

Title: Neandertal and Denisovan DNA from Pleistocene sediments

Authors: Viviane Slon^{1,*}, Charlotte Hopfe¹, Clemens L. Weiß², Fabrizio Mafessoni¹, Marco de la Rasilla³, Carles Lalueza-Fox⁴, Antonio Rosas⁵, Marie Soressi^{6,7}, Monika V. Knul⁸, Rebecca Miller⁹, John R. Stewart⁸, Anatoly P. Derevianko^{10,11}, Zenobia Jacobs^{12,13}, Bo Li¹², Richard G. Roberts^{12,13}, Michael V. Shunkov^{10,14}, Henry de Lumley^{15,16}, Christian Perrenoud¹⁵, Ivan Gušić¹⁷, Željko Kućan¹⁷, Pavao Rudan¹⁷, Ayinuer Aximu-Petri¹, Elena Essel¹, Sarah Nagel¹, Birgit Nickel¹, Anna Schmidt¹, Kay Prüfer¹, Janet Kelso¹, Hernán A. Burbano², Svante Pääbo¹,
Matthias Meyer^{1,*}

Affiliations:

¹Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, 04103 Leipzig, Germany

²Research Group for Ancient Genomics and Evolution, Department of Molecular Biology, Max Planck Institute for Developmental Biology, Tübingen, Germany

³Área de Prehistoria, Department of History, Universidad de Oviedo, Calle Teniente Alfonso Martínez s/n, 33011 Oviedo, Spain

⁴Institute of Evolutionary Biology (UPF-CSIC), 08003 Barcelona, Spain

⁵Departamento de Paleobiología, Museo Nacional de Ciencias Naturales, CSIC, 28006 Madrid, Spain

⁶Faculty of Archaeology, Leiden University, 2333CC Leiden, The Netherlands

⁷Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology,
Deutscher Platz 6, 04103 Leipzig, Germany

⁸School of Applied Sciences, Bournemouth University, Poole, Dorset, UK

⁹Service de Préhistoire, Université de Liège, 4000 Liège, Belgium

¹⁰Institute of Archaeology and Ethnography, Russian Academy of Sciences, Siberian Branch,
Novosibirsk, RU-630090, Russia

¹¹Altai State University, Barnaul, RU-656049, Russia

¹²Centre for Archaeological Science, School of Earth and Environmental Sciences, University of
Wollongong, Wollongong, New South Wales 2522, Australia

¹³ARC Centre of Excellence for Australian Biodiversity and Heritage, University of
Wollongong, Wollongong, New South Wales 2522, Australia

¹⁴Novosibirsk National Research State University, Novosibirsk, RU-630090, Russia

¹⁵Centre Européen de Recherches Préhistoriques de Tautavel, 66720 Tautavel, France

¹⁶Institut de Paléontologie Humaine, 75013 Paris, France

¹⁷Anthropology Center of the Croatian Academy of Sciences and Arts, 10000 Zagreb, Croatia

*Correspondence to: viviane_slon@eva.mpg.de or mmeyer@eva.mpg.de.

Abstract: Although a rich record of Pleistocene human-associated archaeological assemblages exists, the scarcity of hominin fossils often impedes the understanding of which hominins occupied a site. Using targeted enrichment of mitochondrial DNA we show that cave sediments represent a rich source of ancient mammalian DNA that often includes traces of hominin DNA, even at sites and in layers where no hominin remains have been discovered. By automation-assisted screening of numerous sediment samples we detect Neandertal DNA in eight archaeological layers from four caves in Eurasia. In Denisova Cave we retrieved Denisovan DNA in a Middle Pleistocene layer near the bottom of the stratigraphy. Our work opens the possibility to detect the presence of hominin groups at sites and in areas where no skeletal remains are found.

One Sentence Summary: DNA from archaic humans can be retrieved from Late and Middle Pleistocene sediments, even in the absence of their skeletal remains.

Main Text:

DNA recovered from ancient hominin remains enriches our understanding of human evolution and dispersal (*e.g.* (1) and references therein), and has, for example, resulted in the discovery of the Denisovans, a previously unknown group of archaic hominins in Asia who were distantly related to Neandertals (2-4). However, hominin fossils are rare. We therefore decided to investigate whether hominin DNA may survive in sediments at archaeological sites in the absence of macroscopically visible skeletal remains.

Mineral and organic components in sediments can bind DNA (*e.g.* (5-8)) (Figs. S1-S3) and the amplification of short stretches of mitochondrial (mt) or chloroplast DNA from sediments by the polymerase chain reaction (PCR) has been used to demonstrate the past presence of animals and plants at several sites (*e.g.* (9-14)). More recently, DNA extracted from sediments has been converted to DNA libraries, from which DNA fragments were sequenced directly (“shotgun” sequencing) (15, 16). This approach is preferable to PCR as it allows the entire sequence of DNA fragments to be determined. This is important as it makes it possible to detect cytosine (C) to thymine (T) substitutions near the ends of DNA fragments, which are caused by the deamination of cytosine bases (17) and indicate that the DNA is of ancient origin (18-20). However, the abundance of bacterial DNA in sediments and the difficulty in assigning short nuclear DNA sequences to mammalian taxa limit the utility of shotgun sequencing for analyzing DNA from sediments.

Isolating DNA from Pleistocene cave sediments

To investigate whether ancient mammalian DNA, especially of archaic humans, may be preserved in Pleistocene cave sediments, we collected 85 samples from seven archaeological

sites with known hominin occupation, varying in age between ~14 thousand years ago (kya) and ≥ 550 kya (Data file S1) (8). Some samples were collected specifically for the purpose of this study: 4 from Les Cottés (France), 5 from Trou Al'Wesse (Belgium), 1 from El Sidrón (Spain), 1 from Vindija Cave (Croatia), 3 from Denisova Cave (Russia) and 13 from Caune de l'Arago (France). The other samples, 49 from Denisova Cave and 9 from Chagyrskaya Cave (Russia), had been collected previously for luminescence dating. The latter two sites are located in the Altai Mountains, where remains of both Neandertals and Denisovans have been uncovered (3, 21). We extracted DNA from between 38 and 160 milligrams of each sample and converted aliquots of the DNA to single-stranded DNA libraries (8, 22, 23). All libraries were shotgun sequenced and analyzed using a taxonomic binning approach (8). Whereas most of the DNA sequences (79.1%-96.1%) remained unidentified, the majority of those that could be identified were assigned to microorganisms and between 0.05% and 10% to mammals (Figs. S7-S15).

Enrichment of mammalian mtDNA

To determine the taxonomic composition of the mammalian DNA in the sediments, we isolated DNA fragments bearing similarities to mammalian mtDNAs by hybridization capture using probes for 242 mitochondrial genomes, including human mtDNA (8, 24). MtDNA is useful for this purpose because it is present in higher copy numbers than nuclear DNA in most eukaryotic cells and is phylogenetically informative in spite of its small size due to its fast rate of evolution in mammals. Between 3,535 and 3.2 million DNA fragments were sequenced per library (Data file S2), of which between 14 and 50,114 could be assigned to mammalian families with a strategy for taxonomic identification of short and damaged DNA fragments (8) (Fig. S18). To assess whether the sequences were of ancient origin, we evaluated them for the presence of C to

T substitutions at their 5'- and 3'-ends (17, 18) (see Fig. S19 for an example). Additionally, we computed the variance of coverage across the mitochondrial genome for each taxon to test whether sequences mapped randomly across the reference genome (Fig. S20), as would be expected for sequences that are genuinely derived from the taxon they are assigned to. With the exception of 46 sequences from a single sample from Les Cottés, which were originally attributed to procaviids but that mapped only to one restricted region of the genome (Fig. S21), this analysis lent support to the correct taxonomic classification of the sequences we obtained.

Of the 52 sediment samples from the Late Pleistocene, 47 contained mtDNA fragments from at least one family showing evidence of ancient DNA-like damage, while 14 out of 33 Middle Pleistocene samples did so (Figs. 1, S22). Overall, we detected ancient mtDNA fragments from 12 mammalian families, of which the most common were hyaenids, bovids, equids, cervids and canids (Data file S3, Figs. S23-S32). These taxa are all present in the zooarchaeological records of the sites as reconstructed from faunal remains (Fig. S33).

We exploited the known genetic variation within these families to determine the affinity of the sequences we obtained to specific species (8) (Data file S3). In all libraries containing elephantid DNA, the majority (71-100%) of sequences matched variants found in the mtDNAs of woolly mammoths, a species that became extinct in Eurasia during the Holocene (25), but not in other elephantids. Likewise, sequences attributed to rhinocerotids most often carried variants specific to the woolly rhinoceros branch (54-100% support), thought to have become extinct at the end of the Late Pleistocene (25), and show little support (0-6%) for other rhinoceros lineages. In ~70% of libraries containing hyaenid mtDNA, the sequences matched variants of the extinct cave hyena and/or the spotted hyena which exists today only in Africa (26). Lastly, 90% of ursid mtDNA sequences retrieved from Vindija Cave carried variants matching *Ursus ingressus*, an

Eastern European cave bear lineage which became extinct approximately 25,000 years ago (27, 28).

Extraction and DNA library preparation negative controls contained between 32 and 359 mammalian mtDNA sequences. These sequences do not exhibit damage patterns typical of ancient DNA and they originate from common contaminants (24, 29-31), predominantly human DNA, as well as DNA of bovids, canids and suids (Fig. S34).

Targeting hominin DNA

Among the samples analyzed, the only site that yielded sequences from putatively deaminated DNA fragments that could be assigned to hominids (or hominins assuming that no other great apes were present at the sites analyzed here) was El Sidrón. This site differs from the others in that no ancient faunal DNA was identified there (Fig. 1), consistent with the almost complete absence of animal remains at the site (32). To test whether animal mtDNA was too abundant at other sites to detect small traces of hominin mtDNA, we repeated the hybridization capture for all DNA libraries using probes targeting exclusively human mtDNA (8). Between 4,915 and 2.8 million DNA fragments were sequenced per library, out of which between 0 and 8,822 were unique hominin sequences passing our filtering scheme (8). Between 10 and 165 hominin mtDNA sequences showing substitutions typical of ancient DNA were obtained from 15 sediment samples from four sites (Data file S4). To generate sufficient data for phylogenetic analyses, we prepared DNA extracts from additional subsamples of 10 of these samples and used automated liquid handling to generate 102 DNA libraries from these as well as the original extracts (Data file S1, Fig. S22). After enriching for human mtDNA and merging all sequences

from a given sediment sample, 9 samples yielded a sufficient number of deaminated hominin mtDNA fragments (between 168 and 13,207) for further analyses (Data file S4).

Identifying Neandertal and Denisovan mtDNA

We identified “diagnostic” positions in the mtDNA genome that are inferred to have changed on each branch of a phylogenetic tree relating modern humans, Neandertals, Denisovans and a ~430,000-year-old hominin from Sima de los Huesos (8, 33). For eight sediment samples from El Sidrón, Trou Al’Wesse, Chagyrskaya Cave and Denisova Cave, the Neandertal state is shared by 87-98% of sequences overlapping positions diagnostic for Neandertal mtDNA, whereas the modern human, Denisovan and Sima de los Huesos branches are supported by 4-11%, 0-2% and 0-2% of sequences, respectively. In the ninth sample, collected in layer 15 of the East Gallery in Denisova Cave, 84% (16/19) of sequences carry Denisovan-specific variants, compared to 0% (0/10), 5% (1/19) and 0% (0/23) for the modern human, Neandertal and Sima de los Huesos variants, respectively, pointing to a Denisovan origin for these mtDNA fragments (Data file S4, Fig. S40). We note that none of the hominin sequences present in the extraction or library preparation negative controls carry variants specific to the Neandertal, Denisovan or Sima de los Huesos branches (Data file S4).

The average sequence coverage of the mitochondrial genome varied between 0.4- and 44-fold among the nine samples. To be able to reconstruct phylogenetic trees using these sequences, we called a consensus base at positions covered by at least two deaminated fragments and required more than two-thirds of fragments to carry an identical base (34). These relatively permissive parameters were chosen to avoid discarding samples that produced very small numbers of hominin sequences and allowed us to reconstruct between 8% and 99% of the

mtDNA genome (Table S3). Phylogenetic trees relating each of the reconstructed mtDNA genomes to those of modern and ancient individuals (8) (Table S5) show that they all fall within the genetic variation or close to known mtDNA genomes of Neandertals or Denisovans (Figs. 2, S41-S49).

Single vs. multiple sources of hominin mtDNA

We next aimed to assess whether mtDNA fragments from more than one individual are present in a given sediment sample. For this purpose, we identified positions in the mitochondrial genome that are covered by at least ten sequences exhibiting evidence of deamination. Three samples have sufficient data for this analysis (Fig. S50). At each of these positions, nearly all sequences from a sample collected in the Main Gallery of Denisova Cave carry the same base, suggesting that the DNA may derive from a single individual. In contrast, sequences from the El Sidrón sample support two different bases at a single position, as is the case for a second sample from Denisova Cave. Thus, at least two mtDNA genomes seem to be present in both these samples (Fig. S51). The fact that the variable position in the latter sample is a known variant among Neandertal mtDNAs supports the conclusion that more than one Neandertal contributed DNA to it (Table S7).

We then developed a maximum-likelihood approach to infer the number of mtDNA components also in low-coverage data (8) (Fig. S52), allowing us to investigate this issue in four additional samples. We detect only one ancient mtDNA type in the sample from Chagyrskaya Cave and in two other samples from Denisova Cave, while a fifth sample from that site contains mtDNA from at least two ancient individuals (Table S9).

DNA yields from sediments

To assess how much DNA can be recovered from sediment compared to skeletal elements, we counted the number of mtDNA fragments retrieved per milligram of bone (2, 21, 35-38) or sediment originating from the same layers at three archaeological sites. The number of hominin mtDNA fragments retrieved from bone ranges from 28 to 9,142 per milligram, compared to between 34 and 4,490 mammalian mtDNA fragments in sediment (Table S10). Thus, surprisingly large quantities of DNA can survive in cave sediments. We note that most of the ancient taxa we identified are middle- to large-sized (Fig. 1), consistent with larger animals leaving more of their DNA in sediments.

The hominin DNA is present in similar concentrations among subsamples of sediment removed from larger samples (Fig. S53). This suggests that in most cases, the DNA is not concentrated in larger spots but spread relatively evenly within the sediment, which is compatible with it originating from excreta or the decay of soft tissue (9, 39, 40). One exception is a sample from the Main Gallery of Denisova Cave, from which one subsample contains over 500 times more hominin mtDNA fragments than others. As the mtDNA retrieved from it may originate from a single Neandertal (Tables S7, S9), we hypothesize that this is due to an unrecognized small bone or tooth fragment in the subsample. Despite its high content of hominin DNA, the library remains dominated by DNA from other mammals, as only ~7.5% of sequences were attributed to hominins following its enrichment with the mammalian mtDNAs probes. Nonetheless, if such microscopic fragments can be identified and isolated, they may represent a source of hominin DNA sufficiently devoid of other mammalian DNA to allow for analyses of the nuclear genome.

DNA movement across layers

Post-depositional mixing of particles or a saturation of the sediments by large amounts of DNA can potentially lead to movements of DNA between layers in a stratigraphy (40-42). At the sites investigated here, the overall consistency between the taxa identified from DNA and the archaeological records (Fig. S33) suggests the integrity of the spatial distribution of DNA. In Chagyrskaya Cave for example, we recovered abundant mammalian mtDNA fragments showing degradation patterns typical of ancient DNA in layers rich in osseous and lithic assemblages, while no ancient mammalian DNA was identified in an archaeologically sterile layer underneath (43). Additionally, mtDNA sequences attributed to the woolly mammoth and woolly rhinoceros were identified in Late Pleistocene layers, yet they are absent from the layer which postdates the presumed time of extinction of these taxa (25) (Data file S3, Fig. S24). This implies that little or no movement of mtDNA fragments occurred downwards or upwards in Chagyrskaya Cave. However, as local conditions may affect the extent to which DNA can move in a stratigraphy, these need to be assessed at each archaeological site before the DNA recovered can be linked to a specific layer. This may be best achieved by dense sampling in and around layers of interest.

Conclusions

We show that mtDNA can be efficiently retrieved from many Late and some Middle Pleistocene cave sediments using hybridization capture (Fig. 1). Encouragingly, this is possible also for samples that were stored at room temperature for several years (8). Sediment samples collected for dating, site formation analyses or the reconstruction of ancient environments at sites where excavations are now completed can thus be used for genetic studies.

The mtDNA genomes reconstructed from sediments of four archaeological sites recapitulate a large part of the mitochondrial diversity of Pleistocene hominins hitherto reconstructed from skeletal remains (Fig. 2). The recovery of Neandertal mtDNA from El Sidrón, Chagyrskaya Cave and Layer 11.4 of the East Gallery of Denisova Cave is in agreement with previous findings of Neandertal remains at those sites and in those layers (21, 32, 44). At Trou Al'Wesse, where we find Neandertal mtDNA, no hominin remains have been found in the Pleistocene layers. However, Late Mousterian artefacts and animal bones with cut-marks support the use of the site by Neandertals (45). In Denisova Cave, we detect Neandertal mtDNA in layers with Middle Paleolithic stone tools in the Main Gallery (46), in which no Neandertal remains have been found. In the East Gallery, we identify Denisovan as well as Neandertal mtDNA lower in the stratigraphy than where skeletal remains of archaic humans have been discovered (Fig. 3), indicating the repeated presence of both groups in the region.

The absence of identifiable ancient DNA in Middle Pleistocene layers in Caune de l'Arago and Chagyrskaya Cave is not surprising given their age (>300 kya). Although compared to other animals, hominins constitute a rare taxon at most sites, we were able to detect Neandertal DNA in the sediments of four of the six sites containing Late Pleistocene layers. For the remaining two sites, Vindija Cave and Les Cottés, only one and four samples, respectively, were available for this study, suggesting that extensive sampling is necessary at each site to ensure that hominin DNA is detected if present. Fortunately, the automation of laboratory procedures to generate DNA libraries and isolate DNA by hybridization capture (8) now makes it possible to undertake large-scale studies of DNA in sediments. This is likely to shed light on the genetic affiliations of the occupants of large numbers of archaeological sites where no human remains are found.

References and Notes:

1. M. Slatkin, F. Racimo, Ancient DNA and human history. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 6380-6387 (2016).
2. J. Krause, Q. Fu, J. M. Good, B. Viola, M. V. Shunkov, A. P. Derevianko, S. Pääbo, The complete mitochondrial DNA genome of an unknown hominin from southern Siberia. *Nature* **464**, 894-897 (2010).
3. D. Reich, R. E. Green, M. Kircher, J. Krause, N. Patterson, E. Y. Durand, B. Viola, A. W. Briggs, U. Stenzel, P. L. Johnson, T. Maricic, J. M. Good, T. Marques-Bonet, C. Alkan, Q. Fu, S. Mallick, H. Li, M. Meyer, E. E. Eichler, M. Stoneking, M. Richards, S. Talamo, M. V. Shunkov, A. P. Derevianko, J. J. Hublin, J. Kelso, M. Slatkin, S. Pääbo, Genetic history of an archaic hominin group from Denisova Cave in Siberia. *Nature* **468**, 1053-1060 (2010).
4. M. Meyer, M. Kircher, M. T. Gansauge, H. Li, F. Racimo, S. Mallick, J. G. Schraiber, F. Jay, K. Prüfer, C. de Filippo, P. H. Sudmant, C. Alkan, Q. Fu, R. Do, N. Rohland, A. Tandon, M. Siebauer, R. E. Green, K. Bryc, A. W. Briggs, U. Stenzel, J. Dabney, J. Shendure, J. Kitzman, M. F. Hammer, M. V. Shunkov, A. P. Derevianko, N. Patterson, A. M. Andres, E. E. Eichler, M. Slatkin, D. Reich, J. Kelso, S. Pääbo, A high-coverage genome sequence from an archaic Denisovan individual. *Science* **338**, 222-226 (2012).
5. M. P. Greaves, M. J. Wilson, The adsorption of nucleic acids by montmorillonite. *Soil Biol. Biochem.* **1**, 317-323 (1969).
6. M. G. Lorenz, W. Wackernagel, Adsorption of DNA to sand and variable degradation rates of adsorbed DNA. *Appl. Environ. Microbiol.* **53**, 2948-2952 (1987).

7. C. Crecchio, G. Stotzky, Binding of DNA on humic acids: Effect on transformation of *Bacillus subtilis* and resistance to DNase. *Soil. Biol. Biochem.* **30**, 1061-1067 (1998).
8. Materials and methods are available as supplementary materials at the Science website.
9. E. Willerslev, A. J. Hansen, J. Binladen, T. B. Brand, M. T. Gilbert, B. Shapiro, M. Bunce, C. Wiuf, D. A. Gilichinsky, A. Cooper, Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science* **300**, 791-795 (2003).
10. M. Hofreiter, J. I. Mead, P. Martin, H. N. Poinar, Molecular cloning. *Curr Biol* **13**, R693-695 (2003).
11. E. Willerslev, E. Cappellini, W. Boomsma, R. Nielsen, M. B. Hebsgaard, T. B. Brand, M. Hofreiter, M. Bunce, H. N. Poinar, D. Dahl-Jensen, S. Johnsen, J. P. Steffensen, O. Bennike, J. L. Schwenninger, R. Nathan, S. Armitage, C. J. de Hoog, V. Alfimov, M. Christl, J. Beer, R. Muscheler, J. Barker, M. Sharp, K. E. Penkman, J. Haile, P. Taberlet, M. T. Gilbert, A. Casoli, E. Campani, M. J. Collins, Ancient biomolecules from deep ice cores reveal a forested southern Greenland. *Science* **317**, 111-114 (2007).
12. J. Haile, D. G. Froese, R. D. Macphee, R. G. Roberts, L. J. Arnold, A. V. Reyes, M. Rasmussen, R. Nielsen, B. W. Brook, S. Robinson, M. Demuro, M. T. Gilbert, K. Munch, J. J. Austin, A. Cooper, I. Barnes, P. Moller, E. Willerslev, Ancient DNA reveals late survival of mammoth and horse in interior Alaska. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 22352-22357 (2009).
13. D. C. Murray, S. G. Pearson, R. Fullagar, B. M. Chase, J. Houston, J. Atchison, N. E. White, M. I. Bellgard, E. Clarke, M. Macphail, M. T. P. Gilbert, J. Haile, M. Bunce, High-throughput sequencing of ancient plant and mammal DNA preserved in herbivore middens. *Quaternary Sci. Rev.* **58**, 135-145 (2012).

14. M. C. Lydolph, J. Jacobsen, P. Arctander, M. T. Gilbert, D. A. Gilichinsky, A. J. Hansen, E. Willerslev, L. Lange, Beringian paleoecology inferred from permafrost-preserved fungal DNA. *Appl. Environ. Microbiol.* **71**, 1012-1017 (2005).
15. R. W. Graham, S. Belmecheri, K. Choy, B. J. Culleton, L. J. Davies, D. Froese, P. D. Heintzman, C. Hritz, J. D. Kapp, L. A. Newsom, R. Rawcliffe, E. Saulnier-Talbot, B. Shapiro, Y. Wang, J. W. Williams, M. J. Wooller, Timing and causes of mid-Holocene mammoth extinction on St. Paul Island, Alaska. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 9310-9314 (2016).
16. M. W. Pedersen, A. Ruter, C. Schweger, H. Friebe, R. A. Staff, K. K. Kjeldsen, M. L. Mendoza, A. B. Beaudoin, C. Zutter, N. K. Larsen, B. A. Potter, R. Nielsen, R. A. Rainville, L. Orlando, D. J. Meltzer, K. H. Kjaer, E. Willerslev, Postglacial viability and colonization in North America's ice-free corridor. *Nature* **537**, 45-49 (2016).
17. A. W. Briggs, U. Stenzel, P. L. Johnson, R. E. Green, J. Kelso, K. Prüfer, M. Meyer, J. Krause, M. T. Ronan, M. Lachmann, S. Pääbo, Patterns of damage in genomic DNA sequences from a Neandertal. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 14616-14621 (2007).
18. J. Krause, A. W. Briggs, M. Kircher, T. Maricic, N. Zwyns, A. Derevianko, S. Pääbo, A Complete mtDNA Genome of an Early Modern Human from Kostenki, Russia. *Curr. Biol.* **20**, 231-236 (2010).
19. K. Prüfer, M. Meyer, Comment on "Late Pleistocene human skeleton and mtDNA link Paleoamericans and modern Native Americans". *Science* **347**, 835 (2015).
20. C. L. Weiss, M. Dannemann, K. Prufer, H. A. Burbano, Contesting the presence of wheat in the British Isles 8,000 years ago by assessing ancient DNA authenticity from low-coverage data. *Elife* **4**, e10005 (2015).

21. K. Prüfer, F. Racimo, N. Patterson, F. Jay, S. Sankararaman, S. Sawyer, A. Heinze, G. Renaud, P. H. Sudmant, C. de Filippo, H. Li, S. Mallick, M. Dannemann, Q. Fu, M. Kircher, M. Kuhlwilm, M. Lachmann, M. Meyer, M. Ongyerth, M. Siebauer, C. Theunert, A. Tandon, P. Moorjani, J. Pickrell, J. C. Mullikin, S. H. Vohr, R. E. Green, I. Hellmann, P. L. Johnson, H. Blanche, H. Cann, J. O. Kitzman, J. Shendure, E. E. Eichler, E. S. Lein, T. E. Bakken, L. V. Golovanova, V. B. Doronichev, M. V. Shunkov, A. P. Derevianko, B. Viola, M. Slatkin, D. Reich, J. Kelso, S. Pääbo, The complete genome sequence of a Neanderthal from the Altai Mountains. *Nature* **505**, 43-49 (2014).
22. M. T. Gansauge, M. Meyer, Single-stranded DNA library preparation for the sequencing of ancient or damaged DNA. *Nat. Protoc.* **8**, 737-748 (2013).
23. M. T. Gansauge, T. Gerber, I. Glocke, P. Korlevic, L. Lippik, S. Nagel, L. M. Riehl, A. Schmidt, M. Meyer, Single-stranded DNA library preparation from highly degraded DNA using T4 DNA ligase. *Nucleic Acids Res.*, gkx033 (2017).
24. V. Slon, I. Glocke, R. Barkai, A. Gopher, I. Hershkovitz, M. Meyer, Mammalian mitochondrial capture, a tool for rapid screening of DNA preservation in faunal and undiagnostic remains, and its application to Middle Pleistocene specimens from Qesem Cave (Israel). *Quatern. Int.* **398**, 210-218 (2016).
25. Y. V. Kuzmin, Extinction of the woolly mammoth (*Mammuthus primigenius*) and woolly rhinoceros (*Coelodonta antiquitatis*) in Eurasia: Review of chronological and environmental issues. *Boreas* **39**, 247-261 (2010).
26. N. Rohland, J. L. Pollack, D. Nagel, C. Beauval, J. Airvaux, S. Paabo, M. Hofreiter, The population history of extant and extinct hyenas. *Mol. Biol. Evol.* **22**, 2435-2443 (2005).

27. S. C. Münzel, M. Stiller, M. Hofreiter, A. Mittnik, N. J. Conard, H. Bocherens, Pleistocene bears in the Swabian Jura (Germany): Genetic replacement, ecological displacement, extinctions and survival. *Quatern. Int.* **245**, 225-237 (2011).
28. P. Wojtal, J. Wilczynski, A. Nadachowski, S. C. Münzel, Gravettian hunting and exploitation of bears in Central Europe. *Quatern. Int.* **359**, 58-71 (2015).
29. S. Pääbo, H. Poinar, D. Serre, V. Jaenicke-Despres, J. Hebler, N. Rohland, M. Kuch, J. Krause, L. Vigilant, M. Hofreiter, Genetic analyses from ancient DNA. *Annu. Rev. Genet.* **38**, 645-679 (2004).
30. J. A. Leonard, O. Shanks, M. Hofreiter, E. Kreuz, L. Hodges, W. Ream, R. K. Wayne, R. C. Fleischer, Animal DNA in PCR reagents plagues ancient DNA research. *J. Archaeol. Sci.* **34**, 1361-1366 (2007).
31. M. T. P. Gilbert, H. J. Bandelt, M. Hofreiter, I. Barnes, Assessing ancient DNA studies. *Trends Ecol. Evol.* **20**, 541-544 (2005).
32. A. Rosas, C. Martinez-Maza, M. Bastir, A. Garcia-Taberner, C. Lalueza-Fox, R. Huguet, J. E. Ortiz, R. Julia, V. Soler, T. de Torres, E. Martinez, J. C. Canaveras, S. Sanchez-Moral, S. Cuezva, J. Lario, D. Santamaria, M. de la Rasilla, J. Fortea, Paleobiology and comparative morphology of a late Neandertal sample from El Sidrón, Asturias, Spain. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 19266-19271 (2006).
33. M. Meyer, J. L. Arsuaga, S. Nagel, A. Aximu-Petri, I. Martinez, A. Gracia, J. M. Bermúdez de Castro, E. Carbonell, J. Kelso, K. Prüfer, S. Pääbo, Nuclear DNA sequences from the Middle Pleistocene Sima de los Huesos hominins. *Nature* **531**, 504–507 (2016).

34. M. Meyer, Q. Fu, A. Aximu-Petri, I. Glocke, B. Nickel, J. L. Arsuaga, I. Martinez, A. Gracia, J. M. Bermúdez de Castro, E. Carbonell, S. Pääbo, A mitochondrial genome sequence of a hominin from Sima de los Huesos. *Nature* **505**, 403-406 (2014).
35. S. Brown, T. F. Higham, V. Slon, S. Pääbo, M. Meyer, K. Douka, F. Brock, D. Comeskey, N. Procopio, M. V. Shunkov, A. Derevianko, M. Buckley, Identification of a new hominin bone from Denisova Cave, Siberia using collagen fingerprinting and mitochondrial DNA analysis. *Sci. Rep.* **6**, 23559 (2016).
36. R. E. Green, A. S. Malaspinas, J. Krause, A. W. Briggs, P. L. Johnson, C. Uhler, M. Meyer, J. M. Good, T. Maricic, U. Stenzel, K. Prufer, M. Siebauer, H. A. Burbano, M. Ronan, J. M. Rothberg, M. Egholm, P. Rudan, D. Brajkovic, Z. Kucan, I. Gusic, M. Wikstrom, L. Laakkonen, J. Kelso, M. Slatkin, S. Pääbo, A complete Neandertal mitochondrial genome sequence determined by high-throughput sequencing. *Cell* **134**, 416-426 (2008).
37. M. T. Gansauge, M. Meyer, Selective enrichment of damaged DNA molecules for ancient genome sequencing. *Genome Res.* **24**, 1543-1549 (2014).
38. A. W. Briggs, J. M. Good, R. E. Green, J. Krause, T. Maricic, U. Stenzel, C. Lalueza-Fox, P. Rudan, D. Brajkovic, Z. Kucan, I. Gusic, R. Schmitz, V. B. Doronichev, L. V. Golovanova, M. de la Rasilla, J. Fortea, A. Rosas, S. Pääbo, Targeted retrieval and analysis of five Neandertal mtDNA genomes. *Science* **325**, 318-321 (2009).
39. H. N. Poinar, M. Hofreiter, W. G. Spaulding, P. S. Martin, B. A. Stankiewicz, H. Bland, R. P. Evershed, G. Possnert, S. Paabo, Molecular coproscopy: dung and diet of the extinct ground sloth *Nothrotheriops shastensis*. *Science* **281**, 402-406 (1998).

40. J. Haile, R. Holdaway, K. Oliver, M. Bunce, M. T. Gilbert, R. Nielsen, K. Munch, S. Y. Ho, B. Shapiro, E. Willerslev, Ancient DNA chronology within sediment deposits: are paleobiological reconstructions possible and is DNA leaching a factor? *Mol. Biol. Evol.* **24**, 982-989 (2007).
41. L. J. Arnold, R. G. Roberts, R. D. E. Macphee, J. S. Haile, F. Brock, P. Moller, D. G. Froese, A. N. Tikhonov, A. R. Chivas, M. T. P. Gilbert, E. Willerslev, Paper II - Dirt, dates and DNA: OSL and radiocarbon chronologies of perennially frozen sediments in Siberia, and their implications for sedimentary ancient DNA studies. *Boreas* **40**, 417-445 (2011).
42. K. Andersen, K. L. Bird, M. Rasmussen, J. Haile, H. Breuning-Madsen, K. H. Kjaer, L. Orlando, M. T. Gilbert, E. Willerslev, Meta-barcoding of 'dirt' DNA from soil reflects vertebrate biodiversity. *Mol. Ecol.* **21**, 1966-1979 (2012).
43. N. Rudaya, S. Vasiliev, B. Viola, S. Talamo, S. Markin, Palaeoenvironments during the period of the Neanderthals settlement in Chagyrskaya cave (Altai Mountains, Russia). *Palaeogeography, Palaeoclimatology, Palaeoecology* **467**, 265-276 (2017).
44. B. T. Viola, S. V. Markin, A. P. Buzhilova, M. B. Mednikova, M. V. Dobrovolskaya, A. Le Cabec, M. V. Shunkov, A. P. Dereviank, J. J. Hublin, New Neanderthal remains from Chagyrskaya Cave (Altai Mountains, Russian Federation). *Am. J. Phys. Anthropol.* **147**, 293-294 (2012).
45. R. Miller, J. S. Stewart, M. Otte, Résultats préliminaires de l'étude de la séquence paléolithique au Trou Al'Wesse (comm. de Modave). *Notae Praehistoricae* **27**, 41-49 (2007).

46. A. P. Derevianko, *Recent Discoveries in the Altai: Issues on the Evolution of Homo Sapiens* (Institute of Archaeology and Ethnography SB RAS Press, Novosibirsk, 2012).
47. A. M. Moigne, M. R. Palombo, V. Belda, D. Heriech-Briki, S. Kacimi, F. Lacomat, M. A. d. Lumley, J. Moutoussamy, F. Rivals, J. Quilès, A. Testu, Les faunes de grands mammifères de la Caune de l'Arago (Tautavel) dans le cadre biochronologique des faunes du Pléistocène moyen italien. *L'anthropologie* **110**, 788-831 (2006).
48. L. Byrne, Lithic tools from Arago cave, Tautavel (Pyrenees-Orientales, France): behavioural continuity or raw material determinism during the Middle Pleistocene? *J. Archaeol. Sci.* **31**, 351-364 (2004).
49. H. d. Lumley, *Caune de l'Arago, Tautavel-en-Roussillon Pyrénées-Orientales, France, Tome I* (CNRS éditions, Paris, 2014).
50. M. A. d. Lumley, L'homme de Tautavel. Un Homo erectus européen évolué. Homo erectus tautavelensis *L'Anthropologie* **119**, 303-348 (2015).
51. D. Barsky, Les industries lithiques de la Caune de l'Arago dans leur contexte stratigraphique. *Comptes Rendus Palevol.* **12**, 305-325 (2013).
52. A. M. Moigne, D. R. Barsky, "Large mammal assemblages from Lower Palaeolithic sites in France: La Caune de l'Arago, Terra-Amata, Orgnac 3 and Cagny L'EpINETTE" in *The role of early humans in the accumulation of European Lower and Middle Paleolithic bone assemblages* (Monographien des Römisch-Germanischen Zentralmuseums, Mainz, 1999).
53. L. Lebreton, E. Desclaux, C. Hanquet, A. M. Moigne, C. Perrenoud, Environmental context of the Caune de l'Arago Acheulean occupations (Tautavel, France), new insights from microvertebrates in Q-R levels. *Quatern. Int.* **411**, 182-192 (2016).

54. C. Falgueres, Q. Shao, F. Han, J. J. Bahain, M. Richard, C. Perrenoud, A. M. Moigne, D. Lumley, New ESR and U-series dating at Caune de l'Arago, France: A key-site for European Middle Pleistocene. *Quat. Geochronol.* **30**, 547-553 (2015).
55. A. P. Derevianko, M. V. Shunkov, S. V. Markin, *The Dynamics of the Paleolithic Industries in Africa and Eurasia in the Late Pleistocene and the Issue of the Homo sapiens Origin* (Institute of Archaeology and Ethnography SB RAS Press, Novosibirsk, 2014).
56. A. P. Derevianko, S. V. Markin, V. S. Zykin, V. S. Zykina, V. S. Zazhigin, A. O. Sizikova, E. P. Solotchina, L. G. Smolyaninova, A. S. Antipov, Chagyrskaya Cave: A Middle Paleolithic Site In The Altai. *Archaeology, Ethnology and Anthropology of Eurasia* **41**, 2-27 (2013).
57. S. K. Vasiliev, Large Mammal Fauna from the Pleistocene Deposits of Chagyrskaya Cave Northwestern Altai (based on 2007–2011 Excavations). *Archaeology, Ethnology and Anthropology of Eurasia* **41**, 28-44 (2013).
58. M. B. Mednikova, An archaic human ulna from Chagyrskaya Cave, Altai: morphology and taxonomy. *Archaeology Ethnology and Anthropology of Eurasia* **41**, 66-77 (2013).
59. B. Viola, S. V. Markin, A. Zenin, M. V. Shunkov, A. P. Derevianko, “Late Pleistocene hominins from the Altai Mountains, Russia” in *Characteristic features of the Middle to Upper Paleolithic Transition in Eurasia*, A. P. Derevianko, M. V. Shunkov, Eds. (Institute of Archaeology and Ethnography SB RAS, Novosibirsk, 2011), pp. 207-213.
60. A. Derevianko, S. Markin, S. Gladyshev, K. Kolobova, Excavations at the Chagyrskaya Cave, Russia: a Neanderthal Middle Palaeolithic industry in Northern Asia. *Antiquity* **345**, (2015).

61. A. Derevianko, *The Paleolithic of Siberia: New Discoveries and Interpretations* (University of Illinois Press, Illinois, 1998).
62. A. P. Derevianko, M. V. Shunkov, P. V. Volkov, A paleolithic bracelet from Denisova Cave. *Archaeology, Ethnology and Anthropology of Eurasia* **34**, 13-25 (2008).
63. M. B. Mednikova, A Proximal Pedal Phalanx of a Paleolithic Hominin from Denisova Cave, Altai. *Archaeology, Ethnology and Anthropology of Eurasia* **39**, 129-138 (2011).
64. S. Sawyer, G. Renaud, B. Viola, J. J. Hublin, M. T. Gansauge, M. V. Shunkov, A. Derevianko, K. Prüfer, J. Kelso, S. Pääbo, Nuclear and mitochondrial DNA sequences from two Denisovan individuals. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 15696–15700 (2015).
65. C. G. Turner, “Paleolithic teeth of the Central Siberian Altai Mountains” in *Chronostratigraphy of the Paleolithic in North Central, East Asia and America*, A. P. Derevianko, Ed. (Institute of History, Philology and Philosophy, Siberian Branch of the USSR Academy of Sciences, Novosibirsk, 1990), pp. 239-243.
66. E. G. Shpakova, A. P. Derevianko, The interpretation of odontological features of Pleistocene human remains from the Altai. *Archaeology, Ethnology and Anthropology of Eurasia* **1**, 125-138 (2000).
67. S. K. Vasiliev, M. V. Shunkov, M. B. Kozlikin, Preliminary Results for the Balance of Megafauna from Pleistocene Layers of the East Gallery, Denisova Cave. *Problemy archeologii, etnografii, antropologii Sibiri i sopredelnykh territorij* **19**, 32-38 (2013).
68. G. Baryshnikov, Large mammals and Neanderthal paleoecology in the Altai mountains (Central Asia, Russia). *Préhistoire Européenne* **14**, 49-66 (1999).
69. A. K. Agadjanian, The dynamics of bioresources and activity of the paleolithic man, using the example of northwestern Altai Mountains. *Paleontol. J.* **40**, S482-S493 (2006).

70. N. V. Vorobieva, D. Y. Sherbakov, A. S. Druzhkova, R. Stanyon, A. A. Tsybankov, S. K. Vasil'ev, M. V. Shunkov, V. A. Trifonov, A. S. Graphodatsky, Genotyping of *Capreolus pygargus* Fossil DNA from Denisova Cave Reveals Phylogenetic Relationships between Ancient and Modern Populations. *Plos One* **6**, e24045 (2011).
71. A. A. Lissovsky, N. V. Serdyuk, Identification of Late Pleistocene pikas (*Ochotona*, Lagomorpha, Mammalia) of the alpina-hyperborea group from Denisova Cave (Altai) on the basis of the anterior lower Premolar(P-3). *Paleontol. J.* **38**, 680-686 (2004).
72. A. P. Derevianko, S. A. Laukhin, O. A. Kulikov, Z. N. Gnibidenko, M. V. Shunkov, First Middle Pleistocene age determinations of the Paleolithic in the Altai Mountains. *Dokl. Akad. Nauk.* **326**, 497–501 (1992).
73. V. K. Vlasov, O. A. Kulikov, Radiothermoluminescence dating and applications to Pleistocene sediments. *Phys. Chem. Miner.* **16**, 551–558 (1989).
74. M. Roussel, M. Sorresi, “Une nouvelle séquence du Paléolithique supérieur ancien aux marges sud-ouest du Bassin parisien : les Cottés dans la Vienne” in *Le Paléolithique supérieur ancien de l'Europe du Nord-ouest*, P. Bodu, L. Chehmana, L. Klaric, L. Mevel, S. Soriano, N. Teyssandier, Eds. (Mémoire de la Société préhistorique française, Paris, 2013), pp. 283-298.
75. M. Sorresi, M. Roussel, W. Rendu, J. Primault, S. Rigaud, J. P. Texier, D. Richter, S. Talamo, F. Pioquin, B. Larmignat, C. Tavormina, J. J. Hublin, “Les Cottés : nouveaux travaux sur l'un des gisements de référence pour la transition Paléolithique moyen-supérieur” in *Préhistoire entre Vienne et Charente : hommes et sociétés du Paléolithique*, J. Buisson-Catil, J. Primault, Eds. (Association des publications chauvinoises, Chauvigny, 2010), pp. 221-234.

76. M. Frouin, F. Ploquin, M. Soressi, W. Rendu, R. Macchiarelli, A. El Albani, A. Meunier, Clay minerals of late Pleistocene sites (Jonzac and Les Cottés, SW France): Applications of X-ray diffraction analyses to local paleoclimatic and paleoenvironmental reconstructions. *Quatern. Int.* **302**, 184-198 (2013).
77. E. Patte, Le crâne Aurignacien des Cottés. *L'anthropologie* **58**, 450-471 (1954).
78. F. Welker, M. Soressi, W. Rendu, J. J. Hublin, M. Collins, Using ZooMS to identify fragmentary bone from the Late Middle/Early Upper Palaeolithic sequence of Les Cottés, France. *J. Archaeol. Sci.* **54**, 279-286 (2015).
79. S. Talamo, M. Soressi, M. Roussel, M. Richards, J. J. Hublin, A radiocarbon chronology for the complete Middle to Upper Palaeolithic transitional sequence of Les Cottés (France). *J. Archaeol. Sci.* **39**, 175-183 (2012).
80. Z. Jacobs, B. Li, N. Jankowski, M. Soressi, Testing of a single grain OSL chronology across the Middle to Upper Palaeolithic transition at Les Cottés (France). *J. Archaeol. Sci.* **54**, 110-122 (2015).
81. R. Miller, N. Zwyns, M. Otte, Le site du Trou Al'Wesse (comm. de Modave): Campagne de Fouille 2004. *Notae Praehistoricae* **24**, 109-116 (2004).
82. R. Miller, J. Stewart, M. Knul, Y. Waersegers, P. Noiret, K. Wilkinson, The Middle to Upper Paleolithic transition at Trou Al'Wesse: A preliminary overview of stratigraphic units 17 to 15. *Notae Praehistoricae* **35**, 25-34 (2015).
83. R. Miller, M. Otte, J. Stewart, Nouvelles découvertes de la séquence holocène du Trou Al'Wesse: fouilles 2010. *Notae Praehistoricae* **30**, 35-42 (2010).
84. R. Miller, J. Stewart, N. Zwyns, M. Otte, "The stratified Early to Late Mesolithic sequence at Trou Al'Wesse (Modave, Belgium)" in *Chronology and Evolution within the*

- Mesolithic of North-West Europe: Proceedings of an International Meeting, Brussels, May 30th-June 1st, 2007*, P. Crombé, M. Van Strydonck, J. Sergant, M. Boudin, M. Bats, Eds. (Cambridge Scholars Publishing, England, 2009).
85. F. Collin, P. Masy, M. Tinant, La Grotte du Trou Al'Wesse (Province de Liège): Fouilles et découvertes de 1993. *Notae Praehistoricae* **13**, 21-25 (1993).
 86. P. Masy, La sépulture collective néolithique du Trou Al'Wesse à Modave (province de Liège). *Bulletin des Chercheurs de la Wallonie* **33**, 81-99 (1993).
 87. R. Miller, N. Zwyns, J. Stewart, M. Toussaint, M. Otte, Trou Al'Wesse : Campagne de fouilles 2006. *Notae Praehistoricae* **26**, 103-108 (2006).
 88. V. K. Lagerholm, E. Sandoval-Castellanos, A. Vaniscotte, O. Potapova, T. Tomek, Z. M. Bocheński, P. J. Shepherd, N. Barton, M.-C. Van Dyck, R. Miller, J. Höglund, N. G. Yoccoz, L. Dalén, J. R. Stewart, Range shifts or extinction? Ancient DNA and distribution modelling reveal past and future responses to climate warming in cold-adapted birds. *Global Change Biol.* **23**, 1425-1435 (2016).
 89. S. Brace, M. Ruddy, R. Miller, D. C. Schreve, J. R. Stewart, I. Barnes, The colonization history of British water vole (*Arvicola amphibius* (Linnaeus, 1758)): origins and development of the Celtic fringe. *Proc. R. Soc. B.* **283**, 20160130 (2016).
 90. S. Brace, E. Palkopoulou, L. Dalen, A. M. Lister, R. Miller, M. Otte, M. Germonpre, S. P. E. Blockley, J. R. Stewart, I. Barnes, Serial population extinctions in a small mammal indicate Late Pleistocene ecosystem instability. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 20532-20536 (2012).
 91. M. Meiri, A. M. Lister, T. F. G. Higham, J. R. Stewart, L. G. Straus, H. Obermaier, M. R. G. Morales, A. B. Marin-Arroyo, I. Barnes, Late-glacial recolonization and

- phylogeography of European red deer (*Cervus elaphus* L.). *Mol. Ecol.* **22**, 4711-4722 (2013).
92. M. Otte, F. Collin, R. Miller, K. Engesser, Nouvelles datations du Trou Al'Wesse dans son contexte régional. *Notae Praehistoricae* **18**, 45-50 (1998).
93. J. C. M. Ahern, I. Karavanic, M. Paunovic, I. Jankovic, F. H. Smith, New discoveries and interpretations of hominid fossils and artifacts from Vindija Cave, Croatia. *J. Hum. Evol.* **46**, 27-67 (2004).
94. I. Karavanic, F. H. Smith, The middle/upper Paleolithic interface and the relationship of Neanderthals and early modern humans in the Hrvatsko Zagorje, Croatia. *J. Hum. Evol.* **34**, 223-248 (1998).
95. I. Jankovic, I. Karavanic, J. C. M. Ahern, D. Brajkovic, J. M. Lenardic, F. H. Smith, Vindija cave and the modern human peopling of Europe. *Collegium Antropol.* **30**, 457-466 (2006).
96. M. Malez, F. H. Smith, J. Radovic, D. Rukavina, Upper Pleistocene Hominids from Vindija, Croatia, Yugoslavia. *Curr. Anthropol.* **21**, 365-367 (1980).
97. F. H. Smith, D. C. Boyd, M. Malez, Additional Upper Pleistocene Hominid Remains from Vindija Cave. *Am. J. Phys. Anthropol.* **66**, 230-230 (1985).
98. M. H. Wolpoff, F. H. Smith, M. Malez, J. Radovic, D. Rukavina, Upper Pleistocene Human Remains from Vindija Cave, Croatia, Yugoslavia. *Am. J. Phys. Anthropol.* **54**, 499-545 (1981).
99. F. H. Smith, J. C. Ahern, Brief Communication - Additional Cranial Remains from Vindija-Cave, Croatia. *Am. J. Phys. Anthropol.* **93**, 275-280 (1994).

100. R. E. Green, J. Krause, A. W. Briggs, T. Maricic, U. Stenzel, M. Kircher, N. Patterson, H. Li, W. Zhai, M. H. Fritz, N. F. Hansen, E. Y. Durand, A. S. Malaspinas, J. D. Jensen, T. Marques-Bonet, C. Alkan, K. Prufer, M. Meyer, H. A. Burbano, J. M. Good, R. Schultz, A. Aximu-Petri, A. Butthof, B. Hober, B. Hoffner, M. Siegemund, A. Weihmann, C. Nusbaum, E. S. Lander, C. Russ, N. Novod, J. Affourtit, M. Egholm, C. Verna, P. Rudan, D. Brajkovic, Z. Kucan, I. Gusic, V. B. Doronichev, L. V. Golovanova, C. Lalueza-Fox, M. de la Rasilla, J. Fortea, A. Rosas, R. W. Schmitz, P. L. Johnson, E. E. Eichler, D. Falush, E. Birney, J. C. Mullikin, M. Slatkin, R. Nielsen, J. Kelso, M. Lachmann, D. Reich, S. Pääbo, A draft sequence of the Neandertal genome. *Science* **328**, 710-722 (2010).
101. E. M. Wild, M. Paunovic, G. Rabeder, I. Steffan, P. Steier, Age determination of fossil bones from the Vindija Neanderthal site in Croatia. *Radiocarbon* **43**, 1021-1028 (2001).
102. P. T. Miracle, J. M. Lenardic, D. Brajkovic, Last glacial climates, "Refugia", and faunal change in Southeastern Europe: Mammalian assemblages from Veternica, Velika pecina, and Vindija caves (Croatia). *Quatern. Int.* **212**, 137-148 (2010).
103. M. Hofreiter, C. Capelli, M. Krings, L. Waits, N. Conard, S. Munzel, G. Rabeder, D. Nagel, M. Paunovic, G. Jambresic, S. Meyer, G. Weiss, S. Paabo, Ancient DNA analyses reveal high mitochondrial DNA sequence diversity and parallel morphological evolution of late pleistocene cave bears. *Mol. Biol. Evol.* **19**, 1244-1250 (2002).
104. M. Stiller, M. Molak, S. Prost, G. Rabeder, G. Baryshnikov, W. Rosendahl, S. Munzel, H. Bocherens, A. Grandal-d'Anglade, B. Hilpert, M. Germonpre, O. Stasyk, R. Pinhasi, A. Tintori, N. Rohland, E. Mohandesan, S. Y. W. Ho, M. Hofreiter, M. Knapp,

- Mitochondrial DNA diversity and evolution of the Pleistocene cave bear complex. *Quatern. Int.* **339**, 224-231 (2014).
105. D. Brajković, P. T. Miracle, “Middle Palaeolithic and Early Upper Palaeolithic subsistence practices at Vindija Cave, Croatia” in *The Palaeolithic of the Balkans*, A. Darlas, D. Mihailović, Eds. (Archaeopress, Oxford, 2008), pp. 107-116.
 106. K. Zaremba-Niedzwiedzka, S. G. E. Andersson, No Ancient DNA Damage in Actinobacteria from the Neanderthal Bone. *Plos One* **8**, e62799 (2013).
 107. M. Krings, C. Capelli, F. Tschentscher, H. Geisert, S. Meyer, A. von Haeseler, K. Grossschmidt, G. Possnert, M. Paunovic, S. Paabo, A view of Neandertal genetic diversity. *Nature Genet.* **26**, 144-146 (2000).
 108. J. Fortea, M. de la Rasilla, E. Martínez, S. Sánchez-Moral, J. C. Cañaveras, S. Cuezva, A. Rosas, V. Soler, R. Julia, T. de Torres, J. E. Ortiz, J. Castro, E. Badal, J. Altuna, J. Alonso, La cueva de El Sidrón (Borines, Piloña, Asturias): primeros resultados. *Estudios Geológicos* **59**, 159-179 (2003).
 109. D. Santamaría, J. Fortea, M. de La Rasilla, L. Martínez, E. Martínez, J. C. Cañaveras, S. Sanchez-Moral, A. Rosas, A. Estalrich, A. Garcia-Taberner, C. Lalueza-Fox, The technological and typological behaviour of a Neanderthal group from El Sidrón cave (Asturias, Spain). *Oxford Journal of Archaeology* **29**, 119-148 (2010).
 110. C. Lalueza-Fox, A. Rosas, A. Estalrich, E. Gigli, P. F. Campos, A. Garcia-Taberner, S. Garcia-Vargas, F. Sanchez-Quinto, O. Ramirez, S. Civit, M. Bastir, R. Huguet, D. Santamaria, M. T. P. Gilbert, E. Willerslev, M. de la Rasilla, Genetic evidence for patrilocal mating behavior among Neandertal groups. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 250-253 (2011).

111. A. Rosas, A. Estalrich, S. Garcia-Vargas, A. Garcia-Tabernerero, R. Huguet, C. Lalueza-Fox, M. de la Rasilla, Identification of Neandertal individuals in fragmentary fossil assemblages by means of tooth associations: The case of El Sidrón (Asturias, Spain). *Comptes Rendus Palevol.* **12**, 279-291 (2013).
112. S. Castellano, G. Parra, F. A. Sanchez-Quinto, F. Racimo, M. Kuhlwilm, M. Kircher, S. Sawyer, Q. Fu, A. Heinze, B. Nickel, J. Dabney, M. Siebauer, L. White, H. A. Burbano, G. Renaud, U. Stenzel, C. Lalueza-Fox, M. de la Rasilla, A. Rosas, P. Rudan, D. Brajkovic, Z. Kucan, I. Gusic, M. V. Shunkov, A. P. Derevianko, B. Viola, M. Meyer, J. Kelso, A. M. Andres, S. Pääbo, Patterns of coding variation in the complete exomes of three Neandertals. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 6666-6671 (2014).
113. M. Kuhlwilm, I. Gronau, M. J. Hubisz, C. de Filippo, J. Prado-Martinez, M. Kircher, Q. Fu, H. A. Burbano, C. Lalueza-Fox, M. de la Rasilla, A. Rosas, P. Rudan, D. Brajkovic, Z. Kucan, I. Gusic, T. Marques-Bonet, A. M. Andres, B. Viola, S. Pääbo, M. Meyer, A. Siepel, S. Castellano, Ancient gene flow from early modern humans into Eastern Neanderthals. *Nature* **530**, 429-433 (2016).
114. F. L. Mendez, G. D. Poznik, S. Castellano, C. D. Bustamante, The Divergence of Neandertal and Modern Human Y Chromosomes. *Am. J. Hum. Genet.* **98**, 728-734 (2016).
115. C. Lalueza-Fox, M. L. Sampietro, D. Caramelli, Y. Puder, M. Lari, F. Calafell, C. Martinez-Maza, M. Bastir, J. Fortea, M. de la Rasilla, J. Bertranpetit, A. Rosas, Neandertal evolutionary genetics: Mitochondrial DNA data from the Iberian Peninsula. *Mol. Biol. Evol.* **22**, 1077-1081 (2005).

116. T. de Torres, J. E. Ortiz, R. Grun, S. Eggins, H. Valladas, N. Mercier, N. Tisnerat-Laborde, R. Julia, V. Soler, E. Martinez, S. Sanchez-Moral, J. C. Canaveras, J. Lario, E. Badal, C. Lalueza-Fox, A. Rosas, D. Santamaria, M. de la Rasilla, J. Fortea, Dating of the hominid (*Homo neanderthalensis*) remains accumulation from El Sidrón Cave (Piloña, Asturias, North Spain): an example of a multi-methodological approach to the dating of Upper Pleistocene sites. *Archaeometry* **52**, 680-705 (2010).
117. R. E. Wood, T. F. G. Higham, T. de Torres, N. Tisnerat-Laborde, H. Valladas, J. E. Ortiz, C. Lalueza-Fox, S. Sanchez-Moral, J. C. Canaveras, A. Rosas, D. Santamaria, M. de la Rasilla, A new date for the Neanderthals from El Sidrón (Asturias, Northern Spain). *Archaeometry* **55**, 148-158 (2013).
118. P. Cai, Q. Huang, X. Zhang, H. Chen, Adsorption of DNA on clay minerals and various colloidal particles from an Alfisol. *Soil Biol. Biochem.* **38**, 471-476 (2006).
119. A. Ogram, G. S. Sayler, D. Gustin, R. J. Lewis, DNA Adsorption to Soils and Sediments. *Environ. Sci. Technol.* **22**, 982-984 (1988).
120. A. V. Ogram, M. L. Mathot, J. B. Harsh, J. Boyle, C. A. Pettigrew, Effects of DNA Polymer Length on Its Adsorption to Soils. *Appl. Environ. Microb.* **60**, 393-396 (1994).
121. R. E. Green, A. W. Briggs, J. Krause, K. Prufer, H. A. Burbano, M. Siebauer, M. Lachmann, S. Paabo, The Neandertal genome and ancient DNA authenticity. *EMBO J.* **28**, 2494-2502 (2009).
122. M. E. Allentoft, M. Collins, D. Harker, J. Haile, C. L. Oskam, M. L. Hale, P. F. Campos, J. A. Samaniego, M. T. Gilbert, E. Willerslev, G. Zhang, R. P. Scofield, R. N. Holdaway, M. Bunce, The half-life of DNA in bone: measuring decay kinetics in 158 dated fossils. *Proc. Biol. Sci.* **279**, 4724-4733 (2012).

123. J. Dabney, M. Knapp, I. Glocke, M. T. Gansauge, A. Weihmann, B. Nickel, C. Valdiosera, N. Garcia, S. Pääbo, J. L. Arsuaga, M. Meyer, Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 15758-15763 (2013).
124. C. Giguet-Covex, J. Pansu, F. Arnaud, P. J. Rey, C. Griggo, L. Gielly, I. Domaizon, E. Coissac, F. David, P. Choler, J. Poulénard, P. Taberlet, Long livestock farming history and human landscape shaping revealed by lake sediment DNA. *Nat. Commun.* **5**, 3211 (2014).
125. S. A. Bulat, M. Lubeck, I. A. Alekhina, D. F. Jensen, I. M. Knudsen, P. S. Lubeck, Identification of a universally primed-PCR-derived sequence-characterized amplified region marker for an antagonistic strain of *Clonostachys rosea* and development of a strain-specific PCR detection assay. *Appl. Environ. Microbiol.* **66**, 4758-4763 (2000).
126. A. C. Boere, W. I. Rijpstra, G. J. De Lange, J. S. Sinninghe Damste, M. J. Coolen, Preservation potential of ancient plankton DNA in Pleistocene marine sediments. *Geobiology* **9**, 377-393 (2011).
127. P. Korlevic, T. Gerber, M. T. Gansauge, M. Hajdinjak, S. Nagel, A. Aximu-Petri, M. Meyer, Reducing microbial and human contamination in DNA extractions from ancient bones and teeth. *Biotechniques* **59**, 87-93 (2015).
128. J. Dabney, M. Meyer, Length and GC-biases during sequencing library amplification: a comparison of various polymerase-buffer systems with ancient and modern DNA sequencing libraries. *Biotechniques* **52**, 87-94 (2012).
129. M. Kircher, S. Sawyer, M. Meyer, Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform. *Nucleic Acids Res.* **40**, e3 (2012).

130. M. M. Deangelis, D. G. Wang, T. L. Hawkins, Solid-Phase Reversible Immobilization for the Isolation of Pcr Products. *Nucleic Acids Res.* **23**, 4742-4743 (1995).
131. M. Meyer, M. Kircher, Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harb. Protoc.* **2010**, pdb prot5448 (2010).
132. G. Renaud, M. Kircher, U. Stenzel, J. Kelso, freeIbis: an efficient basecaller with calibrated quality scores for Illumina sequencers. *Bioinformatics* **29**, 1208-1209 (2013).
133. G. Renaud, U. Stenzel, J. Kelso, leeHom: adaptor trimming and merging for Illumina sequencing reads. *Nucleic Acids Res.* **42**, e141 (2014).
134. A. Herbig, F. Maixner, K. I. Bos, A. Zink, J. Krause, D. H. Huson, MALT: Fast alignment and analysis of metagenomic DNA sequence data applied to the Tyrolean Iceman. *bioRxiv* (2016).
135. D. H. Huson, A. F. Auch, J. Qi, S. C. Schuster, MEGAN analysis of metagenomic data. *Genome Res.* **17**, 377-386 (2007).
136. Q. Fu, M. Meyer, X. Gao, U. Stenzel, H. A. Burbano, J. Kelso, S. Pääbo, DNA analysis of an early modern human from Tianyuan Cave, China. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 2223-2227 (2013).
137. N. Rohland, D. Reich, Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. *Genome Res.* **22**, 939-946 (2012).
138. T. Maricic, M. Whitten, S. Pääbo, Multiplexed DNA Sequence Capture of Mitochondrial Genomes Using PCR Products. *Plos One* **5**, e14004 (2010).
139. T. Lindahl, Instability and decay of the primary structure of DNA. *Nature* **362**, 709-715 (1993).

140. M. Hofreiter, V. Jaenicke, D. Serre, A. von Haeseler, S. Paabo, DNA sequences from multiple amplifications reveal artifacts induced by cytosine deamination in ancient DNA. *Nucleic Acids Res.* **29**, 4793-4799 (2001).
141. S. Sawyer, J. Krause, K. Guschanski, V. Savolainen, S. Pääbo, Temporal patterns of nucleotide misincorporations and DNA fragmentation in ancient DNA. *PLoS One* **7**, e34131 (2012).
142. M. Rasmussen, Y. Li, S. Lindgreen, J. S. Pedersen, A. Albrechtsen, I. Moltke, M. Metspalu, E. Metspalu, T. Kivisild, R. Gupta, M. Bertalan, K. Nielsen, M. T. Gilbert, Y. Wang, M. Raghavan, P. F. Campos, H. M. Kamp, A. S. Wilson, A. Gledhill, S. Tridico, M. Bunce, E. D. Lorenzen, J. Binladen, X. Guo, J. Zhao, X. Zhang, H. Zhang, Z. Li, M. Chen, L. Orlando, K. Kristiansen, M. Bak, N. Tommerup, C. Bendixen, T. L. Pierre, B. Gronnow, M. Meldgaard, C. Andreasen, S. A. Fedorova, L. P. Osipova, T. F. Higham, C. B. Ramsey, T. V. Hansen, F. C. Nielsen, M. H. Crawford, S. Brunak, T. Sicheritz-Ponten, R. Villems, R. Nielsen, A. Krogh, J. Wang, E. Willerslev, Ancient human genome sequence of an extinct Palaeo-Eskimo. *Nature* **463**, 757-762 (2010).
143. J. P. Noonan, M. Hofreiter, D. Smith, J. R. Priest, N. Rohland, G. Rabeder, J. Krause, J. C. Detter, S. Paabo, E. M. Rubin, Genomic sequencing of Pleistocene cave bears. *Science* **309**, 597-600 (2005).
144. M. L. Carpenter, J. D. Buenrostro, C. Valdiosera, H. Schroeder, M. E. Allentoft, M. Sikora, M. Rasmussen, S. Gravel, S. Guillen, G. Nekhrizov, K. Leshtakov, D. Dimitrova, N. Theodossiev, D. Pettener, D. Luiselli, K. Sandoval, A. Moreno-Estrada, Y. R. Li, J. Wang, M. T. P. Gilbert, E. Willerslev, W. J. Greenleaf, C. D. Bustamante, Pulling out the

- 1%: Whole-Genome Capture for the Targeted Enrichment of Ancient DNA Sequencing Libraries. *Am. J. Hum. Genet.* **93**, 852-864 (2013).
145. C. Gamba, E. R. Jones, M. D. Teasdale, R. L. McLaughlin, G. Gonzalez-Fortes, V. Mattiangeli, L. Domboroczki, I. Kovari, I. Pap, A. Anders, A. Whittle, J. Dani, P. Raczky, T. F. Higham, M. Hofreiter, D. G. Bradley, R. Pinhasi, Genome flux and stasis in a five millennium transect of European prehistory. *Nat. Commun.* **5**, 5257 (2014).
146. K. D. Pruitt, G. R. Brown, S. M. Hiatt, F. Thibaud-Nissen, A. Astashyn, O. Ermolaeva, C. M. Farrell, J. Hart, M. J. Landrum, K. M. McGarvey, M. R. Murphy, N. A. O'Leary, S. Pujar, B. Rajput, S. H. Rangwala, L. D. Riddick, A. Shkeda, H. Sun, P. Tamez, R. E. Tully, C. Wallin, D. Webb, J. Weber, W. Wu, M. DiCuccio, P. Kitts, D. R. Maglott, T. D. Murphy, J. M. Ostell, RefSeq: an update on mammalian reference sequences. *Nucleic Acids Res.* **42**, D756-763 (2014).
147. S. F. Altschul, W. Gish, W. Miller, E. W. Myers, D. J. Lipman, Basic local alignment search tool. *J. Mol. Biol.* **215**, 403-410 (1990).
148. H. Li, R. Durbin, Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics* **26**, 589-595 (2010).
149. H. Li, B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, R. Durbin, S. Genome Project Data Processing, The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**, 2078-2079 (2009).
150. R. C. Team, *R: A language and environment for statistical computing* (R Foundation for Statistical Computing, Vienna, Austria, 2016).
151. D. A. Benson, M. Cavanaugh, K. Clark, I. Karsch-Mizrachi, D. J. Lipman, J. Ostell, E. W. Sayers, GenBank. *Nucleic Acids Res.* **41**, D36-42 (2013).

152. K. Katoh, D. M. Standley, MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* **30**, 772-780 (2013).
153. E. Willerslev, M. T. Gilbert, J. Binladen, S. Y. Ho, P. F. Campos, A. Ratan, L. P. Tomsho, R. R. da Fonseca, A. Sher, T. V. Kuznetsova, M. Nowak-Kemp, T. L. Roth, W. Miller, S. C. Schuster, Analysis of complete mitochondrial genomes from extinct and extant rhinoceroses reveals lack of phylogenetic resolution. *BMC Evol. Biol.* **9**, 95 (2009).
154. C. J. Edwards, D. A. Magee, S. D. Park, P. A. McGettigan, A. J. Lohan, A. Murphy, E. K. Finlay, B. Shapiro, A. T. Chamberlain, M. B. Richards, D. G. Bradley, B. J. Loftus, D. E. MacHugh, A complete mitochondrial genome sequence from a mesolithic wild aurochs (*Bos primigenius*). *PLoS One* **5**, e9255 (2010).
155. A. K. Agadjanian, "Problems of reconstruction of paleoenvironment and conditions of the habitability of the Ancient Man by the example of Northwestern Altai" in *Biosphere Origin and Evolution*, N. Dobretsov, N. Kolchanov, A. Rozanov, G. Zavarzin, Eds. (Springer, 2008).
156. S. G. Turner, N. D. Ovodov, O. V. Pavlova, *Animal Teeth and Human Tools: A Taphonomic Odyssey in Ice Age Siberia* (Cambridge University Press, Cambridge, 2013).
157. W. Rendu, S. Renou, in *Les Cottés (Vienne). Rapport de fouille programmée*, M. Soressi, Ed. (Report deposited at the Service régional de l'archéologie de Poitou-Charentes, 2015), pp. 101-131.
158. K. Tamura, G. Stecher, D. Peterson, A. Filipksi, S. Kumar, MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.* **30**, 2725-2729 (2013).

159. A. Immel, D. G. Drucker, M. Bonazzi, T. K. Jahnke, S. C. Munzel, V. J. Schuenemann, A. Herbig, C. J. Kind, J. Krause, Mitochondrial Genomes of Giant Deers Suggest their Late Survival in Central Europe. *Sci. Rep.* **5**, 10853 (2015).

Acknowledgments:

This study has received funding from the Max Planck Society; the Max-Planck-Förderstiftung (grant P.S.EVANLOMP to S.P.); the European Research Council (ERC) (grant AMD-694707-3 to S.P.); the Australian Research Council (fellowships FT150100138 to Z.J., FT140100384 to B.L. and FL130100116 to R.G.R.); and the Russian Science Foundation (project No. 14-50-00036 to A.P.D. and M.V.S.). The authors declare no conflicts of interest. We wish to thank M.-T. Gansauge, B. Höber and A. Weihmann for laboratory work; A. Hübner, M. Petr, U. Stenzel and B. Vernot for their input on data analysis; V. Aldeias and S. Gur-Arieh for advice on the preliminary study; P. Korlević and C. Piot for graphics; L. Jauregui for comments on the text; V. Wiebe for translations; and T. Devière, E. Flannery, T. Higham, J. Krause, T. Marčić, M. Morley, C. Stringer and B. Viola for their roles in sample acquisition. V.S., C.H., A.A.-P., E.E., S.N., B.N. and A.S. performed the laboratory work; V.S., C.L.W., F.M., K.P., J.K., H.A.B., S.P. and M.M. analyzed the data; M.d.I.R., C.L.-F., A.R., M.S., M.V.K., R.M., J.R.S., A.P.D, Z.J., B.L., R.G.R., M.V.S., H.d.L., C.P., I.G., Ž.K and P.R. provided samples and site-specific expertise; V.S., S.P. and M.M. designed the study and wrote the manuscript with input from all authors. Sequencing data generated for this study are available in the European Nucleotide Archive (ENA) under accession number PRJEB18629. The multiple sequence alignment files used for phylogenetic reconstructions were deposited in the Dryad Digital Repository (<http://dx.doi.org/10.5061/dryad.m2dk7>).

Fig. 1. Ancient taxa detected in Late Pleistocene (LP) and Middle Pleistocene (MP) sediment samples from seven sites. For each time period, the fraction of samples containing DNA fragments which could be assigned to a mammalian family and authenticated to be of ancient origin is indicated. The shaded symbols representing each family are not to scale.

Fig. 2. Cladogram relating mtDNA genomes reconstructed from sediment samples to those of modern and ancient individuals. The branches leading to mtDNA genomes reconstructed from sediments (dashed lines) were superimposed on a neighbor-joining tree relating the previously determined mtDNA genomes of ancient and present-day humans (purple), Neandertals (orange), Denisovans (green) and the Sima de los Huesos hominin (blue) (Table S5). Discrete phylogenetic trees relating each of the mtDNAs reconstructed here and the comparative data are shown in Figs. S41-S49.

Fig. 3. Hominin mtDNAs along the stratigraphy of the East Gallery in Denisova Cave. Layer numbers are noted in gray. The layers of origin for sediment samples and skeletal remains yielding Neandertal (orange) and Denisovan (green) mtDNA genomes are denoted. For details on these and other hominin skeletal remains from other parts of the cave see (8).

Supplementary Materials:

Materials and Methods

Figures S1-S53

Tables S1-S10

References (47-159)

Data files S1-S4