Title: Patterns in palaeontology: An introduction to ancient DNA

Author(s): Peter D. Heintzman[*1]
Volume: 3
Article: 10
Page(s): 10
Published Date: 01/10/2013
PermaLink:
http://www.palaeontologyonline.com/articles/2013/patterns-in-palaeontology-an-introduction-to-ancient-dna

IMPORTANT

Your use of the Palaeontology [online] archive indicates your acceptance of Palaeontology [online]'s Terms and Conditions of Use, available at http://www.palaeontologyonline.com/site-information/terms-and-conditions/.

COPYRIGHT

Palaeontology [online] (www.palaeontologyonline.com) publishes all work, unless otherwise stated, under the Creative Commons Attribution 3.0 Unported (CC BY 3.0) license.

This license lets others distribute, remix, tweak, and build upon the published work, even commercially, as long as they credit Palaeontology[online] for the original creation. This is the most accommodating of licenses offered by Creative Commons and is recommended for maximum dissemination of published material.

Further details are available at http://www.palaeontologyonline.com/site-information/copyright/.

CITATION OF ARTICLE

Please cite the following published work as:

Heintzman, Peter. 2013. Patterns in palaeontology: An introduction to ancient DNA. Palaeontology Online, Volume 3, Article 10, 1-10.

Patterns in palaeontology: An introduction to ancient DNA

by Peter D. Heintzman[*1]

Introduction:

Deoxyribonucleic acid, or DNA for short, is the magical molecule that encodes instructions on how to build organisms, and has been doing so successfully for at least the past 2.5 billion years. Although its function has remained constant throughout this time, the instructions themselves have been slowly modified and upgraded to cope with the changing demands of organisms and the environments in which they live. A modification to DNA is called a mutation, and it is through mutations that we are able to track how organisms have changed, or evolved, through time.

In all multicellular organisms, there are two major types of DNA: mitochondrial (mtDNA) and nuclear (nuDNA) (Fig. 1). These have different histories and can therefore tell us different things. Molecules of mtDNA are relatively small (genome size of ~16,000 base pairs in humans), circular and inherited from only the mother. MtDNA generally mutates more rapidly than nuDNA, and can therefore be useful for telling us about more recent events. By contrast, the nuDNA genome is much larger (3 billion base pairs in humans), tightly packaged into multiple long, thin chromosomes, and inherited from both parents in roughly equal proportions. NuDNA encodes much more information than mtDNA, in part due to its much larger size.

Ancient DNA, or aDNA, is a term used to describe any type of degraded DNA that belonged to a deceased organism or part of an organism. It can include DNA from museum specimens and forensic cases, but only DNA from subfossil remains will be discussed in this article. Ancient DNA is typically broken into short fragments and damaged by exposure to high temperatures, moisture, ultraviolet radiation, oxygen and the enzymatic decay that occurs rapidly after death. Reducing or removing any of these factors usually means that aDNA will be preserved for a longer time. As a result of degradation, recoverable aDNA occurs in much lower concentrations than intact DNA in living tissue.

How do we retrieve ancient DNA?

Ancient DNA can potentially be recovered from nearly all types of biological remains, including bones, teeth, hair, eggshells, insect cuticles (exoskeleton) and seeds — and even sediment and coprolites (Fig. 2). The best environments for finding aDNA are in the permafrost of the arctic, and inside deep caves. Both of these environments are dark, cold and dry, making them ideal for DNA preservation. Importantly, they are also stable through time. In theory, retrievable aDNA

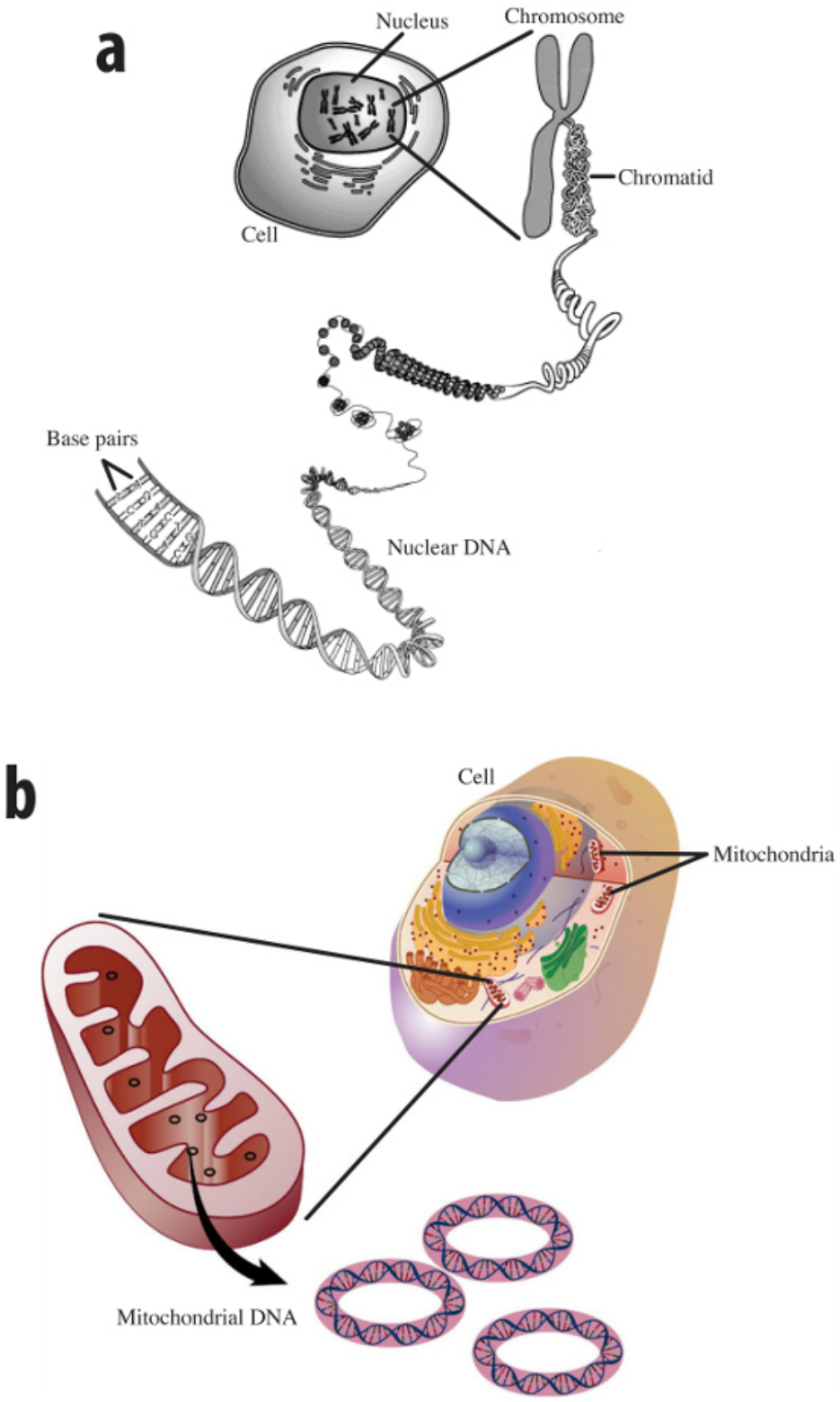

Figure 1 — The two major types of DNA found in all multicellular organisms: (a) nuclear DNA in the cell nucleus and (b) mitochondrial DNA in cell organelles called mitochondria. b. Modified after original by National Human Genome Research Institute.

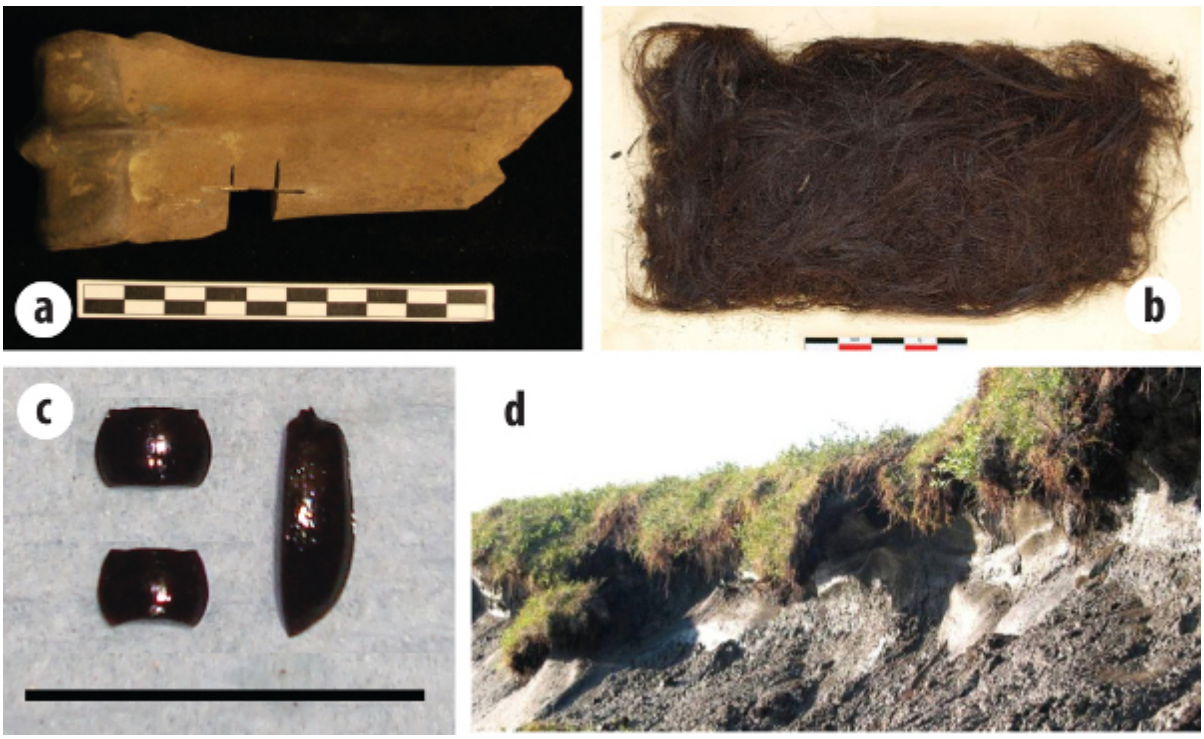

Figure 2 — Sources of permafrost-preserved ancient DNA: (a) bone, (b) hair, (c) insect cuticle and (d) sediment. Scale bars are (a) 10 centimetres, (b) 5 centimetres and (c) 1 centimetre. Sources: (a) Orlando 2013, (b) Rasmussen 2010, (c) photo by the author, (d) University of Florida.

could survive for up to 1 million years under ideal conditions. In practice, however, the oldest recovered aDNA is from the early Middle Pleistocene epoch, collected from a permafrost-preserved horse (up to ~780,000 years old) and from sediment that originated below the Greenland ice sheet (up to ~800,000 years old).

The first step in aDNA research is to recover the DNA from a specimen and to isolate and purify it. This requires digesting the material with enzymes and other chemicals to release the nucleic acids. DNA is then isolated from other undesirable molecules, such as proteins and inhibitors (polyphenols, humics, etc.), which are removed by separation or washing, depending on the extraction method used. Extracted aDNA is usually broken into small fragments of low concentration, so in order to have enough DNA to study, researchers target and amplify short genetic regions using the polymerase chain reaction (PCR). PCR reactions generally target overlapping regions that can be combined to show what the longer sequence looked like. In recent years, however, next-generation sequencing (NGS) technologies have revolutionized aDNA research because they allow researchers to determine the sequences of many short DNA molecules at once, reducing the time and cost of sequencing by many orders of magnitude. It is now possible to sequence 300 million DNA molecules at a time, each up to 300 base pairs long. With NGS, it is also possible to sequence extracted aDNA randomly (shotgun sequencing) to reveal the metagenome of a sample, or the genetic make-up of not only the target organism, but also contaminants and parasites.

Because aDNA is often damaged and found in low concentrations, it is crucial that lab work

(before PCR) is conducted in sterile conditions (Fig. 3). Samples must always be compared with negative controls, or samples that contain no DNA, to test whether they are being contaminated by modern DNA or other sources. Furthermore, aDNA sequence data need to pass basic authenticity tests: they must make evolutionary and biochemical sense, and be reproducible. Reports of geologically ancient DNA sequences from amber-preserved insects, plant fossils, dinosaur fossils and bacteria trapped in rock salt all fail at least one of these tests and therefore should be disregarded.

Figure 3 — Working in sterile, CSI-style conditions is very important for ancient DNA research, to minimize the risk of contamination. Photo by the author, originally published in Elias 2013.

Applications of ancient DNA data:

Information recovered from aDNA sequences has been applied to a broad range of important biological questions that are difficult or impossible to assess using modern DNA or the fossil record alone. Ancient DNA has helped to determine the position of extinct species in family trees (phylogenies), especially when relationships were unclear from anatomical data. Thanks to aDNA, we now know that the closest living relatives of the extinct Eurasian giant deer (*Megaloceros giganteus*) are the fallow deer (*Dama* sp.), and that the extinct cave lion (*Panthera leo spelaea*) and American lion (*Panthera leo atrox*) are more closely related to each other than they are to the living African lion (*Panthera leo*).

When combined with approaches and methods from population genetics, aDNA data can also be used to estimate the timing and impact of important events in the history of a species, such as population expansions and crashes, migrations and population-level extinctions. For example, we now know that the North American bison (*Bison priscus* (extinct) and *Bison bison* (still tasty)) underwent a population expansion that began at least 130,000 years ago, followed by a decline that began around 37,000 years ago, the latter of which is attributed to climatic cooling and

associated environmental changes. During another round of climate change, a period of warming around 12,000 years ago, the distribution of the Eurasian Arctic fox (*Alopex lagopus*) shifted northwards. Ancient DNA data show that the more southerly fox populations became extinct, rather than retreating north to more favourable habitat. Insights such as these have obvious implications for how future climate change may affect Arctic species.

Genetic data from ancient individuals often show 'missing' evolution. This is because the DNA has effectively been frozen in time, and so has not accumulated mutations since the organism was alive. Thus the individual seems to have 'missed out' on evolution experienced by living members of the same or a related species. If there is high confidence in the age of these individuals, they can be used to calibrate the molecular clock, a model that assumes that mutations accumulate at a constant rate. This clock can be used to estimate how long ago the most recent common ancestors (MRCAs) of two organisms lived. Using two ancient horses (one 43,000 years old and the other 560,000–780,000 years old), this approach has been used to demonstrate that the MRCA of all living horses, donkeys and zebras existed 4 million to 4.5 million years ago. Conversely, if the clock is already well calibrated for the group in question, it is possible to use 'missing' evolution to estimate the age of ancient individuals when other methods (such as radiocarbon dating) are not applicable. This has been used to show that a polar bear (Ursus maritimus) jawbone was 130,000 to 110,000 years old, and that an archaic relative of humans called the Denisovan individual lived around 74,000 to 82,000 years ago (Fig. 4).

Future directions for ancient DNA research:

Ancient DNA research has greatly benefited from advances in sequencing technology, refinements in the isolation of aDNA and the discovery of well-preserved samples. Together, these factors are allowing old questions to be re-assessed in unprecedented detail and are opening up completely new avenues of research. Of the latter, the most extraordinary story is that of the archaic relative of humans from Denisova cave in Siberia, Russia. Using only a single incomplete — but incredibly well preserved — little-finger bone (Fig. 5) and cutting-edge methodologies, enough aDNA was recovered to reconstruct a high-quality genome.

By comparing this to the genomes of several modern humans (*Homo sapiens*) and Neanderthals (*Homo neanderthalensis*), researchers showed that Denisovans might represent a distinct species more closely related to Neanderthals than to modern humans. It also turns out that Denisovans interbred with the ancestors of the native peoples of Australia and Southeast Asia, and that Denisovan DNA makes up roughly 6% of the genomes of these peoples. Lastly, the aDNA data suggest that the Denisovan had dark skin, with brown eyes and hair. Amazingly, all of these insights were made possible by the genetic material preserved in a single finger bone! This demonstrates the huge potential of ancient DNA to assess questions related to human evolution, inter-species hybridization (interbreeding) and the physical characteristics of extinct organisms, to name but a few.

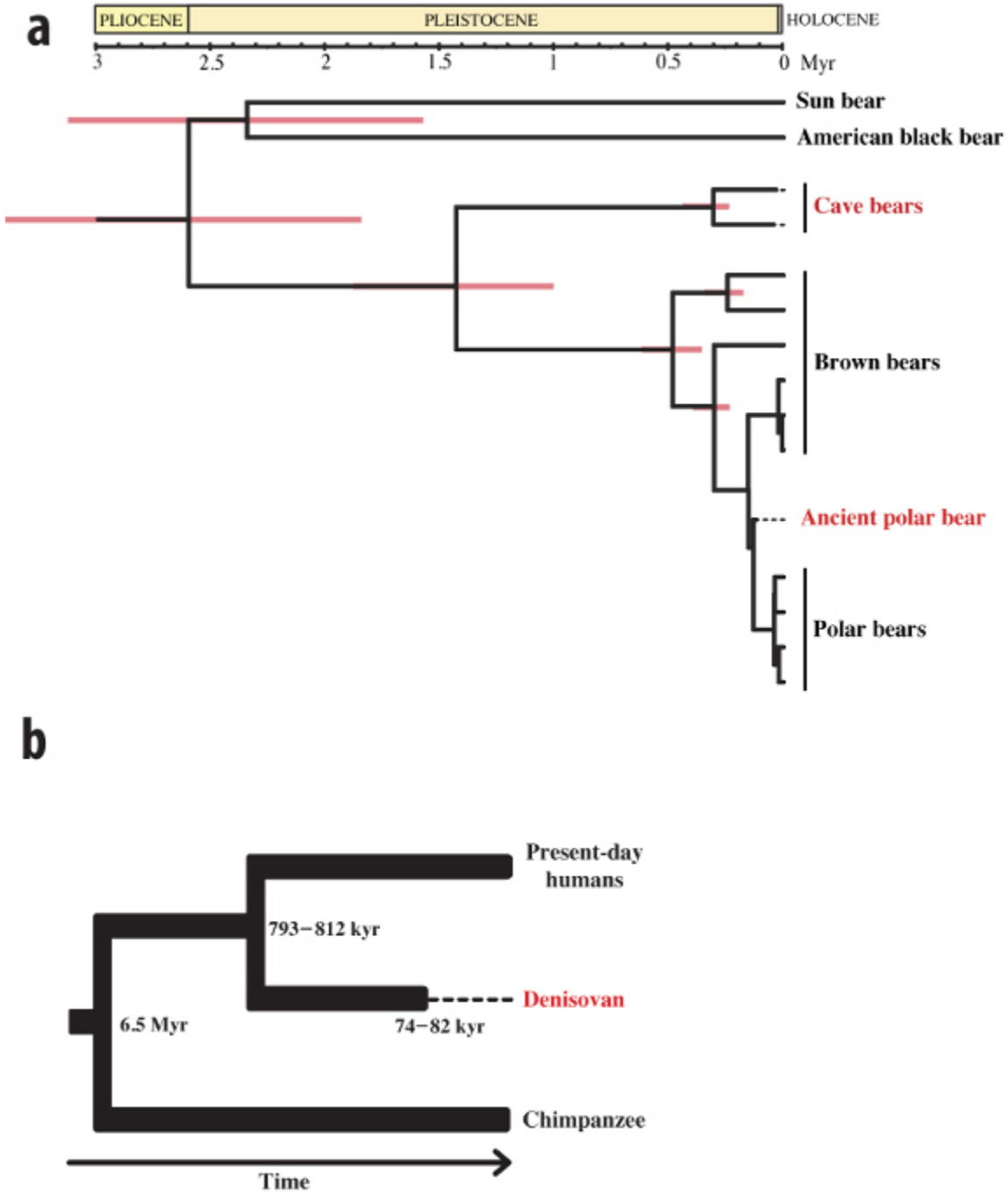

FIGURE 4 — 'MISSING' MOLECULAR EVOLUTION HAS BEEN USED TO ESTIMATE THE AGE OF (A) AN ANCIENT POLAR BEAR AND (B) A DENISOVAN. DOTTED LINES INDICATE 'MISSING' EVOLUTION. ANCIENT INDIVIDUALS ARE IN RED. PINK BARS INDICATE THE UNCERTAINTY SURROUNDING THE TIMING OF DIVERGENCE. NOTE THAT (B) IS NOT TO SCALE. KYR: THOUSAND YEARS OLD, MYR: MILLION YEARS OLD. MODIFIED FROM (A) LINDQVIST 2010, (B) MEYER 2012.

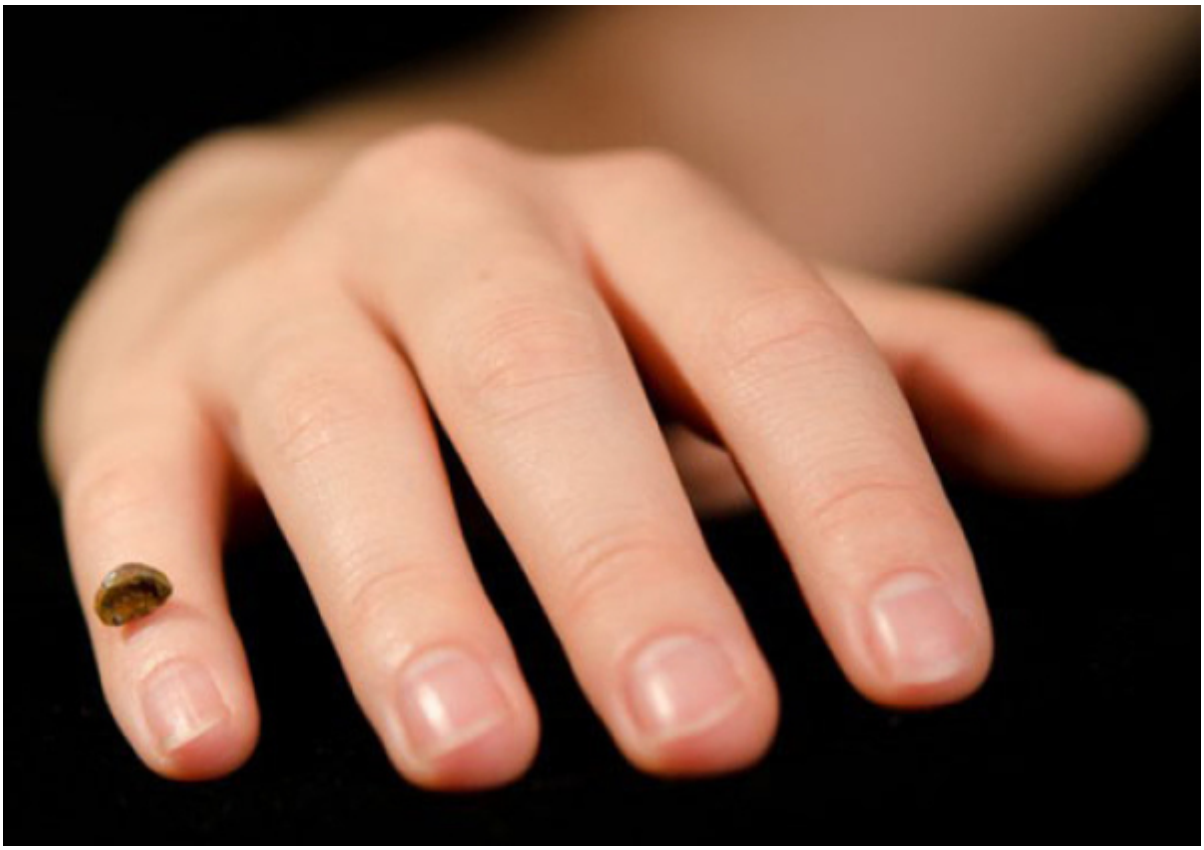

Figure 5 — The Denisovan finger bone from which enough ancient DNA was recovered to construct a high-quality genome. Source: Max Planck Institute for Evolutionary Anthropology.

The concept of resurrecting extinct species, termed de-extinction, has been discussed for decades, but has remained in the realm of science fiction (most famously in Jurassic Park) due to apparently insurmountable technical challenges. However, with advances in our ability to reconstruct the genomes of extinct animals through large-scale aDNA sequencing, as well as developments in cloning technology, de-extinction is now being discussed seriously (see TEDxDeExtinction). Species under consideration include the woolly mammoth (*Mammuthus primigenius*), Tasmanian tiger (*Thylacinus cynocephalus*), great auk (*Pinguinus impennis*) and passenger pigeon (*Ectopistes migratorius*) (Fig. 6). There are still major challenges, both methodological and ethical, that need to be overcome before de-extinction could ever become a reality.

Summary:

This article has provided a brief overview of ancient DNA, including what it is, how we get at it and what we can do with it, as well as future prospects for its research. It is a field that greatly complements and expands our understanding of the palaeobiology of organisms that lived within the past million years, and continues to yield exciting, surprising and insightful information on the histories of species including our own.

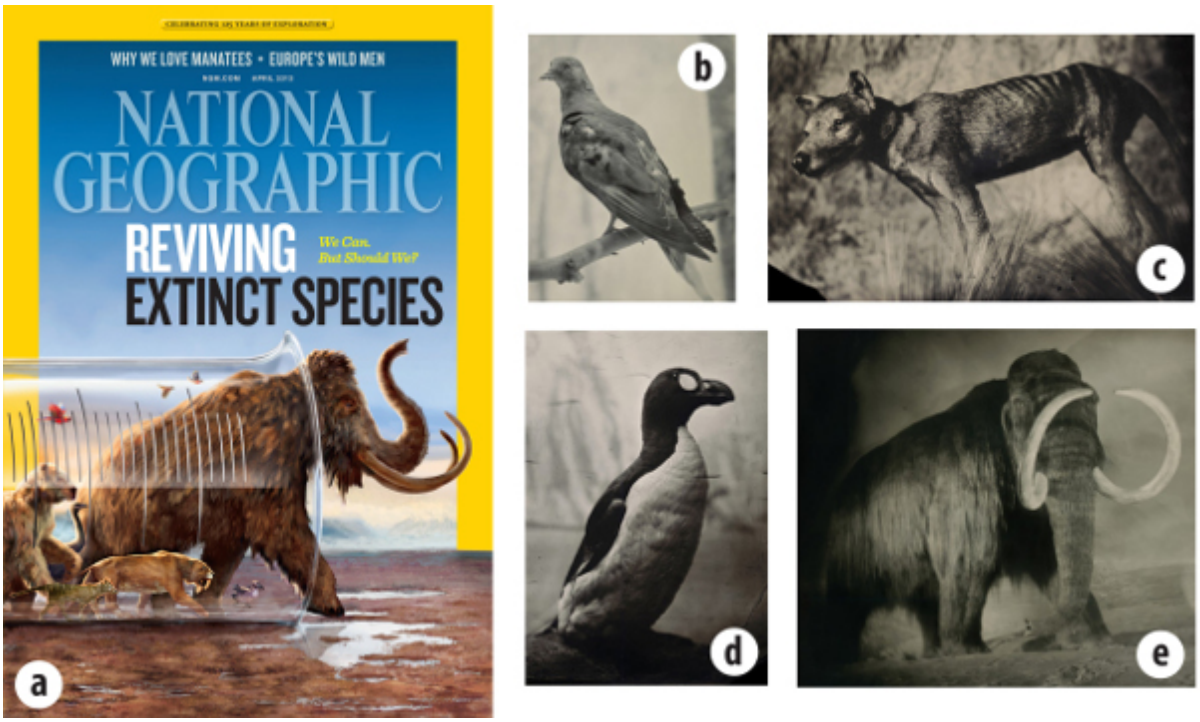

Figure 6 — De-extinction: although we cannot currently make species de-extinct, the idea is being seriously discussed. (a) The April 2013 cover of National Geographic. Candidate species: (b) passenger pigeon, (c) Tasmanian tiger, (d) great auk, (e) woolly mammoth. Modified from images from National Geographic.

Suggestions for further reading:

Austin, J. J., et al. 1997. Palaeontology in a molecular world: the search for authentic ancient DNA. Trends in Ecology and Evolution 12, 303–306. doi:10.1016/S0169-5347(97)01102-6

Cooper, A. & Poinar, H. N. 2000. Ancient DNA: Do it right or not at all. Science 289, 1139. doi:10.1126/science.289.5482.1139b

Hofreiter, M., et al. 2001. Ancient DNA. Nature Reviews Genetics 2, 353–359. doi:10.1038/35072071

Lindahl, T. 1993. Instability and decay of the primary structure of DNA. Nature 362, 709–715. doi:10.1038/362709a0

Orlando, L. et al. 2013. Recalibrating Equus evolution using the genome sequence of an early Middle Pleistocene horse. Nature 499, 74–78. doi:10.1038/nature12323

Reich, D. et al. 2010. Genetic history of an archaic hominin group from Denisova Cave in Siberia. Nature 468, 1053–1060. doi:10.1038/nature09710

Roast, A. 2013. De-extinction: Mammoth prospect, or just woolly? BBC News.

Shapiro, B. et al. 2004. Rise and fall of the Beringian steppe bison. Science 306, 1561—1565. doi:10.1126/science.1101074

Published by: Palaeontology [online]

Figure references:

Elias, S. A. (Ed.) 2013. Beetle records, In: Encyclopedia of Quaternary Science 2nd edn, pp. 161–172. Elsevier.

Lindqvist, C. et al. 2010. Complete mitochondrial genome of a Pleistocene jawbone unveils the origin of polar bear. Proceedings of the National Academy of Sciences of the United States of America 107, 5053–5057. doi:10.1073/pnas.0914266107

Meyer, M. et al. 2012. A high-coverage genome sequence from an archaic Denisovan individual. Science 338, 222-226. doi:10.1126/science.1224344

Orlando, L. et al. 2013. Recalibrating Equus evolution using the genome sequence of an early Middle Pleistocene horse. Nature 499, 74–78. doi:10.1038/nature1232

Rasmussen, M. et al. 2010. Ancient human genome sequence of an extinct Palaeo-Eskimo. Nature 463, 757–762. doi:10.1038/nature08835

[1]Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, Santa Cruz, California, United States