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SEXUAL REPRODUCTION, MATING SYSTEM, AND PROTOPLAST DYNAMICS OF *SEMINAVIS* (BACILLARIOPHYCEAE)[1]

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Cell division, the mating system, and auxosporulation were studied in the marine epipelic diatom *Seminavis* cf. *robusta* Danielidis & D. G. Mann. The interphase protoplast contains two girdle-appressed chloroplasts, each with an elongate bar-like pyrenoid, and also a central nucleus, located in a bridge between two vacuoles. Before cell division, the chloroplasts divide transversely and translocate onto the valves. The nucleus relocates to the ventral side for mitosis. After cytokinesis and valve formation, the chloroplasts move back to the girdle, showing a constant clockwise movement relative to the epitheca of the daughter cell. *Seminavis* cf. *robusta* is dioecious, and sexual reproduction is possible once cells are less than 50 μm. In crosses of compatible clones, gametangia pair laterally, without the formation of a copulation envelope, and produce two gametes apiece. The intensity of sexualization increases as cells reduce further in size below the 50-μm threshold. At plasmogamy, the gametangia dehisce fully and the gametes, which were morphologically and behaviorally isogamous, fuse in the space between the gametangial thecae. The auxospore forms a transverse and longitudinal perizonium. After expansion is complete, there is an unequal contraction of the protoplast within the perizonium, creating the asymmetrical shape of the vegetative cell. Apart from this last feature, almost all characteristics exhibited by the live cell and auxospores of *Seminavis* agree with what is found in *Navicula sensu stricto*, supporting the classification of both in the Naviculaceae. Haploid parthenogenesis and polyploid auxospores were found, lending support to the view that change in ploidy may be a significant mechanism in diatom evolution.

Key index words: **auxosporulation; Bacillariophyceae; breeding system; chloroplast; diatoms; life cycle; Naviculaceae; *Seminavis*; sexual reproduction; systematics**

There is little disagreement among diatomists that the large genus *Amphora* is heterogeneous and requires revision (Round et al. 1990, Mann 1994a): almost all that unites *Amphora* species is extreme asymmetry of the cell about the median valvar plane. This was recognized more than a century ago (Cleve 1895), but no significant changes were made until 1990, when some amphoroid diatoms were transferred to *Seminavis* and *Biremis* (Round et al. 1990). *Seminavis* was erected to accommodate amphoroid species that have semilanceolate valves, two girdle-appressed chloroplasts of unequal size, each containing a single bar-like pyrenoid, a raphe system that possesses an accessory rib running parallel to the raphe internally, apically elongate slit-like areolae, and a small number of plain girdle bands; the wide first band (valvocopula) accounts for most of the width of the girdle and is markedly wider on the dorsal side of the frustule than on the ventral side. The structure of the chloroplasts, raphe, areolae, and girdle (except for the dorsoventrality) all correspond closely to what is found in *Navicula sensu stricto* and its allies (Round et al. 1990, Sullivan 1990), suggesting a close phylogenetic relationship, which is confirmed by *rbc*L sequence data (D. G. Mann and G. E. Simpson, unpublished data).

Among the many "non-siliceous" features recommended for use in investigations of raphid diatom systematics (Mann 1990), few have been investigated for *Seminavis*. Several species have now been transferred to *Seminavis* (Round et al. 1990, Garcia-Baptista 1993, Danielidis and Mann 2002), but the characters used to justify transfer were principally those of the valve and the interphase chloroplast arrangement. Po-

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tentially, one of the richest sources of nonmolecular characters is the sexual phase, which is an obligate part of the life cycle in most diatoms (as yet, no diatoms have been proven to have a strictly asexual life cycle, although this is probable in a very few pennate diatoms: see Mann 1989c, 1999). Details of pairing, gamete formation, plasmogamy, and auxospore development have proved useful taxonomically in several raphid pennate genera, including *Sellaphora* (Mann 1989b), *Navicula* (Mann and Stickle 1989), *Craticula* (Mann and Stickle 1991), *Dickieia* (Mann 1994b), *Amphora* (Mann 1994a), *Placoneis* (Mann and Stickle 1995a), and *Stauroneis* (Mann 1996a, Mann and Stickle 1995b). However, there is no account of sexual reproduction in *Seminavis*, apart from very brief observations of *S. macilenta* (Gregory) Danielidis et D. G. Mann (Danielidis and Mann 2002).

Studies of auxosporulation, besides yielding data for systematics, are an essential step toward understanding the mating system and population biology of diatoms. Until comparatively recently, diatoms were assumed to be predominantly monoecious, and when Drebes (1977) summarized available information on centric and pennate diatoms, only two diatoms—the araphid pennates *Rhabdonema adriaticum* Kützing (von Stosch 1958) and *Grammatophora marina* (Lyngbye) Kützing (von Stosch and Drebes 1964)—were known to be truly dioecious. However, Roshchin and Chepurnov (Roshchin 1994) gave several examples of dioecy in araphid (*Licmophora ehrenbergii* [Kützing] Grunow, *L. abbreviata* C. Agardh, *Striatella unipunctata* [Lyngbye] C. Agardh) and raphid (*Haslea subagnita* [Proshkina-Lavrenko) Makarova et Karayeva, *Nitzschia longissima* [Brébisson] Ralfs) pennate diatoms, and further raphid examples have been added by Davidovich and Bates (1998: *Pseudo-nitzschia multiseries* [Hasle] Hasle, *P. pseudodelicatissima* Hasle) and Mann et al. (1999: *Sellaphora pupula* [Kützing] Mereschkowsky "capitate" deme). Studies of other diatoms have revealed more complex mating systems, with multiple categories of clone (Roshchin 1994, Roshchin and Chepurnov 1999, Chepurnov and Mann 1997, 1999, 2000), but it is as yet unknown how frequent these different types of mating systems are among diatoms.

Recently, we isolated clonal cultures of a *Seminavis* species, which has allowed us to study its protoplast structure, cell cycle, mating system, and auxosporulation.

MATERIALS AND METHODS

A sediment sample containing living cells of *Seminavis* sp. was collected on 23 November 2000 by Dr. Tom Moens at the south edge of the "Veerse Meer," a large brackish water lake in Zeeland, The Netherlands. The Veerse Meer used to be connected to the Oosterschelde and North Sea but has been dammed and is not now subject to significant tidal fluctuations. The Veerse Meer is approximately 22 km long and at its widest measures 1.5 km across. The salinity is typically 20 psu (T. Moens, personal communication) and was 23 psu at the sample station, which was in a very shallow side creek of the lake, in rather muddy sediment (at 51°32′36″ N, 3°48′15″ E).

Sediment was processed using the lens tissue method described by Mann (1989c), and diatoms were harvested via a coverslip put on the lens tissue. The coverslip was then transferred into a Petri dish with culture medium, which was based on filtered and sterilized seawater (32 psu) collected from the North Sea, diluted with distilled water to 20 psu and enriched with nutrients as specified by Roshchin (1994; see also Chepurnov and Mann 1997). Twelve clonal cultures of *Seminavis* were established on 4 December 2000 by isolation of single cells by micropipette into Repli dish wells containing culture medium. Subsequently, monoclonal cultures were transferred to polystyrene 50-mm Petri dishes with 15–20 mL of culture medium and maintained in an incubator at 18° C with a 12:12-h light:dark period and 25–30 μmol photons·m^{-2}·s^{-1} from cool-white fluorescent lights. Reinoculation of cells into fresh medium was performed every 2–3 weeks.

Procedures for experimental crosses, observations of auxosporulation, and slide preparations for photomicroscopy were as described by Mann et al. (1999). In mating experiments, mixed cultures were examined daily for 7–9 days, using a Zeiss Axiovert 135 inverted microscope (Zeiss Gruppe, Jena, Germany), until they reached the stationary phase of growth, when the cells not only stopped dividing but also ceased to be sexually active and stopped moving. Frustules were cleaned by oxidation with hydrogen peroxide and glacial acetic acid and repeatedly washed with distilled water before being mounted in Naphrax. LM was carried out using a Zeiss Axioplan 2 Universal microscope (Zeiss Gruppe, Jena, Germany) equipped with a digital camera Hamamatsu (VIP III) (Photonics Deutschland GmbH, Herrsching, Germany) connected to a computer. Some measurements (e.g. in Table 1) were made with the aid of Scion Image software (Scion, Frederick, MD, USA). Voucher specimens of cleaned material of the original natural sample and the clonal cultures studied are kept in the Laboratory of Protistology and Aquatic Ecology, Gent University, Belgium.

RESULTS

Characteristics of cells from the natural collection. In the original sample, cells were easily recognized by their strong dorsoventrality, semilanceolate ("cymbelloid") valves, and two girdle-appressed chloroplasts. Observations of cleaned valves confirmed their identification as *Seminavis* (Fig. 1, a–c): the striae are uniseriate, the raphe is straight and closer to the ventral side, and the axial area is wider dorsally than ventrally and slightly expanded at the center. Careful focusing reveals a longitudinal rib internally, running parallel to the raphe on its ventral side (Fig. 1a, arrow; cf. Round et al. 1990, p. 573, Figs. f and h).

Valve length was 30.5–60.5 μm (mean = 40.32, SD = 7.0317, $n = 62$). Further data were obtained by using image analysis (Table 1, $n = 25$). Stria density varied within a surprisingly wide range, from 14 up to 21 in 10 μm, the density being consistently less (by 1–2 striae in 10 μm) on the ventral side of the raphe-sternum than on the dorsal side; the density was also higher toward the poles. The dorsal striae were radiate, more so at the apices than at the center. On the ventral side, the striae were visibly radiate but became parallel or nearly parallel toward the poles. Normally, a few central striae were visibly shorter, both dorsally and ventrally. SEM of frustules revealed the ultrastructural characteristics of *Seminavis* (listed above: Round et al. 1990, Danielidis and Mann 2002).

The species studied fits the description of *Seminavis robusta* Danielidis & D. G. Mann (Danielidis and Mann 2002), with some minor deviations concerning the density of both the ventral and dorsal striae. The original description for this species reports a slightly narrower range of values for both characters,

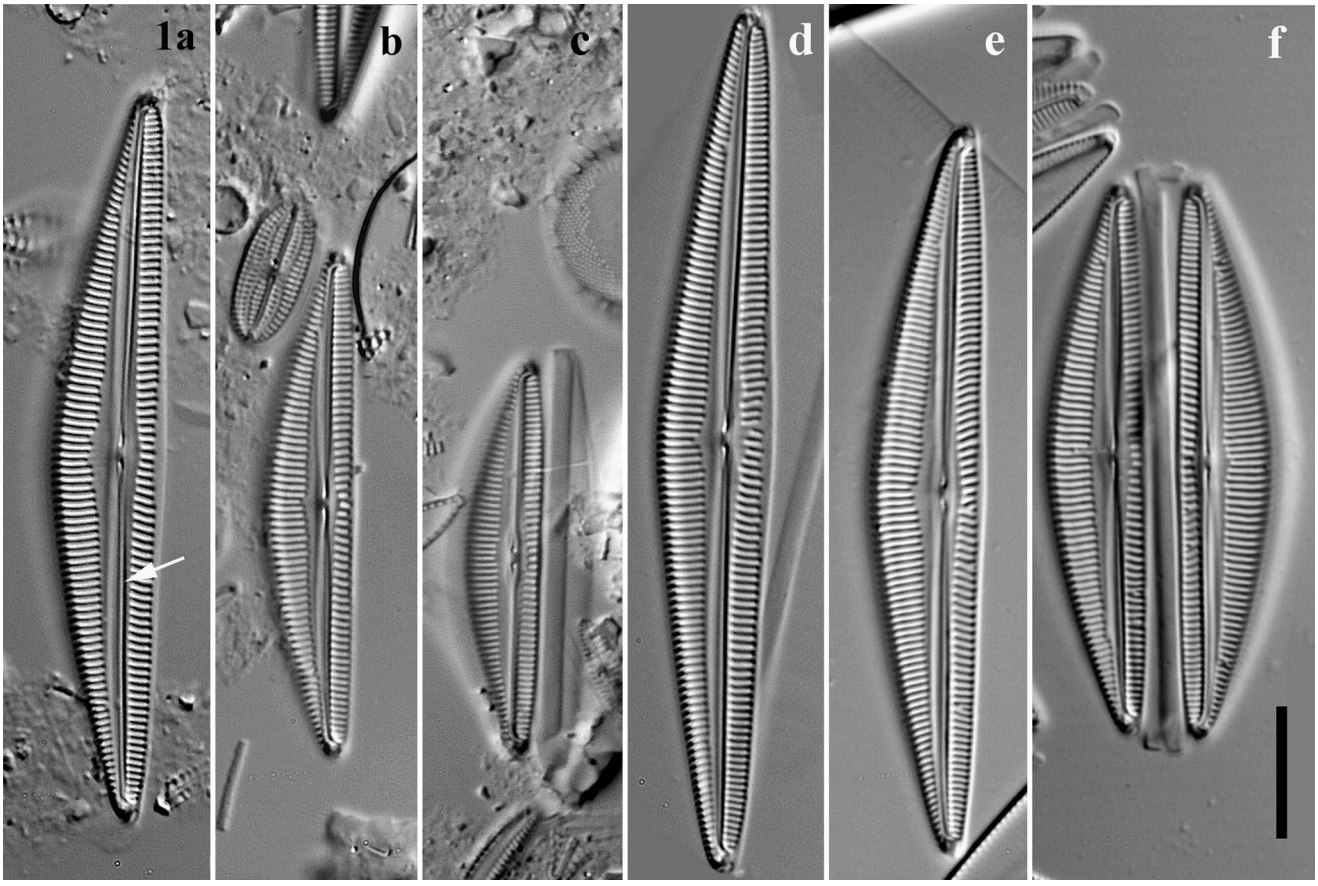

FIG. 1. *Seminavis* cf. *robusta* valves. (a–c) Natural population, Veerse Meer. (d–f) Cultures. (d) Large valve formed after sexual reproduction in mixed culture of clones 79 × 83. (e) Clone 95. (f) Clone 79. Scale bar, 10 μm.

that is, dorsal striae 17–20.5 in 10 μm and ventral 16–19.3 in 10 μm (compare with the data in Table 1). *Seminavis robusta* was based mainly on material coming from the Messolonghi lagoon complex (west Greece). However, misidentified as *Amphora angusta* var. *ventricosa* or *A. ventricosa*, *S. robusta* has been reported from around the world with a wider range of measurements (Danielidis and Mann 2002). Because of the slight mismatch in stria densities between the original description and our material, we refer to our species as *Seminavis* cf. *robusta*, pending further taxonomic study.

TABLE 1. Linear dimensions and stria densities (in 10 μm) in valves of *Seminavis* cf. *robusta* from a natural sample and three clones of different sizes.[a]

	Material studied			
	Sample	Clone 95	Clone 79	Clone 73
Number of observations	25	15	15	15
Length, μm	43.79 ± 7.921 (33.68–60.23)	56.03 ± 0.883 (54.94–58.12)	44.56 ± 0.868 (43.40–46.32)	36.33 ± 0.92 (34.08–37.38)
Width, μm	7.45 ± 0.832 (6.22–9.39)	8.85 ± 0.294 (8.30–9.33)	7.77 ± 0.297 (7.12–8.13)	6.85 ± 0.276 (6.34–7.27)
Stria density				
Center	15.18 ± 1.172 (14–18)	14.1 ± 0.388 (13.5–15)	14.5 ± 0.423 (14–15)	15.3 ± 0.883 (14–17.5)
Ventral				
t(p)	4.308*	6.853*	4.088*	8.704*
Dorsal	16.66 ± 1.068 (15–19.5)	15.83 ± 0.524 (15–16.5)	16.53 ± 0.481 (16–17)	17.5 ± 1.018 (16–20)
t(s)	3.364*	5.177*	2.700**	4.534*
Poles[b]				
Ventral	18.35 ± 0.853 (17–21)	18.25 ± 0.569 (17.5–19)	18.22 ± 0.639 (17–20)	18.45 ± 0.941 (17–20.5)
t(p)	0.017	0.096	0.040	0.113
Dorsal	18.61 ± 0.894 (17.5–21)	18.45 ± 0.0607 (17.5–19.5)	18.47 ± 0.525 (17.5–19.5)	18.67 ± 0.875 (17–20.5)

[a]Values are means ± SD (range). Student's *t* values are given for the differences between the mean striae densities at dorsal and ventral valvar sides (paired comparison [p], separately for central and polar area; the values are arranged between the means compared), at center and poles in general (two-sample comparison [s]), with significance levels: $*P < 0.01$; $**P < 0.05$.

[b]The stria were counted at both poles of every valve.

Clonal cultures. All 12 strains grew well in culture, and no differences were observed when the level of salinity was changed from 16 to 24 psu. During the exponential phase, the growth rate was 2 divisions·d^{-1}. After reaching stationary phase, cultures could remain alive for at least a few weeks. Linear dimensions of the valves and stria densities were measured in three clones (73, 79, 95) that differed visibly in size (Table 1). Clones retained the same morphology in culture that they possessed in nature (Fig. 1, d–f). The first cell measurements (see Fig. 5a) were made 6 weeks after isolation. Periodic measurements showed that the rate of cell size reduction averaged 3–4 μm·mo^{-1} in our culture conditions.

Chloroplasts. The morphology of the chloroplasts and their behavior during the cell cycle were observed while clones were in exponential growth to avoid density-dependent "abnormalities" during stationary phase, when cells can no longer divide and may perhaps arrest at different stages of the cell cycle. During interphase, there were two chloroplasts per cell, each appressed to one side of the girdle (Fig. 2a) and containing a single, straight, bar-like (or rod-like) pyrenoid, which ran almost the whole length of the chloroplast along its central line (Fig. 2b). The dorsal chloroplast was normally larger (in area) than the ventral, corresponding to the difference in width between the two sides of the girdle (Figs. 2, c and d, and 3, a and b). Each chloroplast appeared constricted at the center, as a result of the formation of four subcentral lobes that extended out below the valves (Figs. 2c, 3, a and c, and 4d); those from the ventral chloroplast sometimes reached the dorsal margin (Fig. 2d). The chloroplast was not significantly lobed near the poles, so that a second constriction "roughly halfway from center to poles," as reported for *Seminavis ventricosa* (Gregory) Garcia-Baptista by Danielidis and Mann (2002) and illustrated also by Mann (1996b, Fig. 5), was not present. The sizes of the lobes changed during the cell cycle (see below) and was very variable among cells within a single clone; they seemed to be influenced by external conditions, such as light and the density of cells in culture.

Plastokinesis always preceded mitosis. The chloroplasts divided transversely by further deepening of the central constriction (Fig. 3, a and b); at the same time, the pyrenoid was cut in two (Fig. 3d). Division occurred while the chloroplasts still lay against the girdle (Fig. 3, c and d). Then the two halves of each chloroplast moved as a single unit in the same direction around one fourth of the cell periphery to lie beneath one valve. (Fig. 3, e–h). During this rearrangement, the lobing of the chloroplasts disappeared (Fig. 3, g and h). Once the chloroplast had divided, "bridges" of dense material appeared, traversing the cell lumen and linking the two pairs of daughter chloroplasts (Fig. 3h); these resemble the "capped" ends of the special volutin vacuoles in *Navicula oblonga* (Kützing) Kützing (Mann and Stickle 1989, Figs. 1, 2, 9, 11, and 12). The links persisted during the translocation of the chloroplasts from girdle to valve and may participate actively in this process, because they disappeared soon after the completion of the chloroplast rearrangement.

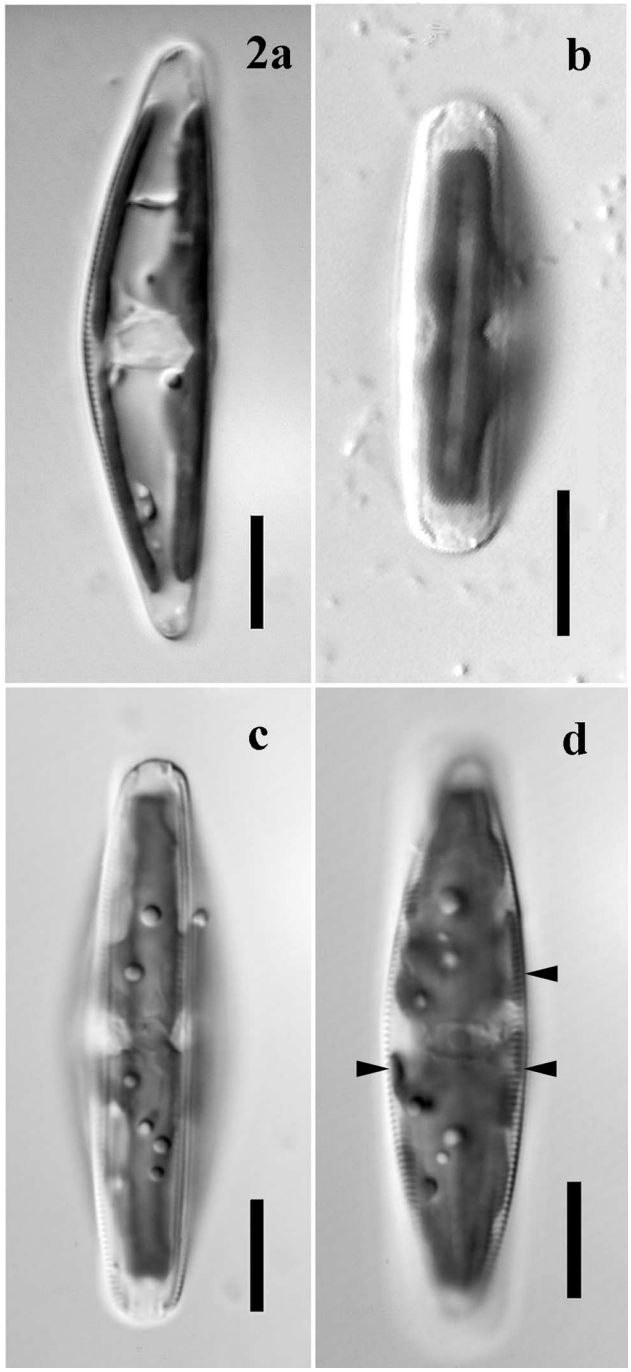

FIG. 2. Interphase cells. (a) Valve view, clone 95, showing central nucleus and two girdle-appressed chloroplasts. (b) Girdle view, clone 71: ventral chloroplast, showing its central linear pyrenoid. (c and d) Two foci of the same cell in girdle view, clone 95. (c) Ventral chloroplast (near focus) with four subcentral lobes. (d) Dorsal chloroplast (distant focus). The margins of three of the four ventral chloroplast lobes are also in focus (arrows). Scale bars, 10 μm.

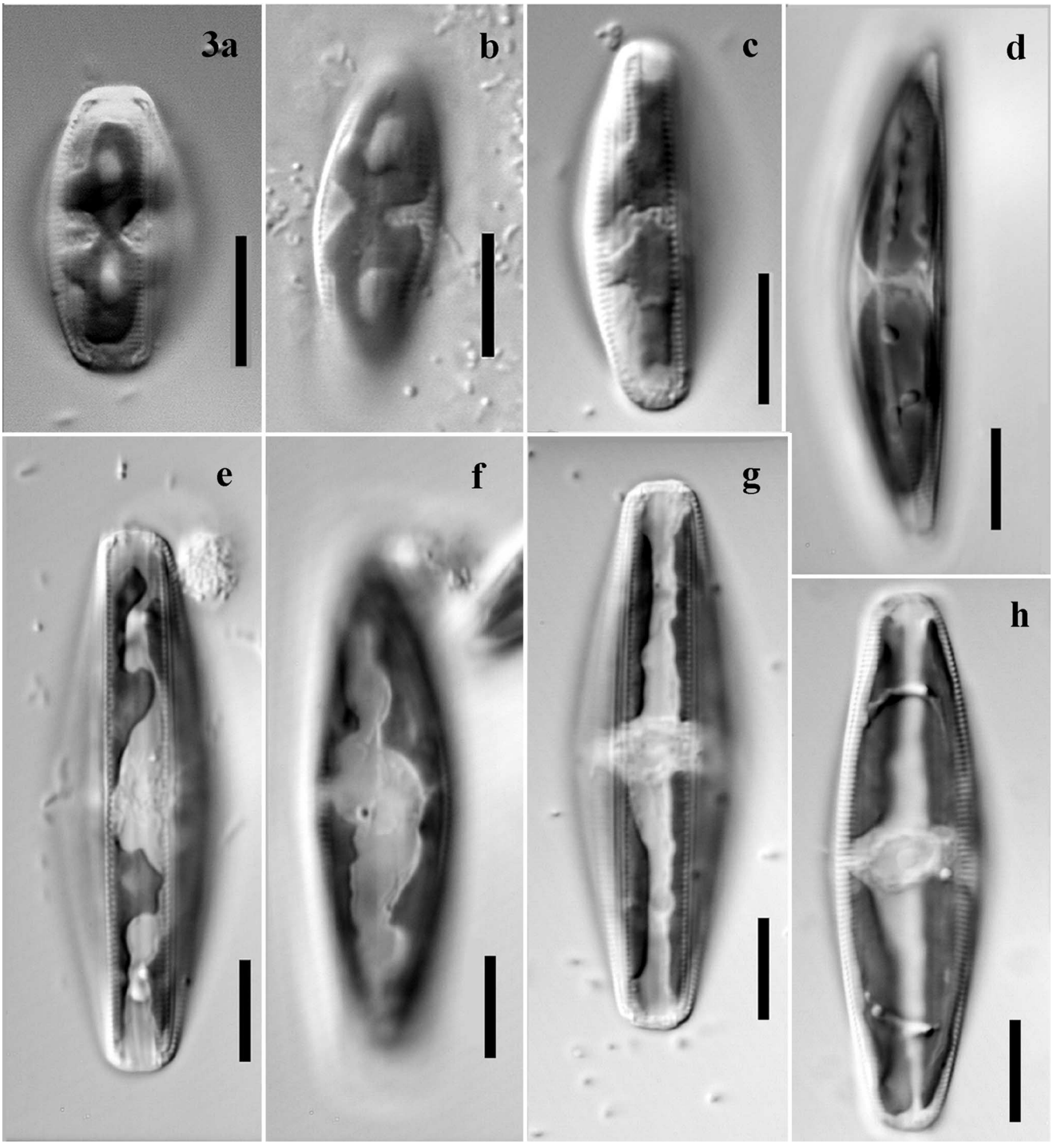

FIG. 3. Chloroplast division. (a and b) Deepening of the central constriction in the ventral chloroplast of clone 68 (a) and the dorsal chloroplast of clone 71 (b). (c and d) Recently divided chloroplasts, still appressed to the girdle, as during interphase: ventral chloroplast of clone 82 (c) and dorsal chloroplast of clone 95 (d). (e and f) Lateral translocation of recently divided chloroplast pairs from the girdle to the valves: girdle view of the same cell, from clone 95, in ventral (e) and dorsal (f) foci. (g and h) Two foci of the same cell from clone 95, in girdle view, in which translocation is complete and lobing of the ventral chloroplast has disappeared: ventral side (g) and central (nuclear) focus (h). Note the "bridges" linking the chloroplasts in (h). Scale bars, 10 μm.

During mitosis and cytokinesis, the chloroplasts did not undergo further rearrangement (Fig. 4a), so that each of the two daughter cells inherited both halves of one of the chloroplasts—dorsal or ventral—present in the interphase cell. During hypotheca formation, the chloroplasts began to move onto the sides of the girdle to reestablish the interphase configuration. Movement of the chloroplast pair represented a

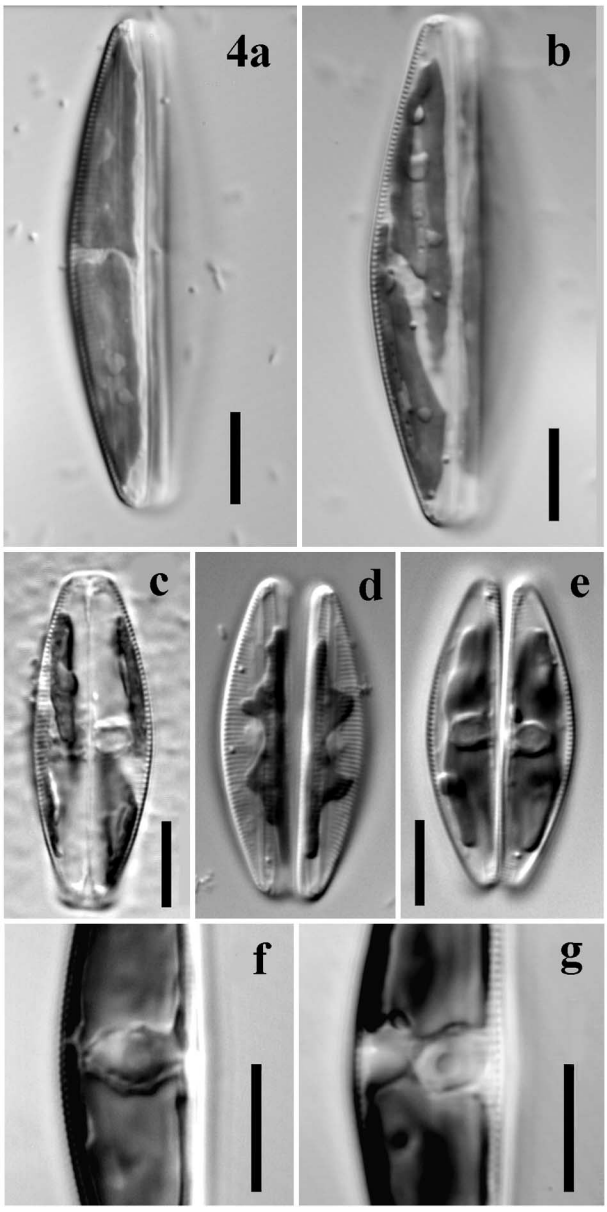

FIG. 4. (a–e) Behavior of the chloroplasts after mitosis in recently divided but unseparated sibling cells. (a) Position of the chloroplasts just after completion of mitotic division: in valve view, clone 95. (b and c) Translocation of the chloroplasts from the epivalve to the girdle, in valve view (b, clone 95) and girdle view (c, clone 75). (d and e) Sibling cells shortly before separation, in which the chloroplasts have attained their final positions, clone 83: focus on the ventral chloroplasts (d) and on the dorsal chloroplasts (e). (f and g) Nuclear movement before mitosis, valve view, clone 95. (f) Interphase. (g) Before mitotic division, with the nucleus shifted to the ventral side of the girdle. Scale bars, 10 μm.

clockwise rotation relative to the epitheca of each daughter cell (Fig. 4, b and c). The chloroplasts elongated as they moved, and once they reached the girdle the characteristic lobing reappeared, generally before the cells split apart (Fig. 4, d and e).

Nucleus. During interphase, the nucleus occupied the center of the cell, lying in a prominent cytoplasmic bridge between two large vacuoles (Fig. 4f). It was transversely elongate in valve view and contained a single prominent nucleolus. Once the chloroplasts had started to divide, the nucleus rounded up and moved toward the ventral side of the girdle (Fig. 4g). This was accompanied by changes in the cytoplasmic isthmus containing the nucleus: the dorsal part of the isthmus disintegrated into separate cytoplasmic strands, whereas the opposite part broadened (Fig. 4g). Mitosis occurred while the nucleus was appressed to the ventral side of the girdle. After cell division, the daughter nuclei remained eccentrically positioned while the epivalve was formed and the chloroplasts were translocated onto the girdle (Fig. 4, c and e). Later, the nucleus moved back to the center of the cell and its transversely elongate oval shape was reestablished.

The time scale for different phases of chloroplast and nuclear division could not be determined because cells remained motile throughout the cell cycle, as in the *Seminavis* cells studied by Danielidis and Mann (2002).

Breeding behavior. None of the 12 clones studied ever exhibited any signs of sexual activity in monoclonal culture. Transition of cells to sexual reproduction was successfully initiated only in mixed cultures of compatible clones. Four sets of crosses were made, involving all the clones in all possible pair-wise combinations; the results of three of these sets are shown in Figure 5. Sexual reproduction involved the pairing and interaction of two cells (gametangia) belonging to different clones. Where size differences were present between the cells of the clones being mated, it was clear that pairing took place only between cells of different clones. For example, in the first set of crosses (Fig. 5a), interclonal mating was proven through size differences between paired gametangia in 6 of the 11 successful combinations (83×79, 83×94, 88×68, 88×73, 95×68, 95×73). The details of sexual reproduction and auxospore development are described in a later section.

The first set of crosses (Fig. 5a), on 18 January 2001, produced positive results only in crosses that included clones 68 or 73 or 83, and they did not allow us to make firm conclusions on how the breeding system is organized. There is a "cardinal point" in the diatom life cycle corresponding to the cell size (which is considered to be species specific) below which the cells are potentially able to reproduce sexually, given appropriate environmental conditions and the presence of appropriate mates. So far, no factors have been discovered that can stimulate sexualization of the cells that are larger than this threshold size (e.g. see Drebes 1977). Our results indicate that on 18 January, 10 of the 12 clones were within the sexually inducible cell size range, although clones 71, 79, 82, 87, and 94 participated in only one successful cross. Sexual reproduction was not observed in any of the

a

68												
71	0											
73	0	0										
75	0	0	0									
79	0	0	0	0								
82	0	0	0	0	0							
87	0	0	0	0	0	0						
93	0	0	0	0	0	0	0					
94	0	0	0	0	0	0	0	0				
83	SS*	SS*	SS	0	SS*	SS	SS	0	S			
88	S	0	S	0	0	0	0	0	0	0		
95	S	0	S	0	0	0	0	0	0	0	0	
Clones	68	71	73	75	79	82	87	93	94	83	88	95
Size (µm) Range	35-38	40-45	34-38	50-54	42-45	37-39	39-42	47-53	45-48	37-40	50-53	55-57
Size (µm) Mean	35.9	42.5	36.0	51.3	43.6	37.8	40.5	49.7	46.0	38.4	51.2	55.7
Size (µm) SD	1.00	1.43	1.33	1.34	1.08	0.79	0.85	1.75	1.06	0.97	0.92	0.68
Initial cells (µm) Range	64-70	65-70			65-73							
Initial cells (µm) Mean	66.4	67.5			68.8							
Initial cells (µm) SD	1.82	1.83			3.34							
Initial cells (µm) N	14	14			30							

b

68												
71	0											
73	0	0										
75	0	0	0									
79	0	0	0	0								
2	0	0	0	0	0							
87	0	0	0	0	0	0						
93	0	0	0	0	0	0	0					
94	0	0	0	0	0	0	0	0				
83	SS	SS	SS	SS	SS	SS	SS	SS	SS			
88	S	S	SS	S	SS	SS	SS	S	SS	0		
95	S	S	S	0	S	S	S	0	S	0	0	
Clones	68	71	73	75	79	82	87	93	94	83	88	95
Size (µm) Range	30-34	37-40	30-33	45-49	39-41	32-36	39-39	45-48	40-43	31-35	45-49	50-53
Size (µm) Mean	31.8	38.9	31.3	47.1	39.4	34.6	36.7	46.0	41.3	33.5	46.6	51.4
Size (µm) SD	1.48	1.00	1.16	1.198	0.70	1.51	1.06	0.94	1.16	1.27	1.58	1.08

c

68												
71	0											
73	0	0										
75	0	0	0									
79	0	0	0	0								
2	0	0	0	0	0							
87	0	0	0	0	0	0						
93	0	0	0	0	0	0	0					
94	0	0	0	0	0	0	0	0				
83	SS	SS	SS	SS	SS	SS	SS	SS	SS			
88	SS	SS	SS	SS	SS	SS	SS	SS	SS	0		
95	SS	SS	SS	SS	SS	SS	SS	SS	SS	0	0	
Clones	68	71	73	75	79	82	87	93	94	83	88	95
Size (µm) Range	25-32	30-36	25-31	39-45	33-39	26-34	30-36	39-43	31-38	26-30	37-44	44-49
Size (µm) Mean	28.4	32.9	26.7	41.5	35.4	29.5	32.7	40.6	34.9	28.2	42.3	45.5
Size (µm) SD	2.59	2.33	2.21	2.46	1.68	2.68	2.00	1.71	2.23	1.48	2.21	1.84

mixed cultures involving clones 75 and 93. The reason is unclear, because these clones had the same cell size as, or were smaller than, clones 88 and 95, both of which participated in successful crosses (Fig. 5a).

In successful combinations, sexual reproduction was not always of the same intensity. In some cultures (83 × 94, 88 × 68, 88 × 73, 95 × 68, 95 × 73; Fig. 5a), only a very small number of cells, representing less than 1% of the total, participated in sexual reproduction, and the first cases of sexual reproduction generally appeared only 5–6 days after cultures were mixed, that is, during the later stages of exponential growth. These crosses were classified simply as successful (S). In contrast, auxosporulation was abundant in all successful crosses involving clone 83 (except 83 × 94): cells were already paired the day after the preparation of mixed cultures, while cell densities were still low. At least 10% of cells were involved in sexual reproduction. Such intensity of auxosporulation was designated as vigorous (SS).

A second set of crosses (Fig. 5b) was undertaken a month after the first, on 19 February, by which time the average cell length had declined by 3.2–4.7 μm. All clones now exhibited sexual activity in at least two crosses, and mating occurred in 25 of the 66 pair-wise combinations: positive results were obtained in all 11 cases where mating had been observed in January but also in 14 new combinations. Clones fell into two groups: a group comprising clones 83, 88, and 95 and another comprising all the remainder. Within each group clones did not mate, but except in two cases (95 × 75 and 95 × 93), clones belonging to different groups were compatible, indicating heterothally (dioecy). Where positive results had already been obtained in January, the intensity of mating tended to increase, for example, producing a change S → SS in 83 × 94 and 73 × 88. Auxosporulation was abundant in six mixed cultures in which it had not occurred at all a month before (83 × 75, 83 × 93, 88 × 79, 88 × 82, 88 × 87, and 88 × 94). In general, weaker responses were seen in crosses involving larger celled clones.

The third set of crosses (Fig. 5c) was begun on 8 April, 7 weeks after the second. Average cell size had declined by a further 3.4–6.4 μm. A clear-cut separation was now present between two groups of clones, representing two different mating types. Auxosporulation was entirely absent in intragroup mixtures but vigorous in all cases when clones belonging to different groups were mixed. There were no exceptions. Hence, clones 83, 88, and 95 are of one mating type, whereas clones 68, 71, 73, 75, 79, 82, 87, 93, and 94 belong to a second mating type.

A fourth confirmatory set of crosses was undertaken immediately after the results of the previous set had been obtained, that is, 8 days after the establishment of the third set of crosses. All results were exactly as in the third set (Fig. 5c).

Auxosporulation. In mixed cultures of sexually compatible clones, cells of opposite sexes became closely associated, usually lying parallel to each other and touching. The most frequent configuration was girdle–girdle (Fig. 6, a and b) but valve–valve (Fig. 6c) and valve–girdle (Fig. 6d) also occurred.

The first sign of differentiation in the gametangia was the rounding off and enlargement of the nucleus, which, by analogy with other pennate diatoms that have been studied in detail (e.g. *Navicula*: Mann and Stickle 1989), corresponds to the transition to prophase of meiosis I (Fig. 6, b and c). Before the first meiotic division, the chloroplasts moved from the girdle to the valves, as during the mitotic cell cycle (Fig. 6, c and d), but they did not constrict in two. Cleavage of the gametangial cell led to the formation of two protoplasts lying on either side of the median valvar plane; neither protoplast produced new siliceous wall elements. Soon after cytokinesis, each nucleus underwent meiosis II, which was not accompanied by cytokinesis. Hence, after meiosis II the gametangium contained two protoplasts, each with two haploid nuclei. The protoplasts differentiated into gametes, contracted (this process began even during meiosis II), and became partially separated from the gametangial thecae (Fig. 6e). Then the gametangia dehisced around their whole circumference (Fig. 6, e and f), and the rounded gametes from different gametangia came into contact and almost immediately fused. Plasmogamy generally occurred while both fusing gametes were still attached to the gametangial thecae (Fig. 6, g and h). In two cases that were observed from the very beginning of gamete contraction up to and including plasmogamy (which occurred nearly synchronously in both pairs of allogamously fusing gametes), the whole process took about 30 min. Occasionally, gametes separated fully from the thecae and became spherical, but these too were capable of fertilization. Once, a gametangium was observed in which the gametes became rearranged to lie either side of the median transapical plane, as is the rule in some other diatoms, for example, *Placoneis* (Mann and Stickle 1995a).

No mucilage envelope could be seen around the paired gametangia or the gametes they released. This

FIG. 5. Results of crossing experiments and apical length measurements. Black filled squares illustrate the absence of intraclonal sexual reproduction; 0, no sexual reproduction in mixed culture; S, infrequent sexual reproduction; SS, vigorous sexual reproduction. Three sets of crosses involving all possible pair-wise combinations of clones were established at different times: (a) 18 January 2001 (apical cell size was measured on 15 January 2001); (b) 19 February 2001 (measured 22 February 2001); (c) 08 April 2001 (measured 08 April 2001). Measurements are ranges (mean ± SD) for 10 cells from each of the clones. The number of initial cells measured in three mixed cultures that exhibited vigorous auxosporulation (*) is indicated (*N*).

may explain why the comparatively strict positioning of the gametangial thecae present during the early stages of sexual reproduction was often lost during the contraction of the gametes and plasmogamy. Sometimes, a moving vegetative cell passed close to or between the copulating gametangia, pushing them apart and preventing successful plasmogamy. Any tiny displacement of the coverslip could cause the same effect. No differences were observed, either in morphology or behavior, either between the two gametes of a single gametangium or between the gametes produced by different paired gametangia. Sexual reproduction is thus isogamous.

In cases where one or both of the fusing gametes remained attached to the gametangial thecae during gamete maturation, this link was lost and the zygote became spherical almost immediately (within a very few minutes) after plasmogamy (Fig. 6i). Later, in developing auxospores, it became obvious that each auxospore contains two chloroplasts and a pair of closely associated nuclei (Fig. 7, a–c); by analogy with other raphid diatoms (e.g. Geitler 1927, 1951, Mann and Stickle 1995a), these two nuclei are unfused gametic nuclei. The two other haploid nuclei from meiosis II seemed to have aborted during the initial phase of zygote development, while the zygote was still spherical. The unfused gametic nuclei lay in a pocket of cytoplasm beneath the auxospore equator on one side and remained in this position while the auxospore was expanding. The chloroplasts also lay on this side and were offset diagonally, one toward each pole (Fig. 7, c–e).

In most cases the auxospores expanded more or less parallel to one another (Fig. 7, c and e). A transverse perizonium of delicate silica bands was laid down around the auxospore as it expanded (Fig. 7d and see below). Each apex of auxospore was covered by a thin two-layered cap, derived through rupture of the organic wall around the unexpanded zygote (Fig. 7f). We do not know whether this wall also contains silica scales like those reported in *Pseudo-nitzschia* by Kaczmarska et al. (2000). Auxospores remain straight throughout expansion.

Karyogamy of the two nuclei was observed once, in an auxospore that had apparently finished expanding (Fig. 7d). Other fully expanded auxospores already contained a single nucleus, so that karyogamy occurred either at a very late stage of auxospore expansion or just after its completion. In fully developed auxospores containing a single diploid nucleus, the contents underwent a contraction, so that the protoplast became separated from the perizonium on both sides but not at the tips (Figs. 7, e and f, and 8, a and b). On the side where the nuclei and chloroplasts lay, the contraction produced an almost flat surface, which later became the ventral side of the initial cell. On the opposite side the contraction was much less at the center than elsewhere, producing a more strongly arched half-rhombic outline; this side corresponded to the future dorsal side of the initial cell (Fig. 7e, lower auxospore). After the contraction, the sequence of events resembled that reported for *Cratic-ula cuspidata* (Kützing) D. G. Mann (Mann and Stickle 1991). The chloroplasts moved from the nucleus toward the opposite side of the auxospore (Fig. 8a) and the zygotic nucleus divided acytokinetically, with abortion of one of the products, and then the initial epivalve was laid down on the side of auxospore adjacent to the remaining nucleus (Fig. 8b). "As the epitheca is completed, the chloroplasts move past each other and onto the side of the auxospore (i.e. the girdle region, as defined by the position of the initial epitheca) to take up a parallel configuration like that present during interphase in vegetative cells. Indeed, the whole behavior of the chloroplasts during the formation of the initial epitheca is reminiscent of vegetative cell division...." (Mann and Stickle 1991) (Fig. 8, c and d). Afterward, the chloroplasts moved beneath the initial epitheca, in preparation for the second acytokinetic mitosis and the formation of the initial hypotheca (Fig. 8e). After the formation of hypotheca, the chloroplasts moved to positions beneath the girdle, completing the formation of the initial cell.

It was only after the contraction of the auxospore protoplast and the formation of the initial cell that it became possible to see clearly the structure of the silicified exoskeleton of the auxospore, comprising the perizonium. The transverse perizonium (Figs. 7e, 8, a–e, and 9a) consisted of a series of well over 30 bands. The primary transverse band (i.e. the one that is deposited first, around the center of the zygote) was visibly broader the others (Fig. 9b), though not as markedly so as in, for example, *Caloneis* (Mann 1989a). During the escape of the initial cell, the transverse perizonium was ruptured at one of the poles. Then one more structure was revealed, the longitudinal perizonium, that appeared to consist of a single, elongate, smooth band (Fig. 9a); however, SEM observations are required to confirm this.

The size of the initial cells was measured in three mixed cultures and ranged from 64 to 73 μm (Fig. 5a), which is somewhat larger than the maximum size found in cells in the natural population (60.5 μm; see above).

Unusual behavior during sexual reproduction. In a few cases, a single unfused gamete developed into an auxospore, which therefore contained a single visible nucleus and a single chloroplast (Fig. 9c). This phenomenon, haploid parthenogenesis, has been reported for various pennate diatoms (reviewed in Mann 1994b). The haploid auxospore was smaller than normal diploid auxospores.

During observations of a cross between clones 83 and 87, it was once observed that, in a pair of normally developing gametangia (Fig. 9d), two gametes released by one gametangium simultaneously contacted a gamete from the other gametangium, whereupon all three fused. The triploid zygote immediately became spherical (Fig. 9e), and although it subsequently touched the remaining haploid gamete, no further fusion occurred. During the next few hours nothing changed,

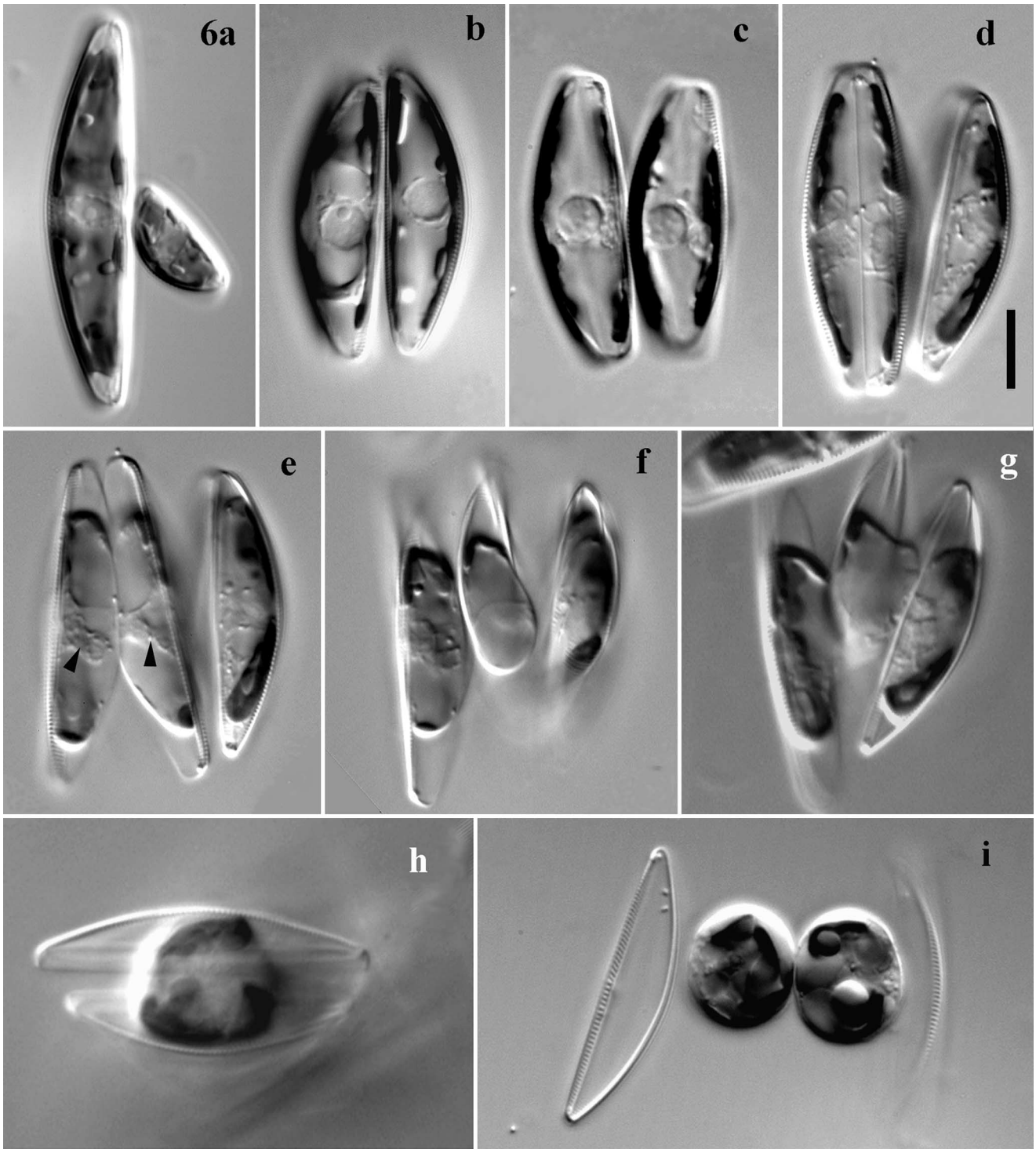

FIG. 6. Gametogenesis and plasmogamy in mixed cultures of *Seminavis* clones of opposite sexes. (a) Just after pairing of sexualized cells: clone 73 (smaller) × 95. In the larger cell (left, clone 95), the nucleus is unexpanded, that is has the "interphase" configuration. The size ratio between the cells of two clones does not correspond to the measurements given in Figure 5, because the subculture of clone 95 used for preparation of the mixed culture was kept for a few months in low light and temperature, where cells divided infrequently and hence reduced in size very slowly. (b and c) A pair of gametangia before karyokinesis of meiosis I; clone 83 (smaller) × 87. Note the enlarged spherical nuclei in meiotic prophase. (b) Valve view. The chloroplasts are still girdle appressed. Bridges of dense material linking the chloroplasts and nucleolus are visible in the left cell. (c) Girdle view. The chloroplasts have already moved onto valves. (d–h) A single pair of gametangia at different times on 29 January 2001, in cross 83 (smaller) × 79. (d) 10.20 hours: meiosis I and cytokinesis just completed. (e) 10.26 hours: meiosis II (dividing nuclei are marked with arrows) and contraction of the gametes. (f) 10.29 hours. (g) 10.35 hours: one pair of gametes has come into contact and will fuse allogamously within a few seconds. (h) 10.51 hours: the other pair of gametes has just fused, producing a somewhat irregular, rounded zygote. The first fusion caused a shift in the position of gametangial frustules, which is why this second zygote is not in focus; now it lies just below the zygote focused. (i) Zygotes lying between thecae of parental cells, 83 (smaller) × 87. Scale bar, 10 μm.

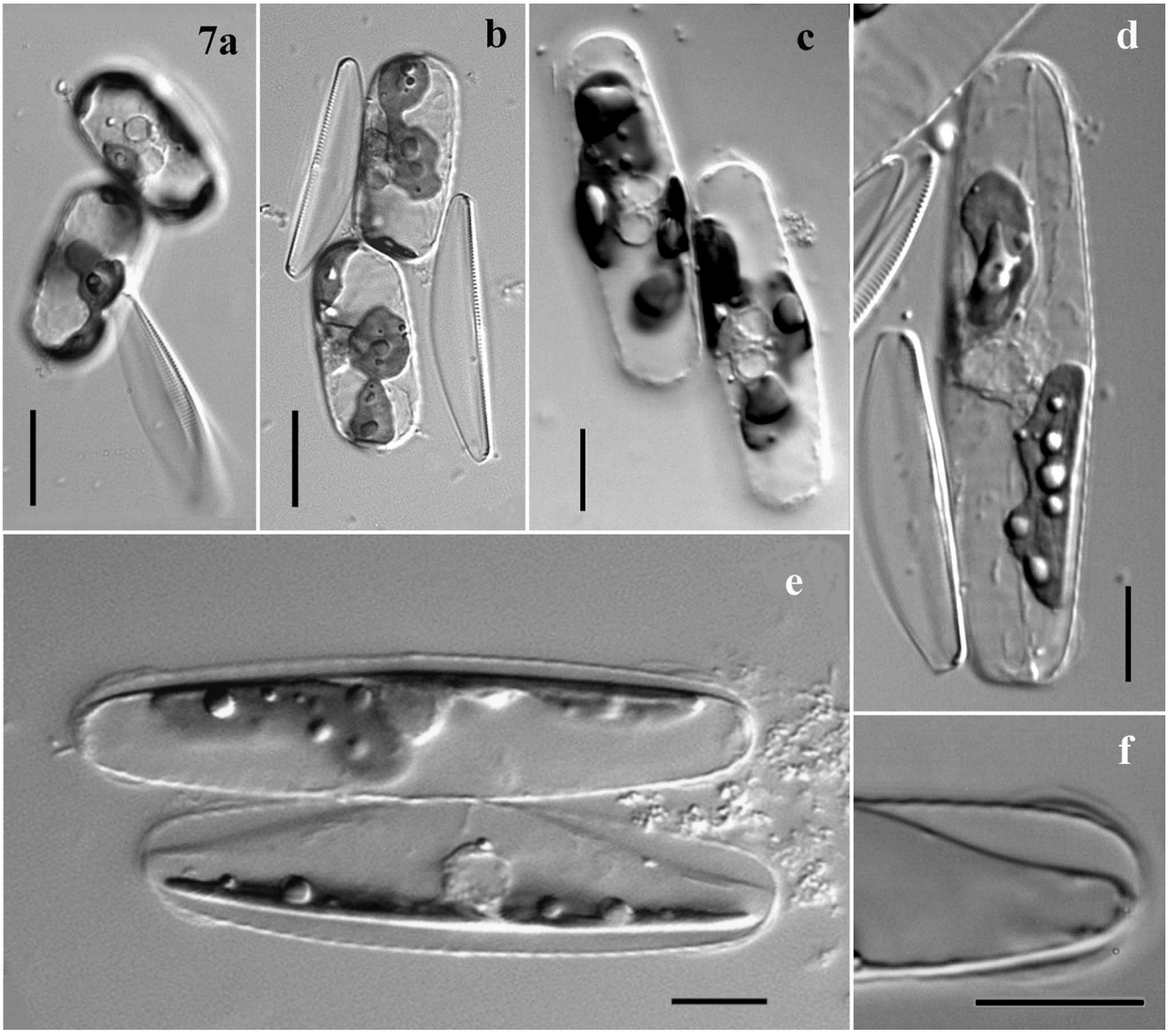

FIG. 7. Developing auxospores, cross 83 × 79. (a and b) Early stages in expansion, with two nuclei visible in the upper cell of (a). Note that the nuclei are appressed to one side of the auxospore (the left sides in b) and that the chloroplasts are centrally constricted chloroplasts (b). (c) Mid expansion: the auxospores still contain two unfused haploid gametic nuclei (at center). (d) Expanded auxospore, before protoplast contraction. The chloroplasts are arranged diagonally and lie on the same side of the auxospores as the fused zygotic nucleus. (e) A pair of fully expanded auxospores, with their contents contracting before initial valve formation; the contraction is unequal (lower auxospore). (f) Auxospore pole, bearing a cap derived from the zygote wall, consisting of two layers. Scale bars, 10 μm.

except that the remaining gamete rounded up and separated from the gametangial thecae. It is unknown whether this polyploid cell was capable of further development into an auxospore. However, on a second occasion, an expanded auxospore was found containing three chloroplasts and a single nucleus with three nucleoli, indicating that it was triploid.

DISCUSSION

Chloroplast structure and systematics. Our data reemphasize (Mann 1996b) that aspects of protoplast structure provide valuable information for diatom systematics. *Seminavis* cf. *robusta* agrees with other *Seminavis* species (Danielidis and Mann 2002) with respect to chloroplast structure and arrangement, differing only in details of chloroplast lobing. The structure, disposition, and division of the chloroplasts agrees well with what is found in *Navicula* (e.g. Mann 1989b, Mann and Stickle 1989, Round et al. 1990). *Seminavis* and *Navicula* have the same type of extremely well-defined linear pyrenoid, which runs the whole length of the chloroplast. In both, plastokinesis is "autonomous" (Mann 1996b), occurring apparently without the in-

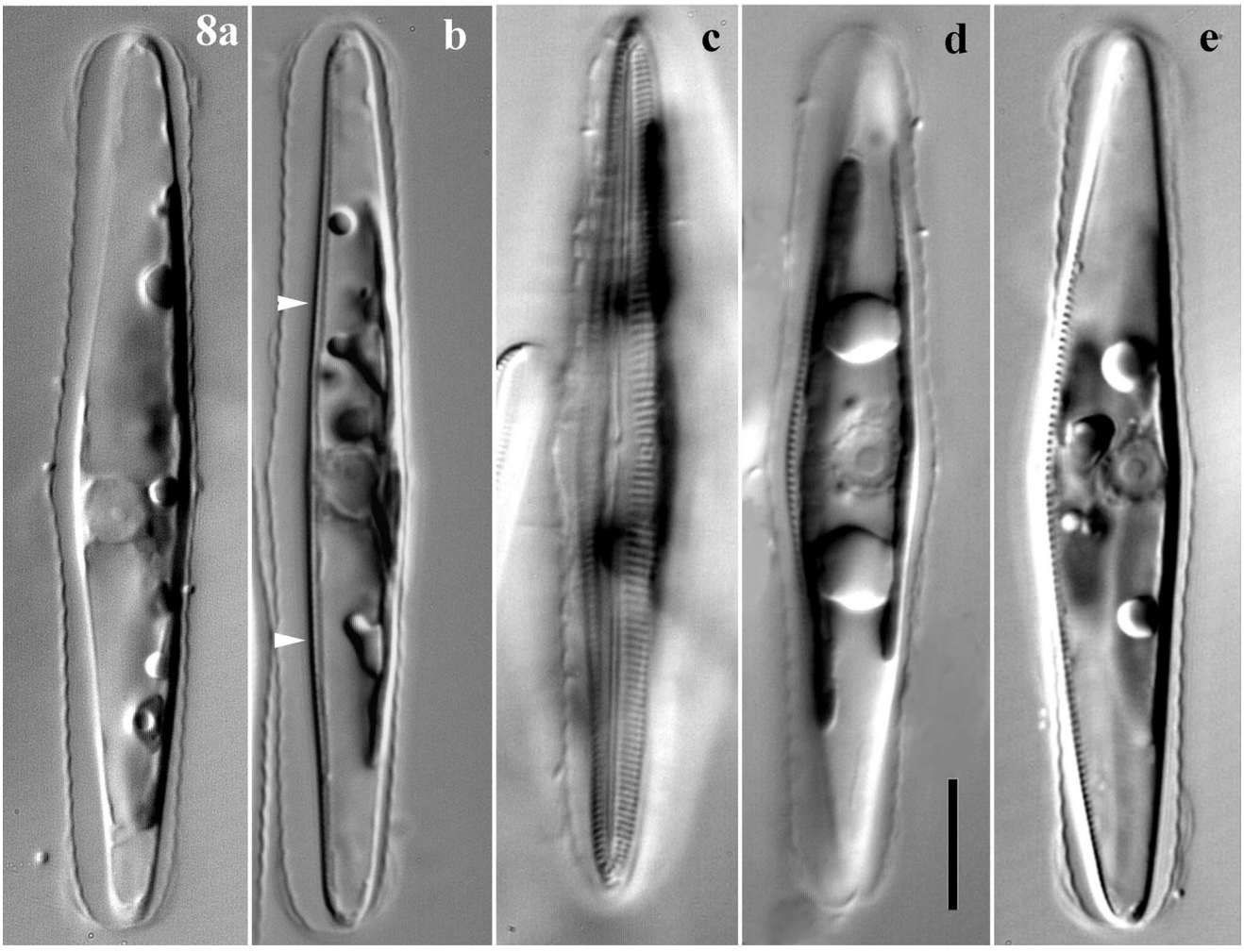

FIG. 8. Initial cell formation, cross 68 × 83. (a and b) Girdle view of a forming initial cell. (a) Just before formation of initial epitheca. The nucleus is closely associated with the (left) side where the epivalve will be laid down; the chloroplast lies opposite. (b) Epitheca (arrows) completed. (c and d) Valve view of a forming initial cell, in which the epitheca is already complete: two foci, of the epivalve (c), and of the nucleus (d), which lies distant from epivalve. The chloroplasts have shifted onto the girdle. (e) Cell just before hypotheca formation, in slightly oblique view: the nucleus lies against the ventral side (near focus, toward right), and the chloroplasts lie against the epivalve (right and distant focus). Scale bar, 10 μm.

volvement of the cleavage furrow or any other organelle (although a contractile ring is presumably present; Kuroiwa and Uchida 1996). By contrast, in some other diatoms plastokinesis is "imposed": the cleavage furrow cuts the chloroplasts in two at cytokinesis (Mann 1996b). Other features linking *Seminavis* and *Navicula* are that each of the two daughter cells inherits both halves of one of the chloroplasts present in the interphase cell (the "unique" type of chloroplast inheritance; Mann 1996b) and that chloroplast division is completed before chloroplast rotation. In *Craticula*, on the other hand, which, like most Naviculaceae, has two girdle appressed chloroplasts (it also resembles *Navicula* in several other respects and is thus an important comparator for Naviculaceae; see Mann and Stickle 1991), chloroplast division and rotation are concurrent, at least in the three species that have been studied (Mann and Stickle 1991). *Craticula* also differs from *Navicula*, *Seminavis*, and other Naviculaceae in its pyrenoids. Schmid (2001) demonstrated by impressive specific staining that a single elongate pyrenoid is present in *C. cuspidata* (she inaccurately reports one of us as stating that several pyrenoids are present in *Craticula*; see Mann and Stickle 1991, p. 81, for our actual remarks), but this is clearly a much more diffuse structure than in the Naviculaceae, because it is difficult or impossible to detect pyrenoids in the living cells of *Craticula* using interference contrast optics. By contrast, in living Naviculaceae the edges of the bar-like pyrenoid are sharp in LM and detection is easy (e.g. see Mann 1989b, Figs. 22 and 23; Mann and Stickle 1989, Fig. 10; Mann 1996b, Fig. 21, Danielidis and Mann 2002, Fig. 7; this article, Fig. 2b). The constant clockwise rotation of the chloroplast pair after cytokinesis in *Seminavis* is a further example of the chirality of chloroplast movements

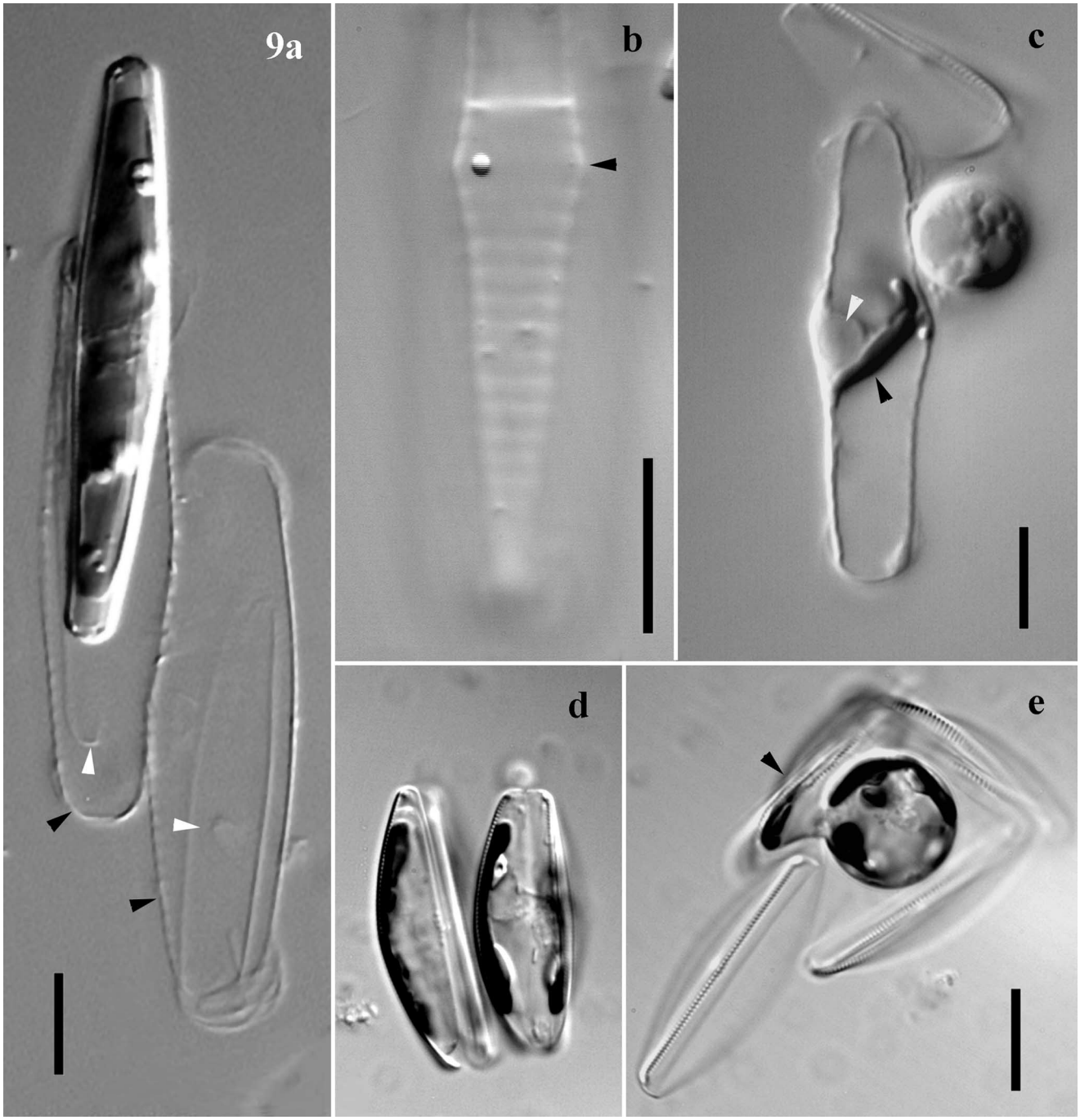

FIG. 9. Perizonium and cases of "abnormal" reproductive behavior. (a) Initial cell escaping from the perizonium (left) and the empty perizonium of its sister cell, cross 79 × 83; note transverse perizonium (black arrows) and longitudinal perizonium (white arrows). (b) Transverse perizonial bands, showing the broader central primary band (arrow); cross 68 × 83. (c) A pair of gametes from a single gametangium. One gamete (spherical) has aborted, whereas the other has developed without fertilization into an auxospore (haploid parthenogenesis). The auxospore contains a single nucleus (white arrow) and a single chloroplast (black arrow). (d and e) Formation of a triploid auxospore in a single pair of gametangia in cross 87 × 83 at different times on 25 February 2001. (d) 16.05 hours: completion of meiosis I. (e) 16.45 hours: a spherical triploid zygote has formed as a result of the simultaneous fusion of three gametes; the fourth gamete (arrow) remains attached to the theca of parental gametangium. Scale bars, 10 μm.

noted in other diatoms (e.g. Rudzki 1964, Thaler 1972, Mann 1985, 1996b).

Nuclear division. In *Seminavis ventricosa*, Danielidis and Mann (2002) noted that, during interphase, the nucleus lies in a small central bridge of cytoplasm between two vacuoles, but they were unable to determine where the nucleus lies during mitosis, because of the motility of the cells throughout cell division.

Based on the generally strong correlation between the ontogenetic asymmetry of the diatom valve and the position of the mitotic nucleus—that the primary side of the valve is formed on the side of the cell where the nucleus divides (Mann 1983, Mann and Stickle 1988, Round et al. 1990)—Danielidis and Mann predicted that mitosis in *Seminavis* occurs on the ventral side, because the terminal raphe fissures are constantly turned to the dorsal side in *Seminavis*, indicating that the dorsal side of the valve is secondary. Our observations confirm Danielidis and Mann's view. In *Navicula*, by contrast, the nucleus oscillates within the cell, dividing on alternate sides of the cell during successive cell divisions (Mann and Stickle 1988). It remains to be established which pattern is primitive within the Naviculaceae clade.

Comparisons between Seminavis and other Naviculaceae. Previous studies of the ultrastructure of the siliceous cell wall and partial information on the chloroplasts have provided strong support for a close relationship between *Seminavis* and *Navicula sensu stricto* and its allies (Round et al. 1990, Sullivan 1990, Danielidis and Mann 2002). *rbc*L gene sequence data also support a link between *Seminavis*, *Pseudogomphonema*, and *Navicula* (D. G. Mann and G. E. Simpson, unpublished data). Our information on the chloroplasts (see above) and on sexual reproduction provides a further opportunity for comparison, because data are available on auxosporulation in several species of *Navicula sensu stricto*, including detailed observations of *Navicula oblonga* (Mann and Stickle 1989, who list previous work on other species).

Mann and Stickle (1989) proposed that seven characters might be typical of sexual reproduction in *Navicula*: 1) gametangia closely associated throughout meiosis but not enclosed within an obvious sheath of mucilage; 2) chloroplast movement from the girdle to beneath the valves early in meiotic prophase; 3) delayed degeneration of the superfluous gametic nuclei, so that young auxospores are 4-nucleate (8-nucleate in taxa producing only one auxospore per pair of gametangia); 4) dehiscence of the gametangia around their whole circumference; 5) morphological and behavioral isogamy, the zygotes therefore being formed between the gametangial thecae, not within them; 6) expansion of the zygotes usually parallel to the apical axes of the gametangia; and 7) flexibility in whether or not the gametes become rearranged within their gametangia before plasmogamy (in those taxa producing two gametes per gametangium). All these characteristics are also found in *Seminavis*, with the exception of 3 (see below). Two of the seven characteristics listed by Mann and Stickle, 1 and 6, are unusual among raphid diatoms, and the correspondence between *Seminavis* and *Navicula* is further reinforced by particular observations. For example, during observations of *N. oblonga* a diatom cell was observed "moving between two paired gametangia at meiosis I" (character 1) and "the bonding between gametes and gametangia, or between zygotes and gametangia, is so feeble that the auxospores often drift far away from the cells that produced them, at least at our mounts" (character 6); these are both phenomena we have also observed in *Seminavis*. However, in *S. macilenta*, Danielidis and Mann (2002) reported a diffuse mucilage envelope around the gametangia, although they did not observe gametogenesis, and so it is possible that the mucilage was secreted not by the gametangia but by the zygote or auxospore.

The only apparently aberrant character in *Seminavis*, relative to the seven characteristics of *Navicula* selected by Mann and Stickle (1989), is 3. Further data are needed, using nuclear staining techniques, but our current view is that the abortion of superfluous nuclei may occur sooner than in *Navicula*, because auxospores of *Seminavis* that had only recently begun to expand always contained only two visible nuclei.

The pattern of reproductive behavior found in *Seminavis* and *N. oblonga* can be classified as type IB2a auxosporulation in the scheme put forward by Geitler (1973): because two isogametes are formed per gametangium, there is generally no rearrangement of the gametes within the gametangia, and there is a tendency for the auxospores to expand parallel to each other and to the long axes of the gametangia. However, the lack of a mucilage envelope and the consequent lack of constraint on the reorientation of gametangia and auxospores (e.g. see Fig. 9e) make *Seminavis* somewhat transitional between type IB2a and IC. Geitler (1973) characterized the auxosporulation of *N. radiosa* Kützing and a race of *Navicula cryptocephala* Kützing as also being of type IB2a.

In two auxospore characteristics, *Seminavis* is notably distinct from *Navicula oblonga*. The first involves the structure of the organic wall that initially surrounds the zygote and then forms caps over the ends of auxospores, after the wall's rupture (controlled breakdown?) at the beginning of auxospore expansion. In *N. oblonga*, the wall is three-layered, with thin outer and inner layers and a thick apparently mucilaginous layer in between. In *Seminavis*, the wall appears to be two-layered and a mucilage layer is apparently absent. The second very distinctive feature of *Seminavis* is the unequal contraction of the protoplast before initial cell formation, which was noted also by Danielidis and Mann (2002) in *S. macilenta*. This contraction creates the characteristic asymmetrical shape of *Seminavis* cells.

Dissimilarities between Seminavis and Amphora. The contrast in auxosporulation between *Seminavis* and *Amphora*, where *Seminavis* was formerly classified, is striking. Although both are morphologically and behaviorally isogamous, in *Amphora* the gametangia and auxospores are kept together by a mucilage sheath and expansion of the auxospores takes place at right angles to the long axes of the gametangia (e.g. Mann 1994a). Furthermore, in *Amphora* the dorsiventrality of the initial cell (and hence of the vegetative cells) is created, not by unequal contraction of the protoplast but by the controlled development of curvature in the

auxospore as it grows, so that the perizonium provides a lunate mould for the initial valves.

Breeding systems in diatoms and their study in culture. Breeding behavior remains a poorly studied field of diatom research. Among vascular plants, which have been studied much more intensively, there are many examples of three basic breeding systems, viz outbreeding, inbreeding (e.g. via selfing), and apomixis, with significant variation within each; in addition, combinations of different mating mechanisms can exist within a single plant species (Barrett 1989, Briggs and Walters 1997, Richards 1997, Holsinger 2000). The limited data show that diatoms are equally varied in their breeding systems, although until recently it was believed that they are almost uniformly monoecious (= homothallic) (e.g. see Drebes 1977) and hence likely to inbreed, especially in sessile species. Our changed perceptions have come from experimental studies of interclonal crosses, which suggest that although inbreeding does occur and is vigorous in some species (Chepurnov and Mann, unpublished observations), pennate diatoms are basically outbreeders, inbreeding often being prevented by dioecy (heterothallism) (e.g. Roshchin 1994, Davidovich and Bates 1998, Roshchin and Chepurnov 1999, Mann et al. 1999). Even where inbreeding is permitted by the mating system, outbreeding may be the rule not the exception, so that enforced inbreeding or selfing causes inbreeding depression (Chepurnov and Mann 1997, 1999, 2000).

We offer the following practical recommendation. If monoclonal cultures of a pennate diatom species fail to exhibit auxosporulation and it is likely (e.g. from observations of auxosporulation in mixed natural populations or rough cultures) that cells are already within the sexually inducible size range, then the diatom is probably dioecious and sexual reproduction should be sought by mixing different monoclonal cultures. There is as yet little evidence that auxosporulation of pennate diatoms requires special inductive conditions (e.g. lack of N), and so lack of auxosporulation in vigorously growing cultures is likely to be significant (e.g. see Drebes 1977, Roshchin 1994, Chepurnov and Mann 1997, 1999, 2000, Davidovich and Bates 1998, Mann et al. 1999, the present investigation). Because of the chance that all clones isolated may be of the same mating type, at least seven clones are necessary before one can be reasonably certain of having both mating types, assuming a bipolar mating system and a 1:1 ratio of mating types in natural populations. Dioecy seems to be obligate in the *Seminavis* cf. *robusta* we investigated, or at least in the population from which the clones were isolated, because no intraclonal reproduction was found.

Changes in the intensity of the sexual response during the life cycle. Our experience of various pennate diatoms has shown that the frequency of auxosporulation is normally vigorous in outbreeders during most of the sexually inducible size range, when compatible clones are crossed. However, in clones of *Seminavis* (Fig. 5) and other diatoms (our unpublished observations), we observed the biggest cells that have only just crossed the sexual size threshold exhibit less frequent sexual reproduction than smaller cells. The intensity of auxosporulation then increases as the clones age and the cells get smaller; intensity increases more quickly in some species and more slowly in others. Two mechanisms may be involved in *Seminavis*. First, clones obey the general MacDonald–Pfitzer rule (e.g. Geitler 1932, Drebes 1977), by which the size distributions of cells in size correspond to a binomial expansion, because only one of the two sibling cells formed as a result of mitotic division is smaller than the parent cell (that sibling inheriting the parental hypotheca). Hence, even if the sexual size threshold is a simple on/off switch, a clone will nevertheless show a gradual increase in sexualization with time. Second, as in various other eukaryotic organisms, cells may be capable of responding to induction signals only during a restricted part of their cell cycle, of variable duration: in the centric diatom *Thalassiosira weissflogii* Grunow the inducible region is located in early G1 phase (Armbrust et al. 1990), the S, G2, and M phases being unresponsive. In *Seminavis*, as well as in other dioecious pennate diatom species, an inductive signal is presumably transmitted from cells of the opposite mating type, and it is possible that the progressive ease of sexualization as cells get smaller reflects a progressively wider "window" for induction within the G1 phase, after they have passed the critical size threshold, which, in *Seminavis* cf. *robusta*, appears to be 50 μm.

Haploid parthenogenesis and polyploidy. The formation of polyploids and the development of unfused gametes into auxospores (haploid parthenogenesis) have now been reported in several pennate diatoms that otherwise exhibit "normal" allogamous reproduction (Mann 1994b, this study). The few chromosome counts for diatoms (reviewed by Kociolek and Stoermer 1989) indicate that polyploidy may be an important mechanism in diatom speciation and evolution, as in higher plants (e.g. Briggs and Walters 1997), because there is wide variation in diploid chromosome numbers (from 8 to 130 in pennate diatoms). The evolutionary significance of haploid parthenogenesis also cannot be excluded, because haploid auxospores can give rise to initial cells and undergo division. This phenomenon has been reported for the araphid species *Licmophora ehrenbergii* (Kützing) Grunow (Roshchin and Chepurnov 1994) and *L. abbreviata* C. Agardh (Chepurnov in Roshchin 1994) and the raphid diatom *Achnanthes longipes* C. Agardh (Chepurnov and Roshchin 1995). In *L. ehrenbergii*, two initial cells derived from haploid auxospores gave rise to clonal cultures that could be maintained and grown for a few months. In *Dickieia ulvacea* Berkeley ex Kützing, Mann (1994b, Figs. 58 and 59) observed the direct reversion of an unfused gamete to become a vegetative cell, accompanied by valve formation, but this was not observed to divide.

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