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STABLE ISOTOPE ANALYSIS OF FOSSIL BONE

ABSTRACT: The biological signal hidden in stable carbon, nitrogen, oxygen and strontium stable isotope ratios in archaeological and fossil skeletal finds give clues to a variety of palaeoecological parameters. In contrast to trace element analysis, these parameters can be assessed on the individual level, which is a major advantage for palaeoanthropology, where single finds prevail over multiple finds at the site of recovery. In this paper, we first review the potential of isotopic analysis of light elements (C, N, O) for the reconstruction of ecological niches of extinct species, followed by a more recent example on how stable strontium isotope ratios can give clues to residence change, migration events and home range assessment in the past. Since modern techniques permit the establishment of valid data even from very small sample sizes, the application of such invasive methods even for valuable fossil finds should be encouraged.

KEY WORDS: Stable isotopes – Palaeodiet – Palaeoecology – Migration

INTRODUCTION

The reconstruction of human ancestry and the further development of human populations requires information on palaeoclimate, palaeodiet, residence change and other related parameters. Several archaeometric approaches aiming at either bone mineral or the organic collagen matrix like multielement or stable isotope analysis have succeeded in the detection of a variety of such past environmental aspects. While trace element profiles established from fossil biological apatite were among the first such applications to unravel ancient dietary behaviour (e.g. Schoeninger 1982, Sillen 1992), the limitation of this method quickly became evident: Due to a high intraindividual variability of bone trace element content even detectable in mammals feeding on a homogenous diet, trace element profiles give clues to palaeodiet on the population level only (Grupe 1992). Among the fossil record, however, individual finds prevail and skeletal series representing a cross section through an ancient population are mostly restricted to anatomical modern *Homo sapiens*. Consequently, trace element analyses were more and more substituted by the investigation of stable isotope ratios of light and heavy elements from both the inorganic and organic bone matrix, since such isotopic data will preserve biological signals characteristic for each individual under study.

Moreover, stable isotopic ratios not only permit the reconstruction of nutritional habits, but give clues to environmental parameters like forest cover, temperature and humidity and, as recent publications have shown, also to migration patterns. Competition over ecological niches is one of the major driving forces in evolution, therefore any such palaeoecological information hidden in fossil finds is of major importance for palaeoanthropology. Investigations of hominid/human and/or animal skeletal remains from a certain geographical region and a defined time level are likely to produce rather detailed images of palaeoecological settings and the living conditions even of extinct species. While isotopic analyses of prehistoric and historic human skeletal series are straightforward, the relative paucity of published data from fossil human and hominid finds may indicate a certain reluctance to sacrificing material for such an invasive method. Technical progress, however, permits the establishment of robust data even from small samples of no more than 50 mg weight, and the far-reaching interpretations made possible from

these archaeometrical data should encourage further research even on very valuable material.

In this paper, we will first summarize the palaeoecological information hidden in stable isotopes in preserved mineralized tissue and will refer to important results obtained in palaeoanthropological research. Later, we shall focus on a still poorly exploited possibility for the reconstruction of residence change, migration patterns and the definition of home ranges by investigation of stable isotopes from heavy elements in biological apatite.

PALAEODIETARY AND PALAEOECOLOGICAL INFORMATION OBTAINED FROM STABLE ISOTOPES OF LIGHT ELEMENTS (CARBON, NITROGEN, OXYGEN)

Stable isotopes of light elements have in common that they differ by one or two mass units only (^{13}C, ^{12}C; ^{15}N, ^{14}N; ^{18}O, ^{16}O), which is a relatively big difference compared to their low atomic weight. Not only are light stable isotopes more volatile than their heavier counterparts, isotopic fractionation occurs in the course of an element's transport through the geo-, hydro- and biosphere since e.g. many enzymes are capable of recognizing these mass differences. As a consequence, different ecosystems (like marine and terrestrial ones, cf. e.g. the pioneering work by Tauber 1981) and even the various components of one ecosystem tend to be isotopically distinct from each other. Differences in the isotopic composition between samples are meaningful but small, therefore stable isotopic ratios are expressed in the conventional δ notation relative to a standard in ‰. The more positive the δ-value, the more is the sample enriched with the heavy isotope of the respective element.

Reconstructing palaeodiet from bone collagen

A major fractionation of carbon stable isotopes occurs in the course of CO_2-assimilation by plants due to different photosynthetic pathways. C_3- and C_4-plants, which largely contribute to the vegetal part of the human diet and the abundance of which is characteristic for different ecological niches, are therefore isotopically distinct from each other. C_3-plant $\delta^{13}C$-values average –27‰, however with a considerable variation from as low as –37‰ under closed canopy conditions until approximately –22‰ under water stress. Isotopic variability is much more restricted in C_4-plants which do not grow under canopies and ranges approximately from –13 to –10‰. These isotopic differences are transferred with a fractionation of +5‰ into the consumer's collagen and hence characterize the consumer as C_3-, C_4- or mixed feeder (cf. Ambrose, Norr 1993). Collagen $\delta^{15}N$-values on the other hand exhibit a pronounced trophic level effect in the course of nitrogen transport through the food web. The more carnivorous the diet, the higher are the collagen $\delta^{15}N$-values of the consumer due to a stepwise enrichment with the heavy ^{15}N isotope, leading to an isotopic fractionation of approximately +4‰ from one trophic level to the next. Collagen $\delta^{15}N$-values therefore characterize the consumer as herbivore, omnivore, primary or secondary carnivore (Ambrose 1993, Schwarcz, Schoeninger 1991).

It is noteworthy that changing climates and environments will lead to different ecological baseline δ-values due to the mass dependent differential mobility of light and heavy isotopes of the same element. Stable isotope ratios are sensitive towards temperature, rainfall and altitude. Especially for carbon, $\delta^{13}C$ has changed since the last ice age (Leuenberger *et al.* 1992) and shifted again by +1.5‰ in its isotopic composition during the last 150 years as a result of fossil fuel burning. $\delta^{13}C$-values from any pre-industrial find must be corrected for this "fossil fuel effect". Because of this variation of ecological baseline isotopic ratios it is highly recommended to perform additional isotopic analyses of skeletal finds of contemporaneous animals with known feeding habits to the human/hominid analysis. Only in doing so will a valid reconstruction of the average daily dietary composition in terms of animal derived and vegetal components and a comparison of dietary behaviour between fossil finds from different time levels and localities be possible. Another limitation of the method is that due to the biological half-life of collagen molecules, isotopic ratios reflect the average long-term ingestion of certain food components. Any marked seasonal dietary pattern will therefore be obscured because δ-values reflect the average year-round diet.

In temperate Europe, C_4-plants are not abundant, therefore palaeodietary studies focus on the trophic level determination by stable nitrogen isotopic ratios. During the Palaeolithic, humans are assumed to have lived as nomadic foragers with a pronounced hunting economy. Isotopic data available from a few Neanderthal finds confirm a "carnivorous" diet by preferential consumption of herbivores from open environments, despite accompanying animal bone analyses revealed that environmental conditions varied considerably with time from temperate and partly forested (Scladina Cave, layer 4, 120,000–80,000 BP; Bocherens *et al.* 1999) to a cold steppe (Marillac site, 45,000 BP; Bocherens 1997). Such a hunting economy still holds for the Mesolithic finds from the Ofnet Cave in Germany (Bocherens *et al.* 1997): In this case, the skeletal finds represent a small population where men, women and children obviously came to death by violence. The conspicuously low variability of the individual $\delta^{15}N$-values of the adults suggest that all individuals belonged to a single tribe and had been killed during a single event. For any omnivore species, the term "carnivorous diet" should be used with caution since no linear relationship exists between the percentage of meat or animal food and collagen $\delta^{15}N$. When human data fall into the range of δ-values from carnivores like wolf and fox, the correct interpretation is a hunting economy with a hunting success sufficient to leave the "meat signal" in bone

collagen. The only "carnivorous" humans are suckling babies. As expected, the youngest children among the Ofnet Cave finds were characterized by the highest $\delta^{15}N$-values, strongly indicating prolonged breastfeeding (Bocherens *et al.* 1997). In pre-Neolithic times, nitrogen isotopic analyses thus permit a firm reconstruction of weaning practices since milk from domestic animals as a substitute for mother's milk was not yet available. For prehistoric and historic times where skeletal finds are representative for a whole population, a monitoring of age-specific stable nitrogen isotope ratios in children's skeletons permit the reconstruction of weaning age and thus important aspects of child care (Dittmann, Grupe 2000).

While stable isotope analysis confirm the assumed hunting economy in the Palaeolithic, the more interesting results are the reconstructions of past ecological niches in terms of which animals were hunted and which were not. For the Neanderthals mentioned above, collagen isotopic ratios were consistent with the consumption of herbivores from open environments. In a recent study of only five Upper Palaeolithic human skeletons from Gough's and Sun Hole Caves in Great Britain, Richards *et al.* (2000) were able to demonstrate by accompanying animal bone analyses that the hunters obviously mainly lived from bovids and deer rather than from horses. Such data are likely to give clues to past human impact on the local fauna.

It is impossible to talk about collagen stable isotope analysis without addressing the issue of decomposition. Although collagen is protected by the bone mineral matrix and thus capable of surviving very long inhumation periods, extractable collagen is usually degraded to a larger or lesser degree. Among the various laboratory protocols, gelatinization is generally recommended for the purification of matrix collagen from bone (cf. Bocherens *et al.* 1997). For any isotopic study, however, a major prerequisite is the testing of the preserved collagen's integrity, e.g. by amino acid analysis or C/N ratios. Experimental collagen decomposition confirmed that bacterial biodegradation is capable of significantly shifting both $\delta^{13}C$ and $\delta^{15}N$ values (Balzer *et al.* 1997). Especially $\delta^{15}N$ can be diagenetically enriched up to +5.8‰ due to ^{15}N enrichment of the substrate in the course of peptide bond cleavage. Such decomposition artifacts mimic a "trophic level effect", and a "carnivorous diet" would be falsely ascribed to the find under study simply because of bad collagen preservation.

Reconstructing palaeodiet and palaeoenvironments from enamel structural carbonate

What is to do when collagen is badly preserved and likely to yield erroneous isotopic data, or is not preserved at all? Alternatively, the measurement of biogenic $\delta^{13}C$-values from structural carbonate within the apatite crystals of dental enamel has been suggested as a means for palaeodietary reconstruction from fossils. Diagenetically added carbonates are troublesome especially for bone, but should be far less a problem for dental enamel. Since the structural carbonate fraction does not contain any nitrogen, a monitoring of trophic level effects is no more possible. Also, the information hidden in carbonate $\delta^{13}C$-values is different from collagen isotopic ratios: While the carbon in collagen molecules is largely derived from the ingested proteins and may substantially underestimate the isotopic composition of non-protein nutrients, carbonate carbon is more or less extracted from all dietary components and therefore also reflects the energy component of the diet (fats, carbohydrates; Ambrose, Norr 1993). As a result, $\delta^{13}C_{carbonate}$ should be enriched by +12‰ compared to the diet and the spacing between $\delta^{13}C_{collagen}$ and $\delta^{13}C_{carbonate}$ should hence average 7‰. The situation becomes more complicated when an organism consumes C_3-protein but C_4-energy components and *vice versa* (Ambrose, Norr 1993). With this in mind, and fossil finds from a locality where both C_3- and C_4-plants are abundant, carbonate $\delta^{13}C$ can give clues to very important palaeoenvironmental parameters.

Tropical Africa is such a region and of course of uttermost interest for palaeoanthropology. The radiation of the genus *Australopithecus* should have led to the occupation of diverse ecological niches by the various species. The specialized masticatory system of the robust forms in terms of dentition, biomechanics and microwear led to the assumption that *Paranthropus* was a vegetarian specialized on hard food. To test this dietary hypothesis, Lee-Thorp *et al.* (1994) analyzed $\delta^{13}C$ in tooth enamel from eight *Paranthropus robustus* specimens from Swartkrans (members 1–3, 1.8–1.0 million years) in comparison with associated faunal remains. The mean hominid $\delta^{13}C$-value of –8.48‰ was interpreted as indicative of a predominant C_3-food with a C_4 contribution of about 25–30%. This interpretation was especially supported by the clear separation of *Papio* and *Theropithecus* fossils from Swartkrans Cave, member 1, by their enamel $\delta^{13}C$-values: While *Papio* specimens were depleted in ^{13}C (range –12.3 till –10.1‰) because of their C_3-diet, *Theropithecus* specimens were enriched with the heavy isotope (range –4.0 till +0.4‰) and thus exhibit a graminivorous C_4-dietary pattern. Since a C_4-signature in $\delta^{13}C$ is not only due to direct ingestion of C_4-plants but also due to feeding on herbivorous grazers, Lee-Thorp *et al.* (1994) conclude that according to the stable isotopic data, *Paranthropus* from Swartkrans was not a specialist frugivorous or graminivorous feeder, but rather an omnivore – an interpretation much in line with the "expensive tissue hypothesis" for encephalized hominids. Less convincing due to small sample size and the nature of trace element analyses (see above), but also with a clear tendency to a more omnivorous dietary behaviour of another eight *Paranthropus* specimens from Swartkrans, member 1, are bone Sr/Ca data established from these fossils by Sillen (1992, Sillen *et al.* 1995). At least at Swartkrans, *Paranthropus* was probably specialized with regard to its vegetal dietary component, but not a specialized vegetarian.

Since carbonate carbon for analysis is basically obtained from bone as CO_2 after acid hydrolysis, it is rather convenient to also take a closer look at the isotopic composition of the oxygen ($\delta^{18}O$; $^{18}O/^{16}O$-ratio relative to a standard) liberated in the course of the same laboratory procedure. $\delta^{18}O$ serves as a "palaeothermometer" since enamel oxygen isotopic ratios are largely a function of the respective ratio in drinking water, which in turn depends from climatic conditions. For warm blooded animals, a rather complex monitoring of the oxygen influx (from drinking water, food and inhalation) and the oxygen efflux (in the form of urine, sweat, and exhaled vapour) is necessary to establish the correct $\delta^{18}O$-climate relationship (Luz, Kolodny 1989). Considerable species-specific differences do exist due to metabolic specifities, since some animals can tolerate water stress while others do not, some animals get most or all of their water from food which in turn exhibits variable $\delta^{18}O$-values (e.g. Sponheimer, Lee-Thorp 1999). Oxygen in bone or tooth mineral is available from the phosphate, the carbonate and also the hydroxyl component. Since the chemical bond between phosphorus and oxygen is stronger, many $\delta^{18}O$-analyses are made from the phosphate group to avoid *post mortem* contamination (methodological considerations e.g. by Stephan 2000). However, the fidelity of structural carbonate $\delta^{18}O$ for the reconstruction of palaeoenvironmental features has also frequently been demonstrated, and carbonate $\delta^{18}O$ analyses, preferentially combined with $\delta^{13}C$, permit the evaluation of important palaeoecological details. Besides the reconstruction of general climatic conditions, the $\delta^{18}O$ variation in animals with different feeding and drinking behaviour, the abundance of such species and hence also the vegetation type of a palaeoenvironment can be assessed. Respective investigations are straightforward again for the Swartkrans area (Sponheimer, Lee-Thorp 1999). Stable isotopic data from only nine animal fossils from the Middle Miocene, hominoid bearing Fort Ternan site in Kenya were sufficient to reject the hypothesis of a Serengeti-typed wooded grassland at that time and rather support the existence of a normal C_3-ecosystem (Cerling *et al.* 1997). Given the importance of ecological settings for hominid evolution, more such data are urgently needed.

Stable strontium isotopes in bone and enamel apatite

New perspectives for palaeoanthropological research are opened up by stable strontium isotope analysis from fossil apatite, which serves as a means for the reconstruction of individual residence chance and migration patterns. In contrast to light elements like C, N and O, Sr is a heavy element and a few mass units difference between its stable isotopes (^{88}Sr, ^{87}Sr, ^{86}Sr, ^{84}Sr) render isotopic fractionations negligible since they always remain lower than the measurement error. Strontium isotopic ratios are therefore not expressed in δ notation. While $^{88}Sr/^{86}Sr$- and $^{84}/^{86}Sr$-ratios are constant (Steiger, Jäger 1977), $^{87}Sr/^{86}Sr$-ratios are geochemically variable. Out of the four stable strontium isotopes, ^{87}Sr is a decay product of ^{87}Rb ($t_{1/2} = 48.8\times10^9$ years), thus the $^{87}Sr/^{86}Sr$-ratio is a function of initial Rb-content and age of rocks. Modern $^{87}Sr/^{86}Sr$ variation of the continental earth crust varies from 0.706 until 1.5 (Geyh, Schleicher 1990).

Given the prerequisite that fossils or skeletons under study are recovered from a geochemically variable area, isotopic differences in such mineralized tissues which had been precipitated at different ontogenetic stages of the respective individual are indicative of residence change. Appropriate tissue samples are dental enamel which is formed during childhood and no more remodelled, and compact bone, the strontium content of which had accumulated during the last few years prior to death. In case an adult skeletal individual exhibits significant differences in $^{87}Sr/^{86}Sr$ isotopic ratios between the enamel of the first permanent molar (enamel formation from shortly before birth until approximately 4 years of age) and compact femoral bone, this individual must have spent the early childhood in a region geochemically different from the site of its recovery. *Post mortem* contamination cannot account for such a difference, because any Sr contaminants must stem from the burial environment and hence are of the local isotopy. Another, however highly unlikely explanation for such a difference would be the import of non-local baby food. In case where compact bone Sr isotopy is different from the burial environment, this individual has either been a recent immigrant, or gathered its food from adjacent, geochemically distinct areas. But it is important to check whether any "local" strontium isotopic ratio is not a contamination artifact by the burial environment. This is preferentially done by an acid pre-treatment of bone analogous to sample processing for trace element analysis (Grupe 1992), accompanied by leaching experiments for the determination of appropriate etching conditions. Another prerequisite is that for the area under study, mobile strontium (in ground and surface water) has the same isotopic composition as the non-mobile strontium from soil. When all these parameters are met, stable strontium isotope ratios are meaningful indicators for residence change and migration on both the individual and the population level.

The research potential of this archaeometric approach was e.g. demonstrated by Grupe *et al.* (1997) in their investigation of Upper Neolithic Bell-Beaker skeletons from Southern Bavaria: Not only could 17.5–25% out of all skeletons with preserved 1st molar/bone sample pairs be identified as immigrants, indicating a considerable mobility of Bell-Beaker people. In addition, overall direction of migration as assumed by the distribution of archaeological Bell-Beaker finds could be confirmed, and the high number of immigrating females and a few cases of residence change during early childhood served as indicators for a migration pattern in small, preferentially family groups. In this example, archaeometric analysis of human skeletal finds was capable of solving the problem whether human artifacts which are recovered as grave goods were distributed by trade or by their manufacturers.

FIGURE 1. Geochemical diversity of Bavaria, and location of the Neuburg site at the bank of the Danube river.

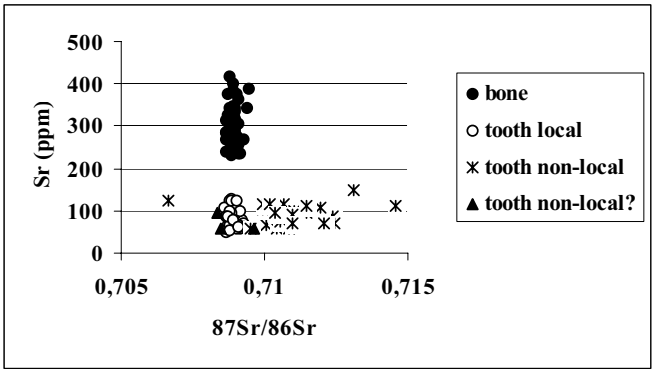

FIGURE 2. Distribution of Sr contents (ppm) and strontium isotopic ratios in bones and teeth from 70 skeletons recovered at the Neuburg site. Non-local individuals have tooth isotopic ratios which differ by more (or less) than 0.001 measurement units from the local (bone) isotopy at the site. Possible additional non-local individuals have tooth isotopic ratios differing by more than two standard deviations from the average local bone isotopic signature.

This definition of "who is who" among a skeletal population is of special importance for any open questions centering around probable migration events, population mixture with expectable consequences for population genetics, and population dynamics in the past. Recently established data concerning the population development in Southern Bavaria after the Roman occupation shall serve as an example for the high amount of information hidden in stable strontium isotope ratios, but also for their current limitations in terms of interpretation. The historical background of the study is the Roman occupation of the southern Bavarian area between the Alps and the Danube river (*Figure 1*) in the course of the 1st century AD, and the building of fortresses along the Limes Romanus at the Danube banks to defend the border. Grave goods in burial sites associated with these fortresses indicate that the respective crew was recruited from several Germanic tribes from various regions. The slow decline of the Roman Empire led to a subsequent withdrawal of Roman troops from the northern alpine provinces, and since 426 AD the remaining soldiers did receive no more salary. In 488 AD, the remaining Romans definitely left the province, and in 551 AD was the hitherto unknown tribe of the "Baiovarii" mentioned for the first time. Current archaeological theory claims that these "Baiovarii" were not a single tribe which settled in the area now devoid of Romans, but rather that the new tribe developed from a population mixture of the original Celtic inhabitants, remaining Germanic soldiers, and perhaps remaining Romans and other parts of the population of this former province (Fischer, Geisler 1988). Unfortunately, grave goods are not very common in such fortress burial sites: especially weapons, normally associated with male burials, were not buried but kept in use. The exact proportion of primarily non-resident males thus cannot be estimated archaeologically. Strontium isotope analyses from skeletons associated with fortress burial sites should give clues to how many people buried there had been immigrants, and where they did come from.

The investigation of 70 skeletons recovered at the burial site associated with the fortress at Neuburg/Donau, Bavaria, dated from 330 until 400 AD, together with the known geochemical variability in this area (*Figure 1*), was capable to support and further refine the current hypothesis of the post-Roman origin of the Bavarian population. Again, sample pairs consisting of the enamel of the first permanent molar and compact femoral bone from one and the same individual were each processed according to the method described by Grupe *et al.* (1997) and forwarded to mass spectrometry.

Since the few non-local grave goods recovered indicate an origin of their owners north-east from their later residence in the northern alpine region which is characterized by $^{87}Sr/^{86}Sr$-ratios between 0.708 and 0.709, their original residence place was assumed to have been the granitic area ($^{87}Sr/^{86}Sr$-ratio >0.710; cf. *Figure 1*). At least, they should have passed this area in the course of their migration and should reveal a mixed isotopic signature in case of individual death shortly after arrival in the Neuburg region. Consequently, early immigrants can be safely identified when their tooth $^{87}Sr/^{86}Sr$-ratios differ by at least 0.001 from their respective bone values. The information on possible origin by grave goods is strongly needed for a correct interpretation of data, since the large area north of the Danube, characterized as "non-uniform"

TABLE 1. Age at death, sex and strontium isotope ratios of individuals archaeometrically defined as immigrants (bold: possible additional immigrants, cf. text) to the Neuburg site.

Grave No.	Sex	Age at death	87Sr/86Sr tooth	87Sr/86Sr bone	Difference in Sr isotope ratios
3	female	young adult	0.713080	0.709443	0.003637
10	female	old adult	0.710532	0.708952	0.001580
12	female	yound adult	0.711048	0.708642	0.002406
13	female	old adult	0.710036	0.708925	0.001111
24	female	old adult	0.711694	0.708921	0.002773
26	male	young adult	0.714224	0.708683	0.005541
33	male	old adult	0.710452	0.708648	0.001804
37	female	juvenile	0.709516	0.708777	0.000739
40	male	old adult	0.709962	0.708953	0.001009
41	male	old adult	0.709962	0.708715	0.001247
42	male	old adult	0.706670	0.708931	–0.002261
44	male	young adult	0.712348	0.709264	0.003084
53	male	old adult	0.710534	0.708679	0.001855
62	male	adult	0.711468	0.709005	0.002463
68	male	young adult	0.710538	0.708793	0.001745
72	male	old adult	0.710684	0.708824	0.001112
74	male	young adult	0.710930	0.709157	0.001773
78	male	young adult	0.710160	0.709094	0.001066
80	?	old adult	0.711024	0.708954	0.002070
90	female	old adult	0.710946	0.708996	0.001950
92	male	old adult	0.712458	0.709068	0.003390
95	male	old adult	0.711945	0.708956	0.002989
96	male	old adult	0.710863	0.708856	0.002007
107	female	young adult	0.711456	0.709013	0.002443
109	female	young adult	0.712043	0.708846	0.003197
116	female	young adult	0.710354	0.708944	0.001410
126	female	old adult	0.710956	0.708926	0.002030
15	**male**	**old adult**	**0.709624**	**0.708836**	**0.000788**
16	**male**	**juvenile**	**0.708483**	**0.708833**	**–0.000350**
31	**male**	**old adult**	**0.708324**	**0.709004**	**–0.000680**

in *Figure 1*, is geochemically extremely variable on a very fine scale. Therefore, without the archaeological information, any individual origin from this non-uniform area cannot be excluded.

Figure 2 shows the distribution of bone and tooth strontium isotopic data in relation to the respective strontium concentrations. While first molar strontium contents are physiologically lower than bone strontium concentrations, a remarkable variability of strontium isotope ratios in teeth in contrast to the very narrow range found in the bone specimens, which all yield the local isotopic signature, is conspicuous. Out of the 70 skeletons analyzed, 26 had tooth isotopic ratios more than 0.001 units higher as opposed to their respective bone values (*Table 1*), another one had an unexpected low tooth value of 0.70667 only (grave No. 42). This latter ratio is consistent with a rather restricted locality further north-east of the granitic area, which is dominated by volcanic metagabbro (*Figure 1*). In sum, 27 out of 70 individuals were archaeometrically identified as immigrants (38.6%), in contrast to only 15 individuals (21.4%) with non-local grave goods.

But is this borderline of 0.001 measurement units, based solely on geochemical data, adequate? From a more biological point of view, any tooth strontium isotopic signature which deviates by more than two standard deviations from the average local signature should be considered significantly different (cf. Grupe *et al.* 1997). For the Neuburg site, all bone $^{87}Sr/^{86}Sr$-ratios are consistent with the local geochemical isotopy and average 0.708912 with a standard deviation of 0.000169 only. Hence, all individuals with a tooth isotopy higher than 0.70925 and lower than 0.708574 may also be considered as primarily non-locals which would add another 3 immigrants and raise the respective figure until 42.9% (*Table 1*). As illustrated in *Figure 2*, these three individuals fall right into the still not precisely defined "grey zone" between clear locals and non-locals. This aspect demonstrates the necessity to find reproducible means to unambiguously define residents from migrants and leads to the conclusion that by remaining on the safe side, the proportion of immigrants is likely to be underestimated. Nevertheless, the number of 38.6% geochemically defined immigrants supports a considerable amount of primarily non-local members of this population. It is noteworthy that more females had been mobile than males: Out of 18 adult females, 12 (66.7%) were

immigrants opposed to 16 out of 41 males (39.0%). Again, these numbers differ considerably from the archaeological data, since archaeometry revealed four additional non-local females and, not unexpected with regard to the paucity of grave goods in male burials, 11 additional males. In contrast, all subadult skeletons recovered at the site showed local isotopies in both enamel and bone sample.

While the high number of mobile females is most plausibly explained by exogamy, does the hypothesis hold that the foreign males had been soldiers recruited by the Romans to protect the Limes? At this stage of the analysis, nothing more can be said than that the non-locals had spent their early childhood distant from their place of residence during their last years of life. Information on the individual age of residence change can only be obtained by serial analysis of tooth enamel from an individual's dentition, since the crown formation of the different tooth types occurs at different ontogentic stages, covering approximately the first 14 years of life. A preliminary investigation of the dentition of four adult males (grave Nos. 26, 40, 42 and 72), which had been identified as immigrants by the deviation of their 1st molar to bone isotopic ratio, demonstrated a change in the isotopy at individual ages ranging from birth until approximately six years respectively, which challenges the hypothesis that non-locals had been hired as soldiers to defend the Roman border. At least these four individuals arrived in the northern alpine region as children and must have been already members of the local population before they entered the troops.

As for the analysis of stable isotope ratios from the light elements C, N and O, applications of the strontium isotope method on fossil remains are scarce, although the examples given here should have demonstrated the quality of information otherwise not extractable from the bone finds. The above mentioned palaeodietary study on *Paranthropus* remains from Swartkrans by Sillen *et al*. (1995) also included the establishment of $^{87}Sr/^{86}Sr$ in addition to Sr/Ca ratios. As far as the geochemical variability of the Swartkrans/Sterkfontein region is concerned, stable strontium isotope analysis should permit the reconstruction of home ranges of these extinct hominids. Interestingly, a mandible fragment of *Paranthropus* find SK 876 was relatively depleted in its bone strontium isotopy which was not consistent with the isotopy of the immediate region. Cautiously, the authors offer the interpretation that this individual may not have been native to the region of its recovery or may have derived its strontium from food with a different isotopic signature (Sillen *et al.* 1997). While for fossil specimens, large distance dislocation by taphonomic processes should also be taken into account as a third possible alternative, and the study mentioned above was carried out on skeletal fragments only and not of paired enamel/bone samples, it nevertheless clearly demonstrates that even single bone fragments alone may yield information with far-reaching implications on early hominid palaeoecology.

CONCLUDING REMARKS

The palaeoecological significance and hence the contribution to the reconstruction of evolutionary processes by stable isotope analysis from fossil and sub-fossil bone finds is out of question. To add a cautionary note, the reader unfamiliar with isotopic methods may believe that it is sufficient to carry out a more or less complicated laboratory protocol, establish reproducible data and simply compare the figures obtained for the various specimens, look for significant differences and choose an interpretation. Besides *post mortem* decomposition and fossilisation which can substantially bias the original biological signal and may well lead to completely erroneous data (e.g. Balzer *et al.* 1997), a vast amount of background information necessary for a correct deciphering of the biological signal hidden in stable isotope compositions is still lacking. This accounts for fine scaled geochemical maps which are not available for every geographical region as well as for the distribution of stable isotopes throughout the diverse components of an ecosystem, let alone the different metabolic pathways an element will pass through when ingested by mammals with different feeding habits and metabolic demands. Past climatic conditions were very different from today's, and so may have been past hunter-prey relationships. It is this routing of stable isotopic meanings which renders their interpretation very complex. Moreover, astonishingly little is known on physiological bone turnover rates and how quickly a change in diet manifests itself in collagen or mineral isotopic ratios. Efforts for a more fine-scaled tuning of stable isotope information in contrast to the reconstruction of long-term events are on their way (e.g. Wiedemann *et al.* 1999), but are likely to open up more problems than insights at the present state of research. However, the review presented here should demonstrate that this background information is stepwise obtained, either by analysis of fossil animal remains, or experimentally. Since modern mass spectrometry requires small sample sizes only, further research including valuable fossil specimens should be encouraged.

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