

In search of new tractable diatoms for experimental biology

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Summary

Diatoms are a species-rich group of photosynthetic eukaryotes, with enormous ecological significance and great potential for biotechnology. During the last decade, diatoms have begun to be studied intensively using modern molecular techniques and the genomes of four diatoms have been wholly or partially sequenced. Although new insights into the biology and evolution of diatoms are accumulating rapidly due to the availability of reverse genetic tools, the full potential of these molecular biological approaches can only be fully realized if experimental control of sexual crosses becomes firmly established and widely accessible to experimental biologists. Here we discuss the issue of choosing new models for diatom research, by taking into account the broader context of diatom mating systems and the place of sex in relation to the intricate cycle of cell size reduction and restitution that is characteristic of most diatoms. We illustrate the results of our efforts to select and develop experimental systems in diatoms, using species with typical life cycle attributes, which could be used as future model organisms to complement existing ones. *BioEssays* 30:692–702, 2008. © 2008 Wiley Periodicals, Inc.

Introduction

The diatoms (Bacillariophyceae), a group of aquatic photosynthetic unicells within the eukaryotic Division Heterokontophyta (Stramenopiles) (Fig. 1),⁽¹⁾ are immensely diverse and ecologically important. They are major players in ocean geochemistry and carry out ~20% of the net primary production of the planet; they form the foundation of the trophic pyramid in many parts of the world ocean.^(2–4) Over a comparatively short evolutionary time (<240 Ma),⁽⁵⁾ they have diversified into hundreds of genera and perhaps 200 000 extant species.⁽⁶⁾ Their trademarks are their beautifully ornate silica shells (Fig. 2A), which are widely used as stratigraphical and environmental indicators.⁽⁷⁾ Intriguingly, the ability of diatoms to make silica shells has recently attracted the attention of materials chemists and molecular biologists, leading to a rapid increase in our understanding of the biochemistry of silicification.⁽⁸⁾ After cytokinesis, silicate is transported into special vesicles in the cell, where it is precipitated and molded to form elaborate structures and regular patterns of ribs and pores (Fig. 2A);⁽⁹⁾ silicification is promoted by protein-bound polyamines and this discovery has already inspired new biomimetic syntheses, e.g. of titanium compounds.⁽¹⁰⁾ The potential of diatoms for silicon technologies and other industrial applications, e.g. biofixation of CO₂, lipid production and bioremediation, is now well established.^(11–14) Equally striking is the life cycle of diatoms, which is characterized by unique features such as a gradual reduction in cell size (Fig. 2B–D) and the presence of species-specific size thresholds (the ‘cardinal points’), including a size threshold for sexualization (see below).⁽¹⁵⁾ Finally, because they belong to a major eukaryotic lineage (the Stramenopiles) that is well separated from other photosynthetic eukaryotes (e.g. the green plants and red algae), and from animals and fungi,^(16,17) studies of their basic biology are of fundamental importance for understanding the evolution of eukaryotic cellular processes. Consequently, diatoms have become a subject of intense studies on many fronts.

Modern experimental biology is largely based on the ‘model-systems’ approach, in which attention is focused on a few favorite ‘model’ organisms and, within these, on a very few laboratory lineages (e.g. <http://www.nih.gov/science/models/>). The choice of model organism is always highly debatable, because uncomfortable compromises must often

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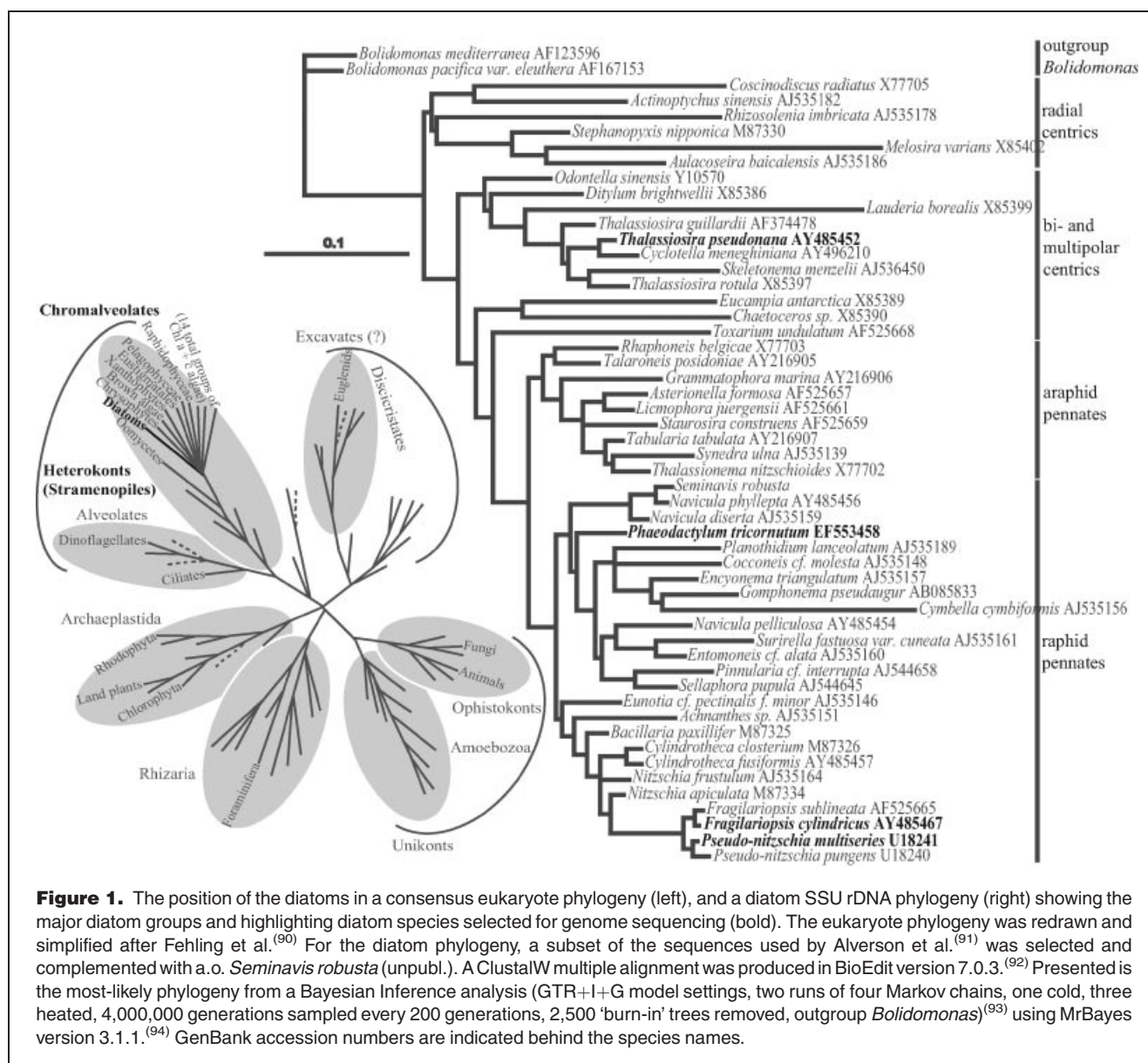
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be made between contradictory requirements (in diatoms, large-celled species would be easier to examine microscopically, but small-celled species often grow faster); this topic has been examined from several points of view—analytical, epistemological and historical.^(18–21) Nowadays it is obvious that studies of 'traditional' and new model systems will make significant progress only if genomic approaches are used and diatom research is no exception to this rule.

The unique cell wall and ecological significance of diatoms led to the decision to sequence the whole genome of a representative species. The marine species *Thalassiosira pseudonana*^(22,23) was chosen and it was the first eukaryote to be sequenced from the phytoplankton. A second diatom, *Phaeodactylum tricorutum*,⁽²⁴⁾ which has also long been a

favourite organism for physiological research, has now been sequenced too. These two diatoms are only distantly related and have the advantage that they represent the two major architectural types within the diatoms: *Thalassiosira* is a 'centric' diatom (a paraphyletic group with radially patterned valves) and *Phaeodactylum* is a 'pennate' diatom (a monophyletic group characterized by their feather-like valve structure) (Fig. 1). As models, these two satisfy some important criteria, including laboratory convenience (short generation time, ease of stock maintenance and experimental tractability), small genome size and molecular genetic transformability.^(25,26) They are also good choices because of the wealth of cytological, physiological and biochemical data that was already available. However, both species suffer from

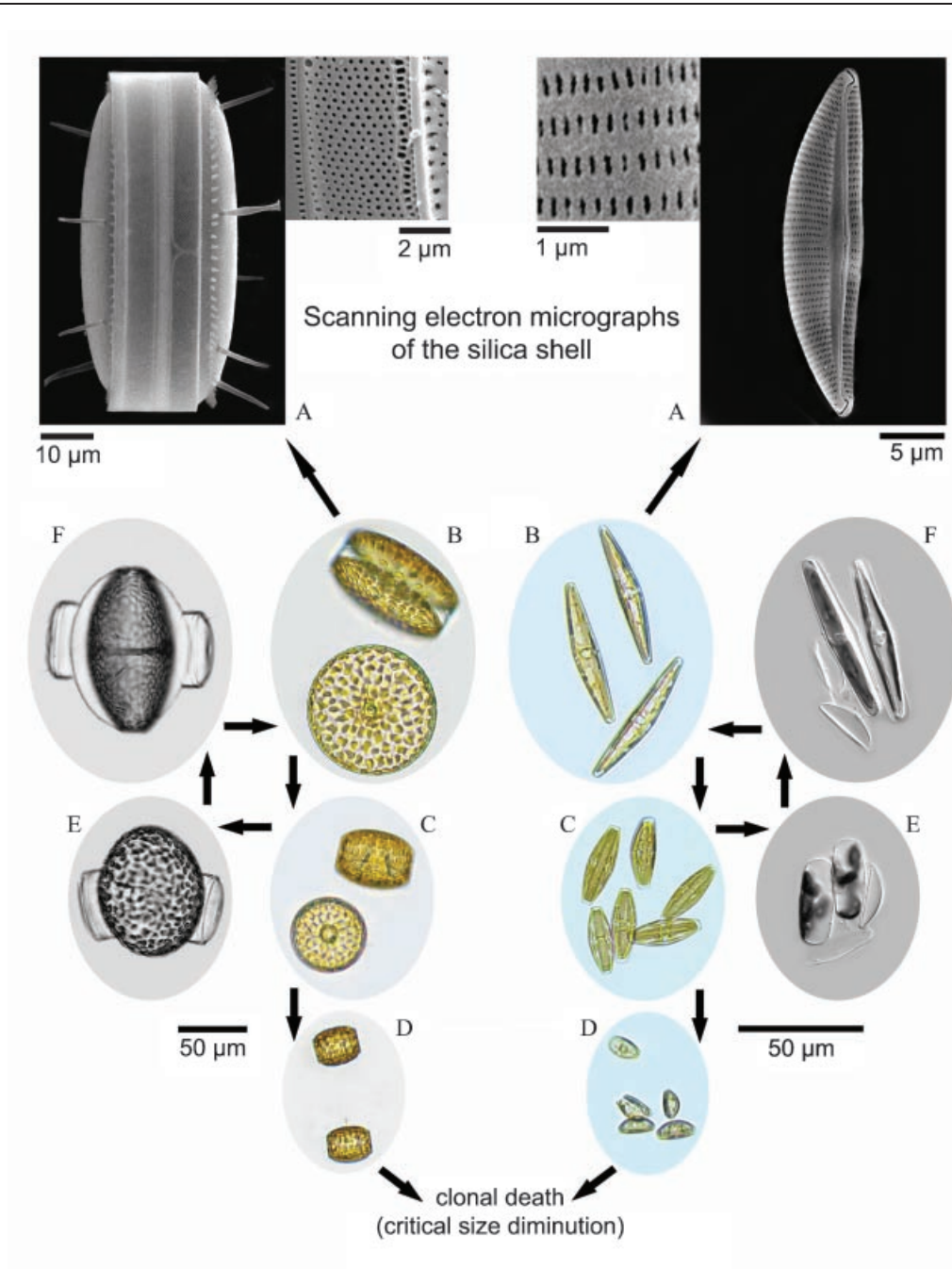


Figure 2. Two evolutionarily distant and ecologically different diatoms: a centric planktonic species (*Thalassiosira punctigera*, left) and a pennate benthic species (*Seminavis robusta*, right). **A:** Morphology of their silica shells, the frustule. **B–F:** Principal stages of their life cycles. **B–D:** Gradual diminution of cells in size in clonal cultures over time. **E:** Auxospores expanding isodiametrically (*Thalassiosira*) and bipolarly (*Seminavis*). Formation of these auxospores resulted from intracolonial sexual reproduction in *Thalassiosira* and from a cross between opposite mating type clones in *Seminavis* (here two sibling auxospores are produced per pair of mating cells). **F:** The contents of developed auxospores transform into enlarged (*initial*) cells, still enclosed within the auxospore cell wall; these then restart a new round of vegetative multiplication.

a problem that was pointed out by Apt et al. in 1996⁽²⁷⁾ “Currently, there are a number of limitations to the use of diatoms as an experimental organism and a commercial resource. A considerable limitation arises from the fact that diatoms grow as diploid, vegetative cells, and no one has been successful in controlling a sexual cycle in culture”. Despite the significant advances made in biotechnological approaches to perform reverse genetics, genetic engineering is not a replacement for ‘conventional’ breeding methods.^(28–30) Without the ability to control sex, useful traits or mutations are not easily combined and, in a diploid organism such as a diatom, generation of knock-out mutants is very difficult (although this disadvantage could be mitigated for some purposes by the use of RNA interference), laboratory studies of inheritance cannot be performed to complete a full functional analysis of the genome, and the full potential of diatoms in biotechnology cannot be realized. According to two recent updates on algal genomics⁽³¹⁾ and transgenics,⁽³²⁾ controlling diatom sex remains a challenge that needs to be met. Here, we look at this problem anew, because we believe that there is now both sufficient theoretical background and reliable practical experience to address and solve the difficulties.

The basic plan of the diatom life cycle

Life cycle traits have been studied in detail in only a tiny minority of diatom species, but these represent many genera and almost all of the principal diatom lineages. Hence it is possible to outline a basic plan for the life cycle that is likely to be valid for the group as a whole^(15,33) and characterizes it as effectively as the siliceous cell exoskeleton.⁽³⁴⁾ There are two principal stages in the life cycle: a prolonged vegetative stage lasting months to years, which is diploid, and a short sexual phase occupying hours to several days (this also includes the development of the zygote into a new vegetative cell). While multiplying mitotically in the vegetative stage, the cells gradually reduce in size (a principle known as the MacDonald–Pfitzer rule, see Ref. 15), because of the special internalized division of diatom cells. The silica wall (frustule) of a diatom cell (Fig. 2A), which determines its shape and size, consists of two overlapping halves (termed thecae) like a Petri dish. After mitotic division, each daughter cell inherits one parental theca, which forms the ‘top’ (epitheca) of its new frustule, and manufactures a new ‘bottom’ (hypotheca) itself during the cell cycle; the key feature is that the hypothecae are initiated—and their sizes determined—while still enclosed by the epithecae that formed the parental wall. As a result, one daughter cell (that inheriting the ‘bottom’ theca of the parental cell) is smaller than the parent, whereas the other is of the same size as the parent. Consequently, with repeated cell divisions, the average size of the population decreases (Fig. 2B–D). Restoration of cell size occurs through development of a specialized cell called an auxospore (Fig. 2E), which is generally formed through sexual reproduction: during

gametogenesis, the old thecae are sloughed off and the zygote matures into an auxospore that is free to expand. After the auxospore has reached its maximum size, a new (initial) cell is formed inside the auxospore envelope, which is approximately two to three times as large (linear dimensions) as the parental cell(s) (Fig. 2F). The initial cell then begins a new round of vegetative multiplication.

Thus two important processes—sexual reproduction and cell size restitution—are uniquely linked in diatoms. Another special feature of the diatom life cycle is that only cells of a particular size range (viz. comparatively small cells) are able to become sexualized. The induction of sex is thus size dependent. One final fact that is essential for our discussion is that cells that have failed to reproduce sexually (e.g. through absence of a necessary environmental cue) will continue to divide mitotically until they reach a critical minimal size, when they die (Fig. 2D). The critical minimal size, together with the maximal size of cells capable of sexual reproduction and the maximal size of initial cells, are fairly strict, species-specific characteristics. They are referred to as the ‘cardinal points’ of the diatom life cycle.^(15,35,36)

Consequences for diatom cultivation

Diatoms, both freshwater and marine, are easily isolated from natural collections by pipette, streaking or any other standard microbiological method and many can be grown in commonly used algal culture media.^(36,37) If all the species that have been grown were counted, the list would be long and diverse. By contrast, the lists of strains published by the major culture collections contain very few diatoms. Given our brief outline of the diatom life cycle, it does not require much imagination to see how a diatom clone is likely to behave in culture. Clearly, a strain that fails to reproduce sexually will ‘disappear’ after a few months or years, once the cells have become critically small (Fig. 2). But if sex and auxospore formation do occur intraclonally, although this will secure the continuation of the culture (at least until the next round of auxosporulation) through the formation of new enlarged cells, recombination will change the genetic structure of the culture, which will not even be clonal unless cells are re-isolated. No other microalgae experience the same difficulties, because none have a similar life cycle. However, there are some diatoms that avoid getting smaller (how they achieve this is unclear, but presumably it involves unusual flexibility of the ‘girdle’ region where the epitheca and hypotheca overlap) or auxosporulate asexually. Such diatoms seem to represent a tiny minority of species, judging by observations of natural populations, but they may comprise a high proportion or even a majority of diatoms that have been held for a long time in culture collections.

Hence it is obvious that, to manage a diatom in culture, we must have information on life cycle dynamics. It would be helpful if culture collections specified the ‘cardinal points’ of the diatom cultures that they supply (to help monitor

progression of the culture through the vegetative phase), practical recommendations on how to cope with sexual reproduction and size restitution, or information that strains are in fact asexual. Our conversations with staff in various algal culture collections and examination of their databases have revealed little or no information of this kind, despite the obvious and genuine commitment of curators to providing the best service they can: it's simply that the information is not available. Because of this, the choice of diatom strains in collections remains poor. Development of cryopreservation techniques (e.g. by CCMP: <http://ccmp.bigelow.org/>) goes a long way to extend the lives of sexual clones but, in the end, it will usually be essential to rejuvenate strains via controlled sexual auxosporulation.

Current experimental models

The two diatoms selected first for complete sequencing were *Thalassiosira pseudonana* clone CCMP1335 and *Phaeodactylum tricornutum* clone CCMP632 (as subculture CCAP1055/1, established in 2003). In addition to having a small genome size, both are good 'laboratory pets', growing easily and rapidly (> 1 division per day). For this reason, these specific strains, or other strains of the same species, have been used in numerous experimental studies (as can be demonstrated via ISI Web of Knowledge). Significantly, both species can be genetically transformed and this is beginning to facilitate major advances in the understanding and manipulation of diatom physiology and silicification,^(22,38,39) which makes *Thalassiosira* and *Phaeodactylum* the preferred models for much current diatom research. Unfortunately, however, neither species exhibits the size reduction–restitution cycle that is so unique for the diatoms; indeed, this is the main reason for their ability to survive in long-term culture (both strains have been in culture for half a century, <http://genome.jgi-psf.org>). Correlated with the absence of a size reduction–restitution cycle, sex and auxosporulation have never been demonstrated in either *P. tricornutum* or *T. pseudonana* cultures, although numerous attempts have been made (including our own efforts) to find or induce them; hence, these diatoms apparently deny the possibility of manipulation through classical genetic methods. Two other pennate diatoms that have been used extensively in laboratory-based studies, *Cylindrotheca fusiformis*^(10,40) and *Navicula pelliculosa*,^(41,42) probably also lack a size reduction–restitution cycle and sexuality.⁽³⁶⁾

The marine centric diatom *Thalassiosira weissflogii* is an instructive example. This is one of the most commonly studied centric diatoms, including the use of molecular approaches.^(43,44) In 2005, Grossmann⁽³¹⁾ briefly discussed the choice of the first diatom genomic model and noted that *Thalassiosira weissflogii* would have been much more ecologically relevant than *T. pseudonana*, but, crucially for the first sequencing project, *weissflogii* “was found to have a

genome that is approximately 20 times larger than that of the latter.” But *T. weissflogii* (as clone “Actin” CCMP1336, which would probably have been selected for sequencing) would have been interesting for other reasons too, because there is good information on the size reduction–restitution cycle⁽⁴⁵⁾ and production of sperm can be reliably controlled experimentally;^(46,47) this led to the successful identification of genes that are up-regulated during spermatogenesis.^(43,48) However, even in this exceptionally well-documented case, we are still far from a full understanding of the life cycle, since production of eggs, fertilization and auxospore formation have never been documented and may not occur.^(49,50)

Two more genome-sequencing projects are currently in progress (www.jgi.doe.gov/sequencing/allinoneseqplans.php). For these, two pennate diatoms have been chosen: the cold-loving *Fragilariopsis cylindricus* and the toxic *Pseudo-nitzschia multiseriis* (Fig. 1).^(23,51) Both occur in the marine plankton but no information is yet available on the biological properties of the clones selected for sequencing. The life cycle attributes of *F. cylindricus* remain almost totally unexplored, although observations of natural populations⁽⁵²⁾ show that a size reduction–restitution cycle is present.

For *P. multiseriis*, the picture is better, since there are data on the life cycles of *P. multiseriis*⁽⁵³⁾ and some of its congeners.^(15,54,55) All *Pseudo-nitzschia* species that have been examined experimentally have undergone typical size reduction during the vegetative phase, with size restoration via sexual auxosporulation. The life of a clone could be extended considerably but not indefinitely if cryopreservation protocols were developed, but our several attempts to do this for *Pseudo-nitzschia* have failed. Furthermore, *Pseudo-nitzschia* species are difficult organisms to monitor microscopically, because cells occur in long chains and offer very few visible markers of progression through the cell cycle or sexualization. Nevertheless, *Pseudo-nitzschia* can be expected to provide valuable insights into the genetic basis of sexual differentiation and life cycle transitions, as well as the production of the toxin domoic acid. The four existing diatom genome sequencing projects will undoubtedly make fundamental contributions to our understanding of diatom biology, and they will greatly facilitate the continued development of modern molecular and genomics-based approaches for environmental research. However, available evidence suggests that additional models are required if the full potential of diatoms for fundamental biological studies and biotechnology is to be realized. To identify other potential models in which the life cycle, and especially sexual crosses, can be reliably controlled, we must first take a closer look at what is presently known about the diatom sexual phase.

Understanding diatom sexuality

Many diatoms are not difficult to grow in vitro, using defined inorganic media,⁽³⁶⁾ and it might be expected, therefore, that

sexual reproduction would often be observed in laboratory cultures. In addition, it is obvious that natural populations must regularly undergo auxosporulation or they would die out. So, why is basic information about the life cycle missing for most diatoms? The reasons are complex and diverse. One is that the size restitution phase is very short relative to the size reduction phase—a few days compared to several months or years,⁽⁵⁶⁾ so that the chances of missing sexual reproduction are high, especially if it is asynchronous in natural populations. Another is the historical accident that most of the classical life cycle studies were described in German or Russian, but neither is now the lingua franca of science. Until the last 10 years, we owed most of what we knew to just three scientists. Lothar Geitler⁽⁵⁷⁾ and Hans-Adolf von Stosch⁽⁵⁸⁾ are legendary among phycologists and also published important work in other botanical fields. Geitler's acute observations provided much of what we know about the life cycles of pennate diatoms, but because most of his work used natural populations rather than clonal cultures, he provided little information about mating systems. Von Stosch, by contrast, worked almost exclusively on cultures. His accounts are full and precise, but cover only a few species, mostly within the centric group. The third name, A.M. Roshchin, is less familiar to phycologists because most of his career was spent at a small biological station in the Crimea, ending during the difficult times of Gorbachev's 'perestroika'.⁽⁵⁹⁾ One of us (VACH) was supervised by Roshchin for a decade and this was a crucial influence and inspiration—so much so that the work survived and prospered despite the collapse of the Soviet Union: even in the most difficult times, diatoms still grew, mated and died on the window sills of the Crimean laboratory.

Despite the slow development of the subject and the historical impediments described above, we are now in a good position to develop model systems that allow conventional genetic analysis and breeding programmes. During the last decade, we have had the chance to study the sexual phase in many diatoms belonging to diverse lineages,^(15,49,55,60–63) extending the observations made by Geitler, von Stosch and Roshchin, and it is clear that the factors controlling sex differ significantly between the two major types of diatom, centrics and pennates.

Only one pattern of allogamous sexual reproduction is known so far in centrics and this is oogamy.⁽¹⁵⁾ Vegetative cells differentiate into gametangia and undergo meiosis either to produce large 'eggs' (1–2 per oogonium) or to form many small motile uniflagellate spermatozooids (several to many spermatocytes per gametangium, each spermatocyte dividing to give four sperm). Given suitable environmental conditions, gamete formation occurs spontaneously in cells that have passed a critical size threshold, the threshold often being higher for production of eggs than for sperm. Cells of the opposite sex do not have to be present for sexualization to

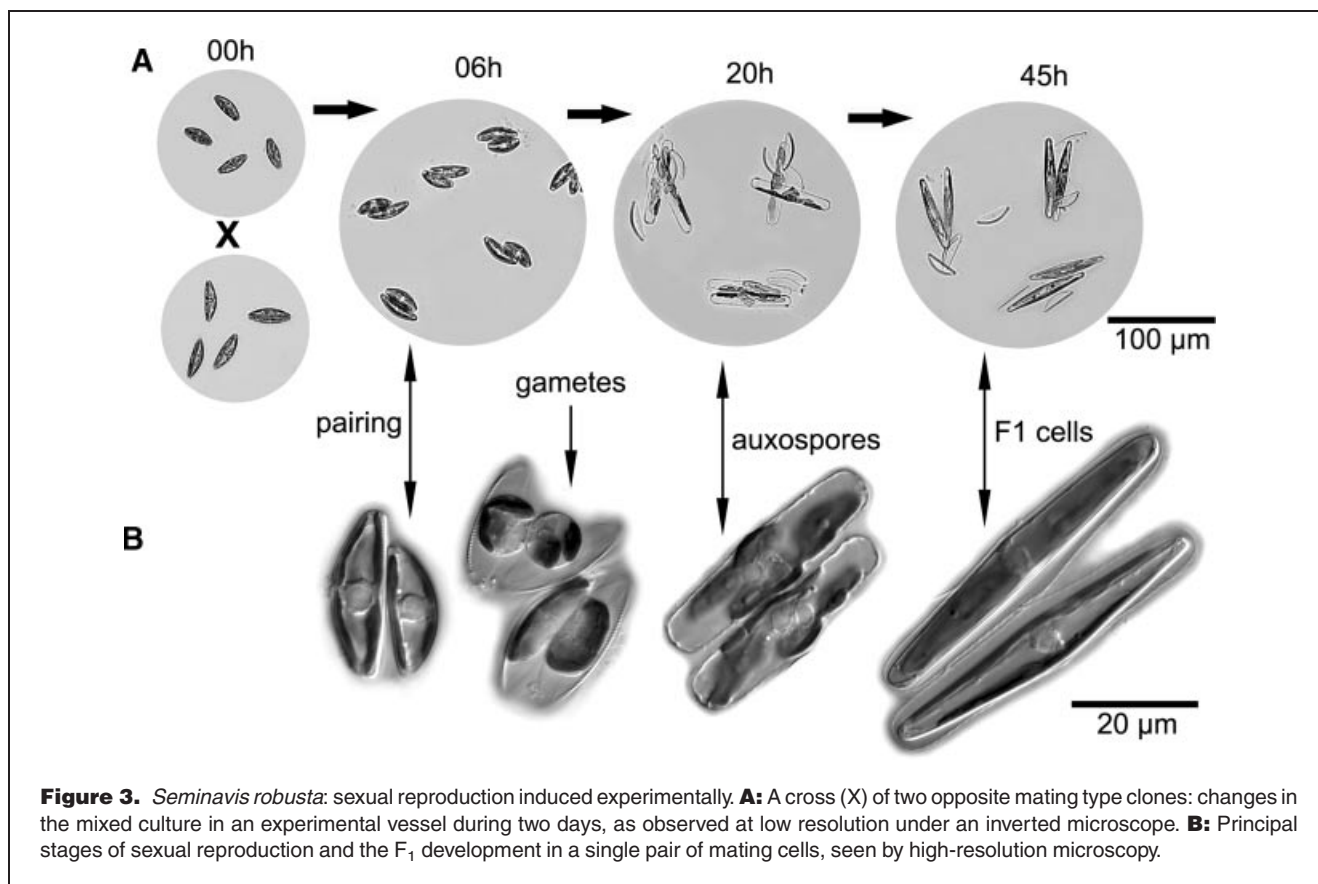
occur, although there is some evidence that gametogenesis is stimulated by the presence of cells differentiating into the opposite sex,⁽⁶⁴⁾ and cultures of small cells sometimes die through mass production of sperm but no eggs. Production of both types of gametes occurs in monoclonal cultures in a wide variety of lineages of oogamous centrics. All of these, then, are homothallic (i.e. 'monoecious'). The opposite type of mating system, heterothally (strict dioecy), has never been reported in centric diatoms.

In contrast, many pennates are heterothallic,⁽¹⁵⁾ and sex determination seems to be genetic and under simple genetic control (our unpublished data). Once within the sexually inducible size range, gametogenesis in allogamous pennates is triggered by cell–cell interaction of compatible cells. However, some pennates are homothallic, and some have decoupled meiosis from cell–cell pairing, becoming paedogamous (fusion of sibling gametes within a gametangium) or autogamous (fusion of gametic nuclei within an undivided gametangial cell).

Selecting a model: solutions

Model organisms must be easy to keep and manipulate. So, our criterion for selecting further model diatoms is experimental convenience coupled with full expression of unique diatom features. We have examined the principal life cycle attributes of tens of diatom species, using our own new isolates and traditional laboratory models including *Thalassiosira pseudonana*, *T. weissflogii*, and *Phaeodactylum tricorutum*. From these, we have selected a few for deeper evaluation and long-term maintenance and among these are several that could be valuable models.

To illustrate our strategy, we will describe the pennate diatom *Seminavis robusta*, which in many ways is one of our favourites. Several clones of this marine benthic diatom were isolated in 2000, when the principal interest was to evaluate a recent taxonomic revision of the genus and its relatives: we wanted information on the cell cycle and sexual reproduction to corroborate a classification based primarily on cell wall morphology. The clones proved to be very healthy in culture and we were soon able to report the cytological and reproductive characteristics of *Seminavis*.⁽⁶⁵⁾ The life cycle proved to be 'typical' of diatoms: there is a size reduction–restitution life cycle and restoration of size takes place via sexual auxosporulation; the potential for sexualization is, as usual, size dependent. The details are that two paired gametangia form two gametes apiece, which then fuse to produce two auxospores (Fig. 3B); this pattern is often termed the 'normal' type of reproductive behaviour among the pennates.⁽⁶⁶⁾ Sexual reproduction is isogamous. The auxospore develops through bipolar expansion, accompanied by the deposition of 'auxospore-specific' siliceous elements (the perizonium), and finally it is transformed into the enlarged cell of the next generation.



A key feature for selecting *Seminavis* as a model is that the mating system is heterothallic. This allows induction of sex to be controlled reliably, because sexual reproduction cannot begin until compatible clones are mixed. Furthermore, pairing and gametogenesis occur with very high frequency and with remarkable efficiency and synchrony (Fig. 3A); no other diatom that we have examined has approached the same success in mating and F₁ development. Furthermore, *Seminavis* cells are reasonably large (up to 80 μm long), and grow on surfaces, e.g. of Petri dishes, where various stages of the cell and life cycles can be recognized easily and monitored directly in experimental vessels even under low magnifications of an inverted or dissecting microscope (Fig. 3A). For example, the chloroplasts move within the cell in preparation for mitosis, providing an unmistakable sign that cell division is imminent.⁽⁶⁴⁾ Although cells move and grow on surfaces, they are only loosely attached and can easily be suspended if required. The life cycle is short and can be completed in a few weeks, if cells are kept growing exponentially in optimal conditions, and the cell cycle is correspondingly short (c. 0.5 days). Finally and significantly, reliable protocols of synchronization and cryopreservation are available.

Given these promising characteristics, we initiated a breeding program over several generations, creating the

first 'long-term' diatom pedigree which currently contains c. 110 clones (Fig. 4). Pedigrees are essential in animal and crop breeding. If lineages are to be selected for heritable characteristics and crosses planned rationally, the history and parentage of each organism must be known. Here again, *Seminavis* has proved to be a 'star pupil'. The high frequency of sex in culture, regardless whether cell densities are high or low, the synchrony of development, and the close association that is maintained between sibling auxospores (i.e. the two auxospores produced by a single pair of gametangia) during their development (Fig. 3A), make it easy to isolate F₁ progeny and to ensure that each isolate is derived from a different fertilization. Establishing descendant clones has become a routine procedure for us. In pennate diatoms like *Seminavis* that have the 'normal' type of sexual reproduction, the two sibling auxospores are the equivalent of 'dizygotic twins' and can potentially provide extra information about inheritance beyond what is available in organisms where haploids are able to combine at random. We have already made progress towards understanding mating type inheritance in diatoms by analyzing pedigree data.

Our controlled breeding program has also demonstrated that *Seminavis* is highly tolerant to inbreeding. Mating between sibling clones of opposite mating type is successful and yields

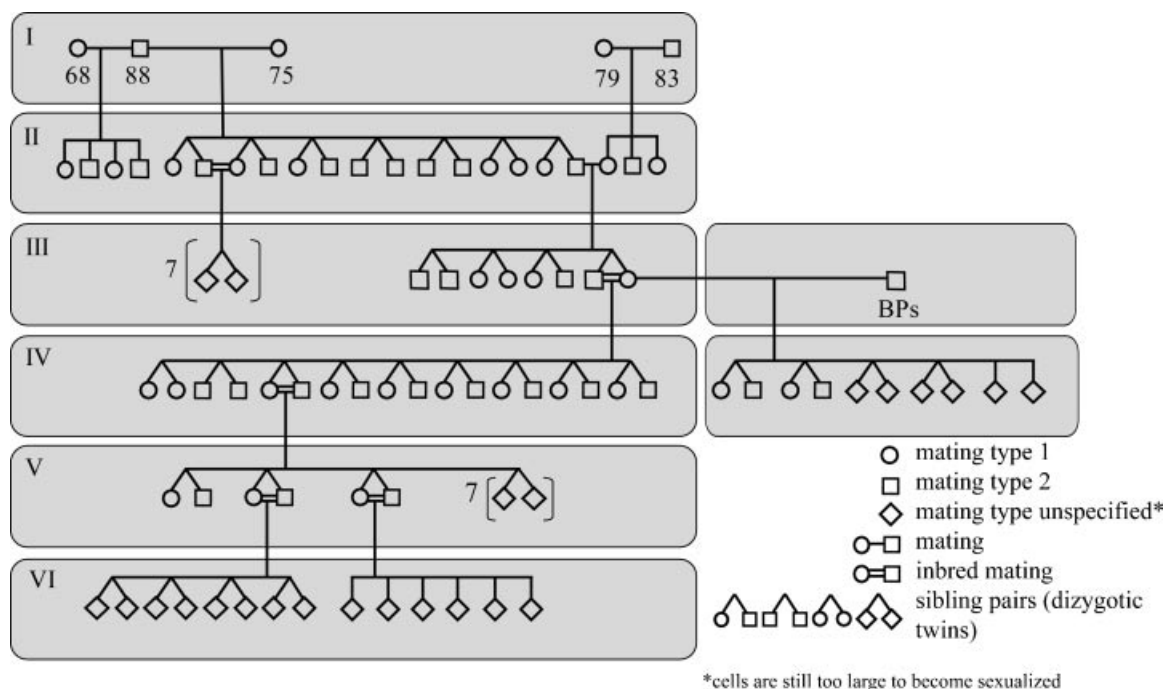


Figure 4. Pedigree of the *Seminavis robusta* laboratory lineage (interclonal crosses, heterothally) which currently involves the clones (most are cryopreserved) of six successive generations (I–VI). Clones are symbolized by diamonds, circles and squares. The natural clones which gave rise to the lineage are 68, 75, 79, 83 and 88 and were isolated from a single sample.⁽⁶⁵⁾ Clone BPs was introduced later and found at a different location. Clones obtained experimentally are linked by vertical lines with the interclonal crossing event that they have originated from.

vigorous progeny, even if the clones were themselves derived from inbred parents. This is a major advantage for long-term maintenance. By contrast, severe inbreeding depression occurs in some other diatoms, even over a few generations.^(15,49,59,67) We have also performed crosses between clones of the 'laboratory lineage' and a new 'wild' genotype of *Seminavis robusta*. These too gave viable offspring and there was also no noticeable 'outbreeding depression'. Furthermore, cultures have repeatedly proved their effectiveness in ecological experiments^(68–70) and practical training courses for students and researchers. The phylogenetic position of a model organism can also be a valuable selling point⁽¹⁹⁾ and, from this point of view, *Seminavis* also scores well, since it belongs to a species-rich and ecologically diverse group, the Naviculaceae, which is very common in both marine and freshwater habitats. Finally, *Seminavis* is an actively mobile benthic diatom, whereas the four sequenced diatoms are all planktonic. In terms of global biogeochemical cycles, planktonic diatoms are far more important than benthic diatoms, but it is in the benthos that diatoms reach the acme of species-level diversity.

One further key feature of a model organism for molecular biologists is its genome size, because this is still a crucial factor in determining whether to pursue the full transcriptomic and

genomic sequencing efforts that would be required for a model to reach its full potential in the modern age. We have determined the *Seminavis* genome size using flow cytometry⁽⁷¹⁾ by comparing its mean G1 DAPI-stained fluorescence with *Phaeodactylum tricornutum* or *Fragilariopsis cylindricus* as internal standard. Assuming that *Seminavis* is diploid, we estimate its haploid genome size to be approximately 153 Mb. This is larger than that of *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*, but considerably smaller than that of *Pseudo-nitzschia multiseries*, which is currently being sequenced. Therefore *Seminavis* also passes this key test as a potential new model diatom.

As technology further advances, sequencing costs are expected to decrease further and this will widen the choice of model systems for genomics. Therefore, it would also be useful to complement *Seminavis* with an equivalent sexual species among the centric diatoms. Here the best candidates are probably the ecologically important and widespread freshwater diatom *Cyclotella meneghiniana* or one of the sexual *Thalassiosira* species (Fig. 2) related to the genomics model *Thalassiosira pseudonana*.⁽⁷²⁾ For studying speciation, variation in morphology, and the evolution of mating systems, three well-studied and easily manipulated diatom groups are

Sellaphora (a freshwater epipelagic diatom, see Refs 60,73), *Eunotia* (a freshwater epiphytic diatom, see Refs 61,74,75) and *Achnanthes* (an attached marine diatom, see Refs 6,76,77). There is considerable information about cytology, phylogenetic relationships, mating systems and reproductive compatibility in *Sellaphora* species (<http://rbg-web2.rbge.org.uk/algae/sellaphora.htm>), and we are developing a collection of clones representing different species and provenances. A comparable effort was recently initiated for *Eunotia*. Two fairly tractable heterothallic diatoms from the large pennate genus *Nitzschia* are also interesting experimental organisms. Clones of *Nitzschia sigma* have been isolated from an extreme environment, the Aral Sea, where it is very common. This species offers the possibility of examining the genetic basis of adaptation to hypersaline conditions. *Nitzschia longissima* is valuable because its enormously long cells (c. 700 μm in some strains) can be manipulated by 'surgical intervention'. Amazingly, they survive being truncated using a razor blade,^(15,59) allowing the effects of cell size on sexualization to be studied in a particularly clear manner and allowing the life cycle to be shortened at will. This diatom could be very useful for examining how diatoms monitor their own size.

Finally, the argument that "... it is often necessary in practice to use 'exceptional' organisms whose exceptional traits make it feasible to study the properties or processes in question"⁽²⁰⁾ is certainly valid. That is why we decided to enrich our culture collection with clones of three other pennate species that exhibit highly unusual behavior during auxospore formation, viz automixis (sex within a single cell) in a *Nitzschia*⁽⁷⁸⁾ and asexual development in *Achnanthes*⁽⁷⁷⁾ and *Eunotia*.⁽¹⁵⁾

Conclusions and perspectives

All that we have discussed above would be idle chatter if there were no concrete plans to assure the secure, long-term maintenance of the model organisms that we have selected. Of course, there are already several excellent culture collections that maintain stocks of diatom species,⁽³⁷⁾ but they do not specialize on this group of organisms. Fortunately, Belgium has considerable experience in the management of microbial resources, in the form of the Belgian Coordinated Collections of Micro-organisms (The BCCM Consortium, <http://bccm.belspo.be>) funded by the Belgian government. This organization has a well-developed strategy and infrastructure for culture collections. Since 2006, the patronage of the BCCM has been extended to the collection of diatoms at Gent University, where our model systems have been developed, and funding has been made available to facilitate transition from an in-house culture collection to a research-based public service collection linked to the BCCM. This will be open for all legitimate users and will operate within a legal framework (expected in 2008).

We are developing the collection with the expectation that the experimental systems that we develop will become attractive, not only for the diatom research 'community', but also for a broad range of researchers studying more general and fundamental biological questions. For instance, in evolutionary biology, the origin and maintenance of sex remain among the biggest unsolved problems.^(79,80) As noted by Birky:⁽⁸¹⁾ "the majority of theory and observation on evolution in general, and on the evolution of sex in particular, deals with vertebrates, insects and plant. . . Nevertheless any general theory of the advantage of sex requires a broader phylogenetic perspective. Invertebrates, fungi and eukaryotic microorganisms have very different and diverse life styles, and the differences may provide insights into advantages and disadvantages of sex." We would add a more positive rider that, if the life cycle is well-understood and reliably controlled, it is much easier to work experimentally with rapidly replicating unicells than with complex multicellular organisms. Furthermore, the range of sexual mechanisms in diatoms makes them ideal for investigating the costs and benefits of different strategies. For example, diatoms show an evolutionary transition from oogamy (the twofold cost of sex is present, see Ref. 80) in centrics to isogamy (no similar cost) in pennates. This is the reverse of the evolutionary trend usually analysed by theorists and present in other eukaryotic lineages.⁽⁸²⁻⁸⁴⁾ There is an accompanying change from haploid mating (copulation between gametes) to diploid mating (copulation between sexualized vegetative cells).

The great achievements made with the previously fully sequenced diatom genomes and the fully sequenced genomes of other eukaryotes, which are appearing at an astonishing rate, have created a bioinformatics treasure trove that we can fully exploit only by utilizing model organisms selected on classical biological criteria, as we have outlined here. The development of new high-throughput sequencing technologies (e.g. 'sequencing by synthesis' or 'pyrosequencing')^(85,86) means that deep transcriptomic and/or full genomic analysis⁽⁸⁷⁻⁸⁹⁾ can rapidly be applied to new, related organisms. Furthermore, the preference for small genome size may soon be relaxed. There is a good prospect, therefore, that diatoms will continue to move from their previous status as intriguing but recalcitrant gems to being experimental work-horses for which both forward and reverse genetic methodologies are widely available. This in turn will accelerate the realization of their immense biotechnological potential.

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