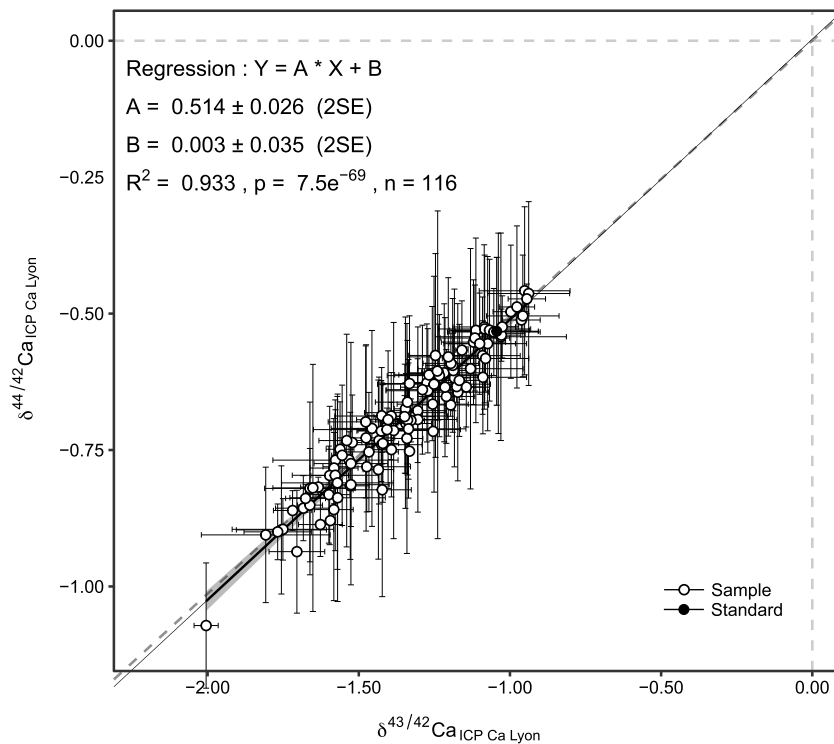


Fig. 1. Three-isotope-plot for all data measured in this study, with $\delta^{43/42}\text{Ca}$ (‰) as a function of $\delta^{44/42}\text{Ca}$ (‰) relative to ICP Ca Lyon bracketing standard. The samples and standards fall on a line with a slope of 0.518 ± 0.028 (2SE), indistinguishable from the 0.507 slope predicted by the exponential mass-dependent fractionation law (red stippled line). Error bars correspond to 2SD. The blue line corresponds to the regression line. The red shaded area corresponds to the 95% confidence interval on the regression line.

$\delta^{44/42}\text{Ca}$ is almost exactly half that of $\delta^{44/40}\text{Ca}$. SRM1486 yielded a value of -1.047 ± 0.013 2SE (± 0.13 2SD, $n = 101$), which is undistinguishable from all SRM1486 samples measured at LGLTPE, with an average value of -1.024 ± 0.006 ‰ ($n = 404$, 2SE; Tacail et al., 2014, 2016, 2017a; Martin et al., 2015, 2017a, 2017b; Hassler et al., 2018) and reported $\delta^{44/42}\text{Ca}$ values in other studies (-1.009 ± 0.026 ‰, 2SE; Heuser and Eisenhauer, 2008; Heuser et al., 2011, 2016a, 2016b). More details on the compositions of reference materials are available in Table S2. All measured samples plotted in a $\delta^{44/43}\text{Ca}$ versus $\delta^{44/42}\text{Ca}$ space fall on a line with a slope of 0.514 ± 0.026 , 2SE, in good agreement with the 0.5067 slope predicted by the linear approximation of exponential mass-dependent fractionation (Fig. 1).

3. Results

Herbivores in both the modern and fossil samples range from browsers ($\delta^{13}\text{C} < -8$ ‰) to grazers ($\delta^{13}\text{C} > -1$ ‰; see discussion in Cerling et al., 2015); hippos are mixed feeders in this modern Tsavo ecosystem. For the Turkana Basin fossil dataset, the time span sampled is from ca. 4 to 1 Myr. A few taxa change their diets through this time period and some taxa at the genus level are present only in the fossil record. The elephantids *Loxodonta* and *Elephas* were grazers in the fossil record, but modern *Loxodonta* is a browser in modern ecosystems in East Africa (see Tables S1 and S2 and discussion in Cerling et al., 1999, 2015) with *Elephas* being extinct in Africa today.

In the savanna mammals of the South African Kruger Park, Sponheimer and Lee-Thorp (2006) observed that grazers have higher Sr/Ca and Ba/Ca ratios than browsers. This observation is not confirmed here in the East African modern mammals of Tsavo (Fig. S2). Noteworthy, we found that rhinos from this sample suite have extremely high Sr/Ca ratios with typical Sr contents that are one order of magnitude higher than in other an-

imals (Fig. S2). In agreement with the literature (Balter, 2004; Peek and Clementz, 2012), however, the Sr/Ca and Ba/Ca ratios are lower in carnivores than in herbivores in the modern dataset (Fig. S2).

Fossil samples at Turkana are affected by diagenesis by the addition of trace metals: there is a strong positive correlation between Ba and Mn concentrations ($R^2 = 0.417$, $p^{***} < 10^{-}$; Fig. S4C; Table 1) and between Sr and U (U; $R^2 = 0.247$, $p^{**} = 0.0004$; Fig. S4D; Table 1). As a consequence, the Sr/Ca and Ba/Ca ratios do not discriminate carnivores from herbivores in this particular fossil assemblage (Fig. S3). In addition, the Sr/Ca and Ba/Ca ratios are correlated at Turkana ($R^2 = 0.225$, $p^{**} = 0.0008$, Fig. S4B) while this correlation is not observed in the recent Tsavo fauna (Fig. S4A).

That the Sr/Ca and Ba/Ca are correlated in fossil samples suggest a common diagenetic process for Sr and Ba. Likely, this diagenetic process involved the addition of a U and Mn-rich phase, which also contains Sr and Ba, explaining the overall increase by a factor of 1.7 and 3.3 of the Sr/Ca and Ba/Ca ratios, respectively, between modern and fossil samples. Mg/Ca ratios are not significantly different between modern and fossil samples. We conclude that our results show that diagenetic processes have altered the concentrations of Sr and Ba, and therefore the potential for isotopic alteration of the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio in fossil materials must be evaluated carefully.

Inversely, the relative sensitivity of trace and major elements to diagenesis can be used to ascertain that little or no diagenesis has occurred for major elements if the trace elements normalized to calcium show ratios similar to modern samples. This is most probably the case for South African Plio-Pleistocene fossils for which original Sr/Ca and Ba/Ca patterns are apparently preserved (Sponheimer and Lee-Thorp, 2006; Balter et al., 2012).

The $\delta^{44/42}\text{Ca}$ values range from -2.00 ‰ to -0.98 ‰ in the modern dataset of Tsavo/Turkana (Fig. 2A) and from -1.77 ‰ to

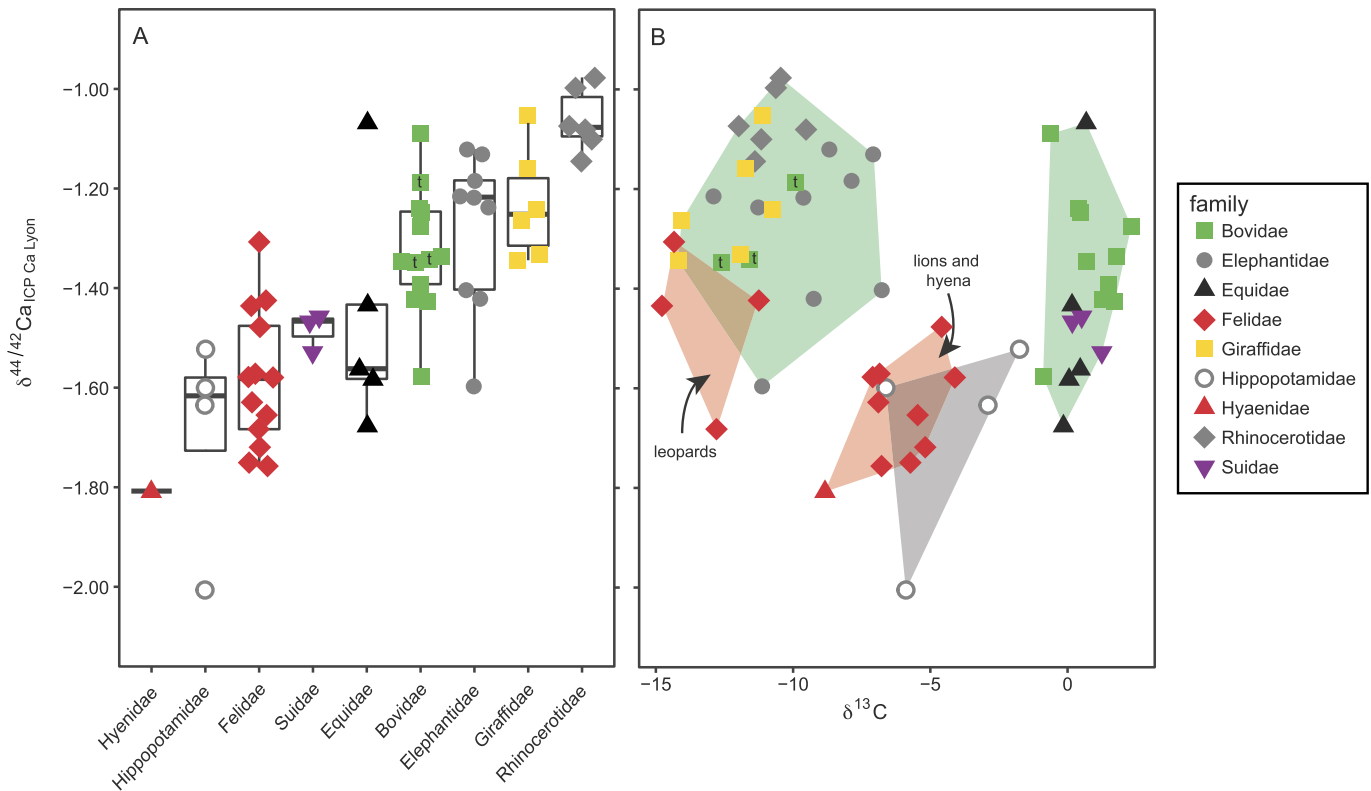


Fig. 2. A. $\delta^{44/42}\text{Ca}$ variability by taxonomic groups (‰, rel. ICP Ca Lyon), arranged by increasing average values, as measured in tooth enamel of a mammalian assemblage from the modern ecosystem of Tsavo, Kenya. B. $\delta^{44/42}\text{Ca}$ as a function of $\delta^{13}\text{C}$ measured from tooth enamel from the same modern assemblage. Note the spatial distinction between the Hyenidae + *P. leo* and *P. pardus*. Abbreviations: t, tragelaphine bovinds. (For interpretation of the colors in the figure(s), the reader is referred to the web version of this article.)

−0.94‰ in the fossil dataset of Turkana (Fig. 3A). In both cases, carnivores exhibit the lowest $\delta^{44/42}\text{Ca}$ values, but hippos also have quite low values, between hyenas and felids. Equids have $\delta^{44/42}\text{Ca}$ values that fall in the variability of the felid carnivores, both in the modern and fossil datasets. In the modern dataset, suids exhibit similar $\delta^{44/42}\text{Ca}$ values to equids, while fossil suids have relatively high $\delta^{44/42}\text{Ca}$ values. Bovids, elephants and giraffes in the modern dataset have $\delta^{44/42}\text{Ca}$ values more positive than equids, carnivores and hippos, although one equid outlier shows a $\delta^{44/42}\text{Ca}$ value close to −1‰. The highest $\delta^{44/42}\text{Ca}$ values are from rhinos and some of the other herbivores such as one giraffe, one equid, a few elephants and bovinds for the modern taxa analyzed (Fig. 2A). In the fossil dataset, however, the fossil giraffes, bovinds, rhinos and elephants have undistinguishable $\delta^{44/42}\text{Ca}$ values (Fig. 3A).

Therefore, diagenesis appears to affect some trace elements (Ba, Mn, possibly Sr) but not Ca-isotope ratios. The existence of a correlation between modern and fossil $\delta^{44/42}\text{Ca}$ values (Fig. S5) implies that diagenesis of the Ca isotope ratios at Turkana is weak otherwise no correlation would have been obtained. Diagenesis of Ca isotopes is expected to be minimal in most cases, because phosphatic tissues are so rich in Ca that only extreme diagenesis (discussion above), which would modify the stoichiometry of CHA, would be able to overprint the original Ca isotope composition (Martin et al., 2017a).

4. Discussion

Recent data of calcium isotope compositions in enamel suggest a strong potential as a paleodietary indicator in marine settings (Skulan et al., 1997; Clementz et al., 2003; Martin et al., 2015, 2017b). On continents, however, data exhibit generally more complex patterning due, probably, to heterogeneous isotopic sources in soils and further fractionation in plants (Skulan and

DePaolo, 1999; DePaolo, 2004; Melin et al., 2014). Melin et al. (2014) analyzed the calcium isotope ratios of 21 bone samples from two modern mammalian communities in northern Borneo and northwestern Costa Rica: they observe a depletion of heavy calcium isotopes up the trophic chains involving two large vertebrate predators (one *Felis bengalensis* individual in Borneo and one *Panthera onca* individual in Costa Rica). Melin et al. (2014) concluded a lack of sensitivity of Ca isotopes to carnivory. Although tooth or bone samples from large predators are indeed difficult to secure, larger datasets including more of them are required to further explore this issue.

Our Ca isotope measurements arise from two modern datasets of mammals living at Tsavo National Park and at Turkana, both from Kenya; these datasets comprise 64 samples from individuals covering 9 different families of large mammals with 18 grazers, 21 browsers, 7 mixed feeders and 18 carnivores (Table 1). We considered several different digestive physiologies in the herbivore mammals of our dataset: ruminant foregut, non-ruminant foregut and hindgut. However, we found no statistically significant differences between groups. Although recent finds have highlighted that fractionation of calcium isotopes in the body mainly occurs from renal activity (Tacail et al. 2017b), it will be worth to expand the dataset and further explore potential links between digestive physiology and isotopic variability. Comparisons of $\delta^{44/42}\text{Ca}$ values with respect to body mass are premature with our current dataset; such studies should also include renal and digestive physiology as well as the C_3/C_4 mix of diet while also comparing for body mass. Here, 1) we discuss a Trophic Level Effect (TLE) as recorded in tooth enamel of modern mammals, underlining significant differences in Ca and C isotope values between some carnivores and herbivores; 2) we highlight that variability in mammal resource use such as plants, soils and waters needs to be considered to account for the observed variability in Ca isotope values of their tissues and may

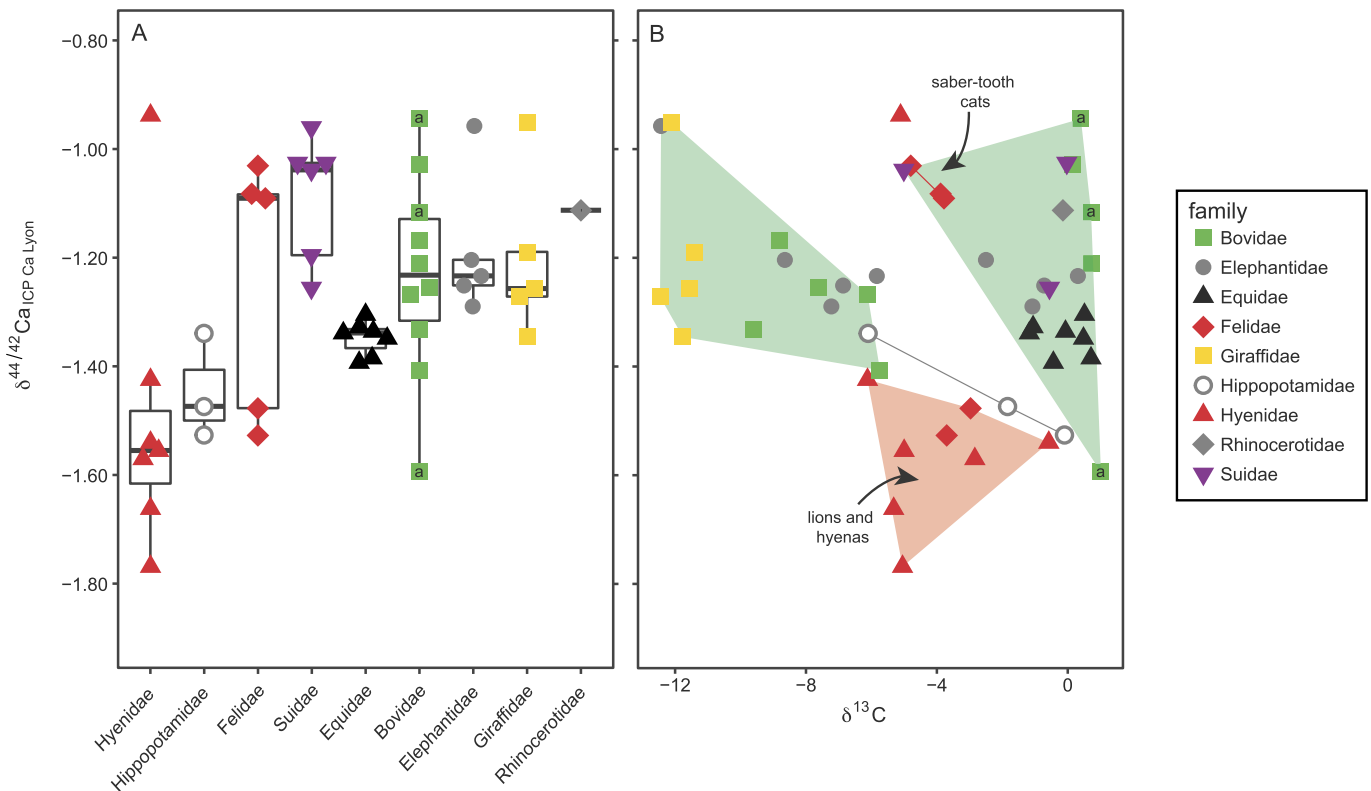


Fig. 3. A, Calcium isotope variability by taxonomic grouping of fossil assemblage of Turkana Basin, Kenya. B, $\delta^{44/42}\text{Ca}$ as a function of $\delta^{13}\text{C}$ measured from tooth enamel from the same fossil assemblage. Abbreviations: a, alcelaphine bovids. (For interpretation of the colors in the figure(s), the reader is referred to the web version of this article.)

be related to differences in calcium isotope ratios between grazers and browsers; and 3) we infer fossil mammal Ca isotopic ecology in light of the knowledge derived from the modern samples.

4.1. $\delta^{44/42}\text{Ca}$ and the Trophic Level Effect (TLE) in modern mammals

Carnivores exhibit an important variability of the Ca isotope composition being the lightest samples of the dataset but also overlapping with herbivore Ca isotope values, except some of the very large herbivores, i.e. rhinos, giraffes and elephants (Fig. 2A). We report a carnivore-prey offset of 0.24‰ when considering all carnivores versus herbivores of the modern dataset, and an offset of 0.33‰ when considering *Panthera leo* and *Crocuta crocuta* from Tsavo versus all modern herbivores. Therefore, a carnivore-prey offset of about 0.3‰ seems to characterize mammalian faunas. For the limited samples we have analyzed, the two modern felids from Tsavo, *Panthera leo* ($n = 9$) and *Panthera pardus* ($n = 4$) have differing $\delta^{44/42}\text{Ca}$ ratios, *P. leo* being the most depleted in heavy Ca isotopes ($-1.63 \pm 0.09\text{‰}$, 1SD) similarly to the single hyenid *Crocuta crocuta* from Tsavo whereas *P. pardus* is enriched in heavy Ca isotopes ($-1.46 \pm 0.16\text{‰}$, 1SD). In the modern dataset from Turkana, *C. crocuta* ($n = 2$) is also the most depleted in heavy Ca but in this ecosystem, *P. leo* ($n = 2$) is notably enriched in heavy Ca (-1.18 ± 0.01 , 1SD), more so than *P. pardus* from Tsavo. Large carnivores are flexible in their diet and their feeding habits may vary from one region to another. Considering only *P. leo* and *C. crocuta* at Tsavo, carnivores possess significant lower $\delta^{44/42}\text{Ca}$ values than all herbivores, except the hippos (see discussion below). Bone is often a significant component of the diet of hyenids but also of *P. leo*. Across felid taxa, proportions of meat versus bone vary (Van Valkenburgh, 1996), indicating bone consumption needs to be considered as a non-negligible supplier of dietary calcium. Even a small amount of dietary bone ingested would shift the values toward light Ca (Heuser et al., 2011), and could explain the low

$\delta^{44/42}\text{Ca}$ values observed in our dataset for *P. leo* and *C. crocuta*. More calcium isotope data-points are needed to test for a potential isotope scattering among carnivores according to their feeding ecology. Based on behavioral observations, a dietary overlap exists between *C. crocuta* and *P. leo* (Hayward, 2006) and may help explain that both taxa display some of the most depleted Ca isotope values of the dataset. In the Pleistocene of France, *C. crocuta* possesses the most $\delta^{44/42}\text{Ca}$ -depleted value of the dataset (Martin et al., 2017a), confirming our observations on *C. crocuta* from modern Kenya.

The $\delta^{13}\text{C}$ distribution clearly distinguishes C_4 from C_3 trophic chains (Fig. 2B) and used with $\delta^{44/42}\text{Ca}$ values, provides further insights into niche partitioning. Browsing herbivores, composed of a few bovids, giraffes, rhinos and elephants exhibit high and low $\delta^{44/42}\text{Ca}$ and $\delta^{13}\text{C}$ values, respectively. They are separated in the $\delta^{44/42}\text{Ca}$ versus $\delta^{13}\text{C}$ space from a group of predators, here represented by leopards, which show similar $\delta^{13}\text{C}$ values but lower $\delta^{44/42}\text{Ca}$ values (Fig. 2B). That *P. pardus* avoids prey living in open habitats has been reported in the wild (Hayward et al., 2006) and the isotopic distribution reported here indicates that some of the herbivores mentioned above, especially small bovids, could represent potential prey of the leopards. The preferred prey of the leopards have body masses not exceeding 25 kg (Hayward et al., 2006) and therefore are not elephants, rhinos or giraffes. Further work is needed to sample and assess $\delta^{44/42}\text{Ca}$ values for forest-dwelling small mammals such as small bovids (e.g., duikers, dik-diks, other neotragins) or primates, all of which are recognized prey of *P. pardus* and known to generally possess depleted $\delta^{13}\text{C}$ values (around -12 to -15‰) (Cerling et al., 2004). On the other hand, another group of herbivores comprising most of the larger bovids, suids and equids occupies a distinct $\delta^{13}\text{C}$ distribution indicating a C_4 source for predators represented by *P. leo*, as indicated by their lower $\delta^{44/42}\text{Ca}$ values, corresponding to the expected dietary shift in $\delta^{44/42}\text{Ca}$ values between consumer and prey.

Hippos do not follow the trends observed in other herbivores and have very low $\delta^{44/42}\text{Ca}$ values typical of carnivores. Such measurements are difficult to reconcile with a TLE given their known grazing ecology (Cerling et al., 2008); however, hippos are semi-aquatic and thus have different physiological adaptations than all the other non-aquatic mammals; influences on bone density and associated bone mass balance may affect their $\delta^{44/42}\text{Ca}$ values. Although hippos have been occasionally observed to exhibit carnivory (Dudley et al., 2016) the observations are so sparse to suggest that carnivory is unlikely to have an observable Ca-isotope effect in hippos.

4.2. Ca isotope variability in environmental sources

Drinking water represents a source of calcium for mammals with concentrations ranging between 15 and 150 ppm in modern-day streams (Tipper et al., 2016). Ca isotopes do not fractionate in a significant extent during geological processes leading to rather homogeneous isotope compositions in rocks, being sedimentary, metamorphic, plutonic or volcanic (Tipper et al., 2016). The Turkana modern and fossil ecosystems are located around Lake Turkana and are comprised primarily of fluvial Quaternary sediments derived primarily from Ethiopian Tertiary and Quaternary volcanic rocks. The Tsavo ecosystem is located between Mombasa and Nairobi and consists of metamorphic basement in the east (all of Tsavo East NP and most of Tsavo West NP) with some Quaternary basalts in the western part of Tsavo West NP. If local Ca-isotope variations are found between different substrates, studies combining $\delta^{44/42}\text{Ca}$ values with $^{87}\text{Sr}/^{86}\text{Sr}$ may be useful to study fossil assemblages.

At the bottom of the trophic structure, mammalian herbivores source most of their calcium from plants. Contrary to geological processes, reviewing the literature shows that Ca isotopes fractionate in a significant extent between monocotyledons (including grasses) and leaves of dicotyledons (Fig. 4), thus representing an important source of isotopic variability. Roots of plants preferentially take up light Ca isotopes, and there is a further fractionation in favor of heavy isotopes with variable amplitude in leaves of dicotyledons, while this process is subdued or absent in monocotyledons (Cenki-Tok et al., 2009; Holmden and Bélanger, 2010). This leads to a difference of $+0.31\%$ ($p^{***} < 10^{-4}$) between whole monocotyledon plant tissue and the leaves of dicotyledons. This $\delta^{44/42}\text{Ca}$ difference implies that grass and sedge consumers, i.e. grazers, should have a $\delta^{44/42}\text{Ca}$ value lower by about 0.3% compared to the browsing leave-eaters; this is generally true for the modern Tsavo dataset (Fig. 2). If different plant parts (i.e., roots, shoots, leaves) have different $\delta^{44/42}\text{Ca}$ values, those differences may be passed on to the consumer and this would be a useful tool for understanding fossil diet partitioning.

This recognized isotopic difference between plant types, being passed on to herbivores, eventually gets passed on to the next trophic level, i.e. carnivores. We expect that carnivores feeding on grazers should exhibit different calcium isotopic compositions than carnivores feeding on browsers and this is supported by our modern dataset with lions and hyenas showing more negative values ($-1.65 \pm 0.10\%$, 1SD, $n = 10$) than leopards ($-1.46 \pm 0.16\%$, 1SD, $n = 4$).

Despite some scattering in $\delta^{44/42}\text{Ca}$ values among predators, it becomes clear that in a modern ecosystem such as Tsavo, *P. pardus* feeds on prey with higher $\delta^{44/42}\text{Ca}$ values, and that *P. leo* and *C. crocuta* primarily feed on prey with lower $\delta^{44/42}\text{Ca}$ values. Remarkably, there is a tight $\delta^{44/42}\text{Ca}$ versus $\delta^{13}\text{C}$ clustering of all the taxa in the modern Tsavo panel, suggesting that Ca and C isotope ratios are driven, at least partially by common processes. The concomitant use of $\delta^{44/42}\text{Ca}$ and $\delta^{13}\text{C}$ values provides for the first time an encouraging perspective on carnivore niche partitioning

between C_3 and C_4 prey. Certainly, more $\delta^{44/42}\text{Ca}$ measurements covering specific feeding ecologies among modern felids are required to further discuss the use of calcium isotopes and decipher niche partitioning among large carnivores.

4.3. Palaeoecological inferences using Ca isotopes

The taxonomic distribution of the Ca isotope ratios have similar ordering for modern and fossil East African faunas (Fig. 2A and Fig. 3A). Comparing the $\delta^{44/42}\text{Ca}$ values in modern Tsavo and fossil Turkana on a family taxon basis leads to a good correlation ($R^2 = 0.621$, $p^* = 0.012$, Fig. 5) with an observed compression in the $\delta^{44/42}\text{Ca}$ range possibly due to differing feeding ecologies between fossil and modern analogues, as evidenced by high $\delta^{44/42}\text{Ca}$ values for fossil suids and saber-tooth cats.

Plio-Pleistocene assemblages from Turkana in northern Kenya are from ca. 4.1 Ma to 1.4 Ma, a period well after the rise of C_4 ecosystems (Cerling et al., 1997), but in a time where there were significant changes in dietary guilds represented in the fossil record (Cerling et al., 2015). The fossil Turkana ecosystem had similar taxonomic lineages as the modern Turkana ecosystem: bovids, elephantids, giraffids, equids, rhinocerotids. Fossil hyenids and felids were analyzed from Turkana; those fossil carnivora taxa include those with no modern analogues such as saber-tooth felids.

The $\delta^{44/42}\text{Ca}$ and $\delta^{13}\text{C}$ relationships are preserved for some taxonomic groups, but not for all groups, when comparing the modern and fossil assemblages (Fig. 2 and Fig. 3). Most taxonomic groups have similar rankings for $\delta^{44/42}\text{Ca}$ for fossil versus modern samples (Fig. 5) suggesting a conservative ecology and/or physiology. Although most taxonomic groups have similar $\delta^{13}\text{C}$ values through time, some notable exceptions, such as elephantids and rhinocerotids, show similar $\delta^{44/42}\text{Ca}$ values in spite of differing $\delta^{13}\text{C}$ values for the data considered here.

Bovids and equids have similar $\delta^{13}\text{C}$ and $\delta^{44/42}\text{Ca}$ values for both modern and fossil faunas although differences are noted. The fossil tragelaphins (*Taurotragus* and *Tragelaphus*) had higher grass components in their diets than the modern ones from Tsavo, and likewise the fossil alcelaphins (*Megalotragus*) had a slightly higher browse content than do modern alcelaphins from East Africa (see Cerling et al., 2015). Equids had similar $\delta^{13}\text{C}$ values for both modern and fossil samples. Fossil and modern bovids have similar $\delta^{44/42}\text{Ca}$ values, but modern equids have $\delta^{44/42}\text{Ca}$ values slightly different than fossil equids.

The comparison between modern and fossil elephantids and rhinocerotids is noted here. Although the $\delta^{44/42}\text{Ca}$ values are comparable, the diets of the studied samples are quite different, unlike all other fossil-modern comparisons in this study. Both elephantid fossil *Elephas* and *Loxodonta* were grazers, but modern *Loxodonta* is primarily a browser (Cerling et al., 2015). The abundant fossil rhinocerotid *Ceratotherium* was a grazer and was analyzed as part of this study; the modern rhinocerotid *Diceros* was a browser (Cerling et al., 2015) and was analyzed as well. For these lineages, $\delta^{44/42}\text{Ca}$ values are similar for fossil and modern comparisons, in spite of the dietary (grazing versus browsing) differences. Clearly, further comparison within the elephantids and rhinocerotids for both modern and fossil faunas is needed to understand why $\delta^{44/42}\text{Ca}$ values in these groups appear to be conservative across dietary differences.

Modern suids, represented by *Phacochoerus aethiopicus*, have $\delta^{44/42}\text{Ca}$ values ($-1.48 \pm 0.04\%$, 1SD, $n = 3$) significantly different from fossil suids ($-1.09 \pm 0.11\%$, 1SD, $n = 3$). The fossil dataset includes three genera (*Kolpochoerus*, *Metridiochoerus* and *Notochoerus*) and there is little variation in their respective Ca isotope values. Modern *P. aethiopicus* are mostly herbivorous, feeding on grass. More specimens of fossil suids, especially contemporaneous lineages are needed to determine if Ca-isotopes can distinguish

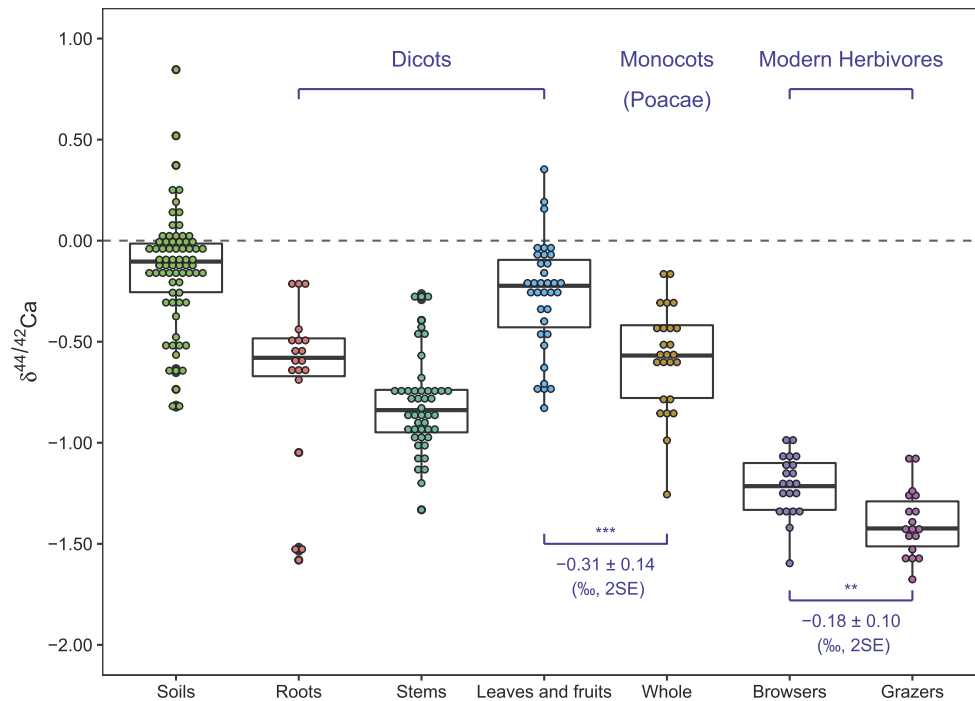


Fig. 4. Calcium isotope variability compared between soils, browser and grazer tooth enamel and their potential source foods, i.e. plant parts including roots, shoots, leaves/fruits and whole Poaceae (data for soils and plants derived from Bagard et al., 2013; Chu et al., 2006; Farkas et al., 2011; Gussone and Heuser, 2016; Heuser et al., 2016a, 2016b; Hindshaw et al., 2013; Holmden and Bélanger, 2010; Moore et al., 2013; Page et al., 2008; Schmitt et al., 2003; Skulan and DePaolo, 1999; Tacail et al., 2014; Wiegand et al., 2005). Student t-test P values are indicated: **P = 0.001–0.01; and ***P < 0.001. (For interpretation of the colors in the figure(s), the reader is referred to the web version of this article.)

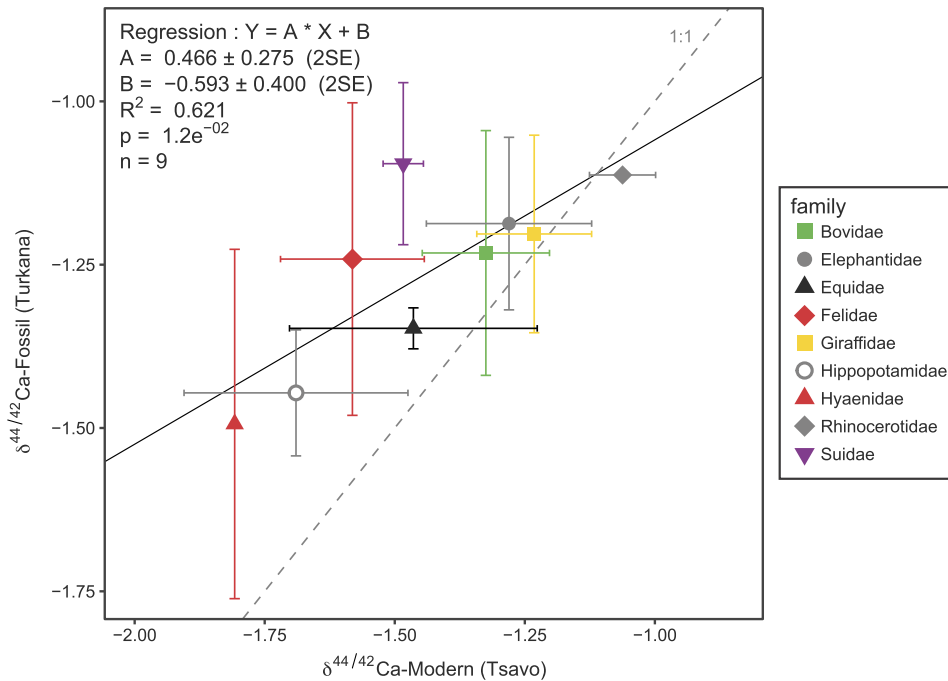


Fig. 5. $\delta^{44/42}\text{Ca}$ in fossil tooth enamel from Turkana Basin compared to $\delta^{44/42}\text{Ca}$ of modern tooth enamel from Tsavo for similar taxonomic groups. (For interpretation of the colors in the figure(s), the reader is referred to the web version of this article.)

different feeding strategies, such as using underground storage organs, versus grass stems or leaves (Fig. 4).

It is noteworthy that the ^{44}Ca -enrichment observed for carnivores between Turkana fossils and modern Tsavo samples is linked to five out of fourteen fossil samples (Fig. 5), with $\delta^{44/42}\text{Ca}$ values above -1.2‰ , which represent very high values even considering the modern Tsavo carnivores. Excluding these five samples, it can

be noted that fossil and recent carnivores have identical $\delta^{44/42}\text{Ca}$ values (Fig. 5) implying that those hyenids and felids already occupied similar niches as modern *C. crocuta* and *P. leo*. There are no pure C_3 carnivores in this dataset of fossil Turkana carnivores that fill the niche of extant leopards. All the fossil Turkana carnivores examined in this paper relied on herbivores with a mixed $\text{C}_3\text{--C}_4$ diet and cover a wide time range.

The group ($n = 5$) of fossil carnivores with extremely ^{44}Ca -enriched values (Fig. 3) includes four felids with two individuals of the genus *Dinofelis* ($-1.17 \pm 0.10\%$ and $-1.03 \pm 0.22\%$), one machairodontid of the genus *Homotherium* ($-1.08 \pm 0.15\%$) and one indeterminate felid ($-1.09 \pm 0.12\%$). Three of them are characterized by saber-shaped canines, the function of which has been interpreted to deliver a weaker bite force than *P. leo* (McHenry et al., 2007). According to our carbon isotope data, this group of felids fed on herbivores that consumed a mixture of C_3 – C_4 plants or the diet was a mix of grazers and browsers. The high $\delta^{44/42}\text{Ca}$ values of Turkana saber-tooth cats imply an absence of bone consumption, probably reflecting adaptation to exclusive flesh-eating. Even considering such a derived feeding preference toward meat-based diet, the $\delta^{44/42}\text{Ca}$ values for this group of felids remain high and applying an offset of about $+0.3\%$ (see Section 4.1) indicates a prey source with a $\delta^{44/42}\text{Ca}$ enamel value around -0.8% , i.e. not measured in our dataset. A provocative explanation would be that these carnivores relied mainly on an unanalyzed group of prey. As tempting as it may seem, two outliers may contradict such hypotheses and are represented by a machairodontine (saber-tooth) with low $\delta^{44/42}\text{Ca}$ value ($-1.53 \pm 0.12\%$) as well as a hyenid with a particularly high $\delta^{44/42}\text{Ca}$ value ($-0.93 \pm 0.13\%$), both of which should be expected to respectively display high and low $\delta^{44/42}\text{Ca}$ values instead. Alternatively, the model of Skulan and DePaolo (1999) could explain high $\delta^{44/42}\text{Ca}$ values in some carnivores if a large proportion of ingested calcium ends up mineralized, in other words resulting in no fractionation between mineral and diet. Clearly, more data are needed to fully cover the range of $\delta^{44/42}\text{Ca}$ variations in modern mammals, but the present study already gives encouraging grounds for first order paleoecological reconstructions. Tighter time intervals for the fossil record would be beneficial for understanding past relationships in $\delta^{44/42}\text{Ca}$ space, and additional studies of modern ecosystems are also needed.

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Appendix A. Supplementary material

Supplementary material related to this article can be found online at <https://doi.org/10.1016/j.epsl.2018.09.026>.

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