Refining techniques for radiocarbon dating small archaeological bone samples

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Chapter Three

Pretreatment and gaseous radiocarbon dating of 40–100 mg archaeological bone

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Published in *Scientific Reports,* 2019, volume 9, article number: 5342
DOI: 10.1038/s41598-019-41557-8

SCIENTIFIC REPORTS

OPEN

Pretreatment and gaseous radiocarbon dating of 40–100 mg archaeological bone

Received: 8 October 2018
Accepted: 11 March 2019
Published online: 29 March 2019

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Radiocarbon dating archaeological bone typically requires 300–1000 mg material using standard protocols. We report the results of reducing sample size at both the pretreatment and ^{14}C measurement stages for eight archaeological bones spanning the radiocarbon timescale at different levels of preservation. We adapted our standard collagen extraction protocol specifically for <100 mg bone material. Collagen was extracted at least twice (from 37–100 mg material) from each bone. Collagen aliquots containing <100 µg carbon were measured in replicate using the gas ion source of the AixMICADAS. The effect of sample size reduction in the EA-GIS-AMS system was explored by measuring ^{14}C of collagen containing either *ca*. 30 µg carbon or *ca*. 90 µg carbon. The gas dates were compared to standard-sized graphite dates extracted from large amounts (500–700 mg) of bone material pretreated with our standard protocol. The results reported here demonstrate that we are able to reproduce accurate radiocarbon dates from <100 mg archaeological bone material back to 40,000 BP.

Bone is one of the most frequently radiocarbon-dated materials recovered from archaeological sites. However, many precious archaeological bones, such as human remains or Palaeolithic bone tools, are too small or valuable for extensive destructive sampling. The reduction of sample size to enable direct dating of precious bone is therefore a key concern for the archaeological community.

In the 1960s and 1970s, gas proportional counters required many grams of bone to produce a radiocarbon date[1,2]. The development and utilisation of Accelerator Mass Spectrometers (AMS) in the 1980s represented a revolutionary step in the reduction of sample size and time required for dating[3]. Routine measurements today typically require 500–1000 micrograms of carbon (µg C) to produce a high precision date. In recent years, several AMS labs have worked on modifications to the graphitisation and AMS measurement process for smaller samples containing <500 µg C[4–13]. However, the graphitisation of small sample sizes is often time consuming and can be prone to large contamination effects[14,15]. A recent study by Cersoy, *et al*.[16] demonstrated that graphite targets containing *ca*. 200 µg C from archaeological bones can be successfully produced and measured using the IonPlus Automated Graphitisation Equipment III (AGE 3)[17] and MIni CArbon DAting System (MICADAS)[18,19] developed at ETH Zurich. However, the hybrid nature of the MICADAS system offers an alternative solution to the complex process of graphitising small samples. Organic samples containing <100 µg C can be placed into an elemental analyser (EA) directly coupled to the gas ion source of the MICADAS via the gas interface system (GIS)[15,18,20–24]. The automated system reduces both sample preparation time and the risk of contamination through handling, and has been successfully utilised in environmental and climatic applications[23,25–28]. In our preliminary study[29] we demonstrated that the gas ion source of the AixMICADAS[30] is suitable for dating bone collagen CO_2 samples of <100 µg C back to 35,000 BP (uncalibrated radiocarbon years before AD 1950).

However, as sample size is reduced the effect of contamination during pretreatment and measurement increases greatly. Sample pretreatment involves the extraction and purification of carbon endogenous to the original bone. Any contamination remaining in the sample at the time of dating can lead to erroneous results. The effects become increasingly catastrophic with the increasing age of the sample due to the minute concentrations of residual ^{14}C. For example, in a bone extract *ca*. 40,000 BP, 1% modern carbon contamination would skew the resulting ^{14}C age by over 7,000 years.

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It is standard practice to extract the proteinaceous portion of bone for ^{14}C measurement, generally referred to as 'collagen'[31]. Although collagen forms around 22% weight of modern bone, degradation following death and burial makes collagen extraction increasingly challenging with advancing age[32]. Whilst the minimum threshold for reliable ^{14}C dating is generally considered to be 1%[32], it is common for the collagen portion of Palaeolithic bone to constitute <10% weight. The lower the level of collagen preservation, the more bone must be pretreated to obtain sufficient material to assess the quality of the extract (i.e. isotopic and elemental analysis) and for ^{14}C dating. Therefore, 300–1000 mg material is commonly sampled for dating Palaeolithic bones.

The majority of ^{14}C labs follow collagen extraction protocols based on Longin[33]. This involves demineralisation of either powdered bone or bone chunks using hydrochloric acid (HCl) followed by gelatinisation of the collagen in weakly acidic water and freeze-drying of the final extract. Different labs vary in the strength of reagents used, the duration of treatments and the inclusion of further decontamination steps. Many studies have been published comparing the collagen yields and isotopic values of the various extraction protocols published in the literature[34–38] as variations in pretreatment conditions can lead to differences in the quantity and quality of the final extracts. The addition of an ultrafiltration step, first proposed by Brown, *et al.*[39] has in particular improved the accuracy of ^{14}C dating of Palaeolithic bones[40]; gelatinised samples are filtered to concentrate large (>30 kDa) molecules to produce a 'cleaner' collagen extract. The technique is not unanimously agreed upon due to the risk of contamination from the humectant-coated filter[41], the effectiveness of the application[37] and the loss of collagen during filtration[34]. However, stringent cleaning steps have been established[42–44] and in many cases the re-dating of ancient bones with ultrafiltration methods has produced much older dates than previous measurements from non-ultrafiltered extracts[40,45,46]. The collagen pretreatment protocol routinely applied to Palaeolithic bone at the Max Planck Institute for Evolutionary Anthropology (MPI-EVA, Leipzig, Germany) is based on a modified Longin plus ultrafiltration protocol[36] and has a strong track record of obtaining high yields of high quality collagen from *ca*. 500 mg samples of Palaeolithic bone[47].

The aim of this study was to determine a suitable method to pretreat <100 mg bone material and further investigate if the gas ion source of the AixMICADAS[29,30] at CEREGE (Centre de Recherche et d'Enseignement de Geosciences de l'Environnement, Aix-en-Provence, France) is suitable for measuring small archaeological bone samples with sufficient accuracy and precision. We investigated the effect of sample size reduction at both the pretreatment and gas measurement stages. Tests were performed on a set of eight archaeological bones ranging from 1% to >10% collagen preservation known to date from >50,000–1,400 BP. Each bone was pretreated multiple times from starting weights of 37–100 mg bone material. Each collagen extract was split and dated multiple times with the gas ion source of the AixMICADAS to test replicability. The gas dates were compared with graphite dates from collagen extracted from >500 mg material of the same bones. We further compared gas dates of *ca*. 30 µg C and *ca*. 90 µg C to explore the effect of sample size on the blank level of the EA-GIS-AMS system. The results demonstrate our ability to obtain accurate and moderately precise radiocarbon dates from <100 µg C extracted from 37–100 mg bone material back to 40,000 BP. The methods described will be used to extract and ^{14}C date collagen from precious archaeological bone artefacts with minimal sample destruction.

Results

Bone pretreatment. Prior to this study, 500 to 700 mg of each bone had been pretreated using our standard collagen extraction protocol[36]. The extracts were analysed by EA-IRMS at the MPI-EVA to assess their suitability for dating (C%, N%, C:N, $\delta^{13}C$, $\delta^{15}N$) and were measured at the Klaus-Tschira-AMS lab in Mannheim, Germany (lab code: MAMS). The same collagen extracts from R-EVA 1489, R-EVA 123 and R-EVA 124 were also dated at the AixMICADAS facility to cross-check the ages[29]. The results were used as a reference for the preparation of small (<100 mg) aliquots of bone.

Modifications to our standard pretreatment protocol were carried out for five bones (Fig. 1): three relatively 'well-preserved' (>10% collagen preservation) archaeological bones (Fig. 1a,b,e) and two 'poorly-preserved' bones (<5% collagen preservation) (Fig. 1c,d). Once we had determined the optimum pretreatment protocol for <100 mg material, we applied this to three more archaeological samples: R-EVA 1489, R-EVA 1905 and R-EVA 1860 (two extracts per bone) (pretreatment information shown in Supplementary Dataset S1).

The standard practice in our lab is to extract large bone aliquots (*ca*. 500 mg material) as a solid piece. Although this method requires a large time investment (demineralisation can take up to four weeks with the HCl 0.5 M changed twice per week), we observe much higher collagen yields using this technique compared to powdered extracts of equal starting weight. Small aliquots (<100 mg) of the test bones were initially pretreated as both fine powder and as solid chunks. For solid pieces of bone, in most cases the collagen yield from small extracts (<100 mg) equalled or exceeded the collagen yields of large extracts (500–700 mg material) and no difference was observed between aliquots of 50 mg bone compared to 70 mg or 100 mg bone material (Fig. 1). In contrast, the powdered aliquots of well-preserved bones generally yielded around half the amount of collagen compared to solid pieces, in line with our observation for large starting weights of bone. Powdered aliquots from the poorly preserved bones either yielded nothing or small amounts (<1 mg) of crumbly yellow material. Due to the poor results from the pretreatment of powdered samples, our protocol for small amounts of bone is based on the extraction of solid pieces as per our standard protocol for larger aliquots. The pretreatment information for powdered extracts is included in the supplementary information.

We initially applied our standard collagen extraction protocol to <100 mg bone material of the well-preserved bones. Three steps of the pretreatment protocol were then modified to see what effect this had on the collagen yield and quality of extracts from small bone aliquots (Fig. 1): step (1) the duration of the demineralisation stage; step (2) the strength of HCl during the demineralisation stage; step (3) the temperature and duration of the gelatinisation stage. Bone collagen yields along with elemental (C%, N% and C:N) and stable isotopic data ($\delta^{13}C$ and $\delta^{15}N$) were used to evaluate the extracts from the different methods. In addition, Fourier Transform Infrared Spectroscopy (FTIR) was used to double check the preservation of the extracted collagen, and to detect

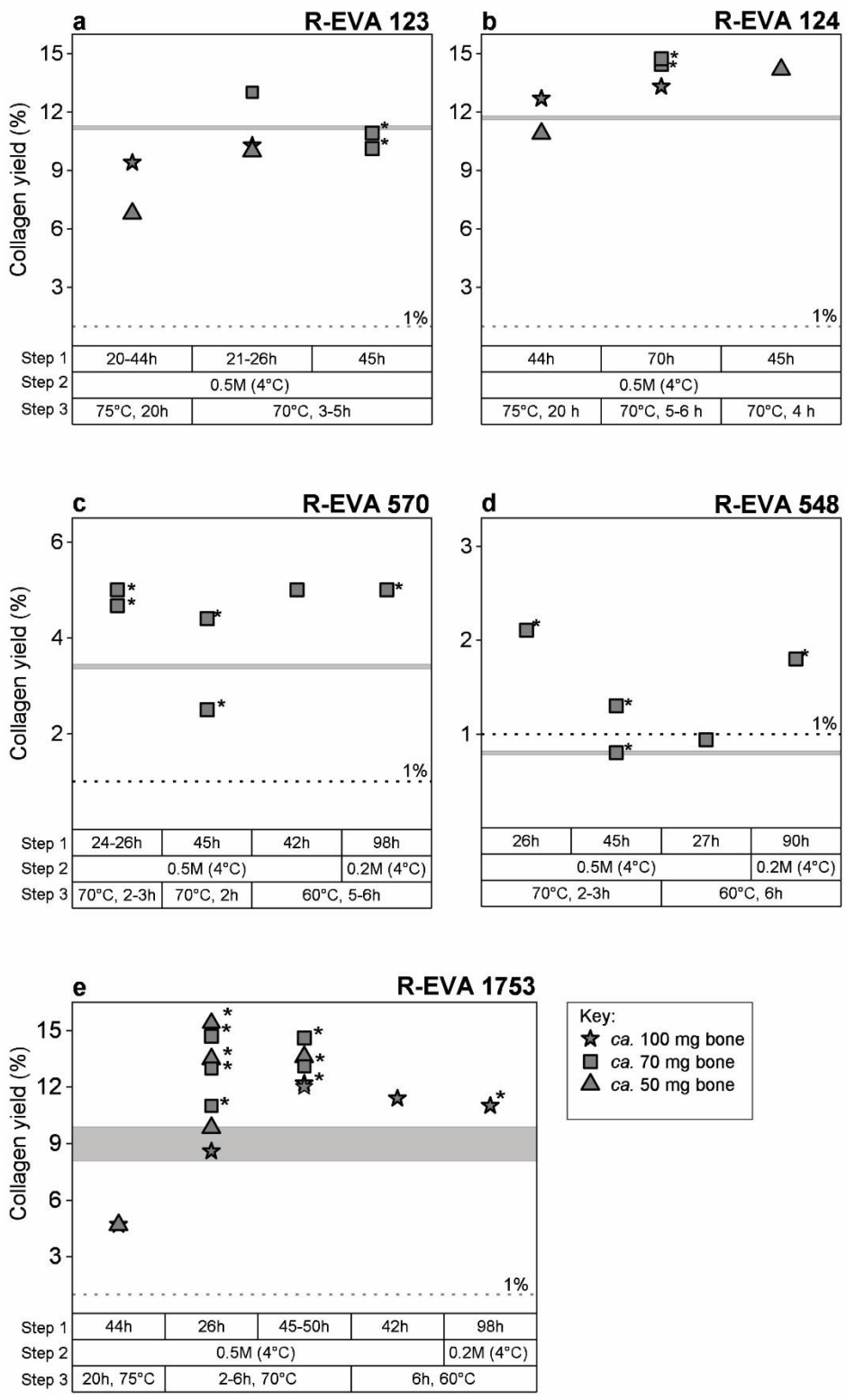

Figure 1. Graphs showing the collagen yields from small aliquots of bone according to variations in pretreatment conditions: (**a**) R-EVA 123, (**b**) R-EVA 124 (**c**) R-EVA 570, (**d**) R-EVA 548 and (**e**) R-EVA 1753. Step 1: duration of the demineralisation stage. Step 2: strength of HCl during demineralisation. Step 3: duration and temperature of the gelatinisation stage (HCl pH3). In (**a**–**d**) the horizontal grey line shows the collagen yield from a large aliquot (>500 mg material) of the same bone. A higher number of data points are present for R-EVA 1753 (**e**) as an aliquot of this bone was extracted alongside each batch of samples. The horizontal grey band in e shows the range in collagen yield of repeated large extractions from the background bone. The dashed lines at 1% show the guideline minimum requirement for reliable ^{14}C dating. Asterisks mark extracts which were dated using the gas ion source (see Fig. 3).

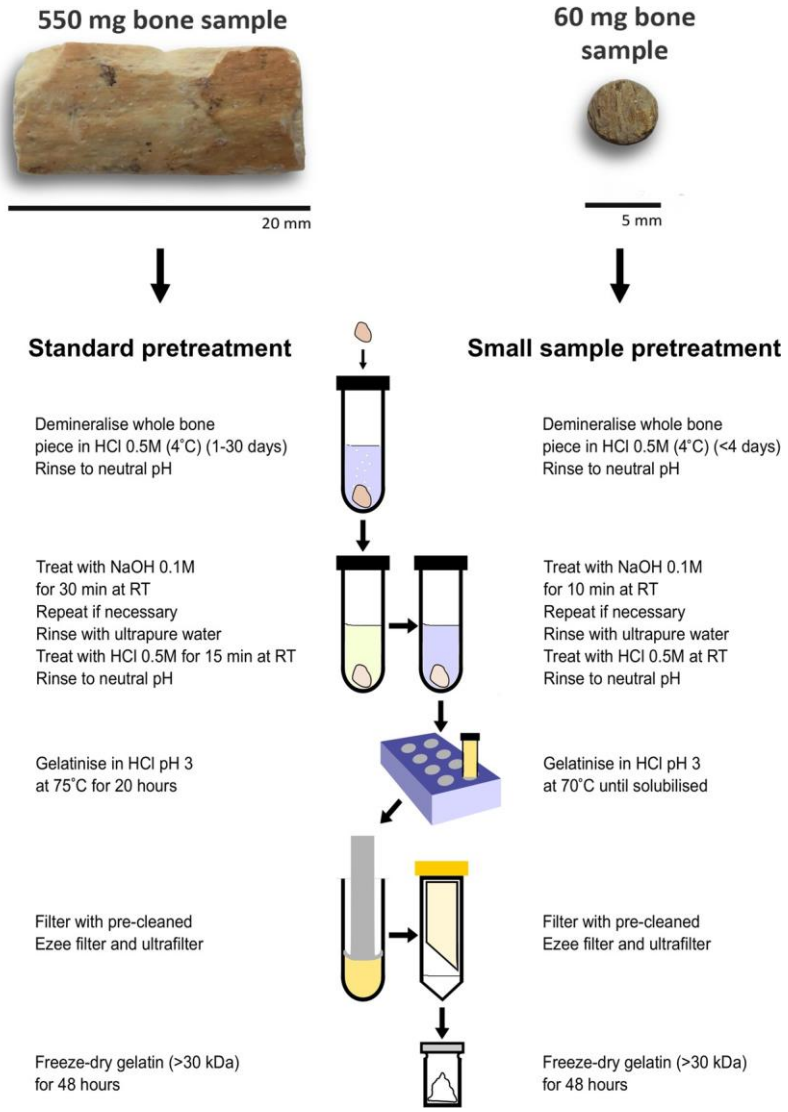

Figure 2. Summary of bone pretreatment protocols used at the MPI-EVA for large (left) and small (right) bone samples.

the presence of possible carbon contaminants[31,48,49]. Detailed pretreatment information for all extracts can be seen in Supplementary Dataset S1.

For the poorly preserved bones (Fig. 1c: R-EVA 570 and Fig. 1d: R-EVA 548) the pretreatment was softened in order to minimise collagen loss during the extraction. The weaker HCl (0.2 M) (step 2) and lower gelatinisation temperature (60 °C) (step 3) required a greater time investment and did not necessarily increase the yield of collagen compared to using stronger acid (HCl 0.5 M) during demineralisation and higher temperatures (70 °C) during gelatinisation. For the poorly preserved samples, demineralisation in HCl 0.5 M generally occurred after one day (4 °C). As Schoeninger, *et al*.[50] observed that one disadvantage of extracting collagen from solid chunks was the tendency for incomplete demineralisation, several extracts were demineralised in HCl 0.5 M for two days. This resulted in lower collagen yields for the poorly preserved bones and in the case of R-EVA 548, the yield of these extracts was so low that the extracts were affected by C contamination to a large extent.

During the gelatinisation stage (step 3), the collagen yield was higher from aliquots which were removed from the heater block as soon as solubilisation had occurred compared to those left on the heater block for 20 h as per our standard protocol for >500 mg. For all bone samples >30,000 BP, solubilisation occurred in <6 h (Fig. 1), whereas R-EVA 1489 and R-EVA 1905 required up to 27 h for full solubilisation (Supplementary Dataset S1).

Of the extracts dated, two (R-EVA 548.13 and R-EVA 548.14) fell close to or under the minimum threshold (1%) for reliable ^{14}C dating (Supplementary Dataset S1). There were small variations in elemental values between pretreatments of the same bone but all values (Supplementary Dataset S1) fell within the accepted ranges of 'well-preserved' collagen[32]. The stable isotopic values were in keeping with the palaeodietary expectations for each animal and were consistent between extracts. Analysis with FTIR was performed for all collagen extracts; each extract dated had a spectra characteristic of well-preserved collagen when compared to library spectra (see

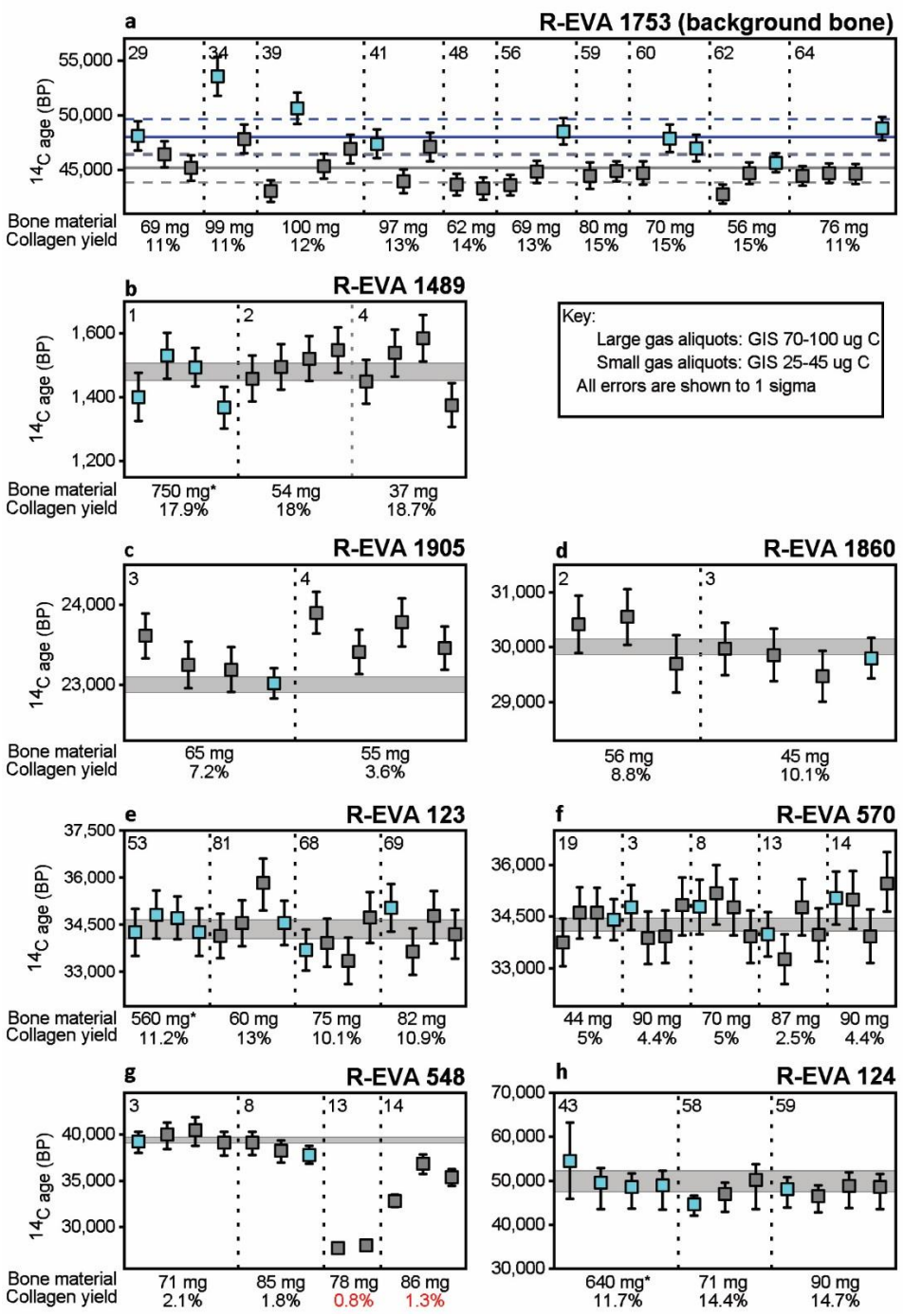

Figure 3. ^{14}C gas measurements of small (25–40 μgC) and large (70–100 μgC) aliquots of collagen extracted from eight bones (**a**–**h**) spanning the ^{14}C time range. Each data point shows the ^{14}C age (BP) and 1σ error (years) of a single EA-GIS-AMS measurement. a) Shows the uncorrected measurements of background bone R-EVA 1753 (>50,000 BP). An aliquot of this bone was prepared alongside every batch of samples from sampling to measurement to monitor contamination introduced during sample preparation. These measurements were used in the age calculation of the other archaeological samples (**b**–**h**), according to session, size (small or large) and type (solid bone extract). The arithmetic mean and associated SD of system blank (IAEA-C1/phthalic anhydrite) measurements are shown as a solid horizontal blue line and dashed blue lines respectively for large 80–100 μgC measurements and as a solid horizontal grey line and dashed grey line for small 25–40 μgC measurements. For all gas measurements in graphs b-h: the absolute error of the blank has been set to 0.001 and an external error of 3.5‰ has been added to all measurements based on the long term standard deviation of standards. Dates >15,000 BP have been rounded to the nearest 10 years. Asymmetrical errors are shown where $F14C \leq 1\sigma*10$. Grey shaded bands show the 1σ range of graphite dates measured from large extracts of the same bone. In a-h, the vertical dotted lines separate different collagen extracts of the same bone with the bone starting weight and collagen yield shown below. The number in the top left of each section is the preparation number of the bone, corresponding to Supplementary Dataset S1. Asterisks mark collagen extracts dated with the gas ion source reported in Fewlass, *et al.*[29].

Supplementary Fig. S3). Considering the collagen yields and ^{14}C measurements, the optimum pretreatment protocol for small aliquots of bone (<100 mg) is shown in Fig. 2.

^{14}C dating. For each of the bones, several collagen extracts (bone weight ranging from 37–100 mg, marked with asterisks in Fig. 1) were dated using the EA-GIS-AixMICADAS (Fig. 3). Each collagen extract was split and measured multiple times. Between two and four replicates were measured containing *ca*. 30–40 µg C, run for the duration of one titanium (Ti) target (*ca*. 12 minutes) and for each bone >20,000 BP, a single aliquot containing *ca*. 80–90 µg C was measured over the duration of three targets to increase precision (see Supplementary Dataset S2). The gas ages obtained were compared to one or more graphite dates measured from collagen extracted from 500–700 mg bone material (Supplementary Dataset S2). Discussed here are measurements made from collagen extracted from solid pieces of bone. Details of measurements made from powdered aliquots (lower collagen yields) are included in the supplementary information.

Figure 3 shows the ages obtained for each bone. The accuracy of the dates generated by the gas ion source is clearly seen in comparison with the graphite dates. Of the 74 new measurements made with the EA-GIS-AMS system shown in Fig. 3b–h, 69 measurements agree within the 95% confidence limit (2σ) of the corresponding graphite dates and 57 agree within 1σ. There are five measurements outside 2σ: four are measurements of the two collagen extracts (R-EVA 548.13; R-EVA 548.14) which fell at or below the minimum threshold of preservation suitable for ^{14}C dating (Fig. 3g), and the last (R-EVA 1905.4.1; Aix-12023.2.1) is slightly older than the other replicates of the same extract (Fig. 3c).

Chi-squared tests (χ^2)[51] were performed using the R_Combine feature in OxCal 4.2[52] using the F^{14}C and associated error for gas replicates of each collagen extract individually and for all replicates per bone. The replicate measurements are statistically indistinguishable for R-EVA 1489, R-EVA 1905, R-EVA 1860, R-EVA 123, R-EVA 570 and R-EVA 124 (output of all statistical tests are included in Supplementary Dataset S2), demonstrating the reproducibility of the measurements and consistency between different pretreatment batches across the range of the ^{14}C timescale. In addition, all of the measurements of R-EVA 1489, R-EVA 123 and R-EVA 124 from this study agree with the EA-GIS-AMS measurements made in 2016 reported in Fewlass, *et al*.[29] (Supplementary Dataset S2).

The exception is the roughly 40,000 year old bone R-EVA 548, which at *ca*. 1% collagen preservation represents the limits of C^{14} dating. The gas dates obtained from the two low yield extracts (R-EVA 548.13 and R-EVA 548.14) were much younger than the other extracts of this bone (Fig. 3g), showing they had been affected by contamination from modern carbon. Due to the low yield, under normal circumstances R-EVA 548.13 would not have been passed for dating following pretreatment. Excluding these two extracts, the replicates from R-EVA 548.3 and R-EVA 548.8 are consistent with the graphite date for this bone.

For background bone R-EVA 1753 (>50,000 BP), the dates from the collagen extracts (Supplementary Dataset S3) were on par with the blank standards (IAEA-C1/phthalic anhydride) of equal size (Supplementary Dataset S4). As expected, the blank level in the EA-GIS system was affected by the reduction in sample size from 90 µg C to 30 µg C (Fig. 3a). The ages of the seven <50,000 BP samples were corrected with background collagen measurements of the same size (*ca*. 30 µg C or *ca*. 90 µg C) and type (solid/powder) measured during the same session.

Discussion

Using a slightly modified version of our standard pretreatment protocol the collagen yield from <100 mg bone material was of equally high quality as extracts from 'large' (>500 mg) bone samples. Decreasing sample size from *ca*. 100 mg to <50 mg bone material also had no detrimental effect on collagen yield. The agreement in age between multiple collagen extracts from different starting weights of bone (Fig. 3) indicates firstly that we obtain reproducible results with the pretreatment protocol and secondly, that the reduction in material during pretreatment did not detrimentally affect the results of ^{14}C dating. In particular, the results indicate that the cleaning steps used for the ultrafilters are sufficient as any C remaining in the filters after cleaning would have a more pronounced effect on reduced sample sizes.

The main alteration to our standard protocol involved reduction in the duration of the gelatinisation stage, with samples removed from the heater block as soon as they had gelatinised (see Fig. 2). Different gelatinisation conditions have been well documented to affect the final extract quality and yield[38,39,53,54]. The higher collagen yields from these extracts supports observations that gelatinised collagen is degraded by prolonged exposure to higher temperatures and acidity[39,53].

R-EVA 548 represents a very challenging prospect for collagen extraction and radiocarbon dating due to the exceptionally low levels of preservation (<1% weight collagen) and old age (*ca*. 39,400 BP), even working with larger sample sizes. The harshest demineralisation (HCl 0.5 M, 2 days, 4 °C) applied to small aliquots of this bone (R-EVA 548.13; R-EVA 548.14) resulted in very low yields of ≤1 mg collagen, likely due to the solubilisation of collagen during the longer demineralisation stage. The resultant underestimated dates clearly show that these aliquots were massively affected by modern carbon contamination. Prior to dating, the consideration of the quality of the extract is crucial in order to obtain reliable dates. Given the low yield of collagen (≤1%) following pretreatment, under normal circumstances these extracts would not been dated or would have been treated with caution. This bone demonstrates the difficulty of pretreatment of poorly preserved bones at the limit of the ^{14}C method.

At such small sample sizes, the consideration of the background correction is crucial. The gas measurements of R-EVA 1489, R-EVA 1905, R-EVA 1860, R-EVA 123, R-EVA 570, R-EVA 548 and R-EVA 124 were all corrected with gas measurements of background bone collagen (R-EVA 1753) of equal size (*ca*. 30 µg C or *ca*. 90 µg C) prepared alongside every batch of samples and measured during the same measurement session to account for any C added during sample preparation and measurement. Figure 3a shows the ages obtained for the

background bone containing *ca*. 25–40 μg C (small) and *ca*. 80–100 μg C (large). The large measurements (mean $F^{14}C = 0.0024$, SD = 0.0006, n = 9, equivalent to 48,600 BP) are on par with the system blank (either IAEA-C1 or phthalic anhydride) measurements of equal size (mean $F^{14}C = 0.0026$, SD = 0.0006, n = 7, equivalent to 48,000 BP) (Supplementary Datasets S3 and S4), indicating that no carbon contamination was introduced during sample preparation. An increased sensitivity to modern ^{14}C is to be expected at lower levels of carbon and it is clear that the smaller background collagen measurements are generally younger. The 25–40 μg C background collagen samples (mean $F^{14}C = 0.0039$, SD = 0.0007, n = 22, equivalent to 44,530 BP) are likewise equal to the system blank measurements of equal size (mean $F^{14}C = 0.0036$, SD = 0.0006, n = 5, equivalent to 45,180 BP) (Supplementary Datasets S3 and S4). These values are lower than previously published values for blank IAEA-C1 samples measured at CEREGE reported in Bard, *et al*.[30] ($F^{14}C = 0.02$ for sample sizes around 30 μg C and $F^{14}C = 0.005$ for samples of 80–100 μg C) and to phthalic anhydride blanks measured at ETH Zurich reported in McIntyre, *et al*.[24] (mean $F^{14}C = 0.0046 \pm 0.0012$, n = 6, size range 84–100 μg C). The results indicate the lower limit of ^{14}C detection with the gas ion source to be around $F^{14}C = 0.004$. As demonstrated by R-EVA 124, beyond this limit the minute levels of ^{14}C can be measured but the uncertainty of the background correction dominates accuracy and precision.

The system blank of the EA-GIS-AMS is affected by the carbon content of the silver cups, cross-talk of the zeolite trap and the cleanliness of the ion source at the time of the measurement[24]. The mass (M_c) and $F^{14}C$ ($F^{14}C_c$) of the constant contamination of the EA + GIS system was deduced by least square regression of modern carbonate and blanks (IAEA-C1) with sample weights ranging between 3 and 100 μg C to be $M_c = 0.55 \pm 0.05$ μg C and $F^{14}C_c = 0.12 \pm 0.03$[55]. The silver cups (5 × 3 mm from Elementar; cleaned at 800 °C, 2 h) had a consistent carbon contribution of 0.049 ± 0.02 μg C. The zeolite trap was heated (450 °C) and the system was flushed with helium between samples to minimize cross-contamination. However, small amounts of C may reside in the zeolite trap after flushing which has been demonstrated to have a large influence on samples <20 μg carbon[23,55]. With this in mind, even our 'small' samples were kept >20 μg carbon. To further alleviate problems of cross-talk, samples were run in order of increasing activity (oldest to youngest) according to the standard practice[55]. Background corrections of samples were applied according to sample size and an external error was added during the age calculation of all samples based on the long term standard deviation of standards and blanks (error 2 described in Fewlass, *et al*.[29]).

In a real life situation, if a small bone sample yielded a high amount of collagen (for example, the mammoth bone R-EVA 123 or the Medieval human bone R-EVA 1489 included in this study), dating with graphite targets would be preferentially undertaken as the precision achieved is much higher and measurements can be made routinely. However, the results of this study demonstrate that the gas ion source can produce an accurate radiocarbon date at low precision from as little as 30 μg C. The precision of the date can be improved when larger sample sizes (up to 100 μg C) are available for measurement over several targets (as demonstrated in Fig. 3). In order to assess variability in handling and blank contribution, in this study we compared multiple measurements of *ca*. 30 μg C with larger aliquots containing *ca*. 90 μg C. When taking the weighted mean and error of the three small aliquots the precision achieved is higher compared to the single large measurement of a roughly equal amount of carbon. However, as the likelihood of contamination being introduced via handling, the EA-GIS or the silver cup is increased for the smaller sample sizes, the preferred method for measuring larger samples would be to measure several targets from a single syringe, rather than splitting a sample into smaller aliquots. Although the measurement of gas samples requires more supervision than graphite targets, the direct coupling of the EA with the GIS significantly reduces sample preparation time by cutting out the graphitisation step which poses a large risk of contamination at such small sample sizes. Therefore in situations where sample size is limited the gas ion source offers an attractive solution for archaeological, as well as environmental, applications.

Even working with the assumption of 1% collagen preservation, in theory sufficient collagen could be extracted from less than 10 mg bone material to obtain a ^{14}C date using the EA-GIS-AMS. However in order to assess the quality of the extract prior to dating and obtain high-resolution stable isotopic data for palaeodietary reconstruction, collagen should also be analysed with an EA-IRMS. At 1%, around 40 mg bone material would supply enough collagen for dating and isotopic analysis. For any sample >1% preservation, excess collagen would be available for further analyses and/or multiple aliquots could be measured with the gas ion source to achieve better counting statistics and thus increase precision. Bearing this in mind, when dating highly precious bone it would be useful to assess the preservation of the artefact prior to sampling or have an understanding of collagen preservation at the archaeological site (for example if other fauna has been sampled for isotopic or ^{14}C dating purposes). Bones of high patrimonial value could be sampled strategically – i.e. for older samples expected to have less than 10% collagen preservation 40 mg bone material could be sampled, whereas for well-preserved Holocene bone much smaller samples could be taken. The case of R-EVA 548 demonstrates that for very old samples (>35,000 BP) with very poor levels of preservation (1–2%), yields falling below 1 mg collagen can be subject to severe contamination issues.

The results presented here provide further confirmation that ^{14}C measurements using the gas ion source of the MICADAS are stable, reproducible and accurate, reaching a level of precision suitable for dating archaeological samples particularly for Palaeolithic samples back to 40,000 BP. In this respect this technique will be highly useful for directly dating precious archaeological bone where limited material is available.

Methods

Sample selection. Eight bones were selected to span the ^{14}C timescale (back to 50,000 BP) at a range of preservation typical for archaeological bones. Collagen extracts from bones R-EVA 124, R-EVA 123 and R-EVA 1489 were previously dated using both graphite targets and the gas ion source in Fewlass, *et al*.[29]. R-EVA 124 was previously labelled as a bison bone but recent aDNA analysis has identified it as belonging to a woolly rhinoceros[56]. R-EVA 548 and R-EVA 570 are two faunal long bones from Teixoneres, Spain. R-EVA 1860 is a faunal long bone excavated from the site of Ranis, Germany and R-EVA 1905 is a predominantly trabecular fragment of horse bone

excavated from Pietraszyn, Poland. R-EVA 1753 is a well-preserved cave bear rib known to date beyond the ^{14}C timescale based on repeated measurements. As standard practice, an aliquot of this bone is extracted and dated alongside every batch of samples to monitor contamination introduced during sample preparation and is used in the age correction of the unknown samples. This is the referred to in the text as the 'background bone'.

Collagen extraction. For each bone, large aliquots (500–700 mg material) were pretreated using our standard acid-base-acid + gelatinisation + ultrafiltration protocol (see Fig. 2) based on Talamo and Richards[36] to produce collagen for dating with graphite targets.

In order to optimise our standard protocol for sample sizes <100 mg, small aliquots of each bone were pretreated multiple times to compare collagen yields and sample quality. Firstly, the outer surface of bone was removed using a sandblaster and aliquots were taken using a rotary drill. Fine diamond grit disc drill pieces were used to remove solid pieces of bone. Fine powder was drilled using round tungsten carbide burs (2.3 mm diameter). Aliquots were weighed via a microbalance into cleaned glass tubes. Solid samples were demineralised in HCl at 4°C with regular visual and mechanical checks and monitoring of CO_2 effervescence. For powdered samples, HCl was added and samples were monitored at room temperature (RT) until CO_2 effervescence had stopped. Following demineralisation, samples were rinsed with ultra-pure Milli-Q water to a neutral pH. Samples were treated with NaOH (0.1 M) at RT for 10 min to remove humic acid contamination and re-acidified with HCl (0.5 M). If a considerable colour change was observed, NaOH was changed and left for another 10 min. Samples were then gelatinised in weak HCl (pH 3) on a heater block set to 60 °C, 70 °C or 75 °C. Samples were either left for 20 h (as per our standard pretreatment), or regularly monitored and removed from the heater block when the sample had fully solubilised. The resultant gelatin was filtered to remove large particles >80 μm (Ezee filters, Elkay labs, UK) and ultrafiltered with Sartorius VivaSpin Turbo 15 (30 kDa MWCO) ultrafilters precleaned according to Brock, *et al*.[43] to separate the high molecular weight fraction (>30kD) for freeze drying (48 h). For details of acid strength, duration of treatment and temperature during pretreatment of samples <100 mg, see Fig. 1 and Supplementary Dataset S1.

Collagen quality assessment. To assess the quality of the collagen, all extracts were analysed via EA-IRMS to obtain elemental (C%, N%, C:N) and stable isotopic data ($\delta^{13}C$ and $\delta^{15}N$). Collagen (*ca*. 400 μg) was weighed into tin cups using a microbalance and measured on a ThermoFinnigan Flash EA coupled to a Thermo Delta plus XP isotope ratio mass spectrometer (IRMS). Stable carbon isotope ratios were expressed relative to VPDB (Vienna PeeDee Belemnite) and stable nitrogen isotope ratios were measured relative to AIR (atmospheric N_2), using the delta notation (δ) in parts per thousand (‰). Repeated analysis of both internal and international standards indicates an analytical error of 0.2‰ (1σ) for $\delta^{13}C$ and $\delta^{15}N$. Where sufficient material was available, collagen (*ca*. 300 μg) was homogenized and mixed with ~40 mg of IR grade KBr powder in an agate mortar and pestle, pressed into a pellet using a manual hydraulic press (Wasserman) and analysed with an Agilent Technologies Cary FTIR Spectrometer with a DTGS detector. Spectra were recorded in transmission mode at 4 cm^{-1} resolution with averaging of 34 scans between 4000 and 400 cm^{-1} using Resolution Pro software (Agilent Technologies). The spectra were evaluated and compared to library spectra of well-preserved collagen and bone to look for evidence of incomplete demineralisation, degraded collagen or the presence of any exogenous material in the extracts.

AMS graphite measurements. Each bone was pretreated as per our standard protocol from approximately 500 mg material. From theses extracts, approximately 3–5 mg collagen was weighed into pre-cleaned tin cups at the MPI-EVA and sent to the Curt-Engelhorn-Centre for Archaeometry Klaus-Tschira-AMS facility in Mannheim, Germany (lab code: MAMS) for graphite dating. The samples were combusted in an EA and the sample CO_2 was converted catalytically to graphite. The samples were dated using the MICADAS-AMS[57]. Age and error calculation of unknown samples was performed using BATS software[58], using background collagen samples and standards measured in the same batch, with an added external error of 1‰ as per their standard practice. Collagen samples measured at CEREGE were weighed into tin cups (*ca*. 2 mg), combusted in a vario MICRO cube EA (Elementar Analysensysteme GmbH, Germany), graphitized using the AGE 3 and dated using the AixMICADAS. Oxalic acid standards and background collagen samples measured in the same session were used to calculate the age of the samples. An external error of 1‰ was also propagated in the error calculation.

AMS gas ion source measurements. Small aliquots (<100 mg) of the same bones were pretreated to purify the collagen. Three or four aliquots of each collagen extract (containing *ca*. 25–40 μg C and a single aliquot per bone containing *ca*. 80–100 μg C) were measured via a microbalance into pre-cleaned silver cups (800 °C, 2 h). These were placed into the auto-sampler of a vario MICRO cube EA which was directly coupled to the gas ion source of the AixMICADAS via the GIS[20,22]. Following combustion, sample CO_2 was adsorbed on a zeolite trap and subsequently expanded to the syringe of the GIS where it was mixed with He (5% CO_2) and introduced to the gas ion source at a flow rate of *ca*. 2 μg C/min. The EA-GIS system was flushed with helium between samples. Pre-cleaned titanium (Ti) gas targets were pre-sputtered for approximately two minutes in the ion source to remove any remaining surface contamination before the sample CO_2 injection. Around 30–40 μg C was consumed by the AMS over the duration of one Ti target[21,55]. For the large aliquots containing *ca*. 80–90 μg C measurements were performed over multiple targets (which can be changed during measurement). Each step was fully controlled via the gas-interface handling software.

The gas measurements in this study were made over two measurement sessions six months apart, both carried out shortly after the ion source had been cleaned. Each measurement session commenced with two oxalic acid II NIST standards (from a gas canister) to normalize and correct samples for fractionation. Blank (^{14}C-free) CO_2 samples (also from a gas canister) were then measured to purge the system and reach a stable operational level ($F^{14}C$ < 0.004) (these measurements were not used in age calculation). In the first session, carbonate reference

material (IAEA-C1) were run prior to the collagen samples to check the background level of the instrument and begin the measurement of old samples under optimal conditions. In the second measurement session, phthalic anhydride was run for the same purpose. In order to alleviate problems of memory effect, the GIS system was flushed with helium between samples and samples were measured in order of increasing activity as per standard procedure (for further discussion, see Tuna, *et al.*[55]). Low energy ion currents for the gas analyses were in the range of 10–15 μA. BATS[58] was used for data reduction. The uncorrected collagen background (R-EVA 1753) measurements of the corresponding type (piece/powder) and equal size were used to correct the archaeological samples measured in the same session (i.e. 'small' sample aliquots were corrected only with 'small' background collagen samples). For all samples, the long term standard deviation of blanks ($F^{14}C = 0.001$) was used as the absolute blank error and an external error of 3.5‰ was added to take into account the long-term variability of standards ('error 2' described in Fewlass, *et al.*[29]).

Data Availability

All data generated or analysed during this study are included in this article and the accompanying supplementary information files.

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Acknowledgements

We would like to thank the Max Planck Society for funding this research. The AixMICADAS was acquired and is operated in the framework of the EQUIPEX project ASTER-CEREGE (PI E. Bard) with additional matching funds from the Collège de France, which also supports the salaries of the authors from CEREGE. We are indebted to Dr. Raquel Silva Maria and Sven Steinbrenner of the Department of Human Evolution at the MPI-EVA for technical assistance. We give kind thanks to Caterina Pangrazzi, Jordi Rosell, Ruth Blasco, Marcel Weiss and Andrzej Wisniewski for access to samples during the course of the study.

Author Contributions

H.F., S.T., B.K., J.-J.H. and E.B. devised the study; H.F. carried out sample pretreatment, FTIR and EA-IRMS analyses under the supervision of S.T.; T.T. and Y.F. performed EA-GIS-AMS measurements; T.T., B.K. and H.F. performed data reduction; H.F. wrote the paper with input from all authors.

Additional Information

Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-019-41557-8.

Competing Interests: The authors declare no competing interests.

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Pretreatment and gaseous radiocarbon dating of 40-100 mg archaeological bone

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Supplementary information:

- Dataset S1 – Pretreatment information for all collagen extracts in the study
- Dataset S2 – Radiocarbon dates for all samples in the study dated with graphite targets and the gas ion source
- Dataset S3 – EA-GIS-AMS data from background bone R-EVA 1753
- Dataset S4 – EA-GIS-AMS data from system blanks
- Figure S1 – Gas dates from background bone R-EVA 1753 according to the amount of C in the EA-GIS
- Figure S2 – Gas measurements of R-EVA 123 and R-EVA 124 from both solid and powdered extracts
- Figure S3 - Example FTIR spectra of collagen extracted in the study

Supplementary Text

Pretreatment and ^{14}C dating of powdered bone samples

Bone aliquots were extracted in two forms: fine powder and solid pieces (as per our standard protocol for *ca.* 500mg bone). We attempted to extract collagen from finely powdered bone to increase our sampling options for precious bones (i.e. a key hole drilling technique). However, the collagen yield of powdered bone was much lower than solid pieces for all samples in the study (Supplementary Fig. S2; Supplementary Dataset S1). Where collagen was recovered often the extracted material appeared poorly preserved with a crumbly texture and was often dark grey or yellow in colour. Where enough material was available for analysis, these extracts were still identified as collagen when analysed with FTIR (Supplementary Fig. S3), although several extracts from the poorly preserved bones showed evidence of incomplete demineralisation. Anecdotally, the striking difference between the two forms was observed at the demineralisation stage; for the older, poorly preserved bones much of the powdered material was lost as soon as HCl was added to the tube. Although the powdered method has the benefit of being faster, increased solubilisation of collagen during demineralisation in powdered bones compared to solid bone sherds was also observed by Schoeninger, et al. [1] and Collins and Galley [2]. As the length of demineralisation is based on visual inspection, a suitable duration is much easier to judge for solid pieces (transparency, softness, buoyancy, CO_2 effervescence).

As a consequence of the low yield of collagen for the poorly preserved bones (R-EVA 570 and R-EVA 548) no powdered extracts from these bones were dated. Despite the lower collagen yield, sufficient collagen was available for gas dating from powdered aliquots of the well-preserved bones, R-EVA 123, R-EVA 124 and R-EVA 1753. The age of the background collagen extracts were slightly younger than their solid counterparts (Supplementary Fig. S1) but it is unknown whether this reflected the limited number of measurements made, the lower collagen yield from these pretreatments and/or the small size of the aliquots measured in the EA-GIS-AMS (*ca*. 25 µg C).The ages of the <50,000 BP samples were corrected with background collagen measurements of the same size (*ca*. 30 µg C or *ca.* 90 µg C) and type (solid/powder) measured during the same session. The exception to this are the large (*ca.* 90 µg C) powder samples from R-EVA 123 and R-EVA 124 (Aix-12002.7.1; 12002.8.1; 12003.8.5; 12003.9.5) which are marked with an asterisk in Supplementary Figure S2. No background measurement of corresponding size/type was made so these were corrected with small (*ca.* 30 µg C) powder backgrounds meaning they are slightly over-corrected. Even with this over-correction, the age of Aix-12002.8.1 is younger than other measurements for this bone. We do not have an explanation for this measurement.

Despite this, there is no difference between the gas measurements obtained from powdered versus solid extracts for R-EVA 123 or R-EVA 124, which all agree within X^2 despite the over-corrected samples (Supplementary Fig. S2; Supplementary Dataset S2). Further, the gas dates from the powdered extracts of R-EVA 123 and R-EVA 124 all agree with the graphite dates

within 2σ. However, due to the reduced collagen yield we will continue our standard practice of extracting collagen from solid chunks of bone (also documented in Tuross [3]).

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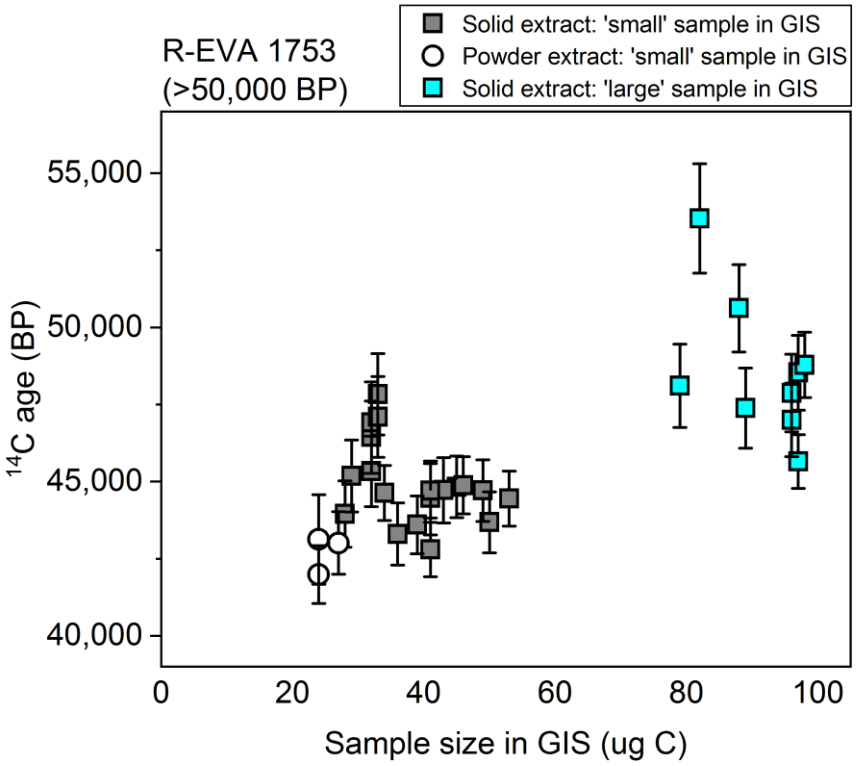
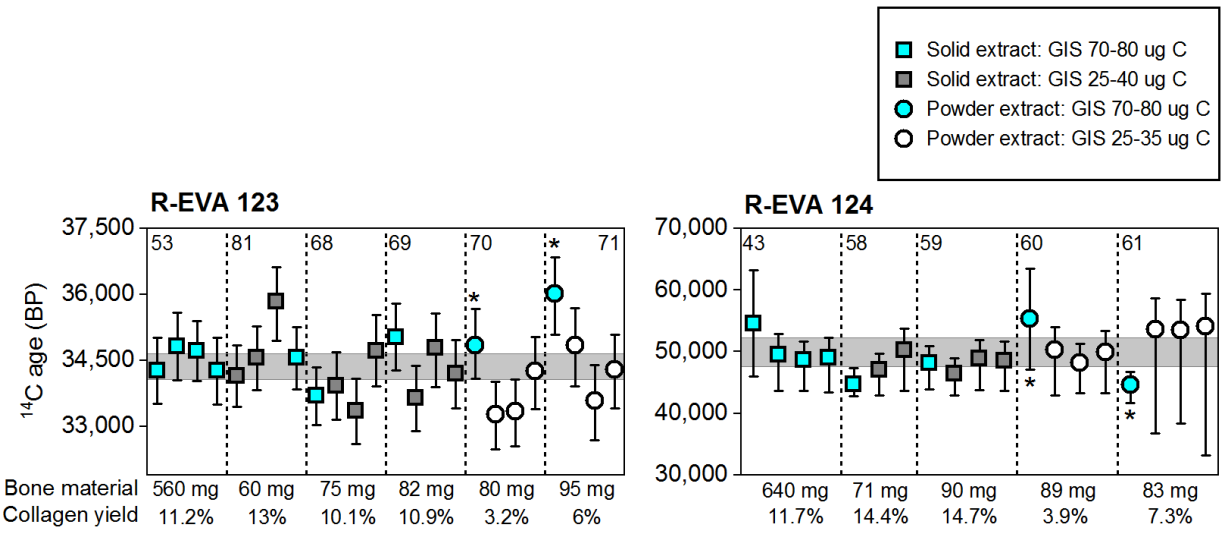
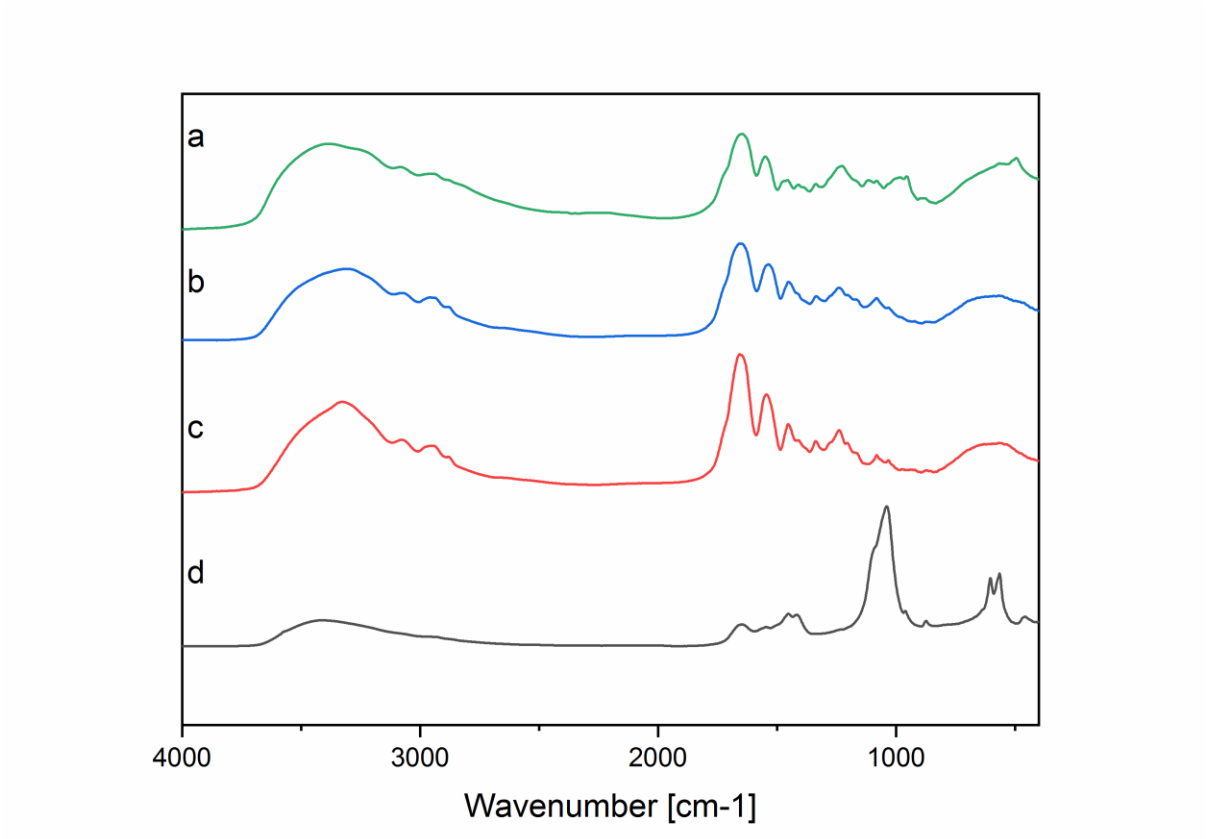


Supplementary figure S1 Gas measurements of collagen from background bone R-EVA 1753 according to the amount of carbon in the EA-GIS system. Error bars are shown to 1σ.



Supplementary figure S2 Gas measurements of collagen from R-EVA 123 and R-EVA 124. Figure amended from Figure 3 in the main text to include gas measurements of collagen extracted from powdered bone.



Supplementary figure S3 FTIR spectra of collagen extracted from a) R-EVA 570.15 (powder) and b) R-EVA 1489.2 (solid) in comparison to characteristic FTIR spectra of c) well-preserved collagen and d) bone.

Pretreatment and gaseous radiocarbon dating of 40-100 mg archaeological bone
Supplementary Dataset S1 – Pretreatment information for all collagen extracts in the study

R-EVA	AMS lab code	prep no	batch	form	bone wt (mg)	Demineralisation			Gelatinisation		Quality control									^{14}C dated
						HCl strength	Temp	Duration	Temp	Duration	Coll yld (mg)	Coll yld (%)	$\delta^{13}C$	$\delta^{15}N$	C%	N%	C:N	FTIR	Collagen appearance	
1753	Aix-12018	2	A	piece	97.1	0.5M	4°C	44h	75°C	20h	4.6	4.7	-22.55	1.45	40.8	14.8	3.2	collagen	white and fluffy	
		3	A	piece	55.4	0.5M	4°C	20h	75°C	20h	2.6	4.7	-22.76	1.26	40.5	14.7	3.2	collagen	white and fluffy	
		4	B	piece	102.5	0.5M	4°C	26h	70°C	4h	8.8	8.6	-22.67	1.44	39.7	14.8	3.1	collagen	white and fluffy	
		5	B	piece	55.9	0.5M	4°C	26h	70°C	2h	5.5	9.8	-22.65	1.59	49.5	18.3	3.2	collagen	white and fluffy	
		29	H	piece	69.2	0.5M	4°C	26h	70°C	6h	7.6	11.0	-22.88	1.64	41.1	15.3	3.1	collagen	white and fluffy	gas
		48	L	piece	62.0	0.5M	4°C	27h	70°C	5h	8.4	13.5	-22.72	1.37	43.9	16.1	3.2	collagen	white and fluffy	gas
		56	P	piece	68.7	0.5M	4°C	29h	70°C	3h	8.9	13.0	-23.24	1.70	45.0	15.6	3.4	collagen	white and fluffy	gas
		60	S	piece	69.3	0.5M	4°C	29h	70°C	3h	10.2	14.7	-22.85	1.44	44.6	16.0	3.3	collagen	white and fluffy	gas
		61	T	piece	40.8	0.5M	4°C	24h	70°C	20h	5.5	13.5	-22.86	1.94	44.8	16.2	3.2	collagen	white and fluffy	gas
		62	U	piece	55.9	0.5M	4°C	32h	70°C	3h	8.6	15.4	-23.21	1.52	45.4	15.6	3.4	collagen	white and fluffy	gas
		39	A1	piece	99.7	0.5M	4°C	50h	70°C	3h	12.2	12.2	-22.94	1.53	43.0	15.9	3.2	collagen	white and fluffy	gas
		40	A1	piece	74.5	0.5M	4°C	45h	70°C	3h	10.1	13.6	-23.16	1.91	43.4	15.9	3.2	collagen	white and fluffy	
		41	A2	piece	97.6	0.5M	4°C	50h	70°C	3h	12.8	13.1	-22.99	1.81	43.7	16.1	3.2	collagen	white and fluffy	gas
		42	A2	piece	92.8	0.5M	4°C	50h	70°C	3h	11.1	12.0	-23.06	2.16	42.3	15.8	3.1	collagen	white and fluffy	
		59	R	piece	79.9	0.5M	4°C	50h	70°C	3h	11.7	14.6	-22.85	1.28	45.0	16.1	3.2	collagen	white and fluffy	gas
		33	J	piece	90.3	0.5M	4°C	42h	60°C	6h	10.3	11.4	-22.31	1.36	43.7	15.8	3.2	collagen	white and fluffy	
		34	J	piece	99.4	0.2M	4°C	98h	60°C	6h	10.9	11.0	-22.28	1.25	44.2	15.7	3.3	collagen	white and fluffy	gas
		6	C	powder	97.4	0.5M	RT	40min	75°C	20h	2.9	3.0	-22.65	1.90	34.5	12.9	3.1	collagen	white and fluffy	
		7	C	powder	51.6	0.5M	RT	40min	75°C	20h	1.7	3.3	-22.63	1.52	38.4	14.2	3.2	collagen	white and fluffy	
		8	D	powder	97.3	0.5M	4°C	2h	70°C	3h	6.2	6.4	-22.35	1.25	37.0	13.5	3.2	collagen	white and fluffy	
		9	D	powder	51.3	0.5M	4°C	2h	70°C	3h	3.4	6.6	-22.57	1.30	42.2	15.4	3.2	collagen	white and fluffy	
		30	H2	powder	68.0	0.5M	RT	10min	70°C	4h	4.2	6.2	-23.04	1.90	41.5	15.4	3.2	collagen	white and fluffy	
		43	A3	powder	80.9	0.2M	RT	10min	70°C	4h	1.9	2.3	-23.20	1.99	35.2	12.9	3.2	collagen	white and fluffy	gas
		44	A3	powder	76.4	0.2M	RT	30min	70°C	3h	1.0	1.3	-23.08	2.73	41.7	15.3	3.2	collagen	white and fluffy	
		45	A4	powder	87.8	0.2M	RT	20min	70°C	6h	5.1	5.8	-22.90	1.85	34.9	12.9	3.2	collagen	white and fluffy	gas
		46	A4	powder	83.3	0.2M	RT	20min	70°C	6h	4.5	5.4	-23.00	1.75	35.8	13.1	3.2	collagen	white and fluffy	

Pretreatment and gaseous radiocarbon dating of 40-100 mg archaeological bone
Supplementary Dataset S1 – Pretreatment information for all collagen extracts in the study

R-EVA	AMS lab code	prep no	batch	form	bone wt (mg)	Demineralisation HCl strength	Demineralisation Temp	Demineralisation Duration	Gelatinisation Temp	Gelatinisation Duration	Quality control Coll yld (mg)	Coll yld (%)	$\delta^{13}C$	$\delta^{15}N$	C%	N%	C:N	FTIR	Collagen appearance	^{14}C dated
124	Aix-12002	43	2016	piece	639.5	0.5M	4°C	16days	75°C	20h	74.6	11.7	-20.00	3.30	45.9	17.2	3.1	-	white and fluffy	graphite + gas
		46	A	piece	106	0.5M	4°C	44h	75°C	20h	13.5	12.7	-20.30	2.90	42.2	15.3	3.2	collagen	white and fluffy	
		47	A	piece	58.8	0.5M	4°C	44h	75°C	20h	6.4	10.9	-20.28	2.93	42.8	15.6	3.2	collagen	white and fluffy	
		48	B	piece	95.5	0.5M	4°C	69h	70°C	5h	12.7	13.3	-20.41	3.05	42.1	15.8	3.1	collagen	white and fluffy	
		49	B	piece	55	0.5M	4°C	44h	70°C	4h	7.8	14.2	-20.34	3.08	46.9	17.6	3.1	collagen	white and fluffy	
		58	A1	piece	70.6	0.5M	4°C	71h	70°C	6h	10.2	14.4	-20.38	4.12	42.6	15.7	3.2	collagen	white and fluffy	gas
		59	A2	piece	90.2	0.5M	4°C	71h	70°C	6h	13.3	14.7	-20.32	3.16	44.6	16.1	3.2	collagen	white and fluffy	gas
		51	C	powder	51.5	0.5M	RT	45min	75°C	20h	1.6	3.1	-20.38	3.14	38.8	14.3	3.2	collagen	black and crumbly	
		52	D	powder	99.6	0.5M	4°C	2h	70°C	3h	5.0	5.0	-20.34	3.08	32.9	11.9	3.2	collagen	dark grey	
		53	D	powder	52	0.5M	4°C	2h	70°C	3h	1.5	2.9	-20.43	3.09	37.6	13.7	3.2	collagen	dark grey	
		60	A3	powder	88.8	0.2M	RT	35min	70°C	4h	3.5	3.9	-20.13	3.26	40.3	14.7	3.2	collagen	grey and fluffy	gas
		61	A4	powder	83.1	0.2M	RT	30min	70°C	6h	6.1	7.3	-20.29	3.08	41.7	15.3	3.2	collagen	white and fluffy	gas
548	Aix-12017	17	2017	piece	597.5	0.5M	4°C	15days	75°C	20h	5.0	0.8	-20.27	4.74	40.4	13.9	3.4	collagen	white and fluffy	graphite
		3	H	piece	71.2	0.5M	4°C	26h	70°C	3h	1.5	2.1	-20.43	4.70	38.5	13.8	3.2	collagen	white and fluffy	gas
		13	A1	piece	78.1	0.5M	4°C	45h	70°C	2h	0.6	0.8	-20.73	4.20	42.9	14.8	3.4	-	white and fluffy	gas
		14	A2	piece	86.4	0.5M	4°C	45h	70°C	2h	1.1	1.3	-20.33	4.33	41.5	14.6	3.3	-	white and fluffy	gas
		7	J	piece	74.7	0.5M	4°C	27h	60°C	6h	0.7	0.9	-	-	-	-	-	collagen	white and fluffy	
		8	J	piece	84.7	0.2M	4°C	90h	60°C	6h	1.5	1.8	-20.29	5.40	41.5	15.1	3.2	collagen	white and fluffy	gas
		4	H	powder	80.6	0.5M	RT	5min	70°C	2h	0.2	0.2	-	-	-	-	-	-	white marks	
		15	A3	powder	82.4	0.2M	RT	10min	70°C	3h	0.5	0.6	-	-	-	-	-	-	white marks	
		16	A4	powder	80.7	0.2M	RT	20min	70°C	4h	0.9	1.1	-	-	-	-	-		white marks	
		9	J	powder	86.7	0.5M	RT	5min	60°C	4h	0.5	0.6	-	-	-	-	-	-	white marks	
		10	J	powder	86.5	0.2M	RT	50min	60°C	4h	0.3	0.3	-	-	-	-	-	-	white marks	

Pretreatment and gaseous radiocarbon dating of 40-100 mg archaeological bone
Supplementary Dataset S1 – Pretreatment information for all collagen extracts in the study

						Demineralisation			Gelatinisation		Quality control									
R-EVA	AMS lab code	prep no	batch	form	bone wt (mg)	HCl strength	Temp	Duration	Temp	Duration	Coll yld (mg)	Coll yld (%)	$\delta^{13}C$	$\delta^{15}N$	C%	N%	C:N	FTIR	Collagen appearance	^{14}C dated
570	Aix-12015	17	2017	piece	451.9	0.5M	4°C	13days	75°C	20h	15.2	3.4	-18.41	7.34	44.5	15.4	3.4	collagen	white and fluffy	graphite
		3	H	piece	62.1	0.5M	4°C	26h	70°C	2h	2.9	4.7	-19.13	7.63	43.3	15.4	3.3	collagen	white and fluffy	gas
		19	U	piece	43.8	0.5M	4°C	24h	70°C	2h	2.2	5.0	-18.43	7.89	44.0	15.1	3.4	collagen	white and fluffy	gas
		13	A1	piece	87	0.5M	4°C	45h	70°C	2h	2.2	2.5	-19.47	7.28	43.9	15.3	3.3	collagen	white and fluffy	gas
		14	A2	piece	90.1	0.5M	4°C	45h	70°C	2h	4.0	4.4	-18.87	7.21	43.1	15.2	3.3	collagen	white and fluffy	gas
		7	J	piece	77.9	0.5M	4°C	42h	60°C	5.5h	3.9	5.0	-18.83	7.67	41.5	14.7	3.3	collagen	white and fluffy	
		8	J	piece	69.8	0.2M	4°C	98h	60°C	5h	3.5	5.0	-18.72	7.86	43.5	15.2	3.3	collagen	white and fluffy	gas
		18	P	piece	53.2	0.5M	4°C	24h	70°C	3h	2.1	3.9	-19.45	7.39	42.6	15.2	3.3	collagen	white and fluffy	
		4	H	powder	62.6	0.5M	RT	5min	70°C	3h	0.4	0.6	-	-	-	-	-	-	tiny white fluff	
		9	J	powder	83	0.5M	RT	5min	60°C	4h	0.9	1.1	-	-	-	-	-	collagen	white and crumbly	
		10	J	powder	72	0.2M	RT	50min	60°C	4h	0.8	1.1	-	-	-	-	-	collagen	white and fluffy	
		15	A3	powder	85.9	0.2M	RT	10min	70°C	3h	0.8	0.9	-	-	-	-	-	collagen + other peaks	yellow and crumbly	
		16	A4	powder	88.1	0.2M	RT	20min	70°C	4h	1.5	1.7	-	-	-	-	-	-	tiny yellow fluff	
123	Aix-12003	53	2016	piece	559.4	0.5M	4°C	12days	75°C	20h	62.6	11.2	-21.10	7.10	45.6	17.2	3.2	-	white and fluffy	graphite + gas
		60	A	piece	100.2	0.5M	4°C	44h	75°C	20h	9.4	9.4	-21.19	6.81	37.9	13.7	3.2	collagen	white and fluffy	
		61	A	piece	51.7	0.5M	4°C	20h	75°C	20h	3.5	6.8	-21.44	6.79	38.8	14.2	3.2	collagen	white and fluffy	
		62	B	piece	101.1	0.5M	4°C	26h	70°C	3.5h	10.4	10.3	-21.37	6.84	42.2	15.6	3.2	collagen	white and fluffy	
		63	B	piece	50	0.5M	4°C	21h	70°C	3h	5.0	10.0	-21.41	6.84	43.2	16.0	3.2	collagen	white and fluffy	
		81	P	piece	60.7	0.5M	4°C	24h	70°C	5h	7.9	13.0	-21.23	6.79	41.8	15.0	3.2	collagen	white and fluffy	gas
		68	A1	piece	75	0.5M	4°C	45h	70°C	4h	7.6	10.1	-21.24	6.86	43.4	15.6	3.2	collagen	white and fluffy	gas
		69	A2	piece	82.4	0.5M	4°C	45h	70°C	4h	9.0	10.9	-21.00	6.92	44.2	15.9	3.2	collagen	white and fluffy	gas
		64	C	powder	100.5	0.5M	RT	50min	75°C	20h	9.4	9.4	-21.33	6.90	37.3	13.8	3.2	collagen	white and fluffy	
		65	C	powder	50.5	0.5M	RT	50min	75°C	20h	2.8	5.5	-21.24	6.88	41.0	15.4	3.1	collagen	white and fluffy	
		66	D	powder	103.3	0.5M	4°C	2h	70°C	3h	7.5	7.3	-21.27	6.80	35.9	13.2	3.2	collagen	white and fluffy	
		67	D	powder	50.3	0.5M	4°C	2h	70°C	3h	4.0	8.0	-21.10	6.84	36.7	13.6	3.2	collagen	white and fluffy	
		70	A3	powder	79.9	0.2M	RT	35min	70°C	4h	2.4	3.0	-21.24	6.82	36.0	13.1	3.2	collagen	grey and fluffy	gas
		71	A4	powder	94.5	0.2M	RT	30min	70°C	6h	5.7	6.0	-21.14	6.98	31.8	11.8	3.1	collagen	grey and fluffy	gas

Pretreatment and gaseous radiocarbon dating of 40-100 mg archaeological bone

Supplementary Dataset S1 – Pretreatment information for all collagen extracts in the study

						Demineralisation			Gelatinisation		Quality control									
R-EVA	AMS lab code	prep no	batch	form	bone wt (mg)	HCl strength	Temp	Duration	Temp	Duration	Coll yld (mg)	Coll yld (%)	$\delta^{13}C$	$\delta^{15}N$	C%	N%	C:N	FTIR	Collagen appearance	^{14}C dated
1860	Aix-12022	1	2017	piece	454.4	0.5M	4°C	10days	75°C	20h	41.1	9.0	-18.04	4.64	42.6	15.4	3.2	-	white and fluffy	graphite
		2	R	piece	55.7	0.5M	4°C	72h	70°C	4h	4.9	8.8	-18.34	4.54	45.2	15.8	3.3	collagen	white and fluffy	gas
		3	S	piece	44.6	0.5M	4°C	24h	70°C	6h	4.5	10.1	-18.11	4.66	43.2	15.3	3.3	collagen	white and fluffy	gas
1905	Aix-12023	1	2017	piece	582.9	0.5M	4°C	26days	75°C	20h + 20h	31.9	5.5	-20.70	5.62	41.4	15.0	3.2	-	white and fluffy	graphite
		3	S	piece	65.0	0.5M	4°C	24h	70°C	27h	4.7	7.2	-20.71	5.53	43.6	15.4	3.3	collagen	white and fluffy	gas
		4	T	piece	54.9	0.5M	4°C	9h	70°C	25h	2.0	3.6	-20.53	5.44	41.1	15.3	3.1	collagen	white and fluffy	gas
1489	Aix-12004	1	2016	piece	753.2	0.5M	4°C	18days	75°C	20h	134.5	17.9	-16.40	8.80	45.4	16.7	3.2	-	white and fluffy	graphite + gas
		2	L	piece	53.9	0.5M	4°C	21h	70°C	11h	9.7	18.0	-16.35	8.79	45.0	16.4	3.2	collagen	white and fluffy	gas
		4	T	piece	37.4	0.5M	4°C	24h	70°C	20h	7.0	18.7	-16.27	8.74	45.1	16.3	3.2	collagen	white and fluffy	gas

Pretreatment and gaseous radiocarbon dating of 40-100 mg archaeological bone

Supplementary Dataset S2 – Radiocarbon dates for all samples in the study dated with graphite targets and the gas ion source

		~500mg extraction, solid graphite target dates							~500mg bone extraction, EA-GIS-AMS dates (Fewlass et al., 2018)					<100mg bone extraction							
R-EVA	Site	R-EVA	Bone wt (mg)	Coll yld (%)	C:N	AMS lab code	^{14}C age	±	R-EVA	AMS lab code	GIS C (µg)	^{14}C age	± (err 2)	Session	R-EVA	Batch	Form	Bone wt (mg)	Coll yld (mg)	Coll yld (%)	C:N
1489	San Martino Lundo Lomaso, Italy	1489.1	753.2	17.9	3.2	Aix-12004.1.1	1490	17	1489.1	Aix-12004.3.1	84	1401	75	Dec-17	1489.2	L	piece	53.9	9.7	18.0	3.2
	Human, long bone					Aix-12004.1.2	1470	17	1489.1	Aix-12004.3.2	74	1530	72								
						MAMS-26317	1481	23	1489.1	Aix-12004.3.3	77	1494	60								
									1489.1	Aix-12004.3.4	74	1368	65	Dec-17	1489.4	T	piece	37.4	7.0	18.7	3.2
1905	Pietraszyn, Poland	1905.1	582.9	2.3/5.5	3.2	MAMS-31228	23000	100						Dec-17	1905.3	S	piece	65.0	4.7	7.2	3.3
	Horse, trabecular													Dec-17	1905.4	T	piece	54.9	2.0	3.6	3.1
1860	Ranis, Germany Unknown fauna, long bone	1860.1	454.4	9	3.2	MAMS-30401	30010	140						Dec-17	1860.2	R	piece	55.7	4.9	8.8	3.3
														Dec-17	1860.3	S	piece	44.6	4.5	10.1	3.3

Pretreatment and gaseous radiocarbon dating of 40-100 mg archaeological bone

Supplementary Dataset S2 – Radiocarbon dates for all samples in the study dated with graphite targets and the gas ion source

R-EVA	<100mg bone extraction, EA-GIS-AMS dates							Adjusted age		Statistical agreement of replicate measurements	Notes
	AMS lab code	Coll wgt (µg)	GIS C (µg)	$F^{14}C$	±	^{14}C age	±	^{14}C age	±	*T is the calculated χ2 value and should be lower than the value given in brackets to be considered contamporaneous at 95% confidence. The degrees of freedom are given by 'df'.*	
1489	Aix-12004.6.1	107	42	0.8339	0.0074	1,459	72	1,459	72		χ2 test including the Fewlass et al 2017 gas measurements:
	Aix-12004.6.2	96	42	0.8302	0.0073	1,495	71	1,495	71	χ^2 test: (df = 3, N = 4) T=0.8 (5% 7.8)	(df = 11, N = 12) T = 11.2 (5% 19.7)
	Aix-12004.6.3	96	42	0.8275	0.0072	1,521	70	1,521	70	weighted mean: 1506 +/- 36	weighted mean: 1480 +/- 20
	Aix-12004.6.4	93	40	0.8248	0.0073	1,548	71	1,548	71		
	AIX-12004.7.1	105	49	0.8349	0.0071	1,449	68	1,449	68		
	AIX-12004.7.2	90	40	0.8257	0.0075	1,539	73	1,539	73	χ^2 test: (df = 3, N = 4) T=5.3 (5% 7.8)	
	AIX-12004.7.3	105	46	0.8209	0.0074	1,585	73	1,585	73	weighted mean: 1484 +/-36	
	AIX-12004.7.4	109	48	0.8426	0.0072	1,376	69	1,376	69		
										χ2 for all replicates: (df = 7, N = 7) T=6.3 (5% 14.1)	
										Weighted mean: 1495 +/-25	
1905	Aix-12023.1.1	102	44	0.0529	0.0019	23,607	283	23,610	280		
	Aix-12023.1.2	94	38	0.0553	0.0020	23,251	293	23,250	290		
	Aix-12023.1.3	87	37	0.0558	0.0020	23,188	283	23,190	280	χ2 test: (df = 3, N = 4) T = 2.9 (5% 7.8)	
	Aix-12023.1.4	245	99	0.0569	0.0014	23,020	192	23,020	190	weighted mean: 23220 +/-130	
	Aix-12023.2.1	101	41	0.0510	0.0017	23,903	264	23,900	260		
	Aix-12023.2.2	93	39	0.0542	0.0019	23,412	279	23,410	280		
	Aix-12023.2.3	99	39	0.0518	0.0020	23,780	304	23,780	300	χ2 test: (df = 3, N = 4) T = 2.3 (5% 7.8)	
	Aix-12023.2.4	97	40	0.0539	0.0018	23,461	275	23,460	270	weighted mean: 23650 +/-140	
										χ2 test for all replicates: (df = 7, N = 8) T = 10.1 (5% 14.1)	
										weighted mean: 23,420 +/-100	
1860	Aix-12022.1.1	91	43	0.0227	0.0015	30,421	518	30,420	520		
	Aix-12022.1.2	101	41	0.0223	0.0014	30,554	509	30,550	510		
	Aix-12022.1.3	90	39	0.0190	0.0015	31,822	625	31,820	630	χ2 test: (df=2, N = 3) T = 1.5 (5% 6.0)	Big discharges inside source during measurement - not included in X2 test
	Aix-12022.1.4	93	38	0.0248	0.0016	29,699	519	29,700	520	weighted mean: 30,250 +/-300	
	Aix-12022.2.1	98	43	0.0240	0.0014	29,972	484	29,970	480		
	Aix-12022.2.2	98	43	0.0243	0.0015	29,865	481	29,860	480		
	Aix-12022.2.3	97	35	0.0255	0.0015	29,473	461	29,470	460	χ2 test: (df = 3, N = 4) T = 0.6 (5% 7.8)	
	Aix-12022.2.4	227	96	0.0245	0.0011	29,800	374	29,800	370	weighted mean: 29780 +/-220	
										χ2 test for all replicates: (df = 6, N = 7) T=3.7 (5% 12.6)	
										weighted mean: 29,950 +/- 180	

Pretreatment and gaseous radiocarbon dating of 40-100 mg archaeological bone

Supplementary Dataset S2 – Radiocarbon dates for all samples in the study dated with graphite targets and the gas ion source

		~500mg extraction, solid graphite target dates							~500mg bone extraction, EA-GIS-AMS dates (Fewlass et al., 2018)					<100mg bone extraction							
R-EVA	Site	R-EVA	Bone wt (mg)	Coll yld (%)	C:N	AMS lab code	^{14}C age	±	R-EVA	AMS lab code	GIS C (µg)	^{14}C age	± (err 2)	Session	R-EVA	Batch	Form	Bone wt (mg)	Coll yld (mg)	Coll yld (%)	C:N
123	Brown Bank, North sea Plains Mammoth, rib	123.53	559.40	11.2	3.2	Aix-12003.1.1	34390	240	123.53	Aix-12003.5.1	89	34260	750	Dec-17	123.81	P	piece	60.7	7.9	13.0	3.2
						Aix-12003.1.2	34320	240	123.53	Aix-12003.5.2	78	34820	770								
						MAMS-26876	34360	300	123.53	Aix-12003.5.3	75	34710	680								
									123.53	Aix-12003.5.4	98	34260	760								
														Jun 17	123.68	A1	piece	75	7.6	10.1	3.3
														Jun 17	123.69	A2	piece	82.4	9.0	10.9	3.2
														Jun 17	123.70	A3	powder	79.9	2.4	3.0	3.2
														Jun 17	123.71	A4	powder	94.5	5.7	6.0	3.2

Pretreatment and gaseous radiocarbon dating of 40-100 mg archaeological bone

Supplementary Dataset S2 – Radiocarbon dates for all samples in the study dated with graphite targets and the gas ion source

R-EVA	<100mg bone extraction, EA-GIS-AMS dates							Adjusted age		Statistical agreement of replicate measurements	Notes
	AMS lab code	Coll wgt (µg)	GIS C (µg)	$F^{14}C$	±	^{14}C age	±	^{14}C age	±	*T is the calculated χ2 value and should be lower than the value given in brackets to be considered contamporaneous at 95% confidence. The degrees of freedom are given by 'df'.*	
123	Aix-12003.10.1	102	45	0.0143	0.0012	34,138	699	34,140	700		χ2 test including the Fewlass et al 2017 gas measurements:
	Aix-12003.10.2	91	38	0.0135	0.0012	34,555	728	34,550	730		(df = 23, N = 24) T = 19.2 (5% 35.2)
	Aix-12003.10.3	105	43	0.0116	0.0012	35,835	829	35,830	+880/-780	χ2 test: (df = 3, N = 4) T = 2.8 (5% 7.8)	weighted mean: 34400 +/- 160
	Aix-12003.10.4	239	98	0.0136	0.0012	34,546	707	34,550	710	weighted mean: 34,730 +/-360	
	Aix-12003.6.6	211	86	0.0151	0.0012	33,694	649	33,690	650		
	Aix-12003.6.7	72	29	0.0147	0.0014	33,919	772	33,920	770		
	Aix-12003.6.8	86	34	0.0157	0.0014	33,347	735	33,350	740	χ2 test: (df = 3, N = 4) T = 1.8 (5% 7.8)	
	Aix-12003.6.9	76	31	0.0133	0.0013	34,725	810	34,720	810	weighted mean: 33,910 +/-360	
	Aix-12003.7.5	205	83	0.0128	0.0012	35,028	765	35,030	760		
	Aix-12003.7.6	72	29	0.0152	0.0014	33,637	742	33,640	740		
	Aix-12003.7.7	78	32	0.0132	0.0014	34,777	835	34,780	+880/-790	χ2 test: (df = 3, N = 4) T = 2.0 (5% 7.8)	
	Aix-12003.7.8	71	26	0.0142	0.0014	34,186	782	34,190	780	weighted mean: 34,430 +/-390	
	Aix-12003.8.5	209	67	0.0131	0.0013	34,842	792	34,840	+830/-760		Large sample size corrected with small size background - Overcorrected
	Aix-12003.8.6	85	25	0.0159	0.0015	33,281	765	33,280	+800/-730		
	Aix-12003.8.7	69	28	0.0157	0.0015	33,347	757	33,350	+800/-720	χ2 test: (df = 3, N = 4) T = 2.7 (5% 7.8)	
	Aix-12003.8.8	79	23	0.0141	0.0014	34,260	824	34,260	+870/-780	weighted mean: 33,970 +/-390	
	Aix-12003.9.5	209	70	0.0113	0.0012	36,013	869	36,010	+920/-830		Large sample size corrected with small size background - Overcorrected
	Aix-12003.9.6	82	25	0.0131	0.0014	34,847	874	34,850	+930/-830		
	Aix-12003.9.7	81	21	0.0153	0.0016	33,589	856	33,590	+900/-810	χ2 test: (df = 3, N = 4) T = 4.5 (5% 7.8)	
	Aix-12003.9.8	79	na	0.0140	0.0015	34,285	837	34,290	+880/-800	weighted mean: 34,820 +/-430	
										χ2 test for all replicates: (df=19, N = 20) T = 18.5 (5% 30.1)	
										weighted mean: 34,380 +/-170	

Pretreatment and gaseous radiocarbon dating of 40-100 mg archaeological bone

Supplementary Dataset S2 – Radiocarbon dates for all samples in the study dated with graphite targets and the gas ion source

		~500mg extraction, solid graphite target dates							~500mg bone extraction, EA-GIS-AMS dates (Fewlass et al., 2018)					<100mg bone extraction							
R-EVA	Site	R-EVA	Bone wt (mg)	Coll yld (%)	C:N	AMS lab code	^{14}C age	±	R-EVA	AMS lab code	GIS C (µg)	^{14}C age	± (err 2)	Session	R-EVA	Batch	Form	Bone wt (mg)	Coll yld (mg)	Coll yld (%)	C:N
570	Teixoneres, Spain Unknown fauna, long bone	570.2	451.9	3.4	3.4	MAMS-34680	34270	190						Dec 17	570.19	U	piece	43.8	2.2	5.0	3.4
														Jun 17	570.3	H	piece	90.1	2.9	4.4	3.3
														Jun 17	570.8	J	piece	69.8	3.5	5.0	3.3
														Jun 17	570.13	A1	piece	87	2.2	2.5	3.3
														Jun 17	570.14	A2	piece	90.1	4.0	4.4	3.3
548	Teixoneres, Spain Unknown fauna, long bone	548.17	597.5	0.8	3.4	MAMS-34677	39390	320						Jun 17	548.3	H	piece	71.2	1.5	2.1	3.3
														Jun 17	548.8	J	piece	84.7	1.5	1.8	3.2
														Jun 17	548.13	A1	piece	78.1	0.6	0.8	3.4
														Jun 17	548.14	A2	piece	86.4	1.1	1.3	3.3

Pretreatment and gaseous radiocarbon dating of 40-100 mg archaeological bone

Supplementary Dataset S2 – Radiocarbon dates for all samples in the study dated with graphite targets and the gas ion source

	<100mg bone extraction, EA-GIS-AMS dates							Adjusted age		Statistical agreement of replicate measurements	Notes
R-EVA	AMS lab code	Coll wgt (µg)	GIS C (µg)	$F^{14}C$	±	^{14}C age	±	^{14}C age	±	*T is the calculated χ2 value and should be lower than the value given in brackets to be considered contamporaneous at 95% confidence. The degrees of freedom are given by 'df'.*	
570	Aix-12015.6.1	95	42	0.0150	0.0013	33,755	689	33,750	690		
	Aix-12015.6.2	104	46	0.0135	0.0013	34,606	748	34,610	750		
	Aix-12015.6.3	98	44	0.0135	0.0012	34,608	724	34,610	720	χ2 test: (df = 3, N = 4) T= 0.9 (5% 7.8)	
	Aix-12015.6.4	223	99	0.0138	0.0010	34,410	600	34,410	600	weighted mean: 34,340 +/-340	
	Aix-12015.5.1	200	82	0.0132	0.0011	34,768	647	34,770	650		
	Aix-12015.5.2	73	na	0.0147	0.0014	33,879	765	33,880	760		
	Aix-12015.5.3	84	32	0.0147	0.0014	33,921	763	33,920	760	χ2 test: (df = 3, N = 4) T = 1.4 (5% 7.8)	
	Aix-12015.5.4	76	31	0.0131	0.0014	34,830	836	34,830	+880/-800	weighted mean: 34,390 +/-380	
	Aix-12015.2.5	199	81	0.0132	0.0013	34,777	790	34,780	790		
	Aix-12015.2.6	78	31	0.0125	0.0013	35,177	862	35,180	+910/-820		
	Aix-12015.2.7	74	31	0.0132	0.0013	34,768	812	34,770	810	χ2 test: (df = 3, N = 4) T = 1.4 (5% 7.8)	
	Aix-12015.2.8	79	32	0.0147	0.0014	33,916	755	33,920	760	weighted mean: 34,670 +/-400	
	Aix-12015.3.5	194	79	0.0145	0.0012	33,988	643	33,990	640		
	Aix-12015.3.6	69	28	0.0159	0.0014	33,274	721	33,270	720		
	Aix-12015.3.7	87	35	0.0132	0.0013	34,772	806	34,770	810	χ2 test: (df = 3, N = 4) T = 2.0 (5% 7.8)	
	Aix-12015.3.8	74	30	0.0146	0.0014	33,971	768	33,970	770	weighted mean: 34,010 +/-370	
	Aix-12015.4.5	209	86	0.0128	0.0012	35,037	772	35,040	770		
	Aix-12015.4.6	86	34	0.0128	0.0013	34,992	835	34,990	840		
	Aix-12015.4.7	77	33	0.0147	0.0014	33,925	779	33,930	780	χ2 test: (df = 3, N = 4) T = 2.0 (5% 7.8)	
	Aix-12015.4.8	69	28	0.0121	0.0013	35,469	863	35,470	+910/-820	weighted mean: 34,870 +/-400	
										χ2 test for all replicates: (df=19, N =20) T=10.7 (5% 30.1)	
										weighted mean: 34,450 +/-170	
548	Aix-12017.2.1	191	71	0.0076	0.0011	39,247	1,145	39,250	+1,240/-1,070		
	Aix-12017.2.2	68	27	0.0069	0.0012	39,986	1,417	39,990	+1,560/-1,310		χ2 test for replicates from R-EVA 570.3 and R-EVA 570.8:
	Aix-12017.2.3	84	31	0.0065	0.0012	40,479	1,521	40,480	+1,690/-1,400	χ2 test: (df = 3, N = 4) T = 0.7 (5% 7.8)	(df = 6, N = 7) T = 3.5 (5% 12.6)
	Aix-12017.2.4	72	26	0.0077	0.0012	39,115	1,272	39,120	+1,380/-1,180	weighted mean: 39,640 +/-660	weighted mean: 39050 +/-460
	Aix-12017.1.2	71	28	0.0077	0.0012	39,129	1,264	39,130	+1,380/-1,170		
	Aix-12017.1.3	70	26	0.0086	0.0013	38,229	1,177	38,230	+1,270/-1,100	χ2 test: (df = 2, N = 3) T = 0.7 (5% 6.0)	
	Aix-12017.1.4	201	81	0.0091	0.0011	37,749	973	37,750	+1,040/-920	weighted mean: 38,300 +/-650	
	Aix-12017.3.1	64	25	0.0316	0.0017	27,747	439	27,750	440	χ2 test: (df = 1, N = 2) T = 0.2 (5% 3.8)	Very low collagen yield
	Aix-12017.3.2	69	29	0.0305	0.0018	28,027	461	28,030	460	weighted mean: 27,880 +/-320	
	Aix-12017.4.1	71	28	0.0168	0.0014	32,833	688	32,830	690		Very low collagen yield
	Aix-12017.4.2	72	28	0.0102	0.0013	36,862	1,040	36,860	+1,110/-980	χ2 test: (df = 2, N = 3) T=12.3 (5% 6.0)	
	Aix-12017.4.3	69	27	0.0122	0.0014	35,378	907	35,380	+960/-860	weighted mean: 34,930 +/-490	
										χ2 test for all replicates: (df = 11, N =12) T = 339.3 (5% 19.7)	

Pretreatment and gaseous radiocarbon dating of 40-100 mg archaeological bone

Supplementary Dataset S2 – Radiocarbon dates for all samples in the study dated with graphite targets and the gas ion source

		~500mg extraction, solid graphite target dates							~500mg bone extraction, EA-GIS-AMS dates (Fewlass et al., 2018)					<100mg bone extraction							
R-EVA	Site	R-EVA	Bone wt (mg)	Coll yld (%)	C:N	AMS lab code	^{14}C age	±	R-EVA	AMS lab code	GIS C (µg)	^{14}C age	± (err 2)	Session	R-EVA	Batch	Form	Bone wt (mg)	Coll yld (mg)	Coll yld (%)	C:N
124	Brown Bank, North sea Plains Woolly Rhino, long bone (Previously labeled as Bison)	124.43	639.5	11.7	3.1	MAMS-26877	50150	+2080/–1650	124.43	Aix-12002.4.1	84		>45430								
						Aix-12002.1.2	49300	+1610/–1340	124.43	Aix-12002.4.2	89		>43770	Jun 17	124.58	A1	piece	70.6	10.2	14.4	3.2
						Aix-12002.1.3	48800	+1530/–1290	124.43	Aix-12002.4.3	80	48610	+4930/-3030								
									124.43	Aix-12002.4.4	74		>43590								
														Jun 17	124.59	A2	piece	90.2	13.3	14.7	3.2
														Jun 17	124.60	A3	powder	88.8	3.5	3.9	3.2
														Jun 17	124.61	A4	powder	83.1	6.1	7.3	3.2

Pretreatment and gaseous radiocarbon dating of 40-100 mg archaeological bone

Supplementary Dataset S2 – Radiocarbon dates for all samples in the study dated with graphite targets and the gas ion source

R-EVA	<100mg bone extraction, EA-GIS-AMS dates							Adjusted age		Statistical agreement of replicate measurements	Notes
	AMS lab code	Coll wgt (µg)	GIS C (µg)	$F^{14}C$	±	^{14}C age	±	^{14}C age	±	*T is the calculated χ2 value and should be lower than the value given in brackets to be considered contamporaneous at 95% confidence. The degrees of freedom are given by 'df'.*	
124	Aix-12002.5.2	213	89	0.0038	0.0011	44,678	2,228	44,680	+2,610/-1,970		χ2 test including the Fewlass et al 2017 gas measurements:
	Aix-12002.5.3	67	27	0.0029	0.0011	46,959	3,156	46,960	+4,010/-2,660	χ2 test: (df = 2, N = 3) T = 1.5 (5% 6.0)	(df=18, N=19) T = 10.1 (5% 28.9)
	Aix-12002.5.4	82	34	0.0019	0.0011	50,193	4,503		>44,150	weighted mean: 47,030 +/-1780	weighted mean: 49210 +/- 930
	Aix-12002.6.1	218	91	0.0025	0.0010	48,114	3,263	48,110	+4,190/-2,740		
	Aix-12002.6.2	74	31	0.0031	0.0011	46,451	2,908	46,450	+3,610/-2,480		
	Aix-12002.6.3	76	32	0.0023	0.0011	48,845	3,745	48,840	+5,040/-3,070	χ2 test: (df = 3, N = 4) T = 0.3 (5% 7.8)	
	Aix-12002.6.4	77	32	0.0024	0.0011	48,528	3,698	48,530	+4950/-3040	weighted mean: 47,900 +/-1680	
	Aix-12002.7.1	201	73	0.0010	0.0010	55,266	8,199		>46,330		Large sample size corrected with small size background - Overcorrected
	Aix-12002.7.2	78	30	0.0019	0.0011	50,253	4,801		>43,940		
	Aix-12002.7.3	67	24	0.0025	0.0011	48,219	3,701	48,220	+4,960/-3,040	χ2 test: (df = 3, N = 4) T = 1.1 (5% 7.8)	
	Aix-12002.7.4	89	33	0.0020	0.0011	49,849	4,479		>43,830	weighted mean: 50,730 +/-2380	
	Aix-12002.8.1	190	75	0.0039	0.0012	44,586	2,446	44,590	+2,920/-2,140		Large sample size corrected with small size background - Overcorrected
	Aix-12002.8.2	81	28	0.0013	0.0011	53,543	7,035		>45,410		
	Aix-12002.8.3	69	33	0.0013	0.0011	53,475	6,813		>45,510	χ2 test: (df = 3, N = 4) T = 3.8 (5% 7.8)	
	Aix-12002.8.4	80	33	0.0012	0.0011	54,116	7,436		>45,700	weighted mean: 50,580 +/-2450	
										χ2 test for all replicates: (df=14, N =15) T = 9.2 (5% 23.7)	
										Weighted mean: 49,030 +/-1010	

Unknown archaeological samples corrected with collagen backgrounds measured in same session according to size (small or large sample size) and type (extracted in solid or powder form)
All powder samples corrected with 30ug powder backgrounds only (no 100 ug C BG)
3.5 ‰ scatter added to all samples
Absolute error of the blank changed to 0.001
For dates >15,000 BP, values have been rounded to 10
*Asymmetrical errors given wherever F14C ≤ 1σ*10*
"Older than" ages have been calculated for samples where F14C < 2σ, according to convention in van der Plicht and Hogg (2006)
"na" shows missing data.

Pretreatment and gaseous radiocarbon dating of 40-100 mg archaeological bone
Supplementary Dataset S3: EA-GIS-AMS data from background bone R-EVA 1753

Session	R-EVA	AMS lab code	Batch	Form	Bone wt (mg)	Collagen yld (%)	Collagen yld (mg)	GIS C mass (ug)	$F^{14}C$	$F^{14}C$ err	^{14}C age (y)	+-(y)
Small aliquots												
Jun-17	1753.29	Aix-12018.1.2	H	Piece	69.2	11.0	83	32	0.0031	0.0005	46,441	1,173
Jun-17	1753.29	Aix-12018.1.3	H	Piece	69.2	11.0	75	29	0.0036	0.0005	45,181	1,166
Jun-17	1753.34	Aix-12018.3.6	J	Piece	99.4	11.0	79	33	0.0026	0.0004	47,829	1,319
Jun-17	1753.39	Aix-12018.4.7	A1	Piece	99.7	12.2	79	32	0.0035	0.0005	45,333	1,143
Jun-17	1753.39	Aix-12018.4.8	A1	Piece	99.7	12.2	76	32	0.0029	0.0005	46,916	1,318
Jun-17	1753.41	Aix-12018.5.2	A2	Piece	97.6	13.1	69	28	0.0042	0.0006	43,951	1,076
Jun-17	1753.41	Aix-12018.5.3	A2	Piece	97.6	13.1	81	33	0.0028	0.0005	47,101	1,311
Jun-17	1753.43	Aix-12018.6.6	A3	Powder	80.9	2.3	76	27	0.0047	0.0006	43,011	1,013
Jun-17	1753.45	Aix-12018.7.2	A4	Powder	87.8	5.8	76	24	0.0054	0.0006	41,989	941
Jun-17	1753.45	Aix-12018.7.3	A4	Powder	87.8	5.8	81	24	0.0047	0.0008	43,127	1,453
Dec-17	1753.48	Aix-12018.9.1	L	Piece	62	13.5	99	50	0.0043	0.0005	43,680	990
Dec-17	1753.48	Aix-12018.9.2	L	Piece	62	13.5	95	36	0.0046	0.0006	43,300	1,010
Dec-17	1753.56	Aix-12018.12.1	P	Piece	68.7	13.0	90	39	0.0044	0.0005	43,600	940
Dec-17	1753.56	Aix-12018.12.2	P	Piece	68.7	13.0	103	45	0.0038	0.0005	44,830	1,000
Dec-17	1753.59	Aix-12018.13.1	R	Piece	79.9	14.6	90	41	0.0039	0.0006	44,470	1,200
Dec-17	1753.59	Aix-12018.13.2	R	Piece	79.9	14.6	105	46	0.0037	0.0004	44,880	930
Dec-17	1753.60	Aix-12018.10.2	S	Piece	69.3	14.7	99	43	0.0038	0.0005	44,720	1,060
Dec-17	1753.62	Aix-12018.14.1	U	Piece	55.9	15.4	93	41	0.0049	0.0005	42,800	880
Dec-17	1753.62	Aix-12018.14.2	U	Piece	55.9	15.4	112	49	0.0038	0.0005	44,710	1,000
Dec-17	1753.64	Aix-12018.15.1	BK	Piece	76.2	11.0	102	53	0.0040	0.0004	44,450	890
Dec-17	1753.64	Aix-12018.15.2	BK	Piece	76.2	11.0	108	41	0.0038	0.0004	44,700	880
Dec-17	1753.64	Aix-12018.15.3	BK	Piece	76.2	11.0	98	34	0.0039	0.0004	44,630	890
								Arithmetic mean:	**0.0039**			
								SD:	**0.00069**			
Large aliquots												
Jun-17	1753.29	Aix-12018.1.1	H	Piece	69.2	11.0	0.207	79	0.0025	0.0004	48,105	1,348
Jun-17	1753.34	Aix-12018.3.5	J	Piece	99.4	11.0	0.198	82	0.0013	0.0003	53,533	1,772
Jun-17	1753.39	Aix-12018.4.6	A1	Piece	99.7	12.2	0.205	88	0.0018	0.0003	50,621	1,417
Jun-17	1753.41	Aix-12018.5.1	A2	Piece	97.6	13.1	0.218	89	0.0027	0.0004	47,386	1,299
Dec-17	1753.56	Aix-12018.12.3	P	Piece	68.7	13.0	226	97	0.0024	0.0004	48,530	1,210
Dec-17	1753.60	Aix-12018.10.3	S	Piece	69.3	14.7	231	96	0.0026	0.0004	47,870	1,260
Dec-17	1753.60	Aix-12018.10.4	S	Piece	69.3	14.7	221	96	0.0029	0.0004	47,000	1,190
Dec-17	1753.62	Aix-12018.14.3	U	Piece	55.9	15.4	224	97	0.0034	0.0004	45,650	870
Dec-17	1753.64	Aix-12018.15.4	BK	Piece	76.2	11.0	252	98	0.0023	0.0003	48,780	1,060
								Arithmetic mean:	0.0024			
								SD:	0.00061			

Pretreatment and gaseous radiocarbon dating of 40-100 mg archaeological bone
Supplementary Dataset S4: EA-GIS-AMS data from system blanks

Session 1: June 2017							
AMS lab code	**Sample**	**GIS C mass (ug)**	**$F^{14}C$**	**$F^{14}C$ err**	**^{14}C age (y)**	**+-(y)**	**Notes**
Aix-10424.2.10	IAEA-C1	103	0.0022	0.0004	48,982	1,337	
Aix-10424.2.11	IAEA-C1	not recorded - large	0.0026	0.0004	47,787	1,177	
Aix-10424.2.12	IAEA-C1	not recorded - large	0.0039	0.0005	44,607	1,068	
Aix-10424.2.24	IAEA-C1	104	0.0024	0.0005	48,487	1,520	
Aix-10424.2.9	IAEA-C1	120	0.0025	0.0004	48,128	1,214	
Aix-10424.2.13	IAEA-C1	not recorded - small	0.0036	0.0005	45,153	1,111	
Aix-10424.2.14	IAEA-C1	39	0.0046	0.0008	43,311	1,330	
Aix-10424.2.16	IAEA-C1	19	0.0089	0.0009	37,896	832	not included due to small size
Aix-10424.2.17	IAEA-C1	14	0.0067	0.0010	40,164	1,158	not included due to small size
Aix-10424.2.21	IAEA-C1	28	0.0032	0.0005	46,039	1,225	

Session 2: December 2017							
AMS lab code	**Sample**	**GIS C mass (ug)**	**$F^{14}C$**	**$F^{14}C$ err**	**^{14}C age (y)**	**+-(y)**	**Notes**
Aix-10109.2.12	Phthalic anhydride	46	0.0039	0.0004	44,648	870	8.00592.0100 from millipore
Aix-10109.2.13	Phthalic anhydride	117	0.0020	0.0004	49,822	1,598	8.00592.0100 from millipore
Aix-10109.2.14	Phthalic anhydride	43	0.0030	0.0004	46,759	1,046	8.00592.0100 from millipore
Aix-10109.2.15	Phthalic anhydride	133	0.0025	0.0004	48,245	1,300	8.00592.0100 from millipore