9 | Fossils of Early Eukaryotes

Eukaryotic organisms have evolved patterns of cell shape and multicellularity unknown in bacteria and archaeans. Fossils with these features suggest that eukaryotes arose early, but emerged as prominent participants in marine ecosystems only late in the Proterozoic Eon, perhaps aided by renewed oxygen increase in the world's oceans.

THE YOUNG MINER taps insistently on my shoulder, gesturing vigorously and shouting (in Chinese) what must surely be instructions. He points toward a nearby truck—half a dozen workers have already dived beneath it. Being no fool, I follow their example, and seconds later an explosion jolts the ground, followed by a hail of rock debris that dents the cab above my head.

We are in Guizhou Province in southern China, visiting one of the many phosphate mines that pock the rugged landscape (figure 9.1). Phosphates find use in fertilizer, and the Guizhou mines play an integral role in the regional economy. What the miners don't know is that Guizhou phosphate, spread on fields from Kunming to Kalamazoo, contains some of the most exquisite fossils ever found in Proterozoic rocks. Moreover, the fossils are predominantly eukaryotic. Sometime between the deposition of the Great Wall and these younger phosphatic rocks, nucleated organisms broke the 2-billion-year ecological hegemony of the bacteria. Prokaryotes didn't go away, of course. They remain the foundation of all functioning ecosystems on this planet. But algae joined and then supplanted cyanobacteria as the principal primary producers in the oceans, and protozoans able to engulf microscopic victims added the complexity of predation and herbivory to food webs.

Figure 9.1. Fossiliferous phosphate rocks of the Doushantuo Formation, exposed in a quarry at Weng'an, China.

Guizhou fossils provide a great introduction to the Proterozoic history of eukaryotes.

The Guizhou phosphates lie along the thin edge of a massive wedge of sedimentary rocks deposited in southern China near the end of the Proterozoic Eon. To the north, where they are exposed in the spectacular Yangtze Gorges, these rocks can be divided into four units that lie one atop another. At the base is a discontinuous blanket of

red sandstones formed by meandering streams as they traversed a coastal plain; volcanic ash found in a thin layer within the sands yields a U-Pb age of 748 ± 12 million years. At the top of the succession is a thick cover of limestones and dolomites that contains Early Cambrian fossils in its uppermost part. In between are two units of particular interest: one provides a record of extreme climate, while the other contains spectacular fossils that have reshaped our understanding of Proterozoic life.

The lower unit, called the Nantuo Tillite, lies directly above the beds of red sandstone. A poorly sorted mixture of boulders, sand, and silt, this formation is widely distributed in southern China. Running water tends to separate sediment particles of differing size and density, so the intimate mixing of silt and football-size boulders suggests a different means of transport—ice. Other sedimentary features confirm a glacial origin for Nantuo rocks. For example, dropstones—isolated pebbles and cobbles plunged into finely laminated silts and muds—record icebergs that rafted coarse debris out onto the ocean before melting and dropping their rocky cargo onto fine-grained sediments below. Pebbles in the tillite display deeply incised striations formed by grinding as rock-studded ice moved across the landscape. Glacial rocks can be seen in younger Proterozoic successions around the world. As we shall see in chapter 12, they provide evidence for a series of ice ages so severe that life itself may have hung in the balance.

As the glaciers melted, rising seas began to deposit the second unit of interest—the fossiliferous Doushantuo Formation. In the Yangtze Gorges region, nearly 1,000 feet of shale, phosphatic rocks, and carbonates accumulated during two cycles of sea-level rise and fall. To the southwest, closer to the ancient shoreline, the formation thins and changes character. In Guizhou, where I got my lesson in mining safety, it is only 140–160 feet thick and consists mainly of phosphatic rocks deposited in near-shore marine environments. Only a few miles farther west, a mere 16 feet of phosphatic sandstone document this interval. To date, no volcanic rocks have been discovered in these beds, but experimental dating based on radioactive uranium and lutetium locked into phosphate crystals as they formed suggests an age of 590 to 600 million years. Encouragingly, this age falls within the range of estimates for Doushantuo deposition (younger than 600 million years and older than

555 million) based on the correlation of its fossils and chemical signatures with those of well-dated successions elsewhere.

The eukaryote-rich rocks of the Doushantuo Formation, then, are much younger than the Great Wall dolomites, and they postdate the fossiliferous rocks of Spitsbergen, as well. Indeed, these rocks are only 50 to 60 million years older than the Cambrian cliffs along the Kotuikan River.[1] Perhaps, then, Doushantuo fossils will not only illuminate the rise of nucleated organisms but hint, as well, of further biological transformation about to begin.

How do we identify a fossil as eukaryotic? For plants and animals, the distinction is easy—no bacteria or archaeans build anything like a leaf or a shell. Microscopic fossils, however, can be more challenging. Biologists find it easy to distinguish between prokaryotes and eukaryotes based on myriad features of cellular organization, genetics, and physiology, but none of these features is available to paleontologists. We have to rely on form.

When Precambrian research was young, paleontologists tried hopefully to recognize eukaryotic microfossils on the basis of size or preserved features of cell biology. Neither worked. It is easy to see the attraction of size—on average, eukaryotic cells are larger than bacteria, and diameter is simple to measure. At the extremes of the scale, size can indeed be informative—bacterial cells more than a millimeter long are unknown, and neither do we know of eukaryotes only 300 nanometers across.[2] But at intermediate sizes (commonly encountered in Proterozoic rocks) there is strong overlap between bacteria and eukaryotes. Tiny green algae in the open ocean are less than a micron in diameter, and—

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[1] My friend Dick Bambach objects to the word "only" in this sentence, reminding me that 50 million years is a very long time. Indeed it is. In 50 million years we could run the history of Egypt, from the pyramids to modern Cairo, more than 10,000 times. Two million human generations could come and go, and so could more than a billion generations of amoebas. My sentence is meant to convey the fact that *relative* to the enormous span of time that separates Great Wall and Doushantuo deposits, 50 million years is pretty short. But Dick's point is a good one. Every now and again, we should sit back and contemplate the immensity of the canvas on which life's early history is painted.

[2] A nanometer is 10^{-9} meter, or one-thousandth of a micron. A 300-nanometer cell would thus be less than a third of a micron long.

recalling those cigar-shaped fossils in Kotuikan cherts—the resting cells of cyanobacteria can be well over 100 microns long.[3] Cyanobacteria that form extracellular envelopes complicate the picture further, because a colony of 10-micron cells can be enclosed in a preservable coat 100 microns across.

If size isn't foolproof, what about preserved details of cell biology? One of the first Proterozoic fossil assemblages to be discovered was that of the 830–810-million-year-old Bitter Springs Formation in central Australia. Bitter Springs fossils occur in chert nodules within carbonates deposited in ephemeral lakes on an arid coastal plain. The cherts contain beautiful cyanobacteria described by UCLA's Bill Schopf, as well as simple spherical fossils about 10 microns in diameter. Some of these spheres are hollow and were originally interpreted as cyanobacteria. Others, although essentially identical, contain small, dark inclusions of organic matter and so were interpreted as eukaryotic algae with preserved nuclei. Nuclei are mostly water, along with highly nutritious proteins and nucleic acids. In consequence, they are quickly and completely obliterated soon after death—so completely that in the entire fossil record only a handful of plausible fossil nuclei have ever been identified. On the other hand, decomposing cyanobacteria and algae commonly contain small balls of organic matter formed as cell contents shrivel. Decaying cytoplasm provides a satisfactory explanation for the "black spots" in Bitter Springs and other Proterozoic microfossils. Some of these fossils may be eukaryotic, but (unlike leopards) we can't tell them by their spots.

What really makes eukaryotic fossils stand out is *morphology*. In chapter 3, we noted that some cyanobacteria have cell shapes and colony forms not duplicated by other bacteria. Similarly, some (but not all) eukaryotic cells display features that are unknown in prokaryotic organisms. Doushantuo fossils illustrate how paleontologists recognize and interpret early fossil eukaryotes.

In and around the Yangtze Gorges, chert nodules occur at two levels within the Doushantuo Formation. The lower cherts are richly fossil-

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[3] The largest bacteria currently known are sulfur-oxidizing cells found in sediments off the coast of South Africa. These giants reach diameters of 500 microns or more, although, in a way, they cheat—the cells are hollow.

iferous, mostly preserving tightly interwoven populations of mat-building cyanobacteria. The upper cherts also contain cyanobacteria, but additionally include distinctively different microfossils. These latter remains are broadly spherical, they are commonly large (up to 600 microns), and—most telling—they display flamboyant ornamentation. Some look like tiny suns, with raylike arms extending in all directions (figure 9.2). Others are festooned with spines, flanges, or knobs. Bacteria don't produce structures like this, but several groups of eukaryotic organisms do. Thus, we have confidence that, using Doushantuo fossils as handholds, we can climb our own branch of the Tree of Life.

The exact biological relationships of these exquisite fossils are unknown, but most appear to be the discarded spore coats of algae. Similar remains are known from Australia, Siberia, Scandinavia, and India; they record a global diversification of marine life in the aftermath of widespread glaciation. Remarkably, this diversification was short-lived. For reasons still under debate (but possibly tied to one last expansion of Proterozoic ice sheets), nearly all of these exuberantly ornamented fossils disappeared within a few million years of their appearance—victims of one of Earth's earliest-known mass extinctions.

There is more treasure in Doushantuo rocks. In 1990, the Chinese paleontologist Chen Menge discovered a second, very different fossil assemblage in black shales high up in the cliffs that line the Yangtze Gorges—fossils large enough to be seen by the naked eye, preserved in great numbers as organic films compressed onto bedding surfaces (plate 5a). Once again, distinctive morphologies indicate that many of the thirty or so populations found so far in these rocks represent eukaryotic algae. In some cases, their biological details are so well preserved that we can almost reanimate them. For example, my former student Shuhai Xiao, now at Tulane University, was able to reconstruct common compressions called *Miaohephyton* (literally, the alga from Miaohe) as seaweeds that formed lawns on the ancient seafloor. Thin, grasslike blades stood erect in the water, anchored by rootlike holdfasts to the muddy bottom—in my mind's eye, I can see them sway gently in slack currents. As these algae grew, they branched now and again by splitting in two at their tips. Reproductive cells formed in wartlike structures that line the upper parts of mature individuals, and disper-
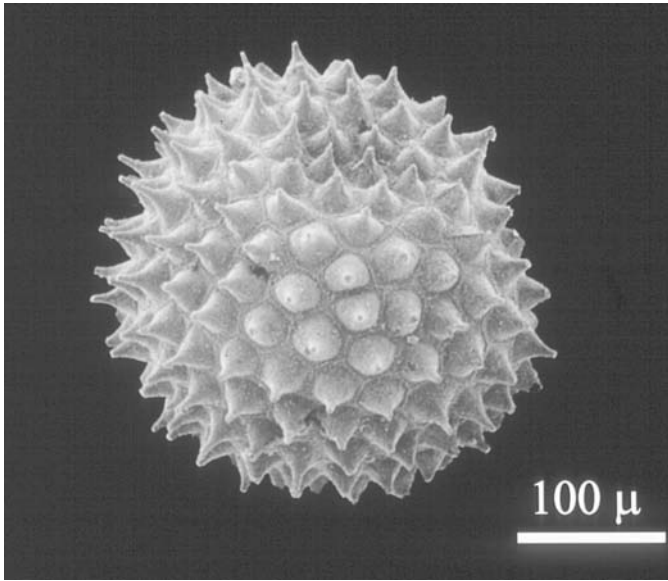
Figure 9.2. A spiny microfossil found commonly in Doushantuo cherts and phosphatic rocks. Such fossils are thought to be the reproductive spores of eukaryotic organisms. Fossil is 250 microns in diameter. (Image courtesy of Shuhai Xiao)

sal occurred both by the release of spores into the water and by the fragmentation of branches along preformed abscission surfaces. A comparable combination of characters can be found today in some brown algae, providing functional and, possibly, genealogical clues to the Doushantuo fossils.

The Maiohe fossils were preserved by rapid burial in fine-grained sediment. Bacteria normally decompose algal tissues soon after death, but at Maiohe they were prevented from completing this task by a veneer of clay that both excluded oxygen and adsorbed the enzymes that break cells apart. As sedimentary layers accumulated, biological remains were pressed like flowers between the pages of a book.

Leaves are commonly compressed in younger mudstones formed in lakes or the floodplains of rivers. In marine rocks, however, organic compressions are rare because burrowing animals irrigate and churn mud and silt layers. Rare, but not unknown. In fact, the most famous of all fossil deposits, the Middle Cambrian Burgess Shale, formed by the

compression of animal carcasses in carbon-rich mudstones.[4] Burgess fossils postdate Doushantuo deposition by only 50–85 million years; thus, the Doushantuo compressions are noteworthy for what they lack as well as what they include. One population of flanged tubes may record simple, sea anemone–like invertebrates (plate 5b), but nowhere do we find evidence of the anatomically and morphologically complex animals that are so conspicuous in Burgess and other Cambrian glimpses through the same preservational window. Evidently, much happened between Doushantuo and Burgess time.

I became involved in the study of Doushantuo fossils at the invitation of Professor Zhang Yun, a kind, cultured, and wonderfully insightful paleontologist at Beijing University. During the 1980s, Zhang collected Doushantuo samples from a phosphate mine near the Guizhou village of Weng'an. Several contained multicellular algae. In 1992, to my ever-lasting good fortune, he asked me to join him in collaborative research. By coincidence, a second Chinese friend, Yin Leiming of the Nanjing Institute of Geology and Palaeontology, invited me to visit China at about the same time. Yin was working on Doushantuo fossils in cherts from the Yangtze Gorges and Zhang was investigating assemblages from the phosphate mines of Guizhou, so I suggested that the three of us join forces to try to understand the full diversity of Doushantuo eukaryotes. Shuhai Xiao, who had completed his bachelor's degree in Beijing under Zhang's guidance, entered Harvard as a graduate student the same year, completing our team.

Doushantuo cherts and shales open two distinct and unusually clear windows on late Proterozoic life, but those Guizhou phosphates provide a third view, even better than the others. In this corner of the shallow Doushantuo seaway, biological remains that entered surface sediments were coated almost immediately by minute crystals of calcium phosphate minerals, preserving both overall morphology and cellular anatomy in remarkable three-dimensional detail. As a result, the Doushantuo phosphates preserve organisms seldom seen in rocks of any age.

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[4] Discovered by Charles Doolittle Walcott in 1909, the Burgess Shale is renowned for its compressions of Cambrian animal remains. See chapter 11 for discussion.

Like cherts along the Yangtze River, the Guizhou phosphates are chock-full of highly ornamented eukaryotic microfossils; in some beds these fossils are so abundant that they literally form sandstones made of phosphatized cells. Other fossils are multicellular, and once again details of morphology show that most represent tissue-forming algae, not bacterial colonies. Particularly informative are small, crust-forming structures made of thick-walled cells arranged in rows that fan outward, like water as it gushes from a spring (plate 5d and e). Biologists call this anatomical organization a "cell fountain," and it is especially common in red algae. Distinctive reproductive structures and anatomically distinct inner and outer tissues strengthen the ties between these fossils and a particular group of reds called the corallines. (Doushantuo fossils don't display all of the features that collectively define coralline algae, but they exhibit enough of them to suggest that these small phosphatic fossils record an early way station in red algal evolution.) Other Guizhou fossils seem to lie halfway between the two major branches of living red algae, again suggesting that Doushantuo phosphates captured the diversity of red algae *in statu nascendi*—as it began to unfold.

Taken together, compressed and phosphatized Doushantuo fossils show that by the time large animals appeared in the oceans, multicellularity was already well established among the algae.

It is exciting to discover algae with cells preserved intact, but the crown jewels of Doushantuo are undoubtedly small balls, 400–500 microns in diameter, found in phosphates near the village of Weng'an (figure 9.3). The balls are uniform in size. Some contain a single cell wrapped in a thick furrowed coat, while others contain multiple cells surrounded by a thin membrane—cells in pairs, quartets, octads, and larger powers of two arranged in a geometric pattern determined by precisely oriented cell divisions. Parts of this division series were reported by Chinese paleontolgists in 1995 and interpreted as colonial green algae, but size, geometry, and envelope formation collectively make such an interpretation unlikely.

Shuhai Xiao discovered new and more informative populations that enabled him to recognize them as animals—specifically, animal eggs and embryos in the early stages of growth. Fossil embryos are rare in the geological record, but they do occur in Cambrian rocks. In fact, some beautiful Cambrian embryos described a year earlier by Stefan Bengtson
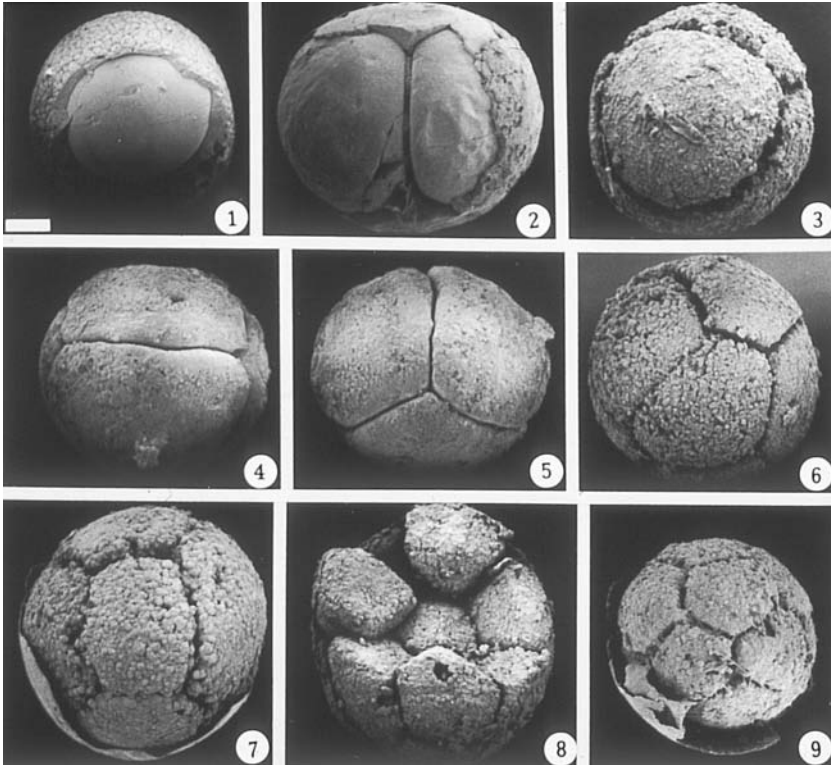
Figure 9.3. Eggs and embryos of early animals, preserved in Doushantuo phosphate. Each fossil is 400–500 microns in diameter. (Images courtesy of Shuhai Xiao)

of the Swedish Museum of Natural History and his Chinese colleague Zhang Yue were Shuhai's inspiration to keep his eyes wide open when sorting Doushantuo fossils.

Later growth stages have not yet been uncovered, so we don't know what kind of adults might have developed from the Doushantuo embryos. Among living animals, arthropods and related invertebrates most closely approximate the egg case and cell cleavage patterns displayed by these fossils, but this doesn't mean that recognizable arthropods plied the Doushantuo seaway. In parallel with Doushantuo shales, Guizhou phosphates contain possible sponges and small tubes likely made by simple coral-like organisms (plate 5c), but they display no evidence of arthropods or any of the other anatomically complex animals found in phosphatized Cambrian rocks. Around 590–600 million years

ago, then, animal evolution may have begun, but the age of animals was still to come. Doushantuo fossils preserve the smoldering fuse of an evolutionary explosion about to begin.

The Doushantuo Formation is a paleontological wonder, our closest approximation yet to a Precambrian Burgess Shale. Zhang Yun, the quiet pioneer who gave us so many of these remains, died in 1999, but his students continue to plumb Doushantuo rocks for new fossils and further insights into early evolution. The prospect that this paleobiological mother lode will be exhausted in my lifetime is remote.

Thinking back on the fossils found in Great Wall cherts and shales, it becomes clear that biology changed radically between 1.5 billion years ago, when the Siberian rocks were deposited, and 590–600 million years ago, when Doushantuo sediments formed. And further remarkable events were imminent, even as Doushantuo rocks accumulated in South China. In the following chapters, we will explore what came next. But, for now, the task is to fill the evolutionary gap between the Great Wall and Doushantuo biotas.

Multicellular red and green algae are common in Doushantuo assemblages. As we first learned in chapter 3, green algae closely related to the extant genus *Cladophora* occur in 700–800-million-year-old shales in Spitsbergen. Indeed, microfossils interpreted as spores of planktonic green algae suggest that the "greening" of the oceans began at least a billion years ago.

Red algae also have a long Proterozoic history. The oldest fossils that can be compared with confidence to any living eukaryotes are beautifully preserved filaments found by Nick Butterfield in cherts about 1.2 billion years old from Somerset Island in arctic Canada (plate 6a and b). Each filament in Nick's population consists of aspirin-shaped cells about 50 microns wide, aligned in a row. The cells are defined by thin, dark walls and united by a thicker but lighter outer wall layer. Cells are clearly grouped into pairs and pairs-of-pairs, providing evidence that these organisms grew by cell division within (rather than at the ends of) the filaments. Cells at the basal end are differentiated into holdfasts that anchored filaments to firm sediments on an ancient tidal flat. Another type of cell division is apparent in some filaments, and it is an unusual one; the aspirin-shaped cells sometimes divided repeatedly to form small reproductive bodies that resemble wedges of pie (plate 6b).

Collectively, these features ally the Somerset fossils with simple red algae (plate 6c and d). This means that the reds must have diverged from other eukaryotes, acquired photosynthesis (via endosymbiosis, as explained in the preceding chapter), and evolved a simple form of multicellularity by at least 1.2 billion years ago. Thus, both reds and greens appeared more than a billion years ago and diversified dramatically by 600 to 590 million years ago. Even heterokont algae born of secondary endosymbiosis may have differentiated early on. Fossils from the Lakhanda Formation in southeastern Siberia contain simple branching filaments comparable in morphological detail to the living heterokont *Vaucheria*. Lakhanda beds are cut by (and, so, are older than) igneous rocks dated at 1,003 ± 7 million years.

Other fossils strengthen the view that eukaryotes rose to prominence during the second half of the Proterozoic Eon. For example, microscopic fossils with distinctly eukaryotic spines or other ornamentation first appear in rocks about 1.2–1.3 billion years old and become increasing commonly as we ascend through the late Proterozoic record (plate 6e–g). More specifically, late Proterozoic biomarker molecules and (more controversially) microfossils record the presence of dinoflagellates, members of another major group of eukaryotes. Other biomarkers extracted from 750-million-year-old shales deep within the Grand Canyon suggest the presence of ciliate protozoans—phylogenetic cousins, actually, of the dinoflagellates.

Grand Canyon rocks document the early budding of one more branch on the eukaryotic tree. Distinctive vase-shaped microfossils entered my narrative early, in the discussion of Spitsbergen cherts and shales. Such fossils are common in upper Proterozoic rocks, often occurring in remarkable numbers, and nowhere are they more abundant than in the Grand Canyon. Working with exquisite populations preserved in carbonate nodules just beneath a bed of volcanic ash dated at 742 ± 6 million years, Harvard student Susannah Porter has demonstrated that these tiny vases were constructed by testate amoebas—amoeboid protozoans that live inside a minute shell, or test, of their own making (figure 9.4).

This discovery fascinates me because it sheds light on aspects of late Proterozoic ecology. Most of the microfossils discussed in this and previous chapters document photosynthetic organisms, either cyanobacte-
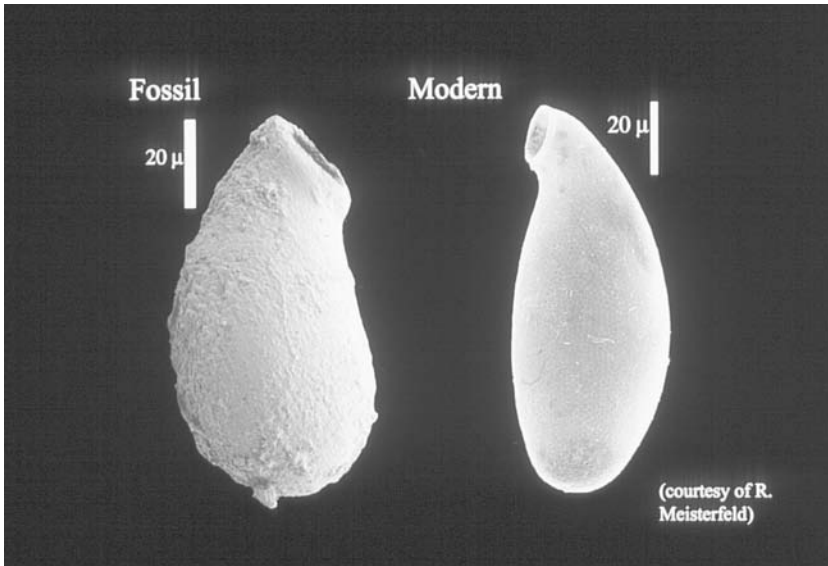
Figure 9.4. Vase-shaped fossil from ca. 750-million-year-old rocks of the Grand Canyon compared with a modern testate amoeba. Note scale on photo. (Figure courtesy of Susannah Porter)

ria or algae. Even the unusual fossils in Gunflint cherts were autotrophic, although they used chemical rather than solar energy to fuel cell growth. In contrast, the vase-shaped organisms were protozoans—heterotrophic eukaryotes that made their living by preying on other microorganisms. The vase-shaped microfossils, thus, tell us of growing ecological complexity in late Proterozoic oceans. Algae and cyanobacteria formed the nutritional base of ecosystems, providing food for untold bacteria. The testate amoebas dined on these algae and bacteria. Moreover, a few vases display hemispherical perforations likely made by other protozoans intent on eating *them*. So, by 750 million years ago, eukaryotes had begun to construct the complex food webs that today form a crown—intricate and unnecessary—atop ecosystems fundamentally maintained by prokaryotic metabolism.

Spiny unicells, multicellular microfossils, compressed macrofossils, eukaryotic biomarker molecules—all can be used to trim the eukaryotic tree with ornaments of time (figure 9.5). They show that as the long Proterozoic Eon moved into its final phase, Earth was becoming a eukaryotic planet.
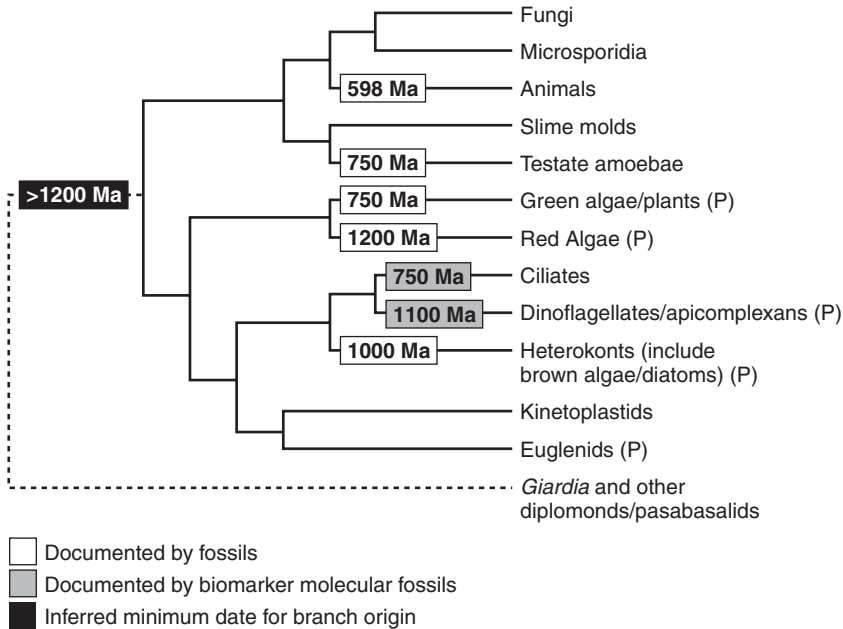
Figure 9.5. The eukaryotic phylogeny first shown in figure 8.2, here trimmed with the dates of early eukaryotic fossils.

In contrast to the cyanobacteria discussed in previous chapters, most eukaryotic fossils don't range through immense intervals of time. Rather, late Proterozoic algal and protozoan species appear in the record, persist for a discrete period, and then disappear, never to be seen again. The familiar pattern of punctuated equilibrium suggests evolutionary dynamics much like those of younger plants and animals, but enacted at a more leisurely pace—the stratigraphic ranges of many Proterozoic eukaryotes appear to be much greater than those of Phanerozoic species. In general, eukaryotic diversity increased through the late Proterozoic Eon and into the Cambrian, but the progress of diversification was halting, punctuated by several intervals of widespread extinction. As noted at the beginning of this chapter, at least some extinctions appear to be linked to latest Proterozoic climatic change.

There is a practical side to this evolutionary pattern—the eukaryotic microfossils in upper Proterozoic rocks can be used to tell time. Boris Timofeev, another of the Russian geologists charged with sorting out the Proterozoic rocks of Siberia, first recognized this potential. But it was

Gonzalo Vidal, a gregarious Spanish-cum-Swedish paleontologist from Uppsala University, who convinced initially skeptical geologists that Proterozoic eukaryotes came and went in a time-ordered fashion. Beginning with sandstones and shales exposed along the shores of Lake Vättern in south-central Sweden, Gonzalo discovered planktonic microfossils in upper Proterozoic rocks throughout Scandinavia. When he demonstrated how the fossils brought stratigraphic order to these rocks, the modern era of Proterozoic biostratigraphy was born.

As noted in the previous chapter, debate about eukaryotic phylogeny continues, but most disagreement focuses on the identity and character of the tree's early branches. There is widespread agreement that much of the eukaryotic diversity seen today began to accumulate during a relatively short interval of rapid divergence. Paleontology seems to be telling us that this "big bang" of eukaryotic evolution began at least a billion years ago.

If that is true, why did this new type of biology take off so late in the evolutionary day? After all, as noted in chapter 6, sterane molecules extracted from 2.7-billion-year-old shales are thought to be molecular signatures of eukaryotic biology. If the course of eukaryotic evolution was set so early in life's history, why should the domain (*our* domain!) have remained subservient to prokaryotes for a billion and a half years before spreading throughout the oceans? No one really knows, but we can think about four types of explanation. The 2.7-billion-year-old biomarker molecules record only one aspect of eukaryotic biology—the ability to make sterol compounds. Eukaryotes have many other distinguishing features, and perhaps the "complete" eukaryotic cell, with its distinctive genes, differentiated nucleus, cytoskeleton, and mitochondria, evolved much later. Alternatively, eukaryotes could have originated early but diverged much later, in the wake of some enabling environmental event. Or, late divergence might reflect biological innovation—sex is the one most commonly invoked. Of course, we must also ask whether the late Proterozoic radiation might be more apparent than real, reflecting a greater volume of rocks and better fossil preservation rather than increased biological diversity.

Shales that lie beneath the rubbly, tick-infested plains of Australia's Top End discount the first and last explanations. Part of the middle

Proterozoic Roper Group, their age fixed by 1,492 ± 3–million-year-old volcanic rocks, these shales contain microfossils whose abundance and quality match the best preservation seen in upper Proterozoic rocks. Yet there are no spiny fossils like those in Guizhou, no tiny vases like the Grand Canyon and Spitsbergen populations, and no branching compressions comparable to those in younger shales from China or Spitsbergen. In short, Roper assemblages display little of the morphological variety that documents eukaryotic diversity in younger Proterozoic beds. There are, however, eukaryotic fossils.

Most Roper microfossils are large, compressed spheres, much like those in the broadly contemporaneous shales that flank Great Wall carbonates in northern Siberia; they are probably, but not demonstrably, eukaryotic. But one small population discovered by Emmanuelle Javaux, a Belgian postdoc in our lab, provides strong evidence of cytologically sophisticated eukaryotes in mid-Proterozoic oceans. The fossils are moderately large spheroids, about 30–150 microns in diameter, distinguished by one to as many as twenty long, slender tubes that arise from their walls (plate 6e). The tubes are irregular in number as well as position, and they sometimes branch. Similar shapes can be seen in some living protists, where tubes develop as extensions of spore walls, allowing reproductive cells that differentiate inside to escape and disperse. By analogy, then, the irregular tubes on the Roper fossils suggest microorganisms that could modify their shape during the lifetime of a single cell. Bacteria don't do this very well, but eukaryotes are masters of the trade—their ability to form and re-form cell shape is conferred by the cytoskeleton, the dynamic internal scaffolding introduced in chapter 8. This being the case, Roper fossils tell us, not only that eukaryotic microorganisms were present nearly 1.5 billion years ago, but also that they already boasted some version of the sophisticated internal organization seen in living eukaryotes.

In addition to microfossils, Roper shales contain molecular fossils, including steranes that provide complementary evidence for eukaryotic life. Even macroscopic fossils of likely eukaryotic origin occur in middle Proterozoic rocks. Observant paleontologists in Australia, China, India, and the United States have uncovered helical compressions an inch or so across in mid-Proterozoic siltstones as well as short strings of beads 1–3 millimeters in diameter preserved as impressions on the surfaces of

sandstone beds. These fossils are hard to classify; conceivably, they record extinct lineages only loosely related to modern eukaryotes.

Collectively, then, paleontological discoveries indicate that the late Proterozoic ascendance of algae and protozoans was not the starting gun for eukaryotic life. Nor is the documented increase in eukaryotic diversity simply an artifact of rock preservation or sampling. *Something*—some biological innovation or environmental shift—must have happened to spur eukaryotic diversification toward the end of the Proterozoic Eon.

If the rise of eukaryotes to ecological and taxonomic prominence came long after the origin of eukaryotic cells, we have to think about triggers for diversification, either biological or physical. What about sex? The undeniable (and, perhaps, not wholly scientific) attraction of this proposition rests on a simple observation and a bit of arithmetic. The observation is taxonomic: about 4,000 bacteria have been given species names; in contrast at least 100,000 protozoans and algae, another 100,000 fungi, some 300,000 land plants, and more than a million animals have been described. The math comes in to bolster the argument that sex promotes diversity by enabling eukaryotes to generate the raw material for evolution—genetic variation within a population—differentially well. Bill Schopf is especially fond of this idea, which he illustrates by means of a simple thought experiment. In bacteria that reproduce by binary cell division, ten mutations that arise in a population will result in a maximum of eleven different combinations of genes. But, let those same ten mutations arise in a sexually reproducing population of eukaryotes, and the possible genetic combinations run into the thousands. No wonder eukaryotes are so diverse. The argument is comfortably simple, it makes intuitive sense, and it relies on natural history of the kind that "every schoolboy knows." In short, it is just the sort of proposition that graduate students love to dismantle.

Let's start with the statistics on species diversity. Named bacterial species have one trait in common—they can be grown in the laboratory. Using new molecular techniques, however, microbial ecologists have found that culturable organisms represent as little as 1 percent of the bacterial diversity present in natural environments. Thus, the true diversity of bacteria may rival that of protozoans and algae.

We also have to poke a bit at the issue of genetic variability, because it makes the important assumption that bacteria have no means of combining genes from two individuals. As noted already in chapter 2, this assumption is spectacularly incorrect. The well-known intestine dweller *E. coli*, for example, practices a form of sex in which two cells become tethered by a tiny tube, allowing genetic material to pass from one to the other. Other bacteria take up small lengths of DNA released to the environment by dying cells. Still others incorporate genetic material transported by viruses. In fact, bacteria exchange genetic material all the time, and they do it not only between two individuals in the same population but between distinct species and even between kingdoms. If sex is defined as the exchange of genetic material between individuals, bacteria are decidedly sexy. Prokaryotes do not suffer from a poverty of genetic variation.

Thus, if we want to entertain the notion of sex as a trigger for eukaryotic diversification, we have to posit that early eukaryotes lacked both sex and the mechanisms for genetic exchange found in bacteria. At present, however, we don't know this. We really don't know when sexual reproduction entered the eukaryotic life cycle, if in fact it wasn't already present in the last common ancestor of all living protists. Perhaps we should approach the problem differently.

The "just add sex" hypothesis assumes that diversity is somehow constrained by the rate at which genetic variation can be generated, but diversity depends as much if not more on biological function and ecology. Prokaryotic diversity reflects the remarkable ability of bacteria and archaea to exploit specific nutrient sources and energy gradients. In contrast, eukaryotes diversified by approaching the world in new ways. As discussed in the preceding chapter, the eukaryotic cytoskeleton and membrane system enables nucleated cells to do something that bacteria can't do at all—swallow particles, including other cells. Thus, as evidenced by the vase-shaped microfossils in the Grand Canyon, eukaryotes introduced grazing and predation into microbial ecosystems. Doggerel by Jonathan Swift captures the consequences nicely:

> So, naturalists observe, a flea
> Has smaller fleas that on him prey;
> And these have smaller still to bite 'em;
> And so proceed ad infinitum.

By expanding ecosystem complexity, eukaryotic cells erected a new scaffolding for diversity.

Eukaryotic organisms do something else that has largely eluded prokaryotes. Plants, animals, fungi, and seaweeds develop via a complex pattern of cell division and differentiation choreographed by molecular signals that pass from cell to cell, switching specific genes on or off as they go. This beautifully coordinated regulatory system may have its origins in single-celled organisms that changed size or shape as they passed through the cell cycle, but it eventually made possible the evolution of complex multicellularity, and in so doing fueled a further expansion of eukaryotic diversity. Ninety-five percent or more of extant eukaryotic species are multicellular.

At the end of the day, it isn't necessary (or, perhaps, wise) to focus too narrowly on any one trait as key to eukaryotic diversification. Sex, cytoskeletons, genetic regulation, and, doubtless, other characters interacted to produce the plethora of eukaryotic forms seen today. No one knows when the modern eukaryotic "tool kit" was assembled, but the small fossils in Roper shales suggest that it was in place long before the diversification recorded in late Proterozoic rocks.

This brings us to the remaining explanation for late Proterozoic diversification—changing environments. Can we envision environmental changes that might have improved the odds of eukaryotic success in a prokaryotic world? If we can, do the rocks provide evidence that the required changes actually took place in concert with eukaryotic diversification? Increasingly, the answer to both questions appears to be yes.

In chapter 6, we discussed evidence for an increase in atmospheric (and surface ocean) oxygen 2.2–2.4 billion years ago. When I was a student in the 1970s, this event was generally accepted as the transition between Earth's two great environmental states: the Archean to earliest Proterozoic, when O_2 was scarce, and the past 2 billion years, during which air and ocean have both been bathed in oxygen. But, as noted in chapter 6, Odense University's Don Canfield has proposed that what the early Proterozoic oxygen revolution ushered in was not the modern world, but rather an alien intermediate marked by moderate oxygen in the atmosphere and surface sea and hydrogen sulfide in deep waters (figure 9.6).
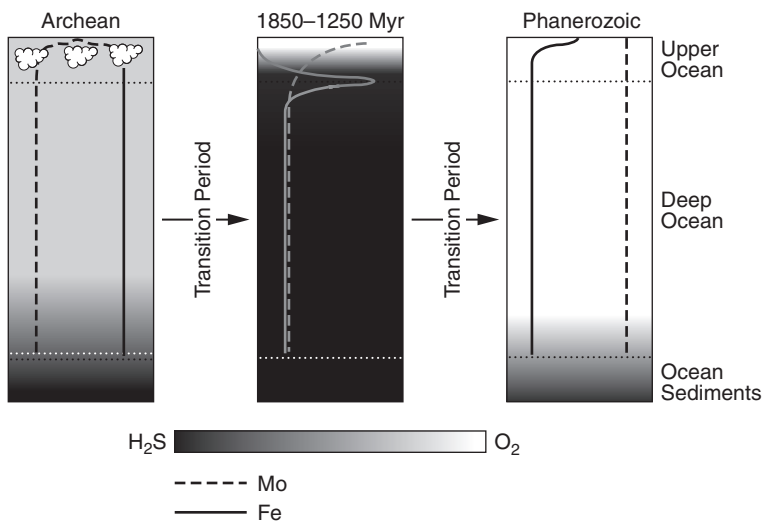
Figure 9.6. Triptych illustrating the three phases of ocean evolution. Early oceans contained little oxygen, but relatively abundant iron. Modern oceans contain abundant oxygen and little iron. In between, during a long-lived state that may have lasted from 1.8 billion years ago until near the end of the Proterozoic Eon, the oceans are thought to have had moderate oxygen in surface waters, but hydrogen sulfide at depth. In such an ocean, biologically important trace elements such as iron and molybdenum (concentrations, higher to the right, illustrated by vertical lines) may have been in seriously short supply. (Reprinted with permission from A. D. Anbar and A. H. Knoll, 2002. Proterozoic ocean chemistry and evolution: a bioinorganic bridge? *Science* 297: 1137–1142. Copyright 2002 American Association for the Advancement of Science)

Those fossiliferous rocks from northern Australia provide a good test of Don's hypothesis (maximizing my scientific return per tick bite). Putting our heads together, postdoctoral fellow Yanan Shen, Don, and I have been able to show that shales deposited in deeper parts of the Roper (and two older) basins preserve chemical signatures similar to those observed today in sediments at the bottom of the Black Sea. The Black Sea is well known to Earth scientists because its oxygen-rich surface layer blankets a much larger volume of water that fairly bristles with H_2S. Independent work by Tim Lyons at the University of Missouri and my former student Linda Kah, now at the University of Tennessee, corroborates and expands this view of distinctive mid-Proterozoic oceans.

Why should this matter to evolving eukaryotes? Ariel Anbar, a talented geochemist at the University of Rochester, pointed out to me that in seawater like that envisioned for the mid-Proterozoic, the essential nutrient nitrogen would be relatively scarce.[5] That is okay for cyanobacteria because they can fix nitrogen and, furthermore, are remarkably adept at scavenging biologically useful forms of nitrogen from their surroundings. But the situation is different for photosynthetic eukaryotes. Algae thrive today where levels of nitrate (NO_3^-, a biologically usable form of nitrogen that is readily available in modern seawater) exceed short-term requirements for growth, allowing cells to stockpile nutrients as a hedge against leaner times. In the mid-Proterozoic ocean, however, this would have been difficult, because nitrate can't build to high levels when oxygen is limited and sulfide lurks beneath the surface ocean. Algae can't fix nitrogen, and they compete poorly against cyanobacteria for the scarce nitrogen compounds that would have been present in mid-Proterozoic seawater. Moreover, to make use of such nitrate as might have been present, algae would have needed the metallic element molybdenum.

"Moly" is an essential ingredient in nitrate reductase, the enzyme that makes nitrate useful to organisms. Today, moly is widely available as a trace constituent of seawater, but in the alien world of the "Canfield" ocean, this element would have behaved somewhat differently. Then as now, moly would have been weathered from continental rocks and carried into the sea by oxygenated rivers. Unlike its distribution today, however, moly would have been common *only* in coastal waters where the rivers entered the sea. Farther from shore, surface waters would have mixed with subsurface water masses, causing molybdenum to be removed by reaction with hydrogen sulfide. Algae that washed into the open ocean would, thus, have faced the insult of molybdenum deprivation to go with the injury of nitrogen limitation.

All this suggests that 1.5 billion years ago, life might have been tough for eukaryotic algae. (Protozoans should have fared better, because they obtain the nitrogen they need from the cells they eat. But remember that

[5] Recall from chapter 2 that nitrogen gas (N_2) permeates air and ocean waters, but cannot be used directly by most organisms. Many prokaryotic microorganisms, including cyanobacteria, can "fix" nitrogen, converting gaseous N_2 into ammonium ion (NH_4^-) that can be incorporated into biological molecules.

the paleontological record provides few recognizable glimpses of early protozoans—with the conspicuous exception of those vase-shaped fossils in Grand Canyon rocks, the fossils that document late Proterozoic eukaryotic expansion are largely algal.) Returning once more to rocks of the Roper Group, we can ask how presumed algae were distributed in this mid-Proterozoic seaway. Our prediction is that photosynthetic eukaryotes should have been most abundant and diverse along the ancient seacoast, where nitrate levels would have been highest and molybdenum most readily available. That, it turns out, is just what we see.

Indeed, it appears that, globally, eukaryotic algae first took root in coastal waters and only later spread across continental shelves. This ecological expansion is poorly documented, but in general it appears to have commenced about 1.2 billion years ago as nitrogen limitation in the "Canfield" ocean began to weaken. Granted new ecological opportunities, both seaweeds and plankton diversified—as we see in the fossil record. Protozoans like those buried deep within the Grand Canyon must have diversified, too, as heterotrophs learned to exploit the new *biological* environments created by the algae.

Thus, as our view of Proterozoic geology and paleontology strengthens, it once again implicates environmental history in the determination of evolutionary pattern. That doesn't let biological innovation off the hook—new functional possibilities, especially those presented by multicellularity, must also have stoked the flame of eukaryotic diversification—but it requires us to seek evolutionary explanation in the *interaction* between genetic possibility and environmental opportunity.

The transition to a fully oxic world appears to have been protracted. As discussed more fully in chapter 11, oceans rich in oxygen from top to bottom may not have developed until the Proterozoic Eon was almost over. When they came, however, the culmination of Earth's environmental transformation paved the way for one last revolution in biology—the rise of animals.