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STABLE ISOTOPE (O, N, C) INTRA-SPECIFIC VARIATIONS IN *B. PRIMIGENIUS* SKELETAL REMAINS FROM A PLEISTOCENE CAVE SEQUENCE: A PROXY FOR DETAILED PALAEOENVIRONMENTAL RECONSTRUCTION

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RIASSUNTO - *Variazione intraspecifica degli isotopi stabili (O, N, C) in resti scheletrici appartenenti alla specie B. primigenius, provenienti da una grotta di età Pleistocenica: dati statistici da usare per ricostruzioni paleoambientali di dettaglio.*
Questa nota vuole essenzialmente fornire dati statistici sulle variazioni intraspecifiche della composizione isotopica dell'ossigeno, carbonio ed azoto in reperti scheletrici fossili provenienti da sequenze stratigraficamente ben definite. Questi dati potranno in seguito essere usati per valutare correttamente le variazioni isotopiche lungo sequenze temporali in termini di effettivi cambiamenti climatici ed ambientali. A questo scopo sono stati misurati 110 reperti scheletrici appartenenti alla specie *B. primigenius* provenienti dal giacimento della grotta Paglicci (Gargano, Puglia) riferibili ad un intervallo temporale compreso tra 32.600 e 13.350 anni BP. I dati hanno effettivamente fornito la variabilità della composizione isotopica del carbonio, azoto e ossigeno anche se in quest'ultimo caso i valori isotopici sono risultati troppo variabili per poter essere usati da un punto di vista statistico. I risultati hanno confermato le ipotesi formulate precedentemente sulle possibili variazioni ambientali durante il Pleistocene superiore nella zona presa in considerazione.

ABSTRACT – *Stable isotope (O, N, C) intra-specific variations in B. primigenius skeletal remains from a Pleistocene cave sequence: a proxy for detailed palaeoenvironmental reconstruction.*
Aim of this note is to provide proxy data for the intra-specific variation of the O, C and N isotopic composition in the case of fossil skeletal remains from well defined stratigraphical sequences. These data can be subsequently used to evaluate the isotopic variations along the temporal sequence in terms of climatic and environmental changes.
110 samples belonging to *B. primigenius* from the Paglicci cave (S. Italy) stratigraphical sequence dating from 32,600 to 13,350 yr BP were measured. The results obtained allowed the evaluation of the intra-specific isotope variability of the studied elements. However, the variability of the oxygen isotope values was too wide to allow their use for statistical considerations, likely because it was probably affected by diagenetic processes. However, the results obtained confirm earlier hypotheses about the environmental changes during Upper Pleistocene in the studied area.

Key-words: Intra-specific variation, stable isotopes, cave sequence, palaeonvironmental reconstruction.
Parole chiave: variazione intraspecifica, isotopi stabili, sequenza stratigrafica di grotta, ricostruzione paleoambientale.

1. INTRODUCTION

When detailed palaeoenvironmental studies are performed the availability of an adequate number of fossil samples, stratigraphically significant for the purpose, is of the utmost importance. This means that, when a stratigraphical sequence is considered, the isotopic composition of several samples from each layer should be measured or, at least, the intra-specific variability of the isotopic values should be taken into account to be able to distinguish the isotopic changes due to real climatic effects from those resulting from diagenetic processes, chemical preparation mistakes, accidental mixing of the samples, etc..

Aim of this paper is the quantitative evaluation of the intra-specific variability of the isotopic composition of oxygen, carbon and nitrogen in the case of a fossil sequence to be used for detailed palaeoenvironmental studies when a large number of remains is not available.

It is well known that the isotopic composition of phosphate oxygen in mammal fossil apatite and that of carbon and nitrogen in collagen from skeletal remains can be used to evaluate quantitatively and/or qualitatively climatic and environmental changes. As regards oxygen, (e.g. Longinelli, 1984; Luz and Kolodny, 1985; D'Angela and Longinelli, 1990; Bryant and Froelich, 1995; Frike et al:, 1998) each mammal species shows its own relationship between δ^{18}Op and the oxygen isotopic composition of environmental water (δ^{18}Ow) as a result of a particular metabolism and dietary behaviour (e.g., Sanchez Chillon et al., 1994; Bryant et al., 1995). Concerning collagen, it is of primary importance that its δ^{13}C and δ^{15}N reflect the mean isotope values of food intake (carbon and nitrogen values deviate from dietary values by about +5 and +3 per mil, respectively). Smith et al., (1986) and Stuiver and Broziunas (1987) presented the water stress and the temperature increase as causes for the observed δ^{13}C increase in plants at the base of the animal food chain. Vogel (1987) and Van der Merwe and Medina (1989) suggested that the $\delta^{13}C$ of the food chain in densely forested areas is drastically decreased by the canopy effect. Heaton et al., (1986) claimed that the $\delta^{15}N$ of mammal collagen increases with decreasing amounts of atmospheric precipitation and this seems to be related to nitrogen metabolism in animals.

Comparing the three isotopic signals (O, C, and N) it may be possible to obtain detailed information on the climatic and environmental conditions during the period considered (e.g., warm and humid or cool and dry or vice versa).

For this study samples belonging to *Bos primigenius* from the Paglicci cave (S. Italy) were taken into consideration.

Data so far available for the isotopic composition of collagen and apatite of mammal skeletal remains from the same cave (Abbazzi et al., 1996; Delgado Huertas et al., 1997; Iacumin et al., 1997) were inadequate for a statistical evaluation as just one sample of each species and layer was measured.

The comprehensive data set herein reported is also intended to lend further support to previously attempted setting of palaeoenvironmental changes over the last Pleniglacial/Late Glacial periods.

2. STUDIED SAMPLES

110 tooth and bone samples belonging to *B. primigenius* were measured, coming from layers from which at least three specimens were available for isotopic measurements. *Bos primigenius* was chosen being quite common in cave sequences of Pleistocene age as well as in archaeological settlements. The samples belong to 29 different layers ranging in age between 32,600 and 13,350 years BP (calibrated ^{14}C ages - Tab. 1). In the case of tooth samples, to reduce the intra-tooth variation related to seasonal climatic changes, the teeth were sampled in vertical bands along the growth axis in order to obtain a mean isotopic value representative of the period of tooth accretion. Enamel was used for the phosphate oxygen measurements and dentine for the carbon and nitrogen measurements in collagen (for the analytical techniques see Delgado Huertas et al. (1997) and Iacumin et al. (1997)).

level	Conv. 14C age BP (1)	Cal. 14C age BP (2)
2	11,440 ± 180	13,355
4	11,950 ± 190	13,955
5	13,590 ± 200	16,290
6	14,270 ± 230	17,105
7	14,820 ± 210	17,730
9	15,270 ± 220	18,200
10	15,320 ± 250	18,245
14b	16,310 ± 350	19,205
15b	16,400 ± 200	19,302
16a	16,450 ± 190	19,365
16b7	17,100 ± 300	20,255
16c2	17,200 ± 300	20,405
17a	17,900 ± 300	21,360
17e	19,600 ± 300	23,190
18b1	20,160 ± 160	23,836
18b2	20,200 ± 350	23,884
19a	20,730 ± 290	24,480
20b	21,260 ± 340	25,095
20c	22,200 ± 200	26,107
20e-d	22,630 ± 390	26,650
21a	23,040 ± 380	27,130
21b	23,470 ± 370	27,600
21c	24,210 ± 300	28,415
21d	24,720 ± 420	28,965
22b	26,800 ± 300	31,243
22f4	28,300 ± 400	32,844
23a	28,100 ± 400	32,633
24a1	29,300 ± 600	33,898
24b2	34,000 ± 800	38,690

Tab.1 - Age of the stratigraphic levels. (1) The conventional ^{14}C ages are from Azzi et al. (1973, 1974, 1977), Evin et al. (1979) and Palma di Cesnola (1992). (2) The calibrated ^{14}C ages are from Delgado Huertas et al. (1997).

Età dei livelli stratigrafici. (1) Le età ^{14}C convenzionali sono quelle riportate in Azzi et al. (1973, 1974, 1977), Evin et al. (1979) and Palma di Cesnola (1992). Le età ^{14}C calibrate sono quelle riportate da Delgado Huertas et al. (1997).

3. DISCUSSION AND CONCLUSIONS

O, C and N isotopic data have no value as palaeoenvironmental indicators if they do not preserve their primitive environmental signature which may be obliterated, collagen and apatite being susceptible of post-mortem alteration. As for the previous works (for details see Iacumin et al., 1997 and Delgado Huertas et al., 1997) all possible checks were done to assess the reliability of the isotopic values. Apparently, the diagenetic processes did not change the pristine isotopic composition of collagen samples. Conversely, by comparing the oxygen isotopic composition of structural carbonate and phosphate of the same samples it as been observed that the values misfit to some extent the carbonate-phosphate equilibrium line. Therefore conclusion is drawn that either carbonate or both carbonate and phosphate were affected by diagenetic processes which partially modified their pristine isotope composition.

For the evaluation of the intra-specific variations it is statistically significant to consider the average of the standard deviations (std) for each layer instead of that for one single layer which could be affected by factors other than real intra-specific variations. For example one specimen might be erroneously related to a certain

level	mean δ^{18}Ow	±std	mean δ^{13}C	±std	mean δ^{15}N	±std
2	-5.9	±0.38	-20.4	±0.20	6.6	±0.20
3	-6.3	±0.38	-		-	
4b	-6,3	±1.14	-20.1	±0.37	6.6	±0.37
4c	-5.7	±0.67	-19.9	±0.52	7.3	±0.23
5a	-6.3	±0.96	-19.6	±0.31	8.1	±0.17
5b	-6.7	±0.82	-20.2	±0.32	9.4	±0.12
5c	-7.4	±0.79	-19.9	±0.24	6.6	±0.31
6	-6.6	±0.39	-19.3	±0.46	7.1	±0.29
7a/b	-7.0	±1.12	-19.0	±0.40	9.1	±0.07
7c	-7.0	±1.08	-19.5	±0.62	9.5	±0.87
8a/b	-5.6	±1.00	-19.8	±0.22	8.0	±1.30
8c	-6.1	±0.44	-19.9	±0.52	7.6	±0.91
8d	-7.1	±0.95	-19.7	±0.71	8.7	±0.51
10a	-6.3	±0.11	-19.1	±0.63	10.4	±1.01
10d	-7.7	±0.67	-18.8	±0.49	9.5	±0.20
10e	-6.7	±0.59	-19.1	±0.20	8.6	±0.64
11a/c	-6.8	±0.72	-19.3	±0.12	8.8	±0.62
12e/f/g	-6.6	±0.85	-19.0	±0.29	8.3	±0.46
14b	-7.5	±1.02	-18.6	±0.19	9.4	±0.22
15b	-6.5	±0.53	-18.9	±0.61	8.3	±0.64
16a	-7.4	±0.76	-18.5	±0.07	8.6	±0.10
17b	-6.2	±0.74	-20.1	±0.47	6.8	±1.00
19a/b	-7.2	±0.08	-20.4	±0.50	-	
20a	-6.6	±0.80	-19.5	±0.18	7.0	±0.09
20c/d	-7,0	±0.82	-19.8	±0.24	6.5	±0.45
22a	-6.9	±1.17	-19.0	±0.29	8.9	±0.34
22d	-7.1	±0.50	-19.5	±0.10	8.0	±0.90
22f	-7.6	±0.65	-19.2	±0.14	7.2	±0.10
23a	-6.5	±0.75	-19.5	±0.10	7.4	±0.28
	mean std	±0.72	mean std	±0.34	mean std	±0.46

Tab. 2 - Stratigraphic levels and δ^{18}Ow, δ^{13}C and δ^{15}N values along with the standard deviation (std) for each level. The δ^{18}_{O} of local meteoric water (δ^{18}Ow) were calculated from the δ^{18}_{O} phosphate values according to the equation reported by D'Angela and Longinelli (1990).

Livelli stratigrafici e valori di δ^{18}Ow, δ^{13}C e δ^{15}N e relativa deviazione standard (std) per ogni livello. I valori di δ^{18}O dell'acqua (δ^{18}Ow) sono stati calcolati dai valori di δ^{18}O del fosfato sulla base dell'equazione riportata in D'Angela e Longinelli (1990).

layer when originally belonging to the upper or lower layer. On the other hand it is hardly affordable to date all the remains and, even in this case, there would be uncertainties mainly related to the instrumental error involved in the age determination technique.

Following the statistical treatment of the isotope data for the deposit it resulted that δ^{13}Cstd show a range (from ±0.07 to ±0.71; mean: ±0.34) narrower than that of δ^{15}Nstd (from ±0.07 to ±1.3; mean: ±0.46). The higher mean value (±0.72) is displayed by δ^{18}Ostd ranging between ±0.08 and ±1.17 (Tab. 2).

This means that we may consider significant along the stratigraphical sequence a δ^{13}C variation greater than 0.7 per mil (2σ). Nitrogen values in natural samples are usually more variable than carbon values: in this case a variation greater than 0.9 per mil (2σ) may be considered significant and really related to environmental changes rather than to fortuitous circumstances. In the case of oxygen only variations greater than 1.4 per mil (2σ) may be considered significant. This range of values is too wide to be of some concern for palaeoclimatic reconstructions. It is apparent that phosphate oxygen too was slightly affected by diagenetic alteration.

In the case of the Paglicci sequence the mean carbon isotope composition of each layer ranges from -20.4 to -18.5 per mil. Such a 2.1 δ units variation is about three times greater than the calculated δ^{13}Cstd intra-specific variability and therefore is quite significant from the environmental and climatological point of view. The overall variation of the nitrogen isotope values, from 6.5 to 10.4 per mil, is even greater and approaches 4 δ units. Consequently, also the nitrogen isotope variations can be considered significant as regards their palaeoenvironmental significance.

Palaeoenvironmental considerations in some detail can be drawn particularly for the period between 13,000 and 20,000 years BP for which a number of samples are available. The variation of the δ^{13}C, δ^{15}N and δ^{18}O of environmental water (δ^{18}Ow) along the stratigraphical sequence is reported in Figure 1, the chronological position of each sample being interpolated from the available ^{14}C ages as already done in Delgado Huertas et al., (1997) and Iacumin et al., (1997). In the case of carbon a trend towards lighter values is apparent after 20,000 BP, as previously found, significant for an environmental change. Besides of fast variations the nitrogen trend show two minima, at ca. 16,300 and 13,200 yr BP (the latter also displayed by C) and two maxima, at some 15,700 and 18,200 yr BP, that likely mirror humid and arid palaeoclimatological phases, respectively. As regards oxygen we can only note that the trend of the mean values after 20,000 BP is quite close to that shown in a previous study where three different mammal species were compared (Delgado Huertas et al., 1997) even though the range of isotope values obtained is considerably broad, probably due to incipient taphonomic and/or diagenetic alteration.

The results of this study emphasise that significance and reliability of the isotope data measured on skeletal remains from chronostratigraphical sequences have to be carefully evaluated before being used for implementing a palaeoenvironmental setting. For the studied deposit only δ^{13}C and δ^{15}N variations exceeding 0.7 and 0.9 per mil, respectively, were statistically significant.

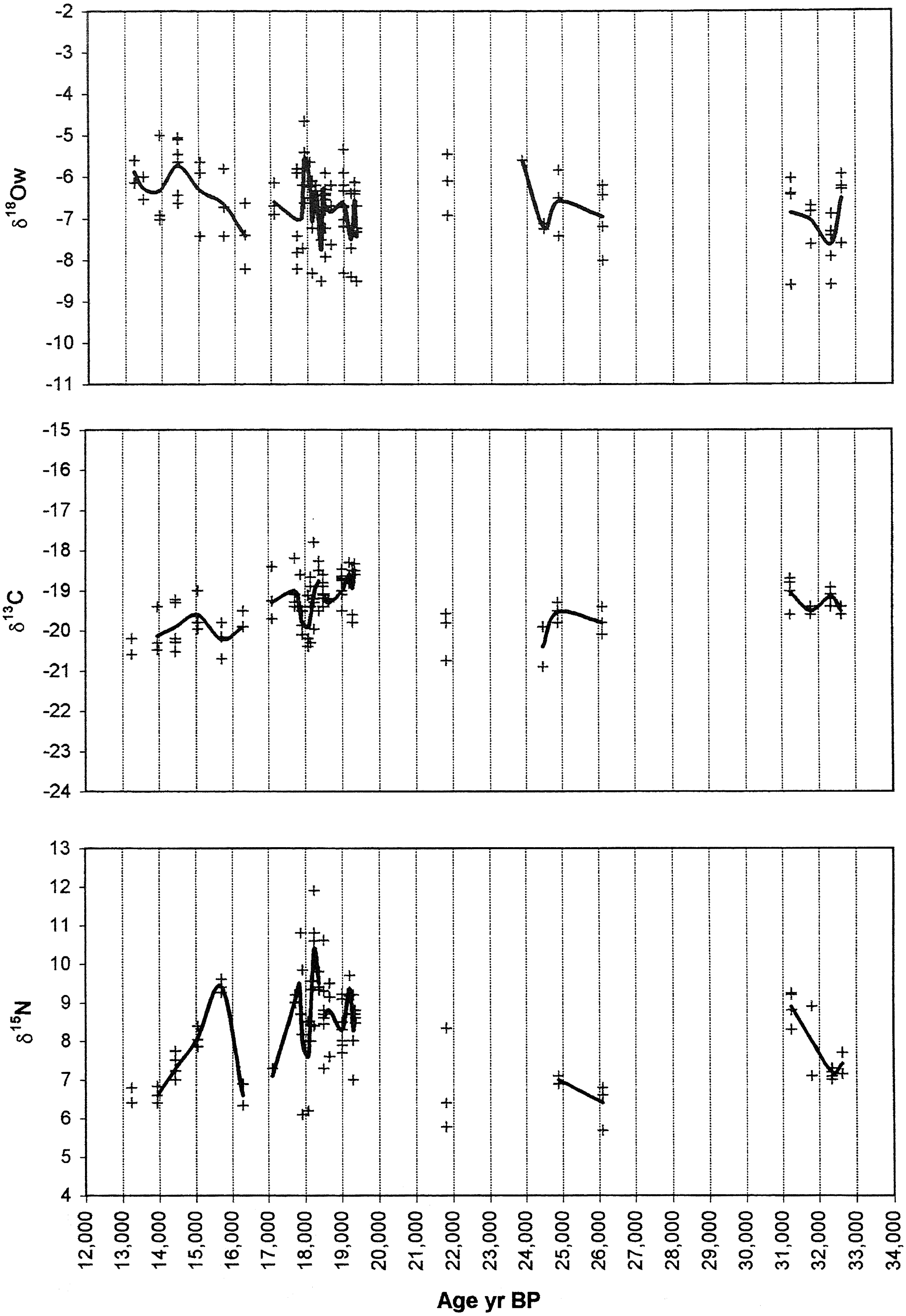

Fig. 1 - Temporal variation in the $\delta^{15}N$ and $\delta^{13}C$ of collagen values and the $\delta^{18}O$ of local meteoric water calculated from the $\delta^{18}O$ phosphate values according to the equation proposed by D'Angela and Longinelli (1990).

Variazione temporale dei valori di $\delta^{15}N$ e $\delta^{13}C$ del collagene e di $\delta^{18}O$ dell'acqua meteorica locale ($\delta^{18}Ow$), questi ultimi calcolati dai valori di $\delta^{18}O$ del fosfato in accordo con l'equazione proposta da D'Angela e Longinelli (1990).

Concerning oxygen, the notable variability and the incipient diagenetic alteration shown by the samples from the Paglicci cave rule out any statistical evaluation of the isotope data. In spite of such constraint the present data are valuable in that support and refine some palaeonvironmental events previously inferred.

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