Experience the precision of time: radiocarbon 3.0

"If not us, who? If not now, when?"

Two questions that eventually formed a quote, inviting you, in the first place, to act now! But if you ask me, an archaeologist who likes to precisely inspect the time of our past to improve our future, I would answer: "What better time than our past to discover how we ended up being who we are today?"

Adobe Stock © *Bruder*

We are the result of finely tuned evolution, which began, perhaps as early as 500 000 years ago (kya BP) in Africa. However, when *Homo sapiens* stepped out of Africa and crossed the Levant, we slowly but inexorably expanded throughout the planet despite the presence of other human species.

At present, in Europe, the dispersals of *Homo sapiens* are associated with several chrono-cultural units in which technological, behavioural and artistic innovations are diachronically divided. The earliest evidence is named Initial Upper Palaeolithic (IUP) (46–42 kya BP), followed by the Protoaurignacian (~42–39 kya BP), the Aurignacian (~39–31 kya BP) and the Gravettian (~33–24 kya BP). The genetic evidence of ancient *Homo sapiens* from Bacho Kiro (Bulgaria), where three human remains have been accurately radiocarbon dated between 42 580 and 45 930 cal BP (calibrated years Before Present, 1950 AD), represents the oldest securely dated *Homo sapiens* individuals in Europe. Those fossils, together with the one from western Siberia, Ust'-Ishim, dated around 45 kya BP, the one from Romania, Oase 1, dated to about 37 to 42 kya BP, and one from Czechia, Zlatý kůň, ca. 45 kya BP have yielded extensive genomic data, which constitutes intriguing evidence of the existence of distinct *Homo sapiens* populations during the Upper Palaeolithic. However, since these human remains are very few compared to, e.g. those of Neanderthals, our understanding of the genetic affinities and population dynamics of *Homo sapiens* spreading across Eurasia is limited. All this evidence points to the decisive role of chronology, a fundamental aspect of Palaeolithic archaeology, and accurate absolute radiocarbon dating is crucial to understanding the timing of cultural events that occurred in the past.

So far, this method does not always provide sufficiently precise and accurate ages to understand the important processes of human evolution. In recent years, new dating methodologies (e.g. optically stimulated luminescence (OSL), electron spin resonance (ESR), or hydroxyproline ^{14}C (HYP)) have been used with larger standard deviation errors on the dates that increase, rather than reduce, the uncertainties on the interpretation of our archaeological record. We all agree that the HYP is a helpful method to elucidate the calibrated range of human fossils or artefacts when the sample is heavily contaminated by glue. However, the results on La Ferrassie 1 Neanderthal skeleton and the *Homo sapiens* skull of Zlatý kůň in Czechia lead us to conclude that even when the important samples stored in museums for long periods are covered with glue, the HYP method does not always work properly. Moreover, performing this method requires an inestimable amount of sample material, thus destroying our most precious artistic and human heritage.

We are well aware of the fact that to propose a reliable chronology, we have to rely on a secure stratigraphy before we can publish our data. We have to be even more careful when we propose a model of the demographic extinction of a human species or discuss the pattern of dispersal of *Homo sapiens* in Europe. As a result, paleogenetics has been the only field of research that has confirmed the interbreeding of Neanderthals, Denisovans and *Homo sapiens*.

The crucial challenge is high temporal resolution chronology, which has so far been severely limited by the low number of dates per site, low resolution of the radiocarbon calibration curve, and limited Bayesian modelling.

This has made us say:

When the going gets tough, the tough get going!

In fact, an advanced evaluation and discussion on the earliest *Homo sapiens* in Europe and their temporal relationship with Neanderthals have been carried out (Talamo *et al.*, 2023). We used:

(i) only dates of samples pretreated in the state-of-the-art methodology
(ii) the most recent advances in the AMS radiocarbon measurement technique
(iii) radiocarbon calibration is now based on a section of high-resolution glacial tree-ring chronologies in the age range of 44 000 and 41 000 calendar years BP (before 1950 AD).

The concise amalgamation of these three aspects, called 'radiocarbon 3.0', leads to a new level of temporal interrelation between *Homo sapiens* at the site of Bacho Kiro, Bulgaria, and, for the first time, a link between the respective presence of modern humans to climatic events (warm and cold phases) in the glacial, documented in Greenland ice cores (Talamo *et al.*, 2023).

Using the high-level radiocarbon dates on bones and the new floating Kauri tree-ring chronology, we confirm the importance of having tree-ring chronology in the calibration curve in the Middle to Upper Palaeolithic period and the importance of using the most refined dates when approaching an archaeological site, as well as the interactions between different disciplines in resolving the chronological dispute in human evolution.

Two new Bayesian models were constructed (two and three phase model; Figure 1 and 2), using the accurate and precise dating of the important site of Bacho Kiro, the only archaeological site with more than 21 high-resolution radiocarbon dates for a single layer and less than 300 years ^{14}C error range for a date around 42 000 years. Four direct dates of *Homo sapiens* were obtained as well. Only the high-precision dates of Bach Kiro allow us to assign the presence of *Homo sapiens* at this site during the cold phase of GS 12 (Talamo *et al.*, 2023). In this way, we have shown that the human occupation at Bacho Kiro did not occur at once, but there were three different occupations (one around 44 650 to 44 430, one at 44 200 to 43 420 and one at 43 110 to 42 700 cal BP) or two different ones (one around 44 650 to 44 430, one at 44 310 to 43 710 cal BP), depending on the ^{14}C dates considered and the Bayesian model used. At present, both scenarios could be supported because it is not yet known whether the IUP may have lasted longer in Bacho Kiro than in the Levant or may have overlapped temporally with the Protoaurignacian dispersal. Moreover, obtaining a small

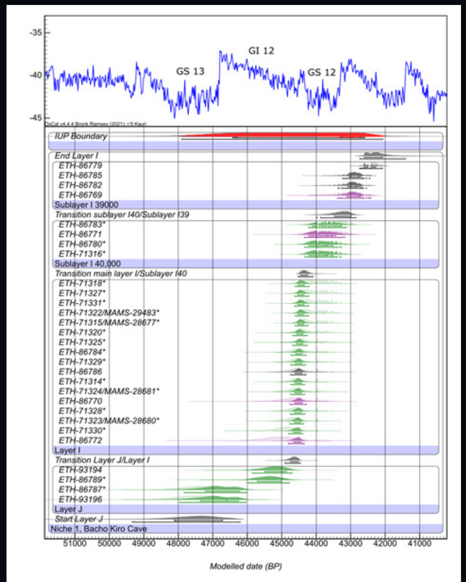

Figure 1: Three phases model. The NGRIP δ^{18}O curve on top is in blue. The IUP Boundary produced by the model is in red. The direct dates of Homo sapiens are in pink. In green are the animal samples dated for the layers. In dark grey are the different boundaries between the different layers.

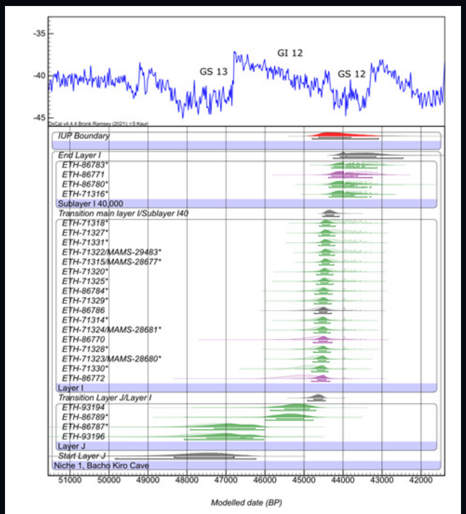

Figure 2: Two phases model: The NGRIP δ^{18}O curve on top in blue. The IUP Boundary produced by the model is in red. The direct dates of Homo sapiens are in pink. In green are the animal samples dated for the layers. In dark grey are the different boundaries between the different layers.

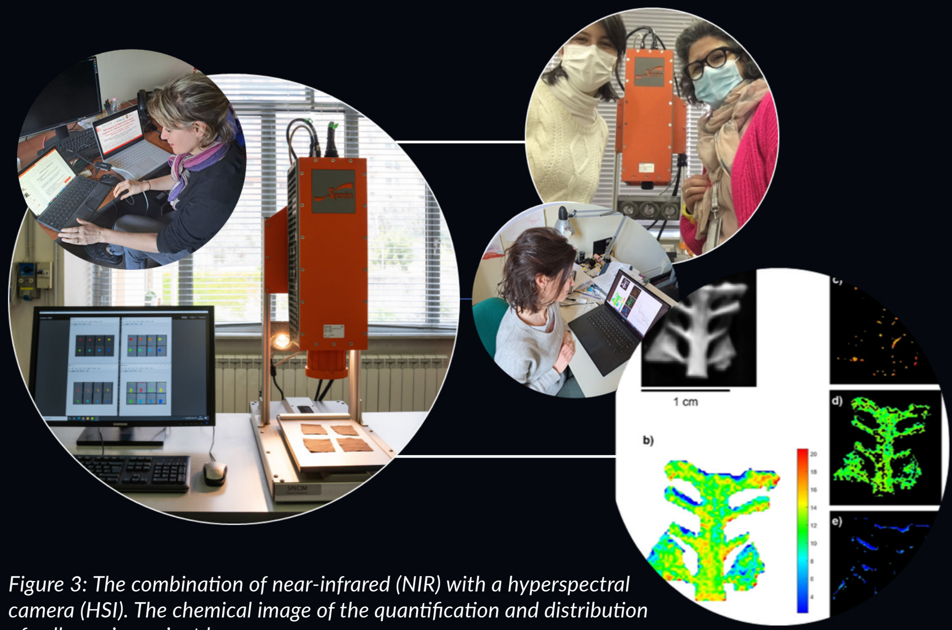

Figure 3: The combination of near-infrared (NIR) with a hyperspectral camera (HSI). The chemical image of the quantification and distribution of collagen in ancient bones.

^{14}C error in a period around 42 000 years ago is a key point of radiocarbon 3.0. The better this error interval is defined and obtained, the more accurate the final age calibration process will be. Furthermore, if we build chronological models using both large (example: ±1700 years) and small (example ±300 years) ^{14}C error intervals, it does not produce valid contributions in the discussion of the interaction between hominids and climate, and even worse, we cannot talk about chronological interactions between different human species in different regions. On the contrary, discussing chronologies obtained from ^{14}C ages with the same tight error intervals leads to an advancement, both in terms of temporal and environmental accuracy. In addition, the extent of the IUP is constrained better by the new models compared to the previous publications (Fewlass *et al.*, 2020). Now we are able to accomplish the definitive high resolution of European key archaeological sites during recurrent climate fluctuations and model the human and faunal species' responses from a diachronic perspective. In this way, we will promote knowledge exchange between archaeology, palaeoclimatology, geochronology and geosciences in general, all essential disciplines in the study of the human past. Even though the radiocarbon community has accepted the discussion of calibrated ranges at 95.4 per cent, we have shown that with the use of the small ^{14}C error range and the advanced calibration curve (IntCal+Kauri), even spatiotemporal archaeological discussion can be brightly addressed using only the 68.3 per cent, and the range overlapping at the 95.4 per cent probability should be considered a maximum probable overlap.

Another recent aspect that is incorporated in the advancement of the radiocarbon method is the reduction of the destruction of the analysed sample. Many archaeological rarest bones (human remains and bone objects) in Prehistory are much too precious and considered a cultural and historical patrimony, and the application of destructive methods—such as ^{14}C—must be as limited as possible. Within this scenario, the advantage of spectroscopic images can be of high impact, in particular regarding the non-destructiveness and the possibility to map, from a spatial extent, the area of the bone in which the proteins are more present and to quantify if the collagen percentage is enough for ^{14}C analysis.

To this aim, a dedicated analytical protocol that combines hyperspectral cameras (working in the near-infrared region of the electromagnetic spectrum, referred to as HSI) with a tailored multivariate image analysis was studied to make the invisible visible, creating chemical images of the quantification and distribution of collagen in ancient bones (Malegori *et al.*, 2023) (Figure 3).

The analytical procedure presented leads to the construction of the quantitative model able to predict the percentage of collagen ascribable to every pixel of the image. To this aim, a set of samples, including both bone powders (44 samples) and fragments (15 samples), were analysed by means of HSI and subsequently submitted to collagen extraction, according to the most modern strategy developed in the BRAVHO Lab. These samples were chosen with the aim of covering a wide range of collagen percentages, from 0 to 20 per cent, and with a focus on low collagen concentration, typical for prehistorical bones.

Once the prediction of the amount of collagen present in the bone is performed, it is possible to build a chemical map of the analyte of interest (here the collagen) in which a false colour bar is used for representing areas with the low (bluish colour) or high (reddish colour) percentage of collagen. Colours are also supported by the numerical prediction of the percentage itself so that the archaeologist can easily understand the real potential of an unknown sample for collagen extraction and consecutive ^{14}C dating (Figure 4).

This innovative and incisive combination of NIR-HSI spectroscopy prescreening and the radiocarbon method provides, for the first time, detailed information about the quantity of collagen on archaeological bones as a crucial step forward for selecting the sampling point for ^{14}C dating. This allows not only selecting the best specimens but also choosing the sampling point in the selected ones based on the amount of collagen predicted. This method helps to drastically reduce the number of samples destroyed for ^{14}C analysis and within the bone, avoiding the selection of areas that may present a quantity of collagen not sufficient for the dating. With this work, we have shown that by using strategic coordination and effective research between spectroscopic methods and radiometric techniques to study prehistoric bones, we can provide a valuable contribution to the preservation, enhancement and protection of our cultural heritage.

Summary

As you may understand, radiocarbon is part of those applied sciences that never stop being improved to be revolutionised in some way. However, it is not always straightforward to make use of these substantial advances because it depends much on the question the archaeologists ask and their expectations.

The widespread application of the radiocarbon 3.0 approach to the European key sites in this timeframe could revolutionise the current scenarios and explain the unsolved questions in this important period of our human history.

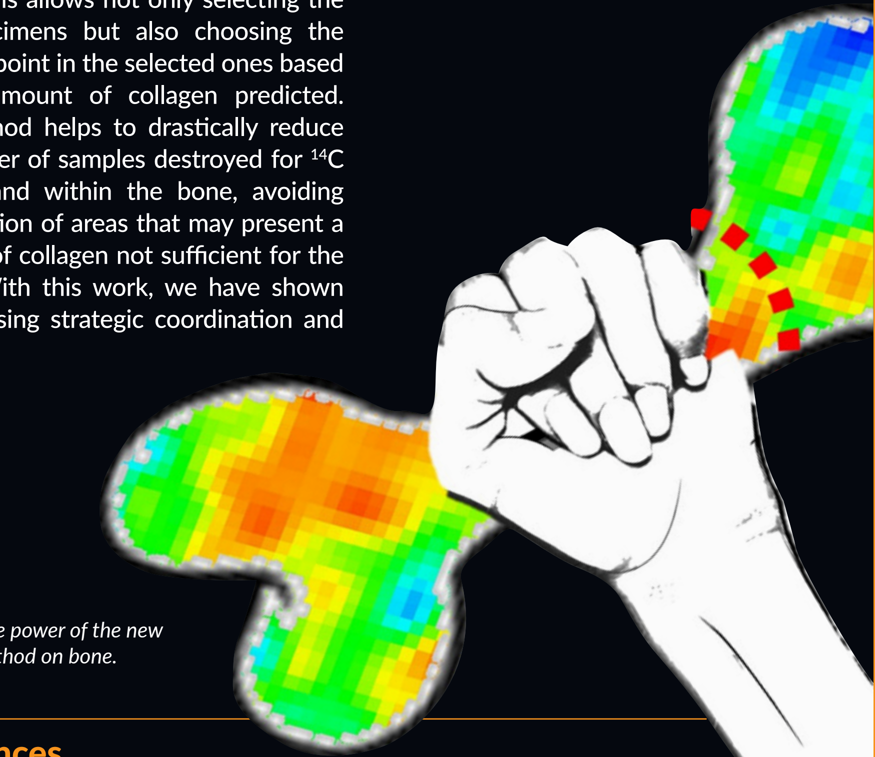

Figure 4: The power of the new NIR-HIS method on bone.

References

Fewlass, H., Talamo, S., Wacker, L., Kromer, B., Tuna, T., Fagault, Y., Bard, E., McPherron, S.P., Aldeias, V., Maria, R., Martisius, N.L., Paskulin, L., Rezek, Z., Sinet-Mathiot, V., Sirakova, S., Smith, G.M., Spasov, R., Welker, F., Sirakov, N., Tsanova, T. and Hublin, J.-J. (2020) 'A 14C chronology for the Middle to Upper Palaeolithic transition at Bacho Kiro Cave, Bulgaria', *Nature Ecology & Evolution*, 4(6), pp. 794–801. doi: 10.1038/s41559-020-1136-3.

Malegori, C., Sciutto, G., Oliveri, P., Prati, S., Gatti, L., Catelli, E., Benazzi, S., Cercatillo, S., Paleček, D., Mazzeo, R. and Talamo, S. (2023) 'Near-infrared hyperspectral imaging to map collagen content in prehistoric bones for radiocarbon dating', *Communications Chemistry*, 6(1), 54. doi: 10.1038/s42004-023-00848-y.

Talamo, S., Kromer, B., Richards, M. P. and Wacker, L. (2023) 'Back to the future: The advantage of studying key events in human evolution using a new high resolution radiocarbon method', *PLOS One*, 18(2), e0280598. doi: 10.1371/journal.pone.0280598.

PROJECT SUMMARY

RESOLUTION aims to achieve an accurate and highly resolved chronology back to 55 000 years BP (Before Present), using some new floating sections of fossil trees. In fact, with tree rings, the resolution will be an order of magnitude higher, and using the most recent advances in the AMS technique, we will obtain confidence intervals of only a few centuries in glacial times.

In this way, we can establish in a precise way the timing of when *Homo sapiens* arrived in Europe, their interaction with Neanderthals and the final cause of the Neanderthal extinction.

The project involves fieldwork in the Mediterranean and Southeast Europe to find more glacial trees, study the existing collection of glacial conifers, exceptional ^{14}C precision for ^{14}C dates in the glacial, and the cutting-edge methodology linking floating tree-ring chronologies to ^{10}Be on the ice core scale.

PROJECT LEAD

Professor Sahra Talamo has been in the Department of Chemistry G. CIAMICIAN at Bologna University since 2019 and is the project lead for RESOLUTION. Talamo is the author of several international scientific papers concerning the chronology and interaction between the Neanderthal and *Homo sapiens*. Director of the new radiocarbon laboratory, BRAVHO (Bologna Radiocarbon laboratory devoted to Human Evolution).

CONTACT DETAILS

Professor Sahra Talamo, Director
Department of Chemistry G. Ciamician,
Alma Mater Studiorum, Bologna University
Via Selmi 2, I-40126 Bologna, Italy

+39 0512 099476
sahra.talamo@unibo.it
www.unibo.it/sitoweb/sahra.talamo
@ERC_RESOLUTION
/erc_resolution

FUNDING

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme under grant agreement No.803147.