Morphological aspects and composition of African elephant (*Loxodonta africana*) ivory

E.J. RAUBENHEIMER

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This study was aimed at determining the origin of the diamond shaped pattern and composition of ivory of the African elephant. Fragments of ivory and tusks were obtained through the National Parks Board from the Kruger Park, Addo Elephant Park, Kaokoveld, Caprivi, Etosha, Kavango and Tembe Elephant Park. Polished surfaces were prepared in different planes and examined with light and electron microscopical techniques. Analyses of the inorganic composition were performed using atomic absorption spectrophotometry, ion selective electrodes and inductively coupled optical emission spectroscopy. The total amino acid composition was determined with the aid of an amino acid analyser. Morphological investigations showed the distinctive diamond shaped pattern of ivory to be caused by the sinusoidal surface to pulpal course followed by odontoblastic tubules. This course is the result of pressure which builds up between tightly packed odontoblasts on their centripetal course along an ever decreasing pulpal circumference during formation of ivory. A total of 17 elements were detected in the inorganic fraction of ivory, some in concentrations as low as 0.25 µg/g. The concentrations of calcium, magnesium, fluoride, cobalt and zinc showed statistically significant differences ($P < 0.007$) between selected regions and may prove valuable in distinguishing chemically between ivory from different geographical locations. The organic content of ivory showed 17 amino acids in varying concentrations. The possible causes of these variations are discussed.

Key words: elephant ivory, morphology, composition

E.J. Raubenheimer, Department of Oral Pathology, MEDUNSA, P.O. Medunsa, 0204 Republic of South Africa (ejraub@mcd4330.medunsa.ac.za).

Introduction

The paired tusks of the African elephant are incisors which develop in the os incisivum of the nasomaxillary complex of bones and which grow continuously throughout life. The unique diamond shaped pattern of elephant ivory, which has not been researched in great detail (Sognnaes 1960; Miles & Boyde 1961; Sikes 1971; Raubenheimer *et al.* 1990) has made it a sought-after product in the manufacturing of works of art. The flourishing illegal trade in ivory has contributed to a significant decrease in elephant numbers on the African continent over the past decades (Armstrong & Bridgland 1989). The Convention on International Trade in Endangered Species (CITES) at its biennial meeting in Lausanne, Switzerland in October 1989 responded by placing a ban on the trade in elephant products. The effect of this ban on well managed elephant populations in Southern African states remains controversial.

Dentine (or ivory) forms the bulk of most teeth and is composed of organic and inorganic fractions. The inorganic composition of elephant ivory has not yet been investigated in detail except for distinguished work done on its carbon and nitrogen isotope ratios. The carbon isotope ratios ($^{13}C:^{12}C$) of ivory distinguishes between elephant roaming woodland and those in dense forests (Van der Merwe *et al.* 1990). The aims of this study were to determine the origin of the chequered (diamond shaped) pattern of elephant ivory and establish a databank on the inorganic and organic composition of ivory from the Southern African region.

Material and methods

Twenty fragments of ivory and five tusks, with masses between 0.7 and 9.3 kg were sectioned and polished in cross sections and sagittal planes and the characteristic diamond shaped pattern studied. One developing tusk was harvested from a full-time fetus 20 minutes after death. Biopsies were taken from the pulpal ivory and soft tissue, cut in thin slices and fixed in 10 % phosphate buffered formalin. Ground sections, 40 µ thick were prepared parallel and perpendicular to the long axis of 12 different fragments of ivory and subjected to light microscopic examination. Routine techniques for the preparation of scanning electron micrographs of hard tissue were employed to visualise the morphology and distribution of dentinal tubules of six different tusks. An image analyser (FIPS, CSIR, Pretoria) was used to measure the distances between the tubules in the light and dark bands respectively.

Sixty four fragments of ivory were obtained through the South African National Parks from Kaokoveld, Etosha National Park, Caprivi, Kavango, Kruger National Park, Tembe Elephant Park and Addo Elephant Park. Specimens weighing between 0.5 g and 1.0 g were prepared by removing the ensheathing layer of cementum with a rotating diamond disc. The specimens were agitated in a weak acid (0.1M HCl) for 10 minutes to remove all traces of metal that may have contaminated the ivory during sample preparation. The fragments were washed for 15 minutes in distilled water and the dry weights of each fragment determined accurately. Each specimen was completely demineralised in 1M perchloric acid. The inorganic composition was determined by atomic absorption spectrophotometry (Perkin Elmer 500; Norwalk, CT, U.S.A.), Astra 8 analyser (Beckman Instruments Inc., Brea, CA, U.S.A.) and ion selective electrodes (Radiometer, Copenhagen, Denmark). The mean values obtained per site of origin as well as the standard deviations (SDs) were expressed in mg/g dry weight and tabulated. The level of significance between the concentrations of each element at different geographical sites was determined with the Student *t*-test for unequal variances. The trace elemental composition of 25 fragments was determined with an inductively coupled plasma optical emission spectroscope (ARL 3400, Boston, MA, U.S.A.). Perchloric acid (1M) was used as a control. The mean concentrations and SDs were expressed in µg/g ivory.

Samples of ivory, weighing between one and three grams, were prepared from 32 fragments of ivory obtained from Kruger National Park (5), Kaokoveld (6), Etosha (15), Tembe (4), and Addo Elephant National Park (2). The samples were hydrolysed in sealed tubes containing 5 ml 6M HCl at 110 °C for 24 hours. The hydrolysates were neutralised, filtered (Millex-GS, 0.22 µ) and diluted 1:1 with citrate buffer (pH 2.2). Calibrants of 43 amino acids were prepared and the amino acid composition of the hydrolysate and calibrants determined using a Beckman 6300 Amino Acid Analyser. The chromatograms were integrated and quantified using a Hewlett-Packard 3390A integrator. The results were tabled as the average of the residues per 100 and the standard deviation for each amino acid calculated.

Results

The pulpal cavity of the tusks were conical in shape, had a smooth surface and a large pulpal opening. The bulk of a large tusk consisted of ivory (Fig. 1a). The entire outer

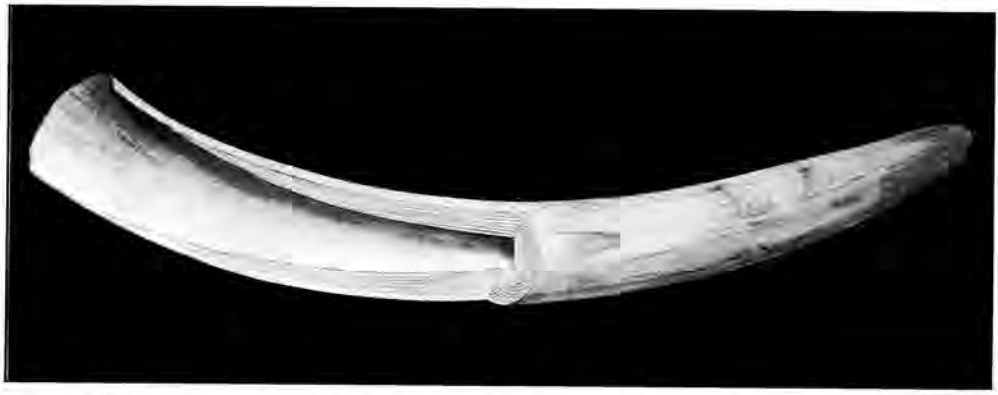

Fig. 1a. Partially dissected tusk exposing the conical pulpal cavity, diamond-shaped pattern (exposed on a polished surface prepared on a cross section) and parallel and alternating light and dark lines (on polished surface prepared in the sagittal plane). Note the large apical opening of the pulp on the left and the solid ivory forming the bulk of the tip of the tusk.

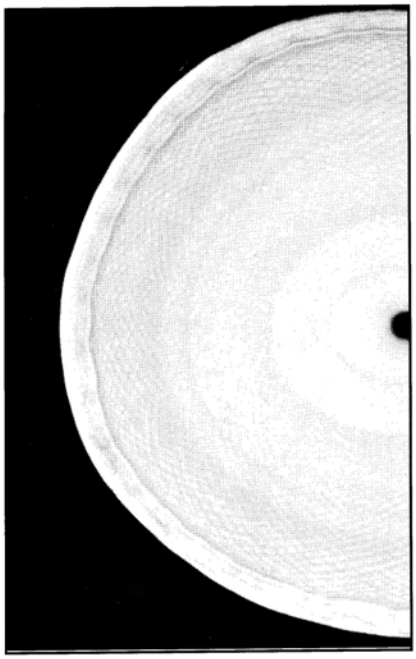

Fig. 1b. Closer view of the diamond-shaped pattern on cross section through the tusk. Note the ensheathing layer of cementum and scalloped ivory to cementum junction.

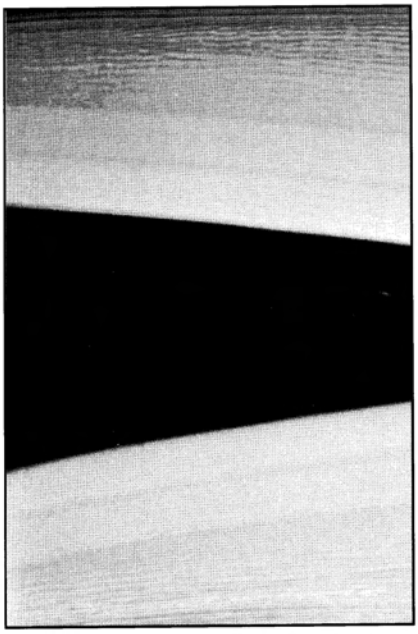

Fig. 1c. Closer view of the sagittal surface showing the alternating dark and light lines.

surfaces of the tusks were found to be covered by a layer of cellular cementum. The cementum to ivory junction was visible as a dark concentric ring. In cross sections, the ivory to cementum junction followed an undulating circular course, forming irregular excrescences alternating with shallow convexities or concavities (Fig. 1b). In this plane, the unique diamond-shaped pattern of the ivory consisted of two systems of alternating light and dark lines which radiate clockwise and anticlockwise, respectively, from the axis of the tusk. The diamond-shaped pattern corresponded to parallel light and dark lines evident on polished surfaces prepared in the sagittal plane (Fig. 1c). The external surface of the tusk followed the contour of the cementum to ivory junction, resulting in parallel longitudinal ridges and troughs which were visible upon external examination of a tusk.

The outermost layer of ivory (mantle layer) consisted of irregularly-spaced odontoblastic tubules which slanted apically and branched extensively. When followed towards the axis of the tusk, the tubules became more evenly spaced and gradually changed their course by curving towards the tip of the tusk. This curvature was found to be the beginning of the regular, sinusoidal course followed by the odontoblastic tubules in a pulpal direction and was present only in sections prepared in the sagittal plane. The convexities and concavities of the sinusoidal pattern corresponded to the alternating light and dark bands seen macroscopically on surfaces prepared in the sagittal plane. The dark bands corresponded with that part of the tubule that curved towards the pulpal opening (Fig. 2a). On high power maginification many dentinal tubules appeared to end blind and others fused, forming one tubule (Fig. 2b). The process of fusion was distinct from the fine lateral branches that seemed to anastomose with those of adjacent tubules. Blind ending tubuli occured more frequently in the dark bands (16 blind ending tubules per 100 tubules, SD 7) than in the light bands (6 blind ending tubules per 100 tubules, SD 3) as counted over 2 500 tubules in each of the bands respectively. Microscopic sections of biopsies of the foetal tusk confirmed the distal slant of the odontoblasts and scattered pyknotic cells, highly suggestive of individual cell death (Fig. 3).

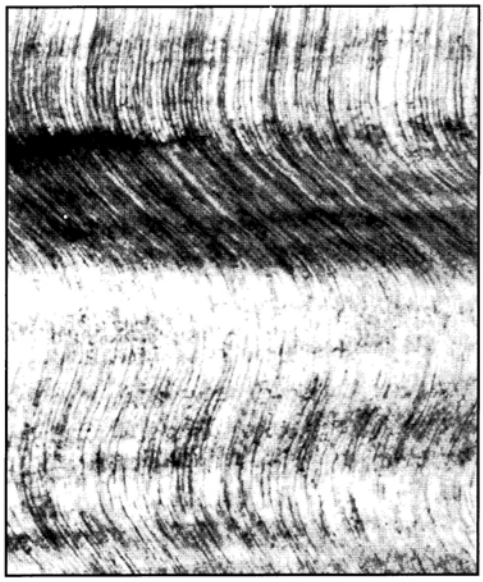

Fig. 2a. The regular sinusoidal curve followed by the odontoblastic tubules. The curve to the right coincides with a dark band (top of figure), an optical phenomenon caused by the increased density of tubules in this region. The tip of the tusk is towards the left. (Unstained section, magnification x180).

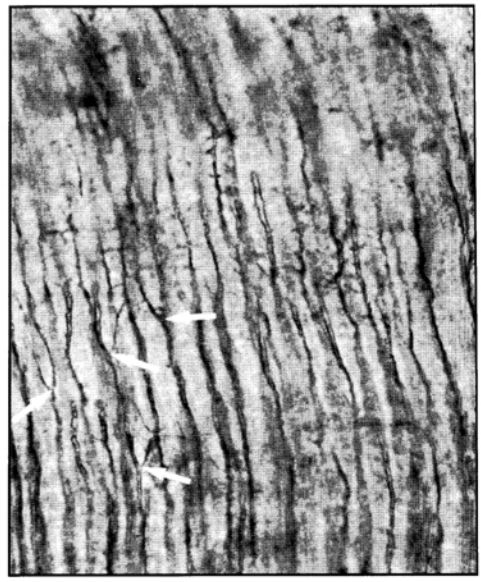

Fig. 2b. Higher power magnification in a dark band, showing fusion of tubles (arrows) (Unstained section, magnification x400).

Scanning electron microscopy showed the pulpal openings of tubules to be oval, with the greatest dimension parallel to the long axis of the tusk (Figs. 4a & 4b). Dentinal tubules were closer packed in areas where they curve towards the pupal opening (i.e. dark bands) (mean distance 4.6 ± 1.7 µm SD) than in the part of the tubule that curves towards the tip of the tusk (i.e. light bands) (mean distance 7.2 ± 2.8 µm SD) (Fig. 2a). This difference was significant ($P < 0.001$). The number of tubules/mm² varied between 31.6 x 103 and 54.3 x 103.

The inorganic composition of ivory is reflected in Table 1. Statistical analyses showed the differences between the respec-

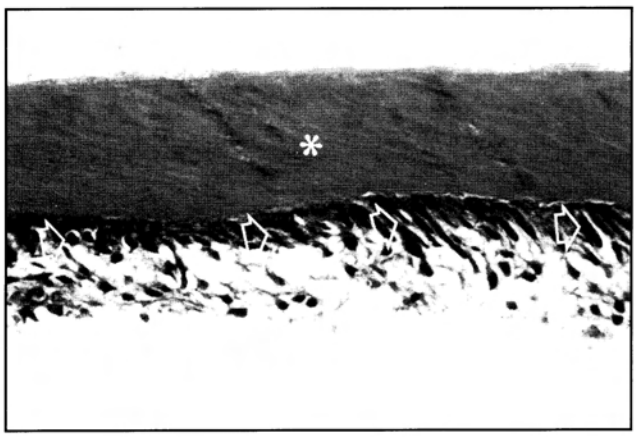

Fig. 3. Microscopic appearance of the foetal tusk. Note the newly formed ivory (asterisk) slanting of the odontoblasts and occasional cells exhibiting cell death (arrows) (H&E stain, magnification x250).

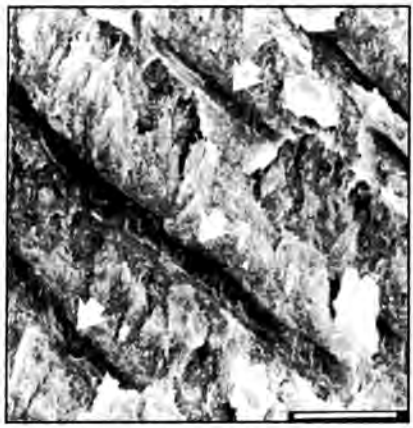

Fig. 4a. Electron micrograph of a fractured surface of ivory. Note the exposed odontogenic tubuli (arrows) (bar = 10 µm).

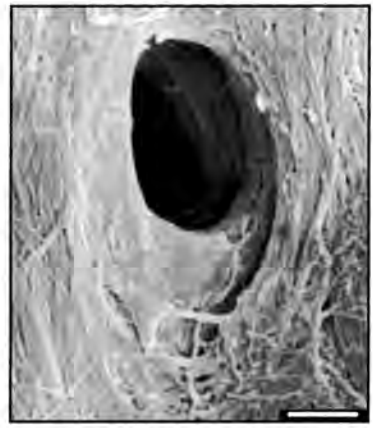

Fig. 4b. High power magnification of the pulpal opening of an odontoblastic tubule (bar = 1 µm).

tive elements in the following geographical locations were highly significant ($P < 0.002$):

Calcium: Addo vs. all other locations, Etosha vs. Caprivi, Etosha vs. Kavango and Caprivi vs. Tembe.

Phosphate: Kruger Park vs. Kaokoveld, Addo vs. Kaokoveld.

Magnesium: Addo vs. Kruger Park, Caprivi and Tembe vs. Addo and Kaokoveld vs. Caprivi.

Fluoride: Kaokoveld vs. all locations except Etosha and Etosha vs. all locations except Kaokoveld.

The following trace elements were detected in ivory (average content expressed in µg/g dry weight, SD in brackets: As:8.0 (1.4), Cd:0.4 (0.04), Cr:3.7 (0.6), Co:0.72 (0.09), Cu:2.2 (1.2), Pb:8.7 (1.2), Mn:0.9 (0.6), Hg:1.4 (0.2), Ni:0.89 (0.1), Zn:20 (10.8), Mo:0.56 (0.06) and Al:6.2 (4.3).

Table 1
Main inorganic elements in ivory, expressed per site of origin (average mg/g dry weight, SD in brackets)

	KNP[1]	Kaoko[2]	Etosha	Caprivi	Kavango	Tembe	Addo
No. of Specimens	13	9	26	6	4	3	3
Ca	195.8 (17)	193.7 (15.9)	192 (16)	208.9 (6.4)	205.9 (1.8)	191.1 (8.9)	170.8 (2.5)
PO_4	115.5 (5)	118 (2.6)	116 (3.5)	114.7 (4)	115.3 (3)	113.1 (5)	113 (1.4)
Mg	14.6 (3.2)	18.2 (4.2)	15.3 (4.2)	13.1 (0.9)	12.2 (2.7)	14.7 (1.2)	17.3 (0.4)
F	0.076 (0.014)	0.106 (0.017)	0.124 (0.029)	0.069 (0.012)	0.058 (0.01)	0.054 (0.009)	0.035 (0.019)

[1] Kruger National Park
[2] Kaokoveld

Ivory obtained from the Kaokoveld was more brittle and hydrolysed more rapidly than those from other regions. The amino acid composition of hydrolysed ivory is expressed in Table 2. The difference in the hydroxylysine content between Kruger and Northern Namibian ivory (Kaokoveld and Etosha) was significant ($P < 0.01$) (Kruger ivory 0.7 ppm, SD 0.1; Etosha 0.4 ppm, SD 0.1; Kaokoveld 0.4 ppm, SD 0.2).

Discussion

The cell responsible for the formation of ivory (or dentine) is the odontoblast. Odontoblasts are derived from the mesenchyme of the dental pulp and after differentiation they move centripetally (i.e. towards the axis of the tusk) and deposit ivory along their pathway. Each odontoblast is responsible for the formation of a cytoplasmic extension. Mineralisation of ivory around this extension forms tubules which traverse the diameter of the ivory. The microporosity of ivory, which is well beyond the resolution of the human eye, is responsible for the absorbent and tactile characteristics thereof, which has made it an unequalled product for the manufacturing of piano keys.

Table 2
Total aminoacid composition of 32 samples of hydrolysed ivory

Aminoacid	Residues/100	(SD)
Aspartic acid	5.1	(0.3)
Hydroxyproline	9.9	(1.0)
Threonine	2.0	(0.2)
Serine	4.0	(0.2)
Glutamine	8.0	(1.3)
Proline	12.2	(1.2)
Glycine	30.8	(1.0)
Alanine	10	(1.2)
Valine	2.3	(0.3)
Methionine	0.4	(0.4)
Isoleucine	1.2	(0.1)
Leucine	3.0	(0.1)
Phenylalanine	1.5	(0.1)
Hydroxylysine	0.4	(0.2)
Lysine	2.7	(0.7)
Histamine	0.7	(0.2)
Arginine	4.6	(0.4)

The tip of the concial pulp becomes solid as ivory depositioning progresses and lengthening of the proximal edge coincides with forward movement and elongation of the tusk.

The centripetal movement of odontoblasts during the formation of ivory leads to a rapid decline in the pulpal circumference. As a result, odontoblasts become progressively more tightly packed and intercellular pressure increases as the pulpal circumference becomes smaller. Morphological manifestations of the increased pressure between odontoblasts are reflected as a significant reduction in the distance between odontoblastic tubules in the dark bands, the oval shape of the tubules as well as the slanting of odontoblastic cell bodies towards the pulpal opening. The only means by which the growing pressure between the centripetally moving odontoblasts on a rapidly decreasing perimeter can be accommodated, is through two processes namely, movement of the odontoblastic cell mass towards the pulpal opening or a reduction in the number of odontoblasts. There is morphologic evidence that both these phenomena occur during the formation of ivory. Movement of the odontoblastic cell bodies towards the pulpal opening coincide with the dark bands (i.e. slanting of the sinusoidal curve of the odontoblastic tubuli towards the pulpal opening). As pressure increases, the number of odontoblasts are reduced through fusion of cells (represented by fusing tubules) and cell death (represented by blind-ending tubules). The occurance of the latter phenomenon is supported by the presence of pyknotic odontoblasts seen in microscopic sections of the rapidly fixed biopsies of the foetal tusk. The relief of intercellular pressure through these mechanisms results in a change in the direction of odontoblastic movement towards the tip of the tusk. This coincides with the anteriorly directed part of the sinusoidal curve. The process of odontoblastic crowding, followed by a bodily movement of odontoblasts towards the pulpal opening and a relief of intercellular pressure through cell fusion and death, with subsequent change in the direction of movement of the cell bodies are probably responsible for the regular sinusoidal

course followed by odontoblasts. This hypothesis is reflected graphically in Fig. 5. The sinusoidal course of the tubules in ivory is reflected as parallel and alternating light and dark bands which are seen on polished surfaces prepared along the axis of the tusk. The light and dark bands were found to be the result of the varying compactness of dentinal tubules between the sectors of the sinusoidal curve of the tubules that slant towards the tip of the tusk or towards the pulpal opening respectively. On cross sections, the diamond-shaped pattern is a reflection of the alternating light and dark bands seen on surfaces prepared in length. This implies that when the layer of odontoblasts are viewed in a radial perspective, movement of odontoblasts towards the pulpal opening with curving of odontoblastic tubules does not occur at the same time but rather as a wave that spreads circumferentially along the peripulpal layer of odontoblasts. On microscopic examination, it was observed that fractures through ivory generally follow the dark bands. The higher number of tubules per unit area probably makes the dark bands weaker than the light bands.

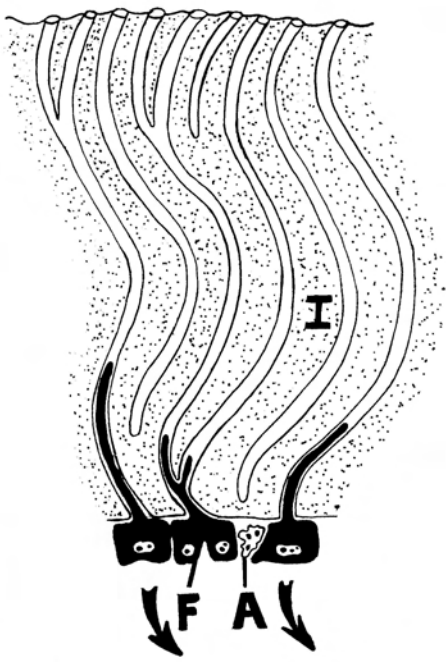

Fig. 5. Schematic representation of the sinusoidal course followed by the odontoblastic tubules (sagittal plane, the tip of the tusk is towards the right, surface towards the top and pulpal cavity towards the bottom of the figure). Note odontoblastic fusion (F) which results in odontoblastic tubules uniting and apoptosis (A) which gives rise to blind ending tubules, thereby effectively reducing the number of odontoblasts during their centripetal movement (arrows indicate the centripetal course followed by the odontoblasts, I - ivory).

Extreme care should be taken when harvesting ivory for chemical analyses. The proximal feather edge of the tusk as well as the layer of mineralised tissue ensheathing the tusk consists of cellular dental cementum and not ivory. Studies which provide no clarity on the exact part of the tusk that was harvested for chemical analyses, should be viewed with caution. During the formation of dentine (or ivory), which is essentially a hydroxyapatite deposited on an organic matrix, over 45 elements could potentially compete for incorporation (Wetherell & Robinson 1973). The inorganic composition of ivory reflects the composition of an animal's diet. Unlike bone, the composition of ivory remains stable and is not subjected to turnover and remodelling after formation thereof (Posner & Tannenbaum 1984). An extensive databank on the composition of ivory from different areas in Africa could assist in tracing the origin of ivory and might play a key role in identifying regions in which illegal ivory harvesting is taking place. This study as well as others (Van der Merwe *et al.* 1988, Van der Merwe *et al.* 1990, Vogel *et al.* 1990), clearly indicate the tracing of the source of ivory on its chemical composition to be a realistic possibility. The techniques used by these groups are expensive and the equipment used is not readily available. Our study indicated that the concentrations of calcium, phosphate, magnesium and fluoride are of potential value in identifying the site of origin of Southern African ivory. Addo, Etosha and Kaokoveld ivory in particular have unique compositions. Addo ivory was distinguished by its low calcium content and Kaokoveld and Etosha ivory by its high fluoride content.

The rapid rate of hydrolisation of ivory from the Kaokoveld and Etosha compared to other regions was of interest. The annual rainfall in these regions are low. The arid environment, characterised by dry savannah and shrub, has led to the term 'desert elephant' to those animals that were driven into the region by human inhabitation of the more arable land (Viljoen, 1987). There is good reason to accept that the diet of these elephant differ significantly from those in the other regions studied. Analyses of ivory from Kaokoveld and Etosha show the highest fluoride concentration, lowest total proline plus hydroxyproline content and under hydroxylation of lysine as unique characteristics. The high fluoride content is likely the result of the water that collects in the closed systems of salt pans and which becomes concentrated due to evaporation. Although excessive fluoride could influence the strength of the hydroxyapatite crystal (Lavelle 1975), the under-hydroxylation of lysine would affect the strength of the organic scaffold of collagen fibres (Chatterjee 1978). Vitamin C, iron and oxygen are cofactors required for the enzymatic hydroxylation of lysine during the biosynthesis of the tropocollagen molecule (Anderson 1992). Insufficient dietary Vitamin C intake, linked to the arid vegetation, is likely to be the main cause of reduced hydroxylation of lysine in the collagen of Kaokoveld and Etosha ivory.

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