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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 ¹⁴C” 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 ¹⁴C-calibration relationship for animal tissues from Africa by using elephant hair of known age. We then report 231 ¹⁴C-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 ¹⁴C from the natural abundance level (Fig. 1B). Since the atmospheric concentration “spike” of ¹⁴C reached at that time, the ¹⁴C/¹²C ratio has been steadily declining from peak values of ca. 1.95 and ca. 1.8 F¹⁴C [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 ¹⁴C 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 ¹⁴C is present primarily as ¹⁴CO₂ and the mixing time of CO₂ in the atmosphere is ~10 y. Thus, by about 1973, the bomb pulse of ¹⁴CO₂ 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 ¹⁴C 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 ¹⁴C 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}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 ⁷	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 Suess effect, the dilution of ^{14}C in the atmosphere by “dead carbon”—fossil fuels have no radiocarbon and combustion of those fuels dilutes the modern atmosphere with carbon (as CO_2) with an F^{14}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 F^{14}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 F^{14}C gradient observed between the NH and SH. Atmospheric samples collected near the equator should have a time- F^{14}C relationship between that of the NH and SH due to mixing across the ITCZ.

Atmospheric ^{14}C (as $^{14}\text{CO}_2$) enters the terrestrial biosphere by photosynthesis; ^{14}C is subsequently incorporated into herbivore tissues as the animals ingest plants for food. The bomb- ^{14}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}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 F^{14}C at any given time. Fig. S1C shows that the clean-air sites used to determine F^{14}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 F^{14}C of dietary foodstuff may lag the F^{14}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 F^{14}C and newly

formed tissue F^{14}C in herbivores. Ehleringer et al. (27) showed a significant range in F^{14}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}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 ~ 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 F^{14}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}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 F^{14}C measurements should focus on obtaining samples near regions of interest; such calibrations will further improve the age estimates of this and other studies.

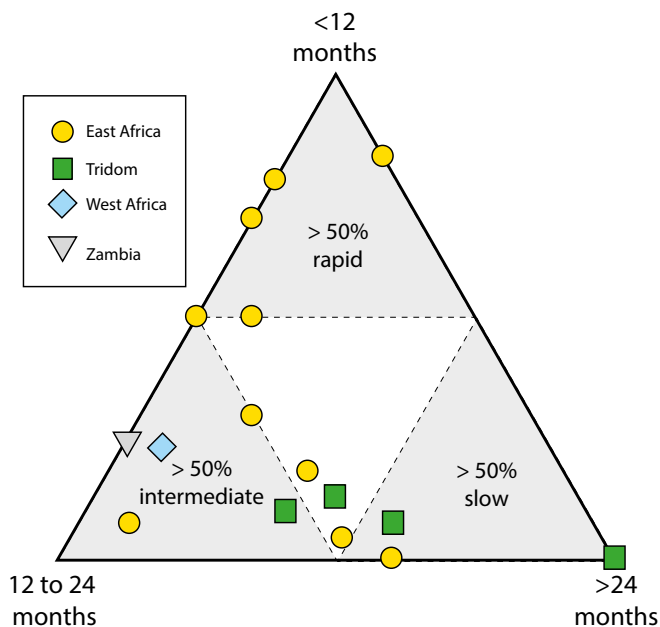


Fig. 4. Lag time distribution shown as a ternary plot for rapid transit (<12 mo lag time), intermediate transit (12–24 mo), and long transit (>24 mo).

we note that the radiocarbon calibration and related uncertainties have large associated errors and that refined regional radiocarbon calibrations may provide better constraints on regional differences in lag times.

Changes in Lag Times from 2002 to 2014. The median lag time for seizures between 2002 and August 2010 ranges from 7 to 13 mo. Lag time increases from 2011 onward, ranging from 13 to 32 mo (Fig. 5). This trend of increasing lag time is most evident in East African seizures where there are a total of nine seizures made over 9 y. The combined dataset from the regions of West Africa ($n = 1$), Southern Africa (Zambia, $n = 1$), and the Tridom ($n = 4$) is smaller than the East Africa dataset but supports the trend of increasing lag time since 2011. The reason for the general increase in lag time since 2011 is unclear. It is likely related to the overall decline in African elephant populations, particularly from 2010 to 2012, where data show population decline due to unsustainable poaching rates, especially in Central and East Africa (3, 4). As a result, it may take longer to accumulate enough large tusks to make a large shipment more profitable. Considering the markedly slower growth rate of tusks of forest elephants, this may also explain the longer lag times we observed for Central African ivory compared with East African ivory. West African forest ivory generally made up a small portion of the tusks in all seizures analyzed and, based on its short lag time, seemed to be added opportunistically at the time of shipment. Factors related to the dynamics of the illegal network of trade or perhaps to changes in the demand for ivory (29, 30) may have also contributed to these trends.

Conclusions

A calibration curve for $F^{14}C$ of modern wildlife tissues in Africa shows that modern elephant hair samples (keratin) have an offset with respect to the bomb curve between about 1 and 2 y from both the NH zone 3 (NH3) and SH zone 3 (SH3) relationships, with the tissue samples having higher $F^{14}C$ values than either the NH3 or SH3 curves. This is likely due to elephant diets that include carbon that was fixed some months to years before ingestion, and also due to recycling of some proteins during keratin or collagen synthesis. The elephant hair calibration curve can be used to determine the date of death of elephants if the $F^{14}C$

of ivory is measured along the pulp cavity of the tusk. It may be necessary to establish taxon-specific calibration curves for date-of-death determinations based on radiocarbon dating of other wildlife products, such as rhinoceros horn, pangolin scales, pelts or furs, or timber. Issues specific to different dietary (i.e., carbon) sources, tissue types, and geographic regions need to be considered for related studies that use $F^{14}C$ measurements to determine the age of wildlife products.

Examination of 231 ivory samples from 14 seizures made between 2002 and 2014 shows that the lag time between elephant death and seizure by law enforcement officials has median values generally ranging between 6 and 35 mo. Specimens originating from East Africa—previously determined via genetic analysis—have the shortest lag times, with ca. 35% of specimens having lag times <12 mo; by comparison, only 7% of ivory specimens originating from the Tridom region of Africa have lag times <12 mo. Only a single specimen analyzed in this study has a lag time >10 y: We find no evidence that long-term government or other stockpiles have been contributing significant amounts of ivory to the illegal ivory trade, emphasizing that poached ivory is being rapidly moved into the illegal trade. This study demonstrates the power of bomb-curve radiocarbon dating for revealing lag times between date of death and seizure, a technique that can be applied to seizures of other wildlife parts, providing critical information to law enforcement, conservation, government, and policy organizations and agencies.

Methods

We report elephant hair and ivory ^{14}C measurements in this paper. Ivory specimens were obtained from seizures in Africa and Asia; specimens were ~10-cm² blocks obtained from the proximal end of the tusk [see ref. 31 and as illustrated in Fig. S2 (32)]. We sampled the innermost dentine in the pulp cavity for samples used to assign date of death to animals (Fig. 2 and Fig. S2); for selected specimens, we also dated the outermost dentine, which often had an outer sheath of cementum that was sampled. Powder was obtained using a high-speed rotary Dremel tool and double-cut carbide bits from the innermost 1–2 mm of the ivory block. Hair samples were obtained during collaring or translocation operations conducted by Save the Elephants and by the Kenya Wildlife Service. The basal 1 cm of hair was analyzed for DNA markers; the subsequent 2 mm (i.e., from 10 to 12 mm) was used for $F^{14}C$ measurements. Dates were corrected to ca. 14 d before the date of collection assuming an average growth rate of 0.8 mm/d (33).

Dentine powder was demineralized with hydrochloric acid to isolate the collagen component; ~1 mg of carbon was used for a single accelerator mass spectrometry (AMS) measurement (ca. 10 mg ivory powder). Delipified hair or demineralized collagen was placed into a precombusted quartz tube with precombusted reagents (cupric oxide and silver), sealed under vacuum, and combusted at 850 °C to convert all organic-bound carbon to carbon dioxide gas (CO₂). CO₂ was cryogenically purified and reduced to graphite; graphite from each

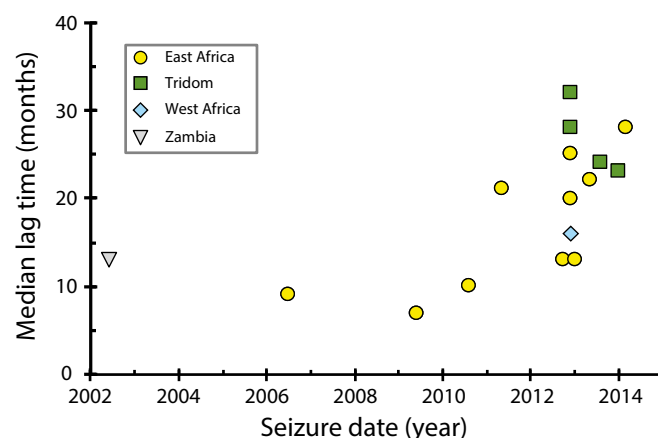


Fig. 5. Median lag time (months) plotted versus seizure date from 2002 to 2014. There seems to be an increase in lag time beginning in 2011.

sample was thereafter pressed into a target for radiocarbon (^{14}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 ± 0.0032 (1σ) (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 ± 1.55 y (± 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 ± 1.71 y (± 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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