Oxygen is supposed to have driven the evolution of complex life. Great story, says biochemist **Nick Lane,** but it's wrong

Genesis revisited

BRIAN LAROSSA

GO WEST, young man! More specifically, go about 200 kilometres west of Crete, then straight down to the bottom of the Mediterranean Sea 3.5 kilometres below. There you will find a lake with some extraordinary inhabitants.

Around 6 million years ago when the Mediterranean nearly dried up, vast amounts of salt were deposited on the sea floor. Some of these deposits were exposed about 30,000 years ago. As this salt dissolves, extra-salty, dense water is sinking to the depths, forming a brine lake 100 metres deep. Even more surprising than the existence of this lake beneath the sea, however, is what lives in it.

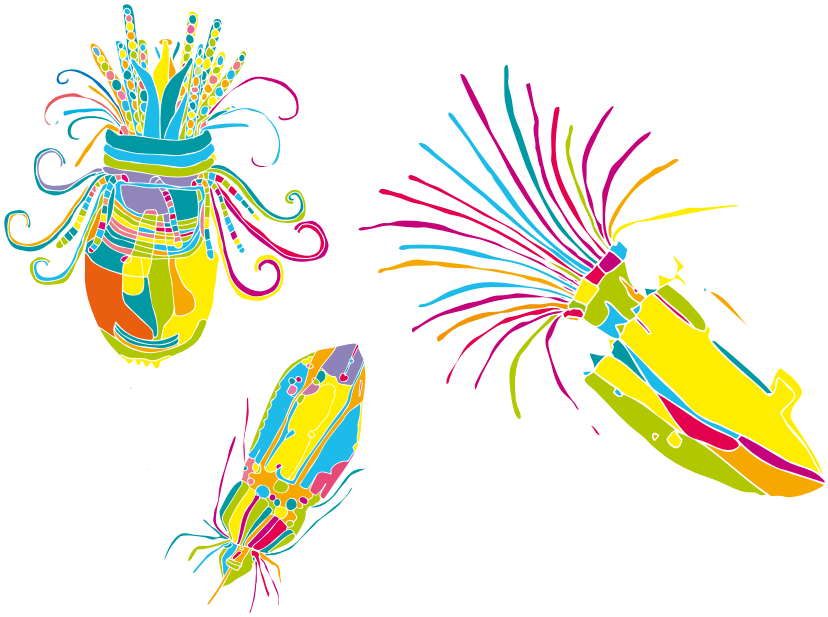
The water in the brine lake does not mix with the water above and so ran out of oxygen long ago. Instead, the toxic gas hydrogen sulphide oozes from the black mud. It's the last place you would expect to find animals. But that's exactly what has been discovered: the first animals, as far as we know, that can grow and reproduce without a whiff of oxygen.

These tiny mud-dwellers are far more than a curiosity. They could be the best pointer yet to the origin of complex cells: the basis of most life on Earth, from amoebae to oak trees.

"The ecology is interesting, but the real significance of these critters is what they say about evolution," says Bill Martin, an evolutionary biologist at the University of Dusseldorf in Germany. For Martin, the discovery is a beautiful affirmation of a radical prediction he made more than a decade ago – that oxygen had nothing to do with the evolution of complex life.

The first kinds of life on Earth, the bacteria and archaea, were simple cells – not much more than bags of chemicals. Eventually, they gave rise to complex cells, or eukaryotes, with sophisticated internal structures, the kind of cells found inside all plants and animals. And one of the most important events in the evolution of complex cells was the formation of a symbiotic union between a host cell and a bacterium – the ancestor of the cellular powerhouses known as mitochondria, which extract energy from food using oxygen.

"Burning" food provides 10 times as much energy as alternative ways of extracting energy from food without oxygen. When complex cells gained this ability, it changed the course of life on Earth: without mitochondria, large active animals might never have evolved (see "Living without breathing", page 39). It is not surprising, then, that most biologists think that the original symbiotic union revolved around oxygen. According to Martin, though, they are utterly wrong. ›



"Clearly, the existence of animals that don't need oxygen means oxygen is not the be-all and end-all of complex life in the universe"

The narrative in the textbooks seems compelling. In the beginning, so the story goes, there was no oxygen. The evolution of photosynthesis changed all that. By releasing their waste – oxygen – into the air, cyanobacteria transformed the globe around 2.3 billion years ago. As oxygen levels rose, the toxic gas caused the first mass extinction, wiping out nearly all existing organisms and paving the way for a new lifestyle: extracting energy from food using oxygen.

The bacteria that evolved this ability were preyed on by other cells. At some point, one cell failed to digest its dinner and instead let the bacteria live on inside it. This host cell, so the story goes, got two huge benefits: protection against oxygen, which was guzzled up by the ancestral mitochondria, and a share of the extra energy its guests could extract from food using oxygen.

It was not until oxygen levels rose even higher, around half a billion years ago, that the oceans could support large multicellular organisms that got their energy by burning food. That led to the Cambrian explosion, when all kinds of animals appeared. The main point about this story is that it sweeps forward with a magisterial inevitability, waiting only on a rising tide of oxygen.

The broad outlines are true. Oxygen levels did rise in two steps; most eukaryotes do generate energy using oxygen, and are normally tolerant of its toxicity; and the earliest fossil animals did appear soon after a big rise in oxygen levels in the oceans. Yet there are grounds to suspect that oxygen was not the puppet master after all.

One is that the initial rise in oxygen did not cleanse the oceans, but converted them into a stinking mess, full of hydrogen sulphide. Far from having few refuges, anaerobes had whole oceans to themselves. What's more, these conditions lasted for more than a billion years, right through the period when the eukaryotes are thought to have evolved.

No free lunch

Another issue is that oxygen is not particularly toxic by itself – it needs to be converted into free radicals before it will react with and destroy proteins and DNA. Mitochondria generate lots of free radicals so, far from protecting their hosts from oxygen, their ancestors would have increased the damage it does. In any case, consuming oxygen merely steepens the diffusion gradient; it's like trying to save yourself from drowning by drinking the surrounding ocean.

Even the power advantage of oxygen is problematic. No bacterium gives away energy for free, so the host cell could not have benefited from oxygen respiration until it had evolved the kit needed to siphon off energy-rich ATP from its guest bacteria. In the meantime, the "symbiosis" would have been a disaster. Thanks to their ability to exploit oxygen, the bacteria would be likely to outgrow the host and end up killing it.

So if the union was not about oxygen, what was it about? Hydrogen, according to Martin and Miklos Müller of The Rockefeller University in New York.

Back in the 1970s, Müller discovered that some single-celled organisms have structures that resemble mitochondria but do something quite different; they generate energy without using oxygen, by breaking food down into carbon dioxide and hydrogen – so Müller called them hydrogenosomes.

Before hooking up with Martin, Müller had gone on to show that hydrogenosomes do not merely resemble mitochondria but are in fact stripped-down mitochondria. They have the same shell, yet completely lack the usual ATP-generating machinery driven by oxygen. Instead, they have machinery that generates ATP while creating hydrogen as waste. The question is, was this different machinery acquired as mitochondria evolved into hydrogenosomes, or was it present all along? And if it was present all along, then what did the bacterial ancestor of the mitochondria actually look like?

Martin and Müller leapt straight in at the deep end. The ancestor of mitochondria, they said, was a versatile bacterium capable of living in a variety of environments – it could use many substances, including oxygen, to produce energy, and it could make hydrogen too. This is hardly an imaginary superbug: existing bacteria like *Rhodobacter* can do all that and more.

The ability of ancestral mitochondria to make hydrogen, rather than use oxygen, was the basis of the primordial pact that gave rise to the eukaryotes, Martin and Müller argued. The bacteria produced hydrogen as waste, and the host cell used it to convert carbon dioxide into methane, gleaning a little energy from the process – just as many archaea, called methanogens, still do. The symbiosis began in an environment with little or no oxygen and only later, after the relationship was well established, did the host cell start exploiting the ability of the ancestral mitochondria to use oxygen.

This idea, known as the "hydrogen hypothesis", was proposed by Martin and Müller in 1998 (*Nature*, vol 392, p 37), but it has never gained widespread acceptance. It was not just up against the gut feeling of most researchers that the rise of the eukaryotes was related in some way to oxygen; on the face of it, what little evidence there was did not support it either.

Most studies of the genes needed to make hydrogenosomes, for example, suggest they evolved repeatedly and independently from mitochondrial genes, with some extra ones being picked up by lateral gene transfer from other organisms along the way. "I think the transformation from aerobic mitochondria to hydrogenosomes has little or nothing to do with the origins of eukaryotes," says microbiologist Mitch Sogin at the Marine Biological Laboratory in Woods Hole, Massachusetts.

Not surprisingly, Martin disagrees. "Single gene studies are subject to so many artefacts that we can conclude almost nothing about

deep evolutionary history from them," he says. "Line up the same genes from the other end and you derive a totally different tree."

What's more, if aerobic mitochondria have evolved into hydrogenosomes on many separate occasions by picking up genes from other organisms, then why do hydrogenosomes always have the same small subset of genes for making hydrogen? They could have picked up all kinds of genes from bacteria, which have an amazing repertoire of metabolic abilities, Martin says, so why pick the same ones each time?

Remarkable abilities

Martin's explanation is simple: they share the same set because they inherited them from a single bacterium – the ancestor of mitochondria. For all its power, this argument is sterile without more evidence one way or another: you either believe it or you don't.

That evidence is starting to emerge. Take *Chlamydomonas*, a unicellular green alga with remarkable metabolic abilities. Ariane Atteia and her colleagues at the Laboratory of Plant Cell Physiology in Grenoble, France, together with Martin, have been studying its mitochondria. Last year, they concluded that the ancestor of these mitochondria was a metabolically versatile bacterium like *Rhodobacter* (*Molecular Biology and Evolution*, vol 26, p 1533).

Meanwhile, Lillian Fritz-Laylin and her colleagues at the University of California, Berkeley, have been looking at *Naegleria gruberi*, a curious shape-changing cell. In the absence of oxygen, its mitochondria appear capable of generating energy by producing hydrogen, the team reported earlier this year, with the help of proteins also found in hydrogenosomes (*Cell*, vol 140, p 631).

And now we have found animals that can live without oxygen lurking in brine lakes at the bottom of the Mediterranean. The three yet-to-be-named species were discovered by marine biologist Roberto Danovaro of the Polytechnic University of Marche in Ancona, Italy and his colleagues (*BMC Biology*, vol 8, p 30). They belong to an obscure group of microscopic animals, the Loricifera, found in ocean sediments around the world.

Little more than a millimetre long, the new species are so inactive that it took a while to prove they were indeed living, if not breathing. What's really striking about them, though, is not just their ability to live without oxygen but the way they manage it: unlike all other animals, including other Loriciferans, they appear to have hydrogenosomes rather than mitochondria.

These recent discoveries are starting to transform people's perspectives. "The simplest explanation is that all the different types of mitochondria inherited their metabolic tool kits from a single versatile ancestor," says Mark van der Giezen at the University of Exeter, UK, who studies the evolution of anaerobic eukaryotes.

And if that is the case, then eukaryotes would have been able to live in anoxic environments right from the start. "Nobody seriously thinks that bacteria dwelling in such habitats only recently adapted to anaerobic niches," points out Martin. "But when it comes to eukaryotes, there is still a curious tendency to assume that they only invaded anaerobic niches of late. There's no logic in that."

Indeed, if the hydrogen hypothesis is right, the implications for complex life are striking. The existence of animals that don't need oxygen means that oxygen is not the be-all and end-all of complex life in the universe. The anoxic oceans a billion years ago might have been full of tiny creatures – as indeed many anoxic basins probably are today, if we look properly – and these animals got larger and more active when oxygen levels rose.

The deeper point relates to the origin of eukaryotes. There was no magisterial progression from simple to complex life as oxygen levels rose; no inevitability about it. Instead, there was a symbiotic union between a bacterium that could make hydrogen and an archaeal host cell that could exploit that hydrogen: a freak event that changed the world. ■

ROBERTO DANOVARO

This little Loriciferan can manage fine without oxygen

LIVING WITHOUT BREATHING

Some fish, mussels and sediment-dwelling worms can live without oxygen for hours or even days. Instead of getting energy by "burning" food, the cells of these animals switch to ways of producing energy that do not require oxygen. Until earlier this year, no animals had been discovered that go their entire lives without oxygen (see main story) – it was thought to be impossible.

Oxygen is not only used for getting energy from food, it is also needed to make compounds like collagen, the "glue" that holds animals together. No oxygen, no collagen; no collagen, no animals, the thinking went. That must be wrong, although we have yet to work out how the newly discovered animals make compounds like collagen without oxygen.

So could there be planets out there with large animals that do not need oxygen? While burning food produces 10 times as much energy as other means like fermentation, in theory an animal might get around that if it could somehow get 10 times as much fuel. The trouble is, fermentation leaves far less energy for predators in ecosystems. With aerobic respiration, there can be five or six links in a food chain before the amount of energy falls below 1 per cent of that available initially. Without oxygen, this happens with just two links.

And with far less scope for predation, animals might not evolve as far or as fast; the need to find prey or dodge predators is thought to have driven the development of features like eyes and mouths and muscles.

Nick Lane is the first Provost's Venture Research Fellow at University College London, and author of *Life Ascending: The ten great inventions of evolution* (Profile, 2009)