

Isotope and microwear analyses on teeth of late Middle Pleistocene *Elephas antiquus* from the Rome area (La Polledrara, Casal de' Pazzi)

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SUMMARY: A multi-approach study was performed on molar teeth of late Middle Pleistocene *Elephas antiquus* from two sites in the Rome area (La Polledrara and Casal de' Pazzi). Different techniques, including SEM study of microwear tracks and stable isotope analyses (carbon, oxygen, and strontium) on tooth enamel, were applied in order to obtain independent information on paleodiet and crosscheck results. Strontium isotope composition ($^{87}\text{Sr}/^{86}\text{Sr}$) helps to define the geographical area where the two populations were living, which is confirmed to be limited to the volcanic province of Latium. Carbon isotope measurements on structural carbonate of biogenic apatite (enamel) suggest a mainly C₃ diet for the two families, La Polledrara being characterised by slightly higher $\delta^{13}\text{C}$ values than Casal de' Pazzi. Preliminary oxygen isotope analyses on enamel phosphate suggest a possible influence of some diagenetic processes on the analysed material, indicating the importance of comparing results from different techniques in order to have a reliable reconstruction, not biased by limits linked to one method. The microwear analysis highlights the need of a very large number of samples to reach reliable conclusions. Preliminary results are consistent with the hypothesis of prevalently browser to intermediate type of diet for late Middle-Pleistocene *Elephas antiquus* in the two sites, Casal de' Pazzi population having a slightly more accentuated grazing attitude. Carbon isotope results suggest drier condition for the La Polledrara population, comparing with Casal de' Pazzi population, which is consistent with published paleoenvironmental reconstruction for the Mediterranean area.

1. INTRODUCTION

The study of alimentary habits of extinct herbivorous gives valuable insights on paleovegetation, allowing paleoenvironmental reconstruction. Methods developed to carry on these researches are various; the most common is to study the isotope composition of vertebrates mineralised tissues, which reflect their diet. Isotopes on herbivorous provide information not only on isotope composition of environmental water and, indirectly, on climate (phosphate oxygen, Longinelli 1995), but also on

prevalent diet and, therefore, on typical local vegetation (carbon on structural carbonate in bio-apatite, Cerling & Harris 1999). In fact, plants are devised in two broad groups characterised by different photosynthetic pathways (C₃ and C₄) having different $\delta^{13}\text{C}$ values ($\delta^{13}\text{C}$ mean value -27‰ and -12‰ respectively): most tree and shrubs use a C₃ photosynthetic pathway, whereas most grasses use the C₄ pathway. Moreover, strontium isotope geochemistry may yield environmental information on the substratum the studied animals were leaving on. Land animals assume their Sr isotope

ratios from the underlying soils and bedrock: the comparison of the Sr isotope ratio measured in the mammal's skeletal remains with the ratios of the soils and bedrock where the fossils were found can be used to distinguish between resident and non-resident animals.

Microwear scars analysis on tooth enamel examined under the Scanning Electronic Microscope can also infer the diet-behaviour of extant-extinct mammals. Pioneering studies focused on the relationships between dental microwears patterns and different kinds of feeding. Microwear analysis on extinct mammals teeth furnish indication on mean alimentary habits, averaging seasonal dietary changes, usually related to important changes in molar microwear patterns, and changes in individual microwear features, which have a very short turnover time (formation/obliviation) of wear patterns. Despite the large amount of studies on elephants, microwear analyses of elephant enamel remain almost unknown.

The aim of this work is to study the paleodiet of Italian Middle Pleistocene *Elephas antiquus* comparing results from different methods in order to test their reliability.

2. MATERIALS AND METHODS

The molars of *Elephas antiquus* have been chosen from fauna assemblages whose structure was widely investigated, and for which hypotheses on environmental conditions have been proposed (Caloi *et al.* 1998). Studied samples come from the late Middle-Pleistocene sites of La Polledrara di Cecanibbio (Torre in Pietra Faunal Unit, Oxygen Isotope Stage 9) and Casal de' Pazzi (Vitinia Faunal Unit, Oxygen Isotope Stage 7) (Anzidei *et al.* 1999; Gliozi *et al.* 1997).

Enamel samples for isotope analyses were manually separated from the central part of molar M_3 teeth, mechanically cleaned from dentine using tungsten-carbide or diamond-impregnated rotary tools, and then finely ground with an agate mortar. Enamel powders for carbon and oxygen isotope analyses of structural carbonate of biogenic apatite were first reacted with NaOCl 2% for 24 h, followed

by reaction with 0.1 M acetic acid for 3 days (Koch *et al.* 1997), then washed with distilled water and dried at 40°C. The resulting enamel powder was further checked under microscope binocular for possible contamination. 150-250 mg of powder were reacted with phosphoric acid in vacuum at 25°C for three days. The cryogenically separated CO₂ was analysed on a Finnigan MAT 252 mass spectrometer. Precision of duplicates of structural carbonate is $\pm 0.1\text{‰}$ (mean standard deviation 1σ) for carbon isotope composition, and $\pm 0.2\text{‰}$ for oxygen isotope composition. The protocol outlined in Crowson & Showers (1991) and Lécuyer *et al.* (1993) was used for oxygen isotope analysis in enamel phosphate. The Ag₃PO₄ crystals were reacted with BrF₅ at c. 600°C for 15h; the oxygen obtained from the reaction was then converted to CO₂ by cycling over hot graphite in the presence of a Pt catalyst. The isotopic measurements were carried out by means of a Finnigan Delta S mass spectrometer. Standard deviation of measurements ranges from about ± 0.1 to $\pm 0.2\text{‰}$ (1σ). Isotopic ratios are reported relative to the isotopic standard PDB-1 for C and V-SMOW for O using the conventional d notation:

$$\delta = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$$

To avoid the diagenetic Sr, which can modify the ⁸⁷Sr/⁸⁶Sr ratios, samples were pre-treated with 1,0 N acetic acid and then washed with bi-distilled water. 100 mg of sample were dissolved in 2.5 N HCl, and Sr chemical separations were performed using standard ion-exchange chromatographic methods. The measured ratios were fractionaction-corrected to an ⁸⁶Sr/⁸⁸Sr value of 0.1194. Repeated analyses of NBS 987 Sr standard gave average ⁸⁷Sr/⁸⁶Sr of 0.71024 +/- 2. Standard deviations are expressed as 2σ on the means.

Microwear analysis were first performed on each enamel loop of two well preserved last lower molar (M_3), from the two sites, as a test to verify the microwear variability. On the base of this test, following analyses were performed only on the averaged-worn laminae. Microwear analysis procedures are described in detail in Capozza (2001).

3. RESULTS

Strontium isotope composition for both La Polledrara and Casal de' Pazzi samples ranges between 0.70983-0.71008, falling within the compositional range of pyroclastic volcanic rocks of the Rome surrounding (0.70980-0.71050, Federico *et al.* 1994).

Figure 1A shows a box plot of carbon isotope composition of the analysed structural carbonates. Fourteen samples, seven per site, for a total of thirty measurements were performed. $\delta^{13}\text{C}$ isotope values for La Polledrara range between $-10.8\text{\textperthousand}$ and $-9.7\text{\textperthousand}$ with a median carbon isotope value of $-10.6\text{\textperthousand}$, whereas Casal de' Pazzi samples has a median $\delta^{13}\text{C}$ value of $-11.9\text{\textperthousand}$ (between -13.7 and $-11.3\text{\textperthousand}$). A reconstruction of *E. antiquus* paleodiet is suggested in Figure 1B, using the carbon isotope fractionation between diet and bioapatite as proposed by

Cerling & Harris (1999). All analysed samples fall in the "C₃ dominated diet" field, although La Polledrara has a relatively ^{13}C -poorer diet than Casal de' Pazzi. Preliminary comparison between oxygen isotope ratios of structural carbonates and phosphates seems to indicate a possible influence of some diagenetic effects for carbonates (Iacumin *et al.* 1996) that need further investigation. For this reason oxygen isotope data are not be presented in this paper.

Molars from La Polledrara have scratches average values between 53.5 and 62.6 in percent (Fig. 2); mean scratch length is 200 μm . Scratches are generally thin, but long and deep tracks are also rarely presents. The molars from Casal de' Pazzi have scratches average values between 69.4 and 77.2 in percent (Fig. 2); they are close to each other, have a mean length ca 600 μm , and their trend is not always parallel. Two to three, sometimes up to four, different

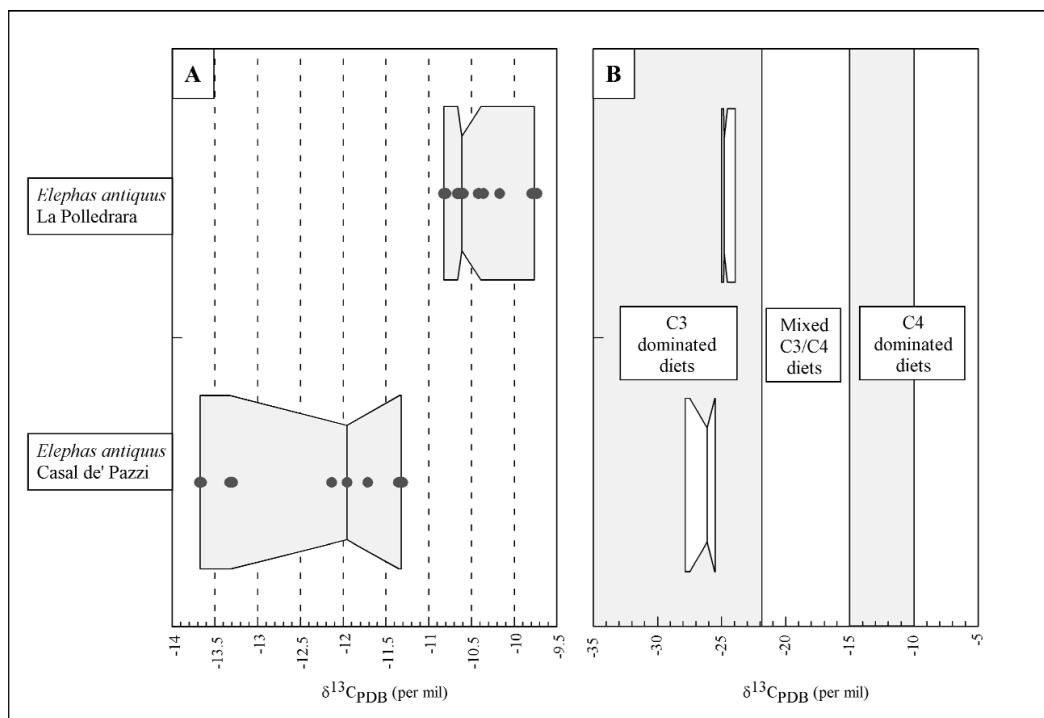


Fig.1 - A) Box plots of carbon isotope composition of analysed samples: dots represent each sample; the box contains all samples for each family; the notch represents the median value. B) Calculation of paleodiet for the two analysed family, following Cerling & Harris (1999).

generations of scratches are visible in the Casal de' Pazzi samples. Pits in both La Polledrara and Casal de' Pazzi samples are grouped and sometimes situated at the scratches cross. Mean microwear values of the averaged-worn laminae of the occlusal surface correspond to mean microwear values of the whole molar, allowing selecting the central part of the tooth for microwear analysis.

The variability ranges of the two molar samples used as test (molar 680 and molar 330) are distinct; average values of the other studied molars fall in these two distinct ranges, depending on the sites to which they belong.

Plotting the mean numbers of pits versus scratches, our *E. antiquus* specimens mainly fall in an area related to browsers and mixed feeders, as defined by Solounias & Moelleken (1992). The younger specimens from Casal de' Pazzi fall in the mixed feeders area, though

some lie in a transition zone towards the grazer feeders field.

4. DISCUSSION AND CONCLUSIONS

Results assess the difficulty to establish the dietary adaptation of fossil elephants only on the basis of one method. Strontium isotope composition ($^{87}\text{Sr}/^{86}\text{Sr}$) helps to define the geographical area where the two populations were living, which is confirmed to be limited to the volcanic province of Latium. Carbon isotope measurements on structural carbonate of biogenic apatite (enamel) suggest a mainly C₃ diet for the two families, La Polledrara being characterised by slightly higher $\delta^{13}\text{C}$ values than Casal de' Pazzi. Preliminary results of oxygen isotope analyses on enamel phosphate suggest a possible influence of some diagenetic processes on the analysed material, which need

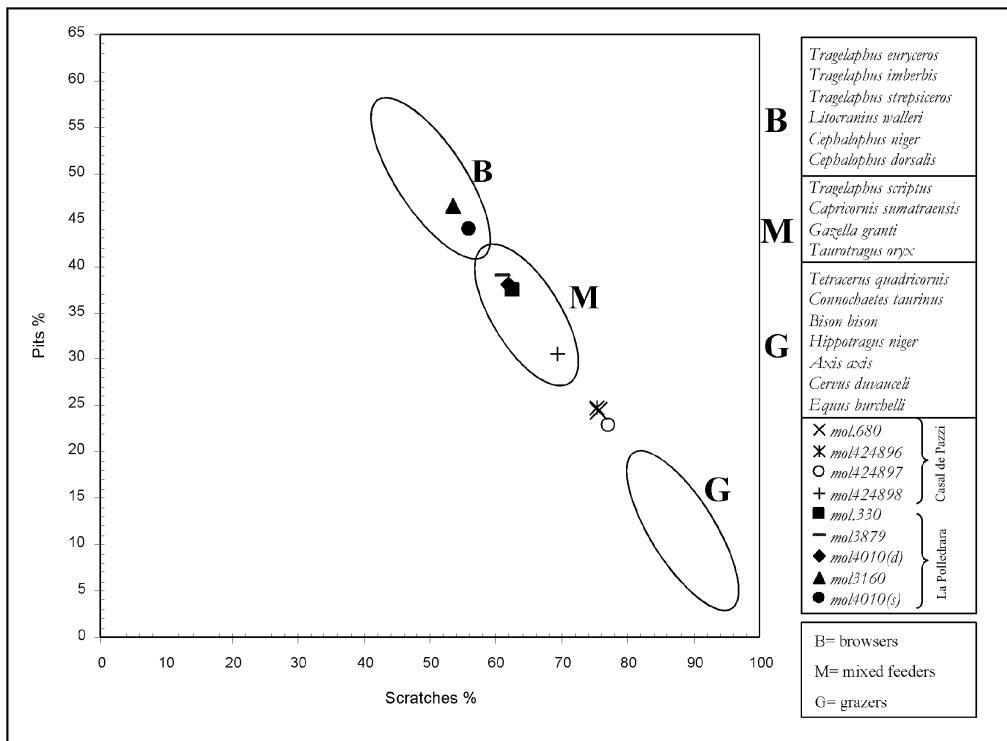


Fig.2 - Plot of microwear values of molars from La Polledrara and Casal de' Pazzi samples; diet subdivision are as proposed by Solounias & Moelleken (1992).

to be further investigated.

For what concern microwear tracks, reliable conclusions are possible only collecting a very large number of data; samples taken from the averaged-worn laminae of the occlusal surface seem to be the most representative of average microwear pattern of each molar. Microwear results fall in the variability field of mixed to grazer ruminants for Casal de' Pazzi, and browser to mixed feeders for La Polledrara.

Preliminary data resulting from our multidisciplinary study indicate interesting differences between the two analysed sites, consistent with the paleoclimatic reconstruction for the two interglacial periods (OIS 9 and 7, Vergnaud Grazzini *et al.* 1990) to which they belong. Late Middle-Pleistocene *Elephas antiquus* of Rome area had a prevalently browser/intermediate type of dietary adaptation. Microwear results suggest that the attitude to eat grass, graminaceous plants or vegetables containing a rather large amount of phytoliths increased in the Casal de' Pazzi group, which is attributed to the Oxygen Isotope stage 7, a more humid, maybe forested, interglacial climatic phase. This attitude of eating more grasses in humid period is also confirmed by research on living elephants (Eltringham 1992). The slightly enriched $\delta^{13}\text{C}$ values of La Polledrara are probably linked to the more arid climate of OIS 9, as C₃ plants tend to have higher carbon isotope composition in xeric climate (Cerling & Harris 1999).

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