Mammoth DNA sequences

SIR — Lindahl points out[1] that the study of ancient DNA requires stringent criteria for establishing the authenticity of sequences, particularly as some claims seem to contradict chemical knowledge about diagenetic processes[2,3]. In addition to criteria suggested previously[4], Lindahl suggests that sequences should be verified by reproduction from different individuals of a species, that negative results should be reported along with the positive ones,

ORIGIN OF FIVE SIBERIAN MAMMOTH SPECIES

Specimen	Age (yr)	Samples		Ref.
		Total	Positive	
Yuribei (1979)	9,700	4	3	10
Dima (1977)	40,000	2	1	11
Sanga-Yuryakh (1908)	40,000	1	0	12
Shandrin (1971)	42,000	2	1	13
Khatanga (1977)	> 50,000	6	2	14

Years in parentheses are the year of discovery of the samples. Total, number of samples available; Positive, number of samples from which amplifiable DNA could be extracted.

and that samples of a moderate age (up to 100,000 years) should be investigated to establish if they contain retrievable DNA. We completely agree and believe that it represents a great danger to the field of molecular archaeology if methods and procedures that allow the confirmation of results are not used. For example, sequences retrieved by molecular cloning (see ref. 5) cannot be reproduced because of the low cloning efficiency of ancient DNA, and are therefore of only limited scientific value.

To show that it is possible to reproduce results from Pleistocene faunal remains, we have extracted[6] DNA from soft tissues of five different mammoths which vary in age from 9,700 to more than 50,000 years old. From four of the animals, more than one sample was available (see table above). Enzymatic amplification of a 93-base-pair fragment of the mitochondrial 16S ribosomal RNA gene yielded an amplification product from four of the five individuals, specifically seven of the fifteen extracts. All amplification products were directly sequenced in both directions. Products stemming from the same individual yielded identical sequences.

In the figure at the foot of the page, the sequences from the mammoths are aligned to the homologous sequences of the two living members of the order Proboscidea, the Indian (*Elephas maximus*) and African (*Loxodonta africana*) elephants, and to two other ungulates. Whereas no insertions or deletions have occurred among the mammoth and elephant sequences, three and four insertions/deletions are needed for an alignment to the cow and horse sequences, respectively. Among the 69 positions where all sequences are unambiguously alignable, the mammoths differ by 0–4 substitutions from the elephants and by 6–12 substitutions from the other ungulates. Thus, the sequences from the mammoth carcasses are not identical to, but are clearly more closely related to, elephant than to the other ungulate sequences presented here. That different but closely related sequences are retrieved from different mammoths further supports the conclusion that they are of ancient origin.

The mammoths differ from each other by 0–5 substitutions whereas the Indian and African elephants, representing two genera, differ by only two substitutions. These preliminary data indicate that the mammoths were highly diverse and may have been divided into geographical or temporal subspecies.

Thus, Pleistocene DNA sequences fulfilling the criteria suggested by Lindahl[1] and others[4] can be retrieved. We suggest that the much older sequences reported in the past few years, for example in amber[7–9], be submitted to the same kind of tests as the faunal remains presented here.

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DNA from ancient mammoth bones

SIR — The recovery of DNA from ancient human and animal remains is a topic of considerable interest. Controversy still surrounds the analysis of DNA sequences from biological samples aged many millions of years, for example from Miocene plants in anoxic sediments or insects in amber[1]. Lindahl has suggested that moderately ancient DNA (about 100,000 years old) should be targeted for analysis to bridge the temporal gap that exists between DNA sequences from relatively recent biological remains and those many millions of years old[2]. We report here the successful polymerase chain reaction (PCR) amplification and sequencing of a fragment of the mitochondrial DNA cytochrome *b* gene, amplified from bones of two Siberian woolly mammoths dated at least 47,000 years before present (BP). To our knowledge, these mammoth bones are the oldest dated vertebrate remains from which DNA has been amplified.

The woolly mammoth, *Mammuthus primigenius*, was widely distributed across northern Eurasia and North America in the late Pleistocene. *Mammuthus* is thought to have originated in Africa about 5 million years ago, where it shared a common ancestor with the two living

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                    10        20        30        40        50        60        70        80        90
Yuribei     AAGAAAAAAA CCTCCGAACG ATATTATAAT TCAGACTTTA CAAGTCAAGA TTCACTAATC GCTTATTGA- CCCAATACTT GATCAACGGA ACA
Dima        .......... .......... .......... .......... .......... .......... .......... .......... .......... ...
Shandrin    .....G.... .......... .......... .....C.... .....A.... .......... .T........ .....T.... .......... ...
Khatanga    .....G.... .......... .......... .....C.... .....A.... .......... .T........ .....T.... .......... ...
Indian elephant .......... .......... .......... .....C.... .....A.... ..T....... .......... .......... .......... ...
African elephant .........T .......... .......... ....T.C... .....A.... ..T....... .......... .......... .......... ...
Cow         ..T.....T. .....G.... ...T..A.GA CT....CC-. .....AT. CA.T....- ...C....T. ..A..A.... .......... ...
Horse       ..C.....C. .....GT... ...T.--A.. C.....A-. .C.....A. .AT..... A........T ..A..CCA.. .......... ...
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Sequence alignment of four mammoths, African and Indian elephant, cow and horse. Dots, sequence identity; dashes, deletions; asterisks, positions that cannot be unambiguously aligned.