

## Ancient DNA

# A mammoth step back in genomic time

Alfred L. Roca

DNA has been retrieved from mammoth specimens that are more than one million years old. Comparing the genomes of these animals and their descendants provides insights into the changes that occurred as one species evolved into another. **See p.265**

In 2013, DNA from a horse that lived sometime between 560,000 and 780,000 years ago was sequenced<sup>1</sup>. It was the most ancient DNA sample ever analysed. But that record has just been smashed by van der Valk and colleagues (page 265)<sup>2</sup>. The authors retrieved DNA from the molars of three mammoths found in north-east Siberia, two of which lived more than one million years (Myr) ago.

The authors isolated the DNA from molars that had been previously collected from the Siberian permafrost. They relied on methods that maximize the recovery of short fragments of residual DNA. The cold temperatures had lessened degradation of the DNA across geological timescales.

The authors dated the mammoths using biostratigraphy, in which faunal remains at the sites where the molars were collected are correlated with fauna at sites for which absolute dates are available. They also estimated the antiquity of the specimens by molecular dating of DNA in a cellular organelle called the mitochondrion, because a higher percentage of the mitochondrial genome was covered by sequencing than was that of nuclear DNA (although similar date estimates were obtained using nuclear DNA from the two more recent specimens).

The mitogenome data revealed that the most recent of the three mammoth specimens, dubbed Chukochoya, lived more than 680,000 years ago (for comparison, the iconic woolly mammoth, *Mammuthus primigenius*, first appeared in the fossil record around 700,000 years ago<sup>3</sup>). In a phylogenetic tree made using nuclear DNA, Chukochoya fell outside a group comprising all woolly mammoths from the Late Pleistocene (129,000–12,000 years ago). This finding is consistent with the morphology of the Chukochoya molar, which identified it as an early form of woolly mammoth.

The second-oldest mammoth, called Adycha, lived about 1.34 Myr ago during the Early Pleistocene (which spanned from about 2.58 million to 773,000 years ago). The authors

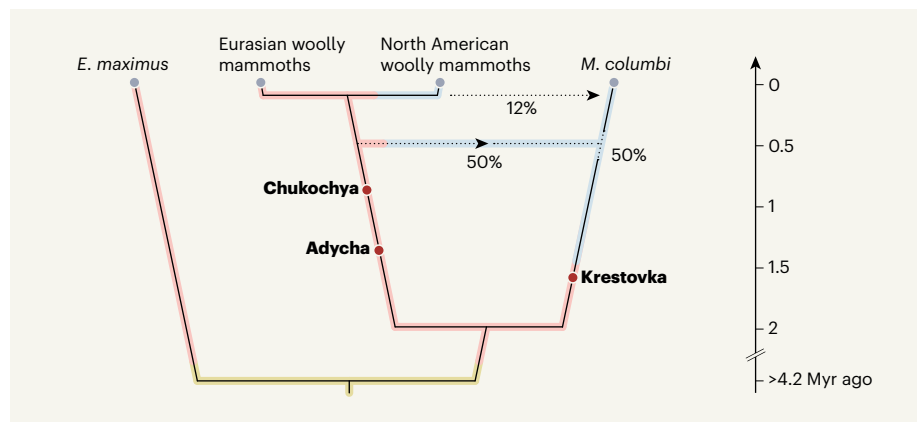
found that Adycha belonged to a population ancestral to woolly mammoths, and which lived before Chukochoya. There were substantial differences between the molar of Adycha and those of Chukochoya and more-recent woolly mammoths, in terms of enamel thickness, number and density of enamel plates, and height of the crowns. We do not yet have a good enough understanding of the developmental genetic programs that underlie these and other morphological traits to be able to identify the genomic changes responsible<sup>4</sup>. Going forward, a better understanding of the genetics of skull and tooth development might enable a closer braiding of mammoth genomics with palaeontology<sup>4</sup>.

The authors went on to compare the genomes of these ancient specimens with those of the animals' woolly mammoth

descendants, to examine how these mammoths had adapted to their cold Siberian environment. Many genetic variants thought to be the result of adaptation to northern latitudes have been identified in woolly mammoths by comparing their genomes with those of African savannah (*Loxodonta africana*) and Asian (*Elephas maximus*) elephants, members of the same mammalian family<sup>5</sup>. Of these variants, van der Valk *et al.* showed that 87% were already present in Adycha and 89% in Chukochoya. This is not surprising, because any lineage preserved in permafrost must already have been adapted to frigid climates. However, the authors also found evidence for further adaptation as the mammoth lineage evolved. For example, the gene *TRPV3*, involved in sensing temperature, carried more variants in Late Pleistocene woolly mammoths<sup>5</sup> than in the ancestral Chukochoya.

The most ancient mammoth was Krestovka, estimated by mitogenome dating to have lived about 1.65 Myr ago (although biostratigraphy suggested a slightly more recent date). A phylogenetic tree indicated that Krestovka was not from a population ancestral to woolly mammoths. Instead, it was part of a lineage that split from the Adycha–Chukochoya–woolly mammoth line roughly 2 Myr ago. The researchers propose that Krestovka was ancestral to the mammoths that entered North America about 1.5 Myr ago<sup>6</sup> and gave rise to the Columbian mammoth (*Mammuthus columbi*) in regions of mild climate in North and Central America (Fig. 1).

The authors also found, in the nuclear genomes of Columbian mammoths, the



**Figure 1 | A timeline of mammoth evolution.** The ancestors of mammoths and Asian elephants (*Elephas maximus*) originated in Africa (indicated by yellow highlight). Both lineages migrated into Eurasia (red) after they had diverged (the date of this divergence is uncertain). Van der Valk *et al.*<sup>2</sup> have sequenced DNA from three ancient mammoth specimens from Siberia, dubbed respectively Krestovka, Adycha and Chukochoya. Genomic analyses found that Adycha and Chukochoya were part of the lineage that gave rise to woolly mammoths. By contrast, Krestovka did not contribute to the woolly mammoth genome, but came from a lineage that diverged roughly two million years (Myr) ago, before migrating into North America (blue). Genomic analyses suggest that hybridization occurred between the Krestovka and woolly mammoth lineages, leading them each to contribute 50% to the ancestry of the North American Columbian mammoth (*Mammuthus columbi*). After woolly mammoths entered North America about 100,000 years ago, they interbred with the Columbian mammoths, replacing 12% of the Columbian mammoth genome. The timescale shown is based on genetic dating. (Figure adapted from Fig. 2c of ref. 2.)

signatures of two admixture (interbreeding) events between the Krestovka and woolly mammoth lineages. The second of these seems to have occurred after woolly mammoths entered North America about 100,000 years ago<sup>6</sup>. In this event, about 12% of the Columbian mammoth genome was replaced by DNA from Late Pleistocene woolly mammoths.

The remainder of the Columbian mammoth genome showed equal contributions from the Krestovka and woolly mammoth lineages, indicative of an earlier admixture event. A 50:50 split of this type might be considered surprising, given that the contributions of two ancestral populations could be in any proportions – 70:30, for instance, or 10:90. The split could be coincidence, but van der Valk and colleagues suggest the possibility of hybrid speciation, in which the offspring of crosses between the two lineages do not themselves breed with either parental lineage<sup>7</sup>.

The authors propose that the initial hybridization occurred around 420,000 years ago, because at this time there seems to have been a transfer of mitogenomes from a woolly mammoth lineage to the Columbian mammoth<sup>2,8</sup>. However, the ancestors of Columbian mammoths entered North America long before this date, and woolly mammoths long after<sup>3,6</sup>, making it difficult to harmonize the date with the fossil record. Mitogenomes transfer readily between species in the elephant family<sup>8,9</sup>, so perhaps the phylogeny of Late Pleistocene mammoths reflects more events than just a single interspecies transfer. Alternatively, the hybridization evident in the nuclear genome might not have occurred at the time estimated using mitogenomes. If DNA could be obtained from Early or Middle Pleistocene mammoth specimens in the permafrost of North America, this might shed further light on the origins of Columbian mammoths.

Many present-day animal species arose during or after the Early Pleistocene. The ability to retrieve DNA from Early Pleistocene specimens means that genomic changes in some lineages can now be tracked across deep time, providing insights into the evolution of modern species. Modern genomes are often used to infer the demographic history of populations across hundreds of thousands of years; these inferences should now be tested for accuracy by examining specimens across real time.

Tracking mitochondrial and nuclear genetic changes across deep time could also reveal the role (if any) of mitochondrial–nuclear interactions in mammoth evolution. When mitochondria are switched between cells from different species *in vitro*, interactions between the proteins encoded by mitochondrial and nuclear genes can be disrupted<sup>10</sup>. It is conceivable that such disruption could drive the evolution or disappearance of mitogenome lineages<sup>9</sup>. This avenue of investigation

is especially pertinent, given that van der Valk and colleagues relied on the transfer of mitogenomes to date the hybridization that gave rise to Columbian mammoths.

Finally, biostratigraphy focuses on the distribution and morphology of small species such as lemmings, pikas and voles. It should now be possible to add a DNA component to biostratigraphy, through genomic analyses of small mammals across sites. Genomics has been pushed into deep time by the giants of the Ice Age – the wee mammals that surrounded them might soon also have their day.

**Alfred L. Roca** is in the Department of Animal Sciences, College of Agricultural, Consumer

and Environmental Sciences, University of Illinois, Urbana, Illinois 61801, USA.  
e-mail: roca@illinois.edu

1. Orlando, L. *et al. Nature* **499**, 74–78 (2013).
2. van der Valk, T. *et al. Nature* **591**, 265–269 (2021).
3. Lister, A. M., Sher, A. V., van Essen, H. & Wei, G. *Quat. Int.* **126–128**, 49–64 (2005).
4. Roseman, C. C. *Am. J. Phys. Anthropol.* **160**, 582–592 (2016).
5. Lynch, V. J. *et al. Cell Rep.* **12**, 217–228 (2015).
6. Lister, A. M. & Sher, A. V. *Science* **350**, 805–809 (2015).
7. Mallet, J. *Nature* **446**, 279–283 (2007).
8. Enk, J. *et al. Genome Biol.* **12**, R51 (2011).
9. Roca, A. L. *J. Genet.* **98**, 83 (2019).
10. McKenzie, M., Chiotis, M., Pinkert, C. A. & Trounce, I. A. *Mol. Biol. Evol.* **20**, 1117–1124 (2003).

This article was published online on 17 February 2021.

## Physics

# Ultra-weak gravitational field detected

**Christian Rothleitner**

An experiment shows that Newton’s law of gravity holds even for two masses as small as about 90 milligrams. The findings take us a step nearer to measuring gravitational fields that are so weak that they could enter the quantum regime. **See p.225**

Four fundamental forces are known in physics: the weak and strong interactions, and the electromagnetic and gravitational forces. The gravitational force is the weakest of these four. For this reason, and because experiments cannot be shielded from Earth’s gravity, measurements of the gravitational field of a test object are difficult to do in the laboratory – even for objects that have multi-kilogram masses. But on page 225, Westphal *et al.*<sup>1</sup> report the detection of the gravitational coupling between two masses of only about 90 milligrams.

The weak, strong and electromagnetic interactions have been unified in the standard model of physics, but the gravitational force cannot be integrated into that model. The best model currently available to describe gravity is the general theory of relativity. This theory has not failed any test so far, but something is odd about it, because it cannot be explained in terms of quantum mechanics.

For most scientific purposes, however, we do not need to use the general theory of relativity to explain gravity – Isaac Newton’s law of universal gravitation<sup>2</sup> works perfectly. Published in 1687, Newton’s law states that the gravitational attraction between two bodies is proportional to their masses and inversely proportional to the square of the distance between them. This has proved to be correct

not only for describing most astronomical observations, but also in laboratory experiments. For example, the trajectory of a freely falling object (such as an apple falling from a tree) can be measured with a precision of less than ten parts in one billion<sup>3</sup>, and the results are in good agreement with what would be expected from Newton’s law.

During the twentieth century, however, doubts emerged about the general correctness of this law: an anomalous velocity distribution of stars in galaxies was observed<sup>4</sup> in the early 1930s, and could not be explained using Newton’s law alone<sup>5</sup>. Even the general theory of relativity cannot account for this phenomenon. One explanation is to postulate the existence of dark matter<sup>6</sup> – an invisible, but gravity-generating, component of the Universe. However, nobody really knows what this dark matter is made of.

Another explanation, which is controversial but easier to integrate into models than is dark matter, is that Newton’s gravitational law needs a correction. One theory that attempts such a correction was proposed in the 1980s, and is called modified Newtonian dynamics<sup>7</sup>. The basis of this theory is that gravitational field strength (the acceleration due to gravity) does not follow Newton’s inverse-square law over large distances.

A further mystery is that the gravitational