Supporting Information

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SI Methods

Background Carbon Assessment. A prerequirement for using the hydroxyproline (Hyp) dating method is the corroboration that significant amounts of exogenous carbon are not added during the process (1). Small graphites, which are likely to be produced when attempting compound-specific AMS dating, are especially sensitive to contamination affecting the AMS date, in particular when the contamination is modern and the samples are very old. Sample pretreatment may add measurable amounts of carbon whose total $^{14}C/^{12}C$ value will depend on its origin. It could potentially come from a variety of sources, including chemical reagents, glassware, column bleed, mobile phases, co-elution of other compounds, and sample carryover of chromatographic impurities from the total collagen sample. ^{14}C contamination from reagents (e.g., dissolved CO_2 in water, unclean tin capsules, or absorbed CO_2 in Chromosorb) or reaction vessel walls may be introduced during the production of CO_2 or during the subsequent graphitization step. To reduce these contamination sources all reagents used are HPLC grade or above and glassware is baked at 500 °C before use. All HPLC lines are metal, where appropriate, and the system is free of organic solvents. Tin capsules are cleaned with cyclohexane and acetone, and Chromosorb is baked at 500 °C. The HPLC column is thoroughly flushed between injections and blanks are collected to make sure no impurities or carryovers are possible. The graphitization and AMS blank of the Oxford Radiocarbon Accelerator Unit (ORAU) is reported to be ~0.15% (52 kaBP) and in the best conditions can be as low as 0.1% (55 kaBP), as measured by graphitizing a gas sample containing no radiocarbon.

The amount, constancy, and age of the background carbon added during the process were tested by checking the accuracy and precision of several Hyp dates of two bones: historically known age pig bone from the *Mary Rose*, Henry VIIIth's flagship that sank in 1545, and very old (>45 ky) Alaskan permafrost bison bone. As shown in Fig. S1, the *Mary Rose* Hyp dates are statistically indistinguishable and produce a combined date of 334 ± 17 yBP, passing a χ^2-test at 95% confidence They are also indistinguishable statistically from the 311 yBP bulk date, passing a χ^2-test at 95% confidence. The Hyp dates are on average about 20 y older than the bulk.

The accuracy of the *Mary Rose* dates suggests that the process introduces a very small amount of blank carbon, which is, at least partially, old. This blank carbon is likely to be the result of column bleed originating from a fossil carbon source (2, 3). Any procedural blank will inevitably contain modern components as well. As already discussed, a modern contaminant will have a significant effect when a very old bone is dated. A bone that is radiocarbon dead will pick up any modern contamination resulting from sample pretreatment and so will be useful for assessing the modern carbon contribution of the procedure blank. The Lemon Mine bison bone has acceptable collagen preservation yet is indefinitely old, i.e., radiocarbon dead. This bone is routinely used at the ORAU to check modern contamination levels. The Hyp fraction of a Lemon Mine bison bone was isolated using the same procedure. Table S1 shows the dates produced for the Lemon Mine Hyp fraction on four different occasions. The modern carbon addition is quite consistent and is calculated to be on average 1.5 ± 0.35 μg C per sample.

Both the *Mary Rose* and the Lemon Mine datasets suggest that the "procedure blank" is insignificant, and the method can be used to date both modern and infinitely old bones.

Correction Algorithm. Although the accuracy and precision of the dates provide evidence that the method is valid for bones of all ages, as the background carbon addition is constant it is possible to correct for any effect on the dates by applying a correction algorithm. The activity measured for a sample can be represented by the equation

$$A_m = f_d * A_d + f_M * A_M + (1 - f_d - f_M) * A_s,$$

in which A_m is the activity measured, f_d is the radiocarbon dead fraction of the contaminant, A_d is the dead fraction activity, which equals 0, f_M is the modern fraction of the contaminant, A_M is its activity, which is taken as 1, and A_s is the activity of the actual sample. This equation can therefore be simplified to

$$A_m = f_M + (1 - f_d - f_M) * A_s$$

and after rearrangement

$$f_d = (f_M + A_s - f_M * A_s - A_m)/A_s.$$

The modern C is assumed to be 1.5 ± 0.35 μg, as calculated from the Lemon Mine dates, and so the f_M is 1.5/graphite size (in micrograms). As the *Mary Rose* samples are of known age, the A_s is known (0.96202), as is the A_m for each sample. f_d can be therefore calculated for each sample (Table S2).

It can therefore be concluded that on average 3.3 ± 1.45 μg C of contaminant are added to each Hyp sample (equaling weight of contaminant, W_c), and its activity is 1.5/3.3 = 0.4545 (±0.2) (equaling activity of contaminant, A_c).

If the activity of the sample measured is now represented by

$$A_m = f_c * A_c + (1 - f_c) * A_s = W_c/W_T * A_c + W_s/W_T * A_s,$$

where f_c is the fraction of contaminant, A_c is its activity, W_c is its weight, W_T is the total weight of the sample plus contaminant, W_s is the weight of the sample, and the rest of the symbols are as above, then the corrected activity of the sample is

$$A_s = (A_m - W_c/W_T * A_c)/(W_s/W_T).$$

The error for the weight of the contaminant is

$$\delta A_s(W_c) = \partial A_s/\partial W_c * \delta W_c = -A_c/W_s * \delta W_c,$$

the error for activity of the contaminant is

$$\delta A_s(A_c) = \partial A_s/\partial A_c * \delta A_c = -W_c/W_s * \delta A_c,$$

and the total error is

$$\sigma^2 = \sqrt{\left[\delta A_s(W_c)^2 + \delta A_s(A_c)^2 + \delta A_s(\mathrm{AMS})^2\right]},$$

where A_s(AMS) is the AMS measurement error.

The corrected *Mary Rose* Hyp dates are presented in Table S2 and Fig. S2.

The same can be done for the Lemon Mine Hyp dates; this time $F^{14}C$, or the activity, is on the *y* axis, as shown in Fig. S3. The resulting corrected Hyp dates for the Sungir and Kostenki samples after applying the correction algorithm are presented in Table S3. The correction makes the dates older, but does not change them radically.

Preservatives Used on the Archaeological Specimens. The Sungir bones are stored in the Gerasimov Laboratory in Moscow. The majority of the bones have been subject to conservation treatment. The laboratory uses a polymer comprising tree sap (termed kanefol), polyvinylbutyral, and phenol/formaldehyde, mixed together with ethanol to produce the conservation product. The Kostenki specimen appears to have been conserved as well but we were not able to establish the chemicals used. During sampling every effort was made to drill away from the bone surface but the high C:Ns measured after preparation of the bone collagen showed that exogenous carbon remained. This result led to us failing the sample for dating as bulk.

1. McCullagh JSO, Marom A, Hedges REM (2010) Radiocarbon dating of individual amino acids from archaeological bone collagen. *Radiocarbon* 52:620–634.
2. Mollenhauer G, Rethemeyer J (2009) Compound-specific radiocarbon analysis–Analytical challenges and applications. *IOP Conf Ser Earth Environ Sci* 5(1):1–9.
3. Shah SR, Pearson A (2007) Ultra-microscale (5-25 g C) analysis of individual lipids by 14 C ams: Assessment and correction for sample processing blanks. *Radiocarbon* 49: 69–82.

Fig. S1. The burial of the Kostenki XIV human.

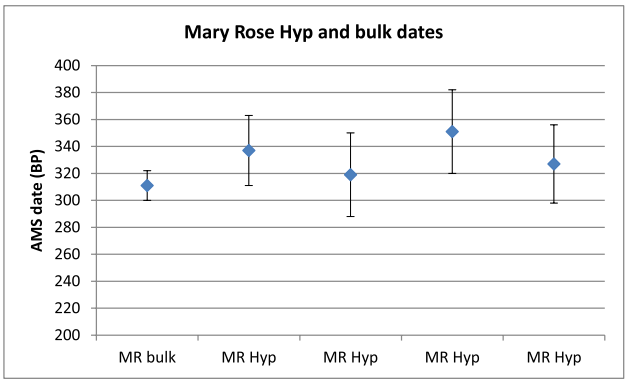

Fig. S2. *Mary Rose* Hyp dates, compared with the bulk date.

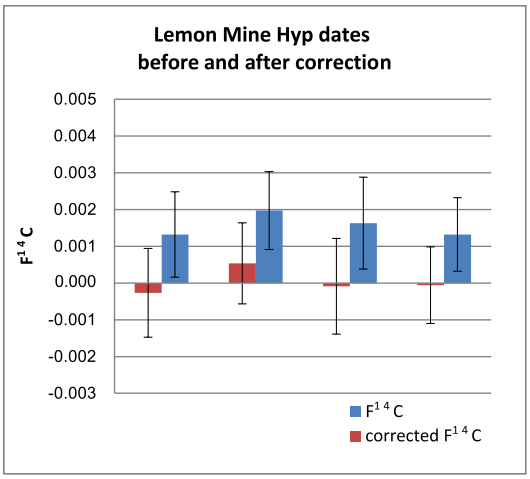

Fig. S3. Four different Lemon Mine Hyp dates after applying the correction algorithm. Note that the activity now is closer to zero, as expected from a radiocarbon "dead" sample.

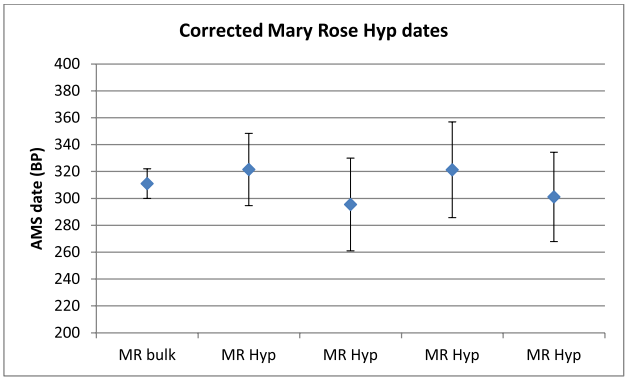

Fig. S4. *Mary Rose* Hyp dates after applying the correction algorithm.

Table S1. Sungir and Kostenki Hyp dates after applying the correction algorithm

Sample	Date BP	±	Corrected date BP	±
Sungir, combined date	30,100	300	30,700	350
Kostenki Markina Gora	33,250	500	33,900	550

Note that the three Sungir dates were combined, as the results suggested they date a single event.

Table S2. Sample extraction yields for the Kostenki and Sungir samples

Sample	Bone sample, mg	Collagen yield, %	Collagen yield, mg	Collagen hydrolyzed, mg	Hyp graphite weight, mg C
Kostenki Markina Gora	710	6.4*	45.3	40	1.17
	500	10.8†	54.0		
Sungir 2	320	6.0*	19.2	30	0.65
	440	9.5†	41.8		
Sungir 3	460	3.4*	15.8	30	0.67
	600	6.1†	36.5		
Sungir mammoth	1,600	2.9*	46.7	30	0.88
	1,420	5.7†	80.5		

*Samples ultrafiltered before AMS dating.
†Samples pretreated with a gelatinization method.

Table S3. Lemon Mine Hyp dates

Sample no.	Date BP	$F^{14}C$	±	Hyp graphite weight, mg C	Modern C, μg
24,707.2 NRC 01 27/5/2010	>45,900	0.00132	0.00100	1.03	1.4
24,707.3 NRC1 01 13/7/2010	>44,100	0.00163	0.00125	0.83	1.4
24,707.4 NRC 01 16/10/2010	>44,200	0.00197	0.00106	0.99	2.0
24,707.4 NRC1 01 16/10/2010	>45,100	0.00132	0.00116	0.90	1.2

The modern C added in the procedure is calculated by multiplying $F^{14}C$, or the activity, by the graphite size. On average the addition of modern C to any Hyp fraction is therefore 1.5 ± 0.35 μg. The error is based on the SD of the replicated values.

Table S4. Calculation of the dead C addition to the *Mary Rose* Hyp dates

Sample no.	Date BP	±	A_m	±	f_M	A_s	f_d	Dead C, μg	Corrected date BP	±
24,705.0 NRC2 01	327	29	0.9601	0.0035	0.0028	0.96202	0.002	1.1	301	33
24,705.1 NRC 01	351	31	0.9573	0.0037	0.0031	0.96202	0.005	2.4	321	36
24,705.1 NRC1 01	319	31	0.9611	0.0038	0.0025	0.96202	0.001	0.6	296	35
24,705.2 NRC1 01	337	26	0.9589	0.0030	0.0017	0.96202	0.003	3.0	322	27

A_m is the activity measured, f_M is the fraction modern, A_s is the activity of the sample, and f_d is the fraction dead. After finding f_d the amount of dead C is calculated by multiplying f_d by the graphite size. On average the addition of dead C to any Hyp fraction is 1.8 ± 1.1 μg. The error is based on the SD of the replicated values. Also presented are the dates after applying the correction algorithm.