Bomb-curve radiocarbon measurement of recent biologic tissues and applications to wildlife forensics and stable isotope (paleo)ecology

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Above-ground thermonuclear weapons testing from 1952 through 1962 nearly doubled the concentration of radiocarbon (14C) in the atmosphere. As a result, organic material formed during or after this period may be radiocarbon-dated using the abrupt rise and steady fall of the atmospheric 14C concentration known as the bomb-curve. We test the accuracy of accelerator mass spectrometry radiocarbon dating of 29 herbivore and plant tissues collected on known dates between 1905 and 2008 in East Africa. Herbivore samples include teeth, tusks, soft tissue, hair, and horn. Tissues formed after 1955 are dated to within 0.3–1.3 y of formation, depending on the tissue type, whereas tissues older than ca. 1955 have high age uncertainties (~17 y) due to the bomb effect.14C dating of these tissues has applications to stable isotope (paleo)ecology and wildlife forensics. We use data from 41 additional samples to determine growth rates of tusks, molars, and hair, which improve interpretations of serial stable isotopic data for (paleo)ecological studies.14C dating can also be used to calculate the time interval represented in periodic histological structures in dental tissues (i.e., perikymata), which in turn may be used as chronometers in fossil teeth. Bomb-curve 14C dating of confiscated animal tissues (e.g., ivory, statue) can be used to determine whether trade of the item is legal, because many Convention of International Trade of Endangered Species restrictions are based on the age of the tissue, and thus can serve as a powerful forensic tool to combat illegal trade in animal parts.

Carbon-14 (14C) is produced in the atmosphere primarily by neutron interaction with 14N through the reaction 14N + n → 14C + p. This occurs naturally from secondary neutron flux generated by cosmic rays and anthropogenically by high neutron flux from nuclear fission in bombs or, to a lesser degree, as nuclear reactors. Atmospheric 14C is oxidized to CO2, which enters the terrestrial biosphere through assimilation into plant biomass. Other living organisms incorporate 14C into their tissues by consuming plants or organisms that consume plants. 14C enters the oceans as CO2 through air–sea exchange and subsequent vertical mixing and becomes part of the biologically available dissolved inorganic carbon pool. Following the inception of thermonuclear weapons testing, periodic measurement of atmospheric 14C concentrations began at stations around the world. These data document the abrupt rise and steady fall of 14C concentration in the atmosphere known as the bomb-curve. The atmospheric 14C concentration and its regional variation have been well known for the last 60 y (1, 2).

Previous studies testing bomb-curve 14C dating are largely limited to tree rings (1, 3) and a small number of mammal tissues (4, 5). Geyh (5) found human bone collagen and animal leather are less suitable for bomb-curve dating than hair, which could be used to determine age of death within about 2 y. Forensics research to determine year of birth has focused primarily on human tooth enamel and dentin (6–10), although proteins in the crystalline portions of eye lenses also provide accurate birth-year estimates (11). Several studies have explored the use of radiocarbon to date tusk ivory (4, 12, 13) but offer only limited data and, in some cases, lower precision than accelerator mass spectrometry (AMS) methods (13).

Here we use animal and plant tissues of known ages to expand significantly on previous studies in the number of samples and tissue types to show that from 1955 to the present 14C-calibrated ages measured by AMS accurately record the date during which the tissues formed. We demonstrate the accuracy of bomb-curve 14C dating based on results from 29apatite, collagen, keratin, soft tissue, and plant samples. Maximum accuracy with respect to the known age is achieved by using tissues that undergo little or no turnover. Samples collected from the proximal, or most recently formed, portion of the tissue can be used to determine date of collection, which is often, but not always, death.

Using an additional 41 14C ages, we determine tissue growth rates by serially sampling along the growth axis of Hippopotamus amphibius (hippo) canines and Loxodonta africana (elephant) tusks, molars, and tail hair. We provide examples of how 14C ages from these mammal tissues can be used in stable isotope (paleo)ecology and wildlife forensics. In stable isotope ecology, growth rates are required to convert distance along the growth axis of a tissue to time, which enables comparison of isotope data with time-series data (e.g., temperature, rainfall, or remote sensing data such as Normalized Difference Vegetation Index).

14C-derived growth rates from extant species can also be used to determine the period (e.g., days or weeks) represented in growth increments in dental tissues, providing a basis for establishing a chronometer in fossil teeth. Chronologic control is imperative in intratooth stable isotope and histological studies that aim to evaluate seasonal variability in past environments. Finally, we demonstrate that 14C dating can be used in wildlife forensics to determine the age of confiscated animal tissues, which in many cases is equivalent to the date of death. For many animal parts, such as ivory and rhino horn, age often determines whether trade of the item is legally permitted.

Results

Fraction Modern Carbon and 14C-Calibrated Ages. 14C data are presented as fraction modern carbon (F14C), where F14C = (A14C/AOX) × (0.975/0.981) × [(1 + δ13COX/1000)/(1 + δ14COX/1000)].


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The \( F^{14}C \) values plotted against the known age reveal that the \( F^{14}C \) in herbivore and plant samples tracks the \( F^{14}C \) of atmospheric CO\(_2\) during the period in which the tissue formed for samples collected before 1955 (Fig. 4). Pertinent information is provided in Dataset S1, Table S1, and all \( F^{14}C \), \( \Delta^{14}C \), and \( 14^{14}C \)-calibrated ages are given in Dataset S1, Table S2. The Northern Hemisphere 3 (NH3) and Southern Hemisphere 1 (SH1) calibration curves (1) are both plotted in Fig. 4 because we sampled animal tissues from both regions. The NH3 data set is appended with the Levin dataset (2, 16) (NH3+Levin) beginning at 1999.50 to permit \( 14^{14}C \) age calibration through 2006. The two curves, NH3+Levin and SH1, differ significantly before ~1970 owing to bomb testing locations and atmospheric circulation, and subtle differences of 4–5‰ persist after 1970. Pre-1970 keratin and plant samples are confirmed (or in several cases presumed) to have been collected from the Southern Hemisphere and track the SH1 curve extremely well (Fig. 4).

Fig. 1B shows the known age versus the calibrated \( 14^{14}C \) age for all samples (n = 22) collected from 1955 to 2006. Fig. 1C includes four samples collected between 1905 and 1953 to illustrate the inaccuracy of calibrated \( 14^{14}C \) ages before 1955. The residual (r) between the \( 14^{14}C \) age (age\(_{14^{14}C}\)) and known age (age\(_{known}\)) is given by \( r = age_{14^{14}C} - age_{known} \) (Fig. S1 and Dataset S1, Table S2). The mean residual of 11 keratin samples collected after 1955 is ~1.3 ± 1.8 (1σ) years. For apatite samples (n = 5), the mean residual is ~0.8 ± 0.7 years; for grasses (n = 3), it is 0.3 ± 0.6 year. For the soft tissue and collagen samples, the residuals are ~0.7 and ~1.2 years, respectively. Variation in mean residuals based on tissue type likely arises from differences in the total number of samples analyzed, in the amount of time integrated in different tissue types, and in the \( F^{14}C \) values (e.g., whether the samples fall on a steep or shallow part of the bomb-curve). Tissues formed during the steeper parts of the bomb-curve tend to have residuals less than 2 years (Fig. S1). The current slope of the bomb-curve is shallower than during the interval from 1955 to ca. 2005, increasing the uncertainty of \( 14^{14}C \)-calibrated ages in tissues formed from ca. 2005 forward (Fig. 4 and Dataset S1, Table S2).

A hair sample (L10830) from a *Cercopithecus mitis* (blue monkey) was reportedly collected in the Congo in 1962 but has a \( F^{14}C \) value of 0.9749 ± 0.0023 (this and all subsequent SDs are 2σ), which clearly indicates it formed before 1955. Nearly 70% of the blue monkey’s diet is fruit and leaves, so significant dietary contribution from older plant material (more than several years old) is unlikely (17). Hair from other primates, including two other *C. mitis*, yield \( 14^{14}C \) ages consistent with known dates, further suggesting that diet is not the cause for the age discrepancy. The most likely explanation is that the date of museum accession, which we use as the known age of the sample, does not reflect the date of death.

**Tissue Growth Rates.** We use multiple \( 14^{14}C \) ages from elephant tusks, molar plates, and tail hair and hippo canines to calculate tissue growth rates (Table 1). A schematic of the general structure of tusks, molars, and canines is shown in Fig. S2. The period of growth for some canines and both tusks continued beyond 1997, when \( 14^{14}C \) data becomes sparse for both the NH3 and the SH1 data sets. Thus, we use the Levin dataset to calibrate \( 14^{14}C \) ages for samples more recent than 1960 with an \( F^{14}C \) ≤ 1.110.

**Elephant tusks.** Tusk growth rates for two female African elephants were determined from collagen-derived \( 14^{14}C \) ages. Growth rates are 4.13 ± 0.39 cm/yr and 5.10 ± 0.74 cm/yr for elephants R37 and Misha, respectively (Fig. 2A and Table 1). We use linear growth rates because they best fit the data from the two tusks, although a second-order polynomial also fits the tusks data from R37. Because there is no calibrated \( 14^{14}C \) age for the youngest data point for Misha, we use September 10, 2008, her known date of death.

Assuming an age at death of 53 ± 5 years for R37 based on molar wear (18, 19), the R37 tusk represents growth from 25 to 53 years of age, whereas Misha’s tusk represents growth from 13 to 28 years of age. Thus, the 20% difference in growth rate between the two tusks may be explained by ontogeny, but may also relate to other factors (e.g., age, sex, and age). Growth rates may not be linear. Mastodon tusks show nonlinear growth rates based on measurements of annual incremental thicknesses and lengths over ~30 years (20, 21). Using the tusk lengths and growth rates for R37 and Misha, we calculate the time represented in the tusks to be 28.0 and 14.8 years, respectively (Table 1). Interestingly, this accounts for 54% and 53% of their total lifespans, respectively, suggesting similar overall rates of wear between the two female elephants.

**Hippo canines.** We calculate growth rates for five hippo canines using a total of 17 enamel \( 14^{14}C \) ages. Length measurements are made along the outer curvature of the canine. Multiple \( 14^{14}C \) ages from a lower (n = 5) and an upper (n = 3) canine of an individual, presumed to be a juvenile or young adult based on canine shape.
and size, give linear growth rates of 3.35 ± 0.25 cm/y and 1.94 ± 0.31 cm/y, respectively (Fig. 2B). Growth rates from three other (lower) canines, presumably from males based on size, range from 4.51 ± 0.21 cm/y to 7.47 ± 0.88 cm/y (Table 1 and Fig. 2C). Passey et al. (22) measured lower canine growth rates in two female hippos from the Toledo Zoo by notching the tooth at the gum line and measuring the distance from the gum line the following year. Growth rates from the 48- and 8-y-old females were 1.35 cm/y and 2.9 cm/y, respectively (Fig. 2), which are lower than the values determined for lower canines in this study (3.35–7.47 cm/y), which is likely due to differences in canine growth rate between male and female hippos and, for the 48-y-old individual, age.

**Elephant molars.** We calculate vertical growth rates along six plates from two molars using a total of 16 14C ages. These growth rates are time-averaged mineralization rates of enamel, which may differ from molar extension rate. The latter is determined by the extension of the molar plate as new dentin and immature enamel are formed, whereas the former represents the difference between the average ages of the enamel volumes sampled at each position along the plate. If molar extension and enamel maturation processes were constant throughout molar formation, then mineralization and extension rates would be equal. Fig. 3A shows sample locations in the third molar (m3) from TE-95. Growth rates were determined in four plates (2, 4, 7, and 9) in sample TE-95 and two plates (7 and 9) in R37’s m3. Growth rates from both molars range from 1.39 ± 0.27 to 1.63 ± 0.14 cm/y (Table 1 and Fig. 3 B and C). The rates fall within the range of those determined histologically for the extinct Columbian mammoth (*Mammuthus columbi*): 1.3–2.2 cm/y (23, 24). The 14C data do not reveal whether growth rates are linear; however, histological data from two extinct proboscidean species, *M. columbi* and *Paleoloxodon cyriotes*, indicate growth rates are highest near the initial occlusal surface and decrease toward the cervical margin (23).

A 14C age on collagen from the mesial root of TE-95 yields an age of 1964.2 ± 0.1, which is the best estimate for the date of death (Fig. 3A). Time represented in unworn molar plates from TE-95 is 7.3 ± 0.6 y, and time represented in an entire elephant molar is ca 0.10 y or more based on 14C ages from TE-95 and R37 (Table 1). The thick enamel and the long time intervals represented in a single plate or entire molar make fossil proboscidean teeth excellent candidates for intratooth stable isotope profiles in paleoecology (e.g., ref. 25).

**Elephant tail hair.** Only one of two tail hairs sampled provides a reasonable growth rate. Sample TSV-171, collected on July 17, 1998, from a female African elephant in Tsavo National Park,
yields a growth rate of 0.81 ± 0.77 mm/d (Table 1). Wittemyer et al. (26) used independent methods to calculate a growth rate of 0.81 ± 0.11 mm/d for female African elephants ($n = 38$). A second tail hair was collected from R37 within days of her death in September 2006. The proximal and distal ends of the 304-mm-long hair have nearly identical $^{14}$C values of 1.0820 and 1.0803, respectively, and the higher value in the proximal end precludes calculating a growth rate. Growth rates determined by independent methods from R37 tail hairs collected between 2001 and 2006 range from 0.56 to 0.62 mm/d.

**$^{14}$C variation based on tissue type and pretreatment.** Four tissue types were sampled at death from two elephants to test for variation in $^{14}$C based on tissue type. Collagen and apatite from tusk dentin sampled from the pulp cavity margin (e.g., the tissue forming at time of death) show indistinguishable $^{14}$C values (Dataset S1, Table S3). The $^{14}$C values can be used to calculate a $^{14}$C-calibrated age for R37, and the collagen and apatite ages fall within a range of less than 0.4 y. We tested whether treating tusk apatite with 3% NaOCl had any effect on $^{14}$C values. Treated and untreated apatite samples from Misha have nearly identical $^{14}$C values, whereas those from R37 differ by 5.6‰ but fall within the range of 2σ uncertainty (Dataset S1, Table S3). The data suggest treatment to oxidize organics before acid digestion is not necessary.

We also analyzed the proximal end of a tail hair (R37-prox-K) and soft tissue from R37’s tusk pulp cavity (R37-PC-tissue). The $^{14}$C value in the tail hair is anomalously high, resulting in an older age than the actual date of death (Dataset S1, Table S3). The soft tissue sample has a $^{14}$C value of 43.7‰, whereas that of R37 differ by 5.6‰ but fall within the range of 2σ uncertainty (Dataset S1, Table S3). The data suggest treatment to oxidize organics before acid digestion is not necessary.

**Discussion**

**Application to Stable Isotope (Paleo)ecology.** $^{14}$C-correlated stable isotope profiles. Serial sampling or intratooth stable isotope profiles of enamel yield information about seasonal change in diet and water use, which relate to seasonality of precipitation and vegetation. This has been suggested as a method for (paleo)dietary and (paleo)ecological reconstruction in modern and fossil mammalian teeth (e.g., refs. 25, 27–31). In ungulate molars, tooth height and growth rate determine total formation time, which is generally no more than 2–3 y (32, 33). However, continuously growing teeth from large mammals (e.g., hippo canines and elephant tusks) and elephant molars form over years to decades and therefore can be used to evaluate long-term dietary and seasonality changes (28, 31, 34) and can capture ontogenetic transitions such as weaning (35).

We show that intratooth stable isotope profiles from two hippo canines of death that died nearly 11 y apart can be concatenated (with sufficient overlap) using $^{14}$C data (Fig. 4). $^{14}$C data come from two canines, one collected in 1996 (TSV-291) and the other in 2008 (K08-201), from the same region near Tsavo National Park. The $^{14}$C values from enamel in the proximal end of the 1996 and the distal end of the 2008 canines are nearly identical and therefore yield similar $^{14}$C ages (Fig. 4). The dominant feature in the $^{14}$C record from the TSV-291 canine is a rapid decrease of 5.4‰, which begins 200 d before death suggests physiological stress preceding death, a pattern observed in other serially sampled hippo canines. Canine K08-201 is from a hippo shot dead on October 10, 2007 as a (crop-raiding) nuisance animal near Mtito Andei.

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**Fig. 3.** (A) Longitudinally cut elephant molar (m3) from individual TE-95 showing calibrated $^{14}$C ages ($\pm 2$σ) for 13 enamel apatite and 2 dentin collagen samples. Sample locations are outlined as ellipses. The molar consists of 11 enamel-covered plates (P1 to P11). (B) Vertical growth rates from four TE-95 molar plates shown in A are calculated from $^{13}$C ages. (C) Vertical growth rates in two molar plates from a lower third molar belonging to R37 (see text). Growth rates ($\pm 2$σ) are calculated from slopes; height along a plate is normalized to the lowest sample location. Age uncertainty is 2σ and if not shown is smaller than the symbol.

**Fig. 4.** Intratooth stable isotope profiles from two hippo canines overlap to provide a continuous 18-y isotope record. $^{14}$C values used as a tie point between the two canines are labeled with arrows indicating sample location. Based on the shape of the $^{14}$C curves, the K08-201 profile has been shifted $\sim 0.3$ y, which is within the 2σ range of uncertainty. Canine TSV-291 was collected in 1996 near the town of Mtito Andei, Kenya. The steep rise in $^{18}$O that begins $\sim 200$ d before death suggests physiological stress preceding death, a pattern observed in other serially sampled hippo canines. Canine K08-201 is from a hippo shot dead on October 10, 2007 as a (crop-raiding) nuisance animal near Mtito Andei.
indicating a switch to (C3) browsing beginning in the latter part of 1995, followed by a 4% increase in δ13C over the last half year of the hippo’s life in 1996 (Fig. 4). The onset of the δ13C shift coincides with beginning of a prolonged drought that persisted until April of 1997. The 2008 canine data suggesting diets persisted among hippos in this region until the year 2000, when the diet returns to predominantly C4 grazing.

Overlapping isotope profiles from multiple teeth based on bomb-curve 14C ages can provide long-term ecological records. These records may be useful for tracking decadal (or longer) scale changes in land-use, climate, or life-history patterns and thus have potential application in wildlife ecology and conservation. Understanding how ecological change, such as periods of drought or seasonal precipitation, affects intratooth isotope profiles in extant taxa provides insight for interpreting profiles in fossil teeth.

Periodicity of incremental growth features. Periodic incremental growth features in tooth enamel and dentin (e.g., perikymata, striae of Retzius, and Andresen lines) can be used as accurate chronometers if the time interval represented by each increment is known (36). By establishing a chronometer, the teeth can provide information about the timing of tooth development and other aspects of life history. The chronometer is critical for interpreting intratooth stable isotope profiles in fossil teeth, where one of the primary goals is to determine the magnitude, duration, and periodicity of diet changes and environmental change in the past. In most human unwisdom of the other hominoid visible on the surface of a tooth represent a period of 7 or 8 d (37). In proboscidean tusks, three hierarchical incremental growth features have been proposed: First-order increments have annual periodicity, second-order are weekly in elephants and mammoths (fortnightly in mastodons), and third-order are daily (38, 39).

We use 14C growth rates to determine the time interval represented in hippo canine perikymata and to confirm the weekly time interval represented in elephant tusk dentin. The distance between perikymata was measured along sections of canine K11- KF using a plugin (Inc Meas v.1.2) in ImageJ software (Fig. S3). Mean increment width is 1.26 ± 0.35 mm (n = 167), and given the growth rate of 45.1 mm/y, each increment represents 10.2 ± 2.9 (1σ) days (Dataset S1, Table S3). Other hippo canines for which 14C growth rates were determined either lacked visible perikymata or photos for making measurements.

In the R37 tusk, we measured the thickness of second-order increments on a transversely cut thin section located 2 mm from the horn of the pulp cavity using the same ImageJ plugin (Fig. S4). The average growth rate determined from histological measurements is 105 ± 1 mm/y (Dataset S1, Table S4). Mean increment width is 2.9 ± 0.35 mm (n = 167), and given the growth rate of 45.1 mm/y, each increment represents 2.9 ± 0.3 (1σ) days (Dataset S1, Table S3). The 14C growth rate provides independent evidence for weekly periodicity of second-order growth increments in elephant tusk dentin.

The period recorded in growth increments in modern teeth and tusks can be applied with caution, because the period between increments may differ between modern and fossil teeth, to similar taxa in the fossil record that cannot be bomb-curve 14C ages to establish a tie point, resulting in an 18-y composite recording of growth. Determining the time represented in periodic growth increments. Determining the time represented in periodic growth increments in teeth of extant taxa provides a potential chronometer in fossil teeth, where knowledge of growth rate is critical to interpretation of intratooth stable isotope profiles or historical data related to life history.

Conclusions

In this study, we show bomb-curve 14C can be used to accurately date keratin, collagen, apatite, and bulk plant tissue, and we provide examples of applications of the technique to stable isotopes and wildlife forensics. Plant and animal tissues that formed between 1956 and 2008 have been accurately dated (−0.9 ± 1.4 y, 1σ) for 21 samples of known age using bomb-curve 14C. Our results from all post-1955 tissues indicate their carbon was derived from recently photosynthesized CO2 (within ca. 1 y of sampling), regardless of tissue type and across a range of biological isotope enrichment factors.

We calculated growth rates of elephant tail hair, tusks, molars, and hippo canines from multiple 14C ages measured along tissue growth axes. 14C measurements from NaOCl-treated dentin, untreated dentin, and hair keratin can help identify where and when tusks yield indistinguishable ages, indicating both apatite and collagen are suitable for bomb-curve 14C dating, and that treatment of dentin apatite to remove organics is not necessary for 14C measurement.

Our results have the following immediate and unique applications to stable isotope (paleo)ecology and wildlife forensics. We concatenated intratooth δ13C and δ18O profiles from two hippo tusks using 14C ages to establish a tie point, resulting in an 18-y composite stable isotope record. Records such as these can be used to study long-term (i.e., multidecadal) population, climate, or ecosystem dynamics that would not be feasible from a single intratooth profile, exclusive of proboscidean tusks. Growth rates from bomb-curve 14C dating can be used to determine the time represented in periodic growth increments. Determining the time represented in periodic growth increments in teeth of extant taxa provides a potential chronometer in fossil teeth, where knowledge of growth rate is critical to interpretation of intratooth stable isotope profiles or historical data related to life history.

14C dating of raw or worked animal tissues can be used to establish sample age and in many cases date of death of an animal, which can determine whether trade is legal according to CITES or other regulations. Poaching for elephant tusks and rhino horn has increased significantly since 2006. Turnaround time and cost of AMS 14C measurements have decreased in the past decades, and therefore it is an accessible wildlife forensics tool. Combined with geolocation (e.g., DNA, stable isotope, and
histological) forensic techniques. $^{14}$C dating of animal parts can help budget-limited government agencies and nongovernmental organizations determine how and where to direct conservation and anti-poaching resources.

Materials and Methods

Sampling and analytical procedures are described in detail in SJ Text. Briefly, inorganic tissues (bioapatite from dentin and enamel) were digested offshore in 10% HCl to obtain $^{14}$C data for carbon cycle modeling and age calibration purposes. Radiocarbon 46(3):1273–1298.


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Supporting Information

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SI Text

Sample Description. Herbivore and plant samples were collected for \(^{14}\)C analysis from the field or museum collections between 1971 and 2009 and span known ages of 1905–2008. Detailed sample information, including sample and species identification, known age, geographic location and coordinates, and specimen location is provided in Dataset 1, Table S1. Hair sampled for this study was determinate body hair from primates and ungulates and indeterminate tail hair from elephants and one ungulate. The horn sample comes from an oryx; the soft tissue sample was from tissue dried onto the pulp cavity of an elephant tusk. Collagen is from elephant tusk and molar dentin. Apatite samples are from elephant molar enamel and tusk dentin and from hippo canine enamel. Three annual grass samples are from Kenya.

Sampling Plan for Determining Growth Rates. The structure and growth processes of hair and teeth were considered in devising sampling plans that minimized the time-averaging in tissues. For elephant tail hair, a 3- to 5-mm segment of hair was cut from the proximal and distal end. Each sample represents -4-6 d based on the growth rate of 0.81 ± 0.11 mm/d for female elephant tail hair determined by Wittemeyer et al. (1).

Elephant tusks are continuously growing, modified incisors made up of dentin with a cementum outer layer. Enamel is deposited at the tip of the tusk, although this is rarely if ever present in adult individuals owing to normal wear. Dentin is deposited throughout life along the conical pulp cavity surface, and incremental growth features representing annual, weekly (or fortnightly), and daily intervals are present in proboscidean tusks (e.g., ref. 2). Dentin was removed from longitudinally cut tusks by drilling along a 1-mm-wide path parallel to growth increments. Distances are measured along the tusk axis from the horn of the pulp cavity to the tip of the tusk (Fig. S24). Sample position is determined by where the growth increments intersect the tusk axis.

Tooth enamel undergoes a more protracted formation process than tusk dentin. An immature enamel matrix is initially secreted by ameloblast cells, followed by a prolonged period of maturation of weeks to months (e.g., table 1 in ref. 3). When enamel maturation is complete, an immature enamel matrix is initially secreted by ameloblast cells, followed by a prolonged period of maturation of weeks to months (e.g., table 1 in ref. 3). Like tooth dentin, enamel contains incremental growth features, and each new layer forms subparallel to the enamel-dentin junction of the tooth (4). We partially mitigate the problem of enamel maturation using the smoothing parameter provided in the \(^{14}\)C age-calibration software, described below. We also minimize the time averaging by drilling ∼1- to 10-mm-wide sample paths through the entire thickness of enamel, oriented parallel to incremental growth features (i.e., perikymata) visible on the outer enamel surface. Elephant cheek teeth (i.e., premolars and molars) consist of a battery of thick, enamel-covered plates. Plates grow from the initial occlusal surface toward the cervical margin, Multiple plates are forming at any given time, exclusive of the very beginning and end of molar formation. When enamel maturation is complete, cementum forms on the outer surface of the plate and eventually all plates are cemented together to form the molar (Fig. S2B).

We use at least two \(^{14}\)C ages per plate to determine vertical growth rates in six plates from two individuals. One molar is from a male elephant, TE-95, presumed to have died around 1970. The elephant’s mandible was stored at a Kenya Wildlife Services facility in Tsavo East National Park and was sampled in 2007. The other is from R37, a female from Samburu National Reserve in Kenya that died on September 26, 2006. Both molars are lower third molars (m3), or sixth molars according to the system devised by Laws (5).

We determine growth rates for five canines (four lower and one upper) from four individuals that died between 1971 and 2007. Distances are measured from the proximal end along the outer curve of the canine because measurement along this surface is most convenient and unambiguous, as opposed to the inner curve or the midline (Fig. S2C).

Sample Preparation. Keratin (hair and horn) samples were wiped with ethanol to remove adhering contaminants. For hair, ∼5 mg were cut from the proximal end of the hair. For horn, ∼4 mg of material was removed from the inner part at the base of the horn using a Dremel tool. Samples were loaded into 9-mm quartz tubes (precombusted to 900 °C) with ∼100 mg of CuO and Ag foil. They were evacuated on a vacuum line, sealed with a torch, and combusted at 850 °C for 4 h. An organic \(^{14}\)C blank, Rio Flo charcoal, was prepared along with the unknowns.

Tusk collagen was isolated from 28 to 50 mg of drilled, powdered dentin by treatment with 0.25 or 0.5 M HCl for at least 4 h. Acid was refreshed at least once during the reaction period. Treated powder was centrifuged, neutralized with NaOH, rinsed five times with ultrapure water, and dried overnight at 60 °C. Collagen was combusted by the same method as the keratin. Apatite from enamel and dentin was removed with a Dremel tool equipped with a 1-mm-diameter bit. Before removing sample powder, an area in excess of the actual milling area was prepared by milling away the outermost ∼0.2 mm of enamel to expose a clean enamel surface. Approximately 90–210 mg of powder was transferred into precombusted glassware, evacuated on a vacuum line, and digested offline with 104% (by density) H$_3$PO$_4$ in sealed vessels at 90 °C for 2 h or until the reaction was complete. Two elephant tusk dentin samples were split and treated with excess 3% NaOCl for 30 min to remove organics. Treated samples were rinsed three times in ultrapure water, dried overnight at 60 °C, and digested in the same manner as untreated samples. An inorganic \(^{14}\)C blank, Carrara marble or IAEA-C1, was prepared along with the unknowns.

Evolved CO$_2$ from combustion or acid digestion was cryogenically extracted on a vacuum line to remove water and other contaminants. SO$_2$, commonly present in enamel samples, was removed by reducing it onto silver: Sample gas was either passed across hot (∼500 °C) Ag-Cu wool or a piece of precombusted Ag foil was placed in the break-seal tube with the extracted CO$_2$ sample and heated at 60 °C for at least 24 h. In most cases, extracted CO$_2$ was split (∼2:1) and sealed into precombusted 6-mm-diameter Pyrex tubes. The larger aliquot was graphitized using the Fe-Zn reduction method and pressed into a target containing ∼1 mg carbon. For smaller sample masses, no split was made, and all extracted CO$_2$ was graphitized.

\(^{14}\)C Vary Based on Tissue Type or Pretreatment? Two elephants with known dates of death provide an opportunity to test for variation in \(^{14}\)C based on tissue type. From R37, we measured \(^{14}\)C in tail hair, soft tissue from the tusk pulp cavity, tusk collagen, 3% NaOCl-treated tusk apatite, and untreated tusk apatite. We sampled the proximal end of a tail hair collected the day after death. The soft tissue sample was from pulp cavity tissue that

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Uno et al. www.pnas.org/cgi/content/short/1302226110 1 of 5
dried onto the surface of the pulp cavity of the tusk. The collagen and twoapatite samples are from the same aliquot of dentin drilled from the tusk pulp cavity margin. We also measured $^{13}$C in collagen, 3% NaOH-treatedapatite, anduntreatedapatite from the same aliquot of dentin from tusk pulp cavity margin of Misha, who died at Utah’s Hogle Zoo in September 2008.

$^{14}$C and $\delta^{13}$C Measurement. A total of 70 samples was analyzed for $^{14}$C concentration on a 2.5-MV General Ionex or a 3.0-MV National Electrostatics Corporation accelerator mass spectrometer (AMS) at the University of Arizona with an external precision of $\sim$0.4%. Stable carbon isotope ratios were determined from the remaining extracted CO$_2$ split at the University of Arizona on a VG Isotech Optima isotope ratio mass spectrometer (IRMS). For smaller $^{13}$C samples that lacked a CO$_2$ split, carbon isotope ratios were determined using online (e.g., Costech 4010 Elemental Analyzer or Finnigan CarboFlo) methods at the University of Utah’s Stable Isotope Ratio Facility for Environmental Research on an MAT-252 IRMS. All samples were analyzed along with internal laboratory standards calibrated to international standards, and precision of sample $\delta^{13}$C values is $<$0.2 $‰$. The $\delta^{13}$C data were used for fractionation corrections of $^{14}$C.

Calculation and Reporting of Bomb-Curve $^{14}$C-Calibrated Ages. All data are reported as fraction modern carbon (F$^{14}$C) following the recommended convention for postbomb $^{14}$C measurements (6) where

$$F^{14}C = (A_{/0.95~A_{OX}}) \times (0.975/0.981)^{\lambda}$$

$$\times \left[ \left( 1 + \delta^{13}C_{OX}/1,000 \right) / \left( 1 + \delta^{13}C_{S}/1,000 \right) \right]^{2}$$, \hspace{1cm} [S1]

where $A$ is the activity or $^{14}$C/$^{13}$C ratio, and the subscripts S and OX are for the sample and the oxalic acid standard, respectively. Where appropriate, we also use the $\Delta^{14}$C notation, expressed in permil notation ($\delta^{13}$C), where

$$\Delta^{14}C = \left[ \left( F^{14}C \right)^{e^{\lambda(\text{year} - \text{year})}} - 1 \right] \times 1,000$$, \hspace{1cm} [S2]

where $\lambda$ is 1/8,267 y and year is the year the sample was analyzed.

Because nearly all samples are from equatorial East Africa and range in age from 1905 to 2008, age calibration necessitates the use of the Northern Hemisphere Zone 3 (NH3), Southern Hemisphere (SH), and Levin datasets (7–9). Grass samples from low-latitude Southern Hemisphere sites (1° to 3° S) have known age range in age from 1905 to 2008, age calibration necessitates the use of the Northern Hemisphere Zone 3 (NH3), Southern Hemisphere (SH), and Levin datasets (7–9). Grass samples from low-latitude Southern Hemisphere sites (1° to 3° S) have known ages that bracket the steep rise in the bomb-curve. These were selected for $^{14}$C measurement to test whether samples from this region fall on the NH3 or SH1 curve. The Northern Hemisphere Zone 2 (NH2) and Levin datasets are used for age calibration on the tusk from Misha. Calibrated $^{14}$C ages were determined using the CALIBomb program (10). The program preposs the bomb calibration data sets with 300 y of INTCAL04 data. Two important parameters used for calculating ages with CALIBomb are resolution and smoothing. Resolution determines the minimum length of time in years that is required to distinguish separate calibrated ranges. We use the default value of 0.2 y for all samples. Smoothing is set to the duration over which the sample forms. We use 0.5 y for teeth and tusks based on our sampling geometry and the time over which these tissues form. The smoothing period for hair is 0.5 y, and this is determined based on the approximate maximum turnover time of carbon in the tail hair (11). For annual grasses a smoothing period of 0.1 y is used.

Selection of $^{14}$C-Calibrated Ages. F$^{14}$C values near or above 1.10 will intersect the bomb-curve twice and yield at least two possible calibrated $^{14}$C ages for the NH2, NH3, or SH1 data sets. This is true for F$^{14}$C values near or above 1.06 using the Levin data set. Resolution and smoothing values less than 0.2 may result in more than two possible ages. In this study, the calibrated $^{14}$C age closer to known ages of the samples was selected. For samples in which the age is uncertain, there are several ways to select the appropriate $^{14}$C age. First, if the approximate date of death or collection is known (e.g., 5–15 y), the correct age can often be selected. For example, using the NH3 dataset and resolution and smoothing values of 0.2 and 0.5, respectively, a hair sample with an F$^{14}$C value of 1.150 $\pm$ 0.002 yields calibrated $^{14}$C age of 1958.72 $\pm$ 0.07 (1σ) or 1990.83 $\pm$ 0.42. If it is known that the sample was collected around or before 1965, then the more recent age, 1990.83, can be rejected.

If there is no information about the date of death or collection, measuring the $^{14}$C content at two or more positions along the growth axis of the hair (or other tissue) can be used to determine the appropriate calibrated $^{14}$C age. Cook et al. (12) and Wang et al. (13) describe this method and show it works well in human teeth using combined enamel apatite and dentin collagen F$^{14}$C values from the same tooth. We provide a brief summary here and show in Results and Discussion that the method is applicable for multiple keratin, apatite, and collagen F$^{14}$C values from a single sample.

Samples from the proximal and distal ends of a growing tissue such as hair, horn, or tooth will give two different F$^{14}$C values. If the proximal F$^{14}$C value is greater than the distal value, then the tissue formed during the rise of the bomb-curve, between 1955 and 1965. Given the opposite, whereby the proximal F$^{14}$C value is less than the distal value, the tissue formed during the fall of the bomb-curve, between 1965 and the present. For samples separated by a very short time period (<1 y), this technique of measuring two or more samples along the growth axis of the tissue may not be able to resolve the appropriate age, especially for tissues formed before ~1995.

Trade Regulations Based on Ivory Age. The Convention of International Trade of Endangered Species (CITES) treaty was enacted in 1973 to prohibit international trade in animal parts from endangered species listed in appendix 1 of the treaty. International trade of raw Asian and African elephant ivory has been banned since they were added to appendix 1 in 1975 and 1989, respectively, with the exception of limited sales of African ivory from selected African countries in 1997, 2002, and, most recently, in 2008.

One way in which recent, illegally procured ivory is brought to market is by cosmetically aging raw or worked ivory. For example, in the United States interstate trade is legal for raw or worked African ivory (e.g., carved statues or figurines) if it was imported before 1989. Worked ivory imported after 1989 must be at least 100 y old for interstate trade to be legal. Trade of raw African ivory imported since 1989 is banned. Laws are similar for Asian elephant ivory, except the cutoff year is 1976. In the European Union there are similar laws with different age criteria established through CITES and the European Commission Regulation 336/97 of 1997. Worked ivory imported before 1947 can be traded within the European Union, whereas all trade of raw ivory is illegal.


Uno et al. www.pnas.org/cgi/content/short/1302226110

![Fig. S1. Residuals (r) by tissue type, where r = age$_{14C}$ − age$_{known}$, plotted by known age of sample.](image-url)
Fig. S2. Schematic diagrams of sampled teeth illustrate structural features, periodic growth increments (in A and C), and sampling strategy. Hatched area in each figure (A–C) shows approximate size and orientation of sampling areas. (A) Upper left: longitudinally-cut elephant tusk with incremental growth features shown at proximal end; Upper right: detail of proximal end of the tusk showing the geometric relationship (angle θ) between the tusk axis and the trace of dentin increments in the plane of longitudinal section. The angle θ is required to compare 14C and histological growth rates (SI Text); Lower right: Transverse view illustrating first- and second-order growth increments. (B) Longitudinally-cut elephant molar comprised of six enamel plates; and (C) a lateral view of a lower hippo canine with perikymata shown over a representative interval (∼2cm). A and B are modified from ref. 1.

Fig. S3. Photograph showing hippo canine (K11-KF) perikymata, which are periodic growth increments on the enamel surface of teeth. Each increment represents $10.2 \pm 2.9$ d. The original digital color photograph has been converted to an enhanced grayscale image to facilitate measurement of increments. Tick marks in the scale at bottom of image are in millimeters.

Fig. S4. Photomicrograph of a transversely cut ivory thin section from R37 showing second-order growth increments at 35x magnification under plane polarized light. Each increment is composed of a dark–light couplet. The mean increment thickness measured along the ~34-mm-thin section is $103 \pm 29 \mu m$ ($n = 334$). The growth rate calculated from $^{14}C$ data are $105 \pm 11 \mu m/wk$, providing independent evidence for weekly periodicity of second-order increments in elephant tusks.

Other Supporting Information Files

Dataset S1 (XLSX)