

4.3 Cell division and auxospore formation

Pfitzer (1871), Lauterborn (1896), Ott (1900) and, more recently, Geitler (1975a) have all investigated various aspects of cell division in Nitzschia. Of these accounts, Geitler's, which largely supercedes those of Pfitzer and Ott, deals with chromatophore division and cytokinesis, while Lauterborn's describes in detail the division of the nucleus. In addition, a paper by Lauritis et al. (1968) outlines the course of cell division and wall deposition, which they followed using ultrathin-section techniques.

Lauterborn illustrated, in a series of excellent lithographs, the stages of mitosis in N. sigmoidea. During prophase the chromosomes condense and then migrate, arranging themselves in two groups about the equator of the spindle, one group lying towards one pole of the diatom, the other lying opposite, towards the other pole. At the same time the nucleoli, of which there are several per nucleus, disappear. The nuclear membrane remains substantially intact throughout most of mitosis.

The chromosomes are fairly closely packed together at metaphase (as is the case in other diatoms; see Lauterborn 1896), but Lauterborn was able to count 16 chromosomes (i.e. $n = 8$, since diatoms are diploid: Drebes 1977).

The spindle is at first somewhat like a truncated ellipsoid, but later, as mitosis proceeds through anaphase and telophase, it elongates and becomes * cylindrical. Lauterborn's illustrations suggest that the nuclear membrane breaks down in telophase, at about the same time as cytokinesis occurs. Subsequently, the daughter nuclei form, the chromosomes uncoil and the nucleoli reappear. The young daughter nuclei are flatly conical in shape (a feature which I have observed in N. linearis also) but later assume the elongated, ellipsoidal shape

characteristic of the interphase nucleus.

Geitler (1975a) noted that in N. palea cytokinesis begins at metaphase and takes about 4 $\frac{1}{2}$ -5 min. The invaginations of the plasma-lemma extend down from the poles and, as they near the polar margins (Geitler's 'distale Ende') of the chromatophores, these too become invaginated. At this stage the inner edge of each chromatophore (i.e. the edge nearest the nucleus) is intact, with no invagination, and it remains so until the furrow extending centripetally from the pole reaches the centre of the plastid; then the inner edge (Geitler's 'proximale Ende') also invaginates. The two furrows extend towards each other at the same speed until the division of the chromatophore is complete. Cytokinesis is completed shortly afterwards. In general, the two chromatophores in a single cell divide synchronously, but sometimes one chromatophore is slightly ahead of the other. The fate of the pyrenoids during division is not known.

In N. palea it appears that the nuclear membrane breaks down during mitosis - 'Im Leben lässt sich der Eintritt in die Mitose daran erkennen, dass der Nukleolus verschwindet und die Kernhülle bzw. die optische Abgrenzung des Kerns unsichtbar wird' (Geitler 1975a).

The recently divided Nitzschia cell has a very distinctive appearance, which has been illustrated by Ott (1900, T.3 f.7, 8) and Geitler (op. cit., Abb.1d) for N. gracilis and N. palea respectively, and which has been observed in many taxa (e.g. N. sigmoidea, N. linearis, various species of the sect. Lanceolatae, etc.: F.277, 492, 501) during the present study. The inner edges of the four daughter plastids form a diamond (as seen in girdle view) which encloses the two conical daughter nuclei. Often, the polar margins also are oblique to the valvar plane, and it would appear from Geitler's study that this arrangement is the direct result of the type of chromatophore division exhibited in these species.

Cytokinesis is followed by the deposition of silica within an intracellular sac bounded by a single unit membrane, the silicalemma, to form the new cell wall (Lauritis et al. 1968). Deposition of the various elements of the frustule does not take place synchronously: in N. alba, for instance, 'the initial silicon deposition vesicle is formed in the central region of the dividing cell' (ibid.).

The study made by Lauritis et al. was accomplished through the preparation and examination of ultrathin sections. This is not, however, the only way by which the course of silica deposition may be followed. Natural populations of diatoms usually contain a proportion of dividing cells, and so, if such a population is fixed and then treated with acids to remove organic matter, it is often possible to trace a series of valves representing the various stages in silica deposition. Observations of this kind have been made on Hantzschia marina, Nitzschia sublinearis, N. sinuata, N. (sect. Fragilariopsis) sp. and Denticula tenuis. In all these the raphe-sternum is one of the first parts to be deposited (F.600-1; 890; 796; 984; 1021, respectively); Lauritis et al. claimed that in N. alba the raphe-sternum is deposited last, but this has been refuted by Chiappino et al., who find it to be deposited first! The transapical costae are deposited as extensions from the raphe-sternum and then the frets are laid down so as to delimit the poroids. In D. tenuis (and probably also in N. sinuata and N. sublinearis) the frets are visible first as expansions of the transapical costae (F.1021), each 'bump' being opposite a 'bump' on the adjacent costa; these expansions grow towards one another and fuse (F.1026-7). The frets are formed sequentially, those nearer the raphe being laid down first (F.1021, 1027). In H. marina and N. (Fragilariopsis) sp. the delimitation of the poroids takes place via a very similar process, even though these species have a different type of valve structure from that of D. tenuis: again, outgrowths of silica

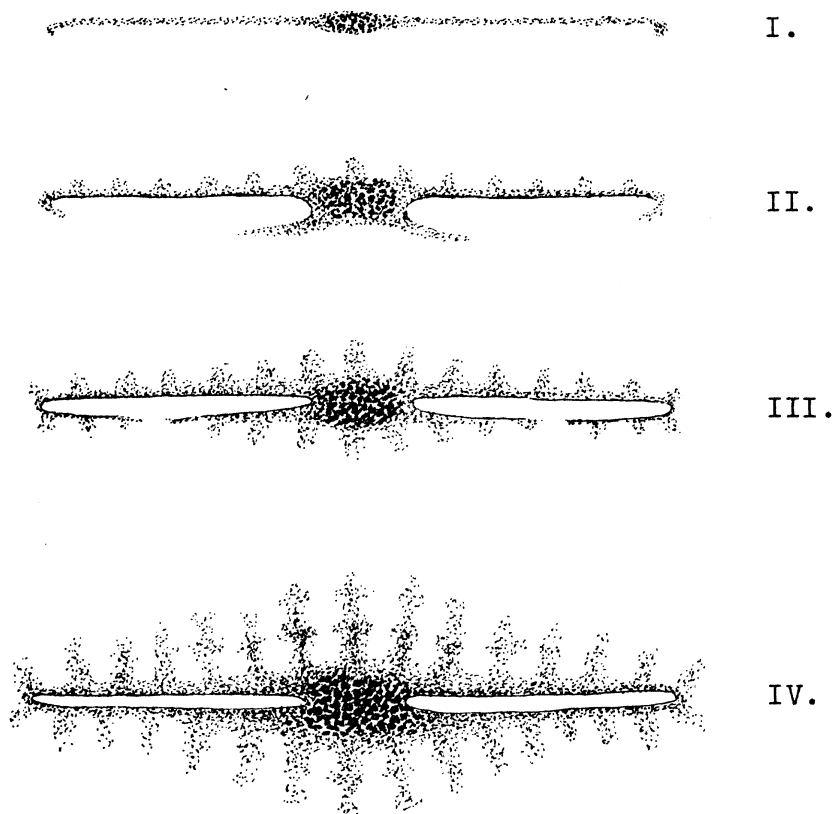
extend out from the transapical costae (the 'hoops' in H. marina) towards one another, but before they fuse each one widens markedly at its tip, so that not one but two rows of poroids are delimited (F.600, 601; 984).

The valve margins and fibulae do not appear until late in the deposition sequence, and the latter seem to be the last elements of the valve to be laid down. In N. sinuata and D. tenuis it has been possible to follow the development of the fibulae; here, the first parts to appear are the two longitudinal ridges which run parallel to the raphe, separating (in the mature valve) the subraphe canal from the remainder of the valve interior (F.1027). Then a series of small bridges are formed (F.796; 1026), which resemble the mature fibulae of H. virgata vars. intermedia and gracilis (q.v.). Indeed, at this stage D. tenuis could easily be mistaken for a species of Nitzschia, since the 'septa' characteristic of the fully formed Denticula valve have not yet appeared. Subsequently, the outer edges of the fibulae (i.e. those nearest the raphe) extend out towards one another and the longitudinal ridges become more pronounced, so that the ovoid portulae are delimited (F.797). At the same time more silica is laid down on the inner side of each bridge, and thus the fibulae gradually extend across the valve (which process is completed in D. tenuis, but not in N. sinuata) and thicken, until they assume their final form. During the deposition of the fibulae, the silicification of the costae, frets, etc. is completed.

The hymena appear to be laid down very soon after the delimitation of the poroids, before the silicification of the frets and costae is complete. Thus, the area of unoccluded hymen per valve is greater during deposition than it is finally (unpubl. obs.).

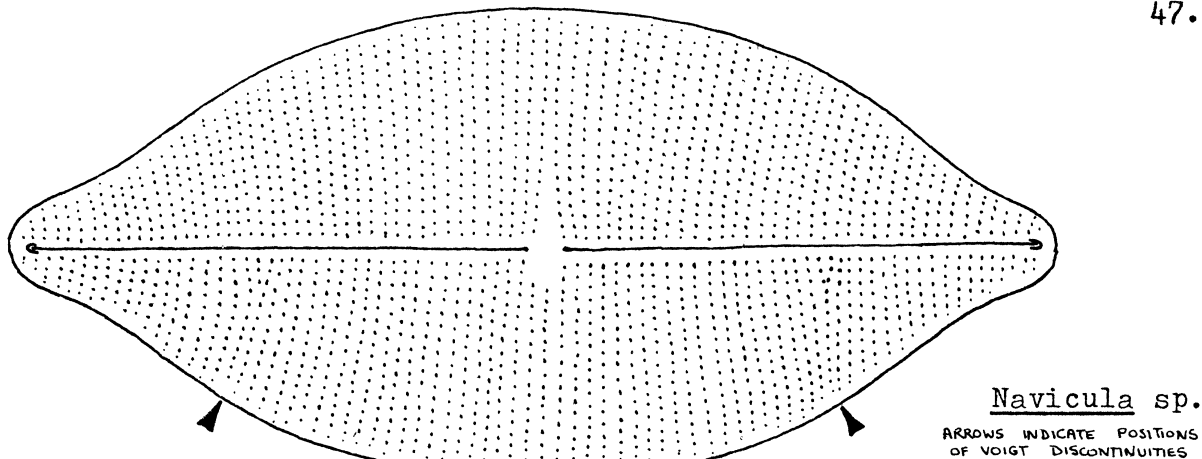
The sequence of deposition in the Nitzschiaceae is similar to that in Navicula pelliculosa, which was investigated in great detail by Chiappino & Volcani (1977). The earliest stages of silicification

in this species, stages which have not yet been observed in any of the Nitzschiaceae, seem to explain how Voigt discontinuities (see below and Chapter 4.5.3) come to be formed. One side of the raphe-



Stages in the development of the valve of Navicula pelliculosa (After Chiappino & Volcani)

sternum appears first (Chiappino & Volcani's 'primary central band'), and bears a thicker and wider central portion, which represents the primordial central nodule. Silicification of the other side of the raphe-sternum then takes place, not synchronously but sequentially, beginning from both poles and from the central nodule. The strips from the poles and those from the central nodule extend towards each other and fuse; the points of fusion are thus on the same side of the raphe-sternum and are each approximately half-way from centre to apex. The deposition of the transapical costae is also sequential and follows closely behind that of the raphe-sternum. Thus, the last transapical costae to appear are those near the points of fusion mentioned above. The Voigt discontinuities (see diagram below) always occur in positions



which correspond to the points of fusion, i.e. both are on the same side of the valve, each one occurring approximately half-way from centre to apex (see Voigt 1942, 1943, 1956, 1962) and this is surely no coincidence. From the information at present available, it seems quite possible that the placing of each costa is determined both by the position of the adjacent, previously formed costa, and by a spacing factor which allows only gradual or no change in the spacing around the valve; at the points of fusion, however, one would expect that the spacing might be somewhat amiss, the degree of disturbance being dependent upon how much the two fusing 'trains' of deposition were out of phase with one another.

The deposition of the valve in those Nitzschia, Denticula, etc. species without central raphe endings may follow a rather different course from that found by Chiappino & Volcani in Navicula pelliculosa, and it is interesting that Voigt discontinuities, if not absent, are at least not obvious in these forms.

With regard to sexual reproduction, it must be admitted that during this study, in which many hundreds of samples have been collected and examined, no sexually reproducing cells of Nitzschia or Hantzschia (nor of any other genera save Melosira, Rhoicosphenia and Anomoeoneis) have been observed. While unfortunate, this experience is not uncommon to diatomists. Details of auxospore formation in the Nitzschiaceae have therefore been gleaned from the literature,

especially from the works of Geitler (1932, 1973 etc.).

Sexual reproduction has been observed in only a small minority of diatom species, and for many genera the method of auxospore formation is either unknown, or known for just one or two species. Certain genera, however, are well-represented among the lists of species for which information is available (see Geitler 1932, 1973), but it is clear that this 'over-representation' is not random, but determined primarily by habitat. Thus, the predominantly epiphytic or epilithic genera (Achnanthes, Cocconeis, Cymbella, Gomphonema, Amphora, Epithemia) are well-investigated, while epipelagic genera (Gyrosigma, Pleurosigma, Navicula, Diploneis, Nitzschia, Hantzschia etc.) are not, at least in relation to the size of the genus. Auxospore formation has been described in only 15 out of several hundreds of Nitzschia species, and the proportion of Navicula species is similar.

The information published so far has been summarised by Geitler in two works (1932, 1973) and indicates that within Nitzschia there is a variety of methods of auxospore formation, in contrast to certain other genera (notably Epithemia, Cymbella and Gomphonema) which are remarkably uniform in this respect. The grounds upon which Geitler has separated the various types of auxospore formation will not be analysed here: comment is restricted mostly to the groupings he recognises and their relation to groupings of species made on the basis of other characters.

N. sigmoidea, N. dissipata and N. flexoides (see Geitler 1949, 1958 and 1968b respectively) all have the same type of auxospore formation (Geitler's type IA la γ ; 1973), which is interesting in view of the similarities between the first two in subraphe construction, presence of external flaps, absence of central raphe endings, etc. (see Nitzschia sects. Dissipatae and Nitzschia). N. flexoides, moreover, is very like N. sigmoidea in overall frustule shape and appear-

ance, and in the absence of central raphe endings; on morphological grounds it would seem to belong in the same group as that species (see sect. Nitzschia).

N. palea, N. subtilis, N. kützingiana and an unidentified species of the sect. Lanceolatae (Geitler 1968b) have a second type of auxospore formation (Geitler's type IA lb), and here again it is noticeable that these species (which all belong to the sect. Lanceolatae) are morphologically very similar.

N. fonticola and N. angustata, on the other hand, which share a third type (Geitler's IC), have never traditionally been classified in the same group; N. fonticola has always been placed in the sect. Lanceolatae, but N. angustata in the sect. Tryblionella. As is shown elsewhere, however, N. angustata does not belong in the sect. Tryblionella but closely resembles N. amphibia and N. cf. hantzschiana, both of which are quite similar to N. fonticola and belong to the sect. Lanceolatae. Indeed, N. angustata shares with N. fonticola a similar valve, raphe and subraphe canal wall construction, and the finding (see Geitler 1973) that the method of auxospore formation is identical need come as no surprise (for details of the morphology of N. fonticola, see Lange-Bertalot 1977).

Until 1969 all investigated Nitzschia species had been found to exhibit one of the three types above. All were allogamous and 'allen ist gemeinsam, dass aus einer Mutterzelle zwei Gameten hervorgehen und daher aus einem Paar zwei Zygoten bzw. Auxosporen entstehen' (Geitler 1969). Types IA la γ , IA lb and IC were separated from one another by the behaviour of the gametes and by the mode of copulation. N. amphibia, however, proved to be quite different (Geitler 1969), producing only one gamete per mother cell and copulating via papillae, which extend out from the poles of the cells, as in some Surirella and Eunotia species. So far, N. amphibia is unique within Nitzschia;

it is perhaps surprising, in view of the morphological similarities between the two species, that it differs from N. angustata in auxospore formation.

In the next year Geitler (1970) published an account of auxospore formation in 'N. frustulum var. perpusilla' (= N. hantzschiana according to Lange-Bertalot 1977), which showed that this species too differs from all others studied thus far. Whereas other species are allogamous, N. hantzschiana is automictic. Cells do not pair: instead, each cell forms two gametes (cytokinesis occurring after the first meiotic division, but not after the second, with one of the haploid nuclei in each gamete becoming pycnotic and taking no further part in auxospore formation) which then fuse to form a zygote.

Of other species, N. longissima and N. hybrida (sects. Nitzschiella and Dubiae) appear to have a type IA la γ or IA lb auxospore formation (Geitler 1932, 1973).

Clearly, our present knowledge is such that details of sexual reproduction are not of much use in taxonomy. Nevertheless, the account given above demonstrates that such details may be useful in the future. It would be nice to have information about N. recta, N. vermicularis and N. flexa, which, from morphological evidence, might be predicted to have similar methods of auxospore formation, methods like those of N. sigmoidea etc., while N. linearis and N. vitrea (which are usually classified near N. recta, in my opinion wrongly) should differ not only from each other, but also from N. recta.

In Hantzschia auxospore formation is so far unknown, while for other genera of the Nitzschiaceae information is also absent, or at best scanty (see Geitler 1932).

With reference to the Epithemiaceae, it is interesting to note that while Denticula vanheurckii has exactly the same method of auxospore formation as have all investigated Epithemia and Rhopalodia

species, a method which is unlike any of those present in the Nitzsch-
iaceae (Geitler 1932), D. tenuis does not, although it is also not
like Nitzschia in this respect. These observations lend support to
the view that the 'Sumatran' Denticula species should at least be
retained in the Epithemiaceae, if not transferred into Epithemia
(see 'Epithemiaceae', Chapter 5). In connection with the question of
the taxonomic position of D. tenuis, it would be useful to have auxo-
spore data concerning species of Nitzschia sect. Grunowia.

One other matter remains to be discussed, namely the alteration
of cell size, shape etc. as a result of vegetative division. Changes
in these parameters are caused by the peculiar method of division
present in the Bacillariophyta, where the new valves are laid inside
the mother frustule so that, unless the parental cincture is somewhat
larger than the parental valves, both transapically and apically, the
new valves must inevitably be smaller in some dimension than the old.
Size reduction has been found to be absent in some diatoms (see Round
1972c), including some Nitzschia species (Wiedling 1943), but from
the variation in length observed in natural populations (e.g. F.367-
371, 379-83, 384-9, 390-5, 458-61) and in cultured material (Wiedling
1943, 1948) it would seem that most Nitzschia and Hantzschia species
do undergo reduction. In the larger and more robust forms it can often
be seen, using the light microscope, that the epivalve is larger than
the hypovalve. This being so, it must be noted that size reduction
does not take place equally in all directions. After making thorough
studies of a number of diatoms, Geitler (1932) formulated certain
'rules' which apply to size and shape changes, and these are worth
quoting in full:

- '1. Die Länge der Apicalachse nimmt nicht nur absolut, sondern
auch relativ stärker als die der Transapicalachse ab; kleinere

Zellen sind daher relativ breiter als grössere' (F.379-83, 384-9, 390-5).

- '2. Die Länge der Apicalachse nimmt absolut und relativ stärker als die Breite der Schalenmantel und Gürtel- (evtl. auch Zwischen-) bänder ab, welche die Länge der Pervalvarachse bestimmt, kleinere Zellen sind daher in Gürtelansicht relativ breiter als grössere.
- '3. Der Schalenriss kleinerer Zellen ist "unkomplizierter" und abgerundeter als der grösserer' (F.384-9).
- '4. Die Zeichnungselemente der Schalen (Streifen, Striche, Punkte) werden im Vergleich zur Verkürzung der Apicalachse wenig verkleinert; ihre Anzahl pro Flächeneinheit bleibt daher innerhalb enger Grenzen konstant' (F.379-83, 384-9, 390-5).
- '5. Die Dicke der Schalen, Gürtel- und Zwischenbänder nimmt im Lauf der Teilungen ab.'

From these rules it is clear that greater caution must be exercised in the use of valve length and outline as taxonomic characters than in the use of valve breadth, depth, stria densities, etc. Valve structure does not appear to alter appreciably during size reduction; the morphology of the initial cell, however, may be radically different from the normal (L. Edgar, unpubl. obs. of Navicula cuspidata).

Geitler (e.g. 1968a) has noted that within a clone the sizes of the initial cells and mother cells are constant within narrow limits. These sizes should be determined, therefore, and used in classification: individual measurements of the sizes of normal, 'inter-auxospore' cells are of much less use taxonomically, but may be all that it is possible to obtain in many cases. Geitler has also found (1968a, 1975b) that species may each contain several 'sibs', separable from one another on the basis of the range in cell size. Thus, in Nitzschia dissipata two 'Sippen' (sibs) may be distinguished - a smaller celled form, with

a length variation of 17-50 μm ., and a larger form which corresponds to Grunow's 'N. media' (Cleve & Grunow 1880), with a variation of 43-72 μm . (Geitler 1968a); Hustedt (1930) failed to separate these forms. In Cymbella cesatii Geitler (1975b) erected a new variety, var. paradoxa, because of differences from the var. cesatii in size and in the mode of pairing. It is questionable, however, whether a difference in size variation alone would have justified the separation of these varieties, and care must be taken not to exaggerate the importance of this character in the same way that symmetry has so often been emphasized in the past.

The change of shape associated with decreasing cell size is a matter which must be studied carefully within each within each species or population. It is easy to mistake different parts of the life cycle for different varieties (or even species?), and this probably explains in part the chaotic state of the taxonomy of Nitzschia sect. Lanceolatae, where few characters can be distinguished with the light microscope and thus valve shape and size have been of relatively greater importance than in certain other groups, e.g. the sect. Panduriformes.