

Teacher's Guide

Diatoms: Life in Glass Houses

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SET-UP: Video Projectors. Video projectors in many lecture rooms are optimized for PowerPoint display. When used with projector settings unchanged, video often appears dark and garish. Picture quality can often be greatly improved if contrast and color saturation levels are lowered while brightness is increased.

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The literature on diatoms is vast and this guide serves only as a brief introduction to the research literature relevant to those topics covered in the video. Many general textbooks on phycology cover diatom biology very well. For an advanced account, see Round et al. (1990).

Diatomite at Lompoc, California. Johns-Manville Corporation (1964) mines some of the large diatomite deposits laid down in Santa Barbara County in the middle to upper Miocene (5-25 million y.a). Many of the current coastal regions, including those now occupied by Los Angeles and San Francisco, were then under the sea. The Lompoc area was probably an archipelago or series of shallow bays and between 3-400 species of fossil diatoms have been recorded here. Rarely, fossils of sea-birds, fish, porpoises and other animals have also been uncovered in the diatomite. The deposits at Lompoc are very rare in being unusually well preserved in their original form; more commonly, such deposits are profoundly altered by geophysical factors into a variety of dense siliceous rocks.

Life within Glass Houses: The Silica Cell Wall: Diatoms are enormously successful organisms as judged by their adaptability, distribution, biomass and relative antiquity. Werner (1977) estimates that they account for up to 25% of the total oxygen production on earth. Their diversity is truly astonishing and the number of species may run to hundreds of thousands (Mann & Droop, 1996; Norton et al., 1996). Robert Andersen memorably described them as the insects of the protist world. Their biological success can be attributed in part to their unique cell walls ("*frustules*") made of silica. A silica wall confers numerous advantages while also creating significant problems. Much of the biology of diatoms devolves around these issues. Here are some of the **advantages**:

1. silica is a most abundant mineral. While an absolute requirement for growth of diatoms, it is not very soluble in water; however, enough is available as orthosilicic acid ($\text{Si}[\text{OH}]_4$) in most watery environments to sustain diatoms and the levels are replenished from sand and suspended silt. Only in the open ocean and large lakes does the availability of silica limit diatom populations (Reynolds, 1984). The total uptake of silica by diatoms from the biosphere is enormous (Reynolds 1986).

One might suppose that once secreted, the wall could go back into solution. However, living diatoms render their own cell walls extremely insoluble, presumably by an organic coating. After cell death the walls do slowly erode, as is evident in the partial etching of frustules sedimenting slowly to great depths in the ocean (Mikkelsen, 1979).

2. silica creates a physically strong and chemically inert, protective covering since frustules cannot be attacked enzymatically. Organisms feeding on diatoms generally have to crush them or evolve strategies for slipping between wall components (e.g., Beakes et al., 1992).

3. uptake of silicon by diatoms is very efficient and precipitating it to form a wall does not require a great investment of metabolic energy, less than that required to create an equivalent organic wall (Raven 1983).
4. this silica secreting machinery has evolved to be enormously flexible morphologically, as the extraordinary diversity of the thousands of species attests. Diatoms are the masters of morphogenesis at the cellular level.

Having cell walls made of silica also creates **problems**. For example:

1. silica is impermeable and walls made of silica would prevent diffusion of nutrients and waste products in and out of the cell. Diatoms solve this problem by permeating the wall with thousands of tiny pores (Chap. 3). From the point of view of diffusion, the wall may not exist as a barrier at all! It appears, however, that the tiny pores are complex and many seem covered by an extremely thin layer whose identity is unknown. It might be premature to assume that all pores are open in living cells (Mann, 1981).
2. silica is inextensible and a wall made of a single unit of silica could not stretch to accommodate cell growth, unlike the polysaccharide walls that enclose many plant cells, To get around this problem, the frustule always consists of two major segments (“*thecae*”) which tightly overlap (Chap. 3) and which slide apart as the cell grows. The major component of each of these thecae is the “*valve*”. When first secreted within the parental frustule after cell division, the new thecae are short as they consist of the valve alone. Thus, they have to grow continuously during cell expansion to maintain the overlap and hence the integrity of the wall. This growth of each theca is accomplished by the addition, one after another, of a series of silica strips called “*girdle bands*” to the edge of the valve (Chap. 18). During sexual reproduction in pennate diatoms, the enlarging cell liberated from the spore protects itself by creating a very complex covering called the “*perizonium*” which also consists of a series of modified girdle bands (Chaps. 19, 20).
3. escape of cells from the confines of the wall is necessary during sexual reproduction. As in cell division, precise control over cell turgor allows the protoplasts to push the thecae apart so as to allow escape of flagellated (Chap. 19) or amoeboid (Chap. 20) gametes.
4. the mechanism of cell growth requires the new valves to be secreted within the parental thecae. Thus, in most species, the new valves are slightly smaller than those of the parent. (For a few exceptions to this principle, see Crawford, 1981). Over many generations, this causes the average size of cells in a population to inexorably shrink. (Fig. 1 below; MacDonald 1869, Pfitzer 1869; reviewed by Rao and Desikachary 1970; Round 1972; Crawford, 1981). This trend towards a decrease in average frustule size in the population is eventually reversed by sexual reproduction (Chaps. 19, 20).

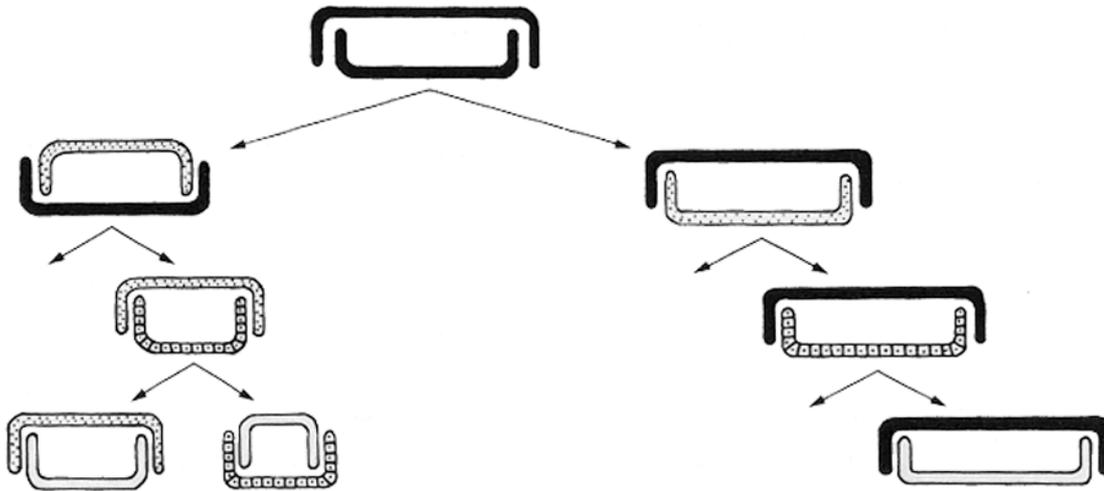


Figure 1 The MacDonal–Pfitzer hypothesis (MacDonal 1869; Pfitzer 1869). At each division, the new valve is always formed within the parental theca, causing the average size of the frustules in a population to slowly decrease.

Symmetry: Centrics and Pennates (Chap. 4) Diatoms are normally viewed from two aspects: “*valve view*”, when the valve is seen in face view; and “*girdle view*”, when the cell lies on its side and one is looking at or through the girdle bands and sides of the valves. The latter aspect is the most useful for watching the events of the life cycle, and so is the aspect most commonly presented in the video.

Many centrics are radially symmetrical although this generalization is not strictly true when smaller morphological characters are taken into account. This symmetry is most obvious in cells seen in valve view, when they appear circular. Many centric species, however, are flattened which is often confusing to students (e.g. the *Odontella/Biddulphia* and *Chaetoceros* shown in this video; incidentally, it is also confusing - and not only to students - how often diatoms are reclassified and change their names!). A further complication is that species such as *Proboscia*, and *Rhizosolenia* (Chaps. 17, 18) have large numbers of girdle bands, and these species invariably lie on their side and therefore can usually be seen only in girdle view.

Pennates have a remarkable variety of simple to very complex symmetries, and are mostly quite easy to tell apart from centrics. Basically, their symmetry is like that of a feather or fern leaf with a main axis bearing more or less parallel sides branches or ribs, whereas in centrics, the pattern is radially organized. Students are recommended to consult a good textbook to view a range of centrics and pennates.

Chloroplasts; Chloroplast Movement (Chap. 5). “*Diatoms exhibit greater variation in chloroplast morphology and arrangement than any other group of algae...*” (Mann, 1996).). One major, but not invariable, difference between pennates and centrics is the morphology of their chloroplasts (Chap. 5). Centrics usually have numerous, small chloroplasts while pennates usually have a few (1-2) large chloroplasts which in many cells, adopt a specific position in the cell dependent on the stage in the division cycle. A few pennates (e.g. the *Ardissonia* shown in Chap. 9,12) have numerous small discoidal chloroplasts like those in centrics (but interestingly, this species may in fact turn out to be a centric- see “Movement”, Chap. 9 below.). Numerous pennates such as *Surirella* appear to have two chloroplasts which in reality are composed of two large lobes connected by a thin isthmus that is broken during cytokinesis. This morphology is also encountered in related flagellates like the golden brown Ochrophyte (Chrysophyte) *Mallomonas*. For a discussion of chloroplasts, see Round et al. (1990).

The larger chloroplasts of pennates become rearranged in the cell before and then just after division; in *Pinnularia*, for example, the two chloroplasts always lie against the girdle bands during interphase, and then rotate through 90°, moving to cover the valves, prior to and during mitosis. These reorganizational events are much more striking in some diatoms than others (Chap. 14, 15) but are a general feature of many pennates. They are intimately related to the overall symmetry of the cell (Mann, 1983, 1984a) and form part of the continuum of events that places the nucleus against the girdle bands for mitosis

Mann (1996) distinguishes two types of chloroplast division: “*autonomous*” and “*imposed*”. The small chloroplasts typical of centrics divide autonomously, i.e., without the intervention of other cytoplasmic agents such as the cleavage furrow which effect the imposed division of chloroplasts of larger pennate diatoms shown in the video. Autonomous division is prolonged, while imposed division is as rapid as cleavage.

Chloroplast movements (Chap. 11) are a common attribute of many algae and some higher plants where they are usually associated with the actin cytoskeleton. Dispersal of chloroplasts has obvious advantages in maximizing the cell’s ability to harvest light energy (Haupt, 1983), although why they should gather in the center of the cell during the dark is less obvious. The clustering of chloroplasts around the nucleus when strongly illuminated (Chap. 11) is probably a protective response, minimizing the possibility of radiation damage to the photosynthetic pigments and perhaps the nucleus. Clustering is interesting in that each chloroplast responds individually, presumably hooking up to a filamentous traction fibre.

Vacuoles; Streaming (Chap. 6). Large centrics and pennates have one or more vacuoles which can, in cells like *Coscinodiscus*, occupy most of the cell volume. The plasmolysis experiment shown in Chapter 6 (to be described in Pollock and Pickett-Heaps, in preparation) explored the possibility that the spatial precision of cytoplasmic events and wall morphogenesis is dependent upon architectural cues supplied by the wall. After plasmolysis, the highly contracted, spherical protoplasts are completely detached from the frustule. The protoplasts initiate recovery by extending active filopodia which seek out and attach to the corners of the frustule (Chap. 6) and after some hours, recovery is complete and the cells continue with division. These filopodia are created by the cells’ microtubule cytoskeleton. Functionally, they may be similar to the microtubule-based cytoplasmic strands that generate major cytoplasmic reorganizations of the nucleus and chloroplast in cells like *Surirella* and *Cymatopleura* (Chap. 15). In both cases, the strands generate tension that is precisely controlled; how and what exactly the strands attach to (presumably the wall) remains enigmatic. Cells plasmolysed during valve secretion ignore partly formed valves and restart morphogenesis of a new valve. This discriminative ability is important since partly-formed valves are structurally free in the cell and useless as spatial cues.

The Microtubule Center (Centrosome); Robert Lauterborn (Chap. 7). Lauterborn’s treatise on diatoms published in 1896 is a remarkable piece of work, well worth tracking down although it is quite rare. His work is an object lesson in what can be achieved with patience and acute powers of observation. For example, he clearly illustrates golgi bodies and microtubules (the latter organelles being technically below the resolution of light microscopy); he illustrated the latter emanating from the conspicuous granule we now call the Microtubule Center. His discussion on the roles of organelles, particularly during mitosis, is remarkable modern.

The Microtubule Center is the focus of the cell’s microtubule cytoskeleton. It is therefore a major focal point for the morphogenetic activities orchestrated by these microtubules. Particularly striking in this context are pre- and post nuclear movement,

valve morphogenesis (Chap. 14), chloroplast reorganization (Chap. 15) and recovery from plasmolysis (Chap. 6).

Motility: Centric Diatoms (Chap. 9). Centric diatoms are normally considered to be immobile. However, a weak form of movement has been reported in some species (Medlin et al., 1986; Pickett-Heaps et al., 1986), apparently related to the secretion of mucilage through the LP. This minor phenomenon becomes important in the context of the proposed evolution of the raphe (next para.) from the labiate process (Hasle, 1974) – a proposition strongly supported if both these features of the valve have the same basic function, the secretion of mucilage, and both are involved in generating movement.

Motility: Pennate Diatoms (Chap. 9). Diatom motility was observed by many early microscopists, including Lauterborn and with the exception below, is confined to those pennates which possess the “*raphe*” a long slit through the wall. The mechanism of motility has generated much speculation and now a good general understanding seems to have been reached (reviewed by Edgar & Pickett-Heaps, 1984). Secretion of mucilage (a proteoglycan: Lind et al., 1997) through the raphe fissure is clearly involved (see video). Actin filaments, usually in two bundles, run the length of the fissure in the cytoplasm immediately adjacent to the cell membrane; there are also numerous mucilage vesicles here. Edgar & Pickett-Heaps propose that mucilage vesicles discharge into the raphe fissure; their contents swell by water uptake while they remain attached to the vesicle membrane, now incorporated into the cell membrane. The attachment region is moved back and forth by interaction with the actin filaments; presumably the bundles have opposite molecular polarity and generate movement in opposite directions. The mucilage emerging through the raphe attaches to particles or substrata. As diatoms move, they leave a trail of this sticky mucilage (Lind et al., 1997; Higgins et al., 2000). Schmid (1997) proposes the alternative that microtubules and MT-based motility systems are involved, but this proposal has not been substantiated by recent work (Wetherbee et al., 1998) which confirms that movement arises from an actin-myosin system, (Poulsen et al., 1999).

While motility is generally said to be confined to those diatoms that possess the raphe, the araphid *Ardissonea* (Pickett-Heaps et al., 1991) is an interesting exception. Jerky, irregular movement typical of raphid diatoms is generated by the active secretion of mucilage through one or two specialised pores at the end of the cell. The mucilage swells by uptake of water and pushes the cell along. This form of motility is quite common elsewhere, for example, in desmids. Recent observations by Kooistra et al., (2003) reveal similar motility in the closely related *Toxarium*; more remarkably, these authors have evidence that both genera are in fact centrics - in spite of their elongated, pennate-like morphology.

This limited ability of diatoms to move can be very important. For example, during sexual reproduction, sexually competent cells have to find each other and then position themselves appropriately (Chap. 20). Some marine benthic species retreat into the protective damp sand or mud when the tide goes out, and emerge again during daylight hours and/or when flooded by the incoming tide (e.g., Jonsson et al., 1994). Other species do the opposite (next para.), retreating into the sand when the tide comes in, to avoid being washed away (Palmer & Round, 1967). The mucilage secreted by motile and attached (Chaps. 9,12) diatoms contributes significantly to the stabilization of sediments (e.g., Sutherland et al., 1998; Underwood & Paterson, 2003).

Phototaxis (Chap. 10). Phototaxis illustrates another way diatom motility is important. The ability to respond to light is obviously critical in allowing diatoms to optimize exposure of their photosynthetic system (including an avoidance response in those

situations where light is intense enough to damage their pigments and/or chloroplast). The photosensitive region seems to be each tip of the cell (Cohn et al., 1999). For an introduction to the complexities of diatom phototaxis, see Cohn et al. (1999) and Cohn & Weitzell (1996)

Phototaxis can be integrated into complex diurnal rhythms set up in ecosystems subjected to both light/darkness and tidal cycles (see previous Section). In intertidal mud and sand flats, Palmer & Round (1967) found that *Hantzschia virgata* emerges from the sand in a diurnal rhythm that can be maintained for up to 11 days in a constant illumination and temperature regime. Moreover, this rhythm is not only linked to tidal activity, being retarded by 40 mins. each day, but also the “clock” responsible for this fine tuning is reset by 12 hours as the emergent phase (low tide) approaches evening at which point the cells switch to an early morning emergence. Thus, phototaxis in these cells appears to be controlled by “an interacting dual-clock system”.

Secretion of Holdfasts and Adhesives. (Chap. 12). Mucilage secretion provides an aid to flotation if mucilage can entrap bubbles of photosynthetically derived oxygen (McConville and Wetherbee, 1983). Secretion of mucilaginous material is integral to both motility (Chap. 9) and attachment of epiphytes to their substrate via pads or stalks secreted at special pores (Chap. 12). A few raphid diatoms (e.g. the monoraphid *Cocconeis*) adhere tightly along their raphe. Thus, the opposite abilities of adhesion and motility involve variations on the same cellular process and, for example, stalk secretion is obviously similar to the mucilage secretion illustrated for *Ardissonea*. The strange behaviour of *Achnanthes* recorded in the video hints at the complexity of behavioral patterns (motility contrasted with attachment) that may accompany these apparently simple events.

Certain diatoms that attach firmly to substrata are a major component of the biofilms that build up on exposed marine surfaces, and thereby contribute to biofouling, a \$billion-a-year problem for ship-owners and navies the world over. Diatoms, along with bacterial flora, are amongst the earliest colonizers of clean surfaces placed into water, and even a newly forming, thin biofilm can significantly affect the drag, and thus the speed of a vessel.

Labiata Processes (“Rimoportulae”). The *Labiata Process* (LP) or “rimoportula” is another of the different types of pores through in the wall. LPs are usually simple slits in the wall, distinguished by the two internal lips (“*labia*”) on each side of the slit. They are always situated in a position(s) characteristic of the species. In a few genera such as *Ditylum* and *Odontella*, a long hollow spine extends externally from the external slit. This extension is, rather confusingly, also called the LP. Morphogenesis of such tubular processes is shown in Chap. 16. LPs are found in centrics and a few araphid pennates where they have been called “jelly pores”. As this name suggest, they have been implicated in mucilage secretion (Pickett-Heaps et al., 1986; Medlin et al. 1986).

Colony Formation. Diatoms form colonies in various ways (Round et al., 1990). *Bacillaria paradoxa* and the various species of tube dwelling diatoms (Chap. 9) provide two interesting examples. Tube diatoms are quite common marine organisms, often mistaken for filamentous brown algae until examined under the microscope. The reasons they form such assemblages is not known. Their biology has been reviewed by Lobban (1989; see also www.uog.edu/classes/botany/Mar_Bot/tube-dwellers.htm).

In *Bacillaria paradoxa* (now *B. paxillifer*), cells are held together along their raphe, but in such a manner that they are still able to undergo their extraordinary coordinated sliding motility (see Kapinga & Gordon, 1992). In a few colonial genera (e.g. some *Chaetoceros*: Chap.16), parts of the valve are physically fused together during

valve deposition. Other colonial species employ strings of chitin fibrils (*Thalassiosira*: Chap. 13) or pads of mucilage, similar to those used for adhesion to substrates (Chap. 12).

Planktonic Diatoms: Effects of Size, Shape and Colony Formation. Planktonic diatoms exhibit a great range of morphologies in the overall shape of the cell, (spherical/tubular etc.), colony formation and spine formation. These morphologies - particularly spines - can significantly increase their resistance to grazing pressure, slow their sedimentation and potentially increase their access to nutrients. For an introduction into literature on the latter two aspects, see Pahlow et al. (1997), Karp-Boss and Jumars (1998) and Huisman et al. (2002).

Chitin Secretion; Strutted Processes (“Fultoportulae”) (Chap. 13). Some centric diatoms secrete chitin, crystalline fibrils of *N*-acetylglucosamine, (Herth, 1979; Herth & Barthlott, 1979), through special pores called “*Strutted Processes*” or “*fultoportulae*” whose disposition is characteristic of the species. The fibrils are flexible and very strong and probably serve to make the cells difficult to eat; they also may aid flotation (e.g., by trapping bubbles produced by wave action or photosynthesis) and delay sedimentation. When Walsby & Xypolyta (1977) removed the fibrils from *Thalassiosira* with chitinase, the cells’ sedimentation rate nearly doubled. Similar chitin fibrils are characteristic of other algae, for example, planktonic green algae such as *Scenedesmus* and *Micractinium*. Chitin is common in the walls of other algae, many fungi and some lower animals (Herth & Schnepf, 1982).

Mitosis and Cleavage (Chap. 14). Diatoms provide superb material for observing mitosis live and Lauterborn (1896) was one of the earliest microscopists to view mitosis and chromosome movement in living cells. We (Pickett-Heaps et al., 1984) have published a partial translation of his chapter on mitosis, including reproductions of some of his figures and we deplore the way this major contribution has been generally ignored in the literature. Using modern electron microscopy, diatoms have allowed analysis of spindle structure and correlation of structure with function at a level not possible with most other cells (reviewed in Pickett-Heaps, 1991). For example, diatoms first demonstrated unequivocally that the mitotic spindle consists of two interdigitated half spindles that slide apart to achieve spindle elongation during late mitosis. Contrary to the consensus prevailing at the time (i.e., that microtubules are nucleated by and grow from kinetochores), diatoms demonstrated that kinetochores attach to microtubules derived from the pole, initially by sliding along them. Another video from Cytographics (“*The Dynamics and Mechanisms of Mitosis*”) gives a more detailed analysis of diatom mitosis.

Valve Morphogenesis (Chap. 15). The means by which diatom cells creates their marvelously sculpted valves after division is extraordinarily complex and largely mysterious. As mentioned in the video, most of what happens is below the resolution of the light microscope and cannot be studied in living cells - a serious drawback with a process that requires so many dynamic events. However, some events which can be followed are shown in Chaps. 15 & 16. Ultrastructural work on valve morphogenesis is extensive and reviewed in Pickett-Heaps et al., (1990). Here are some useful generalizations:

1. valves and girdle bands are invariably secreted within a closed membrane, the Silica Deposition Vesicle (SDV; Darley, 1974). Valve morphogenesis requires the expansion and delicate moulding of this flexible membrane over a quite extended period while silica is secreted within it (Chap. 15).
2. moulding of the SDV is accomplished with the involvement of an extraordinary range of both organelles (MTs, actin, vesicles, etc.) and dynamic cellular

activities (plasmolysis, adhesions, etc.) in different combinations for different species (Pickett-Heaps et al., 1990). This morphogenetic virtuosity is unmatched in any other group of living organisms.

3. in centrics, silicification starts at a specific region, the Primary Silification Site, usually in the center of the valve face (Schmid et al., 1981). This is often the place the MC moves to after cleavage.

4. in raphid diatoms, silicification starts where the raphe is initiated, and the MC adopts a characteristic position on the SDV from the earliest stages; some MTs from it are invariably (so far) found running along the raphe fissure.

5. localised inhibition of silica secretion, e.g., forming pores, is often associated with dense amorphous material of unknown constitution, localized over these sites on the SDV.

6. during morphogenesis of LPs, a striated “*Labiate Process Apparatus*” (LPA: Li and Volcani, 1985a, b) is invariably situated on the SDV precisely where the lips grow. Its role is unknown but it is present whether or not the LP extends an external spine, so it probably plays no part in spine extension.

7. ill-defined, often fibrous (actin?) bands are also associated with the SDV, particularly lining its growing edge; this material may be actively involved in the moulding process and extension of the SDV.

Morphogenesis of Spines; Turgor Pressure (Chap. 16). During valve morphogenesis, the ability of diatoms to create extremely fine, hollow spines of silica exemplifies their superb ability to manipulate silica. This ability raises two immediate and important questions. First: **how is morphogenesis so perfectly controlled?**

The spines are usually delicately tapered, perfectly straight or elegantly curved and with a perfectly even bore. Morphogenesis of the relatively thick spine of *Proboscia* is easiest to understand, and clearly involves both microtubules which maintain its overall straightness, and a collar of actin which generates its conical shape (van der Meene & Pickett-Heaps, 2002). Moreover, growth of the valve is only in one direction, from the tip of the spine back over the body of the cell. In contrast, growing LPs examined under the electron microscope (e.g., Pickett-Heaps et al., 1988) give no indication as to how the SDV and its spine are molded with such precision. In the different spines (“setae”) of *Chaetoceros*, the same question arises, complicated now by the way spine extension can be directionally affected by the geometry of the colony. In both examples, growth of the valve is bi-directional: at the tip of the spine (tip growth) and back over the cell. *Chaetoceros* offers a tantalizing glimpse of a possible model: a fibrous sleeve, probably of actin, lines the outgrowing tip of the SDV and this might act as a self-propelling mold whose shape is controlled by the silica tube just secreted (Pickett-Heaps et al., 1994; Pickett-Heaps, 1998). The function and significance of the extraordinary rotation characteristic of LP extension in *Rhizosolenia* remain to be discovered.

The second immediate question is: **what drives extension of the spine?** Turgor pressure is normally thought to provide the force for driving growth of cells enclosed in extensible walls, and this mechanism forces the rigid thecae apart during normal cell growth (Chaps. 17, 18) and, notably, release of sperm cells (Chap. 19). *A priori*, one might expect turgor to drive spine extension too; however, for the reasons evident in the video, this mechanism cannot apply here. There is also a sound theoretical reason this mechanism cannot apply: the force required to drive elongation of an extensible tube is inversely proportional to its diameter (Pickett-Heaps & Klein, 1998) and for very small tubes such as these spines, the pressure necessary would be enormous. Some other mechanism must be at work, and perhaps extension has more in common with growth of filopodia (Pickett-Heaps, 1998).

Girdle Bands (Chap. 18). As shown in the video, secretion of a series of girdle bands attached to the rim of the valve is essential to allow growth of the cell after division. This is one of the major structural innovations that allowed diatoms to make their wall from a material as rigid as silica. Interestingly, most girdle bands are not continuous strips of silica encircling the cell circumference (however, continuous bands are not uncommon). In most cells, each band is a flat strip of silica curved to follow the contours of the cell and therefore there is a slight gap where the ends of the strip come close to each other (a morphology often referred to as a “*split ring*”). The reason this structural discontinuity exists is not known and may be related to the evolutionary origin of this type of wall construction. Cells arrange the girdle bands so that the gaps lie alternately on different sides of the cell. This arrangement ensures that each gap is overlain by the adjacent girdle band (von Stosch, 1975) and thus, the cytoplasm is completely protected at all times. Careful examination of some of the scanning micrographs in the video will show these gaps in the bands.

Sexual Reproduction (Chaps. 19, 20). Diatoms display remarkable variety in the details of their sexual reproduction and readers should refer to the considerable literature on this subject, well summarized in Round et al. (1990). The primary requirement for stimulating cells into sexual reproduction (Geitler, 1932; Drebes, 1977) is diminution of cell size generated by the MacDonald–Pfitzer mechanism (an example of this variation in size is evident in the *Achnanthes* shown in Chapter 12). Since diminution takes a long time (probably thousands of generations), Lewis (1984) suggests that this process provides a “sex-clock” for inducing sex at long intervals, and one not dependent upon environmental cues. He concludes that it is also adaptive since the basic division mechanism could easily have evolved so that there is no such shrinkage - as indeed is the case in a few species whose girdle bands are flexible enough to allow formation of uniformly sized valves

All diatoms are diploid, rather unusual in simple algae, and therefore two meiotic divisions take place during differentiation of gametes

Sexual Reproduction in Centric Diatoms (Chap. 19).

The mode of sexual reproduction represents a major difference between centrics and pennates. Reproduction in centrics is oogamous and utilizes a sperm cell with a single, forwards-directed flagellum. In differentiating female cells, each meiotic division is followed by the degeneration of one nucleus, so that the egg cell has only one haploid nucleus left when ready for fertilization. Vegetative cells never display flagella and these arise *de novo* in spermatogenous mother cells (Chap. 19). Manton et al. (1970) showed that two centrioles arise at the poles of the spindle in meiosis I; during meiosis II, these separate so that there is only one at each pole of the second meiotic spindle. In many centrics, the basal bodies have already sprouted an active flagellum before meiosis II starts. The flagellum characteristically is “9+0” (i.e., it does not have the central pair of MTs found in other eucaryotic flagella: Manton & von Stosch, 1966; Heath & Darley, 1972).

Fertilization has not been directly observed in the *Ditylum* shown in this video, so it is possible that the subsequent events might be an asexual variant of normal sexual reproduction (see next Chapter). However, the events are representative of other centric diatoms, although there are numerous minor variations in detail. Once fertilized, the diploid protoplast (now usually called an “*auxospore*”) emerges from the frustule if it has not already done so, rounds up, enlarges and secretes a complex wall containing organic material and silica in the form of scales or, in certain species, modified girdle bands (see von Stosch, 1982). (**Erratum:** David Mann has pointed out that the term “perizonium” used in the commentary, is incorrect in that it normally refers only to this wall in *pennate*

diatoms.). The discovery of scales in these walls may be very important because it suggests how the silica wall itself might have originally evolved (Round & Crawford, 1981). Then the cell secretes two new valves inside the wall. A long-standing and well supported hypothesis (Geitler, 1932, 1953a, b; von Stosch and Kowallik, 1969; Drebes, 1972) states that diatom valve formation cannot occur without a preceding mitotic division. Accordingly, while in the auxospore wall, the auxospore undergoes two successive mitoses without cytokinesis. Each is followed by formation of one valve, while one of the two nuclei degenerates. After two such cycles (and therefore with two new valves), the greatly enlarged cell now displays its basic vegetative morphology and it soon divides to establish a new clone. (However, nothing is simple; these “*initial*” valves are in fact slightly different to vegetative valves.)

The interesting appearance of *Ditylum* cells with tri-, tetra- and even pentaradiate symmetry after germination of the auxospore is reminiscent of the changes in symmetry that can be found in desmids (Pickett-Heaps, 1975). This phenomenon has been reported for other centrics too (von Stosch, 1977).

Sexual Reproduction in Pennate Diatoms (Chap. 20). Sexual reproduction is quite different to centrics; with very rare exceptions, gametes are amoeboid and the loss of a flagellated stage is intriguing. Similar reproduction by amoeboid gametes is characteristic of several groups of organisms, notably the Zygnematales in the green algae. One provocative possibility is that this mode of reproduction and loss of flagella evolved initially as an adaptation to an increasingly terrestrial life style, followed by a return to a watery environment (Stebbins & Hill, 1980; Nakahara & Ichimura, 1992).

Sexual reproduction is relatively rare. An excellent early account of conjugation in the genus shown in the video, *Navicula*, is given by Subrahmanyam (1946). (This *Navicula*, and the one shown in the video, is more correctly known now as *Craticula*.) Conjugation starts by pairing or clumping of cells which concurrently invest themselves with copious envelopes of mucilage, presumably a protection for the naked, emerging protoplasts later. Two meiotic divisions are typically followed by one cytokinesis (as shown in the video) and degeneration of one nucleus in each cell. The valves are then separated by swelling of the protoplasts, now highly vacuolate gametes, and these move toward each other; typically, each gamete fuses with one from the adjacent mother cell and thus avoids self-fertilization. Each new zygote contracts and then secretes a polysaccharide wall. This auxospore (zygote) then germinates by splitting the auxospore wall and it now secretes a new type of wall, the *perizonium*, a most complex structure comprised of overlapping siliceous segments (modified girdle bands), and capped at each end by the halves of the split auxospore wall. For a detailed description see Mann (1982, 1984b). Eventually, the two nuclei fuse and the diploid nucleus undergoes two mitoses without cleavage. After each of these mitoses, one nucleus degenerates while the cell secretes one new initial valve. Finally the new cell, much larger than the cells from which it arose, emerges from the perizonium to start a new clone.

Mann and colleagues (Chepurnov et al., 2003) have documented a modified asexual variant in behaviour in which single small cells of *Achnanthes* give rise to auxospores directly; these germinate into new clones without meiosis. Such modified sexual cycles provide reproductive flexibility by short circuiting sexual reproduction and they may be more common than expected in diatoms. Similar asexual variations on the sexual cycle are known elsewhere; one example is the green alga *Hydrodictyon* (Pickett-Heaps, 1975).

Resting Spores. Sexual stages in diatoms are not normally associated with dormancy ((Edlund & Stoermer, 1997). Many diatoms can form thick-walled, characteristically ornamented asexual resting spores for outlasting periods of environmental stress (see

Round et al., 1990; McQuoid & Hobson, 1996). These stages are rarely encountered, and since I have so far been unable to record their formation on video, I will not comment further.

Acknowledgement. I am very grateful to Dr. David Mann who read this document carefully, corrected errors and made many valuable suggestions.

Updates: I appreciate feedback and corrections which I will incorporate into this document as they are sent to me. **Current Update: Dec., 2003**

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