

# Radiocarbon dating of seized ivory confirms rapid decline in African elephant populations and provides insight into illegal trade

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**Carbon-14 measurements on 231 elephant ivory specimens from 14 large ivory seizures (≥0.5 ton) made between 2002 and 2014 show that most ivory (ca. 90%) was derived from animals that had died less than 3 y before ivory was confiscated. This indicates that the assumption of recent elephant death for mortality estimates of African elephants is correct: Very little “old” ivory is included in large ivory shipments from Africa. We found only one specimen of the 231 analyzed to have a lag time longer than 6 y. Patterns of trade differ by regions: East African ivory, based on genetic assignments of geographic origin, has a much higher fraction of “rapid” transit than ivory originating in the Tridom region of Cameroon–Gabon–Congo. Carbon-14 is an important tool in understanding patterns of movement of illegal wildlife products.**

wildlife | forensics | isotopes | Africa | genetics

The illegal trade in elephant ivory has increased significantly in the past decade (1, 2), with studies estimating the current rate of decline of regional African elephant populations to be as high as 8%, primarily due to poaching (3, 4). Central African forest elephant populations decreased by ca. 62% from 2002 to 2011 (5). Forest elephants are particularly vulnerable to poaching because of their slow population growth rates compared with their savanna counterparts (6). Savanna elephants have also experienced massive population declines, particularly in Tanzania and northern Mozambique. The savanna elephant population in the Selous Wildlife Reserve in Tanzania saw a 66% decline from 2009 to 2013 (7). The rapid decline in elephants across Africa has been attributed to the high poaching rates and increased amount of ivory seized over the last decade or so (3). Total global seizures in excess of 40 tons of ivory have occurred in several years since 2010 (8), with over 70% of all ivory seizures exceeding 0.5 ton (hereafter termed “large seizures” or “large ivory seizures”).

We use “bomb <sup>14</sup>C” to determine ages of ivory from 14 different large seizures intercepted by law enforcement officials between 2002 and 2014. These ages represent the date of death of those elephants whose tusks were sampled. This study answers several questions including whether “old” ivory—such as tusks from government stockpiles—is being incorporated into the illegal ivory stream, what the lag time is between animal death and seizure of ivory by law enforcement officials, and whether there are significant differences between the age of ivory originating in different parts of Africa.

We first establish a <sup>14</sup>C-calibration relationship for animal tissues from Africa by using elephant hair of known age. We then report 231 <sup>14</sup>C-calibrated ages of seized ivory and discuss how these ages influence our understanding of the illegal ivory trade in Africa. We use specimens previously studied by Wasser et al. (2), who assigned ivory to its general location of origin using DNA assignment methods.

## Results

**Calibration Curve.** Carbon-14 is a naturally occurring isotope produced by cosmic radiation in the upper atmosphere. Above-ground nuclear weapons testing, primarily in the early 1960s, nearly doubled the concentration of <sup>14</sup>C from the natural abundance level (Fig. 1B). Since the atmospheric concentration “spike” of <sup>14</sup>C reached at that time, the <sup>14</sup>C/<sup>12</sup>C ratio has been steadily declining from peak values of ca. 1.95 and ca. 1.8 F<sup>14</sup>C [fraction modern carbon (9)] in the Northern and Southern Hemispheres (NH and SH), respectively, to the current value of < 1.03; this “bomb-curve” record is well-documented (10–16). The overall higher concentration and peak value of atmospheric <sup>14</sup>C in the NH was due to higher amounts of testing in the NH compared with the SH. Zonal heterogeneity in concentrations was observed in each hemisphere in the 1960s and early 1970s and, as a result, multiple calibration curves (e.g., NH1, NH2, and NH3 and SH1–2 and SH3) were established for each hemisphere (10–12). Atmospheric <sup>14</sup>C is present primarily as <sup>14</sup>CO<sub>2</sub> and the mixing time of CO<sub>2</sub> in the atmosphere is ~10 y. Thus, by about 1973, the bomb pulse of <sup>14</sup>CO<sub>2</sub> was essentially mixed and zonal differences were insignificant; the NH and SH have been each treated as a single zone since 1973 (12).

## Significance

**C-14 dating methods can be used to determine the time of death of wildlife products. We evaluate poaching patterns of elephants in Africa by using <sup>14</sup>C to determine lag time between elephant death and recovery of ivory by law enforcement officials. Most ivory in recent seizures has lag times of less than 3 y. Lag times for ivory originating in East Africa are shorter, on average, than the lag times for ivory originating in the Tridom region (Cameroon–Gabon–Congo). The <sup>14</sup>C data show little or no evidence that large-scale ivory shipments contained ivory stockpiled over long time periods. Little, if any, “old” ivory (i.e., >10 y) seems to contribute to large ivory shipments.**

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**Table 1. Lag time in months for ivory samples from 14 different seizures, grouped by region of origin, as determined by  $^{14}\text{C}$  dating**

Name	Abbreviation	Date of seizure	Region of origin	<i>N</i>	Mean	$\pm 1 \sigma$	Minimum	Maximum	Median
Avocado	AVO	August 22, 2010	East Africa	14	6	9	−10	15	10
HKTA	HKTA	October 17, 2012	East Africa	11	19	7	11	37	18
HKTb	HKTb	October 17, 2012	East Africa	14	14	6	6	21	12
Hong Kong-Kenya	HKK	January 26, 2013	East Africa	10	14	9	1	27	13
Hong Kong-Nigeria	HKN	August 7, 2013	Tridom	11	21	7	8	30	23
Hong Kong-Togo	HKI	July 18, 2013	Tridom	10	39	11	25	62	38
Malawi	M	May 23, 2013	East Africa	11	20	9	5	35	21
Malaysia*	MYS	December 11, 2012	East Africa	39	23	7	4	39	24
Malaysia*	MYS	December 11, 2012	Tridom	28	35	40	5	231	27
Malaysia*	MYS	December 11, 2012	West Africa	13	19	15	5	67	15
Philippines <sup>7</sup>	S7	June 9, 2009	East Africa	14	6	6	−4	18	6
Pili	PIL	May 6, 2011	East Africa	10	18	8	3	31	20
Singapore 2002	LAC	June 28, 2002	Zambia	8	15	6	6	25	14
Singapore 2014	SGP	March 27, 2014	East Africa	10	28	7	20	44	27
Taiwan1	53-	July 6, 2006	East Africa	12	15	20	1	69	8
Togo	TOGI	January 29, 2014	Tridom	16	25	11	9	52	22

\*The Malaysia 2012 seizure had ivory assigned to three different geographic origins: East Africa, Tridom, and West Africa. Results from these different assignments are listed separately.

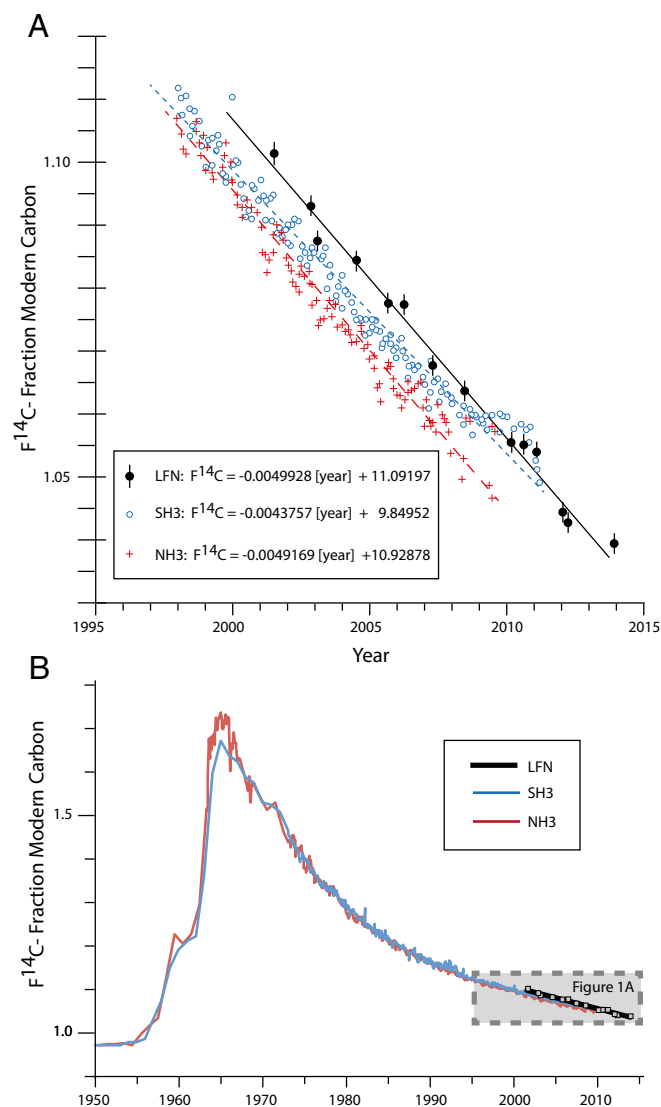
However, differences between the NH and SH persist today due in part to the Sues effect, the dilution of  $^{14}\text{C}$  in the atmosphere by “dead carbon”—fossil fuels have no radiocarbon and combustion of those fuels dilutes the modern atmosphere with carbon (as  $\text{CO}_2$ ) with an  $\text{F}^{14}\text{C}$  value of 0.0. Most of the dead carbon is produced in the NH and mixes across the equator with the SH in the Inter-Tropical Convergence Zone (ITCZ, shown in Fig. S1C); since 1990 this has been the principal driver for differences in  $\text{F}^{14}\text{C}$  between the NH and SH (10–12). The natural exchange of  $^{14}\text{CO}_2$  between the ocean and atmosphere also contributes to the  $\text{F}^{14}\text{C}$  gradient observed between the NH and SH. Atmospheric samples collected near the equator should have a time- $\text{F}^{14}\text{C}$  relationship between that of the NH and SH due to mixing across the ITCZ.

Atmospheric  $^{14}\text{C}$  (as  $^{14}\text{CO}_2$ ) enters the terrestrial biosphere by photosynthesis;  $^{14}\text{C}$  is subsequently incorporated into herbivore tissues as the animals ingest plants for food. The bomb- $^{14}\text{C}$  signal has been used in human forensic studies (17–21) and has also been used previously to date ivory and other animal tissues including hair, horn, and tooth enamel (22–25). Prior work on  $^{14}\text{C}$  dating of ivory has used the atmospheric  $^{14}\text{C}/^{12}\text{C}$  historical record to determine the calibrated age of samples. Both Vogel et al. (23) and Uno et al. (25) note that the  $^{14}\text{C}/^{12}\text{C}$  atmospheric record gives ivory ages that are, in general, 0–2 y earlier than the known age of the sample. This age mismatch likely results from several processes: (i) differing  $^{14}\text{C}/^{12}\text{C}$  ratios between the NH and SH compounded by the lack of atmospheric  $^{14}\text{CO}_2$  measurements in the mixing zone between the hemispheres (i.e., equatorial Africa), (ii) the remobilization of nonstructural carbon during plant growth, (iii) the time lag between C fixation by plants and ingestion of plants by an elephant, and (iv) recycling of proteins in mammals. Each of these is discussed below.

(i) Differing  $^{14}\text{C}/^{12}\text{C}$  ratios between hemispheres: Fig. 1A shows that the “clean-air” sites of the NH and SH have different values for  $\text{F}^{14}\text{C}$  at any given time. Fig. S1C shows that the clean-air sites used to determine  $\text{F}^{14}\text{C}$  of NH and SH are far from the African continent. (ii) Remobilization of nonstructural carbon during plant growth: Muhr et al. (26) have shown that nonstructural carbon in perennial tissues (e.g., branches, stems, and roots) can be mobilized and used for growth several years after fixation and thus  $\text{F}^{14}\text{C}$  of dietary foodstuff may lag the  $\text{F}^{14}\text{C}$  of the atmosphere; because elephants have a high component of perennial plants in their diets, this effect could be important in understanding any offset between atmospheric  $\text{F}^{14}\text{C}$  and newly

formed tissue  $\text{F}^{14}\text{C}$  in herbivores. Ehleringer et al. (27) showed a significant range in  $\text{F}^{14}\text{C}$  for plants collected at a single time from a single region. (iii) Time lag between C fixation by plants and ingestion of plants by an elephant: If carbon in the food that animals eat has been fixed previously, there is likely to be an offset of months or years between the date of ingestion and the date of carbon fixation. This is particularly significant in elephants, because they ingest large quantities of bark and wood, which—if even a small fraction of this carbon is digested and fixed in animal tissues—would skew the  $^{14}\text{C}$ -calibrated age to an earlier date. (iv) Recycling of proteins in mammals: Ayliffe et al. (28) showed that newly formed proteinaceous animal tissues (e.g., collagen and keratin) are composed of about 45% carbon with a turnover time (half-life) of  $\sim 140$  d; thus, proteins should have an “average age” skewed several months before tissue formation.

The combination of these processes may affect the accuracy of age-dating ivory when using the standard NH or SH zonal calibration curves over the past *ca.* 15 y where the slope of the bomb curve is relatively shallow. Therefore, we analyzed 14 elephant hairs with known collection dates between 2001 and 2013 (Methods, Table S1, and Fig. S1B) to develop a calibration curve specific to elephant tissues. Fig. 1 shows the elephant hair relationship for  $\text{F}^{14}\text{C}$  versus date and also the data for the same time interval used to calculate the NH and SH regions NH3 and SH3, respectively, as reported in ref. 16. Dates were assigned to hairs using the segment length collected for measurement and assuming a growth rate of 0.8 mm/d. We use a linear regression for the period 2001–2013 to derive the relationship  $\text{F}^{14}\text{C} = [-0.0049928 \times \text{year} + 11.09197]$  ( $r^2 = 0.988$ ); we note that this linear relationship should not be used for dates before 1995 (Fig. 1B). We assume that the incorporation of  $^{14}\text{C}$  from diet into proteinaceous tissue is the same for elephant hair keratin as it is for elephant ivory collagen and thus use the calibration curve to determine the date of death of the elephant from which each seized tusk originated. If a calibration curve can be generated from ivory of known age—and differs significantly from the calibration curve generated from elephant hair—dates can be recalculated using such new data. All of the calibration samples, and the assigned locations of ivory, fall in (or very near to) the mixing zone across the ITCZ, which separate the NH and SH zones where the “clean-air” sites are located. Future calibrations for ivory, or for other wildlife products, to obtain “age” determinations using  $\text{F}^{14}\text{C}$  measurements should focus on obtaining samples near regions of interest; such calibrations will further improve the age estimates of this and other studies.



**Fig. 1.** (A) Relationship between  $F^{14}C$  and date of collection for elephant hair and for the data used in NH3 and SH3 calibration curves (6). The elephant hair calibration curve is used to calculate date of death from 2001 to 2014. (B) The complete “bomb curve” from 1950 to the present for NH3 and SH3. We note that after 1973 all NH zones are treated as a single zone, and all SH zones are treated as a single zone.

Ivory for date-of-death determination was sampled from the pulp cavity of tusks (the proximal end of the tusk; Fig. 2 and Fig. S2), which is where new ivory (dentine) was forming when the elephant died and thus most accurately records the date of death.

**Radial Growth Rates of Tusks.** For a limited set of tusks we sampled the innermost dentine and also the outermost dentine (Fig. 2 and Fig. S2); in a few cases we also sampled the cementum, which forms outside the outermost dentine. The bomb curve has a “rising” and a “falling” limb: rapidly increasing in the late 1950s, peaking in the early 1960s with the crest in the SH occurring after that in the NH, then gradually diminishing to the current  $F^{14}C$  value of *ca.* 1.02 (Fig. 1B). Thus, an age assignment using  $F^{14}C$  of any postbomb ivory has three solutions, one entirely on the rising limb of the bomb curve (i.e., between *ca.* 1955 and 1965), one with the outer dentine on the rising limb (between 1955 and 1965) and the inner dentine on the falling limb (between 1965 and the present), and one entirely on the falling limb

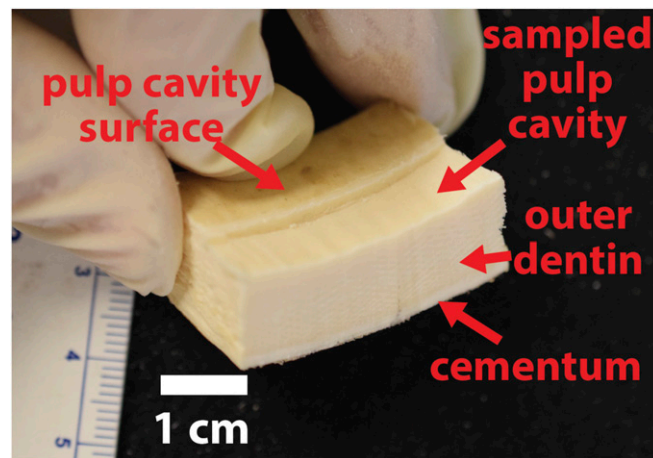
(both after 1965). For ivory entirely on the rising limb, the innermost dentine should have a higher  $F^{14}C$  value than the outermost dentine; data from Table S2 show that this is not the case. For the second case, the outermost dentine would have formed between 1955 and 1965 and the innermost dentine would have formed in the last decade, giving unreasonably slow growth rates for ivory (*ca.* 1 cm thickening per 50 y). Our samples fall in the third case, where both the innermost and outermost dentine  $F^{14}C$  values are on the falling limb, with the innermost dentine having lower  $F^{14}C$  values than the outermost dentine.

All tusks with multiple samples are on the falling limb of the bomb curve, and we thus assume that all other specimens analyzed in this study are on the falling limb. We use the tusks with multiple samples to determine the rate of radial growth of tusks. Radial growth rates were about 7 mm/y for both savanna and forest elephants, ranging from *ca.* 6–14 mm/y (complete results are presented in Table S2 and Fig. S3). Uno et al. (25) measured longitudinal growth rates of *ca.* 5 cm/y for two female African elephants, which correspond to radial growth rates of about 5 mm/y based on a longitudinal/radial growth rate ratio of about 10:1.

**Date-of-Death Determinations of Seized Ivory.** The  $F^{14}C$  values and calculated date of death of 231 ivory specimens (tusks) from 14 large seizures from 2002 to 2014 are summarized in Table 1; complete data are presented in Dataset S1. Details for 12 of the seizures are given in Wasser et al. (2). Two additional seizures were included in our study and were not available at the time of the Wasser et al. (2) study.

Ivory specimens are discussed in the context of lag time, which is the time between when the animals died as determined by  $F^{14}C$  using the calibration discussed above and the time of seizure by law enforcement officials. The lag times for each seizure are shown in Fig. 3, where they are grouped by geographic origin based on DNA analysis (2). Uncertainty for the calculated lag time, defined here as the expanded uncertainty ( $U$ ), incorporates uncertainty in the calibration curve (Fig. 1B) and measurement reproducibility (Dataset S1 and Methods).

Wasser et al. (2) assigned individual ivory specimens to different geographic regions based on genetic data (Fig. S1A). Those assignments have an estimated accuracy of approximately  $\pm 300$  km for both latitude and longitude. Based on those assignments, we group specimens into four distinct regions of origin: East Africa (southern Kenya to northern Mozambique), the Tridom (Cameroon, Republic of Congo, and Gabon, but also including Southwest Central African Republic), West Africa, and



**Fig. 2.** Example of sample from pulp cavity section of specimen SGP-1-2. Arrows indicate the locations of the pulp cavity surface, a sampled area ( $\sim 2$  mm total depth from the pulp cavity surface), outer dentine, and the cementum layer deposited on the outside of the tusk.





sample was thereafter pressed into a target for radiocarbon ( $^{14}\text{C}$ ) measurement. The graphite targets were sent to the W. M. Keck Carbon Cycle Accelerator Mass Spectrometry Laboratory at the University of California, Irvine (UCIAMS) for radiocarbon measurement. Radiocarbon results are reported as fraction modern  $F^{14}\text{C}$  (9), which specifically includes  $\delta^{13}\text{C}$  normalization to  $-25\%$  using the AMS measured  $\delta^{13}\text{C}$  value.

Expanded uncertainty in lag time incorporates uncertainty in the calibration curve (Fig. 1B) and in measurement reproducibility. The latter is estimated by specimen "ivory\_Modern 207," a homogenized powdered ivory sample prepared multiple times (e.g., separate rounds of collagen extraction, combustion, graphitization, and  $F^{14}\text{C}$  measurement) and analyzed within each analytical sequence. Ten analyses of ivory\_Modern 207 gave an average  $F^{14}\text{C}$  value of  $1.1021 \pm 0.0032$  (1  $\sigma$ ) (Dataset S1).  $U$  is calculated from a linear combination of this measurement uncertainty and predicted values from the elephant hair calibration curve, multiplied by a coverage factor ( $k$ ) of 2, which represents ca. 95% uncertainty for the range in lag times. For seized specimens with measured  $F^{14}\text{C}$  values similar to those of the elephant hair used to build the calibration curve (e.g.,  $F^{14}\text{C} \leq 1.1043$ ),  $U$  was  $\pm 1.55$  y ( $\pm 18.5$  mo). One seized ivory specimen had an  $F^{14}\text{C}$  value of

1.1379;  $U$  for this specimen ("ivory\_200;" see Dataset S1) was calculated as  $\pm 1.71$  y ( $\pm 20.6$  mo). Collagen extracted from a fossil bone was used as a blank for AMS measurement (Dataset S1).

Normal distributions to histograms of lag times (Fig. 3) were fit using JMP (SAS Inst., Inc.).

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