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Supplementary Information for
Reevaluating the timing of Neanderthal disappearance in Northwest Europe.

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Supplementary text

Materials

Description of the archaeological sites

Spy Cave

The Betche aux Rotches Cave, more commonly known as the Spy Cave, is located in the village of Spy (Municipality of Jemeppe-sur-Sambre, Province of Namur). The cave opens south-west on the Orneau Valley, a tributary of the Sambre (1). The cave has been explored several times since 1879, when A. Rucquoy started the excavation of the inner part of the cave (2). After Rucquoy's excavations, numerous amateur archaeologists conducted investigations in the cave (3). One of the most important excavation campaigns was led by M. Lohest, a geologist from the University of Liège, and M. De Puydt, an enlightened amateur, in 1885-1886. They explored the terrace of the cave, which led to the discovery of the famous "Men of Spy". This was the first time that fossil human remains were found in a stratigraphic context in association with lithic material and extinct animal remains (4). They collected 89 human remains in the "third fauna-bearing level". Aware of the importance of their discovery, minutes were drawn up, recording the position of the human remains in the stratigraphy on July 11, 1886 (3). For the first time in history, the antiquity of mankind was recorded in a more scientific way. Nevertheless, the spatial relationship between the Neanderthal individuals in the site as a whole and the position of the recovered Neolithic remains (5) are still very difficult to discern. Between 1903 and 1909, Baron A. de Loë and E. Rahir from the RMAH, explored and drew a map of the cave, highlighting the different areas excavated by their precursors (6). They worked in the immediate surroundings of the De Puydt and Lohest excavations, digging on the terrace and the entrance of the cave, including the backfill of those previous excavations. They collected 165 human remains but most of these were not identified as human until the reassessment of the collection (7). Between 1927 and 1933, new excavations were conducted inside Spy Cave by J. Hamal-Nandrin, Ch. Fraipont and S. Leclercq , from the University of Liège (8). Neanderthal remains were found. They are listed in the inventory of the collection of the University of Liège by C. Fraipont and briefly mentioned in (8). F. Twiesselmann, from the RBINS, made a few test pits inside the cave (1950) and systematically excavated the slope in front of the cave (1952-1954), from the terrace to the Orneau River (3). This collection is the largest in terms of human, archaeological and paleontological remains. Despite the numerous profiles sketched, the origin of the deposits remains unclear because they mostly come from the backfill of the previous excavations as demonstrated by refittings made between the De Puydt and Lohest collection and the material unearthed by Twiesselmann (9). For example, the maxillary fragment of the De Puydt and Lohest collection (Spy 11a) fits the 3rd molar still attached to a small alveolar fragment from the Twiesselmann collection (Spy 94a) that is part of this study. The last official excavations were carried out by M. Dewez between 1979 and 1980 in the slope and inside the cave (10). Besides these officially reported excavations, numerous amateurs explored the site throughout the 19th and 20th centuries, excavating the backfill or in situ sediments in the cave, on the terrace or on the slope in front of the cave (3). The latest significant discovery was an accidental surface find in the early 2000's by a local, Ph. Pirson, who discovered a fragmented Neanderthal lumbar vertebra on the slope in front of the cave (11).

The archaeological sequence of Spy Cave appears very rich. While it was initially described as a single culture (4), typological studies have confirmed the presence of 14 distinct archaeological phases, among which at least 10 distinct Palaeolithic assemblages from the Middle Palaeolithic to the Late Upper Palaeolithic (1, 12). The different occupations of the site are evidenced not only by the presence of a very rich lithic collection but are also documented by numerous testimonials of bone industry, including ornaments made in mammoth ivory (13) and Aurignacian split-base points (14). The mixing of the material was demonstrated several times (9), notably by the presence of pottery fragments within the "second fauna-bearing level" or typological studies (15, 16). Together with Goyet Caves, Spy is one of the most important Palaeolithic sites in Belgium and a major site on European scale.

Schmerling Cave (Engis)

The Schmerling Cave, originally known as "Trou Caheur" or "Deuxième grotte d'Engis", is located in the village of Les Awirs (Municipality of Flémalle, Province of Liège). The cave is part of a set of cavities, described by Schmerling. It consists of two caves and a gallery. The two caves were named from East to West by Schmerling: "Première grotte d'Engis" and "Deuxième grotte d'Engis" (17), while the gallery was named as “Troisième caverne d’Engis” by Dupont (18). This gallery was later defined as an extension of the “Deuxième Caverne” (19). The “Trou Caheur” was renamed “Grotte Schmerling” in honor of the famous precursor in 1939 by the “Chercheurs de la Wallonie”, a group of enlightened amateurs (20). Today, the Schmerling Cave opens on the remnants of an alum quarry that deeply modified the landscape, about 750m from the Meuse. Only the back of the cave, emptied by successive excavations during the 19th and 20th Centuries, remains today.

The first excavation was conducted by Ph.-Ch. Schmerling, a medical doctor, during the winter of 1829-1830. This led to the discovery of several human remains, including two calottes: one adult (Engis 1) and one juvenile (Engis 2), the latter being was associated with isolated teeth and a fragment of maxilla. The Engis 1 skull belongs to a Neolithic individual (11): 4590 ± 80 BP (OxA-746); 4920 ± 50 BP (Beta-154814), and was probably part of a plural burial. The juvenile individual was discovered at the bottom of the cave, associated with an Elephantidae tooth (17). For Schmerling, the contemporaneity of human, palaeontological and archaeological remains was asserted by their association at the same site, without regard for stratigraphy or sedimentology (21). This assertion has been discussed several times since the discovery and motivated the resumption of field work, which started almost 40 years later with E. Dupont in 1868 (18). The cave network has been explored numerous times during the 19th Century (22, 23), at the dawn of the 20th Century (15) and on several occasions between 1907 and 1956 (24, 25). No other Neanderthal remains were found.

Première Caverne du Bay Bonnet (Fonds-de-Forêt)

The Bay Bonnet caves, also known as Fonds-de-Forêt caves, are located in the Magne Valley (Municipality of Trooz, Province de Liège), a tributary of the Vesdre river. The two caves open on the left bank of this deep valley, just 1500m upstream of the confluence and are separated from each other by a dozen meters. They were first explored by Ph.-Ch. Schmerling between 1830 and 1831 and, from time to time, by collectors until 1895, when F. Tihon started his exploration of the site leading to the discovery of two human remains in the first cave (the most upstream; (26)). He was also the first to provide stratigraphic observations for the two caves. After Tihon’s excavations, numerous other scholars and collectors excavated the site until a short excavation campaign in 2003 that was carried out prior to the closing of the caves (installation of grids). Based on Tihon’s observation, the first cave is composed of 4 different layers (26). The 3rd layer (from the top) is the most important and contains most of the archaeological material and the two human specimens: a femur and an upper molar. The femur was identified to be Neanderthal since its discovery (26). This layer (named Layer F) was also recognised by A. Rutot, who excavated the site in 1907 (27). The Mousterian artefacts represent the majority of the archaeological material. Based on its faunal remains, this layer has been attributed to the first half of the MIS 3 (50,000-35,000; (28)). However, the presence of several Upper Palaeolithic tools point to a lack of precision of the stratigraphic observations and the association of material from different periods, which occurred frequently in historic excavations (15). Several other excavation campaigns were led by professionals (29) and amateurs (30–33) within the caves and on the terraces. No other Neanderthal remains were discovered.

<u>Chronological information on Scladina, La Naulette, Trou de l’Abîme, Walou Cave, Troisième Caverne de Goyet cave and Trou Magrite</u>

Apart from Spy Cave, Schmerling Cave and Première Caverne du Bay Bonnet, six other caves yielded Neanderthal human remains in Belgium. They were not included in our model for various reasons presented below.

The specimens from Scladina Cave and La Naulette Cave are older than the Late Neanderthal period. They do not inform us of the disappearance of the Neanderthals in Western Europe.

Scladina Cave

The Neanderthal child from Scladina consists of a nearly complete juvenile mandible, a fragment of right maxilla and isolated teeth, unearthed from 1990 onwards (34). The remains of this ca. 8-year-old child (35) were recovered in secondary position in a gully (36). The fossil has been directly dated to 127 +46/−32 ka by Gamma-Ray Spectrometry (37, 38). The combination of bone taphonomy, sedimentogenesis, palaeoenvironmental reconstructions, and chronostratigraphic markers of the whole sequence points to an approximate age of either 87 or 80 ka, during a cold episode of the Weichselian Early Glacial (36).

La Naulette Cave

Several human remains have been unearthed by E. Dupont in 1866 (39, 40) in La Naulette Cave: a human mandible, an ulna, a third metacarpal and a canine. The remains have never been directly dated. However, palaeoanthropological evidence suggests they may be older than “classic Neanderthals”: the mandible presents archaic features that fit within the biometric variability of Neanderthals and their pre-Neanderthal ancestors, while the ulna and third metacarpal show modern morphological and metric characters (41, 42). Moreover, the human remains were lying in the second ossiferous level, below 5 stalagmitic floors (39). Together, these suggest an early age, at least the Weichselian Early Glacial (20).

Other fossils coming from four other sites are probably Late Neanderthals but were either indirectly dated (Trou de l’Abîme and Walou Cave), directly dated but possibly contaminated (Troisième Caverne de Goyet), or undated and deprived of any reliable contextual information (Trou Magrite).

Trou de l’Abîme

A Neanderthal lower right deciduous second molar (LRdm2) was discovered in 1984 at the Trou de l’Abîme (43). As this fossil is a worn tooth that was likely lost during the life of the child (44), it is almost impossible to directly date it without risking its complete destruction. Until recently, it was hypothesized that the archaeological material, the faunal material and the Neanderthal tooth unearthed in 1984-1987 were part of one assemblage, dated around 45,000 BP, even if the dated material was deprived of any anthropic marks (45). Recently, archaeozoological studies confirmed a single short term occupation of the site (46) and allowed to associate the human tooth to anthropogenically modified faunal remains that have been directly dated: 43,600 ± 1,900 BP (OxA-34120) and 43,400 ± 1,800 BP (OxA-34121) using ultrafiltration pretreatment and thus suggestive of this date range for the Neanderthal individual.

Walou Cave

A Neanderthal permanent lower left third premolar was unearthed in Layer CI-8 in 1997 (47–49). This layer also yielded the main Mousterian occupation of the site (50). The detailed study of the premolar led to its attribution to a Neanderthal individual (47). It was not possible to date this tooth directly for this study as it was already partially destroyed during a previous dating attempt at the ORAU which resulted only in isotopic analyses. The chronostratigraphic framework is particularly coherent, as a result of tephrostratigraphy (Rocourt and Laacher See Tephras) and the excellent correlation with the loess sequences of Middle Belgium, strengthened by radiocarbon, TL and ESR dates (48, 51, 52). The Neanderthal tooth is, therefore, highly likely dated to ca. 38-40 ka BP based on this contextual and chronological data (48, 52, 53).

Troisième Caverne de Goyet

The cave was firstly excavated by E. Dupont in between 1868 and 1870 (54) and is, together with Spy, the most important Palaeolithic site in Belgium and of major importance on a European scale. It yielded numerous stone artefacts from the Middle and Upper Palaeolithic, as well as portable art and bone artefacts. From 2004 onwards, the reappraisal of the Dupont’s collections led to the identification of 100 new Neanderthal remains, which belong to a minimum of 5 individuals, showing distinctive anthropogenic modifications (butchery activities and retouchers). Goyet is the first site where multiple Neanderthal bones were identified to have been used as retouchers, as well as the first site with unambiguous evidence of Neanderthal cannibalism in Northern Europe (55–59). These specimens have produced a wide range of radiocarbon dates (55–59) but they likely represent an epiphenomenon. The samples have been dated on collagen and contaminations issues, similar as those observed for Spy, are a plausible explanation for the inconsistency in the dates. These dates may therefore be underestimated. Permission was not granted to resample the material using the HYP pretreatment. We therefore decided to not include these dates in the model until the specimens are redated using the same pretreatment.

Trou Magrite

E. Dupont excavated the site in 1867 and collected a large quantity of archaeological material, including lithic artefacts and art objects (60, 61) as well as faunal remains among which the Neanderthal remains were discovered in 2015 (62). The Neanderthal remains, a juvenile left femoral diaphysis and an upper right permanent canine have not yet been directly dated. Although the femur delivered a mtDNA sequence that falls within the Late Neanderthals genetic diversity, in the absence of clear stratigraphic context and a direct date using the most robust pretreatment (HYP), it is not yet possible to ascertain a secure chronological context for this specimen (62).

Methods

<u>DNA analyses supplementary results</u>

The number of DNA molecules in the libraries, as determined by qPCR, ranged between 5.82×10^{9} and 6.15×10^{10}, and were thus higher than in the extraction and library negative controls (Table S3). After enrichment, mapping to the revised Cambridge Reference Sequence and duplicate removal, between 152,069 and 343,161 unique mitochondrial fragments were obtained from the two specimens (Table S3). Each unique fragment was seen between 2.76 and 8.76 times on average (Table S3), indicating that deeper sequencing would not substantially increase the number of unique mitochondrial fragments.

To determine whether some of these fragments are of ancient origin, we next determined the frequency of cytosine (C) to thymine (T) substitutions at the ends of the alignments. Substantial cytosine deamination was observed only in the femur from Fonds-de-Forêt (Table S4), whereas we did not find any evidence for ancient hominin DNA preservation in the samples taken from the Spy 572a. Moreover, after realignment of the mitochondrial fragments to 242 mammalian mtDNA genomes, we found that between 23,254 and 25,690 unique mitochondrial fragments from Spy 572a were assigned to *Bovidae* (Table S5). This amounts to between 9.7% and 12.8% of all identified mtDNA sequences recovered from Spy 572a (Table S5). The C-to-T substitution frequencies of the bovid fragments ranged from 2.0% (95% confidence interval (CI): 1.5-2.4%) to 2.7% (95% CI: 2.2-3.3%) on 5'-ends, and from 1.3% (95% CI: 0.9-1.7%) to 1.8% (95% CI: 1.4-2.2%) on 3'-ends (Table S6), indicating that these fragments are not of ancient origin. We did not find evidence for *Bovidae* mtDNA in the femur from Fonds-de-Forêt or in the associated extraction and library negative controls (Table S5), suggesting that the bovid mtDNA fragments identified in Spy 572a might originate from animal glue used for conservation.

Of all sequences from the femur from Fonds-de-Forêt that overlap phylogenetically “diagnostic” positions, 94.66% supported Neanderthal state (Table S7). After reconstructing full mitochondrial genome of the femur from Fonds-de-Forêt to an average coverage of 135-fold based on deaminated fragments and comparing it to the mtDNAs of 54 present-day humans, 24 Neanderthals, four Denisovans, Sima de los Huesos hominin and a chimpanzee, we found that it falls within the variation of late Neanderthals (Fig. S3). Moreover, just one base pair differentiates the mtDNA genome of Fonds-de-Forêt from the mitochondrial genomes of Goyet Q57-1, Feldhofer 1 and Vindija 33.25.

<u>Code of the Bayesian model</u>

```
Plot()
{
 Outlier_Model("General",T(5),U(0,4),"t");
 Outlier_Model("SSimple",N(0,2),0,"s");
 Sequence("Belgium Neanderthals")
 {
  Boundary("Start Phase");
  Phase("Belgian N")
  {
   R_Combine("Femur")
   {
    Outlier("General", 0.05);
    R_F14C("OxA-X-2767-13", 0.00796, 0.00084)
```

```
    {
     Outlier("SSimple", 0.05);
    };
    R_F14C("OxA-38322", 0.00734, 0.00103)
    {
     Outlier("SSimple", 0.05);
    };
   };
   Label("Fonds-de-Forêt");
   R_F14C("OxA-38394", 0.007, 0.00149)
   {
    Outlier("General", 0.05);
   };
   Label("Engis");
   R_F14C("OxA-X-2762-6", 0.00567, 0.00167)
   {
    Outlier("General", 0.05);
   };
   R_Combine("Spy 94a")
   {
    Outlier("General", 0.05);
    R_F14C("OxA-X-2762-21", 0.00571, 0.00127)
    {
     Outlier("SSimple", 0.05);
    };
    R_F14C("OxA-X-2778-15", 0.00916, 0.00252)
    {
     Outlier("SSimple", 0.05);
    };
   };
   R_F14C("OxA-38790", 0.0056, 0.00162)
   {
    Outlier("General", 0.05);
   };
   Label("Spy");
  };
  Boundary("End Neanderthals");
 };
};
```

Supplementary Figures

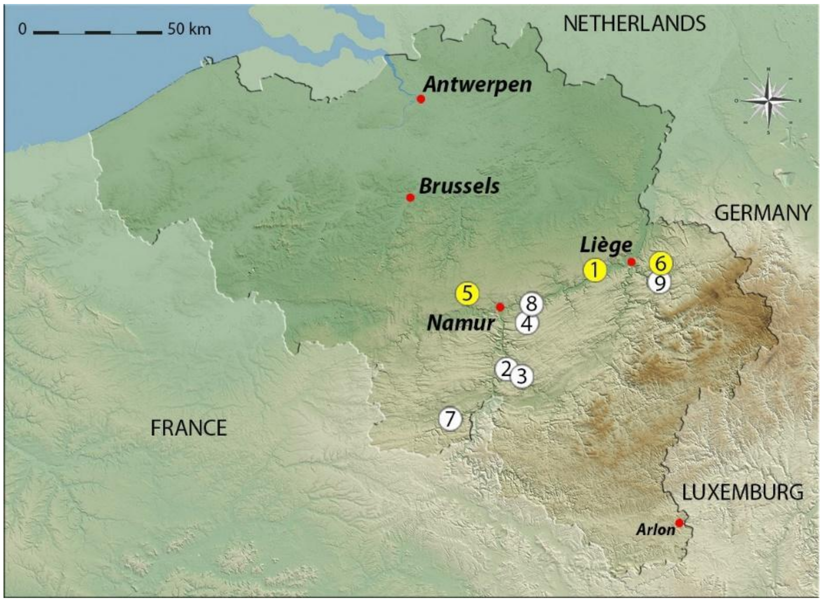

Fig. S1. Map of Belgium showing the location of the sites where Neanderthals were found. Sites are numbered in the order of their discovery (Table S1). [1]: Schmerling Cave (Engis); [2]: La Naulette Cave; [3]: Trou Magrite; [4]: Troisième Caverne de Goyet; [5]: Spy Cave; [6]: Première Caverne du Bay Bonnet (Fonds-de-Forêt); [7]: Trou de l'Abîme; [8]: Scladina Cave; [9]: Walou Cave. New radiocarbon dates are presented in this article for the three sites indicated in yellow.

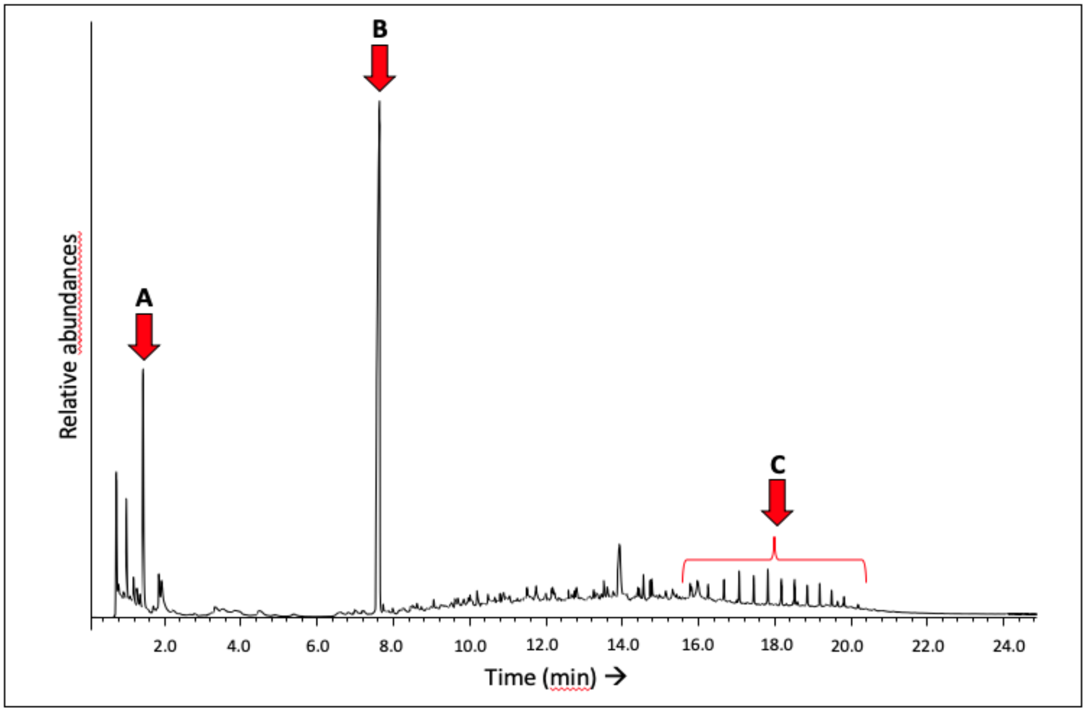

Fig. S2. Pyrogram of the bone powder from the femur of Fonds-de-Forêt showing the presence of paraffin characterised by a series of alkanes [C] and a copolymer of methyl methacrylate [A] and butyl methacrylate [B].

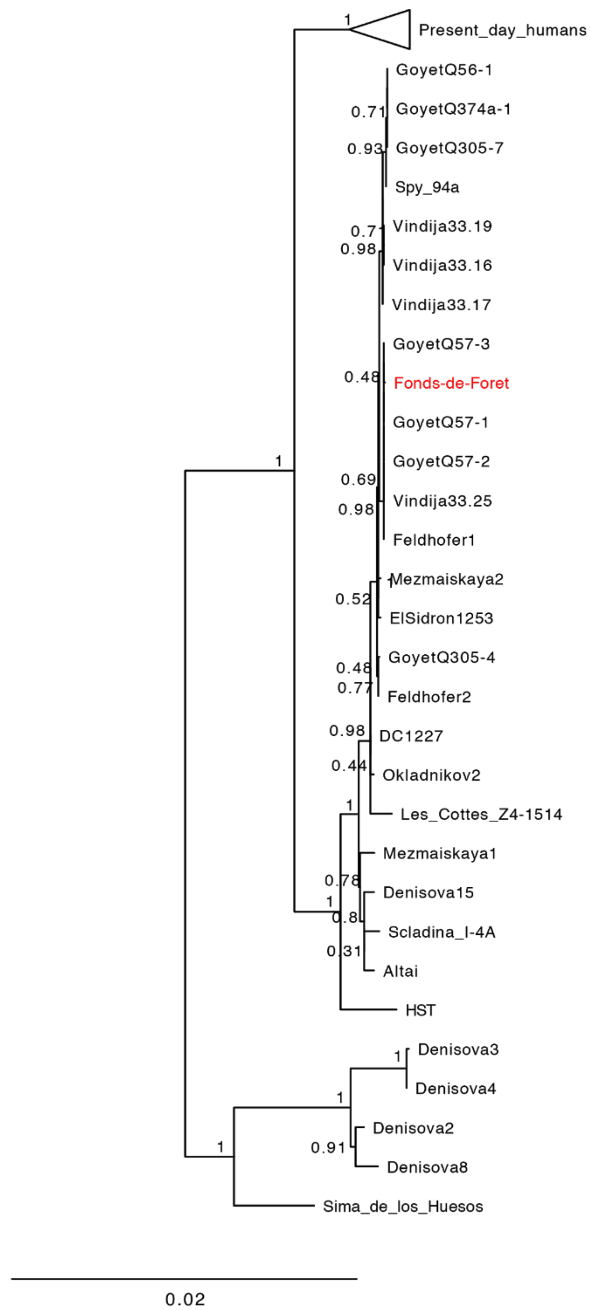

Fig. S3. Maximum likelihood tree relating Fonds-de-Forêt mtDNA to 54 present-day humans, 24 Neanderthals, four Denisovans, one Sima de los Huesos individual and a chimpanzee. The mitochondrial genome of Fonds-de-Forêt femur is highlighted in red. The bootstrap supports are denoted above each branch. A total of 15,854 positions covered in all individuals were used for analysis. Branches leading to present-day humans are collapsed for visualization purposes. A chimpanzee mitochondrial genome was used as an outgroup to root the tree (not shown).

Supplementary tables

Site	Years of the discovery of the first human remains	Years of identification of human remains as Neanderthals
Schmerling Cave	1829-1830	1936
La Naulette Cave	1866	1866 (as ancient hominid)
Trou Magrite	1867	2015
Troisième Caverne de Goyet	1868-1870	2004
Spy Cave	1886	1886
Première Caverne du Bay Bonnet (Fonds-de-Forêt)	1895	1895
Trou de l'Abîme	1984	2009
Scladina Cave	1990	1993
Walou Cave	1997	1999

Table S1. Summary, for the 9 Belgian sites that produced Neanderthal remains, of the years the human remains were discovered and the years they were identified as Neanderthal remains. Sites are listed following the chronological order of the identification of the first human remains.

Specimen		Sample ref	P Code	Sample weight	% Yld	%C	$\delta^{13}C$ (‰) (VPDB)	$\delta^{15}N$ (‰) (AIR)	C/N	Conventional ^{14}C Age (BP)	Lab number	Publication
Spy 92b	Upper left I1	-	-		9.0	41.9	-19.71	-	-	36,350 +310/-280	GrA-32626	(63)
Spy 94a	UM3 Right = Spy I skull	P44561	AF	562	1.5	45.0	-19.7	11.4	3.4	34,650 ± 600	OxA-37681	This article
		P44561	HYP	562	0.4	40.5	-26.2	11.8	5.0	41,500 ± 1,800	OxA-X-2762-21	This article
	Right maxilla frag attached to M3	-	-		7.8	42.5	-19.34	-	-	35,810 +260/-240	GrA-32623	(5)
		P44886	HYP	22	5.5	10.5	-26.0	11.9	5.0	37,700 ± 2,200	OxA-X-2778-15	This article
Spy 430a	Right middle 3rd manual phalanx	-	-		7.4	42.5	-20.14	-	-	33,940 +220/-210	GrA-32630	(63)
		P20982	AF	414	2.4	43.6	-21.6	11.6	3.8	(32,550 ± 400) 31,950 ± 450	(OxA-17916) OxA-21607	(63)
Spy 572a	Fragment of right scapula	P10272	AG	300	8.5	35.3	-19.4	11.2	3.2	23,880 ± 240	OxA-8912	(11)
		P10272	AG*	300	4.1	33.4	-19.0	11.5	3.4	24,730 ± 240	OxA-9213 $^{\$}$	(5)
		-	-		-	41.1	-19.91	-	-	31,810 ± 250	GrA-21546	(11)
		P46379	HYP	272	1.4	40.2	-23.7	13.6	5.0	33,700 ± 550	OxA-38356	This article
Spy 589a	Upper right di1	P20980	AF	155	6.5	42.7	-19.6	14.0	3.4	(34,700 ± 550) 33,950 ± 550	(OxA-17977) OxA-21610	(64)
		P20980	AG	155	12.3	42.3	-19.5	13.9	3.4	33,450 ± 600	OxA-18754	This article
		P20980	HYP	242	0.9	43.0	-23.9	16.3	5.0	41,700 ± 2,300	OxA-38790	This article
Spy 646a	Neanderthal Right hemi-mandible	-	-		4.7	42.5	-21.79	-	-	32,970 +200/-190	GrA-32627	(63)
Spy 737a	fragment of vertebra	P12237	AF	560	4.4	40.4	-18.8	11.5	3.1	36,250 ± 500	OxA-10560	(11)
		P12237	HYP	560	0.3	43.0	-27.3	11.2	5.1	41,600 ± 2,400	OxA-X-2762-6	This article

Table S2. Radiocarbon dates obtained on the Spy Neanderthals. Conventional radiocarbon age is expressed in years BP (65). PCode refers to pretreatment code; 'AG' is collagen; 'AF' is ultrafiltered collagen; and 'HYP' denotes the extraction of hydroxyproline from hydrolysed bone collagen; * indicates that the bone powder was washed with solvents (acetone, methanol and chloroform) prior to decalcification (66, 67). Sample weight is the amount of bone powder used with the exception of P44886 for which it is collagen already extracted. %Yld is the percent yield of extracted collagen (for AF and AG) or hydroxyproline (for HYP) as a function of the starting weight of the bone analysed (or collagen for P44886). %C is the carbon present in the combusted sample (collagen or hydroxyproline). Stable isotope ratios $\delta^{13}C$ and $\delta^{15}N$ are expressed in per mil (‰) relative to vPDB and AIR, respectively, with a mass spectrometric precision of ± 0.2‰ (68). C/N denotes the atomic ratio of carbon to nitrogen and is acceptable if it ranges between 2.9-3.5 in the case of collagen, or 4.9-5.1 in the case of hydroxyproline. Two radiocarbon dates from Spy have been recalculated having been erroneously reported in the scientific literature. Sample Spy 430a (P20982), was initially reported as 32,550 ± 400 BP (OxA-17916) but was recalculated to 31,950 ± 450 BP (OxA-21607). Similarly, Spy 589a (P20980) was initially reported as 34,700 ± 550 BP (OxA-17977) but was recalculated to 33,950 ± 550 BP (OxA-21610). $^{\$}$ This date was erroneously labelled OxA-8913 in Semal et al (2009); the correct label is OxA-9213. Dates obtained on collagen in Groningen are reported in red. Dates obtained on collagen and hydroxyproline at the ORAU are reported in blue and green, respectively.

Specimen	Sample ref	P Code	Sample weight	%Yld	%C	$\delta^{13}C$ (‰) (VPDB)	$\delta^{15}N$ (‰) (AIR)	C/N	Conventional ^{14}C Age (BP)	Lab number	Publication
Engis											
Engis 2_Skull		-		-	39.9	-19.85	-	-	30,460 ± 210	GrA-21545	(11)
	P10254	AG	380	15.2	42.4	-19.3	12.1	3.5	26,820 ± 340	OxA-8827	(11)
		NRC	380	10.5	42.7	-19.4	12.3	3.3	27,670 ± 140	OxA-17503	This article
Engis 2_LRdm2	P46380	HYP	410	0.7	39.9	-23.7	16.1	5.0	39,900 ± 1,700	OxA-38394	This article
Fonds-de-Forêt											
Femur	P44560	HYP	880	0.4	40.4	-24.0	12.8	5.0	39,500 ± 1,100	OxA-38322	This article
		HYP	880	0.5	36.9	-23.4	12.5	5.1	38,800 ± 900	OxA-X-2767-13	This article

Table S3. Radiocarbon dates obtained on the Neanderthal specimens from Engis and Fonds-de-Forêt. Refer to the caption of Table S1 for details. NRC is for Ultrafiltration of collagen obtained with the AG pretreatment.

Specimen	Amount of powder (mg)	Extract ID	Library ID	Number of library molecules	Number of library molecules from oligonucleotide control	Number of molecules sequenced	Number of unique mtDNA fragments ≥ 35bp, MQ≥25	Average number of duplicates	Number of fragments with terminal C→T substitution
Spy 572a [A (surface)]	15.2	E17838	A29250	6.15E+10	1.60E+06	2,457,222	307,161	2.76	4,019
Spy 572a [B (inside)]	24.3	E17839	A29251	3.18E+10	1.31E+06	3,025,719	341,342	3.26	2,409
Spy 572a [C (surface)]	14.7	E17840	A29252	3.10E+10	1.48E+06	3,327,263	343,161	3.98	4,127
Fonds-de-Forêt	26.7	E17841	A29253	5.82E+09	1.34E+06	2,558,821	152,069	8.76	35,406
Extraction negative control		E17842	A29254	1.92E+08	1.61E+06	43,469	1,809	10.27	16
Library negative control			A29243	1.51E+08	1.58E+06	4,842	34	14.44	-

qPCR – quantitative PCR, bp – base pairs, MQ – mapping quality, C – cytosine, T – thymine

Table S4. Characteristics of the DNA libraries enriched for human mitochondrial DNA (mtDNA) and aligned to the revised Cambridge Reference Sequence (rCRS).

Specimen	All fragments 5'-end% [95% CI], 3'-end [95% CI]
Spy 572a [A (surface)]	1.3 [1.2-1.4], 1.1 [1.0-1.1]
Spy 572a [B (inside)]	1.1 [1.0-1.2], 0.6 [0.5-0.6]
Spy 572a [C (surface)]	1.2 [1.1-1.2], 1.0 [0.9-1.0]
Fonds-de-Forêt	32.3 [31.8-32.7], 27.7 [27.3-28.2]
Extraction negative control	0.6 [0.1-1.6], 0.7 [0.2-1.8]
Library negative control	0 [0-52.2], 0 [0-36.9]

CI – confidence interval, C – cytosine, T - thymine

Table S5. Frequencies of C-to-T substitutions at the terminal positions of the mtDNA fragments aligned to the revised Cambridge Reference Sequence (rCRS). Only fragments longer than 35 bp and with a mapping quality of at least 25 were used for this analysis.

Specimen	Number of fragments going into alignment	Fragments ≥ 35bp	Number of unique mtDNA fragments ≥ 35bp	Average number of duplicates	Identified fragments ≥ 35bp	Identified taxa (# fragments assigned to the taxa [% of all identified fragments])	
Spy 572a [A (surface)]	2,448,174	1,141,790	257,497	2.2	181,729	Hominidae (158,384 [87.2%])	Bovidae (23,345 [12.8%])
Spy 572a [B (inside)]	2,963,358	1,345,258	301,380	2.6	237,792	Hominidae (214,538 [90.2%])	Bovidae (23,254 [9.8%])
Spy 572a [C (surface)]	3,280,775	1,649,825	349,562	3.1	265,903	Hominidae (240,213 [90.3%])	Bovidae (25,690 [9.7%])
Fonds-de-Forêt	2,530,679	1,343,300	151,692	6.3	132,892	Hominidae (132,892 [100%])	
Extraction negative control	43,469	18,755	1,650	8.1	1,402	Hominidae (1,402 [100%])	
Library negative control	4,842	557	35	9.6	33	Hominidae (33 [100%])	

bp – base pairs

Table S6. Number and proportion of mtDNA fragments attributed to different families after realignment of the mtDNA data to 242 mammalian mtDNA genomes.

	C-to-T substitution frequencies for identified taxa 5'-end% [95% CI], 3'-end [95% CI]	
Specimen	**Hominidae**	**Bovidae**
Spy 572a [A (surface)]	0.8 [0.7-0.9], 0.6 [0.5-0.6]	2.6 [2.1-3.2], 1.6 [1.2-2.0]
Spy 572a [B (inside)]	0.4 [0.3-0.4], 0.2 [0.1-0.2]	2.0 [1.5-2.4], 1.3 [0.9-1.7]
Spy 572a [C (surface)]	0.7 [0.6-0.7], 0.6 [0.5-0.6]	2.7 [2.2-3.3], 1.8 [1.4-2.2]
Fonds-de-Forêt	31.1 [30.6-31.6], 26.6 [26.1-27.1]	-
Extraction negative control	0.0 [0.0-0.9], 0.7 [0.1-2.0]	-
Library negative control	0.0 [0.0-60.2], 0.0 [0.0-41.0]	-

CI – confidence interval, C – cytosine, T – thymine

Table S7. Frequencies of C-to-T substitutions at the terminal positions of the mtDNA fragments attributed to different families.

	Humans ≠ everyone else		Neanderthals ≠ everyone else		Denisovans ≠ everyone else	
Specimen	**% All fragments [# observations]**	**% Fragments with terminal C-to-T substitutions [# observations]**	**% All fragments [# observations]**	**% Fragments with terminal C-to-T substitutions [# observations]**	**% All fragments [# observations]**	**% Fragments with terminal C-to-T substitutions [# observations]**
Spy 572a [A (surface)]	99.23 [15,352/15,471]	97.60 [122/125]	0.02 [4/16,584]	0.50 [1/199]	0.03 [9/27,588]	0 [0/286]
Spy 572a [B (inside)]	98.29 [19,072/19,404]	90.51 [124/137]	0.03 [6/20,378]	2.19 [3/137]	0.03 [11/33,836]	0 [0/79]
Spy 572a [C (surface)]	99.12 [16,185/16,328]	92.38 [97/106]	0.03 [5/18,437]	0 [0/230]	0.04 [11/29,820]	0 [0/257]
Fonds-de-Forêt	3.68 [232/6,079]	0.69 [9/1,306]	94.66 [5,548/5,861]	98.83 [1,355/1,371]	0.02 [2/12,968]	0.03 [1/2,962]
Extraction negative control	97.62 [41/42]	0 [0/0]	0 [0/60]	0 [0/1]	0 [0/91]	0 [0/2]
Library negative control	100 [2/2]	0 [0/0]	0 [0/1]	0 [0/0]	0 [0/3]	0 [0/0]

C – cytosine, T – thymine

Table S8. Percentage and number of fragments matching the derived state at positions "diagnostic" for modern human, Neanderthal or Denisovan branch in the hominin mitochondrial tree. Fragments obtained by human mtDNA captures and aligned to the rCRS which overlap positions where each of the hominin groups differs from all others (69) were utilized. The results are shown for all fragments and only those fragments with terminal C-to-T substitutions. The number of fragments supporting the derived state and the total number of observations are provided in brackets.

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