

## Genetic data indicating that *Mammuthus primigenius* includes two species

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**SUMMARY:** The data on genetic differentiation within mammoths are considered in comparison with differentiation between extant elephants. Genetic differentiation within mammoths is high, they can be divided into two groups genetic, the distance between which is comparable to the distance between *Loxodonta africana* and *Elephas maximus*. This suggests the existence of two early diverging mammoth lineages, i.e., *Mammuthus primigenius* probably includes two twin species.

During the last years, the methods for amplification and sequencing of DNA fragments were applied to fossil remains and provided a means for comparative genetic studies on extinct and related living forms. In particular, successful PCR amplification and sequencing was performed for the following mitochondrial genes of mammoths and extant elephants: 93-base-pair (bp) fragment of the 16S ribosomal RNA gene (Hoss *et al.* 1994); 961 bp (complete sequence) of the 12S rRNA gene (Noro *et al.* 1998); and fragments of the cytochrome *b* gene of 242 bp, 277 bp (Hagelberg *et al.* 1994), 228 bp (Yang *et al.* 1996), 1005 bp (Ozawa *et al.* 1997) and complete sequence of 1137 bp (Noro *et al.* 1998). The main results of these studies were mutually contradictory. The researchers concluded that the results were unable to resolve conclusively the *Mammuthus-Loxodonta-Elephas* trichotomy (Hagelberg *et al.* 1994) or reported that they resolved the trichotomy in favour of closer relations between mammoths and the Asian elephants (Yang *et al.* 1996; Ozawa *et al.* 1997), or between mammoths and the African elephants (Noro 1998). However, a comparative analysis of all available genetic data (Dubrovo & Rautian 1999; Rautian & Dubrovo 2001) has shown that genetic distances *Mammuthus-Elephas*, *Mammuthus-Loxodonta* and *Loxodonta-Elephas*

non-significantly differ from each other. Thus, mammoth is approximately equidistant from each living elephant. This suggests simultaneous divergence of the lineages of mammoth and living elephants from a common ancestral stock as a result of one and the same event of adaptive radiation. This conclusion agrees with the results of immunological studies (Lowenstein 1985) and comparisons of hair structure (Valente 1983); in these parameters, the distances between three elephantine genera were approximately equal to each other.

The purpose of this paper is a brief review of the data on genetic differentiation within mammoths in comparison with differentiation between extant elephants.

Relatively extensive material was obtained for the fragments of the cytochrome *b* gene examined independently by several research groups in six mammoths and a number of Asian and African elephants (Hagelberg *et al.* 1994; Yang *et al.* 1996; Ozawa *et al.* 1997; Noro *et al.* 1998). We combined the data from these papers and analysed the sequence of 218 bp (from base 96 to 282 and from base 289 to 319), available in each individual. The table shows pair-wise distances computed as the percentage of substitutions corrected by Kimura's two-parameter model (Kimura 1980). These data allow us to compare the differentiation within

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Tab. 1 - Sequence distances among cytochrome b fragments of (M1-M6) *Mammuthus primigenius*, (L1-L6) *Loxodonta africana* and (E1-E6) *Elephas maximus*

	M1	M2	M3	M4	M5	M6	L1, 3,6	L2	L4	L5	E1	E2	E3, 4,5	E6
M1	0	5/0	4/0	6/1	6/1	4/0	4/1	4/1	3/1	4/2	6/0	9/0	6/0	10/0
M2	.024	0	1/0	9/1	9/1	1/0	7/1	7/1	8/1	7/2	7/0	10/0	7/0	11/0
M3	.019	.005	0	8/1	8/1	1/0	6/1	6/1	7/1	6/2	6/0	9/0	6/0	10/0
M4	.033	.048	.043	0	4/0	8/1	6/0	6/0	7/0	7/1	4/1	3/1	4/1	4/1
M5	.033	.048	.043	.019	0	8/1	4/0	4/0	5/0	5/1	4/1	5/1	4/1	6/1
M6	.019	.005	.005	.043	.043	0	6/1	6/1	7/1	6/2	6/0	9/0	6/0	10/0
L1,3,6	.023	.038	.033	.028	.019	.033	0	2/0	3/0	1/1	6/1	9/1	6/1	10/1
L2	.023	.038	.033	.028	.019	.033	.009	0	1/0	3/1	6/1	9/1	6/1	10/1
L4	.019	.043	.038	.033	.024	.038	.014	.005	0	4/1	7/1	10/1	7/1	11/1
L5	.028	.043	.038	.038	.028	.038	.009	.019	.023	0	6/2	9/2	6/2	10/2
E1	.028	.033	.028	.023	.023	.028	.033	.033	.038	.038	0	3/0	1/0	4/0
E2	.043	.048	.043	.019	.028	.043	.048	.048	.053	.053	.014	0	3/0	1/0
E3,4,5	.028	.033	.028	.023	.023	.028	.033	.033	.038	.038	.005	.014	0	4/0
E6	.048	.053	.048	.023	.033	.048	.053	.053	.058	.058	.019	.005	.019	0

The upper right matrix includes the number of transitions/number of transversions. The lower left matrix includes Kimura two-parameter distances.

Primary data on M1 and M2 were obtained by Hagelberg *et al.* (1994); M3, by Noro *et al.* (1998); M4 and M5, by Yang *et al.* (1996); M6, by Ozawa *et al.* (1997); L1-L4, by Noro *et al.* (1998); L5, by Irwin *et al.* (1991); L6, by Yang *et al.* (1996); E1-E4, by Noro *et al.* (1998); E5, by Hagelberg *et al.* (1994); E6, by Yang *et al.* (1996).

(L1,3,6) and (E3,4,5) designate three African and three Asian elephants possessing identical DNA sequences.

mammoths with those of extant elephants and with intergeneric differences.

The mean distances between the pairs *Mammuthus-Elephas*, *Mammuthus-Loxodonta* and *Loxodonta-Elephas* are 0.034, 0.032 and 0.044, respectively. These distances non-significantly differ from each other (Rautian & Dubrovo 2001). The mean values of intraspecies distances in *Mammuthus*, *Elephas* and *Loxodonta* are 0.029, 0.008 and 0.009, respectively. Thus, extant elephants show much lower individual variation than mammoths, whereas differentiation within the latter is comparable to intergeneric differentiation. This was not revealed by the authors of original data because they did not analyse combined primary data but compared their results with only the conclusions of earlier studies.

To create a graphic representation of relationships between considered individuals, we applied the method of multidimensional scaling (Fig.1) to the matrix of genetic distances (Kruskal 1964). The individuals of each extant elephant species form a relatively dense group, whereas mammoths are divided into two groups (M1-M2-M3-M6 and M4-M5). It should be noted that relative positions of mammoths and elephants remain approximately the same in the cases where different numbers of dimensions are used (we tested the variants of two to six dimensions; the stress values were 0.0547, 0.0156, 0.0081, 0.0039, and 0.0024, respectively). The optimum number of dimensions is three that agrees with the presence of four different groups. The mean distance between the members of different mammoth

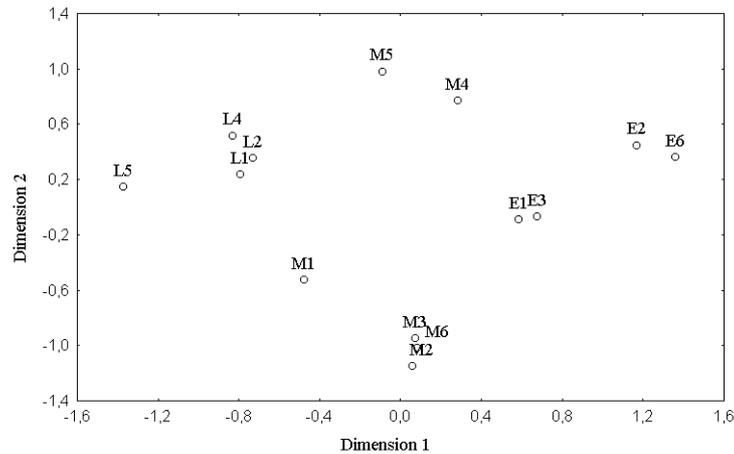


Fig.1 - The positions of mammoths and African and Asian elephants relative to the first two co-ordinate axes of multidimensional scaling of sequence distances based on a 218 base pairs of the cytochrome *b* gene. For designations of individuals, see the table.

groups is 0.042, i.e., approximately the same as between different elephant genera. This was the reason for the high mean distance within mammoths. At the same time, variation in either mammoth group is comparable to those in extant elephants, namely, the distance M4-M5 is 0.019 and the mean distance within M1-M2-M3-M6 is 0.014.

High differences between the mammoth groups could be explained by an extremely high diversity of *M. primigenius* or by belonging to different geographical or (and) temporal forms. The first possibility appears less probable because it implies that mammoths were substantially more diverse than living elephants. In addition, mammoths are divided into two genetic groups and intermediate individuals are absent.

Extremely high genetic differentiation within *M. primigenius* was also revealed in the study of the 16S rRNA gene fragment (Hoss *et al.* 1994). Four mammoths were examined and divided into two groups, the differences between which were comparable to or higher than the differences between Asian and African elephants.

Thus, the results of examination of the cytochrome *b* and 16S rRNA gene fragments

allow us to assume that *M. primigenius* is probably represented by at least two forms (apparent twin species) isolated from each other almost simultaneously with the radiation of lineages of Asian and African elephants and mammoths. Since at the moment of divergence, common ancestor of *Loxodonta*, *Elephas* and *Mammuthus* was more similar in plesiomorphic characters to the African elephant than to the Asian elephant and mammoth (Rautian & Dubrovo 2001); common ancestor of two mammoth branches should resemble the African elephant (lacking the synapomorphies characteristic of the Asian elephant and mammoths) to a greater extent than the Asian elephant. Consequently, the synapomorphies of the Asian elephant and each mammoth branch arose independently in each lineage and were absent in common ancestor (the latter possessed only the potentiality for developing these characters). Additional substantiation of this statements necessitate a thorough morphological examination of common and distinctive features within and between elephantine groups. An evolutionary scheme showing the time of divergence and the extent of morphological advantage in the elephantines considered is shown in figure 2.

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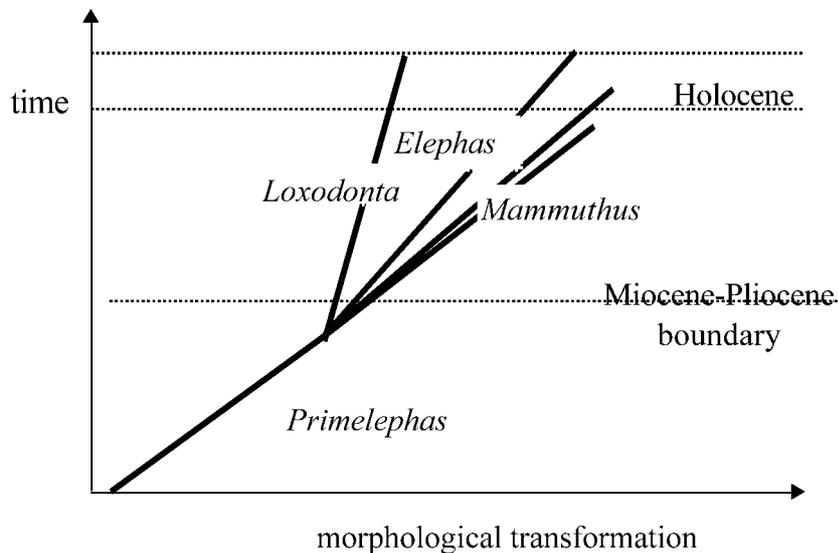


Fig.2 - Scheme of phylogenetic relationships among genetically studied elephantines. Relatively primitive morphological characters of *Loxodonta* are shown by its closer position to the common ancestor (relative to X-axis); similarity between *Elephas* and *Mammuthus* (represented by two lineages) in advanced morphological features are shown by their close position to each other and strong deviation from the ancestor. At the same time, the four forms radiated simultaneously, most probably at the boundary between the Miocene and Pliocene.

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