

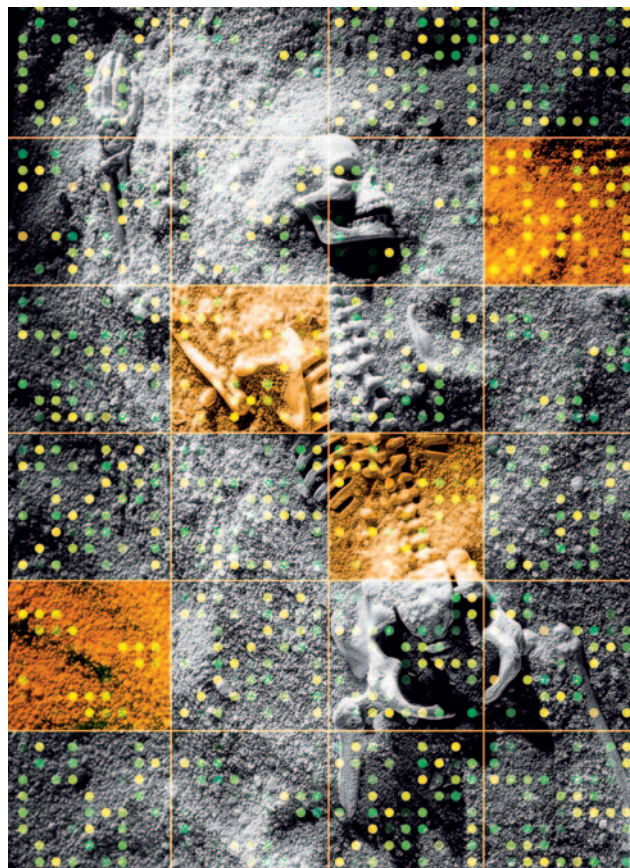
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Biomolecular archaeology provides new insights into past civilizations, cultures and practices

Forensic science has made rapid progress owing to new and improved methods for uncovering and analysing even the tiniest traces of DNA (Hunter, 2006; Benecke, 2002). In addition to helping criminal investigators, these advances in biological, physical and chemical analysis have proven useful to their colleagues in archaeology. In particular, the related field of biomolecular archaeology has benefited from recent interdisciplinary research involving chemistry, bioinformatics, biomechanics, immunological assaying, mass spectrometry and other disciplines, leading to new insights into the history of agriculture, human diseases and civilizations over the past 10,000 years.

Biomolecular archaeology is the study of ancient DNA, recovered primarily from fossilized bones and teeth. These continue to provide valuable information, but new analytical techniques make it possible to extract DNA from other sources, such as the remains of plants, animals, domestic waste and faeces. Other methods adapted from protein biochemistry, immunology or analytical chemistry now allow scientists to investigate the fate of individual human settlements and shed new light on global events such as the history of migrations, civilizations and human biology. "Most of the big questions in archaeology, such as chronology, migration, domestication and urbanization, have all been enhanced by biomolecular investigation," said Matthew Collins, head of BioArch, a joint initiative between the departments of biology, archaeology and chemistry at the University of York, UK.

Biomolecular research has been particularly helpful in investigating how the invention of agriculture affected human populations, health and disease. About 10,000 years ago, humans in



Mesopotamia—what is now Iraq and southern Turkey—first started to domesticate animals and plant food crops, which triggered one of the greatest revolutions in human history. There was never any doubt that the change from a foraging, hunter-gatherer diet to one based on crops and livestock would have profound consequences, but it was generally assumed that the effect on health had been relatively benign—agriculture had, after all, created the conditions for increased population density by sustaining more people using a given area of land. "Traditionally, most have thought that the transition from foraging to farming marked an improvement in the condition of life," said Clark Spencer Larsen, Chair of the Department of Anthropology at Ohio State University (Columbus, OH, USA); however, "studies

by bioarchaeologists have shown that it was definitely a mixed bag."

Larsen went further and described the agricultural revolution as an environmental catastrophe that caused a decline in the quality of life for most human populations (Larsen, 2003). Ironically, similar sentiments have been expressed about the industrial revolution, suggesting that all great changes in human history might initially decrease the quality of human health and life. Without a doubt, the agricultural revolution had both a direct and indirect effect on human health. In most cases, it imposed a more restricted diet by replacing a varied mixture of meat or fish, fruits and vegetables with as few as one or two staple crops. The resulting shortages of key nutrients, and in some cases protein, led to malnutrition. However, in some situations and locations, agriculture actually improved the diet—mostly in marginal areas where naturally occurring food sources were

scarce or only seasonally available—by providing a more reliable source of food.

Work by Collins and colleagues, based on immunological methods to detect protein residues on pottery, established that dairy farming had been surprisingly well developed in the relatively harsh environment of the Outer Hebrides in Scotland during the Iron Age, around 2,500 years ago (Craig *et al*, 2000). It converted inedible fodder, which could be preserved during the winter, into milk as a source of year-round nutrition with beneficial effects on health.

But agriculture also affected human health indirectly. As it facilitated an increase in population densities, it created optimal conditions for the emergence and spread of infectious diseases, perhaps for the first time in human history. Diseases of

biblical fame, such as leprosy, have been tracked in archaeological studies of skeletons, combined in some cases with the sequencing of viral or bacterial DNA obtained from the same sources. Leprosy is now confined to relatively few tropical areas in Africa, Asia and South America, but during medieval times it was endemic in Europe as far north as Scandinavia, in any area with a combination of poor sanitation and overcrowding. Studies of skeletal remains confirmed that the disease originated in the Old World, from where it spread following human migrations (Monot *et al.*, 2005). Several other infectious diseases, including tuberculosis and treponematosi—a form of endemic, non-venereal syphilis—have also been studied using the analysis of DNA sequences of living viruses and bacteria. By correlating these with ancient DNA from bones and teeth, bioarchaeologists have found more evidence that these diseases arose, or at least became more prevalent, around the time agriculture was developed.

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If agriculture was generally bad for human health, conquests and invasions have had an even more dramatic impact on populations, particularly Native American tribes during the era of colonization by Europeans. Written records confirm that many Native American populations were decimated or eliminated by various epidemics in the wake of Christopher Columbus's landing in the Caribbean Islands in 1492. Larsen applied biomolecular archaeology to fill in the gaps of this historical record, which revealed a more complex picture in which disease, combined with changes in diet and lifestyle, ripped apart long-established communities and led to population collapse (Larsen, 2000). In his large-scale study, Larsen was one of the first to apply certain new techniques, such as measuring the ratios of carbon and nitrogen isotopes in skeletons found beneath the floor of missionary churches close to the coast of northern Florida and up into Georgia. He confirmed a dramatic

change in the local diet from a heterogeneous one of seafood and various plants and animals, to one based largely on corn. Apparently, agriculture arrived with a big bang, and its deleterious consequences were amplified further by the diseases imported from Europe.

Biomolecular archaeology is also shedding light on cultural and intellectual aspects of past civilizations, in some cases revising or refining prevailing views that were based on written records and older techniques. For example, renewed analysis of the embalming techniques used in ancient Egypt revealed that this first human civilization was even more scientifically advanced than was previously thought. As Stephen Buckley, who leads the Yemeni Mummy project at York University, pointed out, the ability to preserve bodies after death was the focal point of Egyptian scientific research and resources. "It is important to realize that the need for a well-preserved body for the spirit to recognize was crucial—as they saw it—if they were to pass successfully into the afterlife," said Buckley. "My work has shown that a much more systematic approach...was applied by the embalmers in many cases—the term 'embalmer's art' was in fact much more scientific and sophisticated than we believed. This has implications for our understanding of ancient Egyptian technology. Their abilities have been downplayed."

Buckley's insights into mummification were made possible by various new techniques, such as pyrolysis-gas chromatography combined with mass spectrometry. This method decomposes samples by heating them in an inert gas such as helium, then separates the fragments in a gas chromatograph and finally detects them by mass spectrometry. The advantage is that it works with very small samples of less than 0.1 mg, which can be extracted readily from a mummy. "It is less destructive than the so-called 'non-destructive' techniques of X-raying and [computed tomography] scanning," noted Buckley. "The technique allows the convenient study of both free and bound/polymeric material which can be expected to be present when looking at many of these plant-derived products." In fact, it was this combination of molecular biology and analytical chemistry that enabled archaeologists to identify a number of complex amorphous organic residues and improve their understanding of the embalmer's art.

The techniques used by Buckley were originally developed for more technical applications in protein biochemistry or polymer science rather than archaeology. However, biomolecular archaeology presents unique challenges that are in some respects more demanding than those of other fields. "Arguably, biomolecular archaeology should be on the leading edge of technique development, as the materials are some of the most complex to analyse—mixed, degraded, and at low concentration," Collins said.

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Collins and his team have been working on methods to date archaeological samples based on the rate of protein degradation (Parfitt *et al.*, 2005). As well as using mass spectrometry, proteins can be detected by using immunological methods, and Collins and colleagues have developed a digestion-and-capture immunoassay to detect milk proteins on ceramic pots, which led to their insights into dairy farming in western Scotland.

When it comes to tracing the migration and origin of people, human DNA still gives some of the best clues, although researchers also examine viruses and bacteria that have travelled with their human hosts (Rinaldi, 2007); sources such as bones or teeth restrict analyses to those locations where they can be found. Fortunately, ancient human DNA is not confined to archaeological sites, but occurs far more widely in soils and sediments, thus lending itself to archaeological investigations. By using such samples, a team led by Eske Willerslev, Director of the Centre for Ancient Genetics at the University of Copenhagen, Denmark, sought to determine when humans first arrived in North America from across the Barents Sea. This seems to have occurred slightly earlier than originally believed, after the last ice age about 14,000 years ago. The precise source of human DNA in the soil is usually unknown—according to Willerslev it could have come from intestinal cells in disintegrated faeces, skin cells from footprints or fragments of hair.

Regardless of its source, the main problem with any ancient DNA is contamination with more recent DNA of similar or related origin. Willerslev's team therefore applied a quality control in which soil was spiked with known strains of bacteria before samples were taken. The bacterial DNA can usually be found within the top 1–2 cm of the sample. "We also test the inner part of the sample, from which we do the DNA extraction [for ancient DNA], to test whether there has been any entrance [of bacteria] from the surface to the interior during sampling," said Willerslev. If no DNA from the bacteria used in the quality control is found in this inner part, the researchers conclude that contamination has not occurred during the sampling process. DNA extraction and amplification techniques are then used to locate and sequence the human DNA.

Soil also contains DNA from other organisms that can be used to establish whether the human DNA really was present originally, or leached in from adjacent soil layers. "We can look for animal groups that you know should or shouldn't have been present at the time," said Willerslev. Non-human DNA can also provide information about diet or other aspects of human life at that time, but there is a practical limit to the level of analysis that can be performed. Archaeological sites are usually teeming with potential sources of information at the molecular level, but in practice there are never the resources to analyse more than a small proportion of them, commented Tom

Gilbert, a researcher in ancient DNA and evolution at the University of Copenhagen. "There is always something that can be done, but what is actually done should be based on whether it will provide any useful information and whether the sample is actually preserved well enough to do anything reliable with it," he said.

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Biomolecular archaeologists are also constrained by the circumstances of the locations in which they work. In many cases, the site is unearthed during building development and there is a limited time to perform the analysis. Furthermore, archaeologists often do not consider preserving specimens for subsequent 'post-excavation' analysis, according to Collins, therefore missing out on the opportunity to exploit new techniques in the future.

Despite the progress made with these new tools and technologies, many more questions about human history and the rise of early civilizations remain. Furthermore, these new techniques might produce data that challenges current assumptions about our ancestors, re-opening old debates. For example, the prevailing belief that infectious diseases evolved only during the Neolithic Age might well prove to

be over-simplistic, according to Linda Van Blerkom, Professor of Anthropology at Drew University (Madison, NJ, USA). "While it's true that infectious disease incidence and severity might have increased then, the disease agents themselves appear to be older and in many cases evolved in humans and were then transmitted to animals," she said. Indeed, more studies—not limited to ancient DNA—are needed to unravel the origins of such diseases. Whatever the questions, archaeologists—with the help of their colleagues from biology, physics and chemistry—are now finding more answers, thanks to new and more sensitive diagnostic techniques.

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