NATURE|Vol 457|5 February 2009

BIOGEOCHEMISTRY

Early animals out in the cold

Jochen J. Brocks and Nicholas J. Butterfield

The enduring controversy about the appearance of animals in the evolutionary record takes a fresh twist with an analysis of molecular fossils that places the rise of the sponge lineage before 635 million years ago.

Charles Darwin was famously sceptical about the sudden appearance of fully formed animals (metazoans) in the Early Cambrian fossil record, beginning some 542 million years ago. To a degree, he has been vindicated by the discovery of animal and animal-like fossils extending throughout the preceding Ediacaran Period, which followed the end of the second of the great Cryogenian ice ages around 635 million years ago (Fig. 1). But there the trail runs out. So is this really where metazoan life began? Or is it merely the point at which a capricious fossil record disappears?

In the absence of shells or bones, the fossil record of animals can fade away to localized snapshots, such as the remarkable diversity of early animal-like fossils in the Doushantuo biota of southern China[1]. However, estimates of evolutionary first appearance require a fundamentally more reliable type of data[2]. This is where Gordon Love and colleagues[3] (page 718 of this issue) check in with their analysis of fossil biomarkers — geologically robust and taxonomically distinctive hydrocarbon molecules, derived primarily from the lipid membranes of once-living organisms. And when it comes to tracking primitive animals, the key biomarker is a 30-carbon steroid called 24-isopropylcholestane (24-ipc). The only known sources of this compound are species of the Demospongiae, one of the three main classes of extant sponges (phylum Porifera).

Love *et al.*[3] focused on an unusually complete sequence of sedimentary rock in Oman. They not only document an abundance of 24-ipc throughout the Ediacaran, but also trace it into underlying Cryogenian strata — compelling evidence that the organisms producing this signal were present before the end of the 635-million-year-old glacial event (Fig. 1). Crucially, the authors show that the biomarkers could not have migrated from younger rocks. They achieved this by catalytically cracking the immobile organic matrix in the sediments, releasing 24-ipc biomarkers in similar high abundance to those in the associated soluble extracts.

This rigorous screening procedure was not used in a previous report[4] of 24-ipc from much older rocks, extending back to about 1,600 million years ago. Love *et al.*[3] attribute the conspicuously lower concentrations of the compound in that report to an alternative, non-sponge source, although this interpretation diminishes the value of 24-ipc as a taxonomically diagnostic biomarker. However, there is a strong possibility that the low concentrations of 30-carbon steroids in pre-Cryogenian rocks represent secondary contamination[5]. If so, the new data[3] provide an even crisper signal for a Cryogenian first appearance of 24-ipc and its biological source.

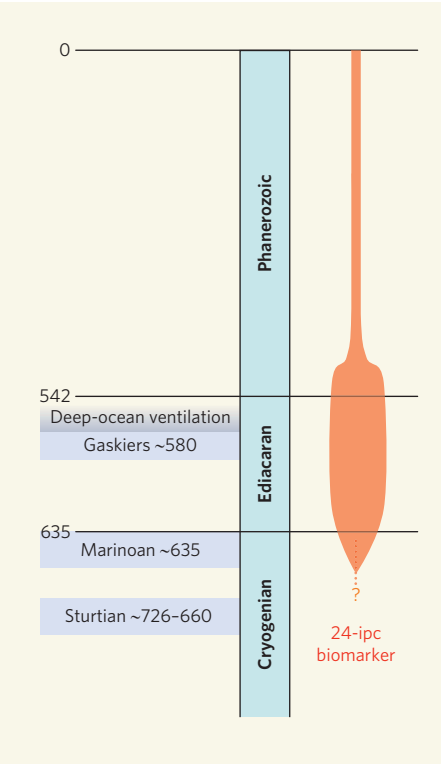

Figure 1 | Early animal evolution and the 24-isopropylcholestane (24-ipc) biomarker. The Cryogenian and Ediacaran were an interval of great environmental upheaval, including severe glaciations (the Sturtian and Marinoan, which may have been global in extent, and the Gaskiers); extreme perturbations of the global carbon cycle; and ventilation of the deep oceans with oxygen. All of these events have been cited as potential triggers for the origin of animals. Love *et al.*[3] add to the evidence of early animal life with their identification of 24-ipc in rocks older than 635 million years. The thickness of the orange line indicates the relative abundance of the 24-ipc biomarker at different times. Numbers indicate time in millions of years ago; not to scale.

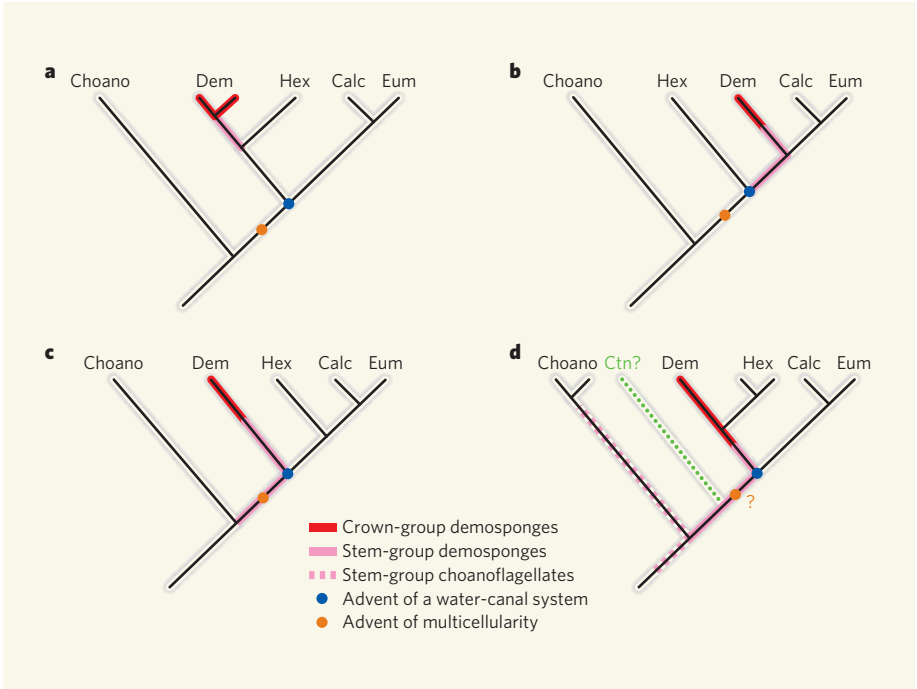

Figure 2 | Biosynthesis of the 24-ipc precursor in different evolutionary schemes. The Ediacaran and Cryogenian occurrences of 24-ipc are probably derived from stem-group demosponges (pink lines); these may or may not represent the true sponges, which exhibit multicellularity and a water-canal system. Indeed, there is no reason to rule out stem-group choanoflagellates now that these unicellular organisms are not considered directly ancestral to sponges[13]. **a**, **b**, 24-ipc is limited to true sponges. **c**, **d**, These schemes allow the possibility of (now-extinct) pre-sponge, non-metazoan organisms as the source; the phylogeny in (**d**) is currently the most strongly supported account of poriferan relationships at the class level[9]. The most recent molecular phylogeny[10] suggests that the most primitive animals are not sponges but ctenophores, a group of animals that superficially resemble cnidarian jellyfish, but that belong to a separate phylum (green dotted line in **d**). Choano, Choanoflagellida; Dem, Demospongiae; Hex, Hexactinellida; Calc, Calcarea; Eum, Eumetazoa; Ctn, Ctenophora.

So, what exactly were the organisms that produced these biomarkers? The most obvious answer, and the one that the authors[3] plump for, is that demosponges had evolved and become ecologically prominent by at least the late Cryogenian. But this conclusion overlooks the evolutionary nature of biological taxa and the incremental assembly of defining characteristics along (now-extinct) 'stem lineages' (Fig. 2). It is only with a full complement of such characteristics — in the last common ancestor of the extant 'crown group' — that modern taxonomic boundaries apply[6]. It is certainly possible, perhaps even likely, that the biomarkers from Oman reflect the existence of true, multicellular sponges with a water-canal system. But this conclusion depends on the evolutionary relationships between extant sponges (represented principally by the demosponges, hexactinellids and calcareans) and their adjacent sister groups (the single-celled choanoflagellates, and the eumetazoans; this latter group includes all metazoans apart from sponges).

A defining characteristic of crown-group demosponges, hexactinellids and calcareans is widely understood to be the development of mineralized skeletal structures, or spicules. The absence of convincing spicules in the Ediacaran or Cryogenian fossil record[7] implies that the modern poriferan classes were not fully defined until the Cambrian — and even then, seemingly bizarre combinations of spicule characteristics in Middle Cambrian fossils[8] suggest a delayed arrival of poriferan crown groups. Assuming that pre-Cambrian 24-ipc biomarkers originated from a sponge stem-group certainly does not rule out their derivation from a true sponge, and some evolutionary scenarios for the distribution of 24-ipc yield this as a unique solution (Fig. 2a,b). Other interpretations, however — including that from the most recent and comprehensively sampled analysis of hexactinellid relationships[9] — allow the biomarker biosynthesis to extend back into stem-group forms that were not sponges, and potentially not even multicellular (Fig. 2c,d). Such a possibility has important implications for the ecological interpretation of 24-ipc and the way it is applied to molecular clocks.

Despite the ambiguities, Love and colleagues' positive identification of 24-ipc in the late Cryogenian marks a considerable advance in resolving early animal evolution — particularly in light of the latest and most comprehensive molecular analysis of metazoan relationships, which no longer identifies sponges as the most primitive living animals[10] (Fig. 2d). The next steps are to find out how far back the signal can be traced in time, and how to interpret negative results. There are currently fewer than half a dozen reports of convincing biomarker occurrences of Cryogenian age, and the conspicuously low abundances of 24-ipc in post-Cambrian sediments stands at odds with the proliferation of presumed demosponge reefs in succeeding periods of Earth history.

Further sampling of the Cryogenian is clearly in order, but so too is the search for independent proxies of early animal life. Like the first predatory animals in the Ediacaran, which seem to have induced a fundamental shift in both organismal morphology and evolutionary dynamics[11], stem-group sponges may have left an indirect ecological fingerprint. It is possible, for example, that the novel feeding habits of sponges — based on the circulation of sea water through a sophisticated water-canal system — may have impinged sufficiently on the marine carbon cycle to register in the biogeochemical record[12]. Combined with new biomarker data and molecular phylogenomics, the identification of such signals promises to pinpoint the first appearance of our earliest animal ancestors. ■

Jochen J. Brocks is at the Research School of Earth Sciences, and the Centre for Macroevolution and Macroecology, the Australian National University, Canberra, 0200 ACT, Australia. Nicholas J. Butterfield is in the Department of Earth Sciences, University of Cambridge, Cambridge CB2 3EQ, UK.
e-mails: jochen.brocks@anu.edu.au; njb1005@cam.ac.uk

1. Xiao, S. & Laflamme, M. *Trends Ecol. Evol.* **24,** 31–40 (2009).
2. Butterfield, N. J. *Integr. Comp. Biol.* **43,** 166–177 (2003).
3. Love, G. D. *et al. Nature* **457,** 718–721 (2009).
4. McCaffrey, M. A. *et al. Geochim. Cosmochim. Acta* **58,** 529–532 (1994).
5. Brocks, J. J., Grosjean, E. & Logan, G. A. *Geochim. Cosmochim. Acta* **72,** 871–888 (2008).
6. Budd, G. *Nature* **412,** 487 (2001).
7. Xiao, S., Hu, J., Yuan, X., Parsley, R. L. & Cao, R. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **220,** 89–117 (2005).
8. Botting, J. P. & Butterfield, N. J. *Proc. Natl Acad. Sci. USA* **102,** 1554–1559 (2005).
9. Dohrmann, M., Janussen, D., Reitner, J., Collins, A. G. & Wörheide, G. *Syst. Biol.* **57,** 388–405 (2008).
10. Dunn, C. W. *et al. Nature* **452,** 745–749 (2008).
11. Peterson, K. J. & Butterfield, N. J. *Proc. Natl Acad. Sci. USA* **102,** 9547–9552 (2005).
12. Sperling, E. A., Pisani, D. & Peterson, K. J. in *The Rise and Fall of the Ediacaran Biota* (eds Vickers-Rich, P. & Komarower, P.) 355–368 (Geol. Soc. Lond., 2007).
13. Carr, M., Leadbeater, B. S. C., Hassan, R., Nelson, M. & Baldauf, S. L. *Proc. Natl Acad. Sci. USA* **105,** 16641–16646 (2008).

COMPUTATIONAL CHEMISTRY

Dances with hydrogen cations

Sotiris S. Xantheas

Life depends on the flow of hydrogen cations in water, yet their dynamic behaviour when in complex with water molecules is unknown. The latest computer simulations cast light on the jiggling of these hydrated ions.

In water, hydrogen cations (H^+) abound, but they exist only as complexes with water molecules. One of the most important of these complexes is the Zundel cation, in which a hydrogen cation is shared by two water molecules. The structure of the Zundel cation has been known for years, owing to evidence from infrared (IR) spectra. But its dynamic behaviour — how the hydrogen cation moves between the two water molecules — is unknown. In *Angewandte Chemie*, Vendrell *et al.*[1] report accurate computer simulations of the IR spectrum of the Zundel ion in the gas phase, and of analogues in which hydrogen atoms have been replaced with deuterium atoms. This allows the first complete characterization of the complex molecular vibrations of Zundel ions, providing information that might contribute to a long-sought-after goal — an accurate computational model of how hydrogen ions are transported through liquid water.

Hydrogen cations are ubiquitous in nature, and are vital components of many chemical and biological environments. For example, they take part in acid–base reactions that determine the formation, fate and transport of the main environmental pollutants that cause acid rain; they are pumped across cell membranes by dedicated proteins, creating gradients in pH and charge that act as energy reservoirs for the cell; and hydrogen-ion movement, when coupled to electron transfer in enzymes, allows bioenergetic conversions to occur, and 'activates' enzyme substrates, readying them to take part in catalytic bond-breaking and bond-making reactions.

Curiously, hydrogen cations seem to diffuse faster through water than do other atomic cations. In fact, hydrogen-cation 'diffusion' in water involves the concerted making and breaking of many bonds in networks of water molecules, in a process known as the Grotthuss mechanism[2] (Fig. 1a). When a hydrogen cation forms a bond to a water molecule, other covalent and hydrogen bonds throughout the network break and re-form until a different hydrogen ion is ejected. Hydrogen cations in water are thus hydrated: they either exist in complex with individual water molecules, forming Eigen ions[3] (H_3O^+), or are shared equally by two water molecules to form Zundel ions[4] ($H_2O–H–H_2O)^+$. The exact form taken by hydrated hydrogen ions — known collectively as hydronium ions — has long received much attention[5–7].

Aqueous hydronium clusters are considered to be effective vehicles for probing the dynamic environment of hydrogen cations in more complex systems such as liquid water[8,9]. The IR spectra of hydronium ions (and of