have been studied in the necessary depth; (only N. amphibia, N. mollis, N. hantzschiana, N. linearis, N. sinuata, N. sigma, N. vitrea). Details, where known, will be described in the section on taxonomy. It will be a long time before comprehensive information is available concerning the girdle of Nitzschia, unless the resolution of the SEM can be improved without an accompanying increase in specimen penetration.

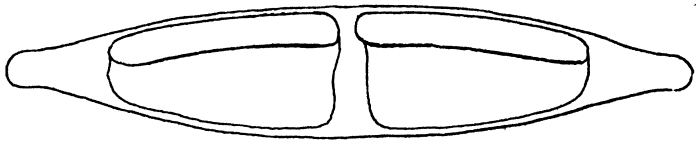
4.6.5 Chromatophores and cytology

Most of the published data concerning the chromatophore structure in Nitzschia species is contained in the works of Karsten (1899), Mereschkowsky (1901, 1903a, b) and Heinzerling (1908). Very little has been published recently, although Geitler (1969, 1970, 1975a) gives some details of the chromatophores of N. amphibia, N. frustulum var. perpusilla and N. palea in the course of discussions about auxospore formation or cell division. Virtually nothing is known about the nucleus, except for the information given by Lauterborn (1896) in an excellent account of nuclear structure and mitosis in N. sigmoidea. In the last twenty years a few papers have been published giving some details of the ultrastructure of Nitzschia cells. Gibbs (1962a, b) illustrated chloroplast and pyrenoid structure in a variety of algae, including N. angularis, while Drum (1963) and Lauritis et al. (1968) gave more thorough accounts of fine structure in N. palea and N. alba respectively. The last-mentioned species is apochlorotic and its structure must therefore be to some extent atypical of Nitzschia. In these studies the usual organelles - dictyosomes, mitochondria, nucleoli, etc. - were found and had much the same structure as in other diatoms (see review by Duke & Reimann 1977).

Mereschkowsky (1903a) distinguished eight types of chromatophore structure occurring within the presently accepted limits of Nitzschia - seven in Nitzschia and two in Nitzschiella, which is now included

in Nitzschia; one of the Nitzschiella types, however, is identical to the first type in Nitzschia. Two of these types were exemplified only in species now classified in other genera: N. paradoxa and N. socialis are now in Bacillaria, and N. vivax was transferred to Hantzschia by Hustedt (1959a). Mereschkowsky's other six groups form a convenient basis for further discussion.

1. 'Premier type. Deux plaques transversales sans fissures longitudinales.'



As Mereschkowsky (1903a) noted, this is the 'standard-type du genre.' The chromatophores are disposed symmetrically about the median transapical plane such that one lies in each polar half-cell. Each chromatophore is not usually placed symmetrically with regard to the apical plane, but lies to the side of the cell, adjacent to one side of the girdle and extending onto one or both valve faces. Occasionally, as in some specimens of N. sinuata, one chromatophore lies against one side of the girdle while the other lies on the opposite side (F.221). Mereschkowsky (1903a) noted that in certain other species (N. linearis, N. denticula, N. debilis) the chromatophore plates can lie diagonally across the cell;'c'est-à-dire qu'elles ne reposent sur aucune des faces mais traversent la cellule d'un angle à l'autre'.

The species in which this type of chromatophore arrangement has been found are listed in Table 10. It should not be thought, however, that the chromatophores are of identical form throughout this group.

TABLE 10

List of Nitzschia species having a type 1 chromatophore arrangement.

SPECIES	Source of information	SPECIES	Source of information
N. acicularis	M1 H *	N. frustulum var. perpusilla	Ch G2
N. actydrophila	?Ha2	N. gracilis	O M1 Ch
N. acuminata	M2 *	N. granii var. subcurvata	?Ha1
N. acus	M1	N. granulata	M2
N.?agnewii	*	N. hantzschiana	Ch *
N. amphibia	G1 *	N. cf.hantzschiana	*
N. angularis	(M,K) *	N. hungarica	M2 *
N. angustata	VH	N. hybrida	K M2
N. apiculata	M2 H *	N. insignis	M2
N. bilobata	K M2 *	N. kützingiana	*
N. biplacata	M1 H	N. lanceolata	K M1,2 *
N. californica	M1	N. linearis	P M2 H Ch *
N. clausii	P *	N. littoralis	?K
N. communis	*	N. longissima (in part)	Cu
var. abbreviata	M1	N. lorenziana	M1 *
N. commutata	K M2 H	N. martiana	M3
N. debilis	*	N. naviculariș	*
N. delicatissima	Cu	N. obtusa	M2 W
N. denticula	M2	var. nana	M1
N. dissipata	*	var. scalpelliformis	*
N. distans var. tumescens	M1	N. pacifica	Cu
N. dubia	P K M2 *	N. palea	P M2 *
N. epithemioides	*	N. paleacea	H Ch *
N. filiformis	*	N. punctata	?K
N. flexa	P *	N. pungens var. atlantica	Cu
N. fonticola	M2 *	N. seriata	Cu Ha2
N. frustulum	Ch *	N. sigma	K M1,2,3 *

TABLE 10 (contd.)

SPECIES	Source of information	SPECIES	Source of information
N. sigmatella	K M3	N. subtilis	K M2
N. sigmoidea	P M2 H *	N. tenuirostris	M1
N. sinuata	*	N. tryblionella	K M2 *
N. solgensis	*	N. turgidula	?Ha2
N. spathulata	*	N. valida	K
var. hyalina	M2	N. vermicularis	H *
N. stagnorum	H	N. vidovichii	M1,2
N. subamphioxoides	*	N. vitrea	Ko *
N. sublinearis	*	N. vitrea var. salinarum	M2 *

Ch = Cholnoky (1926)

Cu = Cupp (1943)

G1 = Geitler (1969)

G2 = Geitler (1970)

Ha1 = Hasle (1964)

Ha2 = Hasle (1965a)

H = Heinzerling (1908)

K = Karsten (1899)

Ko = Kolbe (1927)

M1 = Mereschkowsky (1901)

M2 = Mereschkowsky (1903a)

M3 = Mereschkowsky (1903b)

O = Ott (1900)

P = Pfitzer (1871)

VH = Van Heurck (1896)

W = Williams (1965)

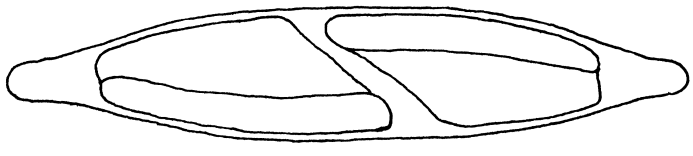
* = my observations

There are, for example, differences between species in the proportion of the cell length occupied by the chromatophore: N. epithemioides always has small chromatophores relative to the cell length (F.350-1), whereas in the larger members of the sect. Tryblionella the chromatophores each extend from centre to pole, (F.166, 168-70). The chromatophore length is not always constant (relative to the cell length) within a species, as noted by Cholnoky (1926), but in many species this seems not to be the case, at least in nature, and the relative chromatophore length may prove to be a useful taxonomic feature, in classification and/or identification.

There is also variation, even within species, in the degree of lobing of the chromatophore margin. Thus, for example, N. sinuata chromatophores have crenulate margins (F.221-2, 224), whereas those of N. communis are smooth (F.500). N. sigma, however, includes some forms with almost smooth margined chromatophores (F.308), and others with extensive lobing (F.309-11): indeed, Mereschkowsky (1903b) proposed the erection of a new variety, var. incisa, to include specimens of the latter type. It seems likely, however, in view of Cholnoky's (1926) observations, that the degree of lobing is as much dependent on environmental factors as on genetic influences.

The two chromatophores of a single cell usually approach each other most closely near their margins, so that between them is a centrally widened cleft (e.g. F.308, but see F.148-51). The nucleus 'sits' in this central widening, though it projects further into the central vacuole than do the chromatophores.

2. 'Deuxième type. Deux plaques obliques, séparées par une fente diagonal.'



notation,'Kompens-Okular' 6, 12 and 18, respectively).

In order to develop the full aperture of the immersion objectives, it was necessary to oil the front lens of the condenser to the back of the microscope slide. This procedure was carried out only when absolutely necessary!

Diatoms were drawn with the aid of a Zeiss camera lucida attachment; an inclined drawing board was unnecessary since the microscope had a vertical, monocular tube.

Occasionally, a Zeiss photomicroscope was used, this being fitted with phase contrast and bright field systems. Photomicrographs were taken using the immersion objectives, (the condenser being immersed where the highest resolution was required), on Pan F film.

2.5.2 Scanning electron microscopy

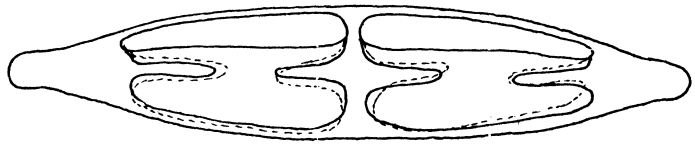
Cleaned or uncleaned material was washed with distilled water and then dried down onto aluminium stubs. In order to prevent charging under the electron beam, it is necessary to coat specimens with thin layers of conducting material. At first, gold/palladium was used for this, the alloy being evaporated from a point source 10 cm. distant from the stub, under high vacuum. The stubs were rotated and tilted with respect to the point source in order that the Au/Pd coating might be as even in thickness and as complete as possible. Later, a sputter-coater was employed. This used a gold target, coating taking place under a low vacuum in an 'atmosphere' of argon. The conducting films of gold thus obtained proved more satisfactory than the Au/Pd coatings (as judged by the frequency of charging under the electron beam). Sputter-coating is also simple and quick. Damblon (1975), who worked on pollen grains, claimed that sputter-coated specimens allowed better resolution of detail, but in the present study other factors (e.g. accelerating voltage, condenser settings) were found to be more

This was found by Mereschkowsky (1901, 1903a) in N. constricta, and in a diatom which he tentatively identified as N. tryblionella var. salinarum. Here one chromatophore lies against one side of the girdle, the other lying diagonally opposite (F.147). The cleft between them is oblique to the transapical plane, though I find it to be less so than was figured by Mereschkowsky (e.g. 1901, T.7 f.16).

This type of arrangement, found in several members of the sect. Panduriformes, is intermediate in form between the usual arrangements in Nitzschia and Navicula, although in some Navicula species, e.g. N. cf.cryptocephala, the chromatophores are offset in a manner reminiscent of N. constricta (unpubl. obs.). In these Navicula species, however, in contrast to N. constricta, very little of the chromatophore abuts the valve face. In other matters there is a vast gulf between these Nitzschiae and Navicula!

Karsten's (1899) illustrations of N. constricta are unusually poor, and his conclusion that there is but a single chromatophore must be regarded with disbelief.

3. 'Troisième type. Deux plaques transversales avec des sinus longitudinaux, divisant la plaque en plusieurs lobes.'



Mereschkowsky (1903a) claimed that this type is 'surtout caractéristique pour la groupe des Spathulatae et notamment pour les espèces N. angularis, N. distans et certaines de leurs varietes.' It may be

seen, however, that N. angularis has been listed among the species displaying a type 1 chromatophore disposition (Table 10). Karsten (1899), moreover, stated that in this species 'ein Chromatophor einer Gürtelseite anliegend, sehr stark zerschlitzt und mit den Rändern bis auf die andere Gürtelseite umgeschlagen.' What explanation is there for these different claims?

Karsten's belief that there is only one chromatophore per cell was explained satisfactorily by Mereschkowsky (1903b), who reexamined material of N. angularis in the light of Karsten's claim and noted that 'les extrémités intérieures des plaques sont ordinairement très rapprochées, leurs bords sont même quelquefois superposés....de sort que, les extrémités se recouvrant, on ne voit plus de fente transversale qui ordinairement sépare la plaque supérieure de l'inférieure.' Certainly, in all cells of N. angularis observed during the present study two well-defined chromatophores have been present (F.234-8). N.B. the overlapping of the chromatophores noted by Mereschkowsky may also be the cause of Karsten's (1899) belief that 'N. punctata-elongata' and N. littoralis have only one chromatophore per cell. Until living material of these taxa is refound, however, this is unconfirmable - hence their listing in Table 10 is only tentative.

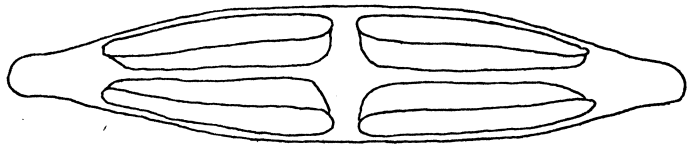
Mereschkowsky (1903b) stated that N. angularis should be split into two species, very similar to each other in frustule morphology, but differing 'entièrement' in their chromatophore arrangement. One species was to correspond to the 'N. angularis' described in his earlier paper (1901), while the other was the 'N. angularis' of Karsten (1899). But the only real difference between the two lies in the degree to which the 'sinus longitudinaux' are developed. In Mereschkowsky's specimens these constrictions of the chromatophores were very marked (1901, T.7 f.1; 1903a, f.70, 71), whereas in Karsten's they are virtually absent and hence these individuals may be said to possess

a type 1 chromatophore arrangement. However, collections of N. angularis from Sandpoint, near Weston-super-Mare, contain some forms which are close to that figured by Karsten (F.237-8), and others in which the sinuses are better developed, intermediate in form between Karsten's specimens and Mereschkowsky's (F.234). Within the Sandpoint forms there is also a dichotomy between those in which each chromatophore extends under the raphe system of each valve at only one point (F.234-6) and those where multiple lobing occurs (F.237-9); Mereschkowsky (1903a, f.71) figures specimens of the former type, while Karsten (1899, f.170) and Mereschkowsky (1903a, f.69, 70) illustrate the latter. No differences have been detected in the valve structure within N. angularis.

N. spathulata often has a slight longitudinal constriction of the chromatophore, but their is much variation within this species (F.250-252). Mereschkowsky (1901) described the chromatophores of N. distans, which also belongs to the sect. Spathulatae, and here again a constriction may (op. cit., T.7 f.6) or may not (T.7 f.5) be present.

There seems little justification for maintaining this group as distinct from type 1, except that it may be intermediate between type 1 and type 4. There is much variation in the degree to which the chromatophore is constricted even within species,and lobing of the chromatophore margin similar to that in N. angularis (in part) occurs in N. vermicularis (F.329) and N. sigma (F.309-11), both of which have a type 1 chromatophore arrangement. That the chromatophores of the sect. Spathulatae would differ in form from those of other Nitzschia species might be predicted from a knowledge that in this section the raphe system is more or less centrally placed on the valve, whereas in most Nitzschia spp. it is eccentric; the basis for this prediction is that chromatophores tend to be absent from the region of cytoplasm directly beneath the raphe (see Mereschkowsky 1903a, p.168; 'Loi gouvernant les phénomènes de l'endochrome').

4. 'Sixième type. Quatre plaques reposant sur les deux connectifs.'

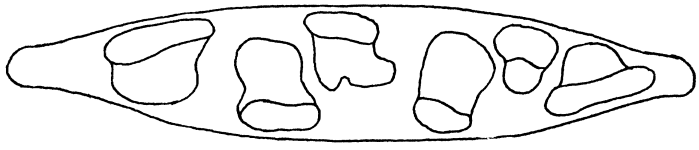


Mereschkowsky (1903a) proposed the separation of two new species, N. tetraplacata and N. fenestrata, from N. distans on the grounds of their different chromatophore structure; the morphology of the siliceous parts was apparently identical throughout. Mereschkowsky gave no formal descriptions or diagnoses, and as a result these species are poorly defined; they are identifiable only by reference to Mereschkowsky's illustrations, of which the scale is unknown!

Cases such as this, where populations almost identical to one another in frustule morphology differ in cytological detail, e.g. in the number of chromatophores per cell, need to be studied very thoroughly. It will be necessary, for instance, to subject N. tetraplacata and N. fenestrata to careful scrutiny, if and when living material of them is found again, in order to eliminate the possibility that each opposing pair of chromatophores is joined, albeit by a very fine connection; sometimes extremely thin connections occur between plates, and these are easily overlooked, e.g. in Scoliopleura tumida (unpubl.obs.). If such connections were present, then these species would fall into place alongside other Nitzschia species exhibiting type 1 chromatophore arrangements, and there would be less reason to separate them from N. distans. On the other hand, there is no reason to assume that individuals with identical frustule morphology must necessarily belong

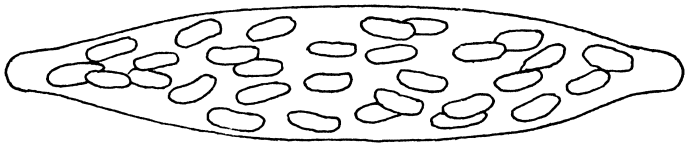
to the same species, and if Mereschkowsky's observations prove to be correct, then perhaps N. tetraplacata and N. fenestrata do indeed deserve specific status.

5. 'Septième type ... Chromatophores en nombre variable, ordinairement plus de 4.'



N. dissipata and N.(Homoeocladia) martiana have been cited as examples (by Mereschkowsky 1903a), but, while the latter is unknown to me, my observations of N. dissipata and those of Geitler (1958) indicate that this species has but two chromatophores per cell, arranged as in Type 1 (F.255). Perhaps Mereschkowsky observed unhealthy or parasitized cells, but it may be that N. dissipata exhibits infraspecific variation with respect to this character, or even that it should be split into two or more species just as Mereschkowsky suggested for N. distans.

6. 'Nitzschiella ... Deuxième type. Endochrome granuleux.'



This type, where there are numerous tiny chromatophores per cell,

is little different from the last, except that the chromatophores are smaller and often bacillar rather than coccoid in form. The chromatophores of N. longissima seem to have been described first by Karsten (1897), but have been illustrated also by Karsten (1899) and Mereschkowsky (1901). Both authors noted numerous, small chromatophores to be present. By contrast, Cupp (1943) depicted a cell of N. longissima in which there were two chromatophores, arranged as in type 1. The specimens observed during the present study were of the Karsten-Mereschkowsky type.

The taxonomy of N. longissima is confused: for instance, Hasle (1964) noted that her specimens, which she referred to N. longissima f. parva (which 'may have a finer transapical structure than the typical form') had 65-70 transapical costae in 10 µm., whereas Cupp (1943), Cleve & Grunow (1880) and others (see Hasle 1964) give linear densities of around 16 in 10 µm. This discrepancy, which is not analysed by Hasle, probably indicates that the present limits of the species are far too wide and all-embracing; thus, one or more species within N. longissima (sensu lato) may have granular chromatophores, while other(s) may have larger, plate-like chromatophores.

In the smaller Nitzschia species there is one pyrenoid per chromatophore (F.179, 491). It usually lies against the girdle and is often visible in girdle view as a paler region within the chromatophore, or in valve view as a thickening of the chromatophore. The pyrenoid is usually elongated, with its long axis parallel to the apical axis of the cell (Heinzerling 1908; for N. palea, see Geitler 1975a), but Karsten (1899) depicted oval pyrenoids in N. hybrida. In N. sigmoidea and N. sigma there are many, elongate pyrenoids in each plastid, which do not, however, all lie with their long axes orientated as above (Geitler 1937, Abb.e-g).

The pyrenoids of Nitzschia species are rarely very obvious in unstained material, unlike those of Hantzschia amphioxys, H. virgata, H. weyprechtii and H. elongata. One exception to this is N. navicularis (F.179, 181-3), in which there is a prominent pyrenoid lying between two lobes of the chromatophore; the lobes lie one against each valve. There is a slight resemblance, therefore, to the arrangement in some Hantzschia species, except that the chromatophore 'plates' in N. navicularis lie in the valvar, not the pervalvar plane; i.e. the arrangement in this species is orientated at right angles to that in Hantzschia. However, in view of the fact that the pyrenoid is always closer to one chromatophore margin than the other (as seen in valve view), and in some cases lies against the girdle, as in other Nitzschia species (F.182), it seems that N. navicularis belongs with the taxa exhibiting a type 1 chromatophore arrangement.

Some Nitzschia species, as has been mentioned before, have no chromatophores and are obligately heterotrophic. Thus, Benecke (1900) described two apochlorotic species, N. leucosigma and N. putrida (see also Karsten 1901), while a third, N. alba, was added by Lewin & Lewin (1967), and a fourth has been found by Round (in preparation).

The nucleus of a Nitzschia cell lies centrally and is often difficult to see, being partly obscured by the chromatophores. The nucleoplasm is fairly homogeneous, not often displaying the granularity so evident in Hantzschia. A single nucleolus can often be distinguished within the nucleus (e.g. F.278), but in some species several nucleoli occur (e.g. in N. sigmoidea, Lauterborn 1896). The nucleus usually stains rather poorly with aceto-carmine: in N. linearis and N. debilis, for instance, even after fairly lengthy 'cooking' in this stain the nucleus is stained only slightly pink. The nucleus is most often near spherical (F.148-51, 223, 234-6, 250-2, 278, etc.), but in N. sigmoidea Lauterborn (1896) showed it to be much elongated in

the apical plane; this is also the case in some N. linearis cells (F.275).

Sometimes, small rod-like structures may be seen, forming a discontinuous ring around the nucleus, which ring may easily be mistaken for the nuclear envelope. Geitler (1975a) has illustrated these in N. palea, and gives details of their behaviour during cell division. Electron microscopic observations demonstrate that these structures are in fact dictyosomes (L.Edgar, unpubl. obs. of N. sublinearis); other diatoms have a similar clustering of dictyosomes around the nucleus (see Duke & Reimann 1977).

The greater part of the interior of a diatom cell is occupied by a central vacuole, so that the cytoplasm is restricted mostly to a thin peripheral layer. Much of the cell interior is sometimes filled with small granules, perhaps as a reaction to particular environmental conditions. Such cells, which have been observed in N. sinuata and N. navicularis (F.179), are motile and show no sign of abnormality. Many tiny granules are sometimes to be observed near the ends of the cells (e.g. in N. sigma), immediately beneath the polar raphe endings, though whether they have anything to do with the mechanism of motility is unknown. Much more prominent masses of such granules, which in vivo exhibit jerky, apparently random oscillations, are to be seen near the poles of Pleurosigma angulatum (unpubl.obs.).

Also present in many, if not all, Nitzschia species are spherical bodies, one lying at the polar end of each chromatophore (F.222, 224, 234, 274, 308-10, 315-6, 490-1, etc.); at this point there is usually a slight depression of the chromatophore margin, in which the body sits. These are the 'Bütschlischen Kugeln' of Lauterborn (1896), which consist of volutin, according to Meyer and Heinzerling (teste Fritsch 1935). They are not equally developed in all species, but are extremely

small in some and very prominent in others. In N. sigmoidea, for instance, the Bütschli globules are small and inconspicuous, whereas in N. sigma they are large. The species studied here have been found to exhibit a fair degree of internal consistency with regard to the relative size and prominence of the Bütschli globules.

4.6.6 The taxonomy of Nitzschia

Grunow's treatment of Nitzschia (1862, and in Cleve & Grunow 1880), in which he divided the genus into over twenty subgroups (usually considered to be sections), has not been left unaltered by subsequent workers (see Table 25 for a summary of the changes). In particular, Hustedt has proposed many modifications of Grunow's system, but unfortunately Hustedt's suggestions are scattered through several papers and it is likely that many workers are unaware of some of his later changes. In this dissertation all the changes suggested by, or sanctioned by Hustedt (and the transfer of Fragilariopsis into Nitzschia proposed by Hasle 1972b), have been implemented and the resultant classification used as a basis for further discussion. The sections remaining are the following:

1. Panduriformes
2. Tryblionella
3. Dubiae
4. Pseudoamphiprora
5. Perrya
6. Insignes
7. Scalares
8. Grunowia
9. Epithemioideae
10. Dissipatae
11. Spathulatae
12. Nitzschia (= 'Sigmoideae')
13. Lineares
14. Lanceolatae
15. Nitzschiella
16. Pseudonitzschia
17. Fragilariopsis