UNEARTHING THE PAST

Molecular analysis of ancient tissue

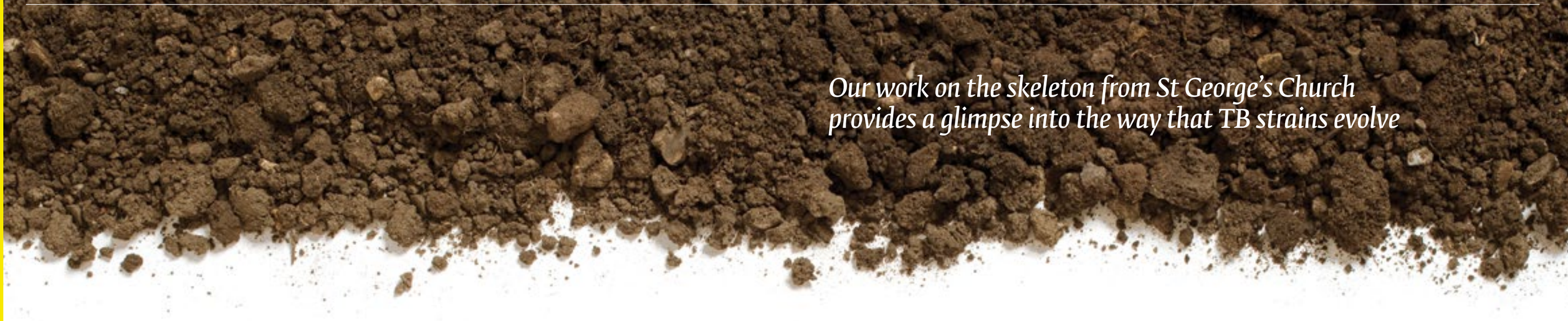
From ancient texts and historic documents, medical historians have uncovered a wealth of information about the history of disease. Now these written records are being supplemented by powerful new methods for studying the impact that diseases have had on people and populations in the past. One of these new methods is ancient DNA analysis, writes Professor of Biomolecular Archaeology **Terry Brown**.

The term "ancient DNA" describes the small amounts of DNA that are sometimes preserved in skeletons, mummies and other dead biological remains. The public has had a longstanding fascination with ancient DNA, beginning 25 years ago when *Jurassic Park* first hit the big screen. Although DNA from dinosaurs is science fiction rather than science fact, ancient DNA has remained in the headlines thanks to real-life projects such as the sequencing of the Neanderthal genome and the identification of the bones of Richard III.

A lesser known, but equally exciting, aspect of ancient DNA is its use to study past diseases. As well as human DNA, some skeletons also contain traces of DNA from infectious bacteria, including ones that quite possibly were the cause of death. By typing these DNA traces we can identify a disease, and by sequencing the DNA we can compare the bacteria with modern strains and start to ask how the disease has evolved over time.

Biomolecular palaeopathology

The use of ancient DNA to study past disease is called biomolecular palaeopathology. In conventional palaeopathology, the skeleton is examined for morphological changes, called lesions, that are indicative of particular diseases. Tuberculosis (TB) is an example of a disease that can be identified, at least tentatively, in this way. TB is mainly a pulmonary disease but in some patients the TB bacilli enter the bloodstream and lymphatic system and spread to the skeleton, where they can cause destruction or remodelling of bony structures. Destruction of parts of the lower thoracic and/or lumbar vertebrae can result in curvature of the spine (kyphosis), and breakdown of the bone surfaces at the hips and knees can also occur. As well as these changes caused by the systemic disease, pulmonary TB can give rise to abnormal bone formation on the inside surfaces of the ribs. These changes can be observed in archaeological skeletons, but none are diagnostic of TB: the changes to the spine can also be



Our work on the skeleton from St George's Church provides a glimpse into the way that TB strains evolve

caused by brucellosis, fungal infections and some types of arthritis, and arthritis can also cause breakdown at the joints. The rib changes are even less diagnostic, as these can be caused by many different pulmonary diseases.

The ambiguity of the palaeopathological indicators of TB leads to the first and most straightforward application of ancient DNA. Clinicians have devised various PCR tests for TB in tissue taken from living patients, these tests directed at genetic loci that are present only in the *Mycobacterium* species that cause the disease. Often two repetitive DNA elements called IS6110 and IS1081 are targeted. One or both of these elements are present in members of the *M. tuberculosis* complex, so a positive PCR signal indicates the presence of TB. Although developed for clinical testing, the same PCRs are equally able to detect TB in samples taken from archaeological bones. There are complications, the main one being that many non-pathogenic *Mycobacterium* species inhabit the soil, and these species can invade buried bones and leave their own DNA traces in the skeleton. Recent work indicates that sequences similar to IS6110 are present in some of these environmental *Mycobacterium* species, leading to possible false-positive detections. But false-positives are almost certainly rare, and reliable detections of TB have been achieved by PCR typing with archaeological bones dating back hundreds of years. PCR typing has also been used to show TB presence in mummified remains.

A rib from the St George's Crypt skeleton. The abnormal bone formation is possibly indicative of TB

Late Victorian TB

In my own laboratory, we used PCR typing to detect the presence of TB in the skeleton of an adolescent female who died in Leeds in the late 19th century and was buried in the crypt of St George's Church. In 2009 the skeletons in the crypt were removed by archaeologists, prior to new building work that was taking place at the church. Before their reburial at a nearby cemetery, the skeletons were examined for signs of disease. The rib lesions sometimes caused by TB were seen on the skeleton of this particular adolescent. We were given permission to take samples from the ribs and these tested positive with the TB PCRs, suggesting that the unfortunate person either succumbed to TB or was suffering from advanced TB when she died of some other cause.

With this particular skeleton we also attempted a more ambitious study. As well as the PCR tests, we used "next generation" DNA sequencing to obtain much more information on the genome of the bacterium. Then, by comparing our DNA sequence with the sequences of strains of *M. tuberculosis* known today, we constructed a phylogenetic tree showing how the late 19th century strain was related to modern varieties. We found the closest similarity with a modern strain called H37, which is quite uncommon in modern TB patients.

This does not mean that the Leeds skeleton had contracted a rare form of TB, because we know that TB strains change over time, both globally and at a local scale, as the bacterium evolves (in part to evade human attempts to eradicate it) and as strains move from one region to another. Interestingly, the original isolate of H37 was collected in 1905 from a patient at the Trudeau Sanatorium in upstate New York. The presence of H37 in New York in 1905 is suggestive, bearing in mind our detection of a very similar strain in Leeds at about the same time. Possibly this strain was relatively common in Europe and North America during the late 19th and early 20th centuries, before being replaced in subsequent years by the strains that are prevalent today.

Evolution of TB

Our work on the skeleton from St George's Church provides a small, tantalising glimpse into the way that TB strains evolve over time. More work is needed, with many more archaeological specimens, to build up a better picture of the way in which the disease has changed over time, and of the factors that have influenced such changes. This work is progressing in research labs around the world, with two fascinating recent outcomes.

The origins of human TB have been debated for many years, a key factor being the similarity between human TB and the bovine version of the disease, which affects a number of different animals but is most closely associated with cattle. One popular theory is that humans first became infected with TB when cattle were domesticated some 10,000 years ago. Farming brought humans into close contact with cows – possibly they shared living quarters – providing the opportunity for the bovine disease to jump to humans. Several diseases that require close contact between individuals in order to spread are thought to have originated in a similar way. However, transmission from cattle now seems less likely, at least for TB. This is because comparisons between *M. tuberculosis* DNA from human skeletons from different age periods have suggested that the disease we recognise today originated much more recently than the beginning of farming, perhaps not until 2,000 years ago. This work still requires confirmation, but if correct it raises intriguing new puzzles about the evolutionary relationships between the types of TB present in humans and other animals.

The final and exciting part of this story concerns the origin of the TB present in the New World before the Europeans arrived. The Native American populations were devastated by diseases brought from Europe by the first colonists. The indigenous groups had little natural resistance to these diseases, incuding TB. Originally it was thought that TB must have been absent from pre-Columbian America, but this view was questioned by archaeological studies that uncovered skeletons with osteological signs of TB from well before the arrival of Europeans. Ancient DNA typing with some of these skeletons has resulted in the remarkable discovery that the bacteria responsible for the ancient American TB are not closely related to modern human *M. tuberculosis*, but are more similar to *M. pinnipedii*, which causes the disease in pinnipeds – animals such as seals, walruses and sea lions.

There are cases of zoo workers catching TB from sea lions, so the transfer of the disease from pinnipeds to humans is not unprecedented, but the idea that TB might have originated in the Americas in this way was unexpected. Like much research, the discovery raises more questions than it answers (did the seals bring TB from the Old World?), but through ancient DNA studies we are likely, in future, to learn much more about this topic and about the history of TB in general. BMS

Ancient American TB is not closely related to modern human TB

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