

The Neanderthal in the family

By Ewen Callaway

Thirty years after the study of ancient DNA began, it promises to upend our view of the past.



Before ancient DNA exposed the sexual proclivities of Neanderthals or the ancestry of the first Americans, there was the quagga.

An equine oddity with the head of a zebra and the rump of a donkey, the last quagga (*Equus quagga quagga*) died in 1883. A century later, researchers published¹ around 200 nucleotides sequenced from a 140-year-old piece of quagga muscle. Those scraps of DNA — the first genetic secrets pulled from a long-dead organism — revealed that the quagga was distinct from the mountain zebra (*Equus zebra*).

More significantly, the research showed that from then on, examining fossils would no longer be the only way to probe extinct life. “If the long-term survival of DNA proves to be a general phenomenon,” geneticists Russell Higuchi and Allan Wilson of the University of California, Berkeley, and their colleagues noted in their quagga paper¹, “several fields including palaeontology, evolutionary biology, archaeology and forensic science may benefit.”

At first, progress was fitful. Concerns over the authenticity of ancient-DNA research fuelled schisms in the field and deep scepticism outside it. But this has faded, thanks to laboratory rigour that borders on paranoia and sequencing techniques that help researchers to identify and exclude contaminating modern DNA.

These advances have fostered an ancient-genomics boom. In the past year, researchers have unveiled the two oldest genomes on record: those of a horse that had been buried in Canadian permafrost for around 700,000 years², and of a roughly 400,000-year-old human relative from a Spanish cavern³. A Neanderthal sequence every bit as complete and accurate as a contemporary human genome has been released⁴, as has the genome of a Siberian child connecting Native Americans to Europeans⁵.

Enabling this rush are technological improvements in isolating, sequencing and interpreting the time-ravaged DNA strands in ancient remains such as bones, teeth and hair. Pioneers are obtaining DNA from ever older and more degraded remains, and glean insight about long-dead humans and other creatures. And now ancient DNA is set to move from the clean-rooms of specialists to the labs of archaeologists, population geneticists and others. Thirty years after the quagga led the way, *Nature* looks to the field's future.

A MILLION-YEAR-OLD GENOME

Ludovic Orlando, an evolutionary biologist at the University of Copenhagen, had low expectations when he started sequencing DNA from a 560,000-to-780,000-year-old horse leg bone. His colleague, Eske Willerslev, had discovered the bone buried in the permafrost of the Canadian Yukon in 2003. Then he had chucked it into a freezer, waiting for technological improvements that would allow the bone's degraded

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DNA to be read. (Freezers in ancient-DNA labs brim with such 'wait and see' samples.)

On a Sunday evening in 2010, Willerslev called Orlando to say that the time had come. Orlando was unconvinced: "I started the project with the firm intention of proving that it was not possible," he says.

Sequencing ancient DNA is a battle against time. After an organism dies, the long strands of its DNA fissure into ever shorter pieces, helped along by DNA-munching enzymes. Low temperatures slow this process, but eventually the strands become so short that they contain little information.

To read the horse's genome, Orlando needed to shepherd useful DNA fragments through the harsh enzymatic treatments used to extract them and ready them for sequencing. Orlando and his team found that the preparation lost vast quantities of fragments. But with a few tweaks to the experimental protocol, such as reducing the extraction temperature, the researchers captured ten times more scraps of DNA than before — and produced a draft of the oldest genome on record².

Using a similar approach, Svante Pääbo, a geneticist at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, and his team turned their attention to 400,000-year-old remains from the Sima de los Huesos cavern in northern Spain, which may have been a burial pit for recent relatives of modern humans called hominins (see 'Hidden heritage'). In the pit, the bones remained at stable, low temperatures, slowing the breakdown of DNA. "If you could have told the hominins where to leave their bones, you may have chosen that site," says Matthias Meyer, a molecular biologist at Pääbo's institute who is leading the efforts.

Last December, the team reported³ roughly

16,300 letters of a Sima de los Huesos individual's mitochondrial genome — the DNA from power-generating structures in its cells. The sequence revealed an unexpected relationship between the Sima de los Huesos remains and the Denisovans, an archaic group of humans that Pääbo's team had discovered in Russia's Altai Mountains thousands of kilometres away. Meyer and his colleagues hope to improve their methods enough to obtain some or all of the Sima de los Huesos individual's nuclear genome, the DNA from the nuclei of its cells. "It must be possible," says Meyer. "I won't rest until this has been done."

It is now a matter of when, not if, someone will produce a genome from an Arctic animal buried in permafrost for longer than 1 million years, says Meyer. But he and Pääbo want to push the limits of ancient DNA in hominin specimens from warmer locales, such as fossils of *Homo erectus*, the common ancestor of humans and Neanderthals, found in Asia. And Orlando says that researchers may have luck using new extraction techniques on previously vexing remains such as Egyptian mummies or *Homo floresiensis*, a small hominin at least 18,000 years old that was found in a cave on the Indonesian island of Flores. "It opens a great number of places where there are lots of important stories going on, such as the Middle East or the tropics," he says.

GHOSTS IN THE CODE

A few years ago, David Reich discovered a ghost. Reich, a population geneticist at Harvard Medical School in Boston, Massachusetts, and his team were reconstructing the history of Europe using genomes from modern people, when they found a connection between northern Europeans and Native Americans. They

proposed that a now-extinct population in northern Eurasia had interbred with both the ancestors of Europeans and a Siberian group that later migrated to the Americas⁶. Reich calls such groups ghost populations, because they are identified by the echoes that they leave in genomes — not by bones or ancient DNA.

Ghost populations are the product of statistical models, and as such should be handled with care when genetic data from fossils are lacking, says Carlos Bustamante, a population geneticist at Stanford University in California. "When are we reifying something that's a statistical artefact, versus when are we understanding something that's a true biological event?"

Sometimes these statistical spectres get a body. Last year, Willerslev's team reported⁵ the genome from 24,000-year-old remains dubbed the Mal'ta boy. The results showed that the boy, who had been found in central Siberia, came from a population related to both modern Native Americans and modern Europeans, matching Reich's prediction (see *Nature* <http://doi.org/r2b>; 2013). "It's a spectacular find," he says.

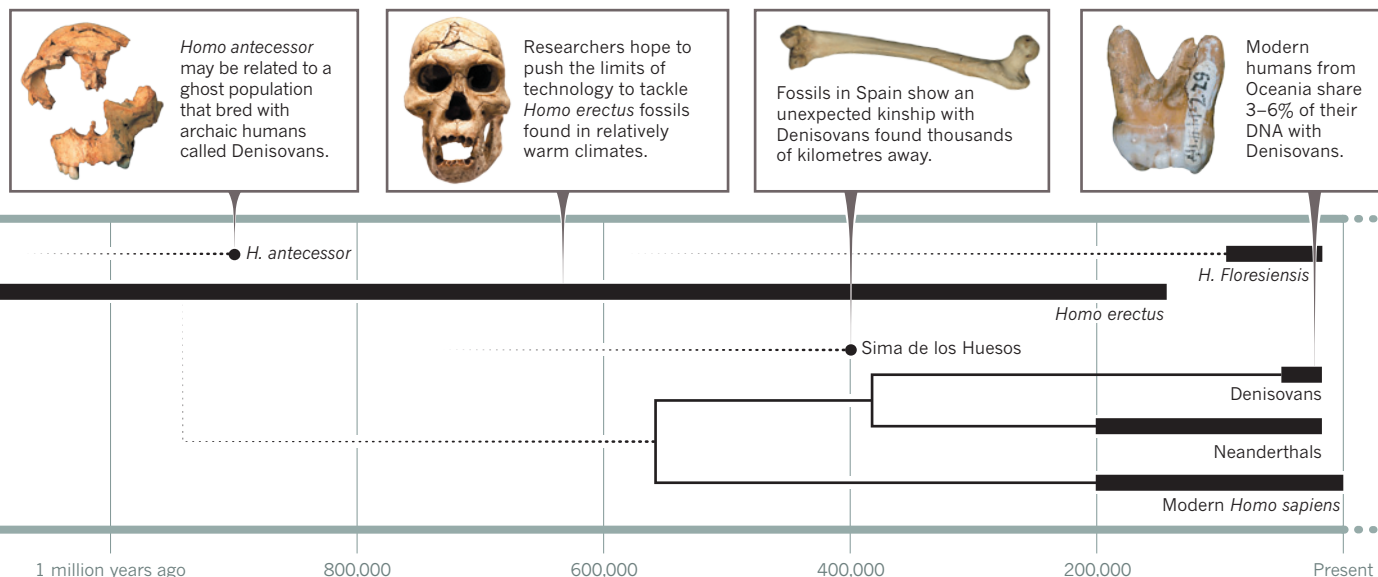
Ghost populations also lurk in ancient DNA. While analysing high-quality genomes of a Neanderthal and a Denisovan, a team led by Reich and Montgomery Slatkin at the University of California, Berkeley, noticed a peculiar pattern: present-day sub-Saharan Africans are more closely related to Neanderthals than they are to Denisovans⁴. But evidence from other ancient genomes suggested that the two archaic groups were equally related to present-day Africans. After weighing the possibilities, the scientists realized that they might have uncovered another ghost population.

The puzzle could be solved, they theorized, if Denisovans had interbred with a species that

PHOTOS (L-R): JAVIER TRUEBA/MSF/SPL; MARKUS SCHIEDER/ALAMY; REF. 3; D. REICH ET AL. NATURE 468, 1053-1060 (2010)

HIDDEN HERITAGE

The study of ancient DNA is revealing connections between archaic humans — and the traces they left behind in modern genomes.



had left Africa perhaps more than 1 million years ago and branched off from the common ancestor of humans, Neanderthals and Denisovans. Subsequent Denisovans would have inherited DNA sequences that present-day Africans lack, explaining why Neanderthals seem to be closer kin to Africans.

Reich's team is analysing genetic signatures in humans with Denisovan DNA to establish when the Denisovans mated with this mystery population — information that could narrow the range of fossils to which it might belong. Genomes studied by Pääbo's lab, principally the Sima de los Huesos remains, may also reveal clues.

Reich is not the only one conjuring ghosts. Chris Stringer, a palaeo-anthropologist at the Natural History Museum in London, has proposed that the 900,000-year-old hominin *Homo antecessor*, known from fossils found near Sima de los Huesos, could be part of the ghost population. If it had interbred with an ancestor of the Denisovans and the Sima de los Huesos hominins, it could explain the relationship between the two groups of remains. Testing that hypothesis would require the elusive Sima de los Huesos nuclear DNA. But Reich is optimistic that Pääbo and his team will pull it off. "They've done miracles before in that lab and they may succeed again."

THE NEANDERTHAL WITHIN

"We don't need bones necessarily to find ancient DNA," says Josh Akey, a population geneticist at the University of Washington in Seattle. "We can find the remnants of ancient DNA floating around in contemporary populations."

If early human populations bred with Neanderthals and Denisovans, their descendants should carry short segments of archaic-human DNA. Researchers such as Akey are beginning to catalogue these segments to learn about the biology of archaic humans. Unlike the hunt for ghost populations, which relies on statistical population models, this approach allows researchers to identify specific regions of the genome acquired by interbreeding.

In January, independent teams led by Akey⁷ and Reich⁸ pieced together a substantial portion — about 20% and 40% respectively — of the Neanderthal genome from bits lurking in the genomes of hundreds of living humans. Their research indicated that some Europeans and Asians had gained genes involved in skin and hair from Neanderthals, possibly helping their ancestors to adapt to cold climates by providing thicker skin, more hair and fewer pores (see *Nature* <http://doi.org/rz9>; 2014). But giant swathes of the modern genomes were devoid of Neanderthal ancestry, hinting that many Neanderthal genes might have been harmful in modern humans. Akey's team identified⁷ one such region around the gene *FOXP2*, which is involved in speech and language. "It's extremely

compelling evidence that there were fitness costs to interbreeding," he says.

These discoveries are only the beginning. The Akey and Reich teams found that the genomes of east Asians possess, on average, slightly more Neanderthal DNA than do people of European

With tools that make sequencing ancient DNA cheaper and easier, the field is becoming more egalitarian.

ancestry. Akey sees this as possible evidence that Neanderthals interbred with ancient humans on at least two separate occasions: once with the ancestors of all Eurasians, and later with a population ancestral only to east Asians. And Akey believes that humans are likely to bear genetic scraps from other extinct species, including some that interbred with the ancestors of humans in sub-Saharan Africa.

ANCIENT DNA FOR THE MASSES

For much of the past 30 years, the sensitivity of the polymerase chain reaction (PCR), the method used to amplify ancient DNA, made it prone to contamination. The field's leaders often greeted the work of outsiders with suspicion, earning some of them the title 'the PCR police'. And in recent years, palaeogenomics has been the domain of specialist labs such as Pääbo's, with the expertise and money to obtain and screen hundreds of fossils to find the few that yield enough DNA to sequence an entire genome.

That is set to change. New procedures mean that researchers can now reliably obtain DNA from all but the most degraded samples, and then sequence only the portions of a genome that they are interested in. "I'm still surprised that there are so few labs in the world that do this," says Johannes Krause, a palaeogeneticist at the University of Tübingen, Germany, who led much of the Denisovan work while in Pääbo's lab. "It's not rocket science."

Gradually, new researchers are entering the field. "If I can break in, then anyone can," jokes Bustamante. His research originally focused on ancestry in current human populations. Then, a few years ago, he got a phone call about a mummy.

An international team had sequenced the genome of Ötzi, a 5,300-year-old frozen corpse found in the Tyrolean Alps of Italy in 1991. The researchers wondered if Bustamante could help them to make sense of the

ice-man's ancestry. Together, they showed that Ötzi was more closely related to humans who now live in Sardinia and Corsica than those in central Europe, evidence that the population of Europe when he was alive looked very different to how it does today⁹.

Bustamante has since plunged into the world of ancient DNA. His team is sequencing samples that chart the arrival of farming in Bulgaria, the transatlantic slave trade in the Americas and dog domestication. The group is developing tools to make sequencing ancient DNA cheaper and easier. "We want to democratize the field," says Bustamante.

Reich, too, sees ancient DNA becoming more egalitarian. His lab's growing interest in areas of human history such as the advent of agriculture or the history of the Indian subcontinent has led it to analyse — often in bulk — remains less rarefied than the scarce Neanderthal samples that first lured him to the field.

Last year, Reich was part of a team that reported¹⁰ an analysis of mitochondrial DNA from 364 European samples between 5,500 and 1,550 years old, to identify major population shifts in Neolithic Europe. Ancient genomics is also set to solve long-standing questions about when and where humans domesticated animals such as dogs, cattle and chickens. A 2013 study¹¹ of 18 mitochondrial genomes from ancient dogs and wolves, for instance, suggested that European hunter-gatherers domesticated wolves from a population that is now extinct.

Researchers are also returning to the questions that launched the field 30 years ago. Around the time that Orlando's team began sequencing the 700,000-year-old horse, it also turned its attention to a much younger sample — from the quagga.

The effort to sequence the full quagga genome is part of large project to understand the evolutionary relationship between living and extinct horses, zebras and donkeys, and to identify the genetic basis for certain traits. "I was thinking it would be cool to do the oldest, but also the first — where ancient DNA started," says Orlando. "It shows the progress the field has made." ■

Ewen Callaway writes for *Nature* in London.

1. Higuchi, R. Bowman, B., Freiberger, M., Ryder, O. A. & Wilson, A. C. *Nature* **312**, 282–284 (1984).
2. Orlando, L. et al. *Nature* **499**, 74–78 (2013).
3. Meyer, M. et al. *Nature* **505**, 403–406 (2014).
4. Prüfer, K. et al. *Nature* **505**, 43–49 (2014).
5. Raghavan, M. et al. *Nature* **505**, 87–91 (2014).
6. Patterson, N. et al. *Genetics* **192**, 1065–1093 (2012).
7. Vernot, B. & Akey, J. M. *Science* **343**, 1017–1021 (2014).
8. Sankararaman, S. et al. *Nature* **507**, 354–357 (2014).
9. Keller, A. et al. *Nature Commun.* **3**, 698 (2012).
10. Brandt, G. et al. *Science* **342**, 257–261 (2013).
11. Thalmann, O. et al. *Science* **342**, 871–874 (2013).