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**Cite this article:** Bibi F, Souron A, Bocherens H, Uno K, Boisserie J-R. 2013 Ecological change in the lower Omo Valley around 2.8 Ma. Biol Lett 9: 20120890.

http://dx.doi.org/10.1098/rsbl.2012.0890

Received: 26 September 2012 Accepted: 20 November 2012

#### Subject Areas:

palaeontology, ecology, evolution

#### Keywords:

stable isotope analysis, palaeoecology, diet, Africa, Pliocene

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Electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2012.0890 or via http://rsbl.royalsocietypublishing.org.



#### Palaeontology

### Ecological change in the lower Omo Valley around 2.8 Ma

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Late Pliocene climate changes have long been implicated in environmental changes and mammalian evolution in Africa, but high-resolution examinations of the fossil and climatic records have been hampered by poor sampling. By using fossils from the well-dated Shungura Formation (lower Omo Valley, northern Turkana Basin, southern Ethiopia), we investigate palaeodietary changes in one bovid and in one suid lineage from 3 to 2 Ma using stable isotope analysis of tooth enamel. Results show unexpectedly large increases in C4 dietary intake around 2.8 Ma in both the bovid and suid, and possibly in a previously reported hippopotamid species. Enamel  $\delta^{13}$ C values after 2.8 Ma in the bovid (*Tragelaphus nakuae*) are higher than recorded for any living tragelaphin, and are not expected given its conservative dental morphology. A shift towards increased C4 feeding at 2.8 Ma in the suid (Kolpochoerus limnetes) appears similarly decoupled from a well-documented record of dental evolution indicating gradual and progressive dietary change. The fact that two, perhaps three, disparate Pliocene herbivore lineages exhibit similar, and contemporaneous changes in dietary behaviour suggests a common environmental driver. Local and regional pollen, palaeosol and faunal records indicate increased aridity but no corresponding large and rapid expansion of grasslands in the Turkana Basin at 2.8 Ma. Our results provide new evidence supporting ecological change in the eastern African record around 2.8 Ma, but raise questions about the resolution at which different ecological proxies may be comparable, the correlation of vegetation and faunal change, and the interpretation of low  $\delta^{13}$ C values in the African Pliocene.

#### 1. Introduction

Four decades of research have addressed the nature and magnitude of environmental change on faunal evolution in Africa in the late Pliocene and early Pleistocene. A major impediment to investigation has been a lack of welldated fossiliferous and sedimentary sequences that continuously sample the 3–2 Ma period and that could be precisely compared with global palaeoclimate proxy records. Under the framework of the Omo Group Research Expedition [1], we here explore palaeoecological change in Plio-Pleistocene eastern Africa using dietary investigation of well-studied, phyletically evolving herbivore species lineages across the 3–2 Ma period.

The suid *Kolpochoerus limnetes* and the bovid *Tragelaphus nakuae* (with its immediate ancestor *Tragelaphus rastafari*) are phyletically evolving species lineages that are well represented and well studied in Plio-Pleistocene deposits



**Figure 1.** Enamel  $\delta^{13}$ C and  $\delta^{18}$ O values for the fossil (*a*) bovid and (*b*) suid specimens sampled. These are differentiated by stratigraphic level within the Shungura Formation. Specimens from units C-4 and younger (i.e.  $< \sim 2.8$  Ma) are significantly enriched in  $\delta^{13}$ C relative to those from units B-10 and older.

of eastern Africa [2–5]. We used precisely dated fossils of these lineages from the Shungura Formation in the lower Omo Valley (northern Turkana Basin, southern Ethiopia), which provides the single most continuous and best-dated fossiliferous sequence of the 3–2 Ma time period anywhere in Africa. We measured tooth enamel stable carbon and oxygen isotope ratios in order to determine whether any significant dietary changes had occurred that might be correlated with local, regional or global palaeoenvironmental signals. We also refer to enamel isotope values for two specimens of the hippopotamid aff. *Hippopotamus protamphibius* reported by Souron *et al.* [6].

#### 2. Material and methods

Twenty-four bovid and 60 suid specimens were sampled at the Authority for Research and Conservation of Cultural Heritage in Addis Ababa. Around 20 mg of fossil enamel from each specimen was treated with buffered acetic acid and reacted with anhydrous H<sub>3</sub>PO<sub>4</sub>, with the resulting CO<sub>2</sub> analysed using a Finnigan MAT 252 isotopic ratio mass spectrometer. Stable carbon and oxygen isotope ratios are reported as  $\delta$ -values relative to the Vienna Pee Dee Belemnite (VPDB) standard using permil (‰) notation, where  $\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 1000; R_{sample}$  and  $R_{standard}$  are the  $^{13}C/^{12}C$  ratios (or  $^{18}O/^{16}O$  ratios in the case of  $\delta^{18}O$ ) in the sample and in the standard, respectively, and the  $\delta^{13}C$  (or  $\delta^{18}O$ ) value of VPDB is defined as 0‰. Full details on materials and methods can be found in the electronic supplementary material.

#### 3. Results

Enamel  $\delta^{13}$ C values in the Shungura bovid, suid and hippopotamid studied became more enriched by an average +4% between units B-10 and C-4/C-5, or sometime between 2.97 Ma and just under 2.74 Ma (figures 1 and 2) [7].  $\delta^{18}$ O values, while showing some changes in the mean and variances among the different stratigraphic levels (especially in

the suids), do not show the type of directional change observed in the  $\delta^{13}$ C data. Full results can be found in the electronic supplementary material.

#### 4. Discussion

We record a pronounced increase in enamel  $\delta^{13}\!C$  around 2.8 Ma in both the bovid and suid lineages studied (figure 1). Although only two Shungura hippopotamid specimens were sampled by Souron et al. [6], the timing, direction and magnitude of the change in enamel  $\delta^{13}C$  match those of the bovid and suid. Such large (greater than +4%) increases in  $\delta^{13}C$ indicate significantly increased dietary intake of C4 plant resources in a relatively short time frame, or not more than (and perhaps much less than) 300 000 years (figure 2). In the bovid lineage, the  $\delta^{13}$ C shift is contemporaneous with a recently recognized taxonomic boundary between chronospecies T. nakuae (younger than around 2.8 Ma) and T. rastafari (older than 2.8 Ma) [3]. However, taxonomic differentiation was made mainly on the basis of braincase morphology, with no obvious indications of any dentognathic changes that might suggest a change in dietary behaviour. Enamel  $\delta^{13}$ C values in *T. nakuae* are also higher than recorded among living tragelaphin species [11], but the significance of this is difficult to interpret without related dental wear data (see the electronic supplementary material, section discussion).

Findings for mixed feeding or grazing behaviours in the suid and hippopotamid are not unexpected given the dietary preferences of these animals today, as well as their dental morphologies, which permit an abrasive and grass-based diet. What is surprising is the rapidity of the dietary shift implied by the  $\delta^{13}$ C data. Dental evolution in *K. limnetes*, such as other suid lineages of the Plio-Pleistocene, is progressive, with gradual lengthening of the third molars, premolar row reduction and increases in hypsodonty and body size indicating long-term adaptation to an increasingly abrasive diet [2,5]. Morphological changes around 2.8 Ma are not especially significant



**Figure 2.** Local palaeoenvironmental indicators in the Shungura Formation from 3.2 to 2.0 Ma. (*a*) Palaeosol stable carbon isotopes [8], grass pollen percentages [9] and proportions of mammals indicative of secondary grassland habitats [10]. (*b*) Fossil enamel stable carbon isotopes show a large increase in enamel  $\delta^{13}$ C around 2.8 Ma indicating palaeodietary change. The  $\delta^{13}$ C increase, however, is not correlated with the expansion of grassland habitats and loss of tree cover, or with proportional increases in grazing mammals. Different ecological proxies, however, may be sampling different spatial and temporal scales.

compared with those observed at other times in this lineage's evolution, and our data suggest a major dietary shift at 2.8 Ma that is decoupled from the long and gradual evolutionary progression indicated by the dentognathic morphological data. Like the suid, the aff. *Hippopotamus protamphibius* lineage shows gradual elongation of its third molars over time [12], and a large dietary shift in this lineage, if confirmed by further sampling, would be similarly significant.

Although similar to previously reported  $\delta^{13}$ C increases in late Miocene herbivores [13], exact dietary interpretations for our data and any resulting inferences about the balance of grasslands to woodlands are confounded by evidence that C<sub>3</sub> grasses may have made up a larger proportion of lowland African vegetation biomass during the Pliocene than they do today [14]. In the absence of further data (e.g. dental wear, phytoliths), it is not possible to rule out the presence and exploitation of C3 grasses in the lower Omo Valley prior to 2.8 Ma. The  $\delta^{13}$ C increase may therefore represent either a significant replacement of trees by grasses (with impacts on herbivore dietary strategies), or a less remarkable replacement of pre-existing C<sub>3</sub> grasses by C<sub>4</sub> grasses (with limited or no change in dietary behaviour). Broader faunal isotopic sampling along with studies of dental wear should be able to further test these scenarios.

That a pronounced increase in dietary  $\delta^{13}$ C took place contemporaneously in two, possibly three, ecologically distinct herbivore lineages opens the possibility of a common environmental driver. Numerous faunal, botanical and palaeoclimate proxy records have identified 2.8-2.5 Ma as a period of drying in Africa [15-17]. In the Shungura mammalian fossil record, 2.8 Ma coincides with a period of elevated ecological and taxonomic turnover that reflects increased aridity and a reduction in humid-forested environments [10,18]. However, mammalian, pollen and palaeosol isotope records also indicate that habitats in the lower Omo Valley during this time remained relatively mesic with no indications of significant grassland habitat expansion until 2.6 Ma or later (figure 2) [8-10]. Some of these differences might be explained by factors affecting the spatial and temporal scaling of the different proxy methods. For example, the stability of the Omo watershed, which supported mesic riparian environments somewhat buffered against regional aridification that more strongly affected areas peripheral to the river itself [8,10,19], or selective feeding by herbivores that might target rare vegetation types or areas peripheral to the river axis. At face value, however, the raw data suggest that vegetation, faunal and evolutionary responses between 3 and 2.5 Ma in the lower Omo Valley may have been decoupled on the order of 100 ka or more.

Similar analyses should be extended to other African fossil faunas in order to determine whether the  $\delta^{13}$ C increase we record was part of a broader pulse of late Pliocene ecological change or was restricted to the lower Omo Valley. Regional comparisons are currently limited, but the available palaeosol and enamel stable isotope records [8,20–23] from the nearby Nachukui and Koobi Fora formations, while

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indicating net increases in aridity and  $C_4$  biomass through time, show no evidence for a large and step-like shift around 2.8 Ma. This suggests that the dietary changes we observe may have been restricted to the lower Omo Valley, providing another example of how these faunas, located within the same depositional basin, differed ecologically.

Neither the rate nor the magnitude of the dietary changes we record (especially in *Tragelaphus*) were to be expected from existing lines of contextual evidence, including dental morphology, faunal turnover, and the palaeosol and palaeobotanical proxy records. Our findings do support previous indications of net change towards increasingly arid habitats in the late Pliocene of eastern Africa, in concert with global and regional climatic records, with the additional indication that major evolutionary responses such as dietary changes,  $C_4$  grassland expansion, faunal reorganization and functional adaptation may not have been tightly coupled, even within a single depositional basin.

We thank B. Steinhilber and C. Wissing for technical assistance at the University of Tübingen; C. Blondel, A. Novello and T. White for discussions; N. Levin, J. Wynn and T. Cerling for palaeosol data, and the editor and three anonymous referees for helping improve an earlier draft of the manuscript. Funding was provided by the Agence National de la Recherche (ANR-09-BLAN-0238 to M. Brunet), the French Ministry of Foreign and European Affairs (Sous-Direction de l'Archéologie/French Embassy in Ethiopia/CFEE), the Fyssen Foundation, a National Science Foundation International Research Fellowship (grant no. 0852975 to F.B.), and a Leibniz-DAAD Research Fellowship (to F.B.). This study was authorized by the Authority for Research, Conservation and Cultural Heritage (Ethiopian Ministry of Culture and Tourism).

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#### References

- Boisserie JR, Guy F, Delagnes A, Hlusko LJ, Bibi F, Beyene Y, Guillemot C. 2008 New palaeoanthropological research in the Plio-Pleistocene Omo Group, lower Omo Valley, SNNPR, Ethiopia. *C R Palevol.* 7, 429–439. (doi:10.1016/j. crpv.2008.07.010)
- Harris JM, White TD. 1979 Evolution of the Plio-Pleistocene African Suidae, p. 128. Philadelphia, PA: American Philosophical Society.
- Bibi F. 2011 *Tragelaphus nakuae*: evolutionary change, biochronology, and turnover in the African Plio-Pleistocene. *Zool. J. Linn. Soc.* 162, 699–711. (doi:10.1111/j.1096-3642.2010.00691.x)
- Gentry AW. 1985 The Bovidae of the Omo Group deposits, Ethiopia (French and American collections). In Les faunes Plio-Pléistocènes de la basse Vallée de l'Omo (Ethiopie); I: Perissodactyles-Artiodactyles (Bovidae) (eds Y Coppens, FC Howell), pp. 119–191. Paris, France: CNRS.
- Cooke HBS. 2007 Stratigraphic variation in Suidae from the Shungura Formation and some coeval deposits. In *Hominin environments in the east African Pliocene: an assessment of the faunal evidence* (eds R Bobe, Z Alemseged, AK Behrensmeyer), pp. 107–127. Dordrecht, The Netherlands: Springer.
- Souron A, Balasse M, Boisserie JR. 2012 Intra-tooth isotopic profiles of canines from extant *Hippopotamus amphibius* and late Pliocene hippopotamids (Shungura Formation, Ethiopia): insights into the seasonality of diet and climate. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 342 – 343, 97 – 110. (doi:10.1016/j.palaeo.2012.05. 007)
- McDougall I, Brown FH, Vasconcelos PM, Cohen BE, Thiede DS, Buchanan MJ. 2012 New single crystal 40Ar/39Ar ages improve time scale for deposition of the Omo Group, Omo-Turkana Basin, East Africa.

*J. Geol. Soc.* **169**, 213–226. (doi:10.1144/0016-76492010-188)

- Levin NE, Brown FH, Behrensmeyer AK, Bobe R, Cerling TE. 2011 Paleosol carbonates from the Omo Group: isotopic records of local and regional environmental change in East Africa. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **307**, 75–89. (doi:10. 1016/j.palaeo.2011.04.026)
- Bonnefille R, Dechamps R. 1983 Data on fossil flora. In The Omo Group: Archives of the International Omo Research Expedition (ed. J de Heinzelin), pp. 191– 207. Tervuren, Belgium: Musée Royal de l'Afrique Centrale.
- Bobe R, Behrensmeyer AK. 2004 The expansion of grassland ecosystems in Africa in relation to mammalian evolution and the origin of the genus *Homo. Palaeogeogr. Palaeoclimatol. Palaeoecol.* 207, 399–420. (doi:10.1016/S0031-0182(04)00049-5)
- Cerling TE, Harris JM, Passey BH. 2003 Diets of East African Bovidae based on stable isotope analysis. J. Mammal 84, 456–470. (doi:10.1644/1545-1542(2003)084<0456:D0EABB>2.0.C0;2)
- 12. Gèze R. 1980 *Les Hippopotamidae (Mammalia, Artiodactyla) du Plio-Pléistocène de l'Ethiopie*. Paris, France: Université Pierre et Marie Curie.
- Uno KT, Cerling TE, Harris JM, Kunimatsu Y, Leakey MG, Nakatsukasa M, Nakaya H. 2011 Late Miocene to Pliocene carbon isotope record of differential diet change among East African herbivores. *Proc. Natl Acad. Sci. USA* **108**, 6509–6514. (doi:10.1073/pnas. 1018435108)
- Rossouw L, Scott L. 2011 Phytoliths and pollen, the microscopic plant remains in Pliocene volcanic sediments around Laetoli, Tanzania. In *Paleontology* and geology of Laetoli: human evolution in context volume 1: geology, geochronology, paleoecology, and paleoenvironment (ed. T Harrison), pp. 201–215. New York, NY: Springer.

- deMenocal PB. 1995 Plio-Pleistocene African climate. *Science* 270, 53–59. (doi:10.1126/science. 270.5233.53)
- Bonnefille R. 2010 Cenozoic vegetation, climate changes and hominid evolution in tropical Africa. *Glob. Planet Change* **72**, 390–411. (doi:10.1016/j. gloplacha.2010.01.015)
- Vrba ES. 1995 The fossil record of African antelopes (Mammalia, Bovidae) in relation to human evolution and paleoclimate. In *Paleoclimate and evolution, with emphasis on human origins* (eds ES Vrba, GH Denton, TC Partridge, LH Burckle), pp. 385–424. New Haven, CT: Yale University Press.
- Hernández Fernández M, Vrba ES. 2006 Plio-Pleistocene climatic change in the Turkana Basin (East Africa): evidence from large mammal faunas. *J. Hum. Evol.* 50, 595. (doi:10.1016/j.jhevol.2005.11. 004)
- Vrba ES. 1988 Late Pliocene climatic events and hominid evolution. In *The evolutionary history of the robust Australopithecines* (ed. FE Grine), pp. 2405–426. New York, NY: Aldine de Gruyter.
- Harris J, Cerling T. 2002 Dietary adaptations of extant and Neogene African suids. *J. Zool.* 256, 45-54. (doi:10.1017/ S0952836902000067)
- Harris J, Cerling T, Leakey M, Passey B. 2008 Stable isotope ecology of fossil hippopotamids from the Lake Turkana Basin of East Africa. *J. Zool.* 275, 323-331. (doi:10.1111/j.1469-7998.2008. 00444.x)
- Cerling TE, Levin NE, Passey BH. 2011 Stable isotope ecology in the Omo-Turkana basin. *Evol. Anthropol.* 20, 228–237. (doi:10.1002/evan.20326)
- Cerling TE *et al.* 2011 Woody cover and hominin environments in the past 6 million years. *Nature* 476, 51–56. (doi:10.1038/nature10306)

#### ELECTRONIC SUPPLEMENTARY MATERIAL

#### to accompany "Ecological Change in the Lower Omo Valley around 2.8 Ma"

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#### **Materials and Methods**

The ratio of carbon 13 to carbon 12 in mammalian herbivore tooth enamel (reported relative to a standard in permil notation as  $\delta^{13}$ C) reflects the proportion of C<sub>4</sub> to C<sub>3</sub> plants ingested during tooth enamel mineralization. At tropical latitudes and low elevations today (<1000m asl), C<sub>4</sub> plants are mainly grasses and sedges found in open or dry habitats, while C<sub>3</sub> plants are mainly trees or herbaceous dicots typically found in shaded or humid habitats [1, 2]. The proportion of grasses that are C<sub>3</sub> at low elevations today in Africa is never more than about 10% [2]. C<sub>3</sub> plants in eastern Africa have a mean  $\delta^{13}$ C value of -27.4 ± 1.5‰ (range of approximately -37 to -23‰), while C<sub>4</sub> plants have a range of -14 to -10%. After ingestion by mammalian herbivores, further fractionation results in a ~14‰ enrichment between diet and enamel [3]. Thus, herbivores that mainly browse on leaves and other C<sub>3</sub> plants sources have low enamel  $\delta^{13}$ C values (<ca. -8‰), while those that mainly graze on C<sub>4</sub> grasses have high enamel  $\delta^{13}$ C values (>ca. -2‰). Accounting for changes in  $\delta^{13}$ C of atmospheric  $CO_2$ , these end members may be adjusted by about +1.4‰ for the Pliocene [4-6]. Modern tropical grasses using the C<sub>4</sub> photosynthetic pathway favour a warm growing season (summer rainfall) and high temperatures and are more efficient at photosynthesis than C<sub>3</sub> grasses under these conditions [7]. C<sub>4</sub> plants also display a competitive advantage in conditions of low atmospheric CO<sub>2</sub> [8], but all evidence suggests CO<sub>2</sub> levels have been relatively constant at preindustrial levels since the late Oligocene [9, 10].

Analyzed specimens were collected during surveys conducted by the International Omo Research Expedition [1967-1976, see 11] and by the Omo Group Research Expedition . For age assignment, we used stratigraphic positions recorded at unit (submember) level [12].

Descriptions of sampling methods and analyses of the hippopotamids are presented in Souron et al. [13] . Enamel sampling of both bovid and suid teeth was performed by A.S. at the Authority for Research and Conservation of Cultural Heritage in Addis Ababa. For bovid and suid teeth, tooth surfaces were cleaned with a tungsten abrasive drill bit and enamel was sampled using a diamond drill bit, each sample of enamel powder weighing around 20 mg. For the suid specimens, treatment and stable isotopic analysis was performed at the University of Tübingen, Germany (Institut für Geowissenschaften, Abteilung Geochemie). Enamel powders were first treated with 2.5% NaOCl for 20 hours to remove organic matter, and rinsed twice with distilled water. They were then reacted with 1M buffer acetic acid-calcium acetate for 24 hours at 20°C to remove diagenetic carbonates, and again rinsed three times with distilled water. They were finally oven-dried for 24 hours at 70°C. Treated bioapatite powders (weighting around 3 mg) were reacted with 100% anhydrous orthophosphoric acid (H<sub>3</sub>PO<sub>4</sub>) at 70°C for 90 minutes. The resulting CO<sub>2</sub> was analysed using a continuous flow system, a ThermoFisher Scientific GasBenchII, coupled to a Finnigan MAT 252 isotopic ratio mass spectrometer (IRMS).

For the bovid specimens, treatment and stable isotopic analyses was performed at the Stable Isotope Ratio Facility for Environmental Research (SIRFER) at the University of Utah. Enamel powders were treated for 30 minutes with 0.1 M buffered acetic acid, rinsed three times with Milli-Q distilled de-ionized water, and dried overnight at 70°C. Treated bioapatite powders (0.4 to 0.6 mg) were reacted with anhydrous H<sub>3</sub>PO<sub>4</sub> at 90°C for 15 minutes using a Finnigan CarboFlo® device coupled to the dual-inlet of a Finnigan MAT 252 IRMS. CO<sub>2</sub> yields from bovid samples show there was no contribution from exogenous carbonate. An acid fractionation factor of 1.00725 is used to convert sample CO<sub>2</sub> back to enamel carbonate  $\delta^{18}$ O values [14].

Stable carbon and oxygen isotope ratios are reported as  $\delta$  values relative to the Pee Dee Belemnite (PDB) standard using permil (‰) notation where  $\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 1000$ , and  $R_{sample}$  and  $R_{standard}$  are the  ${}^{13}C/{}^{12}C$  ratios (or  ${}^{18}O/{}^{16}O$  ratios in the case  $\delta^{18}O$ ) in the sample and in the standard, respectively, and the  $\delta^{13}C$  (or  $\delta^{18}O$ ) value of PDB is defined as 0‰. Results of stable carbon isotopic analyses were corrected using the international standard NBS-19 and analytical precision is ~0.1 ‰ for both  $\delta^{13}C$  and  $\delta^{18}O$  in both laboratories. Statistical tests were performed in SAS JMP 10.0, with significance set at p<0.05.

#### Results

Shapiro-Wilk tests indicate that some of the data distributions differ significantly from normal (p<0.05), so non-parametric tests of significance were used (paired Wilcoxon). The seven bovid teeth from levels B-0, B-2, and B-10 ( $\geq$ 2.97 Ma,  $\bar{x}$ =-8.6 ‰ ) are significantly depleted in  $\delta^{13}$ C compared to those from levels C-8 to C-9 and G-4 to G-13 ( $\leq$ ~2.6 Ma,  $\bar{x}$ =-4.0‰ ) (Wilcoxon, p<0.001). The single bovid tooth from B-12 (~2.9 Ma) has an intermediate  $\delta^{13}$ C value (-5.1‰) and cannot be significantly differentiated from either the pooled older or younger samples (Wilcoxon, p>0.15).

 $\delta^{13}$ C values for the two suid teeth from level B-10 and that from B-12 ( $\geq 2.850$  Ma,  $\bar{x} = -6.4\%$ ) show no overlap with those from levels C-4 and younger ( $\leq 2.740$  Ma,  $\bar{x} = -0.9\%$ ) (Wilcoxon, p<0.01). Suid teeth from units C-4 to F-0 show slight progressive increases in  $\delta^{13}$ C (ca. 0.5‰ mean increases from C-4/C-5 to F-0), with units C-4/C-5 and C-8/C-9 significantly different from unit F0 (Wilcoxon, p<0.01).  $\delta^{13}$ C values from units G-8 and L-9 are also significantly enriched relative to those from units C-4/C-5 (Wilcoxon, p<0.05).

Results of the serial analyses of two fossil hippo canines show that the average  $\delta^{13}$ C value of the canine from level C-9 (2.580 Ma) is enriched by about 5‰ relative to that from level B-9 (3.070-2.951 Ma), with no overlap in the ranges of the serial sample distributions [13].

 $\delta^{18}$ O values, while showing some changes in the means and variances among the different stratigraphic levels (especially in the suids), do not show the type of directional change observed in the  $\delta^{13}$ C data. Furthermore, the direction and magnitude of changes in  $\delta^{18}$ O between successive stratigraphic levels is not correlated among the three herbivorous clades. This implies that individual and clade-specific behaviours, physiologies, and environmentally localised effects are the main determinants of  $\delta^{18}$ O rather than large-scale, extrinsic environmental changes affecting all three herbivores simultaneously. Interestingly, the  $\delta^{18}$ O values from OMO 112/2-10014 are

relatively enriched compared to other eastern African fossil or modern hippopotamids with the exception of some modern Turkana hippos which have enamel  $\delta^{18}$ O values ranging from +2.0 to +2.6 ‰ (n=2 individuals) [15]. This suggests the Omo Member B hippopotamid may have lived some part of its life in an evaporated lake or pond.

#### Supplementary Discussion: Diet of fossil Tragelaphus

The high  $\delta^{13}$ C values recovered for *Tragelaphus nakuae* are unexpected in light of the fact that reported enamel  $\delta^{13}$ C values from living tragelaphins reflect diets almost totally dominated by C<sub>3</sub> vegetation [16-18]. Similarly high  $\delta^{13}$ C values indicative of significant C<sub>4</sub> feeding in tragelaphins come from late Miocene and early Pliocene sites at Gona, Ethiopia [4], the middle to late Pliocene of Laetoli [19], and the middle Pleistocene site of Asbole [20]. Approximating with a simple linear mixing model [13, 21], the most positive  $\delta^{13}$ C values (>-3‰) in these fossil tragelaphins could indicate diets with more than 60% C<sub>4</sub> vegetation. In the Upper Laetolil tragelaphins, dental mesowear indicative of strict browsing combined with enriched enamel  $\delta^{13}$ C values implied the possibility of feeding on C<sub>4</sub> dicots rather than C<sub>4</sub> grasses [19, 22].

The sitatunga (*Tragelaphus spekii*) is an example of a living tragelaphin that inhabits swamps and wetlands and is reported to include significant proportions of grass and aquatic plants in its diet [23, 24], though reported  $\delta^{13}$ C values of its enamel indicate it is a strict browser [16]. Among living deer (Cervidae), chital (*Axis axis*), barashinga (*Cervus duvauceli*), and sambar (*Cervus unicolor*) all subsist largely on grasses [25]. Reported fecal  $\delta^{13}$ C values for the chital indicate wet season C<sub>4</sub>-feeding indifferentiable from values for gaur (*Bos gaurus*) [26]. Like tragelaphins, these deer have relatively low-crowned teeth and simple occlusal enamel configurations, contrasting markedly with the hypsodont teeth with complicated enamel found in grazing bovids in Africa today (Hippotragini, Bovini, Alcelaphini, Reduncini). Plio-Pleistocene fossil tragelaphins such as *T. nakuae* may have engaged in one or a variety of the above scenarios, subsisting on  $C_4$  dicots, wetland grasses, or engaging in seasonal grazing, selecting fresher and greener wet-season grasses that were less abrasive to their low-crowned teeth. The current inability to rule out  $C_3$  grazing in the lower Omo valley prior to 2.8 Ma (see main text) is an additional factor limiting dietary interpretation of enamel isotopic data.

#### References

- Tieszen L.L., Senyimba M.M., Imbamba S.K. & Troughton J.H. 1979 Distribution of C3-Grass and C4-Grass and Carbon Isotope Discrimination Along an Altitudinal and Moisture Gradient in Kenya. *Oecologia* 37(3), 337-350.
- 2 Livingstone D. & Clayton W. 1980 An altitudinal cline in tropical African grass floras and its paleoecological significance. *Quatern Res* **13**(3), 392-402.
- Cerling T.E. & Harris J.M. 1999 Carbon isotope fractionation between diet and bioapatite in ungulate mammals and implications for ecological and paleoecological studies. *Oecologia* 120(3), 347-363.
- Levin N.E., Simpson S.W., Quade J., Cerling T.E. & Frost S.R. 2008 Herbivore enamel carbon isotopic composition and the environmental context of Ardipithecus at Gona, Ethiopia. *Geol Soc Am Spec Pap* 446, 215-234.
- Tipple B.J., Meyers S.R. & Pagani M. 2010 Carbon isotope ratio of Cenozoic CO2: A comparative evaluation of available geochemical proxies. *Paleoceanography* 25(3), PA3202, 3211pp. (doi:doi:10.1029/2009PA001851).
- Passey B.H., Cerling T.E., Perkins M.E., Voorhies M.R., Harris J.M. & Tucker S.T. 2002
  Environmental change in the Great Plains: An isotopic record from fossil horses. *J Geol* 110(2), 123-140.
- 7 Ehleringer J.R., Cerling T.E. & Helliker B.R. 1997 C<sub>4</sub> photosynthesis, atmospheric CO<sub>2</sub>, and climate. *Oecologia* **112**(3), 285-299.

- 8 Ehleringer J.R., Sage R.F., Flanagan L.B. & Pearcy R.W. 1991 Climate change and the evolution of C<sub>4</sub> photosynthesis. *Trends Ecol Evol* **6**(3), 95-99.
- 9 Pagani M., Freeman K.H. & Arthur M.A. 1999 Late Miocene atmospheric CO2 concentrations and the expansion of C-4 grasses. *Science* 285(5429), 876-879.
- Pagani M., Zachos J.C., Freeman K.H., Tipple B. & Bohaty S. 2005 Marked decline in atmospheric carbon dioxide concentrations during the Paleogene. *Science* 309(5734), 600-603.
- Coppens Y. & Howell F.C. 1976 Mammalian faunas of the Omo group: distributional and biostratigraphic aspects. In *Earliest Man and Environments in the Lake Rudolf Basin* (eds. Coppens Y., Howell F.C., Isaac G.L., Leakey R.E.F.), pp. 177-192. Chicago, University of Chicago Press.
- de Heinzelin J. 1983 *The Omo Group: Archives of the International Omo Research Expedition*. Teruvren, Musée Royal de l'Afrique Centrale; 365 p.
- Souron A., Balasse M. & Boisserie J.R. 2012 Intra-tooth isotopic profiles of canines from extant *Hippopotamus amphibius* and late Pliocene hippopotamids (Shungura Formation, Ethiopia): Insights into the seasonality of diet and climate. *Palaeogeogr Palaeoclimatol Palaeoecol* 342-343, 97-110.
- Passey B.H., Cerling T.E. & Levin N.E. 2007 Temperature dependence of oxygen isotope acid fractionation for modern and fossil tooth enamels. *Rapid Commun Mass Spectrom* 21(17), 2853-2859.
- Harris J., Cerling T., Leakey M. & Passey B. 2008 Stable isotope ecology of fossil
  hippopotamids from the Lake Turkana Basin of East Africa. *J Zool* 275(3), 323-331.
- 16 Cerling T.E., Harris J.M. & Passey B.H. 2003 Diets of East African Bovidae based on stable isotope analysis. *J Mammal* 84(2), 456-470.

- 17 Cerling T.E., Harris J.M., Leakey M.G. & Mudida N. 2003 Stable isotope ecology of Northern Kenya, with Emphasis on the Turkana Basin. In *Lothagam: the Dawn of Humanity in Eastern Africa* (eds. Leakey M.G., Harris J.M.), pp. 583-603. New York, Columbia University Press.
- Sponheimer M., Lee-Thorp J.A., DeRuiter D.J., Smith J.M., Van der Merwe N.J., Reed K., Grant C.C., Ayliffe L.K., Robinson T.F., Heidelberger C., et al. 2003 Diets of southern African Bovidae: Stable isotope evidence. *J Mammal* 84(2), 471-479.
- 19 Kingston J.D. 2011 Stable isotopic analyses of Laetoli fossil herbivores. In Paleontology and Geology of Laetoli: Human Evolution in Context Volume 1: Geology, Geochronology, Paleoecology, and Paleoenvironment (ed. Harrison T.), pp. 293-328. New York, Springer.
- 20 Bedaso Z., Wynn J.G., Alemseged Z. & Geraads D. 2010 Paleoenvironmental reconstruction of the Asbole fauna (Busidima Formation, Afar, Ethiopia) using stable isotopes. *Geobios* **43**(2), 165-177.
- Phillips D.L. 2012 Converting isotope values to diet composition: the use of mixing models.
  *J Mammal* 93(2), 342-352.
- Kaiser T.M. 2011 Feeding ecology and niche partitioning of the Laetoli ungulate faunas. In *Paleontology and Geology of Laetoli: Human Evolution in Context Volume 1: Geology, Geochronology, Paleoecology, and Paleoenvironment* (ed. Harrison T.), pp. 329-354. New York, Springer.
- Nowak R.M. 1999 Walker's Mammals of the World, vol. 2. 6 ed. Baltimore, USA, Johns Hopkins University Press.
- Kingdon J. 1982 East African Mammals: An Atlas of Evolution in Africa: Volume III Part C (Bovids). London, Academic Press; 394 p.
- Geist V. 1998 Deer of the World: Their Evolution, Behaviour, and Ecology. Mechanicsburg,
  Stackpole Books; 421 p.

Ahrestani F.S., Heitkönig I.M.A. & Prins H.H.T. 2012 Diet and habitat-niche relationships within an assemblage of large herbivores in a seasonal tropical forest. *J Trop Ecol* **28**(04), 385-394. **Supplementary Table 1.** Specimen numbers, stratigraphic provenience,  $\delta^{13}$ C, and  $\delta^{18}$ O for all

bovid and suid samples, and mean values for serial samples of the two hippopotamid

specimens reported by Souron et al. [13].

					d <sup>13</sup> C	d <sup>18</sup> O
Sample number	Specimen number	Taxon	Tooth Position	Stratigraphic Unit	corrected CaCO <sub>3</sub> vs. PDB	corrected CaCO3 vs. PDB
BO-OM-03	OMO 20/2-10003	Tragelaphus rastafari	Lm3	B-0	-92	0.4
BO-OM-06	W- 99	Tragelaphus rastafari	R m3	B-2	-8.9	-2.7
BO-OM-08	B- 163 b	Tragelaphus rastafari	R m3	B-2	-8.6	-1.1
BO-OM-07	B- 116	Tragelaphus rastafari	R m3	B-2	-8.0	-1.1
BO-OM-01	OMO 28-1970-1853	Tragelaphus rastafari	L M3	B-10	-9.8	0.9
BO-OM-05	L 745-1 (=P610?)	Tragelaphus rastafari	R M3	B-10	-9.4	-3.4
BO-OM-02	OMO 28-1968-2463	Tragelaphus rastafari	L m3	B-10	-6.6	-0.8
BO-OM-04	ОМО 3/0-10030	Tragelaphus rastafari/nakuae	L M3	B-12	-5.1	-1.9
BO-OM-13	ОМО Р 791/S-1970-2985	Tragelaphus nakuae	L M3	C-8	-4.8	-2.8
BO-OM-14	OMO 18-1968-2892	Tragelaphus nakuae	L M3	C-8	-2.8	-3.2
BO-OM-15	OMO 239-1973-4503	Tragelaphus nakuae	Lm3	C-8	-2.7	-2.8
BO-OM-11	L 47-63	Tragelaphus nakuae	L m3	C-8	-2.7	-1.7
BO-OM-16	OMO 18/sup-10035	Tragelaphus nakuae	L M3	C-8/C-9	-4.5	-4.8
BO-OM-16	OMO 18/sup-10035	Tragelaphus nakuae	L M3	C-8/C-9	-4.5	-4.0
BO-OM-12	L 444-1	Tragelaphus nakuae	L m3	C-9	-5.6	-0.3
BO-OM-10	L 218-2	Tragelaphus nakuae	Lm3	C-9	-4.5	-3.5
BO-OM-09	L 572-1f	Tragelaphus nakuae	R m3	C-9	-3.1	-3.1
BO-OM-17	L 35-40 e	Tragelaphus nakuae	L M3	G-4/G-5	-5.5	-1.0
BO-OM-22	OMO 245-1973-4932	Tragelaphus nakuae	L M3	G-6/G-8	-2.8	-2.3
BO-OM-18	ОМО 222-1973-2755	Tragelaphus nakuae	R m3	G-7	-5.1	0.7
BO-OM-20	ОМО 241-1973-4725Е	Tragelaphus nakuae	Lm3	G-11	-6.9	-0.9
BO-OM-19	OMO 254-1973-5217	Tragelaphus nakuae	R M3	G-12-/G13	-2.8	-3.3
BO-OM-21	OMO 249-1973-5028	Tragelaphus nakuae	Lm3	G-13	-3.4	-1.9
BO-OM-23	OMO 257-1973-5341	Tragelaphus nakuae	L M3	G-13	-2.4	-3.9
SU-OM-01	OMO 211-10012	Kolpochoerus limnetes	L M3	B-10	-8.1	-5.9
SU-OM-02	OMO 112/1-10002	Kolpochoerus limnetes	L m3	B-10	-5.2	-4.4
SU-OM-60	L 367-2	Kolpochoerus limnetes	R M2	B-12	-6.0	-2.2
SU-OM-03	OMO 56-10017	Kolpochoerus limnetes	R M2	C-4/C-5	-2.5	-2.7
SU-OM-04	L 335-27	Kolpochoerus limnetes	R M3	C-4/C-5	-1.0	-4.1
SU-OM-05	L 27-50	Kolpochoerus limnetes	L m3	C-4/C-5	-0.6	-7.7
SU-OM-06	OMO 349-10014	Kolpochoerus limnetes	R M3	C-4/C-5	-3.1	-3.9
SU-OM-07	OMO 56-10015	Kolpochoerus limnetes	L m3	C-4/C-5	-1.2	-3.0
SU-OM-08	OMO 56-10021	Kolpochoerus limnetes	L M2	C-4/C-5	-2.8	-2.4
SU-OM-09	L 193-9 e	Kolpochoerus limnetes	L M3	C-8/C-9	-2.0	-5.1
SU-OM-10	OMO 18-1968-2105	Kolpochoerus limnetes	R M2	C-8/C-9	-2.2	-5.6
SU-OM-11	OMO 84-1976-20	Kolpochoerus limnetes	L M2	C-8/C-9	-2.0	-6.1
SU-OM-12	OMO 18-1968-2103	Kolpochoerus limnetes	R m2	C-8/C-9	-1.5	-5.3
SU-OM-13	OMO 18-1971-2823	Kolpochoerus limnetes	L m2	C-8/C-9	-2.0	-4.9
SU-OM-14	OMO 122-10030	Kolpochoerus limnetes	L m3	C-8/C-9	-0.6	-6.3
SU-OM-15	L 144-16 a	Kolpochoerus limnetes	L M2	C-8/C-9	-0.2	-4.3
SU-OM-16	L 271-10001	Kolpochoerus limnetes	R m3	E-1	-1.2	-4.5
SU-OM-17	OMO 71-1969-219	Kolpochoerus limnetes	L M3	E-1	-3.3	-5.9
SU-OM-18	OMO 166-1973-778	Kolpochoerus limnetes	L M2	E-1	-0.5	-5.8
SU-OM-19	OMO 71-1969-213	Kolpochoerus limnetes	Lm3	E-1	-0.1	-4.0

Sample number	Specimen number	Taxon	Tooth Position	Stratigraphic Unit	d <sup>13</sup> C corrected CaCO <sub>3</sub> vs. PDB	d <sup>18</sup> O corrected CaCO <sub>3</sub> vs. PDB
SU-OM-20	OMO 71-1969-335	Kolpochoerus limnetes	R M3	E-1	-0.2	-3.7
SU-OM-21	OMO 71-1969-214	Kolpochoerus limnetes	L m3	E-1	-1.1	-5.5
SU-OM-22	OMO 71-1969-216	Kolpochoerus limnetes	L m3	E-1	-0.2	-2.8
SU-OM-23	OMO 71-1969-218	Kolpochoerus limnetes	R m3	E-1	-1.3	-5.2
SU-OM-24	OMO 71-1969-215	Kolpochoerus limnetes	L m3	E-1	-0.6	-4.5
SU-OM-25	OMO 71-1969-217	Kolpochoerus limnetes	L m3	E-1	-0.3	-4.2
SU-OM-26	OMO 33-1969-329	Kolpochoerus limnetes	R M3	F-0	0.0	-3.6
SU-OM-27	L 747-4	Kolpochoerus limnetes	L M3	F-0	-1.3	-3.6
SU-OM-28	OMO 33-1969-326	Kolpochoerus limnetes	L m3	F-0	1.1	-0.5
SU-OM-29	OMO 33-1970-2915	Kolpochoerus limnetes	L M3	F-0	0.4	-4.1
SU-OM-30	L 398-2260	Kolpochoerus limnetes	L M2	F-0	-0.9	-3.5
SU-OM-31	L 398-1224	Kolpochoerus limnetes	R m3	F-0	0.0	-1.7
SU-OM-32	OMO 33-1974-6603	Kolpochoerus limnetes	R m2	F-0	0.1	-1.9
SU-OM-33	L 398-2018	Kolpochoerus limnetes	R M3	F-0	-0.5	-3.9
SU-OM-34	L 398-1970	Kolpochoerus limnetes	R m3	F-0	-0.6	-0.5
SULOM-35	OMO 33/I3-1973-3356	Kolpochoerus limnetes	R M3	F-0	-0.3	-2.3
SU-OM-36	OMO 310-10029	Kolpochoerus limnetes	I M2	G-8	-0.5	-6.5
SU-OM-30	OMO 47 1970 2167	Kolpochoerus limnetes	D M2	G-8	2.0	-0.5
SU-OM-37	OMO 47-1970-2107	Kolpochoerus limnetes	R M2	G-8	-2.0	-2.5
SU-OM-38	OMO 47-1970-2039	Kolpochoerus limnetes	R M2	G-8	-0.8	-2.5
SU-OM-39	OMO 47-1908-3342	Kolpochoerus limnetes		G-8	-0.5	-2.0
SU-OM-41	OMO 75/Su-1970-405	Kolpochoerus limnetes		G-8	-0.0	-3.0
SU-OM-41	OMO 47-1970-1378	Kolpochoerus limneles		G-8	-4.2	-7.4
SU-OM-42	OMO 47-1968-2127	Kolpochoerus limnetes	L M2	G-8	-0.1	-2.0
SU-OM-43	OMO 47-1968-5555	Kolpochoerus limnetes		G-8	-1.0	-3.4
SU-OM-44	OMO 323-1976-943	Kolpochoerus limnetes	L M2	G-8	-0.3	-4.4
SU-OM-45	OMO 4/-19/3-1486	Kolpochoerus limnetes	K M3	G-8	0.0	-3.2
SU-OM-46	OMO 323-10008	Kolpochoerus limnetes	L m3	G-8	-0.2	-4.9
SU-OM-47	OMO 323-10034	Kolpochoerus limnetes	L M3	G-8	-0.6	-5.1
SU-OM-48	OMO 323-10013	Kolpochoerus limnetes	L m2	G-8	-0.3	-6.8
SU-OM-49	OMO 310-1976-537	Kolpochoerus limnetes	R m3	G-8	-0.4	-6.6
SU-OM-50	OMO 323-1976-923	Kolpochoerus limnetes	R m3	G-8	-0.5	-6.4
SU-OM-51	ОМО 47-1968-2123	Kolpochoerus limnetes	R M3	G-8	-0.4	-1.7
SU-OM-52	ОМО К 7-1969-322	Kolpochoerus olduvaiensis	L m3	L-9	-1.1	-3.9
SU-OM-53	ОМО К 7-1969-4436	Kolpochoerus olduvaiensis	L M3	L-9	-0.3	-5.5
SU-OM-54	ОМО К 7-1969-4430	Kolpochoerus olduvaiensis	m3	L-9	0.5	-0.9
SU-OM-55	ОМО 342-10071	Kolpochoerus olduvaiensis	L m3	L-9	-1.7	-3.3
SU-OM-56	OMO 342-10076	Kolpochoerus olduvaiensis	R m3	L-9	-0.1	-2.3
SU-OM-57	OMO 342-10045	Kolpochoerus olduvaiensis	L M2	L-9	-0.4	-2.4
SU-OM-58	ОМО 342-10134	Kolpochoerus olduvaiensis	R m3	L-9	-0.3	-2.0
SU-OM-59	OMO 342-10088	Kolpochoerus olduvaiensis	L m3	L-9	0.6	-2.1
mean	OMO 112/2-10014	aff. Hippopotamus protamphibius	lower canine	B-9	-7.3	0.4
mean	OMO 331-10003	aff. Hippopotamus protamphibius	lower canine	C-9	-2.3	-5.5