Chapter 2

Molecular clocks and a biological trigger for Neoproterozoic Snowball Earth events and the Cambrian explosion

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ABSTRACT

Two major events occurred in the history of the Earth and its biota during the late Precambrian and Cambrian (750–500 Ma; million years ago): a sequence of global glaciations (Snowball Earth events) followed by the sudden appearance in the fossil record of approximately half of the living animal phyla (the 'Cambrian explosion'). This has fuelled speculation that the two events were associated, perhaps through the generation of biological diversity during periods of isolation in glacial refugia, or in the period subsequent to isolation. However, recent molecular clock analyses have suggested that fungi and plants colonized land in the late Precambrian, considerably earlier than indicated by the fossil record, raising the possibility of a different connection between Snowball Earth events and the Cambrian explosion. These new data suggest a biological rather than geological trigger for the global glaciations, through increased rates of weathering by land fungi (e.g. lichens) and plants, and burial of decay-resistant carbon compounds of early land plants. The weathering and carbon burial would have lowered levels of carbon dioxide, possibly leading to the Snowball Earth events. A biological trigger can also explain the cyclic nature of the glaciations if they reflect cycles of extinction and recovery. Moreover, the fungal photobionts and early land plants may have generated sufficient oxygen in the latest Precambrian and Early Cambrian for animals to evolve larger body sizes and hard parts, explaining the Cambrian explosion. This model can be tested by increased precision of molecular clock estimates of divergence times and by searching for biomarkers and fossils of land fungi and plants in rocks of this time period.

Introduction

The last decade has seen major advances in our knowledge of Earth and biotic history in the late Precambrian, especially the Mesoproterozoic (1600–1000 Ma) and Neoproterozoic (1000–545 Ma). These have come from discoveries in geology and geochemistry, palaeontology, molecular evolution, and developmental biology. These discoveries and their resulting models continue to be debated but are changing our perceptions of the late Precambrian biosphere. For example, the Earth may have passed through several cycles of global glaciations during the period 750–580 Ma, each of which may have been characterized by complete freezing of all of the oceans for 10 myr or longer (Hoffman *et al.* 1998). At the same time, molecular clocks have

suggested that major groups of complex multicellular organisms such as plants, animals, and fungi were present during, if not before, these global glaciations (Wray *et al.* 1996; Feng *et al.* 1997; Wang *et al.* 1999; Heckman *et al.* 2001). Fossils of complex organisms (Wood *et al.* 2002), metazoan embryos (Li *et al.* 1998; Xiao *et al.* 1998), and trace fossils (Rasmussen *et al.* 2002) have been found considerably earlier than expected, lending some support to molecular clock estimates.

On the one hand, these new revelations appear contradictory: an earlier history of complex life in an environment that was much harsher. On the other hand, the contradiction disappears if one is causally connected to the other. A connection that is explored in this chapter is the suggestion that the early colonization of land by fungi and plants became a biological trigger for the Snowball Earth events and the Cambrian explosion (Heckman *et al.* 2001). Firstly, I will review the evidence from molecular clocks for the early diversification of complex life (plants, animals, and fungi), and recent fossil evidence. This will be followed by a discussion of the data supporting Neoproterozoic global glaciations and a proposed geological trigger. Finally, I will discuss the biological trigger model for the initiation of Snowball Earth events and the Cambrian explosion.

Molecular clocks and the early diversification of animals, fungi, and plants

From their conceptual inception three decades ago, molecular clocks have consistently found early divergences for selected animal phyla (Brown *et al.* 1972; Runnegar 1982a,b; Runnegar 1986; Doolittle *et al.* 1996; Wray *et al.* 1996; Feng *et al.* 1997; Bromham *et al.* 1998; Wang *et al.* 1999). In most of these studies, relatively small numbers of genes or proteins were used, and there has been some discussion concerning methodology (Ayala *et al.* 1998; Gu 1998). However, in one case a relatively large number of proteins (50) was used and the vertebrate–arthropod divergence time was estimated at approximately 1000 Ma (Wang *et al.* 1999). The split between cephalochordates (amphioxus) and vertebrates was dated at approximately 750 Ma using nine proteins (Hedges 2001), suggesting that even relatively closely related groups of animals might have deep divergence times. However, most animal phyla have yet to be included in molecular clock analyses because of the paucity of protein sequence data.

The nuclear small subunit ribosomal RNA gene has been used to date the divergence of major groups of fungi, and some splits have been found to be older than 800 Ma (Berbee and Taylor 1993, 2001). However, a relatively young date (965 Ma) for the divergence of fungi and animals was used as a calibration point in those studies, derived from another molecular clock study (Doolittle *et al.* 1996). Subsequently, that split has been dated at 1200 Ma (Feng *et al.* 1997) and 1576 Ma (Wang *et al.* 1999), and the use of those dates as calibrations would proportionately extend the fungal divergence times (Berbee and Taylor 2001) deeper into the Neoproterozoic. In a more recent study, divergences among nine lineages of fungi were dated using 111 proteins and all were found to be Precambrian, with most, including lineages associated with land plants, diverging in the interval 800–1200 Ma (Heckman *et al.* 2001). In that study, the divergence of chlorophytan green algae and seed plants (41 proteins), and between land and vascular plants (50 proteins), were found to be approximately 1100 Ma and 700 Ma, respectively, suggesting an interval during which land plants arose

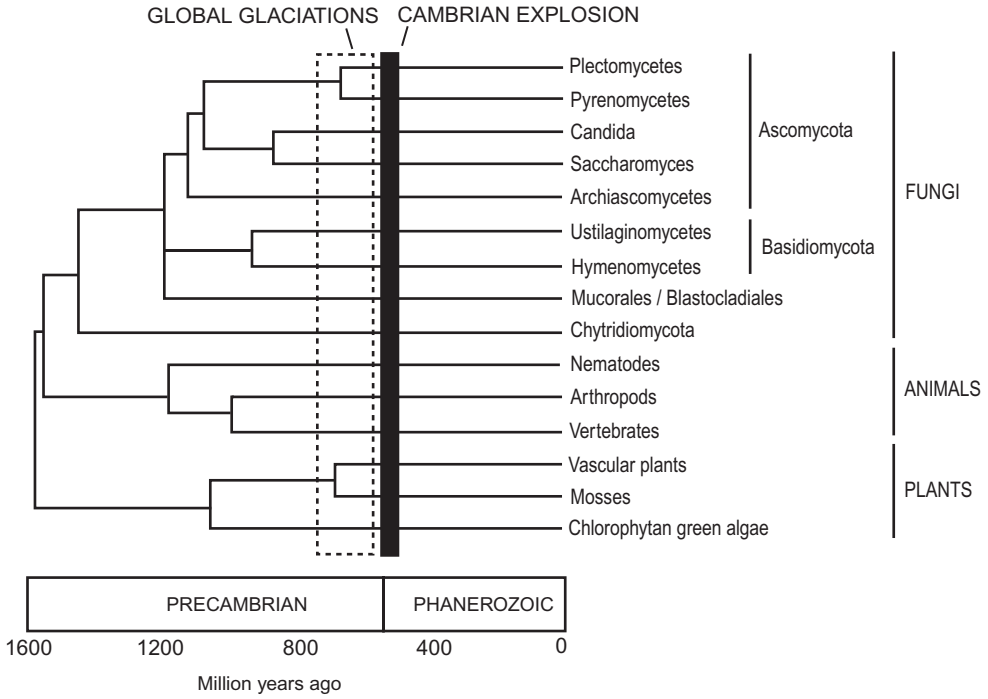

Figure 2.1 A time-calibrated evolutionary tree of fungi, animals, and plants showing times of divergence of selected lineages based on molecular clock studies (Wang *et al.* 1999; Heckman *et al.* 2001; Hedges 2001).

(Figure 2.1). Most of these molecular clock estimates have associated standard errors derived from differences among single protein estimates (Wang *et al.* 1999; Heckman *et al.* 2001). As with other fields of science, different estimates may be obtained with different methods and therefore the error in the estimate does not refer to the difference from the true value (always unknown) but rather the error as estimated under the specific conditions of analysis. In that sense, it is no different from the interpretation of error estimates of phylogenies (Nei and Kumar 2000).

In nearly all of these studies, the molecular clocks have been calibrated ultimately with robust fossil-based estimates of vertebrate divergences or secondary calibrations (molecular time estimates) derived from those primary fossil calibrations. The most commonly employed vertebrate fossil calibration point is the divergence of the mammalian and avian lineages at 310 Ma (Benton 2000). The robustness of this calibration point has been justified elsewhere (Kumar and Hedges 1998; Wang *et al.* 1999) and includes the following factors: (i) the fossils are exceptionally well-preserved, (ii) the earliest representatives of the two lineages are similar in morphology, suggesting that the palaeontological record for the divergence date is not a significant underestimate, (iii) no older remains of either lineage have been found since the mid-1800s, and (iv) earlier branches in the vertebrate tree constrain this divergence from being significantly older. A claim that this divergence should date more appropriately at 288 Ma (Lee 1999) is not supported by others (Carroll 1997; Benton 2000); even if true

this would reduce time estimates by only 7 per cent. If anything, calibrations are likely to be underestimates of the true divergence, but the amount of underestimation is unknown and therefore calibrations are typically presented without errors. If the error in a calibration time is known, such as from variation in the age of the fossils, it could be used as a propagated error in computing each single-gene time estimate (van Tuinen and Hedges 2001). In such instances, the among-gene standard error of the overall estimate would encompass the calibration error.

What are the potential biases in molecular clock analysis that could explain such old time estimates for plants, animals, and fungi? It is widely known that relative rate tests do not detect all rate heterogeneity. Therefore, it is obvious that any undetected heterogeneity could bias the resulting time estimate (Bromham *et al.* 2000). However, in large studies involving many genes, there is no reason to expect a directional bias in the overall time estimate. None the less, we tested this in a study of 658 proteins in vertebrates (Kumar and Hedges 1998) by increasing the stringency of the rate test far beyond the 5 per cent level, effectively removing nearly all rate heterogeneity. Although many more proteins and comparisons were rejected, there was no effect on the overall time estimates indicating that there was no directionality to the rate variation. In specific cases involving species with branches that are consistently short (or long) in many gene trees, some directional bias might be expected, and this would be evident if the stringency of the rate test were increased as described above. However, time may be estimated even in those cases showing rate differences by using lineage specific and variable rate methods (Sanderson 1997; Schubart *et al.* 1998; Thorne *et al.* 1998). The power of the rate test is higher in longer sequences and therefore short sequences distinguished by only one or a few differences should be avoided.

It has been claimed that the well-known statistical bias resulting from averaging ratios might cause an overestimation of time in multiprotein studies, favouring a sequence concatenation approach (Nei *et al.* 2001). Although theoretically correct, its effect on time estimation has been shown to be minimal, probably because large extrapolations are typically avoided and modes are used rather than means (Heckman *et al.* 2001; Hedges *et al.* 2001). In contrast, concatenation may prevent detection of contaminant proteins (paralogues) that are normally detected in the multiprotein approach (Kumar and Hedges 1998). Although some paralogues are easily detected in individual gene trees, especially if different sequences of the same species appear in the tree (clearly indicating the result of gene duplication), other cases of paralogy are not easily detected by such simple inspection. For example, if some gene loss has occurred and multiple sequences of the same species are not present, detection of paralogy might require additional sequences. However, such a gene (without use of additional sequences) would be likely to be an outlier in a multiprotein clock analysis because the branching event being dated would be an earlier event (gene duplication) and not the speciation event in question. Another limitation of the concatenation approach is that, in effect, it gives the fastest evolving proteins the highest weight (i.e. because they contain the highest proportion of variable sites) whereas those proteins may produce the greatest distance estimation errors.

Yet another statistical bias has been attributed to the multiprotein approach (Rodríguez-Trelles *et al.* 2002). This may be a problem with short proteins having low rates of change and estimations involving large extrapolations. In such instances,

the substitutions between closely related sequences (e.g. the calibration) may be underestimated, resulting in an overestimate of divergence time for a distant node. However, simulations (Rodríguez-Trelles *et al.* 2002) have shown that the bias is minimal (~1–2 per cent) for proteins of typical length and rate of change, and, in practice, authors have been aware of this potential bias and have avoided it (Kumar and Hedges 1998; Wang *et al.* 1999). Also, most distributions are not right skewed, and modes (rather than means) have been used for those non-normal distributions (Kumar and Hedges 1998). Some authors have objected to the use of secondary calibration points based on molecular clock estimates as not being 'independent' (Smith and Peterson 2002). However, independence is not a requirement for calibration. Such secondary calibrations are simply used to acquire more proteins or genes for comparison and thus increase the precision of the time estimates.

Another criticism of molecular clocks is that rates may have changed in many lineages concurrently, such as during an adaptive radiation, resulting in consistently biased time estimates (Gingerich 1986; Foote *et al.* 1998; Benton 1999; Conway Morris 2000). For this criticism to be valid the rate change would have to take place to exactly the same degree in the calibration lineages (unlikely) or else the rate test would detect the differences. However, even in such a case, inconsistencies would arise between the fossil record and molecular time estimates that would reveal the distortion in the timescale. As has been pointed out previously (Kumar and Hedges 1998; Easteal 1999), time estimates before and after the Late Cretaceous 'gap' in the fossil record of birds and mammals are largely consistent, suggesting that a widespread increase in molecular rate of change at the Cretaceous–Tertiary boundary was not responsible for the older divergence time estimates (Hedges *et al.* 1996; Kumar and Hedges 1998). In the case of the deep Precambrian divergence times, there are fewer fossil constraints to rule out a uniform rate change, but the occurrence of fossil red algae at 1200 Ma (Butterfield 2000) constrains the plant–animal–fungus divergence to an even earlier date, given that red algae are part of the plant lineage (Moreira *et al.* 2000). Thus, it would not be possible to compress the ~1000 Ma divergences between animal phyla (Wang *et al.* 1999) and fungal lineages (Heckman *et al.* 2001) by 50 per cent, up to the Precambrian–Cambrian boundary, without creating inconsistencies between the molecular and fossil divergence times for plants versus animals and fungi. Moreover, there is no known molecular mechanism to explain such rate acceleration. Typical amino acid substitutions in the housekeeping genes that are employed in these clock studies are considered effectively neutral and are unlikely to be associated with the major morphological changes that take place in adaptive radiations.

Fossil evidence

No widely accepted fossils of animals, land plants, or fungi have yet been collected from deep in the Proterozoic that would corroborate the 1 Ga divergence time estimates calculated in molecular clock studies. None the less, fossils of all three groups have been collected in recent years that have significantly extended their times of origin. In the case of animals, body fossils have been found as early as 555 Ma (Martin *et al.* 2000), embryos to ~570 Ma (Li *et al.* 1998; Xiao *et al.* 1998), and radially symmetrical impressions of possible metazoans at 600–610 Ma (Martin *et al.* 2000). The most convincing evidence for the existence of metazoans prior to this are the

1200 Ma trace fossils of vermiform organisms from rocks in southwestern Australia (Rasmussen *et al.* 2002).

Until recently, the oldest known fossil remains of fungi were from the Rhynie Chert (400 Ma) but, with the discovery of glomalean fungi from Ordovician shallow marine sediments, this has been extended by 60 myr to 460 Ma (Redecker *et al.* 2000). The classification of many Ediacaran organisms remains controversial because they do not resemble animals (Seilacher 1994) and one interpretation is that at least some were marine lichens (Retallack 1994). This was suggested after analysis and consideration of differential compression in animals (e.g. jellyfish) versus lichens. The Ediacaran organisms apparently were more rigid than animals and that durability may have been conferred by chitin (as in lichens and other fungi). Additional support for the interpretation as lichens is the large size of Ediacara at a time when oxygen levels were probably low (Retallack 1994).

The oldest land plants are also known from the Ordovician (Gray and Shear 1992; Wellman and Gray 2000). Although widespread evidence of aerially dispersed land plant spores might be expected in Precambrian strata if land plants were present then, this need not be the case. For example, if Precambrian land plants were restricted in distribution, their spores might not be globally distributed. Also, the spores of the earliest land plants may not have fossilized as well as later spores, and the habitats where they occurred may be under-represented in the exposed Precambrian strata.

Some palaeontologists have argued that the absence of fossil evidence for animals much earlier than the late Neoproterozoic (600–700 Ma) is because they had not yet evolved (Valentine *et al.* 1999; Conway Morris 2000). However, other palaeontologists have entertained the possibility that molecular clock estimates indicating older divergences between animal lineages may be correct (Runnegar 1982b; Xiao *et al.* 1998; Runnegar 2000; Rasmussen *et al.* 2002). Various explanations have been proposed for the absence of metazoan fossils from this earlier period, although the most commonly cited reason is that animals were smaller and soft-bodied (Runnegar 1982a,b; Bengtson and Lipps 1992; Lipps *et al.* 1992; Bengtson 1994; Fedonkin 1994; Weiguo 1994; Davidson *et al.* 1995; Fortey *et al.* 1996; Cooper and Fortey 1998). In fact, there is evidence from trace fossils of a size increase in bilaterian animals and for the acquisition of hard parts at the Proterozoic–Phanerozoic boundary (Bengtson and Farmer 1992; Lipps *et al.* 1992; Valentine *et al.* 1999). Nearly one-third of animal phyla believed to have arisen in the Cambrian, based on phylogenetic relationships, have virtually no fossil record (Valentine *et al.* 1999). All of those phyla are small in size and most are soft-bodied, essentially confirming that such traits can render animals 'invisible' in the fossil record, and lending plausibility to the hypothesis of a long and cryptic history of animal evolution prior to the Cambrian explosion.

Neoproterozoic Snowball Earth events

Several global glaciations (Snowball Earth events) occurred during the Neoproterozoic, from 750–570 Ma (Kirschvink 1992; Kaufman *et al.* 1997; Hoffman *et al.* 1998; Kennedy *et al.* 1998; Walter *et al.* 2000). The number of glaciations continues to be debated, but there is a consensus that there were at least two major episodes, the Sturtian (700 Ma) and the Marinoan (600 Ma); there may have been an additional, smaller, glaciation at 570 Ma (Walter *et al.* 2000). Temporal constraints are poor during this

time period and other glacial episodes may be identified in the future. These Snowball Earth events have been identified primarily by carbon isotopic excursions and from glacial deposits, and they follow the same pattern at localities on different continents (Kaufman *et al.* 1997; Hoffman *et al.* 1998; Walter *et al.* 2000). This and other evidence has led to the following model. First, carbon dioxide levels in the atmosphere declined, causing ice sheets to expand below 30 degrees latitude, triggering a runaway albedo affect, reflecting more solar energy back out to space; this lowered temperatures further and caused all oceans to freeze over (Snowball Earth). Normal volcanic activity continued to contribute carbon dioxide to the atmosphere and, after approximately 10 million years, this was sufficient to warm the Earth and melt the ice. An extreme greenhouse period followed the Snowball Earth state, perhaps for hundreds to thousands of years during which large volumes of limestone were created. After an interlude of millions of years, the cycle began again. A 'soft' Snowball Earth model has been proposed which allows a zone of ice-free equatorial oceans (Hyde *et al.* 2000).

Palaeogeography has been implicated as the trigger for the Neoproterozoic Snowball Earth events (Kirschvink 1992; Hoffman *et al.* 1998). At the time of the Sturtian glaciation, the supercontinent Rodinia straddled the equator and was breaking apart, according to some reconstructions (Meert and Powell 2001). The equatorial position of the continents may have had two affects. Firstly, the greater fraction of the equatorial region having higher albedo (continents) rather than lower albedo (oceans) may have contributed to a lower overall global temperature (Kirschvink 1992). In addition, these tropical landmasses would have weathered more rapidly, lowering carbon dioxide levels more than usual because there were no polar landmasses to provide a buffer (Hoffman *et al.* 1998). The buffer normally works by shutting down continental weathering at an early stage, through freezing of high latitude continental areas, as temperatures begin to drop. This allowed temperatures to increase through build-up of carbon dioxide. Secondly, during the Snowball Earth events, the absence of that buffer allowed weathering to continue until the polar oceans froze and the ice caps extended to equatorial regions (Hoffman *et al.* 1998). This proposed geological trigger is only speculative, and palaeogeography in the Precambrian is not well known. It has been suggested that the increased solar luminosity in the Phanerozoic, coupled with less efficient carbon burial, may explain why no Snowball Earth events have occurred since 570 Ma (Hoffman *et al.* 1998).

The extreme conditions associated with Snowball Earth events, including a post-glacial greenhouse period, would have posed considerable hardships for any life forms, especially eukaryotes. Nevertheless, the fossil record confirms that several groups of eukaryotic algae survived through this period, and members of the animal and fungal lineages must have survived as well if the animal–fungal divergence occurred prior to 750 Ma as is generally believed. If molecular clock estimates are correct, major lineages of animals and fungi survived the Snowball Earth events. Deep sea vents would have provided one possible refuge, and vents and rift zones near the surface, such as in modern Iceland, possibly provided refuge for terrestrial organisms. Continental thermal springs would have been less likely refugia because the water required to charge them may not have been available during these periods of low precipitation. Connections between the Snowball Earth events and animal evolution have been suggested (Kaufman *et al.* 1997), specifically through genetic bottlenecks leading to diversification before and after the last snowball event (Hoffman *et al.* 1998) or through

cycles of allopatric speciation, in refugia, of many lineages that had evolved prior to the glaciations (Hedges 2001).

A biological trigger

I have proposed elsewhere that the presence of fungi and plants on land in the Precambrian, as inferred from molecular clocks, became a biological trigger for Snowball Earth events and the Cambrian explosion of animal diversity (Figure 2.2; Heckman *et al.* 2001). Fungi and plants would have increased rates of weathering and carbon burial, lowering levels of carbon dioxide and global temperatures. At the

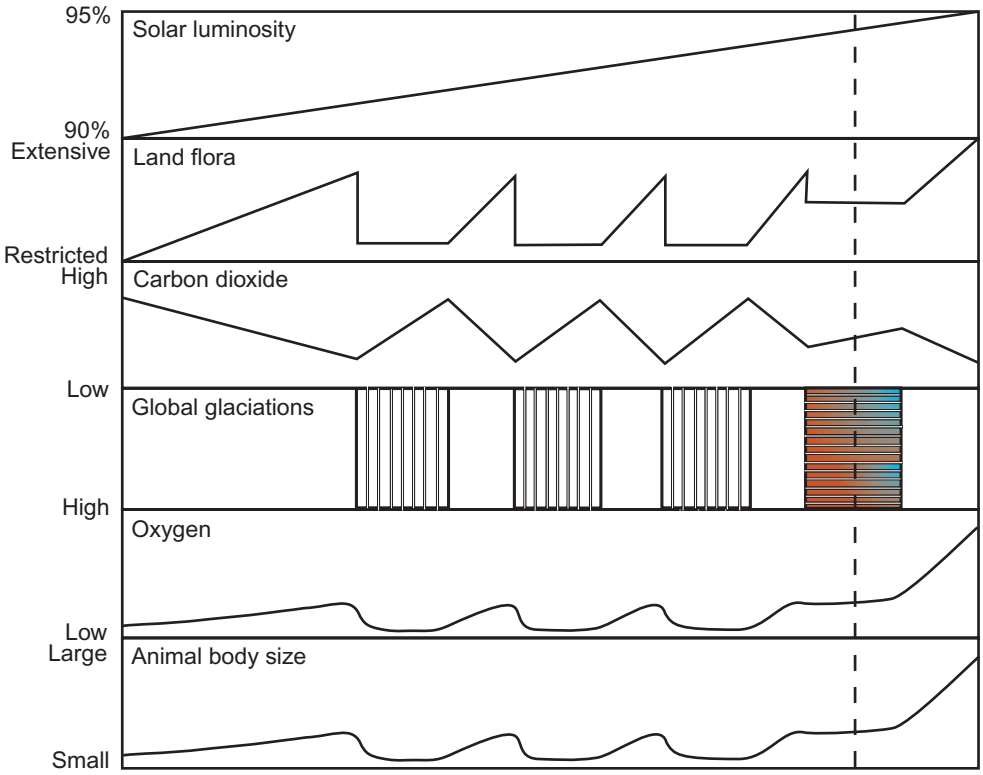

Figure 2.2 Proposed 'Biological Trigger' model for Neoproterozoic Snowball Earth events and the Cambrian explosion (see text). The panels show changes in different components of Earth and biotic history in the interval 1000–500 Ma, but the timescale is not linear because the 10 myr periods of glaciation are expanded to illustrate the model. The first panel (solar luminosity) shows the approximate increase during that period (Kump *et al.* 1999) relative to the present (100 per cent). Other panels show only relative, diagrammatic changes implied by the model and are not quantitative. The land flora includes fungi (e.g. lichens) and primitive land plants. Three Snowball Earth events (vertical lines) are illustrated followed by a pseudo-snowball (horizontal lines) at the Precambrian–Cambrian boundary. Animal body size is largely hypothetical (although size is known to increase in the Early Cambrian), assuming a direct relationship to oxygen levels. The vertical dashed line represents the Precambrian–Cambrian boundary.

same time, the oxygen produced by lichen photobionts and plants would have increased levels of this gas, possibly permitting animals to increase in size and facilitating the develop-ment of hard parts.

The fact that rates of weathering can be enhanced by the presence of organisms on land is well established, and lichens alone may increase rates by 10–100-fold (Schwartzman and Volk 1989; Schwartzman 1999). In general, biologically enhanced weathering during the last two billion years has been claimed to have lowered global temperatures, allowing complex life to develop (Schwartzman 1999). In particular, this mechanism for temperature change has been suggested as a possible cause of the Neoproterozoic glaciations (Carver and Vardavas 1994; Retallack 1994). However, this has been largely speculative because, prior to recent molecular clock studies, there have been no fossils or other evidence of terrestrial eukaryotes during the Precambrian (Horodyski and Knauth 1994; Kenrick and Crane 1997; Redecker *et al.* 2000).

The molecular time estimates for the diversification of major groups of fungi at around 800–1200 Ma, and the origin of land plants at 700–1100 Ma, raised the possibility that these organisms may have triggered the Snowball Earth events (Heckman *et al.* 2001). At first, the appearance of land fungi prior to land plants may come as a surprise because fungi are heterotrophs that acquire their nutrients from absorption, and most species today are involved in symbiosis with land plants. However, lichens represent an ancient ecological form for fungi (Taylor *et al.* 1995), and their symbiotic partners (green algae and cyanobacteria) were present in the Proterozoic. They can withstand severe environmental stresses and live in extreme habitats where neither fungi nor algae could live alone (Ahmadjian and Hale 1973; Gray and Shear 1992; Selosse and LeTacon 1998). Today, lichens form a rock and soil crust flora in harsh terrestrial environments, sometimes in combination with primitive plants (mosses and liverworts) and cyanobacteria. The earlier appearance of fungi (lichens), and associated weathering of rock, would have provided soil for the later colonization of land by plants. Nematodes and tardigrades, which themselves could be classified as extremophile eukaryotes, sometimes are found in symbiosis with lichens and the soil crust. The divergence of nematodes from other animals has been calculated using molecular clocks at approximately 1200 Ma (Wang *et al.* 1999) raising the possibility that nematodes were among the first animals on land. It is possible that terrestrial eukaryotes (fungi, plants, and animals) formed a similar biological crust on exposed land in the Proterozoic.

Besides the increased rates of weathering produced by these terrestrial eukaryotes, the burial of decay-resistant carbon of early land plants would also have contributed to a lowering of temperatures. Both vascular and bryophytic plants have lignins or lignin-like compounds that are decay resistant (Kroken *et al.* 1996) and make up a significant fraction of terrestrially derived carbon deposited in marine sediments (Berner 1999). The burial of such carbon would have lowered levels of carbon dioxide and global temperature. Some prokaryotes, such as cyanobacteria, are adapted to terrestrial life and were probably the first colonists on land (Horodyski and Knauth 1994). The biological trigger model described here might also have occurred simply by the weathering, carbon burial, and oxygen production of these organisms alone, or by marine prokaryotes and eukaryotes. However, that does not explain why the Neoproterozoic Snowball Earth events began at 700 Ma, because organisms (e.g.

cyanobacteria) were present much earlier. An involvement of fungi and land plants in the biological trigger is more likely because their early evolutionary history (Heckman *et al.* 2001) more closely corresponds to the timing of the Neoproterozoic glaciations and Cambrian explosion. In addition, lichens have an enhanced ability to weather the terrestrial environment and land plants uniquely have decay-resistant carbon compounds.

A biological trigger is also a better explanation for the cyclic nature of the Neoproterozoic glaciations than a geological trigger. During each glaciation, most life on land would have disappeared, but the recovery period that followed would have resulted in an increase in productivity and weathering and a concomitant decline in levels of carbon dioxide and global temperature, leading once again to a Snowball Earth episode (Figure 2.2). Carbon isotope excursions reflect this repeated pattern of biotic collapse followed by a recovery period and then another collapse (Kaufman *et al.* 1997; Hoffman *et al.* 1998).

The same mechanism may explain a Neoproterozoic rise in oxygen and the Cambrian explosion of animal diversity. Great attention has been paid to understanding changes in oxygen levels through geological time, but no consensus has been reached aside from a general (though not universal) agreement that there was an initial rise to approximately 1 per cent of present atmospheric levels (PAL), at around 2300 Ma, and a second major increase in the Neoproterozoic (Holland 1994; Canfield and Teske 1996; Ohmoto 1997; Kasting 2001). The second increase has been implicated as a possible explanation for the Cambrian explosion because it would have permitted animals to become larger in size and form skeletons that would more readily reveal their existence in the fossil record (Bengtson and Farmer 1992; Knoll 1992; Bengtson 1994; Knoll 1994).

The carbon isotope record reveals a sharply negative $\delta^{13}C_{carbonate}$ anomaly immediately prior to the Precambrian–Cambrian boundary (545 Ma) that in some ways resembles those negative excursions associated with earlier glacial events, yet there is no evidence that a glaciation occurred at this time (Kaufman *et al.* 1997; Knoll and Carroll 1999). The fact that the Cambrian explosion occurred immediately following this 'pseudo-snowball' event is curious and suggests a possible connection. A geological explanation for this particular carbon isotope excursion is that it is due to the release of methane from oceanic clathrates that were destabilized by combined sea level fall and global warming resulting from volcanic release of carbon dioxide (Walter *et al.* 2000). Alternatively, I suggest that it may have been an extension of the biological trigger model discussed above. Under this model, carbon dioxide levels lowered sufficiently (from biological activity on land) to reduce temperature and productivity (resulting in the carbon isotope excursion) but not enough to cause widespread glaciations or a full Snowball Earth event. As a result, a sufficient diversity of land plants and/or lichens may have survived the episode (in contrast to a full Snowball Earth where most would have perished), permitting a rapid and extensive recovery that generated a major pulse of oxygen. This may go some way towards explaining the Cambrian explosion of animals that took place over the subsequent 40 million years.

This model can be tested by using larger numbers of proteins and taxa, when they become available, to increase the precision of molecular time estimates. In addition, searches should be made for biomarkers or fossils of fungi and land plants from the

late Precambrian and Cambrian. Such searches may require the same methods that have been used to uncover the earliest plant and fungal fossils from the Phanerozoic, such as the examination of shallow marine (nearshore) sediments with acid baths (Gray and Shear 1992; Redecker *et al.* 2000). If the pseudo-snowball event at the Precambrian–Cambrian boundary led to a land plant diversification and expansion, evidence of land plant spores should be recovered from Cambrian sediments.

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