A statistical appraisal of molecular and morphological evidence for mammoth-elephant relationships

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SUMMARY: We present a DNA sequence analysis of the relationship between *Mammuthus, Loxodonta* and *Elephas*, and apply statistical techniques to compare the support for different trees. A *Loxodonta-Mammuthus* clade has the strongest support, but *Elephas-Mammuthus* cannot be ruled out. In morphological characters, several features which have been cited in support of an *Elephas-Mammuthus* relationship are convergent or primitive. The relationship among these three genera is still unresolved.

1. INTRODUCTION

Dental and skeletal morphology have been taken to indicate that *Elephas* and *Mammuthus* are more closely related than either is to *Loxodonta* (Kalb & Froehlich 1995; Shoshani *et al.* 1998). DNA sequence data have produced conflicting results: H.Yang et al. (1996) and Ozawa *et al.* (1997) suggest an *Elephas-Mammuthus* relationship, while Hagelberg *et al.* (1994), Noro *et al.* (1998) and Barriel *et al.* (1999) favoured a *Loxodonta-Mammuthus* clade.

2. MOLECULAR STUDY

DNA was obtained from bone of five *M. primigenius* individuals from Siberian per-

mafrost localities, and blood of 14 *E. maximus* and eight *L. africana*. DNA extraction from *M. primigenius* bone was as described in Hagelberg & Clegg (1991). Further preparation and sequencing was carried out as described in Thomas *et al.* (1997, 1998). A 567 base pair (bp) fragment of the cytochrome *b* gene was amplified from extracted DNA.

Comparison of the 27 sequences revealed ten haplotypes: four *Mammuthus*, three Loxodonta and three Elephas. We first conducted phylogenetic analysis using dugong as the outgroup. Twelve equally parsimonious trees were found (each requiring 163 steps); all had the same basic topology (Fig.1). The bootstrap score of 84% (1000 replicates) suggests that *Loxodonta* and *Mammuthus* are sister taxa. However, the
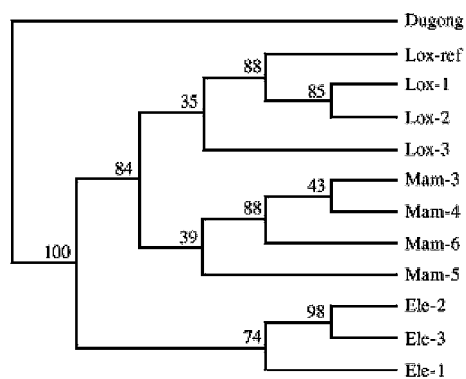

Fig.1 - Bootstrap consensus parsimony tree of elephantid cytochrome *b* sequences. The number at each node represents the percentage with which that group of sequences occurred in the 1000 bootstrap replicates.

Lox = *Loxodonta africana*,
Mam = *Mammuthus primigenius*,
Ele = *Elephas maximus*.

percentages on the nodes delineating each of these two species are low (35 and 39%, respectively).

Maximum-likelihood analysis gave a tree topology identical to one of the most parsimonious trees, and differing from the consensus parsimony tree only in the placement of one mammoth (Thomas *et al.* 2000). By using a Sirenia-Proboscidea split of 65 Ma for calibration, the divergence of the *Elephas* branch was estimated at 14.8 Ma ago, the *Mammuthus-Loxodonta* node at ca. 9.4 Ma ago. In contrast, fossil evidence places the origin of the Elephantidae at ca. 9 Ma ago, the earliest *Loxodonta* at ca. 5.5-6.0 Ma, *Mammuthus* tentatively at 4.0-4.5 Ma, and *Elephas* some 4.0-4.5 Ma (Kalb & Mebrate 1993; Kalb *et al.* 1996; Tassy 1986, 1995).

A possible reason for differences between fossil and molecular dates is lineage sorting, in other words that mtDNA coalescence for the Family Elephantidae, reflected in the cytochrome *b* tree, pre-dates the taxonomic origin of the family. We have shown, using plausible assumptions about divergence dates and population sizes, that there is a significant possibility the gene tree is not congruent with the species tree (Thomas *et al.* 2000). For future work, the use of many independent loci should overcome this problem. Another possible explanation for the high age estimates could be a faster rate of mtDNA evolution in Proboscidea than in Sirenia. To test this, a likelihood ratio test (Felsenstein 1995) compared the tree obtained under the assumption of a molecular clock with an unrooted version of the same tree. The small p-value ($0,05 < p < 0.1$) suggests rate variation among the elephantid lineages, so the DNA-estimated divergence dates must be treated with caution.

Because of the ancient divergence of elephants and dugong, and dissimilarity in their mtDNA transition-transversion ratios, dugong appears problematic as an outgroup. As an alternative way of locating the root of the tree, maximum-likelihood analysis was performed on the ingroup species only, with and without the assumption of a molecular clock and using two substitution models in the PAML package

(Yang Z. 1997). The same tree was obtained under all conditions, and a likelihood ratio test showed no significant difference between the clock and the no-clock models. Thus, within the Elephantidae, the molecular clock assumption holds. The root can therefore be located without an outgroup, and indicates an overall (Ele (Lox, Mam)) topology. Two methods were then used to assess the support for this topology. Using bootstrap proportions (Felsenstein 1995), a monophyletic Lox-Mam clade was supported in 67% of replicates, whereas a monophyletic Ele-Mam clade was supported in 29%. Using the Bayesian method of Yang Z. & Rannala (1997), the posterior probability score for the Lox-Mam clade was only 0.43. Moreover, the second and third best trees, with posterior probabilities of 0.28 and 0.20, respectively, grouped *Mammuthus* and *Elephas*.

To assess claims of resolution of elephantid phylogeny using American mastodon cytochrome b sequences (Yang H. *et al.* 1996), we constructed parsimony (with 1000 bootstrap resamplings) and maximum-likelihood trees using Yang H. *et al.*'s mastodon sequence instead of dugong as the outgroup, and combined all the sequences in our previous analysis, plus the *Mammuthus* and elephant sequences of Yang H. *et al.* (1996) and the *Mammuthus* and *Elephas* sequences of Ozawa *et al.* (1997). Bootstrap support for most ingroup branches was poor. Despite a number of tree topologies with different methods, one consistent feature was that the *Mammuthus* sequences of Yang H. *et al.* (1996) always formed a clade with *Elephas*, whereas the *Mammuthus* sequences of this study and of Ozawa *et al.* (1997) fell outside the *Elephas* clade (see also Derenko *et al.* 1997, Barriel *et al.* 1999). A monophyletic grouping of all the *M. primigenius* sequences is supported in only 0.018% of replicates. This degree of variation among the sequence data obtained from different M. primigenius samples is unexpected and at present unexplained.

3. Morphological study

Morphological characters for *Mammuthus, Loxodonta* and *Elephas*, previously cited as ev-

idence of their relationships, were examined on skulls of *M. meridionalis* (Early Pleistocene), *M. primigenius* (Late Pleistocene), *L. africana* and *E. maximus*.

Some characters are autapomorphous, e.g.

• the 'globular skull' of *Loxodonta* which appears, from Kalb & Mebrate's (1993) analysis, to be an autapomorphy, *Elephas* and *Mammuthus* merely retaining the primitive, narrow-skulled condition.

Other characters are convergent, e.g.

• the high cranium of late *Elephas* and *Mammuthus*, since early skulls of each are lower (Maglio 1973; Boeuf 1983; Lister 1996); see figure 2. Correlation with this cranial expansion may account for several other characters that have been described as synapomorphies of *Elephas & Mammuthus*:

• large dorsal parietal bulges (Tassy & Shoshani 1988; Shoshani *et al.* 1998);

• recessed occipital condyles (Kalb & Froehlich 1995; Kalb *et al.* 1996), due to overlapping by the posterior cranium (Fig. 2);

• concave fronto-parietal region (Kalb & Froehlich 1995; Kalb *et al.* 1996; Shoshani *et al.* 1998), a feature which has been linked (Tassy & Shoshani 1988; Tassy 1996) to the large dorsal parietal bulges.

• numerous enamel lamellae in the molars of late representatives of *Elephas* and *Mammuthus*, mentioned as a synapomorphy by various authors (e.g. Tassy & Shoshani 1988). The earliest representatives of these genera have no more lamellae than the earliest *Loxodonta* (Maglio 1973; Fig. 2).

Three published characters may be phylogenetically informative:

• gracile stylohyoid bone (Tassy & Shoshani 1988; Tassy 1996; Shoshani *et al.* 1998), linking *Elephas & Mammuthus.*

• prominent, close maxillary ridges (inter-alveolar cristae) (Tassy & Shoshani 1988; Tassy 1996; Shoshani *et al.* 1998). Our observations confirm this character as valid in the terminal species *M. primigenius* and *E. maximus*; and illustrations in Boeuf (1983) indicate that it occurs in the more primitive *M. meridionalis* too, linking *Elephas* and *Mammuthus*.

• flared premaxillary bones (Kalb & Froehlich 1995), apparently linking *Loxodonta* and *Mammuthus*. This character appears in the earliest *Mammuthus*, but in later *Mammuthus* the premaxillaries are subparallel and not at all flared (Lister 1996), apparently convergent to *Elephas*. Conversely in some later *Elephas* (e.g. *E. antiquus*) they are very strongly flared
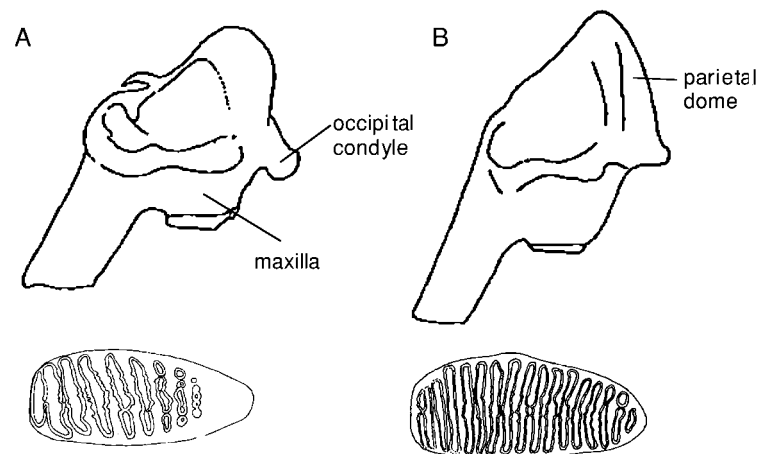

Fig.2 - Skulls and molars of (A) *Mammuthus meridionalis*, (B) *M. primigenius*, showing derived features of expanded parietal domes, less prominent occipital condyles, deep maxilla (and hypsodont molars) and high lamellar frequency, all appear to be convergent to *Elephas maximus*. After Lister (1996).

(Maglio 1973; Osborn 1942), apparently convergent to *Loxodonta*. This illustrates the importance of scoring early representatives of each genus.

4. DISCUSSION

Elephantid phylogeny presents a classic three-taxon phylogenetic problem. The fossil data give a framework of 0-5 Ma for the interval between the first and second nodes within the phylogeny. At the lower limit, the nodes may be so close together that their resolution with either molecular or morphological data is difficult. At the upper limit, with the nodes up to 5 Ma apart, sufficient change should have accumulated to allow their resolution. It is hoped that further molecular and morphological work will resolve this question.

5. ACKNOWLEDGEMENTS

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