

AGE DETERMINATION OF FOSSIL BONES FROM THE VINDIJA NEANDERTHAL SITE IN CROATIA

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ABSTRACT. Vindija cave in Croatia is famous for the Neanderthal bones found in layer G of its sediment profile. Radiocarbon dating has been performed mainly on this layer due to the great interest in its fossils. In addition to Neanderthal remains, the sediment in layer G contains bones from the cave bear. Cave bear bones are found also in other layers of the sediment profile and offer the possibility of studying the bears' evolutionary mode. Therefore, we tried to determine the time span covered by the entire profile. The U/Th age determination method was applied to cave bear bones from different layers of the profile. For the younger part of the profile, the U/Th ages were compared with the results of the ¹⁴C and the amino-acid racemization method. The agreement of the different methods indicates that closed-system behavior can be assumed for the fossil bones from Vindija cave. From this finding it may be deduced that bones from the lower sediment layers are also closed systems and that the U/Th ages of these layers are reliable. This conclusion is corroborated by the stratigraphy of the cave profile.

INTRODUCTION

Vindija cave in Croatia is a very important site for the archaeological, anthropological, and paleontological sciences because the sediment profile covers a large time span (see Figure 1). One layer in the profile (layer G) is of special interest. In this layer, bone material from Neanderthals was found. Recently, many new questions have arisen concerning the fate of the Neanderthals. According to different investigations, their extinction occurred later than previously assumed (e.g. Smith et al. 1999; Barton et al. 1999), and the Neanderthals lived for a relatively long time parallel to modern *Homo sapiens sapiens*, at least in some regions of Eurasia. This fact also led to the questions of whether the Neanderthal and *Homo sapiens sapiens* were coexisting without interbreeding or if they mixed with each other, and also how the Neanderthal disappeared. Recently, DNA analyses have been performed on Neanderthal and modern human DNA material trying to answer these questions (e.g. Ovchinnikov et al. 2000; Krings et al. 2000).

Vindija cave is also an ideal place to study the development of different species of bears. A large number of bear bones from different evolutionary levels (*Ursus deningeri* to *Ursus spelaeus*) were found in sediment layers. We hoped that dating the bear bones would provide information not only about the evolution of the cave bears, but also about the Neanderthal, without destroying the rare and valuable human remains.

U/Th Dating of the Cave Bear Bones

According to the different evolutionary levels of the cave bears, the time span covered by the sediment profile of Vindija cave was expected to exceed the time period datable by the radiocarbon method. Therefore, we applied the U/Th dating method to bear bones originating from the different layers of the sediment profile.

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Uranium-series dating of fossil bones is based on the assumption that the uranium uptake takes place for a short time after the bone is buried in the ground, and that no further uranium exchange between the bone and the environment occurs during burial times (e.g. Ivanovich and Harmon 1988). If these assumptions are not fulfilled, the sample comprises a so-called open system, and dating is only possible by applying models describing the uranium flux through the sample (e.g. Hille 1979; Millard and Hedges 1996). For fossil bones, open system behavior must always be considered, although under special burial conditions, as in cave sediments, closed systems can be expected (Rea and Ivanovich 1986; Leitner-Wild and Steffan 1993). Nevertheless, in the case of bone samples from caves it is also essential for the assessment of the uranium-series data to determine whether open or closed systems must be considered.

A first uranium-series dating run was started in 1989 with samples from layers G to I, taken from a “witness” block of the sediment left unexcavated after the last excavations. The layers K to M were not accessible from the witness block without excavations. Samples from these layers were taken from easily accessible parts of the sediment in front of the cave entrance. Ion exchange separations were used for the chemical isolation of uranium and thorium from the bone matrix. ^{232}U in equilibrium with ^{228}Th was used as a radiochemical spike for the determination of the uranium and thorium yields. The U and Th activities were determined with the alpha-counting technique. Dating results of the first samples are shown in Table 1.

Table 1 Activity ratios and U/Th ages for cave bear bones from the first run. The errors (1σ) are due to counting statistics only. More than one result in one section indicates that several age determinations were performed for the same sample.

Layer	U-content		$^{238}\text{U}/^{234}\text{U}$	$^{230}\text{Th}/^{234}\text{U}$	U-Th age (ka)
	(ppm)	$^{230}\text{Th}/^{232}\text{Th}$			
G	2.5	6.7 ± 0.9	0.9390 ± 0.0160	0.2619 ± 0.0125	32.8 ± 1.9
G	5.0	7.9 ± 0.7	0.9337 ± 0.0168	0.1903 ± 0.0063	22.8 ± 0.9
		7.1 ± 0.9	0.9668 ± 0.0367	0.1745 ± 0.0080	$20.7 +1.1/-1.2$
		9.5 ± 1.0	0.9367 ± 0.0356	0.1765 ± 0.0071	21.1 ± 1.0
H	1.4	7.3 ± 1.2	0.8938 ± 0.0206	0.2930 ± 0.0158	37.5 ± 2.1
		4.0 ± 0.4	0.8926 ± 0.0446	0.2781 ± 0.0136	$35.2 +2.1/-2.3$
H	8.5	7.2 ± 1.2	0.9042 ± 0.0136	0.2299 ± 0.0074	28.2 ± 1.0
		10.3 ± 0.6	0.9524 ± 0.0171	0.2435 ± 0.0066	30.1 ± 1.0
K	3.0	33.2 ± 3.4	0.9191 ± 0.0129	0.6592 ± 0.0152	$114.0 +5.0/-4.8$
K	2.5	— ^a	0.8857 ± 0.0142	0.7158 ± 0.0201	129.8 ± 6.8
L middle part	12.0	— ^a	0.8368 ± 0.0184	1.0597 ± 0.0360	≥ 358
L lower part	0.6	3.2 ± 0.3	0.8496 ± 0.0425	0.8045 ± 0.0418	$105.0 +26.0/-20.0$
L lower part	0.4	5.2 ± 0.6	0.8977 ± 0.0475	0.8850 ± 0.0531	$215.0 +63.0/-38.0$
M	11.0	22.5 ± 1.1	0.8569 ± 0.0360	0.6523 ± 0.0306	$110.6 +10.7/-9.2$
M	12.0	36.0 ± 5.4	0.9058 ± 0.0109	0.6559 ± 0.0262	$112.9 +8.4/-7.8$
M	10.0	35.0 ± 1.8	0.8757 ± 0.0158	0.7310 ± 0.0168	$136.0 +7.0/-6.5$

^aNo ^{232}Th detected

The U/Th ages of the sediment layers L to M are contradictory to the stratigraphic sequence. According to this result, open systems were suspected for the samples. The low $^{230}\text{Th}/^{232}\text{Th}$ ratios of the samples also indicate that some non-authigenic ^{230}Th might be present in the samples, taken up together with ^{232}Th . Later, it turned out that the samples assigned to layers K to M originate from places where the layers are not well defined and where mixing with material from the upper layers might have occurred. As a consequence, bone samples from well documented layers were collected

during a new excavation in 1993 and 1994. Table 2 presents the data from these samples. The U/Th ages of these samples show a normal chronological sequence, with two exceptions in layer K. The $^{230}\text{Th}/^{232}\text{Th}$ ratios of most samples are greater than 20, which means that non-authigenic ^{230}Th can be neglected (Bischoff and Fitzpatrick 1991). The ages of samples with low $^{230}\text{Th}/^{232}\text{Th}$ ratios should be considered as maximum ages, since no correction for initial ^{230}Th was applied.

The data given for the transition of layer H to layer I are from a single bone. The bone was divided into several pieces and a U/Th age was determined for each piece. Portion 5 was one end of the bone, which consisted mainly of spongiosa and was in direct contact with the surrounding sediment. The other portions were compact bone material. From the data we can conclude that spongiosa is not suitable for the age determination if it is in direct contact with the sediment. The determined age of this part of the bone is older than from the compact bone. This may be due to an open-system behavior of the spongiosa. Additionally portion 5—in contrast to the other portions—showed a measurable ^{232}Th content. The determined age may be influenced even by both effects, uranium loss due to open system behavior and a higher ^{230}Th activity brought into the sample together with ^{232}Th . The same can be argued for one of the outliers of layer K. The sample with an age of $102.9 \pm 6.4/-6.1$ ka listed in Table 2 was also spongiosa. An open system should be assumed for the other outlier of layer K as well, although in this case it cannot be explained by the sample type.

The bone samples from the upper sediment layers including the Neanderthal layer G show U/Th ages in the time range, which can be dated with the ^{14}C method. As described above, bone samples are always expected to form open systems, and it is necessary to check whether the U/Th dated samples can be treated as closed systems. The possibility of a crosscheck with the ^{14}C method is obvious, and we compared the dating results of the U/Th method with those derived from the ^{14}C method.

Table 2 Activity ratios and U/Th ages with 1- σ errors determined for cave bear bone samples from the recent excavation. Sample H/I was a large bone which was divided into 5 subsamples (see text).

Layer	U-content (ppm)	$^{230}\text{Th}/^{232}\text{Th}$	$^{238}\text{U}/^{234}\text{U}$	$^{230}\text{Th}/^{234}\text{U}$	U-Th age (ka)
G1	5.1	97.4 ± 23.8	0.8833 ± 0.0168	0.2566 ± 0.0054	33.1 ± 0.8
G1	5.6	49.3 ± 12.1	0.8967 ± 0.0231	0.2272 ± 0.0068	27.9 ± 1.0
G3	1.0	3.8 ± 0.3	0.9113 ± 0.0167	0.3160 ± 0.0056	$41.0 \pm 1.0/-0.9$
H/I, 1	0.9	— ^a	0.8556 ± 0.0265	0.5363 ± 0.0018	$85.2 \pm 4.7/-4.6$
H/I, 2	1.0	— ^a	0.8552 ± 0.0205	0.5596 ± 0.0156	$90.6 \pm 4.3/-4.1$
H/I, 3	1.1	— ^a	0.8697 ± 0.0209	0.5509 ± 0.0142	$88.7 \pm 3.8/-3.7$
H/I, 4	1.1	— ^a	0.8179 ± 0.0344	0.5452 ± 0.0290	$86.4 \pm 7.8/-7.1$
H/I, 5	1.6	30.0 ± 0.2	0.8767 ± 0.0158	0.6179 ± 0.0125	101.9 ± 3.7
I/J	1.3	35.5 ± 3.9	0.9199 ± 0.0204	0.8003 ± 0.0142	$168.3 \pm 8.7/-8.4$
J	5.6	171.4 ± 8.6	0.8518 ± 0.0039	0.7846 ± 0.0041	$156.3 \pm 2.1/-1.8$
J	0.5	— ^a	0.9526 ± 0.0225	0.7745 ± 0.0142	$158.6 \pm 8.1/-7.9$
J	0.5	— ^a	1.0949 ± 0.0288	0.8173 ± 0.0183	$196.0 \pm 20.0/-15.0$
J-K	1.1	11.3 ± 0.1	0.9536 ± 0.0267	0.7983 ± 0.0208	$181.9 \pm 16.3/-13.7$
K	1.9	56.1 ± 7.2	0.9711 ± 0.0134	0.4500 ± 0.0054	$64.7 \pm 1.3/-1.0$
K	0.3	4.67 ± 0.4	0.9264 ± 0.0448	0.7596 ± 0.029	$150.4 \pm 16.2/-13.5$
K	1.3	17.9 ± 1.6	0.9766 ± 0.0197	0.8638 ± 0.0152	$212.2 \pm 16.7/-12.8$
K	1.9	— ^a	0.8884 ± 0.0222	0.7647 ± 0.0184	$159.3 \pm 10.0/-9.5$
K ^b	1.5	10.7 ± 1.1	0.8344 ± 0.0257	0.6089 ± 0.0209	$102.9 \pm 6.4/-6.1$
Basis K	0.2	7.8 ± 0.8	0.9881 ± 0.0468	1.0207 ± 0.0403	>352.6

^aNo ^{232}Th detected

^bMainly spongiosa

Radiocarbon Dating

^{14}C age determinations were performed at various laboratories applying the conventional method and the accelerator mass spectrometry (AMS) method as well. A compilation of all ^{14}C data now available for the Vindija site is given in Table 3. The application of the ^{14}C method to bones from this site is not straightforward because of the poor preservation state of the organic matter in most bones. For one cave bear sample from layer G1, a ^{14}C AMS age of $33,000 \pm 400$ BP (ETH-12714) was determined in Zurich (Karavanić 1995). In a second sample from the same layer, insufficient collagen for an age determination was present. At the VERA (Vienna Environmental Research Accelerator) laboratory, AMS ^{14}C dating was attempted for cave bear bone samples from layers F to H of the cave sediments. It turned out that the collagen of all samples investigated was already highly degraded. For the decay counting technique from samples of layers I and J, some 100 g bone powder was used for the collagen extraction, but the yields were too low for this method. The gelatin produced from these samples was therefore used for AMS ^{14}C determinations.

Table 3 Compilation of radiocarbon data from the Vindija site

Sediment layer	Sample material	^{14}C age (BP)	Lab nr	Reference
E	<i>Ursus Spelaeus</i> bone	$18,500 \pm 300$	Z-2447	Obelić et al. 1994
F	Charcoal	$24,000 \pm 3300$	Z-612	Srdoč et al. 1984
F	Charcoal	$29,700 \pm 2000$	Z-613	Srdoč et al. 1984
F	Charcoal	$27,000 \pm 600$	Z-551	Srdoč et al. 1979
F/d/d	<i>Ursus Spelaeus</i> bone	$26,600 \pm 930$	Z-2433	Obelić et al. 1994
G1	<i>Ursus Spelaeus</i> bone	$18,280 \pm 440$	Z-2432	Obelić et al. 1994
G1	<i>Ursus Spelaeus</i> bone	$33,000 \pm 400$	ETH-12714	Karavanić 1995
G1	<i>Ursus Spelaeus</i> bone	$46,800 + 2300/-1800$	VERA-1428	
G1	Neanderthal bone	$29,080 \pm 400$	OxA-8296	Smith et al. 1999
G1	Neanderthal bone	$28,020 \pm 360$	OxA-8295	Smith et al. 1999
G3	Neanderthal bone	$>42,000$	Ua-13873	Krings et al. 2000
H	<i>Ursus Spelaeus</i> bone	$33,400 + 2000/-1600$	VRI-1125	E Pak, pers. com. 2000
I	<i>Ursus Spelaeus</i> bone	$(37,000 \pm 600)$	VERA-0109	
J	<i>Ursus Spelaeus</i> bone	$(34,700 \pm 500)$	VERA-0105	

Even though the ages derived by AMS measurements for these samples are in the same time range as the age determined for the layer G1 sample in Zurich ($37,000 \pm 600$ BP [VERA-0109] for layer I and $34,700 \pm 500$ BP [VERA-0105] for layer J), they should be treated with caution. These “young ages” may be due to an insufficient cleanup of the samples. When the collagen is highly degraded (to less than 5% of the initial organic content) the ratio between carbon from the collagen and carbon from contamination present in the bone material can be rather disadvantageous and special chemical pretreatment methods (not available for these samples) would be necessary to remove all impurities (Hedges and van Klinken 1992). On the other hand, we must note that for a cave bear bone from the layer above—layer H—a ^{14}C age (VRI-1125) of $33,400 + 2000/-1600$ BP could be determined with the radiometric method. This sample was relatively well preserved with 10% of the initial collagen still present (E Pak, personal communication 2000).

Another attempt was made to find bone samples with enough organic material in layer G1. The nitrogen content of bone samples gives an estimate of the amount of collagen still present in a sample. Therefore, the N-content of 11 cave bear bone samples of layer G1 was determined with an elemental analyzer. From the investigated samples, only one sample was suitable for an age determination. A N-content of 1.7 weight percent of total bone and a C/N ratio of 3.1 was determined for this

sample. These values indicate a good preservation state of the bone. It can be estimated that about 30% of the initial organic matter is still present. This value is well above the 5% level, which is recommended as a limit for the applicability of the standard chemical pretreatment method for fossil bones (Hedges and van Klinken 1992). An AMS ^{14}C age of $46,800 \pm 2300/-1800$ BP (VERA-1428) was determined for this sample.

The now available ^{14}C ages of the bears from layer G1 show a relatively large scatter from about 18 ka to 46 ka (see Table 3), and the Neanderthal time period cannot be determined exactly by dating of the bear bones. For this purpose, bone material directly from the Neanderthal must be used, although this material is very valuable and rare. This material was dated by Smith et al. (1999) in Oxford. As with the cave bears, the organic material of the human bones was highly degraded and only two of seven bone samples available for the dating had enough collagen. Ages of $29,080 \pm 400$ BP (OxA-8296) and $28,020 \pm 360$ BP (OxA-8295) were determined for these samples. Also in layer G3 of the cave sediment bones from the Neanderthals were found. Recently an age of greater than 42,000 BP (Ua-13873) for a Neanderthal bone originating from this layer was determined in Uppsala (Krings et al. 2000).

DISCUSSION OF THE DATING RESULTS

Figure 1 shows a scheme of the sediment profile from Vindija cave. It also shows the U/Th ages from bone samples of the different layers (except layers K–M of the first dating run) and the ^{14}C results.

The agreement of the U/Th and ^{14}C data of the samples from layer G1 is evident. This indicates that the U/Th data of the bone samples are reliable and that the samples can be treated as closed systems. The ages determined for the human bones are remarkably young as described in Smith et al. (1999). Up to now, the Vindija Neanderthals belong to the most recent dated Neanderthals. As mentioned above, the ages of the cave bear samples from layer G1 originate from a large time interval. The Neanderthal ages fit very well into this period. It cannot be expected that the cave was used by the humans and the bear at the same time. Therefore, there must be a difference in the ages of the human and the bear bones. From the data, we can conclude that the cave was inhabited by the bear before and after its usage by the humans.

A U/Th age of $41,000 \pm 1000/-900$ BP was determined for the cave bear bone from layer G3. The $^{230}\text{Th}/^{232}\text{Th}$ ratio of this sample is very low. As discussed above, some non-authigenic ^{230}Th may contribute to the determined ^{230}Th activity and the age should be considered as maximum age. This age is in agreement with a bear-bone amino acid racemization age of $42,400 \pm 4300$ BP for the same layer (Malez et al. 1984). An age of more than 42,000 BP was determined recently for a Neanderthal bone sample from this layer. At the first glance, this looks like a disagreement with the cave bear ages, but in relation with the $46,800 \pm 2300/-1800$ BP age of the cave bear sample from layer G1, a Neanderthal age of greater than 42,000 BP is not in contradiction to the determined cave bear ages.

The ^{14}C age of layer H ($33,400 \pm 2000/-1600$ BP) also verifies the U/Th ages for bone samples of this layer determined during the first U/Th dating run.

Table 3 and Figure 1 also show the ^{14}C ages determined in Croatia for charcoal and bear bone samples from layers E, F, and G1. The same ages (18 ka) were determined for cave bear bones from layers E and G1, whereas charcoal and bone samples from the intermediate layer F are older (see Figure 1).

We must note that in the discussion above, a very important point concerning the ^{14}C age determination has not been considered. All ages determined with the ^{14}C method are given as ^{14}C ages since

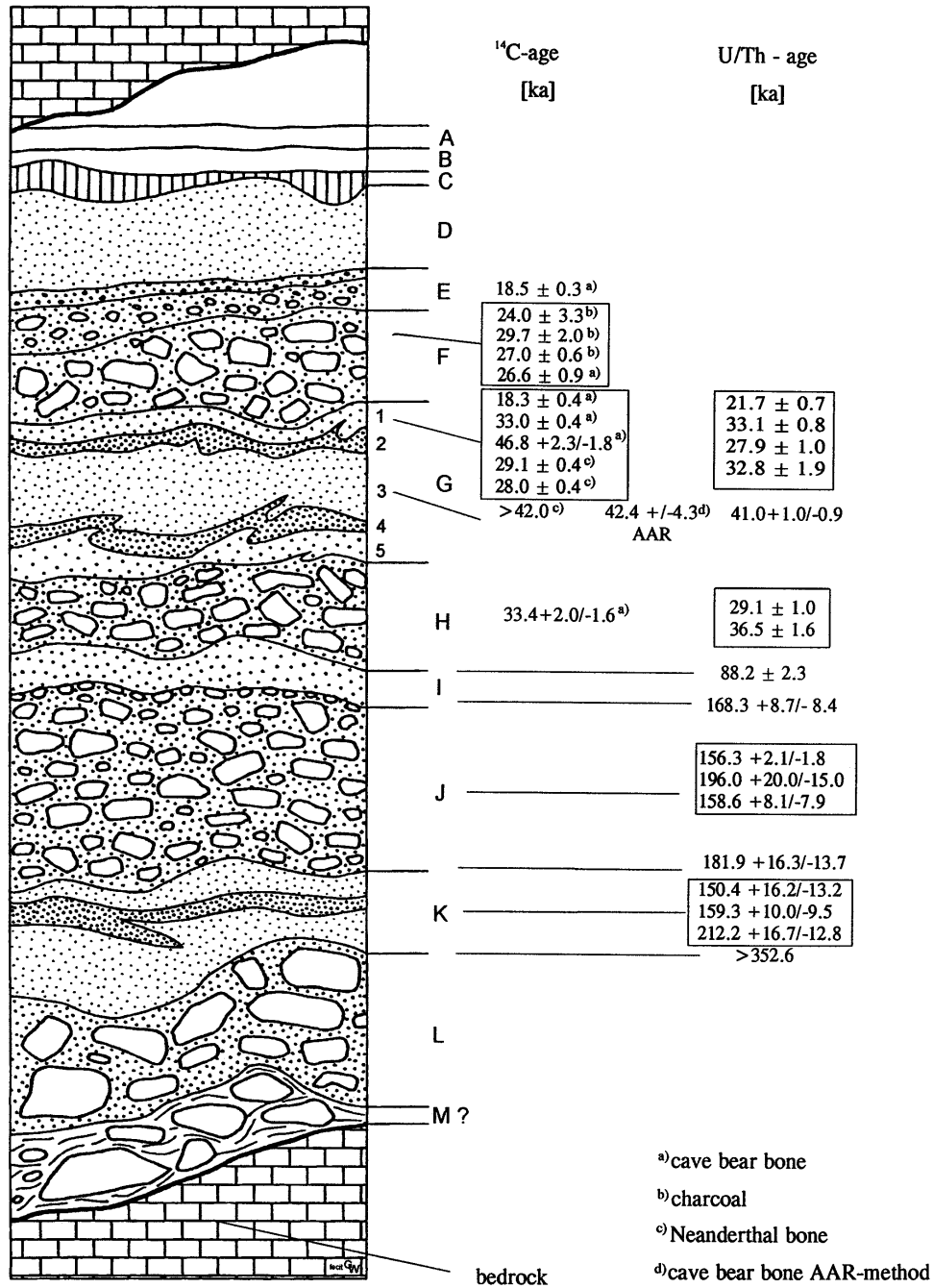


Figure 1 Schematic view of Vindija cave profile. Ages in units of 10³ yr (ka) from the ¹⁴C (see Table 3), AAR (Malez et al. 1984) and U/Th methods are indicated together with the 1-σ errors. Weighted mean values are used for cases where more than one age determination from the same bone was performed. The result of portion H/I 5 was not used for the mean value of sample H/I.

no accepted calibration curve (INTCAL98) is available for the time period before 24,000 cal BP. This means that the given ages must be taken as minimum ages, and an age offset of several thousand years is possible. Paradoxically, it is even possible that the scatter of the ages will be smaller after calibration, if one considers the steep decrease of $\Delta^{14}\text{C}$ between 30,000 and 35,000 cal BP measured in laminated sediments from Lake Suigetsu (Kitagawa and van der Plicht 1998) as real.

At the present state, divergent values for the absolute time of the main ^{14}C excursions were determined from marine, ice, and terrestrial cores (Beck et al. 2001). The calibration data from Lake Suigetsu go back to a ^{14}C age of 45 ka, and peaks in the $\Delta^{14}\text{C}$ occur at 23 and 31 cal ka BP. But it is possible that there are varves missing in the varve chronology and calibrated ages beyond 20 ka should be considered as minimum ages (van der Plicht 1999). If one uses the calibration curve from Lake Suigetsu despite this uncertainty, samples with a ^{14}C age of about 28 ka would be shifted to about 32 ka, whereas the calibration of the 33 ka ^{14}C ages would only lead to a much smaller time shift. The 46 ka bear bone age lies outside the calibration range. If the ^{14}C data were calibrated in this way, the ^{14}C method and the U/Th method would agree again, also supporting the closed system assumption for the Vindija bones.

However, the above discussion must be considered highly speculative, because the ^{14}C calibration beyond 24,000 cal BP is far from settled (Beck et al. 2001).

From the results obtained with the different dating methods, we can conclude that layers E to H were formed between around 20,000 and 45,000 BP. The scatter of the data indicates that the sediment layers are disturbed. Despite the calibration for the ^{14}C ages, a large number of age determinations and cluster analysis would be necessary to achieve more precise information about the individual time intervals during which the cave was inhabited.

From the agreement of the AAR-, U/Th-, and ^{14}C ages of the bone samples from layers G and H, we may conclude that the bone samples from these layers may be treated as closed systems. The U/Th ages of the underlying sediment layer I to the base of layer K are older than the upper part of the sediment. If a closed system behavior—which is proven for the upper layers—is also assumed for bones from these layers, the data indicate that the sediment of this part of the profile is also reworked. The ages from layers J and K scatter in the same time range. The bone sample from the transition of layers H to I yielded an age of 88 ka, which is between the time range of the upper and the lower sediment profile. The age determined for the base of layer K is beyond the U/Th datable time range. The chronological sequence of the data reflects the stratigraphic situation of the profile with parts of the profile reworked. Cryoturbation of layers E to I was already determined during the first excavations by Malez and Rukavina (1979).

CONCLUSION

The application of both the U/Th method and the ^{14}C method was difficult for Vindija cave because of the sample material available from this site. For the U/Th method in general, dating bone material is problematic due to a possible open-system behavior. The application of the ^{14}C method was further complicated because the organic substances in most of the bones were highly degraded. As a consequence, for ^{14}C dating of the younger part of the cave profile, samples with a suitable collagen content had to be found by monitoring the N-content of various bones. Despite these difficulties, we could determine a chronology for the entire cave profile. Our study shows that the U/Th method can be applied to bone samples from Vindija cave. We compared U/Th ages with those derived by completely independent age determination methods, the ^{14}C method, and the AAR method. The concordance of the ages determined with these three methods justifies the closed-system assumption for

the Vindija site. The reliability of the U/Th ages of the older samples from the Vindija profile is also supported by the stratigraphy. Therefore we conclude that—although problematic—U/Th dating of bone samples can provide useful age information for time periods not accessible to the ^{14}C method, if the closed system assumption is carefully checked.

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