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.

Matthias Höss
Svante Pääbo
Institute of Zoology,
PF 202136,
D-80021 Munich,
Germany
N. K. Vereshchagin
Institute of Zoology,
Russian Academy of Sciences,
Universitetskaya Nab.,
St Petersburg 199034,
Russia

1. Lindahl, T. *Nature* **365**, 700 (1993).
2. Pääbo, S. & Wilson, A. C. *Curr. Biol.* **1**, 45–46 (1991).
3. Lindahl, T. *Nature* **362**, 709–715 (1993).
4. Pääbo, S., Higuchi, R. G. & Wilson, A. C. *J. biol. Chem.* **264**, 9709–9712 (1989).
5. Pääbo, S. *Nature* **314**, 644–645 (1985).
6. Höss, M. & Pääbo, S. *Nucleic Acids Res.* **21**, 3913–3914 (1993).
7. DeSalle, R., Gatesy, J., Wheeler, W. & Grimaldi, D. *Science* **257**, 1933–1936 (1992).
8. Cano, R. J., Poinar, H. N., Pieniazek, N. J., Acra, A. & Poinar, G. O. Jr *Nature* **363**, 536–538 (1993).
9. Poinar, H. N., Cano, R. J. & Poinar, G. O. Jr *Nature* **363**, 677 (1993).
10. Arslanov, C. A., Ljadov, V. V., Filonov, B. A. & Tjernov, S. B. in *Yuribeiskij Mamont* (ed. Sokolov, V. E.) 35–36 (Nauka, Moscow, 1982).
11. Schilo, N. A. & Losjkin, A. V. in *Magadanskij Mamentjenok* (eds Vereshchagin, N. K. & Michelson, V. M.) 48–49 (Nauka, Leningrad, 1981).
12. Heintz, A. Ye. & Garutt, V. Ye. *Dokl. Akad. Nauk SSSR* **154**, 1367–1370 (1964).
13. Solonevitch, N. G., Tichomirov, B. A. & Ukraintseva, V. V. *Trudi Zool. Inst. Akad. Nauk SSSR* **63**, 277–280 (1977).
14. Arslanov, C. A., Ljadov, V. V., Tertitjnaja, T. V. & Filonov, B. A. *Trudi Zool. Inst. Akad. Nauk SSSR* **111**, 17–18 (1982).

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

```
                    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.. .......... ...
                                  ******** **  ***       **  ********        *
```

Sequence alignment of four mammoths, African and Indian elephant, cow and horse. Dots, sequence identity; dashes, deletions; asterisks, positions that cannot be unambiguously aligned.

```
Lox  T TTC GGC TCA CTA CTA GGA GCA TGC CTA ATT ACA CAA ATC CTA ACA GGA TTA TTC CTA GCC ATA CAT TAT
Mam1 . ... ... ... ... ... ... ... ... ... ... ..c ..g ... ... ... ... ... ..t ... ... ... ... ...
Mam2 . ... ... ... ... ... ... ... ... ... ... ..c ... ... ... ... ..g ... ..t ... ..t ... ... ...
Ele  . ... ... ... ... ... ... ... ... ... ... ..c ... ... ... ... ... ... ... ... ... ... ... ..c

Lox  ACA CCC GAC ACA ATA ACT GCA TTT TCA TCT ATA TCC CAT ATT TGC CGA GAT GTA AAC TAC GGC TGA ATT
Mam1 ... ..t ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..c ... ... ... ... ...
Mam2 ... ..t ... ... ... ... ... ... ... ... ... ... ... ..c ... ... ... ..c ... ... ..t ... ...
Ele  ... ..t ... ... ... ... ... ... ... ... ... ... ... ..c ... ... ..c ..c ... ... ... ... ...

Lox  ATT CGA CAA CTA CAC TCA AAC GGA GCA TCC ATT TTC TTC CTC TGC CTA CAC ATT GGA CGA AAC ATC TAC
Mam1 ... ... ... ... ... ... ... ... ... ... ..t ... ... ... ... ... ... ... ... ... ... ... ...
Mam2 ... ... ... ... ... ... ... ... ... ... ..t ... ... ... ... ... ... ... ... ... ... ... ...
Ele  ... ... ... ... ... ... ... ... ... ... ..t ... ... ... ... ... ... ... ... ... ... ... ...

Lox  TAT GGG TCC TAC CTA TAC TCG GAA ACT TGA AAT ACC GGC ATT ATA TTA CTA CTA ATC ACC ATA GCC ACC
Mam1 ... ... ... ... ... ... ... ... ... ..c ... ... .
Mam2 ... ... ... ... ... ... ... ... ... ..c ... ... ... ... ... ... c.. ... ... ..a ... ... ... ...
Ele  ... ..a ... ... ... ... ..a ... ..c ... ... ..a ... ... ... ... ... ... ... ..t ... ... ... ...
```

Alignment of DNA sequences obtained from the two Siberian mammoths (Mam 1 and 2) and one Asian elephant (Ele) with the published African cytochrome *b* sequence (Lox)[8] (GenBank accession number X56285); identity to this sequence is denoted by a full stop (.). The sequence was read from bases 96 to 378 of the cytochrome *b* gene[8].

species of elephantid, the African elephant *Loxodonta africana* and Asian elephant *Elephas maximus*[3]. Mammoth remains include not only bones and teeth from thousands of localities, but also whole carcasses frozen in Siberian and Alaskan permafrost.

We extracted DNA from bones of two frozen Siberian woolly mammoths, and subsampled both specimens for accelerator mass spectrometry (AMS) radiocarbon dating at the Institute of Geological and Nuclear Sciences, New Zealand. The first individual, the Khatanga mammoth, consisted of the partial carcass of an adult male excavated in 1977 from alluvial sand in the eastern Taimyr peninsula[4]. We cut a wedge of cortical bone from one humerus (no. 31829) at the Zoological Institute, St Petersburg. Previous conventional radiocarbon dating on a sample of trunk and skin yielded dates of ≥40,340 years BP (LU-750) and ≥53,170 years BP (LU-1057), respectively. Both these dates are consistent with the new bone AMS date of >47,000 years BP (NZA-3711). As the specimen is beyond the range of finite radiocarbon dating, the age of this mammoth must be 47,000 years or older.

The second mammoth sample was from a mandible (no. PIN 3657-181) excavated in 1975 by AVS from the Allaikha river, northeast Siberia[5]. It was found in permafrost 16 m below the surface in section ANV-1, beneath a horizon radiocarbon dated to >46,000 years BP on autochthonous plant vegetation remains (GIN-1681). We removed a piece of bone for DNA analysis, and a subsample yielded an AMS date of >47,000 years BP (NZA-3712). The predominant fossils in the fauna horizon were of the extinct lemming *Dicrostonyx simplicior*, an evolutionary precursor of the extant collared lemming *D. torquatus*. This suggests a late Middle Pleistocene age for the mammoth, not less than 150,000 years BP.

We extracted DNA from the two mammoth bones (0.7 g bone) as described previously[6], in parallel with three forensic and three prehistoric human bone samples, and an extraction blank with no bone. DNA was amplified from the bone extracts using the PCR with the highly conserved primers L14841 and H15149, which define a 375-base-pair fragment of the cytochrome *b* gene[7]. All the bone extracts yielded phylogenetically meaningful DNA sequences. We performed amplification reactions in a laboratory where elephant DNA had not been handled before. Following amplification, we determined the DNA sequences of both strands and compared them with the previously published African elephant cytochrome *b* sequence[8] (see figure above), as well as with the orthologous sequences of eight African and six Asian elephants (one of which is shown in the figure). Preliminary phylogenetic analysis of the sequences from all these individuals shows that the mammoths fall within the elephantid clade, with the close divergence times for the three genera reflected in a tight clustering of the *Mammuthus*, *Loxodonta* and *Elephas* sequences.

Previous studies using protein analysis have failed to resolve the trichotomy between the three genera. Using a radioimmunoassay, Lowenstein and colleagues found the albumins of *Mammuthus*, *Loxodonta* and *Elephas* to be 99% immunologically homologous, consistent with an evolutionary divergence between 3 and 5 million years ago[9]. Our results are unable to resolve this trichotomy conclusively, although parsimony and maximum likelihood analysis with dolphin and rhinoceros as outgroups suggest that *Mammuthus* and *Loxodonta* could be sister taxa in a monophyletic clade, in contrast to the anatomical evidence. Sequencing data from additional informative mtDNA regions and a larger number of individuals, from both the living and extinct genera, are needed before the evolutionary relationships of the elephantids can be fully understood.

Erika Hagelberg
Mark G. Thomas
Charles E. Cook Jr
Department of Biological Anthropology, University of Cambridge, Cambridge CB2 3DZ, UK
Andrei V. Sher
Institute of Evolutionary Animal Morphology and Ecology, 117071 Moscow, Russia
Gennady F. Baryshnikov
Zoological Institute, 199034 St Petersburg, Russia
Adrian M. Lister
Department of Biology, University College London, London WC1E 6BT, UK

1. Lindahl, T. *Nature* **362**, 709–715 (1993).
2. Lindahl, T. *Nature* **365**, 700 (1993).
3. Lister, A. M. *Trends Ecol. Evol.* **4**, 362–363 (1989).
4. Vereshchagin, N. K. & Nikolaev, A. I. *Trudy Zoologicheskovo Instituta* **111**, 3–17 (1982).
5. Kaplina, T. N. *et al. Bull. Comm. Quat. Res.* **50**, 73–95 (1980).
6. Hagelberg, E. & Clegg, J. B. *Proc. R. Soc.* B**244**, 45–50 (1991).
7. Kocher, T. D. *et al. Proc. natn. Acad. Sci. U.S.A.* **86**, 6196–6200 (1989).
8. Irwin, D. M., Kocher, T. D. & Wilson, A. C. *J. molec. Evol.* **32**, 128–144 (1991).
9. Shoshani, J., Lowenstein, J. M., Walz, D. A. & Goodman, M. *Paleobiology* **11**, 429–437 (1985).

Peptide design

SIR — Jameson *et al.*[1] reported the rational design of a modified cyclic peptide from the CDR3 region of CD4 receptors of T cells which displayed considerable therapeutic effects both *in vitro* and *in vivo* in the experimental mouse model for allergic encephalomyelitis. The significant *in vivo* biological activity of this reverse-sequence peptide probably results from the fact that it contains all D-amino acids.

Brady and Dodson[2] summarized, in an associated News and Views article, how such a peptide analogue with reversed peptide bonds can introduce stability to proteolysis and at the same time stereochemically preserve the topological surface of the side chains in the original L-amino-acid sequence.

Readers should be made aware that this general approach to modification of the peptide backbone has a rather rich history in the areas of peptide medicinal chemistry and bioorganic chemistry. Cyclic retro-inverso peptides[3] and linear, partial retro-inverso pseudopeptides[4] have long been used as relatively stable peptidomimetics for numerous bioactive peptides. A recent review by Chorev and Goodman[5] provides entry into the literature of retro-inverso isomers in peptide design and synthesis.

David B. Glass
Departments of Pharmacology and Biochemistry, Emory University School of Medicine, Atlanta, Georgia 30322, USA

1. Jameson, B. A., McDonnell, J. M., Marini, J. C. & Korngold, R. *Nature* **368**, 744–746 (1994).
2. Brady, L. & Dodson, G. *Nature* **368**, 692–693 (1994).
3. Prelog, V. & Gerlach, H. *Helv. chim. Acta* **47**, 2288–2294 (1964).
4. Goodman, M. & Chorev, M. *Acc. chem. Res.* **12**, 1–7 (1979).
5. Chorev, M. & Goodman, M. *Acc. chem. Res.* **26**, 266–273 (1993).