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SEARCHING FOR NEW PRE-SCREENING CRITERIA OF ^{14}C DATING OF OSSEOUS MATERIALS. AN ADAPTATION FOR ROMANIAN ARCHAEOLOGICAL SAMPLES

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Abstract. An enlargement of criteria concerning the chances for extracting collagen for radiocarbon dating by AMS method is presented. PAST 5 program is used, based on statistical analysis of the pre-screening data obtained on raw bone, searching for specific features for Romanian osseous materials, to assign the most appropriate pretreatment method.

Keywords: osteoarchaeology, radiocarbon dating, statistical analysis of data, pre-screening methods, diagenesis processes

1. INTRODUCTION

Extracted Degraded Archaeological Material that has undergone diagenetic alteration (EDAM) [1] is derived from Type I collagen representing one of the organic parts of osseous materials, having great contribution in archaeology [2].

The living protein differs from the protein extracted from bones immediately after death, but also from that extracted from bones/osseous materials that have undergone various diagenesis processes in the post-depositional stage [3].

Radiocarbon dating of osteological material is focused on this last type of protein. Since 1949 to the present days, the information tries to predict the chances of extracting reliable collagen, datable by radiocarbon method. An important landmark was provided by the Oxford Radiocarbon Accelerator Unit (ORAU) in 2010, by publishing an overview article on the pre-screening techniques for osteological materials from UK, to choose suitable samples for this type of analysis [4]. The most reliable and accessible method proved to be the elemental analysis that establishes the mass percentages %C, %N, as well as the C:N atomic ratio.

If the raw bone powder falls within the ranges established by these studies, then there is a maximum 85% chance of obtaining, through the ORAU methods, over 1% overall yield or/and a minimum of 10 mg of EDAM – formally associated with protein residues larger than 30 kDa [5]. These ranges were defined by analyzing correlation factors and establishing linear regression equations [4].

Given the environmental conditions at certain archaeological sites in Romania, only a maximum of 40% of the archaeological bone samples statistically fall within the recommended ranges of these three parameters. The recent large number of samples from various archaeological sites in Romania has created the opportunity to develop pretreatment methods other than ORAU. By adapting pretreatment methods used worldwide to the RoAMS laboratory practices, the chances of extraction increased sometimes to near 100%, even for specimens that should have been rejected according to the ORAU criteria.

The set of samples that will be presented in this paper has two characteristics: i) the bone samples have very different degrees of preservation; ii) a large number of specimens, almost 100, were selected, to reach a statistical threshold. Therefore, it offered the opportunity to develop a study using a program for analyzing the main parameters defined by the ORAU criteria.

3. ARCHAEOLOGICAL CONTEXT (1), MATERIALS (2) AND METHODS (3)

1. The Iron Gates section of the Lower Danube valley along the border between Romania and Serbia has an unparalleled record of Mesolithic and Early Neolithic settlement spanning the period from the Final Pleistocene to the middle Holocene (ca. 12,700 to 5,600 cal BC). Over 50 caves and open-air sites were identified during archaeological surveys in advance of dam construction in the 1960s and 1980s, and follow-up rescue excavations revealed numerous burials and architectural remains and produced rich inventories of faunal and lithic material as well as pottery and portable artifacts.

Most sites are no longer accessible, submerged beneath the reservoirs created by the Iron Gates I and II dams. Until now, over 300 single-entity AMS dates (human, animal and vegetal) have been published for mainly Stone Age contexts at 15 sites on both banks, with only a few at RoAMS. This number does not include the dates we are discussing here.

2. It was previously established that ^{14}C dates on human bone collagen from the Stone Age sites in the Iron Gates carry a reservoir offset owing to large inputs of fish into the diet [6]. Thus, the ^{14}C dates discussed here were measured on samples collected mainly from herbivores *(Bos* and *Cervus* sp.*).*

A representative set of ca. 100 samples has been chosen here that originate from different eight Romanian sites in this region and were selected based on their different conditions of osteological preservation (Fig. 1). Such sites were located both in caves and in the open air (on the riverbank or on islands). Their chronology and preservation conditions of the faunal remains in general (as visually assessed) are presented and shortly described in Table 1.

The terraces on which the archaeological sites lay consist of silty Early Holocene River alluvium resting on older, poorly sorted river gravels, probably deposited in periglacial environments. The alluvial sediments have been affected by soil formation processes during most of the Holocene and the soil has been decalcified to various depths.

Most of the cave sites developed in Upper Jurassic (Tithonian) limestones. The Late Quaternary sediments formed over the natural bedrock floors and were of variable thicknesses. Larger and smaller fragments of the cave ceiling occur frequently in these sediments, enriching them with calcium carbonate.

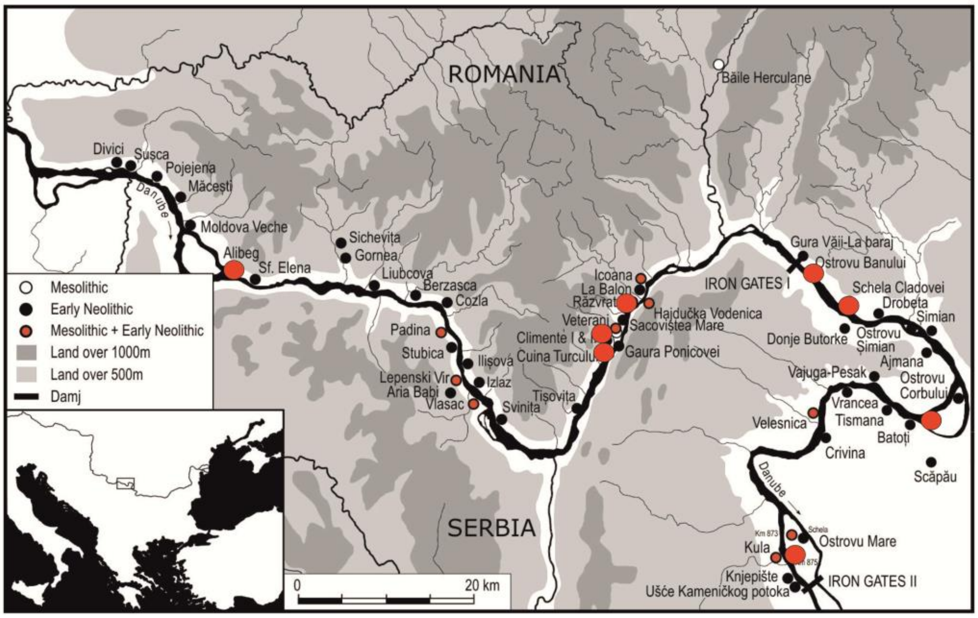

Fig. 1 – Map of the Iron Gates Mesolithic and Early Neolithic sites. Sites mentioned in the text are presented in Table 1.

3. The selected bone sample is mechanically cleaned, washed in MilliQ8 ultrapure water in an ultrasonic bath, and dried for 4 hours at 60 degrees Celsius in a forced-ventilation oven. Then, from the inside to the outside in triangulation, approximately 5 - 10 mg of raw bone powder is extracted. The bone powder is weighed on an analytical balance with precision to the fifth decimal place, and introduced into the Elementar (vario Micro, GmbH). Total thermal combustion takes place in an oxygen atmosphere. The gases are separated, purified, dried, and the equipment software expresses the carbon and nitrogen content in mass percentages and mass ratios. Finally, the C:N atomic ratio is calculated.

Table 1

Short characterization of the archaeological sites mentioned in the text

Site name	Site type	Chronology	Bone preservation at the site
Alibeg	Open air	Final Mesolithic, Early Neolithic	Poor (frequent site flooding)
Cuina Turcului	Rock shelter	Early/Middle Mesolithic, Early Neolithic	Good (calcium carbonate)
Climente II	Cave	Early Mesolithic, Early Neolithic	Poor (thin stratigraphy and presence of calcium carbonate)
Răzvrata	Open air	Final Mesolithic, Early Neolithic	Good
Ostrovul Banului	Open air (island)	Middle Mesolithic, Final Mesolithic, Early Neolithic	Poor
Schela Cladovei	Open air	Late Mesolithic, Early Neolithic	Good (calcium carbonate precipitation)
Ostrovul Corbului	Open air (island)	Late/Final Mesolithic, Early Neolithic/Mesolithic	Good
Ostrovul Mare	Open air (island)	Late, Final Mesolithic	Poor to fair

4. RESULTS AND DISCUSSION

4.1. ELEMENTAL ANALYSIS AND EXPERIMENTAL DATA INTERPRETATION

The averaged results of the elemental analysis, for each archaeological site are presented in Table 2.

According to the ORAU criteria [4], %N must be at least equal to 0.76% (0.80% for Paleolithic), %C recommended is over 10%, C:N atomic ratio recommended is in the range of 2.90 - 3.60; minimum 10 mg collagen and/or minimum 1% global pretreatment yield.

These values are recommended for the standard working method from ORAU and its derivatives (some methods from the Zürich radiocarbon dating laboratory, for example).

At RoAMS laboratory in IFIN-HH, applying these criteria over 10 years established a percentage of datable Romanian bones of 30 - 40%, but which percentage is fined with a maximum of 85% chance of success (even if the values are within intervals) [4].

For this particular set of 95 samples, according to the %N criterion, 81 samples should be processed; according to the %C criterion, only 11 samples should be processed; according to the C:N atomic ratio criterion, only 14 samples should be processed.

Taking into account all three criteria, out of 95 samples, only 7 samples should be processed.

Table 2

Correlation between the site characteristics and the archaeologist's observation concerning bone

Archaeological site	Number of samples per site	%N, average ± Standard Deviation	%C, average ± Standard Deviation	C:N atomic ratio, average ± Standard Deviation
Schela Cladovei	34	1.66 ± 0.78	6.15 ± 1.99	4.81 ± 1.60
Cuina Turcului	29	3.54 ± **4.70**	7.02 ± 2.23	4.00 ± 1.75
Răzvrata	2	0.37 ± 0.20	3.42 ± 1.40	11.17 ± 1.73
Ostrovu Banului	4	1.26 ± 0.23	4.66 ± 0.51	4.34 ± 0.39
Alibeg	5	0.81 ± 0.19	4.30 ± 0.95	6.18 ± 0.54
Climente II	3	1.23 ± 0.96	4.51 ± 1.62	7.68 ± 6.75
Ostrovu Corbului	9	1.25 ± 0.52	4.98 ± 1.54	4.85 ± 0.68
Ostrovu Mare	9	1.41 ± 0.64	5.43 ± 1.57	5.12 ± 1.97

The worst pre-screening results were obtained for Răzvrata, where preservation based on visual inspection and bone survival at the site is quite good. The best pre-screening results were obtained for the site at Cuina Turcului, where archaeological bone preservation was deemed poor. It is observed that in some cases a large standard deviation appears, which suggests a high variability in sample quality within the same batch.

After evaluating the dating chances, a strategy to apply beyond the ORAU recommendations or archaeological criteria was applied. Some samples were processed once, others several times, iteratively developing combined methods. Some of these methods are taken from literature, others developed during experiments. In the end, only 11 samples out of 95 could not be effectively dated after treated by the 7 methods employed: 2 ORAU and 5 non-ORAU. In order to streamline the dating strategy in the future, we sought pre-selection means that would show more realistically how many samples and which method could lead to a successful collagen extraction. Table 3 represents a short description of the pretreatment methods.

Table 3

Methods used in bone pretreatment

No	Sample processing method	Abbreviation of the method name	Comments
1	Zurich; Acid-Collagenization-Ultrafiltration [7]	ETHZ	Clean, well-preserved bone, in sufficient quantity; easy to remove carbonate deposits and demineralize

No	Sample processing method	Abbreviation of the method name	Comments
2	Acid (HF)-Collagenization-Ultrafiltration [7-9]	ETHZ-HF	Clean, well-preserved bone, in small quantity; crusts resistant to short-time acid attack
3	Oxford Radiocarbon Accelerator Unit; Acid-Base-Acid-Collagenization-Ultrafiltration [9]	ORAU	Dirty bones (contaminated with humic matter), well-preserved and in sufficient quantity
4	Complexation with EDTA-Collagenization-Ultrafiltration [9,10]	EDTA	Clean or dirty, poorly preserved bones (showing the phenomenon of diagenesis), in sufficient quantity
5	Acid (HF)- Complexation-Collagenization-Ultrafiltration [7-10]	EDTA - New	Clean or dirty, poorly preserved bones (showing the phenomenon of extended diagenesis), in sufficient quantity
6	Acid (EDTA and acetic acid)-Ultrafiltration [9,11]	EDTA - min	Clean, poorly preserved bones (showing the phenomenon of extended diagenesis), in quantity under 1 g
7	Acid (acetic acid)-Collagenization-Ultrafiltration [9,12]	CB	Clean, highly mineralized/carbonated bones relatively well preserved, in sufficient quantity

4.2. STATISTICAL ANALYSIS

As has been defined as a free software package, PAST (PAleontological STatistics) is focused on statistical paleontological data analysis including "functions for data management, data visualization through graphics, univariate and multivariate analysis procedures as well as linear and non-linear modelling. There are also functions for diversity calculation, time series analysis, geostatistical and stratigraphic analysis." [13].

Using multivariate analysis, combined with 2D and 3D data visualization through graphics, the search directions are: which samples cannot be dated by any method; which samples or groups of samples can be successfully dated by a specific method.

For starting a database, all these 95 samples were selected by RoAMS codes even if they provided collagen or not. Their associated graphic codes depending on archaeological sites and methods are represented in Figures 2 and 3.

PAST 5 graphically arranges the results after establishing the degree of correlation. There are 3D and 2D representations of the combinations along the coordinate axes. In Figure 2, Component 1 numerically expresses on the 0X axis the combination of the three parameters %N, %C, C:N. Component 2 numerically expresses on the 0Y axis how strong their combination is, according to the experimental results. Component 3 numerically expresses on the 0Z axis the other direction of variability. The Eigenvalues obtained in each case determine the length of the graphic segment for the three quantities with physical significance: %N, %C and C:N atomic ratio. Three projections appear in the plane, oriented differently, depending on the angle at which the combination between the components is seen.

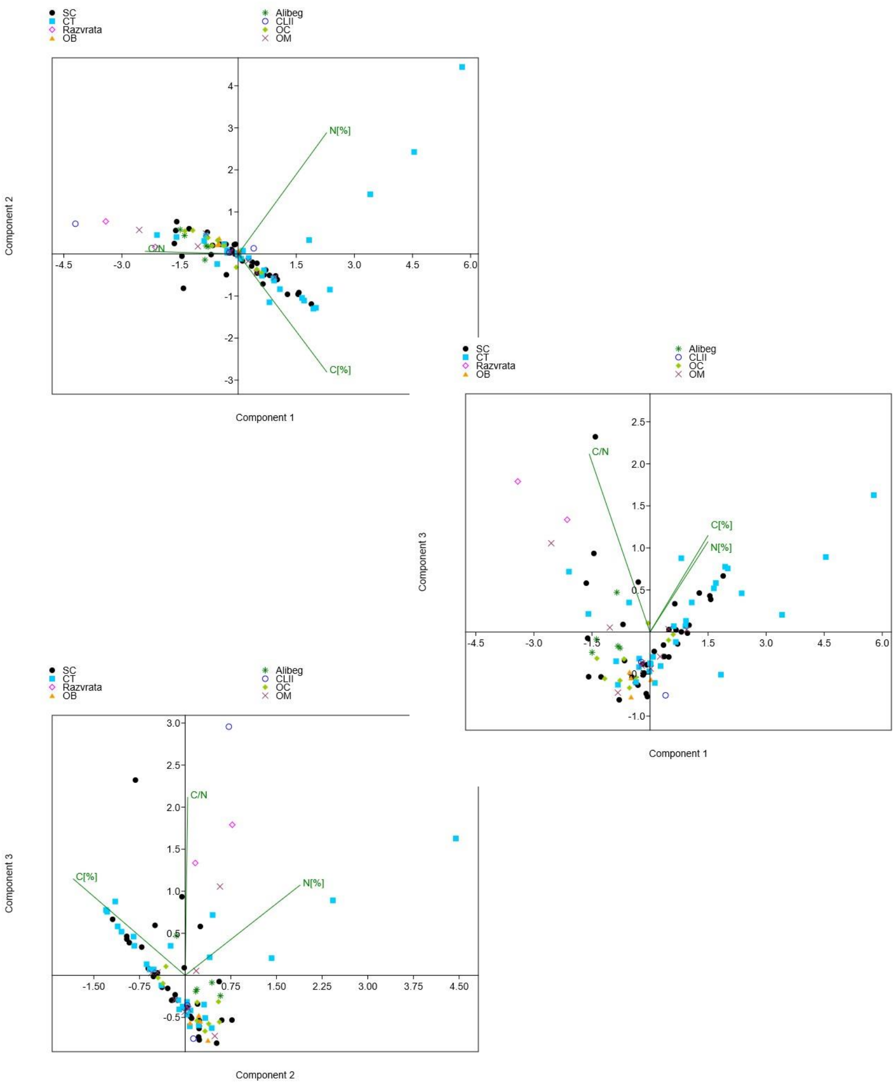

Fig. 2 - Plane projection of the results offered by combining %N, %C, C:N according to the three Principal Components that ensure variability, depending on archeological site.

The three 2D projections according to the 7 pretreatment methods previously presented in Table 3 each help to establish the optimal processing strategy (Fig. 3).

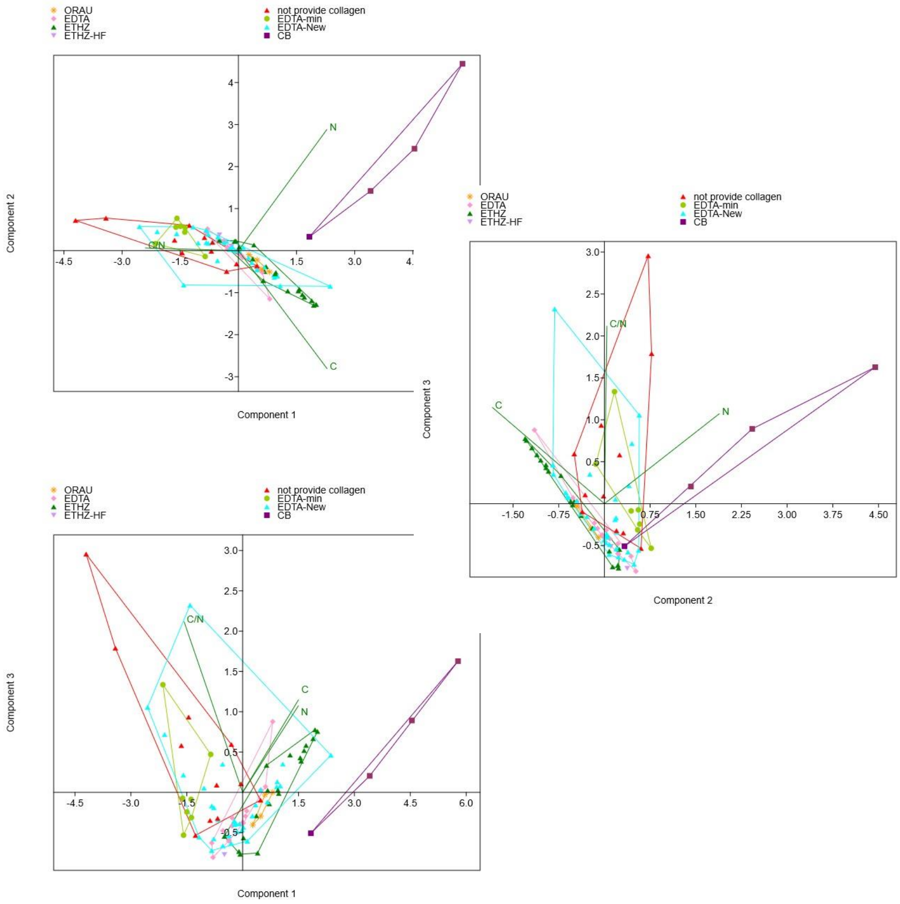

Fig. 3 - PAST 5 Program results; preliminary identification of best fitting of samples with pretreatment methods.

4.6. DISCUSSION

According to Figure 2 and Component definitions, the best grouping of samples that did not yield collagen is better viewed by 2D projection of the variability established by Component 1 and Component 3. These 11 samples are delimited from the others. For selected Romanian bones, C:N atomic ratio criterion is decisive, more than the %N criterion considered decisive at ORAU. Its Eigenvalue is almost double that for %N and %C.

Concerning pretreatment methods, the most suggestive graphic is the same projection in which the methods are quite well delimited. It is observed that for samples with special characteristics EDTA-min and CB methods are indicated, while the EDTA-New method offers the greatest coverage. The ORAU, ETHZ and derivatives methods cover a small number of samples. The ETDA-New method also managed to cover most samples from different sites, over a wide range of %N, %C and C:N parameters.

5. CONCLUSIONS

We extended the Oxford Radiocarbon Accelerator Unit (ORAU) predictability criteria on the success of collagen extraction from osteological material, in the particular case of bones from Romania. By guiding the pretreatment strategy, we managed to obtain radiocarbon dates for 84 samples out of a total of 95. Otherwise, respecting all ORAU criteria, only 7 samples could have been successfully dated. Respecting the criterion (%N > 0.80%), only 81 samples could have provided collagen, if the success rate would have been 100%, and not up to 85% as stipulated by ORAU, i.e., a maximum of 69 results.

When the ORAU criterion for osteological materials (%N > 0.80%) is not enough to differentiate between datable and non-datable Romanian bones, the C:N atomic ratio becomes dominant.

As for the types of methods that could be applied depending on the pre-screening data, PAST 5 managed to successfully separate them. There are samples that can be classified as special methods. The most promising method remains EDTA-New. The ORAU/ETHZ methods and their derivatives manage to cover a small number of situations, in accordance with the %N criterion.

Moreover, the %N, %C, C:N values can lead to false positive or false negative conclusions. The formation of a representative database, developed with the help of a statistical calculation program such as PAST 5, will allow the introduction of pre-screening values for the set of samples before pretreatment stage. Their placement in the generated graphs, depending on the previous data, will lead to a better assessment of the dating chances, but also of the best pretreatment method to be applied. The development of complementary methods will lead to an increase in the area of applicability predictions.

This is a unique project in Romania radiocarbon dating practices using AMS method, under construction. In the next stage, a statistical analysis on the collagen obtained after applying the pretreatment methods will be explored using same program by generating a secondary database. Also, the description of the original parts of the pretreatment methods will be published separately.

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