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DETERMINING THE SOURCE-AREA OF IVORY THROUGH ISOTOPIC ANALYSIS

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INTRODUCTION

Knowledge of the area of origin of ivory is an essential part of the strategy proposed by the Southern African Centre for Ivory Marketing (SACIM) for controlling renewed trade in ivory from Botswana, Malawi, Namibia, Zambia, and Zimbabwe. Before trade is resumed, it must be demonstrated that ivory from these countries can be unambiguously distinguished from other, illegal sources of ivory. Dr. Nikolaas J. van der Merwe (University of Cape Town and Harvard University), Dr. John C. Vogel (CSIR, Pretoria), and their colleagues have proposed that it might be possible to determine source-area of ivory from its isotopic composition. In their pilot studies, Vogel, van der Merwe, and colleagues demonstrated that African elephants from different regions had unique isotopic compositions. Isotopic techniques are established tools in archaeology, paleontology, geology, and ecology, and the factors controlling the isotopic relationships between an animal and its environment have been extensively studied. However, isotopic source-area determination must have a secure scientific basis that can withstand legal scrutiny. The studies by van der Merwe, Vogel, and colleagues were exploratory, and did not attempt to address every significant factor that may complicate isotopic source determination. Until these factors are thoroughly investigated, the isotopic method will not supply the unambiguous source-area determinations needed to control renewed trade in ivory. We consider the empirical foundations of the method, evaluate its current status as a forensic tool, and propose a protocol for rigorously verifying the limits of the technique.

WHAT ARE ISOTOPES, AND HOW ARE THEY ANALYZED?

Most elements in nature exist as two or more isotopes. Isotopes are forms of a chemical element that differ in atomic mass. Isotopes are useful because their abundances vary in response to biological and chemical processes. Stable isotopes are those that do not undergo radioactive decay. For example, any natural sample of carbon contains two stable isotopes, the light isotope, ^{12}C, which is very abundant, and heavier isotope, ^{13}C, which occurs at trace levels. Because stable isotopes of an element have different masses, they are processed at different rates in chemical and metabolic reactions. This favouring of one isotope over the other during chemical reactions generates variations in stable isotopic ratios. Other isotopes, such as ^{14}C and ^{87}Rb, are unstable; they decay radioactively with time to form other isotopes or elements. ^{87}Rb, for example, decays to form ^{87}Sr. Consequently, the ratio of a radioactively-produced isotope (e.g., ^{87}Sr) to a stable isotope of the same element (e.g., ^{86}Sr) can vary, depending on the geologic age of the sample and the amount of the parent isotope present (e.g. ^{87}Rb).

Variations in the isotope ratios of carbon ($^{13}C/^{12}C$), nitrogen ($^{15}N/^{14}N$), strontium ($^{87}Sr/^{86}Sr$), and lead ($^{206}Pb/^{204}Pb$) were examined in the studies of ivory source-areas. Carbon and nitrogen were examined in collagen, the dominant protein in bones and ivory. Carbon and nitrogen isotopic analysis of has three steps: 1) dissolution of bone or ivory mineral to isolate collagen, 2) combustion of collagen to release carbon dioxide and nitrogen gases, and 3) analysis of the isotopic composition of these gases on a gas source mass spectrometer. Carbon isotope analysis of collagen has been automated by coupling a gas chromatograph to a mass spectrometer. Automated nitrogen isotope analysis using such a system is not yet routine. Overall, the techniques for analysis of carbon and nitrogen isotopes are relatively straightforward, inexpensive (probably = $50 US dollars/sample), and unlikely to be plagued by sample handling and contamination problems.

Strontium and lead exist as trace elements in the mineral hydroxyapatite, which is a constituent of bones and ivory. Isotopic analysis of these elements involves 1) combustion of collagen to isolate mineral, 2) dissolution of the mineral in acids, 3) wet chemical isolation of Sr or Pb, and 4) analysis of isotopic composition on a solid source mass spectrometer. This technique is more difficult and much more expensive ($150 US dollars/sample). In addition, because the amount of Sr and Pb in bones and ivory is very low, sample contamination is a serious issue. To minimize contamination, samples are usually processed and analyzed in "clean labs" with filtered air and ultra-high purity reagents and water.

FACTORS CONTROLLING THE ISOTOPIC COMPOSITION OF MAMMALS

Carbon, nitrogen, strontium, and lead chiefly enter animals through food plants. An animal's organic and mineral matter have an isotopic composition with geographic region because of changes in the isotopic ratio of food plants, and possibly because of changes in the way animals process nutrients under different climatic regimes. The factors that control variation in each isotope are considered below.

Carbon: During photosynthesis, plants convert atmospheric carbon dioxide and water into acids, and ultimately, plant tissues. There are tow major biosynthetic pathways that plants use to fix carbon. In one, known as the C_3 pathway, carbon dioxide is first fixed as a 3-carbon molecule, whereas a 4-carbon molecule is the first product in the C_4 pathway. In African ecosystems, C_3 plants include all trees, and nearly all shrubs and herbs, and the grasses that occur in high altitudes and in shaded, dense woodland environments, whereas the grasses in most warm or dry settings are C_4 plants. When plants fix carbon using either pathway, they preferentially incorporate the light isotope, $_{12}C$. This discrimination between isotopes during photosynthesis differs between C_3 and C_4 plants. C_3 plants have a carbon isotope value that is substantially lower than C_4 plants. The carbon isotope composition of C_3 plants in dense woodlands is especially low, because these plants fix carbon dioxide that already has a low isotope value due to input of gas from rotting C_3 vegetation.

The diets of African herbivores have been studied using isotopes by many researchers. There is a small, but consistent difference in isotopic value between an animal's food and the collagen it deposits in bones and teeth. The food available to herbivores in woodland habitats generally has a low carbon isotopic value, due to the dominance of C_3 plants. In open habitats, where C_4 grasses are more abundant, the carbon isotopic value of available plant foods is higher. Because elephants eat plants roughly in proportion to their availability in the environment, their carbon isotopic values track these ecological differences. Thus carbon isotopes allow differentiation between elephants from woodland versus grassland environments.

Nitrogen: There is a negative correlation between rainfall abundance and the nitrogen isotopic value of plants in African ecosystems, with isotope values in arid regions and low values in wet regions. The isotopic compositions of animals track these dietary differences. However, as with carbon, there is a small offset between the isotopic value of food and an animal's collagen. This offset is greater in arid regions than in areas with high rainfall. There is no consensus as to the cause for this change in offset, but it may relate to adaptations for water conservation or to the effects of nutritional stress on mammals in arid regions. In any case, nitrogen isotopes generally allow discrimination between elephants in wet versus dry habitats.

Strontium and Lead: Animal bones and teeth have the same Sr and Pb isotopic compositions as their food plants, and plants, in turn, directly record the isotopic composition of underlying soils and bedrock. ^{87}Sr and ^{206}Pb are produced by the radioactive decay of ^{87}Rb and ^{238}U, respectively. As a consequence, soils derived from very old rocks, and rocks which were rich in rubidium and uranium, have high $^{87}Sr/^{86}Sr$ and $^{206}Pb/^{204}Pb$. In Africa, there is a dichotomy between regions with old, granitic crust and high Sr and PB isotope ratios versus areas with either young, volcanic rocks or marine-derived sediments and low isotope ratios.

The variations in isotopic ratios for carbon, nitrogen, strontium, and lead are controlled by different factors: forest cover, rainfall, geology. Because these factors vary independently, these isotopes may provide a powerful tool for discriminating among elephant populations. Vogel, van der Merwe, and colleagues clearly demonstrated segregation of many African populations using samples of bone and sometimes ivory (Fig. 1). However these pilot studies had several limitations. The number of individuals examined in each park was too low (typically) to permit confident assessment of isotopic variability in these populations. This paucity of data prevented rigorous statistical tests of isotopic discrimination among populations. We consider three potential complications in greater detail: sources of within-population isotopic variability, limitations in geographic coverage, and the potential for high isotopic variability within individual tusks.

SOURCES OF WITHIN-POPULATION ISOTOPIC VARIABILITY

The studies by van der Merwe, Vogel, and colleagues could not explore many of the factors contributing to potential isotopic variability within elephant populations, such as migration, ecological change within a region, or seasonal dietary shifts. For example, van der Merwe et al. (1990) and Vogel et al. (1990) obtained completely non-overlapping carbon and nitrogen isotope values for the elephants from Addo, South Africa, indicating a very high degree of within population variability.

Clearly, if elephants move into different regions in response to habitat degradation, drought, hunting, or space competition, their isotopic compositions could change. Van der Merwe and Vogel demonstrated that elephants from very closely spaced parks were isotopically distinct, suggesting that the isotopic map of Africa is very "fine-grained", with major differences over short distances. If individuals move between adjacent regions, their isotopic signals will be homogenized, and isotopic discrimination could be impossible in such cases.

Ecological and meteorological changes can alter the flora in a region. A change in the proportion of trees would definitely affect the carbon isotopic composition of elephants. Dramatic changes in tree cover have been documented in many parks, due in part to intense browsing by elephants. Likewise, changes in rainfall abundance, water availability, or nutritional status might influence elephant nitrogen values. These changes could be particularly acute in drought-affected populations.

Elephant diets can vary dramatically with season. In some deciduous woodlands, dry season forage consists chiefly of leaves, twigs and bark, whereas in the wet seasons, elephants eat mostly grasses (e.g., Ruaha N.P., Tanzania). The reverse pattern was reported for a savannah population (e.g., Rwenzori N.P., Uganda). Consequently, portions of a tusk grown in a wet season may differ isotopically from portions grown in a dry season, and these seasonal patterns will vary among elephant populations.

LIMITATIONS OF THE EXISTING GEOGRAPHIC DATABASE

Some complications due to within-population variation could be addressed if the studies by Vogel, van der Merwe and their colleagues were expanded to include a larger number of individuals from the same populations. However, the geographic coverage of elephant populations must also increase. A variety of herds from southern Africa were analyzed, but coverage in eastern African was sparse. Tanzania, for example, has a large number of elephants, yet there were no isotopic data reported for elephants from this nation.

A thorough collection of data from eastern Africa is essential. Prior to the Appendix I listing of elephants in 1989, east African herds suffered extreme losses from poaching. The isotopic variations in ivory from the remaining vulnerable herds must be well characterized, so that this ivory can be unambiguously distinguished from the ivory derived from all other elephants and excluded from the world market. This could be accomplished by collecting ivory from natural deaths. In addition, because southern African nations cull their elephant herds, parks and preserves in these countries have not experienced extreme losses of woodlands due to intense elephant browsing. Thus, elephants from southern African parks are not expected to show a high degree of variability due to floral change. The isotopic effects of woodland loss must be investigated in eastern African elephants.

ISOTOPIC VARIABILITY WITHIN INDIVIDUAL TUSKS

Elephant bones were the chief sample materials in the studies by van der Merwe, Vogel, and their colleagues. The general isotopic relationship between diet and collagen or mineral are the same for bones and ivory. However, bones and tusks are formed in very different ways. Bone collagen and mineral are initially deposited in young animals, then continuously remodelled as an animal grows and ages. Typical isotopic samples (20 to 50 mg of either collagen or mineral) contain material that has been homogenized over much of an animal's life. Tusks, on the other hand, grow by continuous addition of new layers of ivory along the pulp cavity, with little subsequent remodelling. Many millimetres of ivory are added to the growing surface each year. At the same time, the tusk erupts from the tooth alveolus, carrying previously formed ivory towards the tip, while making room for new material at the tusk base. Thus a typical isotopic sample of tusk contains material grown over a brief interval, perhaps totalling only one or two years.

Because bone samples are averaged over much of an animal's life, isotopic shifts due to migration, ecological change, or seasonal dietary shifts will be obscured. In contrast, ivory samples may be quite sensitive to these shifts. van der Merwe and colleagues examined a single tusk and reported low carbon and nitrogen isotopic variability. However, in our own work on extinct relatives of elephants (mastodonts and mammoths), we discovered substantial isotopic variability across tusks. Such variability may ultimately prove the greatest limitation to isotopic source-area

determination. If tusks are highly variable due to the causes discussed above, there may be isotopic overlap between tusk samples collected from different populations that is not apparent from bone samples. The variability in ivory samples must be studied further.

Recommended protocol for further studies

It has not been unequivocally demonstrated that elephants from different living populations are isotopically distinct. We have developed a protocol for future investigations of isotopic sourcing that will rigorously test the power of the method. In addition, this protocol may provide unique insight into seasonal diets, migration patterns, and other significant aspects of African elephant ecology.

- Elephants from all extant African populations must be analyzed.
- Data for the geologically-controlled isotopes of Sr and Pb are currently scarce. They must be consistently reported for all specimens. Sr and Pb isotopes are very important, given that carbon and nitrogen isotopes are more susceptible to short term ecological and meteorological changes.
- Ivory must be the primary material examined for all populations. Research collections of ivory should be preserved for all living African elephant herds. The age, sex, and date of death of each individual should be recorded. Ivory for examination should be collected as bulk samples from near the pulp cavity. This material was grown in the two to three years prior to death.
- For several populations, a very large number of individuals should be analyzed (50). Attempt to examine equal numbers of each sex. Perform statistical analyses on these populations to determine the minimum number of individuals that must be analyzed to adequately characterize the mean and standard deviations of a population.
- Wherever possible, examine at least the minimum number of individuals as determined from the statistical studies.
- For several individuals from each population, analyze multiple samples (20) collected from the tip to the base of single tusks to assess variability.
- Provide a statistical treatment of isotopic discrimination among the herds.

If all samples from each population fall into isotopically-discrete clusters, then isotopic sourcing could be successfully applied to all ivory guaranteed to be from living African herds. Alternatively, populations may overlap due to high isotopic variability within tusks, but be isotopically-discrete when only samples from the pulp cavity are considered. Given this result, isotopic sourcing could be used only for ivory samples that retained traces of the pulp cavity. This would prevent most forensic applications to carved ivory, and prohibit verification of source-areas in countries receiving carved ivory. Finally, even isotopic samples from the pulp cavity may overlap between populations. If this occurs between vulnerable populations and controlled, southern African elephant populations, the isotopic method would not permit discrimination between what might be illegal and legal ivory.

The pilot studies by Vogel, van der Merwe, and their colleagues are exciting, because they suggest a basis for discriminating members of endangered African elephant populations. However, these studies are clearly preliminary and do not claim to have carefully characterized all the significant sources of variation in elephant populations or even in individual elephant tusks. Until the full range of this variability is known, isotopic analysis cannot provide the unambiguous determinations of source needed to monitor a renewed ivory trade.

Suggested Readings

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