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The Precambrian emergence of animal life: a geobiological perspective

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ABSTRACT

The earliest record of animals (Metazoa) consists of trace and body fossils restricted to the last 35 Myr of the Precambrian. It has been proposed that animals arose much earlier and underwent significant evolution as a cryptic fauna; however, the need for any unrecorded prelude of significant duration has been disputed. In this context, we consider recent published research on the nature and chronology of the earliest fossil record of metazoans and on the molecular-based analysis that yielded older dates for the appearance of major animal groups. We review recent work on the climatic, geochemical, and ecological events that preceded animal fossils and consider their portent for metazoan evolution. We also discuss inferences about the physiology and gene content of the last common ancestor of animals and their closest unicellular relatives. We propose that the recorded Precambrian evolution of animals includes three intervals of advancement that begin with sponge-grade organisms, and that any preceding cryptic fauna would be no more complex than sponges. The molecular data do not require that more complex animals appeared well before the recognized fossil record; nor, however, do they rule the possibility out, particularly if the interval of simpler metazoan ancestors lasted no more than about 100 or 200 Myr. The geological record of abrupt changes in climate, biogeochemistry, and phytoplankton diversity can be taken to be the result of changes in the carbon cycle triggered by the appearance and diversification of metazoans in an organic carbon-rich ocean, but as yet no compelling evidence exists for this interpretation. By the end of this cryptic period, animals would already have possessed sophisticated systems of cell–cell signalling, adhesion, apoptosis, and segregated germ cells, possibly with a rudimentary body plan based on anterior–posterior organization. The controls on the timing and tempo of the earliest steps in metazoan evolution are unknown, but it seems likely that oxygen was a key factor in later diversification and increase in body size. We consider several recent scenarios describing how oxygen increased near the end of the Precambrian and propose that grazing and filter-feeding animals depleted a marine reservoir of suspended organic matter, releasing a microbial 'clamp' on atmospheric oxygen.

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INTRODUCTION

The story of animal life began in the Precambrian geological era prior to 543 Ma, in a world that we would scarcely recognize as our own. Atmospheric carbon dioxide (CO_2) was many times the modern value, but molecular oxygen (O_2) was much less abundant than today (Kasting & Catling, 2003). The land was devoid of plants, and the dominant visible forms of life were mats of microorganisms forming laminated, organic–inorganic structures called stromatolites. We live in a very different world, one transformed by the activity of multicellular life; animals (Metazoa) as well as land plants. The extant animal kingdom is composed of five major groups: the Porifera (comprising multiple clades of sponges), the enigmatic Placozoa (consisting of a single described species), Cnidaria (jellyfish, anemones, corals, hydrozoans, and sea pens), Ctenophora (comb jellies), and the Bilateria (animals displaying bilateral symmetry). The last three groups are collectively known as the Eumetazoa. With the possible

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exception of acoels, which may be basal, the Bilateria are divided into two groups based on major differences in embryonic development: the Protostomata (e.g. flatworms, roundworms, arthropods, molluscs, annelids), and the Deuterostomata (vertebrates, tunicates, lancelets, hemichordates, echinoderms). Most of the Protostomata have been further assigned to two 'superphyla', the Lophotrochozoa and the Ecdysozoa (Halanych, 2004).

The transformation from the strictly microbial world to one shared by animals began in the Neoproterozoic Era (1000–543 Ma), and fossils of the Cambrian Period (543–490 Ma) include representatives of nearly all readily fossilized modern animal phyla (except bryozoans), as well as putative examples of now-extinct groups. Many authoritative reviews have described and attempted to explain the geologically rapid diversification of animal life recorded by Early and Middle Cambrian fossils such as those of the Chengjiang and Burgess Shale deposits. For example, Marshall (2006) examined potential environmental, developmental, and ecological explanations for the Cambrian 'explosion' and proposed that the diversification of animal body plans was the result of an increase in the number of factors determining the fitness of organisms as species began to interact with one another. Peterson *et al.* (2005) proposed that one particular interaction – macrophagy – was the most likely culprit. At the other end of the spectrum, Kirschvink & Raub (2003) speculated that the Cambrian explosion was triggered by a catastrophic geophysical event – the inertial interchange of the Earth's rotation axis. Several syntheses, e.g. Valentine *et al.* (1999), Knoll & Carroll (1999), and Erwin (1999), have also addressed the nature of the Cambrian radiation.

The oldest indisputable animal-like fossils are found at least several tens of million years before the Precambrian–Cambrian boundary, but the time of emergence of the Metazoa as a distinct clade has not been established. Currently, all widely accepted metazoan fossils are confined to the latter half of the Ediacaran period. [The Ediacaran spans geological time between the terminus of a major glacial event 635 Ma and the Precambrian–Cambrian boundary (Knoll *et al.*, 2004).] But one could argue *ad uniformitarian* that significant evolutionary change requires lengthy geological time and thus recorded animal history must have a substantial prelude. Indeed, some molecular-based calculations of divergence times between extant metazoan groups yield ages that are much older than the oldest animal fossils (Wray *et al.*, 1996). Although the established fossil record of animals is preceded by dramatic changes in Neoproterozoic climate, atmospheric composition, the carbon cycle, and phytoplankton fossils, any relationship of these to the emergence of animal life remains conjectural.

In an insightful review, Budd & Jensen (2000) critiqued several scenarios that attempted to explain how Precambrian animals might have escaped fossilization, e.g. by being too small, too rare, or by occupying habitats where preservation did not occur. They argued that important traits of Early Cambrian body plans could not have evolved in organisms too small or too strictly planktonic to produce trace fossils, and concluded that the appearance of such traits cannot be used to argue for an earlier, cryptic fauna. [Valentine (2002) made essentially the same point.] Budd & Jensen (2000) explained the Cambrian 'explosion' by assigning many Cambrian fossils to 'stem' groups that preceded the radiation of the 'crown' groups (modern phyla), thus effectively minimizing the amount of evolutionary change required to produce them. Conway Morris (2006) also critiqued the evidence for a prelude and argued that the 'explosion' was real and likely the inevitable result of complex (but unspecified) ecological interactions. Early Cambrian fossils that can be unambiguously assigned to crown groups (or their sister groups) could eventually test these scenarios, but the fossil record is meager, e.g. Butterfield (1994) and Siveter *et al.* (2001).

The early emergence of animal life remains a dynamic area of study and new technologies such as whole-genome and expressed sequence tag (EST) sequencing, multicollector ion-probe mass spectrometry, and X-ray tomography are used to query the genomic and fossil record in ways that were hitherto impossible. Much of this research can be organized as addressing four primary questions: Did the Metazoa have a lengthy Precambrian history? What were the climatic, biogeochemical, and ecological conditions during that interval, and how might the geological record be used to infer the presence or absence of animals before trace or body fossils? To what extent can the physiological and genetic traits of extant animal groups and their closest unicellular relatives be used to infer the major evolutionary steps in an ancestral animal line of descent? How might emerging metazoans have adapted to and altered their environment, i.e. the microbial biosphere, biogeochemical cycles, and, inevitably, other animals? This review is organized accordingly.

THE RECORD OF PRECAMBRIAN ANIMAL LIFE

The fossil record

Recent palaeontological studies have refined the chronology of Precambrian animal life, although they have not dramatically changed the overall picture. All uncontested animal fossils are no older than *c.* 600 Ma and thus pre-date the Chengjiang (Early Cambrian) and Burgess Shale (Middle Cambrian) faunas by no more than 70–90 Myr. There are reports of much older disks, burrows, and necklace-like forms that might have been left by animals (Hofmann *et al.*, 1990; Seilacher *et al.*, 1998; Rasmussen *et al.*, 2002b; Fedonkin, 2003), but alternative interpretations such as microbial 'mat' structures have been argued (Rai & Gautam, 1999; Seilacher *et al.*, 1999; Conway Morris, 2002; Rasmussen *et al.*, 2002a) and such finds await further substantiation, identification, and/or dating (Rasmussen *et al.*, 2002c).

Among the oldest fossils of animal life are the remarkable phosphatized forms of the Doushantuo Formation in China that have been traditionally assigned an age of around 580 Ma. The Doushantuo fossils include forms resembling benthic eggs and embryos – probably those of marine invertebrates (Chen *et al.*, 2000; Xiao *et al.*, 2000), rather than of algae (Xiao, 2002). Recent taphonomic experiments with sea urchin embryos demonstrated that animal embryos could have been preserved long enough for mineralization to occur (Raff *et al.*, 2006). An alternative interpretation of these forms as giant vacuolate bacteria (Bailey *et al.*, 2007) is not supported by the presence of eukaryotic cyst-like organic vesicles around some fossils (Yin *et al.*, 2007). More controversial are reports of cellular remains and body fossils, including forms that resemble epidermal cells, porocytes, amoebocytes, and sclerocytes of sponges, as well as monaxid siliceous spicules that are characteristic of certain demosponges, e.g. (Li *et al.*, 1998a); these may instead be fossil algae or products of diagenesis (Li *et al.*, 1998b; Zhang *et al.*, 1998). The purported spicules, in particular, lack axial canals or excess silicon (Yin *et al.*, 2001). Putative fossils resembling the embryonic and adult forms of cnidarians have been described (Chen *et al.*, 2002), and cross-sections of adult bilaterian bodies exhibiting a central gut bordered by paired coeloms have been reported (Chen *et al.*, 2004b), but these interpretations are controversial, again because the forms may represent diagenesis rather than biology (Bengtson & Budd, 2004; Chen *et al.*, 2004c). X-ray tomography of Doushantuo fossils failed to show any of the epithelial organization shared by all extant metazoans (Hagadorn *et al.*, 2006), suggesting that these embryos represent earlier stem groups.

Recent radiometric dating and chemostratigraphy have improved estimates of the age and duration of the Doushantuo formation. The formation unconformably overlies a glacial deposit that has been correlated to a 635 Ma unit in Namibia (Hoffmann *et al.*, 2004). U-Pb dating of zircons from volcanic ash beds that bracket the entire Doushantuo in one section indicates that it formed between 635 and 551 Ma (Condon *et al.*, 2005). Zhang *et al.* (2005) dated an ash bed above the fossil-bearing uppermost phosphorites in another section to 555 ± 6 Ma. Other dates are more equivocal. Whole-rock Pb–Pb dating of the stratigraphically older and younger parts of the upper (fossil-bearing) phosphorite unit yielded ages of 599 ± 4 Ma (Barfod *et al.*, 2002) and 576 ± 14 Ma (Chen *et al.*, 2004a), respectively. Alternatively, Condon *et al.* (2005) correlated the subaerial exposure surface dividing the lower unit (devoid of fossils) and upper Doushantuo unit with an episode of glaciation 580 Ma, in conflict with the older Barford *et al.* (2002) date, and placing the fossils between 550 and 580 Ma. Kaufman (2005) notes that an unconformity and negative excursion in the isotopic composition of inorganic carbon at the *top* of the Doushantuo could instead be correlated with the same glaciation, making the fossils between 635 and 580 Ma. The age difference between these different correlations is thus as much as 85 Myr.

The oldest body fossils are imprints of diverse soft-bodied organisms (the Ediacaran fauna) whose taxonomic affinities remain controversial. Seilacher (1989) proposed that the Ediacaran biota represent a sister group (the 'Vendazoa') to the Metazoa or at least a unique animal phylum (Buss & Seilacher, 1994) with synapomorphies such as a 'quilted' structure. However, at least some of these organisms may have been members of extant metazoan phyla (Narbonne, 2005). The oldest reported fossils are of the frond-like *Charnia* that appear immediately over a 575 ± 1 Ma ash bed in the Drook unit of the Avalon Formation in Newfoundland (Bowring *et al.*, 2003; Narbonne & Gehling, 2003) (Fig. 1). *Charnia* was originally interpreted as a macrophytic alga; that taxonomic assignment has been discounted based partly on its deep-water setting, and instead it has been identified as a pennatulacean cnidarian (sea pen). Nevertheless, macrophytic algae have been reported in waters as deep as 268 m (Littler *et al.*, 1985), and alternative assignments of *Charnia* (as sponge or colonial prokaryote) have been proposed (Steiner & Reitner, 2001). Ediacaran taxonomy is controversial, and Brasier & Antcliffe (2004) recently questioned the basis (analogy) on which many fossils, especially *Charnia*, are classified. They underscored the importance of distinguishing between morphological diversity due to speciation or evolution within a group, and separation of body parts or a life cycle. With the exception of the Drook *Charnia* fossils, all Ediacaran fossils are younger than about 565 Ma (Waggoner, 2003).

In a review of the history of the Ediacaran fauna, Narbonne (2005) made two salient points: First, before 560 Ma, ediacarans were immobile forms of limited diversity and a poriferan or cnidarian level of body plan (i.e. largely frond-like rangeomorphs such as *Charnia*). Investigators have argued that a few of these are stem-group ctenophores (Dzik, 2002; Shu *et al.*, 2006). Second, mobile bilaterians formed part of a younger (560–542 Ma), more diverse Ediacaran fauna that potentially contained representatives of all three bilaterian superphyla (Fig. 1). For example, *Spriggina* was an arthropod or arthropod-like form (Ecdysozoa), annelid worm burrows, and probably the apparent mollusc *Kimberella* represented early Lophotrochozoa. Less certain is the presence of early Deuterostoma: *Ernettia*, with chambered walls, may have been a chordate (Dzik, 1999), and *Tribrachidium*, with its distinctive three-fold symmetry, has been seen as an echinoderm, but also as a cnidarian (Ivantsov & Fedonkin, 2002).

Disturbance or manipulation of sediments by animals, e.g. tracks, burrows, and changes in sediment fabric due to bioturbation, appear at least by 555 Ma (Martin *et al.*, 2000) but no earlier than 560 Ma (Droser *et al.*, 2002; Jensen, 2003; Jensen *et al.*, 2005) (Fig. 1). The oldest unequivocal trace fossils are exclusively simple, unbranched, horizontal traces that formed close to the sediment–water interface, often just beneath microbial mats (McNaughton & Narbonne, 1999; Jensen *et al.*, 2005; Seilacher *et al.*, 2005). Sulfidic conditions beneath the microbial mats that covered much of the seafloor

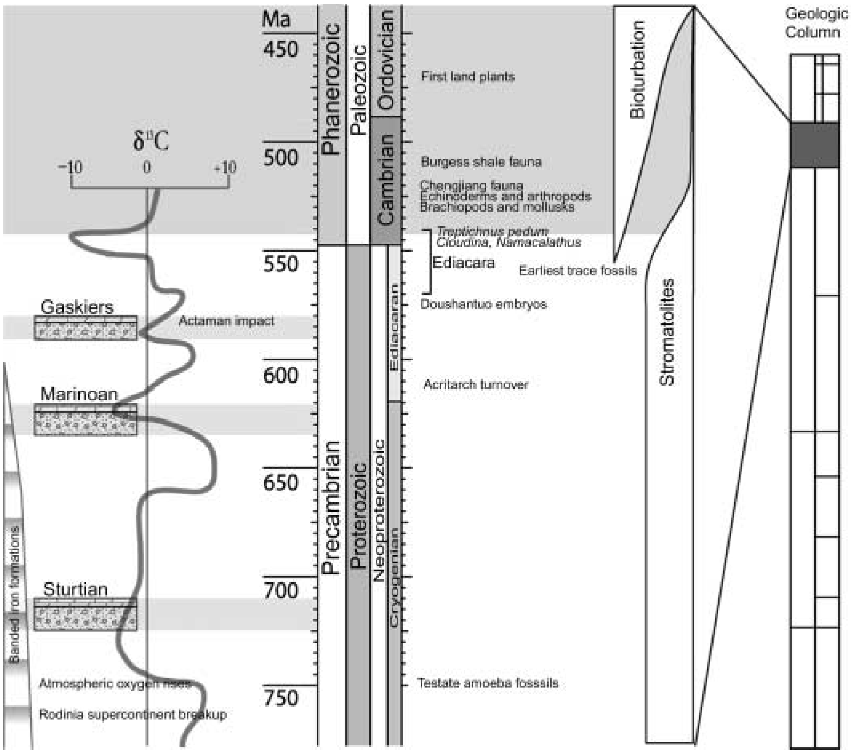

Fig. 1 A timeline of late Precambrian and early Phanerozoic history, styled after Narbonne (2005), with major geological (left-hand side) and biological (right-hand side) events. The former includes major tectonic and climate events such as the three major Neoproterozoic glacial intervals, and changes in the atmosphere. A schematic of the inorganic carbon isotope record is included. Shaded horizontal bars represent the best available age constrains on the glacial intervals. Biological history includes highlights of the earliest animal fossil record, as well as changes in sediment texture due to animal activity. The place of this interval in the entire geological column of Earth history is represented on the far right.

during that time appear to have restricted burrowing animals to shallow depths within the sediment, and the absence of deep burrows until the end of Neoproterozoic time suggests that bilaterians had not yet evolved physiologies allowing them to contend with these conditions, let alone benefit from them by feeding on sulfide-consuming bacteria as some taxa do today (Bailey *et al.*, 2006). Slightly more complex burrows are known only from the latest Neoproterozoic (Jensen *et al.*, 2000). One such trace fossil, *Treptichnus pedum*, defines the base of the Cambrian (Landing, 1994) and coincides with a significant increase in infaunal activity (Droser *et al.*, 1999) (Fig. 1).

Skeletal biomineralization in animals first appeared in the Ediacaran but was restricted to silica and to hexactinellid sponges (Brasier *et al.*, 1997), and possibly demosponges (Li *et al.*, 1998a) (but see above). One of the earliest calcareous fossils was *Namapoikia*, consisting of somewhat irregular tubes packed closely together. It encrusted walls of fissures in microbial reefs of the Nama Group, Namibia, *c.* 549 Ma, and is considered likely to be a poriferan or cnidarian (Wood *et al.*, 2002). The small shelly forms *Cloudina* and *Namacalathus* make a brief appearance immediately before the Precambrian–Cambrian boundary at 542 Ma (Amthor *et al.*, 2003). The shell of *Cloudina* is an irregular tube of stacked funnels. Despite its millimetre size, it is commonly found bored through, presumably by a smaller predator (Bengtson & Zhao, 1992; Hua *et al.*, 2003), an observation supporting the hypothesis that biomineralization in animals was a defensive adaptation. *Namacalathus* is a goblet-shaped form attached to microbial reefs that has been interpreted to represent a metazoan with a cnidarian-like body plan (Grotzinger *et al.*, 2000).

In summary, a parsimonious interpretation of the fossil record is that sessile, metazoan stem-group organisms lacking epithelial organization and the ability to conspicuously disturb sediments appeared by ~600 Ma and perhaps as early as 635 Ma. Members of some crown groups appeared by 580 Ma or shortly thereafter. Bilateria (or their stem-group representatives) appeared *c.* 560 Ma among the later Ediacaran fauna and as evidenced by bioturbated sediments. Biomineralizing taxa appeared in the last few million years of the Precambrian and rose to prominence by the Early Cambrian. Thus Precambrian animal life may have undergone three successive intervals of evolutionary change, each lasting about 15–20 Myr and involving (1) diversification and epithelial organization (by 580 Ma); (2) bilateral symmetry and mobility (by 560 Ma); and (3) biomineralization and predation (by 545 Ma). That the fossil record appears to resolve these advances suggests that earlier, unrecorded forms would have belonged to the first interval and been limited to organisms of a complexity no greater than non-biomineralizing sponges. Such a soft-bodied fauna would have escaped fossilization and left no trace fossils. Lower oxygen levels may also have restricted the benthos to facies where sediment reworking made preservation less likely (Dornbos *et al.*, 2006).

Molecular chronometry

In the method of the 'molecular clock' the rate of change in the sequence of a gene is estimated using the divergence of copies in taxa whose first appearance in the fossil record is known: the rate is then used to estimate the dates of deeper divergences. Evolutionary genetics predicts clock-like evolution only for 'neutral' genes or parts of genes that are not subject to selection, but this condition is usually not satisfied in cases of interest and clock-like behaviour is empirically demonstrated instead. The first published divergence times between major metazoan groups using this method pre-date the oldest animal fossils by as much as a factor of two (Runnegar, 1982a; Wray *et al.*, 1996). Molecular divergence times should, in principle, be older than fossil dates (Bromham, 2003). Fossils give a *latest* time for the appearance of a new taxon – the Jaanusson or Sppil-Rongis Effect (Jaanusson, 1976) – and the actual ages of the calibration taxa and therefore the taxa of interest are older. Although possibly important in the turnover of individual species on million-year timescales during extinctions, the effect cannot explain discrepancies of tens to hundreds of million years. Another explanation is the divergence of neutral genes between isolated populations within a species before actual speciation and morphological divergence takes place. However, the amount of molecular variation involved is very small compared to the genetic divergence between the major animal groups.

This leaves two possibilities: the molecular clock calculations are incorrect, or a cryptic fauna with a long evolutionary history went unrecorded as fossils. Recent investigations have attempted to improve divergence time calculations by including more taxa and more genes (Douzery *et al.*, 2004; Peterson *et al.*, 2004; Blair & Hedges, 2005b), employing more calibration points (Peterson *et al.*, 2004; Pisani *et al.*, 2004), and using Bayesian statistics to account for uncertainties in, or the use of lower limits for calibration times (Blair & Hedges, 2005b). This attention has not produced broad agreement (Benton & Ayala, 2003); rather, it has revealed serious problems with the molecular clock approach and its (mis)application, including the neglect of calibration and derivation errors (Graur & Martin, 2004), dependence on prior assumptions (Welch *et al.*, 2005), and uncertainties in the underlying evolutionary models (Roger & Hug, 2006).

Moreover, the assumption that the rate of molecular evolution is a constant independent of taxon or geological time is now widely recognized as untenable (Bromham & Hendy, 2000; Bromham, 2003; Glenner *et al.*, 2004; Ho *et al.*, 2005; Pereira & Baker, 2006). A major criticism of early work was that calibrations based on slowly evolving vertebrate genes were used to date the divergences of their rapidly evolving invertebrate counterparts, producing spuriously ancient ages (Ayala *et al.*, 1998). Peterson *et al.* (2004) and Peterson & Butterfield (2005) reported analyses that accounted for relative rates and yielded dates for the protostome–deuterostome divergence in much better agreement with the fossil record, although they arrived at this by rejecting the less congruent of two models. Blair & Hedges (2005a) showed that older divergence times are recovered using fossils ages as *minimum* rather than fixed calibration times. Although this is strictly true, they argue (circularly) that because divergence times of clades can be much older than the oldest fossils, fossils themselves provide only a lower bound to calibration times; this dramatic relaxation of the calibration dates naturally produces much more ancient divergence time for the uncalibrated clades.

Aris-Brosou & Yang (2003) went further, completely relaxing the molecular clock assumption and replacing it with Bayesian prior models of rate change and speciation along a tree. They dated the protostome–deuterostome divergence to 581 ± 112 Ma, in agreement with the fossil record. Douzery *et al.* (2004) used a similar analysis and reported an older but not significantly different time (761–642 Ma). Blair & Hedges (2005a) pointed out that these calculations also produce other divergence times that violate the fossil record, and Welch *et al.* (2005) showed that the ages calculated with this method may be unduly influenced by the assumptions of the model. While it seems certain that the molecular clock does not run true, there is either not enough information in our data to reliably account for its vagaries, or we do not yet know how to extract such information (Bromham, 2006).

Taken together, these problems suggest that published confidence intervals of molecular clock dates are greatly underestimated and that deep Precambrian divergence times for the major animal groups do not constitute a compelling argument for a lengthy pre-Ediacaran interval with a cryptic animal biota. Nor, however, can the molecular data or fossil record completely rule such an interval out, specifically one involving organisms not much more complex than sponges and lasting no more than one or two hundred million years. What was the environment of these hypothetical earliest metazoans, and might there be nonfossil clues to their presence in the geological record?

THE LATE PRECAMBRIAN WORLD BEFORE ANIMAL FOSSILS

Supercontinents and snowballs

Neoproterozoic climate was influenced by a lower solar luminosity, a phenomenon well founded in astrophysical theory (Gough, 1981), and compensatory greenhouse gases, whose past levels are much less certain. Most direct records or proxies of atmospheric composition are unavailable for this era: However, Kaufman & Xiao (2003) interpreted carbon isotopes in Mesoproterozoic (*c.* 1.4 Ga) eukaryotic microfossils as indicating CO_2 concentrations 10–200 times the present atmospheric level (PAL). Such estimates are very uncertain because carbon isotope fractionation depends on many factors besides pCO_2, including light level and the physiology and growth rate of the fractionating species (e.g. Laws *et al.*, 1997). Elevated levels of methane could also have provided an enhanced greenhouse effect (Pavlov *et al.*, 2003).

The late Precambrian Earth was not always warm, however. Harland (1964) first interpreted worldwide Neoproterozoic glacial deposits as evidence for intervals of global glaciation, but only recently have these been interpreted to reflect extreme 'Snowball Earth' events, in which an ice-albedo feedback of the climate system drove glaciation and pack ice formation to equatorial latitudes (Kirschvink, 1992; Hoffman *et al.*, 1998). This explanation has proved to be as controversial as it is dramatic, and the geological evidence is intensely debated (e.g. Allen & Hoffman, 2005a; Jerolmack & Mohrig, 2005; Allen & Hoffman, 2005b). Only a few accurate ages are available and more are clearly needed, but at least three major intervals of glaciation have been identified: the Sturtian (*c.* 700 Ma) (Fanning & Link, 2004), the Marinoan (*c.* 635 Ma) (Hoffmann *et al.*, 2004; Zhou *et al.*, 2004), and the possibly less severe Gaskiers glaciation (580 Ma) (Calver *et al.*, 2004) (Fig. 1).

An important part of the controversy is the verification of the glacial origin and global synchronicity of diamictite units. Eyles & Januszczak (2004) argued that many of the sedimentological features taken as evidence for Neoproterozoic glaciation (e.g. diamictites and lonestones) are not unambiguously glaciogenic and could have formed in mass flows on unstable continental slopes during rifting. They propose that the unusually synchronous and global appearance of such sediments was a consequence of the accommodation space created by tectonic rifting during the break-up of the equatorial supercontinent Rodinia, rather than climatic and glacioeustatic change. In their view, the glacial sedimentary record is a convolution of cold climate and tectonic extension, and the age and number of actual glacial episodes may not be amenable to correlative dating (Kennedy *et al.*, 1998). The break-up of Rodinia was previously thought to have occurred at around 750 Ma; however, new palaeomagnetic data suggest that its disintegration began 50–100 Myr earlier (Fig. 1) and was complete by 750 Ma (Torsvik, 2003). This is too early to explain the formation of glacial sedimentary units by the creation of accommodation space (Eyles & Januszczak, 2004). The earlier date also challenges several proposed trigger mechanisms for major Neoproterozoic glaciations based on tectonics associated with the rifting of the supercontinent (Hoffman, 1999; Ridgwell *et al.*, 2003; Donnadieu *et al.*, 2004). Nonetheless, an earlier demise of Rodinia may have initiated a cooler period in which the Earth was more susceptible to glaciation.

Also controversial is the latitudinal extent of glaciation and whether oceans were completely ice-covered (the 'hard' snowball scenario) or thin ice or open seas persisted at the equator (the 'slushball' scenario) (Warren *et al.*, 2002; Goodman & Pierrehumbert, 2003; Pollard & Kasting, 2005). Negative feedbacks in the climate models that include the global hydrological cycle and the effects of clouds resist extreme cooling such that the most severe snowball state is difficult to enter (Hyde *et al.*, 2000) or escape from (Pierrehumbert, 2004). Two different, apparently contradictory lines of evidence concerning ice cover have been recently reported. The first involves the presence of biomarkers, specifically 2-α-methylhopanes (from cyanobacteria) and alkylated 2,3,6-trimethylbenzenes (derived from the isorenieratene pigments of green sulfur bacteria), in glacial deposits in a core from South America (Olcott *et al.*, 2005). The former constitute evidence that photosynthesis was taking place in the glacial oceans, while the latter indicates the presence of sulfide in the photic zone. The presence of phototroph biomarkers in the glacial units is not entirely surprising, given that the canonical snowball scenario posits an interval of intense glaciation (and tillite deposition) before the climactic snowball event in which the oceans freeze over, the hydrological cycle shuts down, and marine sediments stop accumulating. A 'hard' snowball interval itself is represented by a hiatus in the sedimentary record and is not amenable to traditional analysis.

In another study, elevated concentrations of iridium (Ir) at or near the base of carbonates overlying Sturtian and Marinoan glacial deposits in Africa are interpreted as the result of accumulation of extraterrestrial matter over several millions of years of snowball glaciation (Bodiselitsch *et al.*, 2005). This Ir is supposed to have fallen in interplanetary dust particles onto the pack ice and to have subsequently been released into the water column when the ice melted and marine deposition resumed. There are, of course, terrestrial sources of platinum-group elements such as Ir, and concentration spikes in marine sediments can be produced by changes in sedimentary redox conditions (Colodner *et al.*, 1992). In principle, a terrestrial origin has been excluded by the behaviour of multiple platinum-group elements (Bodiselitsch *et al.*, 2005), but measurements of other indicators of an extraterrestrial source, e.g. osmium isotopes (Peucker-Ehrenbrink & Ravizza, 2000), are clearly needed. Ir spikes could also be produced by one or more large impacts; one such event created the post-Marinoan Acraman impact structure in Australia (Grey *et al.*, 2003).

The origin of 'cap' carbonates – dolostones that directly overlay glacial units and formed in a marine transgression – is undoubtedly one key to understanding the nature of Neoproterozoic glaciations (Shields, 2005). They were originally explained by the postglacial overturn of anoxic oceans and injection of deep-water CO_2 into the surface ocean (Grotzinger & Knoll, 1995). In the classical snowball scenario, they are instead the product of intense weathering during the hothouse conditions that terminated the glacial period, and the resulting abiotic precipitation of accumulated atmospheric CO_2 as carbonates (Hoffman *et al.*, 1998). Shields (2005) argued that this and other models do not explain the global uniformity and complexity of cap carbonate sequences, and proposed instead that the carbonates were precipitated by algal blooms in a massive, transient freshwater layer produced by rapid glacial melting. A less catastrophic explanation of these enigmatic units is that they are the consequence of increased alkalinity in a glacial ocean with greatly reduced neritic (shelf) area at a time when calcifying organisms were not present to drive precipitation of carbonates in the pelagic zone (Ridgwell *et al.*, 2003). One means to distinguish between these hypotheses – one in which carbonates were precipitated under high temperatures and the others in which they were precipitated under near-freezing conditions – might be carbonate palaeothermometry, e.g. a search for ikaite pseudomorphs (glendonites) such as those that have been found in carbonates between the glacial intervals (James *et al.*, 2005).

Another explanation for the cap carbonates is that their carbon originated as biogenic, isotopically light methane in permafrost that was released and oxidized to CO_2 by flooding during a postglacial marine transgression. Kennedy *et al.* (2001) noted that the amount of carbon needed to produce the cap carbonates is consistent with that required to produce contemporaneous, large negative carbon isotope excursions, provided that all the carbon is from isotopically light biogenic methane ($\delta^{13}C \approx -60‰$ relative to the Pee Dee belemnite, see discussion of the carbon isotope record in the next section). They argued that a colder climate, sea-level drawdown, and additional shelf area in the post-Rodinia world meant that the Neoproterozoic methane hydrate reservoir was at least as large, if not larger than that on the modern Arctic shelf. However, sufficient organic matter may not have accumulated in soils in the absence of land plants, or in marine sediments exposed during the preceding glacial regression. Also, this scenario does not explain why the carbonate units invariably cap the glacial units, as glacial activity could continue even as continental ice sheets retreated and sea level rose. Hoffman & Schrag (2002) also claimed that sulfate might have limited anaerobic oxidation of the methane, although the amount of methane oxidized (8×10^{17} mol) is dwarfed by the total sulfur budget of the oceans (4×10^{19} mol).

The carbon cycle

Late Neoproterozoic carbonate rocks, including the 'cap' carbonates, also contain the largest known fluctuations (11–15‰) of carbon isotopic composition ($\delta^{13}C$) in Earth history (Fig. 1). These follow a relatively featureless billion-year period in the carbon isotope record, described by Roger Buick as the 'boring billion'. Isotopic excursions associated with the Sturtian and Marinoan glaciation can be explained by a 'hard' snowball scenario in which primary production is shut off by pack ice (Hoffman *et al.*, 1998). However, new models suggest that ice may have been thin or absent at low latitudes (Warren *et al.*, 2002) and that the glacial oceans would have been nutrient rich, enhancing productivity. The snowball scenario also does not explain those excursions not associated with glaciations, i.e. at 550 Ma and at the Precambrian–Cambrian boundary itself (Aharon, 2005; Zhang *et al.*, 2005).

Ocean overturn and mixing are another possible explanation of the excursions in inorganic $\delta^{13}C$ (Aharon, 2005). However, this requires a large isotopic gradient with depth, and the residence time of inorganic carbon in the shallow ocean (the source of the carbonates) is years (Kump, 1991) and far too short to explain anomalies that are estimated from subsidence rates to have persisted for 0.1–1 Myr. Regardless, the effect on the ratio of organic matter to inorganic burial rate, and hence the inorganic carbon isotopic composition would be small (Halverson *et al.*, 2002).

Injection of isotopically light carbon into the ocean–atmosphere system could explain the anomalies (Bartley *et al.*, 1998). Halverson *et al.* (2002) proposed a prolonged release of biogenic light methane from clathrate hydrates to the atmosphere where it was oxidized to CO_2. Walter *et al.* (2000) link destabilization of marine clathrates to warming at the end of glaciations: One problem with this scenario is that warming is accompanied by a eustatic rise that stabilizes clathrates (a 100-m rise offsets a 3 °C increase), and the break-up of Rodinia would have exacerbated the problem by further elevating sea level. As discussed above, Kennedy *et al.* (2001) instead invoked flooding and thawing of a terrestrial permafrost reservoir which they argue could have been more than a hundred times larger than that found at present.

Organic matter is also isotopically light ($\delta^{13}C \approx -25‰$): Rothman *et al.* (2003) showed that the Neoproterozoic carbon isotopic record cannot be explained by carbon cycle models that have been successfully applied to Phanerozoic time. They proposed instead that the Neoproterozoic oceans contained 2–3 orders of magnitude more reactive (dissolved or suspended particulate) organic matter than present, and that negative carbon isotopic anomalies could be explained by biotic remineralization of this reservoir and exchange with inorganic carbon in the ocean and atmosphere. Elevated level of reactive organic matter were permitted by ocean stratification and increased organic matter suspension, lower oxygen, and the absence of filter-feeding and/or predation by animals.

In fact, the billion years preceding the late Neoproterozoic isotope events might include important but undetectable changes in the carbon cycle and not be boring after all. Bartley & Kah (2004) argued that a much larger dissolved inorganic carbon (DIC) reservoir in the ocean–atmosphere system would have masked any perturbations to the carbon cycle. Such a reservoir would have existed if ocean pH was constant (Grotzinger & Kasting, 1993) but atmospheric CO_2 was higher because of a silicate weathering feedback acting under a fainter Sun (Walker *et al.*, 1981). A large marine DIC pool would also have buffered changes in the partial pressure of CO_2 (pCO_2) and its greenhouse effect, explaining the absence of glaciations before the Neoproterozoic. As pCO_2 and the size of the marine DIC reservoir declined, the sensitivity of the system to fluctuations in the rate of remineralization of dissolved organic carbon (DOC) increased, particularly if DOC far exceeded the modern reservoir (Rothman *et al.*, 2003).

Oxygen and ocean chemistry

The availability of molecular oxygen is widely considered to have been the pacemaker of geochemical change and biological evolution during Precambrian time. The near-consensus of palaeo-Earth scientists is that free O_2 was virtually absent from the early Precambrian atmosphere but appeared in small but geochemically significant concentrations (at least 0.1% PAL) by about 2.4 Ga (Canfield, 2005). A geochemical model based on sedimentary rock abundances and isotopes of C and S predicts that the atmospheric O_2 did not vary by more than a factor of two during the Phanerozoic (543 Ma to present) (Berner *et al.*, 2003). pO_2 during the intervening (Proterozoic) time is therefore most pertinent to the rise of animals but is also poorly constrained. Euxinic conditions in the ocean limit Proterozoic O_2 to 3% PAL, but this estimate depends on the assumed phosphorus concentration and rate of ocean turnover (Kasting, 1987). Enhanced rates of organic matter burial inferred from carbon isotope analysis suggest pO_2 rose at 800–580 Ma (Des Marais *et al.*, 1992). This timing (Fig. 1) is consistent with an increase to perhaps 5–18% PAL inferred from a depletion of ^{34}S in biogenic marine sediments and a molecular clock estimate of the proliferation of nonphotosynthetic sulfide-oxidizing bacteria (Canfield & Teske, 1996). However, given the serious problems with molecular chronometry, the second of those arguments should be set aside.

Changes in ocean redox state, thought to reflect atmospheric oxygen, are recorded in the iron content of Precambrian sedimentary rocks. Banded iron formations (BIFs) are cosmopolitan Precambrian sedimentary units consisting of alternating layers of iron oxides (magnetite and haematite) and chert. The trace and rare earth element patterns in Proterozoic BIFs are consistent with a deep-sea hydrothermal source (Klein, 2005). In the anoxic deep oceans of the early Precambrian (prior to 1.8 Ga), Fe existed in its soluble ferrous form and was readily transported to shallow continental shelves, where it reacted with oxygen (produced by marine cyanobacteria) and precipitated as ferric iron. [An alternative explanation involves anoxygenic photosynthesis by bacteria using ferrous iron as an electron donor (Kappler *et al.*, 2005).] Banded iron formations are thus an indication of the past chemical state of the deep oceans.

The disappearance of BIFs after 1.8 Ga (until their brief re-appearance in association with Neoproterozoic glaciations) was originally attributed to oxygenation of the deep sea and immobilization of iron (Beukes & Klein, 1992). Canfield (1998) proposed an alternative explanation in which the deep Proterozoic ocean remained anoxic and became sulfidic after 1.8 Ga: Increased subaerial weathering of sedimentary pyrite (FeS_2) under an O_2-containing atmosphere led to elevated marine sulfate, production of sulfide, titration of iron from the oceans as pyrite, and suppression of BIF formation (Fig. 2). Evidence for the 'Canfield Ocean' includes elevated sedimentary pyrite at 1.84 Ga (Poulton *et al.*, 2004) and an increase in sulfur isotopic fractionation between sedimentary pyrite and gypsum beginning 2.2–2.3 Ga – attributed to the action of sulfate-reducing bacteria on sulfate concentration above 1 mM (Canfield, 1998). Still, high inferred rates of S isotope change in Proterozoic sediments suggest that sulfate levels did not exceed a few mM and were still an order of magnitude below the modern value (Shen *et al.*, 2003; Kah *et al.*, 2004). Biomarker data also support Canfield's scenario: Logan *et al.* (1995) argued that the unusually complete biodegradation of algal compounds during the Proterozoic was in part due to the action of sulfate-reducing bacteria. Early Proterozoic sedimentary rocks also contain carotenoids derived from phototrophic purple sulfur bacteria and green sulfur bacteria, indicative of euxinic conditions (Brocks *et al.*, 2005) (Fig. 2).

The reappearance, after a billion-plus year hiatus, of iron-rich sediments with Neoproterozoic glacial deposits and extreme carbon isotope excursions can be interpreted either as a return to total global marine anoxia due to the isolation of the oceans from the atmosphere (Kirschvink, 1992), or, alternatively, a decrease in the weathering rates of sedimentary pyrite and flux of sulfur as sulfate into the oceans as a consequence of a colder climate (Canfield & Raiswell, 1999). The residence time of sulfur is about 3 Myr in the modern ocean (and perhaps shorter in the Proterozoic), and thus marine sulfur concentration could have been responsive on that timescale.

The fossil record of phytoplankton and its implications

In the absence of obvious abiotic triggers, it is tempting to relate Neoproterozoic perturbations in the carbon cycle (and perhaps climate) to events in biological evolution. Logan *et al.* (1995) proposed that the rapid removal of organic matter from the water column in the form of faecal pellets, beginning in Cambrian time, resulted in the improved preservation of algal lipids. In the Precambrian, smaller particles sank more slowly and organic matter would have been rapidly degraded in the water column. Logan *et al.* (1995) compared hydrocarbons (the diagenetic products of organic matter from planktonic microorganisms) in sedimentary rocks of Neoproterozoic age with those from the Phanerozoic. They found that hydrocarbons derived from heterotrophic bacteria dominate in Neoproterozoic sedimentary rocks but are largely replaced by those derived from photosynthetic algae in younger rocks. Autotrophic metabolism produces C_{14}-C_{18} *n*-alkyl carbon chains that are ^{13}C-depleted compared to co-occurring isoprenoids pristane and phytane, both of which are photosynthetic degradation products. Heterotrophic metabolism produces the opposite isotopic shift (Hayes, 2001). A distinct ^{13}C depletion in *n*-alkyl compounds is observed through the Precambrian–Cambrian boundary, indicating a shift from efficient carbon cycling in a heterotrophic microbial loop to movement of that carbon to higher trophic levels, e.g. grazers

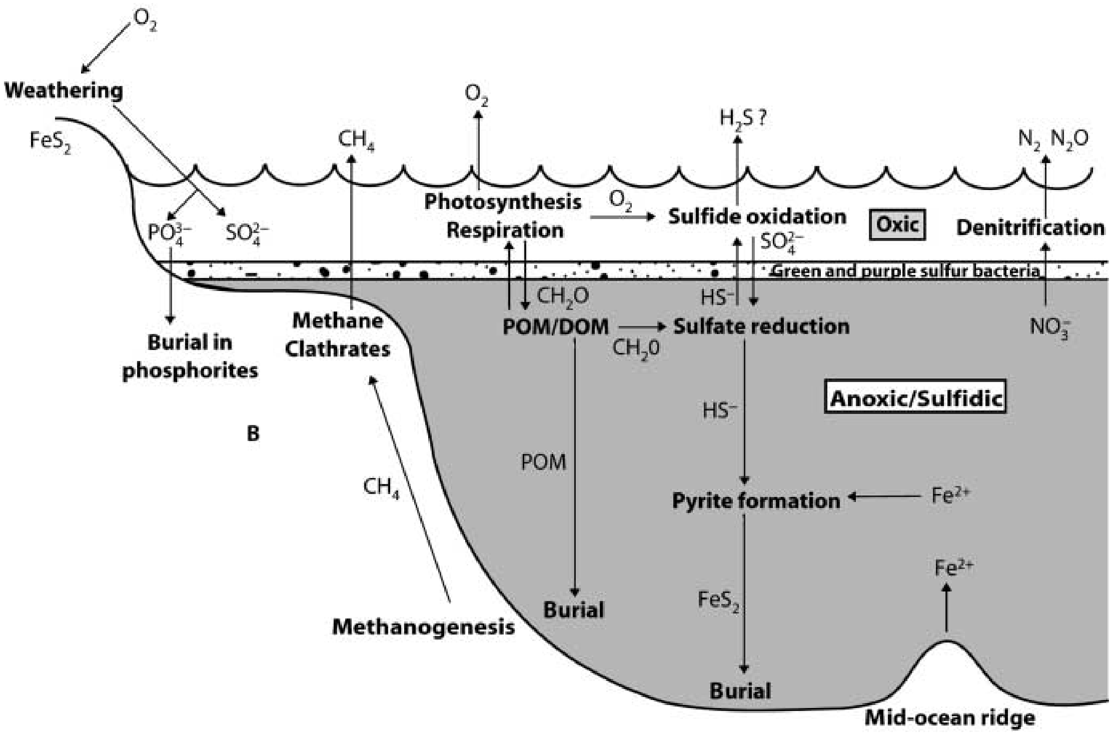

Fig. 2 Important biogeochemical processes in a hypothetical Neoproterozoic ocean and in terrestrial weathering: in this schematic, arrows identify chemical flows connecting these processes. The deep ocean is assumed to be anoxic and sulfidic; euxinic presence of sulfide in the photic zone is exploited by anoxygenic phototrophs. The oceans may have been an important source of atmospheric H_2S and the greenhouse gases CH_4 and N_2O. POM = particulate organic matter; DOM = dissolved organic matter.

and predators, and its eventually loss to the seafloor as faecal pellets (Logan *et al.*, 1997). An opposite shift accompanies the mass extinction of animals at the Permo–Triassic boundary (Grice *et al.*, 2005). This is consistent with the proposal of Rothman *et al.* (2003) that a large marine reservoir of reactive organic carbon disappeared in the late Neoproterozoic, perhaps as a consequence of the evolution of filter-feeding, predation, and/or the development of animals with guts capable of faecal pellet production.

Clues to the carbon cycle–animal connection might lie in the Neoproterozoic fossil record of eukaryotic phytoplankton, which consists largely of acritarchs, organic-walled planktonic microfossils of problematic and probably polyphyletic taxonomic affinity. Their record reflects global environmental changes that presumably influenced the early diversification of animal life as well. Furthermore, phytoplankton and the animals that consumed them must have influenced each others' evolution and extinction (Butterfield, 2007). Prior to the Marinoan glaciation, individual acritarch species typically persisted for hundreds of millions of years. At around 600 Ma, species diversified in size and morphology, and longevities declined to 15–50 Myr (Knoll, 1994; Grey *et al.*, 2003; Huntley *et al.*, 2006). The immense longevity of the pre-Marinoan acritarch species may have resulted from saturation of the photic zone by populations of cyanobacteria and phytoplankton in the absence of effective predation and herbivory. Under such conditions, nutrients would have limited population size and intense competition would have led to severe niche partitioning, leaving few opportunities for new species (Stanley, 1976). The emergence of metazoan zooplanktors sometime after the Marinoan glaciation but before the earliest record of the Ediacaran fauna may have relaxed nutrient limitation, allowed new species to invade, and applied new selection pressures, producing the observed shift in morphotype diversity and longevity (Butterfield, 1997; Peterson & Butterfield, 2005). Indeed, one acritarch morphotype corresponds to the embryo-containing vesicles in the Doushantuo, suggesting that metazoans may have existed by 632 Ma (Yin *et al.*, 2007).

Acritarchs declined dramatically in both diversity and morphologic variety at about the time of appearance of the Ediacaran fauna (*c.* 575 Ma), and they failed to recover until Cambrian time (Knoll, 1994). The earliest members of the Ediacaran fauna included sessile suspension feeders (i.e. rangeomorphs) (Clapham *et al.*, 2003) and thus a causal connection between these biotic events, one entailing a group of producers and the other a group of primary consumers, is a plausible, albeit hypothetical scenario. It is also possible that environmental change somehow simultaneously favoured the evolutionary radiation of metazoans, while being detrimental to acritarchs. Grey *et al.* (2003) correlated the acritarch decline with the Acraman impact in Australia, but it is not obvious why such an impact would not have also affected adversely animal life.

The record of Proterozoic carbonaceous compressions interpreted to be macroscopic algae also suggests stepwise changes in diversity and morphology contemporaneous to those in the acritarch record (Xiao & Dong, 2006). Because of the difficulty of distinguishing algal from microbial carbonaceous compressions and of determining whether such changes were driven by animal herbivory or by some other factor such as nutrient limitation, caution should be exercised in interpreting the fossil macroalgae record. Nevertheless, the geological record of the Neoproterozoic does contain unambiguous indications of dramatic changes in the carbon cycle, some temporally connected to glaciations, and it also provides intriguing evidence that a reorganization of the marine food web occurred. If ancestral animals were somehow involved in these changes, what may have they been like? In the absence of a fossil record we must turn to the physiologies, life cycles, and genomes of living animals for clues.

THE LIVING RECORD OF EARLIEST ANIMAL EVOLUTION

Extant organisms are a rich source of information about the origins and early evolution of animals, one that is complementary to the fossil record. This information can be used in three ways: First, extant single-celled organisms can be studied as analogs to the eukaryotic ancestors of metazoans. Second, comparisons of metazoans with their closest unicellular relatives can be used to infer genetic traits of a hypothetical 'protometazoan', the last common ancestor of both groups and the starting point of uniquely metazoan evolution. Finally, comparisons among all extant animal groups can be used to infer the properties of the 'urmetazoan' and eumetazoan ancestors of all extant metazoans and of all nonsponge metazoans, respectively.

A unicellular beginning

The oldest eukaryotic fossils with a taxonomic affinity of any certainty are of the red alga *Bangiomorpha* from 1.2 Ga rocks (Butterfield, 2000; Javaux *et al.*, 2001), although some older macroscopic fossils may be of eukaryotes (Walter *et al.*, 1990; Kumar, 1995). Steranes, the saturated degradation products of sterols, have been found in 2.7 Ga shales (Brocks *et al.*, 1999). These are widely thought to be of eukaryotic origin, although the possibility that they are from cyanobacteria has been raised (Volkman, 2005) (but see Summons *et al.*, 2006). Thus, unicellular eukaryotes were present at least half a billion and perhaps as long as 2 billion years before the emergence of metazoans. The Neoproterozoic fossil record of eukaryotic taxa thought to be closely related to the Metazoa is poor (Fig. 1): Fossil testate amoebas have been found in the *c.* 750 Ma Chuar Group in Grand Canyon (Porter & Knoll, 2000; Porter & Knoll, 2003). The oldest established fossils of fungi (the major sister group to the Metazoa) are of Ordovician age (Redecker *et al.*, 2000), although possible Neoproterozoic examples have been reported (Butterfield, 2005; Yuan *et al.*, 2005).

Phylogenetic studies place the origin of the Metazoa among a group of unicellular protists, some of which have colonial lifestyles (see following section). However, multicellularity or at least colonial behaviour has evolved in multiple eukaryotic groups and in some bacteria, presumably due to the advantage such lifestyles provide for feeding, dispersion and protection from predation (Bonner, 2000; Kaiser, 2001; King, 2004; Kirk, 2005). One idea is that multicellularity emerged by adaptive resolution of conflict between competing cells, i.e. cooperation enforced by sanctions against 'cheaters' (Buss, 1999). Colonial cells produce signals, i.e. small molecules that are mediated by the environment and processed by a signal transduction system involving transmembrane proteins such as histidine and tyrosine kinases. They also communicate by direct interactions between transmembrane proteins and by gap junctions that directly connect the cytoplasm of adjacent cells (Kaiser, 2001). Hazkani-Covo *et al.* (2004) compared the complete genomes of the unicellular yeast *Saccharomyces cerevesia* to those of the bilaterians *Drosophila melanogaster* and *Caenorhabditis elegans*. In the latter two organisms they found a proliferation of genes encoding proteins targeted to the cell membrane and exterior. They interpreted this as the result of selection for more sophisticated cell–cell communication during the emergence of animals.

Cellular differentiation is also widespread in protozoans and algae. It has been studied in detail in the colonial alga *Volvox carteri*, which displays germ-soma division of labour (Kirk, 2005), the social amoeba *Dictyostelium discoideum*, which forms fruiting bodies and mobile 'slugs' (Eichinger *et al.*, 2005), and the unicellular protozoan *Naegleria gruberi*, which can repeatedly alternate between amoeboid and free-swimming flagellate forms (Fulton, 1993). It seems likely that the unicellular lineage leading to the Metazoa was already capable of cell–cell communication and cellular differentiation. Müller (2003) proposed that these were two of many steps in the evolution of an autonomous, unicellular eukaryote into a primitive metazoan ancestor, an integrated colony or individual composed of cooperating, differentiated cells with the organizational grade of a simple sponge.

The protometazoan ancestor: from many, one

Molecular phylogenetic analyses show that the Choanoflagellates, a monophyletic group of protists of which some possess colonial lifestyles, are the closest sister group to the Metazoa, with which they form a clade to the exclusion of the fungi (Medina *et al.*, 2001). Choanoflagellates also have a morphological resemblance to the flagellated choanocytes of sponges. Maldonado (2004) argued that choanoflagellates are not originally unicellular but are instead highly derived sponges that have reverted to a single-celled existence. One test of this hypothesis is the relative size of the mitochondrial genomes of choanoflagellates, sponges, and other metazoans, which are thought to be under strong selective pressure for downsizing: If choanoflagellates are extremely derived sponges, they should have mitochondrial genomes the same size or smaller than those of sponges. Instead, the mitochondrial genomes of two sponge species are intermediate in size between those of choanoflagellates and other metazoans (Lavrov *et al.*, 2005), consistent with sponges evolving from a choanoflagellate-like ancestor. Two choanoflagellate species, *Monosiga brevicollis* and a *Proterospongia*-like species, express homologs to animal genes for cell–cell signalling and adhesion such as tyrosine kinases and G protein-coupled receptors. At least some animal cell adhesion factors, i.e. cadherins, and C-type (calcium-dependent) lectins are present in choanoflagellates and thus predate metazoans (King *et al.*, 2003). Protein–protein interaction domains also previously thought to be unique to metazoans have been found in choanoflagellates (King, 2004). It would appear that many of the biological building blocks for a multicellular existence were already present in the last common ancestor of the choanoflagellates and metazoans.

A second group occupying a pivotal phylogenetic location with the choanoflagellates near the animal–fungal node is the Mesomycetozoea, a group of parasitic or saprophytic microorganisms (Mendoza *et al.*, 2002). Some members of this group have characteristics of both protists and fungi, and complex lifecycles driven by interactions with their hosts. However, it is not known if the phylogenetic placement of mesomycetozoeans has been somehow affected by their parasitic lifestyle, as appears to be the case for some fast-evolving parasites such as *Giardia lamblia*. Metazoans, choanoflagellates, and mesomycetozoans are sometimes collectively termed the Holozoa, but it is not yet established that this grouping represents a monophyletic clade.

Reconstructing the urmetazoan ancestor

Reconstruction of evolutionary events within the Metazoa is contingent on an accurate phylogeny. The use of protein and eventually gene sequences and other genetic markers in quantitative approaches (Zuckerkandl & Pauling, 1965) has permitted a systematic, although not necessarily complete or incontrovertible view of animal phylogeny (Adoutte *et al.*, 2000; Halanych, 2004). Recent technical advances in genomics and computational biology have greatly expanded the phylogenetic repertoire of genetic markers, taxa, and analytical tools. The last includes the improvement of computational economy in maximum likelihood techniques (Schmidt *et al.*, 2002) and the development of methods based on Bayesian analysis (Huelsenbeck *et al.*, 2001).

The different methods yield variation in the placements of phyla within the overall tree of animal life, but have consistently resolved several major groupings; the Bilateria, Cnidaria, Ctenophora, multiple clades of poriferans, and Placozoa (Fig. 3). The phylogenetic relationships between these five clades are still not resolved (Zrzavy *et al.*, 1998; Wallberg *et al.*, 2004), although most analyses place poriferans at the base of the animal tree. Recent work indicated that sponges are paraphyletic and that the Eumetazoa are sister to the calcareous sponges, to the exclusion of the hexactinellid sponges and demosponges (Borchiellini *et al.*, 2001; Peterson & Butterfield, 2005). The branching order of cnidarians, bilaterians and ctenophores is unresolved, although molecular data on the last group are only now becoming available. The placement of placozoans is likewise problematic: One analysis placed them as sister to the Bilateria (Wallberg *et al.*, 2004), while a recent analysis of the placozoan mitochondrial genome concluded that placozoans are basal to sponges – and all other metazoans (Dellaporta *et al.*, 2006). The resolution of these relationships will be crucial to constructing an explanatory history of evolution of the Metazoa.

The lack of resolution among basal groups of metazoans raises the question of whether this polytomy has evolutionary significance or is simply a reflection of the limits of our data and/or methods. Using independent simulations, Levinton *et al.* (2004) and Rokas *et al.* (2005) found that radiations which occurred in a relatively short period of time in the distant past will likely result in irresolvable molecular phylogenies. It is possible that our inability to resolve the precise relationships between several groups of phyla indicate that they diverged rapidly. It is also possible, however, that these unresolved polytomies are an artefact of the selection of the genes and taxa used in the studies (Jermiin *et al.*, 2005).

Setting aside the question of the position of the Placozoa, phylogenetic reconstructions taken at face value suggest that eumetazoans are derived and that the urmetazoan ancestor would have many traits found in poriferans. Some bilaterian cell signalling and adhesion genes are expressed in the sponge *Oscarella carmela* (Nichols *et al.*, 2006). Larroux *et al.* (2006) reported that some developmental genes found in bilaterians are also expressed during development in the demosponge *Reniera*, even though sponges are thought to possess a primitive grade of developmental complexity (Brusca & Brusca, 2003). Such conserved gene functionality suggests that the urmetazoan ancestor had the potential for specification of multiple cell types, formation of fixed body axes, and simple tissue development (Larroux *et al.*, 2006). The urmetazoan ancestor would have had mechanisms to control growth and eliminate unwanted cells in those tissues (Müller, 2003). Multicellular organisms possess a variety of mechanisms that regulate their size or that of their constitutive tissues (Gomer, 2001), but animals have widely adopted apoptosis (programmed cell death) as an integral part of tissue development and size regulation. Apoptosis involves the synthesis of enzymes such as caspases, a family of cysteine proteases that break down cells. Caspases are present in both sponges and cnidarians (Wiens *et al.*,

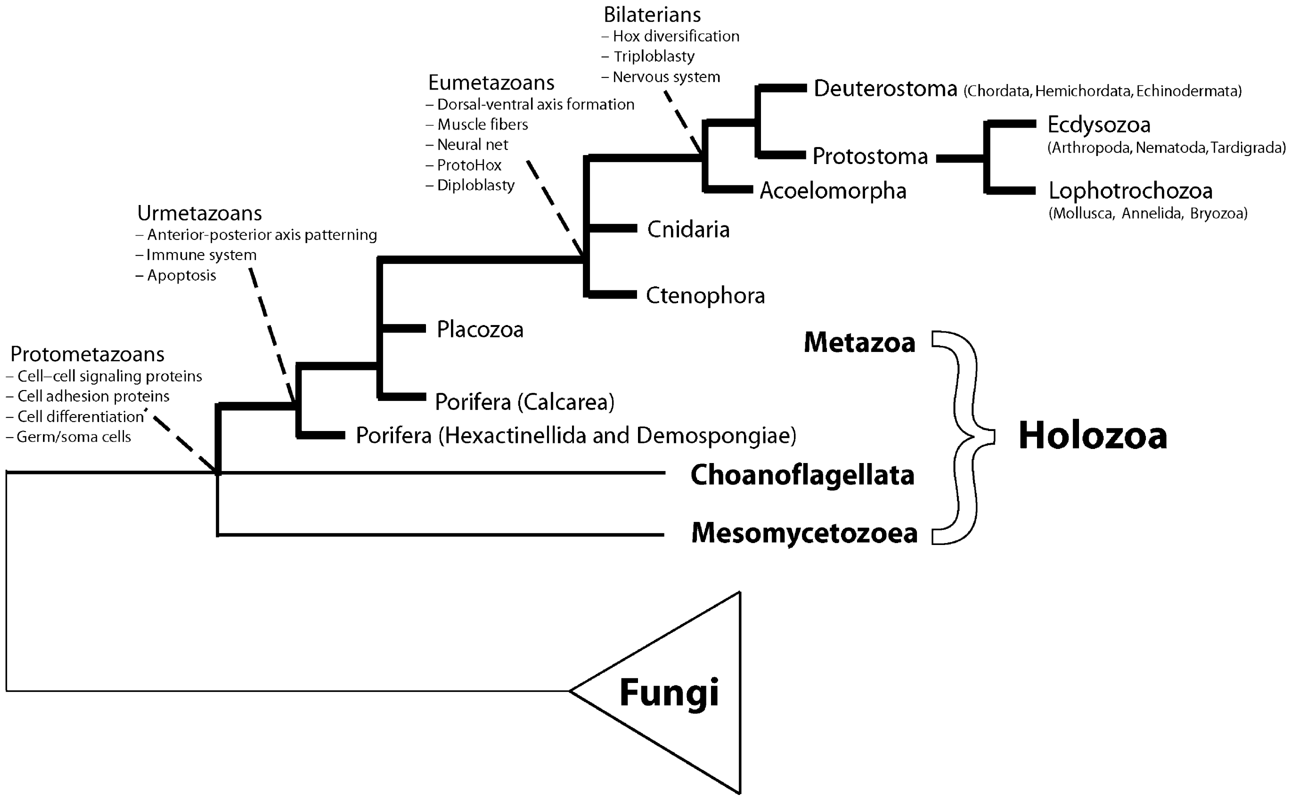

Fig. 3 Schematic phylogeny of the major extant groups of the Metazoa and their closest relatives, the choanoflagellates and mesomycetozoan protists. The three groups are collectively termed the Holozoa. The Fungi is the sister clade to the Holozoa, to the exclusion of plants and all other unicellular eukaryotes. The phylogenetic placement of Placozoa is controversial, with different analyses placing it basal to all other Metazoa, basal to the eumetazoan ancestor, or as a sister group to Bilateria. The evolutionary relationships between Bilateria, Cnidaria, and Ctenophora are also not established, and the paraphyletic nature of sponges is still tentative.

2003a; Wiens & Müller, 2006) but their origin is unclear. No true caspases have been yet found in eukaryotes outside Metazoa although caspase-like activity has been documented in plants, fungi, protists, and some bacteria, and genes with significant sequence similarity to caspases have been discovered in these groups (Boyce *et al.*, 2004). [The animal form of caspase may in fact be the result of horizontal transfer from bacteria (Koonin & Aravind 2002)]. The sponge-like urmetazoan ancestor would also have had segregated germ cells. Animals segregate germ cells by two mechanisms; epigenesis (signal induction) and preformation by inherited determinants. The former is used to the exclusion of the latter in basal groups such as sponges and cnidarians and would appear to be ancestral (Extavour & Akam, 2003). In these organisms, pluripotent cells can produce both germ and somatic stem cells, allowing for regeneration as well as both sexual and asexual reproduction.

From one, many

Most of metazoan diversity is based on developmental pathways in which stem cells differentiate into multiple somatic cell types in specific spatial patterns. This often takes place during embryogenesis from zygote to adult, although sponges and jellyfish and some bilaterians retain such totipotent cells in the adult form and can reproduce by budding. Since the pioneering 19th century work of T. H. Huxley and E. Haeckel, and later research by Hyman (1940), differences in the physiology of extant organisms as well as the evolution of body plans have been cast in terms of differences in development and of the evolution of regulatory genes involved in development (Goodman & Coughlin, 2000).

The introduction of genomic and proteomic techniques has revitalized the study of developmental evolution. A typical approach is to compare the pattern of spatial expression of developmental genes in two evolutionarily distant organisms and to infer the function of the gene in their last common ancestor (Martindale *et al.*, 2004; Martinelli & Spring, 2004; Larroux *et al.*, 2006). However, conservation of spatial expression pattern does not necessarily imply conservation of function (Nielsen & Martinez, 2003; Svensson, 2004). While it is relatively easy to verify gene function in model organisms such as *Drosophila melanogaster* which have well-developed genetic tools, such finesse is not available for many invertebrates.

In such cases, gene silencing by small interfering RNA (siRNA) (Hannon, 2003) could prove to be a useful probe of developmental gene function. Interpretation of experiments also depends on the phylogenetic placement of the organisms of choice, something that is controversial for many basal metazoan taxa. Martindale (2005) notes that much of the work has been done on highly derived organisms that may have lost many primitive traits relative to basal taxa.

A common conclusion of studies so far is that developmental genes and pathways (or their homologs) are highly conserved across all major phyla, including basal nonbilaterians. The widespread conservation of both embryogenesis pathways and developmental regulatory genes also suggests that the core of developmental gene regulatory networks was 'frozen' early in animal evolution (Davidson & Erwin, 2006) and that evolutionary events responsible for different body plans and phyla happen by comparatively modest rearrangements or duplications of existing genetic machinery (Gilbert, 2003).

Members of the Hox gene family of homeodomain proteins play a key role in pattern formation during bilaterian embryogenesis, and the diversification of triploblastic (bilaterian) animal body plans has been interpreted in terms of the changing number, specificity, and expression of clusters of these genes (Gellon & McGinnis, 1998; Peterson & Davidson, 2000; Garcia-Fernández, 2005a, b). The picture has been complicated by the discovery of the ParaHox sister group (Brooke *et al.*, 1998) and the finding that cnidarians, diploblasts with only rudimentary (or vestigial) symmetry, possess both Hox and ParaHox genes (Finnerty & Martindale, 1999). Hox/ParaHox genes appear to be unique to the metazoans, but non-Hox homeoproteins are found in plants and fungi where they play a role in mating type regulation. The presence of Hox and ParaHox genes in both the Bilateria and the Cnidaria indicates that an ancestral 'ProtoHox' gene cluster diverged in an even more distant metazoan ancestor (Chourrout *et al.*, 2006; Ryan *et al.*, 2007). Searches for ProtoHox analogs in ctenophores and placozoans have uncovered promising candidates (Finnerty *et al.*, 1996; Jakob *et al.*, 2004); there is at least one homeoprotein in sponges (Perovic *et al.*, 2003), but so far no Hox genes have been found. If further genome sequencing in additional poriferans and representatives of the Ctenophora and Placozoa bear out this picture, then either the Hox/ParaHox group was independently lost in the different poriferan lineages (unlikely), or animal Hox genes arose from a single homeoprotein ancestor in a eumetazoan ancestor (Bharathan *et al.*, 1997).

In addition to the discovery of Hox genes, studies of development in sea urchins, jellyfish, and hydra also provide tantalizing evidence that bilateral symmetry and triploblasty may also have its origins in a common ancestor to bilaterians and cnidarians (Finnerty *et al.*, 2004; Finnerty, 2005; Kamm *et al.*, 2006; Matus *et al.*, 2006). It remains to be seen whether the Ctenophora share this ancestry. All eumetazoans share a common developmental pathway involving the formation of germ layers and both anterior-posterior and dorsal-ventral patterning as the foundation for body plan formation during embryogenesis. In contrast, the larval forms of sponges may have anterior-posterior organization but lack a dorsal-ventral axis (Martindale, 2005). Thus the ultimate origin of both Hox/ParaHox genes and axial patterning may lie in the eumetazoan ancestor.

A plausible scenario of animal emergence based on the traits of extant organisms involves a unicellular protometazoan ancestor resembling a choanoflagellate and having a colonial lifestyle and a complement of cell–cell signalling and adhesion genes. Later adaptations that evolved in a sponge-like urmetazoan ancestor were components of structural integrity, e.g. fibrous collagen proteins, fibronectin, and integrins (Müller, 2003; Wiens *et al.*, 2003b), caspase-based apoptosis, and segregated germ cells. The development of a relatively simple body plan would have been regulated by a progenitor set of homeoproteins; axial patterning would emerge only with the appearance of the Hox/ParaHox gene family in the eumetazoan ancestor. Sly *et al.* (2003) suggested that the ancestral form of bilaterians was a direct developer exhibiting gradual development of the adult body plan without metamorphosis. This ancestral form then developed a planktonic larva that could disperse and move to a wider variety of habitats, leading to an adaptive radiation in the earliest Cambrian.

CONNECTING GEOLOGY AND GENOMES

Undoubtedly, the greatest challenge in the study of animal emergence is to connect biological evolution and geological change in the late Precambrian. For example, what was the impact of a 'snowball Earth' climate state on life, and is there any connection with the appearance of the Metazoa? Complete entombment of the oceans by thick ice represents obvious metabolic challenges for life, particularly complex organisms (Gaidos *et al.*, 1999). Negative excursions in the isotopic record of carbon in carbonates are suggestive of, but not uniquely interpretable as, severe attenuation of the biosphere during Neoproterozoic glaciations (Kirschvink, 1992). However, the chemofossil and microfossil record does not support a scenario of mass extinction (Olcott *et al.*, 2005; Corsetti *et al.*, 2006) and, unless a much older age for the Doushantuo is accepted, the oldest unequivocal evidence for animal life still postdates the last interval of Neoproterozoic glaciation (Narbonne & Gehling, 2003). The persistence of ice-free oases at the equator would have permitted presnowball planktonic organisms to persist, including, presumably, the urmetazoan or eumetazoan ancestor. Runnegar (2000) speculated that blue-water refugia during a snowball event would have selected for organisms with extended planktonic larval stages. Such long-lived, yolk-feeding (lecithotrophic) larvae would have included extra 'set-aside' cells that after deglaciation underwent adaptive evolution in benthic habitats (Davidson *et al.*, 1995; Peterson & Davidson, 2000). However, Peterson (2005) has recently shown that the lecithotrophic larval form evolved independently in multiple groups by the Early Cambrian, perhaps as a result of selection by benthic predation, and was not ancestral.

Atmospheric O_2 is an important link between geological process and biological evolution. The appearance of abundant O_2 has long been related to the development of animal life, e.g. (Nursall, 1959; Runnegar, 1982b), and new data show that the appearance of the Ediacaran biota is contemporaneous with oxygenation of the deep ocean (Fike *et al.*, 2006; Canfield *et al.*, 2007). Although anoxic environments persist on the modern Earth, metazoans capable of sustained anaerobic metabolism are invariably small and derived from aerobic ancestors (Hochachka *et al.*, 1973). In the latest of many papers on the subject, Catling *et al.* (2005) argued for the uniqueness of oxygen as an ideal inorganic electron acceptor in respiratory metabolism. They also estimated the relative energy yields of anaerobic vs. aerobic metabolism of glucose (3–12%), and the relative growth yields per energy-bearing molecule of aerobic vs. anaerobic organisms (six times). They concluded that an oxygen concentration of 10% PAL was required for millimetre-sized organisms lacking circulatory systems: higher concentrations would have been necessary for organisms to attain centimetre sizes, and still larger organisms would have required complex respiratory systems (Catling *et al.*, 2005).

However, it is at least possible that the earliest interval of metazoan evolution occurred under conditions of low oxygen, perhaps a few percent PAL. An O_2 level of 5% PAL by 600 Ma is sufficient to explain the late Neoproterozoic sulfur isotope data (Canfield & Teske, 1996). Flat worm-like ‘animals’ such as *Dickinsonia* that required a slightly higher O_2 concentration (Runnegar, 1991) and trace fossils indicative of animal activity did not appear until about 560 Ma. Earlier organisms, including Ediacaran *Tribrachidium* and rangeomorphs, may have had a passive planktonic or sessile lifestyle like those of jellyfish or sponges, one that minimized their O_2 requirement. Lack of a circulation system may not have inhibited the evolution of large animals; sponges can grow to meter sizes, relying on a simple system of internal water circulation to provide oxygen to cells. The first species may have relied exclusively on passive filter-feeding of phytoplankton, bacterioplankton, and particular organic matter rather than predation. More speculatively, algal symbionts could have provided an additional source of energy to animal hosts under conditions of low oxygen. Such symbionts are found in extant cnidarians (notably corals), ctenophores, acoels and at least one species of mollusc (Rumpho *et al.*, 2000).

Whenever it happened, the mechanism(s) by which O_2 levels rose to approximately the modern value is not known. O_2 levels in Phanerozoic time (543 Ma to Recent) are thought to have been controlled by the balance between the burial of organic matter and pyrite in marine sediments and weathering of these reduced forms of carbon and sulfur in older, uplifted sedimentary rocks (Lasaga & Ohmoto, 2002; Berner *et al.*, 2003). In this picture, an increase in O_2 requires either an enhancement in organic matter burial or an attenuation of weathering. The relatively constant $\delta^{13}C$ in carbonates over Earth history (with some exceptions as discussed above) has been traditionally interpreted as indicating a constant ratio of organic to inorganic carbon burial rate (Schidlowski, 1988). Because the total burial rate must match the total flux of CO_2 from volcanoes and metamorphism of carbonate rocks, which is assumed to be stable or only slowly decreasing, an approximately constant burial rate of organic carbon is also inferred. Nevertheless, variations in $\delta^{13}C$ are consistent with elevated rates of organic carbon burial and a concomitant increase in O_2 during the Neoproterozoic (Des Marais *et al.*, 1992). Furthermore, Bjerrum & Canfield (2004) argued that calculations of organic/inorganic burial ratio must account for removal of inorganic carbon by hydrothermal weathering of seafloor and that this new accounting shows a significant increase in the burial rate of organic matter over Earth history.

Any Neoproterozoic increase in organic matter burial in turn demands a mechanistic explanation: Some scenarios invoke the action of animals themselves to explain increased burial efficiency, e.g. the production of faecal pellets (Logan *et al.*, 1995), or burrowing to escape predators. The latter is a circular argument as burrowing itself probably requires elevated seawater O_2; animal-independent evidence for increasing O_2 pre-dates sediment disturbance by tens of million years or more (Canfield & Teske, 1996; Droser *et al.*, 2002; Fike *et al.*, 2006; Canfield *et al.*, 2007). The faecal pellet scenario is tenable only if the animal gut evolved before a benthic lifestyle (and the advent of trace fossils). This possibility is suggested by the acritarch record (Peterson & Butterfield, 2005), but settling of organic matter to the seafloor is only the first step in its diagenesis in marine sediments and eventual preservation – or not. Searches for pellets or organic microtextures in pre-560 Ma sedimentary rocks (Robbins *et al.*, 1985; Krinsley *et al.*, 1993; Brasier & McIlroy, 1998) could support or rule out the faecal pellet scenario.

Changes in the cycling of other elements may have contributed to the increase in oxygen. Bjerrum & Canfield (2002) proposed that the concentration of phosphorus in the late Archean (3.8–2.4 Ga) and early Proterozoic oceans was suppressed by adsorption onto the iron oxide particles in BIFs. Phosphorus, derived almost exclusively from weathering of terrestrial rocks, is thought to be the limiting nutrient on geological timescales (Benitez-Nelson, 2000) and thus low P concentrations would have limited net primary productivity and hence O_2 production. Following the cessation of BIF deposition, the concentration of marine P, and hence O_2 production would have increased. Elevated phosphorite deposition did occur across the Proterozoic-Phanerozoic transition (Cook & Shergold, 1984), but modern phosphorite formation is a consequence of benthic microbial biogeochemistry at sites of ocean upwelling and not necessarily a reflection of seawater phosphate concentration. Adsorption on ferric oxyhydroxides may also have been moderated by competition with elevated levels of silica (Konhauser *et al.*, 2007).

In contrast to phosphorus, nitrogen can be fixed from the atmosphere into a bioavailable form by some bacteria and prokaryotes and does not impose the same absolute limits on marine productivity. However, the N-fixing enzymes nitrogenase and nitrogenase reductase use metal cofactors such as Fe and Mo, which can form insoluble sulfides under reducing conditions, or V, which is also depleted in anoxic ocean basins (Emerson & Huested, 1991). Deepwater anoxia in the Neoproterozoic may have removed these and other bioessential metals from solution, thus limiting primary production (Anbar & Knoll, 2002) (Fig. 2); oxygenation of ocean deep water would have removed this sink. However, cyanobacterial growth could have been robust to changes in marine chemistry, as experiments with modern organisms suggest (Zerkle *et al.*, 2006), by increased uptake affinity, preferential use of less insoluble metals, e.g. V, or changes in cellular C:N ratio. During low but nonzero levels of oxygen in the Neoproterozoic, bioavailable nitrogen may also have been limited by the absence of ammonia and depletion of nitrate by prokaryotic denitrification (anaerobic reduction of nitrate to N_2O and N_2) the Neoproterozoic (Fenndel *et al.*, 2005) (Fig. 2). The lack of fixed nitrogen limited primary production, which in turn inhibited the rise of O_2.

However, all of these describe positive *feedbacks* in the oxygen cycle, rather than triggers. In search of a trigger, attention has recently turned to terrestrial drivers of change in the marine carbon cycle during Neoproterozoic time. Lenton & Watson (2004) proposed that preferential leaching of phosphorus from continental rocks by terrestrial fungi or lichens may have enhanced the flux of this important nutrient into the Neoproterozoic oceans (Taunton *et al.*, 2000; Welch *et al.*, 2002). If marine productivity is phosphorus-limited on geological timescales and carbon is buried with a fixed C: P ratio, an increased flux of P into the oceans would have permitted increased organic matter burial and allowed atmospheric O_2 concentrations to rise. Fungal acceleration of silicate weathering rates would also have lowered atmospheric CO_2 and potentially initiated glaciations (Schwartzman & Volk, 1991; Heckman *et al.*, 2001; Lenton & Watson, 2004). Kennedy *et al.* (2006) pointed to a Neoproterozoic shift in marine sediment composition and a rise in radiogenic $^{87}Sr/^{86}Sr$ as evidence of increased continental weathering and transport of clay particles to the ocean. Because clay minerals are generally produced in biologically active soils, they interpret this as a signal of the proliferation of a hypothetical terrestrial biota in the Neoproterozoic. They proposed that close association of organic particles with clay minerals enhanced their preservation in marine sediments, and increased net organic carbon burial and atmospheric O_2.

One weakness of these scenarios is the absence of evidence for a Proterozoic terrestrial biome capable of enhancing weathering, i.e. plants or fungi. There are no undisputed land-plant body fossils or spores older than the mid-Ordovician, *c.* 476 Ma (Kenrick & Crane, 1997) (Fig. 1). One molecular clock analysis placed the origin of land plants at 700 Ma (Heckman *et al.*, 2001), but, as was discussed earlier, such estimates are highly problematic. Yuan *et al.* (2005) recently reported associations between fungal hyphae and algae or cyanobacteria in the Doushantuo formation; however, these are thought to be of *marine* origin. The oldest indisputable terrestrial eukaryotic microfossils are 1.2 billion years old (Horodyski & Knauth, 1994), although there is geochemical evidence for microbial activity in older soils (Watanabe *et al.*, 2000; Prave, 2002; Watanabe *et al.*, 2004), but see also Melezhik *et al.* (2004). Fungi have been shown to enhance weathering of silicate rocks and leaching of nutrients (Gislason *et al.*, 1996; Brady *et al.*, 1999; Etienne & Dupont, 2002). Searches for evidence of early land fungi, perhaps in the form of unique chemofossils, are of obvious merit. Of course, a terrestrial connection would only defer the question of what ultimately drove the timing of global change. There are no obvious environmental factors that would limit the emergence of terrestrial biosphere to the Neoproterozoic: the small amount of oxygen required to produce an ozone layer that would shield surface life against harmful UV radiation was probably present after the initial oxygenation event *c.* 2.4 Ga (Kasting, 1987).

Another concern is that increased weathering would also have accelerated the liberation and oxidation of reduced carbon and sulfur from sedimentary rocks, thus enhancing the consumption of O_2. However, volcanic rocks, not sedimentary rocks (whose minerals have already experienced some degree of weathering), provide most of the Ca^{2+} and Mg^{2+} ions to the oceans and drive carbonate deposition on geological timescales. Igneous rocks, particularly basalts, are also relatively rich in phosphorus. Preferential weathering of these rocks can lower atmospheric CO_2 levels, cool the planet, suppress the rate of weathering of all other rock types (including kerogen- and pyrite-containing sedimentary rocks), and thereby simultaneously increase organic matter burial and decrease O_2 consumption by weathering. In this way, weathering of volcanic provinces emplaced during the break-up of Rodinia and the opening of the Pacific and Iapetus oceans (Frimmel *et al.*, 2001; Li *et al.*, 2003) may have ushered in a colder Neoproterozoic climate regime as well as boosted oxygen levels.

It is also possible that Proterozoic O_2 was set by microbial respiration in an organic-rich ocean, rather than abiotic weathering of reduced carbon and sulfur on land. In this scenario, the advent of filter feeding and grazing in Ediacaran taxa as suggested by the acritarch record (Peterson & Butterfield, 2005) also removed the reactive marine organic matter that was the substrate for microbial respiration, allowing O_2 to rise. On long timescales, the *rates* of oxidation of volcanic gases and terrestrial reduced compounds must balance burial of organic matter in marine sediments. But if these rates are only weakly sensitive to O_2 *concentration*, as they may be (Betts & Holland, 1991; Lasaga & Ohmoto, 2002), then the latter will be determined primarily by the balance between rates of primary production and aerobic respiration. Over an organic-rich Neoproterozoic ocean, the equality between production and consumption would have occurred at lower pO_2 because of the high oxygen affinity of aerobic bacteria. That large organic reservoir could have been the same reservoir of reactive carbon proposed by Rothman *et al.* (2003) to explain the Neoproterozoic oscillations in the carbon cycle. Since the advent of animals and the disappearance of that reservoir, the balance between production and consumption of oxygen has occurred at higher pO_2. The transition between those states involved a lower efficiency of remineralization and higher rate of carbon burial, as suggested by the carbon isotope record. Hypothetically, efficient filter feeding of sponge-like ancestral metazoans by 560 Ma would have produced oligotrophic conditions in the oceans, lessening microbial activity, and allowing atmospheric O_2 to rise. The virtual disappearance of suspended organic matter would have led to an ecological crisis that selected for complex body plans able to predate or filter feed on larger particles (Vacelet & Duport, 2004). At the same time, higher oxygen levels would have allowed increased body size and permitted colonization of the benthos, leading to the advent of trace fossils. In this scenario, animal evolution was both the cause and effect of increased atmospheric oxygen.

Such a dynamic is one example of how our animal ancestors and our planet may have coevolved through microbial intermediaries. Numerous interactions between modern animals and microbial symbionts, commensals, and parasites have been documented, and there is no reason why such interactions were not equally well developed among the Ediacaran and Cambrian faunas (McMenamin, 1986; Fortey, 2000). Microorganisms mediate many, if not most of the steps in biogeochemical reactions important to ocean chemistry, atmospheric composition, and climate. Microbial mats dominated the shallow seafloor of the late Precambrian and served as the primary habitat of benthic animals before being overthrown in the 'Cambrian substrate revolution' (Bottjer, 2005). Close associations between animals (particularly sponges) and bacteria may even have provided some of the genetic source material for metazoan evolution (Lakshminarayan *et al.*, 2004).

Animal evolution and diversification subsequent to 555 Ma could have been more strongly influenced by animal–animal interactions than by the abiotic and microbial environment (Peterson *et al.*, 2005; Marshall, 2006). Among many other innovations that accompanied the emergence of animal life, the development of the nervous system is arguably one of the most important, as it allowed the rapid receipt, processing, and transmission of information about an animal's environment and enabled complex behaviour on which natural selection could have acted. Sponges have no nerve cells, but cnidarians possess a neuronal network that includes a ring-shaped concentration of cells in some taxa. The central nervous system appears to have arisen in the common ancestor of the bilaterians (Holland, 2003) (Fig. 3). The emergence of complex behaviour radically changed the environment of animals as they become predators, prey, mates, or competitors of other animals (Stanley, 1976). Ecological interactions become the most dynamic part of the environment, leading to the 'evolutionary arms race' of further selection, specialization, and diversification that continue today.

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