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OPINION

WHY SILICA OR BETTER YET WHY NOT SILICA? SPECULATIONS AS TO WHY THE DIATOMS UTILISE SILICA AS THEIR CELL WALL MATERIAL

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The diatoms (Bacillariophyta) are one of the most successful microalgal groups on earth. Diatoms are ecologically significant and are present in nearly all aquatic habitats (Round 1981, Mann 1999, Graham & Wilcox 2000). Several species exist in unusual niches: e.g. sea ice brine channels, on the skin of copepods and whales, on bird talons, in hot springs or highly basic or acidic ponds (Round 1981, Round *et al.* 1990). A few species occur as dinoflagellate (Chesnick *et al.* 1997) or foraminifera (Chai & Lee 1999, 2000) endosymbionts. Knowledge of the evolutionary history of the diatoms can provide evidence as to how they have invaded all these habitats, e.g. Chesnick *et al.* (1997).

Diatoms are nearly all autotrophic: hetrotrophic members are rare (see e.g. Li & Volcani 1987). As primary producers in the world's oceans, they are keystone species in global nutrient cycling. About 20% of the total carbon and silica sequestered are fixed by less than a few hundred species in the marine phytoplankton (Guillard & Kilham 1978, Goldman 1993, Hasle & Syvertsen 1996, Mann 1999). The marine food web and the oceanic biogeochemical cycles are fuelled by diatom primary production, and thus models that seek to predict the effects of increased levels of atmospheric carbon dioxide (Falkowski *et al.* 1998, Smetacek 1998) need to take account of the ecophysiology of these organisms. Knowledge of the geological age of the diatoms gives palaeoclimatologists a clue as to how long these organisms have been modifying the biosphere (Kooistra & Medlin 1995, Siever 1991).

Diatoms are used as indicators of water quality, either in recent or geological times (Kilham *et al.* 1996, Theriot *et al.* 1997, Mann 1999, Stoermer and Smol 1999). Diatom frustules often preserve well, with their intricate microscopic details intact, and can be identified to species even after millions of years (e.g., Gersonde & Harwood 1990, Harwood & Gersonde 1990, Barron & Mahood 1993). Diatom fossils are used for stratigraphic calibration (Baldauf 1992, Gersonde & Bárcena 1998, Stoermer & Smol 1999). Morphological markers that are used to link fossil and extant species and to characterise palaeoconditions can assess phylogenies. Phylogenies based on extant diversity can be compared with those independently derived from fossil data.

Diatoms are responsible for a major part of oceanic biogeochemical cycles expanding the range of topics linked to these organisms. Diatom frustules derived from fossil deposits have been mined and used in commercial products as abrasives and filters (Stoermer & Smol 1999). Extant diatoms are a source of food or valuable biochemical compounds (Falciatore & Bowler 2002). In addition, diatoms provide materials scientists with models for the synthesis of silica-based materials at ambient temperatures and pressures (Kröger *et al.* 2000, Vrieling *et al.* 2000, Brott *et al.* 2001). Phylogenetic knowledge will undoubtedly improve our understanding of how morphological features have developed and evolved, leading to refined ontogenetic models, e.g. of silica utilisation in diatoms.

It is clear that the diatoms belong to the pigmented heterokont algae, and with their sister group, the Bolidophyceae, are part of the initial radiation of the pigmented heterokont algae (Fig. 1). Molecular clock calculations based on linearised trees from both nuclear and plastid genes and first appearance dates of the diatoms indicate that it is unlikely that the diatoms existed before the Permian–Triassic boundary (Medlin *et al.* 1997). These studies also suggested that the secondary endosymbiosis giving rise to the modern groups of the phytoplankton, the chlorophyll *a+c* algae, is specifically related to this event.

The evolution of the silica appears to have occurred since the divergence of the diatoms from the Bolidophyceae because no traces of silica scales have been found in this sister group of the diatoms, or in the earlier divergences of the Heterokonta. Just exactly why the diatoms utilise silica has been an issue frequently debated among diatomists (see discussion on the diatom list server http://www.indiana.edu/~diatom/silica.dis). Silicon in the form of SiO_2 is the second most abundant element in the earth's crust and hydroxylated surfaces of quartz would have been the most abundant substrates for selective polymerisation and aggregation of biomers, especially after periods of extensive volcanism (Hench 1989). Such surfaces have been implicated as playing an important role in the origin of life because organic molecules, i.e. amino acids, can be selectively adsorbed onto their surface for further condensation into proteins. Although defence is often invoked as the most likely reason for silica metabolism and wall evolution in the diatoms (see discussion on the diatom list server), other reasons for silica metabolism in the diatoms may be found among vertebrate cell lines (Carlisle 1976, Birchall 1978, Hench 1989), where silica is necessary to prevent cell ageing, to induce normal formation of connective tissues, to reduce the effects of toxic metals and to inhibit fungal attack. Mammalian cells can be retained in a prolonged resting state when seeded onto bioactive glass surfaces (Seitz *et al.* 1982). Hench (1989) also reported prolonged resting states for other fibroblast cell lines (unpublished data). In mammalian connective tissue, Si concentration decreases with age. Thus, increased Si levels have been linked to retardation of diseases that increase with age, such as arteriosclerosis and rheumatoid arthritis. Within a cell, highest levels of Si have been found inside the ER (Hench 1989). In the diatoms, the silica deposition vesicle (SDV) in which the new valve is formed is constructed from coalescing vesicles presumably derived from the ER (Schmid 1994).

As a unicell, the life expectancy of a diatom could be considered indefinite, assuming it was not grazed or did not enter the reproductive window. At least the cell population retaining the epitheca in each diatom cell division will never get smaller and can never enter the reproductive window. Essentially it is these cells that could live indefinitely and would most likely benefit from any process preventing cell ageing or placing them in a prolonged resting state providing that they are not grazed or die from other causes. The resting spore stages of the diatoms and the chrysophytes are silica-walled. Many diatoms in the Antarctic are known to have resting cells that predominate in winter (not spores, see Fryxell *et al.* 1994). These cells are more heavily silicified and one could argue that increased silica metabolism is needed to keep the cells in a prolonged resting state during winter when light and nutrients are limiting.

Glass bioactive substrates also initiate some cell types to activate their genetic code, i.e. a special cell function (Hench 1989). It is known that in diatom cultures net cell protein production ceases within four hours after silicon is depleted (Coombs *et al.* 1967). Clearly in the diatoms, Si is closely linked with all metabolic processes (see Darley & Volcani 1969) and it should be investigated as to whether or not Si is complexed in some way in the ribosomes to facilitate protein production or whether there is an abundance of non-ribosomal protein synthesis in the diatoms, such as in the polyketide toxins known to be produced by a variety of microalgae, not only in some restricted species of *Pseudonitzschia*. Proteins composed of unusual amino acids (e.g. polyketides) are known to be formed outside the ribosomes (Neilan *et al.* 1999). Many unusual amino acids are known to be present in the diatom wall matrix and are likely involved with silica polymerisation (Lobel *et al.* 1996, Kröger & Sumper 1998), thus offering more evidence to suggest that silica may play a role in condensation of proteins in the diatoms.

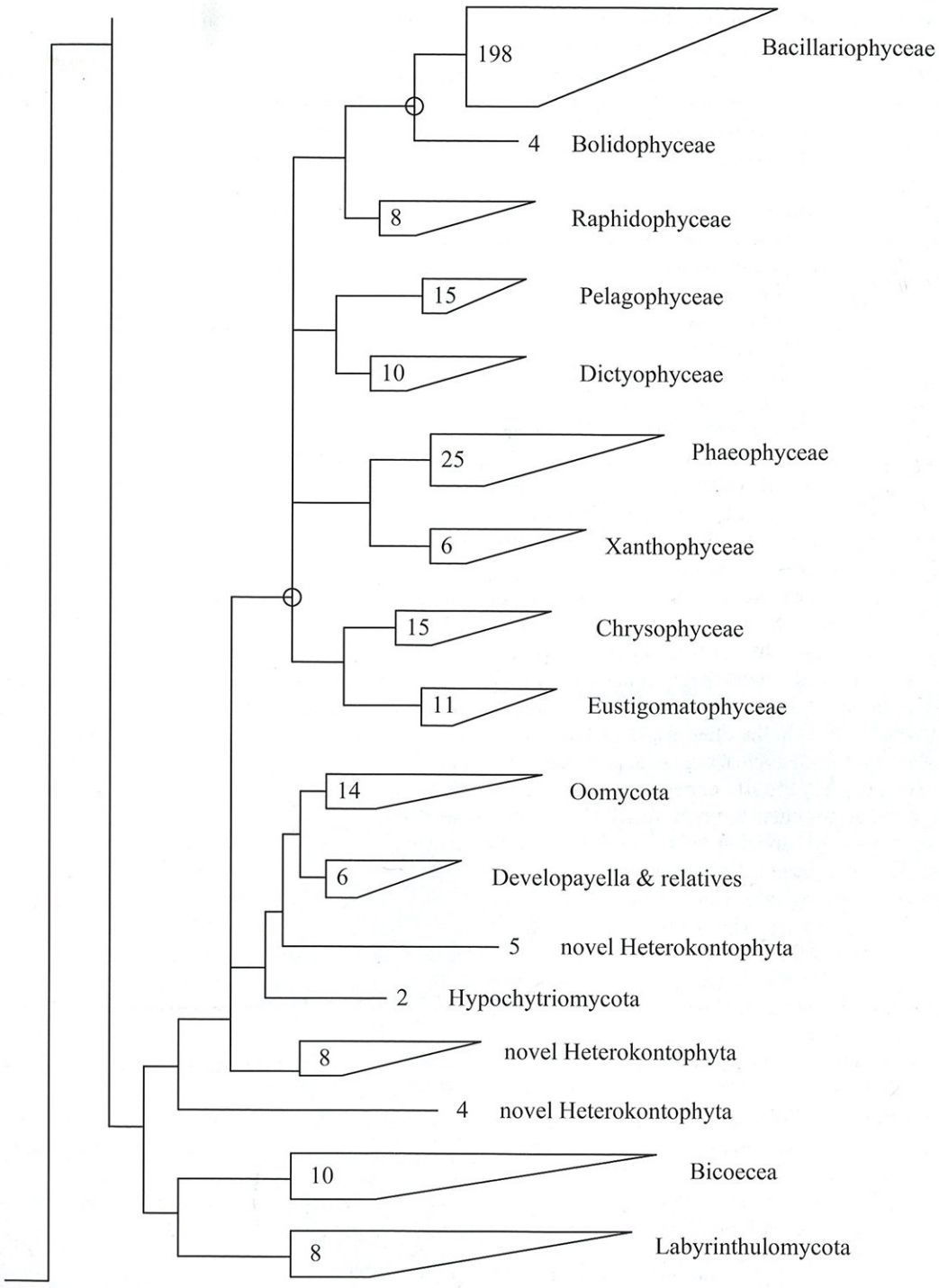

Fig. 1 Phylogenetic tree showing full Heterokontophyta lineage with the Bolidophyceae as sister group to the diatoms. The size of each triangle is proportional to the number of taxa consolidated to form the triangle. The actual number of taxa is presented inside each triangle.

It is also known from artificial systems inducing silica polymerisation that silicic acid will naturally polymerise in an acidic environment (Geddes & Birch 2000). We now know that the internal lumen of the silica deposition vesicle is acidic (see references in Vreiling *et al.* 2000) and we can infer that the nannoscale uniformity of the pore architecture is likely controlled by the pH of the vacuole as demonstrated in artificial systems (Geddes & Birch 2000). We also know that structures resembling known features of diatom cell walls, e.g., the macrolabiate process, can be formed naturally as in a Belousov–Zhabotinskii reaction and thus can be considered self-organising three dimensional structures formed by simple fluid mechanics (Madore and Freedman 1987) (Fig. 2). Parkinson *et al.* (1999) have also shown by computer simulation based on a diffusion-limited aggregation algorithm that microtubules are able to localise the deposition of new siliceous material. They also showed that physical factors, such as surface mobility, surface tension and temperature, may also influence the growth pattern of an aggregate.

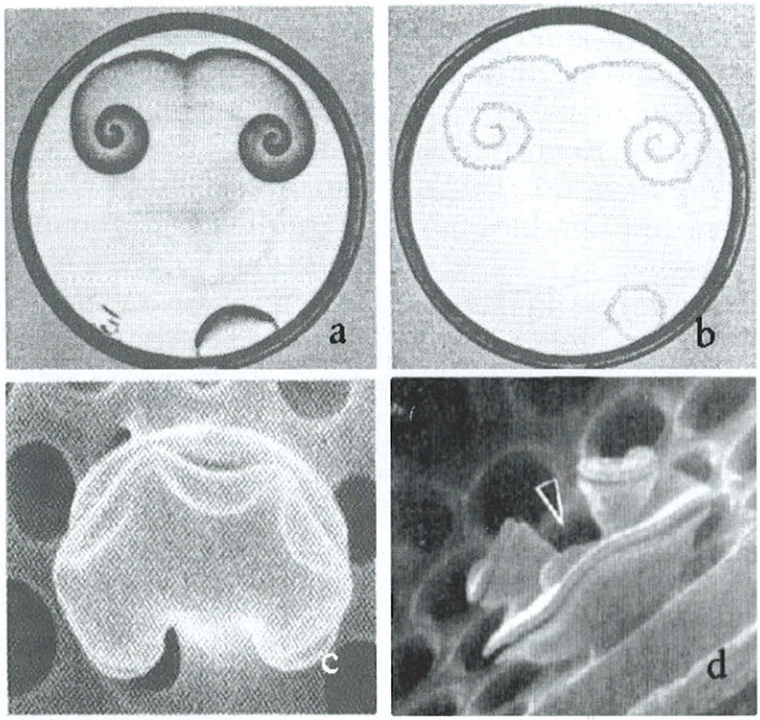

Fig. 2. Structures derived from spontaneous chemical activity and a computer simulation of that activity (A & B) (modified from Madore & Freedman 1987) and a comparison to a similar structure, the macrolabiate process, in the diatom cell wall (C & D). D, courtesy G.A. Fryxell.

Thus, the following scenario can be hypothesised for the evolution of silica metabolism in the diatoms. A simple naked bi-flagellate cell evolved silica metabolism, which conferred advantages, e.g. preventing ageing the cell and placing it in a prolonged resting state. Silica became involved in the metabolic processes of the cell presumably by supplying bioactive surfaces for reactions to take place. As silica accumulated in the cells, presumably in highest abundances in the ER, it would follow, naturally that it might be sequestered within a vacuole whose internal pH was acidic. Silica entry must occur fast enough to maintain the required internal concentration despite cell growth; catalysed entry of silicic acid, e.g. by aquaporin-like channels, are required (Raven 2001). The silica began to polymerise and the polymerised silica was extruded from the cell because in a polymerised state the silica was

inaccessible to the chemical reactions needed to prevent cell ageing. The use of silica as a wall material then releases photosynthesis from involvement in wall material and photosynthetic products can enter the biochemical cycle of other cell materials, such as lipids, etc. Thus, the cell evolved a continuous need to replenish its internal silica pool. In this respect the diatoms have coupled an absolute requirement for silica before the cell can divide (Darley & Volcani 1969). The species-specific cell wall structure of the diatoms has evolved as the cell utilised both membrane-mediated morphogenetic and macromorphogenetic mechanisms to mould the wall features (Kröger & Sumper 1998) and to guide the naturally mediated silica polymerisation induced by the acidic conditions of the silica deposition vesicle. Thus, the evolution of silica metabolism conferred advantages to the life of the unicell and the cell wall evolved as a waste product of silica metabolism, which later conferred additional protection to the cell.

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