Revised direct radiocarbon dating of the Vindija G_1 Upper Paleolithic Neandertals

Tom Higham*, Christopher Bronk Ramsey*, Ivor Karavanić†, Fred H. Smith‡, and Erik Trinkaus§¶

*Oxford Radiocarbon Accelerator Unit, University of Oxford, 6 Keble Road, Oxford OX1 3QJ, United Kingdom; †Odsjek za arheologiju, Filozofski fakultet, Sveučilišta u Zagrebu, Ivana Lučića 3, HR-10000 Zagreb, Croatia; ‡Department of Anthropology, Loyola University Chicago, 6525 North Sheridan Road, Chicago, IL 60626; and §Department of Anthropology, Washington University, Campus Box 1114, St. Louis, MO 63130

Contributed by Erik Trinkaus, November 17, 2005

The 1998/1999 direct dating of two Neandertal specimens from level G_1 of Vindija Cave in Croatia to ≈28,000 and ≈29,000 radiocarbon (^{14}C) years ago has led to interpretations concerning the late survival of Neandertals in south-central Europe, patterns of interaction between Neandertals and in-dispersing early modern humans in Europe, and complex biocultural scenarios for the earlier phases of the Upper Paleolithic. Given improvements, particularly in sample pretreatment techniques for bone radiocarbon samples, especially ultrafiltration of collagen samples, these Vindija G_1 Neandertal fossils are redated to ≈32,000–33,000 ^{14}C years ago and possibly earlier. These results and the recent redating of a number of purportedly old modern human skeletal remains in Europe to younger time periods highlight the importance of fine chronological control when studying this biocultural time period and the tenuous nature of monolithic scenarios for the establishment of modern humans and earlier phases of the Upper Paleolithic in Europe.

Aurignacian | early modern humans | Europe | Late Pleistocene

The period between ≈40,000 and 28,000 radiocarbon (^{14}C) years ago (B.P.) in Europe witnessed a complex series of shifts in human biological and behavioral evolution. It saw the cultural transition from late Middle Paleolithic technocomplexes to those of the earlier phases of the Upper Paleolithic, a transition that took place in a temporal and geographical mosaic across the northwestern Old World. The period experienced the dispersal of early modern humans into the region, their probable contemporaneity within Europe with late Neandertal populations, and the eventual disappearance of the Neandertals through geographically variable population processes. As the best documented sequence for the Late Pleistocene archaic to modern human and the Middle to Upper Paleolithic biocultural transition, the European record continues to be the focus of attention, debate, and disagreement over the cultural and biological processes and the biocultural interactions that were involved in the transition. Moreover, because it involved, by its end, the final establishment of humans morphologically similar to and whose cultural behaviors were close to those of ethnohistoric foraging populations, this transition continues to generate interest as the final "event" in the sequence of our predecessors becoming "human."

Our perceptions of the biocultural processes involved in this transitional period have been deeply affected in the past decade by the application of accelerator mass spectrometry (AMS) radiocarbon dating to both late Neandertals and early modern humans (1, 2). Beginning in the 1990s, several early modern humans have been directly dated to >28,000 B.P. (3–9). In addition, a suite of purportedly pre-28,000 B.P. modern human remains has been assigned to either later phases of the Upper Paleolithic [Cro-Magnon (France), La Rochette (France), and Koněprusy (Czech Republic) (10–12)] or the Holocene [Engis (Belgium), Hahnöfersand (Germany), St. Prokop (Czech Republic), Velika Pećina (Croatia), and Vogelherd (Germany) (13–18)]. Direct and indirect dating has placed several Neandertal specimens at the beginning and the middle of this chronological period [Arcy-sur-Cure (Grotte du Renne, France), Feldhofer (Germany), Saint-Césaire (France), and Zaskalnaya (Ukraine) (19–22)] and has placed others toward the more recent end in the cul-de-sac of Iberia [Cabezo Gordo (Spain), Columbeira (Portugal), Figueira Brava (Portugal), and Zafarraya (Spain) (23–25)]. Moreover, two Neandertals from Vindija Cave in Croatia yielded radiocarbon determinations at the end of this transitional period (15).

Although revised dating and technological considerations have placed the beginnings of the initial Upper Paleolithic ≈40,000 B.P. and the emergence of the Aurignacian *sensu stricto* close to 37,000 B.P. (26), the revised dating of human fossils has removed any clear association of diagnostic human remains with the Aurignacian before ≈34,000 B.P.; only the French early modern human remains from Brassempouy, La Quina, and Les Rois are securely associated with Aurignacian assemblages, and they are all <34,000 ^{14}C years old (27, 28). The Romanian Peştera cu Oase modern humans, dated to ≈35,000 B.P., are dated earlier, but they have no archeological association (7, 29). As a consequence, what was once perceived as a smooth transition of culturally Aurignacian early modern humans replacing Middle Paleolithic and initial Upper Paleolithic Neandertals across Europe has become more complex and ambiguous. Although the resolution of these processes depends on the analysis of the paleoanthropological record and the discovery of diagnostic human remains with secure archeological associations in this time period, it is apparent that the changing chronological interrelationships of the humans and the technocomplexes continue to play a critical role in the decipherment of these processes.

In this context, the late Neandertal fossil remains from level G_1 of Vindija Cave in northern Croatia have a pivotal role. In 1998 and 1999, two specimens, Vi-207 and Vi-208, were directly AMS dated to ≈29,000 B.P. (29,080 ± 400; OxA-8296) and ≈28,000 B.P. (28,020 ± 360; OxA-8295), respectively (15). Vi-207 is a right posterior mandible, and Vi-208 is a parietal fragment. Both have distinctively Neandertal affinities (30, 31). The dating of these specimens made them the most recent known Neandertals and documented an extensive temporal overlap between Neandertals and early modern humans in Central Europe.

In addition to these human biological relationships, level G_1 at Vindija Cave yielded a small archeological assemblage containing technotypologically Middle and earlier Upper Paleolithic lithic artifacts plus several distinctively early Upper Paleolithic bone points (31). It has been argued that the mix of Neandertals, Middle Paleolithic tools, and Upper Paleolithic technology was

Conflict of interest statement: No conflicts declared.

Abbreviations: B.P., radiocarbon (^{14}C) years before present; AMS, accelerator mass spectrometry; C:N, atomic ratio of carbon to nitrogen; ORAU, Oxford Radiocarbon Accelerator Unit.

¶To whom correspondence should be addressed. E-mail: trinkaus@wustl.edu.

ANTHROPOLOGY

Table 1. Past and current results of direct radiocarbon dating of the Vindija G_1 (Vi-207 and Vi-208) Neandertal remains

Sample (P) no.	OxA/OxA-X no.	Fraction	^{14}C age B.P., yr	C:N	δ^{13}C, ‰	δ^{15}N, ‰	Bone wt, mg	Collagen wt, mg	% C	% N
			Sample 12 Vi-208(110)							
9663	8295	AG	28,020 ± 360	3.2	−19.5	10.6	233.9	15.2	37.1	13.5
	2082–09	AG	29,200 ± 360	3.6	−19.8	11.4	229.9	10.9	42.7	14.0
	2089–06	AF >30 kDa	32,400 ± 800	3.4	−20.2	10.3	229.9	4.8	42.3	14.6
	2094–10	AF <30 kDa	31,390 ± 220	3.3	−19.5	10.0				
			Sample 14 Vi-207(136)							
9665	8296	AG	29,080 ± 400	3.6	−20.5	11.3	229.2	9.7	36.6	11.8
	2082–10	AG	29,100 ± 360	4.1	−22.8	12.2	128.8	6.7	41.6	11.7
	2089–07	AF >30 kDa	32,400 ± 1,800	4.3	−24.6	11.1	128.8	2.1	39.0	10.7

OxA and OxA-X numbers are given. The latter are experimental, nonroutine chemistry prefixes with OxA-X-*nnnn-xx* corresponding with the AMS wheel and position number in that wheel.

the result of cryoturbation and *Ursus spelaeus* (cave bear) activity in level G_1, with elements mixing into level G_1 from both the Upper Paleolithic F complex above and the Middle Paleolithic level G_3 below (32, 33). However, the lack of evidence for such disturbances in the primary area yielding these fossils and artifacts makes that explanation unlikely (34, 35). Yet, the technotypological mix in level G_1 remains unexplained. It is the only such complex known, and the situation is further complicated by the association of the artifacts with Neandertals dated to the period of the latest Aurignacian and the transition to the Gravettian in Central Europe.

The resolution of the scenarios concerning the behavioral and reproductive interactions of Neandertals and early modern humans, scenarios central to our understanding of the dispersal of modern humans and the ultimate fate of the Neandertals, are ultimately dependent upon the accurate temporal ordering of these human groups within geographical regions. Because of the critical importance of the dates on the Vindija 207 and 208 Neandertals, and given recent technical and chemical pretreatment advances in AMS radiocarbon dating since 1998 (36), it seemed appropriate to reassess these crucial, directly dated, late Neandertals.

Radiocarbon Redating of Vindija 207 and 208

Previous AMS radiocarbon dates from Vindija Cave were characterized by a high failure rate which resulted from the lack of recoverable collagen and the poor preservation of the material that yielded collagen (15). Reliably dating bone under these circumstances is challenging and requires a careful assessment of the quality of the extracted collagen before and during the dating process (see *Methods*). Resultant suspicions about the late dates from the initial Vindija results prompted one of us (E.T.) to contact the Oxford Radiocarbon Accelerator Unit (ORAU) and ascertain whether more work using current ultrafiltration sample preparation protocols (36) might be feasible. Fortunately, not all of the initially sampled material from the Neandertal specimens Vi-207 (mandible) and Vi-208 (parietal) had been used in the original analysis. The amount of archived bone powder was small, however; only 129 mg remained from Vi-207, and 230 mg remained from Vi-208. These weights are 0.5–0.25% of the routinely required sample starting weight (see *Methods*).

The analytical data and AMS determinations for these specimens are reported in Table 1. Different codes are used to designate different pretreatments in Table 1. AG refers to filtered gelatin (i.e., the pretreatment method as applied in 1998 and 1999), and AF refers to ultrafiltration of the extracted gelatin. The Vi-208 sample (P9663) produced a reasonable yield of AG filtered gelatin (4.7 wt % collagen), indicating that as much as 20% of the original collagen may remain. Not all of the collagen is extractable in poorly preserved bone (range 20–50%). The atomic ratio of carbon to nitrogen (C:N) for this sample was poor (3.6) and therefore outside our range of acceptance. The AMS result (OxA-X-2082-09) of 29,200 ± 360 B.P. is slightly older than the original AMS date of OxA-8295 (28,020 ± 360 B.P.).

We then rehydrolyzed and ultrafiltered 7.0 mg of the remaining gelatin. The >30-kDa fraction was retained and lyophilized, yielding 4.8 mg of gelatin. The C:N ratio (3.4) of this higher-molecular-weight material indicates collagen of an acceptable quality, as do the percentage carbon and nitrogen yields upon combustion. The AMS determination for the >30-kDa fraction was 32,400 ± 800 B.P. (OxA-X-2089-06). This date is older than both of the previous gelatin dates and mirrors the pattern of older radiocarbon determinations for bones that are ultrafiltered, compared with determinations of the same bone pretreated by using less rigorous methodologies (36, 37). We also analyzed the <30-kDa fraction of the ultrafiltered gelatin. In this example, the results are consistent with the improved removal of low-molecular-weight contaminants of a more modern age that would otherwise be incorporated in the unfiltered gelatin, although the age difference is not significant. The shift in the age indicates that the fraction of removed contaminants comprises traces of ^{14}C of a more modern age.

The Vi-207 sample was pretreated in an identical manner. The sample's starting weight was, however, only 129 mg. The C:N ratio for the AG (filtered gelatin) fraction of the sample was 4.1 and therefore outside our acceptance range and indicative of contamination with exogenous carbon. The AMS date of 29,100 ± 360 B.P. (OxA-X-2082-10) is identical to the first date for the same specimen (OxA-8296), which was treated by using the same method and which also had an unacceptably high C:N ratio. This finding casts additional doubt on the reliability of the first analysis (OxA-8296). The >30-kDa fraction was very small (2.1 mg) and produced a C:N ratio of 4.3, which similarly indicates a high-molecular-weight contaminant. The δ^{13}C value of −24.6‰ suggests a humic/sediment source. The nitrogen yield values are also in the lower range of values expected for intact collagen. The radiocarbon determination we obtained was 32,400 ± 1,800 B.P., which, with the high standard error, is indistinguishable in age from the AG (filtered gelatin) fraction (the high standard error is a result of the small size of the sample). We consider this age to be indicative of the age of the fragmentary remaining bone collagen and a proportion of the higher-molecular-weight noncollagenous material, which may, in fact, be unbroken cross-links with collagen. This age is not demonstrably accurate despite the apparent similarity with the age obtained from the other specimen.

Discussion

The Vindija G_1 Age. These redates show conclusively that the original direct AMS determinations on the Vindija 207 and 208

Table 2. Direct and associated AMS radiocarbon dates for European early modern humans >28,000 B.P.

Specimen	^{14}C age, yr	Lab no.	Ref.
Oase 1	>35,200	OxA-1711	7
	34,290, +970, −870	GrA-22810	
	(34,950 +990, −890)		
Kostenki 1	32,600 ± 1,100	OxA-7073	4, 6
Mladeč 1	31,190 +400, −390	VERA-3073	8
Mladeč 2	31,320 +410, −390	VERA-3074	8
Mladeč 8	30,680 +380, −360	VERA-3075	8
Mladeč 9a	31,500 +420, −400	VERA-3076a	8
Kent's Cavern 4	30,900 ± 900	OxA-1621	3
Muierii 2	30,150 ± 800	LuA-5228	5, 9
Cioclovina 1	29,000 ± 700	LuA-5229	5, 9

Ages are provided in ^{14}C years B.P. All dates are direct human fossil dates. The date in parentheses for Oase 1 is the combined result of the two determinations. The original Kent's Cavern 4 date is provided, although it is almost certainly too young (43), and its morphology may not be diagnostic of early modern humans.

remains were too young and that the "true" ages should be in the vicinity of 32,000 B.P. or slightly older. Previous attempts to radiocarbon date *U. spelaeus* bones from level G_1 yielded disparate results, from 18,280 ± 440 B.P. (Z-2432), to 33,000 ± 400 B.P. (ETH-12714), to 46,800 + 2,300, −1,800 B.P. (VERA-1428) (38–40). The first is clearly too young for the stratigraphy, and the last is either too old due to preservation problems or may derive from the underlying level G_3 (35). The date of ≈33,000 B.P. is in close agreement with the new results from the level G_1 Neandertal remains, although all of the results should not be used to infer more than that the level G_1 human remains and associated archeological debris date in the vicinity of 32,000–34,000 B.P. and perhaps somewhat earlier. If the dates on the humans and the cave bear are the best approximations of the age of level G_1, the dates would place the now substantial, if fragmentary, sample of Neandertal remains from this level (30, 35, 41) in the middle of the biocultural transition period in Europe.

The European Context. Turning our attention to dated early modern human remains from Europe, it is worth acknowledging two principal problems and areas of uncertainties. First, ORAU's experience of redating Middle Upper Paleolithic specimens shows that in many instances, residual contaminants may have affected the previous determinations. This contamination is particularly evident in low-collagen, poorly preserved bones. Second, there is a dearth of human fossil specimens from the critical time period between 40,000 and 28,000 B.P., let alone well dated and morphologically diagnostic specimens. This lack of specimens may be due to unknown but unusual methods by which early modern humans disposed of their dead (28, 42, 43) or to extensive erosion in cave sites during this period (1). Therefore, for both reasons, any conclusions concerning the temporal relationship between early modern humans and late Neandertals on the basis of the current data (including the new Vindija G_1 dates) must be tentative.

The only well dated European early modern human remains that preceded the Vindija G_1 fossils are those from the Peştera cu Oase. A direct radiocarbon age of ≈35,000 B.P. was determined on the Oase 1 mandible on the basis of two direct dates, one using the ultrafiltration pretreatment (Table 2). However, the Peştera cu Oase is in the region of the Iron Gates, ≈500 km east of Vindija. In Central Europe, the oldest directly dated diagnostic early modern human remains are those from Mladeč, Czech Republic, where four individuals have yielded radiocarbon dates of ≈31,000 B.P. (Table 2). These determinations did not include the pretreatment methods described here, and the true radiocarbon ages of the specimens (despite consistency across four separate specimens) could be slightly older than the current results.

In Western Europe, the oldest directly dated purportedly modern human is the Kent's Cavern 4 maxilla with a radiocarbon age of ≈31,000 B.P. (Table 2); however, recent redating of material stratigraphically bracketing this specimen at ORAU shows that the initial AMS determination is inaccurate and that the material's "true age" is probably between 35,000 and 37,000 B.P. (37). Moreover, it is extremely fragmentary (3, 44) and may be undiagnostic as to its Neandertal versus modern human affinities. A series of modern human isolated teeth and phalanges from Brassempouy are associated with dates between ≈30,000 and ≈33,500 B.P. (28). There is an undescribed juvenile partial mandible from an Aurignacian level at La Quina Aval in central France which is morphologically modern on the basis of its protuberant tuber symphyseos. The basal Aurignacian level has provided a radiocarbon date of ≈32,000–33,000 B.P. (27); this date should provide a maximum age for the specimen. There are also diagnostic modern human remains associated with a later Aurignacian assemblage at Les Rois à Mouthiers, France (45, 46), but they and the site are undated and unlikely to predate 32,000 B.P.

The only other European early modern human remains >28,000 B.P. with direct radiocarbon dates (Table 2) are a fragmentary and undescribed tibia and fibula from Kostenki, Russia, which date ≈32,000–33,000 B.P., cranial and postcranial remains from Peştera Muierii, Romania, which date ≈30,000 B.P. (47), and the cranium from Peştera Cioclovina, Romania, which dates ≈29,000 B.P. (48). These three specimens are all substantially east of Vindija. The only Central and Western European late Pleistocene modern human fossils that may be contemporaneous with or earlier than the Vindija G_1 remains are a couple of the early modern human Brassempouy pieces. The Mladeč remains may overlap them in time, depending upon the whether they are indeed older than their current age of ≈31,000 B.P. and whether the Vindija G_1 fossils are indeed close to the ≈32,000 B.P. minimum age provided by the new dates.

As a consequence of this current status of the European pre-28,000 B.P. early modern human fossil record, it is difficult to argue for the strict contemporaneity of Neandertals and early modern humans within a portion of Europe, despite the temporal overlap between the Oase fossils in the east and Neandertals considerably further to the west. It may, therefore, be possible to maintain a scenario of a time-trangressive replacement of a Neandertal morphology by a morphology of early modern humans (by a variety of population processes) from east to west across Europe, beginning at least 35,000 B.P. in the lower Danube basin and ending ≈30,000 B.P. in Iberia. However, this leaves open the question of who was responsible for the Aurignacian assemblages known across most of Europe by at least 36,000 B.P. Did modern humans leave it behind, or were Neandertals responsible for much of the earlier Aurignacian?

This situation currently makes it difficult to use an archeological complex, such as the Aurignacian, as a correlate for the spread of modern humans across Europe during this biocultural evolutionary transitional time period (in contrast to ref. 49). Several factors play into this ambiguity. It is possible that the dating difficulties described above with reference to Vindija may be more widespread than hitherto anticipated, and that the 4,000-year gap between the earliest directly dated modern humans and the earliest Aurignacian is a function of radiocarbon accuracy on the few dated specimens. The gap may be a function of the scarcity of Aurignacian human remains and the low number of dated specimens. Alternatively, it may be a result of semiindependence of the spread of the Aurignacian (a cultural process) and the westward dispersal of early modern humans (a biological population process) (cf. ref. 31).

Conclusion

The application of direct dating to late Neandertal and early modern human fossil remains in Europe, combined with ongoing advances in radiocarbon sample preparation techniques, has altered our perceptions of this biological transition between these groups of humans and the penecontemporaneous emergence and spread of earlier Upper Paleolithic technocomplexes. This change is reflected in the evolving age of the Vindija G_1 late Neandertal fossils. The current situation also highlights the tenuous nature of monolithic explanations of human biocultural change (ones strictly equating human biological and cultural entities) during this time period in Europe. Writing 45 years ago on the European Upper Paleolithic, Movius (50) wrote that "time alone is the lens that can throw it into focus." This statement remains as true now as it was then. An increasingly refined radiometric chronological framework remains central to the resolution of these issues.

Methods

At the ORAU, attempts are made to identify problematic bones before ^{14}C AMS measurement by analyzing a suite of analytical parameters that are indicative of the bone collagen preservation (51). Among these are the atomic ratio of carbon to nitrogen (C:N), the percentage collagen as a function of the starting weight (wt % collagen) compared with average modern bone, the carbon and nitrogen yield of the collagen upon combustion, and the deviation of stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) from those expected (indicating gross contamination only).

Modern bone should yield a C:N atomic ratio of just under 3.2. We accept a bone if its C:N ratio falls between 2.9 and 3.5 (36). Variation in the C:N value is due to deamination of the bone (lower C:N values) or to the addition of exogenous carbon atoms (higher C:N values). The purified collagen we extract is usually around C:N 3.2 until the pretreatment yield falls below either 5 mg or 1 wt % collagen (10 mg of collagen per g of bone), at which point the C:N begins to increase, probably because of the degraded nature of the collagen and the increased chance of being affected by contamination. Bone that is composed of less than 1 wt % collagen is not dated because as the collagen weight declines, the possible influence of contaminants increases. Chemically characterizing extracted material as wholly collagenous and autochthonous when it falls below this threshold is particularly difficult. Without the accompanying analytical data described above, little confidence can be expressed in AMS radiocarbon determinations with pretreatment yields that are low relative to their starting weight.

Yields of extractable collagen in three of the seven samples initially analyzed from Vindija Cave were poor (≈3–7 wt % collagen), and the C:N ratios indicated probable contamination in the bone. The remainder of the human samples and the bone points from level G_1 yielded no extractable collagen and/or retained insoluble contaminants and therefore could not be dated (15). The pretreatment applied was routine and comprised the decalcification of the bone, rinsing in dilute NaOH, and denaturing of the raw collagen in weakly acidic water (pH 3) at 75°C [the latter the so-called "Longin" collagen method (52)]. Gelatinization and simple filtering of the hydrolysate is effective in removing much of the contamination from bones in the majority of cases because it excludes most nonproteinaceous and insoluble materials, which include sediment particulates and insoluble contaminants. However, in some problematic cases, up to 8–10% contamination may remain (53). For samples of 5–10 half-lives, this contamination can be highly significant in terms of the measured ^{14}C age. In the dating of poorly preserved bones such as these, then, gelatinization techniques alone may not prove to be reliable.

Since the original Vindija dates were obtained, a crucial ultrafiltration step has been added (36, 54). The bone is initially physically cleaned by using an aluminum oxide shotblaster and then powdered. Between 0.5 and 1.0 g is routinely sampled by using tungsten carbide drills. A sequence of acid, base, and acid is added to the bone powder in a test tube, interspersed by rinsing with ultrapurified water (MilliQ, Millipore) between each reagent. The crude collagen is gelatinized in pH 3 solution at 75°C for 20 h. The gelatin solution is filtered by using an 8-μm polyethylene Eezi-filter (Elkay Laboratory Products, Basingstoke, U.K.), and the insoluble residues are discarded. The filtered gelatin is then pipetted into a specially precleaned ultrafilter (36) (30-kDa molecular-mass cutoff, Vivaspin 15, Sartorius) and centrifuged at 2,500–3,000 rpm in a Centaur 2 MSE until 0.5–1 ml of the >30-kDa gelatin fraction remains. The ultrafilter retains the >30-kDa molecular mass fraction, which will include undegraded collagen α chains, which have a molecular mass of ≈97–110 kDa. The <30-kDa fraction should contain low-molecular-mass components such as salts, degraded and cleaved collagen fragments, and sometimes soil-derived, low-molecular-mass contaminants and is discarded. The >30-kDa fraction is lyophilized, and if the wt % collagen is >1%, the sample is then combusted and analyzed by using a Europa Scientific ANCA-MS system consisting of a 20-20 IR mass spectrometer interfaced to a Roboprep CHN sample converter unit, operating in continuous flow mode. This procedure enables the measurement of $\delta^{15}N$ and $\delta^{13}C$, nitrogen and carbon content, and C:N ratios. Quality assurance acceptance of these analytical parameters results in the sample being passed for subsequent AMS dating. Graphite is prepared from the carbon dioxide before AMS radiocarbon measurement by using published techniques (55, 56).

The ultrafiltered gelatin usually produces collagen of a demonstrably improved quality, as shown by the C:N ratios (36). Ultrafiltration appears to be an effective method for removing low-molecular-weight contaminants from bone collagen before AMS dating in the majority of cases. Comparisons between ultrafiltered and nonultrafiltered early Upper and Middle Paleolithic collagen determinations show older and, in most cases, more accurate AMS results when ultrafiltration is used. This difference is particularly evident when dating low-yielding bones (37, 54). Ultrafilters will not remove higher-molecular-weight contaminants such as humics cross-linked with collagen where those cross-links are not broken during chemistry. A base wash is included in the pretreatment of the bone collagen to solubilize humics, and gelatinization further denatures the collagen triple helix, resulting in the removal of humics. By assessing the quality of the extracted gelatin by using the parameters described above and applying ultrafiltration, this approach is used to screen problem bones before dating; however, there is scope for further development of this technique insofar as humic-contaminated bone is concerned.

We are grateful to the late Maja Paunović and Jadranka Mauch Lenardić and the staff of the Institute of Paleontology and Quaternary Geology, Croatian Academy of Science and the Arts in Zagreb for continued access to the Vindija remains. We thank the staff of the ORAU for their careful sample preparation, and we particularly thank C. Tompkins, C. Stringer, and J. Ahern for their helpful comments. The original sampling and dating of the Vindija remains was supported by the Wenner–Gren and L.S.B. Leakey Foundations.

1. Churchill, S. & Smith, F. H. (2000) *Yearb. Phys. Anthropol.* **43,** 61–115.
2. Trinkaus, E. (2005) *Annu. Rev. Anthropol.* **34,** 207–230.
3. Stringer, C. (1990) in *Hominid Remains: An Update. British Isles and Eastern Germany*, ed. Orban, R. (Université Libre de Bruxelles, Brussels), pp. 1–40.
4. Sinitsyn, A. (2004) *Etud. Rech. Archéol. Univ. Liège* **106,** 237–244.
5. Păunescu, A. (2001) *Paleoliticul şi mezoliticul din spaţiul transilvan* (Editura AGIR, Bucharest, Romania).
6. Richards, M. P., Pettitt, P. B., Stiner, M. C. & Trinkaus, E. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 6528–6532.
7. Trinkaus, E., Moldovan, O., Milota, Ş., Bîlgăr, A., Sarcina, L., Athreya, S., Bailey, S. E., Rodrigo, R., Gherase, M., Higham, T., *et al.* (2003) *Proc. Natl. Acad. Sci. USA* **100**, 11231–11236.
8. Wild, E. M., Teschler-Nicola, M., Kutschera, W., Steier, P., Trinkaus, E. & Wanek, W. (2005) *Nature* **435,** 332–335.

9. Olariu, A., Skog, G., Hellborg, R., Stenström, K., Faarinen, M., Persson, P. & Alexandrescu, E. (2005) in *Applications of High Precision Atomic and Nuclear Methods*, eds. Olariu, A., Stenström, K. & Hellborg, K. (Editura Academiei Române, Bucharest, Romania), pp. 222–226.
10. Henry-Gambier, D. (2002) *Bull. Mém. Soc. Anthropol. Paris* **14,** 89–112.
11. Orschiedt, J. (2002) *Paléo* **14,** 239–240.
12. Svoboda, J., van der Plicht, J. & Kuželka, V. (2002) *Antiquity* **76,** 957–962.
13. Hedges, R. E. M., Pettitt, P. B., Bronk Ramsey, C. & van Klinken, G. J. (1996) *Archaeometry* **38,** 181–207.
14. Stringer, C. (1996) *Archaeometry* **38,** 183.
15. Smith, F. H., Trinkaus, E., Pettitt, P. B., Karavanić, I. & Paunović, M. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 12281–12286.
16. Terberger, T., Street, M. & Bräuer, G. (2001) *Archäol. Korrespond.* **31,** 521–526.
17. Svoboda, J., van der Plicht, J., Vlček, E. & Kuželka, V. (2004) *Anthropologie (Brno)*, **42,** 161–166.
18. Conard, N. J., Grootes, P. M. & Smith, F. H. (2004) *Nature* **430,** 198–201.
19. Mercier, N., Valladas, H., Joron, J. L., Reyss, J. L., Lévêque, F. & Vandermeersch, B. (1991) *Nature* **351,** 737–739.
20. Hublin, J. J., Spoor, F., Braun, M., Zonneveld, F. & Condemi, S. (1996) *Nature* **381,** 224–226.
21. Pettitt, P. B. (1998) *Étud. Rech. Archéol. Univ. Liège* **85,** 329–338.
22. Schmitz, R. W., Serre, D., Bonani, G., Feine, S., Hillgruber, F., Krainitzki, H., Pääbo, S. & Smith, F. H. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 13342–13347.
23. Hublin, J. J., Barroso Ruiz, C., Medina Lara, P., Fontugne, M. & Reyss, J. L. (1995) *C. R. Acad. Sci. Sér. IIA* **321,** 931–937.
24. Antunes, M. T., Santinho Cunha, A., Schwartz, J. H. & Tattersall, I. (2000) *Mem. Acad. Cièncc. Lisb. Cl. Cièncc.* **38,** 283–317.
25. Walker, M. J. (2001) in *A Very Remote Period Indeed: Papers on the Paleolithic Presented to Derek Roe*, eds. Milliken, S. & Cook, J. (Oxbow Books, Oxford), pp. 153–159.
26. Zilhão, J. & d'Errico, F., eds. (2003) *Trabalhos de Arqueologia* **33,** 1–355.
27. Dujardin, V. (2003) *Antiq. Natl.* **33**, 21–26.
28. Henry-Gambier, D., Maureille, B. & White, R. (2004) *Bull. Mém. Soc. Anthropol. Paris* **16,** 49–87.
29. Trinkaus, E., Zilhão, J., Rougier, H., Rodrigo, R., Milota, Ş., Gherase, M., Sarcina, L., Moldovan, O., Bălteanu, I., Codrea, V., *et al.* (2006) in *Neanderthals and Modern Humans Meet?*, ed. Conard, N. J. (Kerns, Tübingen, Germany), in press.
30. Wolpoff, M. H., Smith, F. H., Malez, M., Radovčić, J. & Rukavina, D. (1981) *Am. J. Phys. Anthropol.* **54,** 499–545.
31. Karavanić, I. & Smith, F. H. (1998) *J. Hum. Evol.* **34,** 223–248.
32. Kozłowski, J. (1996) in *The Lower and Middle Paleolithic*, eds. Bar-Yosef, O., Cavalli-Sforza, L. L., March, R. & Piperno, M. (International Union of Prehistoric and Protohistoric Sciences, Forli, Italy), pp. 205–218.
33. Zilhão, J. & d'Errico, F. (1999) *J. World Prehist.* **13,** 1–68.
34. Karavanić, I. & Smith, F. H. (2000) *Curr. Anthropol.* **41,** 838–840.
35. Ahern, J. C. M., Karavanić, I., Paunović, M., Janković, I. & Smith, F. H. (2004) *J. Hum. Evol.* **46,** 27–67.
36. Bronk Ramsey, C., Higham, T. F. G., Bowles, A. & Hedges, R. (2004) *Radiocarbon* **46,** 155–163.
37. Jacobi, R. M., Higham, T. F. G. & Bronk Ramsey, C. (2006) *J. Quat. Sci.*, in press.
38. Obelić, B., Horvatinčić, N., Srdoč, D., Krajar Broni, I. & Sliepčević, A. (1994) *Radiocarbon (Zagreb)* **36,** 303.
39. Karavanić, I. (1995) *J. Anthropol. Res.* **51,** 9–35.
40. Wild, E. M., Paunović, M., Rabeder, G., Steffan, I. & Steier, P. (2001) *Radiocarbon* **43,** 1021–1028.
41. Smith, F. H. & Ahern, J. C. M. (1994) *Am. J. Phys. Anthropol.* **93,** 275–280.
42. Svoboda, J. (2000) *J. Hum. Evol.* **38,** 523–536.
43. Zilhão, J. & Trinkaus, E. (2002) *Trabalhos de Arqueologia.* **22,** 519–541.
44. Keith, A. (1927) *Trans. Proc. Torquay Nat. Hist. Soc.* **5,** 1–2.
45. Mouton, P. & Joffroy, R. (1958) *Gallia Suppl.* **9,** 1–117.
46. Vallois, H. V. (1958) *Gallia Suppl.* **9,** 118–137.
47. Nicolăescu-Plopşor, D. (1968) *Seventh Int. Congr. Anthropol. Ethnol. Sci. Moscow* **3,** 381–386.
48. Rainer, F. & Simionescu, I. (1942) *Anal. Acad. Romane Mem. Secţ. Stinţ., Seria III* **17,** 489–503.
49. Mellars, P. (2005) *Evol. Anthropol.* **14,** 12–27.
50. Movius, H. L., Jr. (1960) *Curr. Anthropol.* **1,** 355–392.
51. van Klinken, G. J. (1999) *J. Archaeol. Sci.* **26,** 687–695.
52. Longin, R. (1971) *Nature* **230,** 241–242.
53. van Klinken, G. J. & Hedges, R. E. M. (1995) *J. Archaeol. Sci.* **22,** 263–270.
54. Brown, T. A., Nelson, D. E., Vogel, J. S. & Southon, J. R. (1988) *Radiocarbon* **30,** 171–177.
55. Bronk Ramsey, C. & Hedges, R. E. M. (1999) *Nucl. Instr. Meth. Phys. Res. B* **123,** 539–545.
56. Bronk Ramsey, C., Pettitt, P. B., Hedges, R. E. M., Hodgins, G. W. L. & Owen, D. C. (2000) *Archaeometry* **42,** 459–479.