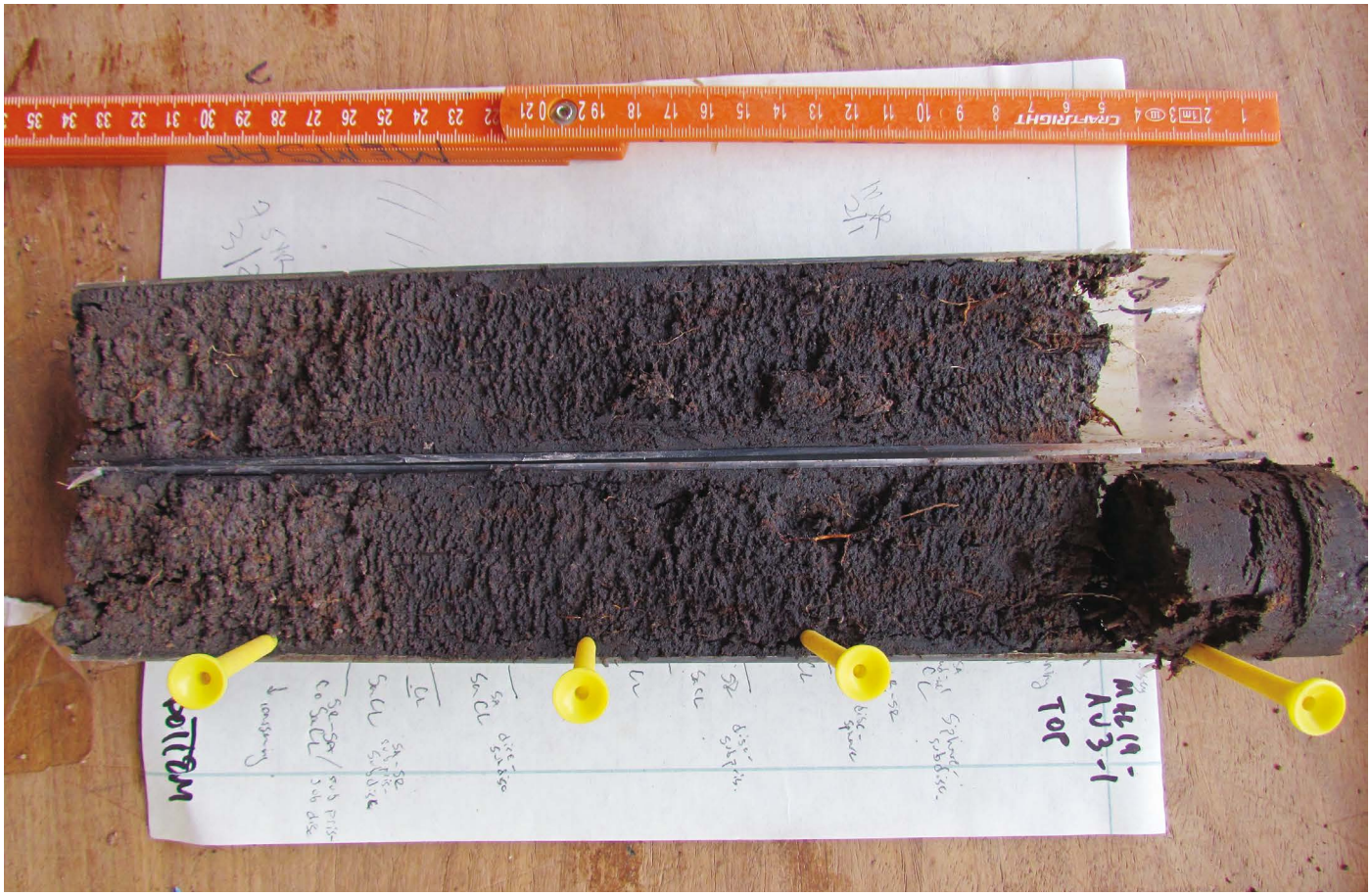


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A sediment core that was extracted about 11 kilometres from Mount Hora in Malawi, a site with a rich history of human occupation.

# MOLECULAR FOSSILS: POO AND POLLUTANTS GIVE CLUES TO PAST

Biochemical techniques enable archaeologists to mine ancient sediments for insights into the people who once lived there. **By Katarina Zimmer**

In 2019, environmental archaeologist David Wright at the University of Oslo visited what was, at first glance, an extremely unappealing field site: a cluster of leech-infested swamps in northern Malawi. At times ankle-deep in the grassy water, he and his colleagues set up a percussion auger — an instrument used in the extraction of cylinders of mud by hammering a long plastic tube into the ground. The hard part was getting the cores back out again. With his knees bent, Wright would brace the auger against his shoulder and push against the ground. In one swamp, the sediment was so sticky that the auger snapped in half. "It's pretty tough work," Wright says, and the leeches settling around the team's ankles were an added bonus.

Why all the effort over mud? These swamps surround Mount Hora, a granite hill located about 60 kilometres west of Lake Malawi, which separates the country from Mozambique and Tanzania. The site has a rich history, from the oldest-known human cremations roughly 9,500 years ago to 2,000-year old iron-smelting sites. The muddy layers of accumulated organic material, Wright hoped, would hold further clues about ancient groups of people. Pieces of preserved charcoal might reveal fire-making habits, while fossilized pollen could help researchers to understand the environment in which people lived.

But Wright's particular interest was more scatological: coprostanol, a molecule in human faeces that could shine a light on human population trends. "They're durable little suckers," Wright says of such biomarkers. Combined with other evidence, "you get a much clearer picture of what there was in the past".

Archaeologists have long focused on bones and other relatively macroscopic artefacts, but they are now teaming up with geochemists, palaeoecologists and biologists to unearth molecular lines of evidence. Technological and methodological advances over the past few decades have made it possible to detect minute traces of organic compounds in ancient sediments, complementing conventional pollen and charcoal studies. Such molecular fossils include leaf waxes and fats from bacterial membranes that contain clues about past climates, while coprostanol and pollutants from fires can help to reconstruct ancient human activities. DNA lingers in sediments, too, revealing rich detail about plants, animals and humans. Although the methods

used to detect these molecules are complex – and each marker has its limitations – they're already providing valuable clues about times long gone.

One of Wright's swamp cores, for instance, revealed an increase in coprostanol around Mount Hora that began at least 1,000 years ago, suggesting an influx of farming and herding communities that brought a new way of life to the region, Wright's team reported in 2024 (ref. 1). For researchers who learn to dissect ancient sediments, Wright says, "it's a really powerful tool".

## Scrolling back in time

Many sediments – from permafrost to cave floors – are biological archives of the past. Lakes and swamps can be particularly useful to researchers because they gradually accumulate material that washes down from a wide area and contains deep, oxygen-deprived layers that slow the breakdown of organic matter. To reach those sediments, scientists can cut profiles into cave floors or extract cores using hand-held tools, such as Wright's auger or even specialized drilling platforms for deeper sediments. A 380-metre-long core from Lake Malawi recorded 1.3 million years of history[2].

In the laboratory, scientists first scrutinize a core's layers, which can help to pinpoint time intervals at which, say, sediment is missing because a lake dried out or experienced an uneven deposition of material. Taking care not to contaminate samples with modern material – by wearing gloves and extracting sediment from inner parts of a core, for instance – plant macrofossils from different layers are sent to specialized labs that use techniques such as radiocarbon dating to estimate their age.

Fossilized pollen and charcoal can last for millions of years inside such sediments. Palaeoecologist Rahab Kinyanjui at the National Museums of Kenya in Nairobi extracts pollen using strong acids and salts to remove organic matter, carbonates, sand and silts, and charcoal with salts to coax particles apart and allow the material to float to the surface. Studying the species-specific morphology of pollen under a light microscope can help researchers to reconstruct a region's flowering plants, whereas charcoal concentration patterns can reveal periods of human-caused fire.

Pollen studies have led Wright to conclude that, after farming communities arrived around Mount Hora at least 1,000 years ago, its forested landscape became grassier. A concurrent rise in charcoal concentrations suggests that those people might have burned the forests to clear land for agriculture. "They brought a whole new way of interacting with the landscape," he says.

## Reconstructing ancient climates

A region's ancient vegetation can provide hints about its climate. But molecular biomarkers can reveal more detail. The waxes that protect leaf surfaces, for example, contain long-lasting organic molecules called *n*-alkanes. Variation among the individual carbon and hydrogen atoms of these molecules hold climatic clues. For hydrogen atoms, the ratio of heavy versus light hydrogen isotopes can indicate the strength of rainfall that plants experienced in their lifetime. Heavy rains are generally enriched with a light hydrogen isotope because heavier ones are the first to condense into water droplets. The heavier it rains, "there's less and less of the heavy isotope left and more and more of the light isotope", explains geochemist James Russell at Brown University in Providence, Rhode Island.

Organic geochemist Kate Freeman at Pennsylvania State University in University Park, says that some colleagues have jokingly called leaf waxes the 'Honda Civics' of biomarkers because – like the popular car – they are relatively straightforward to work with. Analysing these isotopes begins by extracting fats from sediment samples, often using an accelerated solvent extractor followed by liquid chromatography to separate the leaf waxes from other lipids.

The sample is then injected into a gas chromatograph coupled to an isotope ratio mass spectrometer. The chromatograph separates each *n*-alkane according to its boiling point. One at a time, each batch of molecules undergoes pyrolysis – a reaction that breaks them down into hydrogen gas. The mass spectrometer then uses magnetic fields to separate heavy isotopes from light ones and measures their concentrations.

Some palaeoclimatologists also mine sediments for bacterial membrane components called branched glycerol dialkyl glycerol tetraethers (brGDGTs). Bacteria can adjust the abundance of these forked molecules to prevent them from packing too closely together as temperatures change. By studying the composition of membrane lipids in different climates, scientists have effectively created a 'palaeothermometer' that can estimate past temperatures to within 2 °C.

Because brGDGTs aren't volatile enough to be vaporized in a gas chromatograph, they're usually separated using ultra-high-performance liquid chromatography, which

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**Assistants on the edge of a pit (left) that was dug to retrieve a broken auger at a sampling site near Mount Hora (right).**

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**PhD student Anneke ter Schure collects an ancient-DNA sample in southern Armenia in 2019.**

separates molecules, in part, on the basis of their polarity. This is coupled to a quadrupole mass spectrometer, which can be set to selectively analyse molecules with specific mass-to-charge ratios, allowing scientists to quantify the 15 brGDGTs that are currently used for environmental reconstructions, says palaeoclimatologist Tobias Schneider at the Swiss Federal Institute of Aquatic Science and Technology in Dübendorf.

Membrane lipid compositions and leaf-wax hydrogen isotope ratios must be interpreted cautiously, Russell says, because they're also influenced by other factors, such as the number and distance of a region's moisture sources. But they can help to answer important questions – such as why Norse settlers left southern Greenland 500 years after arriving there in AD 985. In a 2022 lake-core analysis[3], Schneider and his colleagues concluded that it wasn't solely colder weather that drove the settlers away, as some had suggested. Rather, a decline in rainfall might have contributed to the settlers' decision, because this would have hampered the production of the hay they needed to get their sheep through winter.

## Investigating past human activity

Other molecular markers can also reveal clues to ancient people's activities. Scientists are finding small, multi-ring molecules known as polycyclic aromatic hydrocarbons (PAHs) – which arise from the incomplete combustion of organic matter – to be useful complements to charcoal in studying ancient fire activity. Because PAHs are also used to assess modern-day atmospheric pollution, "there is a lot of science about them", says organic geochemist Elena Argiriadis at the Institute of Polar Sciences in Venice, Italy.

For instance, the precise make-up of PAHs can indicate the origin, intensity and sometimes even the source of a given fire; the PAH retene, for example, originates only from soft-wooded trees such as conifers. In a study published in December[4], scientists used PAHs alongside 400,000-year old stone tools from a UK field site as the earliest evidence for human-made fire, probably stemming from early Neanderthals. An analysis of sediments didn't reveal charcoal, which had probably washed away, but it did show a lot of heavy PAHs. This suggested that fire burned at that location and was intense enough to create heavy molecules. Finding higher concentrations of lighter, wind-carried PAHs would have indicated there was a lower-temperature wildfire farther away, the authors concluded.

**"They're durable little suckers, you get a much clearer picture of what there was in the past."**

An important consideration, says Tyler Karp, a palaeoecologist at the University of Chicago in Illinois, is that PAHs are also produced through the gradual heat- and pressure-driven transformation of carbon into fossil fuels. "That can actually confound your palaeo-fire interpretations," Karp says. To correct for this, Karp compares the concentrations of alkylated forms of PAHs – which are preferentially produced by slow and low-temperature processes – with the non-alkylated forms that arise from fire.

Coprostanol, the biomarker that Wright used as a proxy for people in the study in Malawi, presents similar complexities.

Coprostanol stems from the breakdown of cholesterol and is present at high concentrations in the guts of humans (and other primates, as well as in pigs). But many other animals also excrete small amounts of coprostanol, as well as other molecules that are produced when gut bacteria convert plant-derived compounds called sterols into a particular configuration called 5β-stanols. Sediment samples can also contain 5α-stanols, signifying that sterols were broken down in the environment rather than in animals' guts, explains geochemist Jago Birk at Georg-August University of Göttingen, Germany, who performed the faecal biomarker analysis in the Malawi study.

Distinguishing between these stanols – which are analysed together using a gas chromatograph–quadrupole mass spectrometer – is important to determine their origin, Birk says.

To isolate the human-specific signal from that of other animals, Birk compares coprostanol concentrations with those of other 5β molecules. And including the ratio between 5β- and 5α-stanols can help to control for situations in which coprostanol seems elevated because there's a lot of organic matter in a particular sediment layer. "I myself always work with biomarker ratios because no biomarker is really completely specific," Birk says. The same is true for bile acids, another faecal biomarker that is preserved in sediments and that can provide clues about past organisms.

## Digging up ancient DNA

Increasingly, scientists are setting their sights on sedimentary ancient DNA (sedaDNA), which binds to minerals and can survive for thousands – or in rare cases, millions – of years. Because it originates at least partly from leaves, roots, skin, hair, blood or urine that organisms once shed into their environment, sedaDNA is detectable over much larger geographical areas than fossilized remains. "A mammoth dies in one place, but it roams a large area, and it pees all over that place," says evolutionary biologist Sanne Boessenkool at the University of Oslo.

Metabarcoding is the most established method for identifying sedaDNA. This involves amplifying specific segments of genetic material that can then be sequenced to identify the species. In one 2022 study[5], Boessenkool used plant-specific DNA primers to amplify genetic material from sedaDNA extracts from a cave in the Armenian Highlands – an important site for modern humans tens of thousands of years ago. Boessenkool compared these sequences to a reference database to identify the plants that these humans probably carried into the cave; some of them, other evidence suggests, had medicinal properties or have been used to dye textiles.

One downside of examining only specific

DNA segments is that it doesn't capture the damage that happens as DNA degrades over time, making it hard to distinguish between ancient DNA and modern DNA that might have contaminated sediment samples. Another method, shotgun sequencing, does detect this damage, because it involves sequencing all the DNA in a sample. However, this produces enormous quantities of data, much of them unidentifiable or from organisms that aren't of interest. "You're really only analysing a very, very small fraction of DNA that you've sequenced," says Lucas Elliot, a molecular ecologist at the Arctic University of Norway in Tromsø.

It also requires computationally intensive methods to identify the species by matching each sequence with whole-genome reference sequences, which don't exist for every organism. And, the method often lacks species-level specificity; a 2025 analysis of lake sediments near an 8,900-year old campsite in northern Sweden, for instance, documented a broad range of organisms — from willow trees to bears — but couldn't distinguish whether an organism was a dog or a wolf[6].

For human DNA, which is extremely rare in sediments, shotgun sequencing might not even detect sufficient quantities for analysis. "You can't even necessarily be sure that [it's] human," says population geneticist Benjamin Vernot at the University of Vienna. In a 2017 *Science* study[7], one research team tested a different method to search for human DNA in cave-floor sediments across Eurasia. Called DNA hybridization capture, the method uses magnetic beads coated with single-stranded DNA probes that are specific to the sequences of interest — in this case, sequences derived from the mitochondrial genomes of modern humans and our Neanderthal and Denisovan relatives. Any DNA in the sample that complements the probes sticks to them and can then be sequenced, revealing Neanderthal and Denisovan DNA in layers dating back several hundred thousand years.

In 2021, Vernot and his colleagues at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, took this approach a step further[8] by using probes that target human nuclear DNA, which contains more species- and population-specific sequences than mitochondrial DNA. This allowed the researchers to discriminate between Neanderthal populations that once inhabited the Galería de las Estatuas cave in what is now northern Spain. SedaDNA results must always be interpreted cautiously, Vernot says: a single positive result could be an outlier, and the absence of sedaDNA could stem from poor preservation conditions rather than an organism's absence.

Such limitations are why research teams are increasingly studying organic markers in combination. Last year, researchers including Wright and Boessenkool received a €12-million (US$14-million) grant from the European Research Council to study ancient DNA, coprostanol, PAHs, pollen and charcoal in lake and swamp cores across Africa and the Middle East, to explore how humans have transformed their environment over the past 6,000 years.

Future scientists might be able to glean even more detail. Researchers are already mining existing biomarkers for new kinds of information — such as plants' growth conditions based on carbon isotopes in leaf waxes — as well as exploiting new biomarkers, such as palmitone, which is found in leaf waxes of the crop taro (*Colocasia esculenta*). The use of inorganic markers, such as sulfur and lead, to improve records of volcanic activity[9] and ancient metal-making processes is also forging ahead.

Armed with an ever-growing arsenal of techniques, it's an exciting time for the field, Schneider says. He is looking forward to seeing increasingly interdisciplinary teams pushing the boundaries of discovery to answer even more important archaeological questions. "Every study is showing something new — a new aspect, new interpretation, new proxies or new molecules — that helps further and further refine the picture of the past."

TOP: JAVIER TRUEBA/MADRID SCIENTIFIC FILMS; BOTTOM: MAX PLANCK INST. FOR EVOLUTIONARY ANTHROPOLOGY

**Top: The Galería de las Estatuas cave site in northern Spain. Bottom: Matthias Meyer in the clean laboratory at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany.**

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