Chapter 18

NON-DESTRUCTIVE DETERMINATION OF ^{87}SR/^{86}SR ISOTOPE RATIOS IN EARLY UPPER PALEOLITHIC HUMAN TEETH FROM THE MLADEČ CAVES – PRELIMINARY RESULTS

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Introduction

Strontium isotope ratio analysis was applied successfully within the last decade as one method of choice to reconstruct migration events in past human populations (Runia, 1985; Price et al., 1994, 2000; 2002; Grupe et al., 1997; Ezzo et al., 1997; 2002; Latkoczy et al., 1998; Sillen et al., 1998; Budd et al., 2000; Teschler-Nicola et al., 1999; 2001; Chiaradia et al., 2003; Bentley et al., 2002; Kutschera and Müller 2003; Schweissing and Grupe, 2003; Hodell et al., 2004). ^{87}Sr/^{86}Sr ratios are functions of the local environment. Sr incorporation in human bones and dentine undergoes a periodical turnover, since these tissues equilibrate with the individual metabolism. Therefore a later stage of an individual's life is recorded herein. Dental enamel, on the other hand, does not undergo a turnover and can be considered an "archive of childhood" (Grupe et al., 1997; Lee et al., 1999), since it is formed during the early years of the life of an individual (see Hillson, 1996). Under favorable preservation conditions a significant difference in the Sr isotope signature between enamel and dentine (or bone) can point to a residential change of an individual (Lee et al., 1999; Beard and Johnson, 2000). But the preservation status of human skeletal remains, diagenetic changes or contamination have to be taken into consideration in order to avoid conclusions drawn from artifacts (Sillen, 1986; Sillen and Le Geros, 1991; Budd et al., 2000; Latkoczy et al., 2001; Prohaska et al., 2002; Chiaradia et al., 2003; Trickett et al., 2003). Most of the original biogenic Sr is preserved in enamel (Lee-Thorp and Sponheimer, 2003). Investigations of solubility distributions of hydroxyapatite lead to the same conclusion, namely that biogenic Sr is sufficiently conserved in enamel over long term periods (Shellis and Wilson, 2004). This is an important prerequisite for the present pilot study of Sr-isotope ratios of the Mladeč Upper Paleolithic specimen.

The strontium isotope ratio technique has rarely been used for identifying locals and non-locals in the Paleolithic (Sillen et al., 1995). So far, most investigations have focused on tooth/bone pair analysis of prehistoric and historic populations (see above). One of the reasons might be that the common technologies for the determination of Sr-isotopes have been of an invasive nature. For the present pilot study, we applied the laser ablation technique (Prohaska et al., 2002) in combination with a multi collector inductively coupled plasma mass spectrometer (LA-MC-ICP-MS). The coupling of laser ablation enhances the isotope ratio analysis capabilities of ICP-MS by introducing a direct solid sampling system with high spatial resolution (spot diameter down to a few µm), which enables direct "quasi non-destructive" isotope ratio analysis of solid surfaces. Single collector ICP-MS in combination with laser ablation has so far been limited for its achievable total combined uncertainties on the final ratio (Prohaska et al., 2002). MC-ICP-MS instruments allow the assessment of Sr isotope ratios with an improved instrumental precision up to three orders of magnitude. Several publications report the capabilities of resolving Sr isotopic variations within different samples on a

10–100 µm scale by LA-MC-ICP-MS (Bizzarro et al., 2003; Schmidberger et al., 2003; Ramos et al., 2004) and a correction for systematic bias which has to be taken into account (Waight et al., 2002). Laser ablation in combination with MC-ICP-MS is therefore the method of choice for direct isotope ratio determination of valuable human remains.

Within this study, we analyzed ^{87}S/^{86}Sr isotope ratios of small teeth fragments of the most representative specimens of Mladeč 1, Mladeč 2 and Mladeč 8. The results contribute to shed light on whether early modern humans of Mladeč changed their residence or stayed as autochthones in this area.

Material and site description

Material

Analysis was performed on teeth of individuals Mladeč 1, Mladeč 2 and Mladeč 8 (Table 1). The samples were excavated by Josef Szombathy at the end of the 19th century and have since then been inventorised in the osteological collection of the Anthropological Department at the Naturhistorisches Museum Wien (Szombathy, 1925). We used fragments of about 1–3 mm^3 of enamel and/or dentine. The majority of the fragments were taken in the course of the radiocarbon dating procedure carried out recently (Wild et al., 2005).

From the specimen of Mladeč 1, most probably a female, we used a small section of the right maxillary second molar (M^2), consisting mainly of enamel. Because of the insufficiently preserved dentine in this fragment, we also took 2 mm^3 of dentine from the partly preserved left first maxillary premolar (P^3). In the latter, the tooth crown is more or less completely destroyed post mortally and dentine is exposed in the whole area. We had to follow curatorial concerns, since all other teeth are well and completely preserved or restored.

In case of Mladeč 2, also a young female, an approximately 3 mm^3 sample was taken from the left third maxillary molar (M^3), containing both a very small enamel section as well as the enclosing dentine section.

From the Mladeč 8 specimen, a male individual, we took 2–2,5 mm^3 of dentine from the root of the left maxillary second molar (M^2). A further sample was taken from the right second maxillary incisor (I^2), which exhibited fracture cracks and minimal post mortem damage, allowing an accurate sampling of approximately 2 mm^3 of tooth material, mainly composed of dentine. The adjacent enamel was not adequate for subsequent analysis. Additional sampling of enamel has not be performed so far due to curatorial concerns.

All samples had fresh and uncontaminated cleavage areas, which were used for further LA-ICP-MS analysis.

Table 1. Specification of the enamel and dentine samples from Mladeč 1, Mladeč 2 and Mladeč 8 taken for LA-ICP-MS analysis of Sr-isotope ratios

Specimen no.	Sex	Sample material
Mladeč 1	Female	Enamel/right M^2
Mladeč 1	Female	Dentine/left P^3
Mladeč 2	Female	Enamel/left M^3
Mladeč 2	Female	Dentine/left M^3
Mladeč 8	Male	Dentine (1)/ right I^2
Mladeč 8	Male	Dentine (2)/ left M^2

Since the Mladeč Caves are located in an area of variable geology and unknown whole rock, soil and water Sr-isotope ratios, the local signal of Sr isotopes has first to be determined for the different potential Sr reservoirs. Within this pilot study, two drop water samples obtained from the "Witch Cave" and the "Virgin Cave" have been analyzed so far. The sampling sites are indicated in the map given in Fig. 1. It might be relevant that both samples are from dripping water; there is no running water within the cave system today.

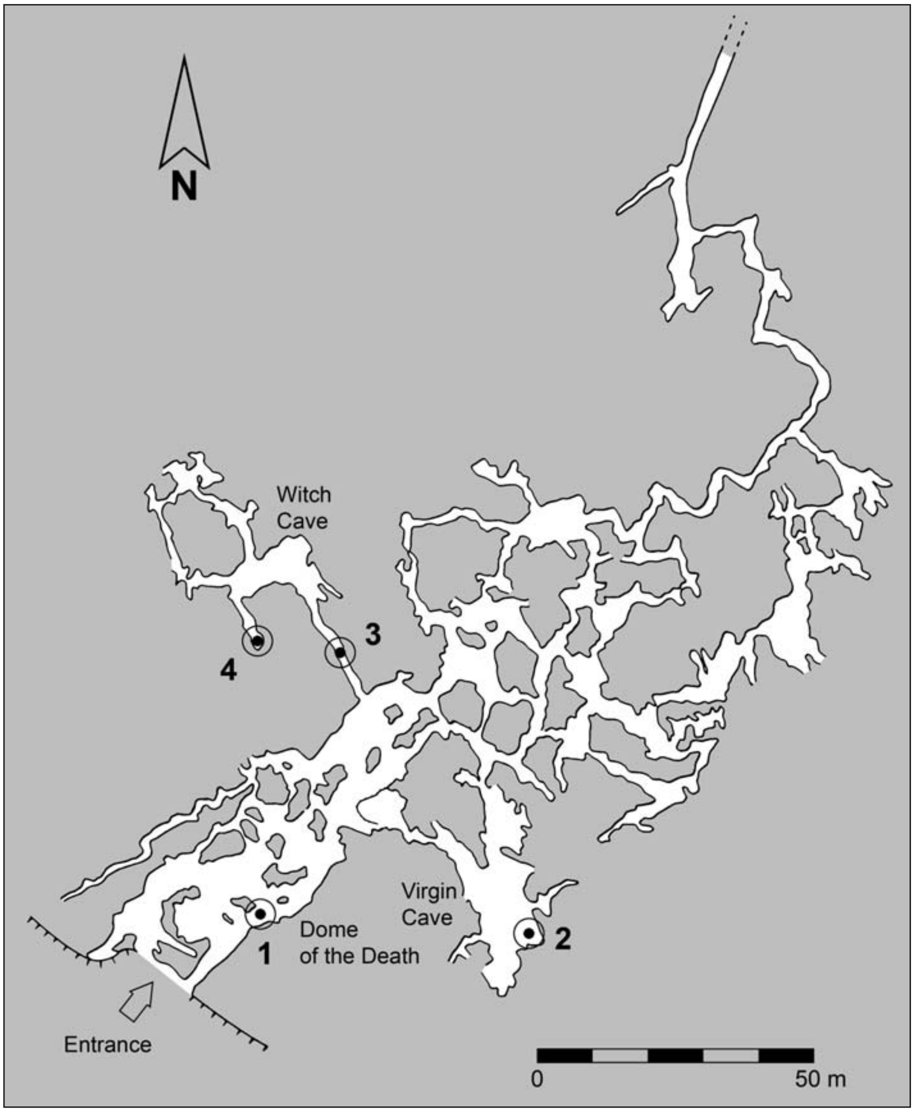

Fig. 1. Map of the Mladeč Cave system showing the sampling sites (2 and 4 = water samples; 1 and 3 = soil samples)

Geomorphology of the site

The Mladeč Caves have developed in a small area (2.4 x 1.5 km) of Devonian limestones near the town of Litovel (central/northern Moravia). They comprise an intricate maze of fissure passages, domes, chimneys filled with sediments and large boulders. From the geomorphological point of view, limestones form the Třesín hill are situated at the eastern margin of the Zábřeh Highland. The highest point (345 m) is more than 105 m above the large flat fluvial plain of the river Morava and its branches (the Upper Moravian Basin). The limestone area represents a fault block surrounded by Lower Carboniferous siliciclastic rocks (graywackes, siltstones, shales). The Devonian and Carboniferous rocks are sunken in the East along a NW – SE tending fault and are covered by fluvial Holocene sandy clays, by Pleistocene sandy gravels and by Pliocene varied sandy clays. Especially the southern slopes of the Třesín hill including the entries to the caves were covered by thick banks of loess prevalently in the Late Pleistocene (Würm). The loess and older laterite products of limestone weathering were washed into the caves through chimneys and fissures, together with limestone fragments. Sedimentary filling was formed up to 3–4 m in thickness and alternates with layers of calcite travertine in several places.

Devonian limestone

The light-gray massive Devonian limestones of the Mladeč Karst were slightly metamorphosed and recrystalized during the Variscan orogeny. General lithological qualities and one isolated fossil finding allow to compare them with the Vilémovice limestones of the classical Moravian Karst development. As a consequence, they can be considered to be uppermost Devonian (Frasnian, roughly 385–375 My). Their thickness is estimated to about several hundred meters. Štelcl and Zimák (1999) reported very pure limestones (98–99% $CaCO_3$) with low content of $MgCO_3$ (0.52–0.73%) and $FeCO_3$ (up to 0.1%). $MnCO_3$ occurs only in traces and the insoluble part forms 0.27–0.65%.

Lower Carboniferous siliciclastics

The nearest Lower Carboniferous siliciclastics occur about 2 km west or 1 km south of the Mladeč Caves. They consist of graywackes in alteration with siltstones and shales. According to the study of heavy mineral assemblages in the graywackes, they are assumed to be a continuation of the Protivanov Formation in the Drahany Upland (Otava, 1997). In some places Lower Carboniferous shales are folded into the Devonian limestones.

Würmian loess

The Würmian loess forms a thick sedimentary cover of up to 15 meters on the Devonian limestones just around the Mladeč Caves. It is distinctly calcareous, partly with lime nodules.

Analytical setup

Analysis of tooth samples was performed at the test site of NU Instruments by using a NU plasma multi collector inductively coupled plasma mass spectrometer (MC-ICP-MS) (NU Instruments Ltd., Wrexham, UK) in combination with a 213 nm UV laser (New Wave Research Co. Ltd., USA). Laser ablation and MC-ICP-MS parameters are summarized in Table 2.

Five replicate single spot analyses using a beam diameter of 100 µm were performed on each sample. Laser parameters were adjusted in order to obtain a constant signal of about 2 minutes per shot. Data evaluation was performed on the transient signal after the Sr signal had reached a stable

Table 2. Laser ablation and MC-ICP-MS instrumentation parameters

MC-ICP-MS and laser ablation parameters	
RF Power	1300 W
Argon gas flow rates	
Cooling	13 L/min
Auxiliary	0.35 L/min
Cones	Ni
Collector arrangement	
Sr	^{89}Sr:H5 ^{88}Sr:H4 ^{87}Sr:H2 ^{86}Sr:Ax ^{84}Sr:L3
Rb	^{85}Rb:L2
Kr	^{83}Kr:L4 ^{82}Kr:L5
Data acquisition mode	Time resolved analysis (TRA)
Dwell time	0.2 sec
Laser ablation parameters	
Wavelength	213 nm
Beam diameter	100 µm
Ablation mode	Single spot
Pulse energy	75% (≈ 27,5 J/cm^2)
Repetition rate	10 Hz
Ablation gas flow rates	
Mixing gas 1 He	0.7 L/min
Mixing gas 2 Ar	0.5 L/min

maximum. All intensities were corrected for blank including a Kr correction. Therefore, a gas blank from the purging gas of the ablation cell was measured for 20 seconds prior to each ablation. ^{87}Sr was corrected for minor ^{87}Rb interferences via the ^{85}Rb signal. The ^{87}Rb contribution was calculated via the ^{85}Rb-signal intensity by using the natural abundances as recommended by the IUPAC (Coplen, 2001). All raw ratios were corrected for mass bias using the ^{88}Sr/^{86}Sr signal applying a power law mass bias correction. The total combined uncertainty (calculated via propagation of errors to the final result) was 0.04% RSU for ^{87}Sr/^{86}Sr.

The Sr isotopic composition of the drop water sample from the “Witch Cave” and the “Virgin Cave” of the Mladeč Cave system was analyzed by static MC-TIMS (Triton, Finnigan, Bremen, Germany) after complete Rb/Sr separation and purification using conventional ion exchange procedures (Klötzli et al., 2001).

Results and discussion

In this pilot study, we obtained a stable distribution of LA-ICP-MS data within the investigated areas, which covered a field of approximately 2 mm^2. The final mean ^{87}Sr/^{86}Sr ratios, polled from five individual/replicate spot analyses, are summarized in Table 3 and Fig. 2.

The results show a noticeable variability: Mladeč 1 reveals a statistically significant difference between the isotope ratios of dentine of the first left premolar (0.7108) and the enamel (0.7119) of the second right molar. The latter represents the highest value within the Mladeč samples. Mladeč 2 possesses a similar pattern: The Sr-isotope ratio of the enamel of the third left molar (0.7113) is statistically significantly higher than the ratio obtained from dentine of the same tooth (0.7106). The Mladeč 8 dentine samples of the second permanent incisor (0.7104) and the left second molar (0.7106) show

Table 3. $^{86}Sr/^{87}Sr$ isotope ratios of enamel and dentine samples of individuals Mladeč 1, Mladeč 2 and Mladeč 8 (mean of five replicate analyses) and the water samples of the "Witch Cave" and the "Virgin Cave"

Individual	Tooth	Tissue	Analytical technique	$^{87}Sr/^{86}Sr$	SU
Mladeč 1	right M^2	Enamel	LA-MC-ICP-MS	0.7119	0.0003
Mladeč 1	left P^3	Dentine	LA-MC-ICP-MS	0.7108	0.0003
Mladeč 2	left M^3	Enamel	LA-MC-ICP-MS	0.7113	0.0003
Mladeč 2	left M^3	Dentine	LA-MC-ICP-MS	0.7106	0.0003
Mladeč 8	right I^2	Dentine (1)	LA-MC-ICP-MS	0.7104	0.0003
Mladeč 8	left M^2	Dentine (2)	LA-MC-ICP-MS	0.7106	0.0003
Sample name	**Cave**	**Type**			
Mladeč W01	Witch Cave	cave water	TIMS	0.710545	0.000003
Mladeč W02	Virgin Cave	cave water	TIMS	0.71056	0.00008

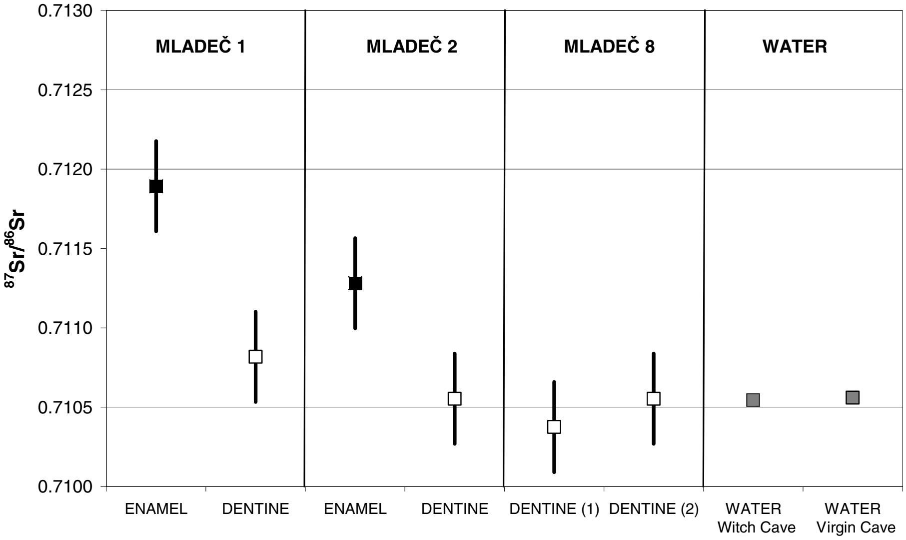

Fig. 2. $^{86}Sr/^{87}Sr$ isotope ratios of enamel and dentine samples of Mladeč 1, Mladeč 2 and Mladeč 8 and the water samples of the "Witch Cave" and the "Virgin Cave"

no intra-individual variation and exhibit nearly identical, very low Sr isotope ratios which are consistent with the dentine ratios of the other Mladeč specimen, in particular Mladeč 2.

The Mladeč 1 and Mladeč 2 specimens show an analogue Sr isotope ratio pattern, namely a higher ratio in the enamel samples and a statistically significantly lower ratio in the dentine (Fig. 2). According to theoretical considerations and the examined potential of the Sr isotope ratio technique for the study of prehistoric migration (Price et al., 2001; 2004; Bentley et al., 2002; 2003; 2004), it seems plausible at first glance that both individuals could represent non-locals. But one of the problems arising in such investigation is the determination of the local Sr reservoirs and the corresponding Sr isotope ratio signals as a necessary prerequisite for distinguishing migrants from locals. Although it has been suggested to investigate local animals for that purpose (Price et al., 2002), the study of Neolithic animal teeth by Bentley et al. (2003) demonstrated that this approach cannot solve the problem in a satisfactory way. Moreover, we do not have a sufficient number of recommended 'ideal' faunal remains within the preserved fossil record of the Mladeč Caves. In particular, teeth of stationary animals are missing (see Pacher, this volume, chap. 6). Another problem is due to the fact that the Mladeč Caves are situated in an area where Devonian limestone, Lower Carboniferous siliciclastics and Würmian Loess occur. Since Sr isotope ratio mapping of that area does not exist, we have used as proxy one water sample from the "Witch Cave", located in an cave area which is not accessible to the general public. The second water sample is from the 'Virgin Cave' of the Mladeč Cave, where public access is allowed (nonetheless, this fact did not influence the Sr isotopic composition, even if tourists are used to throw coins into the water). The Sr isotope ratios of the "Witch Cave" and the "Virgin Cave" water are 0.710545 and 0.71056, respectively. These values are in accordance with all dentine values of the investigated specimens. Moreover, one of the Mladeč 8 dentine samples from the right maxillary second incisor shows a (very low) Sr isotope ratio, which is practically identical to the value obtained in a single sample from the Bell Beaker period site Moravská Nová Ves, located in South Moravia on "Quaternary deposits of loess, fluviatile sediments and Cretaceous deposits". Price et al. (2004) reported a Sr ratio of about 0.7103 in the bone and 0.71009 in the enamel sample, "suggesting a locally born individual". The results given by Price at al. (2004) seem to underline the assumption that the dentine values of the Mladeč samples represent the local signal.

It is established that biogenic Sr is well preserved in enamel (Lee-Thorp and Sponheimer, 2003; Shellis and Wilson, 2004), whereas dentine or bone can exhibit remarkable diagenetic alterations. Therefore, even if the Sr isotope ratios of the dentine from the Mladeč samples correspond to the local signal, we cannot assume a priori that this Sr was incorporated *intra vitam*. Post mortem diagenetic alterations have to be taken into account as well. In this context it might be of interest that analytical scanning electron microscopy (JEOL JSM6400 equipped with an energy dispersive system) of embedded small block preparates of several human postcranial elements, including Mladeč 20 (a rib fragment), Mladeč 25a (a right proximal radius) and Mladeč 28 (a left proximal femur) have provided evidence for the presence of calcite ($CaCO_3$) with minor amounts of Mg, Mn and Fe (see Teschler-Nicola, this volume, chap. 5). A similar composition was reported by Štelcl and Zimák (1999) for the almost pure limestone of the Mladeč Karst. Moreover, SiO_2, typically for siliciclastics, which represent a further component of the Mladeč geomorphology, was also detected in parts of the human bone block preparates.

Interestingly, the high enamel values obtained from the Mladeč1 and 2 specimens correspond to ratios which Price et al. (2004) determined for individuals recovered from Velke Prilepy, a multi-component site in Bohemia located more north and westward (Price et al. 2004).

In sum, the results of our non-invasive Strontium isotope ratio investigation on the early Upper Paleolithic specimens from the Maldeč Cave could provide first direct measures of the assumed mobility in hunter-gatherer populations. But they must be seen as preliminary: not only because of the insufficient number of samples, but also because of our inadequate knowledge on the variability of the local bedrock and water signals, the sparse reference dates, specifically the lack of supra-regional Sr isotope ratio signals, and the problems arising from diagenetic alterations in fossil human remains.

Future investigations must include further samples of the local site, water samples of the immediate vicinity, faunal remains unearthed from the cave as well as enamel and dentine samples of two other human tooth samples (Mladeč 8, Mladeč 9b). The nature and degree of post mortem alterations will be of additional concern. Referring to the highly valuable material, it will not be possible to solve this question by using leaching procedures and therewith differentiate biogenic and geogenic apatite in the nearest future. In this context, multi-elemental pattern analysis might be of interest. For this reason, we are currently investigating the elemental distribution and diffusion profiles of major and trace elements in tooth tissues in comparison to the elemental pattern of the adjacent repository material.

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