

THE FOSSIL RECORD OF EARLY EUKARYOTIC DIVERSIFICATION

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ABSTRACT—The Cambrian explosion can be thought of as the culmination of a diversification of eukaryotes that had begun several hundred million years before. Eukaryotes – one of the three domains of life — originated by late Archean time, and probably underwent a long period of stem group evolution during the Paleoproterozoic Era. A suite of taxonomically resolved body fossils and biomarkers, together with estimates of acritarch and compression fossil diversity, suggest that while divergences among major eukaryotic clades or ‘super-groups’ may have occurred as early as latest Paleoproterozoic through Mesoproterozoic time, the main phase of eukaryotic diversification took place several hundred million years later, during the middle Neoproterozoic Era. Hypotheses for Neoproterozoic diversification must therefore explain why eukaryotic diversification is delayed several hundred million years after the origin of the eukaryotic crown group, and why diversification appears to have occurred independently within several eukaryotic super-groups at the same time. Evolutionary explanations for eukaryotic diversification (the evolution of sex; the acquisition of plastids) fail to account for these patterns, but ecological explanations (the advent of microbial predators) and environmental explanations (changes in ocean chemistry) are both consistent with them. Both ecology and environment may have played a role in triggering or at least fueling Neoproterozoic eukaryotic diversification.

ALTHOUGH THE CAMBRIAN explosion is certainly a significant event in itself, it can be seen as part of a larger diversification of eukaryotes that had begun millions of years before. Neither multicellularity nor biomineralization was invented in the Cambrian explosion; both were present in several clades long before the close of the Proterozoic (Allison and Hilgert, 1986; Butterfield, 2000; Porter et al., 2003). Similarly, predatory behavior, often cited as one of the possible triggers of Cambrian radiation (e.g., Stanley, 1973), first made its appearance several hundred million years before, albeit in microbial organisms. Eukaryotic diversification reached an acme in latest Neoproterozoic and early Cambrian time, but substantial increases in diversity characterize the earlier Neoproterozoic Era as well (Knoll 1994; Vidal and Vidal-Moczydlowska, 1997). Here I discuss the Proterozoic diversification of eukaryotes, focusing, in particular, on evidence from the late Mesoproterozoic and Neoproterozoic fossil record.

WHAT IS A EUKARYOTE?

Before discussing the fossil record of eukaryotes, it would be useful to define what a eukaryote is. Eukaryotes are one of three major groups or ‘domains’ of life. They are distinct from the other two domains – Archaea and Bacteria – in that they have a more complex cell structure: their cells possess a membrane-bound ‘true’ nucleus, a cytoskeleton, a complex endomembrane system, and organelles such as mitochondria and plastids. Eukaryotes are probably best known for their multicellular representatives — indeed, multicellularity evolved at least seven times within the eukaryotes, in animals, fungi, red algae, slime molds, brown algae, and at least twice in green algae. A substantial component of eukaryotic diversity, however, is microbial, comprising taxa traditionally grouped within the protozoa and algae. It is these microbial groups that primarily contribute to the early fossil record of eukaryotes.

CURRENT VIEWS OF EUKARYOTE PHYLOGENY

Our view of eukaryote phylogeny has undergone significant change in the last decade. Early phylogenies based on rRNA painted a compelling picture: complex eukaryotes, such as animals, plants, and fungi, diverged rapidly and late in eukaryotic evolution, while simpler, often bizarre, mitochondrion-lacking eukaryotes diverged in regular succession much earlier (Sogin, 1991). Several studies now show that this pattern is an artifact of long branch attraction (e.g., Philippe et al., 2000), and that the early branching, amitochondriate clades are in fact highly derived, their mitochondria secondarily lost or reduced (e.g., Tovar et al., 2003; Sutak et al., 2004). Recent phylogenies based on molecular and ultrastructural data are starting to converge on a different pattern. These indicate that most eukaryotes fall within one of several ‘super-groups’ (Fig. 1; Simpson and Roger, 2002; Baldauf, 2003; Nikolaev et al., 2004): the opisthokonts, including the animals, choanoflagellates, and fungi; the Amoebozoa, including most slime molds and the lobose amoebae; the plants, including the red and green algae and the land plants; the chromalveolates, including the diatoms, brown algae, ciliates, dinoflagellates, chrysophyte algae, and, possibly, the haptophytes (the group that includes the coccolithophores); the Rhizaria, including the foraminifera, the filose testate amoebae, and the radiolaria; and the excavates, including the euglenids and several parasitic taxa. Relationships among these taxa are not well resolved, although recent work based on gene fusion data suggests that the tree can be rooted between two larger clades, the opisthokonts + amoebozoa on one side, and the rest of the eukaryotic super-groups on the other (Stechmann and Cavalier-Smith, 2002, 2003). If correct, this would represent a radical departure from the conventional view of eukaryotic evolution, implying that the group that includes the animals and fungi may have branched off early in eukaryotic evolution.

THE EARLIEST EUKARYOTES

Late Proterozoic rocks record the diversification of eukaryotic super-groups, but the eukaryotic clade itself appears to have originated much earlier. Sterane molecules, whose biochemical precursors, sterols, are widespread and diverse in living eukaryotes, have been found in ~2700 Ma shales from Australia (Brocks et al., 1999), suggesting that eukaryotes may have evolved by late Archean time. Sterols have also been found in several bacterial groups as well (see, e.g., Cavalier-Smith, 2002a), although none exhibit the structural diversity indicated by the Archean steranes (Brocks et al., 2003). Plausible eukaryotic body fossils — problematic macrofossils and structurally complex microfossils first appear ~1000 m. y. later, in late Paleoproterozoic and early Mesoproterozoic rocks. Problematic macrofossils include the ~1400-1800 Ma *Grypania* and ~1000-1600 Ma “strings of beads” structures, both interpreted as photosynthetic eukaryotes (Grey and Williams, 1990; Han and Runnegar, 1992; Kumar, 1995). If these interpretations are correct, they would imply that the eukaryotic super-groups must have already diverged by early Mesoproterozoic time, as plastids, the site of photosynthesis in eukaryotes, were acquired *after* the plant supergroup had diverged from its closest relative (although see Andersson and Roger, 2002). More convincingly eukaryotic fossils include the ~1500 Ma structurally complex microfossil *Tappania plana*, whose irregularly branching processes and bulbous protrusions suggest the presence of a cytoskeleton — a character unique to eukaryotes (Javaux et al., 2001; see also Javaux et al., 2003).

With the possible exception of problematic macrofossils like *Grypania*, these early fossils may best be interpreted as stem group eukaryotes: taxa that branched off the ‘main line’ early in eukaryotic evolution, prior to the last common ancestor of all living eukaryotes [in contrast, ‘crown group eukaryotes’ refer to those eukaryotes that are descended from the last common ancestor of all living eukaryotes (Fig. 1); see, e.g., Budd and Jensen (2000) for further discussion of ‘stem’ and

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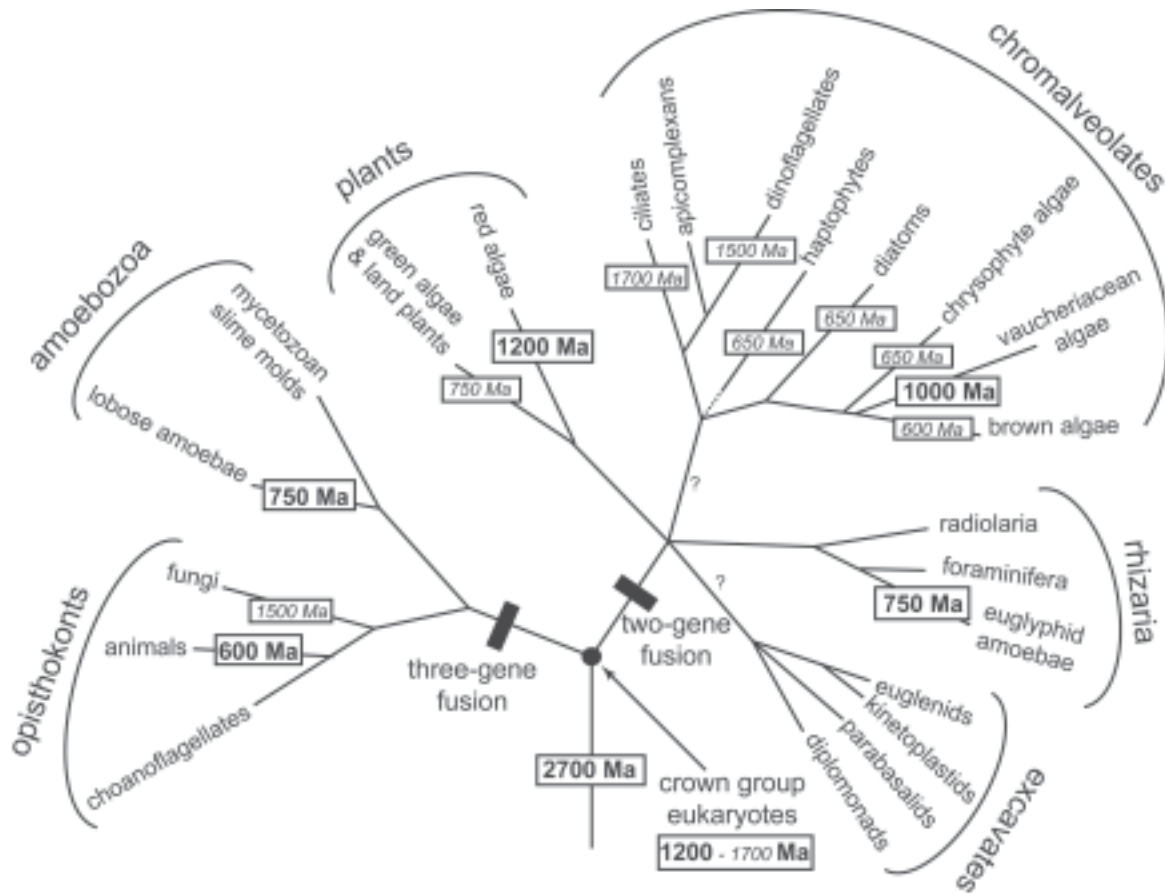


FIGURE 1—A current view of eukaryote phylogeny, based on a consensus of molecular and ultrastructural data [modified from Simpson and Roger (2002), Baldauf (2003), and Nikolaev et al. (2004)]. Question marks associated with excavates and chromalveolates indicate that monophyly of these clade is in question. The dotted line indicates uncertainty with respect to the inclusion of the haptophytes in the chromalveolate clade. Rooting of the tree is based on gene fusion data from Stechmann and Cavalier-Smith (2002, 2003). The three-gene fusion uniting the opisthokont and Amoebozoa super-groups refers to genes involved in pyrimidine synthesis; the two-gene fusion uniting the rest of the super-groups refers to the dihydrofolate reductase and thymidylate synthase genes (Stechmann and Cavalier-Smith, 2003). Dates are derived from fossils discussed in the text, and in most cases represent broad estimates. As discussed in the text, many of these taxonomic assignments are uncertain; dates associated with the more questionable assignments are in italics; those associated with the more convincing assignments are larger and in boldface (see also Table 1).

‘crown’ groups]. As stem groups, these fossils provide a minimum age constraint on the origin of the eukaryotic clade itself and on the acquisition of important eukaryotic synapomorphies (e.g., sterols and the cytoskeleton). The eukaryotic crown had originated by the time we see the first representatives of a eukaryotic super-group (~1200 Ma; though possibly as early as 1500-1700 Ma; see Fig. 1 and below), but not until the early to middle Neoproterozoic Era do fossils representing a diversity of eukaryotic super-groups appear.

EUKARYOTIC DIVERSIFICATION

Two lines of evidence indicate that the diversification of eukaryotes was underway by middle Neoproterozoic time. The first, and most compelling, comes from a suite of body fossils and molecular biomarkers that, as discussed in the following sections, can either be plausibly or convincingly assigned to modern eukaryotic clades (see Table 1). The second comes from acritarchs – problematic organic-walled microfossils – and macroscopic carbonaceous compression fossils. Although taxonomically unresolved, these provide useful information about general trends in Neoproterozoic eukaryote evolution.

Taxonomically resolved body fossils.— The earliest body fossil that can be assigned with confidence to a eukaryotic super-group is late Mesoproterozoic in age: *Bangiomorpha pubescens*, a multicellular filament found in the ca. 1200 Ma Hunting Formation, arctic Canada (Fig. 2.1-2.2; Butterfield et al., 1990; Butterfield, 2000). Although *Bangiomorpha* specimens exhibit a number of characters found within both the red and green algal and cyanobacterial clades, they exhibit one feature – an unusual pattern of cell division (Fig. 2.1) – not known outside the red algal family Bangiaceae (Butterfield et al., 1990; Butterfield 2000). It is possible this character is “well within the capacity of a filamentous bacterium to evolve” (p. 38, Cavalier-Smith, 2002a), but it is more parsimonious (based on morphological characters alone) to suggest the fossils are indeed early

representatives of red algae. Additional evidence for multicellular photosynthetic eukaryotes comes from the somewhat younger filamentous microfossils *Paleovaucheria clavata* (German, 1981) and *Proterocladus* sp. (Butterfield, 1994). *Paleovaucheria* (Fig. 2.3), which occurs in both the >1000 Ma Lakhanda Suite, Siberia (German, 1981, 1990; Woods, et al., 1998), and the ~750 Ma Svanbergfjellet Formation, Spitsbergen (Butterfield, 2004), exhibits several characters that collectively indicate an affinity with the vaucheriacean algae, relatives of the brown algae, and members of the chromalveolate clade (Potter et al., 1997; Fast et al., 2001). These include sparsely branching thalli with few septa that tend to concentrate at the bulbous termini of the filaments; circular openings at the ends of the termini; and filaments of two distinct size classes (German, 1990; Woods et al., 1998; Butterfield, 2004). *Proterocladus* sp. (Fig. 2.5), also from the Svanbergfjellet Formation, is a simple, uniseriate filament that exhibits occasional branching and intercellular septa, morphology reminiscent of the modern green alga, *Cladophora* and *Cladophoropsis*, though also observed in some simple red algae (Butterfield et al., 1994).

Vase-shaped microfossils (VSMs), abundant, diverse, and globally distributed in rocks ~750 Ma in age, provide evidence for two additional eukaryotic super-groups (Porter and Knoll, 2000; Porter et al., 2003). At least 17 species of VSMs have been described. These include *Paleoarcella athanata* (Fig. 2.7), whose test is indistinguishable from that of the modern lobose testate amoeba, *Arcella*, a member of the amoebozoan super-group (Fig. 1); and *Melicerion poikilon* (Fig. 2.6) whose test, apparently covered in mineralized scales, is closely comparable to those of euglyphid amoebae in the rhizarian super-group (Fig. 1). [Other VSM species (e.g., Figs. 2.8-2.9) exhibit characters found in both modern taxa and thus cannot be definitively assigned to either.] Thus, VSMs indicate that both the Rhizaria and the Amoebozoa had begun to diverge by ~750 Ma. They also provide the earliest definitive fossil evidence for heterotrophic eukaryotes.

TABLE I—Taxonomically resolved body fossils and biomarkers preserved in Proterozoic rocks. Ma = Millions of years. “Confidence” refers to confidence in interpretation: (+) = interpretation is based on compelling evidence; (~) = the interpretation is plausible. For more details, see discussion in text.

Fossil	Occurrence	Age	Interpretation	Confidence	Super-group	References
<i>Bangiomorpha pubescens</i>	Hunting Formation, Canada	~1200 Ma	Bangiophyte red alga	+	Plants	Butterfield et al., 1990 Butterfield, 2000
<i>Palaeovaucheria clavata</i>	Lakhanda Suite, Siberia Svanbergfjellet Fm, Spitsbergen	~1000 Ma ~750 Ma	Vaucheriacean alga	+	Chromalveolates	German, 1981, 1990; Woods et al., 1998; Butterfield, 2004
<i>Proterocladus</i> sp.	Svanbergfjellet Fm, Spitsbergen	~750 Ma	Cladophoralean green alga	~	Plants	Butterfield et al., 1994
<i>Palaeoarcella athanata</i> (VSM)	Chuar Group, USA	~750 Ma	testate amoebae	+	Amoebozoa	Porter et al., 2003
<i>Melicerion poikilon</i> (VSM)	Chuar Group, USA	~750 Ma	Euglyphid testate amoeba	+	Rhizaria	Porter & Knoll, 2000; Porter et al., 2003
Other VSMs	Numerous localities	~750 Ma	Lobose and filose testate amoebae	~	Amoebozoa & Rhizaria	Porter & Knoll, 2000; Porter et al., 2003
<i>Tappania</i> sp.	Shaler Supergroup, Canada Roper Group, Australia	~900-800 Ma ~1500 Ma	Fungi	~	Opisthokonta	Butterfield, in press
* scale microfossils*	Tindir Group, Canada	~700-600 Ma	Diatoms, haptophytes, & chrysophytes	~	Chromalveolates	Allison & Hilgert, 1986; Kaufman et al., 1992
Gammacerane (biomarker)	Chuar Group, USA Tuanshanzi Fm, China	~750 Ma ~1700 Ma	Ciliates	~	Chromalveolates	Summons et al., 1998; Summons & Walter, 1990; Peng et al., 1998
Dinosterane (biomarker)	Chuar Group, USA McArthur Group, Australia	~750 Ma ~1500 Ma	Dinoflagellates	~	Chromalveolates	Moldowan et al., 2001
<i>Miaohephyton bifurcatum</i>	Doushantuo Formation	~600 Ma	Brown algae	~	Chromalveolates	Xiao et al., 1998a
<i>Megaspheera</i> sp. <i>Parapandorina</i> sp.	Doushantuo Formation	~600 Ma	Animals	+	Opisthokonta	Xiao et al., 1998b; Xiao and Knoll, 2000

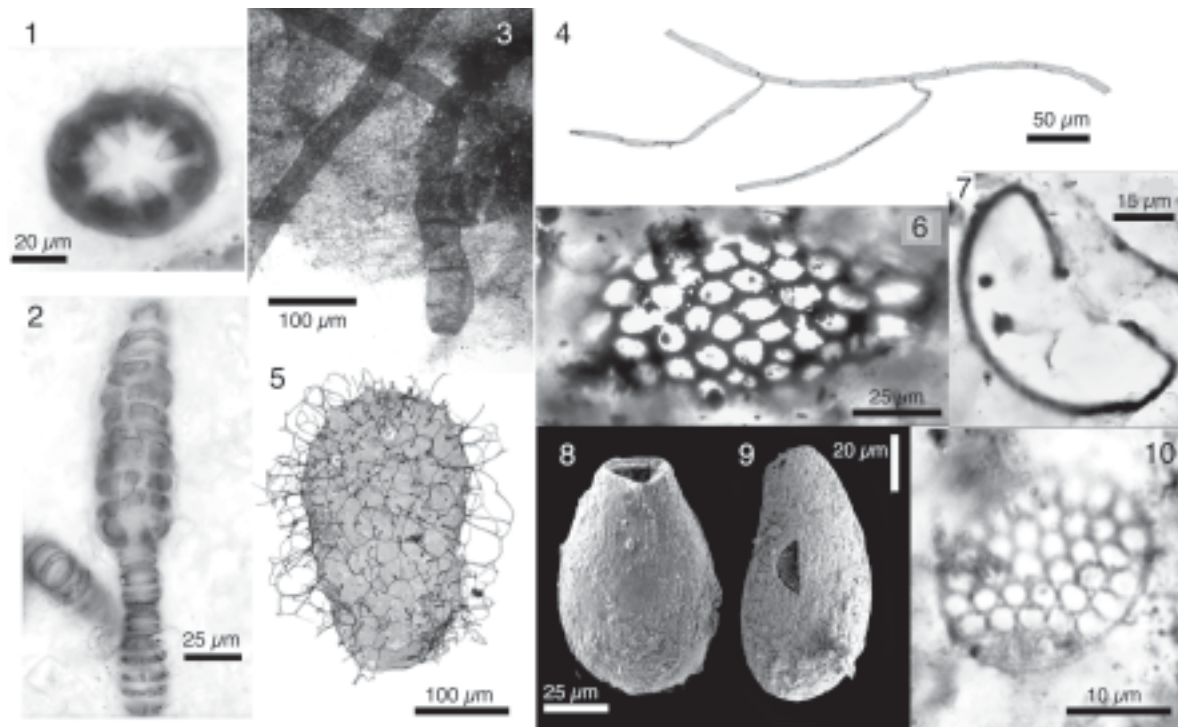


FIGURE 2—Representative images of early eukaryotic body fossils. 1, 2) *Bangiomorpha pubescens* from the ca. 1200 Ma Hunting Formation, arctic Canada, interpreted to be a bangiophyte red alga; 1) cross-section of *Bangiomorpha* filament, showing longitudinal intercalary cell division, a character unique to modern bangiophytes; 2) side view of *Bangiomorpha* filament; 3) *Paleovaucheria clavata* from the >1000 Ma Lakhanda Suite, Siberia, interpreted to be a vaucheriacean alga, related to the brown algae; 4) *Proterocladus* sp. from the ~750 Ma Svanbergfjellet Formation, Spitsbergen, interpreted to be a green alga; 5) *Tappania* sp. from the ~800 Ma Shaler Supergroup, arctic Canada, interpreted to have possible affinities with the fungi; 6-9) Vase-shaped microfossils (VSMs) from the ~750 Ma Chuar Group, Grand Canyon, interpreted to be the remains of filose and lobose testate amoebae; 6) *Melicerion poikilon*, interpreted to be a euglyphid testate amoeba; 'holes' indicate location of mineralized scales; 7) *Paleoarcella athanata*, interpreted to be a lobose testate amoeba (arrow indicates test opening); 8-9) VSMs whose relationships to specific modern filose and lobose testate amoeban clades remain unclear; 8) *Trigonocyrrillium horodyskii* and 9) *Bonniea pytinaia*. Note the semicircular hole in *B. pytinaia*, possibly the work of predators; 10) the 'scale' microfossil, *Chilodictyon caliporum* var. *striatimarginatum* from the ~700-600 Ma Tindir Group, northwest Canada, interpreted to have possible affinities with the chrysophyte algae, a member of the chromalveolate clade. Images 1,2,4, and 5 courtesy of N. J. Butterfield; image 3 courtesy of A. H. Knoll; image 10 courtesy of S. M. Awramik.

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An intriguing new report provides additional evidence for eukaryotic heterotrophs in early Neoproterozoic rocks. Butterfield (in press) describes fossils assigned to the genus, *Tappania*, which exhibit branched, septate processes capable of secondary fusion – characters seen today in the higher fungi (Fig. 2.5). If correct, and if the fossils, from the ~900-800 Ma Shaler Supergroup, northwest Canada, are related to those of the same name from the much older Roper Group (Javaux et al., 2001; Butterfield, in press), it would extend the record of fungi well back into early Mesoproterozoic time.

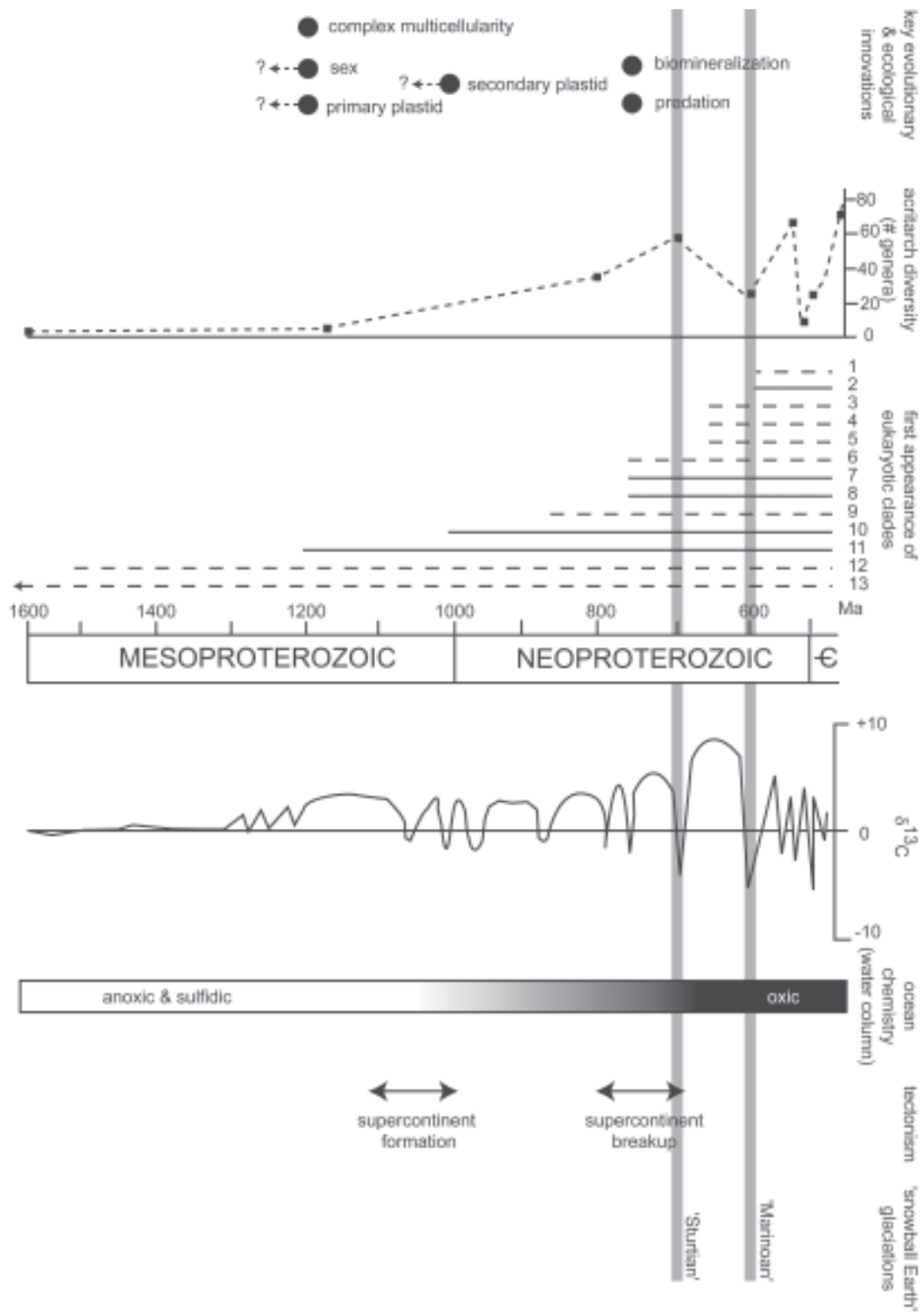
The final body fossil evidence for eukaryotic clades by middle Neoproterozoic time comes from the ~700-600 Ma Tindir Group, northwest Canada, which preserves an exceptional assemblage of diverse (26 species) ‘scale microfossils,’ round to elliptical siliceous structures ~5-85 μm in diameter (Fig. 2.10; Allison and Hilgert, 1986; Kaufman et al., 1992). Some specimens with radially oriented perforations and raised rims resemble the valves of centric diatoms, while other scales exhibit characters reminiscent of the chrysophyte and haptophyte algae (Allison and Hilgert, 1986). Interestingly, all three taxa appear to be closely related (Fig. 1); it is possible the scale microfossils represent stem groups of one or more of these taxa.

Biomarkers.—Biomarkers — biologically informative organic compounds preserved in sedimentary rocks – provide possible evidence for additional eukaryotic clades in Proterozoic oceans. Gammacerane, a biomarker whose biochemical precursor, tetrahymanol, is known from ciliates (Ourisson et al., 1987; Harvey and McManus, 1991), has been found in the ~750 Ma Chuar Group, Grand Canyon, and in the ~1.7 Ga Tuanshanzi Formation, China, suggesting that ciliates may have evolved by late Paleoproterozoic time (Summons et al., 1988; Summons and Walter, 1990; Peng et al., 1998). Tetrahymanol is also known, however, from photosynthetic sulfur bacteria (Kleemann et al., 1990); it is thus possible the biomarkers reflect the presence of these prokaryotes instead. Tetrahymanol has also been found in ferns (Zander et al., 1969; Kamaya, et al.,

1991), and while it is highly unlikely that ferns had evolved by Proterozoic time, the presence of the compound in this group suggests that it may be even more taxonomically widespread – and thus less taxonomically useful — than currently thought.

Dinosterane molecules potentially provide evidence for the presence of Proterozoic dinoflagellates – a group whose body fossil record, interestingly, only extends back to the middle Triassic (Moldowan et al., 2001; and references therein). Dinosterol, the biochemical precursor of dinosterane, is known almost exclusively from dinoflagellates, although a report of dinosterol in diatoms underscores the possibility that, as with tetrahymanol, the compound may be taxonomically more widespread (Volkman et al., 1993). Steranes from the ~800 Ma Bitter Springs Formation and late Neoproterozoic Pertatataka Formation, Australia, include probable dinosteranes, but their abundances are too low to rule out contamination (Summons and Walter, 1990). Similarly, the low concentration of dinosteranes from the ~1100 Ma Nonesuch Formation, Michigan (Pratt et al., 1991), also calls into question their provenance (Summons et al., 1992). More recently, Moldowan et al. (2001) have reported dinosteranes from the ~750 Ma Chuar Group, Grand Canyon, and the ~1500 Ma McArthur Group, Australia, with particularly high abundances in a sample from the McArthur Group. If confirmed, this would suggest that the dinoflagellate clade may have diverged by ~1500 Ma.

Acritarchs + carbonaceous compression fossils.— Acritarchs are, by definition, taxonomically problematic, although most would agree they represent eukaryotes of some kind (Javaux et al., 2003), and thus provide useful information about patterns of eukaryotic diversification and abundance through time. Studies of acritarch and compression fossil diversity paint the same picture as that suggested by biomarkers and taxonomically resolved body fossils: following a long interval of low diversity during the Mesoproterozoic Era, eukaryotes underwent a significant expansion in the middle Neoproterozoic Era, ~800-700 Ma (Fig. 3; Vidal



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and Knoll, 1983; Schopf, 1992; Knoll, 1994; Vidal and Moczydlowska-Vidal, 1997; Hofmann, 1994).

Summary.— Pinning down the timing of eukaryotic diversification is, of course, hindered by the incompleteness of the fossil record. Most taxonomically resolved body fossils rely on exceptional circumstances for their preservation, and in many cases their record consists of just one occurrence (Table 1). They thus provide only minimum age constraints on the origin of the eukaryotic crown group and divergences among eukaryotic super-groups (see Fig. 1), and should not be read to indicate the first appearance of any particular super-group. Nevertheless, they suggest that the earliest divergences within the eukaryotic crown group — and thus the origin of the eukaryotic crown group itself — had occurred by 1200 Ma (red algae), and possibly as early as 1700 Ma (ciliate biomarkers; Fig. 1), consistent with recent molecular clock analyses (Wang et al., 1999; Yoon et al., 2002; Yoon et al., 2004). More robust and less facies-dependent fossils such as acritarchs, carbonaceous compressions, and vase-shaped microfossils suggest, however, that eukaryotes did not undergo significant diversification until several hundred million years later, during the middle Neoproterozoic Era, consistent with the appearance of several additional eukaryotic super-groups at this time (Figs. 1 and 3; Table 1). Thus, the

diversification of eukaryotes may mirror the diversification of animals (e.g., Knoll and Carroll, 1999): in both, it appears that early divergence among major clades was followed millions of years later by diversification *within* the clades themselves. It is this later diversification *within* clades that is preserved, in the case of animals, as the ‘Cambrian explosion’, and, in the case of eukaryotes, as middle Neoproterozoic diversification. If true, then hypothesized causes for eukaryotic diversification must explain why diversification was delayed long after the origin of the eukaryotic crown group, and why, when diversification did occur, it took place within several different super-groups, independently, at the same time.

WHAT DROVE EARLY EUKARYOTIC DIVERSIFICATION?

Hypotheses that seek to explain causes of eukaryotic diversification fall into three categories: those that invoke evolutionary innovations; those that invoke ecological innovations; and those that invoke environmental change.

Evolutionary innovations.— The evolution of sex — and thereby enhanced genetic variability — has long been implicated in the diversification of eukaryotes (e.g., Schopf, 1973; Knoll, 1992). If

FIGURE 3—Eukaryotic diversification in the context of important environmental, evolutionary, and ecological events. Carbon isotope curve modified from Kah et al. (1999) and Bartley and Kah (2004); acritarch diversity modified from Vidal and Moczydlowska-Vidal (1997); squares indicate binned data; dashed line represents linear interpolation between data points. Tectonic constraints discussed in Karlstrom et al. (2001), age constraints on glaciations discussed in Hoffman and Schrag (2002); patterns of ocean chemistry modified from Anbar and Knoll (2002). Age constraints for first appearances of taxa and for ‘key’ ecological and evolutionary innovations discussed in text. The key innovation, ‘complex multicellularity’ refers to multicellular organisms with differentiated cells (Butterfield, 2000). Numbered eukaryotic clades are: 1, brown algae; 2, animals; 3, haptophytes; 4, diatoms; 5, chrysophyte algae; 6, green algae; 7, lobose amoebae; 8, euglyphid amoebae; 9, fungi; 10, vaucheriacean algae; 11, red algae; 12, dinoflagellates; 13, ciliates. Dashed lines indicate those fossil groups whose taxonomic assignments are questionable.

true, one would expect that the origin of sex, a probable synapomorphy of crown group eukaryotes (or at least very early evolving in the eukaryotic tree; Cavalier-Smith, 2002b), would coincide with eukaryotic diversification. As discussed above, however, the origin of the eukaryotic crown group (and thus sex) likely preceded Neoproterozoic diversification by several hundred million years. Thus, sex is unlikely, by itself, to have acted as a trigger of Neoproterozoic diversification.

More recently, Butterfield (2000), noting that the earliest fossil evidence for sex coincides with the earliest evidence for multicellularity (*Bangiomorpha pubescens*; Fig. 3), has argued that sex played an indirect role in driving diversification by allowing complex multicellularity to evolve. Given that many of the eukaryotic clades represented in the diversification – not to mention the acritarchs – are single celled and small, it is unlikely that multicellularity *per se* was the key innovation that spurred diversification. It is possible, however, that the ecological *side effects* of multicellularity (e.g., tiering; cf. Bottjer and Ausich, 1986) may have played a role (Butterfield, 2000).

Others have suggested that the acquisition of plastids – the site of photosynthesis in eukaryotic cells – may have triggered eukaryotic diversification (Knoll, 1992; Brasier and Lindsay, 1998). The earliest convincing evidence for primary plastids – plastids derived from an endosymbiosis between a photosynthetic prokaryote (a cyanobacterium) and a eukaryotic host – comes from the ~1200 Ma fossil *Bangiomorpha pubescens* (Fig. 3). Because plastids are plesiomorphic to the red algae, *Bangiomorpha*, if indeed a red alga, must have had plastids. Secondary plastids – those derived from an endosymbiotic relationship between a photosynthetic *eukaryote* and a eukaryotic host – appeared soon after: secondary plastids are plesiomorphic to the chromalveolate clade (Fast et al., 2001; Yoon et al., 2002), thus the vaucheriacean algal fossil, *Paleovaucheria*, implies that secondary plastids had evolved by ~1000 Ma (Fig. 3). Because chromalveolate plastids are derived from red algae (Fast et al., 2001; Yoon et

al., 2002), earlier evidence for chromalveolates suggested by possible dinoflagellate biomarkers in the McArthur Group, Australia, would push back the timing of both secondary *and* primary plastid acquisition (and indeed, the origin of the red algal clade) to ~1500 Ma. In either case, the fact that secondary plastids appear to have been acquired shortly after the acquisition of primary plastids underscores the advantages of photosynthesis for eukaryotes. The timing of plastid acquisition relative to eukaryotic diversification, however, and the fact that most eukaryotes are non-photosynthetic, suggests that plastid acquisition *per se* was not the key innovation that drove eukaryotic expansion.

Ecological innovations.—Food web complexity must have increased significantly with the appearance of animals (e.g., Conway Morris, 1986); indeed, it has been proposed that the advent of metazoan predators triggered the Cambrian explosion (Stanley, 1973) or at the very least was responsible for the nearly simultaneous, independent acquisition of animal skeletons (e.g., Bengtson, 1994). Similarly, an increase in the complexity of *microbial* food webs may have had a hand in fueling eukaryotic diversification. Ecological theory predicts that the appearance and diversification of organisms at one tier in the food web will trigger diversification at lower tiers (Paine, 1966). Thus the evolution and diversification of predators should fuel diversification of their prey. The relatively spartan Precambrian fossil record makes it difficult to test this prediction, but available evidence is at least consistent with such a scenario. Fossil evidence for lobose and filose testate amoebae, bolstered by possible evidence for ciliates and fungi in similarly aged rocks, indicate that predatory microbes were abundant and diverse by 750 Ma (Fig. 3). [Phagotrophy — the ability to ingest particles — must have evolved even earlier, as eukaryotic algae acquired their plastids through phagocytosis (Gibbs, 1992). Although a necessary prerequisite to a predatory lifestyle, however, the evolution of phagotrophy does not by itself indicate that predators were abundant or widespread.] Further possible evidence for predation at ~750 Ma

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comes from unusual, semicircular holes in the tests of some vase-shaped microfossils (Fig. 2.9). The origin of these holes is unclear, but it is possible they represent the actions of predators attacking the test to get at the cell inside (Porter et al., 2003). Additional evidence for predatory activity by 750 Ma comes from the means used to defend against it: eukaryotic biomineralization also first appears at this time (Porter et al., 2003; Fig. 3).

Interestingly, the appearance of abundant, diverse populations of microbial predators at ~750 Ma coincides with an increase in primary productivity — suggested by increasing average $\delta^{13}\text{C}$ values (Fig. 3) — and an increase in acritarch diversity. This was initially interpreted to suggest that increased primary productivity and diversity allowed microbial predators to flourish (Porter and Knoll, 2000; Porter et al., 2003), but it is possible that the converse is true: the appearance of microbial predators *fueled* — rather than was *fueled by* — diversification of primary producers. This would explain not only why eukaryotic diversification was delayed, but also why diversification occurred independently and coincidentally in several eukaryotic clades. It might also explain the appearance of several multicellular clades shortly thereafter (e.g., Xiao et al., 1998a, b; Xiao and Knoll, 2000; Xiao et al., 2002; Xiao et al., 2004), as several studies suggest that predators may have played a selective role in the evolution of multicellularity (see, e.g., Boraas et al., 1998; cf. Stanley, 1973).

Environmental change.—Early eukaryotic diversification coincides with significant shifts in Earth's environment, including changes in biogeochemical cycling, global tectonics, and ocean chemistry. Starting around ~1.3 Ga, after a long period in which $\delta^{13}\text{C}_{\text{carb}}$ values hovered near zero, average values shifted to ~3.5‰, with variability in $\delta^{13}\text{C}$ increasing into the Neoproterozoic Era (Fig. 3; Buick et al., 1995; Kah et al., 1999; Bartley et al., 2001; Frank et al., 2003). This shift is matched by a reorganization in global tectonics: the last part of the Mesoproterozoic Era saw the formation of the supercontinent, Rodinia, while the early to middle Neoproterozoic witnessed

its breakup (Fig. 3; Karlstrom et al., 2001). At the same time, the degree of carbonate saturation of the oceans declined (Grotzinger, 1989; Kah and Knoll, 1996; Bartley et al., 2000; Bartley and Kah, 2004), and the deep ocean, thought to be anoxic and sulfidic, became increasingly oxygenated (Fig. 3; Canfield, 1998).

Recently, Anbar and Knoll (2002) suggested a link between these environmental changes and eukaryotic diversification. They argued that eukaryotic diversity and abundance was restricted before Neoproterozoic time by limitation in the availability of trace elements, in particular, molybdenum and iron, important for biological nitrogen fixation. Dissolved forms of both elements, they noted, would be scarce in an anoxic, sulfidic ocean (Canfield, 1998; Anbar and Knoll, 2002). In this hypothesis, eukaryotes were able to diversify only when increasing oxygen levels associated with increased organic carbon burial (and thus higher average $\delta^{13}\text{C}$) ventilated ocean depths (Anbar and Knoll, 2002). Changes in organic carbon burial may reflect increased tectonic activity (although see Karlstrom et al., 2001), and/or an increase in the abundance, diversity, and distribution of eukaryotic organisms (Frank et al., 2003). The shift may thus reflect a positive feedback loop, perhaps initiated by increased tectonism, whereby eukaryotic diversification both drives and is driven by increases in ocean oxidation (Frank et al., 2003).

At least one prediction of this hypothesis — that pre-Neoproterozoic eukaryotic diversity should be restricted to shallow, nearshore environments, where trace elements would have been available from riverine input (Anbar and Knoll, 2002) — has been borne out by paleoenvironmental data on acritarchs from the early Mesoproterozoic Roper Group (Javaux et al., 2001). [Conversely, Neoproterozoic eukaryotic communities have their greatest diversity farther offshore (Knoll et al., 1991; Butterfield and Chandler, 1992).] Further tests will require more detailed data on the early to middle Neoproterozoic pattern of eukaryotic diversification, depositional setting, and the timing of ocean ventilation.

Among the most significant of Neoproterozoic environmental events are, of course, the Neoproterozoic glaciations, often referred to as ‘snowball Earth’ glaciations (Kirschvink, 1992; Hoffman et al., 1998; Hoffman and Schrag, 2002). It is important to note, however, that both divergence among and diversification within the eukaryotic super-groups occurred *before* the advent of the Neoproterozoic glacial age. Thus, although snowball Earth glaciations almost certainly shaped eukaryotic diversification, it did not trigger them (that is not to say that subsequent diversifications were unrelated to ‘snowball’ glaciation, of course; although see Grey et al., 2003). An additional, and perhaps more significant, corollary to this observation is that a high number of eukaryotic lineages – both autotrophic and heterotrophic – must have survived these extreme glaciations, calling into question the extent to which snowball Earth created a severe bottleneck in life history (Runnegar, 2000).

EUKARYOTES AND THE EVOLUTION OF ANIMALS

Eukaryotes continued to diversify through the end of the Proterozoic and into the early Cambrian – in fact, the Cambrian explosion can be thought of as a diversification of *eukaryotes*, albeit one dominated by a single clade, the animals. The first appearance of animals in the fossil record (in the ~600 Ma Doushantuo Formation, of China; Xiao et al., 1998b; Xiao and Knoll, 2000) coincides with first appearances of a diverse array of multicellular algae (including possible brown algae; Xiao et al., 1998a, Xiao et al., 1998b, Xiao et al., 2002; Xiao et al., 2004), suggesting that whatever triggered the evolution of multicellularity in the ancestor to animals may have also influenced the evolution of multicellularity in various algal clades. Similarly, increases in acritarch diversity accompany latest Neoproterozoic through early Cambrian animal radiation (Knoll, 1994; Vidal and Moczydlowska-Vidal, 1997), suggesting that these diversifications are somehow linked. In addition, the appearance of biomineralization in several diverse animal clades at the Proterozoic-Cambrian boundary is

mirrored by the appearance of biomineralized or agglutinated skeletons in other eukaryotes as well (Bengtson and Conway Morris, 1992). Thus, hypotheses that seek to explain the Proterozoic-Cambrian animal ‘revolution’ would benefit from viewing the event in the wider context of eukaryotic evolution.

CONCLUSIONS

The fossil record indicates that stem group eukaryotes had appeared by late Archean time, and that substantial steps in cell evolution probably occurred during the Paleoproterozoic Era. A suite of well preserved body fossils and molecular biomarkers indicate that the earliest divergences among eukaryotic super-groups – and thus the origin of the eukaryotic crown group – had occurred by ~1200 Ma and possibly even earlier, consistent with molecular clock analyses. Diversification *within* eukaryotic super-groups was underway by the middle Neoproterozoic Era, and culminated in the Cambrian explosion. It is unlikely that evolutionary factors such as the origin of sex or the acquisition of plastids played a role in triggering eukaryotic diversification. Diversification did coincide, however, with environmental and ecological shifts; both may have played a role in either triggering or fueling diversification.

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