THE HIDDEN HISTORY OF ANCIENT PLAGUES

A finding that Vikings carried smallpox virus shows how genetics is changing our knowledge of past diseases. **By Laura Spinney**

The death date of smallpox is clear. After killing more than 300 million people in the twentieth century, it claimed its last victim in 1978; two years later, on 8 May 1980, the World Health Assembly declared that the variola virus, which causes smallpox, had been eradicated. But the origins of this devastating virus are obscure. Now, genetic evidence is starting to uncover when smallpox first started attacking people.

Humans as far back as AD 600 carried variola, an international research team reported this week[1] after years of fishing for viral DNA in ancient human remains. The analysis also implies that the virus was circulating in humans even earlier: at least 1,700 years back, in the turbulent period around the fall of the Western Roman Empire, when many peoples were migrating across Eurasia.

The research pushes DNA evidence of smallpox back by a millennium. In 2016, researchers had dated it to the seventeenth century, using DNA extracted from a Lithuanian mummy[2]. "We've shown that 1,000 years earlier, during the Viking Age, variola was already quite widespread in Europe," says Martin Sikora, an evolutionary geneticist at the University of Copenhagen and a member of the team.

Smallpox is only the latest example of a serious infectious disease whose history has been suddenly and substantially rewritten by ancient-DNA analysis in the past decade. Earlier this year, a study[3] reported that the measles virus — thought to have emerged in humans around the ninth century — might have jumped to people in the first millennium BC, which is when its sequence seems to have diverged from the related (and now-eradicated) rinderpest virus, which infected cattle. In 2018, Sikora's team showed that hepatitis B had been infecting humans since the Bronze Age, 5,000 years ago[4]; in 2015, the team reported a similarly early origin for the plague, which is caused by the bacterium *Yersinia pestis*[5].

Not all genetic studies have moved disease origins backwards in time, however: in 2014, a German-led group reported that tuberculosis had been infecting humans for less than 6,000 years, not 12,000 as the consensus had been, let alone 70,000 years as had previously been suggested[6].

These findings are shaking up researchers' understanding of how diseases have affected human populations throughout history, says Ann Carmichael, a plague historian at Indiana University in Bloomington. The DNA evidence suggests that diseases such as plague and hepatitis B are associated with major prehistoric migrations — something that seems now to be true of variola too. Whether migrations brought the diseases to new areas or the emergence of disease triggered people to move is a question that archaeologists, historians and geneticists hope to be able to answer.

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The DNA evidence has also provided insights into the virulence of ancient smallpox: the latest work suggests that the Vikings, for instance, carried an extinct variola lineage quite different from the modern strain. Integrating the genetics with history and archaeology is the work that lies ahead, says archaeologist Søren Sindbæk of Aarhus University in Denmark. "We can begin to nail these events down to the human scale," he says. "Going forward, high-definition timing will be critical to rewriting human history."

Ancient pathogen genomes

Before the ancient-DNA revolution, researchers had to rely on examining skeletons — or, more rarely, mummies — for visible evidence of disease, spotting the telltale signs of leprosy or syphilis, for instance, and cross-referencing with historical records. But many infections don't leave visible marks on bone. Other, indirect clues to the age of some diseases have come from estimating the age and geographical distribution of protective mutations in humans: people whose red blood cells lack the 'Duffy antigens', for example, enjoy some protection against the malaria parasite *Plasmodium vivax*.

Researchers have been able to fish out fragments of pathogen DNA from remains since the 1990s. And in the past decade, next-generation DNA sequencers that can read myriad short snippets — useful for sequencing DNA damaged after hundreds or thousands of years — have helped researchers reconstruct entire genomes of ancient pathogens. In 2011, scientists published the first such genome[7], of *Y. pestis*, gathered from four skeletons in a London graveyard where thousands of Black Death victims were buried in the fourteenth century.

It is now routine to screen ancient human remains for known pathogens, says Eske Willerslev, an evolutionary geneticist at the University of Cambridge, UK, who worked on the smallpox study. This began as an offshoot of a project[8] to chart the Viking diaspora of the late first millennium AD, but grew into a much larger analysis. Researchers screened DNA collected from 1,867 individuals who lived in Eurasia and the Americas between 32,000 and 150 years ago. They found stretches of DNA that resembled modern variola strains in 26 of them; for 13, they were able to go back to the original remains and extract more variola DNA through targeted capture, a technique that uses laboratory-synthesized DNA to pick out similar strands from bones or teeth. (Researchers have homed in on the petrous bone — a part of the skull close to the ear — as

Remains of a smallpox carrier buried in Öland, Sweden, between AD 800 and 1050.

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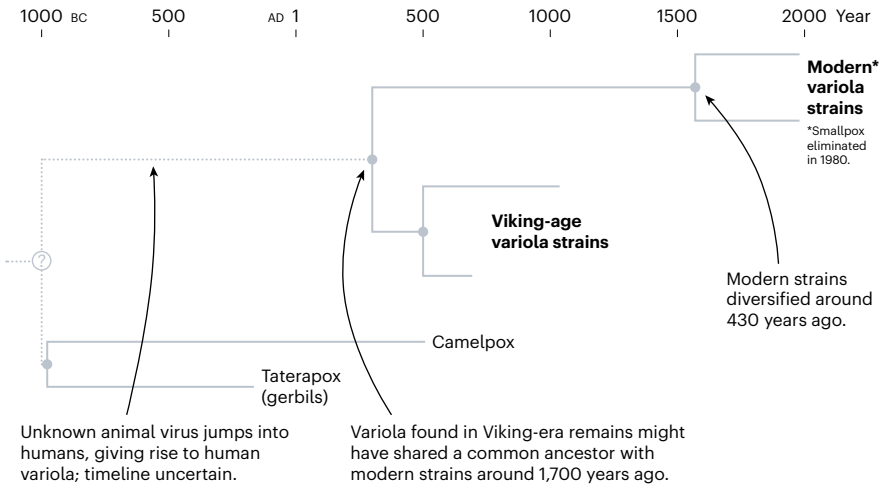
ANCIENT SMALLPOX

The discovery of the variola virus, which causes smallpox, in Viking-era remains shows that the disease has been present in humans for at least 1,700 years.



SOURCE: B. MÜHLEMANN, M. SIKORA & T. JONES/REF. 1

a good source of ancient DNA, because it is the densest mammalian bone and so preserves human DNA well. But pathogens are more likely to show up in the teeth, because more blood flows through them, says Willerslev.)

Eleven of these individuals dated from around AD 600 to 1050, overlapping the Viking Age, and they hailed from present-day Scandinavia, Russia and the United Kingdom. One was unearthed from a mass grave in Oxford, UK, and is thought to have died in the St Brice's Day Massacre of 1002, when the English king Ethelred the Unready ordered the extermination of people identified as Danes. Four Viking-era individuals provided enough viral DNA for researchers to reconstruct near-complete variola genomes, which they compared with modern variola sequences. Surprisingly, the lineage infecting the Viking-era samples was not a direct ancestor of nineteenth- and twentieth-century lineages. "It's a separate evolutionary trajectory that died out at some point and, as far as we know, is not present any more today," Sikora says.

The researchers traced this family tree using a 'molecular clock' approach: they measured how much the ancient and modern lineages differed, and used the rate at which genetic differences accumulate to calculate how much time had elapsed since the lineages split. This analysis suggests that their most recent common ancestor lived around 1,700 years ago (see 'Ancient smallpox').

That doesn't mean the disease first reached humans at that time, says Terry Jones, a computational biologist based at the Charité Hospital in Berlin and the University of Cambridge, who worked on the project; it is simply the date of the coalescence of all sampled diversity so far. Willerslev says he thinks his group has screened enough individuals from the Bronze, Neolithic and Mesolithic Ages (stretching from around 15000 to 1200 BC) — without finding variola — to say that it's unlikely smallpox was circulating widely before 3,000–4,000 years ago.

Other researchers have surmised that variola was infecting humans well before 1,700 years ago. Historical records suggest that a smallpox-like disease has been with us for more than 3,000 years, and might even have killed the young pharaoh Rameses V in the twelfth century BC — although nobody can be certain that he had smallpox or that, if he did, the disease killed him. The latest DNA evidence doesn't shed any light on that idea, but an Egyptian project to analyse the DNA of royal mummies is scheduled to report in 2022.

Scientists not involved in the variola study are impressed by the work. "This new paper is showing that there were lineages that we were completely ignorant about," says Michael Worobey, an evolutionary biologist at the University of Arizona in Tucson. But Hendrik Poinar, a palaeogeneticist at McMaster University in Hamilton, Canada, who worked on the 2016 smallpox study, says that large differences between the Viking and the modern lineages make it possible that the Vikings might not have had smallpox as we would recognize it.

That might be true, says Jones. There is some evidence, for example, that cumulative gene inactivation in the virus made it more virulent. "We can't be sure, but there's a good argument that before the seventeenth century, smallpox was endemic and mild," he says.

Rewriting disease history

Studies of ancient pathogens such as plague, hepatitis B and smallpox have proved it's possible to detect pathogens in remains that don't show signs of disease, so scientists need not confine their analyses to remains in plague pits. That gives a more comprehensive picture of pathogens' impact in the ancient world.

The distribution of pathogen genotypes and the way they change over time might shed new light on how people moved in the past. The discovery of *Y. pestis* in the preserved teeth of Yamnaya herders who came to Europe from the eastern European steppe, for example, has given rise to the theory that these invaders accelerated the decline of Neolithic farming societies after 3500 BC by spreading plague among them. But this is still a contested idea because there is archaeological evidence that a decline was already under way 1,000 years before the Yamnaya arrived, says Detlef Gronenborn, an archaeologist at the Leibniz Research Institute for Archaeology in Mainz, Germany.

But because only around 200 complete ancient-pathogen genomes have been sequenced[9] — and only a few for each pathogen — conclusions that can be drawn from phylogenetic analysis are limited for now. Even in the current pandemic, with tens of thousands of SARS-CoV-2 genomes analysed, researchers have sometimes drawn erroneous conclusions about the path the virus took during its spread[10]. The further researchers go back in time and the sparser the samples, Poinar says, the greater the risk of over-interpretation.

Researchers say that investigations into the evolutionary history of viruses might also be helpful in protecting people in the future. "It's very hard to predict where virus evolution is going to go," says virologist Lasse Vinner of the University of Copenhagen, another author on the paper, "but knowing where it has been, we get a better idea of the possibilities of variation." Andrea McCollum, an epidemiologist at the Centers for Disease Control and Prevention in Atlanta, Georgia, who has studied smallpox, says that such a family tree could be informative about the protection that remaining stocks of smallpox vaccine would afford against related orthopox viruses.

Disease historians, meanwhile, are recognizing that they have new questions to answer. "We really need to start over," Carmichael says. The 2011 confirmation that *Y. pestis* gave rise to the Black Death laid to rest debate over the cause of that pandemic. And because the Black Death strain was very similar to modern *Y. pestis*, it has led historians to pose a new question: why was plague so much more lethal in the pre-modern world than in the modern one? Co-morbidities and ways of life might partly explain it, but the answer is not yet clear. "Addressing that is a historical question, not a genetic one," she says.

Laura Spinney is a science writer in Paris.

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