

## CHAPTER 1



### The Missing Microbes

A few years ago, I was given the opportunity to work on a research ship on the Black Sea off the north coast of Turkey. The Black Sea is a fascinating and unique body of water: below the upper 150 meters or so, there is no oxygen. The focus of my work was to study the photosynthetic microbes in the upper 150 meters.

Photosynthetic microbes use the energy of light from the Sun to make new cells. Throughout the world's oceans, there are microscopic photosynthetic organisms, the *phytoplankton*, that produce oxygen. They are the forerunners of higher plants but evolved much earlier in Earth's history. After several days, the instrument my research group used to detect phytoplankton, a special type of fluorometer that we had developed years earlier, recorded some strange signals that none of us had ever seen. The signal was quite deep in the water column: just at the location where all oxygen is gone and the light intensity is very low. As we worked, I realized that the organisms responsible for the strange fluorescence signal occupied a very thin layer, perhaps only a meter or so thick. They were photosynthetic microbes, but unlike the phytoplankton higher up in the water column, they could not produce oxygen. These microbes were representatives of an ancient group of organisms that evolved long before phytoplankton. They were living relics of life at the time before there was oxygen on the planet.

Working on the Black Sea had a profound influence on how I think about the evolution of life on Earth. In my mind, sampling deeper into the water column was like going back in time to find microbes that had once dominated the oceans and are now confined to a very small fraction

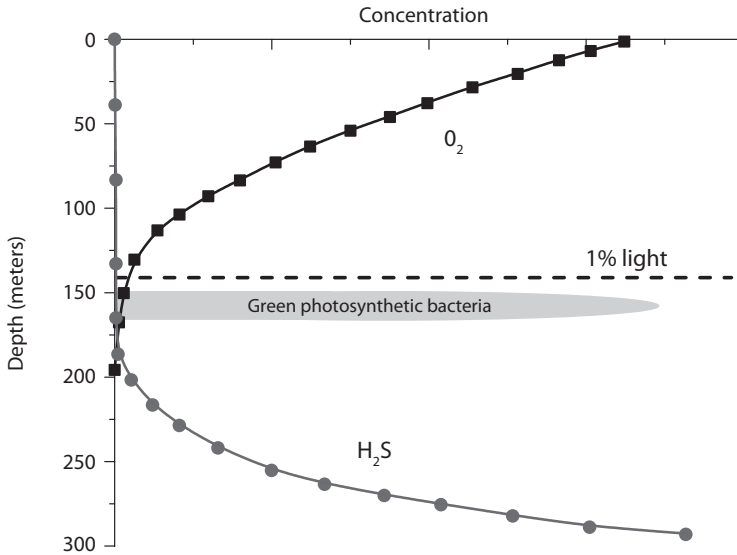


FIGURE 1. An idealized profile of dissolved oxygen and hydrogen sulfide gas (which smells like rotten eggs) in the upper 300 meters of the Black Sea. This body of water is unique in the ocean; in most ocean basins and seas, oxygen is detectable to the seafloor. Just below the depth at which 1% of the sunlight from the surface remains, there is a very narrow layer of photosynthetic bacteria that split the hydrogen sulfide with energy from the Sun, for their own growth. The metabolism of these organisms is extremely old; it probably evolved more than three billion years ago, when oxygen concentrations on the Earth's surface were extremely low.

of their former habitat. The photosynthetic green sulfur bacteria, which turned out to be the organisms responsible for the strange fluorescence signal, are obligate anaerobes; they use energy from the Sun to split hydrogen sulfide ( $H_2S$ ) and use the hydrogen to make organic matter. These organisms can live at very low light intensities but cannot tolerate exposure to even small amounts of oxygen.

As we traversed the Black Sea over the next several weeks to sample different areas, we saw dolphins and fish in the upper ocean, but there were no multicellular animals below the upper 100 meters or so. Animals can't live for long without oxygen, and there appeared to be none

in deeper waters. Microbes had altered the environment of the Black Sea. They produced oxygen in the upper 100 meters but consumed the gas further down. In so doing, they made the interior of the Black Sea their exclusive home.

After about a month at sea, I found myself back in port in Istanbul, admiring Turkish rugs. Mount Ararat, in northeast Turkey, is famous for its woven rugs depicting the story of Noah's ark. The kilims from that region are rich tapestries with pairs of giraffes, lions, monkeys, elephants, zebras, and all sorts of familiar animals woven into them. As the merchants unrolled their wares and provided endless cups of sweet tea, I started to think about how the story of the ark has influenced our distorted understanding of life on Earth. On one hand, the story is about destruction and resurrection. On the other hand, it is about how God made humans stewards of life. In neither case do microbes appear as creators nor destroyers of life.

The word "evolution" literally means "to unroll," but as the merchant unrolled the beautiful rugs for me, I began to see how the biblical story of the ark failed to provide a clue about how life evolved. Was all life on Earth preserved by Noah and taken on the ark? Could some organisms have been left behind? Although the story of the ark is deeply embedded in Western culture, it fails to inform us about the origins of life. To begin to understand the origin of life requires another perspective, one based on science and, especially, its application to the evolution of microbes.

To a large extent, science is the art of finding patterns in nature. Finding patterns requires careful observations, and inevitably we are biased by our senses. We are visual animals, and our perceptions of the world are based primarily on what we see. What we see is determined by the tools we have. The history of science is closely tied to the invention of novel tools that allow us to see things from different perspectives, but ironically, the invention of tools is biased by what we see. If we don't see things, we tend to overlook them. Microbes were long overlooked, especially in the story of the history of evolution.

The first few chapters in the ongoing story of the evolution of life on Earth were written largely in the nineteenth century by scientists

who studied the fossils of animals and plants—fossils they could easily see. The patterns in nature they observed ignored microbial life for two simple reasons: there was no obvious fossil record of microbes in rocks, and the pattern of microbial evolution could not be easily discerned by looking at living organisms. The tools for finding fossils of microbes barely existed; however, even if there had been such tools, the roles these organisms played in shaping the evolution of Earth would not have been appreciated until new tools became available in subsequent decades. The patterns of evolution observed in animals and plants were historically inferred from the shapes and sizes of their fossils and the arrangement of these fossils through geological time. That approach does not work nearly as well when applied to microbes.

In sum, the oversight of microbes, in both the literal and figurative senses, distorted our worldview of evolution for more than a century, and including microbes in our understanding of evolution is still a work in progress. In as much as science is the art of discovering patterns in nature (and that is difficult enough), it is also about discovering patterns that we cannot see with our naked eyes.

But first, let us briefly examine the story of evolution as it emerged in the nineteenth century. That was when many of our scientific concepts about life came to be formed. The ideas were largely based on what could be seen and framed by biblical stories of the creation, including the flood and the story of Noah's stewardship of God's animals, such as the stories woven into Turkish rugs.

In the early 1830s, a gentleman scientist, Roderick Impey Murchison, and a charismatic Cambridge University professor, Adam Sedgwick, reported that there were fossils of animals deep in the ground at a site in Wales. Fossils had been known for centuries, but their significance was not well understood. Many people realized that these were impressions of organisms that had died long ago—but how long ago was not clear, nor was it clear how the impressions were preserved.

Sedgwick was one of the foremost authorities on fossils in Britain, and one of the students who attended his lectures was Charles Darwin. In the summer of 1831, barely yet twenty-two years old, Darwin went with Sedgwick into the field in north Wales to learn about fossils firsthand.

That experience changed Darwin's life forever. Darwin not only helped Sedgwick find fossils of animals in the rocks, he also learned basic principles of geology, and those observational skills would be very useful to him for the rest of his life.

The fossils found in the rocks in England and Wales by Sedgwick and Murchison were also found elsewhere in Europe, and a system of classification based on the sequences of the fossils in the rocks began to take hold. Often the physical appearance of the fossils resembled familiar animals that lived in the oceans, such as clams, lobsters, and fish; however, some of the fossils were incredibly bizarre, and no one had ever seen anything like them in the oceans of the times. There was tremendous controversy about the meaning of the fossils, but the discoveries clearly suggested a sequence of changes in animal forms from lower to higher levels in the layers that these ancient marine sediments formed. At the time, it was generally understood that rocks deeper down in a sequence were older than the rocks above.

The discovery of animal fossils in rocks was hardly new. Probably the most famous early description of fossils was recorded by a Danish scientist, Nicolas Steno, in 1669. He had found objects that looked very much like shark's teeth in rocks in Italy, but how objects from once-living organisms could be so preserved puzzled him. Steno, however, thought carefully about how the fossils were arranged in the rocks. They were arranged in layers, and it appeared to him that the older layers lay below younger layers. This notion, called *superposition*, is one of the primary rules in sedimentary geology and strongly influenced the interpretation of the fossil record by Sedgwick more than a century later. Steno eventually abandoned science and entered the Church to devote his life to God. His early work on fossils was largely forgotten, and he himself believed that life began as it was described in Genesis.

To me, the logic of the idea that the fossils preserved in rocks are arranged in some accordance with time was an extraordinary insight, but it was not easily supported, because basic geological information was not yet available. To a large extent, the effort of finding patterns in fossils awaited the great mind of Charles Lyell, one of Darwin's intellectual mentors and a close friend. Lyell, a Scottish barrister turned naturalist,

is often credited with founding a new area of science, which he called *geology*. Lyell, like Steno, realized that there was a logical sequence in the fossil record; however, unlike Steno, Lyell expounded on geological processes, such as erosion, volcanism, and earthquakes, to help explain the sequences observed in the fossil record. Indeed, his elucidation of the fossils in the rock sequences would later inspire Darwin to muse upon how organisms change over time. The lifelong friendship between Lyell and Darwin was a legendary symbiosis in science.

On December 27, 1831, as Darwin was beginning his voyage on the HMS *Beagle*, a ninety-foot, ten-gun brig with seventy-four people on board, he was allowed to have very few books in the very cramped chart room, which was his assigned sleeping quarters. He slept in a hammock in the 9- by 11-foot room, which had a 5-foot ceiling; it was dark and uninviting, and he had to share the quarters. Among other things, he took with him the first volume of the first edition of Lyell's new book, *Principles of Geology*, which had been published in 1830. He also took his personal copy of the King James Bible. On ships I work on, I have a hot shower every day, and while I sometimes share a small cabin, there is a library on most research vessels. Perhaps, then, it should not be too surprising that Darwin used seasickness as an excuse to leave the *Beagle* at almost every opportunity and to wander across the continents to meet the ship at another port of call.

Lyell took on the hard task of explaining to an interested public how animal fossils could wind up in the Alps in central Europe, as well as in the hills of Scotland and throughout the British Isles. One of the basic problems was time and how the Earth came to be formed.

Several arguments had been put forth over the centuries. One, from medieval times, was that that God made rocks to look like familiar organisms to test the faith of his flock. As absurd as it is, the notion still has many proponents, especially in parts of the United States. A second idea was that in ancient times, volcanoes exploded and carried animals from the oceans onto land, where they died and their skeletons were preserved in the rocks. A third concept was that the animals died after the Great Flood, when the sea level dropped. Indeed, this diluvian origin of fossils appealed to Sedgwick himself. There were several other

ideas, which Lyell recounted eloquently and with precision, as a barrister might present a case to a jury.

Lyell proposed the radical idea that the fossils from marine animals were found in rocks on land because a long time ago the rocks were under water. Over time, the rocks were somehow uplifted and deposited on land. That notion, tested many different ways, is actually correct, but the processes responsible would not be uncovered until more than a hundred years later. One of the major problems Lyell faced was accounting for the age of the Earth. How long was “a long time ago”?

The age of the Earth had been meticulously calculated by James Ussher, the Archbishop of Amargh, in the book *Annales Veteris Testamenti*, which was published in 1654. It was taken by virtually every educated British citizen as the most accurate estimate of the time of creation. On the basis of a literal interpretation of the Bible, Ussher had determined that the Earth was formed at nightfall of the Sunday preceding October 23, 4004 BCE in the Julian Calendar; that is, about 6000 years ago.

As a student of law, Lyell had been trained in argumentation and was amused by some of the illogical and sometimes irrational thought processes used to explain the existence of and changes in fossil animals. He understood the power of argumentation and wrote that “the system of scholastic disputations encouraged in the Universities of the middle ages had unfortunately trained men to habits of indefinite argumentation, and they often preferred absurd and extravagant propositions, because greater skill was required to maintain them; the end and object of such intellectual combats being victory and not the truth.” But even talented barristers can’t win arguments against the written word of God.

Lyell didn’t know anything about how evolution might work, let alone how to measure geological time. He thought Jean-Baptiste Lamarck’s theory—that traits were acquired by animals during their lifetime and somehow passed on to future generations—was as good as any and more rational than most. Indeed, Lamarck’s work on animal forms (he was the world’s leading authority on animals without backbones—the invertebrates) led to him to propose that organisms could be arranged along a chain in time, from the simplest to the most complex forms. Lamarck set in motion the idea that organisms somehow change—that is,

evolve—over time. Indeed, although now largely unjustifiably ridiculed or ignored in biology texts and classes, Lamarck was the intellectual father of a field he called *biology*.

The idea that fossils of animals were arranged in layers of rocks along an arrow of time got Darwin thinking about life on time scales he could barely imagine and could not easily quantify. If the oldest fossils were many meters beneath other fossils, how long had it taken for the layers of rock to build up?

Darwin was extremely puzzled by the early fossils that Murchison and Sedgwick had found. He knew that beneath the layers of rocks with fossil animals were layers that contained no fossils, but he could not understand why. The record of animals appeared to come out of nowhere, and their evolution appeared to be relatively rapid. But how rapid? And why, all of a sudden, were there fossils of fish, but in the rocks below there were only organisms that looked like invertebrates? And even further below, why were there no fossils of animals at all? It was the geological equivalent of unrolling a Turkish rug depicting the story of the ark, but half or more of the rug had no animals. Darwin needed to explain these issues first to himself and then to his colleagues. To answer these questions, he needed to try to date the rocks, and for that he needed a clock.

On September 7, 1859, the bells in the clock tower housing Big Ben rang for the first time. The clock was meticulously designed and is extraordinarily accurate; indeed, it is an iconic symbol of English engineering and craftsmanship at the dawn of the Industrial Revolution. Two months after that historic event, on November 24 to be precise, John Murray, III, the venerable London publisher on Albemarle Street, sent Charles Darwin's new book, *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*, to market.

In Chapter 9 of *The Origin of Species* (the title was later shortened), Darwin attempted to account for the time required for extinct fossil animals to have changed, or evolved, to become the modern forms. The problem was not straightforward. Lyell and his predecessor, the Scottish physician James Hutton, had proposed that the Earth was infinitely old. Darwin did not know whether that concept was true, but he certainly believed that it had to be more than 6000 years old. To obtain a more



realistic age, he developed a rather interesting, if not downright ingenious, approach to measuring geological time.

Darwin's clock was based on a geological phenomenon: the rate of erosion of sedimentary rocks, the kind that contain fossils. He specifically chose the Weald, a well-studied chalk and sandstone cliff abutting the sea in Kent, England. Darwin calculated that this formation eroded about one inch per century, and based on the size of the formation at the time, he calculated that the "denudation of the Weald must have required 306,662,400 years; or say three hundred million years."

Darwin didn't account for the time required for the formation of the cliff itself, but that was a detail. Moreover, he didn't account for the rocks below the Weald, which would have only made the age of the cliff even older, and possibly infinitely old, as thought by Lyell. Darwin's estimate of the age of the cliff certainly was a bold speculation, and without a better constraint, it was apparently based on a rational, physically verifiable idea. The implication was obvious. The Earth was very old. It was much, much older than Ussher had calculated, and it was a lot older than most people could possibly have imagined at the time. And while the date of the origin of life on Earth had not been determined (and remains unclear to this day), that there were rocks that had no fossils beneath those above implied that Darwin's estimate of the age of the Earth was conservative.

Regardless, millions of years are not the history in the Bible, and they certainly didn't fit what everyone had been taught at school. Darwin clearly knew his estimate was going to be met with skepticism, but he had no way of knowing what was to come. Besides assaulting the Biblically held, seventeenth-century calculations of the Archbishop of Armagh, Darwin's estimated age of the Earth was assailed by a fellow scientist, the Einstein of the day, the physicist William Thomson, later to become Lord Kelvin. Thomson set out to put the record straight, based on first principles of physics.

Thompson argued that the age of the Earth could be accurately determined by assuming that the planet began as a molten rock and subsequently cooled. Using measurements of the change in temperature with depth through the Earth's crust and experiments he performed about the conduction of heat by rocks, he developed an equation for how fast

the Earth had cooled to its present state. In 1862, Thomson proclaimed that the Earth was about 100 million years old. He admitted a huge uncertainty of between 20 and 400 million years, but as time went on, he became increasingly dogmatic and convinced that the actual age was closer to 20 million years. This estimated age appeared to be too short to allow evolution, as Darwin envisaged it, to proceed. Thomson became one of the harshest critics of Darwin's new ideas regarding evolution, not because he did not believe in evolution *per se*, but rather, because as a physicist, he did not believe the calculations of the age of the Earth based on geological processes such as rates of erosion. Ultimately, the contrarian views of Thomson forced geologists to develop better models for the age of the Earth, but doing so would take almost another century.

If Darwin was even remotely correct, then life evolved on Earth over a very, very long time—much, much longer than anyone imagined. But how did it evolve? In a doodle on page 36 in Notebook B from 1837, Darwin sketched a tree of life in which he had the radical idea that organisms were related to each other from a common ancestor and that their relationship could be discerned from similarities in physical appearance. That basic notion was similar to Lamarck's concepts that had been developed more than fifty years earlier; however, Darwin had a different idea as to how the process occurred.

The changes in the animal forms were subtle and, based on the distance between fossils in the rock record, appeared to be slow. In addition, for the proposal to work, some organisms that appeared earlier in the fossil record had to go extinct and be replaced by new species, or the Earth would have an ever-increasing number of animal and plant species. In other words, once an organism becomes extinct, it should never reappear later in the fossil record.

Darwin realized that this remarkable, revolutionary idea would be challenged; and so it was. The fossils were clearly relics of animals and plants, but there were no bones of humans in the rocks. If that were true, then Darwin clearly understood the implications of the “missing” humans; like animals in the fossil record, we must also have arisen by some process that allows one organism to evolve into another over some undefined, but prolonged, time.

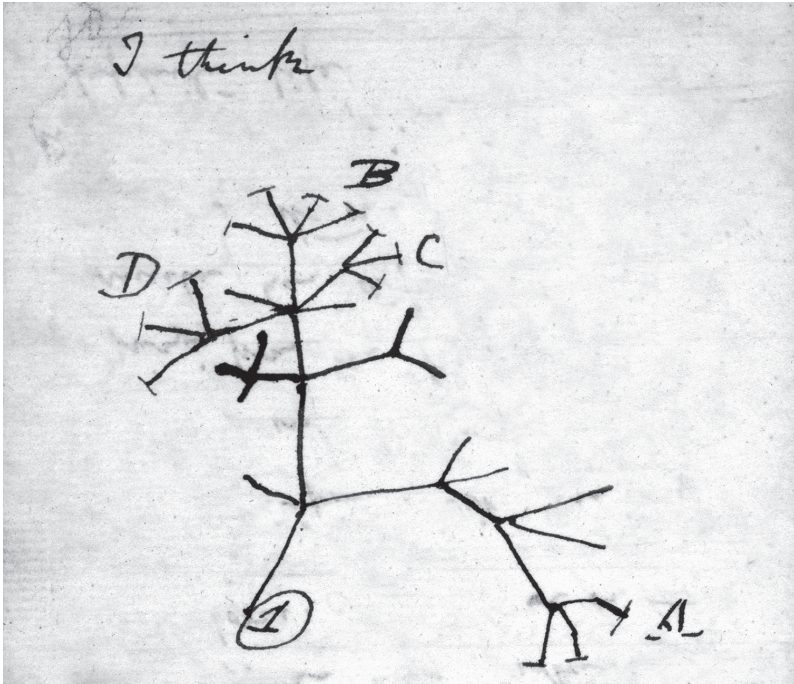


FIGURE 2. A reproduction of the doodle that Darwin sketched in Notebook B between 1837 and 1838. The basic idea is that extant species are descended from extinct species but are also related to other extant species to form a historical tree of life. This doodle was the kernel for the theory of descent with modification followed by selection—the core of Darwinian evolution. (With permission from Cambridge University Press and thanks to Peter and Rosemary Grant. Copyright © 2008 The Committee for the Publication of Charles Darwin's Notebooks.)

The concept of genes and the basis of the physical inheritance of traits were totally unknown to Darwin, or anyone else at the time. (Gregor Mendel would not present his work on inheritance of traits in peas until more than six years after the publication of the first edition of *The Origin of Species*, in 1866). Indeed, despite the confusion in most biology texts, Darwin would not have had a major problem accepting Lamarck's basic concept that organisms can inherit traits from their environments; however, Darwin's major contribution was the idea

that within all species there is natural variation that can be selected. Breeders of dogs and pigeons did this all the time. However, in nature, Darwin proposed that the traits are selected by the environment in which the species lives. Selection either enhances the ability of the organism to reproduce, or not. If it does, then the traits most suitable for the particular environment are passed on to subsequent generations. The concept of descent with variation followed by selection occupies six chapters in the *Origin*. It was one of the most remarkable scientific ideas ever put forth, and to this day, it remains a core, unifying principle of biology.

There is a single illustration in the *Origin*, at the end of the book, of a hypothetical origin of taxa, which is loosely based on the doodle from Notebook B. Curiously, the figure does not show a single origin for all taxa but, rather, many origins giving rise to new species. The concept of origin, as in the origin of life, was in the back of Darwin's mind but not explicitly discussed in the book.

More than a decade after the publication of the *Origin*, in a letter to Joseph Hooker dated 1871, Darwin mused that life arose in a "little warm pond with some sorts of ammonia and phosphoric salts,—light, heat, electricity, etc. present, that a protein compound was chemically formed, ready to undergo still more complex changes, at the present day such matter would be instantly devoured, or absorbed, which would not have been the case before living creatures were formed."

Eighty years after that notion was put forth, a young chemist, Stanley Miller, and his Nobel Laureate advisor, Harold Urey, actually did make amino acids (the building blocks of proteins) in a laboratory at the University of Chicago. They used ammonia gas, methane, hydrogen, and water and a spark to simulate lightning. That experiment, which was published in 1953, gave great hope that an understanding of the origin of life was imminent. However, there is a huge gap between making the chemical constituents of organisms and making the organisms themselves. In even the simplest organisms, the chemical constituents are organized into microscopic machines that give rise to metabolic processes and allow the cell to replicate. No one has yet created a living organism from scratch, but that is not to say it is impossible.

The simplest organisms are microbes, organisms of which Darwin was surely aware but not certain how to accommodate in his theory. Indeed, Darwin took a microscope with him on the *Beagle*. (Along with his Bible and natural history books, he also took two pistols, twelve shirts, two books to help him learn Spanish, and a coin purse.) But because microbes do not leave a fossil record that is clearly visible to the naked eye, Darwin could not have known that the rocks beneath the visible fossils were not from a period in Earth's history that was before the origin of life—but rather a period before animals or plants. Even if Darwin had observed fossil microbes, he would almost certainly not have understood what their relationship to plants or animals was. Darwin, and virtually every other scientist in the nineteenth century, would have been profoundly surprised to learn that plants and animals had all descended from microbes over a period of time that was completely unimaginable in the nineteenth century—far longer than 300 million years. Indeed, microbes are not mentioned in the Bible, except indirectly in reference to diseases like the plague. They certainly weren't deliberately taken by Noah on the ark, nor are they woven into the Turkish tapestries depicting the story of the Great Flood.

While we have made great progress in the 150 years since the publication of the *Origin*, scientists are still struggling to understand whether life began in a small warm pond, at a deep-sea hydrothermal vent, or somewhere else. How might it have started? How did it get going? How did microbes lead to the evolution of plants and animals? How were these organisms missed for so long in our search for the origins and evolution of life?

The answers to these questions are complex, and many aspects are still far from fully understood, but we have learned a lot because of the tools developed during the last century. If Darwin had been on an oceanographic research voyage in the Black Sea early in the nineteenth century, he might have observed that there were no animals below the upper hundred meters and concluded that the deep water was lifeless. But had he been a microbiologist, our understanding of the origins of species would have been very different. Although microbes were well known in the nineteenth century, it took another century before they

were included in our understanding of the evolution of life on Earth. Microbes were missed because of our observational biases. They had been on this planet for billions of years before the first animal arose.

Let's meet the missing microbes and see how they played an outsized role in making this planet function. Without microbes, we would not be here.