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Opportunities and constraints for reconstructing palaeoenvironments from stable light isotope ratios in fossils

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Stable light isotope ratios ($^{13}C/^{12}C$ and $^{18}O/^{16}O$) in fossil teeth provide key archives for understanding ecology of past faunal communities and the evolution of environments during the Plio-Pleistocene. Given the inevitable processes of diagenesis during fossilisation, the integrity of isotopic information and the degree of detailed information that can be extracted, remain important issues in all fossil studies. The most appropriate tests are those intrinsic to isotopic abundances in ecosystems. They are easier to develop for $^{13}C/^{12}C$ in savanna environments where large $^{13}C/^{12}C$ differences exist between C_4 tropical grasses and C_3 trees and shrubs. Validating $^{18}O/^{16}O$ ratios in fossil carbonate or phosphate is more difficult, but patterned variability, mainly tracking water-related behaviour, within modern faunal communities has been replicated in several fossil assemblages. The identification of seasonal variation in $^{13}C/^{12}C$ and $^{18}O/^{16}O$ along the growth axis of a tooth crown, also applicable in areas composed solely of C_3 plants, fills a dual role as a test and for providing data on seasonal amplitude. The results of studies from low- and mid-latitude African sites suggest that isotopic variation in rainfall on short timescales and ecological differences amongst animals, dominate over smaller differences in $^{18}O/^{16}O$ composition due to temperature.

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INTRODUCTION

Under appropriate conditions the mineral component of vertebrate bones and teeth survives for millions of years. Fossilised bones and teeth preserve information about many of the processes and conditions to which that animal was subject during its lifetime, and which can be accessed via chemical indicators, most notably, the stable light isotope composition. One major constraint is that the effects of alterations induced by decay and fossilisation processes on these biochemical indicators must not obscure the original composition, at least, not to the extent that it can no longer be interpreted. The extent to which these natural processes might alter biogenic isotopic compositions is a source of long-standing controversy.

Recently it has become apparent that isotopic degradation does not necessarily follow physico-chemical alteration (Stuart-Williams *et al*., 1996; Lee-Thorp and Sponheimer, 2003). In other words, processes such as recrystallisation that are, after all, bound to occur to some degree during fossilisation, do not inevitably lead to poor isotopic integrity. The isotopic data from fossils are often surprisingly robust. There are limits to this generalisation, however, some isotopic applications (most notably in palaeoclimatology) require a great deal of precision for meaningful interpretation. The question then becomes what are the limits imposed by diagenesis and natural variability in those cases requiring precision in isotopic data? In order to assess this, it is important to understand (1) the constraints imposed by the nature of biological minerals on fossilisation pathways and (2) natural variability in ecosystems, both past and present. In this paper, we assess current understanding of fossil calcified tissue chemistry in the light of these problems and go on to address the limitations and advantages that natural variability imposes on our interpretations of past environments.

NATURE OF BIOLOGICAL MINERALS AND DIAGENESIS

Bones and teeth can survive for so long precisely because the mineral component of these tissues — a series of calcium phosphate-based minerals called biological apatites (or bioapatites) — is stabilised by postmortem chemical changes. These changes integral to the fossilisation process, alter the mineral structure immediately postmortem and thereafter; alteration or diagenesis, occurs whether or not burial takes place (Tuross *et al*., 1989; Person *et al*., 1995).

The minerals in mammalian skeletal tissues are collectively known as biological apatites: hexagonal calcium phosphates, resembling (but not identical to) hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] in structure. Differences compared to synthetic or geologically occurring apatites include non-stoichiometry, high isomorphic substitution and adsorption, lattice distortions, and small crystal size (LeGeros, 1991). Biological apatites are a continuum of structures differing in kind and degree of substitutions, function and crystallinity[1]. All of these factors affect the properties and function of the mineral in some degree, both in life and in death.

The location of phosphate within the apatitic structure is well-understood, but carbonate ions may occur in several locations, both as adsorbed ions on crystal surfaces, and within the unit cell substituted mainly in the phosphate position (sometimes called structural or "B" carbonate) and to a lesser extent in the hydroxyl position ("A" carbonate) (Rey *et al*., 1991). Most CO_3^{2-} is substituted for PO_4^{3-}, (usually in combination with Na^+ substituted for Ca^{2+} to preserve net charge), while minor amounts of HPO_3^-, carbonates and bicarbonates are adsorbed on hydration layers in bone apatite.

As a result of enhanced surface area/mass ratios due to small crystal size, reactive surfaces and adsorbed ions, and high number of substitutions that raise solubility, bone apatite is highly reactive (Driessens *et al*., 1978; LeGeros, 1991). Enamel apatite differs from bone and dentine: it has fewer substitutions, less distortion, greater long-range order and crystals are about an order of magnitude larger (LeGeros, 1991). About half the amount of substituted CO_3^{2-} (~3%) is present compared to bone apatite (~6%).

Other differences are in higher-order structures and in the organic matrix. Deposition and orientation of bone and dentine apatite is regulated by collagen fibril periodicity, whereas enamel forms as rods on templates of tubules (Boyde, 1967). The tubular organic matrix, made up of phosphoproteins and amelogenins, decreases during enamel maturation to negligible amounts (<1%), whereas the proportion of collagen remains high (~20–30%) in bone and dentine. Formation and turnover times differ; bone is constantly remodelled during life whereas enamel forms during a (relatively) discrete, early period in the individual's life. Hence isotopic ratios in phosphate or carbonate compartments of bone apatite reflect conditions integrated over many years, whereas those in enamel reflect conditions at the time of mineralisation of a particular tooth. It is important to note, however, that enamel matures over several months — the first phase of relatively rapid mineralisation of tubules is followed by a longer period of maturation of several months duration (Boyde, 1967; Balasse, 2003). Therefore, isotope values obtained from even extremely tiny sampling increments cannot reflect discrete, short episodes, no matter how small the sampling intervals or attempts to sample along enamel prisms (for a review see Balasse, 2003). The isotopic signal is inevitably mixed and dampened, although efforts are currently underway to disentangle the smearing by application of mathematical algorithms to, initially, enamel formed under controlled conditions (Passey and Cerling, 2002; Passey *et al*., 2003).

It is widely recognised that enamel preserves both chemical structure and isotopic composition far better than bone mineral (Lee-Thorp and van der Merwe, 1987), a phenomenon that can be ascribed to its higher crystallinity and less porous microstructure. In living bone, the mineral is actively maintained in a paracrystalline state, because one of its key functions is to provide a reservoir of Ca^{2+} and CO_3^{2-} ions readily accessible to the blood supply. In the absence of these in vivo molecules and in the presence of a ready supply of the raw materials for growth (principally Ca^{2+} and PO_4^{3-} ions), bone apatite will "grow" by processes of recrystallisation and Ostwald "ripening" (scavenging of smaller by larger crystallites; Eanes and Posner, 1970). Essentially the trajectory is towards stabilisation and greater crystallinity (Tuross *et al*., 1989; Person *et al*., 1995; Sponheimer and Lee-Thorp, 1999*a*). In the process numbers of foreign ions compatible with the apatite structure may be added or exchanged from the surroundings. Contrary to earlier beliefs by phosphate practitioners (e.g. Longinelli, 1984; Luz and Kolodny, 1985), these processes can incorporate new PO_4^{3-} ions. Enamel is already relatively stable and essentially preconditioned, therefore possibilities for recrystallisation and crystal growth are minimised. Ionic or isotopic exchange processes, however, can continue in both tissues.

These properties need also to be considered in the chemical pretreatment methods commonly used to eliminate intrusive material or to minimise alteration in fossils. For instance, use of too strong acid solutions or too lengthy immersions in even weaker acid solutions, will induce recrystallisation and isotopic effects (Lee-Thorp and van der Merwe, 1991; Koch *et al*., 1997; Sponheimer, 1999; Balasse *et al*., 2002). The pathways of alteration — addition of extraneous material, recrystallisation and ripening — are common to both phosphate- and carbonate-based studies. The main exception is that oxygen bound to phosphate is not easily exchangeable, whereas oxygen bound to carbonate is. However, the phosphate bond is not immune to post-mortem attack by enzymatically-catalysed microbial attack (Blake *et al*., 2001) and alteration of isotopic ratios in biogenic phosphate has been demonstrated (Sharp *et al*., 2000). There seems to be little evidence from Fourier Transform Infrared studies that carbonate itself is any more or less mobile that phosphate (Sponheimer and Lee-Thorp, 1999*a*; Lee-Thorp and Sponheimer, 2003); both ions undergo subtle reorganisation during fossilisation. In spite of these subtle changes, however, consistent offsets between $^{18}O/^{16}O$ composition in these two ions suggest that isotopic compositions are robust (Bryant *et al*., 1996; Iacumin *et al*., 1996; Sharp *et al*., 2000).

Having established the framework imposed by the nature of the minerals, we can proceed to consider some of the limits and

[1]Crystallinity denotes both crystal size and a lack of defects or distortions.

possibilities of carbon and oxygen isotope-based tools offer for palaeoecology and palaeoenvironmental reconstruction.

CARBON ISOTOPE ABUNDANCES IN FOSSIL FOODWEBS

The carbonate substituted within biological apatites is derived from blood bicarbonate, which is in turn derived from dietary carbon. The expected enrichment in $\delta^{13}C$ of about 10‰ in the system CO_2-HCO_3^--CO_3^{2-} at equilibrium is matched by isotopic differences of *ca*. 10–14‰ between dietary carbon and apatite carbonate[2] (Lee-Thorp *et al*., 1989; Cerling and Harris, 1999). Early scepticism about the use of mineral (e.g. Schoeninger and DeNiro, 1982) has been largely overcome especially in the case of enamel (for a review see Lee-Thorp, 2000). A crucial demonstration showed that expected differences in $\delta^{13}C$ of browsers and grazers eating isotopically distinct C_3 and C_4 plants were maintained for millions of years (Lee-Thorp and van der Merwe, 1987). Typical values for modern browsers and grazers are modified slightly in fossils; a small positive $\delta^{13}C$ shift of ~2–3‰ is partly attributable to diagenesis and partly to the "fossil fuel effect" that has seen $\delta^{13}C$ of entire modern ecosystems decrease. Nonetheless, values for fossil fauna are clearly identifiable to C_3, C_4 or mixed diets, even at millions of years remove.

This finding opened the way for a plethora of palaeodietary and palaeoenvironmental applications ranging widely in age. The most straightforward application is in biomes where the large distinction between C_3 and C_4 feeders provides both the means to interrogate the environment, and an intrinsic test for reliability. Other kinds of biomes are also amenable, but published applications are fewer.

GLOBAL C_4 GRASS EXPANSION

One important application to investigate vegetation and climate shifts in older material, is to the major global expansion of C_4 grass systems between ~8 and 5 million years ago (Cerling *et al*., 1997). This phenomenon had been first detected in soil isotope studies (Quade *et al*., 1989; Cerling, 1993) but the patterns are clearer and less ambiguous in fossil fauna, allowing detailed tracking of the expansion across latitudes (Cerling *et al*., 1997). C_4 grasses first began their expansion in lower latitudes, spreading over the next few million years to mid-latitudes in Africa, North and South America, Australia and parts of Eurasia (Cerling *et al*., 1997). The exceptions are also instructive; some mid-latitude regions remained resolutely C_3. Lack of any evidence for C_4 plants in Greece (Quade *et al*., 1994) and Turkey (Quade *et al*., 1995) and the southwestern Cape of South Africa (Franz-Odendaal *et al*., 2002) strongly suggests that winter-rainfall, Mediterranean-type ecosystems were already in place in these regions in the late Miocene and early Pliocene.

The near-synchronous expansion of C_4 grasslands across large parts of the world must have a global driver, but the exact causes have remained curiously elusive. A popular and plausible hypothesis (Cerling *et al*., 1997) proposed that plummeting CO_2 levels towards the end of the Miocene favoured C_4 plants, since they are adapted to cope with lower pCO_2 levels (Ehleringer *et al*., 1997). But more recent evidence from alkenones in marine cores suggests that no fall in pCO_2 levels occurred at that time, since they were already low (Pagani *et al*., 1999), has led to a re-evaluation of all the evidence. Since new continental-based carbon isotope evidence supports the marine evidence (Segalen *et al*., 2002), the major forcing mechanisms may reside in a combination of tectonic and solar insolation drivers. Resolution of the problem likely requires a good deal more detailed isotopic and other evidence of the transition period in various regions.

EVOLUTION OF OPEN HABITATS IN SOUTHERN AFRICA

A good deal of work on Plio- and Pleistocene faunas has been done in East and South Africa, and more recently a large-scale project in Chad (Zazzo *et al*., 2000). The results showed that significant proportions of C_4 grasses were present in the earliest periods represented by the South African sites, from about 3.3 Ma onwards (Lee-Thorp and van der Merwe, 1987; Sponheimer and Lee-Thorp, 1999*b*). Later studies were able to refine the picture for various periods, but this data merely shows presence or absence of C_4. In the Chad case, this was very useful, since the sites spanned major changes from the late Miocene to the Pliocene (more woody vegetation) to the more open habitats of the Pliocene (Zazzo *et al*., 2000).

In cases where good data for a large cross-section of the faunal assemblage exists, it has been possible to develop a "C_3/C_4" index from the faunal $\delta^{13}C$ data. The index, essentially calculated from the numbers of genera or individual specimens falling into one of a grazer, mixed feeder or browser, category, provides an indicator of how closed or open the landscape was. Importantly the index makes no assumptions about the dietary habits of the fauna, it is based rather on the assumption that in a closed habitat more browsers will find appropriate forage, while grazers will be favoured in open grassy landscapes (Sponheimer and Lee-Thorp, 2003).

The index was applied to a series of sites, differing in age, in order to construct a view of the longterm evolution of open habitats on the central inland plateau of southern Africa (Luyt and Lee-Thorp, 2003). The faunal $\delta^{13}C$ provides a more direct reflection of actual diet rather than one assumed from taxonomic considerations, as a result, a new view of landscape evolution emerges that differs from earlier scenarios in one important respect. It shows that the largest and most significant floral change towards an open grassy landscape occurred about 1.7–1.8 Ma, rather than in stepwise fashion from 3 Ma ago as earlier suggested (Fig. 1). This result is concordant with the emergence of very open grassy Serengeti-like ecosystems in East Africa at about this time.

[2]Stable light isotope abundances are by convention expressed in the δ format in relation to an international standard, in parts per mil. For $^{13}C/^{12}C$ the expression is: $\delta^{13}C$ (‰) = $(R_{sample} - R_{std})/R_{std} \times 1000$ where: $R = {}^{13}C/{}^{12}C$. The standard is Peedee Belemnite carbonate (VPDB). The same expression applies to $^{18}O/^{16}O$ but the standards are VPDB or Standard Mean Ocean Water (VSMOW). The former is used throughout this paper.

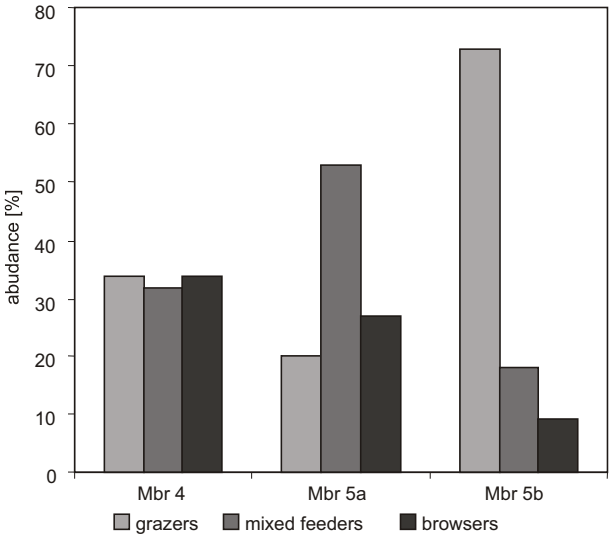

Fig. 1. Histogram showing strong shifts in the relative proportions of grazers, mixed feeders and browsers derived from tooth enamel $\delta^{13}C$ data in 3 fossil faunal assemblages

The fauna are from members 4, 5a and 5b at Sterkfontein Cave, Gauteng Province, South Africa and represent periods at approximately 2.5, 2, and 1.7 Ma, respectively. Plotted in this way, it is clear that the landscape becomes significantly more open with time. The largest shift occurs with the transition to 5b at about 1.7 Ma; data from Luyt and Lee-Thorp (2003)

OXYGEN ISOTOPE VARIABILITY IN ECOSYSTEMS

SYSTEMATICS OF $^{18}O/^{16}O$ IN CALCIFIED TISSUE MINERALS

Until quite recently applications of oxygen isotopes in calcified tissues have been based mostly on apatite phosphate rather than apatite carbonate. This was because of the exceptional strength of the P–O bond, which is unlikely to exchange oxygen atoms with water, in contrast to carbonate. Hence it was generally assumed that phosphate oxygen isotope composition (δO_p) is stable and that "the record is nearly perfectly retained after death" (Luz *et al*., 1984, p. 256). One problem with this assertion is that it neglects a basic feature of calcified tissue chemistry, the tendency to recrystallise, and isotopic alteration of phosphate has been demonstrated (see above).

Phosphate-based $\delta^{18}O$ studies have from the start focused on extraction of palaeoclimate-related information, and in particular, on isotopic composition of palaeowaters and the estimation of palaeotemperatures in continental contexts. The link to palaeotemperature derives from the major effect of latitude on meteoric rainfall oxygen isotope ratios, values become increasingly depleted at higher cooler latitudes (Dansgaard, 1964). However, the relationship is in fact rather complex and subject to a whole range of influences which may dominate on a regional basis.

$\delta^{18}O_p$ (and likewise $\delta^{18}O_{carb}$) is formed in equilibrium with blood plasma ($\delta^{18}O_{bw}$), which is in turn determined by the isotopic mass balance for the body (input *vs*. output) and strongly influenced by the isotopic composition of environmental waters ($\delta^{18}O_w$) (Longinelli, 1984). Luz and colleagues derived flux models for oxygen through the mammalian body, supporting their models by experimental data on rats (Luz and Kolodny, 1985) and $\delta^{18}O_{bw}$, and $\delta^{18}O_p$ observations from modern and historical humans respectively (Luz *et al*., 1984). They found simple linear relationships between $\delta^{18}O_w$, $\delta^{18}O_{bw}$ and $\delta^{18}O_p$, but that deviations depended primarily on the ratio between rate of drinking to rates of drinking and respiration, and production of metabolic water. A third prediction, that environmental effects other than isotopic composition of water sources were small, was later revised (Luz and Kolodny, 1989) following observations that where significant amounts of body water come from leaf water, aridity caused further enrichment in $\delta^{18}O_p$ via leafwater evapotranspiration isotope effects. Luz and Kolodny (1985, 1989) proposed that those species whose water consumption was large relative to energy expenditure were more suitable indicators of environmental $\delta^{18}O_w$. This is the model that has been followed in palaeotemperature studies, with a focus on data from "well-behaved" species (according to Luz and Kolodny's criteria), such as equids.

PALAEOCLIMATE APPLICATIONS BASED ON PHOSPHATE ANALYSES

Subsequently, numbers of studies have explored and enlarged on the palaeotemperature/palaeoclimate theme. Several aimed to establish environmental $\delta^{18}O_w$ and hence temperature sequences from large herbivorous mammals such as equids (e.g. Sanchez-Chillon e*t al*., 1994; Bryant *et al*., 1996) in contexts ranging in age from Pleistocene to Miocene or older. Ayliffe and Chivas (1990) showed that, even if not reliable indicators of $\delta^{18}O_w$ are available, $\delta^{18}O_p$ of drought-tolerant macropods could be used to infer relative humidity in the past. Fricke *et al*. (1995) examined climate shifts from $\delta^{18}O_p$ in Viking settlers to determine likely causes of their disappearance from Greenland during the 14th century AD.

With wider application came the realisation that reasonable environmental water $\delta^{18}O_w$ or temperature results could not be extracted in many cases, or the results were inexplicably variable (see Sanchez-Chillon *et al*., 1994), or the calculations required assumptions about $\delta^{18}O_w$ to derive temperatures, a practice problematic even in the recent (Holocene) materials being studied (Stephan, 2000). The possibility of diagenesis was raised seriously for the first time. Ayliffe *et al*. (1994) demonstrated that enamel $\delta^{18}O_p$ from Pleistocene elephant material was intact but bone and dentine $\delta^{18}O_p$ from the same individual was not. However, emerging complex intra-assemblage and intra-individual variability were all too easily attributed to diagenesis, to the neglect of other possible influences. In the face of unexplained variability, the lack of an obvious, intrinsic test for oxygen isotopic fidelity meant that diagenesis could not be ruled out as a major influence. It has, however, transpired that the troublesome variability, once better understood, holds a key to these tests and may provide new information about ecosystems and their natural variability.

ISSUES OF VARIABILITY — INTERSPECIFIC

A comparative study of a small faunal assemblage from Turkana, Kenya, suggested a strong relationship between $\delta^{18}O_p$ and diet, and physiology in addition to drinking behaviours (Kohn, 1996; Kohn *et al*., 1996). A handful of comparative studies using $\delta^{18}O_{carb}$ have shown that the distribution of oxygen isotopes, in one place, and at one time, is strongly differentiated. One advantage of carbonate over phosphate analyses is that larger numbers of samples can be easily analysed, leading to more robust data sets from a greater diversity of environments. The Amboseli study, which focussed on large-bodied herbivores to meet the Luz and Kolodny criterion, found large differences of up to 6‰ in the means (not individual values, the variation amongst which differs by up to10‰) and the means for large-bodied grazers alone differed by ~3‰ (Fig. 2A). Similarly large differences were observed in two modern assemblages from southern Africa, Takatshwane in Botswana and the Morea Estate in Klaserie, in eastern South Africa (Fig. 2B, C). Takatshwane is located in the Central Kalahari near Ghanzi and therefore represents a warm open desert environment with sparse Kalahari thornveld vegetation. Klaserie lies immediately to the east of the Kruger National Park, near the escarpment and represents a warm, relatively moist, woodland environment. Explanations offered for these large differences invoke water-related and cooling behaviours, although some of the details may differ. There seems little argument that consistently low $\delta^{18}O_{carb}$ values for *Hippopotamus amphibius* (hippopotamus) are linked to their habits of immersion under water during the day and foraging at night. This behaviour means that they do not need to cool down by mechanisms such as panting, and plant waters are depleted in ^{18}O at night (Bocherens *et al*., 1996). The differences amongst the grazers, which are mostly obligate drinkers, likely reflect subtle differences in feeding and drinking behaviour. For instance, grazers that prefer to live near open water sources, such as *Kobus ellipsoprymnus* (waterbuck) are consistently depleted in ^{18}O compared to other grazers. Interestingly, the mean value for the equids (the "well-behaved" grazer) is ~1‰ higher than another large-bodied grazer, *Syncerus caffer* (African buffalo) at Amboseli.

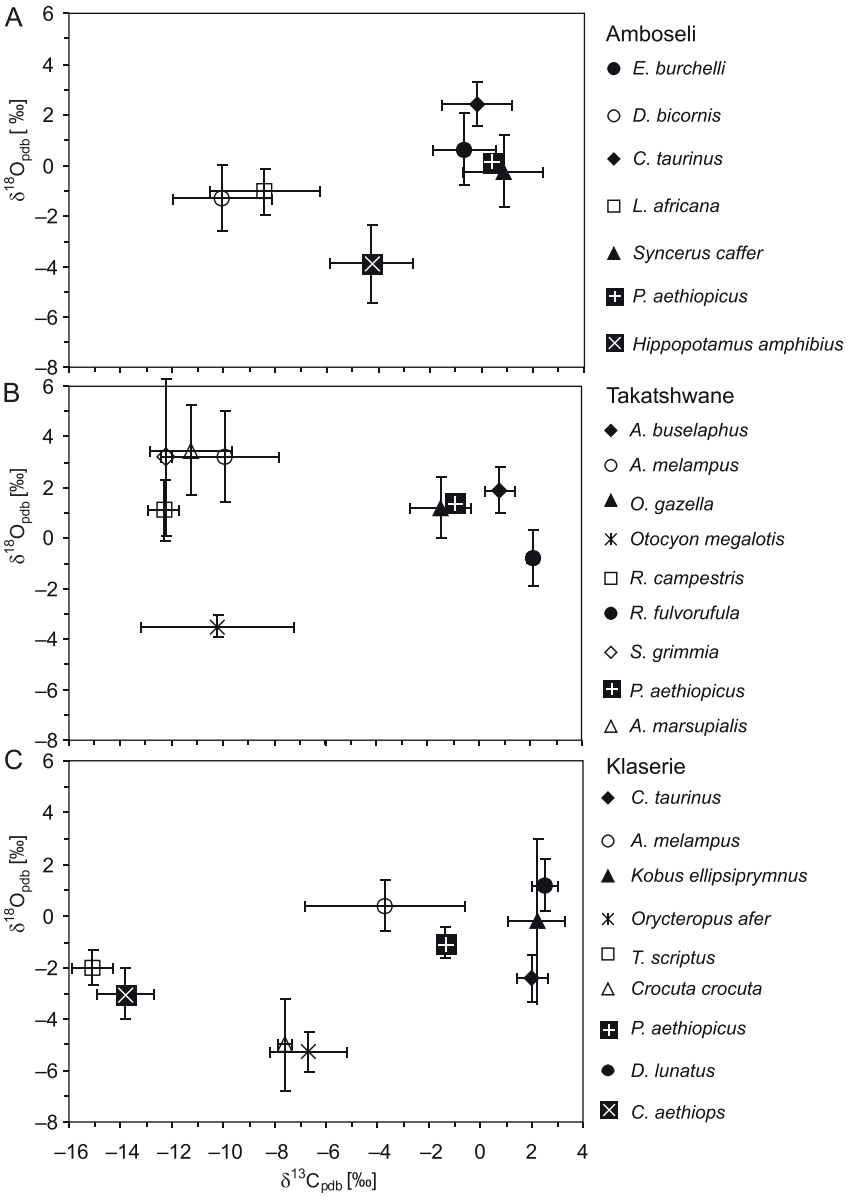

Fig. 2. Plots of $\delta^{18}O$ *versus* $\delta^{13}C$ (both from tooth enamel carbonate) in 3 modern faunal assemblages from Amboseli, Kenya (A), Takatshwane, Botswana (B) and Klaserie, South Africa (C)

Takatshwane is located in the Central Kalahari, at approximately 22°S, 22°E, while Klaserie is located far to the East, at approximately 23°S, 31°E. These two sites form part of the same warm-season rainfall system sourced in the Indian Ocean. $\delta^{13}C$ values distinguish C_3 from C_4 feeders in a predictable way; $\delta^{18}O$ shows high interspecific and even intra-specific variability as discussed in the text; data from Bocherens *et al*. (1996, Amboseli), Sponheimer and Lee-Thorp (2001, Klaserie) and Lee-Thorp and Sponheimer (unpubl. data, Takatshwane)

Oxygen isotope distribution, unexpectedly, also reflects trophic behaviour. In Takatshwane and Klaserie, the faunivores, *Otocyon megalotis* (bat-eared fox), *Crocuta crocuta* (spotted hyena) and *Orycteropus afer* (aardvark), are significantly depleted in ^{18}O compared to the herbivores. ($p = 0.0004$; ANOVA and *t* test). Low values for faunivores are likely related to higher dietary protein levels, since proteins are relatively depleted in ^{18}O compared to carbohydrates (Kohn, 1996; Sponheimer and Lee-Thorp, 2001). There are other patterned differences of interest, for instance, suids and most primates have relatively lower $\delta^{18}O$, although not so low as the faunivores (Fig. 2C).

These patterns are essentially replicated in fossil faunal assemblages (Bocherens *et al*., 1996; Sponheimer and Lee-Thorp, 1999*c*; Luyt *et al*., 2000; Cerling, pers. comm.). Giraffids in Miocene sites were consistently enriched, an observation ascribed to feeding in the upper canopy where leaf-water values are high (Cerling *et al*., 1997). Bocherens *et al*. (1996) showed that hippos retained their predictable low $\delta^{18}O_{carb}$ values in Pleistocene sites, they further suggested that the large differences between most herbivores and hippos could be used as tests for integrity of $\delta^{18}O$ from enamel carbonate. The idea was tested at the 5 Ma site of Langebaanweg on the southwestern coast of South Africa and inter-specific indeed the extinct hippopotamids were significantly lower than the other herbivore fauna. This observation was particularly valuable at this purely C_3 site, since carbon isotope distinctions could not be used to test for isotopic integrity (Franz-Odendaal, 2002).

Given these high levels of inter-specific variation at any one site, representing one time and place, can useful palaeoclimate information be derived? Some information about prevailing, overall $\delta^{18}O_w$ is available from a bulk view of each dataset. Figure 2 shows that each of these sites has a different average value for the entire fauna. The highest values, on average, are found at the interior Kalahari site of Takatshwane. The results suggest that evaporation plays a strong role in rainfall events and/or on environmental water supplies available to animals. Given the site's location, the average $\delta^{18}O_w$ values at this site are likely to be a composite of two isotope effects working in opposition: the continental effect (since the vapour source is in the Indian Ocean far to the east) and evaporation. Average values for Klaserie are more negative, although this site is nearer the vapour source. Rainfall at both of these sites (and likely Amboseli as well) show high coefficients of variation on interannual and decadal scales, so one might expect that longer term faunal collections would show even higher variability (these were all short-term collections over no more than a few years). High resolution stalagmite records from the Makapans Valley in the same general region as Klaserie, demonstrate very high annual-, decadal- and centennial-scale variability in rainfall $\delta^{18}O_w$ (Lee-Thorp *et al*., 2001; Holmgren *et al*., 2003). Serial isotope data from elephant ivory from the Kruger National Park nearby suggests that $\delta^{18}O_w$ and rainfall amount variability at sub- and decadal-scales are reflected in ivory $\delta^{18}O_{carb}$ (Codron, unpubl. data).

Inter-specific variability amongst the grazers, although presenting possible sources of information about palaeoecology of ancient and extinct species, clearly represents a conundrum for those wishing to extract detailed palaeoclimate information and especially for calculating palaeotemperatures. The issue, even when confronted with a modern assemblage of animals, is simply one of which well-behaved species to choose? This is even more problematic when confronted with fossil faunal assemblages, where a high proportion of the faunal assemblage are extinct and of (largely) unknown habits.

ISSUES OF VARIABILITY — INTRA-INDIVIDUAL

Several phosphate- and carbonate-based studies have shown that inter- and intra-tooth variation could be used to infer variables such as seasonal climatic amplitude, season of birth and other biological variables (e.g. Bryant *et al*., 1996; Fricke and O'Neil, 1996; Sharp and Cerling, 1998; Stuart-Williams and Schwarcz, 1998; Balasse *et al*., 2002; Franz-Odendaal *et al*., 2003). The existence of seasonally distinct patterns at the same time provides a means for assessing whether the original oxygen isotope signals were preserved or not. This was one of the tactics used in addressing the issue at the early Pliocene site (5 Ma) of Langebaanweg, where it was unclear that biogenic could be extracted from material of that age (using enamel carbonate; Franz-Odendaal *et al*., 2002).

Intra-individual variability can provide short time series of sequential information about range and amplitude of seasonality. This may be accomplished in several ways. Some studies have concentrated on continuously growing teeth such as beaver incisors (e.g. Stuart-Williams and Schwarcz, 1998), the challenge here being the methods required to deal with phosphate analyses of very small samples. Others have focussed on tooth rows and/or multiple analyses of hypsodont or large, mature teeth (e.g. Balasse *et al*., 2002; Fig. 3).

Figure 3 shows the results of several studies documenting seasonal patterns from the Western Cape, South Africa. It is a coastal region with a winter-rainfall, Mediterranean-type climate and the rainfall sources are located in the SE Atlantic. Evidence suggests that this climate regime has been in existence for millions of years (Franz-Odendaal, 2002), therefore comparison across large periods of time remains reasonably appropriate. The sequences (composed of the 2nd and 3rd molars) for the modern *Raphicerus campestris* (steenbok), a small mixed feeder, show a range in $\delta^{18}O_{carb}$ of 4–5‰ across a nearly complete seasonal cycle (Fig. 3A). This is a reasonable representation of the seasonal rainfall cycle, although values are undoubtedly dampened and averaged because of the limitations imposed by the nature of mineralisation and sampling (Balasse, 2003). Low points correspond to low $\delta^{13}C_{carb}$, indicating that lowest points for $\delta^{18}O_{carb}$ reflect winter. Data for the approximately 1000-year old sheep 3rd molars (Fig. 3B), from the pastoralist site of Kasteelberg, show a lower seasonal range (~3‰) with values slightly positively offset. This "shrinking" could be taken as evidence of some diagenesis, except that there appears to be a considerable range in the seasonality indicators of different individuals from the archaeological site (Balasse *et al*., 2002). Moreover, the domestic animals were managed and therefore might not always show the extremes indicated by wild animals. The Pliocene sivatheres (Fig. 3C) show a similar seasonal range (2–3‰) to the archaeological sheep, but the average values resemble the modern steenbok more closely. These samples were sampled more crudely (lower sample numbers per tooth) that those in Figure 3A and B, so it is likely that more time-averaging occurred. The small positive offset for the archaeological sheep might indicate slightly drier or warmer conditions at this time, but clearly there is a great deal of intra- and inter-individual variability, likely representing changing conditions over the time period of accumulation. Firmer conclusions would require much larger sample numbers.

In spite of the differences in ranges of seasonal amplitude represented at these sites, obtaining information about seasonal range would seem to be a more attainable (and possibly, useful) goal than determining large-scale sequences of $\delta^{18}O$ of meteoric water, which may vary on short or longer timescales for

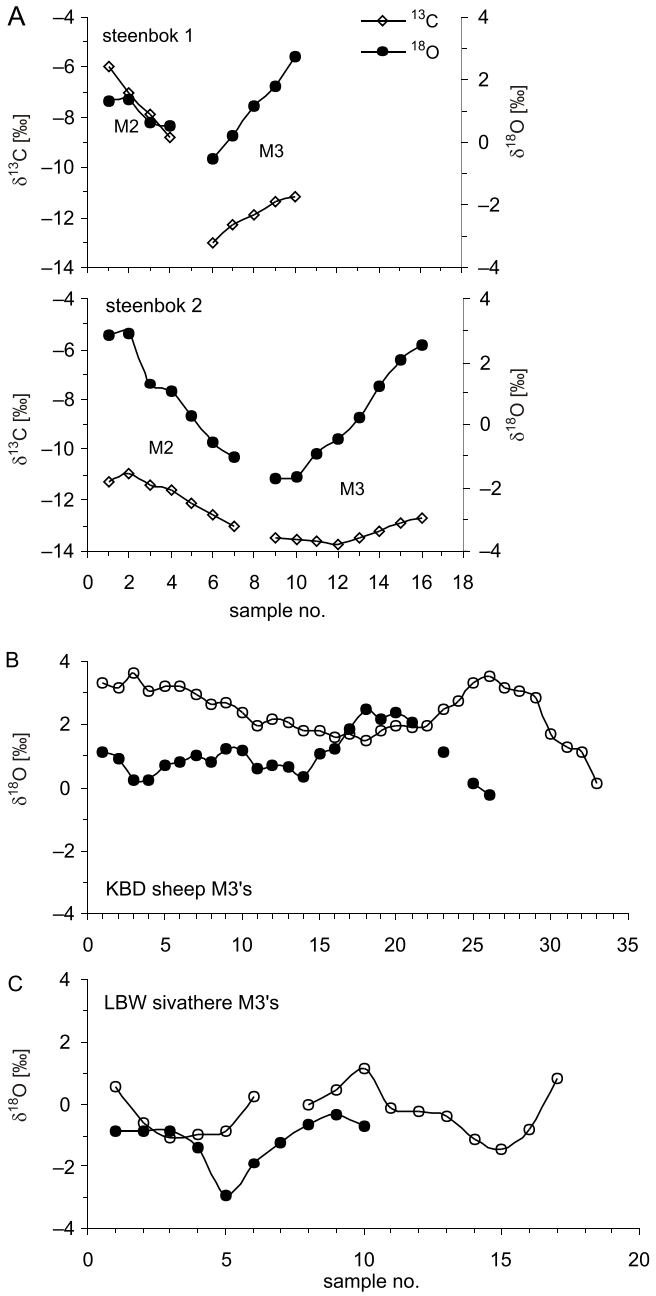

Fig. 3. Intra-tooth variability and seasonality in modern, archaeological and fossil (Pliocene) teeth in the Southwestern Cape

A — modern steenbok, **B** — sheep from the archaeological herder site of Kasteelberg(KBD), C — sivatheres from Pliocene age Langebaanweg (LBW). Two examples are given in each case to show that similar intra-tooth patterns prevail. $\delta^{18}O$ values are expressed relative to VPDB. The range of variation in $\delta^{18}O_{carb}$ is broadly similar for all three sets, but it can be seen that intra- or inter-tooth variation in one animal (modern or ancient) is very large. The M2's and M3's for the steenbok, which have small teeth, provide part of a seasonal sequence, while the M3's of the larger animals represent a longer time period, a year in the case of the sheep and >1 year for the sivatheres. Data are from Balasse *et al*. (2002) and Franz-Odendaal (2002) for Langebaanweg

any one of a number of reasons. Intra-individual, inter-individual and inter-specific variability is an area that promises to reveal information about both seasonal climatic amplitude and animal or human behaviour. It's not at all clear that attempting to derive $\delta^{18}O$ of environmental waters and temperatures, under the circumstances described above where high annual or sub-decadal variability exists in rainfall patterning, and consequently, in $\delta^{18}O$ of environmental waters. Estimating palaeotemperatures from these kinds of highly variable data is even more problematic. There are several impediments, they include firstly, identifying species that best represent ambient environmental water conditions, in the absence of filters, and secondly, high isotopic rainfall variability where short-term shifts in $\delta^{18}O_w$ are high and would provide vastly over-inflated estimates of temperature shifts.

CONCLUSIONS

$\delta^{13}C$ and $\delta^{18}O$ from either phosphate or carbonate in fossil tooth enamel, provide manifold indicators of past environmental and climate conditions. $\delta^{13}C$ data is informative about dominant floral systems in past environments and where appropriate, the strong distinction between C_3 and C_4 plants double-up as indicators of isotopic integrity. Just two examples of applications have been dealt with in this paper, many others exist. The wide range of palaeoenvironmental applications is greatly assisted by the ease in obtaining large amounts of data and the level of detail or analytical precision required to establish the necessary information is not excessively high. On the other hand, the level of precision required for palaeotemperature estimations would seem to be frequently unattainable in the face of high environmental and biological variability. Variability ascribed to different animal behaviours, while presenting alternative means for testing isotopic integrity, presents impediments to the goals of extracting $\delta^{18}O_w$ and palaeotemperature information. Further variability across short timescales suggests strong influences of highly variable rainfall $\delta^{18}O$ at the low- to mid-latitude sites discussed in this paper.

We have discussed inter- or intra-tooth shifts in $\delta^{18}O$, representing seasonal changes in $\delta^{18}O_w$, largely for the purposes of highlighting the problems presented by variability. Nevertheless, it is appropriate to stress the positive aspects as well. Firstly, obtaining sequential information about the range and amplitude of seasonality seems to present a more easily attainable, and perhaps ultimately more useful, goal for palaeoclimate research. Secondly, patterned variability, whether reflecting biological species differences or seasonal shifts, offers a robust means for testing the reliability of isotope values from either the carbonate or phosphate compartments. The issues of variability raised here also clearly demonstrate the need for larger-scale analyses of faunal material and in a greater variety of settings, than has previously been carried out.

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