

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- | | |
|-------------------------------------|--|
| n/a | Confirmed |
| ☐ | ☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ A description of all covariates tested |
| ☒ | ☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☐ | ☒ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	For measurement of steroid concentrations in tusk samples using an Agilent system including an Agilent 6495 triple quadrupole mass spectrometer and Agilent 1260 and 1290 dual front-end HPLC/UPLC, the Agilent MassHunter software package version 10. For CT data collected using the Nikon X TH 225 ST microCT scanner, we used the Nikon X-Tek software package (V5.1.6054.18526) that includes Inspect-X for data acquisition and CT Pro for data reconstruction. CT data produced on the Phoenix Nanotom S nanoCT used the acquisition software Datos Acquisition and reconstruction software Datos.rec. The metadata for the files do not contain information related to software version numbers and the lab that produced the scan was not able to track down the information. The work was performed on 16 April 2016, before the current technician's time in the lab and the information was not documented by the previous lab technician. CT data collected using the SCANCO Medical µCT100 used Scanco uCT Tomography v6.4-2 for data acquisition and uCT Evaluation Program v6.6 for reconstruction. CT data produced on the Aracor ICT-1500 used the software package CATS v10.2 for data acquisition and reconstruction.
Data analysis	Steroid data were analysed using the Agilent MassHunter Workstation Software for Qualitative and Quantitative analyses version 10. CT data were analysed using Amira v. 2019.3. Stable isotope data were analyzed using the software package ThermoScientific ISODAT NT 3.0.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Steroid and isotope data generated and analysed during the current study are included in this published article and its supplementary files. CT scan data are available on the University of Michigan library's repository for digital data, Deep Blue Data. DOI addresses for CT scan data are listed in the Supplementary Notes.

Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender

N/A

Population characteristics

N/A

Recruitment

N/A

Ethics oversight

N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description

This study used serial sampling of dentin growth layers in elephant and mammoth tusks to investigate approximately monthly changes in steroid hormone concentrations (pg steroid/mg dentin). Using steroid hormones preserved in tusk records from a male African elephant, a male woolly mammoth, and a female woolly mammoth, we found that the woolly mammoth male experienced testosterone increases consistent with musth.

Research sample

The research sample included tusks from an adult male African elephant, an adult male woolly mammoth, and an adult female woolly mammoth. The African elephant tusk had a known time of death and was used to identify (with a high degree of certainty) steroid hormone changes associated with musth. With the male woolly mammoth tusk we tested for the presence of a testosterone pattern similar to the "musth" pattern identified in the elephant tusk. The female woolly mammoth tusk (along with early-life years in the male woolly mammoth tusk) were used as controls to verify that the 'musth' pattern was present only in adult years for the males.

Sampling strategy

Serial sampling of tusk dentin involves collection of samples representing contiguous intervals of dentin growth. Each sample is unique and represents a discrete time of life. Serial analyses using a single sample for each interval of growth are commonly used to detect patterns in growth records because the relevant data is how records diverge from those that are likely to be produced randomly. A pattern in the data that is unlikely to be produced randomly can be interpreted as meaningful. Stochastic modeling was not used in this study. Although a biologically meaningful pattern made up of many points is less likely to occur randomly than one made up of few points, the real determinant of sampling resolution and number of samples in this study was practical feasibility. In order to detect changes that occur at a monthly scale, we aimed to collect samples that represented monthly growth intervals.

Data collection

Steroid hormone data were collected using LC-MS/MS and were recorded by the lead author, MDC. Stable isotope data were collected using IR-MS in the University of Michigan Stable Isotope Lab.

Timing and spatial scale

The observable outward expressions of musth in elephants typically lasts between one and a few months, and usually appear during most years (at about the same time of year each time) after the elephant reaches musth onset. The scale of dentin samples was selected to provide approximately 12 serial samples for each annual growth increment. To achieve this roughly monthly analysis, sample intervals were approximately 0.5-0.7 mm thick. An annual growth layer in an elephant or mammoth tusk is typically between six and 10 mm thick.

Data exclusions	No data were excluded from the analysis.
Reproducibility	Control samples included with each analytical run of samples showed reproducibility in steroid quantitation. Resampling of portions of the growth record (and double sampling of the overlapping growth record in slabs taken from different locations in the tusk) were used to test for reproducibility of the steroid patterns detected. Data demonstrating such reproducibility are displayed in the male woolly mammoth figure in the main text and are included in the supplementary information.
Randomization	To eliminate the potential for machine and procedural effects to produce 'synthetic' patterns in the data (e.g., if carryover between analyses or 'degradation' of the analytical column were to produce directional drift in the data), we randomized the order of analyses.
Blinding	Although the process of LC-MS/MS analysis provides little room for bias effects, blinding was nevertheless used to prevent the possibility of analytical bias in interpretation of signal curve integration (which was often done manually). Blinding was achieved through random ordering of samples and sample analyses with the investigator unaware of each sample's position relative to others in the sequence. This addressed the potential that an investigator's decisions during manual integration of MS signals could be affected by a tendency to want to see patterns, thus producing patterns in the data where none actually existed.

Did the study involve field work? ☐ Yes ☒ No

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☐	☒ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Palaeontology and Archaeology

Specimen provenance	UMMP 118186 was collected and exported to the US in 1963 prior to CITES regulations on elephant ivory. UMMP 118711 was collected under a standing permit issued to the Almazy Anabara commercial venture in Yakutsk, Sakha Republic, Russian Federation. UMMP 118710 was collected under permit from the Wrangel Island Nature Refuge to co-author Sergey L. Vartanyan, as a consequence of his employment as a research scientist at the Reserve. Both of these specimens from the Russian Federation exported more than 15 years ago and the original permits are not available. However, the Russian co-authors on this manuscript, who are most knowledgeable about the specifics, have certified that these specimens were collected and exported under appropriate permits.
Specimen deposition	All specimens are deposited in the collections of the University of Michigan Museum of Paleontology and are available to researchers following appropriate channels.
Dating methods	Radiocarbon dates for two tusks are provided. One date (for the female tusk from Wrangel Island) was previously published, but the calibrated date was updated here using a more recent calibration curve. The other date was provided by the University of Arizona AMS Laboratory using collagen from a block of previously unexposed dentin removed from the tusk. Calibrations were calculated using OxCal 4.4 (Bronk Ramsey, 2009), using the IntCal20 calibration curve (Reimer et al., 2020).
☒	Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.
Ethics oversight	Study was completed with oversight from the University of Michigan Museum of Natural History following best practices for specimen curation. No additional ethics oversight was required because study specimens were nonsensitive materials that were existing museum specimens obtained legally and not linked to the ivory trade or were otherwise culturally significant or sensitive remains.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	The study did not involve laboratory animals.
Wild animals	Tusk of a wild male African elephant killed in 1963, imported to the US prior to CITES, and donated to the museum for research was used in this study.
Reporting on sex	Only male elephants experience musth, the condition linked to a dramatic increase in testosterone that is the focus of this study. Thus, we analysed a tusk of a male woolly mammoth to assess the preservation of testosterone records in tusk dentin. One tusk of a female woolly mammoth was analysed to provide a contrasting record with the expectation that testosterone would not display the fluctuations associated with musth.
Field-collected samples	The study utilized specimens in the existing collections of the University of Michigan Museum of Natural History and did not involve any new field collection.
Ethics oversight	No ethics oversight was required because the only samples that were not from long-dead animals were historical plasma samples provided by the Toledo Zoo from its long-term storage.

Note that full information on the approval of the study protocol must also be provided in the manuscript.