Observations on the microstructure of fossil tusks from the Charkadio cave (Tilos, Dodekanese, Greece)

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SUMMARY: Two fossil tusks of the dwarf elephants from the Quaternary locality of Tilos from (45 ka BP to 4-3.5 ka BP) were studied in regard to the microstructure of the dentine part. Measurements were taken of the dentinal tubule density and of the Schreger angles. These were compared to known values for mammoths, mastodones, and the two extant elephant species. These measurements suggest a closer relation of the dwarf elephants from Tilos to the extant species than to mammoths. Also the dentinal tubule density of the two specimens features a wide range that requires further study, especially regarding the ontogenetic stage of the animal.

1. Scope, Material and Methodology

The scope of this study is to increase our knowledge of the internal structure of the dwarf elephant tusks from the Charkadio Cave on the island of Tilos. The skeletal parts of the species first appear in the sediments of the cave about 45 ka BP and become extinct almost 4-3.5 ka BP (Symeonides 1972, Bachmayer *et al.* 1976, 1984, Theodorou 1983, 1988). The material had originally been designated to *Palaeoloxodon antiquus falconeri* Busk (Symeonidis 1972). However, this name refers to the dwarf elephants from the island of Malta. As a result, since no migration route between the two islands can be proved, this name should not be used when referring to the elephant remnants from Tilos. Theodorou (1983) has accepted the temporal use of this name, until further material can be examined (Theodorou & Symeonidis 2001, this volume).

The samples were selected according to the degree of completeness and preservation of the tusk. Two tusks were selected, one from a juvenile individual (T.00/144), and one from an adult one (T.00/53). Both tusks are lacking tip and base and appear extremely fragmented and brittle. However the relative position of the fragments can still be indicated. The tusk sample T.00/144 has a length of 20.55 cm from proximal to distal end (arch), while the same measurement for the tusk sample T.00/53 is 47.00 cm. The maximum and minimum diameters at the middle of the tusk are 4.05 cm and 3.65 cm respectively, for the tusk sample T.00/144. The same diameters for the tusk sample T.00/53 measure at 9.50 cm and 7.50 cm respectively. The mean width of the cementum layer is 3 mm for T.00/144 and 4 mm for T.00/53.

The sample T.00/144 was studied by optical analysis of the Schreger angles (OASA). In the preparation of the sections two aspects had to be considered: the fragility of the tusk and the consequent examination under the SEM. In order to avoid embedding, the tusk was encased in plaster. Ten transverse sections were made, recording the distance of each section from the proximal end of the tusk. The surfaces created were sufficiently flat, so that no polishing would be required. Afterwards, the sections were scanned, with a resolution of 1000 dpi, and the images were processed with a photo-processing program. The Schreger angles were then measured at high magnification.

The dentinal tubule density can be measured on microphotographs of fractured surfaces (Forssell-Ahlberg *et al.* 1975), chosen parallel to the periphery of the tusk.

Thirty stub samples were taken from several points along the inner surface of the cementum-dentine junction (CDJ), of both tusks, in order to observe the circumferential surface of the tusk under the SEM. The sample surface was covered with a conductive gold layer to evacuate charges.

2. Observations

On sample T.00/144, we can easily distinguish the Schreger lines intersecting to form concave and convex angles. A total of 145 measurements of convex angles were taken at varying distances from the CDJ.

From the stubs examined under the SEM, the dentinal tubule density was calculated on a total of 26 distinct areas on the 15 stub samples of the tusk sample T.00/144 and 24 distinct areas on the 15 stub samples of the tusk sample T.00/53 (Tab. 1).

3. Results

According to Hildebolt *et al.* (1986) *"tubule densities measured at specific distances from the pulp vary according to how much dentine has formed"*. As a result, we would expect the dentinal tubule density in tusks to increase proximally. This is not the case in the tusk samples examined here, where the density does not seem to correlate with the distance from the proximal end. This may be explained if we also consider the degree of branching of the tubules towards the CDJ.

The mean dentinal tubule density of the tusk of the juvenile individual is almost half the mean density of the tusk of the adult animal, although the measurements in both cases were taken near the cementum-dentine junction. This could be indicative of a dependency on age, although it may not necessarily be so. Garberoglio & Brannstrom (1976) in their study of human dentinal tubules, state that "no great difference was observed between old and young teeth". Also in a preliminary research by J. Trapani (pers. comm.) the dentinal tubule densities in samples of mammoths (*Mammuthus primigenius, M. columbi*) ranged between 24,100-41,400 dentinal tubules per square millimetre (d.t./sq mm). While, in the same study, samples from *Mammut americanum* measured between 15,200-41,600 d.t./sq mm. Obviously, such a wide range could also exist in the tusks of the dwarf elephants of Tilos. In any case, we should examine this feature in relation to the ontogeny of the animal.

According to Espinoza & Mann (1992) the

Tab.1

T.00/144		T.00/53	
Distance from proximal end cm	Mean dentinal tubule density d.t./sq mm	Distance from proximal end cm	Mean dentinal tubule density d.t./sq mm
4.6*	14,645	3.0	25,816
9.4	13,835	3.2	24,896
10.6**	18,880	14.0	37,501
12.3	14,673	15.0*	28,240
13.3	17,253	15.3	35,530
15.1	14,241	17.0	36,730
16.5	16,553	28.0	38,574
		29.5	22,344
		30.0	38,146
		37.0	26,353
		38.5	37,598
		44.0**	45,088
Mean value	15,726	Mean value	33,069
Minimum value	11,524*	Minimum value	21,322*
Maximum value	21,810**	Maximum value	45,088**

*, ** The asterisks connect the minimum and maximum values to the corresponding distances from the proximal end.

mean outer Schreger angles for *Mammuthus primigenius* is 73.21°, while the same mean for *Loxodonta africana* and *Elephas maximus* (undifferentiated) is 124.15°, and these values overlap between 90° and 115°. Also Fisher *et al.* (1998) measuring maximal angles on tusk specimens give a mean value of 87.1° for mammoths (*M. primigenius, M. columbi* and *Mammuthus* sp.) and a mean of 124.7° for mastodons (*Mammut americanum*). In our case, the sample analysed measure outer angles (distance from CDJ < 2 mm) ranging from 119° to 158.5°, with a mean of 142°. In their study, Fisher *et al.* (1998) also state that the maximal angle increases proximally. By plotting the angle measurements on our sample against the distance from the proximal end of the tusk, we observe that the same occurs here (Fig. 1).

Poulakakis (unpubl. MSc Thesis) compared DNA samples of the "*Palaeoloxodon antiquus falconeri*" of Tilos to those from *Mammuthus primigenius* and the extant Proboscidean species. He places the dwarf elephants of Tilos in closer affinity to the *Elephas maximus*. Indeed the measurement of Schreger angles would tend to place the dwarf elephants of Tilos closer to the extant species than mammoths, although the dentinal tubule densities do not provide any differentiation at this point. In order to reach a more definite conclusion, we should compare the mean values of the angle, at any given position, using more specimens from adult individuals.

4. Discussion

Although the dentinal tubule density plotted against the distance from the proximal end does not provide a distinctive pattern, this should be examined further in more tusk samples. The fact that the Schreger pattern, which is directly related to the distribution of the dentinal tubules (Miles & Boyde 1961), is "taxon – specific" (Trapani 1998) must be considered. The branching of the dentinal tubules near the CDJ may complement for greater distance from the pulp to maintain the density of the tubules relatively stable. Even so, plotting tubule density against distance from the pulp at any given position could provide a differentiating method.

Evidently, the many issues that arise from this study require further examination. The use of more tusk samples from adult individuals

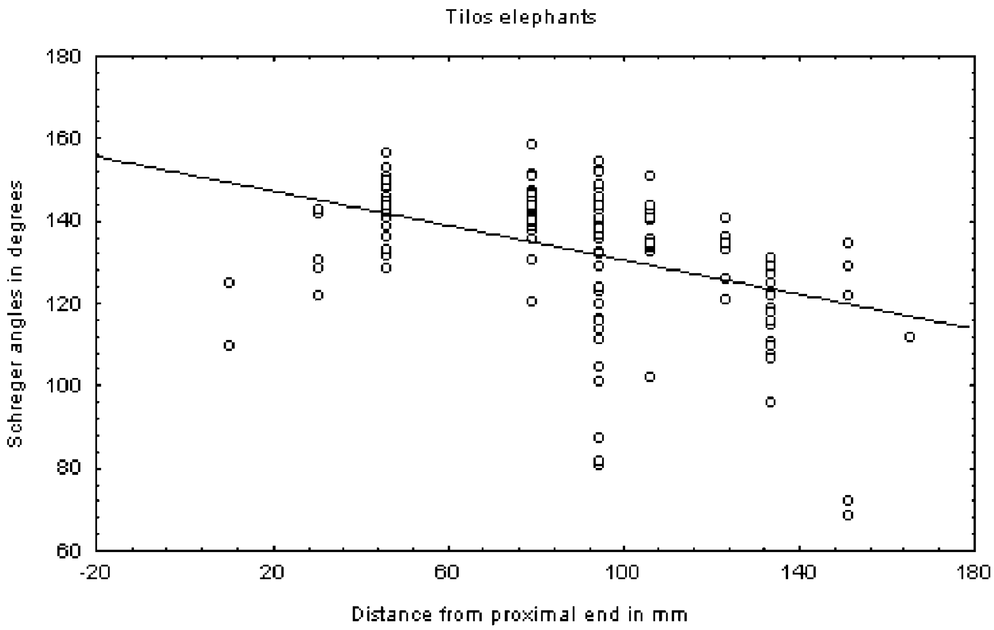

Fig.1 - The Schreger angle increases proximally.

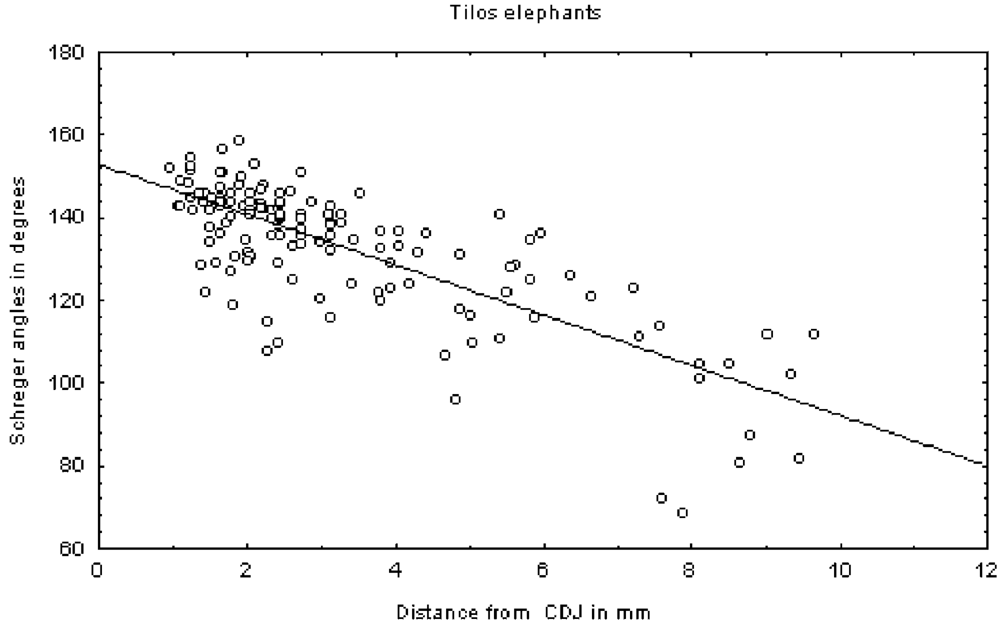

Fig.2 - The Schreger angle increases with greater distance from the pulp cavity.

appears necessary, in order to reach more definite conclusions. Of course, the rarity of the fossil tusk samples and the destructiveness of the method have to be considered as well.

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6. References

Bachmayer, Fr., Symeonidis N., Seeman R., Zapfe H. 1976. Die Ausgrabungen in der Zwergelefantenhohle “Charkadio” auf der Islel Tilos (Dodekanes, Griechenland) in der Jahren 1974 und 1975. *Ann. Naturhistor. Mus. Wien* 80: 113-144.

Bachmayer, Fr., Symeonidis N., Zapfe H. 1984. Die Ausgrabungen in der Zwergelefantenhöhle der Insel Tilos (Dodekannes, Griechenland) im Jahr 1983, *Sitzungsberichten der Österr. Akademie der Wissenschaften Mathem.-naturw. Kl.*, Abt.I. 193: 6-10.

Bradford, E.W. 1967. Microanatomy and Histochemistry of Dentine. In A.E.W. Miles (ed.), *Structural and chemical organization of teeth*, Vol.2: 3-33. London: Academic Press.

Boyde, A. & Lester K.S. 1967. An electron microscope study of fractured dentinal surfaces. *Calcified Tissue Research* 1: 122-136.

Espinoza, E.O. & Mann, M.J. 1992. *Identification guide for ivory and ivory substitutes*. WWF Publications.

Espinoza, E.O. & Mann, M.J. 1993. The history and significance of the Schreger Pattern in Proboscidean ivory characterization. *Journal of the American Institute for Conservation* 32: 241-248.

Fischer, D.C., Trapani, J., Shoshani, J., Woodford, M.S. 1998. Schreger angles in mammoth and mastodon tusk dentin. *Current research in the Pleistocene* 15: 105-107.

Forssell-Ahlberg, K., Brannstrom, M., Edwall L. 1975. The diameter and number of dentinal tubules in rat, cat, dog and monkey. *Acta Odont. Scand.* 33: 243-250.

Garberoglio, R. & Brannstrom, M. 1976. Scanning electron microscopic investigation of human dentinal tubules. *Archives of Oral Biology* 21: 355-362.

Hildebolt, C.F., Bate, G., McKee, J.K., Conroy G.C. 1986. The microstructure of dentine in taxonomic and phylogenetic studies. *American Journal of Physical Anthropology* 70: 39-46.

Miles, A.E.W. & Boyde, A. 1961. Observations on the structure of elephant ivory. *Journal of Anatomy* 95: 450.

Symeonidis, N.K. 1972. Die entdeckung von Zwergelefanten inder Hohle "Charkadio" auf der Insel Tilos (Dodekanes, Griechenland), *Ann. Géol. des Pays Hellén.* 24: 445-461.

Theodorou, G. 1983. *The dwarf elephants of the Charkadio cave on the island of Tilos (Dodekanese, Greece).* PhD Thesis. University of Athens.

Theodorou, G. 1988. Environmental factors affecting the evolution of island endemics: The Tilos example from Greece. *Modern Geology* 13, 183-188.

Trapani, J. & Fisher D.C. 1998. Schreger angle gradients in Proboscidean tusk dentin. *Journal of Vertebrate Paleontology* 18 (3): 2A.