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Using morphometric and analytical techniques to characterize elephant ivory

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Abstract

There is a need to characterize Asian elephant ivory and compare with African ivory for controlling illegal trade and implementation of national and international laws. In this paper, we characterize ivory of Asian and African elephants using Schreger angle measurements, elemental analysis {X-ray fluorescence (XRF), inductively coupled plasma-atomic emission spectroscopy (ICP-AES), and inductively coupled plasma-mass spectroscopy (ICP-MS)} and isotopic analysis.

We recorded Schreger angle characteristics of elephant ivory at three different zones in ivory samples of African ($n = 12$) and Asian ($n = 28$) elephants. The Schreger angle ranged from 32° to 145° and 30° to 153° in Asian and African ivory, respectively.

Elemental analysis (for Asian and African ivory) by XRF, ICP-AES and ICP-MS provided preliminary data. We attempted to ascertain source of origin of Asian elephant ivory similarly as in African ivory based on isotopes of carbon, nitrogen and strontium. We determined isotopic ratios of carbon ($n = 31$) and nitrogen ($n = 31$) corresponding to diet and rainfall, respectively. Reference ivory samples from five areas within India were analyzed using collagen and powder sample and the latter was found more suitable for forensic analysis. During our preliminary analysis, the range of $\delta^{13}C$ values ($-13.6 \pm 0.15‰$ and $-25.6 \pm 0.15‰$) and $\delta^{15}N$ values ($10.2 \pm 0.15‰$ and $3.5 \pm 0.15‰$) were noted.

Keywords: Elephant ivory; Schreger angle; Morphometry; Inductively coupled plasma analysis–MS; Isotopic analysis; X-ray fluorescence

1. Introduction

India being one of the 12 identified mega-biodiversity nations has 8% of the world's biodiversity with 60% of the world's tigers, 50% of Asian elephants, 70% of Asian rhinos and harbours the only population of Asiatic lion in the wild [1]. Illegal trade in wildlife and its products is a major threat and concern for conservation of endangered species throughout the world. Major illegal wildlife trade exists in skin, ivory, horn, antler, bone, live animals, feathers, nails, claws and pod. The illegal wildlife trade has been estimated to be worth US$ 5 billion which in economic terms ranked second after the drugs [1].

Over the years, poaching of megavertebrate species has depleted their numbers. In India, 75 mammal species out of 129 mammals listed in various Schedule categories under Wildlife (Protection) Act 1972 are under threat from illegal trade, of which 25 mammal species are included under the endangered categories of Schedule I and II.

Ivory being one of the highly priced article is illegally traded and the estimated annual world demand for ivory during the 1980s was 500–700 tonnes [2]. The African elephant (*Loxodonta africana*) was initially placed in Appendix II of CITES and its ivory was permitted for trade in the global market this was justified because of its large population and the considerable volumes of ivory generated from the presence of tusks in both males and females. However, was the Asian elephant (*Elephas maximus*) was listed in Appendix I of CITES and in Schedule I of the *Indian Wildlife* (*Protection*) *Act* 1972

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due to its endangered status. In spite of such legal protection, ivory from Asian elephant has always been found in the illegal trade. This is a major cause of concern for the conservation of the Asian elephant as large number of elephants were being poached every year [3].

The ban on trade in African elephant ivory in 1989 by CITES was a reaction to the significant decline in its populations from poaching [3]. However, the ban was relaxed for three African countries namely Botswana, Namibia and Zimbabwe in 1997 to permit a one-time sale of 60 tons of ivory [4]. The continued poaching of elephants in India [3] is a clear indication that illegal ivory trade is still in existence from Asian sources. This is probably because Asian ivory is more valuable than African [3]. Due to the demand for Asian ivory, widespread poaching has skewed the sex ratio in several populations [5]. The adult male to female ratio of the Nilgiri elephant population in India shifted from 1:5 in 1981 to 1:15 in 1998 [5] and further to about 1:25 by 2005 [6].

Ivory of African and Asian elephants is indistinguishable, particularly in the processed form, and thus it is almost impossible to trace the origin of tusks [7]. Enforcement of wildlife protection laws is often hampered by lack of proper methods to identify the species as well as source of the ivory [8]. Therefore, the present study aims to characterizing elephant ivory using different techniques to help enforcement agencies in getting rapid and reliable identification of seized materials and presentation of evidence in courts on the origin of such material.

Morphometry has been used extensively to differentiate species. Schreger angle of ivory is one of the important morphometric characters to be used for differentiation [9]. Elemental analysis could potentially help in distinguishing Asian from African ivory. Isotopic analyses is also useful in determining the source of origin and hence, has important role in forensics [9]. Isotopes have been successfully used to determine the source of African ivory [10]. For instance, carbon, nitrogen and strontium isotopes provide information on feeding habits, water stress and geology, respectively.

2. Materials and methods

Ivory reference samples of Asian and African elephants were used for analysis by different morphological and analytical techniques (Table 1).

Table 1
Different techniques used in characterizing Asian and African ivory

Techniques applied	Asian ivory	African ivory
Schreger angle	28	12
X-ray fluorescence (XRF)	5	5
Inductively coupled plasma-AES	5	3
Inductively coupled plasma-MS	3	3
Isotopic study	31	–

3. Morphometric technique

The Schreger angle pattern is a characteristic structural feature of the dentine portion of elephant tusk (Fig. 1). These are sets of intersecting lines radiating in spiral fashion from the axis of the tusk [9]. The angles are formed when dentinal tubules, produced by odontoblasts move towards the tusk axis during dentine deposition [11]. The Schreger angle are either centripetal or centrifugal. The Schreger angle technique has been used by in USA and by CITES to distinguish tusks of African ivory and mammoth to prevent illegal trade. Schreger angle measurements have widely been used to distinguish ivory of different species [9,12,13].

Schreger angles were examined on polished transverse section of tusks. For getting the best Schreger angle photographs, xeroxing and scanning of sample were tried. Scanning gave the best visibility of angles. A 10–20 angles were measured manually or by using software [14], at three different regions (central, middle and periphery) of Asian elephant ivory ($n = 28$) and African elephant ivory ($n = 12$).

4. Analytical techniques

4.1. X-ray fluorescence

X-ray fluorescence is an non destructive technique for elemental analysis. The X-ray spectrum reveals a number of characteristic peaks. The energy of the peaks leads to identification of the elements present in the sample (qualitative analysis) and intensity provides the relevant elemental concentration.

Five Asian and five African ivories were used for this analysis. Cross-section of ivory samples were cut and polished. Small pieces of 35–40 mm diameter and less than 10 mm thickness were directly used for analysis. Samples were desiccated overnight and analyzed under X-ray spectrometer (Siemens SRS 3000). X-ray fluorescence technique is a dry technique which gives intensity of various elements present.

4.2. Inductively coupled plasma-atomic emission spectrometry (ICP-AES)

ICP-AES is a powerful analytical tool for determinative elemental analysis. Detection limit is around 1 ppm. Three to five samples of Asian and African ivory were analyzed. Powder samples were digested by six treatments of 15 ml mixture of hydrofluoric acid (HF) and perchloric acid ($HClO_4$) each. Followed by two treatments of Perchloric acid and after adding 15 ml 10% HCl to the dried sample it was heated. This solution was made-up to 100 ml by adding distilled water. Prepared solution was analyzed through ICP-AES (Jobin Yvon JY 70 plus) spectrometer. Instrumental concentrations of various elements were transformed into parts per million using standard formula.

4.3. Inductively coupled plasma mass spectrometry (ICP-MS)

Inductively coupled plasma mass spectrometry is one of the advanced techniques to know the elemental details of the

samples analyzed as its detection level is very low, typically in parts per billion and parts per trillion. Teflon crucibles were cleaned and labelled for various samples. Approximately 0.1 g of powder sample was measured and 10–15 ml of mixture of concentrated nitric acid and hydrofluoric acid in 1:2 ratio was added. These sample was boiled with the lid on the crucible, until the sample dried. Again the same mixture was added and the sample was boiled further without the lid on the crucible. 5 ml of perchloric acid was added to these samples and boiled without lid. Same step was followed and completely dried samples were extracted with 20% HCl. The sample solution was made-up to 100 ml by adding distilled water and kept ready in plastic bottles for instrumental analysis using Perkin-Elmer SCIEX ICP-Mass Spectrometer model ELAN DRC-e.

4.4. Isotopic analysis

Isotopic ratio mass spectrometer (CE Instruments Flash EA, 112 series, ThermoQuest) was used. Standard used for the analysis were Pee Dee Belemnite for carbon analysis and atmospheric nitrogen for nitrogen analysis. Tusk samples from five states, Uttaranchal, North East, Tamil Nadu, Karnataka and Kerala were collected. Collagen was extracted from powdered ivory samples [15]. We also measured isotopic ratio using powdered samples directly. Initially collagen as well as powder from four ivory samples were analyzed for carbon isotopes in isotopic ratio mass spectrometer. The carbon isotopic values of both of these (collagen and powder form) were highly correlated and thus, we analyzed only the powder form of the samples as this was quicker. In total 31 samples were analyzed for carbon and nitrogen isotopes.

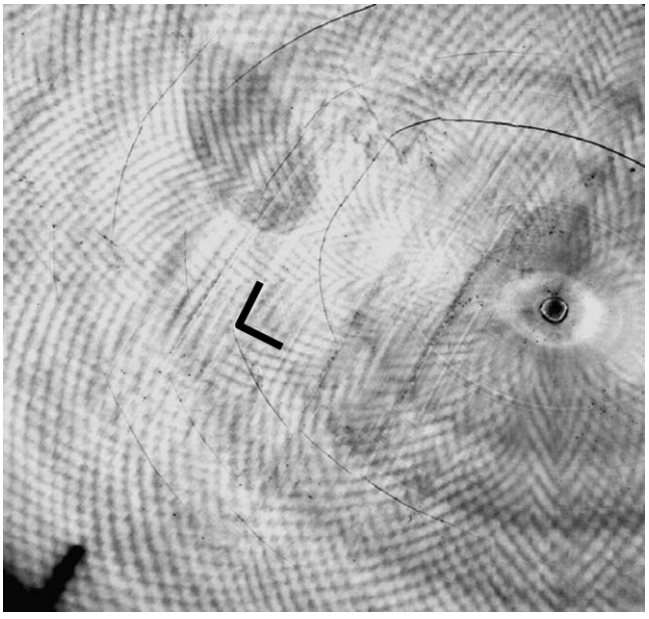

Fig. 1. Tusk dentine portion showing concave Schreger angles.

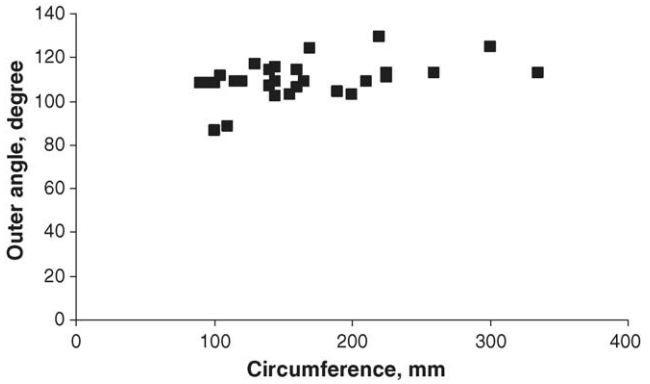

Fig. 2. Relationship of elephant tusk circumference with outer Schreger angle.

5. Results

5.1. Schreger angle

Circumference of tusk (an indicator of age of the animal) and the value of Schreger angle were plotted to know the relationship between them in case there was a change in the angle with age. There was no relationship between circumference of elephant tusk and Schreger angle values measured at the outer portion of the tusk (Fig. 2).

When all angle values of various ivory samples from three different zones namely central, middle and outer were plotted in 3-D graph, the mean value of outer Schreger angle for African ivory above are 120° and for Asian ivory these are below 120° (Fig. 3). Fig. 4 indicates mean of Schreger angle values taken at all the three portions in Asian (91.1 ± 0.70) and African (103.6 ± 1.35) ivory.

Variation in Schreger angle values at various zone of African and Asian ivory indicates that outer angle value of African ivory was higher (>120°) as compared to Asian ivory (<120°) (Fig. 5). But the middle and inner angle mean values were found to overlap in the two species.

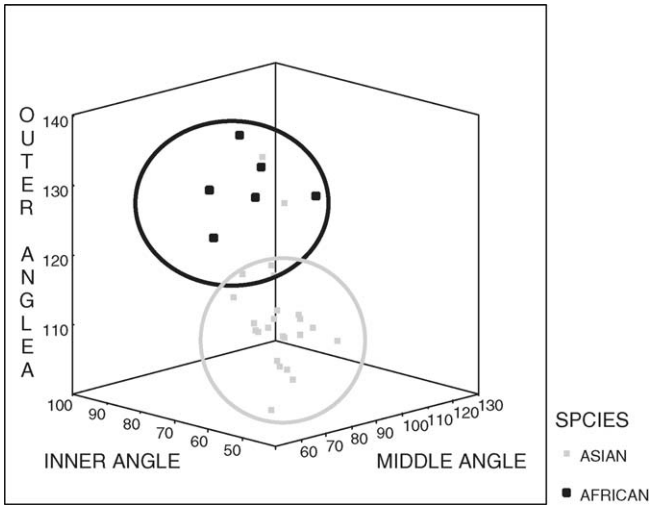

Fig. 3. Use of Schreger angle for species differentiation.

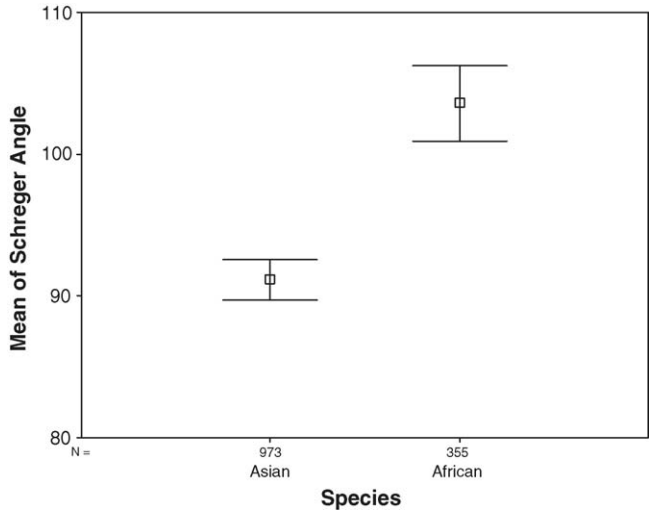

Fig. 4. Mean of Schreger angle (from all three portions of tusk) Asian and African ivory.

5.2. X-ray fluorescence

Intensities of elements such as Fe, Ni, Cu, Zn, Nd, Hf, Os, Cr, Ce, Dy, Ca, K, Cl, S, Sr, P, Cs, Ti, Mg, Al, Si and Na were obtained using X-ray fluorescence techniques. Fig. 6 reveals that the intensity of strontium in Asian ivory ranges from 1.24 to 1.5 and in African ivory from 1.8 to 2.04 kcps. Intensity of hafnium in Asian ivory ranges from 0.24 to 0.27 and 0.38 to 0.43 kcps in African ivory. Strontium and hafnium intensity were thus higher in African ivory than in Asian ivory. However, there were only marginal differences in intensity of phosphorous, calcium and halogen.

5.3. Inductively coupled plasma-atomic emission spectrometry

Mean elemental concentrations (ppm) in Asian ($n = 5$) and African ivory ($n = 3$) indicate that phosphorous, magnesium and chromium have higher concentrations in Asian elephant ivory as compared to the African elephant ivory, whereas concentrations of silicon, calcium, strontium, barium, iron, manganese and zinc were found to be higher in African ivory as compared to Asian ivory (Table 2 and Fig. 7). Concentrations of barium, strontium, silicon, iron, manganese, zinc, phosphorous and magnesium were relatively consistent in all samples of the Asian ivory. On the other hand, only barium concentration was found to be consistent in African ivory.

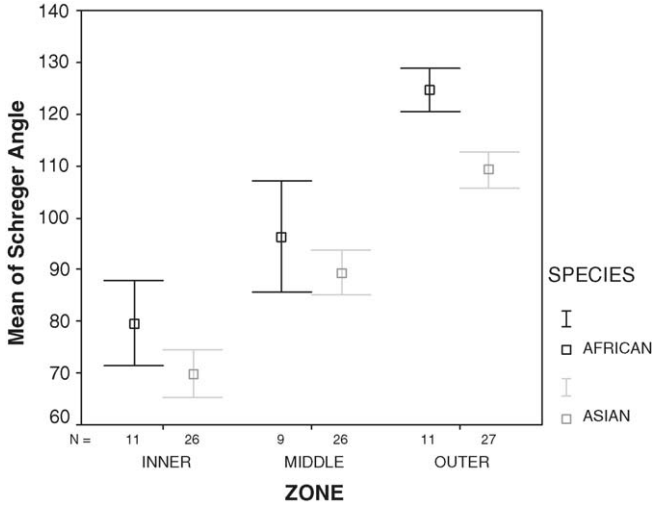

Fig. 5. Variation in Schreger angle at various zone of African and Asian ivory.

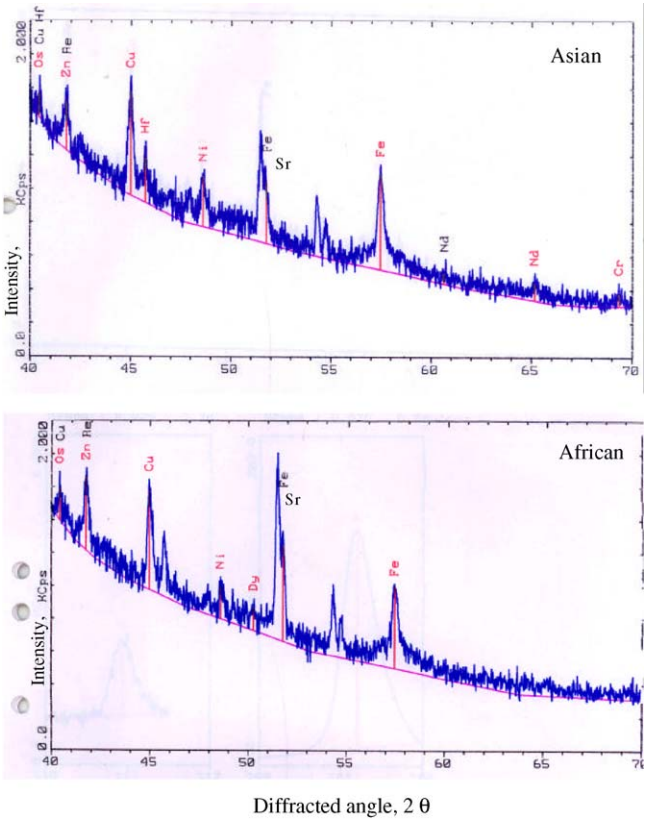

Fig. 6. XRF spectrum output of Asian and African ivory.

5.4. Inductively coupled plasma mass spectrometry

Analysis through ICP-MS reveals that the concentration of vanadium (V), samarium (Sm), europium (Eu), gadolinium

Table 2
Mean elemental concentration of Asian ($n = 5$) and African ivory ($n = 3$) calculated through ICP-AES in parts per million

Elements	Asian	African
P	89536.2 ± 3238.29	83364.2 ± 6214.68
Si	72.64 ± 2.64	117.76 ± 1.93
Ca	158585.5 ± 3443.55	177840.3 ± 48053.2
Sr	113.3 ± 19.81	221.3 ± 85.78
Ba	34.1 ± 9.47	56.9 ± 2.67
Mg	16797.1 ± 2278.26	12901.9 ± 9122.66
Fe	3306.7 ± 864.66	4927.81 ± 2379.99
Mn	16.9 ± 5.86	27.93 ± 20.93
Zn	35.9 ± 2.07	54 ± 28.49
Cr	23.7 ± 13.68	21.7 ± 14.23

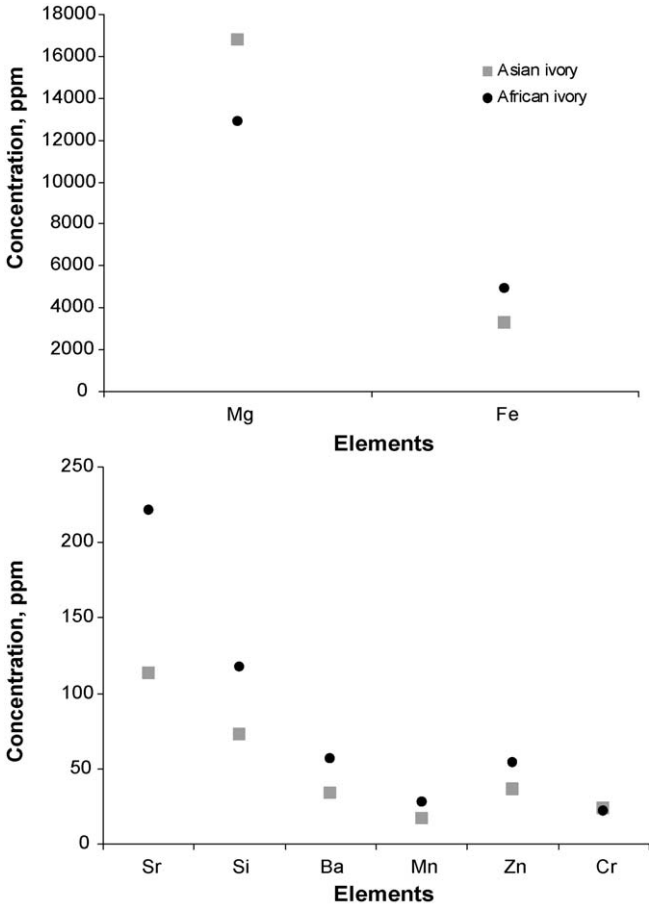

Fig. 7. Mean concentration of Fe, Mg, Sr, Si, Ba, Mn, Zn and Cr determined by ICP-AES in Asian and African ivory.

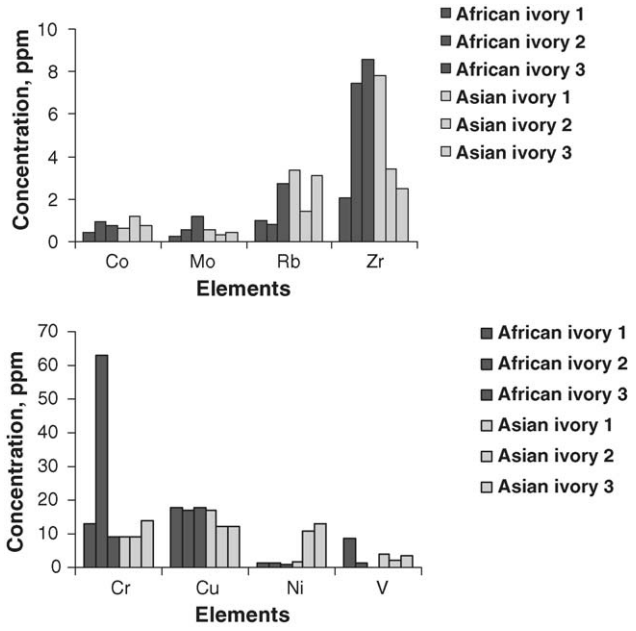

Fig. 8. Concentration of Co, Mo, Rb, Zr, Cr, Cu, Ni and V in Asian and African ivory by ICP-MS.

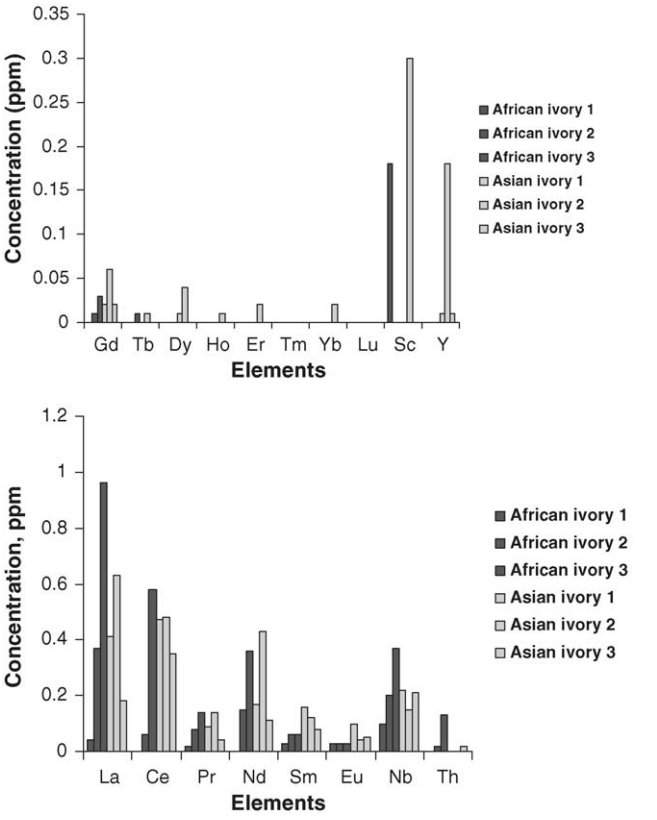

Fig. 9. Concentration of La, Ce, Pr, Nd, Sm, Eu, Nb, Th, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Sc and Y in Asian and African ivory by ICP-MS.

(Gd) and scandium (Sc) were higher in the Asian ivory whereas molybdenum (Mo) and nickel (Ni) were higher in the African ivory (Figs. 8 and 9).

Some rare earth elements, dysprosium (Dy), holomium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb) and yttrium (Y) were present only in Asian ivory and not detectable in African ivory. Yttrium was consistently found in all samples of Asian ivory (Fig. 9).

5.5. Isotopic ratio analysis

The δ^{13}carbon values of collagen and tusk powder (Fig. 10) were found to be highly correlated ($R^2 = 0.9943$). The mean δ^{13}C for African ivory (-19.4 ± 0.38 per mil) is higher than Asian ivory (-21.2 ± 0.58 per mil) with slight

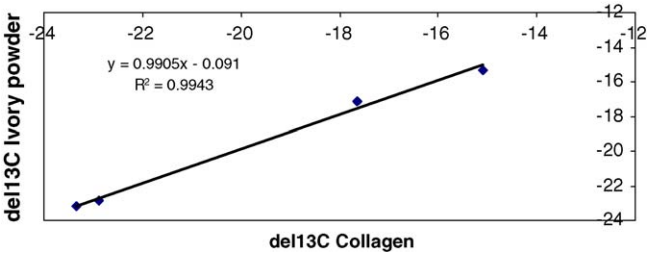

Fig. 10. Relationship between δ^{13}C of collagen and tusk powder of Asian elephant.

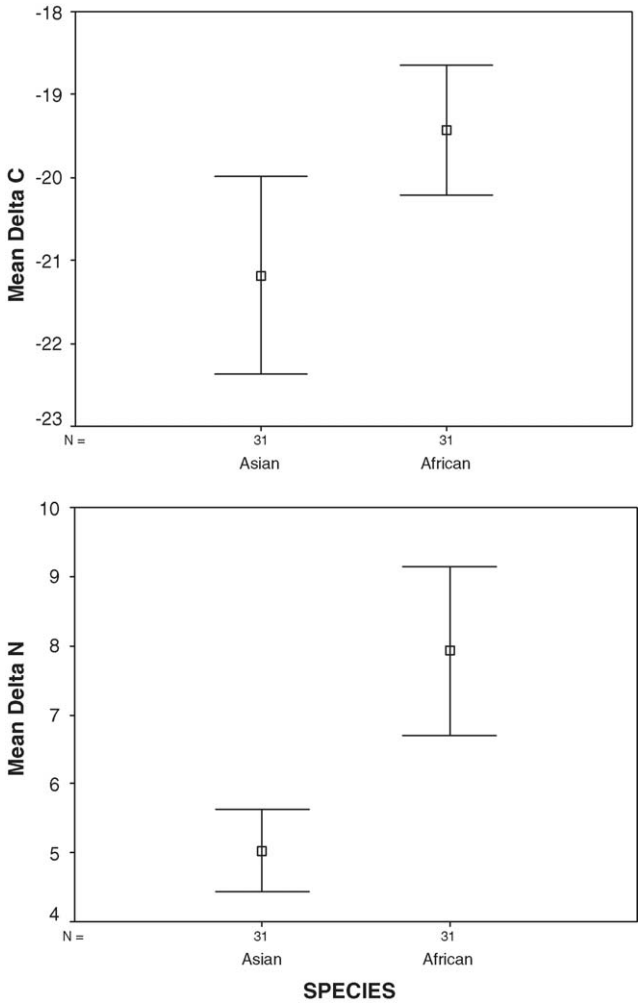

Fig. 11. Mean and standard error of carbon and nitrogen isotope of Asian and African ivory.

overlaps (Fig. 11). Mean δ^{15}N for African ivory (7.9 ± 0.59 per mil) is higher than the Asian ivory (5.03 ± 0.29 per mil) with no overlaps (Fig. 11). Values of δ^{13}C and δ^{15}N are significantly different between Asian and African elephant

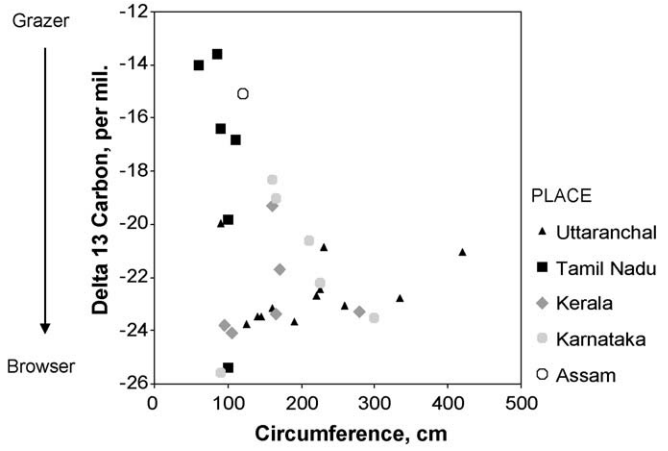

Fig. 12. Relationship between circumference of tusk and diet of Asian elephants in grass dominating habitat.

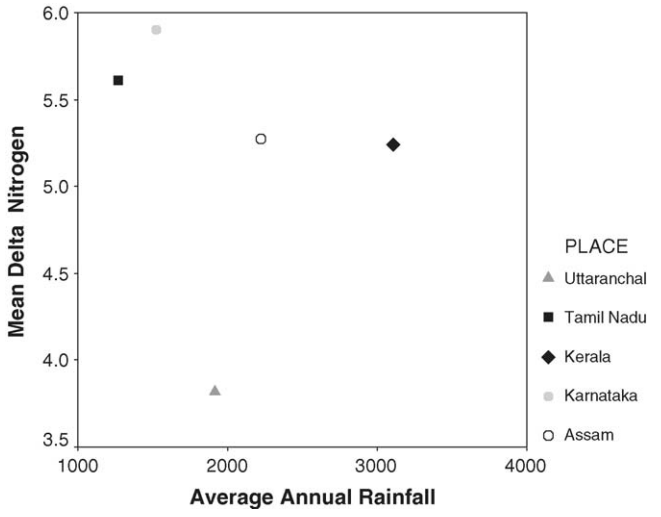

Fig. 13. Scatter plot between mean δ^{15}N and average annual rainfall.

($p < 0.001$). African ivory dataset used from van der Merwe et al. [16].

Fig. 12 reveals relationship between circumference of the tusk and diet of elephants in grass dominating habitat, where younger elephants had carbon isotopes values ranging from -18 to -13 per mil, where as adult elephants had values ranging from -20 to -26 per mil.

Scatter plot between mean value of δ^{15}N and average annual rainfall indicates that different regions forms separate cluster (Fig. 13). However, the mean values of δ^{15}N of Assam and Kerala were found overlapping.

Ivory from State of Karnataka has medium to high nitrogen isotopic ratio and the Uttaranchal has low nitrogen isotopic ratio. Whereas, ivory from Assam, Kerala and Tamil Nadu had medium nitrogen isotopic ratio (Fig. 14).

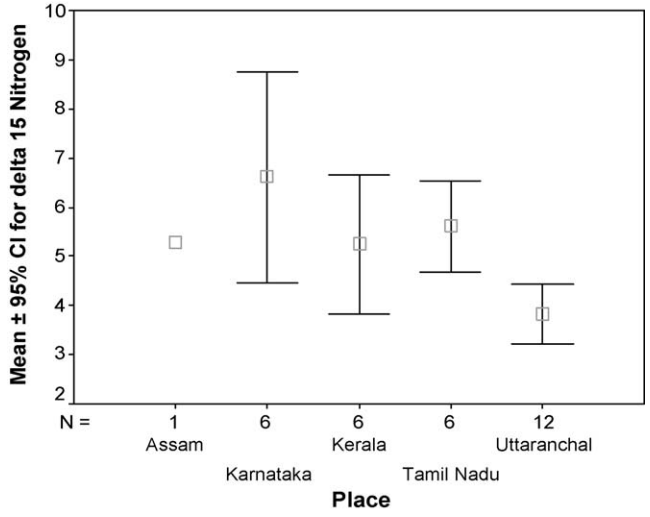

Fig. 14. Error bars showing Asian elephant tusk having high and low nitrogen isotopic ratio.

6. Discussion

6.1. Schreger angle

Identification of ivory and its substitutes is important to strengthen enforcement agencies to curb the illegal trade of ivory. Although there have been some attempts to identify African ivory from various regions [12,17] there is insufficient work on identification of Asian ivory. Espinoza and Mann [17] tried identifying ivory and its substitutes through their cross sections, and found that each ivory type is morphologically distinct [9]. Hanfee [18] in her handbook gives keys to identification of ivory of Asian and African elephants and also ''fake ivory'' based on visual observation. Schreger [19] in his paper is credited with the description of Huntre–Schreger bands in enamel. The presence of unique pattern of Schreger lines in transverse sections of proboscidean ivory created by the structure of microscopic dentinal tubules [20] is a distinguishing characteristic from other ivory forms. The intersection of these lines forms concave and convex angles; the concave angles open medially and the convex angles open towards the periphery. Two zones are visible of which the outer one is more distinct. Mammoth has Schreger angles less than 90° whereas extant African and Asian elephants have angle greater than 105° [9].

This study showed that mean Schreger angle value on the outer portion of ivory is more than 120° in African and less than 120° in Asian elephant (two outliers have to be rechecked). Schreger angle of mammoth was reported to be $73.21^\circ \pm 14.71$ and of African ivory was $124.15^\circ \pm 13.35$ [21]. We have attempted in this study to identify ivory in intact, pieces or processed forms. We observed that Schreger angle value is not dependent on age of the elephant and that Schreger angles on the outer portion are most suitable to distinguish African and Asian ivory. The ability to distinguish tusk dentin of proboscidean taxa on the basis of Schreger angle will be useful for forensic scientists, wildlife conservationist and archaeologists [12]. This is also a nondestructive forensic method for identification [9].

6.2. X-ray fluorescence

Ivory of elephants comprises chiefly of dentine which are made by the inorganic compound known as dahllite with the general formula $Ca_{10}(PO_4)_6(CO_3)H_2O$ [16]. Intensities of 22 elements were obtained using X-ray fluorescence techniques. The results show that strontium and hafnium was higher in concentration in African ivory than in Asian ivory. However, there were marginal differences in intensities of phosphorous, calcium and halogen. The concentration of strontium and hafnium could serve as species specific signatures to identify African and Asian ivory wherever morphometric techniques are not useful.

6.3. Inductively coupled plasma-atomic emission spectrometry

The analysis shows that phosphorous, magnesium and chromium has higher concentration in Asian elephant ivory as compared to African elephant ivory whereas concentration of silicon, calcium, strontium, barium, iron, manganese and zinc were higher in African ivory as compared to the Asian ivory. The concentration of barium, strontium, silicon, iron, manganese, zinc, phosphorous and magnesium were consistent in all samples of the Asian ivory. On the other hand, only barium concentration was found consistent in the African ivory. Difference in the ranges of the above elements in African and Asian ivory will be useful in distinguishing the two ivories.

6.4. Inductively coupled plasma mass spectrometry

Concentration of vanadium, samarium, europium, gadolinium and scandium are higher in the Asian ivory where as molybdenum and nickel are higher in the African ivory. Other rare earth elements dysprosium, holomium, erbium, thulium, ytterbium and yttrium are merely detectable only in Asian ivory and not detectable in African ivory. The consistence of yttrium concentration in all samples of Asian ivory and not detectability of this element in African ivory can be useful to distinguish ivories of the two continents.

6.5. Isotopic analysis

Scientist have analyzed isotopic ratios for ivory of African elephant for determining source area [16]. This technique has yet not used in Asian ivory to know their origin though, some isotopic work on bones of Asian elephants has been done [22]. The trivariate isotopic analysis namely stable carbon isotope ratio ($^{13}C/^{12}C$) in elephant bone collagen shows the mixture of C_3 foliage and C_4 grasses in the diet, and are directly proportional to the density of C_3 browse [23], nitrogen isotope ratio ($^{15}N/^{14}N$) in bone collagen is related to rainfall and water stress [24,25] and strontium isotopes ($^{87}Sr/^{86}Sr$) reveal local geology [25,26].

This study shows that isotopic ratio from tusk powder and collagen is highly correlated. Thus, forensics examination from powder samples is more suitable as sample preparation is limited and results can be obtained quickly as compared to collagen. Some distinction across regions may be possible on the basis of browser versus grazer populations; for instance elephants from Karnataka, Kerala and Uttaranchal seem to browse to a greater extent than those in Tamilnadu and Assam but this could also be due to rapid turnover of collagen and fluctuating isotopic signatures in younger animals [27]. Thus, tracing the source of ivory from carbon isotopic ratios alone is not possible.

Mean $\delta^{13}C$ for African ivory is higher than Asian ivory but there is overlap between the values from the two continents. Mean $\delta^{15}N$ for African ivory is higher than Asian ivory with no overlaps in the standard errors; this could thus serve as a tool to distinguish the species. Mean $\delta^{15}N$ and average annual rainfall shows that different states can be separated into different cluster. However mean of $\delta^{15}N$ values of Assam and Kerala are overlapping might be due to low sample size. The presence of low nitrogen isotopic ratio in Uttaranchal elephants tusk,

medium to high in Karnataka elephants tusk, and medium nitrogen isotopic ratio in Assam, Kerala and Tamil Nadu elephant tusk are due to presence of different plant type and food species in different states.

We conclude that it is possible to differentiate Asian ivory from African ivory using Schreger angle, isotopic study, XRF, ICP-AES and ICP-MS. With such database, different techniques can be applied depending on the type of seized material received such as whole tusk, piece, powder and artifact. Isotopic study will be useful for wildlife managers and law enforcement authorities to know the origin of seized material and take measures to curb poaching. Strontium isotope data will add to the robustness of these results.

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