The Black Death Decoded

The genome of a *660-year-old* bacterium is revealing secrets from one of Europe's darkest chapters.

By Ewen Callaway

As word of a brutal pestilence raging across Europe reached London, its residents started digging. In 1348, Ralph Stratford, Bishop of London, dedicated acres of land that had been purchased to bury the legions of Black Death victims who would overwhelm existing churchyard cemeteries. Within two years, one-third to one-half of the city's 40,000–100,000 residents succumbed, and many thousands were buried in two newly dug cemeteries at East and West Smithfield. At the height of the scourge, 200 bodies were interred each day.

East Smithfield, originally called the Churchyard of the Holy Trinity, is one of a handful of burial sites known to have been used only during the Black Death. In the 1980s, excavation of this 'plague pit' turned up nearly a third of the 2,400 bodies estimated to be buried there, some piled five deep. Despite the urgency of the time, the bodies were placed purposefully, oriented east to west, some with charcoal, possibly to absorb the fluids released during putrefaction, and many with coins and trinkets of their former lives. Such foresight not only helped keep corpses from piling up in the streets, but also, it seems, afforded some Black Death victims a dignified Christian burial. Six-and-a-half centuries later, it would also give scientists the opportunity to dissect the disease that laid waste to Europe (see 'Death on the march').

This month, geneticists reported that they have reconstructed the genome of *Yersinia pestis*, the bacterium that causes bubonic plague , recovered from remains at East Smithfield[1].

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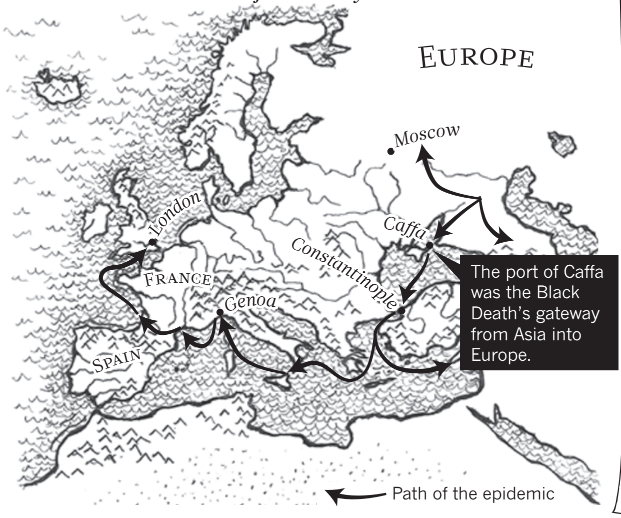
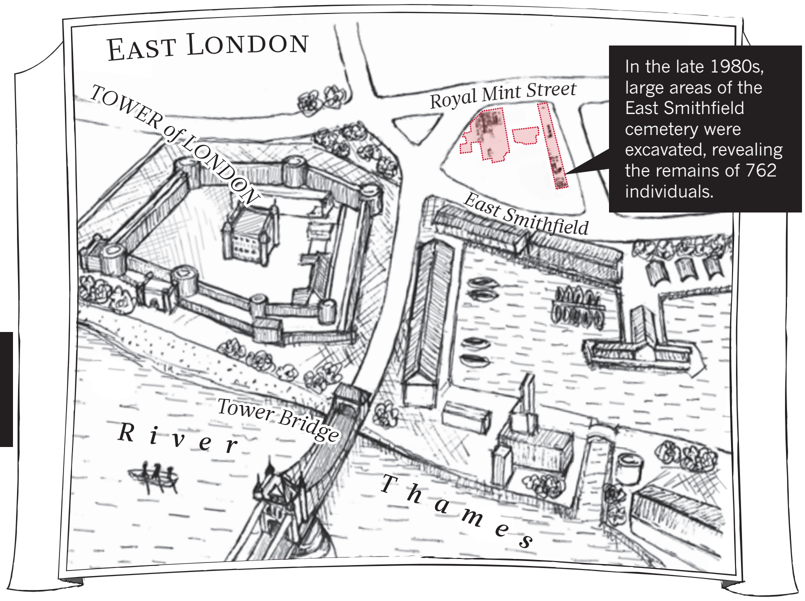
Death on the march

In the 1340s, a pestilence originating in Western Asia spread rapidly across Europe. Before it overtook London in 1348, land was set aside in East Smithfield to bury the dead.





The sequence — the first from an ancient bacterial pathogen — may help to explain how a disease could wreak so much havoc. It also marks a renaissance in genetic studies of ancient diseases, a field that has suffered a controversial history but that is now being revitalized. "There will be a race now for all the ancient pathogens," says Hendrik Poinar, a palaeogeneticist at McMaster University in Hamilton, Canada, who co-led the sequencing efforts.

Plagued with disbelief

When Alexandre Yersin linked *Y. pestis* to bubonic plague in 1894, many scientists surmised that the pathogen was behind not only the Black Death, but also a spate of earlier mass die-offs. The sixth-century Justinian plague devastated Constantinople and killed millions in Europe and the Near East. Plagues reared their heads periodically for the next two centuries. Black Death itself reappeared several times, even into the nineteenth century.

Clues tying *Y. pestis* to these outbreaks came largely from historical accounts of their symptoms, such as Giovanni Boccaccio's description of the Black Death in *The Decameron,* written around 1350: "It first betrayed itself by the emergence of certain tumours in the groin or the armpits, some of which grew as large as a common apple, others as an egg."

But some modern historians and scientists came to doubt that *Y. pestis* caused these ancient outbreaks. Bubonic plague epidemics known to have been caused by *Y. pestis* in the past century seemed too mild to have been caused by the same culprit as the Black Death: they killed fewer people and spread more slowly. Some 'plague revisionists' have argued that fleas, which spread *Y. pestis* to humans, would have struggled to survive the cold temperatures reported during the Black Death. And there was the speed with which it killed — Boccaccio reported that death often occurred within three days of the first symptoms appearing. Anthrax or a haemorrhagic-fever-causing virus similar to Ebola would be more likely than plague to cause such a rapid demise, say critics.

DNA evidence would seem to offer a definitive answer. In 2000, a team led by Didier Raoult, a microbiologist at the University of the Mediterranean in Marseilles, France, said it had proved the link between the bacterium and the disease. The researchers reported[2] that they had successfully recovered *Y. pestis* DNA from the teeth of a child and two adults dug up from a fourteenth-century mass burial site in Montpellier. The team identified the bacterium using a sensitive technique called the polymerase chain reaction (PCR) to amplify a portion of a gene from *Y. pestis* called *pla*. "We believe that we can end the controversy," the team wrote[2]. "Medieval Black Death was plague."

But several critics raised concerns about contamination. The PCR might instead have amplified DNA from modern *Y. pestis* used previously in the lab, or possibly the sequences from a closely related soil-dwelling bacterium. "I could never, ever replicate it," says Thomas Gilbert, an evolutionary geneticist at the University of Copenhagen in Denmark. In 2004, Gilbert and his colleagues reported no trace of *Y. pestis* DNA in 108 teeth from 61 individuals found in plague pits in France, Denmark and England (including East Smithfield)[3].

Raoult says that there was no contamination and that Gilbert's methods did not accurately replicate his[4]. Still, those who were already sceptical of the suggestion that *Y. pestis* caused the Black Death latched on to Gilbert's study.

Other studies of microbial DNA extracted from ancient human remains — including those affected by tuberculosis, syphilis and malaria — were also being scrutinized. In several cases, researchers could not replicate results, or they found methodological shortcomings. Critics said that DNA from these samples was too degraded by heat, moisture and time to detect, and the field soon divided into believers and sceptics.

"There was a complete schism," says Ian Barnes, a palaeogeneticist at Royal Holloway University of London, who says he spent two-and-a-half years trying — unsuccessfully — to find DNA evidence of syphilis or tuberculosis in bones dating from the nineteenth and early twentieth centuries[5]. "People largely ignored each other," he says.

Digging up answers

Although Poinar was dubious of claims about ancient microbial DNA, he was intrigued by the bones from East Smithfield. Nearly all of the remains are from Black Death victims, many of whom were cut down during the prime of their lives.

In a bright ground-floor laboratory of the Museum of London, a short walk from East Smithfield, osteoarchaeologist Jelena Bekvalac examines the nearly complete skeleton of one of the plague pit's former residents. Wearing a black silk scarf dotted with white skull-prints, Bekvalac handles a pelvic bone and determines that it belonged to a man who died in his late teens or early twenties. Apart from some plaque on his teeth and a gash in his skull that shows some signs of healing, the man's skeleton offers no outward evidence of Black Death.

His remains, and those from hundreds of others, represent a snapshot of life and death in London during the epidemic. Since the site's excavation, researchers have descended on the bones in search of information.

In the late 1990s, Poinar met Sharon DeWitte, then a graduate student at Pennsylvania State University in State College, who was working on a demographic analysis of the remains suggesting that Black Death preferentially killed those who were already frail. The two considered drilling into teeth and bones to find *Y. pestis* DNA, but Poinar wasn't satisfied with the available detection tools, which were still based on PCR. "We sort of sat on the samples for a few years waiting for all the stars to align," says DeWitte, now at the University of South Carolina in Columbia.

That alignment came from next-generation DNA sequencers, machines that read short snippets of DNA. The technology was perfect for sequencing DNA that has been damaged by spending hundreds of years underground.

The sequencers allowed Svante Pääbo, a palaeogeneticist at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, and his team to sequence a draft of the Neanderthal genome[6]. But finding and sequencing ancient pathogens in a human skeleton is much harder — like finding "needles in the football field", Poinar says — because their genomes are 1,000 times shorter than that of the Neanderthal and closely resemble those of soil microbes that have infiltrated the bones.

Another technology helped narrow the search. Pääbo and his team developed a technique, called targeted capture, in which they used lab-synthesized 'bait' DNA to snag ancient DNA strands from a bone sample[7], leaving soil-microbe and other sequences behind. "It's pretty much like fishing in a pond," says Johannes Krause, a palaeogeneticist at the University of Tübingen in Germany, who worked with Pääbo on the Neanderthal genome and co-led the Black Death project with Poinar.

In a proof-of-principle experiment published in August of this year, Krause and Poinar's team used sequences from a contemporary plague strain to fish out *Y. pestis* DNA from the teeth of victims buried at East Smithfield. From this, they sequenced a short loop of DNA, called the pPCP1 plasmid, that is partially responsible for bubonic plague's ability to infect humans.

Their results[8], along with a paper published last year[9] that found *Y. pestis* sequences in different Black Death bone samples, have convinced most scientists that bubonic plague was involved in the Black Death.

In their most recent paper[1], Poinar and Krause completed the ancient genome and showed that it sits at the root of an evolutionary tree that comprises 17 contemporary strains of *Y. pestis*. This indicates that the Black Death strain spawned many of the forms of *Y. pestis* that infect humans today.

This strain, Krause adds, probably emerged not long before the Black Death started its rampage across western Asia and Europe in the fourteenth century. "That, for me, was the biggest surprise," he says. It suggests, the authors argue, that earlier plagues were caused by either a now-extinct strain of *Y. pestis* or by an entirely different pathogen.

Mark Achtman, a plague-evolution expert at University College Cork in Ireland, calls this interpretation "absolute nonsense". Krause and Poinar's team did not consider a number of modern plague strains found in central and east Asia, which are thought to have earlier origins than the East Smithfield strain, Achtman says. Genome sequences for these strains were not available to his team, says Krause, but he is eager to see how they are related.

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For more on Black Death research, see *Nature*'s video:
go.nature.com/hxbtel

Mysterious scourge

Just as puzzling, however, is that *Y. pestis* seems to have changed very little over the past 660 years. The genome of the Black Death strain differs from that of the modern *Y. pestis* 'reference' strain by about 100 nucleotides, but each of these genetic differences can be found in at least one contemporary strain. "We can't find anything that makes the Black Death special," Krause says.

Historical descriptions of the Black Death have helped link *Yersinia pestis* with the disease.

The team is now looking for other genetic changes that could account for the Black Death's ferocity, such as rearrangements in the genome, which are difficult to determine from the short fragments of DNA available. To better understand how the plague worked, researchers could try to resurrect the Black Death pathogen by modifying the genomes of contemporary *Y. pestis* strains. Although this might sound alarming, research on *Y. pestis* is already carefully controlled, and even an accidental infection with such a strain could be easily treated with modern antibiotics.

Moreover, Poinar says, the Black Death was not just about the bacterium. Environmental and epidemiological factors must have aided in its vicious tear through Europe. Sick soldiers returning to Europe from Caffa, the Black Sea port that was the plague's gateway from Asia, unleashed the disease on a population that would have been weakened by malnourishment and years of cold, wet weather, he says.

Achtman says that it is possible that Black Death was not spread by rat-dwelling fleas, as *Y. pestis* is today, but by other animals, which could have enhanced transmission. Or another circulating pathogen could have contributed, as in the 'Spanish flu' pandemic that killed up to 100 million people worldwide in 1918-19, often with the help of bacterial pneumonia.

Whatever questions remain about the Black Death, scientists are now keen to apply the latest sequencing methods to other ancient epidemics. "I've completely gone from thinking, 'ancient pathogens are a load of crap,' to 'hold on, maybe some of this stuff works'," says Gilbert, whose team has started to sequence DNA from pathogens that plagued ancient crops. Researchers could identify ancient microbes and chart their spread and their evolutionary relationships with contemporary strains. For example, Europeans who travelled to the New World may have introduced new forms of tuberculosis to North America and brought syphilis back to Europe.

Ancient pathogens may help scientists understand current and future outbreaks, says Terry Brown, a biomolecular archaeologist at the University of Manchester, UK. He and Charlotte Roberts, of Durham University, UK, are charting the evolution of tuberculosis strains in Britain and Europe. "By looking over the past 1,000 years of disease in British cities, we can understand problems occurring in the Third World, where more and more people are crowding into cities," he says. Similarly, the sequencing and resurrection of the influenza strain responsible for the 1918 pandemic[10] has helped researchers to interpret the sequences of contemporary flu strains.

For all its ferocity, the Black Death left few visible marks on London. Today, the plague pit at East Smithfield is in the heart of London's financial district, buried under modern office suites and the old Royal Mint building. The only visible remnants are the crumbled ruins of St Mary Graces, a Cistercian abbey built near the site in 1350.

London may have seen its last significant bubonic plague outbreak, but catastrophic epidemics are a rule of human history, not an exception. Centuries from now, what traces will the next great scourge leave? Future archaeologists chronicling its history may find memorials, graves and probably even the bodies of victims. But another story will also lurk in its DNA, just waiting to be read. ■

Ewen Callaway *writes for* Nature *from London.*

1. Bos, K. I. *et al. Nature* **478,** 506–510 (2011).
2. Raoult, D. *et al. Proc. Natl Acad. Sci. USA* **97,** 12800–12803 (2000).
3. Gilbert, M. T. P. *et al. Microbiology* **150,** 341–354 (2004).
4. Drancourt, M. & Raoult, D. *Microbiology* **150,** 263–264 (2004).
5. Barnes, I. & Thomas, M. G. *Proc. R. Soc. B* **273,** 645–653 (2006).
6. Green, R. E. *et al. Science* **328,** 710–722 (2010).
7. Briggs, A. W. *et al. Science* **325,** 318–321 (2009).
8. Schuenemann, V. J. *et al. Proc. Natl Acad. Sci. USA* **108,** E746–E752 (2011).
9. Haensch, S. *et al. PLoS Pathog.* **6,** e1001134 (2010).
10. Taubenberger, J. K. *et al. Nature* **437,** 889-893 (2005).