Radiocarbon dating casts doubt on the late chronology of the Middle to Upper Palaeolithic transition in southern Iberia

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It is commonly accepted that some of the latest dates for Neanderthal fossils and Mousterian industries are found south of the Ebro valley in Iberia at *ca.* 36 ka calBP (calibrated radiocarbon date ranges). In contrast, to the north of the valley the Mousterian disappears shortly before the Proto-Aurignacian appears at *ca.* 42 ka calBP. The latter is most likely produced by anatomically modern humans. However, two-thirds of dates from the south are radiocarbon dates, a technique that is particularly sensitive to carbon contaminants of a younger age that can be difficult to remove using routine pretreatment protocols. We have attempted to test the reliability of chronologies of 11 southern Iberian Middle and early Upper Paleolithic sites. Only two, Jarama VI and Zafarraya, were found to contain material that could be reliably dated. In both sites, Middle Paleolithic contexts were previously dated by radiocarbon to less than 42 ka calBP. Using ultrafiltration to purify faunal bone collagen before radiocarbon dating, we obtain ages at least 10 ka ^{14}C years older, close to or beyond the limit of the radiocarbon method for the Mousterian at Jarama VI and Neanderthal fossils at Zafarraya. Unless rigorous pretreatment protocols have been used, radiocarbon dates should be assumed to be inaccurate until proven otherwise in this region. Evidence for the late survival of Neanderthals in southern Iberia is limited to one possible site, Cueva Antón, and alternative models of human occupation of the region should be considered.

Since the early 1990s, it has been widely acknowledged that the region south of the Ebro River and Cantabrian Cordillera in Iberia, here defined as southern Iberia, provided a refugium for the final Neanderthals (1–5). The earliest stages of the Aurignacian, an Upper Paleolithic lithic industry widely linked with an anatomically modern human (AMH) authorship (6–8), are absent from the region (5). In contrast, in northern Iberia the Aurignacian appeared around 42 ka calBP (calibrated radiocarbon date ranges, years before 1950), shortly after the disappearance of the Mousterian, a Middle Paleolithic lithic industry usually associated with Neanderthals (5). If the relationship between industry and species holds, this finding suggests a pause in AMH dispersal, providing a vacuum in which Neanderthals in southern Iberia could have survived (5, 9). Numerous Mousterian and Neanderthal assemblages in this region have yielded post-42 ka calBP dates supporting such a scenario, most famously at Carihuela (10), Gorham's Cave (11, 12), and Zafarraya (13). A period of overlap between Neanderthals and AMHs in neighboring regions has implications for the inevitability of acculturation (1), interbreeding (14), and understanding the role of the environment in the spatial distribution of human populations (15). It is therefore crucial that the chronologies on which the pattern of occupation in Iberia is based are accurate.

Unfortunately, the chronology of the transition between the Middle and Upper Paleolithic has been clouded by doubts over the reliability of the chronological methods used, the extent to which taphonomic influences have blurred the association between the objects dated and the archaeological evidence, and the fact that the latest lithic assemblages are small and often undiagnostic, making them difficult to assign to the Mousterian (5, 16, 17). Because of this doubt, there is considerable debate regarding the extinction date of the "late" Neanderthals in Iberia and thus the length of any overlap with AMHs. Zilhão (5, 18, 19), for example, has suggested that Neanderthals survived across the southern region through Heinrich Event 4 (H4) until *ca.* 32–30 ka BP (*ca.* 36.7–34.5 ka calBP), and has termed this pattern the Ebro Frontier model. Zilhão suggests that this pattern can be explained, at least in part, by the cultural adaptation of AMHs to the open steppe-like environments found north of the Cantabro-Pyrenean chain. Only when woodland was replaced during the climatic deterioration of GI7 (*ca.* 35.5 ka) did they spread south. Others (2, 12, 20) have envisaged a gradual retreat of Neanderthals into the extreme southwestern region around Gibraltar and southern Portugal, with an extinction date of less than 30 ka BP (34.9–34.5 ka calBP). A minority (16, 17, 21) regard the evidence as too weak, and have entirely rejected the late survival of Neanderthals.

Here we show that many of these studies have underestimated the problematic nature of the radiocarbon dates in southern Iberia, which comprise two-thirds of the post-42 ka calBP dates for the Mousterian and Neanderthals. The significant effect that young contaminants have on the accuracy of Paleolithic radiocarbon chronologies has become apparent with the development and application of rigorous pretreatment protocols designed to more effectively remove exogenous sources of carbon, such as ABOx-SC of charcoal and ultrafiltration of bone collagen (22). With 1% modern carbon contamination, a sample of 50 ka BP will appear *ca.* 37 ka BP. Using improved pretreatment protocols, the existing Paleolithic chronologies at sites such as Fumane, Italy (23), Abri Pataud, France (24), Geissenklosterle, Germany (25), and Kent's Cavern, United Kingdom (8) have lengthened by several millennia. Moreover, upon application of rigorous pretreatment protocols, the radiocarbon dates for these sites yielded results consistent with stratigraphy and in agreement with nonradiocarbon chronologies.

To test the reliability of the radiocarbon evidence for late survival of Neanderthals in southern Iberia, our study focused on radiocarbon dating several purportedly late Neanderthal, Middle, and early Upper Paleolithic assemblages using a rigorous pretreatment method for cleaning bone collagen involving ultrafiltration (22, 26). Because of the poor preservation of organic material in southern Iberia, the nitrogen content (%N) of

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bone was measured to identify which bones were most likely to contain enough collagen for radiocarbon dating (27). Of 215 bones screened, only 27 contained enough nitrogen to attempt collagen extraction (*SI Text*).

Site Description

Two sites have been dated: Jarama VI, which provides the primary evidence for a late Mousterian in central Iberia, and the Cueva del Boquete de Zafarraya, containing the latest Neanderthal fossils in Europe. A further nine sites were examined, but no suitable materials for radiocarbon dating were found (see *Description of Sites and Samples That Could Not Be Radiocarbon Dated* and *%N Screening Results* in *SI Text*, Figs. S1 and S2, and Table S1).

The rock shelter of Jarama VI (Fig. 1*A*) is located in a steep-sided limestone gorge in the Jarama valley on the southern flank of the Sistema Central. An area of 16 m^2 was excavated between 1989 and 1993 (28). Unit I, formed of sands, silts, and products of gelifraction of the cave walls, was found in one corner of the excavation (Fig. 1*B*) and contained a rich Upper Paleolithic assemblage with blade and bladelet blanks. This unit is separated from the underlying fluvial deposit by an erosive event. Levels 2.1, 2.2, and 3 contain Mousterian assemblages with points, side-scrapers, burins, notches, and denticulates. The Levallois technique is present, and the complete reduction sequence is represented with cores and debitage products dominating the assemblages. Some of the sparse assemblage in level 2.2 is arranged around a hearth feature. A human metatarsal, tentatively identified as Neanderthal, was recovered from this level (29). Units 2.2 and 3 are separated by a thick flood deposit (level 2.3) and a second erosive event (28).

Charcoal from level 2.1 (29500 ± 2,700 BP Beta-56638, 44090–29830 calBP), the hearth feature in 2.2 (32600 ± 1,800 BP Beta-56639, 42260–34510 calBP), and a burrow, thought to relate to level 1 (23380 ± 500 BP Beta-56640, 29370–26920 calBP), has

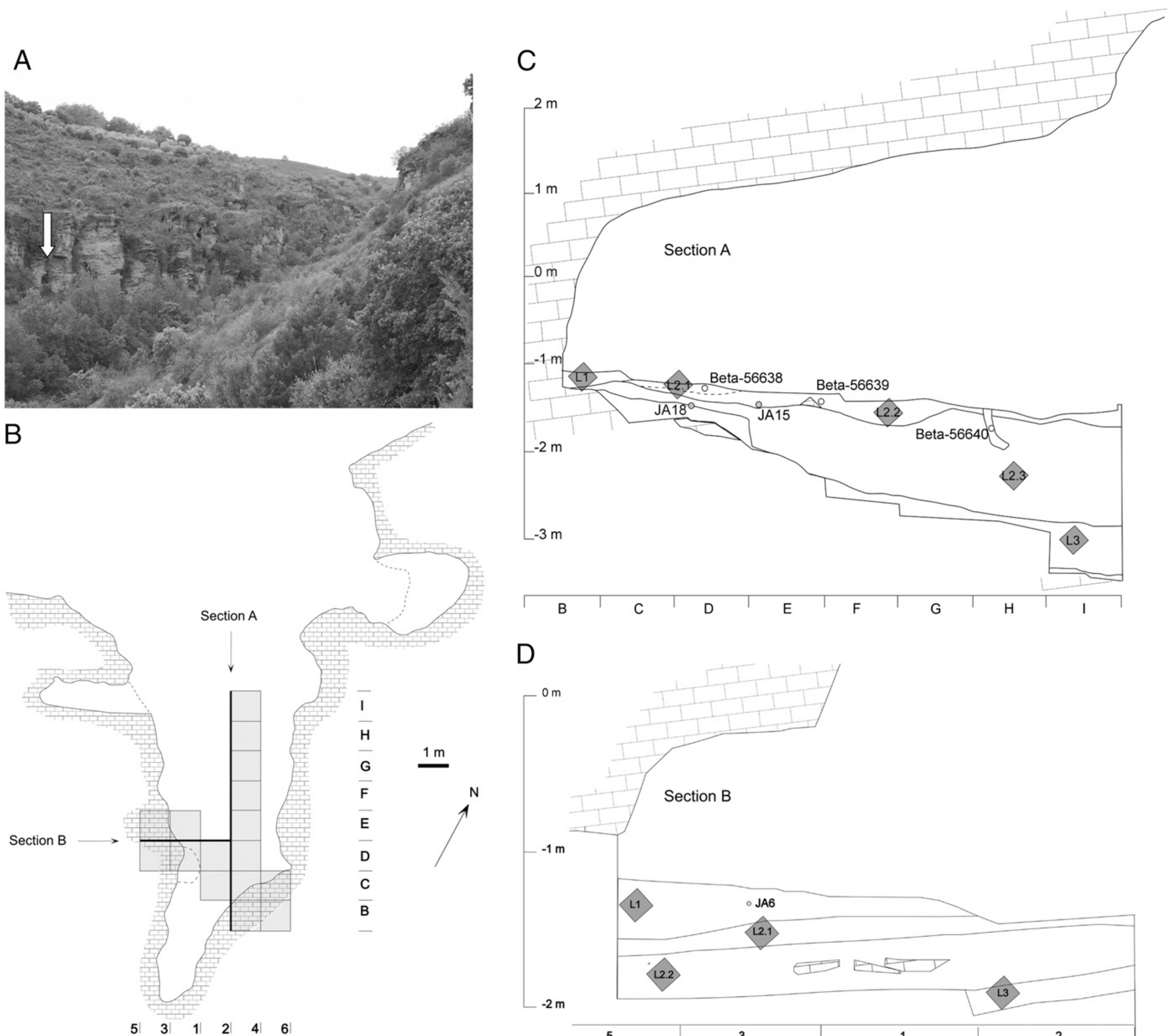

Fig. 1. The site of Jarama VI (40°56′50,56′′N 3°19′1,06′′W, Valdesotos, Castilla-la-Mancha, 822 m above sea level). (*A*) The location of the cave in the Jarama canyon is marked with a white arrow. (*B*) Plan of the cave. (*C* and *D*) Stratigraphy and location of samples radiocarbon dated. Open circles represent published dates and closed circles represent dates from this work.

been conventionally radiocarbon dated (28) (Table S2). The site features prominently in discussions of the chronology of the final Mousterian because of the statistical agreement between the two Mousterian dates and the secure association of one date with human activity (3, 5, 19, 30).

Excavations at the montane site of Cueva del Boquete de Zafarraya (Fig. 2*A*) in 1981–1983 and 1990–1994 (31) produced a remarkable collection of cutmarked, fragmented, and burned Neanderthal remains. The site has been, and is still, regarded by some as providing the latest secure evidence for Neanderthals (4, 13), but others (19), although more cautious, still consider it late.

The cave is divided into the Sala de Entrada and the Sala del Fondo (Fig. 2*B*). Clandestine excavations in the early 20th century left a deep (5–6 m) hole in the Sala del Fondo and contributed to a level of disturbed deposits, removed before excavation. The in situ sediments are split into three units, the middle of which is formed of a silty-sand matrix with limestone fragments and contains a lithic assemblage assigned to the Typical Mousterian alongside the Neanderthal fossils (32). Upper Paleolithic bladelets have been recovered in this unit in the Sala del Fondo to a depth of 118 cm, but were found in an area where sediment was loosely packed, and are thought to be intrusive (33).

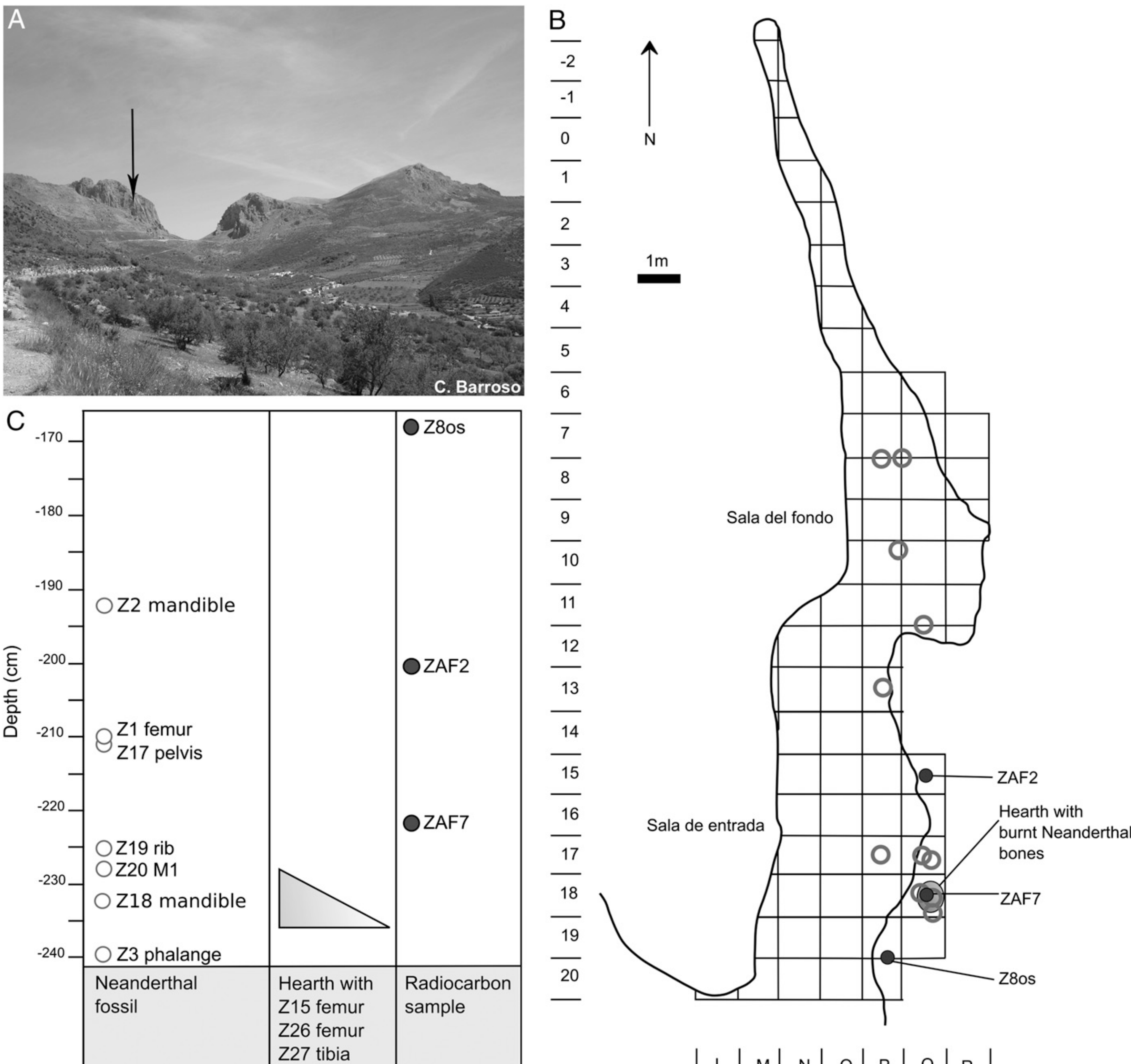

Fig. 2. The site of Zafarraya (36°57′05 ″N, 4°07′36′′W, Ventas de Zafarraya, Andalucia, 1,100 m above sea level) (*A*) located in the Boquete de Zafarraya. (*B*) Plan and location of samples (filled circles) and Neanderthal remains (large open circles) [modified from Barroso-Ruiz et al. (34)]. (*C*) Stratigraphic relationship between Neanderthal remains in P-Q 17-18 and radiocarbon dates. Note that Z6 (scapular) found in Q18 has not been plotted in *B* and *C* because coordinates are not available, the mandible Z18 was found in three fragments split between squares Q18 and P17, and four of the five Neanderthal remains in the Sala del Fondo were found in the level of disturbed deposits that capped the site. The location of samples dated in this study are denoted with filled circles and the location of Neanderthal fossils with open circles. Mousterian lithics were found 1 m above these radiocarbon dates, but no suitable samples for radiocarbon dating were found at a similar depth.

Of the 55 Neanderthal and AMH fossils from Zafarraya, a cluster of 10 Neanderthal remains, including two highly diagnostic mandibles, was recovered from the Sala de Entrada between 190 and 240 cm depth (Fig. 2 *B* and *C*) (34). Three of these remains, two femora and one tibia, are associated with a hearth [context D(Sm)] and are calcined and highly fragmented.

Two attempts have been made at directly dating the Neanderthal bones. U-Series dating by γ-spectroscopy was unsuccesful (35, 36) and no collagen could be recovered from two bones (Z1 and Z2) (Fig. 1*C*) for radiocarbon dating. The chronology is therefore based on bones of the most abundant fauna, *Capra pyrenaica* (37). Hublin et al. (13) dated three bone samples from the Sala del Fondo with Uranium Series (U-Series), and conventional radiocarbon after extracting collagen with the Longin (*SI Methods*) protocol. The deepest sample was found at a similar depth to the Neanderthals in the Sala de Entrada, and results ranged between 33.4 ± 2 ka and 28.9 ± 4.2 ka (see *Published Radiometric Dates from Jarama VI and Zafarraya* in *SI Text* and Table S2). These results were accepted (13) as the two methods agreed, the dates were in stratigraphic order, and collagen yields were high (>1%). However, this late chronology has been cast into doubt (35, 36, 38) by a series of 42 radiocarbon, U-Series, electron spin resonance, and amino acid racemisation dates on bone and teeth that are inconsistent within and between methods, and compared with depth (see *Published Radiometric Dates from Jarama VI and Zafarraya* in *SI Text* and Table S2). This result is unsurprising given that, in addition to the uncertainties regarding radiocarbon dates in this period, U-Series, electron spin resonance, and amino acid racemisation can be unreliable when dating the open systems of osseous remains (39–41).

Results

Of 30 anthropogenically modified bones screened for %N at Jarama VI, two contained >0.8%N, suggesting enough collagen was present to obtain a date, and a further five were considered marginal (*%N Screening Results* in *SI Text*). Three samples were dated: both samples containing >0.8%N (levels 1 and 3) and the only sample with a marginal content of nitrogen from unit 2 (Fig. 1*C*).

Two bones produced infinite radiocarbon ages and one (OxA-X-2310-22), a finite age with an error of 3,700 ^{14}C years (Table 1). This date should be regarded as a minimum age as the bone contained little collagen (<1%) and has an error approaching 4,000 ^{14}C years, the point at which radiocarbon dates become infinite (*SI Methods*). The bone from level 1 was found in the uppermost spit and may result from local disturbance or bioturbation (28).

Together, the three new dates show that the radiocarbon dates on charcoal are severe underestimations. No bones with an adequate content of nitrogen from level 2.1 exist to date the final Mousterian occupation of the site. However, with the removal of the charcoal dates from discussion, there is no evidence for a late Mousterian occupation at Jarama VI.

At the Cueva del Boquete de Zafarraya, sampling was undertaken in two stages. To test the accuracy of the published radiocarbon dates, bones previously dated (35, 36, 38) were resampled for %N analysis (Table S3). All but one sample contained insufficient nitrogen for the ultrafiltration protocol to be attempted. In addition, previously undated *Capra pyrenaica* bones were examined. Reflecting the punctuated human occupation of the cave (37), cut-marks and evidence of smashing was not observed, and so unmodified bones were selected for screening. Although all eight samples from the Sala del Fondo failed the screening test (*%N Screening Results* in *SI Text*), 4 of 11 samples from the Sala del Entrada contained sufficient nitrogen to proceed with the ultrafiltration treatment (*%N Screening Results* in *SI Text*).

The %N analysis of bones previously dated correlates well with the quality assurance data obtained at the time of dating (see *Published Radiometric Dates from Jarama VI and Zafarraya* in *SI Text*). All except one bone, Z8os, failed the %N test, and all but Z8os produced collagen with unrealistic δ^{13}C, %C, and %N values (42) (*SI Methods*), suggesting that degradation and or contamination of the protein was significant. Collagen was originally extracted from Z8os with a protocol that, except for the ultrafiltration step, was identical to the ultrafiltration protocol (laboratory code AG) (*SI Methods*) (36, 38), and a result of 33300 ± 1,200 BP (OxA-8999) was obtained. When redated with the ultrafiltration protocol, its age increased to >46700 BP (OxA-23198 and OxA-26440) (Table 1). All radiocarbon dates on bone from the site, including those from the apparently well-preserved bones in Hublin et al.'s (13) study, should therefore be viewed with extreme caution.

Two of the four bones treated for the first time here contained >1% collagen, and add further weight to this conclusion. ZAF7 gave an infinite date (OxA-21813) and ZAF2 a date of 46300 ± 2,500 BP (OxA-21810) (Table 1) close to the limit of the radiocarbon method. Z8os and ZAF2 were found stratigraphically above the Neanderthal remains in the Sala de Entrada, and ZAF7 was found within the hearth feature (Fig. 2 *B* and *C*). The dated bones were not cut-marked, and only date the context of the Neanderthal fossils and Mousterian lithics. Although these results can only be used to tentatively suggest that these remains date close to or beyond the limit of radiocarbon, they cast into doubt the previous post-42 ka calBP chronology. This collection of Neanderthals should no longer be cited as providing evidence for the southern Iberian Neanderthal late refugia.

Table 1. Radiocarbon dates of bones from Jarama VI and Zafarraya

Sample reference	Treatment	OxA-	Date (BP)	Yield (mg)	Yield (%)	%C	δ^{13}C (‰)	δ^{15}N (‰)	C:N	Comment or source
Jarama VI										
JA-6	AF	21,714	>50,200	14.0	1.4	46.9	−19.3	9.5	3.4	
JA-15	AF	X-2310–22	49,400 ± 3,700	5.4	0.5	43.2	−18.2	7	3.2	Low % yield. Close to background.
JA-18	AF	X-2,290–56	>47,000	7.4	0.7	45.3	−18.8	10.3	3.3	Low % yield
Zafarraya										
Z8os	AG	8,999	33,300 ± 1,200	3.9	1	32.5	−19.1	5.3	3.3	Michel et al. (36, 38)
	AF	23,198	>46,700	62.1	5.4	44.5	−18.9	4.6	3.3	
	AF*	26,440	>46,700	15.3	1.9	41.2	−19.1	5.2	3.2	
ZAF2	AF	21,810	46,300 ± 2,500	21.3	2	44.6	−19.7	7	3.3	
ZAF3	AF*			2.2	0.3					Failed on low yield
ZAF7	AF*	21,813	>49,300	13.8	1.4	44.3	−18.9	5	3.4	
ZAF8	AF			0.7	0.1					Failed on low yield

AF denotes the ultrafiltration pretreatment protocol, AG dontes a protocol identical except for the omission of ultrafiltration and, * denotes an additional solvent treatment. Z8os was dated with and without a solvent treatment to assess whether ultrafiltration concentrated hydrocarbons affecting the date. To obtain a reliable radiocarbon date, bone should contain >1% collagen, δ^{13}C between −22 to −18‰, δ^{15}N between 2‰ and 12‰, C:N 3.9–3.4 and % C >30% (42). All errors are given at 1SD. Typical 1SD errors for stable isotope values and %N measurement are ± 0.2‰ and ± 0.1%, respectively.

Discussion

The warm temperatures across much of southern Iberia today (43) are not favorable for the preservation of the organic remains required for radiocarbon analyses, as reflected in the scarcity of datable bone (*%N Screening Results* in *SI Text*). The absence of well-preserved organic remains has lead to the dating of inappropriate sample types [e.g., sediment in the case of Carihuela (10)], the application of particularly gentle pretreatment protocols, and the acceptance of dates on material that would otherwise be considered too degraded (e.g., Zafarraya). The significance of these concerns is well illustrated by the difference of more than 10 ka radiocarbon years between radiocarbon dates on material pretreated with routine protocols and the more rigorous ultrafiltration bone pretreatment method at Jarama VI and Zafarraya. Unless radiocarbon dating has been undertaken on samples treated with a protocol demonstrated to give accurate dates on equivalent material, dates must be assumed to be inaccurate unless proven otherwise.

Bearing this finding in mind, alongside taphonomic factors, a review of sites thought to postdate 42 ka calBP is given in Table S4. None of these sites provide strong evidence for the late survival of Neanderthals or the Mousterian. For example, in support of the Ebro frontier hypothesis, Zilhão et al. (30) claim the most robust evidence for the late survival of Neanderthals comes from Sima de las Palomas and Cueva Antón (Murcia), Gruta da Oliveira (Portugal), Gorham's Cave (Gibraltar, Natural History Museum Excavations), and Jarama VI. Of these sites, we can now remove Jarama VI. The other four sites have significant shortcomings that limit their reliability. Gorham's Cave (11) has been dated by radiocarbon using charcoal pretreated with a deliberately gentle pretreatment (RR) (*SI Methods*) designed to ensure that some carbon survives the procedure and can be dated. Sadly, this method is even less likely (26) to remove contaminants than the routine protocol (acid-base-acid or ZR) (*SI Methods*) found to be problematic at other sites of a similar age (8). The chronologies of Sima de las Palomas (44) and Gruta da Oliveira (45) are based on radiocarbon dating of burnt bone and U-Series dating of bone. Burnt bone of Paleolithic age can sometimes produce unreliable radiocarbon dates because the origin of the carbon extracted is unknown (22). To obtain a reliable U-Series date episodes of uranium uptake and leaching must be identifiable (40). At Sima de las Palomas, two radiocarbon dates on burnt bone, associated with disarticulated Neanderthal fossils and Mousterian lithics, postdate 42 ka calBP (Table S4). From the same stratigraphic unit, there are two significantly older results: a U-Series date on bone where the uranium uptake history could not be established (43.8 ± 0.75 ka APSLP4) and an optically stimulated luminescence date (54.7 ± 4.7 ka X2509) (44). Given the problematic nature of these contradictory dates, the age of this unit and its Neanderthal fossils remains uncertain. Oliveira level 8 contains a Mousterian assemblage with consistent radiocarbon and U-Series dates, suggesting an age of less than 42 ka calBP (45). Despite this consistency, the dates suffer from the same methodological problems as those seen in Sima de las Palomas, and must again be used with care.

The only site to contain a date obtained using a method known to give reliable results is Cueva Antón, level Ik (46). A radiocarbon date on charcoal pretreated with the rigorous ABOx-SC method (32890 ± 200 BP OxA-21244, 38440–36810 ka calBP) (26) suggests that the unit probably formed shortly after 38.5 ka calBP. However, the dated charcoal is not functionally associated with the archaeology and the "handful" (46) of lithics is not yet fully published. No typical Upper Paleolithic artifacts have been uncovered (46), but the small size and largely undiagnostic nature of the published lithic assemblage means that it can only be tentatively assigned to the Middle Paleolithic. Although the ongoing excavation (46) of Cueva Antón may uncover a larger and more diagnostic lithic assemblage, in our view the assemblage cannot currently be used as the only evidence for the late survival of Neanderthals.

At Pego do Diabo, a single cutmarked bone has been dated, using ultrafiltered collagen, to 38750 ± 750 BP (44300–42100 calBP, OxA-15004) (30) and provides the last evidence of human activity before H4. This bone is not associated with a lithic industry, so it is not known whether it indicates a Neanderthal or AMH presence. The next reliable date is from Cueva Antón, level Ik (46), leaving a gap of at least 4,000 y during which no accurate dates exist (Fig. 3). Crucially, this period spans H4, the period during which the Ebro Frontier model (5, 18, 19) predicts Neanderthals were living in southern Iberia while AMHs were to the north. The first evidence of modern humans in southern Iberia is from the Evolved Aurignacian (5, 9). This industry is poorly dated in southern Iberia (Table S5), but in comparison with sites further north, is expected to fall after H4 (5, 24).

Three scenarios should be considered when examining the Middle to Upper Paleolithic transition in Iberia. These are: (*i*) the southern region of Iberia was abandoned by both Neanderthals and AMHs; (*ii*) Neanderthals existed in southern Iberia; and (*iii*) AMHs were present, spreading into the southern regions soon after they arrived in northern Iberia.

In our view, too few sites have been well dated to test these scenarios, and all may be possible. Given the scarcity of accurately dated sites, it possible that both AMHs and Neanderthals were present in the region during H4, and that the region was entirely abandoned. The latter theory is difficult to assess because an unconformity or hiatus between Middle and Upper Paleolithic exists in many sites (5, 51, 52). Given these uncertainties, when studying this period in Iberia, assemblages dated to <42 ka calBP should not be assumed to be produced solely by Neanderthals unless diagnostic lithic assemblages, or preferably fossils, are present.

The late survival of Neanderthals has played a role in discussion regarding the inevitability of acculturation, the duration over which interbreeding may have occurred and the role of the environment in the Middle to Upper Paleolithic transition. With doubt cast over the late survival of Neanderthals, the place of southern Iberia in these arguments must be viewed cautiously. It is crucial that efforts are directed toward testing existing chronologies, and the construction of new chronological datasets must continue before further conclusions can be drawn.

Methods

Bone samples were screened for %N before radiocarbon dating (27). If a bone contain more than 0.8%N, in around 70% of cases more than the 1% collagen required for dating (42) can be extracted with the ultrafiltration protocol (27). Bone with 0.5–0.7%N was considered marginal and only selected for dating if better preserved material was not available.

All samples were dated at the Oxford Radiocarbon Accelerator Unit. Radiocarbon dating followed the methods described in Brock et al. (26). Bones were treated with the ultrafiltration protocol, involving a series of HCl and NaOH washes to remove bone mineral, exogenous carbonates, and humic acids, followed by gelatinization, filtration (45–90 μm Ezee filter), and ultrafiltration (Vivaspin 15 30-kDa MWCO ultrafilter) to further clean the protein. As a cautionary measure, samples from Zafarraya were given a series of

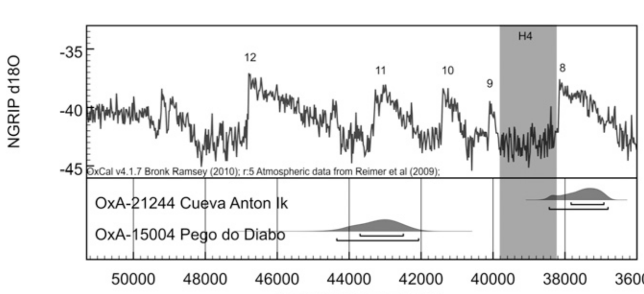

Fig. 3. The interval between reliable radiometric age estimates in the south of Iberia. OxA-15004 (30) from Pego do Diabo is a cut-marked bone treated with the ultrafiltration protocol, and OxA-21244 (46) is a fragment of charcoal from a level containing lithics reminiscent of the Middle Paleolithic at Cueva Antón. Although the date's relationship to human activity may be questioned, it is currently the only accurate date near H4 in southern Iberia. H4 is shown in gray and the Greenland Interstadials are numbered against the NGRIP $\delta^{18}O$ record (47, 48). Radiocarbon dates are calibrated against IntCal09 (49) in OxCal v4.1 (50).

solvent washes before collagen extraction as glues were applied to some bones during excavation. The purified collagen was freeze-dried and combusted in an elemental analyzer (e.g., Carlo Erba NA 2000) linked to a continuous flow-isotope ratio mass spectrometer (CF-IRMS; e.g., Sercon 20–20). A portion of gas was fed into the CF-IRMS and the remaining CO_2 was collected, converted to graphite, and dated by accelerator mass spectrometry (53) (*SI Methods*). Other pretreatment protocols discussed in the text are described in Table S6. Radiocarbon dates have been calibrated using the IntCal09 calibration curve (49) in OxCalv4.1 (50). IntCal09 extends to 50 ka calBP, and calibration is impossible for samples older than *ca.* 45 ka BP. Throughout the text conventional radiocarbon (BP) and nonradiocarbon dates are given at 1 SD, and calibrated radiocarbon date ranges (calBP) at 95% probability.

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Supporting Information

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Description of Sites and Samples That Could Not Be Radiocarbon Dated

Bones from 11 sites were screened for the preservation of collagen. Nine sites yielded no datable bone and are described below. Site locations are given in Fig. S1 and the percentage of nitrogen (%N) contents are given in Fig. S2.

Cendres, 38°41′N 0°08′E (Teulada-Moraira, Alacant), Elevation 50 m. Excavations since 1995 have uncovered a rich sequence of Upper Paleolithic deposits (1). Beneath levels containing Gravettian assemblages, a potential initial Upper Paleolithic was uncovered in 2005. We aimed to date this and the overlying initial Gravettian occupation. The surfaces of bones were poorly preserved. Three bones without cut-marks or other evidence of butchery were sampled. All failed the %N screening protocol.

Mallaetes, 39°00′44′′N 0°18′04′′W (Barx, Valencia), Elevation 500 m. Mallaetes contains a small collection of Aurignacian remains, including a massive based point and a lozangic point that are indicative of the Evolved Aurignacian, at the base of an Upper Paleolithic sequence (2). The site was excavated by Pericot and Jordá between 1946 and 1949 and Fortea and Jordá cleaned sections and excavated a small area in 1970 (3). The majority of lithic and bone artifacts were found in the earlier excavations. The chronology of this assemblage is based on a single conventional radiocarbon date on a mixture charcoal and sediment (29690 ± 560 BP KN-1926, 36120–32920 calBP) from the 1970s excavation (3).

Given the age of Pericot and Jordá's excavation and the low occupation density, only modified remains were sought from the collection held at the Museu de Prehistòria de València. The two bone points, on display, were considered likely to have been conserved, and a third bone point fragment, not on display, was small (<1 g) and consolidated. One cutmarked bone was found, but failed the %N screening test. A second bone passed the %N test, but the single striation was not regarded as a convincing cut mark and was therefore not dated.

El Niño, 38°33′N 2°4′W (Ayna, Albacete), Elevation 800 m. Initially noted for its Upper Paleolithic rock art (4), El Niño was excavated in 1973 (5–7), uncovering a sequence containing a limited assemblage, likely to be Middle Paleolithic, below the lower eboulis (level 6). As part of a project aiming to re-excavate this poorly known Middle Paleolithic site lead by Alejandro Garcia (Universidad de Cantabria), fauna was sampled to provide a first estimate of the age of the deposit. Twenty-three bones were screened for nitrogen content, only two of which showed clear signs of anthropogenic modification. All failed the screening protocol.

Quebrada, 39°44′51′′N 0°59′35′′W (Chelva, Valencia). Excavations since 2006 at the rock shelter of Quebrada have uncovered layers containing Mousterian assemblages (8). Two radiocarbon dates on charcoal suggest that one combustion feature was created just before 42 ka calBP in the uppermost Mousterian level (layer III) (40500 ± 530 Beta-244003, 45250–43450 calBP: 43930 ± 750 BP Beta-244002, extends beyond limit of calibration curve). Nine bones were sampled from spits (capas) 5 and 2 to test the existing dates. The surface of the bones was too poorly preserved to identify cut-marks and all failed the %N test.

El Salt, 38°41′13′′N 0°30′32′′W (Alcoy, Alcant), Elevation 680 m. El Salt contains a stratified sequence of Middle Paleolithic layers with a rich material record including large lithic and faunal assemblages and numerous combustion structures constituting a succession of archaeological palimpsest deposits (11–14). In the basal layers (S.U.XI–XII) human occupation was intense, and starts to diminish in S.U.X, currently undergoing excavation. Between VIII and V lithics are scarce, but five Neanderthal teeth were found in layer V. A radiocarbon date on bone found close to these teeth was treated with the ninhydrin method (Table S6). The bone yielded little carbon, and so the date of 37100 ± 320 BP (code unknown, 42470–41430 calBP) may be an underestimate. Taken at face value however, the date suggests that level V was forming shortly before the arrival of anatomically modern humans (AMHs) in northern Iberia.

This study attempted to test the chronology of units VIII to V. Of 40 bones selected with Bertila Galván and Cristo Hernández (Universidad de la Laguna) at El Salt and the Museu Arqueològic Municipal d'Alcoi, eight had a marginal nitrogen content and five, including a Neanderthal tooth, passed the screening test. Radiocarbon pretreatment was attempted on three bones and one tooth, but none yielded enough collagen for dating. Polyvinal acetate consolidation of the remaining bones, applied at the time of excavation, was suspected after examination under a microscope and FTIR spectroscopy. Regrettably, therefore, no further work was possible on bone.

Sima de las Palomas, 37°47′59′′ N0°53′45′′W (Torre Pacheco, Murcia), Elevation 123 m. Sima de las Palomas is a karstic shaft that was mined in the 19th century. The Upper Cutting, excavated since 1992, contains at least four Neanderthal individuals associated with a Mousterian assemblage, which Walker et al. (9) claimed post-dates the first occurrence of the Aurignacian north of the Ebro. The radiocarbon dates were on burned bone and the U-series dates on bone that had reached equilibrium in uranium uptake, and both sets of dates must be regarded with caution. Given these problems, Walker et al. (10) have expressed concern that the chronology may be inaccurate. To test the age of this deposit a Neanderthal phalange was screened. Sadly, the phalange did not past the screening test.

Cueva Bajondillo, 36°37′10′′ N 40° 29′ 33′′ W, (Torremolinos, Málaga, Andalucia), Elevation 10 m. Cueva Bajondillo contains one of the few Evolved Aurignacian assemblages (layer 11) in southern Iberia, overlying a sequence of layers containing Middle Paleolithic industries (layers 17–14) (15). Layers 12 and 13 fill a depression in the top of layer 14, and both may contain redeposited material (Zilhão, 2006; see ref. 47). The site was subject to a rapid rescue excavation in 1989, but the standing section has since been extensively studied by Cortés Sánchez (15). Thermoluminescence dates on burnt flint are anonymously young throughout the stratigraphy, and the chronology is based on radiocarbon dates on charcoal/sediment removed from the standing section from levels 14, 13, and 11 (15). Considering their large errors, these dates fall broadly in line with the expected age of the Evolved Aurignacian and place the Middle Paleolithic beyond 42 ka calBP. However, the sample type is far from ideal and this chronology requires testing.

The few bone fragments recovered by Cortés Sánchez (15) from layers 14–11 of the standing section were examined. Fragments were small (maximum dimension < approximately 5 cm) and thin (< approximately 0.5 cm); they were not identifiable to species and were too degraded to preserve signs of anthropogenic modification. Given the extremely low chance of collagen preservation, the fragments were not screened for %N. The only alternative samples for radiocarbon are marine shells; however, none have been recovered from the standing section from the units of interest. Cortés Sánchez (15) noted that the excavated lithic assemblages from layers 14–11 appear to be mixed, and so these shells samples were not selected for dating.

Nerja, 36°45′44′′N 3°50′42′′W (Maro-Nerja, Málaga, Andalucia), Elevation 158 m. A series of six radiocarbon dates on charcoal from the Gravettian levels 13 and 12 suggest that Nerja contains one of the earliest Gravettian ages in southern Mediterranean Spain (16), with a Bayesian model predicting a start date of 31680–28654 cal BP at 95% significance. To test, and hopefully increase the precision of this boundary, 25 faunal bones from capas XIII, 13, and 12 were selected. Unfortunately, all of these bones contained less than 0.5%N.

Gorham's Cave, 36°07′N 5°21′W (Gibraltar), Elevation 14 m. Gorham's cave has been subject to three main excavations: by Waechter [1948–1954 (17, 18)], the Natural History Museum (NHM) [1995–1998 (19)], and the Gibraltar Museum [1997–ongoing (20)]. The first two excavations were toward the front of the cave and the most recent toward the rear. Radiocarbon dates have been obtained from all excavations. Those from Waechter's excavation were measured conventionally and removed from a section in 1957, long after the excavation had ceased and should be viewed with caution (21).

Eighteen samples of charcoal and two burned bones have been dated from the NHM excavation. All charcoal fragments were treated with the gentle RR protocol at the Oxford Radiocarbon Accelerator Unit (ORAU) (Table S6), with the exception of OxA-6075 from context 4d/22, which was pretreated with the ABA protocol. Evidence of occupation is exceptionally scarce within the layers containing the purported final Middle Paleolithic (contexts 11–24) (22). When first described, this sample was considered largely undiagnostic, poorly associated with the dated charcoal, and potentially mixed, containing a rejuvination flake from a prismatic core and Levallois flake (19, 22). Dates from this context, of around 30 ka BP can therefore only provide a *terminus ante quem* for the abundant Mousterian assemblages in level 18 and level 19, which both contain charcoal dated beyond 40 ka BP, as first suggested by Pettitt and Bailey (21). The most recent excavations have been dated with radiocarbon on 22 charcoal samples treated with an ABA protocol (20). These samples place the final Mousterian between 32–24 ka BP (*ca*. 36–29 ka calBP), although Finlayson et al. (20) suggest a minimum age of 28 or 24 ka BP (*ca.* 32 or *ca.* 29 ka calBP). Zilhão and Pettitt (23) doubt the reliability of the Finlayson et al. (20) dates, questioning both the ability of the pretreatment to remove contamination and the association of the charcoal fragments with the small assemblage of lithics.

We attempted to date modified bone from both the NHM and Gibraltar Museum excavations. Of 49 cut-marked bones screened, none contained sufficient nitrogen to attempt a date.

%N Screening Results

The %N contents of all bones screened in this study are presented in Table S1. These results are compared with %N contents of bones from northern Iberia, obtained using identical methods, in Fig S2. Preservation of collagen is significantly worse south of the proposed Ebro frontier, most likely because of higher temperatures.

Published Radiometric Dates from Jarama VI and Zafarraya

Both Jarama VI and Zafarraya have been dated before this study (Table S2). Bones from Zafarraya dated by Michel et al. (26, 27) were screened for nitrogen as part of this study (Table S3). The results closely match the carbon and nitrogen stable isotope and elemental data of collagen obtained by the ORAU at the time of dating. Collagen samples that did not meet the criteria set by Van Klinken et al. (47) also did not contain sufficient nitrogen to suggest success by dating using the ultrafiltration protocol. These dates should therefore be viewed with caution.

Critique of Dates from Middle Paleolithic/ Neanderthal Assemblages Thought to Be <42 k cal BP in Southern Iberia

See Table S4 for a critique of dates from Middle Paleolithic/Neanderthal assemblages thought to be <42 k cal BP in southern Iberia.

Critique of Dates from Radiometrically Dated Evolved Aurignacian Assemblages in Southern Iberia

See Table S5 for a critique of dates from radiometrically dated Evolved Aurignacian assemblages in southern Iberia.

SI Methods

All bone samples were screened for nitrogen content before radiocarbon dating. The majority of nitrogen in bone is contained within collagen, the fraction selected for radiocarbon dating, and so can provide in indication of whether a bone can be dated. When collagen constitutes less than 1 wt% of the bone, quality assurance indicators, such as the C:N ratio, %C, and $\delta^{13}C$ often suggest that the protein is of poor quality (either being degraded or contaminated) and not suitable for dating (47). Brock et al. (24, 48) have shown that when bone contains more than 0.8% nitrogen, more than 1% collagen could be extracted with the ultrafiltration protocol in around 70% of cases. The method is minimally destructive requiring 5 mg of bone powder obtained with a dentist's drill after removal of the bone surface. The sample is loaded into a tin capsule and the amount of nitrogen remaining measured in an automated carbon and nitrogen analyzer (e.g., Carlo Erba NA 2000) coupled to a continuous flow-isotope ratio mass spectrometer (CF-IRMS) (e.g., Sercon 20/20) (24, 48).

The use of an ultrafilter to remove the smallest contaminants from bone collagen was first suggested in 1988 (49). When applied to bone of Paleolithic-age, the process often produces ages that are significantly older than collagen extracted and purified with other procedures (50, 51). This increase in age suggests improved removal of contaminants because young contamination has a much larger effect on the measured age than ancient contaminants containing no ^{14}C (*ca.* 80 ^{14}C years for ancient vs. *ca.* 13000 ^{14}C years for 1% modern contamination added to a sample of 50000 BP).

Radiocarbon dating followed the methods described in Brock et al. (48). Bones were treated with the ultrafiltration protocol, denoted by the laboratory code AF. After sequential washing in HCl to remove the mineral phase, NaOH to remove base soluble organic components and HCl to remove secondary carbonates, the collagen was gelatinized. Large insoluble contaminants were then removed from the soluble gelatin with a 45- to 90-μm Eeze filter before ultrafiltration with a Vivaspin 15 30-kDa MWCO ultrafilter to remove the smallest contaminants. The residue was then freeze-dried. As a precautionary measure, if it was possible that glues or consolidants had been applied to the bone, a sequence of methanol, acetone, and chloroform washes were applied before pretreatment.

Collagen was combusted in an automated carbon and nitrogen analyzer coupled to a CF-IRMS as described for %N measurement, allowing measurement of carbon and nitrogen abundance and stable isotopes. The remaining gas was collected cryogenically for conversion to graphite using an iron catalyst (52) and radiocarbon dated by accelerator mass spectrometry (53).

Dates have been calculated according to the conventions of Stuiver and Polach (53, 54) and corrected for pretreatment and combustion backgrounds (55). Samples are defined as infinite when the $F^{14}C$ is less than twice its error from 0 and thus indistinguishable from background (54). The infinite date is equivalent to twice the size of the error. For example, if a sample has an $F^{14}C$ of 0.0022 ± 0.0012 (equivalent to $49200 \pm 4{,}400$ BP), it is regarded as infinite and ascribed a date of 0.0024 $F^{14}C$ or >48500 BP. In terms of the conventional ages that are presented here, any dates with an error of 4,000 ^{14}C years or greater are considered infinite.

A summary of methods and laboratory codes is given in Table S6. This table also contains descriptions of methods no longer in use at the ORAU, and methods mentioned in the text that have been used by other laboratories.

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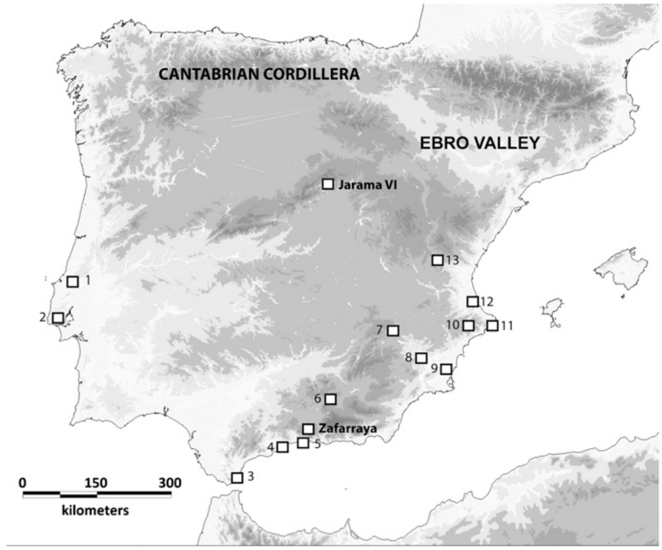

Fig. S1. The location of sites discussed in the main text and sampled in this study, alongside major topological features relating to the Ebro frontier hypothesis. 1, Gato Preto; 2, Pego do Diabo; 3, Gorham's Cave; 4, Bajondillo; 5, Nerja; 6, Carihuela; 7, El Niño; 8, Cueva Antón; 9, Sima de las Palomas; 10, El Salt; 11, Cendres; 12, Mallaetes; 13, Quebrada.

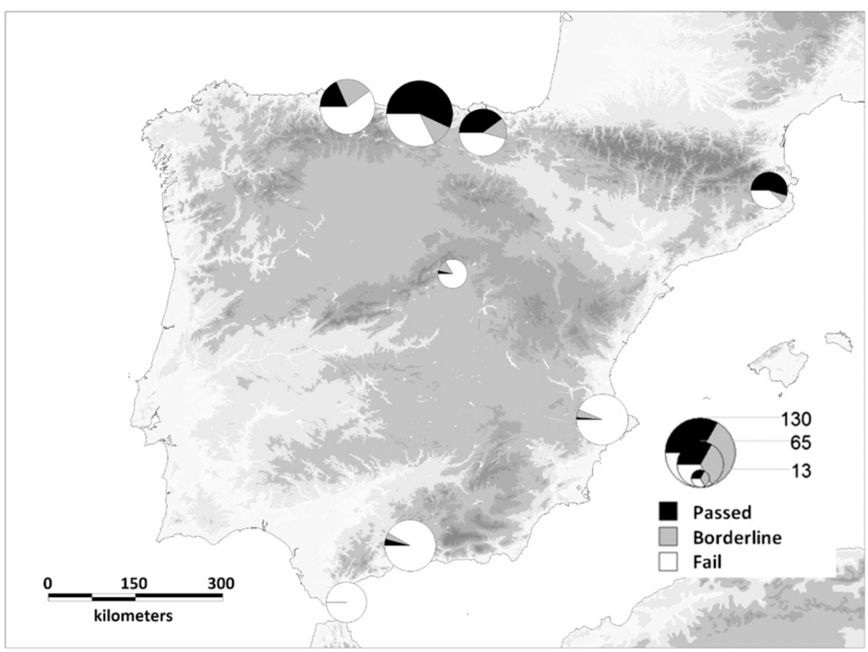

Fig. S2. The %N content of bone across Iberia. Nitrogen contents are higher in north of the Ebro, indicating markedly better preservation of collagen than in the south. Bones are ranked <0.5% N "fail," 0.5–0.7 "borderline," and >0.8% "pass" on the basis of how likely >1% collagen can be extracted from the bone with the ultrafiltration protocol (24). Each pie chart represents one administrative region. Data for southern Iberia is from *%N Screening Results* and northern Iberia from Wood (25).

Table S1. Results of the %N screening test for all samples in sites discussed in the main text and *Description of Sites and Samples That Could Not Be Radiocarbon Dated*

Table S1

If a sample of bone powder contains >0.7%N there is an 80% chance of the sample containing sufficient collagen for radiocarbon date (24) using the methods described in *SI Methods*. Samples containing 0.5–0.7%N are considered borderline here, and those containing <0.5%N were failed. Error on measurement is 0.2%N at 2 SD.

Table S2. Published radiometric dates from Jarama VI and Zafarraya

Table S2

14C denotes a conventionally measured radiocarbon date and AMS, a radiocarbon date measured by accelerator mass spectrometry. For laboratory pretreatment codes, please refer to Table S6. EU refers to an Electron Spin Resonance or U-Series date that assumes early uptake of uranium and LU, linear uptake. U-Series Alpha refers to U-Series dates measured by α-spectroscopy and TIMS, thermal ionization mass spectroscopy.

Table S3. The %N content of bones dated by Michel et al. (28, 29) and the quality assurance data of the dated collagen

Table S3

All but one sample have isotopic or elemental data that suggest collagen was very degraded or contaminated, and low nitrogen contents. To obtain a reliable radiocarbon date, bone should contain >1% collagen, $\delta^{13}C$ between −22‰ and −18‰, $\delta^{15}N$ between 2‰ and 12 ‰, C:N 2.9–3.4 and % C >30% (55). Error on stable isotope values is typically ± 0.2‰ and %N measurement ± 0.2%, 2 standard deviations.

Table S4. Critique of dates from Middle Paleolithic/Neanderthal assemblages thought to be <42 ka calBP in southern Iberia

Table S4

All uncalibrated dates are given at 1 SD, and all calibrated date ranges at 95% probability. Errors for nonradiocarbon dates are given at 1 SD.

Table S5. Critique of dates from radiometrically dated assemblages that are thought to be Aurignacian in southern Iberia

Table S5

All uncalibrated dates are given at 1 SD, and all calibrated date ranges at 95% probability. Errors for nonradiocarbon dates are given at 1 SD.

Table S6. Summary of radiocarbon pretreatment methods mentioned in the main text and *SI Text*

Table S6

Where a method is used at the ORAU (48, 56), the associated laboratory code is given.
*Method no longer in use at the ORAU.

ORAU Pretreatment code	Material	Generic name	Reference	Description
AF	Bone	Ultrafiltration	48	Acid, base and acid washes are followed by gelatinisation of the insoluble residue and filtration and ultrafiltration of the gelatin.
AG*	Bone		48	Acid, base and acid washes are followed by gelatinisation of the insoluble residue and filtration of the gelatin.
AC*	Bone		56	Acid wash followed by hydrolysis of the insoluble residue and purification of the amino acids with activated charcoal and ion exchange chromatography
RR*	Charcoal		48	Acid wash, ultrasonication in ultrapure water and an acid wash.
ZR	Charcoal	ABA	48	Acid, base and acid washes.
XR	Charcoal	ABOx-SC	48	Acid and base washes are followed by chemical oxidation and partial combustion.
Not used that the ORAU	Bone	Longin method	57	Acid wash followed by gelatinisation of the insoluble residue and filtration of the gelatin to remove large insoluble materials.
Not used that the ORAU	Bone	Ninhydrin method	58	Ninhyrin is used to remove the carbon from the carboxylic acid group of amino acids. After demineralisation in acid, ninhydrin removes any free amino acids from the collagen. The protein is then hydrolysed and the carbon released from a subsequent ninhydrin treatment collected for dating.