LETTERS

Direct dating of Early Upper Palaeolithic human remains from Mladeč

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The human fossil assemblage from the Mladeč Caves in Moravia (Czech Republic)[1] has been considered to derive from a middle or later phase of the Central European Aurignacian period on the basis of archaeological remains (a few stone artefacts and organic items such as bone points, awls, perforated teeth)[2], despite questions[3] of association between the human fossils and the archaeological materials and concerning the chronological implications of the limited archaeological remains[4]. The morphological variability in the human assemblage, the presence of apparently archaic features in some specimens, and the assumed early date of the remains have made this fossil assemblage pivotal in assessments of modern human emergence within Europe[5–7]. We present here the first successful direct accelerator mass spectrometry radiocarbon dating of five representative human fossils from the site. We selected sample materials from teeth and from one bone for ^{14}C dating. The four tooth samples yielded uncalibrated ages of ~31,000 ^{14}C years before present, and the bone sample (an ulna) provided an uncertain more-recent age. These data are sufficient to confirm that the Mladeč human assemblage is the oldest cranial, dental and postcranial assemblage of early modern humans in Europe and is therefore central to discussions of modern human emergence in the northwestern Old World and the fate of the Neanderthals.

The Mladeč site has significance for both human evolutionary and archaeological issues[3,8,9], and the relevance of its remains has increased as a result of the recent dating of the purportedly Aurignacian-age modern human remains from Velika Pećina (Croatia)[10], Hahnöfersand (Germany)[11] and Vogelherd (Germany)[3] to the Holocene epoch, the remains from Koněprusy (Czech Republic)[9] to the Magdalenian period, and those from Cro-Magnon (France)[12] and La Rochette (France)[13] to the Gravettian period. The only directly dated European modern human fossils of Aurignacian age are the Peştera cu Oase (Romania) mandible and cranium at ~35,000 ^{14}C years before present (that is, ~35 ^{14}C kyr BP)[14], the Kent's Cavern (UK) maxilla at ~31 ^{14}C kyr BP[15], the Peştera Muierii (Romania) remains at ~30 ^{14}C kyr BP[16], and the Peştera Cioclovina (Romania) cranium at ~29 ^{14}C kyr BP[16], none of which has a secure and diagnostic archaeological association. Moreover, at least the Oase fossils overlap in time with late Neanderthals from for example, Vindija (Croatia), which is at present dated to ~29 ^{14}C kyr BP[10] and Arcy-sur-Cure (France) at ~34 ^{14}C kyr BP[17]. The assessment of whether the Mladeč fossils are indeed Aurignacian in age, and if so, their chronological position within the Aurignacian time span, has become central to understanding early modern humans in Europe.

The Mladeč human remains are universally accepted as those of early modern humans since the analysis of Szombathy[1]. However, there is an ongoing debate as to whether they exhibit distinctive archaic features, indicative of some degree of regional Neanderthal ancestry, or are morphologically aligned solely with recent humans and therefore document only a dispersal of modern humans into Europe. The purportedly archaic, or Neanderthal, features include aspects of the sagittal cranial profile and robust supraorbital regions

Figure 1 | Dated human fossil specimens from Mladeč. a, Upper jaw fragment Mladeč 8, male, exhibiting modern human features and some 'archaic' features such as dental dimensions. **b**, Cranium Mladeč 1, female, exhibiting derived modern human features. The graduation marks on both scales indicate centimetres. Copyright for the photographic material: Wolfgang Reichmann (2004), Naturhistorisches Museum, Anthropologische Abteilung, Burgring 7, 1010 Vienna, Austria.

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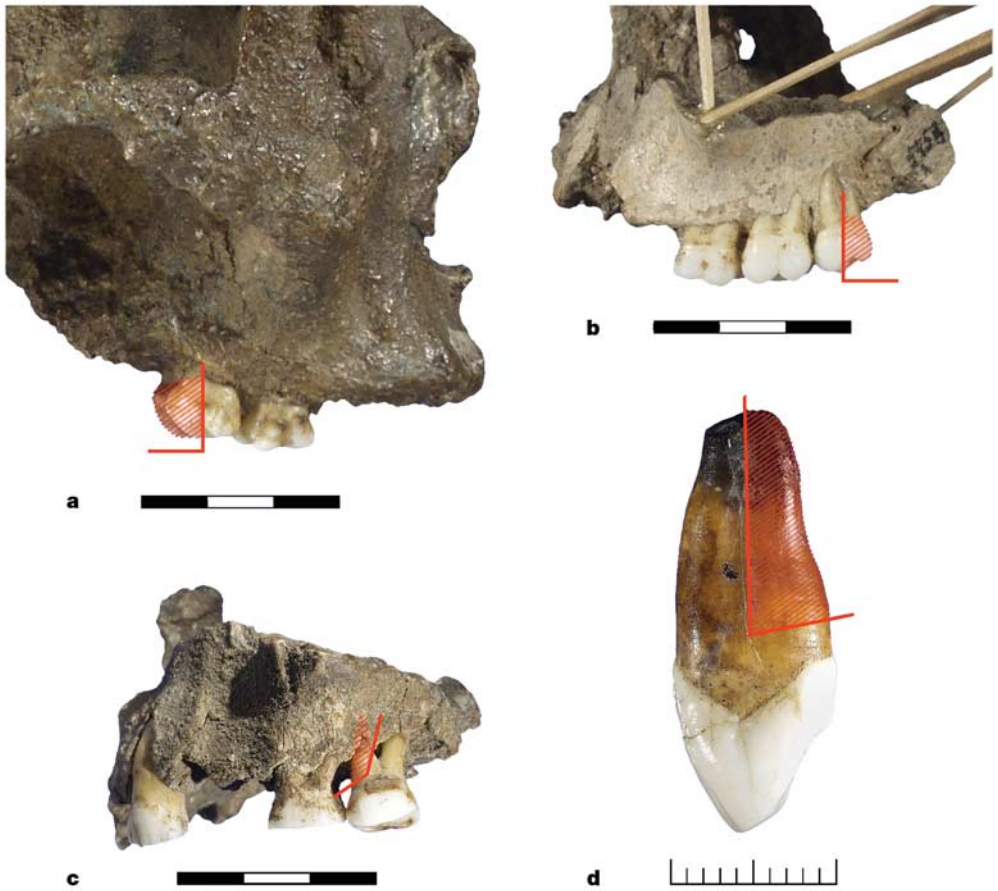

Figure 2 | Documentation of the dated specimens showing the sampled parts in red. **a**, Mladeč 1, lateral view from right. **b**, Mladeč 2, lateral view from left. **c**, Mladeč 8, lateral view from left. **d**, Mladeč 9a, right maxillary canine, mesial view. A centimetre scale is displayed for **a** to **c**; for **d** the minor graduation marks on the scale indicate 1 mm. Copyright for the photographic material: Wolfgang Reichmann (2004), Naturhistorisches Museum, Anthropologische Abteilung, Burgring 7, 1010 Vienna, Austria.

in the Mladeč 5 and 6 males, distinctive occipital bunning in Mladeč 3, 5 and 6, large palatal and dental dimensions of Mladeč 8 (Fig. 1a), the large crowns of the Mladeč 9a, 10 and 51 canines, and articular hypertrophy of some of the postcrania. Moreover, although they are robust compared to recent females, the Mladeč 1 (Fig. 1b) and 2 crania exhibit few of these features[5,6]. And most recently, ancient DNA from Mladeč 2 and 25c failed to show evidence of the mitochondrial (mt)DNA sequences found in some Neanderthals[18]. The Neanderthal affinities of some of the anatomical features have been questioned[7], but ultimately the implications of the Mladeč assemblage for human population dynamics with the transition to modern humans in Europe are dependent on whether they can be dated close to the transitional time period.

Several efforts have so far been made to obtain reliable and relevant ^{14}C dates from the Mladeč fossils, but all of them failed. These dating attempts were followed at the Centre for Isotope Research Radiocarbon Laboratory in Groningen by ^{14}C dating of carbonate samples from remnants of the crust that appear to have sealed the layer containing the human bones and artefacts in the 'Dome of the Dead'. From the results (GRN-26333: $34,160^{+520}_{-490}$ ^{14}C yr BP and GRN-26334: $34,930^{+520}_{-490}$ ^{14}C yr BP) it was concluded that the minimum age of the bones is 34–35 ^{14}C kyr BP[9]. Another attempt to date the human fossils was performed at the Vienna Environmental Research Accelerator (VERA) Laboratory in Vienna. Curatorial considerations to save the

Table 1 | Results of EA/IRMS quality checks of the Mladeč samples

Laboratory number	Sample name	C content (% DW)	N content (% DW)	C/N ratio	δ^{13}C (‰)	δ^{15}N (‰)
VERA-2736*	Mladeč 25c	6.44 ± 0.20	0.47 ± 0.06	13.7 ± 1.7	−24.6 ± 0.2	10.0 ± 0.5
VERA-3073†	Mladeč 1	11.8	3.2	3.7	−19.1	10.6
VERA-3074†	Mladeč 2	6.4	1.4	4.7	−20. 6	10.3
VERA-3075‡	Mladeč 8	10.7 ± 0.1	2.3 ± 0.2	4.7 ± 0.4	−21.4 ± 0.3	11.7 ± 0.4
VERA-3075‡	Mladeč 8, collagen	44.3 ± 0.3	16.1 ± 0.7	2.7 ± 0.1	−20.1 ± 0.4	10.9 ± 0.7
VERA-3076A‡ and VERA-3076B‡	Mladeč 9a, right maxillary canine	9.6 ± 0.6	2.4 ± 0.4	4.0 ± 0.3	−19.7 ± 0.2	9.6 ± 0.6

*Values represent the mean value of three EA/IRMS measurements and one standard deviation (s.d.) of the mean.
†Only a single EA/IRMS measurement was performed.
‡Values represent the mean value of two EA/IRMS measurements and one s.d. of the mean.
DW, dry weight. The δ^{13}C and δ^{15}N values are defined as the relative deviation (in ‰) of the ^{13}C/^{12}C and ^{15}N/^{14}N ratio of a sample from the ^{13}C/^{12}C of the V-PDB (Vienna-Pee Dee Belemnite) standard and the ^{15}N/^{14}N of the atmospheric N_2 standard, respectively. The s.d. of the mean values given in the table for multiple measured samples include uncertainties due to sample inhomogeneities. The reproducibility of the measurements of the laboratory standard was 0.1‰ (s.d.) for δ^{13}C and <0.2‰ (s.d.) for δ^{15}N. The δ^{13}C of the untreated samples reflects the isotopic composition of the total carbon present in the sample originating from the organic and the inorganic sample fraction, as well as from exogenous carbon. Similarly, the δ^{15}N reflects the isotopic composition of the total nitrogen.

Table 2 | Radiocarbon ages determined for the human remains from the Mladeč site

Laboratory number	Sample name	Sample material	^{14}C-age (yr BP)*
VERA-2736	Mladeč 25c	Ulna	$26{,}330 \pm 170$
VERA-3073	Mladeč 1	Right M2, distal half of the crown	$31{,}190^{+400}_{-390}$
VERA-3074	Mladeč 2	Left M3, distal half of the crown	$31{,}320^{+410}_{-390}$
VERA-3075	Mladeč 8	Left M2, mesial-buccal root	$30{,}680^{+380}_{-360}$
VERA-3076A	Mladeč 9a, right maxillary canine	Lingual half of the root (white-coloured collagen)	$31{,}500^{+420}_{-400}$
VERA-3076B	Mladeč 9a, right maxillary canine	Lingual half of the root (brown-coloured collagen)	$27{,}370 \pm 230$

*Errors are one-sigma uncertainties.

human bones necessitated first ^{14}C dating of the animal remains from Mladeč with accelerator mass spectrometry (AMS), thereby dating the human remains indirectly. Out of eight selected samples from different species, only five could be dated successfully. The uncalibrated ^{14}C ages of these specimens yielded a wide range from 8.5 ^{14}C kyr BP to about 42 ^{14}C kyr BP (see Supplementary Table 1)—so an accurate indirect dating of the human remains was impossible. Therefore we needed to date the human remains directly.

We used a proximal ulna fragment, Mladeč 25c, and tooth samples from the most prominent specimens kept at the Naturhistorisches Museum in Vienna, that is, Mladeč 1, 2, 8 and the isolated maxillary right canine Mladeč 9a (samples from Mladeč 2 and Mladeč 25c were also used in the DNA study, see above). We assumed that the collagen in dentine is preserved from degradation and contamination (for example, consolidants) in non-abraded teeth with an intact enamel layer (Mladeč 1, 2) or in tooth roots covered by an intact alveolar bone (Mladeč 8). The isolated canine Mladeč 9a was in an excellent state of preservation in general and was therefore selected for dating as well. Before sampling, casts of the teeth were made to preserve the morphological information. Approximately one-half of each crown or part of the root (see Fig. 2) was taken for the radiocarbon determinations.

Amino-acid analysis of bone samples performed in the course of the DNA study indicated the variable preservation state of the human fossils. Amino acids of Mladeč 2 and 25c were identified as well preserved and fulfilled the criteria for DNA analysis, whereas the collagen of other bone samples, including Mladeč 8, appeared not suitable[18]. Although these results show that the collagen of some of the Mladeč fossils is well preserved, the suitability for ^{14}C dating of the selected samples was tested directly. A good preservation of the collagen was detected for all teeth samples via carbon and nitrogen elemental and isotopic analysis (see Methods and Table 1). Only the ulna appeared less well preserved and probably contaminated.

To remove possible superficial contaminants from the ^{14}C samples the tooth surface was abraded, leaving 350 to 200 mg of sample material for further processing. We used a method similar to that of ref. 19 for the chemical pre-treatment of the teeth (see Methods). The pretreated collagen from the human teeth and gelatine produced from the ulna and the animal bones were subjected to the routine sample preparation and measurement procedure used for ^{14}C dating of archaeological samples at VERA[20,21].

The ^{14}C ages of all human Mladeč samples dated in this study are listed in Table 2. All uncalibrated ages of the teeth agree at $\sim$31 ^{14}C kyr BP within uncertainties (except for sample VERA-3076B). The ^{14}C age of $\sim$26 ^{14}C kyr BP of the ulna is significantly younger. It is not certain that contamination in the ulna, detected in the quality check (see Methods), was completely removed by the applied chemistry. In the case of the isolated canine, a dark brown colour of the root apex was detected. Samples VERA-3076A and VERA-3076B both originate from the canine, but from different fractions of the extracted collagen—coloured white and brownish, respectively. The younger age of the dark collagen (VERA-3076B) supports our hypothesis that the colour of the apex resulted from contamination that was at least partially present after the chemical processing.

The ages determined for the Mladeč samples all lie within a time period for which a generally agreed calibration curve for the transformation of uncalibrated ^{14}C ages $>$20 kyr BP into calendar time ranges is not yet available. According to the existing, albeit divergent, ^{14}C records for this period determined in different archives, a shift of the 'true ages' by several thousand years towards higher ages might be possible[22]. (See refs 23 and 24 for further discussion on this issue.)

The AMS ^{14}C dating of the Mladeč human remains confirms that they derive from the time period of the middle to late Aurignacian of Central Europe. With the presence of multiple individuals, males and females, adult and immature with cranial, dental and post-cranial elements, the Mladeč assemblage is the oldest directly dated substantial assemblage of modern human remains in Europe. Only the $\sim$35 ^{14}C kyr BP Peştera cu Oase mandible and cranium, from two individuals, are securely older among European early modern humans, and they currently lack postcranial remains and an archaeological association. Moreover, the Mladeč dates on both robust 'males' (Mladeč 8 and 9a) and less robust 'females' (Mladeč 1 and 2) fall into the same time period, reinforcing the idea that the variability within the assemblage reflects not only the original population variability but probably also its level of sexual dimorphism.

METHODS

Quality test of the sample material. In addition to the ^{14}C samples, small samples ($\sim$10 mg) of the dentine as well as some material from the ulna were acquired for combined elemental analysis/stable isotope ratio mass spectrometry (EA/IRMS). The measurements were performed with an elemental analyser (EA 1110, CE Instruments) coupled to a gas isotope ratio mass spectrometer (Delta^{PLUS}, Finnigan MAT) operating in the continuous flow mode.

We measured the carbon and nitrogen content and deduced the C/N ratios of the powdered samples. The C/N ratio comprises the ratio of the total carbon (from the inorganic matrix, the organic material and possible contaminants) to the total nitrogen (from amino acids and possible contaminants). This quality check was suggested by Hedges and van Klinken[25]. They argue that C/N ratios $\gg 4$ in bone samples may result from deamination (a diagenetic consequence) or may indicate the presence of large amounts of exogenous carbon. The C/N ratios of the untreated teeth lie between $\sim$4.7 and $\sim$3.7, and they are therefore within the accepted range or only slightly enhanced. This indicates that no significant carbon contamination is present in the samples.

The nitrogen content of fresh, defatted and dried compact bones lies between 4% and 5% DW (dry weight) (ref. 26). The good preservation state of the collagen of all teeth samples was denoted by N contents above 1.4% DW. Although mainly applied for collagen extracts and appraised as not very sensitive for the detection of contaminants, the δ^{13}C and δ^{15}N values of the untreated dentine support the good preservation of the collagen and the absence of large amounts of exogenous carbon (see Table 1).

The collagen yield of the ^{14}C sample Mladeč 8 (25 mg collagen extracted from $\sim$300 mg sample material) was sufficiently high to enable an EA/IRMS measurement of a subsample from the purified collagen also. A C/N ratio of $\sim$2.7 was determined for this sample. This value is comparable with C/N ranges from 3.5–2.9 (refs 27, 28) and 3.4–2.6 (ref. 29) for gelatinized collagen from well-preserved bones. The values of the N content and δ^{13}C and δ^{15}N values (see Table 1) also

indicate that the collagen extracted from Mladeč 8 can be considered well preserved.

The low N content of the unprocessed ulna (~0.5% DW) and the low collagen yield during sample preparation show that the degradation of the collagen is already advanced, but the extant amount is still higher than 5% of the collagen content in recent bones. This 5% level is considered a prerequisite for reliable ^{14}C dating of bones[25]. A C/N ratio of about 14 indicates the presence of exogenous carbon in this sample.

Chemical pretreatment of ^{14}C teeth samples. We extracted the collagen from the teeth by dissolving the inorganic material in dilute HCl. In the case of the crown samples, where the major part of the enamel was removed mechanically, the rest of the enamel still present was dissolved in this step. (Collagen yields cannot be taken as a measure of the collagen preservation in the crowns because an unknown variable part of the enamel, which is very low in organic material, was present in these samples.) In addition to the demineralization step, we treated the samples with a dilute NaOH solution, and thereafter again with HCl. After each step the collagen was washed with bi-distilled water.

The gelatine production—frequently used for the preparation of ^{14}C-bone samples[26]—was omitted in the treatment of the human teeth to avoid unnecessary loss of the valuable sample material which comes along with each clean-up step. As mentioned above, for collagen originating from 'protected' dentine a smaller risk for contamination can be assumed. Even the NaOH treatment of the collagen for the removal of humic acids would not have been necessary, because the collagen appeared as a white coloured substance (except in one case, see above) after demineralization. The NaOH solution stayed uncoloured during this treatment, which indicates that no significant amount of humic acids was present in these samples.

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