

Report

Out of America: Ancient DNA Evidence for a New World Origin of Late Quaternary Woolly Mammoths

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Summary

Although the iconic mammoth of the Late Pleistocene, the woolly mammoth (*Mammuthus primigenius*), has traditionally been regarded as the end point of a single anagenetically evolving lineage, recent paleontological and molecular studies have shown that successive allopatric speciation events must have occurred within Pleistocene *Mammuthus* in Asia, with subsequent expansion and hybridization between nominal taxa [1, 2]. However, the role of North American mammoth populations in these events has not been adequately explored from an ancient-DNA standpoint. To undertake this task, we analyzed mtDNA from a large data set consisting of mammoth samples from across Holarctica (n = 160) and representing most of radiocarbon time. Our evidence shows that, during the terminal Pleistocene, haplotypes originating in and characteristic of New World populations replaced or succeeded those endemic to Asia and western Beringia. Also, during the Last Glacial Maximum, mammoth populations do not appear to have suffered an overall decline in diversity, despite differing responses on either side of the Bering land bridge. In summary, the “Out-of-America” hypothesis holds that the dispersal of North American woolly mammoths into other parts of Holarctica created major phylogeographic structuring within *Mammuthus primigenius* populations, shaping the last phase of their evolutionary history before their demise.

Results and Discussion

Conventionally, the mammoth diversity of the New World is thought to have originated in two discrete waves. In the first, the Eurasian species *Mammuthus trogontherii* entered North America circa 1.8–1.5 mega-annum (Ma); there, according to most paleontological interpretations, it later evolved into the temperate-adapted Columbian mammoth, *M. columbi*. A second independent wave would have introduced woolly mammoths into northwestern North America circa 200 kiloannum (ka), a date that remains poorly constrained [3–5]. Although re-evaluations of Eurasian paleontological records [6, 7] have replaced the anagenetic view of mammoth evolution with one involving bouts of allopatric speciation, vicariance, and introgressive hybridization over a lengthy interval, the contribution of New World populations to this model has remained unclear.

To shed new light on the presumed nature of species succession, phylogeography, and diversity of mammoths in the Late Pleistocene Holarctic, we extracted DNA from 135 securely identified samples of *Mammuthus*. After assessing the level of preservation of host DNA in each with a quantitative

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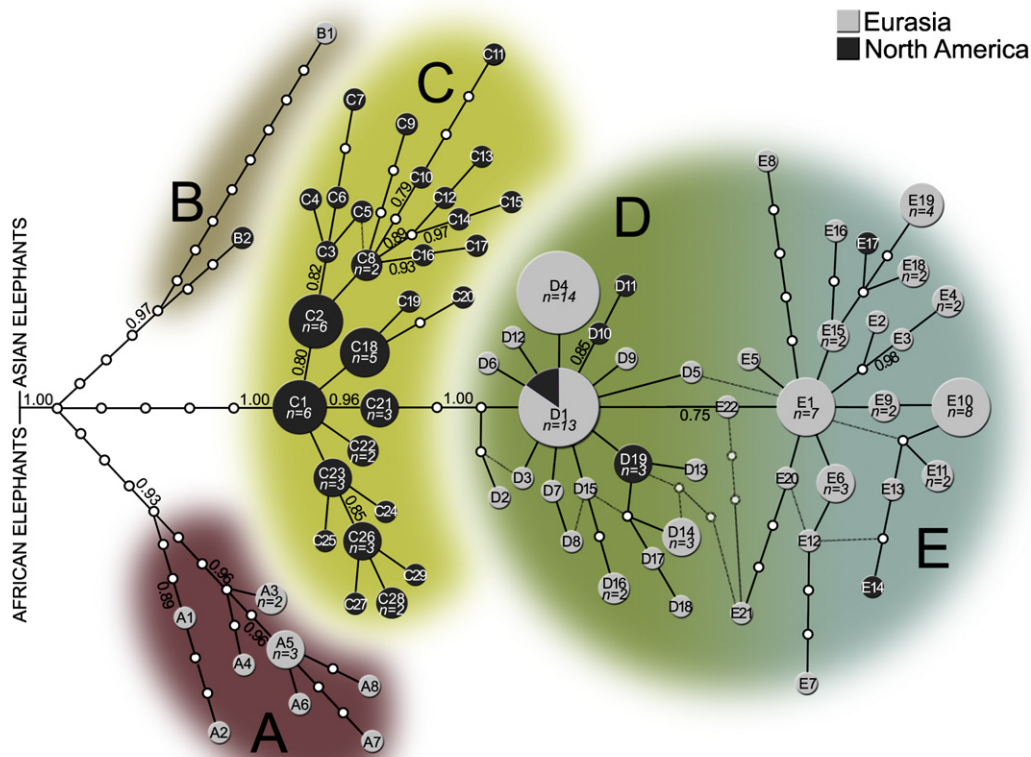


Figure 1. A Rooted Reticulated Tree for All Haplogroups

RRT distribution of 80 haplotypes within five haplogroups (A–E). Posterior probabilities ≥ 0.75 are indicated at nodes. Empty circles represent missing haplotypes. The size of each circle is proportional to its frequency within the data set. Both the preferred pathway (i.e., compatible with the Bayesian analysis; indicated by black branches) and alternative reticulations (indicated by shaded branches) are shown.

PCR assay (Supplemental Data available online), we amplified, cloned, and sequenced 743 bp of the hypervariable region mtDNA from 108 individuals (Table S1). To this data set we added 52 previously published sequences [8, 9] that shared 705 bp with our fragment. Of the combined total (160 samples), 63 stem from northwestern North America and 97 from Eurasia (Supplemental Data). Of 131 ^{14}C -dated samples (see Supplemental Data for date calibrations in ka), 105 returned finite estimates and may thus be placed within “radiocarbon time” (i.e., within the last 50 kiloyears before present [ky B.P.]). Apart from a relative excess of old ^{14}C -dated specimens, the distribution of our sequences documents the continuous occupation of both continents throughout radiocarbon time until their extinction in mainland Eurasia and North America circa 10 ky B.P. (~11.5 ka). Extinction occurred considerably later on some high-latitude islands. The molecular data reported here also include the first Holocene sequence from a Wrangel Island mammoth.

To establish whether our data set comprises a truly representative sample, we constructed a Bayesian analysis of the Stirling probability of theoretically expected haplotype richness (Supplemental Experimental Procedures and Figure S2). When applied to the current dataset (80 distinctly different haplotypes), this analysis shows that we sampled 70%–89% of the theoretical haplotype richness expected for Late Pleistocene mammoths from Beringia—an adequate basis for predictive modeling (Supplemental Data).

Phylogeographic Pattern and Spatiotemporal Distribution

To analyze the phylogenetic structure and distribution pattern of mitochondrial (mt) diversity, we produced a rooted reticulated tree (RRT) utilizing both Bayesian and network methods (Figure 1; see Supplemental Data for methodological considerations). On the basis of internal branch support and sampling of high-frequency stem haplotypes, we partitioned this diversity on the RRT as five haplogroups (A–E, Figure 1), clustered into three main clades (A, B, and C+D+E) bounded by high posterior probabilities (PP = 0.93–1.00). The clade C+D+E shows additional structuring, with a D+E clade (PP = 1.00) arising from a paraphyletic C (PP for monophyly of group C = 0.00). As the color coding in Figure 1 indicates, group A haplotypes were found to be exclusively Asian in distribution, whereas those in group C occurred exclusively in North America throughout radiocarbon time; the remaining groups (B, D, and E) were unevenly represented on both sides of the Bering Strait. The predominantly Asiatic distribution of haplogroups D and E and the endemism of haplogroups A and C together indicate a high level of intercontinental differentiation, perhaps because of filtering effects operating within the Bering land bridge [10].

This pattern is even more striking when the temporal spread of the full set of dated sequences is considered together with the spatial distribution of haplotypes (Figure 2). Group C was the only haplogroup present in northwestern North America during the first period, which covers finite ages greater than

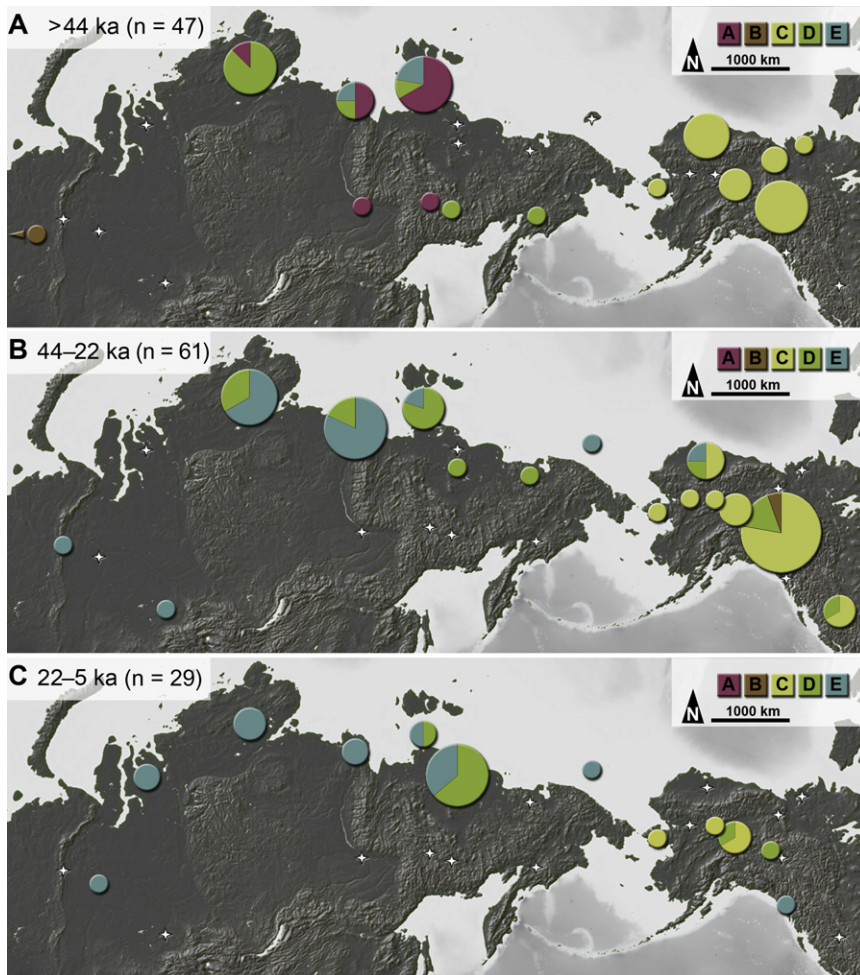


Figure 2. Geographic Distribution of Radiocarbon-Dated Mammoth Samples

Haplotypes (including finite and infinite dates) are plotted on the map in three successive time intervals. Groups' sizes are proportional to the number of samples.

44 ka and infinite estimates (see Figure 2A). Mammoth populations were more varied in Eurasia at this time: Haplogroups A and D were represented at high frequencies in Asia (44% each), with few E haplotypes (12%) and only a single occurrence (in Estonia) of group B (Table S5).

For subsequent intervals (44–22 ka, Figure 2B; 22–5 ka, Figure 2C), the distribution pattern is notably different: All mammoth samples fall within groups C (74% in North America versus 0% in Asia), D (18% versus 36%), and E (5% versus 64%), except for a single specimen from Yukon belonging to group B (Table S5). In Siberia, groups D and E apparently replaced the formerly abundant group A, as previously determined by Barnes et al. [8]. During the interval prior to the final extinction of mammoths (Figure 2C), group D became restricted to eastern Yakutia and Chukotka, whereas in northwestern North America, groups C, D, and E persisted until the regional disappearance of mammoths in mainland Alaska, circa 13–12 ka [11].

New World Origins of Terminal Pleistocene Siberian Populations

It is important to examine how the dating of haplogroup differentiation affects interpretation of the emerging phylogeographical picture. To investigate this, we derived divergence dates with the software BEAST (version 1.4.6 [12]) under a relaxed molecular-clock model, using all dated mammoths as tip calibrations in combination with two internal calibrations: (1)

first appearance of elephantids circa 7.5 Ma, as suggested by paleontological records [13] and confirmed by molecular analyses of complete mt genomes within Elephantoidea [14], and (2) a Bayesian estimate of the differentiation date of 11 dated complete mammoth mt genomes derived from our analyses (see Supplemental Data).

Three differentiation nodes portrayed in Figure 3 are especially pertinent to our analysis. Node I separates the branches leading to the three major clades (A, B, and C+D+E), node II marks the differentiation of clade C+D+E, and node III marks that of clade D+E.

Because most of the mt diversity found among Late Pleistocene Holarctic mammoths is almost exclusively accounted for by clade C+D+E (92% of all samples), we begin analysis with node II and the origin of ancestral group C. The singular North American distribution of this group (Figures 1 and 2), its paraphyletic structure (Figure 3 and Figure S3), and the sampling of putative ancestral haplotypes C1 and C21 (Figure 1) strongly point to the differentiation

of this clade within North America at some point during the Middle Pleistocene (node II, 95% highest posterior density [HPD]: 749–281 ka, median age 464 ka). Although the coalescent date almost certainly predates actual North American differentiation, it does provide an upper bound. Notably, the median of the Bayesian estimate is more than twice as old as the conventional paleontological estimate for the first appearance of *M. primigenius* in northwestern North America [3, 4], whereas the younger bound is only moderately older. This apparent conflict needs to be placed in context: There are far fewer identified Middle Pleistocene sites in northwestern North America than in Eurasia [2, 6, 7]. Those that have been found have been difficult to date securely, further hindering comprehensive taxonomic review of North American mammoths (P.M., unpublished data). In any case, the disparity in these first-appearance estimates should shrink in the future with better sampling [15].

Lineages within North American group C gave birth to clade D+E, which expanded back into Eurasia circa 301 ka (node III, 95% HPD: 398–214 ka) to become thereafter the predominant haplogroup within terminal Pleistocene mammoth populations. The westward migration of the precursor of clade D+E and its successful expansion in Siberia are not anticipated by paleontological models, which, in the absence of any morphological evidence to the contrary, have tacitly assumed that Eurasian mammoth populations received no more than minor contributions from North American vicars [2, 13]. Barnes

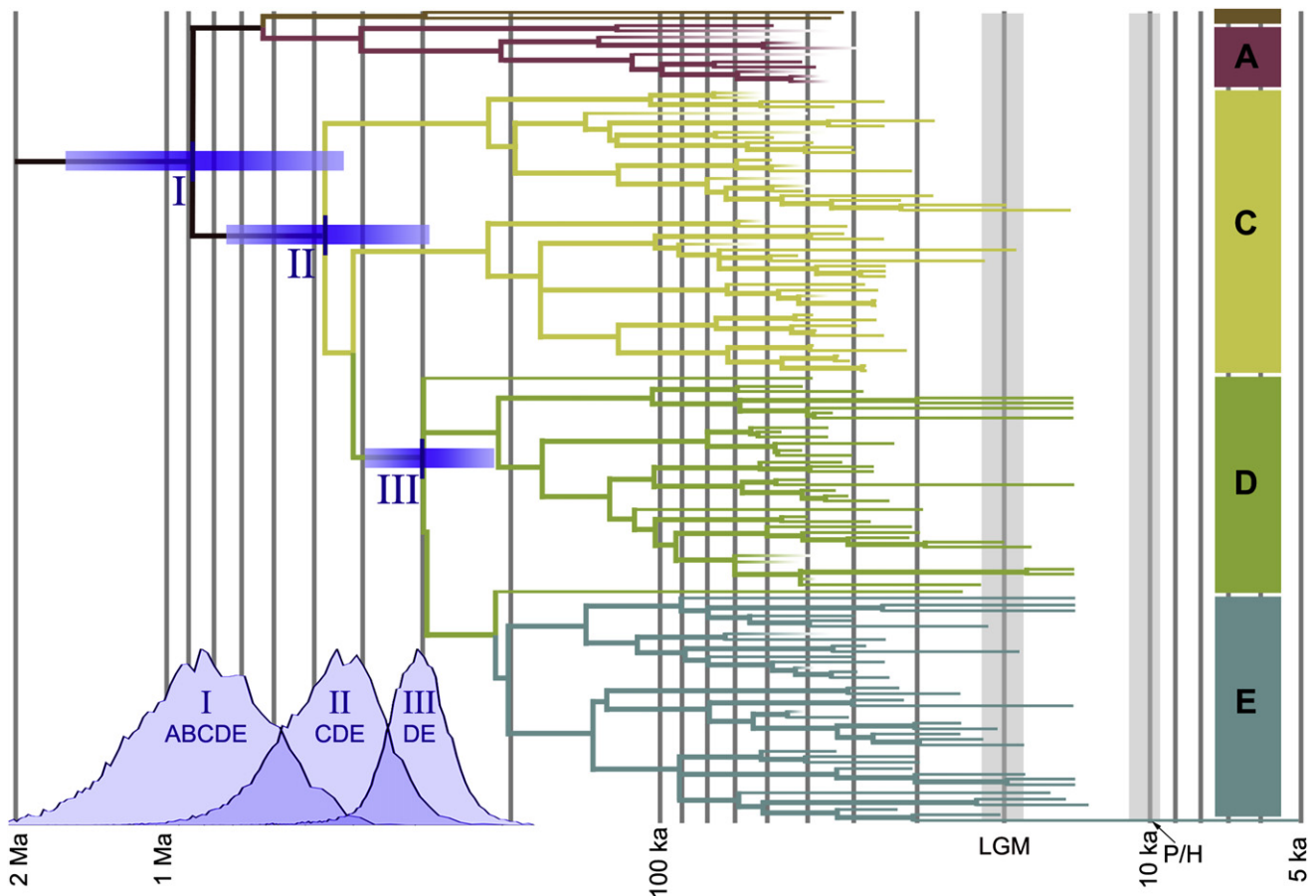


Figure 3. Maximum Credibility Tree Showing Dates of Divergence

All five haplogroups are shown with their associated 95% HPD interval for the three main splitting events (Nodes I, II, and III; see main text) given in calendar years before present. LGM, Last Glacial Maximum; P/H, Pleistocene/Holocene boundary. Time is displayed in logarithmic scale.

et al. [8] reported a possible instance of expansion from eastern to western Beringia, but their sample size was small ($n = 6$, only 3 of which supported expansion). Our data strengthen this hypothesis and show unequivocally that haplogroup C, an endemic and dominant group of North American mammoths, was basal to all remaining Asian mammoth populations (including that on Wrangel Island).

We emphasize that our molecular results are not in conflict with morphological evidence for the origin of Middle Pleistocene mammoths in northeastern Siberia [7]. However, they are clearly inconsistent with the assumption that the diversity recovered in Late Pleistocene Siberia was exclusively and persistently endemic. It is relevant to note that these results are consistent with those derived for Late Pleistocene *Bison* [16]: Siberian and Asian populations of this taxon also share a North American origin. Whether population histories involving wholesale replacement were the exception or the rule among Pleistocene Holarctic megafauna is a very interesting question, which should be addressable in the near future.

Endemic Siberian Mammoth Turnover: Drift versus Replacement

When mammoths from North America migrated westward through Beringia circa 300 ka, they were confronted with autochthonous Asian populations from which they had been separated for a long time. The median estimate of the date of

origin of these populations, i.e., when clades A and B parted from C+D+E (node I in Figure 3), is 888 ka, although the range is wide (95% HPD: 1588–429 ka). Our date for this node is notably consistent with recent estimates obtained from whole mt genomes (95% HPD: 2430–380 Ka, median circa 1.0 Ma [1]), which independently justify the calibrations used for our analyses (Supplemental Data). The information available for haplogroup B ($n = 2$) is too limited for useful analysis.

It may be reasonably assumed from the exclusive Siberian distribution of haplogroup A that this latter group must have inhabited this part of Eurasia through much if not all of the Middle Pleistocene (Figure S4). Its long duration, widespread distribution, and relative frequency ($f = 44\%$) in northeastern Siberia prior to 44 ka (Figure 2A) contrasts sharply with its subsequent complete disappearance (Figures 2B and 2C). This situation has two possible explanations. The simpler of the two is demographic, i.e., genetic drift, as suggested previously by Barnes et al. [8]. However, although we did not detect a persistent demographic bottleneck by using Bayesian skyline analysis as implemented in BEAST [12], we did find indications of a general overall population decline around this time, whether samples were considered as panmictic or separated by geography (Figure 4). Here, haplogroup A might be seen as having suffered natural decline, with later and unrelated replacement by immigrants stemming from North America. A less likely scenario, competitive replacement, may warrant

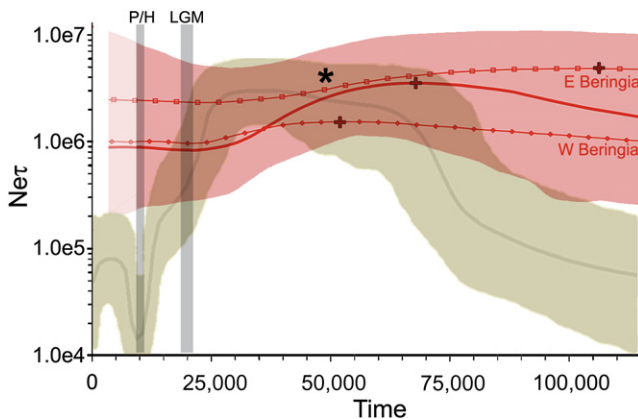


Figure 4. Skyline Plot of the Mammoth Data Set Spanning the Late Pleistocene

The thick red line corresponds to the average $Ne\tau$ for all dated mammoths (95% HPD shown as the red shaded area). Averages obtained for either western Beringia (represented by squares) or eastern Beringia (represented by diamonds) are shown for comparison. The “+” signs indicate the maximum $Ne\tau$ recovered for each set of mammoths. The asterisk marks the putative extinction of A. The underlying skyline plot (in gray) is redrawn from that for ancient Bison (after Drummond et al. [22]).

consideration if only because the timing of clade D+E’s expansion and clade A’s collapse in both distribution and diversity during the Late Pleistocene seem unlikely to have been coincidental.

Although our estimate of the age of the split between clades A and C+D+E (node I, Figure 3) only slightly predates the earliest fossils tentatively assigned to *M. primigenius* (circa 800–600 ka [7]) from northeastern Siberia, it is significantly older than the time (circa 400 ka) at which typical *M. primigenius* morphology first becomes clearly dominant in the Siberian fossil record [7, 17]. Although the paleontological chronology is not based on secure dating, it nevertheless raises some interesting points concerning the systematic implications of our molecular analyses. The co-occurrence of both A and D+E clades within Late Pleistocene Siberian mammoth populations could in theory have two alternative explanations. Our results are most easily interpreted as the consequence of early introgression events, such as those presumed on morphological grounds to have occurred between derived early *M. primigenius* and more primitive mammoths in Eurasia [2, 7]. In this interpretation, the genetic signatures of the C+D+E clade are in fact mitochondrial “fossils” of ancestrally distinct lineages (such as *M. trogontherii*) that had been maintained within successor populations of *M. primigenius*. Long-term retention of ancient polymorphisms in sister taxa has been detected in African [18, 19] and Asian elephants [20], in which philopatric matriarchal herds, low evolutionary rates, and large long-term effective population sizes help to preserve mitochondrial polymorphisms that might otherwise have been lost through drift [19] or other processes.

Alternatively, the taxon to which group A haplotypes belonged may have actually corresponded to one of the earlier chronospecies (*M. trogontherii* or some other primitive mammoth). Although dates derived from whole mtDNA genomes [1], as well as statistical analysis of molar features of Eurasian mammoths, provide some incidental support for this argument [6], it is clear that more work is needed before this explanation can be seriously entertained.

Mammoth Population Dynamics through Time

Our Bayesian skyline analysis (Figure 4) reveals that, after slow and regular growth up to and during Marine Isotope Stage (MIS) 4, the effective size of mammoth populations decreased significantly (approximately 3-fold) during MIS 3 (65–25 ka). This overall decrease seems to be the result of two independent trends: a slow negative growth affecting western Beringian populations early in the Late Pleistocene, potentially compensated by a positive growth in eastern Beringian populations until circa 50 ka, when their population size also started to decrease (Figure 4). However, given the wide confidence intervals of the skyline analysis, a high and constant effective population size during the Late Pleistocene cannot be ruled out, and thus the results should be treated with caution.

The estimate of effective population size (Ne) inferred with BEAST is dependent on the generation length (τ) of the taxon analyzed ($Ne\tau$ in Figure 4). With an assumed average of 20 years generation time in mammoths, comparable to modern elephants [21], the average Ne for the Late Pleistocene in the study by Barnes et al. [8] ranged between 1500 and 3500. By contrast, our average Ne was 40,000–150,000, or 10- to 100-fold higher (see Figure 4 and Supplemental Data). Interestingly our analysis recovers a $Ne\tau$ for mammoths similar to that recovered for ancient bison [16, 22]. These estimates are an order of magnitude lower than a recent estimate by Nogués-Bravo et al. [23], who concluded that several millions of mammoths lived contemporaneously in Beringia.

It is of equally great interest that both bison [16, 22] and mammoths exhibited population expansion during MIS 4 and contraction during the interstadial conditions of MIS 3. An identical flux has also been documented in bears [24] and horses [25], all of which suggests that demographic studies can and do reveal the effects of climate change on natural populations during this period. However, it is also true that mammoths apparently reacted earlier and more drastically to change than did bison populations, which remained static until the Last Glacial Maximum (LGM). Thereafter, roles reversed: Bison experienced a sharp and rapid decrease in numbers in the period just prior to the Pleistocene/Holocene boundary, whereas mammoths appeared to have passed through the LGM and its aftermath with only limited effects on their population size and diversity (Figure 4).

The classical understanding of Pleistocene mammoth populations, typically told as a tale of two continents, should be revised to take these dynamic patterns into account, thus revealing a clearer picture of the tempo and mode of both evolution and extinction in *Mammuthus*.

Experimental Procedures

DNA Extraction, Amplification, Cloning, and Sequencing

DNA was extracted from 135 mammoth samples distributed over their Holarctic range. Samples of 100 mg ($\pm 20\%$) were extracted in batches of 12 (including one extraction blank) in a dedicated clean room separated from PCR, post-PCR, and sequencing facilities (<http://socserv.mcmaster.ca/adna/labs.htm>), following a protocol previously published [26]. After concentration to a final volume of 100 μ l in 0.1X TE (pH 8.0), the extracts were screened for mammoth DNA with a specific 84 bp qPCR assay (Supplemental Data).

A 743 bp fragment of mtDNA that spanned the cytochrome *b*, tRNA Thr and Pro, and the Hypervariable Region I was obtained for 108 specimens (containing between 20 and 200,000 copies of the qPCR fragment per μ l of extract) with one to five overlapping PCR amplicons. All nucleotide positions were covered by a minimum of two clones each from two independent PCRs, yielding a total of 1392 clones (Supplemental Data). Sequences were aligned with the 705 bp in common from 52 previously published sequences

[8, 9, 27, 28]. Of the total sample (160 mammoths), 63 stem from northwestern North America and 97 from Eurasia.

Phylogenetic Methodology

The construction of the RRT relies on both network analysis (JMP method [29]) for the network structure and Bayesian phylogenetic analysis under a no-clock model (with MRBAYES; [30]) for rooting and statistical support (see Supplemental Data).

The dating of the main cladogenesis, as well as the spatial distribution of specimens, relies on radiocarbon-dated specimens. We show that because of fragment-length limitations, the methodology using only tip calibration of the Bayesian analysis with BEAST [12] yields erroneous rate and date estimates (see Supplemental Data for a detailed analysis). Therefore, in addition to tip calibrations, two node calibrations were used to derive a Bayesian estimate of the age of the nodes discussed here.

Population-Biology Analyses

The coalescent analyses with BEAST derived the demographics of the mammoth populations on the basis of a Bayesian skyline model [22]. The coalescent analysis was performed on the total 138 dated mammoths exclusively, as well as on the mammoths separated into geographic partitions (western Beringia versus eastern Beringia).

Accession Numbers

The GenBank accession numbers for the Pleistocene/Holocene sequences reported in this paper are FJ015093–FJ015152.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures, Supplemental Discussion, five figures, and five tables and can be found with this article online at <http://www.current-biology.com/cgi/content/full/18/17/1320/DC1/>.

Acknowledgments

We thank the Vuntut Gwitchin Government of Old Crow for their support of this research, D. Liberles and A. Devault for constructive criticism, and J. Christmas and A. Sekuler for institutional support. We thank A. Lister, P. Tassy, and an anonymous reviewer for constructive criticism on an earlier version of this manuscript. R.D. was supported by a fellowship from the Human Science Frontier Program (HFSP-00285C/2005). H.N.P. thanks the Natural Science and Engineering Research Council (grant #299103-2004), the Social Sciences and Humanities Research Council (grant #410-2004-0579), the Canadian Research Chairs program, Discovery Communications (a Quest grant), and McMaster University. R.D.E.M. thanks the National Science Foundation (OPP 0117400), the Niarchos Foundation, and Dr. Janet Asimov for grants in support of his research on Quaternary megafauna. D.R.G. was supported by the Natural Science and Engineering Research Council (grant #288321). H.N.P. and R.D.E.M. designed the project. B.B., D.F., D.G., C.d.M., P.M., A.T., and G.Z. provided access to the collections and/or the samples. C.F., R.D.E.M., H.N.P., D.P., and A.T. sampled the Cerpelex mammoth collections in the “ice cave” in Khatanga (Central Siberia). K.B., G.C., R.D., C.E.K., M.K., H.N.P., and C.S. designed and performed the aDNA lab experiments. P.S. and D.R.G. extracted the collagen from all samples selected for carbon dating (performed by J.S.). D.F. and G.Z. provided critical assessments of results and paleoenvironmental interpretation. R.D. and H.N.P. analyzed the data. R.D., H.N.P., and R.D.E.M. wrote the manuscript.

Received: June 21, 2008

Revised: July 16, 2008

Accepted: July 17, 2008

Published online: September 4, 2008

References

- Gilbert, M.T., Drautz, D.I., Lesk, A.M., Ho, S.Y., Qi, J., Ratan, A., Hsu, C.H., Sher, A., Dalen, L., Gotherstrom, A., et al. (2008). Intraspecific phylogenetic analysis of Siberian woolly mammoths using complete mitochondrial genomes. *Proc. Natl. Acad. Sci. U.S.A.* 105, 8327–8332.
- Lister, A.M., and Sher, A.V. (2001). The origin and evolution of the woolly mammoth. *Science* 294, 1094–1097.
- Harington, C.R. (1977). Pleistocene mammals of the Yukon Territory. Ph.D. thesis, University of Alberta, Edmonton, Canada.
- Péwé, T.L. (1975). Quaternary Geology of Alaska, Volume 835 (Washington, D.C.: U.S. Government Printing Office).
- Sher, A.V., Kuzmina, S.A., Kuznetsova, T.V., and Sulerzhitsky, L.D. (2005). New insights into the Weichselian environment and climate of the East Siberian Arctic, derived from fossil insects, plants, and mammals. *Quaternary Science Reviews* 24, 533–569.
- Foronova, I. (2007). Thin-enamel dental specialization in mammoth evolution: An example of direction selection. *Quat. Int.* 169–170, 95–104.
- Lister, A.M., Sher, A.V., van Essen, H., and Wei, G. (2005). The pattern and process of mammoth evolution in Eurasia. *Quat. Int.* 126–128, 49–64.
- Barnes, I., Shapiro, B., Lister, A., Kuznetsova, T., Sher, A., Guthrie, D., and Thomas, M.G. (2007). Genetic structure and extinction of the woolly mammoth, *Mammuthus primigenius*. *Curr. Biol.* 17, 1072–1075.
- Gilbert, M.T., Tomsho, L.P., Rendulic, S., Packard, M., Drautz, D.I., Sher, A., Tikhonov, A., Dalen, L., Kuznetsova, T., Kosintsev, P., et al. (2007). Whole-genome shotgun sequencing of mitochondria from ancient hair shafts. *Science* 317, 1927–1930.
- Guthrie, R.D. (2001). Origin and causes of the mammoth steppe: A story of cloud cover, woolly mammal tooth pits, buckles, and inside-out Beringia. *Quaternary Science Reviews* 20, 549–574.
- Guthrie, R.D. (2004). Radiocarbon evidence of mid-Holocene mammoths stranded on an Alaskan Bering Sea island. *Nature* 429, 746–748.
- Drummond, A.J., and Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* 7, 214.
- Shoshani, J., and Tassy, P. (2005). Advances in proboscidean taxonomy & classification, anatomy & physiology, and ecology & behavior. *Quat. Int.* 126–128, 5–20.
- Rohland, N., Malaspinas, A.S., Pollack, J.L., Slatkin, M., Matheus, P., and Hofreiter, M. (2007). Proboscidean mitogenomics: Chronology and mode of elephant evolution using mastodon as outgroup. *PLoS Biol.* 5, e207.
- Agenbroad, L.D. (2005). North American proboscideans: Mammoths: The state of knowledge, 2003. *Quat. Int.* 126–128, 73–92.
- Shapiro, B., Drummond, A.J., Rambaut, A., Wilson, M.C., Matheus, P.E., Sher, A.V., Pybus, O.G., Gilbert, M.T., Barnes, I., Binladen, J., et al. (2004). Rise and fall of the Beringian steppe bison. *Science* 306, 1561–1565.
- Stuart, A.J., Sulerzhitsky, L.D., Orlova, L.A., Kuzmin, Y.V., and Lister, A.M. (2002). The latest woolly mammoth (*Mammuthus primigenius* Blumenbach) in Europe and Asia: A review of the current evidence. *Quaternary Science Reviews* 21, 1559–1569.
- Debruyne, R. (2005). A case study of apparent controversy between molecular phylogenies: The interrelationships of African elephants. *Cladistics* 21, 31–50.
- Roca, A.L., Georgiadis, N., and O'Brien, S.J. (2005). Cytonuclear genomic dissociation in African elephant species. *Nat. Genet.* 37, 96–100.
- Fleischer, R.C., Perry, E.A., Muralidharan, K., Stevens, E.E., and Wemmer, C.M. (2001). Phylogeography of the Asian elephant (*Elephas maximus*) based on mitochondrial DNA. *Evolution* 55, 1882–1892.
- Haynes, G. (1991). *Mammoths, Mastodonts, and Elephants: Biology, Behavior, and the Fossil Record* (Cambridge: Cambridge University Press).
- Drummond, A.J., Rambaut, A., Shapiro, B., and Pybus, O.G. (2005). Bayesian coalescent inference of past population dynamics from molecular sequences. *Mol. Biol. Evol.* 22, 1185–1192.
- Nogués-Bravo, D., Rodríguez, J., Hortal, J., Batra, P., and Araújo, M.B. (2008). Climate change, humans, and the extinction of the woolly mammoth. *PLoS Biol.* 6, e79.
- Barnes, I., Matheus, P., Shapiro, B., Jensen, D., and Cooper, A. (2002). Dynamics of Pleistocene population extinctions in Beringian brown bears. *Science* 295, 2267–2270.
- Guthrie, R.D. (2003). Rapid body size decline in Alaskan Pleistocene horses before extinction. *Nature* 426, 169–171.
- Poinar, H.N., Schwarz, C., Qi, J., Shapiro, B., Macphee, R.D., Buigues, B., Tikhonov, A., Huson, D.H., Tomsho, L.P., Auch, A., et al. (2006). Metagenomics to paleogenomics: Large-scale sequencing of mammoth DNA. *Science* 311, 392–394.
- Krause, J., Dear, P.H., Pollack, J.L., Slatkin, M., Spriggs, H., Barnes, I., Lister, A.M., Ebersberger, I., Pääbo, S., and Hofreiter, M. (2006). Multiplex amplification of the mammoth mitochondrial genome and the evolution of Elephantidae. *Nature* 439, 724–727.

28. Rogaev, E.I., Moliaka, Y.K., Malyarchuk, B.A., Kondrashov, F.A., Derenko, M.V., Chumakov, I., and Grigorenko, A.P. (2006). Complete mitochondrial genome and phylogeny of Pleistocene mammoth *Mammuthus primigenius*. *PLoS Biol.* 4, e73.
29. Cassens, I., Mardulyn, P., and Milinkovitch, M.C. (2005). Evaluating intraspecific “network” construction methods using simulated sequence data: Do existing algorithms outperform the global maximum parsimony approach? *Syst. Biol.* 54, 363–372.
30. Huelsenbeck, J.P., and Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17, 754–755.