Mitochondrial genomes of Pleistocene megafauna retrieved from recent sediment layers of two Siberian lakes

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Abstract

Ancient environmental DNA (aeDNA) from lake sediments has yielded remarkable insights for the reconstruction of past ecosystems, including suggestions of late survival of extinct species. However, translocation and lateral inflow of DNA in sediments can potentially distort the stratigraphic signal of the DNA. Using three different approaches on two short lake sediment cores of the Yamal peninsula, West Siberia, with ages spanning only the past hundreds of years, we detect DNA and assembled mitochondrial genomes of multiple mammoth and woolly rhinoceros individuals—both species that have been extinct for thousands of years on the mainland. The occurrence of clearly identifiable aeDNA of extinct Pleistocene megafauna (e.g., > 400K reads in one core) throughout these two short subsurface cores, along with specificities of sedimentology and dating, confirm that processes acting on regional scales, such as extensive permafrost thawing, can influence the aeDNA record and should be accounted for in aeDNA paleoecology.

Key words: ancient environmental DNA, sediment core, Arctic, herbivore, mammal

Introduction

Sedimentary deposits constitute highly valuable archives of past ecosystem changes as they contain dateable layers with organismic remains including ancient DNA (aDNA). Such remains are typically assumed to represent the ecosystem of the time around which the respective stratum was deposited. aDNA from sediments has yielded remarkable insights regarding paleoecology, phylogeography, and extirpation and extinction events of keystone taxa such as mammoths (Haile *et al.*, 2009; Boessenkool *et al.*, 2012; Graham *et al.*, 2016; Murchie, Monteath, *et al.*, 2021). Based on such ancient environmental DNA (aeDNA), a recent study proposed that the wooly mammoth (*Mammuthus primigenius*) may have survived in Eurasia for much longer than previously assumed, as the authors retrieved mammoth DNA sequences in sediment layers that were approximately 4.6—7 thousand years (kyr) younger than the most recent mammoth fossils (Wang *et al.*, 2021); however, in response to this interpretation, Miller and Simpson (2022) opined that these results may be more likely due to taphonomic processes leading to release of aDNA from the remains of long-dead organisms from permafrost, where it is well preserved.

Conclusions derived from aDNA isolated from sediment cores rely on the stratified nature of the remains in question and dating of the respective layer by radiometric methods. However, in theory, various physical and geochemical processes such as translocation of DNA through sediment strata (Haile *et al.*, 2007), re-deposition of older sediment carrying DNA of extinct organisms (Arnold *et al.*, 2011), and preservation bias (Boere *et al.*, 2011) can distort the biological signal of aDNA and thus bias the accuracy of allocation of taxa to specific time periods (Armbrecht *et al.*, 2019). For lake sediments, deposited under aquatic conditions, studies have suggested that leaching is not a concern (Parducci *et al.*, 2017), but it has been observed in soils and cave sediments (Haile *et al.*, 2007). The question of obtaining last appearance dates of extinct taxa using aDNA in dated sediment layers (Haile *et al.*, 2009) is under discussion, but the surprisingly young records published to date still date to multiple thousands of years before present and thus lie within a timeframe of possible late survival.

Results and Discussion

In 2019, we retrieved short subsurface sediment cores from two Arctic thermokarst lakes (LK-001 and LK-007, located approximately 5 km apart, over massive permafrost; **Table 1**; Dvornikov *et al.*, 2016) on the Yamal peninsula, Siberia, to extract DNA and assess changes in mammal abundances in the Arctic over the past decades and centuries. From lake LK-001, we collected a secondary core which was sliced in the field at 1-cm steps for Pb^{210} radiometric dating, which indicated that the sediments at the top of this core were deposited recently, and that the core spanned the past few

centuries (Table S5). The cores for DNA extraction were closed in the field immediately after retrieval and were then transported to the dedicated aDNA laboratories of the University of Konstanz, Germany. In this lab facility, established in 2020, no other samples from the Arctic or from any large mammals had been processed previously. From core LK-001, we isolated DNA and produced genomic double-stranded libraries from 23 samples according to standard procedures. The core was opened and all subsequent steps until index PCR setup were carried out under customary aDNA laboratory conditions. In particular, the core opening facilities and the lab are located in buildings separated from the downstream molecular genetic analyses, the ventilation of the aDNA lab is based on a HEPA filter system and positive air pressure, and the lab is subjected to nightly UV radiation. Work in the lab is conducted under strict aDNA precautions, adhering to established aDNA protocols (Fulton and Shapiro, 2019). We enriched the libraries for mammalian DNA using a custom RNA bait panel produced from complete mitogenome sequences of 17 mammal species that currently or previously occurred in the Arctic (adapted from Murchie, Kuch, *et al.*, 2021). The enriched libraries were sequenced, and we mapped the sequences against a database of 73 mammal mitogenomes, followed by BLASTn alignment against the complete NCBI nt database. We thus retrieved mitogenomic sequences of mammals that were expected during the age range covered by the core, e.g., reindeer (*Rangifer tarandus*), Arctic lemming (*Dicrostonyx torquatus*), and hare (*Lepus*); however, throughout the entire core, there were abundant sequences of two species that have been extinct for several thousand years, i.e., mammoth (*Mammuthus primigenius*) and woolly rhinoceros (*Coelodonta antiquitatis*). Twenty-two of the 23 LK-001 libraries produced > 1,000 reads, each, assigned to *Mammuthus*, with read counts ranging from 2,852 to 72,919 (mean 21,140 ± 17,296). Negative controls (extraction and library blanks) did not produce any reads assigned to mammals. In the sample with the highest *M. primigenius* read counts (31.5 cm depth, dated to 81 years), the coverage of the reference mitogenome (NCBI accession NC_007596.2) was 95.3%, (434 (± 213)-fold). Across all samples, 465,080 reads assigned to mammoth were produced, with 98.3% coverage (2,762- ± 1,176-fold). Read lengths ranged from 28 to 289 bp (mean 100 ± 44 bp; **Fig. 1**). The number of wooly mammoth reads decreased from lower samples towards the top of the core (**Fig. 1**), and mapping suggested that the sequences throughout the core originated from multiple individuals. Signatures of post-mortem DNA decay were comparably minor (**Fig. 1**), with reference to an *M. primigenius* genome downloaded from NCBI (accession NC_007596.2). The proportions of bases did not suggest a substantial deviation from those in the reference genomes or in the closest extant relative of *Mammuthus*, the Asian elephant (*Elephas maximus*). Twelve of the 23 libraries produced > 100 reads, each, assigned to wooly rhinoceros, with a total of 2,737 reads and a cumulative coverage of 44% (assembled to NC_012681.1 2).

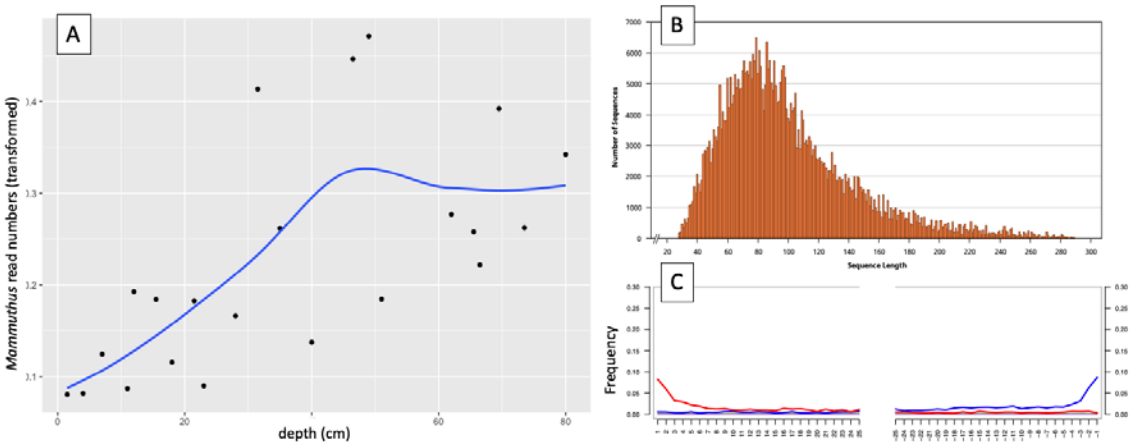

Fig. 1. aDNA of *Mammuthus* in recent lake sediments. (**A**) Read counts assigned to *Mammuthus* (square-root-transformed proportion of the respective number of raw reads per library) after hybridization capture enrichment of ancient DNA of core LK-001 (shown are results of 22 libraries; one library was excluded as it did not produce any reads assigned to mammals). Sample depths are indicated on the horizontal axis (in cm); the solid line indicates the general trend. (**B**) Fragment length distribution and (**C**) damage patterns across the 22 libraries.

As these results were entirely incongruent with the temporal occurrence of these species, we employed several methods to confirm their validity, i.e., conventional PCR and Sanger sequencing of a mammoth COI fragment, mammal metabarcoding, and droplet digital PCR (ddPCR) of a mammoth cytochrome b fragment. We performed these analyses on core LK-001 and, to evaluate whether this is a locally isolated phenomenon, on a second short core, LK-007, from a nearby lake, which had been opened and processed under the same conditions indicated above to prevent contamination. Additionally, we screened the core LK-001 for plant macrofossils, of which three were sent for ^{14}C AMS dating.

Conventional PCR and Sanger sequencing confirmed amplification of a COI fragment of *M. primigenius*. Mammal metabarcoding produced mammoth sequences (74 bp) in 13 samples of core LK-001 and 9 samples of core LK-007. In the LK-001 core, the highest *M. primigenius* read count occurred at 31.5 cm (2,992 reads), and in the LK-007 core at 26 cm *M. primigenius* (3,580 reads). ddPCR produced *M. primigenius* sequences in 14 samples of each core. Metabarcoding and ddPCR patterns across the cores were similar, although not completely congruent, as ddPCR appeared to be more sensitive (Fig. S1). The dates retrieved by radiocarbon dating were not congruent with the initial age inference suggested by Pb^{210}. While the lowest and topmost sample (with ages of 1,547 ± 228 and 339 ± 79 uncal. yrs BP respectively) suggest relatively young ages and agree in their temporal succession, the middle sample, at 36,5 cm, yielded a radiocarbon age of 8,677 +/- 132 yrs.

The mammoth was abundant throughout most of Eurasia during the Pleistocene, but populations declined at the end of the Pleistocene, with the species going extinct in the mid-Holocene (Nogués-Bravo *et al.*, 2008). The youngest fossils from mainland Siberia have been dated to 9,650 years (Stuart *et al.*, 2002). The wooly rhinoceros was a cold-adapted megaherbivore, which was abundant from western Europe to north-east Siberia during the Middle to Late Pleistocene (Kahlke and Lacombat, 2008). This species was predominantly grazing, probably resorting to browsing only due to seasonal vegetation restrictions (Kahlke and Lacombat, 2008; Rey-Iglesia *et al.*, 2021; Stefaniak *et al.*, 2021). The reasons underlying the extinction of this species at ca 14 ka BP are not entirely clear, but it is largely attributed to sudden climate warming and subsequent habitat unsuitability due to vegetation changes (Stuart and Lister, 2012; Lord *et al.*, 2020; Wang *et al.*, 2021) in the Bølling-Allerød interstadial warming.

The present data, which implies frequent and abundant Pleistocene megafaunal DNA throughout a sediment core deposited in the lake over the past centuries suggests that physical processes, rather than presence of live organisms, are responsible for the recovery of this DNA. While not in itself fully conclusive, our data suggests the source of the DNA of Pleistocene mammals from older permafrost deposits, either from a carcass or from sedimentary materials carrying the DNA. The numbers of mammoth sequences increased with depth downcore, with comparably low abundances over the most recent 11 cm of the core (aged < 30 years), pointing to a decrease of input of this DNA through time. The apparently rather limited extent of aDNA damage in the mammoth sequences suggests that the source of this DNA has been preserved exceptionally well, which also suggests an origin from permafrost, and the specific dynamics of thawing and redeposition of material in the study area offer an explanation.

Here, the active thermokarst likely began during the climatic optimum of the Holocene (9000–3000 BC; (Savvichev *et al.*, 2021). The LK-001 and LK-007 lake basins are interbedded mainly into the IVth marine plain, formed in the Kazantsevskaya, Marine Isotopic Stage 5. The permafrost of these lake catchments was formed no earlier than 70—60 kyr BP after the Kazantsevskaya transgression in more sub-aerial conditions and with mean annual temperatures 6 to 7 °C lower than modern temperatures (Baulin *et al.*, 1981). As this area was under coastal-marine conditions for a long period, these lake basins may be paleo-marine remnants, or they were formed later as a result of thermokarst over the segregated or tabular (massive) ground ice during the Holocene climatic optimum (Kachurin, 1961). The area is also subject to abrupt permafrost thaw (thermo-denudation), resulting, for example, in the formation of retrogressive-thaw slumps (thermocirques) and the

transport of a large amounts of thawed terrestrial material into the lake water (Dvornikov *et al.*, 2018). Such abrupt permafrost thaw processes normally appear adjacent to lakes and can form specific geomorphological elements,. i.e., thermo-terraces (Kizyakov *et al.*, 2006) within lake catchments and lakes; they are normally polycyclic processes appearing due to the extension of a seasonally thawed layer (active layer) up to the top of massive ice and more favorable thermal conditions within the existing forms (Leibman and Kizyakov, 2007; Kokelj *et al.*, 2009). Traces of past thermo-denudation can be observed within both lake catchments. In catchments of five neighboring lakes, large retrogressive thaw slumps appeared in recent years (2012-2013) accompanied by the thaw and lateral transport of modern and late Pleistocene deposits into lakes. Additionally or alternatively, thermo-erosion of upper geomorphological levels and transport via stream networks could transport ancient material into the modern lacustrine sediments. However, the two studied lakes are headwater lakes (with outlet, no apparent inlet) and this option can only be considered in terms of small thermo-erosional valleys within the catchments.

An alternative mechanism for the redistribution of Late Pleistocene material in the sediments is related to subcap methane emission (bubbling) from degrading permafrost beneath the lake bottom. In-lake bubbling can be observed in a circum-Arctic scale: in North-East Siberia, Alaska and Canada. This is common especially in lakes with a depth exceeding two meters, which do not freeze entirely up to the bottom in winter, leading to the formation of a talik. The expansion of the talik may further trigger subcap methane emission, which can reach 40-70 kg yr^{-1} of pure (94-100%) methane in neighboring lakes (Kazantsev *et al.*, 2020). The constant methane seepage doesn't allow ice to be formed in winter (whereas the normal winter ice thickness is approximately 1.5 m) and can potentially disturb the stratigraphy of lake sediments. Additionally, dramatic emissions of methane can form craters in terrestrial and lacustrine environments (Dvornikov *et al.*, 2019). In this case Late Pleistocene sediments will well be re-distributed within the water-body and the entire stratigraphy will be mixed.

In the case reported here, the simultaneous finding of a Pb^{210} chronology indicating recent sediment deposition and of plant macrofossils that dated to > 8000 yrs BP in a sample from 36.5 cm, suggest lateral input of ancient material, including the mammalian DNA, putatively related to permafrost thawing processes. Numerous studies on aDNA discussed possible leaching through sedimentary strata of the DNA itself, yet it was typically considered not an issue as most of these studies were conducted under stable permafrost or similar conditions (eg, Hansen *et al.*, 2006; Haile *et al.*, 2007, but see Andersen et al. 2012). Permafrost thawing and redeposition of material adds a new dimension to this problem of temporal interpretation. The fact that we retrieved mammoth sequences from both cores of two lakes located approximately 5 km apart suggests that this is not an isolated phenomenon but occurs on a regional or even larger scale. Given the wide spread of abrupt

permafrost thaw processes in the Arctic plains (West Siberia, Taimyr, Chukotka, Alaska, Canadian Arctic (Kizyakov *et al.*, 2006 and references therein), the phenomena of disturbed stratigraphy of lacustrine sediments can potentially be observed at a pan-Arctic scale. While this indicates that temporal interpretation of sedimentary aDNA records should be exercised with caution, our study also demonstrates that a careful evaluation of available information on the site and ecosystem in conjunction with the use of independent dating techniques can uncover incongruencies. This is more difficult in older time periods, where artefactual stratigraphies caused by equivalent processes acting for a limited time will not be detected as easily as in our case of long extinct species. The same applies to extant taxa or those which have undergone extinction or extirpation more recently, the presence of which cannot as easily be excluded as in the current example. However, we suggest that the inclusion of robust dating techniques and knowledge of local geophysical processes can provide good arguments to evaluate the reliability of aeDNA records.

Materials and Methods

Field sites, DNA isolation and hybridization capture enrichment

In 2019, sediment cores (6 cm diameter; Table 1) were retrieved from two lakes (LK-001 and LK-007, respectively, Table 1) on the Yamal peninsula, Siberia, using a UWITEC piston corer (UWITEC, Mondsee, Austria). The lakes were located approximately 5 km apart. The cores were transported to the aDNA laboratories of the University of Konstanz, Germany; from lake LK-001, a secondary core was taken which was sliced in the field at 1-cm steps for radiometric dating from 0 to 39.5 cm depth, performed at the Environmental Radioactivity Research Centre, University of Liverpool (supplementary material). Additional 14C dating of three specimens of plant remains, extracted at 36.5 cm, 51 cm and 74 cm, was performed (supplementary material).

Table 1. Sediment cores retrieved from two lakes on the Yamal peninsula, Siberia.

lake	coordinates	m above sea level	area	water depth	core length
LK-001	70°16'45.6" N, 68°53'02.8" E	28	38 ha	17 m	80 cm
LK-007	70°16'02.8" N, 68°59'35.7" E	36	39 ha	14 m	75 cm

Sedimentary DNA was isolated from 23 samples of core LK-001 and from 16 samples of core LK-007 using commercially available kits with modified protocols (supplementary material). The extracts of core LK-001 were subjected to library preparation for capture enrichment. Enrichment probes were designed from mitogenomes of 17 herbivorous mammal species that currently or previously

occurred in the Arctic (Table S1) and few lichen sequences (Table S2). Genomic libraries were produced according to (Li *et al.*, 2013) with some modifications (Seeber *et al.*, 2019) (see supplementary material). Filtered reads were mapped to mammalian mitogenomes, followed by BLASTn alignment against the complete NCBI nucleotide database and subsequent metagenomic analyses using MEGAN (Huson *et al.*, 2016). Reads assigned to *Mammuthus* were mapped to a complete *M. primigenius* reference mitogenome (NCBI accession NC_007596.2); reads assigned to *Coelodonta antiquitatis* were mapped to the NCBI reference mitogenome NC_012681.1 2.

Conventional PCR, mammal metabarcoding, and ddPCR

Based on the enriched fragments with the highest coverage, PCR primers specific to *M. primigenius* were designed using Geneious Prime 2022.1.1 (Kearse *et al.*, 2012), i.e., mamm801 (5`-CCCATGCAGGAGCTTCTGTAGA-3`) and mamm800r (5`-GACATAGCTGGAGGTTTTATGT-3`) to produce a 121-bp amplicon of the CO1 gene. The specific PCR conditions are described in the supplementary material. Mammal metabarcoding PCRs were performed on 21 DNA extracts of core LK-001 and 27 samples of core LK-007, with eight independent replicates, each. Each batch of PCRs included one non-template control. Established metabarcoding primers were used (Giguet-Covex *et al.*, 2014), and human blocking primers (Garcés-Pastor *et al.*, 2021) were included. PCR conditions are described in the supplementary material. Sequencing was performed on an Illumina NovaSeq platform, with 2 x 150 reads. The raw data were processed as described in the supplement. We used the sample LK-001_66.5 of core LK-001 which had produced one of the highest *Mammuthus* read counts after enrichment. The specific target amplified by the primer pair were used to design a probe (5`-GGATACTCCTGCAAGGTGAAGTG-3`). With this probe, ddPCR was performed with 24 extracts of core LK-001 and with 30 extracts of core LK-007, with three replicates, each (supplement).

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Competing interests

The authors have no competing interests to declare.

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