Forensic entomology has different applications from DNA profiling, but can complement it in murder investigations. The former might help identify the suspect, while the latter can be used to identify the time of death based on the developmental stage of, for example, blow fly maggots. Similarly to DNA profiling, forensic entomology is now being applied across an increasing range of cases, including neglect, where it can establish that wounds have not been cared for by analysing the remains of insects in the wound area.

Forensic entomology has different applications from DNA profiling, but can complement it in murder investigations

In turn, forensic applications are having a growing impact on the course of entomological research, according to Mark Benecke, an independent forensic consultant in Cologne, Germany. "There are much improved mathematical models now, and tons of experiments carried out by students that give us much better insight into the diversity of habitats, so that in court we can give much better replies to questions about possible errors in the evidence for example," Benecke said. "Also, in my case, I find the police seem to like the stuff, and there is just a generally heightened interest in sending in entomological evidence from the crime scene, even if it is just a wing."

While DNA profiling largely dominates the headlines, forensic entomology has made some notable achievements too, according to Adriana Oliva from the Laboratorio de Entomología forense, Museo Argentino de Ciencias Naturales, in Buenos Aires, Argentina. "In 1994 there was a resonant case where a conscript was killed and the Army tried to hush the thing up," he said. "My expertise showed that the death had occurred much earlier than they claimed. Public indignation resulting from this case led eventually to the abolition of compulsive military service in Argentina."

Yet, while forensic entomology and DNA profiling can be combined in single investigations, they have radically different skills bases. "Forensic biology and forensic entomology are very different," said Gail Anderson, director of undergraduate programmes at the School

of Criminology at Simon Fraser University in Vancouver, Canada. "In today's forensic world, forensic biology really means DNA, and you just need a BSc honours in molecular biology or biochemistry to be taken on by a lab and then trained there. There are then a good number of jobs, but a million kids who want them. Almost all forensic entomologists, however, are not solely [forensic scientists], but [are] also university professors who do these cases on the side as police consultants. There are presently 16 of us Board certified in North America, including two in Canada, so there are very few jobs there."

Nevertheless, there is a growing sense that forensic skills in general are seeing wider applicability, as they involve the judgement and evaluation of different and conflicting sources of evidence, according to Tomberlin. "We are finding our graduates

can apply forensic skills anywhere from the courtroom to the boardroom," he said. "We teach critical thinking skills and how to evaluate information as presented, which can be applied to making processes more efficient, or developing a product, as well as to solving crimes."

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History in a single hair

Museums are treasure troves of ancient DNA. Modern extraction and sequencing technology is key to putting the secrets of genetic history to use

Howard Wolinsky

When strolling through museums, his own included, Kristofer Helgen, curator of mammals at the Smithsonian's National Museum of Natural History (Washington, DC, USA), said he is often shocked by the state of some specimens. "I see glaring holes or really major pieces sawn out of bones over the last 10 or 15 years by scientists who were trying to extract DNA out of bone," said the mammalogist, who oversees one of the world's largest collections comprising about 600,000 specimens of skins, skulls and skeletons from extinct animals.

However, new sequencing technologies and new techniques for collecting DNA from ancient specimens now mean that far less material is needed—only a few milligrams or less. "As curator, part of my job is to protect our specimens. Another part is making our collection available for research," Helgen said. "With the changes in methods, we're getting more out of less. Although this type of sampling is called 'destructive,' it doesn't

have to be very destructive at all." Destructive or not, the convergence of new sequencing and sampling technologies and the availability of more samples from museums have advanced palaeontology and the emerging field of palaeogenomics. The knowledge of ancient genes is helping researchers to understand the fate of extinct animals and to analyse the function of specific genes and their evolution over time. Some scientists are already speculating about putting together the genome of extinct species as a first step to reviving animals such as the mammoth or the Tasmanian tiger.

One of the biggest breakthroughs in recent years has been the discovery that hair samples, once considered useless in terms of DNA, are in fact a rich source of mitochondrial DNA (mtDNA). Stephan Schuster, Professor of Biochemistry and Molecular Biology at Pennsylvania State University (USA) and Eske Willerslev's group at the Centre of Excellence in GeoGenetics

at the University of Copenhagen (Denmark), realized that the tough keratin surrounding the hair shaft actually protects a significant quantity of mtDNA. “The amount of mitochondria is almost 20-fold over what you find in bone,” said Schuster, a pioneer in research on woolly mammoths from Siberia, and thylacines—Tasmanian tigers—from Australia. “This brought down the cost [of sequencing] by almost a factor of 10, if not more, and then allowed us to do a whole series of mammoth mitochondria.” Schuster coined the term ‘museomics’ for using ancient materials from museums, including hair, fur, hooves and feathers, in cutting-edge DNA research.

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The Museum of Zoology in Saint Petersburg, Russia, provided Schuster and his colleagues with hair from the Adams mammoth—the first complete woolly mammoth skeleton to be discovered—which was recovered from Siberia in 1806 by botanist Michael Adams. Altogether, the Penn State team and Willerslev’s group obtained hair from 10 woolly mammoths from a wide arc across northern Siberia (Figs 1,2). The mtDNA from the hairs showed the existence of two distinct groups of mammoth (Gilbert *et al*, 2007).

Willerslev and his team reported another hairy discovery earlier this year. They sequenced the first complete nuclear genome from the hair of a 4,000-year-old Palaeo-Eskimo—the first people to settle in the New World Arctic (Rasmussen *et al*, 2010). Willerslev, an evolutionary biologist who established the first ancient DNA sequencing facility in Denmark, had been searching for samples of human material in a remote area of Greenland four years ago. Frustrated, he asked a curator at the Natural Museum of Denmark in Copenhagen—only a bicycle ride from his home—if they had anything from which it might be possible to extract DNA. He recalled that the curator would not submit bones to destructive testing, but mentioned that there were some hair samples—possibly tossed by an ancient barber—lying around in a drawer

Fig 1 | The forefoot of the Berezovka mammoth after initial preparation, showing exceptional preservation of the shin and hair. Courtesy of Smithsonian Institution.

in the museum basement. The analysis of the DNA extracted from the hair gave Willerslev a range of phenotypic information about the 4,000-year-old man, including his skin and eye colour, blood type and the consistency of his earwax—dry, like Asians and Native Americans. “If I had known, I would have taken the hair to begin with,” quipped Willerslev.

Museum collections are treasure troves for palaeogenomics. According to Schuster, every specimen used for taxonomic purposes could potentially act as a source for mitochondrial and even nuclear markers. As Helgen commented, it requires only a small amount of hair, such as the small handful used to document the full mitochondrial genomes of two thylacine specimens. “If I showed you one of those specimens here in the Smithsonian, neither you nor I would have any idea where those hairs had been taken,” he said. “It hasn’t caused any damage to the appearance or integrity of the specimen.”

Helgen added that samples sitting for decades or even centuries in museum drawers, cabinets and closets are making it possible

for researchers to understand how animals evolved over time. “All of a sudden every day we’re getting multiple requests from researchers around the world to take hairs or little bits of skin or muscle or tissue or bone [from] specimens in our collection for any number of imaginable studies,” he said.

Ross MacPhee, curator of mammals at the American Museum of Natural History in New York is another palaeontologist looking for DNA samples—though not in museums, but in sediments. Willerslev and MacPhee rewrote history last year when they reported that mammoths and ancient horses lived longer in mainland America than suspected previously—until at least 10,500 years ago (Haile *et al*, 2009). The researchers also concluded that mammoths and horses overlapped with humans for several millennia in a region where humans first entered North America, which challenges theories that megafauna went extinct within centuries of human arrival through rapid overkill. Their findings were based on DNA found in soil samples from locations on the banks of the Yukon River near Stevens Village in Central Alaska.

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Willerslev had already demonstrated that DNA from animals and plants is preserved in ancient sediments, even in the absence of macrofossils (Willerslev *et al*, 2003). “Whilst an animal leaves only a single corpse when it dies, it leaves quantities of DNA traces through urine and faeces (left in the soil) while it is still alive,” he said. “It is these DNA traces which we find in the soil. [...] It’s actually possible now to reconstruct major parts of past ecosystems by going into sediments and obtaining mitochondrial and chloroplast DNA.”

Hanks of hair and pieces of bone and teeth along with ‘crusties’—dried flesh adhering to ancient bones—horns, hooves and nanofossils from sediments have vastly enriched the pool from which researchers can extract ancient DNA. But many experts agree that it was the arrival of next-generation sequencers from 454 Life Sciences (Branford, CT, USA) and Illumina

(San Diego, CA, USA) that has given the field a boost.

Schuster, in fact, bet his career on the new technology. In 2004, he moved his lab to Penn State from the Max Planck Institute for Developmental Biology in Tübingen, Germany. "The 454 changed everything. We started to work with them in late 2004 and then got one of the first instruments in 2005 and the mammoth paper came out in December 2005," he said.

Further development of the technology, Schuster said, has made it possible to validate and compare sequences and to achieve much higher fidelity. However, gaining acceptance of the new technology and persuading journals to publish articles based on it proved to be an uphill battle. Skeptics considered the 454 impractical because it only reads about 100 base pairs. But Schuster said the platform turned out to be ideal for reading ancient DNA, which typically is broken into short fragments.

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"We demonstrated that you could get complete mitochondria from an extinct animal using this technology. We thought this was a huge accomplishment and we tried to publish it," he recalled. "Reviewers rejected our work several times by saying, '\$36,000 cost for extinct mitochondria is too high.' We never thought cost was a factor in peer review. And then we tried to think about how could we bring down the cost." The push to reduce costs led eventually to using hair shafts, which turned out to have a 20-fold greater amount of mtDNA than bone. The latest-generation sequencers are even producing a million-fold more data, which allows scientists to move from mtDNA to nuclear DNA (Miller *et al*, 2008).

In addition to new sequencing platforms, improved methods for extracting DNA from bones and other tissues are also paying off. Adrian Briggs, a researcher at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, and his colleagues published a report in 2009 on a new technique called primer extension capture (PEC), which extracts more information from samples such as poorly

Fig 2 | The Berezovka mammoth cadaver after preliminary excavation, pictured with German–Russian paleontologist Eugene Pfizenmayer and an unidentified member of the expedition. Courtesy of Smithsonian Institution.

preserved Neanderthal bones (Briggs *et al*, 2009). "In a typical sample, only one in 100,000 molecules may be Neanderthal mtDNA, while most of the DNA comes from bacteria, fungi and other organisms that lived in the bone sometime during the past 30,000 years. We have a way now of pulling the targeted molecules out quite efficiently before sequencing, enriching targets," Briggs said.

In late March, his colleague Johannes Krause and an international team reported in *Nature* that they had identified a full mtDNA sequence from a previously unknown hominid, who shared a common ancestor about one million years ago with anatomically modern humans and Neanderthals (Krause *et al*, 2010). The DNA was sequenced from a 30mg sample from a finger bone found in 2008 in the Altai Mountains in southern Siberia. The ground-up bone was converted into an Illumina sequencing library before PEC was applied to isolate mtDNA fragments. "We have recently screened many hominid bones very quickly using this method to find ones with preserved ancient DNA. PEC allowed us to do this much faster and in much higher resolution than previous methods," Briggs commented.

Extracting and sequencing ancient DNA are, however, only the first steps towards understanding the evolution

of extinct animals and hominids. James Noonan of the US Department of Energy's Joint Genome Institute (Walnut Creek, CA, USA) and his colleagues took the next step and reported in 2005 that they had cloned cave bear and Neanderthal DNA molecules in *Escherichia coli*. Along the same lines, in 2008 Andrew Pask and his team, then at the Department of Zoology at the University of Melbourne, Australia, reported the cloning in mice of a gene involved in cartilage formation from the extinct thylacine.

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The extinction of thylacine, a carnivorous marsupial with a striped back, was a relatively modern event. The last known Tasmanian tiger died in captivity in Hobart Zoo in 1936. Pask, now at the University of Connecticut (Storrs, CT, USA) thus described these samples as 'not-so ancient DNA'. "People often refer to this work as ancient DNA, but I don't like to call thylacine stuff ancient because we're really only talking about 100 years or so." The researchers

obtained DNA from more than 100-year-old ethanol-fixed tissues from pouched baby thylacines at the Museum Victoria in Melbourne and found many intact DNA fragments of about 500 base pairs and fewer in the samples.

Pask explained that he is interested in the skeletal development of the thylacine because it is similar to that of dogs. Tasmanian tigers and dogs held similar ecological niches and have similar skeletons, but have not shared a common ancestor for more than 148 million years. "Most people wouldn't be able to tell the difference between a dog skeleton and a thylacine skeleton. It's such a great example of convergent evolution," he said. "The thylacine gives us a really good opportunity to start to look at which genes we think are very important for laying down that body plan."

Exploring the function of ancient genes could yield valuable insights into how animals and our own species evolved over time

Exploring the function of ancient genes could yield valuable insights into how animals and our own species evolved over time. One major step regarding human evolution was the finding that the *FOXP2* gene, a transcription factor, is crucially involved in the development of language; it might well have been just a few mutations in this gene that enabled humans to acquire and use language (Enard *et al*, 2002). Last year, researchers from the evolutionary genetics group at the Leipzig Max Planck Institute for Evolutionary Anthropology reported they had inserted the gene from Neanderthals into mice to further analyse its function.

If ancient genes can be cloned and revived, it should also be possible to reconstruct entire genomes and resurrect extinct animals—and this has already happened. In January 2009, Spanish researchers reported bringing back the Pyrenean ibex, a wild mountain goat that had been declared

extinct in 2000. Researchers took DNA from frozen skin samples to replace genetic material in eggs from domestic goats to clone the ibex. The kid was born alive but soon died from lung problems.

Meanwhile, attempts to clone the thylacine and the woolly mammoth have come and gone. In 1999, Australian researchers launched a project to clone the Tasmanian tiger but dropped it six years later. Frank Howarth, Director of the Australian Museum in Sydney told the Australian Broadcasting Corporation that the quality of the DNA was too poor to work with and that the museum lacked the necessary facilities and skills.

The outlook is more sanguine for the mammoth. Unlike the thylacine, the mammoth has a close living relative, the Asian elephant. Its genome could theoretically be used as a chassis to recreate the mammoth genome and an elephant could also, theoretically, carry a mammoth embryo. However, Schuster thinks that new technologies are needed to put about four million changes between the mammoth and the elephant genome back in place. He expects that reviving the mammoth will occur as "a side product of technology that we will develop for other purposes".

Yet, not everybody is excited about the possibility of bringing back extinct species. "If you can give birth to a mammoth with a mammoth genome, that's really only part of it. All you're getting is the living genome [but] not the living animal," Helgen warned. "So even if it proves possible to bring a mammoth back, it will be a sideshow. It will not be a conservation tool."

Pask agreed, but suggested that the case of the thylacine might be different because humans had a role in its extinction and its habitat still exists. The new sequencing systems would certainly make it possible to get a complete genome. Still, he commented, there are limitations, because it is questionable whether a thylacine cell can be made and the technology is not yet available in marsupials.

Schuster commented that the point of his work is to save species on the brink of extinction rather than bringing back animals that

...reviving the mammoth will occur as "a side product of technology that we will develop for other purposes"

have gone naturally extinct. Robert Millar, a director of the Institute for Breeding Rare and Endangered African Mammals (Utrecht, the Netherlands), agrees and considers any diversion of funds to revive the mammoth "an absolute tragedy". "Bringing back the mammoth is a gimmick because we're living on a planet where the mammoth [suffered] natural extinction," he said, pointing out that currently, "[o]ne in three amphibians is threatened with extinction. One in eight birds is known to be in jeopardy and one in four mammals. Let's look at ways of preserving them."

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