

New mitochondrial data demonstrating a close relationship between *Mammuthus primigenius* (Blumenbach, 1799) and *Loxodonta africana* (Blumenbach, 1797)

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SUMMARY: The question of the phylogenetical relationships between recent Elephantidae has been raised on morphological but also molecular grounds. Mitochondrial data (cytochrome b gene particularly) either support or question the classical scheme which associates *Mammuthus primigenius* with the Indian elephant, *Elephas maximus* Linné 1758. Recent studies depict a closer relationship between mammoth and African elephant, *Loxodonta africana*. We report the partial sequencing of two mitochondrial markers from a 49,000 years old Siberian mammoth. The comparison of its sequence with 10 other mammoths and with African (both bush and forest forms) and Asian elephants gives weight to this hypothesis. However, a previous study of the same specimen (Yang *et al.* 1996) produced a highly divergent sequence that clusters with Indian elephants. The accuracy of the two alternative sequences is evaluated and leads us to the conclusion that the woolly mammoths form the monophyletic sister-group of African elephants on molecular grounds.

1. EXTRACTION AND SEQUENCING OF DNA FROM THE LYAKHOV MAMMOTH

We performed DNA extraction on the only complete specimen of Siberian mammoth conserved out of Russian territory. This specimen, a male adult that lived on Lyakhov main island (North-East Siberia) more than 49,000 years ago, was offered to the Muséum National d'Histoire Naturelle in 1912. Its skeleton is visible at the Galerie de Paléontologie (MNHN, Paris, France). Samples of dry skin and a sesamoide bone of the right foot were collected and submitted to a phenol/chloroform extraction protocol (Hassanin *et al.* 1998). No positive extraction was carried out for skin samples although a previous study, led by Yang *et al.* (1996), had used the same tissue from this specimen as a valid source of DNA prior to direct sequencing of partial cytochrome b gene.

On the other hand, positive extracts were obtained with the bone. We determined the maximal size of amplifiable DNA; thanks to a

complete range of PCR length from 100 to 250 bp, we yielded usable products up to 180 bp. These results are consistent with other studies on similar material concerning fragmentation of DNA through time. They are also far less enthusiastic than those of Yang *et al.* (1996) who retrieved fragments up to 400 bp using extracts of DNA from skin.

The repeatable positive PCR we obtained were sequenced manually with 15 primers determining overlapping domains. 566 bp of cytochrome b and 128 bp of 12S ribosomal DNA were finally considered.

2. COMPARISON BETWEEN THE TWO SEQUENCES OF CYTOCHROME B FROM LYAKHOV MAMMOTH

Yang *et al.* (1996) sequenced a 228 bp domain of cytochrome b that our sequence (Debruyne *et al.*, in prep.) completely overlaps. Along this domain, these two sequences differ on 9 positions (Fig. 1). For each of these positions, our sequence displays the typical charac-

	111111111111222222222222333
	1344556678801122246678011
	7245062513976935864705025
<i>M.p.</i> Noro	AAGTTCTTTTTCTCTCTATTCCATG
<i>M.p.</i> Ozawa
<i>M.p.</i> Thomas (a)
<i>M.p.</i> Thomas (b)
<i>M.p.</i> Thomas (c)T.....
<i>M.p.</i> Thomas (d)	.GA.....T....C.....
<i>M.p.</i> Derenko
<i>M.p.</i> Greenwood	.G.....
<i>M.p.</i> this study (Lyakhov)
<i>M.p.</i> Yang (b) (Lyakhov)	G.A.C..C...T.G..CG.N...A
<i>M.p.</i> Yang (a)	G.A.C..C...TCG..C.CC....
<i>E.m. indicus</i> (India)	G.A.C..C...C...CG.CT...A
<i>L.a. africana</i> (S.-Afr)	..A.C...C...T.G..C.C.....
<i>L.a. cyclotis</i> (S.-Leone)	..ACC.CCC...A.TC.....G.A
<i>M. americanum</i> Yang	..A.C...CCC...ATC.CC.T.CA

Fig.1 - Display of variable positions between the two sequences of Lyakhov mammoth (in boldface). *M.p.* : *Mammuthus primigenius*; *E.m.*: *Elephas maximus*; *L.a.*: *Loxodonta africana*; *M. americanum*: *Mammuth americanum*.

ter found among sequences of mammoths. On the other hand, the sequence of Yang *et al.* (1996) shows the same character states as Asian elephants for 7 of these positions (the 2 other ones being shared with African elephants only).

When unweighted-parsimony analysis is applied to this fragment, these two sequences are kept separated: our sequence clusters with 8 sequences of other mammoths from 5 different studies (Derenko *et al.* 1997; Greenwood *et al.*

1999, Noro *et al.* 1998; Ozawa *et al.* 1997; Thomas *et al.* 2000) while the other sequence of Lyakhov mammoth branches deeply within Asian elephant sequences (Fig. 2).

Considering the material studied, the conditions of DNA extraction (our inability to reproduce extracts using skin samples is notable), the differential length of fragments amplified by Yang *et al.* (1996) and this study, and the discrepancy between the two sequences of cytochrome b for Lyakhov mammoth, we consider that Yang *et al.*'s sequence is the result of a contamination by non-identified exogene DNA.

3. AFFINITIES OF THE MAMMOTH CLADE

With the exception of Yang *et al.*'s sequences, all the sequences of woolly mammoths form a clade (Fig. 2). The 8 longest sequences (including ours) cluster and are highly supported by both statistical indices and Bremer score (more details in Debruyne *et al.* in prep.).

Nevertheless, the sister-group association of this mammoth clade with African elephants is not so well supported. To determine whether this monophyly is robust and reliable, we per-

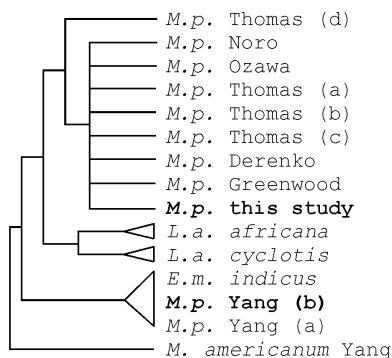


Fig.2 - Summary of the analyses of cytochrome b gene (acronyms see Fig. 1).

formed several treatments on the matrix:

(1) application of a general-reversible model of likelihood analysis with heterogeneity in the variation of the positions.

(2) Hassanin *et al.* (1998) weighting was used to estimate the impact of mutational saturation on topology.

(3) decompositions of the matrix following the ordination of values for retention index of the positions demonstrated the reliability of the basal node of Elephantids.

(4) application of the PRN method (Lecointre *et al.* 1994) was used to estimate whether the molecular data available for woolly mammoths are sufficient to establish the close relationships of this species towards extant elephants.

None of these treatments was able to dismiss the association of *Mammuthus* and *Loxodonta* in a clade.

3. REFERENCES

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