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Refining techniques for radiocarbon dating small archaeological bone samples

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Chapter six

Conclusion

Project 1

Testing the suitability of the MICADAS gas ion source for dating Palaeolithic collagen

We established a collaboration with Professor Edouard Bard and his team at CEREGE, Aix-Marseilles University, to test the accuracy, reproducibility and precision of the gas ion source of the AixMICADAS (Bard et al., 2015) to ^{14}C date small archaeological bone collagen samples. The pilot study in Chapter 2 represented the first use of a MICADAS gas ion source for dating archaeological bone collagen and for dating samples of Pleistocene age (Fewlass et al., 2017). The preliminary study was carried out on large collagen samples split into multiple aliquots. This was done to rule out any variation arising through pretreatment so we could focus the test on the instrumental accuracy, precision and reproducibility. We used three techniques of producing CO_2 from bone collagen to see what effect these had on the measurements. We determined that the optimal method of CO_2 production was the EA and zeolite trap directly coupled to the gas ion source (Wacker et al., 2013). The method is fast and automated, and the results indicated that the zeolite trap did not contribute to the instrumental background at the sample size measured. Measurements of ^{14}C from the gas ion source were statistically indistinguishable from measurements made with graphite targets. The first results demonstrated that the gas ion source system could produce accurate, reproducible results for sample sizes $<100 \mu\text{g C}$ back to 35,000 $^{14}\text{C BP}$ (Fewlass et al., 2017).

Building on the successful preliminary tests of the gas ion source, further measurements were carried out on small collagen aliquots ($<100 \mu\text{g C}$) extracted from small pieces (40 – 80 mg) of bone at varying levels of collagen preservation (Chapter 3; Fewlass et al., 2019b). This was to test the gas ion source for a wider range of samples (age, collagen preservation), explore the effect of sample size reduction in the gas interface system and determine if the extraction of small bone aliquots produced accurate and consistent results.

In the expanded study, we reduced the C sample size to determine the effect on the background level of the EA-GIS-AMS system. We compared measurements of $30 \mu\text{g C}$ to $90 \mu\text{g C}$ measured over the duration of multiple titanium targets. At both $30 \mu\text{g C}$ and $90 \mu\text{g C}$, the bone collagen

background ^{14}C measurements were equivalent to the instrument background level of equal size demonstrating that no significant carbon contamination resulted from the pretreatment. As expected, we saw a systematic effect on the background level with reduction in sample size, likely arising from the carbon contribution of the silver cups used to introduce the collagen into the EA. This can be accounted for by measuring background collagen samples of equal size to the unknown samples and using these measurements in the age correction. We observed a significant improvement in the instrumental background level of the EA-GIS-AMS system in the second study (0.4 pMC) compared to the pilot study (0.65 pMC) (see Table 6 in Chapter 2 compared to Supplementary Dataset S3 in Chapter 3). Following the pilot study, a leaking capillary in the gas interface system was identified and fixed, which improved the instrumental background level. The results reported in chapter 3 demonstrate that the limit of the gas ion source for dating samples is approximately 45,000 BP with any measurements older than this being infinite.

Although the precision achieved with the gas ion source is lower than graphite targets due to the lower ion currents, the level of precision now achievable with the MICADAS gas ion source is nevertheless useful for addressing archaeological questions, particularly for the Palaeolithic. For example, for mammoth collagen extract R-EVA 123.53, compare graphite date Aix-12003.1.1: 34390 ± 240 ^{14}C BP (988 μg C) with CO_2 date Aix-12003.10.4: 34550 ± 710 ^{14}C BP (98 μg C), measured from ten times less carbon (Fewlass et al., 2017; Fewlass et al., 2019b). In fact, the error ranges achieved with the gas ion source in these studies is similar to error ranges that have been quoted for graphite dates in the same time range over the past two decades (e.g. Trinkaus et al., 2003; Higham et al., 2011; Pleurdeau et al., 2016), although we are now moving towards unprecedented levels of precision from graphitised samples (see Chapter 4; Fewlass et al., in review).

The research described in chapters 2 and 3 clearly demonstrates the high level of accuracy and reproducibility of ^{14}C measurements with the gas ion source and the moderate level of precision which can be achieved. The results demonstrate the suitability of the gas ion source of the Aix-MICADAS for dating archaeological collagen in situations where sample material is limited (e.g. collagen yield of 1-3 mg).

Project 2

Pretreatment of <100 mg bone samples

The work detailed in Chapter 3 was undertaken in the labs at the MPI-EVA to optimize our standard collagen extraction protocol for <100 mg bone material (Fewlass et al., 2019b). Consistent yields of high quality collagen were obtained with the reduction of bone material from 500 mg to 100 mg to <50 mg. We confirmed previous observations that pretreatment of whole pieces of bone results in higher yields of collagen compared to pretreatment of powdered bone. This may imply that collagen is damaged by heat during the drilling of bone powder and/or is increasingly solubilised or lost during the various steps of pretreatment. The most significant alteration to our standard protocol for ~500 mg bone is a reduced duration of the gelatinisation step. Regular monitoring and removal of <100 mg samples from the heater block as soon as gelatinisation occurred resulted in higher collagen yields compared to leaving samples for 20 hours as per standard practice. This modification is more labour-intensive than the standard protocol, necessitating smaller numbers of samples to be prepared in tandem. However, the reduction in sample size and modifications to the pretreatment protocol means that collagen extraction and filtration of <100 mg bone can be completed in ~1 week compared to the ~2-4 weeks generally required for well preserved samples of ~500 mg bone.

Notably, the ^{14}C measurements of collagen extracts from 40-100 mg 'background' bone samples (>50,000 BP) indicate that no significant C contamination was introduced in the lab during pretreatment. This implies that the cleaning steps we routinely use for the ultrafilters sufficiently removed the humectant coating on the filter and no exogenous carbon was introduced to the >30 kDa gelatin fractions. Due to the high sensitivity of small samples to contamination, we pretreat three aliquots of the background bone (>50,000 BP) of varying sizes <100 mg alongside <100 mg samples (in order to achieve approximately the same amount of collagen as the samples) and measure them in the same batch to monitor lab based contamination.

The dates obtained from the small bone extracts were accurate and reproducible across the range of the ^{14}C timescale at various levels of collagen preservation. The dating results demonstrate that <100 mg bone samples can be successfully and consistently pretreated without introducing additional modern carbon contamination during lab work and handling, which is a key concern in the reduction of sample size.

Project 3

Pretreatment and dating of human remains from Dolní Věstonice II and Pavlov I, Czech Republic

The methods established during projects 1 and 2 were applied to small fragments of human bone from Dolní Věstonice II and Pavlov I, Czech Republic (Fewlass et al., 2019a). Extensive analysis of the human skeletal material from these sites has yielded fascinating insights into the morphology and behaviour of Gravettian populations. Human bones representing both ritual human burials and disarticulated remains were sampled for aDNA analysis in 2013, contributing a large amount of genetic information to the study of ancient European *Homo sapiens* populations (Fu et al., 2016).

Following their excavation in the 1950s and 1980s, the human remains were not directly dated in order to preserve the material from destructive analysis. However, small amounts of bone material were left over from seven individuals following the aDNA analysis in 2013. Very small aliquots of bone (37-203 mg) were sampled and pretreated using the methods described in chapter 3. Elemental and stable isotopic analysis indicated that samples were well preserved and analysis with FTIR did not show any sign of external contaminants, indicating that the extracts were suitable for ^{14}C dating. The collagen yields were sufficiently high for the ages to be cross-checked with both the gas ion source of the AixMICADAS and with solid graphite targets. The results confirm the Gravettian origin of the human bones and are in keeping with their archaeological context and previous ages obtained from the site. The replicate measurements are in agreement with each other and in some cases with dates on associated charcoals, lending confidence to their reliability. It appears that some charcoal samples from the site radiocarbon dated in the 1980s were affected by contamination, leading to underestimation of their ages. The study serves as further evidence of the suitability of the gas ion source for producing accurate results from small amounts of bone. The direct dates from the human remains will allow a more nuanced discussion of the occupation of these sites and, within a wider context, the chronology of occupation of the Middle Danube region during the Gravettian.

Project 4

Pretreatment and dating of small bone fragments from Bacho Kiro Cave, Bulgaria

A comprehensive program of radiocarbon dating was undertaken to establish a new, reliable site chronology for Bacho Kiro Cave, Bulgaria (Fewlass et al., in review). The latest methods and instrumentation in ^{14}C dating were applied to a large, high quality dataset of newly excavated material to produce a robust, reliable site chronology at exceptional levels of accuracy and precision. Ninety-five new AMS dates set the range of occupation at the site from >51,000 BP to ~35,000 cal BP, spanning the Middle to Upper Palaeolithic transition. The Initial Upper Palaeolithic (IUP) assemblage is now securely dated from 46,930-43,830 cal BP (95% probability).

The pretreatment methods established during the course of this research (chapter 3) were applied to six fragments of human bone excavated from Bacho Kiro Cave in 2016, four from the IUP layers and two from the Upper Palaeolithic layers. The bone fragments, identified through ZooMS screening, were characteristically small, leaving limited material available for direct radiocarbon dating and further molecular analysis (aDNA, palaeoproteomics). Small aliquots of the human bones (80-110 mg) were pretreated and the resulting high quality collagen extracts were dated, along with the fauna from the site, at exceptionally high precision with graphite targets at ETH Zurich in collaboration with Dr Lukas Wacker. The two human collagen extracts from the Upper Palaeolithic layers were dated with the gas ion source of the AixMICADAS to corroborate the graphite dates and further confirm the reliability of the CO_2 method, producing ages of 35,960 - 35,150 cal BP at 95% probability (F6-597; $31,660 \pm 140$ ^{14}C BP) and 34,810 - 34,210 cal BP at 95% probability (BK-1653; $30,570 \pm 120$ ^{14}C BP) (Fig. 4; Chapter 5).

The direct radiocarbon dates demonstrate that the four bone fragments from the IUP layer are the earliest remains of Upper Palaeolithic *Homo sapiens* known in Europe, dating between 46,790 - 42,810 cal BP (95% probability) in full agreement with the other dates from the IUP assemblage. Their secure association with a high density of IUP artefacts and the new robust site chronology make Bacho Kiro Cave crucial in the discussion of the early occupation of Europe by *Homo sapiens* in the Upper Palaeolithic (Hublin et al., in review).

NIR spectroscopy: a non-destructive pre-screening method for collagen preservation

We recently collaborated with Professor Matt Sponheimer (University of Colorado, Boulder) on a pilot study establishing a non-destructive method of assessing collagen preservation in bone using near infra-red (NIR) spectroscopy (Sponheimer et al., In press). This technique enables entirely non-destructive and fast pre-screening of bone to ascertain if sufficient collagen is preserved for radiocarbon dating. The proof-of-concept study demonstrates a high level of agreement between predicted and actual collagen yields following extraction with an error of prediction of $\pm 2\%$, which likely reflects the inter-lab reproducibility of replicate collagen extractions from a single bone ($\sim 1.7\%$). The NIR instrument is ruggedized and small enough to take as hand luggage during travel so the analysis can take place onsite at excavations or museums, circumnavigating the complex issue of exporting precious material or removing them from the safety of museums. This method was successfully utilized for the human burial remains from Dolní Věstonice II and Pavlov I, described in chapter 4. In future, this innovation will allow us to selectively sample bone where chances of successful collagen extraction are high and has profound implications for minimising destruction to precious bone artefacts.

Archaeological implications

We can successfully and reproducibly pretreat <100 mg Palaeolithic bone material for radiocarbon dating. When collagen extraction produces suitably high yields (>3mg), ^{14}C dates at very high precision can be achieved with graphite targets using the MICADAS AMS. When the extraction of extremely small amounts of bone material or low levels of preservation yield 1-3 mg collagen, the gas ion source of the MICADAS offers an accurate and reproducible method of ^{14}C measurement, but the quality of each sample should be carefully assessed before measurement on a case by case basis.

Using much smaller amounts of bone for radiocarbon dating (Fig. 1) greatly increases the possibilities for directly dating precious artefacts. The research described in this thesis contributes 13 more directly dated individuals to the collection of reliably dated Upper Palaeolithic *Homo sapiens*, including the earliest remains yet identified, in Europe (Fig. 2). By minimising sample destruction, these methodologies have great potential for further applications to small or precious bone artefacts with a high patrimonial value to address significant archaeological questions.

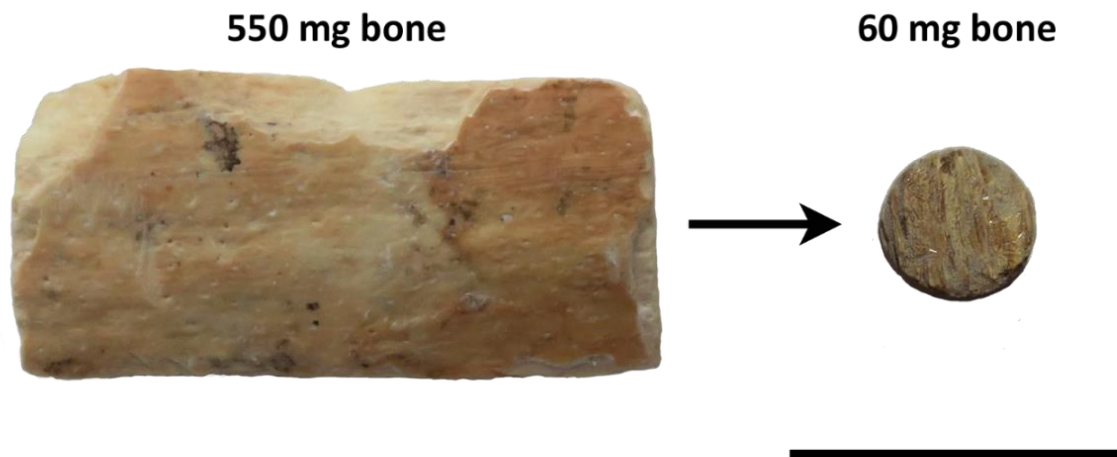


Figure 1. Comparison of the amount of Palaeolithic bone material pretreated with our standard protocol (left) and with the method detailed in Chapter 3 that can be used to radiocarbon date small or precious bone samples (right). Scale bar is 1 cm.

The Middle to Upper Palaeolithic Transition

The makers of the so-called 'Transitional' industries present in sites straddling the Middle-to-Upper Palaeolithic transition across Eurasia is a topic of much discussion (D'Errico et al., 1998; Churchill and Smith, 2000; Mellars, 2005; e.g. Hublin, 2013; Hublin, 2015). These industries are stratigraphically sandwiched between underlying Middle Palaeolithic assemblages, produced by Neanderthals, and overlying Upper Palaeolithic assemblages, made by *Homo sapiens*. One such industry, the Châtelperronian, known in western and south-western France and north-eastern Spain, consists of blades, pigments, bone tools and personal ornaments. Whilst the Châtelperronian has an Upper Palaeolithic character, it shows similarities with the Mousterian of Acheuleun Tradition type B (see Soressi and Rousset, 2014) and is associated with Neanderthal remains at several sites (Lévêque and Vandermeersch, 1980; Hublin et al., 1996; Bailey and Hublin, 2006; Welker et al., 2016).

The Châtelperronian layers at Grotte-du-Renne at Arcy sur Cure are notable for the presence of numerous Neanderthal teeth and other fragmented bones alongside a significant number of decorated bone tools, personal body ornaments and large amounts of pigment. However, it has been suggested that the association is the result of vertical mixing between layers. Bar-Yosef and Bordes (2010) suggested that the association is the result of re-working of Neanderthal fossils from the underlying Mousterian layers, whereas Higham et al. (2010) interpreted an inconsistent series of radiocarbon dates from the site as evidence of Upper Palaeolithic artefacts moving downwards through the stratigraphy. This was subsequently challenged based on the stratigraphic integrity of the lithic assemblages at the site and the inconsistent ¹⁴C results were attributed to poor collagen preservation and incomplete sample decontamination (Caron et al., 2011). A more recent series of dates on un-consolidated samples selected for good collagen preservation produced stratigraphically consistent results, supporting the association of the Neanderthal fossils and Châtelperronian assemblages at Grotte du Renne (Hublin et al., 2012). A palaeoproteomic study in 2016 identified 28 additional Neanderthal bone fragments from the Châtelperronian layers at Grotte du Renne, and direct ¹⁴C dating of one such specimen (Fig. 2) firmly placed it within the Châtelperronian age range (Welker et al., 2016). Direct dates from small amounts of material from the Châtelperronian ornaments could resolve the question of the contemporaneity of the personal ornaments with the Neanderthal remains.

A fragmented maxilla and three teeth (re-fitted post-excavation) were excavated from Kent's Cavern, UK, in 1927 and since their discovery have been identified as Upper Palaeolithic *Homo sapiens* (Keith, 1927). In the 1980s, the maxilla (KC4) was directly AMS radiocarbon dated to 30,900 ± 900 ¹⁴C BP (OxA-1621; 37,430-33,410 cal BP at 2σ), which supported its Upper Palaeolithic assignment and, at the time, made it the oldest hominin to have been directly dated by ¹⁴C methods (Hedges et al., 1989). It has since been argued that the direct date of KC4 was an

under-estimate of its true age due to incomplete removal of conservatives during sample pretreatment (Jacobi et al., 2006; Higham et al., 2011). In 2011, a second attempt to obtain a direct date using ultrafiltration failed when a small sample of tooth root yielded very little collagen (89 mg dentine powder resulted in 0.38 mg collagen [0.4% weight]) (Higham et al., 2011). As a second direct date was not possible, Higham et al. (2011) used Bayesian techniques to estimate an age for KC4 of 44,180-41,530 cal BP (2σ) based on dates from ultrafiltered collagen from fauna located above and below the maxilla. The validity of this strategy has been strongly questioned based on the lack of reliable contextual information from the 1920s excavation and it has been suggested that the original direct date is more in keeping with the archaeological evidence (White and Pettitt, 2012; Zilhão, 2013). White and Pettitt (2012) stated that “Radiocarbon dating of unmodified fauna from sites with questionable stratigraphies should not be used to suggest the apparent age of human taxa. [...] Without a new direct ultrafiltration date, [...] the age of KC4 will [...] never be conclusively resolved.” The authors have defended their techniques and, after incorporating further AMS dates of associated fauna into their model, have provided an even more precise estimate for the age of KC4 from 42,350-40,760 cal BP, although they acknowledge that the new AMS dates indicate some post-depositional mixing between layers likely occurred (Proctor et al., 2017). They conclude that estimate can only be tested by direct dating of the maxilla, which “...will not be possible until further technical developments for dating very small samples are more routinely available” (Proctor et al., 2017).

As KC4 is the only *Homo sapiens* fossil from north-western Europe $\geq 35,000$ cal BP its age is crucial in determining the duration and range of overlap between *Homo sapiens* and Neanderthals in this region. The original date for KC4 demonstrates that some collagen is preserved in the maxilla, although details on the chemistry are not provided in the 1989 datelist (Hedges et al., 1989). The results described in Chapter 3 (Fewlass et al., 2019b) demonstrate that in general much lower yields of collagen result from the pretreatment of small amounts of powdered bone compared to whole bone, which likely contributed to the failure of pretreatment outlined in Higham et al (2011). A re-dating program for KC4 could employ NIR pre-screening to assess the level of collagen preservation across the maxilla. The sampling of a tooth root, as attempted by Higham et al. 2011, may somewhat circumnavigate the issue of conservatives and would be less visually invasive. As the fragmented maxilla was found separately from the three teeth, the direct dating of the KC4 bone or tooth should be conducted on the same sample where any possible future DNA sampling would occur. An ultrafiltered collagen extract from <100 mg bone/dentine could provide an accurate radiocarbon date, either through AMS dating with a graphite target or with the gas ion source of the MICADAS. A reliable direct date would resolve the on-going controversy over the early presence of *Homo sapiens* in north-western Europe.

Prior to the discovery of *Homo sapiens* remains in the IUP layers at Bacho Kiro Cave (Fewlass et al., in review; Hublin et al., in review), the oldest known remains of our species in Europe was the

Pestera cu Oase 1 mandible recovered from a cave in Romania in 2002. Morphological analysis identified the mandible as *Homo sapiens* with some archaic features indicative of admixture with Neanderthals (Trinkaus et al., 2003). aDNA analysis later showed that 6-9% of the Oase 1 nuclear genome was derived from Neanderthals, indicating a *Homo sapiens*-Neanderthal admixture event occurred 4-6 generations (<200 years) before Oase 1 lived (Fu et al., 2015).

No archaeology accompanied the human remains so direct radiocarbon dating was necessary to establish the age of the fossil. The first attempt at Oxford (350 mg bone) using ultrafiltration produced a very low yield of collagen (1.5 mg/0.4%) with an acceptable C:N value (C:N=3.3) which produced a minimum age of >35,200 ¹⁴C BP (OxA-11711). A second attempt to date the mandible at Groningen (706 mg bone) without ultrafiltration resulted in a higher collagen yield (28.5 mg/4%) but with a C:N value outside the range generally considered suitable for reliable ¹⁴C dating (C:N=2.6). The collagen extract was dated to 34,290 +970, -870 ¹⁴C BP (GrA-22810). The two dates were combined (34,950 +990, -890 ¹⁴C BP), giving a wide calibrated range of 41,760-37,310 cal BP (Trinkaus et al., 2003; Trinkaus, 2013).

The face and fragmented cranium of another *Homo sapiens* individual (Oase 2) was also found in the cave. The first two attempts to directly date the cranium failed due to very poor collagen preservation and a third attempt yielded a minimum age of 28,980 +180, -170 ¹⁴C BP (GrA-24398), although the authors suggest that Oase 2 is roughly contemporary with Oase 1 (Rougier et al., 2007; Trinkaus, 2013).

As the current dates confirm the early Upper Palaeolithic origin of the Oase fossils, further sampling is considered unnecessarily destructive (Trinkaus, 2013). In light of the early direct dates of *Homo sapiens* remains from south-eastern Europe at Bacho Kiro Cave and forthcoming improvements in resolution of the calibration curve in this time range (Talamo et al., 2017; Cheng et al., 2018; Reimer et al., 2018), a high precision direct date from <100 mg bone from Oase 1 would play an important role in determining the duration of overlap between *Homo sapiens* and Neanderthals in central Europe (Fig. 2).

Whilst the Aurignacian technocomplex is widely accepted as a proxy for the presence of Upper Palaeolithic *Homo sapiens* in Europe, very few human remains have been found in secure association with diagnostic assemblages (Churchill and Smith, 2000; Mellars, 2006a). The rare (and relatively large) assemblage of human fossils from Mladeč (Czech Republic) has been directly dated (without ultrafiltration) to ~31,000 ¹⁴C BP (Wild et al., 2005), but the majority of human remains associated with Aurignacian contexts are isolated teeth or fragmentary bones and few have been directly dated (see Ahern et al., 2013).

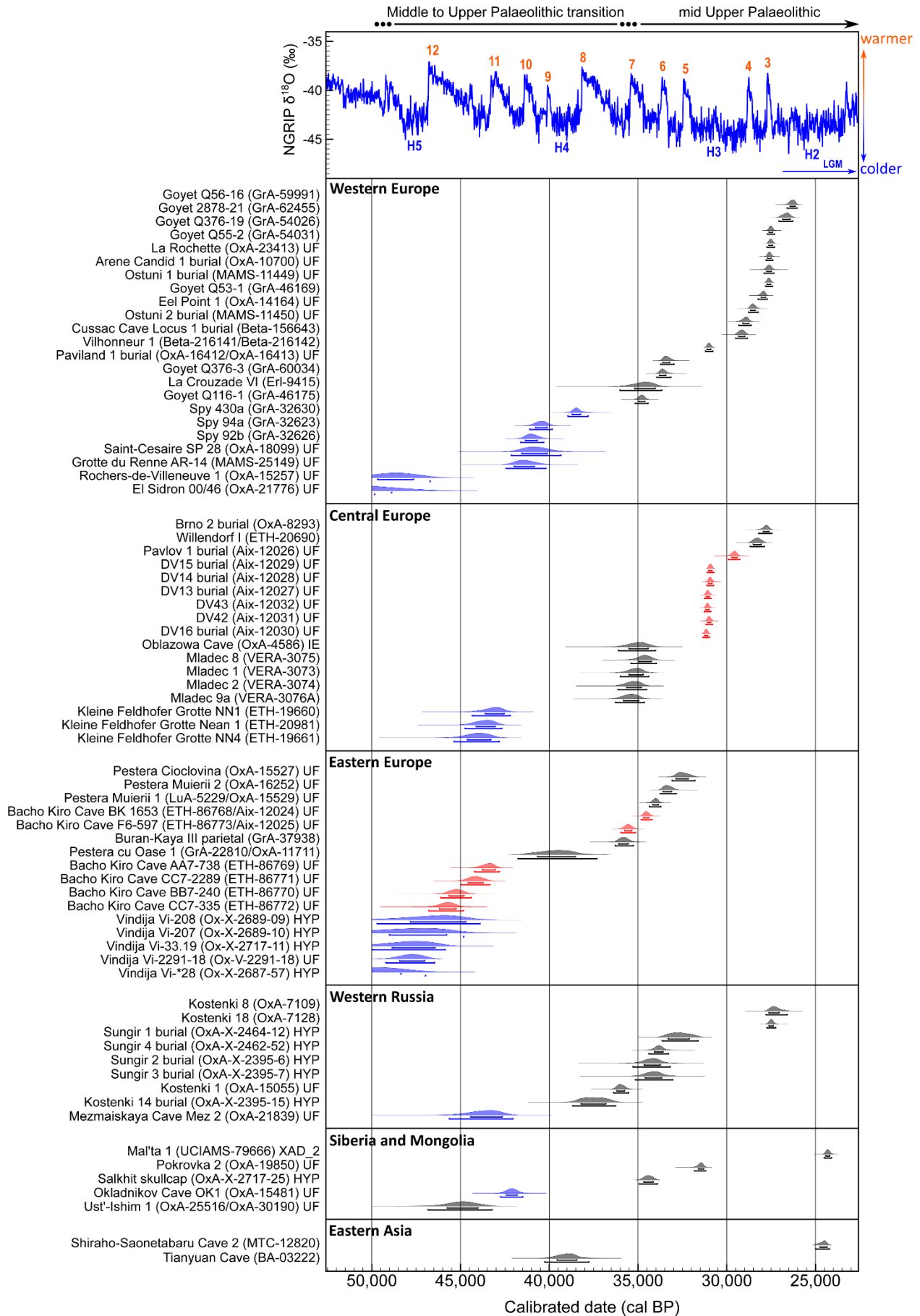


Figure 2. Calibrated ranges of direct ^{14}C AMS dates of human remains in Eurasia dating between 50,000-25,000 cal BP on bulk collagen, filtered collagen (UF/XAD_2/IE) or isolated amino acids (HYP) (where specified in source publication). Homo sapiens are shown in black (existing dates) and red (this thesis) and Neanderthals are shown in blue. Sample ID and AMS lab number shown on the left. Dates were calibrated using the IntCal13 dataset (Reimer et al., 2013) in OxCal 4.3 (Bronk Ramsey, 2009). Where two statistically indistinguishable dates are available from one bone the dates have been combined (R_Combine) in OxCal. Dates are shown in comparison to the NGRIP (GICC05) $\delta^{18}\text{O}$ record (Svensson et al., 2008) which is a proxy for Northern Hemisphere palaeoenvironmental conditions (Greenland Interstadial numbers, Heinrich events (H5, H4, H3, H2) and Last Glacial Maximum (LGM) are indicated). References and pretreatment information are included in Appendix 1.

Mid Upper Palaeolithic

In comparison to the preceding early Upper Palaeolithic, mid Upper Palaeolithic human remains are relatively abundant. The discovery of both ritualistic and isolated human remains from Gravettian contexts across Eurasia have provided a wealth of morphological, behavioural and genetic insights into Gravettian life and have in particular sparked much discussion about variation in funerary practises (Pettitt, 2011; Trinkaus and Buzhilova, 2018). However, the wider interpretation of these remains is hindered by a lack of accurate, precise direct radiocarbon dates.

The Gravettian technocomplex is wide-spread across Europe and similarities in burial practises (grave goods, ochre, multiple internments) have been observed across large areas (Pettitt, 2011). A trend of increasing richness in burial goods over time was observed by Svoboda (2008), in particular reference to the exceptionally rich single burials at Brno in Moravia (Oliva, 1999; Pettitt and Trinkaus, 2000) and Arene Candide, Italy (Pettitt et al., 2003) and the spectacular burials discovered at Sunghir, Russia, all of which were originally dated to the later Gravettian period (Trinkaus et al., 2014). Over the past two decades, nearly 20 radiocarbon dates ranging from ~30,000-20,000 ^{14}C BP have been made from the four Sunghir burials using various collagen extraction methods (Pettitt and Bader, 2000). The most recent dates suggest the burials date to the early-mid Gravettian (Marom et al., 2012; Kuzmin et al., 2014; Nalawade-Chavan et al., 2014), which conflicts with the theory of a temporal trend in increasing burial richness. Direct dating has demonstrated that several human burials originally assumed to be Gravettian are in fact Holocene intrusions (Trinkaus and Pettitt, 2000; Svoboda et al., 2002; Tillier et al., 2009).

Recent excavations at Borsuka Cave, Poland, uncovered six deciduous human teeth and 112 pendants made of herbivore teeth spread across 4x3 m² (Wilczyński et al., 2016). The assemblage was interpreted as a disturbed infant burial. Two of the pendants were radiocarbon dated to 27,350 ± 450 ^{14}C BP (Poz-32394: 68.2%: 31,640-30,930 cal BP) and 25,150 ± 160 ^{14}C BP (Poz-38236: 68.2%: 29,400-28,980 cal BP) and a reindeer metatarsus from the same layer was dated to 26,430 ± 180 ^{14}C BP (Poz-38237) (Wilczyński et al., 2012; Wilczyński et al., 2016). Although the layer lacked diagnostic lithics, the burial was associated with the Pavlovian culture based on the

contemporaneity of the dates with the burial contexts at Dolní Věstonice, Pavlov and Predmosti (see Chapter 5). The lack of agreement between the ^{14}C dates from the two pendants (outside 2σ) raises the question of the association of the pendants to each other, and further, the human teeth with the pendants which forms the basis of the interpretation of a burial. Considering the lack of diagnostic lithics, direct dating of small samples of dentine from the human teeth and pendants could not only confirm whether the human remains fall within the Gravettian time period but also resolve the question of the contemporaneity of the human remains with the pendants, providing a more robust foundation for the inclusion of this burial in the wider discussion of Gravettian funerary practices.

Radiocarbon dating: an evolving field

The absolute nature of radiocarbon enables us to explore broad patterns of human behaviour across time and space (e.g. Mellars, 2006b; Hublin, 2015; Bae et al., 2017). Yet bearing in mind the problems associated with dating in the Palaeolithic period, large-scale statistical models built on existing dates of varying reliability have limited use. In order to circumnavigate these problems, large-scale dating and re-dating programs have been undertaken to generate new AMS radiocarbon dates using rigorous pretreatment methods and robust quality criteria (Higham et al., 2014). The integration of radiocarbon data with other dating techniques and chronometric markers are further approaches undertaken to improve the robusticity of large-scale analyses (Lowe et al., 2012; Davies et al., 2015). Recently, Staubwasser et al. (2018) inferred patterns of depopulation and re-population based on climatic cycles by linking cold, arid periods recorded in stable isotopes in speleothems from the Carpathians with archaeologically sterile layers in Eurasian Middle to Upper Palaeolithic sites. Improved accuracy and higher precision in archaeological chronologies (Fewlass et al., in review) will facilitate closer links between human presence and specific climatic events at increasingly high resolution.

Whilst the extension of the calibration curve back to 50,000 BP (Reimer et al., 2009) represents a huge achievement for researchers working on the chronology of the late Middle and Upper Palaeolithic, the low precision of the curve beyond the dendrochronological record has been the ultimate limit to the chronological resolution possible from high precision measurements. The improvements to the forthcoming calibration curve IntCal19 (Cheng et al., 2018; Reimer et al., 2018) and future work to extend the dendrochronological portion of curve beyond 14,000 BP should greatly increase the accuracy and precision of calibration in this period. This adds greater significance to the need to obtain accurate and precise radiocarbon measurements directly from important Palaeolithic human remains and artefacts. Accuracy and precision at both the ^{14}C measurement and calibration stage are essential for refining the chronology of the arrival and

spread of *Homo sapiens* across Eurasia during the Upper Palaeolithic (Hublin, 2012; 2015; Bae et al., 2017).

As demonstrated over the past 70 years, radiocarbon dating is a continually evolving field, driven forwards by developments in both technology and understanding. The MICADAS represents a huge advance in accuracy and precision for radiocarbon dating in archaeology. An increasing number of AMS facilities across the globe now house a MICADAS, thanks to its compact size and relatively low maintenance costs, meaning that the methods explored in this dissertation have the potential for wide spread application. The field will continue to benefit from improvements in instrumentation and pretreatment methods and will likely see further advances in accuracy and precision from decreasing sample sizes. The results of this project are intended to contribute to a more robust chronological framework for the Upper Palaeolithic period whilst preserving precious archaeological material for future generations.

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