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Ancient DNA: the first three decades

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Introduction

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When Russell Higuchi, Allan Wilson and co-workers reported the molecular cloning of a small fragment of DNA from a piece of dry tissue of a quagga, an extinct member of the horse family [1], they could hardly imagine that 30 years later several hundred scientists would meet at The Royal Society in London[1] to mark the anniversary of the event, and review the astonishing progress of the field of old DNA studies, including the sequencing of whole genomes of extinct megafauna and of our human ancestors. As Alec Jeffreys pointed out in a *News and Views* written to accompany the 1984 quagga article, his own attempts to recover DNA from a long-dead animal, in his case from preserved mammoth tissue, had not been hopeful: most of the DNA was from recent microbial contamination, and original, elephant-like sequences were present in tiny amounts, highly degraded and altered by post-mortem modifications. While Jeffreys admitted it was too early to give up trying, he argued that the prospects of combining molecular biology and palaeontology 'into a grand evolutionary synthesis by studying fossil DNA, still look like nothing more than a glorious dream' [2].

Despite Jeffrey's caution and the technical difficulties of ancient DNA studies (as studies on old DNA became known), the huge range of potential applications of ancient DNA research in many fields, including archaeology, anthropology, evolutionary biology and conservation, combined with developments in molecular biology in the mid-1980s, including the polymerase chain reaction (PCR) [3], novel molecular loci such as mitochondrial DNA (mtDNA) [4,5] and advances in forensic identification by Jeffreys himself [6,7] inspired a steady stream of ancient DNA research projects in the subsequent decade. The 1984 quagga article was followed shortly afterwards by a report of the detection of human DNA in an extract of muscle from a pre-Dynastic Egyptian mummy using DNA hybridization [8]. PCR soon became the technique of choice in ancient DNA studies, and was applied to the study of ancient human brain tissue [9], maize remains [10], human archaeological bones [11], dry skins of the extinct marsupial wolf and kangaroo rats [12,13], New Zealand moas [14] and fossilized remains of plants and insects aged millions of years [15,16]. By the early 1990s, the field had grown rapidly, and in July 1991, over 60 researchers met in Nottingham, UK, for the first international meeting on ancient DNA, while a newsletter devoted to ancient DNA research was launched to help researchers share information.

The developments in ancient DNA research were not uncontested. Some scientists argued that the spontaneous depurination of DNA after death would limit the survival of informative DNA sequences to a few millennia [17]. While this proved not to be the case, and there are now numerous instances of DNA survival in Pleistocene remains recovered from cold regions, most notably the recent sequencing of genomic DNA from a 700 000 year old horse [18], it is true that studies of so-called antediluvian DNA sequences from plant and animal fossils and amber were shown eventually to be unrepeatable and irreproducible [19].

The demonstration that DNA could be recovered from bones [11,20] meant that researchers were no longer limited to scarce soft-tissue remains, and bone DNA typing had almost immediate applications in human forensic identification [21–23], as well as opening the door to genetic studies on past human populations, such as Pacific Islanders and American Indians [24,25]. Unfortunately, human

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DNA studies became the most contentious area of ancient DNA research, owing to the difficulties in discriminating authentic ancient DNA sequences from recent contamination [26–28], and many researchers concluded that human remains would not be amenable to genetic investigations. During the middle and later part of the 1990s, researchers turned their attention to other, presumed more straightforward or informative ancient DNA studies, including nuclear sequences of plants [29], and mitochondrial and nuclear DNA sequences of extinct fauna [30–33]. To a large extent, these efforts were driven by the availability of well-preserved remains, for example megafauna preserved in permafrost, and the desire to broach phylogenetic and evolutionary questions without the formidable obstacles faced by human ancient DNA studies.

The second decade of ancient DNA research was marked by technical innovations in methods of DNA extraction, and the analysis of DNA from a variety of remains, including coprolites [34], and diverse genera, such as extinct ground sloths, [32,35], Ice Age brown bears [36], elephantids [37,38], New Zealand moas [39] and many others. Ancient DNA applications in archaeology remained scarce, whereas geneticists and forensic scientists continued to develop and apply novel genetics systems and techniques, suited to both human evolutionary studies and forensic science, including autosomal short tandem repeats (STRs), Y chromosome biallelic markers and short tandem repeats (Y-STRs), and the sexually dimorphic amelogenin gene [40–44].

One of the most important achievements of the late 1990s was the sequencing of DNA from the Neanderthal-type specimen by Svante Pääbo and co-workers [45]. Owing to the scientific and public importance of the project, the work was performed with extreme safeguards against contamination, numerous controls and in two different laboratories. The study helped formulate a series of standards for ancient DNA studies, later expanded to include several specific criteria of authenticity [46,47]. While the standards were a practical reaction against a number of erroneous studies in preceding years, they also had negative consequences, as they discouraged some researchers from embarking on or publishing ancient DNA studies and stifled open discussion, with the result that human ancient DNA studies lost prominence, or at times were even discredited. Fortunately, the huge interest in human evolution stimulated additional studies on hominid remains [48–50], and led eventually to a resurgence of research on ancient human populations. With their 2005 study on Neolithic human samples, Haak *et al.* [51] reminded sceptics that population studies on ancient human populations were indeed possible. It is important to remember, however, that the bulk of these studies involved the PCR amplification of a small section, typically a few hundred base pairs in length, of multi-copy mtDNA, and in very few cases Y chromosome polymorphisms or autosomal STRs [52,53]. It would require a technological paradigm shift before ancient DNA studies came of age.

Developments in sequencing technology opened the floodgates to new studies. Shotgun sequencing was used to recover 27 kilobases (kb) of nuclear DNA from a cave bear fossil [54], a finding immediately superseded by the introduction of next-generation sequencing techniques [55] and the first application of these techniques to the large-scale sequencing of mammoth DNA [56]. This was accompanied by the publication of complete mitochondrial genomes of mammoths [57,58] and the first analysis of a functional gene of an extinct species, also in mammoths [59]. These results were dwarfed a few years later by the publication of the first genome sequence of a long-dead human, a 4000 year old palaeo-Eskimo, recovered from a bunch of hair kept at a museum [60] and a draft sequence of the Neanderthal genome [61]. Since then, ancient DNA findings have been reported at increasingly short intervals. At the time of writing, complete or partial genomes have been generated for several anatomically modern humans, starting out from so-called ancient samples, to shed light on the expansions and migrations of humans in different parts of the world [62,63], and several archaic humans, including the Denisovan, the first archaic human group to be identified by its genome sequence [64]. Moreover, the time depth of ancient DNA analyses has long surpassed the limit of 100 000 years imagined by the research community, to an astounding 700 000 years for bones recovered from permafrost [18] and about 400 000 years for bones in regions outside permafrost [65,66].

This *Theme Issue* contains a cross section of current research in ancient DNA, three decades after the birth of the field. After bitter debates about the feasibility or even desirability of studies on ancient human populations, researchers have gained renewed confidence, maybe simply as a result of the large interest in human evolutionary history. While ancient DNA techniques will sometimes provide limited results owing to the poor preservation of human skeletons in warm and humid climates, even short fragments of mtDNA are able to shed light on the origins of populations such as the Caribs of the Antilles [67]. Short fragments of mtDNA, combined with Y chromosome data, and information based on stable isotopes and archaeological context can help identify past individuals, as in the graves of Mongol nobles [68]. Today, individual identification and paternity testing in forensic practice are carried out using commercial multiplex kits that amplify 16–24 autosomal STR markers, including amelogenin for sex identification, in a single reaction. The increased sensitivity of these kits has allowed old forensic cases to be solved using small amounts of stored biological material, but can also be applied to the study of the social organization of poorly understood populations such as the Yakuts [69].

The advent of next-generation sequencing has helped researchers develop and apply new methods for authenticating ancient human DNA sequences, and these techniques have been applied to the study of the Neolithic transition in Scandinavia [70] and the migrations of Norwegian Vikings [71]: and while next-generation sequencing promises to revolutionize the genetic study of the Neanderthals and other potential human ancestors, it is important to remember that painstaking analyses based on PCR and conventional DNA sequencing paved the road for these exciting studies [72]. Human history is tightly associated with the history of the pathogens that plagued us through the ages, and new ancient DNA studies on pathogen DNA promise to help us understand the evolution of diseases that were important in the past and are threatening to resurface owing to antibiotic resistance, increased urbanization, and efficient air travel. The DNA of these pathogens, including those for tuberculosis, leprosy and plague, may survive for centuries and be amenable to analysis [73,74]. But other, less deadly pathogens have also colonized our bodies through history: dental calculus contains a huge number of different bacterial species, and

next-generation sequencing is an ideal tool to reveal the taxonomic structure of this and other human microbiomes, and reveal changes attributed to diet or disease [75].

The development of human societies is closely tied with the fates of the animals and plants that served them as food. Ancient DNA researchers have used DNA sequences from domestic animals [76,77] and plants [78] either as a proxy for the study of human evolution, or as a way to understand the processes involved in domestication, and have identified curious sources of ancient tissue, such as parchment from old manuscripts, as a source of DNA from historical livestock [79].

Palaeogenomics will continue to develop at an accelerated rate and has many applications in the study of extinct animals, human evolution and palaeopathology, as reviewed by Orlando and co-workers [80]. The techniques will be ideally suited to population studies on species in parts of the world, including Antarctica, where low temperatures permit the excellent preservation of abundant biological remains [81]. Conversely, new molecular techniques will make population studies on rare and valuable human samples much easier by permitting the initial screening of large numbers of bone samples, and help save time and effort by aiding the selection of samples that contain usable DNA [82]. Palaeogenomics also has an important role to play in the study of past environments, including climate change and species extinctions, but the research is fraught with difficulties. Nevertheless, environmental samples, such as sediments, ice and water, can help reveal changes in the distribution of animal and plant taxa through space and time [83,84].

For much of its history, ancient DNA research was driven first and foremost by what was technically achievable, and by the need to avoid contamination by modern DNA. The progress in ancient DNA has been marked by mileposts such as the first extinct animal's DNA, the first human DNA, the first bone DNA, the oldest DNA, the first Neanderthal sequence, the first ancient mitochondrial genome, the first draft of an ancient genome. In the past, a large number of ancient DNA studies were either purely technical, or one-off historical puzzles but, as we can see from the contributions to this *Theme Issue*, this is no longer the case, and ancient DNA researchers are now addressing a growing number of important scientific questions.

Ancient DNA is an exciting and fascinating subject, and offers huge possibilities for scientific research. Ancient DNA techniques were instrumental in the development of important areas of forensic identification and species conservation. We now have at our disposal molecular and bioinformatics techniques that were undreamt of three decades ago, and can look forward to the maturity of ancient DNA research and new applications in archaeology, evolution, climate research and many other areas.

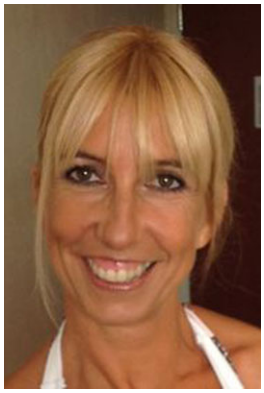
Endnote

[1]'Ancient DNA: the first three decades', The Royal Society, London, 18–19 November 2013. Followed by 'Ancient DNA applications in human evolutionary history', The Royal Society, Chicheley Hall, 20-21 November 2013.

Guest editor biographies

Erika Hagelberg has a PhD in biochemistry from Cambridge University, and a master in history and philosophy of science from University College London. From 1987 to 1992, she worked at the Institute of Molecular Medicine, University of Oxford, on the analysis of DNA from ancient bone. While in Oxford, she pioneered the earliest applications of bone DNA typing in forensic identification and human evolutionary history. After holding teaching positions at Cambridge University and the University of Otago, New Zealand, in 2002 she was appointed professor of evolutionary biology at the University of Oslo, Norway. Her main research interests are in ancient DNA, the human settlement of the Pacific and the history of genetics.

Michael Hofreiter studied biology in Munich, then moved to the Max Plank Institute for Evolutionary Anthropology in Leipzig, completing his PhD and a post doc working on various aspects of ancient DNA including population analyses of Pleistocene species, DNA extraction methods and palaeogenomics. From 2005 he ran an independent research group at the MPI looking at mammoth and mastodon phylogenetics, functional ancient DNA analyses, and adapting Next Generation Sequencing for work with ancient DNA and multiple samples. From 2009 to 2014 he was Professor of Evolutionary Biology and Ecology at the University of York, and is now Professor for Evolutionary Adaptive Genomics at the University of Potsdam.



Christine Keyser is currently Full Professor at the University of Strasbourg (France). She received her PhD in Molecular and Cellular Biology and her Habilitation in Genetics from the University of Strasbourg, and has since taught as an Assistant Professor from 2003 to 2011. In 2001, she was appointed legal expert by the Court of Appeal of Colmar and until recently she worked as forensic DNA expert for the French Ministry of Justice. She has both teaching and research duties, mainly in the fields of population genetics, forensic genetics, and molecular anthropology. Her research, conducted within the Institute of Legal Medicine of Strasbourg (AMIS laboratory), has been pioneering in the use of molecular techniques applied to forensic human identification for the study of ancient human remains.

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