Stable isotope analyses of tooth enamel carbonate of large herbivores from the Tugen Hills deposits: palaeoenvironmental context of the earliest Kenyan hominids

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Abstract

Understanding shifts in past climatic and environmental conditions are crucial for throwing light on human evolution. Available reconstructions of the palaeoecology of faunal and floral assemblages indicate that the earliest Kenyan hominids, including *Orrorin tugenensis*, are associated with forest landscapes. In this study, we present stable isotope data of tooth enamel carbonate of large herbivores associated with these hominids in order further to evaluate their environmental context. Fossil teeth were sampled in the Lukeino Formation (6.1-5.7 Ma, Late Miocene) and the Mabaget Formation (5.3-4.5 Ma, Early Pliocene) at the foot of the Tugen Hills (Great Rift Valley). Despite chemical changes in fossil enamel, preservation of the palaeoenvironmental signals is supported by the difference in $\delta^{18}\text{O}$ between hippos and terrestrial fauna. Moreover, the giraffid specimen from the Mabaget Formation exhibits one of the highest $\delta^{18}\text{O}$ values, as expected for a drought-tolerant animal. The $\delta^{13}\text{C}$ of large herbivores indicates that the earliest Kenyan hominids inhabited a mixed $\text{C}_3\text{-C}_4$ environment as did *Ardipithecus* (Ethiopia) and *Sahelanthropus* (Chad), two early putative hominids. The Tugen Hills might have been a wooded grassland with patches of woodland. There is no record of closed-canopy forest at the time of deposition. We emphasize differences in food habits among herbivore groups. Deinotheres, nearly all elephantids and the giraffid individual had a C_3 diet which reflected browsing behaviour. The intake of C_4 plants prevailed for equids and gomphotheres and was consistent with grazing habits. Most hippos and suids were mixed $\text{C}_3\text{-C}_4$ feeders and consequently were generalist herbivores. Bovids and rhinos had a diet ranging from C_3 to C_4 plants. Variations in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ among faunal assemblages suggest changes in local climate and vegetation. Moister conditions likely occurred in the Early Pliocene and the latest Miocene (ca. 5.7 Ma) than before. The C_3 plant biomass would have increased in the latest Miocene. There were probably denser stands of trees within the depositional environment during the Early Pliocene than in the Late Miocene, which is consistent with palaeontological studies.

1. Introduction

The study of climate and environmental change is crucial for understanding human evolution. It has traditionally been based on the ecological features of faunal and floral assemblages and the depositional context of fossil remains, but recently stable isotope analyses of tooth enamel and pedogenic carbonate have proved useful for exploring African Neogene ecosystems (Cerling et al., 1997, 2011; Kingston and Harrison, 2007; Ségalen et al., 2007; Uno et al., 2011).

Throughout the Neogene, faunas and floras in Africa were influenced by major climatic changes. The asynchronous growth of polar ice caps caused latitudinal shifts of climatic belts and therefore modified continental environments. In the Early Miocene, tropical forest in Africa was widespread on either side of the equator, but with the growth of the Antarctic ice sheet, the tropical belt was displaced northwards and reached the southern part of Europe during the Middle Miocene (Pickford and Morales, 1994; Pickford and Senut, 2003). Later, it shifted southwards to the current position when the Arctic ice cap expanded during the Pliocene (Kennett, 1995; Zachos et al., 2001). The aridification of Africa began in the southwest with the formation of the Namib Desert (17-16 Ma), much earlier than the region of the present-day Sahara (8-7 Ma) (review in Senut et al., 2009). Development of the Great Rift Valley modified atmospheric circulation patterns in East Africa and led to unstable environmental conditions (Coppens, 1983; review in Senut, 2006a). The earliest documented grassland ecosystem is in Uganda some 17.5 Ma ago (Pickford, 2002). Plants which use C₄ pathway emerged around 10 Ma in Kenya (Uno et al., 2011) and expanded in the Plio-Pleistocene when East African environments began going dry (review in Ségalen et al., 2007). Several animal lineages in eastern and northern Africa were replaced by arid-adapted groups immigrating from southern Africa and Eurasia. Other East African lineages withdrew westwards as the tropical forest cover shrank (review in Senut et al., 2009).

Furthermore, hominids appeared in the Late Miocene in Kenya and possibly in Chad and Ethiopia (review in Senut, 2006b, 2011). The origin of bipedalism is a major issue in the study of human evolution as it is one of the markers of our lineage. For decades it was said to have emerged in an open environment, and that the transition from quadrupedalism to bipedality took place in savanna

(savanna hypothesis). However, Pliocene hominids were partly arboreal, but were bipedal when on the ground (review in Senut, 2006b, 2011). Trees were present in the environment (Bonnefille, 2010). The discovery of *Orrorin tugenensis*, a Late Miocene biped, challenged the commonly accepted savanna hypothesis because its postcranial bones indicated that it was a part time tree-dweller (Senut et al., 2001; Pickford et al., 2002; Galik et al., 2004; Gommery and Senut, 2006). Palaeontological studies of the faunal and floral assemblage suggest a well vegetated environment (Pickford and Senut, 2001; Senut, 2006b; Pickford, 2011; Bamford et al., 2013). It was thus important to test these ideas using an alternative approach.

Our study provides a perspective of the environmental context of the earliest Kenyan hominids (including *Orrorin tugenensis*) through stable isotope analyses of teeth of large herbivores associated with the hominid fossils. Tooth remains were collected in deposits in the eastern foot of the Tugen Hills in the Baringo Basin of the Great Rift Valley (**figure 1**). The sampling localities fall within the Lukeino and Mabaget Formations, dated from the Late Miocene to Early Pliocene (**figure 2**). Fossil teeth of large herbivores were selected according to their abundance in the deposits and their potential for yielding useful isotopic data. We investigated the stable isotope ratios of the biogenic carbonate in enamel. Carbonate (CO_3^{2-}) is incorporated in the phosphate (PO_4^{3-}) and hydroxyl (OH^-) structural sites of biological apatite which is the main constituent of teeth and bones. Bioapatite is a calcium phosphate mineral, the chemical formula of which can be simplified to $\text{Ca}_5(\text{PO}_4, \text{CO}_3)_3(\text{OH}, \text{CO}_3)$ (review in LeGeros and LeGeros, 1984). It is known to record the stable carbon isotope composition of the diet (Krueger and Sullivan, 1984; Cerling and Harris, 1999; Passey et al., 2005) and the stable oxygen isotope composition of the body water which depends on climatic and ecophysiological factors (Longinelli, 1984; Luz and Kolodny, 1985; Kohn et al., 1996). Thus tooth enamel carbonate of wild herbivores can be used to determine aspects of vegetation and climate of their ecosystems.

We analysed tooth enamel rather than bone or dentine because it is more resistant to diagenesis (Lee-Thorp and van der Merwe, 1991; Quade et al., 1992; Ayliffe et al., 1994; Wang and Cerling, 1994; Koch et al., 1997). Compared with bone and dentine, enamel is more mineralized and exhibits a higher crystallinity and a lower porosity (LeGeros and LeGeros, 1984; Asscher et al., 2011). As a result, the recrystallization of bioapatite and the pore-space infilling of secondary minerals are

more limited in enamel than in bone and dentine in the same taphonomic context (Michel et al., 1996; Kohn et al., 1999).

In this paper, we assessed the state of preservation of fossil enamel from the Lukeino and Mabaget Formations before using isotopic data to make palaeoenvironmental interpretations. Fluorine (F), iron (Fe) and manganese (Mn) concentrations were measured in fossil specimens. They are trace elements in modern teeth and bones (LeGeros and LeGeros, 1984; Elliott, 2002; Skinner, 2005). Diagenetic F, Fe and Mn enrichment was observed in herbivore enamel from other Mio-Pliocene deposits in the Kenyan Rift Valley (Kohn et al., 1999; Schoeninger et al., 2003a; Passey et al., 2007). It is reported to result from pore-space infilling by Fe-Mn oxyhydroxides and dissolution/recrystallization processes of bioapatite in which F^- replaces OH^- . We verified that chemical alteration of fossil specimens had no significant effect on palaeoenvironmental signals of biogenic carbonate.

Based on the $^{13}C/^{12}C$ ratio ($\delta^{13}C$), we propose a dietary reconstruction of large herbivores associated with the earliest Kenyan hominids from which we build a picture of the local vegetation. Study of variation in $^{18}O/^{16}O$ ratio ($\delta^{18}O$) was aimed at exploring climate change in the Tugen Hills from the Late Miocene to the Early Pliocene. Lastly we compared our stable carbon isotope data with those obtained from herbivore faunas associated with early putative hominids in Chad and Ethiopia.

2. Background

2.1 Geological context

The Lukeino Formation is of Late Miocene age (Bishop et al., 1971; Pickford, 1975, Pickford and Senut, 2001). Magnetostratigraphy and radiometric dating yielded ages from 6.1 to 5.7 Ma (Hill et al., 1985; Deino et al., 2002; Sawada et al., 2002). This formation is about 100 m thick. It overlies the Kabarnet Trachyte Formation and is covered by the Kaparaina Basalt Formation (**figure 2**). The sediments are fluvio-lacustrine and consist mostly of sandstones, siltstones, tuffs and diatomites. The Kapsomin Basalt and the Rormuch Sills (trachyte intrusions) are points of reference within the Lukeino

Formation, and enable us to distinguish three units: the Kapgoywa Member (ca. 6.1-5.8 Ma), the Kapsomin Member (ca. 5.8 Ma) and the Kapcheberek Member (ca. 5.7 Ma). Remains of the hominid *Orrorin tugenensis* were found in all three members (Pickford, 1975; Senut et al., 2001; Sawada et al., 2002).

The Mabaget Formation is of Early Pliocene age (Pickford et al., 1983, 2004; Hill, 1985) and overlies the Kaparaina Basalt Formation (**figure 2**). Its thickness varies throughout the basin. For instance, there are about 150 m of sediments exposed at Pelion (type section) and 40 m at Mosionin. Deposits are mainly sandstones, siltstones and tuffs, deposited in a fluvio-lacustrine system. Radiometric dating provided ages from 5.3 to 3.8 Ma within the Mabaget Formation at Tabarin (Deino et al., 2002). However, a minimum age of ca. 4.5 Ma would fit with the faunal evidence (Pickford et al., 1983, 2004). This formation yielded remains of an indeterminate hominid, possibly *Australopithecus praegens* or *Ardipithecus ramidus* (Ferguson, 1989; Pickford et al., 2004). A hominid mandible discovered at Tabarin (Hill, 1985) was dated at 4.5-4.4 Ma by Deino et al. (2002).

2.2 Stable carbon isotopes

For wild large herbivores, bioapatite increases in $\delta^{13}\text{C}$ by 12-14‰ relative to plants that they usually eat (Lee-Thorp et al., 1989; Cerling and Harris, 1999). Terrestrial plants have differences in $\delta^{13}\text{C}$ which are related to the photosynthetic pathways, known as C_3 , C_4 and Crassulacean Acid Metabolism (CAM) (Bender, 1971; Deines, 1980). In East African environments, $\delta^{13}\text{C}$ values range from -35 to -23‰ for C_3 plants, and vary between -15 and -11‰ for C_4 plants. CAM plants have intermediate values between C_3 and C_4 plants (Koch et al., 1991; Cerling and Harris, 1999; Cerling et al., 2003c). Trees and most shrubs and forbs use C_3 pathway. The majority of East African grasses are C_4 plants, except for those growing at high altitude, above 2500-3000 m (Tieszen et al., 1979; Livingstone and Clayton, 1980). C_4 plants are better adapted than C_3 plants to low atmospheric CO_2 concentrations (<500 ppmv), high temperatures (>30°C), high irradiance levels and prolonged water stress (Ehleringer, 1979; Ehleringer et al., 1991, 1997). Plants which use CAM pathway are mainly succulents and favour arid environments due to their high water-use efficiency (Ehleringer, 1979).

The $\delta^{13}\text{C}$ of terrestrial plants is controlled by that of CO_2 from which they form (Farquhar et al., 1989). The isotopic composition of atmospheric CO_2 ($\delta^{13}\text{C}_{\text{atm}}$) has been decreasing since the industrial revolution (1800-1850 AD), due to the combustion of ^{13}C depleted fossil fuels. The pre-industrial $\delta^{13}\text{C}_{\text{atm}}$ values (from 1000 to 1800 AD), determined from air trapped in ice cores, are around -6.5‰ (Friedli et al., 1986; Francey et al., 1999). The reconstruction of herbivore palaeodiet and palaeovegetation based on the $\delta^{13}\text{C}$ of fossil teeth needs to take account of $\delta^{13}\text{C}_{\text{atm}}$ values over geological time. Estimates of $\delta^{13}\text{C}_{\text{atm}}$ based on benthic foraminifera $\delta^{13}\text{C}$ records range between -6.7 and -6.1‰ from the Late Miocene to the Early Pliocene (7-4 Ma) (Levin et al., 2008; Tipple et al., 2010), which is comparable to pre-industrial values.

Besides the isotopic composition of source air, the $\delta^{13}\text{C}$ of C_3 and C_4 plants can be influenced by light levels and water availability (Ehleringer et al., 1986; Ehleringer and Cooper, 1988; Farquhar et al., 1989; Stewart et al., 1995; Buchmann et al., 1996). Variations in $\delta^{13}\text{C}$ are also observed within C_4 plants due to different photosynthetic subpathways: the nicotinamide adenine dinucleotide-malic enzyme (NAD), the nicotinamide adenine dinucleotide phosphate-malic enzyme (NADP) and the phosphoenolpyruvate carboxykinase (PCK). For instance, isotopic values average -13‰ for NAD and PCK grasses and -12‰ for nearly all NADP grasses in East Africa (Cerling et al., 2003c). Furthermore, C_3 plants growing in dense tropical forest exhibit lower $\delta^{13}\text{C}$ values than those growing in savanna and bushland. They are subjected to the “canopy effect” which combines the fixation of ^{13}C depleted CO_2 from soil respiration (-25 to -28‰) with the low light levels beneath the canopy (Medina and Minchin, 1980; van der Merwe and Medina, 1991; Jackson et al., 1993). In tropical Africa, the $\delta^{13}\text{C}$ mean value of C_3 plants is around -27‰ in savanna and bushland whereas it varies from -31 to -34‰ in understory of closed-canopy forest (Cerling et al., 2003c, 2004).

To reconstruct palaeodiets of large herbivores, we used the mixing C_3 - C_4 model established by Cerling et al. (2005). The $\delta^{13}\text{C}$ values of herbivore enamel are divided into three dietary categories. The predominantly C_3 diet is characterized by values lower than -8‰. The predominantly C_4 diet exhibits values higher than -2‰. The mixed C_3 - C_4 diet includes values between -8 and -2‰. CAM plants are not considered in the interpretation of $\delta^{13}\text{C}$ values because they are much less common than C_3 and C_4 plants. They do not make a significant contribution to diets of extant large herbivores in

East Africa, except perhaps for black rhinos (*Diceros bicornis*) in arid and semi-arid environments (Cerling and Harris, 1999).

2.3 Stable oxygen isotopes

The $\delta^{18}\text{O}$ of bioapatite depends on the temperature and the $\delta^{18}\text{O}$ of the fluid from which it precipitates (Longinelli and Nuti, 1973; Kolodny et al., 1983; Amiot et al., 2007). As mammals are homeothermic animals, they show a constant $\delta^{18}\text{O}$ offset between body water and PO_4 component in bioapatite (Luz and Kolodny, 1985), and between PO_4 and CO_3 components in bioapatite ($\Delta \approx 9\%$) (review in Martin et al., 2008). The $\delta^{18}\text{O}$ of body water of herbivores is chiefly controlled by the $\delta^{18}\text{O}$ of ingested water (*i.e.* drinking water, water contained in food) and the physiological mechanisms of water conservation (review in Kohn and Cerling, 2002).

The stable oxygen isotope composition of meteoric water ($\delta^{18}\text{O}_{\text{mw}}$) depends on temperature, quantity of precipitation and the $\delta^{18}\text{O}$ of atmospheric water vapour. Precipitation is depleted in ^{18}O from the equator to the poles ("latitude effect"), and with increasing altitude and distance from coastlines (Dansgaard, 1964; Rozanski et al., 1993; Fricke and O'Neil, 1999; Gonfiantini et al., 2001; Poage and Chamberlain, 2001). In the tropics, seasonal variations in $\delta^{18}\text{O}_{\text{mw}}$ are ascribed to the "amount effect". A negative correlation is observed between isotopic values and rainfall amounts (Dansgaard, 1964; Rozanski et al., 1993, 1996). Moisture in the air is derived primarily from evaporation of the ocean surface but, in some regions, water vapour supplied by evapotranspiration of the land surface and terrestrial plants is a substantial source of precipitation (Brubaker et al., 1993) and can affect the $\delta^{18}\text{O}_{\text{mw}}$. For instance, transpired moisture from the Sudd and the Congo Basin is likely responsible for the high isotopic values of rainfall in Ethiopia (Levin et al., 2009).

A rise in temperature and a fall in relative humidity increase the rate of evaporation and plant transpiration, and lead to the ^{16}O depletion of surface water, soil water and leaf water (Craig and Gordon, 1965; Dongman et al., 1974). Leaf water is depleted in ^{16}O relative to soil water from which it is derived (Yakir, 1992; Yakir and Sternberg, 2000). Body water of mammals is also affected by ^{16}O depletion due to the loss of water vapour through sweating and panting (Kohn and Cerling, 2002). As

a result, the PO_4 and CO_3 components in herbivore bioapatite are expected to decrease in $\delta^{18}\text{O}$ when the environment evolves to more humid or colder conditions (« cooler » is a more appropriate word for tropical regions). Variations in moisture source may also impact the isotopic values of bioapatite.

Two isotopic patterns are commonly observed in bioapatite of large herbivores from East Africa. The $\delta^{18}\text{O}$ is lower for hippos than for terrestrial fauna including bovids, elephants, equids, giraffids, rhinos and suids (Bocherens et al., 1996; Cerling et al., 2003b, 2008). Hippos wallow in the water during the day and forage for grass and other fodder at night (Estes, 1991; Kingdon, 1997). It is argued that their amphibious lifestyle restricts the ^{18}O enrichment of body water by reducing the loss of water vapour from body cooling. Night-time intake of plants by hippos could also limit this isotopic enrichment because plant transpiration is reduced during this period (Bocherens et al., 1996; Clementz et al., 2008). Furthermore, $\delta^{18}\text{O}$ values of herbivores with different behaviours record different aspects of the same environment. The obligate-drinking species (e.g. black rhino, elephant, hippo) track the $\delta^{18}\text{O}_{\text{mw}}$ more faithfully than the drought-tolerant species (e.g. dikdik, giraffe, oryx). The latter obtain most of their water from leaves and their isotopic values are impacted by changes in relative humidity. The highest $\delta^{18}\text{O}$ values are commonly observed in drought-tolerant herbivores at the ecosystem level (Kohn et al., 1996; Cerling et al., 2003b, Levin et al., 2006).

3. Materials and methods

We studied 181 molars and premolars of large herbivorous mammals from sixteen localities of the Lukeino and Mabaget Formations (**figure 2**). Teeth from the three members of the Lukeino Formation were collected. Fossil specimens are distributed among nine families within three orders: artiodactyls (bovids, giraffids, hippos and suids), perissodactyls (equids and rhinos) and proboscideans (deinotheres, elephantids and gomphotheres). Deinotheres and gomphotheres are extinct groups. Some teeth were identified at the species or genus level: *Nyanzachoerus jaegeri* (suid) in the Mabaget Formation, *Nyanzachoerus tulotos* (suid) in the Lukeino Formation, *Anancus kenyensis* (gomphothere), *Deinotherium bozasi* (deinothere), *Eurygnathohippus* (formerly *Hipparion*, equid) and *Hippopotamus* “sensu lato” (hippo) in both formations. Updated faunal lists of these formations were reported by Pickford et al. (2009).

An enamel powder was obtained from each tooth with a drill and a diamond burr. After cleaning the tooth surface, samples were taken along a vertical groove on the crown. Bulk samples cover several months to a few years of biomineralization, depending on the rate of enamel growth in the species (see table 1 in Kohn, 2004) and the size of the tooth remains.

Measurements of F, Fe and Mn concentrations were performed on nineteen specimens from the Lukeino Formation and twenty from the Mabaget Formation. These individuals are bovids (n=7), deinotheres (n=2), elephantids (n=1), equids (n=2), giraffids (n=1), hippos (n=20), rhinos (n=2) and indeterminate proboscideans (n=4). To provide comparative chemical data, we analysed four molars and premolars of extant bovids (goats/sheep) from the Tugen Hills. These teeth were collected in the field or in the vicinity of dwellings. Before performing the analyses, bulk samples were treated with 0.1 M acetic acid for 10 min to remove diagenetic carbonate. They were rinsed with deionized water and then dried at 60-70°C. After that step, samples were dissolved in 30% nitric acid at 100°C for 1h. Some 30 mg of sample were commonly used for these analyses. The fluorine content was analysed with a fluoride ion selective electrode (*Mettler Toledo* electrode). The iron and manganese concentrations were tested by ICP-AES (*Jobin Yvon 2000* spectrometer). Relative uncertainty of measurements was about 5%. Results are presented in **Appendix 1**, Supplementary Material.

Stable isotope measurements were made on the CO₃ component of all fossil teeth (n=181). Bulk samples were pretreated with 2% bleach for 15 min and 0.1 M acetic acid for 10 min. These two reactions were aimed at removing organic contaminants and diagenetic carbonate, respectively. Enamel powders were rinsed after each treatment and then dried at 60-70°C. Samples weighing 0.8-1.2 mg were reacted with 100% phosphoric acid in single vessels under vacuum at 70°C for 12 min. The resultant CO₂ was analysed by dual-inlet isotope-ratio mass spectrometry (GV Instruments Isoprime spectrometer). The stable oxygen isotope ratio of carbonate in bioapatite was calculated using the temperature-dependent fractionation factor between calcite and CO₂ produced as a result of phosphoric acid digestion (Swart et al., 1991). The ¹³C/¹²C and ¹⁸O/¹⁶O ratios were reported in the δ notation relative to the international standard PDB, in parts per mil, as: $\delta^{13}\text{C} (\text{‰}) = [({}^{13}\text{C}/{}^{12}\text{C})_{\text{sample}} / ({}^{13}\text{C}/{}^{12}\text{C})_{\text{standard}} - 1] \times 1000$ and $\delta^{18}\text{O} (\text{‰}) = [({}^{18}\text{O}/{}^{16}\text{O})_{\text{sample}} / ({}^{18}\text{O}/{}^{16}\text{O})_{\text{standard}} - 1] \times 1000$. Corrections were

based on working carbonate standards calibrated to the reference material NBS-19. Analytical precision of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ was 0.1‰ for repeated measurements of the standards. Results are listed in **Appendix 2**, Supplementary Material.

Statistics were performed using the *R* software. We used the Mann-Whitney test (1947) for comparisons between two independent groups. The latter are significantly different if the p-value is lower than the significance level α , fixed at 0.05 (5%).

4. Results and discussion

4.1. State of preservation of fossil enamel

All fossil specimens show higher F, Fe and Mn concentrations than extant individuals (**table 1**). On average, fossil enamel contains about 40 times more fluorine, 35 times more iron and 120 times more manganese than modern enamel. Comparable F, Fe and Mn concentrations were measured in herbivore enamel from other Mio-Pliocene deposits in the Kenyan Rift Valley (Kohn et al., 1999; Schoeninger et al., 2003a; Passey et al., 2007). No significant difference was observed between individuals from the Lukeino Formation (n=19) and those from the Mabaget Formation (n=20) (p-value=0.59 for [F], 0.43 for [Fe] and 0.73 for [Mn]). Moreover, the chemical composition of fossil enamel is not significantly different between bovids (n=7) and hippos (n=20) (p-value=0.13 for [F], 0.20 for [Fe] and 0.69 for [Mn]). The other families were excluded from statistical comparisons due to the low number of analysed specimens (see section 3). Trace element enrichment in fossil enamel reflects the climatic conditions and tectono-volcanic activity of the East African Rift. Tropical weathering of basaltic lava flows (*i.e.* mafic rocks) could be responsible for large amounts of Fe and Mn in diagenetic fluids. High F concentrations in these fluids probably result from hydrothermal alteration in the Baringo Basin (Renaut et al., 2002; Tarits et al., 2006). At Cheboit and Kapsomin in the Lukeino Formation, there are extensive tufa deposits which accumulated around hot springs (Pickford et al., 2009).

Previous studies emphasized chemical alterations of herbivore enamel from the Lukeino and Mabaget Formations. Cathodoluminescence observations of fossil specimens other than those analysed indicated an incorporation of rare earth elements and manganese into the apatite lattice (Ségalen et al., 2008). Infrared spectroscopy highlighted a loss of structural carbonate and a gain in crystallinity (Roche et al., 2010). In addition, fossil enamel exhibits a higher calcium/phosphorus mass ratio than modern enamel (Roche et al., 2010).

Despite chemical alterations of fossil enamel, no correlation was observed between isotopic ratios of biogenic carbonate and F, Fe and Mn concentrations. The stable carbon and oxygen isotope ratios of fossil specimens fall within the statistical range of extant large herbivores from East Africa (Bocherens et al., 1996; Cerling et al., 2003b; Levin et al., 2008). We measured values from -14.0 to 1.2‰ for $\delta^{13}\text{C}$, and between -5.8 and 4.9‰ for $\delta^{18}\text{O}$. Preservation of the palaeoenvironmental signals in tooth enamel carbonate of large herbivores from the Lukeino and Mabaget Formations is supported by the fact that the $\delta^{18}\text{O}$ values are consistent with the amphibious lifestyle of hippos and the drought tolerance in giraffids. In each formation, hippos are significantly depleted in ^{18}O compared with terrestrial fauna including bovids, equids, giraffids, proboscideans, rhinos and suids (p-value<0.001) (**figure 3**). This isotopic difference was expected (see section 2.3). However, all $\delta^{18}\text{O}$ values of fossil hippos, except one, are included in the statistical range of terrestrial herbivores, which is not observed in modern counterparts (Bocherens et al., 1996; Cerling et al., 2003b, 2008). A possible explanation for this is that hippos had a lower affinity for water habitat in Mio-Pliocene time than today. In the Lukeino Formation, the $\delta^{18}\text{O}$ values average $-2.6 \pm 1.2\text{‰}$ for hippos (n=16) and $-0.3 \pm 2.5\text{‰}$ for terrestrial herbivores (n=55). The mean values are $-2.0 \pm 1.3\text{‰}$ for hippos (n=32) and $-0.5 \pm 2.2\text{‰}$ for terrestrial herbivores (n=78) sampled from the Mabaget Formation. The giraffid specimen from this formation exhibits one of the highest $\delta^{18}\text{O}$ values (4.4‰), as expected for a drought-tolerant animal. Extant giraffes are non-obligate drinkers (see section 2.3) and commonly have high $\delta^{18}\text{O}$ values, up to 11‰ (Cerling et al., 2003b, 2008; Kingston and Harrison, 2007).

Furthermore, the $\delta^{18}\text{O}$ values average $0.0 \pm 2.5\text{‰}$ for bovids (n=32), $-1.9 \pm 1.0\text{‰}$ for deinotheres (n=8), $-3.1 \pm 1.9\text{‰}$ for elephantids (n=8), $2.1 \pm 1.9\text{‰}$ for equids (n=13), $0.2 \pm 1.1\text{‰}$ for gomphotheres (n=8), $0.4 \pm 1.5\text{‰}$ for rhinos (n=25) and $-1.4 \pm 1.6\text{‰}$ for suids (n=21) in the Lukeino and

Mabaget Formations. Difference in $\delta^{18}\text{O}$ is significant between elephantids and equids which represent the extremes ($p\text{-value} < 0.001$). This is consistent with isotopic data of modern counterparts (Bocherens et al., 1996; Cerling et al., 2008). We also calculated a mean $\delta^{18}\text{O}$ value of $-1.5 \pm 2.1\text{‰}$ for unidentified fossil specimens ($n=17$) which are either elephantids or gomphotheres.

4.2. Palaeodiets of large herbivores

The $\delta^{13}\text{C}$ values of herbivore enamel indicate the presence of C_3 plant eaters, C_4 plant consumers and mixed feeders in the Lukeino and Mabaget Formations (**figure 4**). These dietary categories were previously observed in other Late Miocene and Early Pliocene faunas in East Africa (Cerling et al., 2003a; Levin et al., 2008; White et al., 2009; Uno et al., 2011).

Bovids from the Lukeino and Mabaget Formations exhibit $\delta^{13}\text{C}$ values from -14.0 to 0.7‰ . There are ten specimens with a C_3 diet, nine with a C_4 diet and thirteen with a mixed diet. The distribution of isotopic values displays a gap of $5\text{--}6\text{‰}$ between two groups in each formation. One guild comprises C_3 plant eaters whereas the second consists of C_4 plant consumers and mixed feeders, except for one individual in the Lukeino Formation close to C_3 plant eaters ($\delta^{13}\text{C} = -7.7\text{‰}$). A discontinuous range of $\delta^{13}\text{C}$ values for extant bovids is observed in East African environments and results from browsing and grazing behaviours among species (Cerling et al., 2003c).

The giraffid specimen from the Mabaget Formation has a $\delta^{13}\text{C}$ value of -13.1‰ which indicates a C_3 diet. The latter is observed in extant giraffes (*Giraffa camelopardalis*) and reflects the intake of tree leaves (Cerling and Harris, 1999).

Fossil specimens of *Hippopotamus* “sensu lato” have $\delta^{13}\text{C}$ values between -13.1 and 0.2‰ . Most individuals (30 out of 48) are mixed feeders. There are nine specimens with a C_3 diet and another nine with a C_4 diet. Difference in $\delta^{13}\text{C}$ is not significant between individuals from the Lukeino Formation ($n=16$) and those from the Mabaget Formation ($n=32$) ($p\text{-value}=0.23$). The mean value is $-5.3 \pm 3.4\text{‰}$ for all specimens. Two individuals from the Mabaget Formation are clearly separated from the others and exhibit the lowest $\delta^{13}\text{C}$ and highest $\delta^{18}\text{O}$ values (**figure 4a**). These ratios would not be

ecologically discrepant because such an isotopic variation among fossil hippos was previously observed in Pliocene deposits in the Turkana Basin, a tectonically controlled lake system in northern Kenya and southern Ethiopia (Schoeninger et al., 2003b). The heterogeneity of stable isotope values for fossil hippos may reflect differences in their habitat (grassland vs. woodland, river vs. lake), as observed in their modern counterparts (Boisserie et al., 2005; Cerling et al., 2008). These isotopic studies also indicate that most extant hippos (*Hippopotamus amphibius*) are mixed feeders with a predominant intake of C₄ plants.

The $\delta^{13}\text{C}$ values of suids from the Lukeino and Mabaget Formations vary between -11.5 and -1.4‰. Most individuals (17 out of 21) are mixed feeders. Three specimens have C₃ diet and one a C₄ diet. Values average $-6.6 \pm 2.3\text{‰}$ for *Nyanzachoerus tulotos* (n=8) in the Lukeino Formation, and $-4.8 \pm 1.9\text{‰}$ for *Nyanzachoerus jaegeri* (n=13) in the Mabaget Formation. Although the interspecific difference is not significant (p-value=0.10), the highest values (above -4‰) were only observed in *Nyanzachoerus jaegeri* (4 out of 13 individuals). *Nyanzachoerus jaegeri* could have a higher intake of grasses (C₄ plants) than *Nyanzachoerus tulotos*. This interpretation is in agreement with their dental morphology. *Nyanzachoerus tulotos* has brachyodont cheek teeth (*i.e.* low-crowned teeth) whereas *Nyanzachoerus jaegeri* is characterized by hypsodont cheek teeth (*i.e.* high-crowned teeth) (Harris and Cerling, 2002). The latter species is better adapted to the intake of grasses which are very abrasive plants due to their high phytolith content (Piperno, 2006). Published $\delta^{13}\text{C}$ data of nyanzachoeres provide additional support for our palaeodietary reconstruction (Harris and Cerling, 2002; Levin et al., 2008).

Specimens of the equid *Eurygnathohippus* (formerly called *Hipparion*) show $\delta^{13}\text{C}$ values from -4.8 to 0.2‰ for both formations. The majority of individuals (9 out of 13) has a C₄ diet and the remaining specimens are mixed feeders. This result is consistent with the dentition of these fossil equids which have hypsodont cheek teeth reflecting their grazing habits (Bernor et al., 2010). Other isotopic studies also demonstrate the predominant intake of C₄ plants in *Eurygnathohippus* (Levin et al., 2008; White et al., 2009; Uno et al., 2011). No significant difference in $\delta^{13}\text{C}$ was observed between individuals from the Lukeino Formation (n=8) and those from the Mabaget Formation (n=5) (p-value=0.88). The mean value is $-1.4 \pm 1.5\text{‰}$ for all specimens.

Rhinos from the Lukeino and Mabaget Formations have $\delta^{13}\text{C}$ values from -13.7 to -0.8‰. Among the individuals analysed, there are ten with a C_3 diet, four with a C_4 diet and eleven with a mixed diet. The range of $\delta^{13}\text{C}$ values is discontinuous in each formation. For specimens from the Mabaget Formation, a gap was observed between -5.8 and -3.4‰, within the category of mixed feeders. There are two guilds: one toward the C_3 diet ($n=14$), the other toward the C_4 diet ($n=4$). We noticed three groups in the Lukeino Formation: C_3 plant eaters ($\delta^{13}\text{C}$ lower than -11‰, $n=2$), C_4 plant consumers ($\delta^{13}\text{C}$ higher than -2‰, $n=2$) and mixed feeders ($\delta^{13}\text{C}$ between -8 and -5‰, $n=3$). The heterogeneity of $\delta^{13}\text{C}$ values for fossil rhinos could reflect interspecific variation in diet. *Brachyotherium lewisi*, *Ceratotherium praecox*, *Diceros bicornis* and *Diceros cf. pachygnathus* were reported in faunal lists of the Lukeino and Mabaget Formations (Pickford et al., 2009). In Africa today, there are two rhino species with different dietary habits. The black rhino (*Diceros bicornis*) is a browser and consequently a C_3 plant eater whereas the white rhino (*Ceratotherium simum*) is a grazer characterized by a C_4 diet (Bocherens et al., 1996; Cerling et al., 2003b).

The $\delta^{13}\text{C}$ values of *Deinotherium bozasi* vary between -12.7 and -11.9‰ for both formations. All specimens have a C_3 diet, which is consistent with their dentition. Deinotheres have brachyodont and lophodont cheek teeth which are adapted to browsing (Shoshani and Tassy, 1996). They are reported to have maintained a C_3 diet throughout the entire record in East Africa (Cerling et al., 1999; Uno et al., 2011). One individual was analysed in the Lukeino Formation. Its $\delta^{13}\text{C}$ value is included in the statistical range of the seven specimens from the Mabaget Formation. The mean value is $-12.2 \pm 0.3\text{‰}$ for all individuals.

For *Anancus kenyensis*, the $\delta^{13}\text{C}$ values range from -2.1 to 1.2‰. Almost all individuals have a C_4 diet (7 out of 8). One specimen from the Lukeino Formation is a mixed feeder, very close to C_4 plant eaters. The cheek teeth of *Anancus kenyensis* are brachyodont and bunodont with thick enamel and the crown is usually heavily invested by cementum. On the basis of the dental morphology, this gomphothere species was reported to be adapted to the intake of nuts (tough fruits) and/or grasses (abrasive plants) (Shoshani and Tassy, 1996). Isotopic data of *Anancus kenyensis* are in agreement with grazing habits. They cannot be explained by a prevalent intake of nuts which leads to a C_3

signature of bioapatite. The predominantly C₄ diet of *Anancus* is also confirmed by other isotopic studies (Levin et al., 2008; White et al., 2009; Uno et al., 2011). Three individuals were analysed in the Mabaget Formation. Their $\delta^{13}\text{C}$ values range within those of the five specimens from the Lukeino Formation. The mean value is $-0.1 \pm 1.1\text{‰}$ for all individuals.

Elephantids from the Lukeino and Mabaget Formations exhibit $\delta^{13}\text{C}$ values between -12.4 and -3.2‰. Nearly all specimens are C₃ plant consumers (7 out of 8). One individual from the Mabaget Formation has a mixed diet, rich in C₄ plants. Stable isotope analyses of extant African elephants (*Loxodonta*) indicate diets dominated by C₃ plants, even though some specimens show evidence for a mixed C₃-C₄ diet (Cerling et al., 1999).

Another 17 specimens of proboscideans from the two formations were investigated but could not be identified due to the small size of the tooth remains. They are either elephantids or gomphotheres (*Anancus kenyensis*). Their $\delta^{13}\text{C}$ values vary from -12.4 to 0.7‰. Values of C₃ plant eaters (n=5) plot apart from those of mixed feeders (n=4) and C₄ plant consumers (n=8). The C₃ diet group is likely composed of elephantids whereas the C₄/mixed diet guild may comprise gomphotheres and elephantids.

4.3. Past vegetation and climate of the Tugen Hills

The Lukeino Formation mammalian fauna is dominated by impalas and colobus monkeys, and suggests that the environment comprised thicket, woodland and forest (Pickford and Senut, 2001). Remains of water chevrotains, tree civets, fruit bats and lorises attest to the presence of forest areas (Pickford et al., 2009, Pickford, 2011). Some fossil leaves from this formation are associated with woodland or dry evergreen forest, others suggest a more humid forest (Senut, 2006b; Senut et al., 2010; Bamford et al., 2013). The faunal assemblage of the Mabaget Formation, including water chevrotains and peafowls, suggests that the environment was heavily vegetated (Pickford et al., 2004, 2009).

The $\delta^{13}\text{C}$ values of fossil large herbivores indicate that the earliest Kenyan hominids inhabited a mixed C_3 - C_4 environment. They are comparable to those of extant large herbivores living in East African savanna and bushland, taking into account the correction for the anthropogenic ^{13}C depletion of atmospheric CO_2 (Bocherens et al., 1996; Cerling et al., 2003b; Levin et al., 2008). By contrast, extant herbivores living in dense tropical forest - C_3 ecosystem marked by the “canopy effect” - exhibit much lower isotopic values than those measured (Cerling et al., 2004). However, a minority of fossil specimens ($n=17$) show $\delta^{13}\text{C}$ values lower than -12‰ which may reflect C_3 diets in wooded areas. Such negative values were observed in bovids ($n=5$), giraffids ($n=1$), hippos ($n=1$), proboscideans ($n=8$) and rhinos ($n=2$) from the Mabaget Formation (**figure 4a**) and the Kapcheberek and Kapsomin Members of the Lukeino Formation (**figure 4b,c**). Trees did not form a continuous cover in the Tugen Hills during the Late Miocene and Early Pliocene but they could have formed fairly dense stands where water supply was optimal. Given the depositional context of the fossil remains, patches of woodland were probably located around the Lukeino and Mabaget palaeolakes and along the rivers which drained into the lakes (*i.e.* riparian vegetation).

C_3 diets of hippos, rhinos and suids from the Lukeino Formation were only observed in the Kapcheberek Member, the uppermost unit (**figure 4b**). Nearly all bovid specimens from this member were C_3 plant eaters unlike those from the lower stages of the formation (**figure 4c,d**). These results suggest that there was more C_3 vegetation within the Tugen Hills in the latest Miocene (*ca.* 5.7 Ma) than before (*ca.* 6.1-5.8 Ma). A greater C_3 plant biomass could reflect a higher woody cover (*i.e.* more trees and shrubs). The lowest values of C_3 plant consumers were observed in ten individuals from the Mabaget Formation (-12.5 to -14.0‰) (**figure 4a**). $\delta^{13}\text{C}$ values of C_3 plants decrease in shaded and watered conditions (Ehleringer et al., 1986; Ehleringer and Cooper, 1988; Farquhar et al., 1989; Stewart et al., 1995). A possible explanation is that the Tugen Hills were more wooded (leading to less light on the ground) and/or more humid during the Early Pliocene (*ca.* 5.3-4.5 Ma) than in the Late Miocene (*ca.* 6.1-5.7 Ma). This is consistent with palaeontological studies based on faunal assemblages (Pickford et al., 2004, 2009). Pollen data from sediments in the Gulf of Aden and offshore the Niger delta suggest a tree expansion in tropical Africa from roughly 5.5 to 3.5 Ma (Bonnefille, 2010). A decline of C_4 grass biomass in northeastern Africa by 4.3 Ma is indicated by decreased $\delta^{13}\text{C}$ values of plant leaf wax from marine deposits of the Gulf of Aden (Feakins et al.,

2013). Moreover, a shift to more negative $\delta^{13}\text{C}$ values in pedogenic carbonate from Awash and Turkana Basins suggests that peaks of woody vegetation occurred between 3.9 and 3.2 Ma in Northeast Africa (Cerling et al., 2011).

There are fifteen individuals from the Mabaget Formation and eleven from the Kapcheberek Member which show lower $\delta^{18}\text{O}$ values than those from the lower members of the Lukeino Formation. These values range from -3.3 to -5.8‰ (**figure 4a,b**) and they were observed in bovids (n=5), hippos (n=7), proboscideans (n=10), rhinos (n=1) and suids (n=3). They could reflect a stronger “amount effect” on $\delta^{18}\text{O}_{\text{mw}}$, implying that moister conditions occurred in the Tugen Hills during the latest Miocene and the Early Pliocene than before. Rainfall patterns in East Africa today are controlled by major air streams (*i.e.* northeast and southeast monsoons, westerlies), the position of convergence zones (*i.e.* Intertropical Convergence Zone, Congo Air Boundary), topography and sea surface temperature (SST) fluctuations in the Indian and Atlantic Oceans (Nicholson, 1996). Observational studies emphasize that warmer Indian Ocean SST are currently associated with more rain in East Africa (review in Cane and Molnar, 2001). Such conditions might have occurred in the latest Miocene and the Early Pliocene. Increased moisture would have led to more C_3 vegetation in the latest Miocene, as suggested by $\delta^{13}\text{C}$ values. Specimens from the Mabaget Formation do not exhibit more negative $\delta^{18}\text{O}$ values than those from the Kapcheberek Member. Local moisture in the Early Pliocene would have been comparable to that of the latest Miocene. This suggests that the shift to lower $\delta^{13}\text{C}$ values in C_3 plant eaters from the Mabaget Formation is a result of vegetation change. Denser stands of trees - where understory grew in lower light conditions - likely appeared within the Tugen Hills in the Early Pliocene. Statistical comparisons between fossil assemblages at the species level are necessary to confirm or refute these hypotheses; a larger dataset of fossil enamel samples, particularly from the Miocene deposits, is required for these comparisons. Furthermore, the aridity index established by Levin et al. (2006) could not have been applied to fossil assemblages. It takes account of enamel $\delta^{18}\text{O}$ values of giraffid, dikdik and oryx (two bovid taxa) which are evaporation sensitive herbivores. Unfortunately, we had only one giraffid individual (from the Mabaget Formation) and bovid specimens in both formations were too fragmented to be identified at the genus or species level.

4.4. Large herbivores associated with early potential hominids in Chad and Ethiopia

The $\delta^{13}\text{C}$ values of fossil large herbivores from the Tugen Hills were compared with those for counterparts associated with early putative hominids: *Ardipithecus kadabba*, *Ardipithecus ramidus* and *Sahelanthropus tchadensis* (**figure 5**). In these species, the claimed bipedalism - a distinctive trait of the family Hominidae in the study of hominoid evolution - needs to be reassessed because of insufficient evidence (Senut, 2006b, 2011). Isotopic data of herbivore enamel were compiled from Zazzo (2001), Boissarie et al. (2005), Levin et al. (2008) and White et al. (2009).

Remains of *S. tchadensis* were discovered in the Toros Menalla deposits in the Djurab Desert (Chad) and they were dated at 7-6 Ma on the basis of the faunal assemblage (Brunet et al., 2002). Radiometric dating yielded ages between 7.2 and 6.8 Ma for the sediments thought to contain the hominid fossils (Lebatard et al., 2008), but their stratigraphic position is unclear (Beauvilain, 2008). A detailed study of anthracothere remains from Toros Menalla indicated that the deposits probably span the period 10 to 6 Ma (Pickford, 2008). Palaeontological and sedimentological studies suggest that *S. tchadensis* inhabited a perilacustrine vegetation including gallery and islet forests, wooded savanna and grassland close to a sandy desert (Vignaud et al., 2002; Brunet et al., 2004; Schuster et al., 2006).

In the Awash River Valley (Ethiopia), the remains of *A. kadabba* (5.8-5.2 Ma) and *A. ramidus* (4.5-4.3 Ma) were found in the Late Miocene Adu-Asa Formation and the Early Pliocene Sagantole Formation, respectively (White et al., 1994, 1995; Haile-Selassie, 2001; Semaw et al., 2005; Simpson et al., 2007). Palaeontological studies indicate that *Ardipithecus* might have lived in a woodland with forest patches (WoldeGabriel et al., 2001, 2009; Haile-Selassie et al., 2004; White et al., 2009) whereas the $\delta^{13}\text{C}$ values for pedogenic carbonate suggest a more open ecosystem (Cerling et al., 2010, 2011).

The $\delta^{13}\text{C}$ values for large herbivores range from -11.3 to 2.0‰ in the Toros Menalla sites (n=46), and vary between -15.7 and 3.3‰ in the Adu-Asa and Sagantole Formations (n=286). They indicate that *Sahelanthropus* and *Ardipithecus* lived in mixed $\text{C}_3\text{-C}_4$ environments as did the Mio-Pliocene hominids from the Tugen Hills. Values lower than -12‰ were not observed in large

herbivores from Toros Menalla. One bovid from the Adu-Asa Formation and some deinotheres and giraffids from the Sagantole Formation show such negative values. These observations suggest that woodland represented a scarce or patchy cover within these depositional environments. There is no record of closed-canopy vegetation.

Deinotheres from the Sagantole Formation had a C₃ diet. Their values are close to those of coeval specimens from the Mabaget Formation. Nearly all giraffids from the Chadian and Ethiopian deposits were C₃ plant eaters. One individual from the Sagantole Formation is reported to have had a C₄ diet (Levin et al., 2008). The intake of C₄ plants prevailed for equids and gomphotheres from the Toros Menalla sites and the Adu-Asa and Sagantole Formations. Fossil elephantids from the Awash River Valley had a C₄ or mixed diet whereas those from the Tugen Hills were C₃ plant consumers or mixed feeders. C₃, C₄ and mixed diets were observed in bovids, hippos, rhinos and suids from the Chadian and Ethiopian deposits.

The highest $\delta^{13}\text{C}$ values of bovids, equids, rhinos and suids are recorded by specimens from the Sagantole Formation and they emphasize the relevance of the C₄ grass biomass within the depositional environment. Maximum values are about 1‰ for suids, 2‰ for equids and suids, and 3‰ for bovids. It is worth noting that variations in relative abundance of the NAD, NADP and PCK grasses within ecosystems may cause slight differences in $\delta^{13}\text{C}$ among C₄ plant eaters. Herbivore enamel associated with a diet of NADP grasses is ^{13}C enriched by about 1‰ compared to that associated with diet of NAD/PCK grasses (Cerling and Harris, 1999). The latter favour xeric habitat whereas NADP grasses are more common in mesic habitat (Hattersley, 1992; Cerling et al., 2003c). Water stress and light levels can also affect $\delta^{13}\text{C}$ values of C₄ grasses (Buchmann et al., 1996) and consequently the isotopic composition of herbivore enamel. C₄ grasses generally exhibit the highest $\delta^{13}\text{C}$ values in sunny-watered conditions (Buchmann et al., 1996).

A minority of hippos from the Lukeino and Mabaget Formations had a C₃ diet (9 out of 48 specimens). We observed no C₃ plant eating hippos from the Adu-Asa and Sagantole Formations and only one individual from Toros Menalla is reported to have had a C₃ diet (Boisserie et al., 2005). These

observations suggest that there was a greater C₃ plant biomass (*i.e.* possibly a higher woody cover) near rivers and lakes within the Tugen Hills than in the other depositional environments.

Lastly we notice that Early Pliocene suids (Mabaget and Sagantole Formations) had higher $\delta^{13}\text{C}$ values than their Late Miocene counterparts (Adu-Asa and Lukeino Formations and Toros Menalla sites). Increased intake of C₄ grasses among suids is correlated with the development of hypsodonty during the Plio-Pleistocene (Harris and Cerling, 2002).

5. Conclusion

The aim of this study was to explore the environment of the earliest Kenyan hominids, using the stable carbon and oxygen isotope composition of tooth enamel carbonate of large herbivores. The $\delta^{13}\text{C}$ values show differences in diet among taxa between 6.1 and 4.5 Ma (**figure 6**). Deinotheres, giraffids and nearly all elephantids had a C₃ diet that reflects a browsing behaviour. Equids and gomphotheres were mainly C₄ plant eaters, which indicates grazing habits. Most hippos and suids were mixed C₃-C₄ feeders and they were therefore generalist herbivores. Bovids and rhinos had a diet which varied from C₃ to C₄ plants.

The presence of C₃ plants (trees, shrubs and forbs) and C₄ plants (tropical grasses) in the Tugen Hills during this period indicates a complex of heterogeneous habitats for the fauna including hominids. The palaeontological studies of the faunal assemblages from the Lukeino and Mabaget Formations and the leaf remains from the Lukeino Formation suggested that there was forest at the time of deposition (Pickford and Senut, 2001; Pickford et al., 2004; Senut, 2006b; Pickford, 2011; Bamford et al., 2013). However, the $\delta^{13}\text{C}$ of herbivore enamel emphasizes the significance of C₄ grass biomass in the Tugen Hills, indicating that trees did not form a continuous cover. Isotopic data would fit with a savanna ecosystem (*i.e.* a wooded grassland as defined by White, 1986). But, fairly low isotopic values (between -12‰ and -14‰) do not exclude the presence of patches of woodland (**figure 6**). The latter were likely located in perilacustrine and riparian areas, given the depositional context of the fossil remains.

We observed variations in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ among faunal assemblages which may reflect changes in local climate and vegetation. The $\delta^{18}\text{O}$ data suggest that the Tugen Hills experienced more humid conditions in the Early Pliocene (ca. 5.3-4.5 Ma) and the latest Miocene (ca. 5.7 Ma) than before (ca. 6.1-5.8 Ma). The $\delta^{13}\text{C}$ values indicate that the C_3 plant biomass might have increased within the depositional environment during the Late Miocene. The Tugen Hills were probably more wooded in the Early Pliocene than before, which is consistent with palaeontological studies (Pickford et al., 2004, 2009). It is worth noting that our study focussed on large herbivores which represent a part of the fauna discovered in the two formations. Integrating other groups (e.g. gastropods, rodents, primates) in the isotopic approach is necessary to refine the environmental context of the earliest Kenyan hominids.

Postcranial studies show that *Orrorin tugenensis* was a bipedal hominid which could also climb trees (Senut et al., 2001; Pickford et al., 2002; Galik et al., 2004; Gommery and Senut, 2006). This suggests that human bipedalism originated in a mixture of grassy and wooded areas. Patches of woodland provide food such as fruits but also the possibility to escape from savanna predators. Variations in altitude, latitude and tectonics played a role in the distribution of diverse ecosystems (Bailey et al., 2011) and ultimately in the distribution of hominoids (including hominids) and locomotor behaviours, which happen to be more diverse than previously expected. Reconstructing detailed environments in which hominids emerged is still a challenge and further geochemical studies need to be performed in late Middle Miocene deposits, a period in which the dichotomy between the great apes and the hominids might have occurred (Arnason et al., 2000; Senut and Pickford, 2004; Langergraber et al., 2012).

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References

- Amiot R., Lécuyer C., Escarguel G., Billon-Bruyat J.-P., Buffetaut E., Langlois C., Martin S., Martineau F., Mazin J.M. (2007). **Oxygen isotope fractionation between crocodilian phosphate and water**. *Palaeogeography, Palaeoclimatology, Palaeoecology* 243: 412-420.
- Arnason U., Gullberg A., Schweizer Burguete A., Janke A. (2000). **Molecular estimates of primate divergences and new hypotheses for primate dispersal and the origin of modern humans**. *Hereditas* 133: 217-228.
- Asscher Y., Weiner S., Boaretto E. (2011). **Variations in atomic disorder in biogenic carbonate hydroxyapatite using the infrared spectrum grinding curve method**. *Advanced Functional Materials* 21: 3308-3313.
- Ayliffe L.K., Chivas A.R., Leakey M.G. (1994). **The retention of primary oxygen isotope compositions of fossil elephant skeletal phosphate**. *Geochimica et Cosmochimica Acta* 58: 5291-5298.
- Bailey G.N., Reynolds S.C., King G.C.P. (2011). **Landscapes of human evolution: models and methods of tectonic geomorphology and the reconstruction of hominin landscapes**. *Journal of Human Evolution* 60: 257-280.
- Bamford M.K., Senut B., Pickford M. (2013). **Fossil leaves from the Upper Miocene Lukeino Formation (Baringo, Kenya)**. *Geobios* 46: 253-272.
- Beauvilain A. (2008). **The contexts of discovery of *Australopithecus bahrelghazali* (Abel) and of *Sahelanthropus tchadensis* (Toumai): unearthed, embedded in sandstone, or surface collected?** *South African Journal of Science* 104: 165-168.
- Bender M. (1971). **Variations in the $^{13}\text{C}/^{12}\text{C}$ ratios of plants in relation to the pathway of photosynthetic carbon dioxide fixation**. *Phytochemistry* 10: 1239-1244.

- 669 ➤ Bernor R.L., Armour-Chelu M.J., Gilbert H., Kaiser T.M., Schulz E. (2010). **Equidae**. In Werdelin
670 L., Sanders W.J. (eds.). *Cenozoic Mammals of Africa*. University of California Press, Berkeley:
671 691-727.
- 672 ➤ Bishop W.W., Chapman G.R., Hill A., Miller J.A. (1971). **Succession of Cainozoic vertebrate**
673 **assemblages from the Northern Kenya Rift Valley**. *Nature* 233: 389-394.
- 674 ➤ Bocherens H., Koch P.L., Mariotti A., Geraads D., Jaeger J.-J. (1996). **Isotopic biogeochemistry**
675 **(¹³C, ¹⁸O) of mammalian enamel from African Pleistocene hominid sites**. *Palaios* 11: 306-318.
- 676 ➤ Boisserie J.-R., Zazzo A., Merceron G., Blondel C., Vignaud P., Likus A., Mackaye H.T., Brunet
677 M. (2005). **Diets of modern and Late Miocene hippopotamids: evidence from carbon isotope**
678 **composition and micro-wear of tooth enamel**. *Palaeogeography, Palaeoclimatology,*
679 *Palaeoecology* 221: 153-174.
- 680 ➤ Bonnefille R. (2010). **Cenozoic vegetation, climate changes and hominid evolution in tropical**
681 **Africa**. *Global and Planetary Change* 72: 390-411.
- 682 ➤ Brubaker K.L., Entekhabi D., Eagleson P.S. (1993). **Estimation of continental precipitation**
683 **recycling**. *Journal of Climate* 6: 1077-1089.
- 684 ➤ Brunet M., Guy F., Boisserie J.-R., Djimdoumbaye A., Lehmann T., Lihoreau F., Louchart A.,
685 Schuster M., Tafforeau P., Likus A., Mackaye H.T., Blondel C., Bocherens H., de Bonis L.,
686 Coppens Y., Denys C., Düringer P., Eisenmann V., Flisch A., Geraads D., Lopez-Martinez N.,
687 Otero O., Pelaez Campomanes P., Pilbeam D., Ponce de Leon M., Vignaud P., Viriot L., Zollikofer
688 C. (2004). «**Toumaï** », **Miocène supérieur du Tchad, le nouveau doyen du rameau humain**.
689 *Comptes Rendus de l'Académie des Sciences « Palevol »* 3: 275-283.
- 690 ➤ Brunet M., Guy F., Pilbeam D., Mackaye H.T., Likus A., Ahounta D., Beauvilain A., Blondel C.,
691 Bocherens H., Boisserie J.-R., de Bonis L., Coppens Y., Dejax J., Denys C., Düringer P.,
692 Eisenmann V., Fanone G., Fronty P., Geraads D., Lehmann T., Lihoreau F., Louchart A.,
693 Mahamat A., Merceron G., Mouchelin G., Otero O., Pelaez Campomanes P., Ponce de Leon M.,
694 Rage J.C., Sapanet M., Schuster M., Sudre J., Tassy P., Valentin X., Vignaud P., Viriot L., Zazzo
695 A., Zollikofer C. (2002). **A new hominid from the Upper Miocene of Chad, central Africa**.
696 *Nature* 418: 145-152.
- 697 ➤ Buchmann N., Brooks J.R., Rapp K.D., Ehleringer J.R. (1996). **Carbon isotope composition of**
698 **C₄ grass is influenced by light and water supply**. *Plant, Cell and Environment* 19: 392-402.

- 699 ➤ Cane M.A., Molnar P. (2001). **Closing of the Indonesian seaway as a precursor to east**
700 **African aridification around 3-4 million years ago.** *Nature* 411: 157-162.
- 701 ➤ Cerling T.E., Harris J.M. (1999). **Carbon isotope fractionation between diet and bioapatite in**
702 **ungulate mammals and implications for ecological and paleoecological studies.** *Oecologia*
703 120: 347-363.
- 704 ➤ Cerling T.E., Harris J.M., Hart J.A., Kaleme P., Klingel H., Leakey M.G., Levin N.E., Lewison R.L.,
705 Passey B.H. (2008). **Stable isotope ecology of the common hippopotamus.** *Journal of Zoology*
706 276: 204-212.
- 707 ➤ Cerling T.E., Harris J.M., Leakey M.G. (1999). **Browsing and grazing in elephants: the isotope**
708 **record of modern and fossil proboscideans.** *Oecologia* 120: 364-374.
- 709 ➤ Cerling T.E., Harris J.M., Leakey M.G. (2003a). **Isotope paleoecology of the Nawata and Apak**
710 **Formations at Lothagam, Turkana Basin, Kenya.** In Leakey M.G., Harris J.M. (eds.). Lothagam:
711 the Dawn of Humanity in Eastern Africa. Columbia University Press, New York: 605-624.
- 712 ➤ Cerling T.E., Harris J.M., Leakey M.G. (2005). **Environmentally driven dietary adaptations in**
713 **African mammals.** In Ehleringer J.R., Cerling T.E., Dearing M.D. (eds.). A History of Atmospheric
714 CO₂ and its Effects on Plants, Animals, and Ecosystems. *Ecological Studies* 177. Springer, New
715 York: 258-272.
- 716 ➤ Cerling T.E., Harris J.M., Leakey M.G., Mudida N. (2003b). **Stable isotope ecology of northern**
717 **Kenya, with emphasis on the Turkana Basin.** In Leakey M.G., Harris J.M. (eds.). Lothagam: the
718 Dawn of Humanity in Eastern Africa. Columbia University Press, New York: 583-603.
- 719 ➤ Cerling T.E., Harris J.M., MacFadden B.J., Leakey M.G., Quade J., Eisenmann V., Ehleringer J.R.
720 (1997). **Global vegetation change through the Miocene/Pliocene boundary.** *Nature* 389: 153-
721 158.
- 722 ➤ Cerling T.E., Harris J.M., Passey B.H. (2003c). **Diets of East African Bovidae based on stable**
723 **isotope analysis.** *Journal of Mammalogy* 84: 456-470.
- 724 ➤ Cerling T.E., Hart J.A., Hart T.B. (2004). **Stable isotope ecology in the Ituri Forest.** *Oecologia*
725 138: 5-12.
- 726 ➤ Cerling T.E., Levin N.E., Quade J., Wynn J.G., Fox D.L., Kingston J.D., Klein R.G., Brown F.H.
727 (2010). **Comment on the Paleoenvironment of *Ardipithecus ramidus*.** *Science* 328: 1105d.

- 728 ➤ Cerling T.E., Wynn J.G., Andanje S.A., Bird M.I., Korir D.K., Levin N.E., Mace W., Macharia A.N.,
729 Quade J., Remien C.H. (2011). **Woody cover and hominin environments in the past 6 million**
730 **years**. *Nature* 476: 51-56.
- 731 ➤ Clementz M.T., Holroyd P.A., Koch P.L. (2008). **Identifying aquatic habits of herbivorous**
732 **mammals through stable isotope analysis**. *Palaio* 23: 574-585.
- 733 ➤ Coppens Y. (1983). **Les plus anciens fossiles d'Hominidés**. In Chagas C. (ed.). Recent
734 Advances in the Evolution of Primates. *Pontificiae Academiae Scientiarum Scripta Varia* 50.
735 Pontificia Academia Scientiarum, Vatican City: 1-9.
- 736 ➤ Craig H., Gordon L.I. (1965). Deuterium and oxygen-18 variations in ocean and the marine
737 atmosphere. In Tongiorgi E. (ed.). Stable Isotopes in Oceanographic Studies and
738 Paleotemperatures. Consiglio Nazionale delle Ricerche, Laboratorio di Geologia Nucleare, Pisa:
739 9-130.
- 740 ➤ Dansgaard W. (1964). **Stable isotopes in precipitation**. *Tellus* 16: 436-468.
- 741 ➤ Deines P. (1980). **The isotopic composition of reduced organic carbon**. In Fritz P., Fontes
742 J.C. (eds.). The Terrestrial Environment. *Handbook of Environmental Isotope Geochemistry* 1.
743 Elsevier, Amsterdam: 329-545.
- 744 ➤ Deino A.L., Tauxe L., Monaghan M., Hill A. (2002). **$^{40}\text{Ar}/^{39}\text{Ar}$ geochronology and paleomagnetic**
745 **stratigraphy of the Lukeino and lower Chemeron formations at Tabarin and Kapcheberek,**
746 **Tugen Hills, Kenya**. *Journal of Human Evolution* 42: 117-140.
- 747 ➤ Dongmann G., Nürnberg H.W., Förstel H., Wagener K. (1974). **On the enrichment of H_2^{18}O in**
748 **the leaves of transpiring plants**. *Radiation and Environmental Biophysics* 11: 41-52.
- 749 ➤ Ehleringer J.R. (1979). **Photosynthesis and photorespiration: biochemistry, physiology and**
750 **ecology implications**. *HortScience* 14: 217-222.
- 751 ➤ Ehleringer J.R., Cerling T.E., Helliker B.R. (1997). **C_4 photosynthesis, atmospheric CO_2 , and**
752 **climate**. *Oecologia* 112: 285-299.
- 753 ➤ Ehleringer J.R., Cooper T.A. (1988). **Correlations between carbon isotope ratio and**
754 **microhabitat in desert plants**. *Oecologia* 76: 562-566.
- 755 ➤ Ehleringer J.R., Field C.B., Lin Z., Kuo C. (1986). **Leaf carbon isotope and mineral**
756 **composition in subtropical plants along an irradiance cline**. *Oecologia* 70: 520-526.

- 757 ➤ Ehleringer J.R., Sage R.F., Flanagan L.B., Pearcy R.W. (1991). **Climate change and the**
758 **evolution of C₄ photosynthesis**. *Trends in Ecology & Evolution* 6: 95-99.
- 759 ➤ Elliott J.C. (2002). **Calcium Phosphate Biominerals**. In Kohn M.J., Rakovan J., Hughes J.M.
760 (eds.). Phosphates: Geochemical, Geobiological and Material Importance. *Reviews in Mineralogy*
761 *and Geochemistry* 48. Mineralogical Society of America, Washington D.C.: 427-453.
- 762 ➤ Estes R.D. (1991). **The Behavior Guide to African Mammals: including Hoofed Mammals,**
763 **Carnivores, Primates**. University of California Press, Berkeley, 611p.
- 764 ➤ Farquhar G.D., Ehleringer J.R., Hubick K.T. (1989). **Carbon isotope discrimination and**
765 **photosynthesis**. *Annual Review of Plant Physiology and Plant Molecular Biology* 40: 503-537.
- 766 ➤ Feakins S.J., Levin N.E., Liddy H.M., Sieracki A., Eglinton T.I., Bonnefille R. (2013). **Northeast**
767 **African vegetation change over 12 m.y.** *Geology* 41: 295-298.
- 768 ➤ Ferguson W.W. (1989). **Taxonomic status of the hominid mandible KNM TH 13150 from the**
769 **Middle Pliocene of Tabarin, in Kenya**. *Primates* 30: 383-387.
- 770 ➤ Francey R.J., Allison C.E., Etheridge D.M., Trudinger C.M., Enting I.G., Leuenberger M.,
771 Langenfelds R.L., Michel E., Steele L.P. (1999). **A 1000-year high precision record of $\delta^{13}\text{C}$ in**
772 **atmospheric CO₂**. *Tellus* 51B: 170-193.
- 773 ➤ Fricke H.C., O'Neil J.R. (1999). **The correlation between $^{18}\text{O}/^{16}\text{O}$ ratios of meteoric water and**
774 **surface temperature: its use in investigating terrestrial climate change over geologic time**.
775 *Earth and Planetary Science Letters* 170: 181-196.
- 776 ➤ Friedli H., Löffler H., Oeschger H., Siegenthaler U., Stauffer B. (1986). **Ice core record of the**
777 **$^{13}\text{C}/^{12}\text{C}$ ratio of atmospheric CO₂ in the past two centuries**. *Nature* 324: 237-238.
- 778 ➤ Galik K., Senut B., Pickford M., Gommery D., Treil J., Kuperavage A.J., Eckhardt R.B. (2004).
779 **External and internal morphology of the BAR 1002'00 *Orrorin tugenensis* femur**. *Science*
780 305: 1450-1453.
- 781 ➤ Gommery D., Senut B. (2006). **La phalange distale du pouce d'*Orrorin tugenensis* (Miocène**
782 **supérieur du Kenya)**. *Geobios* 39: 372-384.
- 783 ➤ Gonfiantini R., Roche M.-A., Olivry J.C., Fontes J.C., Zuppi G.M. (2001). **The altitude effect on**
784 **the isotopic composition of tropical rains**. *Chemical Geology* 181: 147-167.
- 785 ➤ Haile-Selassie Y. (2001). **Late Miocene hominids from the Middle Awash, Ethiopia**. *Nature*
786 412: 178-181.

- 787 ➤ Haile-Selassie Y., WoldeGabriel G., White T.D., Bernor R.L., DeGusta D., Renne P.R., Hart W.K.,
788 Vrba E., Stanley A., Howell F.C. (2004). **Mio-Pliocene mammals from the Middle Awash,**
789 **Ethiopia.** *Geobios* 37: 536–552.
- 790 ➤ Harris J.M., Cerling T.E. (2002). **Dietary adaptations of extant and Neogene African suids.**
791 *Journal of Zoology* 256: 45-54.
- 792 ➤ Hattersley P.W. (1992). **C₄ photosynthetic pathway variation in grasses (Poaceae): its**
793 **significance for arid and semi-arid lands.** In Chapman G.P. (ed.). *Desertified Grasslands: their*
794 *Biology and Management.* Academic Press, London: 181-212.
- 795 ➤ Hill A. (1985). **Early hominid from Baringo, Kenya.** *Nature* 315: 222–224.
- 796 ➤ Hill A., Drake R., Tauxe L., Monaghan M., Barry J.C., Behrensmeyer A.K., Curtis G., Jacobs B.F.,
797 Jacobs L., Johnson N., Pilbeam D. (1985). **Neogene palaeontology and geochronology of the**
798 **Baringo Basin, Kenya.** *Journal of Human Evolution* 14: 759-773.
- 799 ➤ Jackson P.C., Meinzer F.C., Goldstein G., Holbrook N.M., Cavelier J., Rada F. (1993).
800 **Environmental and physiological influences on carbon isotope composition of gap and**
801 **understory plants in a lowland tropical forest.** In Ehleringer J.R., Hall A.E., Farquhar G.D.
802 (eds.). *Stable Isotopes and Plant Carbon-Water Relations.* Academic Press, San Diego: 131-140.
- 803 ➤ Kennett J.P. (1995). **A review of polar climatic evolution during the Neogene, based on the**
804 **marine sediment record.** In Vrba E.S., Denton G.H., Partridge T.C., Burckle L.H. (eds.).
805 *Paleoclimate and Evolution, with Emphasis on Human Origins.* Yale University Press, New Haven:
806 49-64.
- 807 ➤ Kingdon J. (1997). **The Kingdon Field Guide to African Mammals.** Reprinted with corrections by
808 A&C Black, London, 476p.
- 809 ➤ Kingston J.D., Harrison T. (2007). **Isotopic dietary reconstructions of Pliocene herbivores at**
810 **Laetoli: implications for early hominin paleoecology.** *Palaeogeography, Palaeoclimatology,*
811 *Palaeoecology* 243: 272-306.
- 812 ➤ Kleinsasser L.L., Quade J., McIntosh W.C., Levin N.E., Simpson S.W., Semaw S. (2008).
813 **Stratigraphy and geochronology of the Late Miocene Adu-Asa Formation at Gona, Ethiopia.**
814 *In* Quade J., Wynn J.G. (eds.). *The Geology of Early Humans in the Horn of Africa. Special Paper*
815 *446.* The Geological Society of America, Boulder: 33-65.

- 816 ➤ Koch P.L., Behrensmeyer A.K., Fogel M.L. (1991). **The isotopic ecology of plants and animals**
817 **in Amboseli National Park.** *Annual Report of the Director of the Geophysical Laboratory,*
818 *Carnegie Institution of Washington 1990-1991: 163-171.*
- 819 ➤ Koch P.L., Tuross N., Fogel M.L. (1997). **The effects of sample treatment and diagenesis on**
820 **the isotopic integrity of carbonate in biogenic hydroxylapatite.** *Journal of Archaeological*
821 *Science* 24: 417–429.
- 822 ➤ Kohn M.J. (2004). **Comment: Tooth enamel mineralization in ungulates: Implications for**
823 **recovering a primary isotopic time-series, by B. H. Passey and T. E. Cerling (2002).**
824 *Geochimica et Cosmochimica Acta* 68: 403–405.
- 825 ➤ Kohn M.J., Cerling T.E. (2002). **Stable isotope compositions of biological apatite.** *In* Kohn
826 M.J., Rakovan J., Hughes J.M. (eds.). *Phosphates: Geochemical, Geobiological and Material*
827 *Importance. Reviews in Mineralogy and Geochemistry* 48. Mineralogical Society of America,
828 Washington DC: 455-488.
- 829 ➤ Kohn M.J., Schoeninger M.J., Barker W.W. (1999). **Altered states: effects of diagenesis on**
830 **fossil tooth chemistry.** *Geochimica et Cosmochimica Acta* 63: 2737-2747.
- 831 ➤ Kohn M.J., Schoeninger M.J., Valley J.W. (1996). **Herbivore tooth oxygen compositions:**
832 **effects of diet and physiology.** *Geochimica et Cosmochimica Acta* 60: 3889-3896.
- 833 ➤ Kolodny Y., Luz B., Navon O. (1983). **Oxygen isotope variations in phosphate of biogenic**
834 **apatites, I. fish bone apatite-rechecking the rules of the game.** *Earth and Planetary Science*
835 *Letters* 64: 398-404.
- 836 ➤ Krueger H.W., Sullivan C.H. (1984). **Models for carbon isotope fractionation between diet and**
837 **bone.** *In* Turnland J.F., Johnson P.E. (eds.). *Stable Isotopes in Nutrition. ACS Symposium Series*
838 *258.* American Chemical Society, Washington D.C.: 205-220.
- 839 ➤ Langergraber K.E., Prüfer K., Rowney C., Boesch C., Crockford C., Fawcett K., Inoue E., Inoue-
840 Muruyama M., Mitani J.C., Muller M.N., Robbins M.M., Schubert G., Stoinski T.S., Viola B., Watts
841 D., Wittig R.M., Wrangham R.W., Zuberbühler K., Pääbo S., Vigilant L. (2012). **Generation times**
842 **in wild chimpanzees and gorillas suggest earlier divergence times in great ape and human**
843 **evolution.** *Proceedings of the National Academy of Sciences of the United States of America* 109:
844 15716-15721.

- 845 ➤ Lebatard A.-E., Bourlès D.L., Durringer P., Jolivet M., Braucher R., Carcaillet J., Schuster M.,
846 Arnaud N., Monié P., Lihoreau F., Likius A., Mackaye H.T., Vignaud P., Brunet M. (2008).
847 **Cosmogenic nuclide dating of *Sahelanthropus tchadensis* and *Australopithecus***
848 ***bahrelghazali*: Mio-Pliocene hominids from Chad.** *Proceedings of the National Academy of*
849 *Sciences of the United States of America* 105: 3226-3231.
- 850 ➤ Lee-Thorp J.A., Sealy J.C., van der Merwe N.J. (1989). **Stable carbon isotope ratio differences**
851 **between bone collagen and bone apatite, and their relationship to diet.** *Journal of*
852 *Archaeological Science* 16: 585-599.
- 853 ➤ Lee-Thorp J.A., van der Merwe N.J. (1991). **Aspects of the chemistry of modern and fossil**
854 **biological apatites.** *Journal of Archaeological Science* 18: 343-354.
- 855 ➤ LeGeros R.Z., LeGeros J.P. (1984). **Phosphate minerals in human tissues.** In Nriagu J.O.,
856 Moore P.B. (eds.). *Phosphate Minerals*. Springer-Verlag, New York: 351-385.
- 857 ➤ Levin N. E., Zipser E.J., Cerling T.E. (2009). **Isotopic composition of waters from Ethiopia and**
858 **Kenya: insights into moisture sources for eastern Africa.** *Journal of Geophysical Research*
859 114, D23306.
- 860 ➤ Levin N.E., Cerling T.E., Passey B.H., Harris J.M., Ehleringer J.R. (2006). **A stable isotope**
861 **aridity index for terrestrial environments.** *Proceedings of the National Academy of Sciences of*
862 *the United States of America* 103: 11201-11205.
- 863 ➤ Levin N.E., Simpson S.W., Quade J., Cerling T.E., Frost S.R. (2008). **Herbivore enamel carbon**
864 **isotopic composition and the environmental context of *Ardipithecus* at Gona, Ethiopia.** In
865 Quade J., Wynn J.G. (eds.). *The Geology of Early Humans in the Horn of Africa. Special Paper*
866 446. The Geological Society of America, Boulder: 215-234.
- 867 ➤ Livingstone D.A., Clayton W.D. (1980). **An altitudinal cline in Tropical African grass floras and**
868 **its paleoecological significance.** *Quaternary Research* 13: 392-402.
- 869 ➤ Longinelli A. (1984). **Oxygen isotopes in mammal bone phosphate: a new tool for**
870 **paleohydrological and paleoclimatological research?** *Geochimica et Cosmochimica Acta* 48:
871 385-390.
- 872 ➤ Longinelli A., Nuti S. (1973). **Oxygen isotope measurements of phosphate from fish teeth and**
873 **bones.** *Earth and Planetary Science Letters* 20: 337-340.

- 874 ➤ Luz B., Kolodny Y. (1985). **Oxygen isotope variations in phosphate of biogenic apatites, IV.**
875 **Mammal teeth and bones.** *Earth and Planetary Science Letters* 75: 29-36.
- 876 ➤ Mann H.B., Whitney D.R. (1947). **On a test of whether one of two random variables is**
877 **stochastically larger than the other.** *The Annals of Mathematical Statistics* 18: 50-60.
- 878 ➤ Martin C., Bentaleb I., Kaandorp R., Iacumin P., Chatri K. (2008). **Intra-tooth study of modern**
879 **rhinoceros enamel $\delta^{18}\text{O}$: Is the difference between phosphate and carbonate $\delta^{18}\text{O}$ a sound**
880 **diagenetic test?** *Palaeogeography, Palaeoclimatology, Palaeoecology* 266: 183–189.
- 881 ➤ Medina E., Minchin P. (1980). **Stratification of $\delta^{13}\text{C}$ values of leaves in Amazonian rain**
882 **forests.** *Oecologia* 45: 377-378.
- 883 ➤ Michel V., Ildefonse P., Morin G. (1996). **Assessment of archaeological bone and dentine**
884 **preservation from Lazaret Cave (Middle Pleistocene) in France.** *Palaeogeography,*
885 *Palaeoclimatology, Palaeoecology* 126: 109-119.
- 886 ➤ Nicholson S.E. (1996). **A review of climate dynamics and climate variability in eastern Africa.**
887 *In* Johnson T.C., Odada E.O. (eds.). *The Limnology, Climatology and Paleoclimatology of the East*
888 *African Lakes.* Gordon and Breach Publishers, Amsterdam: 25-56.
- 889 ➤ Passey B.H., Cerling T.E., Levin N.E. (2007). **Temperature dependence of oxygen isotope acid**
890 **fractionation for modern and fossil tooth enamels.** *Rapid Communications in Mass*
891 *Spectrometry* 21: 2853–2859.
- 892 ➤ Passey B.H., Robinson T.F., Ayliffe L.K., Cerling T.E., Sponheimer M., Dearing M.D., Roeder B.L.,
893 Ehleringer J.R. (2005). **Carbon isotope fractionation between diet, breath CO_2 , and bioapatite**
894 **in different mammals.** *Journal of Archaeological Science* 32: 1459-1470.
- 895 ➤ Pickford M. (1975). **Late Miocene sediments and fossils from the Northern Kenya Rift Valley.**
896 *Nature* 256: 279–284.
- 897 ➤ Pickford M. (2002). **Early Miocene grassland ecosystem at Bukwa, Mount Elgon, Uganda.**
898 *Comptes Rendus de l'Académie des Sciences « Palevol »* 1: 213-219.
- 899 ➤ Pickford M. (2008). ***Libycosaurus petrocchii* Bonarelli, 1947, and *Libycosaurus anisae***
900 **(Black, 1972) (Anthracotheriidae, Mammalia): nomenclatural and geochronological**
901 **implications.** *Annales de Paléontologie* 94: 39-55.
- 902 ➤ Pickford M. (2011). **Lorisine Primate from the Late Miocene of Kenya.** *Journal of Biological*
903 *Research* n.1, volume LXXXIV - Rubbettino - Soveria Mannelli: 47-52.

- 904 ➤ Pickford M., Johanson D., Lovejoy C.O., White T., Aronson J. (1983). **A hominoid humeral**
905 **fragment from the Pliocene of Kenya.** *American Journal of Physical Anthropology* 60: 337-346.
- 906 ➤ Pickford M., Morales J. (1994). **Biostratigraphy and palaeobiogeography of East Africa and**
907 **the Iberian peninsula.** *Palaeogeography, Palaeoclimatology, Palaeoecology* 112: 297-322.
- 908 ➤ Pickford M., Senut B. (2001). **The geological and faunal context of Late Miocene hominid**
909 **remains from Lukeino, Kenya.** *Comptes Rendus de l'Académie des Sciences « Sciences de la*
910 *Terre et des planètes »* 332: 145-152.
- 911 ➤ Pickford M., Senut B. (2003). **Geology and palaeobiology of the central and southern Namib,**
912 **volume 2: palaeontology of the Orange River Valley, Namibia.** Geological Survey of Namibia,
913 Memoir 19, 398p.
- 914 ➤ Pickford M., Senut B., Cheboi K. (2009). **The geology and palaeobiology of the Tugen Hills,**
915 **Kenya.** *Geo-Pal Kenya* 1. Egerton University, Egerton: 1-134.
- 916 ➤ Pickford M., Senut B., Gommery D., Treil J. (2002). **Bipedalism in *Orrorin tugenensis* revealed**
917 **by its femora.** *Comptes Rendus de l'Académie des Sciences « Palevol »* 1: 191–203.
- 918 ➤ Pickford M., Senut B., Mourer-Chauviré C. (2004). **Early Pliocene Tragulidae and peafowls in**
919 **the Rift Valley, Kenya: evidence for rainforest in East Africa.** *Comptes Rendus de l'Académie*
920 *des Sciences « Palevol »* 3: 179-189.
- 921 ➤ Piperno D.R. (2006). **Phytoliths: a Comprehensive Guide for Archaeologists and**
922 **Paleoecologists.** AltaMira Press, Lanham, 239p.
- 923 ➤ Poage M.A., Chamberlain C.P. (2001). **Empirical relationships between elevation and the**
924 **stable isotope composition of precipitation and surface waters: considerations for studies**
925 **of paleoelevation change.** *American Journal of Science* 301: 1-15.
- 926 ➤ Quade J., Cerling T.E., Barry J.C., Morgan M.E., Pilbeam D.R., Chivas A.R., Lee-Thorp J.A., van
927 der Merwe N.J. (1992). **A 16-Ma record of paleodiet using carbon and oxygen isotopes in**
928 **fossil teeth from Pakistan.** *Chemical Geology* 94: 183-192.
- 929 ➤ Quade J., Levin N.E., Simpson S.W., Butler R., McIntosh W.C., Semaw S., Kleinsasser L.,
930 Dupont-Nivet G., Renne P., Dunbar N. (2008). **The geology of Gona, Afar, Ethiopia.** *In* Quade
931 J., Wynn J.G. (eds.). *The Geology of Early Humans in the Horn of Africa. Special Paper* 446. The
932 Geological Society of America, Boulder: 1-31.

- 933 ➤ Renaut R.W., Jones B., Tiercelin J.-J., Tarits C. (2002). **Sublacustrine precipitation of**
 934 **hydrothermal silica in rift lakes: evidence from Lake Baringo, central Kenya Rift Valley.**
 935 *Sedimentary Geology* 148: 235-257.
- 936 ➤ Renne P.R., WoldeGabriel G., Hart W.K., Heiken G., White T.D. (1999). **Chronostratigraphy of**
 937 **the Miocene-Pliocene Sagantole Formation, Middle Awash Valley, Afar rift, Ethiopia.**
 938 *Geological Society of America Bulletin* 111: 869-885.
- 939 ➤ Roche D., Ségalen L., Balan E., Delattre S. (2010). **Preservation assessment of Miocene-**
 940 **Pliocene tooth enamel from Tugen Hills (Kenyan Rift Valley) through FTIR, chemical and**
 941 **stable-isotope analyses.** *Journal of Archaeological Science* 37: 1690-1699.
- 942 ➤ Rozanski K., Araguas-Araguas L., Gonfiantini R. (1993). **Isotopic patterns in modern global**
 943 **precipitation.** In Swart P.K., Lohmann K.C., McKenzie J., Savin S. (eds.). *Climate Change in*
 944 *Continental Isotopic Records. Geophysical Monograph* 78. American Geophysical Union,
 945 Washington D.C.: 1-36.
- 946 ➤ Rozanski K., Araguas-Araguas L., Gonfiantini R. (1996). **Isotopic patterns of precipitation in**
 947 **the East African region.** In Johnson T.C., Odada E.O. (eds.). *The Limnology, Climatology and*
 948 *Paleoclimatology of the East African Lakes.* Gordon and Breach Publishers, Amsterdam: 79-93.
- 949 ➤ Sawada Y., Pickford M., Senut B., Itaya T., Hyodo M., Miura T., Kashine C., Chujo T., Fujii H.
 950 (2002). **The age of *Orrorin tugenensis*, an early hominid from the Tugen Hills, Kenya.** *Comptes*
 951 *Rendus de l'Académie des Sciences « Palevol »* 1: 293-303.
- 952 ➤ Schoeninger M.J., Hallin K., Reeser H., Valley J.W., Fournelle J. (2003a). **Isotopic alteration of**
 953 **mammalian tooth enamel.** *International Journal of Osteoarchaeology* 13: 11-19.
- 954 ➤ Schoeninger M.J., Reeser H., Hallin K. (2003b). **Paleoenvironment of *Australopithecus***
 955 ***anamensis* at Allia Bay, East Turkana, Kenya: evidence from mammalian herbivore enamel**
 956 **stable isotopes.** *Journal of Anthropological Archaeology* 22: 200-207.
- 957 ➤ Schuster M., Düringer P., Ghienne J.F., Vignaud P., Mackaye H.T., Likius A., Brunet M. (2006).
 958 **The age of the Sahara Desert.** *Science* 311: 821.
- 959 ➤ Ségalen L., de Rafélis M., Lee-Thorp J.A., Maurer A.F., Renard M. (2008).
 960 **Cathodoluminescence tools provide clues to depositional history in Miocene and Pliocene**
 961 **mammalian teeth.** *Palaeogeography, Palaeoclimatology, Palaeoecology* 266: 246-253.

- 962 ➤ Ségalen L., Lee-Thorp J.A., Cerling T. (2007). **Timing of C₄ grass expansion across sub-**
963 **Saharan Africa.** *Journal of Human Evolution* 53: 549-559.
- 964 ➤ Semaw S., Simpson S.W., Quade J., Renne P.R., Butler R.F., McIntosh W.C., Levin N.,
965 Dominguez-Rodrigo M., Rogers M.J. (2005). **Early Pliocene hominids from Gona, Ethiopia.**
966 *Nature* 433: 301-305.
- 967 ➤ Senut B. (2006a). **The “East Side Story” twenty years later.** *Transactions of the Royal Society*
968 *of South Africa. Special Issue: a Festschrift to H.B.S. Cooke* 61: 103-109.
- 969 ➤ Senut B. (2006b). **Bipédie et climat.** *Comptes Rendus de l'Académie des Sciences « Palevol »* 5:
970 89-98.
- 971 ➤ Senut B. (2011). **Fifty years of debate on the origins of human bipedalism.** *Journal of*
972 *Biological Research* n.1, volume LXXXIV - Rubbettino - Soveria Mannelli: 37-46.
- 973 ➤ Senut B., Pickford M. (2004). **La dichotomie grands singes-homme revisitée.** *Comptes Rendus*
974 *de l'Académie des Sciences « Palevol »* 3: 265-276.
- 975 ➤ Senut B., Pickford M., Gommery D., Mein P., Cheboi K., Coppens Y. (2001). **First hominid from**
976 **the Miocene (Lukeino Formation, Kenya).** *Comptes Rendus de l'Académie des Sciences*
977 *« Sciences de la Terre et des planètes »* 332: 137-144.
- 978 ➤ Senut B., Pickford M., Ségalen L. (2009). **Neogene desertification of Africa.** *Comptes Rendus*
979 *de l'Académie des Sciences « Géoscience »* 341: 591-602.
- 980 ➤ Senut B., Pickford M., Ségalen L., Roche D., Gommery D. (2010). **Paléoenvironnements**
981 **néogènes et évolution des hominoïdes.** In Barbault R., Foucault A. (eds.). *Changements*
982 *Climatiques et Biodiversité.* Editions Vuibert-AFAS, Paris: 125-144.
- 983 ➤ Shoshani J., Tassy P. (1996). **The Proboscidea, Evolution and Paleontology of Elephants and**
984 **their Relatives.** Oxford University Press, New York, 472p.
- 985 ➤ Simpson S.W., Quade J., Kleinsasser L., Levin N., MacIntosh W., Dunbar N., Semaw S. (2007).
986 **Late Miocene hominid teeth from Gona project area, Ethiopia.** *American Journal of Physical*
987 *Anthropology* 132: 219.
- 988 ➤ Skinner H.C.W. (2005). **Biominerals.** *Mineralogical Magazine* 69: 621-641.
- 989 ➤ Stewart G.R., Turnbull M.H., Schmidt S., Erskine P.D. (1995). **¹³C natural abundance in plant**
990 **communities along a rainfall gradient: a biological integrator of water availability.** *Australian*
991 *Journal of Plant Physiology* 22: 51-55.

- 992 ➤ Swart P.K., Burns S.J., Leder J.J. (1991). **Fractionation of the stable isotopes of oxygen and**
 993 **carbon in carbon dioxide during the reaction of calcite with phosphoric acid as a function**
 994 **of temperature and technique.** *Chemical Geology (Isotope Geoscience Section)* 86: 89-96.
- 995 ➤ Tarits C., Renaut R.W., Tiercelin J.-J., Le Hérissé A., Cotten J., Cabon J.Y. (2006). **Geochemical**
 996 **evidence of hydrothermal recharge in Lake Baringo, central Kenya Rift Valley.** *Hydrological*
 997 *Processes* 20: 2027-2055.
- 998 ➤ Tieszen L.L., Senyimba M.M., Imbamba S.K., Troughton J.H. (1979). **The distribution of C₃ and**
 999 **C₄ grasses and carbon isotope discrimination along an altitudinal and moisture gradient in**
 1000 **Kenya.** *Oecologia* 37: 337-350.
- 1001 ➤ Tipple B.J., Meyers S.R., Pagani M. (2010). **Carbon isotope ratio of Cenozoic CO₂: a**
 1002 **comparative evaluation of available geochemical proxies.** *Paleoceanography* 25, PA3202.
- 1003 ➤ Uno K.T., Cerling T.E., Harris J.M., Kunitatsu Y., Leakey M.G., Nakatsukasa M., Nakaya H.
 1004 (2011). **Late Miocene to Pliocene carbon isotope record of differential diet change among**
 1005 **East African herbivores.** *Proceedings of the National Academy of Sciences of the United States*
 1006 *of America* 108: 6509–6514.
- 1007 ➤ van der Merwe N.J., Medina E. (1991). **The canopy effect, carbon isotope ratios and**
 1008 **foodwebs in Amazonia.** *Journal of Archaeological Science* 18: 249-259.
- 1009 ➤ Vignaud P., Düringer P., Mackaye H.T., Likius A., Blondel C., Boisserie J.-R., de Bonis L.,
 1010 Eisenmann V., Etienne M.-E., Geraads D., Guy F., Lehmann T., Lihoreau F., Lopez-Martinez N.,
 1011 Mourer-Chauviré C., Otero O., Rage J.-C., Schuster M., Viriot L., Zazzo A., Brunet M. (2002).
 1012 **Geology and palaeontology of the Upper Miocene Toros-Menalla hominid locality, Chad.**
 1013 *Nature* 418: 152-155.
- 1014 ➤ Wang Y., Cerling T.E. (1994). **A model of fossil tooth and bone diagenesis: implications for**
 1015 **paleodiet reconstruction from stable isotopes.** *Palaeogeography, Palaeoclimatology,*
 1016 *Palaeoecology* 107: 281– 289.
- 1017 ➤ White F. (1986). **La végétation de l'Afrique.** Mémoire accompagnant la carte de végétation de
 1018 l'Afrique, Unesco/AETFAT/UNSO, 384p.
- 1019 ➤ White T.D., Ambrose S.H., Suwa G., Su D.F., DeGusta D., Bernor R.L., Boisserie J.-R., Brunet M.,
 1020 Delson E., Frost S., Garcia N., Giaourtsakis I.X., Haile-Selassie Y., Howell F.C., Lehmann T.,

1021 Likius A., Pehlevan C., Saegusa H., Semprebon G., Teafor M., Vrba E. (2009). **Macrovertebrate**
1022 **paleontology and the Pliocene habitat of *Ardipithecus ramidus***. *Science* 326: 50-56.

1023 ➤ White T.D., Suwa G., Asfaw B. (1994). ***Australopithecus ramidus*, a new species of early**
1024 **hominid from Aramis, Ethiopia**. *Nature* 371: 306-312.

1025 ➤ White T.D., Suwa G., Asfaw B. (1995). **Corrigendum: *Australopithecus ramidus*, a new**
1026 **species of early hominid from Aramis, Ethiopia**. *Nature* 375: 88.

1027 ➤ WoldeGabriel G., Ambrose S.H., Barboni D., Bonnefille R., Bremond L., Currie B., DeGusta D.,
1028 Hart W.K., Murray A.M., Renne P.L., Jolly-Saad M.C., Steward K.M., White T.D. (2009). **The**
1029 **geological, isotopic, botanical, invertebrate, and lower vertebrate surroundings of**
1030 ***Ardipithecus ramidus***. *Science* 326: 41-45.

1031 ➤ WoldeGabriel G., Haile-Selassie Y., Renne P.R., Hart W.K., Ambrose S.H., Asfaw B., Heiken G.,
1032 White T. (2001). **Geology and paleontology of the Late Miocene Middle Awash valley, Afar**
1033 **Rift, Ethiopia**. *Nature* 412: 175-178.

1034 ➤ WoldeGabriel G., White T.D., Suwa G., Renne P., de Heinzelin J., Hart W.K., Heiken G. (1994).
1035 **Ecological and temporal placement of Early Pliocene hominids at Aramis, Ethiopia**. *Nature*
1036 371: 330-333.

1037 ➤ Yakir D. (1992). **Variations in the natural abundance of oxygen-18 and deuterium in plant**
1038 **carbohydrates**. *Plant, Cell and Environment* 15: 1005-1020.

1039 ➤ Yakir D., Sternberg L.S.L. (2000). **The use of stable isotopes to study ecosystem gas**
1040 **exchange**. *Oecologia* 123: 297-311.

1041 ➤ Zachos J., Pagani M., Sloan L., Thomas E., Billups K. (2001). **Trends, rhythms, and aberrations**
1042 **in global climate 65 Ma to present**. *Science* 292: 686-693.

1043 ➤ Zazzo A. (2001). **Validation méthodologique de l'utilisation des compositions isotopiques**
1044 **(¹³C, ¹⁸O) des bioapatites fossiles pour la reconstitution des paléoenvironnements**
1045 **continentaux**. PhD dissertation. Pierre-and-Marie-Curie University, Paris, 263p.

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Figure captions

Figure 1: Location of the Tugen Hills deposits (red square).

Figure 2: Stratigraphic section of the Lukeino and Mabaget Formations. Radiometric dates and geomagnetic reversal ages are reported in Pickford et al. (1983), Hill et al. (1985), Deino et al. (2002) and Sawada et al. (2002).

Figure 3: Box and whisker plots of $\delta^{18}\text{O}$ values for hippos and terrestrial herbivores from the Lukeino and Mabaget Formations. The number of specimens is given in brackets for each group. Outliers are shown as filled circles. “MB” = Mabaget Formation (5.3-4.5 Ma), “LK” = Lukeino Formation (6.1-5.7 Ma).

Figure 4: $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of large herbivores from the Mabaget Formation (a), the Kapcheberek Member of the Lukeino Formation (b), the Kapsomin Member of the Lukeino Formation (c) and the Kapgoywa Member of the Lukeino Formation (d). The number of specimens is given in brackets for each group. “indet. probos.” = indeterminate proboscideans.

Figure 5: $\delta^{13}\text{C}$ values of fossil large herbivores from the Tugen Hills (Kenya), the Awash River Valley (Ethiopia) and the Djurab Desert (Chad). The number of specimens is given in brackets for each group. Box and whisker plots are used for groups including at least five individuals, and outliers are shown as filled circles. “MB” = Mabaget Formation (5.3-4.5 Ma), “LK” = Lukeino Formation (6.1-5.7 Ma), “SG” = Sagantole Formation (5.2-3.9 Ma), “AA” = Adu-Asa Formation (6.4-5.2 Ma) and “TM” = Toros Menalla deposits (7-6 Ma). Stable isotope data of Chadian palaeofauna are taken from Zazzo (2001) and Boisserie et al. (2005). Ages of Toros Menalla sites are indicated in Vignaud et al. (2002) and Brunet et al. (2004). Isotopic data of Ethiopian palaeofauna are compiled from Levin et al. (2008) and White et al. (2009). Ages of Adu-Asa and Sagantole Formations are reported in WoldeGabriel et al. (1994, 2001), Renne et al. (1999), Kleinsasser et al. (2008) and Quade et al. (2008).

1077 **Figure 6:** Dietary reconstruction of large herbivores from the Lukeino and Mabaget Formations, based
1078 on $\delta^{13}\text{C}$ values of tooth enamel. Individuals are classified according to age and families. The
1079 unidentified proboscidean specimens are not represented in the figure.

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1081 **Table captions**

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1083 **Table 1:** Summary of fluorine, iron and manganese concentrations in tooth enamel of extant and fossil
1084 large herbivores from the Tugen Hills. "Fm." = formation, "SD" = standard deviation.

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1086 **Figure 1**

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Figure 2

Figure 3

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1106 **Table 1**

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		Modern specimens (n=4)	Specimens from the Mabaget Fm. (n=20)	Specimens from the Lukeino Fm. (n=19)
[F] ppm	Range	149 to 334	4 845 to 19 946	4 072 to 18 862
	Mean (± SD)	241 (± 67)	9 879 (± 4 274)	9 405 (± 4 512)
[Fe] ppm	Range	98 to 180	1 069 to 12 909	600 to 19 648
	Mean (± SD)	125 (± 33)	4 441 (± 3 188)	4 163 (± 4 500)
[Mn] ppm	Range	10 to 15	226 to 3 008	453 to 2 132
	Mean (± SD)	11 (± 2)	1 322 (± 820)	1 409 (± 557)

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