Report

Current Biology

Sourcing Elephant Ivory from a Sixteenth-Century Portuguese Shipwreck

Highlights

- The largest 16th-century cargo of shipwreck ivory was analyzed using DNA and isotopes
- The tusks derived from ≥17 different herds of forest elephants from West Africa
- The formative stages of global trade relied on extensive West African networks
- Historical habitat use and genetic diversity data provide conservation benchmarks

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In Brief

de Flamingh et al. analyze ivory from a sixteenth-century shipwreck. DNA establishes that the tusks were from ≥17 different herds of West African forest elephants. Isotope analyses find that the elephants were from different habitats, but not deep rainforests. The data provide insights into global commodity circulation and historical elephant ecology.

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Sourcing Elephant Ivory from a Sixteenth-Century Portuguese Shipwreck

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SUMMARY

The oldest known shipwreck in southern Africa was found in Namibia in 2008.[1–4] Forty tons of cargo, including gold and silver coins, helped identify the ship as the *Bom Jesus*, a Portuguese *nau* (trading vessel) lost in 1533 while headed to India.[4–6] The cargo included >100 elephant tusks,[7] which we examined using paleogenomic and stable isotope analyses. Nuclear DNA identified the ivory source as African forest (*Loxodonta cyclotis*) rather than savanna (*Loxodonta africana*) elephants. Mitochondrial sequences traced them to West and not Central Africa and from $\geq$17 herds with distinct haplotypes. Four of the haplotypes are known from modern populations; others were potentially lost to subsequent hunting of elephants for ivory. Stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) indicated that the elephants were not from deep rainforests but from savanna and mixed habitats. Such habitats surround the Guinean forest block of West Africa[8] and accord with the locations of major historic Portuguese trading ports.[9,10] West African forest elephants currently range into savanna habitats;[11–13] our findings suggest that this was not consequent to regional decimation of savanna elephants for their ivory in the 19th and 20th centuries. During the time of the *Bom Jesus*, ivory was a central driver in the formation of maritime trading systems connecting Europe, Africa, and Asia. Our integration of paleogenomic, archeological, and historical methods to analyze the *Bom Jesus* ivory provides a framework for examining vast collections of archaeological ivories around the world, in shipwrecks and other contexts.

RESULTS AND DISCUSSION

Ivory on the *Bom Jesus* Derived from Forest Elephants in West Africa

The shipwreck and its contents are well preserved (Figure 1),[1] and we were able to extract DNA successfully from 44 of 62 (71%) tusks available. Using targeted amplicon sequencing on the shipwreck ivory DNA, we examined three short unlinked chromosome segments for single-nucleotide polymorphisms (SNPs) fixed between African savanna (*Loxodonta africana*) and African forest (*L. cyclotis*) elephants.[14] The character states detected were always diagnostic of forest and never of savanna elephants (Table S1). This indicated that the ivory derived from forest elephants, which historically ranged across the entire Guinean and Congolian tropical forest blocks of West and Central Africa (Figure 2).[15]

Female elephants remain with natal social groups (“herds”) in relatively restricted geographic ranges. Thus, mitochondrial (mt) DNA, which is maternally transmitted, can often identify the geographic provenance of elephants.[16,17] A ~436-bp region of D-loop mtDNA was amplified for 44 shipwreck ivory samples (Table S2). African elephant mtDNA is geographically structured into 8 subclades (Figure 3A).[18] In a network with published sequences representative of these 8 subclades (Figure 3B; Table S2),[18] 23 of the 44 ivory samples grouped with the Western mtDNA subclade, carried only by elephants from West Africa. The other 21 grouped within the West-Central subclade, carried by elephants in both West and Central Africa (Figure 3A). For 17 of 44 shipwreck ivory samples, complete mitogenomes were assembled (see STAR Methods). Each of the 17 carried a distinct mitogenome haplotype, and when compared to 11 published mitogenomes (Table S2), the shipwreck ivory grouped with elephants from West Africa (Figure 3D).

Each mtDNA subclade consists of distinctive sequence haplotypes, many with very limited geographic distributions.[18] A 336-bp segment of the mtDNA D-loop control region of the shipwreck

Figure 1. The *Bom Jesus* Shipwreck Cargo
Top: gold 10-*cruzado* coins (cross insignia), minted under the reign of King João III of Portugal in 1525 and withdrawn in the 1530s, helped to date and identify the ship.[4] Bottom: the shipwreck cargo included more than 100 unworked elephant tusks. Images: Amy Toensing; National Geographic Image Collection license.

ivory was compared to the 37 published mtDNA sequences within the West-Central (n = 29) and Western (n = 8) mtDNA subclades (Table S2; Figure 3C). The ivory exactly matched 3 haplotypes reported only from West Africa, with one exact match to a haplotype found in both West and Central Africa and no matches to haplotypes carried exclusively by Central African elephants (Figure 3C). Additionally, 16 novel geographically referenced elephant samples collected across Africa in the late twentieth century were sequenced for the same mtDNA region as the ivory (Table S2). The shipwreck ivory haplotypes grouped with haplotypes of the newly sequenced elephants that were from West Africa (Figure S1).

The shipwreck ivory samples lacked haplotypes from Northern-savanna, Savanna-wide, and Southeast-savanna subclades, present only among savanna elephants (see Figure 3A), consistent with the *Bom Jesus* ivory being exclusively from forest elephants. The shipwreck ivory carried no haplotypes from the North-Central, East-Central, or South-Central subclades, which would almost certainly have been present among tusks harvested in Central Africa (Figure 3A). Thus, all lines of evidence consistently indicated a West African forest elephant origin for all of the shipwreck ivory. The West African origin for the tusks also accords with historical records. Raw and carved ivories were exported from the Atlantic coast of Africa to Portugal from the mid-fifteenth century.[19,20] Of three major regions believed to export ivory to Portugal,[1,3,21] our genetic findings were consistent with an origin in Senegambia or the Gulf of Guinea (Figure 2) but rule out the Loango coast in Central Africa.[22]

The Ivory Derived from Forest Elephants outside of Deep Rainforests

To further examine the geographic source(s) of the ivory, carbon and nitrogen stable isotope ratios were measured (successfully for 97 of 100 tusks available) to determine the diets and habitats of the elephants. Stable carbon isotope ratios measured in ivory originate from the plant food the elephant consumed, which in turn is an indication of their habitats. Ivory has more positive $\delta^{13}C$ values than plant food due to further partitioning of carbon isotopes during digestion and tissue synthesis, with a diet-tissue enrichment of $\sim 5.5‰$ reported in modern and fossil proboscideans.[23] Published $\delta^{13}C_{collagen}$ for wild African elephants range from $-27‰$ for pure C_3 feeders in deep rainforests to $-11‰$ for savanna elephants consuming substantial quantities of C_4 grass (Figure 4). In the shipwreck ivory collagen, $\delta^{13}C$ values averaged $-20.4‰ \pm 1.2‰$, with a range from $-22.2‰$ to $-17.1‰$ (Figure 4; Table S3). Shipwreck ivory $\delta^{13}C$ values are more positive than those of modern elephants from Cameroon, Democratic Republic of Congo, and Liberia, which have ^{13}C-depleted values reflecting pure C_3 diets in deep forest/rainforest habitats with continuous tree canopy.[24–27] Shipwreck samples with the most positive $\delta^{13}C$ values fall within the range of modern comparative samples from open or shrub savanna environments, where elephants consume substantial proportions of C_4 grasses.[28–30]

Variation of ^{15}N in elephants is driven primarily by nitrogen cycling in the soil, which is strongly influenced by moisture availability.[33–36] Published $\delta^{15}N_{collagen}$ values for wild African elephants range from $2‰$ in moist areas to $17‰$ in arid areas.[29,30,31,37–41] In the shipwreck ivory, $\delta^{15}N$ values averaged $6.8‰ \pm 0.8‰$, with a range from $4.8‰$ to $9.0‰$ (Figure 4). Such values typically derive from mesic terrestrial environments, falling in the middle of the range of $\delta^{15}N$ values documented for African elephants and encompassing the values for elephants living in habitats in Angola, Benin, Burkina Faso, Chad, Central African Republic, and Niger. The shipwreck ivory $\delta^{15}N$ is lower than those (above $10‰$) of elephants in open, arid grassland savanna environments (Angola, Namibia, and South Africa) or living in dense forests (Democratic Republic of Congo).[28,30]

Values for the shipwreck ivory extend over 46% of the total variation in $\delta^{13}C$ and 49% of the total variation in $\delta^{15}N$ across African elephants (Figure 4).[30,40] The broad range suggests that the ivory was sourced from elephants from different habitats with different rainfall regimes and vegetation, although not from deep rainforests or arid environments. For four tusks, we took additional incremental samples to examine within-individual dietary variation over time (Figure S2). The $\delta^{13}C$ values from the incremental sampling for all four of these tusks are indicative of elephants that lived in mixed savanna and woodland habitats during the time the tusks were growing. The longest sequence derives from tusk B6082: $\delta^{13}C$ and $\delta^{15}N$ values show a cyclical pattern of increasing and decreasing over a series of 22 growth

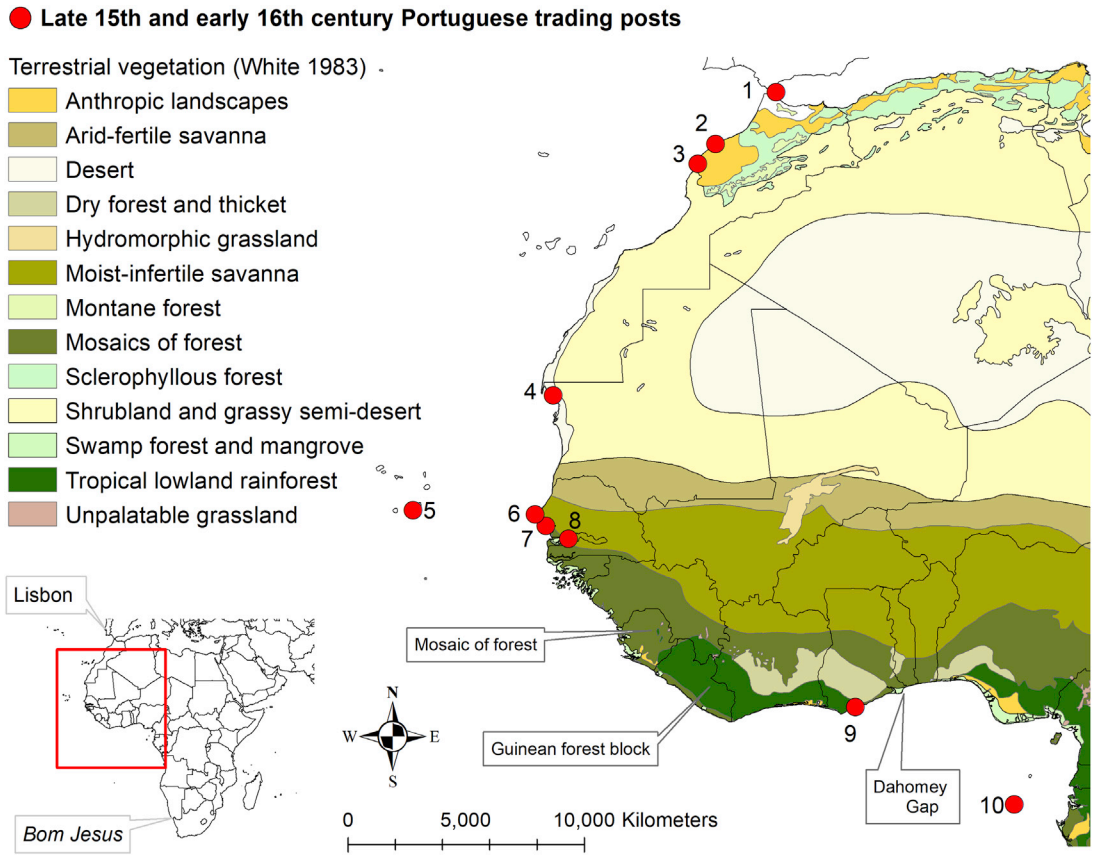

Figure 2. Terrestrial Vegetation Types of West Africa

West African terrestrial vegetation types (color coded) include dense tropical lowland rainforests and mosaics of forests, as well as savannas.[8] In the early sixteenth century, Portuguese merchants traded at ports (red circles) along the West African coast (4): 1-Ceuta; 2-Azemour; 3-Safi; 4-Arguim and Cape Blanco; 5-Cape Verde; 6-Bezeguiche (Dakar), 7-Joal, 8-Sutuco and Gambia River; 9-Elmina (São Jorge de Mina); and 10-São Tomé and Príncipe. Inset map shows Lisbon and the shipwreck site in Namibia.

increments, indicative of switching between C_3 browse and C_4 graze in the dry and wet seasons, respectively.[28,29,42,43] The range of $\delta^{13}C$ values (6‰) in this tusk is consistent with a habitat outside of the deep tropical forest, for which seasonal variation in $\delta^{13}C$ is typically $\leq 2‰$.[43]

The Sourcing of Ivory from West Africa to Portugal

In West Africa, dense tropical lowland rainforests of the Guinean forest block are surrounded by dry forest and thicket and a mosaic of forest and savanna habitats (Figure 2).[8] Based on combined genetic and isotope analyses, the elephants hunted for the ivory cargo of the *Bom Jesus* originated from West African forest elephants in habitats outside the Guinean forest block. In 1482, the Portuguese built a fort at an established trading post in West Africa, São Jorge de Mina, or Elmina, on the south-western edge of the Dahomey (or Benin) Gap.[9,10] This is a long-established[44] area of savanna and drier-type forest vegetation that provided an important corridor for transporting goods (including ivory) from the interior to the coast, thus avoiding travel through dense forest. Settlements near the Dahomey Gap then expanded as Elmina became an important entrepôt.

Due to the difficulty of maneuvering large long-distance trading vessels and the dangers of sailing close to the shore, outgoing ships on the India route typically did not tack along the West African coastline but would sail from Portugal southwest across the Atlantic and then southeast on the trade winds.[21,45] Ivory from West Africa was frequently shipped to the islands of Cape Verde and São Tomé to be counted, weighed, and sent via smaller vessels to Casa da Índia in Lisbon, the central clearing house for African and Indian imports to Portugal.[22,45,46,47] Centralized loading of outgoing long-distance trading vessels (*naus*) in Lisbon enabled tight control of the valuable cargo. Although the Great Lisbon earthquake and fire of 1755 destroyed many of the archives of the Casa da Índia, the consolidation of ivory from different localities within West Africa accords with our findings: the range of isotope values suggests multiple different habitats, as does the presence of 17 distinct mitogenome haplotypes, indicating that the *Bom Jesus* ivory derived from at least 17 different "herds" of elephants.

Implications for the Ecology and Conservation of West African Elephants

In West Africa, the historic range of savanna elephants was likely continuous across the Sahelian/Sudanian savanna habitat belts north of the Guinean forest block.[15] The current distributional

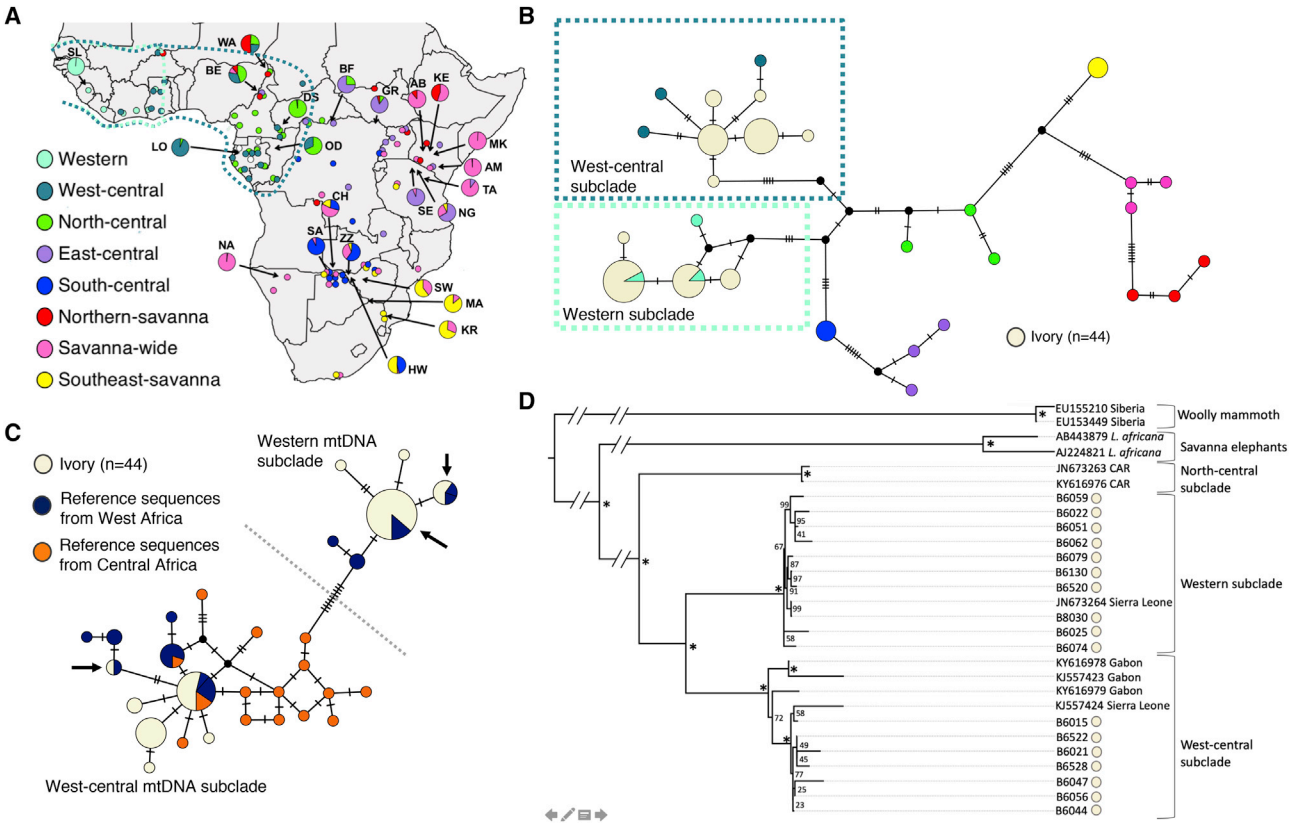

Figure 3. The Shipwreck Ivory Originated in West Africa

(A) African elephant mtDNA groups into 8 well-supported subclades (color coded). Modified from Ishida et al.[18] (permission: https://creativecommons.org/licenses/by-nc/3.0/legalcode).

(B) The shipwreck ivory mtDNA grouped with only the West-Central subclade and Western subclades—dashed lines show distributions in (A).[18] Subclades common only among savanna elephants or Central African forest elephants were not detected in the shipwreck ivory.

(C) Shipwreck ivory carried mtDNA haplotypes found only in West Africa (blue) or in both Central (orange) and West Africa but never haplotypes found only in Central Africa.

(D) For 17 of the ancient shipwreck ivory samples, complete mitogenomes grouped with modern elephants from West Africa. Bootstrap values are shown (asterisk indicates 100%).

Median joining network (B and C) cross-hatches indicate mutational differences; circles represent haplotypes.

See also Figures S1 and S3 and Tables S1 and S2.

range of forest elephants includes habitats both in tropical forest and in nearby savannas.[11–13,48] Before our analyses, the recent distribution of forest elephants in West Africa outside of tropical forest habitats could be attributed to decimation of savanna elephants in West Africa in the 19th and 20th centuries.[49,50] Our combined genetic and isotope results instead indicate that utilization of savanna habitats by forest elephants in West Africa preceded the removal of savanna elephants and dates back to at least the sixteenth century.

The *Bom Jesus* tusks are of varying length and size (from 2 to 33 kg), and the elephants may have been hunted indiscriminately, both males and females, young and old alike.[1,7] Among the mtDNA haplotypes identified from the sixteenth-century tusks, only four have been reported among contemporary populations (Figure 3C), likely reflecting the impact of the ivory trade and reduction of historic elephant range by at least 93% in West Africa.[51] Decrease in population size and genetic diversity are associated with negative conservation outcomes, such as expression of deleterious alleles, reduced reproductive fitness, and increased risk of population extirpation.[52] Our geo-referenced sequences from late-twentieth-century elephants in the University of Cape Town (UCT) collection, and the newly sequenced shipwreck ivory sequences, add substantially to the previously small body of isotopic and genetic information for West African elephants, with the potential to aid in the sourcing of confiscated illegal ivory.[40,53] Improving the ability to trace poached ivory can help guide optimal allocation of scarce law enforcement resources.

Conclusions

The *Bom Jesus* was one of 80 vessels that sailed from Lisbon on the India route between 1531 and 1540.[54] This was one of the most strategic and lucrative commercial routes of the time, linking to established trading networks between Africa and Asia.[55,56] The large number of tusks recovered from the *Bom Jesus* is evidence of ivory acquisition and circulation driving the formative stages of globalization.[57] With a resolution not possible using any single approach, our

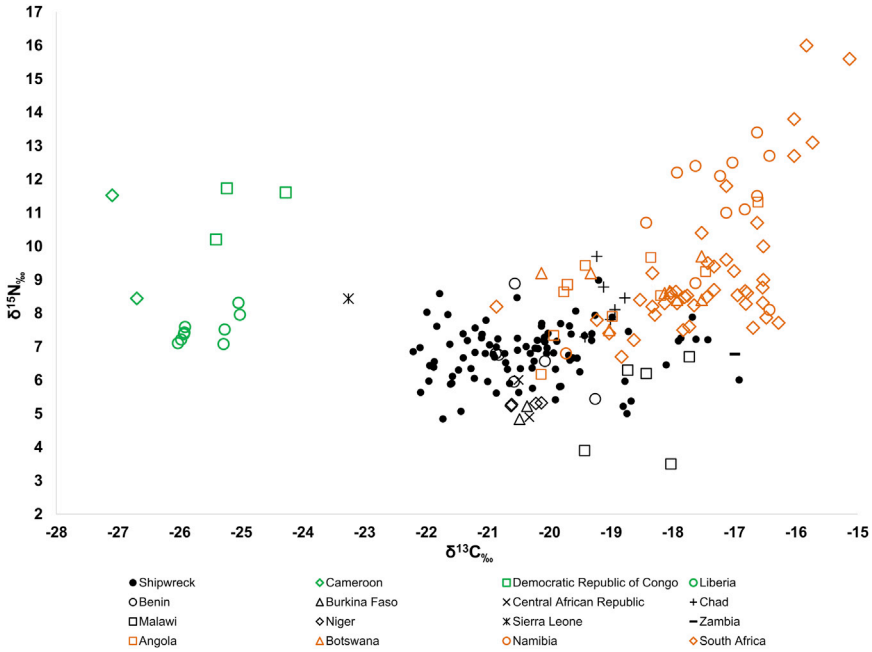

Figure 4. Shipwreck Ivory $\delta^{13}C$ and $\delta^{15}N$ Values, with Reference Samples from Elephants across Africa

Orange open icons represent elephants in drier, open savanna habitats; green open icons represent closed canopy forest habitats.[29–31] Black icons represent elephants from mesic shrub and wooded savanna environments;[29–32] the shipwreck ivory (black filled circles) cluster with these. The broad range of values for the shipwreck ivory suggests sourcing from multiple locations with different rainfall and vegetation patterns.
See also Figure S2 and Tables S3–S5.

interdisciplinary methodologies revealed the long-term genetic diversity and habitat use of the African forest elephant, helpful for conserving this iconic species.[43] To refine the sourcing of archeological and historical ivory, future work can utilize the combination of genetic and isotope methods presented here and additional approaches in both fields as they are further developed.

Unworked elephant tusks, ivory working debris, and finished objects made from ivory have been recovered from numerous archaeological contexts worldwide, including, but not limited to, shipwrecks with ivory cargo reported from the Mediterranean Sea and the Atlantic, Pacific, and Indian Oceans.[58–62] Our methods are applicable to the vast collections of historic and archaeological ivories in museums across the globe.[63–65] Analyzing historic and archaeological ivories affords a window into human-animal relationships across thousands of years and can reveal the formative and changing patterns of exchange between people who lived oceans apart.[30,66–70] Our study on the largest archaeological cargo of African ivory ever found provides a framework for examining one of the world's most important raw materials throughout human history.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead Contact
  - Materials Availability
  - Data and Code Availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Shipwreck ivory samples
- METHOD DETAILS
  - DNA extraction
  - Mitochondrial DNA sequencing
  - Single nucleotide polymorphisms for nuclear DNA gene regions
  - Stable carbon and nitrogen isotope analysis
- QUANTIFICATION AND STATISTICAL ANALYSIS
  - Mitochondrial DNA D-loop analyses
  - Mitogenome analyses
  - GIS data for cartography

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.cub.2020.10.086.

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AUTHOR CONTRIBUTIONS

Conception, A.d.F., A.C., J.S., S.C., R.S.M., and A.L.R.; Samples and Facilities, A.C., J.S., S.C., A.D.S.B., N.M.L.-M., and R.S.M.; Shipwreck Curation, N.M.L.-M.; Analyses, A.d.F. and A.C.; Interpretation, A.d.F., A.C., J.S., S.C., A.D.S.B., R.S.M., and A.L.R.; Drafting, A.d.F., A.C., and J.S., with contributions from all.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological Samples		
Shipwreck elephant ivory samples (*Loxodonta cyclotis*)	~2 g of ivory permitted under Namibian export heritage permit 07/2013 and CITES permits 166360 and 152037	N/A
20th century historical reference samples (*Loxodonta* spp.)	University of Cape Town Collection, South Africa	N/A
Critical Commercial Assays		
NEBNext® Ultra II™ DNA Library Prep kit	New England Biolabs	Cat# E6177S
NEBNext® Multiplex Oligos (Unique Dual Indexes) for Illumina®	New England Biolabs	Cat# E6440S
Deposited Data		
Novel ivory mitogenome sequences	This paper	BioProject: PRJNA668700 as part of the NCBI Sequence Read Archive (SRA: SRR12809510 - SRA: SRR12809526)
Ivory D-loop sequences	This paper	GenBank: MT576485- GenBank: MT576528
20th century historical reference sample sequences	This paper	GenBank: MW115961 - GenBank: MW115976
Complete mitochondrial genome subclade dataset	Selected sequences from[18]	Sequence identifiers listed in Table S2
Western and West-Central mtDNA subclade dataset	Selected sequences from[72–75]	Sequence identifiers listed in Table S2
Nuclear DNA SNP data	This paper	Table S1
Stable carbon and nitrogen isotope data	This paper	Tables S3 and S4
Loxodonta africana genome assembly	https://www.broadinstitute.org/elephant/elephant-genome-project	Loxafr 3.0
Oligonucleotides		
5′GTATA AGACATTACA ATGGTC3′	From[76]	*Laf CR1*
5′AGATGTCTTATTTAA- GAGGA3′	From[76]	*Laf CR2*
5′TGTAAAACGACGGCCAGTTTCCAAACAGGGTCACATCC3′	From[14]	*BGN-s2F-M13F*
5′CAGGAAACAGCTATGACAGCCAATTTCCTTTTGTCTGTT3′	From[14]	*BGN-s2R2-M13R*
5′TGTAAAACGACGGCCAGTTGTGCTCTCCTGTTGCTTTG3′	From[14]	PHK-s1F-M13F
5′CAGGAAACAGCTATGACACCTACTTGTTGCTGACTTTTGAA3′	From[14]	*PHK-s1R-M13R*
5′TGTAAAACGACGGCCAGTTCCTGCAATAAGCAGCAACA3′	From[14]	*PHK-s2F-M13F*
5′CAGGAAACAGCTATGACTTTCCCAAAGATGATGAAAACA3′	From[14]	*PHK-s2R2-M13R*
5′TGTAAAACGACGGCCAGTGATTGAGTTGGAGCCTCTGC3′	From[14]	*PLP-s1F-M13F*
5′CAGGAAACAGCTATGACCTCAAGCAGTGTCAAATCAAAA3′	From[14]	*PLP-s1R2-M13R*
Software and Algorithms		
MUSCLE	[77]	https://www.ebi.ac.uk/Tools/msa/muscle/
POPART (Population Analysis with Reticulate Trees)	[78]	http://popart.otago.ac.nz/index.shtml
AdapterRemoval v. 2.1.7	[79]	https://github.com/MikkelSchubert/adapterremoval
Bowtie2 v. 2.3.2	[80]	http://bowtie-bio.sourceforge.net/bowtie2/index.shtml
SAMtools v. 1.5	[81]	http://www.htslib.org

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Geneious R7	https://www.geneious.com	https://www.geneious.com
BWA v. 0.7.15	[82]	http://bio-bwa.sourceforge.net
mapDamage v. 2.0.9	[83]	https://ginolhac.github.io/mapDamage/
RAxML v. 8.2.12	[84]	https://cme.h-its.org/exelixis/web/software/raxml/
Jmodeltest v. 2.1.7	[85]	http://evomics.org/learning/phylogenetics/jmodeltest/
FigTree v. 1.4.4	[86]	http://tree.bio.ed.ac.uk/software/figtree/
ArcMap v. 10.7.1	ESRI 2011, ArcGIS Desktop: Release 10. Redlands, CA: Environmental Systems Research Institute	https://desktop.arcgis.com/en/arcmap/10.7/get-started/main/get-started-with-arcmap.htm

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, ALR (roca@illinois.edu)

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

Novel mitogenome sequences, ivory D-loop sequences, and 20th century reference sample sequences generated during this study are available in the NCBI Sequence Read Archive (mitogenomes) and GenBank (ivory D-loop and 20th century reference sequences). Mitogenome bam files can be found under BioProject: PRJNA668700 as part of the NCBI Sequence Read Archive (SRA: SRR12809510 - SRA: SRR12809526); ivory D-loop sequences can be found under GenBank: MT576485 - GenBank: MT576528; and 20th century reference sample sequences can be found under GenBank: MW115961 - GenBank: MW115976. Sequences generated for this manuscript were from ancient DNA templates, which are subject to the effects of DNA damage (Figure S3).

Previously published reference sequences are available from GenBank (see Table S2 for GenBank accession numbers).

The nuclear DNA SNP data generated during this study are available in Table S1.

The stable carbon and nitrogen isotope data generated during this study are available in Tables S3–S5.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Shipwreck ivory samples

The elephant ivory samples were exported under Namibian export heritage permit 07/2013 and CITES permits 166360 and 152037.

METHOD DETAILS

DNA extraction

DNA was extracted from 62 ancient ivory samples using an ancient DNA extraction protocol previously developed and optimized by the Malhi Ancient DNA Laboratory[87] at the Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign (UIUC). All shipwreck ivory DNA extractions were conducted in this facility in a laboratory dedicated to the analysis of ancient DNA. DNA was extracted from 20th century geo-referenced samples from the University of Cape Town (UCT) collection in a dedicated Bio-Safety Level 2 laboratory at the University of Pretoria (UP), South Africa. The UP laboratory was decontaminated with DNA-off prior to the initiation of the project, and the entire laboratory was UV sterilized on a daily basis using a UV ceiling light for at least 1 hour. Using swabs, we sampled multiple surfaces in the laboratory and none of the surface samples yielded PCR amplicons for elephant mtDNA. No samples other than the 20th century historical reference samples were processed in this laboratory for the duration of this project. PCR amplification of both the shipwreck ivory and the 20th century historical reference samples was carried out in laboratories that were isolated from other laboratories in which DNA extractions were carried out. To ensure that external or cross-sample contamination was avoided, extraction and PCR-negative controls were included with each round of sample processing, and not more than 8 samples were processed at any one time.

Mitochondrial DNA sequencing

A 441 bp fragment was amplified for 44 of 62 available ivory samples, and a 316 bp fragment was amplified for 16 twentieth-century geographically-referenced samples from the UCT collection, using published mtDNA D-loop primers *Laf CR1* an d *Laf CR2*.[76] Genomic libraries were constructed for 17 ancient ivory samples using the NEBNext® Ultra II™ DNA Library Prep kit and NEBNext® Multiplex Oligos (Unique Dual Indexes) for Illumina®. Libraries were pooled and shotgun sequenced on a HiSeq 4000 and NovaSeq 6000 platform at the UIUC Core Sequencing Facility.

Single nucleotide polymorphisms for nuclear DNA gene regions

To determine the species identity of the elephants from which the tusks had been harvested, we amplified short nDNA regions from three genes (*BGN*, *PHK*, *PLP*) that contain single nucleotide polymorphisms (SNPs) that show fixed character state differences between forest and savanna elephants[14] following the amplification procedure described in[14,87] (Table S1) and using primers *BGN-s2F-M13F, BGN-s2R2-M13R, PHK-s1F-M13F, PHK-s1R-M13R, PHK-s2F-M13F, PHK-s2R2-M13R, PLP-s1F-M13F* and *PLP-s1R2-M13R*.

Stable carbon and nitrogen isotope analysis

Elephant tusks grow continuously and incrementally.[88] To average possible seasonal and annual variation, we removed small pieces of ivory extending across multiple growth layers. Sampling attempted to minimize damage to the tusks. In the laboratory, sample surfaces were cleaned by sanding with a Dremel hand drill fitted with an emery disc. Collagen was extracted by demineralising in 0.3M HCl at room temperature for several days to 2 weeks, then rinsed with distilled water to neutrality. Acid was changed every few days. Samples were soaked in 0.1M NaOH overnight to remove base soluble contaminants and again rinsed with distilled water to neutrality. The samples were then put into pH 3, 0.01M HCl and heated to 70°C for 48 hours to denature the collagen ('gelatinization'), then filtered through 60-90 μm Ezee® filters and lyophilized. Dentine collagen was successfully extracted from 97 of 100 shipwreck ivory samples.

We were allowed to sample four tusks incrementally, as these tusks had pieces which were damaged or broken and were therefore amenable to sampling a transverse section of the tusk along multiple growth layers. We sampled these four tusks from transverse sections, with incremental samples taken at every 1mm from the cementum – dentine junction inward toward the pulp cavity, following.[29] Approximately 10 mg of powder were drilled from each increment using a Microdrill. Collagen was extracted from each powder by demineralising in 0.3M HCl at room temperature overnight, then rinsing with distilled water to neutrality, centrifuging between rinses. Due to sample size constraints, only a single $\delta^{13}C$ and $\delta^{15}N$ measurement was obtained for each powder.

Approximately 0.5 mg of each extract were weighed into a tin capsule, placed in a Thermo Flash Elemental Analyzer 2000, and combusted at ~1650°C. The resultant CO_2 and N_2 gases were passed into a Delta V Plus mass spectrometer for measurement of carbon and nitrogen isotope ratios as well as elemental compositions (%C, %N). The results are expressed in the delta (δ) notation in parts per thousand (‰), relative to the international standards Vienna Pee Dee Belemnite (VPDB) for carbon and Ambient Inhalable Reservoir (AIR) for nitrogen. All samples other than serial samples were run in duplicate and the values averaged. The standard deviation of repeated-measurements (n = 40) of homogeneous standard materials was ≤ 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$. The $\delta^{13}C$ values of reference elephant samples have been corrected for depletion of ^{13}C in atmospheric CO_2 since the Industrial Revolution, due to burning of fossil fuels, to enable direct comparison with the shipwreck archaeological samples.[89]

QUANTIFICATION AND STATISTICAL ANALYSIS

Mitochondrial DNA D-loop analyses

Mitochondrial D-loop sequences were compared to those of eight previously reported African elephant mtDNA subclades.[18] We downloaded three 4258-base pair (bp) reference sequences for each of the eight mtDNA subclades from the original dataset used by[18], aligned our shorter 441 bp sequences to the longer reference sequences using the program MUSCLE[77], and trimmed the sequences so that only regions present in both the reference sequences and the ivory sequences were included in the final alignment. The alignment was then used to construct a median-joining network in the software POPART (Population Analysis with Reticulate Trees)[78] that compared shipwreck ivory to mtDNA subclade reference sequences (Figure 3B), to novel twentieth-century geographically-referenced and published mtDNA sequences from West and Central African elephants (Figure S1). The genomic libraries used to assemble complete mitogenomes were constructed using multiple DNA extractions from the same samples as those used for the shorter D-loop analysis. For all DNA extractions per sample, the complete mitogenome and D-loop analyses resulted in consistent mtDNA subclade assignments for the sample.

A geographically-referenced database was compiled for haplotypes within the two mtDNA subclades that the ivory grouped with (Western and West-central mtDNA subclades) by downloading previously reported D-loop mtDNA elephant sequences from GenBank and coding them to reflect the geographic origin (West Africa or Central Africa) of those elephants. We aligned[77] the 441 bp ancient ivory sequences to the geographically-referenced database (336 bp), and trimmed the data so that only overlapping regions were present in the final alignment. The alignment was then used to infer a pairwise-distance based median-joining network[78] (Figure 3C). It is important to note that some studies reporting reference sequences failed to list the frequency at which each haplotype was observed. We encourage future researchers to report both the haplotype sequence as well as the frequency at which haplotypes were observed in the population. However, the current study sought to determine only whether the ivory matched

geographically-referenced haplotypes from West or from Central Africa, and thus the frequency at which haplotypes were observed in populations was not of concern.

Mitogenome analyses

All bioinformatic analyses were performed using the Biocluster2 supercomputer of the Carl R. Woese Institute for Genomic Biology. Reads were de-multiplexed and trimmed using AdapterRemoval[79] to have a minimum sequence length of 25 bp. Reads were aligned to the assembled African elephant genome (*Loxodonta africana* assembly Loxafr 3.0) and to a published forest elephant mitogenome[90] using bowtie2[80] with the local alignment option, and capping fragment length at 1000 bp. Aligned sequences were transformed to BAM format in SAMtools v. 1.1.[81] Using SAMtools, BAM files were filtered to remove unmapped reads and reads with a quality score less than 30, were sorted and indexed, and PCR duplicates were removed. Consensus sequences were generated from the de-duplicated alignment files in Geneious R7 (https://www.geneious.com) using a minimum read coverage of 3X and the "Highest Quality" algorithm that takes the relative residue quality into account when building majority consensus sequences. We were able to reconstruct mitogenomes for 17 ivory samples with 2.5-36.55 X average coverage (Table S2), and annotated them using the program GeSeq.[91]

Ancient DNA damage patterns were verified by aligning trimmed reads to the African forest elephant mitogenome with BWA[82] and quantifying damage in mapDamage2[83] using a fragment size of 90 bp. The shipwreck ivory DNA showed damage patterns typical of ancient DNA (Figure S3) with increased nucleotide misincorporations toward the terminal ends of the DNA molecules.

Consensus sequences of ancient ivory mitogenomes were compared to 11 previously published African elephant mitogenomes (Table S2) by inferring a maximum likelihood (ML) tree in RaXML.[84] Jmodeltest[85] indicated that the GTRGAMMA substitution model best fit the data. GTRGAMMA was therefore used in ML analysis which was repeated 1000 times with 100 bootstrap iterations for each run. The best ML tree was identified using the rapid bootstrapping algorithm in RaXML[84] and FigTree[86] was used to visualize the tree using a mid-point root. These reference sequences included published complete mitogenomes from two woolly mammoths (*Mammuthus primigenius*) with GenBank accession numbers EU155210 and EU153449 [92], two African savanna elephants (*Loxodonta africana*) with GenBank accession numbers NC000934[93] and AB443879[94], and seven African forest elephants (*Loxodonta cyclotis*) with GenBank accession numbers KY616976, KY616978-9, KJ557423-4, JN673263-4.[90,95,96]

GIS data for cartography

We used ArcMap (ESRI 2011, ArcGIS Desktop: Release 10. Redlands, CA: Environmental Systems Research Institute) to create a geographic map of West Africa using the World Countries (Generalized) layer from ESRI and the UNESCO/UNEP vegetation layers.[8] Trading post locations are indicated with current country names (Figure 2).