TEACHER'S GUIDE

DNA to Darwin Case Study

The evolution of mammoths and their living relatives

Version 2.0

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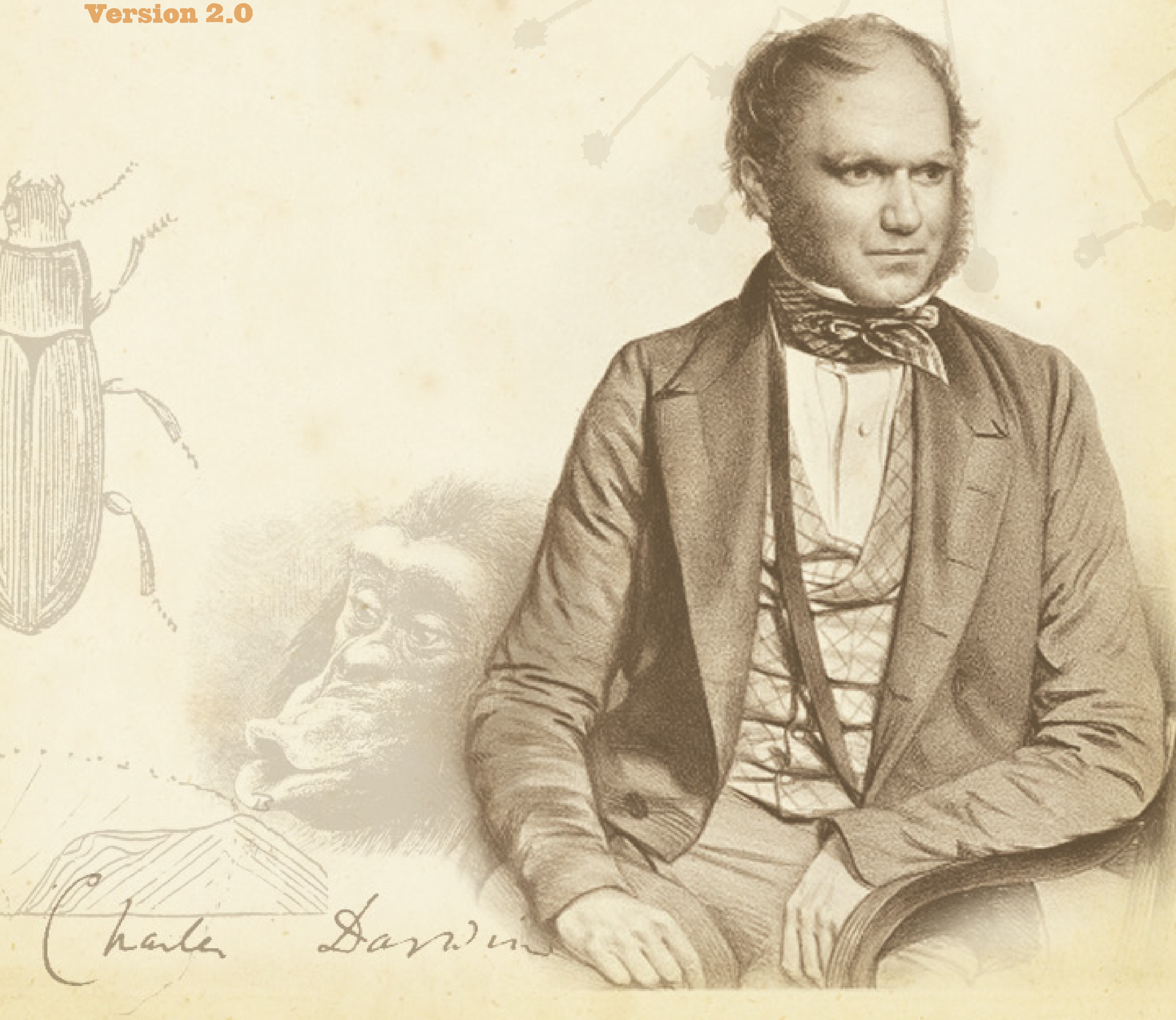


Evolution of mammoths

In this activity the evolutionary relationship of the extinct Woolly mammoth (*Mammuthus primigenius*) to modern elephants and other species is investigated. Complete mitochondrial DNA (mtDNA) sequences are used for this purpose.

DNA, ancient and modern

DNA can usually be obtained from living species only, but there are some exceptions. The Woolly mammoth (*Mammuthus primigenius*) was the first extinct species to have its nuclear genome sequenced (Miller *et al*, 2008).

Woolly mammoths became extinct about 6 000 years ago, but the specimen used for the nuclear DNA sequencing was about 20 000 years old, and had been recovered from the Siberian permafrost. The choice of a mammoth, and such an old specimen too, may seem odd because there are many species that have become extinct within living memory. For example, the 'marsupial wolf' or Thylacine (*Thylacinus cynocephalus*), became extinct in 1936, when the last animal died in Beaumaris Zoo (now Hobart Zoo), Tasmania. There are hundreds of Thylacine specimens preserved in museums around the world and also the remains of many more extinct species.

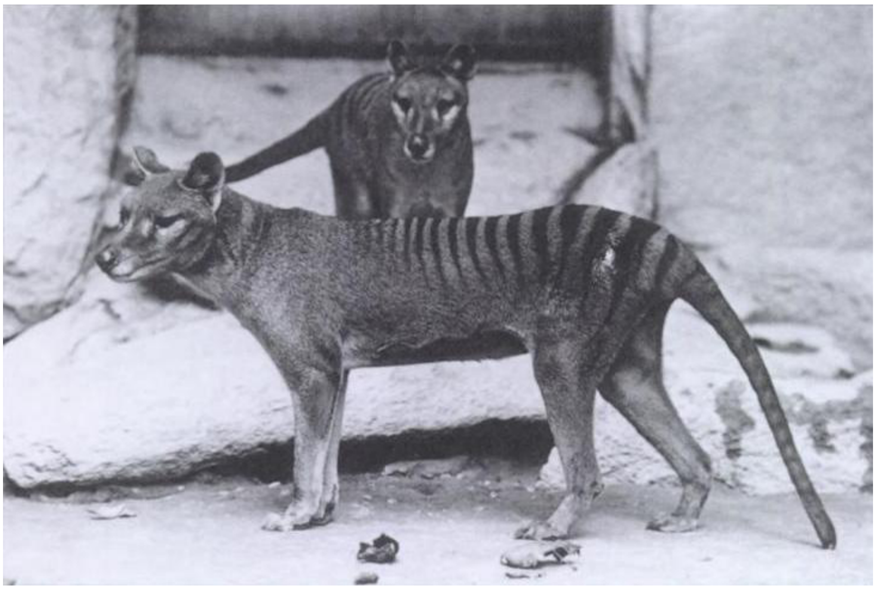


IMAGE FROM: E.J. Keller, Smithsonian Institution archives.

The extinct marsupial wolf (Thylacinus cynocephalus) in Washington D.C. National Zoo, c. 1906. An attempt to sequence the Thylacine genome was abandoned in 2005.

The invention of the polymerase chain reaction (PCR) in the mid-1980s sparked an interest in recovering and amplifying DNA from such museum specimens, and not just in the scientific community: stories like 'Jurassic Park' caught the wider public imagination. The prospect of resurrecting extinct species remains very remote, however — it would be impossible with our current biological understanding and technologies.

Unfortunately, DNA usually degrades rapidly after death, particularly if it has been kept at relatively warm temperatures or stored, like most museum specimens, in alcohol. An attempt to sequence the Thylacine genome from a pup preserved at the Australian Museum was officially abandoned in 2005 for this reason. DNA from inside the bones and teeth of specimens often proved to be contaminated with modern human and microbial material, even when scrupulous precautions had been taken.

While innovative technologies may allow more extinct species to be sequenced in the future (for example, a draft of the Neanderthal genome has been announced (Green *et al*, 2009), to date greater success has been achieved in recovering and analysing the relatively short stretches (~1700 bp) of mitochondrial DNA (mtDNA) (Miller *et al*, 2009). Not only is the mtDNA less likely to be fragmented than the nuclear genome, but it is far more abundant and therefore easier to recover (~1000 copies of the mitochodrial genome are present in each cell, compared with two of the nuclear genome). In addition, such short sequences are easy for beginners with low-powered computers, such as school students, to analyse.

General reading

The making of the fittest. DNA and the ultimate forensic record of evolution by Sean B. Carroll (2009) Quercus Books (Paperback) ISBN: 978 1847247247.
A popular lay account of some of the molecular evidence for evolution.

Reading the story in DNA: A beginner's guide to molecular evolution by Lindell Bromham (2008) Oxford University Press (Paperback) ISBN: 978 0199290918. *An engaging textbook on molecular evolution, which assumes no specialist mathematical knowledge and takes the reader from first principles. Although it's aimed at undergraduates, this superb book contains sufficient detail for PhD students, yet parts will appeal equally to 16–19 year-olds.*

Scientific publications

Most of these documents can be accessed free-of-charge, online.

Cooper, A. (2006) The year of the mammoth. *PLoS Biology*, 4(3): e78. doi: 10.1371/journal.pbio.0040078.
This is an easy-to-follow primer that places the sequencing of mammoth mitochondrial genomes in context and outlines the methods used to date.

Gross, L. (2006) Reading the evolutionary history of the woolly mammoth in its genome. *PLoS Biology*, 4(3): e74. doi: 10.1371/journal. pbio.0040074.
A simple report which explains the findings of the Rogaev et al. paper, in language which 16–19 year-old students should understand.

Miller, W. *et al* (2008) Sequencing the nuclear genome of the extinct woolly mammoth. *Nature*, 456, 387–390. doi: 10.1038/nature07446
This research paper describes how, for the first time, the nuclear genome of an extinct species was sequenced.

Rogaev, E.I. *et al* (2006) Complete mitochondrial genome and phylogeny of pleistocene mammoth *Mammuthus primigenius*. *PLoS Biology*, 4(3): e73. doi: 10.1371/journal.pbio.0040073. *This is one of the papers that the current educational exercise is based on.*

Rohland, N. *et al* (2007) Proboscidean mitogenomics: Chronology and mode of elephant evolution using mastodon as outgroup. *PLoS Biology*, 5(8): e207. doi: 10.1371/journal.pbio.0050207. *The mastodon sequence data was obtained from this research.*

Sedwick, C. (2008) What killed the woolly mammoth? *Science in School*, Issue 9, 18–21. Available from: **www.scienceinschool.org**

Rohland, N. *et al* (2010) Genomic DNA sequences from mastodon and woolly mammoth reveal deep speciation of forest and savanna elephants. *PLoS Biology*, 8(12): e1000564. doi: 10.1371/journal.pbio.1000564. *Nuclear DNA shows that African elephants are two different species: Forest and the Savanna elephants, which diverged 2.6–5.6 million years ago.*

Other genomes of extinct species

Green, R.E. *et al* (2008) A complete Neandertal mitochondrial genome sequence determined by high-throughput sequencing. *Cell*, 134, 416–426. doi: 10.1016/j.cell.2008.06.021

Miller, W. *et al* (2009) The mitochondrial genome sequence of the Tasmanian tiger (*Thylacinus cynocephalus*). *Genome Research*, 19, 213–220. doi: 10.1101/gr.082628.108

Requirements

Software

Geneious can be downloaded free-of-charge from: **www.geneious.com**. The software is available for Windows, Macintosh and Linux operating systems. Only the free, 'basic' version of the software is required here.

DNA sequence data

For the *Initial exercise*, students will need the *Geneious* document containing three aligned DNA sequences (Mammoth, Asiatic elephant and African elephant): **Elephants_and_mammoth.geneious** plus the same data with Neanderthal DNA as an outgroup: **Elephants_mammoth_Neanderthal.geneious**

For the *Extension activities*, students will need either to download sequences from the Internet, or be provided with two *Geneious* documents containing the ready-aligned sequences: **Dugong_Hyrax_Mammoth_Elephants.geneious** and **Plus_Mastodon.geneious**.

Students' worksheets

Students will require copies of worksheet pages 2–6 for the *Initial exercise*. The *Extension activities* are described on worksheet pages 7–12.

Presentations

The *QuickTime* animation and *PowerPoint* or *Keynote* presentations may be helpful for introducing this exercise. *QuickTime* may be downloaded free-of-charge from the Apple web site: **www.apple.com/quicktime**.

Educational aims

The activity reinforces students' understanding of DNA structure and mutation. It introduces the principle of alignment and encourages students to compare aligned mtDNA sequences by eye and by using statistics generated by the software.

Students use computer software to produce a phylogeny for three closely-related genera that evolved in the African Pliocene: *Mammuthus* (mammoth); *Elephas* (Asian elephant) and *Loxodonta* (African elephant).

Possible extension activities include investigating the relationship of two extant species, the Dugong (*Dugong dugon*) and the Rock hyrax (*Procavia capensis*), to elephants and speculating about the relationship of the extinct North American Mastodon (*Mammut americanum*) to these species before testing this idea using mtDNA sequence data.

Sequences for these additional species may be downloaded from the *GenBank* database and aligned by the students, or they can be provided to the students ready-aligned.

Prerequisite knowledge

Students will need to understand the structure of DNA, including the base-pairing mechanism. They will need to know that mitochondria have their own DNA and to be taught the principle of sequence alignment.

Other useful resources

Walking with beasts (2002) BBC Worldwide Ltd.
Region 2, PAL: two DVDs. 2 Entertain Video. ASIN: B00005UBMG.
The complete series presented by Kenneth Branagh. 173 minutes.
Part of programme six, 'Mammoth journey', where a mammoth falls through the ice into a frozen lake, can be used as an introduction to this activity.

Walking with cavemen (2003) BBC Worldwide Ltd.
Region 2+4, PAL: one DVD. 2 Entertain Video. ASIN: B000087LOS.
The final episode, 'The survivors' shows how mammoths were hunted by early humans, although the scientific content of this series has been criticised.

Scale models of Asiatic and African elephants and Woolly Mammoths are available from toy shops such as *Toys 'R' Us*, and occasionally from natural history museum and science centre gift shops. Such models can be useful when students are initially considering the possible evolutionary relationships between the three species, and can provide a better impression of their appearence than photographs and drawings.

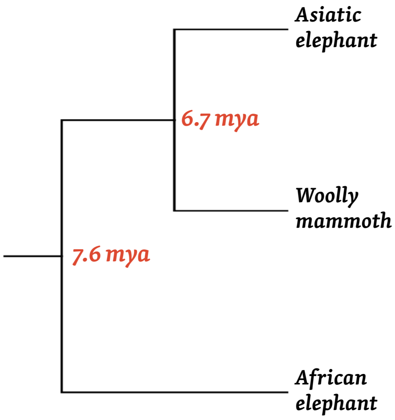
Answers to the questions on the worksheets

Page 4

1. In DNA sequences, transitions — base pair changes from a purine to a purine (A→G or G→A) or a pyrimidine to a pyrimidine (C→T or T→C) — occur more often.
2. Transitions are more likely to occur as they require only minor changes to the structure of the bases.

Page 6

1. The relationship between the three species was disputed for many years because the phenotypic (physical) evidence was contradictory. Initially, the DNA evidence was not much more helpful. The Woolly mammoth and the Asiatic elephant separated from their common ancestor about 6.7 million years ago, very shortly after its divergence from the line leading to the African elephant, about 7.6 million years ago. Consequently all species' DNA sequences are very similar, and fairly long stretches of DNA (the entire mitochondrial genome) had to be sequenced before the evolutionary relationships could be resolved.
2. Tree C is correct.



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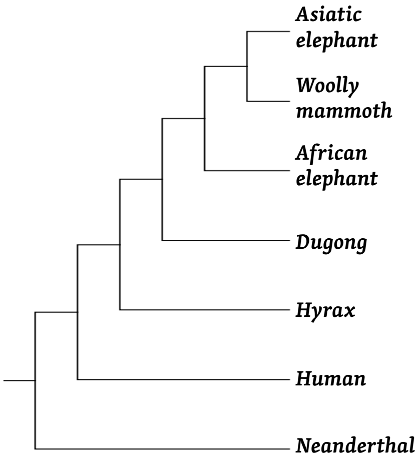
1. The Rock hyrax and Dugong are separate branches of the tree. The Dugong appears to be more closely related to the elephants and mammoth than the Rock hyrax.
2. The tree *does not* indicate how closely related the pair are to modern elephants and to the Woolly mammoth. (As long ago as 1798, it was, however, suspected that Rock hyraxes might be related to elephants, based on skeletal similarities.)
3. If human mtDNA was added to the alignment and used to generate the tree, humans would probably be placed close to the Neanderthal, with the Dugong and Hyrax being shown as more closely related to the elephants (you can check this by using the sample human mtDNA sequence provided with *Geneious* — see the diagram on the right).



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1. Physically, the North American mastodon looks as though it is more closely-related to Woolly mammoths than to modern elephants. The sites from which the mammoth and mastodon DNA samples were recovered (Eastern Siberia and Western Alaska, respectively) could also suggest a close relationship between these two species. Students may therefore be tempted to place the North American mastodon on a branch of the tree close to the Woolly mammoth.

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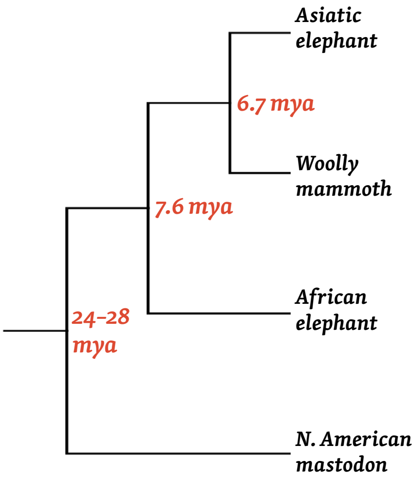
1. Students may group the Woolly mammoth and Mastodon together.
2. The mtDNA evidence suggests that the evolutionary lineage giving rise to the North American mastodon split from the modern elephants and the Woolly mammoth 24–28 million years ago. Therefore the Woolly mammoth is more closely related to modern elephants than it is to the North American mastodon — appearances can be misleading!



Note that the horizontal scales in these diagrams are not proportional to time.