

When did mammoths go extinct?

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A unique challenge for environmental DNA (eDNA)-based palaeoecological reconstructions and extinction estimates is that organisms can contribute DNA to sediments long after their death. Recently, Wang et al.¹ discovered mammoth eDNA in sediments that are between approximately 4.6 and 7 thousand years (kyr) younger than the most recent mammoth fossils in North America and Eurasia, which they interpreted as mammoths surviving on both continents into the Middle Holocene epoch. Here we present an alternative explanation for these offsets: the slow decomposition of mammoth tissues on cold Arctic landscapes is responsible for the release of DNA into sediments for thousands of years after mammoths went extinct. eDNA records are important palaeobiological archives, but the mixing of undatable DNA from long-dead organisms into younger sediments complicates the interpretation of eDNA, particularly from cold and high-latitude systems.

All animal tissues, including faeces, contribute DNA to eDNA records², but the durations across which tissues can contribute genetic information must vary depending on tissue type and local rates of destruction and decomposition. On high-latitude landscapes, soft tissues and skeletal remains of large mammals may persist, unburied, for millennia^{3–5}. For example, unburied antlers of caribou (*Rangifer tarandus*) from Svalbard (Norway) and Ellesmere Island (Canada) have been dated^{3,4} to between 1 and 2 cal kyr BP (calibrated kyr before present). Elephant seal (*Mirounga leonina*) remains near the Antarctic coastline^{5,6} can persist for more than 5,000 years. This is in contrast to bones in warmer settings, which persist for only centuries or decades^{7,8}. Because bones are particularly resistant to decay, quantifying how their persistence changes across environments enables us to constrain the durations that dead individuals generally contribute to eDNA archives. To do this, we consolidated data on the oldest radiocarbon-dated surface-collected bones from different ecosystems. We included bones that we are reasonably confident persisted without being completely buried ('never buried'), and bones for which exhumation cannot be confidently excluded ('potentially never buried'). Pairing bone persistence with mean annual temperatures (MAT) from their sample localities, we find a strong link between the local temperature and the logged duration of bone persistence (Fig. 1, never buried bones: $R^2 = 0.94$, $P < 0.01$; potentially never buried bones: $R^2 = 0.95$, $P < 0.01$). Millennial-scale bone persistence is probably ubiquitous in Arctic ecosystems, particularly those with low sedimentation rates. Bone persistence increases with body size⁷, so although the persistence of Arctic mammoth bones is unknown, results based on smaller-bodied organisms in warmer modern temperatures (Fig. 1) are probably underestimates of bone persistence for Pleistocene megafauna living in colder settings. Of note, bones and other biological tissues in cold environments are frozen for much of each year and even weather-worn specimens can produce viable DNA⁶.

eDNA, like all other sedimentary records, incorporates inputs from many sources and ages^{2,9}. Although this temporal mixing is frequently ignored in deference to inputs from living individuals, dead remains also contribute DNA as they decay. The magnitude of temporal mixing in eDNA must, therefore, largely depend on the decay durations of bones and other tissues. Because DNA cannot be directly dated, the degree of temporal mixing cannot be estimated for an individual eDNA sample. However, even diminutive antlers of female caribou can persist on tundra surfaces for more than 3,000 years (Fig. 1). Beyond extended bone persistence, Arctic settings are often characterized by ice-driven (for example, frost-heaving and cryoturbation) and geomorphological processes that release ancient fossils to the surface, thereby expanding the magnitude of temporal mixing within eDNA¹⁰. Wang et al. themselves reported mammoth DNA from surface samples adjacent to mammoth bones eroding out of nearby sediments¹. Although they interpret this as contamination today, if this same temporal mixing occurred during the formation of sediment layers from the deeper past, it would go unnoticed.

How much temporal mixing can we expect in eDNA records? Arguably, the best time to evaluate this question is following a species extinction, after which contributions of DNA into sediments shift from a mix of live- and dead-sources to dead-only sources. The timing of extinction is unlikely to coincide with the last occurrence of that species¹¹, but the temporal distribution of body fossils or eDNA can be used to estimate extinction timing. Mammoth body fossils found in Northeast Siberia, Northwest and Central Siberia, and northern North America ($n = 101$, 468, and 394, respectively; Supplementary Methods and Supplementary Data 3) are known semi-continuously from around 50 cal kyr BP until their last occurrences. Thus their predicted extinction intervals¹² (Supplementary Methods) are tightly constrained (Fig. 2). Using eDNA records, we find that extinction intervals are poorly constrained and, for Northwest and Central Siberia, includes the modern day (Fig. 2). More importantly, the mean extinction estimate for Northwest and Central Siberia is 2.7 cal kyr BP. On the basis of the temperature of the most recent mammoth DNA-bearing site (MAT = -13.3 °C), we would expect bone persistence times of between 2.26 and 4.19 kyr (mean and upper 95% confidence intervals for never buried bones) to more than 8.0 kyr (upper 95% CI for potentially never buried bones). Thus, using eDNA time series at face value implies that bones of the last mainland Siberian mammoths might still be persisting on today's landscapes. Yet, in the face of concerted efforts, the most recent mammoth fossils in this region are no younger than 11 cal kyr BP and are generally entombed in permafrost^{10,13}. This differs from Wrangel Island (expected bone persistence between 1.96 kyr and 3.53 kyr (mean and upper 95% confidence interval for never buried bones) to more than 6.66 kyr upper 95% confidence interval for potentially never buried bones), where

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Matters arising

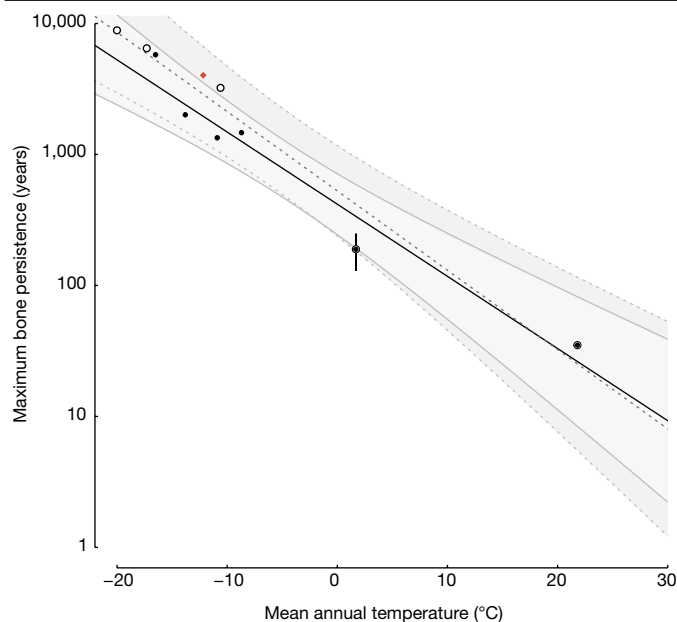


Fig. 1 | Duration of exposed bone persistence on landscapes as a function of the bone location's MAT. Persistence estimates (regressions and their 95% confidence intervals) are shown for bones that have probably remained at least partially exposed for their entire post-mortem history (never buried; filled points, solid lines, $R^2 = 0.94$, $P < 0.01$) and bones that were found exposed, but have more ambiguous post-mortem histories (potentially never buried; open circles, dashed lines, $R^2 = 0.95$, $P < 0.01$). For locations with more limited sampling, the same bones were used for both regressions (filled points surrounded by open circles). The most recent mammoth bone found exposed on Wrangel Island is shown (red diamond), but is not included in the regressions. Error bars are 2σ and generally smaller than the points.

mammoths persisted until 4 cal kyr BP, Middle Holocene sediments are thin and their bones lie exposed on the ground¹⁴.

One possibility is that millennial-scale gaps between the last mammoth fossils and the youngest eDNA samples highlight the inherent incompleteness of fossil records. This seems to be an unlikely driver, given the near-continuous record of mammoth fossils (Fig. 2) that terminate without a recognized sedimentological shift. eDNA might

also be recording individuals immigrating from Holocene mammoth populations on Wrangel Island or the Pribilof Islands. This too seems unlikely, given the wide oceanic crossings that would be required¹⁵. Instead, we consider the most parsimonious explanation to be that mammoth-bearing Middle Holocene sediments incorporated genetic information from well-preserved remains still lying on landscapes or introduced from exhumed remains of even more ancient individuals. This explanation is corroborated by our finding that the ages of all Siberian sediments containing mammoth DNA are within the expected interval between the last mammoth occurrences and the durations those remains could persist on Siberian landscapes (Fig. 2). Although two North American sediments containing mammoth DNA are younger than expected, exhumation of remains from deeper sediments could explain the genetic occurrence of this extinct species.

Nevertheless, eDNA records of mammoths extend beyond their fossil records. As Wang et al. claim¹, a possible reason is that mammoths survived on mainland North America and Eurasia into the Middle Holocene. However, the combined evidence indicates that this

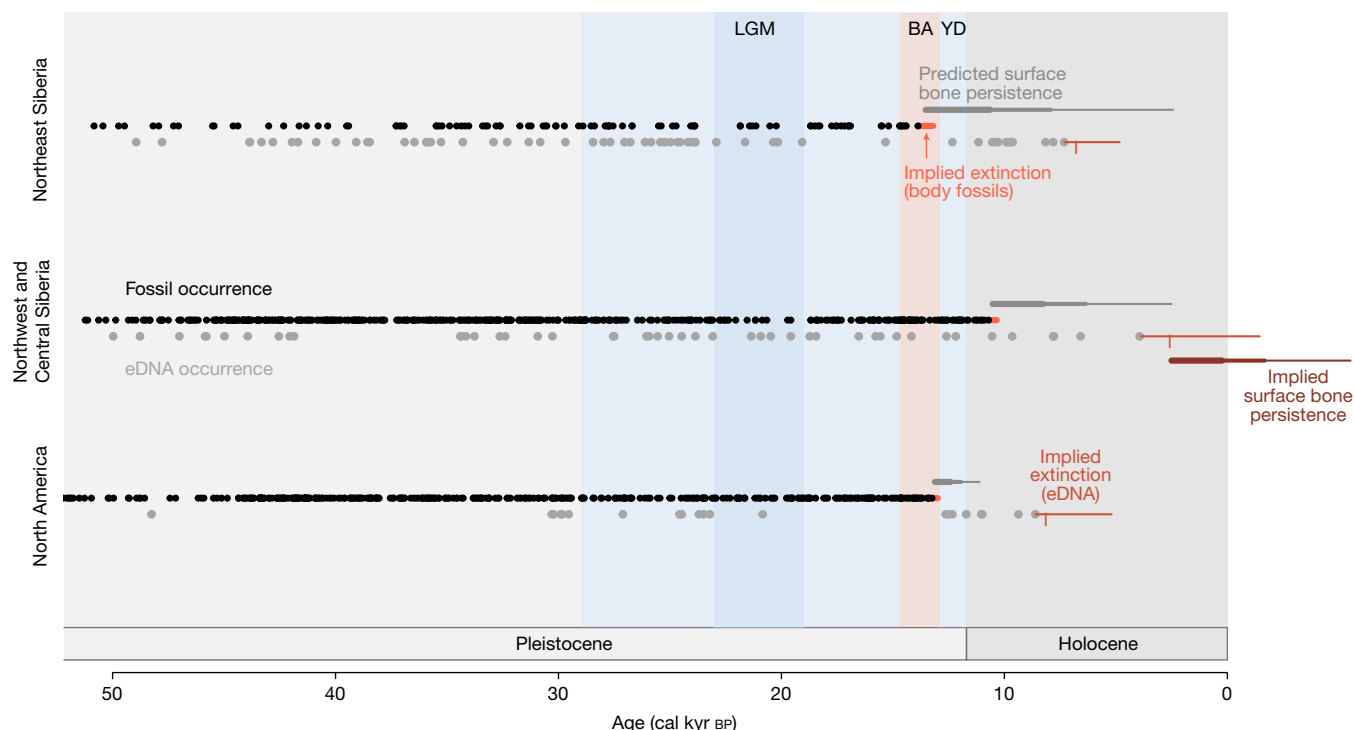


Fig. 2 | Time series of mammoth body fossils and eDNA records. Body fossils (black points) and eDNA (grey points) are illustrated separately. The 95% confidence intervals for mammoth extinctions are estimated¹² separately for fossil and eDNA records¹ in each region (red horizontal lines; vertical line is mean extinction estimate using eDNA records). Predicted persistence of mammoth bones for each region extends from the median of the bone-informed extinction

estimate. Thick grey horizontal lines, mean prediction based on never buried bones; medium grey horizontal lines, upper 95% confidence interval based on never buried bones; thin grey horizontal lines, upper 95% confidence interval for potentially never buried bones. LGM, last glacial maximum; BA, Bølling Allerød; YD, Younger Dryas.

pattern can be explained by Arctic environmental and taphonomic conditions that increase the persistence of DNA-bearing tissues on landscape surfaces and permit the release of long-dead tissues exhumed from permafrost. The mixing of DNA from long-dead organisms into younger sediments complicates the interpretation of eDNA, but we can start to control for this challenge by assessing the lengths of time across which DNA of extinct species are incorporated into sedimentary records.

Methods

To evaluate how bone persistence durations change with environment, we aggregated literature records of the ages of bones collected from landscape surfaces. For the purposes of this study, we only included the oldest bone from each region. To diversify the environmental settings included in the dataset, we added three accelerator mass spectrometry radiocarbon-dated bones from Arctic Alaska (two caribou antlers from the Coastal Plain, Arctic National Wildlife Refuge, USA) and temperate North America (one elk (*Cervus elaphus*) femur from Yellowstone National Park, USA; Supplementary Methods and Supplementary Data 1). For a full description of methods used, see Supplementary Information.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-022-05416-3>.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All data generated or analysed during this study are included in the Article and its supplementary information.

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Author contributions J.H.M. and C.S. designed the project. J.H.M. contributed data, conducted the geographic information system (GIS) analyses and wrote the paper. C.S. conducted all non-GIS analyses, made the figures and edited the paper.

Competing interests The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41586-022-05416-3>.

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Software and code

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Data collection We estimated the mean annual temperature of different locations around the world using the 2.5 minute BioClim1 raster (WorldClim2) and QGIS (version 3.4). Values were extracted using the 'raster' (version 3.0-7) package in R (version 4.0.3).

Data analysis Data were analyzed using the open-source software R (version 4.0.3). Radiocarbon dates were calibrated using the 'rcarbon' (version 1.4.2) package in R. To estimate the timing of extinction, we used the OLE function in the R package 'sExtinct' (version 1.1).

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Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	This study uses radiocarbon ages of bones sitting on landscapes around the world to estimate how mean annual temperature impacts the duration that bones can persist (unburied) in different environmental settings. We compare the resulting expectation of bone persistence duration to evaluate whether middle Holocene sediments containing mammoth DNA may have come from long-dead individuals as their remains decompose (as opposed to DNA contributed by late-surviving mammoths populations).
Research sample	The most severely weathered bones known from Yellowstone National Park and the Arctic National Wildlife Refuge, Alaska are included in this analysis, along with all literature records appropriate for estimating the persistence of bones on different landscapes around the world. Additionally, we include all previously dated and published mammoth bones from Siberia and North America (which come from previously published compilations).
Sampling strategy	Data presented here represent an exhaustive survey for all subjects. This includes known data on bone persistence around the world, all known mammoth bones from Siberia and North America, and all mammoth-bearing eDNA records published from Wang et al. 2021.
Data collection	Data collection on bones from Yellowstone National Park and the Arctic National Wildlife Refuge were collected by Joshua Miller using a standardized data collection scheme. Data on geographic location and taphonomic setting were taken in the field using a GPS and a field notebook. Additional observation on taphonomic state were evaluated in the lab through visual analysis. Miller collected all data related to bone persistence.
Timing and spatial scale	Most of the data used in these analyses come from efforts across many decades (from which all appropriate data are used). The eDNA data come directly from Wang et al. 2021 (all mammoth-bearing eDNA sediments from Siberia and North America are used; https://www.nature.com/articles/s41586-021-04016-x). The bone persistence data similarly come from all available data (which includes samples collected across 63 years).
Data exclusions	No data were excluded from this study.
Reproducibility	Lab experimentation was not part of this work. However, by incorporating data from decades of work, the reproducibility of different portions of our results (e.g., the time period after which mammoth fossils are no longer recovered in Northeast Siberian, Northwest and Central Siberia, and northern North America) is highlighted. Further, all methods are divulged to encourage additional, directly comparable work.
Randomization	Randomization was not relevant for our study. This study specifically evaluates differences in the distribution of fossils and eDNA samples within three specific geographic regions.
Blinding	Blinding was not relevant for our study. This work is largely a reevaluation of published data.
Did the study involve field work?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

Field work, collection and transport

Field conditions	Fieldwork (Yellowstone National Park, Arctic National Wildlife Refuge) was conducted on fair-weather days (e.g., not activity raining).
Location	For Yellowstone National Park, bones were evaluated across the Northern Range (approximately: 44.95 latitude, -110.50 longitude). For the Arctic National Wildlife Refuge, bones were evaluated across the Coastal Plain (approximately: 69.63 latitude, -141.43 longitude). Mammoth bones were evaluated across northern North America, and Siberia.
Access & import/export	All new collections highlighted in this work were done in accordance with all local, state, and federal laws. Collection from Yellowstone National Park was done under permit YELL-2007-SCI-5486, approved by Tom Olliff (Chief, Yellowstone Center for Resources) in 2007. Collections from the Arctic National Wildlife Refuge were approved in 2018 by Steve Berendzen (Manager, Arctic National Wildlife Refuge).
Disturbance	No discernible site disturbance was caused by this study. All study sites were approached on foot and all efforts were done to minimize impact during the work.

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Methods

n/a	Involved in the study
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<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Palaeontology and Archaeology

Specimen provenance	All new collections highlighted in this work were done in accordance with all local, state, and federal laws. Collection from Yellowstone National Park was done under permit YELL-2007-SCI-5486, approved by Tom Olliff (Chief, Yellowstone Center for Resources) in 2007. Collections from the Arctic National Wildlife Refuge were approved in 2018 by Steve Berendzen (Manager, Arctic National Wildlife Refuge).
Specimen deposition	All specimens are curated at public repositories. Newly published bone specimens highlighted here are available at the Department of Geology's collections, University of Cincinnati, Cincinnati, OH, USA.
Dating methods	AMS radiocarbon dates were generated from collagen extracted using standard acid/base pretreatments. Dates were acquired from the Center for Accelerator Mass Spectrometry (Lawrence Livermore) and University of California Irvine Keck-CCAMS facility. Quality assurance was monitored using internal lab standards and radiocarbon blanks. Radiocarbon dates were calibrated using the 'rcarbon' (version 1.4.2) package in R and the IntCal20 calibration curve.
<input checked="" type="checkbox"/> Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.	
Ethics oversight	Because all specimens were dead at the time of collection, no ethical oversight or guidance was required for this work.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Reply to: When did mammoths go extinct?

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REPLYING TO J. H. Miller & C. Simpson. *Nature* <https://doi.org/10.1038/s41586-022-05416-3> (2022)

Since the inception of ancient environmental DNA (eDNA) research, considerable attention has been paid to the depositional and diagenetic processes of DNA molecules in different sediments and settings¹. Understanding those processes is critical to determine whether the recovered DNA is of the same age as the deposit in which it is found. It is therefore not unreasonable to ask, as Miller and Simpson have² in response to our recently published eDNA study of 50,000 years of Arctic ecosystem changes³, whether remains of long-dead megafauna might have contributed older DNA to younger deposits. They propose that this may account for our finding that mammoths persisted into the Holocene epoch in the continental Arctic.

The basis for Miller and Simpson's proposal is that mammoth remains could have persisted on the surface of cold Arctic landscapes for millennia after the species' extinction, and while decomposing, released DNA into younger sediment layers. Their argument assumes that surface skeletal persistence is predominantly temperature-related, based on a correlation between mean annual temperature and the time unburied bones appear to persist. Leaving aside the limited sample size ($n = 10$) on which their correlation is derived, and the fact that not all the dated bones in the model have been on the surface since the animals' death (for example, the Wrangel Island mammoths were evidently released from permafrost only a few years before their discovery⁴), there can be little doubt that temperature is a factor in bone preservation in the Arctic. However, it is not the sole or even dominant factor. Instead, this is a region where multiple factors work against ubiquitous, millennia-long preservation, including carnivore and scavenger activity, moisture effects, seasonal freezing and thawing, strong ultraviolet radiation, and a range of biogeochemical processes that lead to enzyme digestion and organic matter decomposition^{5,6}. Mammoth individuals, being large, would require wide geographic ranges⁷. The expected average density of mammoth fossils per unit area would therefore be extremely low, and so too would the likelihood that these rare remains contributed DNA to our sampling sites. Given that mammoth DNA was found in 23 Holocene samples from 14 different sites (Fig. 1a), these late survivals are highly unlikely to be a result of DNA released from dead remains.

Furthermore, the eDNA that we obtained from surface samples belonged solely to species present on the landscape presently,

indicating that secondary contamination from fossil material is minor. However, it is well understood that some depositional settings (for example, riverbanks and thaw lakes) may be affected by complex processes, whereby older material (not only eDNA but the sediment strata) can be redeposited within younger sediments. This applied for one site (an actively eroding riverbank setting) of our original study that did not meet our criteria of an unmixed section with clear sedimentological and chronological contexts for eDNA sampling (described in the supplementary information of ref. ³), which was therefore excluded from the analysis. This reinforces the well-known caution that fluvial settings require particularly stringent sampling and dating protocols⁸.

Although Miller and Simpson rightly note that there is a near-continuous record of dated mammoth fossils, that record is not a reliable estimator of extinction timing. The youngest dated fossil marks the last time a species was abundant on the landscape⁹, rather than its last occurrence, which is highly likely to go undetected when a species is declining toward extinction, especially across the large geographic range of the vast Arctic landmass. Given the patchy nature of both the fossil and radiocarbon records, there can be centuries-long gaps between dated specimens (figure 1 in ref. ³). Those gaps would only increase as species declined and shifted their ranges to smaller portions of their former area¹⁰. Mammoths may have survived in refugia—such as the last pockets of the steppe-tundra landscape to which they were adapted—long after the date of the last known fossils, and most probably also after their last recorded occurrence in eDNA. However, there is a greater chance of detecting the lingering presence of an animal with eDNA than with its fossils, because an animal releases millions of DNA molecules onto the landscape on a daily basis over the course of its lifetime, but only leaves one skeleton, which is far less likely to be preserved, found and dated.

Notwithstanding limitations in Miller and Simpson's model and the lack of evidence for redeposition of DNA in our samples, it is reasonable to ask what we might expect to see if the slow decomposition of mammoth tissues on cold Arctic landscapes released DNA into sediments ubiquitously millennia after mammoth extinctions.

First, if redeposition of ancient DNA were widespread, we would expect to see mammoth eDNA in many sampling sites across the

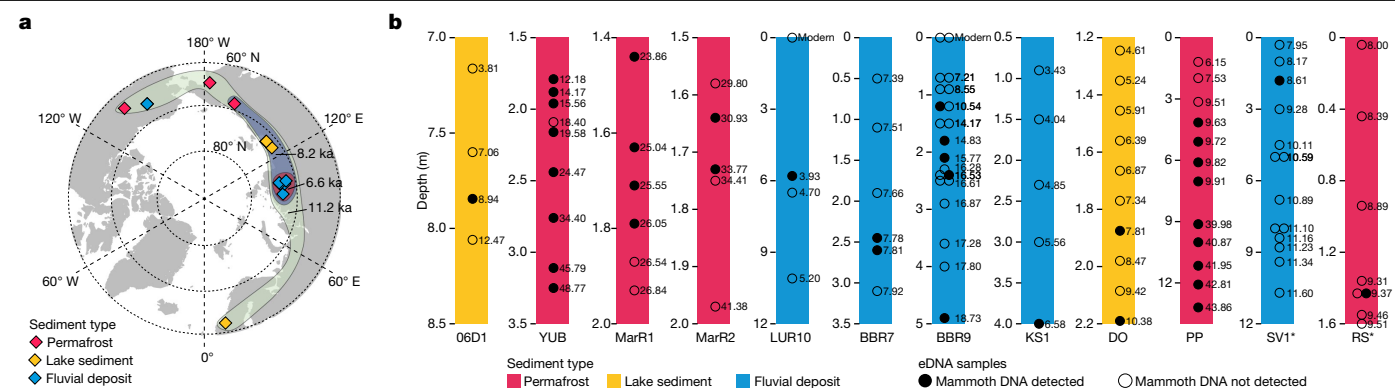


Fig. 1 | The geographical distribution of late-surviving mammoths, and the vertical distribution of eDNA samples and the identified mammoths in sediment profiles. a, Mammoth eDNAs were identified in 23 out of 192 Holocene samples, from 14 out of 32 sites covering the Holocene, and originated from 3 different sediment contexts. The 3 coloured regions show the shrinking distribution of mammoth in the Holocene: green, blue and red correspond to

species' full range during the late Pleistocene epoch, and not only restricted to particular regions in the Holocene. Yet, we instead found evidence of later surviving populations—mammoths younger than the Pleistocene/Holocene boundary (11.7 thousand years before present (kyr BP))—in only 23 out of the 192 Holocene samples, in different depositional contexts from 14 out of the 32 sites covering the Holocene (Fig. 1a). The Holocene-age mammoth eDNA occurs in distinct spatial and temporal patterns. It disappears first from the North Atlantic and North American regions, and finally from Siberia, especially northwest and central Siberia (Fig. 1a). These patterns are highly unlikely to have resulted from mammoth bones persisting on the ground surface or being exhumed from below then releasing DNA—if that were the case, the pattern of Holocene ages of mammoth eDNA would be unlikely to be so geographically uneven or to become geographically restricted over time.

Second, if mammoth DNA was continually 'leaking' into deposits, it would probably be detected in most (if not all) of the stratigraphic layers that formed after its DNA first found until the remains (whether preserved on the surface or exhumed from below) had disappeared altogether. Thus, mammoth DNA would not be restricted to time-specific depositional layers within sites, but would instead be 'smeared' across successive layers. We do not see this either—there is no evidence of mammoth DNA being smeared throughout a section, either horizontally or vertically (Fig. 1b). Instead, the DNA of mammoths and other animals is usually restricted to specific strata and separated by layers where their DNA is absent, including fluvial sites that can harbour eDNA from geographically wider catchments and upstream DNA sources that may feed them⁸. In many cases, mammoth DNA is detected only in some—and not all—of the samples from the same stratum (Fig. 1b), indicating that it has not diffused through a horizontal layer.

Third, if mammoth DNA was an artefact of redeposition, the signal would probably be random with respect to changes in vegetation and climatic conditions. That is not the case. Our eDNA results were embedded in a comprehensive reconstruction of past Arctic ecosystems, which revealed continental and regional associations between mammoth eDNA and (1) eDNA of other animals, (2) the steppe-specific herbaceous plants, and (3) palaeo-climate panels reconstructed independently from different climate models (figures 2 and 4 in ref. ³). Our results show that the range of where mammoth eDNA has been found shrinks through the Holocene along with the shrinking of the steppe-tundra vegetation and the climatic and hydrogeological conditions to which the species was adapted to in the Pleistocene¹¹, thereby supporting the geographically uneven and increasingly restricted

11.2, 8.2 and 6.6 kyr BP, respectively. **b,** Sites ($n = 12$) where mammoth eDNA was detected in at least one sample and with available sampling depths. For sites with only height available (marked with an ending asterisk in the site name), the sampling heights have been converted to relative depths. The number next to each eDNA sample indicates the age (in kyr BP) of that sample. More details can be found in ref. ³.

pattern just noted. If lingering mammoth bones had leached older eDNA ubiquitously, we should not have seen spatiotemporal co-occurrences of mammoth, steppe vegetation, and the cold and dry Pleistocene-like climate conditions.

Finally, if redeposition of DNA in younger deposits was a problem, the eDNA of late-surviving mammoths ought to reflect the full range of clades present in mammoth populations in the late Pleistocene. They do not. Instead, we find a consistent decline of mammoth mitochondrial haplogroup diversity from the Pleistocene into the Holocene to the point where only Clade 1DE remained, both on isolated islands and on continental Siberia (figure 4 in ref. ³). It is highly unlikely that this reduction in genetic diversity was because individuals harbouring the same haplogroup were the only ones whose DNA was being released into younger sediments over time. This finding instead conforms to a pattern of a species' decline towards extinction.

In sum, we find all evidence pointing to the validity of the eDNA identifications of late-surviving Arctic megafauna reported in our original study³. However, we acknowledge the possibility that unburied or exhumed animal fossils can contribute DNA to younger sediment layers, and this should always be considered (along the lines we described in ref. ³). This is particularly important in cases in which the animal species targeted were abundant and widely distributed on the landscape, for fine-resolution reconstructions, and for studies relying primarily on fluvial sediments as the eDNA source.

Reporting summary

Further information on experimental design is available in the Nature Portfolio Reporting Summary linked to this Article.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-022-05417-2>.

Data availability

All data analysed in this study are included in this article or have been published previously.

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Additional information

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