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# MIDDLE PLEISTOCENE HUMAN AND ANIMAL MOBILITY AT ISERNIA LA PINETA: A STRONTIUM AND OXYGEN ISOTOPE PERSPECTIVE

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ABSTRACT: In this work, we explored the isotopic composition of faunal (rodents, rhinoceros and bison) and human skeletal remains from the Middle Pleistocene layers of Isernia la Pineta (Molise, Italy). We particularly focused on high spatial resolution isotope analyses of tooth enamel by laser ablation MC-ICP-MS for strontium isotopes and by micro-drilling sampling for oxygen isotopes. Results from bone specimens were compared with the isotope variability of modern plants collected in the area surrounding the site, in a radius of about 30 km. While the human group seems local, macro-mammals show a higher degree of mobility.

KEYWORDS: Isotopes, middle Pleistocene, deciduous tooth, strontium isotopes, laser ablation, oxygen isotopes

#### 1. INTRODUCTION

In recent years, the use of isotope ratios (e.g.  $^{87}{\rm Sr}/^{86}{\rm Sr}$  and  $\delta^{18}{\rm O})$  to decrypt past human and animal migratory patterns has been an important tool in archaeological and paleontological research. In particular, the discovery and significance of natural variations in the isotopic composition of strontium in biological and inorganic materials, has made Sr isotopes one of the best geochemical indicators for provenance studies (Bentley, 2006). As an alkaline earth metal with chemical-physical properties similar to those of calcium, strontium is commonly found in natural calcium carbonate (e.g. shells and speleothems) and phosphate (e.g. bones and teeth). The ratio between the isotopes 8 and 86Sr can be employed as a provenance marker typically deriving from a precise geological area depending on the age and on petrochemical features of the local rocks. Thus, Sr enters the ecosystem reaching plants and animals, mainly through water, following erosion and weathering of the bedrock. Sr is mainly fixed in the hydroxyapatite of teeth and bone tissue substituting calcium, with concentrations ranging between c.a. 50 to 1000 ppm. In this sense, tooth enamel becomes the best target tissue to perform Sr isotope analysis. This is because of two different reasons. First, being a ~96% mineralized tissue (mainly hydroxyapatite Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub>), enamel is guite resistant to diagenetic alterations, usually preserving the biogenic isotopic signature of the individuals. Second, enamel forms during childhood and does not remodel during the life of the individual, therefore, its Sr isotopic composition should reflect the signature of the place where the individual has spent its youth. This makes teeth the perfect target to unravel human/animal migrations (Lugli et al., 2017a).

Similarly, oxygen is fixed within carbonate and phosphate groups of the hydroxyapatite. The  $\delta^{18}\text{O}$  composition of tooth enamel reflects the isotope composition of the body water, which, in turn, varies linearly with the local meteoric water. Consequently, the  $\delta^{18}\text{O}$  value of skeletal remains is an important tool the decrypt the palaeoclimatic proxies, but also to track possible movements of an individual between places with different environmental conditions (Pellegrini et al., 2011).

In this work, we used isotope techniques to explore the mobility patterns of humans and animals from the Middle Pleistocene layers of Isernia la Pineta (Molise, Italy; Peretto et al., 2015). In particular, the most ancient human deciduous tooth ever found in Italy (~580 ka) years was studied employing the micro-destructivity offered by the laser ablation MC-ICP-MS. The mobility of the human was thus compared with macro-mammal (bison and rhinoceros) mobility patterns and with the local bioavailable Sr isotope composition obtained from the analysis of modern plants grown in areas away from Holocene contaminations and on and micro-mammals from the Middle Pleistocene layers of the site (i.e. rodents; Lugli et al., 2017b). Moreover, to get new insights

80 Lugli F. et al.

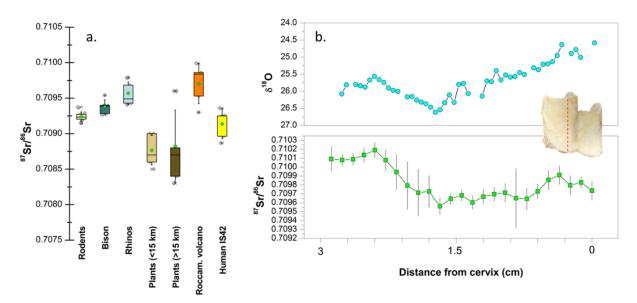


Fig. 1 - (a) Strontium isotope data from human, fauna and modern plant specimens of the Isernia La Pineta site and surroundings. (b) High spatial resolution analyses of strontium isotopes and oxygen isotopes of a single *S. hundsheimensis* tooth.

into the *Stephanorhinus hundsheimensis* migratory patterns and to unravel possible routes taken by this animal, we measured Sr and oxygen isotopes of the outer enamel surface in a high spatially resolved approach.

#### 2. MATERIAL AND METHODS

The <sup>87</sup>Sr/<sup>86</sup>Sr ratios of the IS42 human and of a Stephanorhinus hundsheimensis tooth were determined by LA-MC-ICP-MS technique. We sampled several portions (linear transects) of the external enamel surface. following a cervical-incisal direction (human enamel n = 5; rhino enamel n = 25). Sr data were acquired with a double focusing MC-ICP-MS Neptune™ (Thermo Fisher Scientific), coupled to a 213 nm Nd:YAG laser ablation system (New Wave Research™), housed at the Centro Interdipartimentale Grandi Strumenti (CIGS) of the University of Modena and Reggio Emilia. The detailed protocol is described in Lugli et al. (2017a). In order to maximize the ion beam size stability, we chose to use dynamic linear ablation (500 x 100 µm), which provides more precise results compared to static spot ablation. We employed a laser frequency of 10 Hz and an energy of ~20 J/cm<sup>2</sup>. The following masses were collected using nine Faraday detectors:  $^{88}\rm{Sr},~^{87}\rm{Sr},~86.5,~^{86}\rm{Sr},~^{85}\rm{Rb},~85.5,~^{84}\rm{Sr},~^{83}\rm{Kr},~^{82}\rm{Kr}.~A~60~s~background~signal~for~each$ mass was collected before the analysis to correct for Kr interferences. The presence of doubly-charged Dy, Yb and Er was negligible, also suggesting a low content of rare earth elements within the enamel tissue. Data were acquired in single blocks of 200 cycles, with an integration time of 0.5 s. To correct for the formation of  $^{40}\text{Ca}^{13}\text{P}^{16}\text{O}$  and  $^{40}\text{Ar}^{13}\text{P}^{16}\text{O}$ , we employed a set of inhouse bioapatite reference materials (a swine tooth, a bovine tooth and a shark tooth) building a calibration curve. The analysis of our human tooth in-house reference material (ROCht42) yielded an average (n = 10) LA  $^{87}$ Sr/ $^{86}$ Sr ratio of 0.70865  $\pm$  0.00040 (2 $\sigma$ ) and an accuracy of 0.005% (16 ppm) compared with the true value obtained by dissolution MC-ICP-MS of 0.70864  $\pm$  0.00001 (2se).

Faunal enamel samples and modern plant specimens were analyzed after chemical dissolution and chromatographic separation of Sr. Samples preparation follows the protocol presented in Lugli et al. (2017a). 5-10 mg of enamel and about 15 mg of plant ashes were digested in 1 ml of 14N HNO3 and re-dissolved in 3N HNO<sub>3</sub> after evaporation. The Sr separation uses columns with a ~300ul volume filled with Eichrom Sr specresin. Sr isotope ratios were determined using the MC-ICP-MS Neptune at the CIGS - UNIMORE. A [blank/ standard/blank/sample/blank] bracketing sequence was adopted to monitor any instrumental drift. Data were normalized through exponential law to a 88Sr/86Sr ratio of 8.375209. During this analytical session, the standard yielded a  $^{87}$ Sr/ $^{86}$ Sr ratio of 0.71025 ± 0.00002 (2 $\sigma$ ; n = 33). The Sr ratios were corrected for instrumental bias to a NBS-987 value of 0.71026 ± 0.00002.

High resolution (0.5 mm) sampling for stable isotope analysis was done on the outer enamel surface of a  $S.\ hundsheimensis$  tooth specimen (apex-cervix direction), using a micro-drilling system. Fifty-eight samples were obtained this way. The  $\delta^{18}O$  isotope composition was thus determined at the Max Planck Institute for Chemistry (Mainz, Germany) on a Thermo V mass spectrometer, equipped with a GasBench II preparation device and a cold trap. In this configuration, tooth enamel samples of <100 microgram (equivalent of <5 microgram CaCO3) can be measured with <0.15% precision (1SD).

### 3. RESULTS

The human tooth yielded an average  $^{87}$ Sr/ $^{86}$ Sr ratio of 0.70914 ±0.00038 (2 $\sigma$ ; 5 ablation lines). Rodent teeth, analyzed by dissolution MC-ICP-MS, showed an

average Sr isotope composition of 0.70924  $\pm 0.00013$  ( $2\sigma$ ; n =16). Seven bison teeth yielded  $^{87}$ Sr/ $^{86}$ Sr ratios ranging between 0.70927 and 0.70954 (average 0.70938  $\pm 0.00018$ ;  $2\sigma$ ; n =7). Rhinoceros teeth, measured by dissolution MC-ICPMS, showed an  $^{87}$ Sr/ $^{86}$ Sr ratio ranging from 0.70941 and 0.70979 (average 0.70958  $\pm 0.00030$ ;  $2\sigma$ ; n =9). One rhinoceros tooth was also analyzed by LA-MC-ICP-MS to explore its intratooth variability, yielding  $^{87}$ Sr/ $^{86}$ Sr ratios ranging between 0.71019 to 0.70956 (0.70982  $\pm 0.00038$ ;  $2\sigma$ ; n = 25). On the same tooth, high resolution oxygen isotope analyses yielded  $\delta^{18}$ O<sub>C</sub> (SMOV) values between 24.6 and 26.6%. These values were then converted in  $\delta^{18}$ O phosphate values and in  $\delta^{18}$ O water values using regression from Pellegrini et al (2011) and D'Angela & Longinelli (1990).

#### 4. DISCUSSION AND CONCLUSION

When compared with micro-mammals and local plants, the human Sr isotope composition falls within the supposed local isotopic variability (Fig. 1). This suggests that the human group from Isernia likely exploited local resources, within a limited supply area (c.a. 10-15 km), similarly to modern hunter-gatherer collectors (Lugli et al., 2017b). On the contrary, given that the Sr isotope composition of macro-mammals seems affected by a higher radiogenic Sr signature, we infer that both rhinos and bisons grazed on a different supply area. We suggest that the higher <sup>87</sup>Sr/<sup>86</sup>Sr ratio derives from the contamination with volcanic soils and are likely related to seasonal migrations in the south-west direction, toward the fertile grounds of the Roccamonfina volcano area (Conticelli et al., 2009). The high-resolution isotope analysis of a S. hundsheimensis tooth agrees with this interpretation. In fact, both Sr and O isotope ratios show clear fluctuations correlated with the growth of the tooth enamel within a period of ca. 400 days (Fig. 1). These variations can be related to seasonal movements between places with different oxygen and Sr isotope composition (e.g. from Isernia to Roccamonfina/Albani Hills and vice-versa).

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