Biomarkers

ROUND 251 million years ago, in the blink of a geological eye, up to 95 per cent of marine species and 85 per cent of those on land went extinct. It was the greatest mass extinction the world has ever endured, and it marked the end of the Permian period. Life took 10 million years to recover.

This cataclysmic event is often portrayed as the time when "life nearly died", but that is hardly fair. The oldest and most successful life forms on Earth - the bacteria and archaea - sailed through virtually unharmed. The Permian extinction is better seen as a time where life almost went back to normal - when biological conditions that had prevailed on Earth for more than 3 billion years briefly re-established themselves. The microbes did not merely survive; it now appears that they played a leading role in the extinctions.

This new view of the Permian comes not from studying fossilised bones, teeth and shells, but from biochemicals that have been trapped inside rocks for billions of years. This emerging branch of science is called biomarker analysis, or chemical palaeontology, and it is now so powerful that it threatens to make classical palaeontology obsolete. It provides nothing less than a brand new perspective on the history of life on Earth. We now know exactly when the first multicellular organisms evolved, and have made startling insights into the mass extinctions that came to plague them.

Biomarker research has its roots in oil exploration. While palaeontologists dedicated themselves to discovering and describing extinct organisms, geologists were breaking down sedimentary rocks and searching for organic molecules that would reveal where to look for oil. All over the world, they found golden liquid residues inside sedimentary rocks rich in organic matter.

Eventually they learned how to extract these liquids even from sedimentary rocks with very little organic content, including some of the oldest on Earth, at 3 billion years old or more. Most of these liquids were made up of hydrocarbons already familiar to oil geologists, such as ethane. But there were also some strangers, including long-chain alkanes and polycyclic molecules called hopanes. Where did they come from?

The answer came from biochemists studying living organisms. They discovered that if you subject certain biological molecules - mainly lipids found in cell membranes and other structures - to heating, cooling and pressure of the kind that would be encountered after burial in sedimentary rock, they break down into highly stable, organic compounds. So stable, in fact, that some can remain unchanged for billions of years. Equally important is that, unlike many other organic molecules, they are not made by any known inorganic processes.

What's more, some of the compounds turn out to be unique to certain groups of organisms, and many classes of molecules have now been definitively linked to specific groups of microbes, plants and animals. These are known as biomarkers. For example, the C_{28} to C_{32} polyenoic fatty acids were recently found to be a unique biomarker for sponges.

The discovery of these "molecular fossils" means that ancient organisms that otherwise left no trace in the fossil record can now be identified. The stage was set for biomarkers to tackle their first major mystery - the origin of complex life.

The organisms of our world are divided into two very different kinds: the simple prokaryotes, including bacteria and archaea, and the more complex eukaryotes. The main difference between the two is that eukaryotes have a nucleus and other organelles within the cell. Eukaryotic cells are also bigger and lend themselves to integrated multicellular life. But when did they evolve?

The fossil record is no help here. Fossilised single-celled organisms, presumably prokaryotes, first appeared some 3.5 billion years ago. But it is not possible to tell from fossils alone when eukaryotic cells appeared. The first eukaryote would have been a single-celled organism and, unlike some single-celled eukaryotes such as the microscopic plants that fossilise in vast numbers to form chalk, would not have had a microscopic skeleton.

Enter the biomarker crew. In the late 1990s, Roger Summons of the Australian Geological Survey Organisation in Canberra and Roger Buick of the University of Sydney went searching for biomarkers called steranes - telltale signs of eukaryotic organisms - in Australia's ancient sedimentary rocks. They traced the biomarker back to rocks 2.7 billion years old but no older. So it appears that eukaryotes first appeared around that time (*Science*, vol 285, p 1033).

Summons and Buick also discovered a discernable change in biomarkers over time. The earliest eukaryotic steranes are neither abundant nor diverse. Instead, the rocks are dominated by biomarkers called l-isoprenoids, which are signs of bacteria and archaea. By 800 million years ago, however, the biomarker profile had changed. It became dominated by hopanes, which reveal the presence of cyanobacteria (once erroneously called blue-green algae).

Cyanobacteria are key components of stromatolites; the layer-cakes of bacteria and sediment that were the most complex form of life on Earth before the evolution of multicellularity. The eukaryotic steranes also become more diverse and abundant around this time, and other eukaryotic biomarkers for the red and green algae as well as large, single-celled protozoans such as amoebas are now found. All of this reveals that large, plant-like algae such as kelp and sea lettuce had evolved but that the forerunners of animals were still in the single-celled stage.

Following the Cambrian explosion about 542 million years ago, when most animal groups appeared, the profile changed again, and for the first time we find the rich and complex record of biomarkers associated with animals. The newly evolved animals literally ate the stromatolites out of existence.

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Biomarkers are also helping with another long-standing thorn in palaeontologists' sides - the mass extinctions. The modern study of mass extinctions began in 1980 with the sensational announcement by Luis Alvarez and his son Walter, both at the University of California, Berkeley, that the extinction that killed the dinosaurs was caused by an asteroid impact - an event now known as the K/T extinction. This extraordinary claim demanded extraordinary evidence, but the fossil record was equivocal. If the impact actually happened, surely it would have been accompanied by a simultaneous and sudden extinction. However, the fossil record told a tale of a gradual extinction, rather than a catastrophically sudden one.

In 1982, Phil Signor and Jere Lipps of the University of California, Davis, showed that the fossil record would always appear to be gradual even after a sudden extinction because of the difficulties of sampling rare species. Even so, most palaeontologists stuck to the idea of a gradual extinction and it wasn't until 1990, when the Chicxulub crater was found in Yucatan, Mexico, that the impact hypothesis was widely accepted.

Palaeontologists later did painstaking work showing that the ammonites and other marine invertebrates went extinct at exactly the same time as the dinosaurs. Despite this, the fossil record was left with a reputation as being too unreliable to solve many important geological and biological conundrums.

Following the K/T debate, the study of mass extinctions became fashionable, and palaeontologists fanned out to work on the other members of the "big five" - the Ordovician, Devonian, Permian and Triassic mass extinctions. The new overriding paradigm was that mass extinctions were caused by impacts but, once again, research into the timings and nature of species extinctions was equivocal. New tools were needed, and biomarkers stepped up.

In 2005, a team led by Tetsuya Arinobu of Aichi Medical University School of Medicine in Japan took a new look at one of the most famous K/T sites, the thick white chalk of Caravaca in Spain, which was laid down in the sea. When the team sampled rocks across the K/T boundary, they made a discovery that was consistent with a sudden extinction: a thin layer, deposited soon after the K/T boundary, rich in biomarkers diagnostic of land plants, including shrubs and trees (see Diagram). This showed that there had been a short-lived deluge of dead plant material into the sea, consistent with the sudden destruction of entire forests (*Palaeogeography, Palaeoclimatology, Palaeoecology*, vol 224, p 108). Similar biomarker evidence has since been found at other K/T sites in Japan and elsewhere. For instance, a study in Hokkaido found a sudden decrease in the abundance of terrestrial plants starting right after the K/T event and lasting for 7000 years.

These studies were just the opening act for the use of biomarkers in the study of mass extinctions. After all, the K/T asteroid hypothesis is already supported by many lines of evidence. Yet what of the other extinctions?

Soon, researchers were extracting organic liquids from rocks of the late Permian, the time of the largest mass extinction of all and the subject of major debate as to its cause. Like the K/T event, this mass extinction has sometimes been attributed to an impact. But biomarker results suggest otherwise.

In 2005 Summons, now at the Massachusetts Institute of Technology, teamed up with geochemist Kliti Grice of Curtin University of Technology in Perth, Western Australia. Working with cores of sedimentary rock from China and Western Australia, they identified an interesting organic biomarker known as isorenieratene.

Today, the precursors of this molecule is found only in the cell walls of two very specific groups of microbes - green and purple sulphur bacteria. These peculiar microbes make a living by photosynthesis but cannot tolerate oxygen and do not extract electrons from water as most photosynthetic organisms do. Instead they use hydrogen sulphide, the "rotten egg" gas which is highly toxic to plants and animals.

Summons and Grice realised that the presence of these microbes indicated an ocean environment that was shallow enough for light to penetrate and photosynthesis to occur, yet was completely lacking in oxygen. Instead, it was saturated with hydrogen sulphide. It seemed that the seas of the late Permian were awash with deadly poison.

The team has since found the sulphur bacteria biomarker at a dozen late-Permian sites around the world, good evidence that the hydrogen sulphide oceans were a global phenomenon. In 2005 Summons, Grice and others combined their biomarker evidence in an influential paper that eliminated asteroids as a suspect for the Permian extinction and pointed the finger at a mass poisoning (*Science*, vol 307, p 706).

Biomarkers have since become the main supporting evidence for a new hypothesis for the extinction - death by hydrogen sulphide. This was proposed by a team led by geochemist Lee Kump of Pennsylvania State University in University Park. Kump suggested that so much hydrogen sulphide was produced in an ocean devoid of oxygen that it escaped into the atmosphere and poisoned plants and animals, as well as depleting the ozone layer that protects Earth from the sun's harmful UV radiation.

Stagnant seas

The hydrogen sulphide was produced by bacteria that lived in stagnant, deoxygenated water. These bacteria left no trace in the sedimentary record - there are no known biomarkers that reveal them - but their presence can be inferred from the abundance of purple and green sulphur bacteria, which need hydrogen sulphide to survive.

So how did the stagnant ocean come about? The ultimate culprit was intense global warming triggered by massive emissions of greenhouse gases from one of the largest and most sustained volcanic eruptions ever known, the Siberian Traps flood basalt. As in today's greenhouse world, the higher latitudes warmed fastest, lessening the difference in temperature between the polar regions and the tropics. This differential is the driver of the oceanic and air currents that keep the oceans oxygenated. In the hothouse of the late

Permian, temperatures at the high latitudes were barely different from those at the equator, currents ground to a halt and the ocean stagnated, ultimately causing a huge and lethal build-up of hydrogen sulphide produced by anaerobic bacteria.

It is not only the Permian extinction that has been tied to hydrogen sulphide. The biomarker isorenieratene has now been identified in rocks dating from the Devonian and Triassic extinctions. It is beginning to look as if the K/T mass extinction was unique in having been caused by an impact.

Having contributed to mass extinctions, microbes then take advantage. Triassic rocks from Meishan, China, contain evidence of a "microbial takeover" event in the form of a molecule called 2-methylhopane, which is diagnostic of the stromatolite-building cyanobacteria. In the sediments that mark the Permian extinction around the world, diverse coral-reef fossils give way to microbialites, stromatolite-like structures built up of sediments deposited on biofilms made by masses of bacteria.

An even more dramatic example is found in Western Australia's Canning Basin, where late-Devonian fossil coral reefs are overlain by reefs formed entirely of microbial deposits. The Devonian mass extinction was another case of microbes wresting the world's ecosystems away from animals for a million years or more.

Biomarker evidence has thus contributed to a startling new view of mass extinctions, one that suggests that we humans are unknowing combatants in an endless struggle for supremacy, fought between the "native" earthlings - bacteria and archaea - and the Johnny-come-lately animals and plants. For the aeons of the Precambrian, the planet was the exclusive domain of microbes. The rise of complex plants and animals rendered that world obsolete - but it reappears periodically in the aftermath of mass extinctions. And bacteria are not just the innocent beneficiaries of such events, they actively contribute to bringing them about.

A few years ago, the drama of the K/T event supported a widespread assumption that mass extinctions were caused by asteroid impacts. It now seems that the pendulum has swung away again. Only the K/T mass extinction was caused by an impact; the rest were the result of microbial hostile takeover. At the end of the Permian, the microbes nearly prevailed. Their day will come again.

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From issue 2642 of New Scientist magazine, 09 February 2008, page 40-43