



Sediment DNA can revolutionize archaeology—if it is used the right way

Vera Aldeias^{a,1}  and Mareike C. Stahlschmidt^{b,c,1} 

Affiliations are included on p. 5.



The possibility of fingerprinting ancient organisms using the DNA they leave behind is a revolution in our understanding of the past. Paleogenetic studies are rewriting our knowledge on ancient hominin genomes, hominin dispersals, interbreeding events, and ancient DNA is also a key tool in biodiversity monitoring efforts. Of particular interest: We can now retrieve DNA directly from sediments and soils (sedaDNA) (1–3) in a variety of modern and past depositional environments, including permafrost, lakes, marine sediments, or caves settings.

As archaeologists, and particularly as geoarchaeologists (i.e., scientists using earth sciences tools to address archaeological questions), we could not be more excited about the unprecedented level of insight that sedaDNA can provide, especially given that sediments are ubiquitous, whereas hominin fossils are extremely rare. However, to properly interpret sedaDNA, we need to understand its context and association within the archaeological record.

Obtaining information about ancient humans, vegetation, or animal communities requires the excavation and retrieval of buried material. During excavation, we create associations between materials by observing changes in their sedimentary context and defining individual layers. We can then date the sediments or individual components and assign the same age to materials enclosed in the same layer. Much depends on this association, and no debate is more heated in the field than the question of context: Does an artifact or fossil belong to a certain layer or not?

To ensure that soil and sediment DNA is a trustworthy paleogenetic tool, it's important that we understand the microscopic sources and potential movement of sedaDNA. We propose future pathways that, in our view, will be crucial in developing reliable collection and interpretations of sedaDNA data. Pictured is the archaeological site at Satsurblia Cave in the nation of Georgia, which yielded sedaDNA of ancient humans, wolves, and bison. See Ref 4. Image credit: Mareike C. Stahlschmidt.

Author contributions: V.A. and M.C.S. designed research and wrote the paper.

The authors declare no competing interest.

Copyright © 2024 the Author(s). Published by PNAS. This article is distributed under [Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 \(CC BY-NC-ND\)](https://creativecommons.org/licenses/by-nc-nd/4.0/).

Any opinions, findings, conclusions, or recommendations expressed in this work are those of the authors and have not been endorsed by the National Academy of Sciences.

¹To whom correspondence may be addressed. Email: valdeias@ualg.pt or mareike.stahlschmidt@univie.ac.at.

Published June 20, 2024.

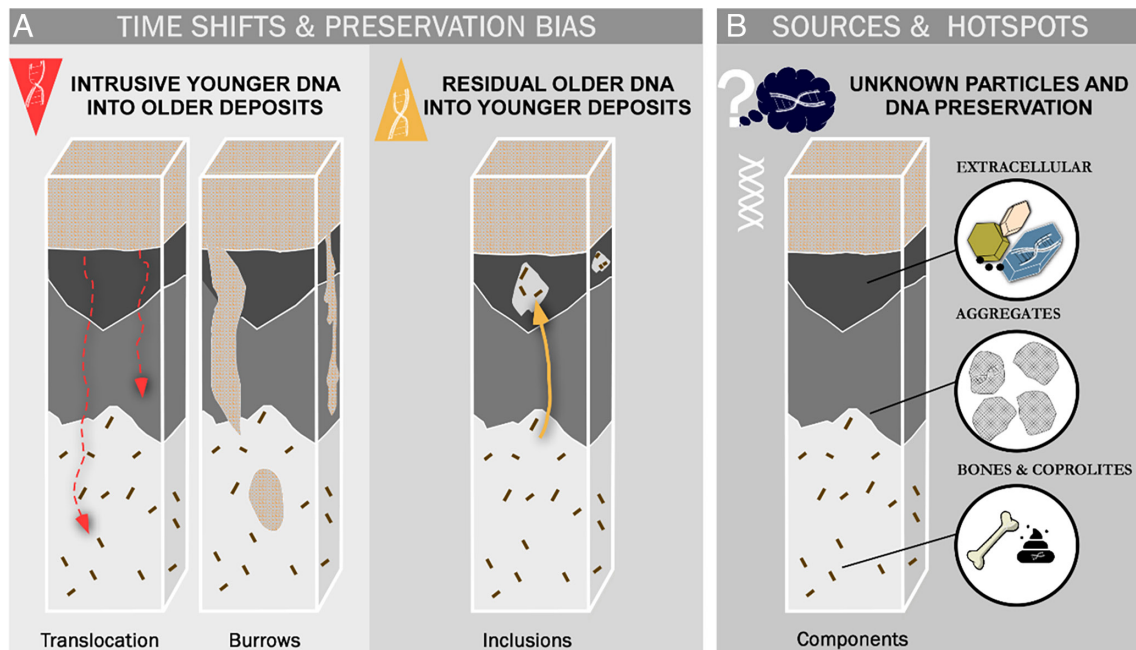


Fig. 1. Schematic drawing showing time shifts where younger sedaDNA can migrate to older sediments by translocation (e.g., clays migrating downward) or burrowing, or older DNA being reworked into younger sediments (A); unknown sources that can contain sedaDNA at a microscopic scale: adsorbed to minerals or contained in micro-sized soil aggregates, bones, and coprolites (B).

A related question is how a particular layer formed: Does it result from a quick episode of deposition preserving everything in situ, or are the sediments disturbed or even redeposited, mixing materials of variable ages?

We argue here that these familiar issues of stratigraphic context apply also to sedaDNA. DNA molecules are not immune to movement after burial (that is, taphonomy) (5–8). Since these biomolecules are nanoscale materials contained in microremains or adsorbed to minerals, we cannot rely solely on field observations to evaluate where the DNA is stored and whether genetic material has moved since its original deposition time. Hence, it is important that we understand the microscopic sources and potential movement of sedaDNA. We propose future pathways that, in our view, will be crucial in developing reliable collection and interpretations of sedaDNA data. We call for using micro-contextual analysis to clarify the stratigraphic integrity and taphonomy of sedaDNA (a microcontextual sedaDNA approach, or MiCoDNA).

A Key Advance

Retrieving genetic data from archaeological sediments opens the possibility of extracting data from sources that are invisible during excavation and can be linked to sand-sized bone fragments, plant biomass, excreta, or organic tissue (9–12). SedaDNA can thus go beyond standard archaeogenetic research rooted in fossils, which is necessarily a death assemblage. It may instead present a life assemblage (13), reflecting on aspects of human site use, population dynamics, and structuring of domestic spaces (14). But to transcend the question of “who was here,” we need to develop and employ analytical approaches that acknowledge and take advantage of the highly variable sedimentary record.

SedaDNA opens the possibility to reconstruct ancient occupation histories. Zavala et al. (12) and Vernet et al. (15)

present excellent examples of this, differentiating between various hominin and animal groups that occupied sites through time, while Gelabert et al. (4) obtained low-coverage nuclear DNA from sediments comparable to what we can retrieve from bones. The recovery of hominin DNA in the absence of macrofossils holds particular importance during periods of population turnover or when researchers debate the identity of the makers of a specific archaeological assemblage—for instance, during the transition from Neanderthals to anatomically modern humans. Given the rapid increase in resolution of sedaDNA studies, there is reasonable hope that we will maximize the sedaDNA output even further, making it feasible to use millimeter-thick stratified sediments to zoom into hominin population dynamics at an unprecedented level (e.g., kinship relationships between dwellings). Similarly, we can use (micro)stratigraphic analysis of faunal, floral, and hominin sedaDNA to investigate human–animal dynamics (e.g., introduction of domesticated species, husbandry practices, or turnover between human and carnivore occupations at cave sites). Plant sedaDNA can considerably augment archaeobotanical records (16–19) by providing data on both the paleo-vegetation around a site and on plant materials used by past humans. Understanding animal and plant turnovers can, in turn, yield valuable insights into environmental changes or ecosystem responses to natural events (e.g., postglaciation changes, volcanic eruptions, earthquakes) that would have also influenced ancient hominin’s adaptations.

The genomic signature of human-made sediments and features adds a new and unprecedented dimension to their life history—e.g., preserving information on activity areas for animal or plant processing and the people producing, using, and discarding sediments. This deposition of microscopic cultural sediments and particles (microscopic bones, skin cells, hair, and excreta) can carry molecular information. While cultural deposits are more abundant in later historical

periods, even in older Pleistocene-aged periods, thin lenses formed by distinct human-made activities can preserve snapshots of human behavior (20–23). Performing genetic analysis on these microcontexts will enable a deeper understanding of how they were formed and can link individuals or groups to their creation.

Tracing Origins and Sources

The specific formation history of sedaDNA, however, remains an intriguing challenge. Issues of contamination from modern DNA have been largely overcome through the identification of damage patterns signaling ancient DNA—namely, chemical modification at the ends of the molecules due to deamination (24) and the fragmented base-pair length of ancient sequences (25). Still, it is challenging to determine how “ancient” these ancient DNA signatures are, due to their susceptibility to both time-related changes and environmental conditions; for instance, damage occurs faster in hotter climates (26). While these key signatures indicate that the extracted sequences are not modern contaminants, they do not exclude the possibility that ancient DNA sequences were derived from multiple sources or different time periods (Fig. 1). If we are interested in events of human occupation or biodiversity changes, or want to derive phylogenetic information from sedaDNA, we need external validation of the integrity of the data. This external validation depends on the microstratigraphic integrity of the sampled area within a particular layer.

To fully realize the potential of sedaDNA, we propose four intersecting guidelines rooted in a microcontextual approach to sedaDNA studies.

Currently, we do not have a good grasp of which particles within the sediments are contributing genetic data, nor do we know the ideal preservation contexts. Genetic information can be stored in small, microscopic bones embedded in the sediments, in microscopic fecal material (coprolites), or as extracellular DNA (fragments separated from their parent cell). Extracellular DNA is free to be adsorbed by minerals, especially clay minerals, or other minute components of the sediments (7, 27, 28), which also shields DNA from degradation. In this respect, archaeological stratigraphies, composed of heterogeneous particles derived from geogenic, biogenic, and anthropogenic processes, are particularly challenging, since all these potential sources of sedaDNA are present (Fig. 2A). Current samples intended for sedaDNA analysis typically consist of a few grams of bulk sediment that necessarily include a mixture of microscopic components. To disentangle the spatial variations and possible sources of sedaDNA at archaeological sites, Massilani et al. (10) performed a sedaDNA study on undisturbed sediment blocks that had been impregnated with resin, a by-product of thin sections manufactured for archaeological soil micromorphological analysis. For Denisova Cave sediments, sedaDNA was preserved throughout the studied blocks, but bones and coprolites provided greater yields of DNA molecules compared to the matrix samples (10). In the study by Kjær et al. (2), however, extracellular DNA bound to smectite clays appears to have been the main source of sedaDNA (see

also refs. 29–31). While there are general factors (e.g., UV exposure, temperature imbalance, time, geochemical processes) that govern the preservation of sedaDNA, each site and layer is formed by a specific and interconnected combination of processes that need to be taken into account when evaluating the preservation potential and stratigraphic integrity of sedaDNA. In our opinion, a case-by-case analysis is needed for each site and stratum.

SedaDNA cannot be directly dated. This differs from bones that can be simultaneously dated and sequenced for ancient DNA (34). Instead, the age of sedaDNA relies on its stratigraphic association. Thus, we need to make sure that the genetic data originate from intact or undisturbed deposits and that they are coeval with the layer from which they are recovered. Recent sedaDNA studies work under these two assumptions, but it would be overly optimistic to assume that sedaDNA is never reworked from one layer to another, since sediment particles can be moved from their original depositional context. If localized reworking has occurred at submillimeter scales, the integrity of the recovered sedaDNA can be difficult or impossible to assess in the field. For instance, while clays can adsorb DNA (29, 31), these minute minerals can also be easily translocated down into older strata (illuviation or burrowing) or be reworked into younger strata (5, 8), resulting in time shifts (Figs. 1 and 2B). Indeed, microscopic or nanoscale components have a greater chance of being remobilized from their original contexts than larger bones, ceramics, or stone tools. Even in sites with established sedaDNA preservation, the recovery success rate can be highly variable (1, 12). This uneven sedaDNA distribution further highlights the need for greater understanding of both the depositional and postdepositional processes affecting sedaDNA preservation.

A Microcontextual sedaDNA Approach

Given these caveats, we need to closely combine sedaDNA data with the site formation processes taking place at each site and sample unit. Otherwise, we risk treating sedaDNA as a “black box,” where it is unclear whether genetic data originate from intact or disturbed (micro)contexts and whether their age is the same as the layer from which they were retrieved. To fully realize the potential of sedaDNA, we propose four intersecting guidelines rooted in a microcontextual approach to sedaDNA studies.

1. **Microcontext:** We need to contextualize sedaDNA at the scale on which it occurs—encased in or adsorbed onto microscopic material. Systematically combining sedaDNA sampling with geochemical techniques (e.g., X-ray fluorescence, infrared spectroscopy) and archaeological soil micromorphology will enable the identification of formation processes affecting DNA preservation and integrity. Micromorphology is particularly key to assessing (micro)stratigraphic integrity, as this technique enables the observation of sediments or soils in their original geometry, allowing for the identification of key features, such as the presence of clay illuviation, diagenesis, relocation of soil particles, heat impact, redoximorphic processes, or reworking (35). Practically, we propose sampling in association with, or

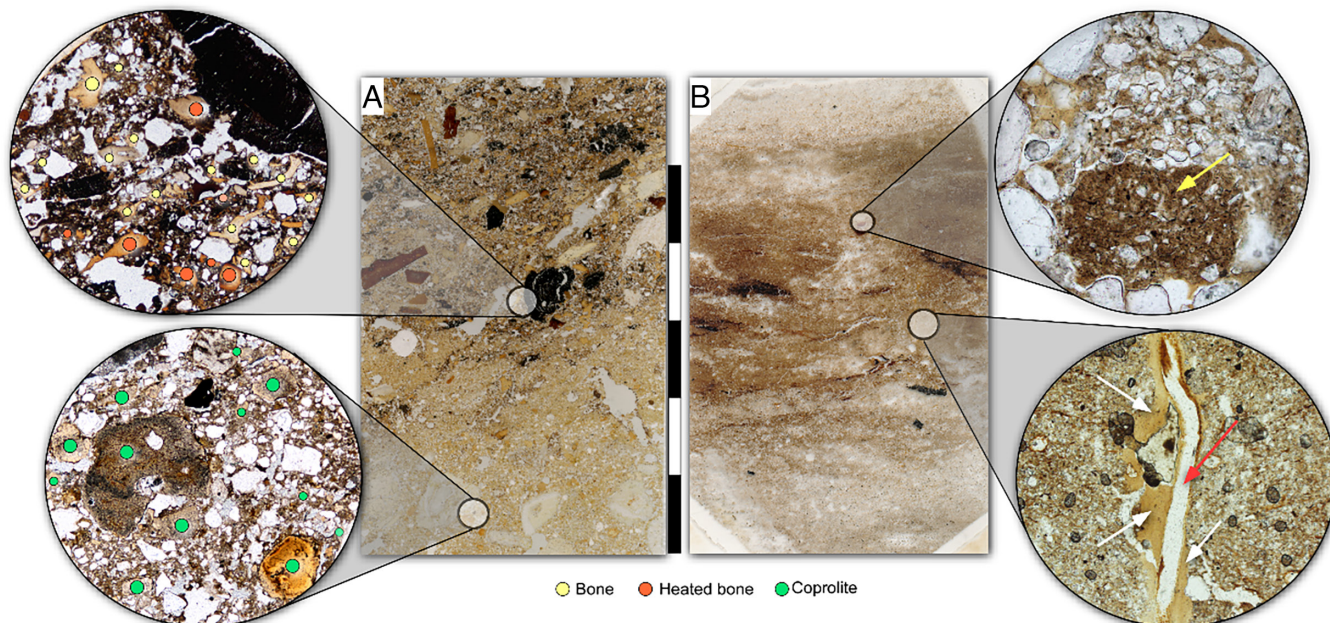


Fig. 2. View of archaeological deposits in soil micromorphology thin sections dated to ~46 thousand years ago (ka) from Bacho Kiro Cave (32) in Bulgaria (A) and dated to ~57 to ~71 ka at the open-air site of Lichtenberg (33) in Germany (B). Note the compositional heterogeneity of the deposits rich in human inputs at Bacho Kiro, combining distinct microscopic particles, such as bone fragments (marked as yellow circles), with several degrees of heating (red circles) and carnivore coprolites (green circles). The deposits from Lichtenberg show the interplay of sediments from different ages at the microscale with redeposited (older) soil particles (yellow arrow) and a root canal (red arrow) partially infilled with (younger) illuviated clays (white arrows). Such inclusions are commonly too small to be separated in bulk sampling and are spatially variable within a site and within a layer. (Scale bar for both thin sections: 1-cm increments.)

directly on the blocks collected for, micromorphology. As a first step to assess DNA integrity and preservation, we strongly advise sampling from resin-impregnated blocks produced during processing for micromorphology, as this allows for microstratigraphic control of the sampling process, and it has been shown that resin impregnation does not affect DNA preservation or extraction (10). Building on this first assessment, samples can be collected from the exposed backside of blocks directly in the field and in lateral extensions from the blocks to address particular questions. Only for sites with very good microstratigraphic control would we then recommend bulk sampling strategies. We also advise systematically testing archaeologically sterile and diagenetically affected sediments to test assumptions about DNA movement and taphonomy.

2. Experiments: We urgently need research into the taphonomy of sedaDNA in terrestrial contexts, with studies addressing preservation and mobility dynamics within sediments and soils. Fundamental experimental studies should focus on processes linked to preservation and contextual integrity dynamics to address questions such as: Under what geochemical conditions can we expect DNA binding and preservation to persist? What is the depositional history of the DNA adsorbing materials, before and after DNA absorption? And how do postdepositional alterations to the sediments affect sedaDNA preservation and movement?
3. Interdisciplinarity: We retrieve sedaDNA from a sedimentary matrix, and, thus, as with any other archaeological analysis, the very start of this analysis needs to be rooted in geoarchaeology. We envision sampling

and interpretation as a united geo- and archaeogenetic endeavor, led by archaeological questions, guided by geoarchaeological considerations, and controlled by genetic contamination precautions. Researchers and institutions should invest in enhancing communication among these three fields, including joint workshops, field work, and cosupervision and training of researchers.

4. Systematic Reporting: Contextual sedimentary information needs to be systematically reported. Such information should include sediment type, mineralogy, pH, salinity, depositional and postdepositional history, and environmental background. These data can then be used alongside standardized reporting of the characteristics of the genetic data: number and lengths of reads, deamination rates, composition of the identified taxa, pipelines, codes used, and sharing of “negative” results—i.e., the absence of any DNA, ancient DNA, or of targeted species. Only if sedaDNA teams employ similar protocols and language in the reporting of their results can we move beyond the investigation of individual sites and address events and processes across scales of time and space.

SedaDNA is poised to become an integral part of any research dealing with ancient sediments, from reconstructing human evolution, to paleoenvironments, to biodiversity and conservation studies. By positioning sedaDNA within a well-established microcontextual framework, we will be able to enhance our comprehension of sedaDNA hotspots with higher yields, along with better understanding of preservation mechanisms and integrity. Carrying out these studies under rigorous guidelines of systematic integration of genetic sampling with stratigraphic and geological studies will allow

us to contextualize and correctly interpret sedaDNA. The breadth of applications of sedaDNA will undoubtedly provide us with unprecedented insights into the past.

ACKNOWLEDGMENTS. This article stems from a meeting supported by the scheme Fast Output in Frontier Archaeology (FOFA) of the Interdisciplinary Center for Archaeology and Evolution of Human Behaviour (University of Algarve, Portugal), funded by the Portuguese Foundation for Science and Technology under Program UIDP/04211/2020. This research is funded by the European Union: M.C.S. by the European Research Council (ERC) MicroStratDNA Project 101042570; and V.A. by the ERC MATRIX Project 101041245. Views

and opinions expressed are, however, those of the authors only and do not necessarily reflect those of the European Union or the ERC Executive Agency. Neither the European Union nor the granting authority can be held responsible for them. We are also grateful to Shannon McPherron, Paul Goldberg, Dennis Sandgathe, Richard Roberts, Godefroy Devevey, Hussein Jaafar Kanbar, and Susanna Sawyer for comments on an earlier draft of this manuscript.

Author affiliations: ^aInterdisciplinary Center for Archaeology and Evolution of Human Behaviour, Universidade do Algarve, Faro 8005-139, Portugal; ^bDepartment of Evolutionary Anthropology, University of Vienna, Vienna 1010, Austria; and ^cHuman Evolution and Archaeological Sciences, University of Vienna, Vienna 1010, Austria

1. V. Slon *et al.*, Neandertal and Denisovan DNA from Pleistocene sediments. *Science* **356**, 605–608 (2017).
2. K. H. Kjær *et al.*, A 2-million-year-old ecosystem in Greenland uncovered by environmental DNA. *Nature* **612**, 283–291 (2022).
3. E. Willerslev *et al.*, Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science* **300**, 791–795 (2003).
4. P. Gelabert *et al.*, Genome-scale sequencing and analysis of human, wolf, and bison DNA from 25,000-year-old sediment. *Cur. Biol.* **31**, 3564–3574 (2024).
5. J. Haile *et al.*, Ancient DNA chronology within sediment deposits: Are paleobiological reconstructions possible and is DNA leaching a factor? *Mol. Biol. Evol.* **24**, 982–989 (2007).
6. K. K. Sand, S. Jelavić, K. H. Kjær, A. Prohaska, Importance of eDNA taphonomy and sediment provenance for robust ecological inference: Insights from interfacial geochemistry. *Environ. DNA* **6**, e519 (2024).
7. C. L. Freeman *et al.*, Survival of environmental DNA in sediments: Mineralogic control on DNA taphonomy. *Environ. DNA* **5**, 1691–1705 (2023).
8. C. Giguët-Covex *et al.*, New insights on lake sediment DNA from the catchment: Importance of taphonomic and analytical issues on the record quality. *Sci. Rep.* **9**, 14676 (2019).
9. D. Haouchar *et al.*, Thorough assessment of DNA preservation from fossil bone and sediments excavated from a late Pleistocene–Holocene cave deposit on Kangaroo Island, South Australia. *Quat. Sci. Rev.* **84**, 56–64 (2014).
10. D. Massilani *et al.*, Microstratigraphic preservation of ancient faunal and hominin DNA in Pleistocene cave sediments. *Proc. Natl. Acad. Sci. U.S.A.* **119**, e211366118 (2022).
11. K. Andersen *et al.*, Meta-barcoding of 'dirt' DNA from soil reflects vertebrate biodiversity. *Mol. Ecol.* **21**, 1966–1979 (2012).
12. E. I. Zavala *et al.*, Pleistocene sediment DNA reveals hominin and faunal turnovers at Denisova Cave. *Nature* **595**, 399–403 (2021).
13. L. Whitmore *et al.*, Inadvertent human genomic bycatch and intentional capture raise beneficial applications and ethical concerns with environmental DNA. *Nat. Ecol. Evol.* **7**, 873–888 (2023).
14. K. T. Özdoğan *et al.*, Archaeology meets environmental genomics: Implementing sedaDNA in the study of the human past. *bioRxiv* [Preprint] (2023). <https://doi.org/10.21203/rs.3.rs-3568244/v1> (Accessed 9 November 2023).
15. B. Vernot *et al.*, Unearthing Neanderthal population history using nuclear and mitochondrial DNA from cave sediments. *Science* **372**, eabf1667 (2021).
16. L. Parducci *et al.*, Ancient plant DNA in lake sediments. *New Phytol.* **214**, 924–942 (2017).
17. E. Capo *et al.*, Lake sedimentary DNA research on past terrestrial and aquatic biodiversity: Overview and recommendations. *Quaternary* **4**, 6 (2021).
18. A. T. M. ter Schure *et al.*, Anthropogenic and environmental drivers of vegetation change in southeastern Norway during the Holocene. *Quat. Sci. Rev.* **270**, 107175 (2021).
19. A. T. M. Ter Schure *et al.*, Sedimentary ancient DNA metabarcoding as a tool for assessing prehistoric plant use at the Upper Paleolithic cave site Aghitu-3, Armenia. *J. Hum. Evol.* **172**, 103258 (2022).
20. P. Goldberg *et al.*, Bedding, hearths, and site maintenance in the Middle Stone Age of Sibudu Cave, KwaZulu-Natal, South Africa. *Archaeol. Anthropol. Sci.* **1**, 95–122 (2009).
21. L. Wadley *et al.*, Middle Stone Age bedding construction and settlement patterns at Sibudu, South Africa. *Science* **334**, 1388–1391 (2011).
22. P. Goldberg *et al.*, New evidence on Neandertal use of fire: Examples from Roc de Marsal and Pech de l'Azé IV. *Quat. Int.* **247**, 325–340 (2012).
23. M. M. Haaland *et al.*, Geoarchaeological investigation of occupation deposits in Blombos Cave in South Africa indicate changes in site use and settlement dynamics in the southern Cape during MIS 5b–4. *Quat. Res.* **100**, 170–223 (2021).
24. J. Dabney, M. Meyer, S. Pääbo, Ancient DNA damage. *Cold Spring Harb. Perspect. Biol.* **5**, a012567 (2013).
25. 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).
26. T. Lindahl, B. Nyberg, Heat-induced deamination of cytosine residues in deoxyribonucleic acid. *Biochemistry* **13**, 3405–3410 (1974).
27. K. M. Nielsen, L. Calamai, G. Pietramellara, "Stabilization of extracellular DNA and proteins by transient binding to various soil components" in *Nucleic Acids and Proteins in Soil*, P. Nannipieri, K. Smalla, Eds. (Springer, Berlin Heidelberg, 2006), pp. 141–157.
28. W. Wackernagel, "The various sources and the fate of nucleic acids in soil" in *Nucleic Acids and Proteins in Soil*, P. Nannipieri, K. Smalla, Eds. (Springer, Berlin Heidelberg, 2006), pp. 117–139.
29. P. Cai, Q.-Y. Huang, X.-W. Zhang, Interactions of DNA with clay minerals and soil colloidal particles and protection against degradation by DNase. *Environ. Sci. Technol.* **40**, 2971–2976 (2006).
30. M. Franchi, J. P. Ferris, E. Gallori, Cations as mediators of the adsorption of nucleic acids on clay surfaces in prebiotic environments. *Orig. Life Evol. Biosph.* **33**, 1–16 (2003).
31. C. A. I. Goring, W. V. Bartholomew, Adsorption of mononucleotides, nucleic acids, and nucleoproteins by clays. *Soil Sci.* **74**, 149–164 (1952).
32. J.-J. Hublin *et al.*, Initial upper Paleolithic Homo sapiens from Bacho Kiro Cave, Bulgaria. *Nature* **581**, 299–302 (2020).
33. M. Weiss *et al.*, Neanderthals in changing environments from MIS 5 to early MIS 4 in northern Central Europe—Integrating archaeological, (chrono)stratigraphic and paleoenvironmental evidence at the site of Lichtenberg. *Quat. Sci. Rev.* **284**, 107519 (2022).
34. P. Korlević, S. Talamo, M. Meyer, A combined method for DNA analysis and radiocarbon dating from a single sample. *Sci. Rep.* **8**, 4127 (2018).
35. M. A. Courty, P. Goldberg, R. Macphail, *Soils and Micromorphology in Archaeology* (Cambridge University Press, Cambridge, UK, 1989).