Feature

The mysteries of the diatoms

Understanding the physiology of these unique and spectacularly successful algal species could lead to substantial benefits in a wide range of areas from nanotechnology to climate change. **Michael Gross** reports.

Diatoms — single-celled algae typically enshrined in a cell wall made of intricately laced silica — have fascinated researchers with a whole range of mysteries, from their evolutionary origins through to their morphogenesis and reproduction. They entered the plant kingdom rather late in evolution, and through an unusual entry. Researchers believe they are secondary endosymbionts, meaning that their precursor was a eukaryote that engulfed another eukaryote, resulting in a quadruple membrane around the chloroplasts the diatom gained from this act of piracy.

The evolutionary success story of diatoms only begins some 200 million years ago, but they have spread around the globe and diversified into hundreds of genera and around 100,000 species in this short fraction of the geological timescale. Today, they are present wherever there is liquid water, in the oceans, in freshwater, and even in soil. They have already played a significant role in the global cycles of carbon and nitrogen, and are responsible for large sediments of silica including diatomaceous earth.

Why have diatoms been so successful? Is it to do with their silica wall, as research from Paul Falkowski at Rutgers University has suggested? Silica cell walls are energy efficient to produce and unlike the carbonate biominerals of other species are not sensitive to ocean pH. Fossil traces of diatoms' silica shells (frustules) can be dated back to 185 million years ago, and their rise seems to have been unstoppable since then.

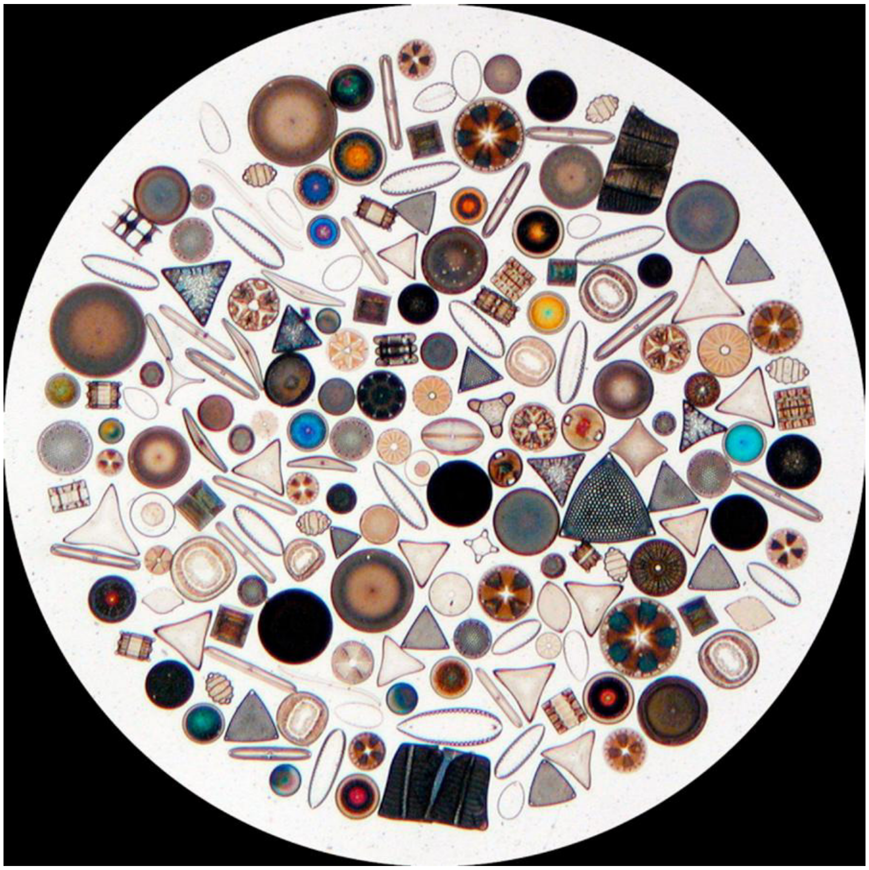
Alternatively, results from Christian Wilhelm at Leipzig show that they have a very efficient way to dissipate excess solar energy, known as non-photochemical quenching. Some experts believe that may be a crucial factor explaining their success.

Genomes growing apart

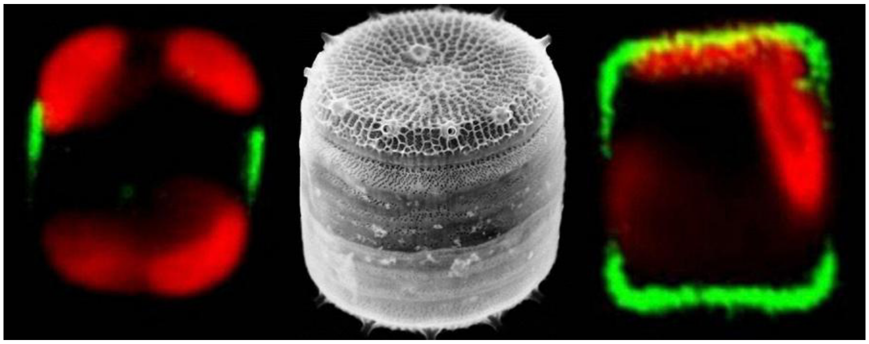
In recent years, complete genome sequences of four diatom species have become available. In 2004, the group of Virginia Armbrust at the University of Washington in Seattle reported the genome of the diatom *Thalassiosira pseudonana*, which was followed by *Phaeodactylum tricornutum*, *Fragilariopsis cylindrus* and *Pseudo-nitzschia multiseries* (Nature (2009) *459*, 185–192). Of these, *Thalassiosira* belongs to the group of centric (radially symmetric) diatoms, while the other three are raphid pennates, where the defining 'raphe' is a slit along the bottom that enables motility on surfaces.

The first two genome sequences showed that, in their relatively short evolutionary history, diatom species have grown apart much more than comparable groups. *T. pseudonana* and *P. tricornutum*, for instance, only parted company around 90 million years ago, but their genomes are as different as human and fish, which evolved separately for 550 million years.

The genomes also shed light on the unusual endosymbiotic origin and gene mixing of diatoms. Primary endosymbiosis, the process that gave us green algae and higher plants, happened around 1.5 billion years ago when an ancestral eukaryote acquired a cyanobacterium, which became the ancestor of today's chloroplasts.



Diatom diversity: In a time span of less than 200 million years, diatoms have branched out into a multitude of species, which can be as genetically different as humans and fish. (Photo: Wikimedia Commons.)



Morphogenesis molecules: Transformant strains of *Thalassiosira pseudonana* expressing fluorescently labelled cingulins and silaffins. (Photo: Nils Kröger.)

In secondary endosymbiosis, by contrast, a eukaryote engulfed another eukaryote, namely a red alga, complete with its chloroplasts, mitochondria, and its nuclear genome. The alga in question may also have been infected by intracellular bacteria. The descendants of this more complicated merger, which happened only around one billion years ago, include diatoms, brown macroalgae, and oomycetes, important plant pathogens.

In the diatom genomes, researchers found a very eclectic mixture of genes, some resembling plants, others animals or bacteria. “One might say diatoms are animals with chloroplasts,” says Nicole Poulsen from the B CUBE Centre at the Technical University Dresden. Chris Bowler from the Ecole Normale Supérieure at Paris thinks this clash of concepts just represents our anthropocentric and simplistic world view. “While we might want to call diatoms ‘plantimals,’ these things are much more complex than we think,” he says.

Like animals, for instance, diatoms possess a complete urea cycle, inherited from the heterotrophic host of the secondary endosymbiosis. The group of Chris Bowler together with Andrew Allen at the J. Craig Venter Institute in San Diego, California, has recently used knockdown studies of urea cycle enzymes to show that the cycle enables diatoms to recover quickly after prolonged periods of nitrogen limitation (Nature (2011) *473*, 203–207). This fits in with numerous observations of the diatoms’ ‘bloom and bust’ response, i.e. their ability to outcompete all other species as soon as a nutrient limitation is removed. The study also shows an intriguing interaction of the ‘animal-like’ urea cycle with other metabolic reactions enabled by the products of genes acquired by horizontal gene transfer from bacteria, e.g., for the biosynthesis of polyamines. Allen’s group also conducted the first comprehensive analysis of small RNAs in a diatom, *Thalassiosira pseudonana* (PLoS ONE *6*, e22870). The researchers found that the RNAi machinery of diatoms most closely resembles that of plants.

Diatoms, suggests Chris Bowler, seem to have mastered the art of combining physiological reactions from different sources to create something new, such as the polyamines generated from the urea cycle, which enable them to build their silica shells, as discussed below. Another example is the complex interaction between their chloroplasts and mitochondria, both likely derived from different sources during diatom evolution, which Bowler and colleagues have analysed in a recent review article (Journal of Experimental Botany (2012) doi:10.1093/jxb/err441). This ability to create a mix-and-match physiology may also be part of the explanation for their spectacular success.

These complexities show, says Bowler, “that model organisms give us only a very biased view of how life works.” *Escherichia coli*, *Drosophila*, *Arabidopsis* and yeast have provided us with deep insights into how biology works in these particular systems, but they don’t help us much to understand the physiology of diatoms. And understanding this is becoming more and more important, as diatoms have a huge influence on geochemical cycles and our climate.

Riding the global cycles

Diatoms fix as much carbon dioxide as all the rainforests of the world combined, and they may very well have been the main architects of our current, moderately cool climate situation. The rise of the diatoms began in a much warmer climate than ours, with no ice caps on the poles. The atmospheric carbon dioxide concentration was much higher than today. In the Cretaceous, around 100 million years ago, when diatoms began to become widespread and developed great diversity, the carbon dioxide level was fivefold higher than today, and oxygen was lower. Remarkably, diatoms managed to thrive and expand during a period of extreme climate change, and they also seem to have come through the mass extinction that saw off the dinosaurs without too many problems. The opening of the Drake Passage around 40 million years ago, which created the continent of Antarctica, was particularly beneficial for the diatoms because they began to proliferate dramatically in the cold turbulent waters of the Southern Ocean.

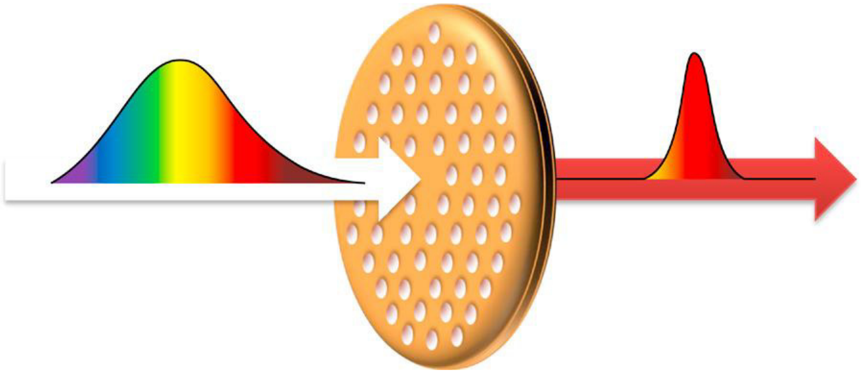
As diatoms evolved thicker and denser cell walls and spread across the oceans, it became more likely that dead diatoms might sink to the ocean floor and thus sequester their carbon. This became a significant cause of carbon dioxide reduction at the planetary level in the next tens of millions of years, until carbon dioxide approached the level that we used to have before the Industrial Revolution. Currently, by burning fossil fuels such as petroleum that were generated in the past by diatoms and other plankton, we are undoing much of the carbon sequestration work that these diatoms did.

Recognition of the importance of diatoms for the Earth’s carbon cycle has led to the idea of encouraging them to step up their current sequestration activities in the oceans. Iron is a limiting nutrient in large parts of the oceans, so fertilisation with iron could lead to diatom blooms (Curr. Biol. (2009) *19*, R143–R144). Large-scale experiments designed to test the idea indicated that the expected diatom bloom duly arrived, but that only negligible parts of the carbon dioxide used by the diatoms were sequestered to the sea floor. Instead, initial studies showed most of the carbon just entered the food chain and was recycled back into the atmosphere. By contrast, a new study published in July 2012 suggests that at least half the bloom biomass sinks below a depth of 1,000 metres, from which the authors conclude that a substantial portion is likely to reach the ocean floor (Nature (2012) *487*, 313–319).

To better understand the role of diatoms in our climate, and to find out whether they can help us to avert climate change, says Chris Bowler, we need to find out a lot more about them. "We need a better understanding of their physiology at the molecular level in order to understand how they affect planetary level processes," Bowler says. "For example, we need to understand how they take up nutrients, and their capacity to store and metabolise iron, nitrogen, silicon and carbon, and we need to learn much more about their biogeography and the factors that govern their sudden proliferation in response to episodic nutrient upwellings, and their subsequent demise following bloom events."

For instance, the iron storage protein ferritin is present only in a small group of pennate diatoms (Nature (2009) *457*, 467–470), and it is less obvious how other species cope with variable concentrations of the metal. In a recent study, Adrian Marchetti, Virginia Armbrust and colleagues have investigated the transcriptional response to increased iron availability in an environmental sample containing diatoms. They found that hundreds of diatom genes responded quickly to the change in nutrient availability, linking iron uptake to many other metabolic reactions including photosynthesis, nitrate assimilation, the urea cycle, and carbohydrate synthesis (Proc. Natl. Acad. Sci. USA (2012) *109*, E317–E325). This massive transcriptional response also helps to explain the very rapid blooming of diatoms after large-scale iron fertilisation experiments.

As researchers are only beginning to understand the complex physiology of diatoms and its interaction with global geochemical cycles, it is not yet possible to predict how climate change will affect diatoms — in the worst case scenario they could produce a positive feedback effect. For instance, says Bowler, if bigger diatoms go extinct and are replaced by smaller phytoplankton species, these will be less efficient at carbon sequestration via the biological carbon pump, so the net effect will be a further increase in atmospheric carbon dioxide concentrations.



Light work: Extraordinary optical transmission through a gold *C. asteromphalus* diatom frustule replica (Photo: Ken Sandhage).

Mysteries of morphogenesis

One of the most intriguing mysteries of the diatoms is the morphogenesis of their most conspicuous feature, the silica cell wall. Shapes and sizes of these microscopically small frustules vary widely. In centric diatoms they are radially symmetrical, while in pennate diatoms they have bilateral symmetry. In all cases, however, the frustule consists of two halves (theca), which fit together like a Petri dish and its lid (hypo- and epitheca), and they have lace-like patterns of nanometre-scaled pores which are species-specific and thus presumably genetic. The search for the biomolecules that determine these patterns turned out to be extremely challenging.

In 1990, Nils Kröger started the search for morphogenesis proteins in his undergraduate work with Manfred Sumper at the University of Regensburg, and found nothing. He persisted in his PhD and postdoctoral work with rather more drastic methods, such as dissolving the frustules in anhydrous hydrogen fluoride, and eventually discovered a group of proteins now called the silaffins, as they have a marked affinity to silica and promote its precipitation from solutions of silicic acid.

Initially, the silaffins resisted characterisation by routine methods such as peptide sequencing. This problem could eventually be pinned down to the presence of a large number of side-chain modifications, mainly consisting of phosphate residues and unusual polyamines. Further research showed that the sequence of the silaffins as such is less important for their function than the presence and the nature of these polyamines, and that, under certain conditions, suitably structured polyamines without any protein component can take over silaffin-style functions.

While the silaffins and polyamines are able to generate nanoscopic particles of silica from silicic acid solutions, they still don't explain the genetically inherited patterns in silica shells. Only in February 2011 could Kröger report the discovery of a new class of proteins that appear to be crucial cornerstones in the molecular puzzle of silica morphogenesis.

"We thought about how we could search for silaffin-like proteins in the genome of *Thalassiosira*, given that silaffin sequences are highly variable and apparently not very important for their function," says Kröger, who has just relocated his lab from the Georgia Institute of Technology in Atlanta to the newly set-up B CUBE Centre at the Technical University of Dresden. "We decided to look for silaffin-like amino acid composition instead, in combination with relevant signal sequences. We identified 86 silaffin-like proteins and concentrated on six of these. Hybridising them with GFP we could show that they are located in the girdle band, or cingulum, of *Thalassiosira*, so we called them cingulins."

Messy business: Biofouling on the hulls (and even propellers) of ships is a major economic problem. (Photo: Gail Ashton, Smithsonian Environmental Research Center.)

By contrast, the earlier silaffins are located in the top and bottom parts of the frustule. In earlier preparations of silaffins, the cingulum had been left aside as an insoluble residue. Now the GFP-fluorescence showed that the molecules of interest were precisely in this residue. Attempts to solubilise the cingulins by dissolving the silica (with ammonium fluoride) and digesting the chitin that is also part of the matrix revealed microscale rings that closely resemble the morphology of the girdle band where they are found (Proc. Natl. Acad. Sci. USA (2011) *108*, 3175–3180). Intriguingly, the protein matrix of the girdle band survives these harsh procedures intact. Specifically, the rings show dark, parallel stripes which occasionally fuse or branch out, a pattern that is reflected in the anatomy of the girdle band in the live cell wall.

Thus, cingulins are the first biomolecules from diatoms that offer not only the catalytic activity to precipitate silica, but also the scaffold to arrange it in the desired form. The researchers have also discovered analogous compounds in several other diatom species and hypothesise that they are generally involved in the morphogenesis of the girdle band.

Kröger's new colleagues at Dresden, Eike Brunner and Karl-Heinz van Pée, are also studying further structural biomolecules from diatoms, including the silacidins originally discovered in Sumper's lab at Regensburg, and chitin, which they suspect to play a structural role in diatom frustules. Although the chitin does not seem to influence silica deposition directly, it may act as a scaffold that enables the appropriate display of chitin-binding proteins or protein complexes that carry silica-forming domains (ChemBioChem (2011) *12*, 1362–1366).

Applications

The silica frustules with their intricate nanoscale patterns can make any nanotechnologist jealous. Nature can produce such structures at ambient temperature and under benign conditions, an achievement that our technology cannot match yet. However, while they are waiting for the 'recipe' to emerge from the fundamental research into morphogenesis, nanotechnologists can use the natural structure as a template, either to coat it with other substances, or to replace the silica with other materials.

The group of Ken Sandhage at the Georgia Institute of Technology has developed methods for the reactive conversion of an existing structure to a new chemical identity, producing frustule replicas made of materials such as titanium dioxide, magnesium oxide, silicon, or carbon.

Recently, the group developed a wet chemical process for applying thin, shape-hugging layers of various nanocrystalline metals (e.g. copper, silver, gold, nickel) onto diatom frustules. Selective dissolution of the underlying silica then yielded 3D metallic replicas of the starting frustules (Adv. Funct. Mater. (2012) *22*, 2550–2559). "As one example, we converted the frustule of the *Coscinodiscus asteromphalus* diatom into a nanocrystalline gold replica that retained the quasi-periodic pore structure of the frustule," Sandhage explains. "These gold replicas were found to exhibit a phenomenon called 'extraordinary optical transmission'. That is, infrared light of a particular wavelength could pass through these gold replicas, even though the wavelength of the IR light was larger than the diameter of the pores in the replicas. Such metallic diatom frustule replicas could be used for optical filtration and, when properly functionalized, for the detection of various analytes in fluids."

In a similar approach, the group of Eike Brunner at Dresden has coated diatom frustules with noble metals (silver, gold, platinum) and with cadmium telluride nanoparticles. The researchers foresee applications in surface-enhanced Raman spectroscopy (SERS), scanning electron microscopy and catalysis (Chem. Asian J. (2012), *7*, 85–90).

Another aspect of diatom biology of interest for possible applications is the glue that raphid pennates, such as *Amphora coffeaeformis*, secrete through their raphe while they glide across a surface. Nicole Poulsen at Dresden is currently spending much of her time scraping diatom glue off surfaces, to get sufficient material for proper molecular analyses. "It took a long year, just to get a method to obtain pure material," Poulsen says, but now she's getting results, so watch this space.

Diatom adhesives are of interest for two opposite reasons — some may want to mimic bioadhesives like these to produce better glues that work under difficult conditions, for instance under water. Others want to stop diatoms from sticking to things under water, such as ships. The problem is, Poulsen explains, that the glue left behind by a moving diatom, together with other microorganisms (e.g. bacteria), primes the surface, allowing for the attachment of other organisms, and thus leads to the formation of a biofilm and ultimately to the attachment of larger macroorganisms (e.g. seaweeds, barnacles). This coating adds significantly to the drag of large ships and thus increases fuel consumption. The only chemical solution known to prevent this from happening is tributyl tin, which was banned in the late 1980s on environmental grounds.

Jim Callow at the University of Birmingham is among the very small number of people investigating this phenomenon and looking for new solutions. “Our main test species is the green macroalga (seaweed) *Ulva* but we also use the unicellular diatom *Navicula* specifically because diatoms show opposite adhesion preferences,” Callow explains. “*Ulva* tends to adhere most strongly to hydrophilic coatings while diatoms such as *Navicula* adhere most strongly to hydrophobic coatings, especially those that are silicone-based. The frontiers of this subject lie in the development of the next generation of marine antifouling/fouling-release coatings based on amphiphilic surface-active block copolymers coatings that are able to resist both types of algae.”

A final application of diatoms brings us back to climate change — some species of diatoms are investigated with the aim of developing them for the industrial production of biofuels. This sounds surprising, as their most conspicuous attribute is their silica shell, which doesn’t help with the biofuel production and would have to be recycled.

However, as Kröger explains, some species can survive without silicon. *Phaeodactylum tricornutum*, for instance, can switch between three different morphotypes in response to specific environmental conditions, and only one of the forms needs silicon (Protist (2011) *162*, 462–481).

“The idea is that if we remove certain nutrients like nitrogen or silicon from the medium, the diatoms accumulate lipids. The challenge is to find conditions where they do that and still grow to good yields,” Kröger explains. Here, as in the geochemical role of diatoms discussed above, progress is limited by the incomplete understanding of the molecular physiology of diatoms. More research into these intriguing organisms is definitely needed.

Michael Gross is a science writer based at Oxford. He can be contacted via his web page at www.michaelgross.co.uk

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Q & A

Edvard and May-Britt Moser

Interviewed by Nancy Bazilchuk and Hege J. Tunstad

Edvard and May-Britt Moser are the founding directors of the Norwegian University of Science and Technology’s (NTNU) Centre for the Biology of Memory and the Kavli Institute for Systems Neuroscience (KI/CBM). They grew up on two small but adjacent islands off the west coast of Norway. They first met in high school, but didn’t really get to know each other until their paths crossed as students at the University of Oslo. They found out that they had similar interests and decided to go into psychology together, graduating with degrees in Psychology from the University of Oslo in 1990. At the same time, they found out they had an interest in each other as well.

The pair obtained their PhDs in Neurophysiology from the University of Oslo in 1995, under the supervision of Per Andersen. They have also worked periodically with Richard Morris at the Centre for Neuroscience, University of Edinburgh from 1992–1996, and with John O’Keefe at the University College of London in 1996. Both were appointed associate professors at NTNU in 1996, and full professors in 1998 (E.M.) and 2000 (M.B.M.). Their work at NTNU has focused on how spatial location and spatial memory are computed in the brain. Their most famous achievement to date is probably the discovery in 2005 of entorhinal grid cells, which points to the entorhinal cortex as a hub for the brain network that enables us to find our way. In conjunction with researchers at the KI/CBM, they have also shown how a variety of functional cell types in the entorhinal microcircuit contribute to representation of self-location, how the outputs of the circuit are used by memory networks in the hippocampus, and how episodic memories are separated from each other in the early stages of the hippocampal memory storage.

Photo credit: Ned Alley.

Both are members of the Norwegian Academy of Science and Letters and have been elected to the European Molecular Biology Organization (EMBO). They have also been recognized with a wealth of scientific and research prizes over the years, including the 2011 Louis-Jeantet Prize for Medicine and the 2011 Anders Jahre’s Award for Medical Research.

What turned you on to biology in the first place?

Edvard: For my part it was quite random because I was interested in lots of things. I wanted to start with nuclear physics, and I was interested in geology, and evolution. It was actually quite random. I began in chemistry, inorganic chemistry, and I thought that was boring, so then I turned to psychology. And met May-Britt. We soon found out that the few pages in our textbooks that were about neuroscience were the most interesting. And then we turned to the brain. This was in the early 1980s.

What was it like to make the transition from studying traditional psychology to studying neuroscience, as you have done?

May-Britt: There was no transition. When we decided to start in psychology, both of us had this