

Supporting Information

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SI Text: Amino Acid Differences Between the M1 and M25 mtDNA Genomes

Sequence data from 13 mammoth mitochondrial proteins showed between 1 and 5 sequence differences between individuals M1 and M25 (one case, *ND4*, showed a C-terminal insertion of five residues). In all cases, the substitutions were conservative. That is, all involve substitutions within the set of hydrophobic residues, extended to include Thr and Ser, except for one: an Asn-Asp change in *ND4*. It was possible to predict structures for four of the proteins by homology modeling techniques [using SWISS-MODEL (1, 2)]. Table S3 shows the details of these calculations. The sequence similarities between parent and target molecules were all at least 78% residue identity, suggesting that the quality of the models should be quite high. (All four parent structures were bovine homologues.)

Pictures of the modeled structures show that the sites of mutation are on the surface. Fig. S2 shows the sites of mutation in the structures for *Cytb* and *COX1*.

Comparison with Elephant Sequences. Alignment of *COX1* and *Cytb* sequences between the two mammoth individuals and two of the extant elephant species showed alignments with no insertions or deletions. The sets of sequences are very similar, with almost all

positions constant. When judging only from the *COX1* and *Cytb* protein sequences, the data suggest that mammoths are more closely related to African rather than Asian elephants. In this regard, we find 7 out of 16 sequence differences in *Cytb* and 3 out of 7 in *COX1*, at which the two mammoths and *L. africana* have a common residue but *E. maximus* has a different one. However, at position 371 of the *Cytb* alignment, both mammoths and *E. maximus* have Met, and *L. africana* has Val.

The alignments are shown in Fig. S3. At position 14 of the *CYTB* alignment, M25 has the same residue as the extant elephants, different from M1. At positions 159 and 238 of the *CYTB* alignment, both mammoths have the same residue (159 Asp, 238 Phe), and both extant elephants have the same residue (159 Asn, 238 Leu); a similar distribution appears at residues 329, 330, and 334. At position 303 of the *Cytb* alignment, M25 and M1 have different residues (Ile and Met, respectively), whereas the two extant elephants have Leu. At position 345, M1 and *L. africana* have Tyr but M25 and *E. maximus* have His.

The four *COX1* sequences are very similar until near the C terminus, where the extant elephants have a C-terminal extension.

Conclusion. There is no reason to suggest that any of the sequence changes is non-neutral.

1. Guex N, Peitsch M.C (1997) SWISS-MODEL and the Swiss-PdbViewer: An environment for comparative protein modelling. *Electrophoresis* 18:2714–2723.

2. Schwede T, Kopp J, Guex N, Peitsch PE (2003) SWISS-MODEL: An automated protein homology-modeling server. *Nucleic Acids Res* 31:3381–3385.



Fig. S2. Sites of mutation in the structures for Cytb and Cox1.

Cytochrome b

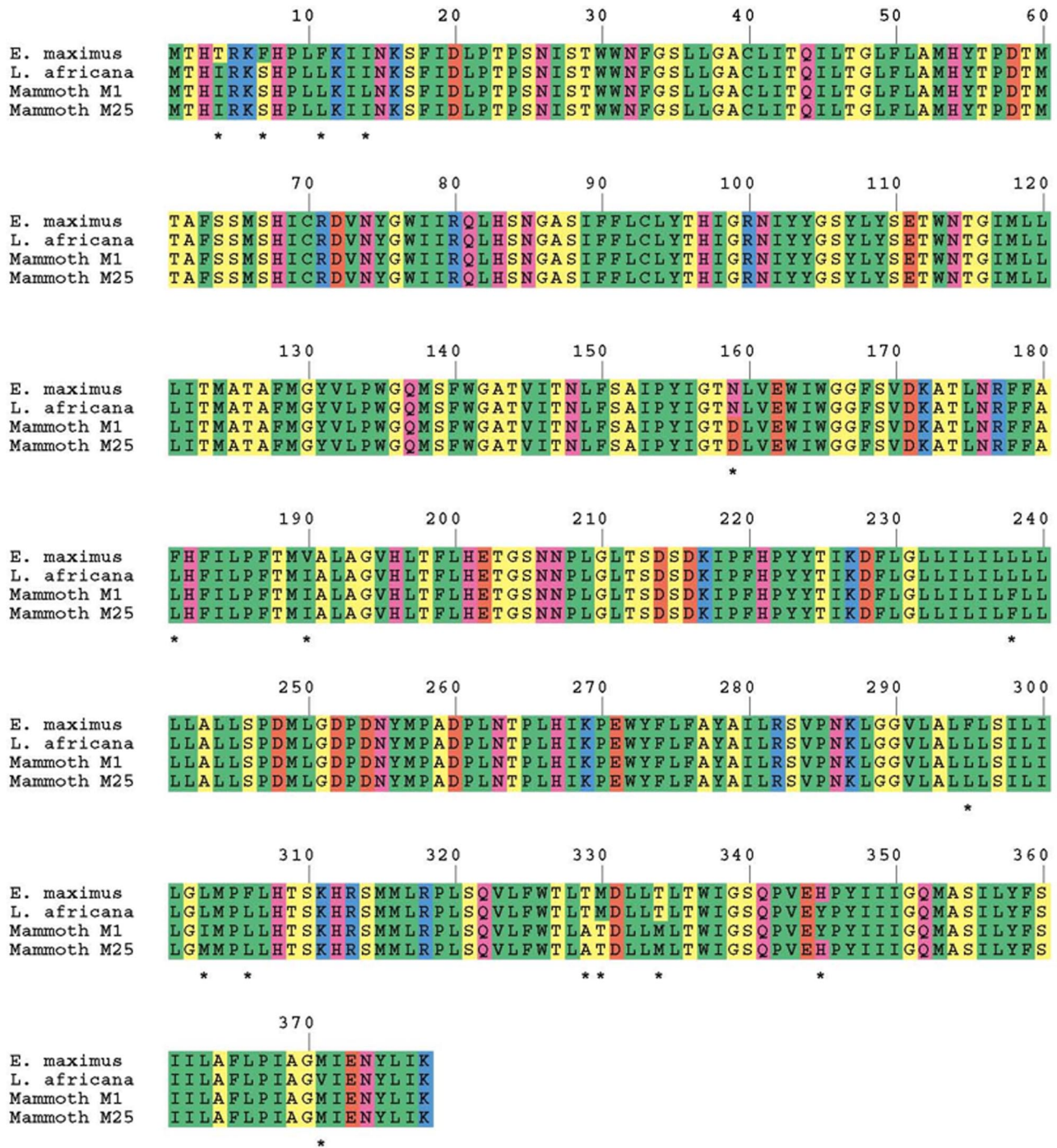


Fig. S3. (Figure continues on the next page.)

COX1

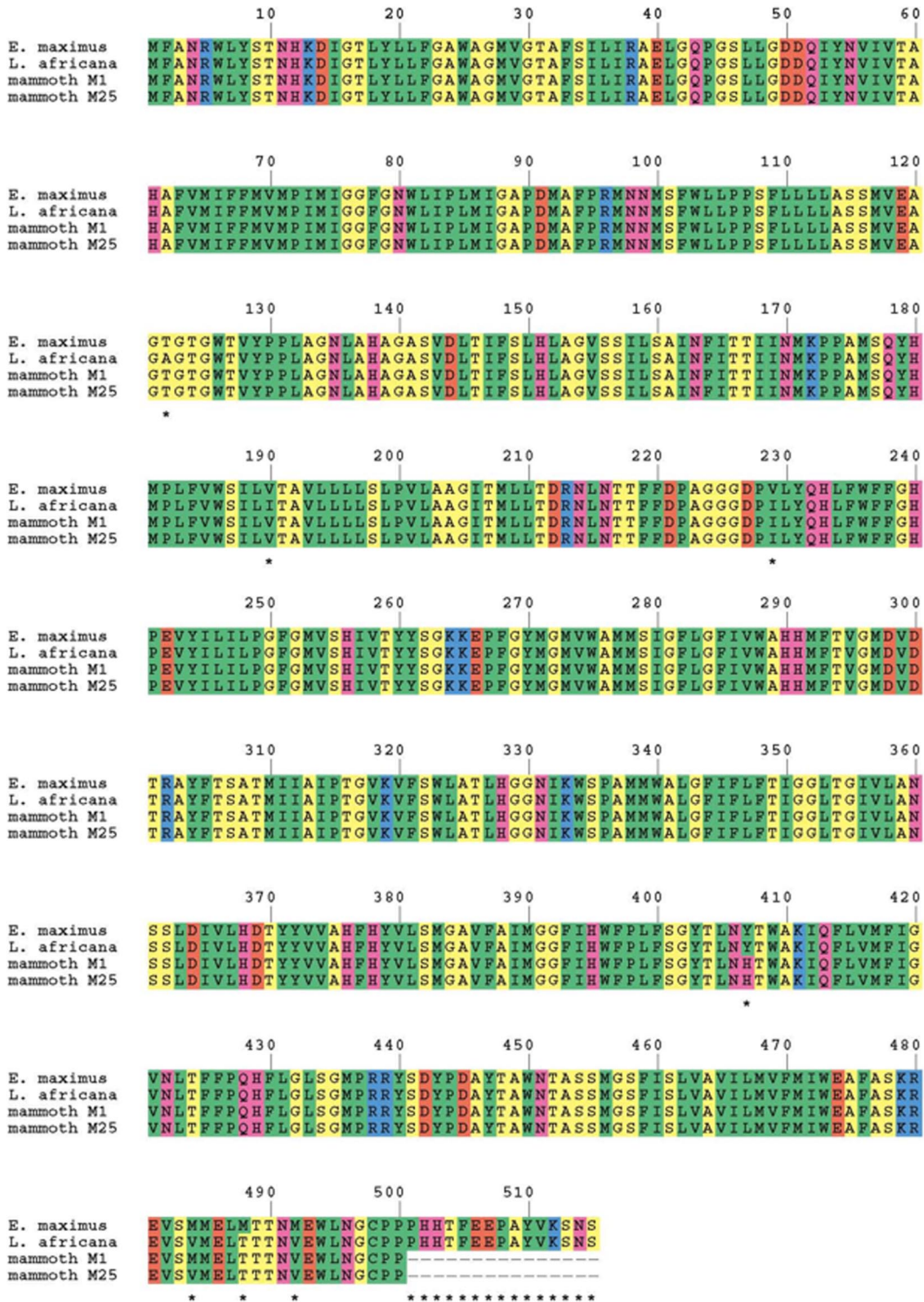


Fig. S3. Alignments of elephant and mammoth *Cytb* and COX1 sequences.

Table S1. Sample details

Sample	Museum/ other ID	Geographic origin	Sample source	Amount of material used, g	Size of digestion, ml	GPS east	GPS north	¹⁴ C accession no./source	¹⁴ C age	Collected by	Collection year	Sampled by
M15	Site 5 (ref. 1)	Ayon Island, Russia	Universita Degli Studi Di Parma, Italy	0.43	15	169	69.8	OXA-19605	13,995 ± 55	No information available	No information available	P. Iacuminn
M19	Yukagir Mammoth	Yukagir Village, Yakutsk, Russia	Zoological Institute, Russian Academy of Sciences	0.6	15	140.34.873	71.52.988	GrN-28258, GrN-28259, GrN-24288 (ref. 2)	18,560 ± 50	No information available	2003	A. Tikhonov
M20	LDR-P72	Bolshoy Lyakhovskiy Island, Russia	Lena Delta Reserve	0.35	15	142.89	73.64	OXA-19608	>63,500	F. Selyakhov	2000	T. Kuznetsova
M21	LDR-P73	Bolshoy Lyakhovskiy Island, Russia	Lena Delta Reserve	0.2	15	143.6	73.21	OXA-19609	>58,000	I. Mikolauskas	2001	T. Kuznetsova
M25	F-0299	Bolshaya Chuokhya River, Russia	Ice Age Museum	5.2	30	157.7	69.79	OXA-19610	59,300 ± 2,700	F. Shidlovskiy	2003	A. Sher

1. Iacumin P, Davanzo S, Nikolaev V (2006) Spatial and temporal variations in the ¹³C/¹²C and ¹⁵N/¹⁴N ratios of mammoth hairs: Palaeodiet and palaeoclimatic implications. *Chem Geol* 231:16–28.
 2. Aptroot A, van Geel B (2006) Fungi of the colon of the Yukagir Mammoth and from stratigraphically related permafrost samples. *Rev Palaeobot Palynol* 141:225–230.

Table S2. Primers and regions PCR amplified and sequenced for specimen M25 to confirm SNPs that differ from the Krause reference mammoth

Primer set	Forward primer 5' → 3'	First base of F primer*	Reverse primer 5' → 3'	Amplicon size	Annealing temperature
1	TGTAGCTTAAAAAAAAGCAAGG	6	GGCGAGACGTCATGAGCTAC	215	60
3	GACGGTCAAAGATGGGCTAC	775	AATCTGTAATGATTGTTTGATGTGG	191	60
4	AGTACCGCAAGGGAATGATG	1212	GGCTCGATTGGTATTTCCACC	225	60
6	GATCGGATGCCCACTGATAG	1782	GGCCGTTTAACTTGGTCA	221	60
7	TAACAATTTTGGTTGGGGTGA	2235	TCGAGGTCGTAACCCTGCT	190	60
8	AGCCGCTATTAAGGGTTCGT	2452	CTGTTCTGGATTGGGTGGT	210	60
9	GCAGTAGCCTTCCTCACCTT	2786	TCAGACTTGATATTGCGAGAATAAAA	289	60
10	ACCATATCCCCTAATTAACCTAAATCT	3013	GGTTAGTGTAAGGACCCATTATT	217	60
13	AGACAACCAATCCACGATCC	4042	TGGTGCTAATTTTGTGAGGTT	278	60
14	GGAATGATCTTATAAACCCTGACAAAA	4284	TGAAGTTGTGAGGGTATTGTCT	300	60
15	TCCCTAACTAATATGAAACAAAAACA	4590	AGATAGGGTGATTAGAGTCGGTAA	315	60
17	TCTTGAGACGACCAAAATCT	5471	TGCTGTCCCAGCTTCTACT	230	60
18	CACCTTGACAGGATATCCTCTATT	5781	CCTGCAGGGTCAAAGAAAAGT	221	60
19	CTACCCTTCATGGCGGTAATA	6304	GAATTTTGGCTCATGTATGGTTT	264	60
20	TTTATCGGTGTTAATTTGACATTCTT	6582	CGTTTGTGTTGTGAGTTCTATCA	223	60
21	CAAGCCAACTCTATAACCTTTATGC	6911	TGGACGTTTATTATTTGTGTGGA	286	60
22	TCCACACAAATATAATAAACGTCCA	7172	AGCTCCAGAATCATTGGTGTC	171	60
23	CCTACAGATCTCCAGTTCCG	7453	TGCTGACCAGCTTTCAAAGTA	240	60
24	TCTCCTTAATGAATGGAACGAA	7762	TCGAAGAAGGCGGATAGTTC	202	60
25	TCCAACCTCAAATCGCCTAA	8015	TTTATATCGGAAGCCAGGA	268	60
26	TCACCAAACACATGCCTATCA	8608	AAAAGGCTCAGAAGAAACCTG	299	60
27	CAGGTTTCTTCTGAGCCTTTTA	8886	GCCATAGATACCGTCGGAGA	299	60
28	ACCTGAGCTCATCACAGCTTAAT	9035	ACGTCTACGAAATGTCAATATCAA	308	60
29	CATTTACTGATGAGGATCCTATTTCTT	9367	GCGATTTCTAGGTCAAATAGGAGA	299	60
30	TTTACCCTGAGCTATCCAAGC	9678	ATTGAGGGATAATAGTGCATTTAAG	346	60
31	TGTTTACCTTAAATGCACTATTATCC	9992	GGCTAGTTAGACTAATTGCGAAGC	287	60
32	CCTCCGGATCAACTTTTCAG	10417	AATGGATTTGGCTAGGTATGG	305	60
33	GGATCTCTCCCCTACTAGTAACC	10629	AAGGCTATGTGGCTGACTGAA	422	60
34	CCATGGCCTAACTTCCTCAA	11117	TGTTCTCTCGGGTAAATGAAGG	350	60
35	TTTACCCGAGAGAACACCTTG	11451	GAAAGCCACATTGTTAGATGAGG	216	60
36	TCAACCCAATCTCAACCCTA	11741	CTGTTTCGTCCTGCCATC	486	60
37	ACGGACGAACAGACGTAAC	12215	TGATGGAAGTCACGGATGAA	230	60
38	ACCCTCATCCGCTTTTATCC	12514	GCTGTTGAAGTAATGGGTATTGTTT	341	60
39	GGTTTTACTCAAAGACCCAATTAT	12902	CGGAATAGTATTTATTGAGATGAAGA	260	60
40	CGCATACAACACCCCAAATA	13160	GGCTTAGGTAGGGCAGGATT	204	60
41	CCAAAACCTCCCTTCAACAAAG	13675	GGGGGATCTTTCTTGGGTCT	260	60
42	CCACTATCATCCCACCGAAA	13877	TGGTAGGAGTCCATGTAGAGGT	220	60
43	GACTAATGATCTGAAAAACCATCG	14097	TGCTCCGTTTGTAGTGTAGTTG	315	60
44	CCTACCTATACTCGGAAACCTGAA	14467	AGCCTGTTTCGTGAAGGAAG	297	60
45	ACCTTCCTCACGAAACAGG	14742	ATCGTAGGATGGCGTAAGCA	256	60
46	CCTAGCCCTACTCCTATCAATCC	15023	GGGTTACTTAATGAGGTAGTTTTCCG	268	60
47	GGTATTCAGGGAAGAGGTCCA	15349	CACGAATATGACTTGACACATTGA	255	60
49	CCATCTTCGTGTCCTCTTC	15784	ACCAAATGCATGACACCACA	200	60

*Numbering with reference to the revised Krause sequence [Krause J, *et al.* (2006) Multiplex amplification of the mammoth mitochondrial genome and the evolution of Elephantidae. *Nature* 439:724–727], GenBank entry DQ188829.2

Table S4. Structure prediction calculations

Gene name	Parent structure	% sequences similarity to parent	Residues predicted
<i>COX1</i>	1occA	93	1–511
<i>COX2</i>	1v54B	84	2–226
<i>COX3</i>	1v54C	87	3–261
<i>Cytb</i>	2fyuC	78	2–378
<i>ATP8</i>	n.a.		
<i>ATP6</i>	n.a.		
<i>ND1</i>	n.a.		
<i>ND2</i>	n.a.		
<i>ND3</i>	n.a.		
<i>ND4L</i>	n.a.		
<i>ND4</i>	n.a.		
<i>ND5</i>	n.a.		
<i>ND6</i>	n.a.		