Variation in the sexual behaviour of *Achnanthes longipes* (Bacillariophyta). III. Progeny of crosses between monoecious and unisexual clones

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(*Received 6 September 1999; accepted 5 May 2000*)

The allogamous raphid diatom *Achnanthes longipes* C. A. Agardh possesses a complex breeding system involving interactions between three types of clone: monoecious, unisexual and bisexual. Previous studies showed that these three types can be crossed with each other, with a tendency for sexual characteristics to be inherited: inbred monoecious lineages gave rise to monoecious or, very rarely, to bisexual clones, while inbred unisexual lineages yielded unisexual and bisexual clones. The current paper reports on the progeny of crosses between monoecious and unisexual clones and their inbred offspring. All three types of clone appeared in the F_1 and F_2, although unisexual clones of opposite sex to the parental clone were not found. Inbreeding depression was observed and also a tendency for 'normal' auxosporulation (producing two auxospores per pair of gametangia) to be replaced by 'reduced' or 'intermediate' auxosporulation (producing one auxospore per pair). In addition, patterns of incompatibility were observed that were not seen during earlier studies of clones isolated directly from nature. These included the inability of some F_1 clones to mate with each other, in spite of compatibility with all other clones examined (unisexual, bisexual and monoecious).

Key words: *Achnanthes*, auxosporulation, Bacillariophyta, breeding system, diatom, inbreeding, sexual reproduction

Introduction

This paper continues a series devoted to the life cycle and sexual reproductive biology of the cosmopolitan marine diatom *Achnanthes longipes* C. A. Agardh (Roshchin, 1984, 1994*a*, *b*; Roshchin & Chepurnov, 1992, 1999; Chepurnov & Roshchin, 1995; Chepurnov & Mann, 1997, 1999). This species has proven to have a very complex and variable reproductive biology. Clones isolated from natural populations along the northern Black Sea coast at Karadag, Crimea, can be classified into three groups (Chepurnov & Roshchin, 1995; Chepurnov & Mann, 1997). Monoecious clones exhibit relatively high levels of intraclonal reproduction, are compatible with each other, and are also compatible with other types of clone (i.e. they are panmictic). Unisexual clones exhibit low levels of intraclonal reproduction and occur as one of two sexes; among unisexual clones, clones of one sex will only mate vigorously if mixed with clones of the opposite sex. Bisexual clones also exhibit low levels of intraclonal reproduction, but can mate with either type of unisexual clone or with monoecious clones. By contrast, most previous studies of breeding systems among diatoms have revealed much simpler reproductive systems, usually involving monoecy (Geitler, 1932; Wiese, 1969; Drebes, 1977), but with increasing evidence for dioecy in pennate genera (e.g. Roshchin, 1994*a*; Davidovich & Bates, 1998; Mann *et al.*, 1999).

Achnanthes longipes exhibits considerable variation in the pattern of sexual reproduction, even in crosses between the same pair of clones or among intraclonal pairs in monoecious clones, and automixis, polyploidy and parthenogenesis have also been recorded (references as above). There are three allogamous methods of auxosporulation, which differ in the numbers of gametes and zygotes produced; all of them involve isogamy. In the first variant, two gametes are produced per gametangium, which fuse to produce two zygotes (auxospores), provided that none of the gametes abort. This is the 'normal' type of auxosporulation as defined by Hustedt (1930) and Geitler (1932, 1973) (see also Mann, 1993). In the second variant, only one gamete is produced by each gametangium and hence only one auxospore is formed; this is the 'reduced' type of auxosporulation (Mann, 1993). Finally, there is an intermediate type of auxosporulation, in which one of the gametangia produces two gametes while the other produces one. In this case, as in the 'reduced' type, only one diploid auxospore is formed; the superfluous gamete aborts or, very occasionally, develops into a stunted auxospore through haploid parthenogenesis (Chepurnov & Roshchin, 1995). The 'intermediate' mode of sexual reproduction is not among the many types of auxosporulation listed by Geitler (1973).

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We have already reported on the reproductive and other characteristics of the progeny of dioecious (Roshchin, 1994*b*; Chepurnov & Roshchin, 1995) and monoecious lineages (Chepurnov & Mann, 1999). In the progeny of dioecious lineages we only obtained dioecious or bisexual clones; in the progeny of monoecious clones, resulting either from intraclonal reproduction or from crosses between different monoecious clones, there were only monoecious or, very rarely, bisexual clones. In the present paper we complete this preliminary analysis of the inheritance of sexual characteristics in *A. longipes* by reporting the results of crosses between monoecious and unisexual clones.

Material and methods

The clones used in these experiments were derived from the same clones studied previously (Chepurnov & Mann, 1997, 1999), which were isolated from Black Sea microphytobenthos at Karadag, Crimea (Ukraine) in April and May 1993. Their sexual characteristics were determined through observations of monoclonal cultures and by exhaustive crossing experiments (Chepurnov & Mann, 1997, 1999). For the present paper, we studied the progeny of crosses between the monoecious clone 6 and the unisexual clone 10.

The sexuality of progeny clones was determined by mating them with the parental clones and also with other clones of known sexuality (Tables 3, 5). These comprised clones 4 (monoecious), 7 and 8 (unisexual clones of opposite sex to clone 10), and three monoecious clones, MI(1A), MI(2A) and MI(4A), which were F_1 clones derived through monoecious reproduction of clone 6 (Chepurnov & Mann, 1999).

The procedure for obtaining new clones after auxosporulation was as follows. Commonly, each pair of gametangia is attached to the substratum (in this case, the bottom of the Petri dish) by a mucilage stalk formed by one of the gametangia (Roshchin, 1994*b*; Chepurnov & Roshchin, 1995). The mucilage stalk elevates the gametangia into the water column and so it is fairly easy to detach the pair of cells using a microneedle (while observing the process with a binocular microscope) and transfer them into another dish by glass micropipette. The two initial cells produced during 'normal' auxosporulation generally begin to move at different times and hence do not leave the perizonium simultaneously. This makes it easier to separate the initial cells and isolate sibling clones. To obtain the F_1 of clone 6 × clone 10, we isolated four pairs of initial cells into separate Petri dishes between 08.00 and 08.30 hours on 6 December 1993. The pairs were then observed hourly until the first of the initial cells left the perizonium and moved away from the other initial cell and the gametangial frustules. As soon as this occurred, the remaining initial cell was transferred into another dish, while still 'attached' to the gametangia. By 17.00 hours each of the eight initial cells had been transferred to its own separate dish.

Other methods, including culture conditions and media, protocols for crossing experiments and microscopical techniques, have been described by Chepurnov & Mann (1997). Descriptions of the monoecious lineages derived from clone 6, including clones MI(1A), MI(2A) and MI(4A), have been given by Chepurnov & Mann (1999). Mean cell lengths are based on measurements of 10 cells.

Results

First generation (*MU1*)

On 2 December 1993, monoecious clone 6 and unisexual clone 10 were inoculated together into a mixed culture. By then, cell lengths in these clones were 19–32 μm (mean = 24·1, SD = 3·82) and 33–42 μm (mean = 36·8, SD = 2.53), respectively. By 5 December, vigorous pairing had occurred between cells of the different clones and in some cases zygotes were present, or even expanding auxospores. Although the two clones differed markedly in mean cell length, the largest cells of clone 6 were almost the same size as the smallest cells of clone 10. So, even though intraclonal reproduction (within clone 6) was much rarer than pairing between different clones, we restricted our observations to those pairs in which one gametangium was longer than *c.* 35 μm (and must therefore have belonged to clone 10) and the other was smaller than *c.* 30 μm (undoubtedly clone 6).

In crosses between clones 6 and 10, the 'normal' type of reproductive behaviour was predominant. Examination of 204 pairs of gametangia on 6 December 1993 revealed that 77·5% had performed 'normal' sexual reproduction, with the production of two auxospores (Chepurnov & Mann, 1997, table 3). The 'reduced' type of auxosporulation occurred in only 1·5% of pairs, the remainder (21%) being examples either of the 'intermediate' type of auxosporulation, or of 'normal' auxosporulation in which one auxospore had aborted.

Four pairs of initial cells were isolated (see Material and Methods) and gave rise to clones MU1(1A) and MU1(1B), MU1(2A) and MU1(2B), MU1(3A) and MU1(3B), and MU1(4A) and MU1(4B). Each clone proved to be viable and at first there were no obvious differences between them in the rate or manner of growth. In each case the clones grew mainly as motile solitary cells, sometimes forming stalks and rarely giving rise to short chains of less than 10 cells. Cell length was measured at intervals, from 21 December onwards. To avoid having to wait for months while the cells reduced in size sufficiently to pass the sexual size threshold, we employed methods to shorten the life cycle, through abrupt size reduction (Roshchin & Chepurnov, 1992; Chepurnov & Mann, 1997). New subcultures were then isolated and their sizes are given in Table 1.

After abrupt cell size reduction there were some changes in the character of growth, with tufted aggregations of cells appearing in seven of the eight clones. In a previous study we found similar tufts in cultures of

Table 1. Characteristics of F_1 (MU1) generation clones of *Achnanthes longipes* obtained after crossing two natural clones, monoecious clone 6 and unisexual 10

	Cell lengths[a] (μm)				Monoecious reproduction	
Clone	At isolation	After abrupt cell size reduction	When cells first introduced into mixed culture	Maximum for successful interbreeding	Presence	Maximum length of cell (μm) reproducing monoeciously[a]
MU1 (1A)	134·4 (1·18, 133–137)	77·9 (0·92, 74–80)	71·5 (1·64, 69–74)	65·4 (3·31, 61–70)	0	
MU1 (1B)	130·9 (1·80, 128–134)	45·0 (0·82, 44–46)	40·1 (2·43, 36–44)	40·1 (2·43, 36–44)	+	40·1 (2·43, 36–44)
MU1 (2A)	137·0 (0·67, 136–138)	60·0 (1·64, 58–63)	53·5 (1·91, 50–55)	53·5 (1·91, 50–55)	+	47·7 (1·71, 45–50)
MU1 (2B)	134·0 (2·27, 130–137)	53·1 (1·00, 52–55)	46·9 (2·08, 44–50)	46·9 (2·08, 44–50)	0	
MU1 (3A)	133·5 (1·78, 130–135)	30·1 (1·11, 29–32)	30·1 (1·11, 29–32)	30·1 (1·11, 29–32)	+	30·1 (1·11, 29–32)
MU1 (3B)	132·8 (1·04, 132–135	40·7 (2·87, 35–44)	40·7 (2·87, 35–44)	40·7 (2·87, 35–44)	0	
MU1 (4A)	135·4 (0·97, 133–136)	75·7 (1·78, 73–78)	62·1 (2·99, 57–67)	62·1 (2·99, 57–67)	+	47·6 (3·18, 44–51)
MU1 (4B)	122·7 (2·54, 118–125)	48·1 (2·34, 44–50)	42·1 (1·53, 40–45)	42·1 (1·53, 40–45)	0	

+, monoecious (intraclonal) reproduction occurs; 0, intraclonal reproduction entirely absent.
[a] Lengths are the mean (SD, range) for 10 cells. Maxima apply to the *cultures* in which monoecious reproduction or interbreeding occurred.
All clones were isolated on 6.12.93.

monoecious clones (Chepurnov & Mann, 1997, fig. 2) but these were less diffuse and had more densely clustered stalks than the tufts formed by the MU1 clones. Only one clone, MU1(2B), continued to grow in the same manner as before abrupt size reduction. Clones MU1(3B) and MU1(4A) formed very long ribbon-like colonies, sometimes containing hundreds of cells, like those illustrated by Chepurnov & Mann (1999, fig. 2) in the F_1 generation derived from crosses of two monoecious clones. The new patterns of growth remained constant within each clone until they reached the size range for vegetative enlargement (below 20 μm in length), when the cells tended to lose their motility and also the ability to produce mucilage stalks (see Roshchin & Chepurnov, 1992; Roshchin & Chepurnov in Roshchin, 1994*a*; Chepurnov & Mann, 1997).

Monoecious reproduction in the MU1 generation. In each of the four pairs of sibling clones, one of the clones proved to be capable of limited monoecious (intraclonal) sexual reproduction while the other did not (Table 1). In clones MU1(1B) and MU1(3A) monoecious reproduction took place soon after abrupt size reduction, when cell lengths were *c*. 40 μm and 30 μm long, respectively. However, in clones MU1(2A) and MU1(4A) monoecious reproduction was delayed. Clones MU1(2A) and MU1(4A) had been reduced much less by abrupt size reduction, to *c*. 60 and 76 μm, respectively, and intraclonal reproduction did not occur until the cells had reduced in length to *c*. 48 μm.

In the four clones where monoecious reproduction occurred, monoecy occurred regularly and predictably after re-inoculation of cells into fresh medium. However, monoecious reproduction was generally infrequent and quite often there were only a few pairs of gametangia within each 90 mm diameter Petri dish. This stands in sharp contrast to the frequencies we have observed in monoecious clones isolated from nature, where up to 16% of cells can be involved in intraclonal reproduction at any one time. Indeed, the levels of monoecious reproduction found in clones MU1(1B), MU1(2A), MU1(3A) and MU1(4A) were similar to those found in bisexual and unisexual clones (Chepurnov & Mann, 1997, 1999). Clones MU1(1B), MU1(2A) MU1(3A) and MU1(4A) all stopped reproducing monoeciously when the cells had decreased to below 20 μm in length, i.e. when they had reached the size range for vegetative enlargement (Roshchin & Chepurnov, 1992; Chepurnov & Mann, 1997).

During intraclonal reproduction the commonest type of auxosporulation was the 'normal' type (Table 2), but both the other types of behaviour ('reduced' and 'intermediate') were also found. In our earlier studies of intraclonal reproduction in monoecious clones of *Achnanthes longipes* we found that, where gametangia produce two auxospores, one of them is always non-viable (Chepurnov & Mann, 1999). The same rule seems to apply in the four monoeciously reproducing MU1 clones, since in a high proportion of pairs exhibiting 'normal' auxosporulation one of the auxospores began to degenerate before expansion was complete (Table 2; contrast the interclonal crosses shown in Table 4). However, in some cases both auxospores develop, at least to the stage where initial cells are formed. Two pairs of initial cells were isolated

Table 2. *Achnanthes longipes*: patterns of auxosporulation during monoecious reproduction of clones MU1(1B) and MU1(2A) in pure culture

				Type of auxosporulation			
				Type IC ('normal')			
				Two expanded auxospores[b]			
Date	Clone	Range of cell length[a] (μm)	n	Both developed	One degenerating	One expanded auxospore[b, c]	Type IIA ('reduced')
28.2.94	MU1(1B)	36–44	9	22%	33%	33%	11%
28.3.94	MU1(2A)	44–48	28	36%	43%	10.5%	10.5%
8.5.94	MU1(2A)	26–38	29	21%	21%	3%	55%

[a] Measurements of cell length ($n = 10$) were made within ± 2 weeks of the date on which observations of auxosporulation were made.
[b] Counts refer to pairs of copulating cells, not individual auxospores.
[c] The single expanded auxospore was accompanied either by an aborted zygote or two non-copulating gametes, indicating that the type of auxosporulation was of type IC ('normal'), or by an aborted gamete, reflecting the 'intermediate' type of auxosporulation, where one of the paired gametangia forms two gametes and the other only one; the presence of a second zygote or superfluous gamete demonstrated clearly that auxosporulation was not of the reduced type where each gametangium produces only one gamete.

Table 3. *Achnanthes longipes*: results of crosses among clones of the MU1 generation, and between MU1 clones and other clones of known sexuality

		Clone							
Clone	Sexuality	MU1(1B)	MU1(2A)	MU1(3A)	MU1(4A)	MU1(1A)	MU1(2B)	MU1(3B)	MU1(4B)
MU1(1B)		[trace]							
MU1(2A)		++	[trace]						
MU1(3A)		++	++	[trace]					
MU1(4A)		++	++	++	[trace]				
MU1(1A)		+	+	+	+	[0]			
MU1(2B)		++	++	++	++	++	[0]		
MU1(3B)		++	++	++	++	++	0	[0]	
MU1(4B)		++	++	++	++	++	0	0	[0]
4	Monoecious	++	++	++	++	++	++	++	++
MI(1A)	Monoecious	++	++	NC	++	++	++	++	++
MI(2A)	Monoecious	++	++	NC	++	++	++	++	++
MI(4A)	Monoecious	++	++	++	++	++	++	++	++
7	Unisexual-1	++	++	++	++	++	++	++	++
8	Unisexual-1	++	++	++	++	++	++	++	++
10	Unisexual-2	++	++	++	++	0	++	++	++

Unisexual-1, unisexual clone of sex 1; unisexual-2, unisexual clone of sex 2.
NC, cross not made; +, infrequent interclonal mating; ++, vigorous interclonal mating; 0, clones incompatible; [trace], very limited monoecious reproduction in monoclonal culture; [0], monoecious reproduction is absent.

following 'normal' auxosporulation during monoecious reproduction in clone MU1(4A). In both cases only one initial cell was able to multiply successfully, while the other died without dividing.

Interclonal reproduction of MU1 clones. Soon after the MU1 clones had been manipulated to reduce their size through abrupt size reduction, they were mated with each other and with other available clones. Seven of the eight were immediately able to reproduce sexually in at least some combinations on 28 February 1994, but clone MU1(1A) showed no signs of sexualization during attempts to cross it with other clones. Thus, for example, it failed to interact at all with the monoecious, and hence panmictic, clone 4 (a clone isolated directly from nature), whereas clone 4 mated successfully with all of the other seven MU1 clones. MU1(1A) was then 69–74 μm long and was the largest-celled of the clones. By 21 March, MU1(1A) had reduced to 61–70 μm and it was then able to cross with clone 4 and other clones. The largest gametangium of MU1(1A) found in March was 69 μm; hence it is likely that MU1(1A) was still just above the sexual size threshold in February, immediately after abrupt size reduction.

Each of the four MU1 clones exhibiting limited monoecious reproduction in monoclonal culture (clones MU1(1B), MU1(2A), MU1(3A) and MU1(4A)) was able to mate with any other clone, regardless of its sexuality, and auxosporulation was always vigorous (Table 3). Of the

Table 4. *Achnanthes longipes*: patterns of auxosporulation in crosses between MU1 clones and various other clones of known sexuality

						Auxosporulation			
						Type IC ('normal') or 'intermediate'			
						Two expanded auxospores[b]			
Date	MU1 clone	Range of cell length[a] (μm)	Partner clone	Range of cell length[a] (μm)	n	Both developed (%)	One degenerating (%)	One expanded auxospore[b, c] (%)	Type IIA ('reduced') (%)
Crosses between unrelated clones									
28.3.94	MU1(1A)	57–63	4 (monoecious)	27–35	125	70·4	0	24·0	5·6
28.2.94	MU1(1B)	36–44	4 (monoecious)	38–43	146	74·0	2·0	19·9	4·1
25.2.94	MU1(2A)	58–63	4 (monoecious)	38–43	25	60·0	0	28·0	12·0
28.2.94	MU1(2B)	44–50	4 (monoecious)	38–43	167	91·6	0	5·4	3·0
21.3.94	MU1(3A)	20–26	4 (monoecious)	27–35	120	71·8	1·7	20·0	7·5
15.3.94	MU1(3B)	27–36	4 (monoecious)	38–43	49	71·4	0	22·4	6·2
28.2.94	MU1(4A)	57–67	4 (monoecious)	38–43	112	71·4	2·7	24·1	1·8
21.2.94	MU1(4B)	44–50	4 (monoecious)	38–43	106	84·9	0	12·3	2·8
14.3.94	MU1(2A)	45–50	7 (unisexual-1)	18–27	48	77·1	0	16·7	6·2
28.2.94	MU1(2B)	44–50	7 (unisexual-1)	18–27	149	61·8	1·3	29·5	7·4
21.2.94	MU1(4B)	44–50	7 (unisexual-1)	18–27	77	51·9	0	36·4	11·7
28.2.94	MU1(2B)	44–50	8 (unisexual-1)	27–35	162	59·9	1·9	33·3	4·9
4.3.94	MU1(4B)	40–45	8 (unisexual-1)	27–35	22	50·0	0	45·5	4·5
Crosses between MU1 clones and related inbred clones									
28.3.94	MU1(1A)	57–63	MI(1A) (monoecious)	48–54	45	46·7	2·2	42·2	8·9
14.3.94	MU1(2A)	45–50	MI(1A) (monoecious)	59–63	44	25·0	0	59·1	15·9
21.3.94	MU1(2B)	34–44	MI(1A) (monoecious)	48–54	94	54·2	0	30·9	14·9
25.3.94	MU1(1A)	57–63	MI(2A) (monoecious)	24–28	22	22·7	0	63·5	13·6
28.4.94	MU1(2A)	25–38	MI(2A) (monoecious)	25–31	98	37·8	1·0	37·8	23·5
28.2.94	MU1(2B)	44–50	MI(2A) (monoecious)	30–34	47	31·9	0	42·6	25·5
21.2.94	MU1(4B)	44–50	MI(3A) (monoecious)	36–42	72	54·2	0	33·3	12·5
28.2.94	MU1(2B)	44–50	MI(4A) (monoecious)	42–46	32	46·9	0	37·5	15·6
21.3.94	MU1(3A)	20–26	MI(4A) (monoecious)	28–37	83	36·1	6·0	33·7	24·1
Backcrosses to parental clone 10									
21.3.94	MU1(1B)	24–31	10 (unisexual-2)	33–38	126	1·6	4·8	42·1	51·6
7.3.94	MU1(2A)	50–55	10 (unisexual-2)	15–27	54	18·5	27·8	37·0	16·7
21.3.94	MU1(3A)	20–26	10 (unisexual-2)	33–38	88	8·0	1·1	34·1	56·8
21.3.94	MU1(3B)	27–36	10 (unisexual-2)	33–38	115	27·8	1·8	37·4	33·0
7.3.94	MU1(4A)	57–67	10 (unisexual-2)	15–27	71	46·5	5·6	43·7	4·2
Crosses between MU1 clones									
28.3.94	MU1(1A)	57–63	MU1(2B)	34–44	68	36·8	10·3	33·8	19·1
25.3.94	MU1(1A)	57–63	MU1(3B)	27–36	67	68·7	1·5	17·9	11·9
28.3.94	MU1(1A)	57–63	MU1(4B)	29–36	46	32·6	4·3	52·2	10·9
10.3.94	MU1(1B)	36–44	MU1(2A)	45–50	42	40·5	19·0	35·7	4·8
28.2.94	MU1(1B)	36–44	MU1(2B)	44–50	79	43·0	25·3	22·8	8·9
21.3.94	MU1(1B)	24–31	MU1(3A)	20–26	86	16·3	11·6	38·4	33·7
21.2.94	MU1(1B)	44–46	MU1(4B)	44–50	54	44·4	29·6	22·2	3·7
28.2.94	MU1(2A)	50–55	MU1(2B)	44–50	224	53·6	24·1	17·0	5·4
15.3.94	MU1(2A)	45–50	MU1(3B)	27–36	94	54·3	2·1	31·9	11·7
21.3.94	MU1(2A)	45–50	MU1(4A)	48–58	54	46·3	18·5	31·5	3·7
21.2.94	MU1(2A)	58–63	MU1(4B)	44–50	53	49·1	34·0	9·4	7·5
21.3.94	MU1(3A)	20–26	MU1(4A)	48–58	41	61·0	2·4	26·8	9·8
21.3.94	MU1(3A)	20–26	MU1(4B)	29–36	66	33·3	7·6	34·8	24·2
15.3.94	MU1(3B)	27–36	MU1(4A)	48–58	24	29·2	0	50·0	20·8
4.3.94	MU1(4A)	57–67	MU1(4B)	27–35	38	73·7	0	21·1	5·3
28.4.94	MU1(4A)	44–51	MU1(4B)	12–23	119	55·5	1·7	28·6	14·3

[a] Measurements of cell length ($n = 10$) were made within ± 2 weeks of the date on which observations of auxosporulation were made.

[b] Counts refer to pairs of copulating cells, not individual auxospores.

[c] The single expanded auxospore was accompanied either by an aborted zygote or two non-copulating gametes, indicating that the type of auxosporulation was of type IC ('normal'), or by an aborted gamete, reflecting the 'intermediate' type of auxosporulation, where one of the paired gametangia forms two gametes and the other only one; the presence of a second zygote or superfluous gamete demonstrated clearly that auxosporulation was not of the reduced type where each gametangium produces only one gamete.

Table 5. *Achnanthes longipes*: results of crosses among clones of MU2 generation, and between MU2 clones and various clones of known sexuality

		Clone					
Clone	Sexuality	MU2(1A)	MU2(6A)	MU2(3A)	MU2(4A)	MU2(5A)	MU2(2A)
MU2(1A)		[0]					
MU2(6A)		0	[0]				
MU2(3A)		++	++	[+]			
MU2(4A)		++	++	0	[0]		
MU2(5A)		++	++	0	0	[0]	
MU2(2A)		++	++	+	0	0	[0]
MU1(1A)		0	0	++	++	++	++
MU1(1B)		++	++	+	+	0	+
MU1(4A)		++	++	++	++	++	++
MU1(4B)		++	++	++	++	++	++
4	monoecious	++	++	NC	++	++	++
6	monoecious	NC	++	++	++	++	++
7	unisexual-1	++	++	++	++	++	++
8	unisexual-1	NC	++	NC	NC	++	++
10	unisexual-2	0	0	++	++	++	++

Unisexual-1, unisexual clone of sex 1; unisexual-2, unisexual clone of sex 2.
NC, cross not made; +, infrequent interclonal mating; ++, vigorous interclonal mating; 0, clones incompatible; [+], limited monoecious reproduction in monoclonal culture; [0], monoecious reproduction is absent.

clones that were incapable of monoecious reproduction, three (MU1(2B), MU1(3B) and MU1(4B)) had identical mating patterns, all of them being compatible with any of the monoecious and unisexual clones (of either sex) and with any of the MU1 clones; however, they were incapable of mating with each other (Table 3).

Clone MU1(1A) behaved unlike all the other clones (Table 3). It was compatible with the monoecious clones 4, MI(1A), MI(2A) and MI(4A), and also with the unisexual clones 7 and 8, which are of the same sex, but it never mated with the parental clone 10, a unisexual clone of opposite sex to clones 7 and 8. Furthermore, interbreeding was always very rare between MU1(1A) and the four MU1 clones capable of limited monoecious reproduction (MU1(1B), MU1(2A), MU1(3A) and MU1(4A)) and was sometimes absent altogether. However, it mated vigorously with the non-monoecious MU1 clones (MU1(2B), MU1(3B) and MU1(4B)).

Table 4 contains data on the frequency of different kinds of auxosporulation in crosses between the MU1 clones and other clones. One group of crosses, comprising those between MU1 clones and natural clones 4, 7 and 8, involve quite unrelated clones. Another group represents crosses between the MU1 clones and related clones – either the parental clone 10 (unisexual) or F1 derivatives of parental clone 6 (the MI clones), obtained through monoecious reproduction. The third group comprises crosses between the MU1 clones themselves. As in Table 2, four variants are recognized: 'normal' auxosporulation producing two fully formed, apparently healthy auxospores; 'normal' auxosporulation with one fully formed auxospore and one auxospore that had begun to expand but then aborted before producing an initial cell (Chepurnov & Mann, 1999, fig. 1); gametangia with a single expanded auxospore, accompanied either by an aborted zygote or the remains of two gametes (indicating 'normal' auxosporulation of type IC: see above), or by a single aborted gamete (indicating the 'intermediate' type of auxosporulation). Chi-square analysis indicates heterogeneity within each type of cross (unrelated, related, inter-MU1) with respect to the proportions of the different patterns of auxosporulation, which is perhaps not unexpected given that each clone is the product of a different sexual act and is almost certainly genetically distinct from all other clones included within the study. Furthermore, the crosses were carried out at different times, in different culture conditions (although temperature was held constant, the cultures were subjected to variable natural lighting from a north-facing window), introducing further sources of variation unrelated to the mating types of the clones being crossed. As a result of the heterogeneity within each type of cross, we have not attempted detailed statistical analysis of the data in Table 4; instead, we will only comment on the most obvious trends within the data.

Several different patterns of behaviour can be distinguished from Table 4. In crosses between unrelated clones (between MU1 clones and monoecious clone 4, unisexual clone 7 or unisexual clone 8) degenerating auxospores were absent or rare. They were also rare in crosses between the MU1 and MI clones, their frequency reaching a maximum of 6% of all pairs in the cross between MU1(3A) and MI(4A). By contrast, in backcrosses between the MU1 clones and the parental clone 10, and in crosses between the MU1 clones themselves, it was much more common to find one of the auxospores degenerating, the proportion reaching 34% of all pairs in the cross between MU1(2A) and MU1(4B) (Table 4).

Table 6. *Achnanthes longipes*: patterns of auxosporulation in crosses between MU2 and various other clones

Date	MU2 clone	Range of cell length[a] (μm)	Partner clone	Range of cell length[a] (μm)	*n*	Type of auxosporulation			
						Type IC ('normal')			Type IIA ('reduced')
						Two expanded auxospores[b]		One expanded auxospore[b, c]	
						Both developed	One degenerating		
3.6.94	MU2(3A)	49–52	7 (unisexual-1)	48–50	97	60·8%	0	23·7%	15·5%
6.6.94	MU2(3A)	49–52	MU1(4A)	30–37	78	74·4%	7·7%	10·3%	7·7%
6.6.94	MU2(3A)	49–52	MU1(4B)	36–41	44	52·3%	6·8%	29·5%	11·4%

[a] Measurements of cell length ($n = 10$) were made within ± 2 weeks of the date on which observations of auxosporulation were made.

[b] Counts refer to pairs of copulating cells, not individual auxospores.

[c] The single expanded auxospore was accompanied either by an aborted zygote or two non-copulating gametes, indicating that the type of auxosporulation was of type IC ('normal'), or by an aborted gamete, reflecting the 'intermediate' type of auxosporulation, where one of the paired gametangia forms two gametes and the other only one; the presence of a second zygote or superfluous gamete demonstrated clearly that auxosporulation was not of the reduced type where each gametangium produces only one gamete.

There was a corresponding trend in the proportions of 'normal' and 'reduced' auxosporulation. Combining all counts (because of heterogeneity within each group, the combined counts are illustrative only), in crosses between MU1 clones and unrelated clones (clones 4, 7 and 8), 69% of pairs exhibited fully 'normal' auxosporulation, producing two healthy auxospores, while 6% of pairs showed 'reduced' auxosporulation; the remaining 25% exhibited 'normal' or 'intermediate' behaviour with degeneration of gametes or zygotes to yield only one auxospore per pair of gametangia. In crosses between MU1 clones, the proportions were 46% 'normal', 42% degenerate 'normal' or 'intermediate' auxosporulation and 12% 'reduced' auxosporulation. Finally, in crosses between MU1 clones and related clones derived from monoecious clone 6 (monoecious clones MI(1A)–MI(4A)) or the parental unisexual clone 10, the proportions were 33% fully 'normal', 45% degenerate 'normal' or 'intermediate' and 23% 'reduced'.

Second generation (*MU2*)

On 5 April 1994, eight pairs of initial cells were isolated from a mixed culture of sibling clones MU1(4A) and MU1(4B), where vigorous interbreeding had occurred (Table 3). In one of the eight pairs both initial cells were viable and multiplied successfully. In all seven other clones only one of the initial cells survived and grew. The other initial cell remained alive for 1–2 days, moving over the substratum and sometimes forming a mucilage stalk, but then died without dividing, except in one case, where one division occurred. The surviving cell was always the one that had left its perizonium first. From the same culture, four initial cells were isolated that had formed after the 'reduced' type of auxosporulation. All four died without dividing.

On 10 May 1994, four pairs of initial cells were isolated from a mixed culture of sibling clones, MU1(2A) and MU1(2B), which mated vigorously, like the MU1(4) clones. In three of the pairs only one initial cell was viable and grew, while the other died; in the fourth pair, both initial cells died. Finally, on 6 June 1994, eight pairs of initial cells were isolated following mating between clones MU1(1A) and MU1(1B). In each case, one initial cell survived, grew and divided, while the other died.

From the viable clones of the MU2 generation, six were retained. Three were from the MU1(4A) × MU1(4B) cross and the others were from the MU1(1A) × MU1(1B) cross; they were designated MU2(1A)–MU2(3A) and MU2(4A)–MU2(6A), respectively. Ideally, all the MU2 clones would have been retained and studied but this was impractical. The six MU2 clones studied grew at similar rates and exhibited growth characteristics similar to those of the MU1 clones. Ribbon-like colonies were uncommon and chains longer than 10 cells occurred very rarely. As with the MU1 clones, abrupt size reduction was used to shorten the life cycles of the MU2 clones. No changes

in growth characteristics were noted following size reduction.

Monoecious reproduction in the MU2 generation. Among the six MU2 clones, only one (MU2(3A)) was able to reproduce monoeciously. It began to form auxospores in monoclonal culture when the mean cell length had reached 46·6 μm, following a short period of purely vegetative growth after abrupt size reduction (Table 1). Monoecious reproduction was relatively common, especially within tufts of cells, and occurred after every subculturing until cells reached the size range for vegetative enlargement (< 20 μm).

Interclonal reproduction of MU2 clones. The MU2 clones were crossed with each other, with natural monoecious and unisexual clones, and with the parental MU1 clones. The mating reactions were more complex than in previous studies of *Achnanthes longipes*, but some patterns emerge from careful scrutiny of Table 5.

Clones MU2(1A) and MU2(6A) could not mate with each other and exhibited identical behaviour when crossed with other clones. Both were incompatible with the unisexual clone 10 and with MU1(1A), but could mate vigorously with unisexual clones 7 and 8. This suggests that clones MU2(1A), MU2(6A), MU1(1A) and 10 were all unisexual clones of sex 2. Consistent with this, all of them could mate with the monoecious clones 4 and 6. The behaviour of the other MU2 clones was fairly consistent but bizarre. They showed no tendency to interbreed with each other, except very rarely in mixtures of MU2(2A) + MU2(3A), but mated vigorously with any other clone, whether it was monoecious or unisexual, natural or from the MU1 generation, apart from clone MU1(1B), where interactions were feeble or absent (in the MU1(1B) + MU2(5A) mixture).

Patterns of auxosporulation were not studied in great detail in the MU2 generation. In the three crosses for which data were available, the 'normal' type of auxosporulation was predominant (Table 6).

Discussion

We have previously demonstrated compatibility between *Achnanthes longipes* clones with different reproductive characteristics: monoecious, unisexual and bisexual clones can mate with each other in any combination, except unisexual clones of the same sex (see Introduction). We have now shown that the progeny of crosses between monoecious and unisexual clones are viable and can mate successfully with other clones to produce an F_2 generation. Hence *A. longipes* appears to constitute a single reproductive community at Karadag, Crimea. It remains to be seen whether the Karadag populations are typical of the species or abnormally variable in their sexual characteristics. We already know, however, that Karadag clones are compatible with clones from the North Sea, near Edinburgh, and with a clone from Panama, provided by Dr Linda Medlin. All the non-Crimean clones have been able to reproduce monoeciously and they have all exhibited vegetative enlargement, within the same size range as in Black Sea clones.

Our results for the MU1(2A) and MU1(4A) clones (Table 1) are consistent with earlier data showing that there is a difference in the upper size threshold for monoecious reproduction and outcrossing (Chepurnov & Mann, 1997, table 1). The upper limit for monoecious reproduction is *c.* 50 μm, while interclonal reproduction can occur when cells are 65–70 μm long. Furthermore, in the MU clones, as in other clones that have been studied, monoecious reproduction was never vigorous compared with crossing between different, compatible clones. Thus, although *A. longipes* is capable of extreme inbreeding, with sexual reproduction possible within a single clone, its reproductive system is strongly biased towards outbreeding.

The inheritance of reproductive characteristics does not on the whole follow a simple pattern and hence, without studies of many more crosses between clones and their progeny, it is still impossible to develop a genetic theory of sexuality in *A. longipes* (see also Chepurnov & Mann, 1999). However, the present paper completes our preliminary observations of mating within and between dioecious clones (including unisexual and bisexual clones) and monoecious clones, and we will therefore attempt to summarize all our findings to date.

We have observed in several different types of cross that the two initial cells produced by a single pair of gametangia often differ with respect to their sexuality or viability. Thus, for example, in the second inbred generation obtained through the monoecious reproduction of monoecious clone MI(3A), Chepurnov & Mann (1999) found that only one of the initial cells in each pair was viable. This was true also of the monoeciously produced progeny of clone 6, the parent of the MU1 clones used in the current study. In the MU1 clones themselves, one out of each pair of sibling clones was able to reproduce monoeciously whereas the other was not. Such observations suggest that some aspects of sexuality may be under simple allelic control.

The progeny of intraclonal reproduction within monoecious clones or of crosses between different monoecious clones have been shown to include monoecious clones and bisexual clones but not unisexual clones (Chepurnov & Mann, 1999). Crosses between unisexual clones, on the other hand, produced only unisexual and bisexual clones and when these were mated, again, no monoecious clones were produced (Chepurnov & Roshchin, 1995). Our new results, presented above, show that when monoecious and unisexual clones are mated, the progeny in the F_1 and F_2 can include clones of all kinds – unisexual clones (MU1(1A), MU2(1A) and MU2(6A)), bisexual clones (MU1(2B), MU1(3B), MU1(4B)) and monoecious clones (MU2(3A), although this clone showed certain unusual features: see below). In addition, some clones were found

that were basically bisexual but showed a limited capacity for monoecious reproduction (clones MU1(1B), MU1(2A), MU1(3A) and MU1(4A)). However, in these the intensity of intraclonal reproduction is very low compared with the monoecious clones studied previously, including clones 4 and 6 (Chepurnov & Mann, 1997, 1999). Perhaps coincidentally in view of the small numbers of clones studied, no unisexual clones of sex-1 were found in the MU1 and MU2 generations; the three unisexual clones (MU1(1A), MU2(1A) and MU2(6A)) were all of the same sex as the parent unisexual clone 10.

The present study has revealed types of behaviour not found in earlier studies. Clones MU1(2B), MU1(3B) and MU1(4B) exhibited no intraclonal reproduction (and hence are not monoecious clones) and were compatible with either type of unisexual clone, as well as with monoecious and bisexual clones (Table 3); taken on its own, this evidence would lead to their classification as bisexual clones. However, these three clones were incapable of interbreeding amongst themselves. Likewise, clones MU2(2A), MU2(4A) and MU2(5A) could mate with the related unisexual clones MU2(1A) and MU2(6A), or with any of the unrelated clones used in the study, suggesting that they are bisexual, but they too were incapable of interbreeding amongst themselves. Similarly, although it was compatible with any unrelated clone and able to reproduce intraclonally as intensely as natural monoecious clones, clone MU2(3A) would not mate with its close relatives MU2(4A) and MU2(5A) and showed very little response to clone MU2(2A). Finally, clone MU1(1A) showed only weak interactions with the four bisexual MU1 clones showing limited monoecious reproduction (clones MU1(1B), MU1(2A), MU1(3A) and MU1(4A)). In all these cases, the exceptional behaviour is only manifest in crosses between very closely related clones. We found similar 'unexpected' behaviour in crosses between related clones in previous studies (Chepurnov & Roshchin, 1995; Chepurnov & Mann, 1999). But when unrelated, natural clones are studied, the picture is apparently simple: clones are either monoecious, bisexual or unisexual and the success or failure of mating can be predicted accordingly. Furthermore, in mixtures of unrelated clones, interbreeding is either vigorous or absent, according to whether the clones are compatible or incompatible.

In our earlier studies (Roshchin, 1994*b*; Chepurnov & Roshchin, 1995; Chepurnov & Mann, 1999), we showed that, even though intraclonal reproduction is possible in *A. longipes* (very rarely in unisexual and bisexual clones and more frequently in monoecious clones), there are severe limits to inbreeding. There was a rapid decrease of viability in inbred lineages derived from unisexual and monoecious lineages, accompanied by a reduction in the number of gametes produced by each gametangium from two to one. This was responsible for the increasing proportions of the 'intermediate' and 'reduced' types of auxosporulation. It was nearly impossible to obtain viable progeny from the second inbred generation. The same trends can be seen in the data presented here. During monoecious reproduction in the MU1 generation, it appears that at most only one of the two auxospores produced by pairs exhibiting the 'normal' type of auxosporulation is viable. Crosses among related clones, such as between the MU1 clones themselves or between MU1 clones and the parental clone 10, also show high levels of abortion, at least when compared with crosses between unrelated clones. They also exhibit higher frequencies of the 'intermediate' and 'reduced' types of auxosporulation. Even when mature initial cells are formed, they may not be able to give rise to a new vegetative generation, as in the four initial cells isolated following 'reduced' auxosporulation in crosses between MU1(4A) and MU1(4B).

The data reported in the present paper allow some statistical comparisons with earlier data using the same clones. Chepurnov & Mann (1999) reported the frequencies of different types of auxosporulation during monoecious reproduction of MI clones, which represent the inbred first generation progeny of the natural clone 6. These can be compared with the frequencies of different types of auxosporulation in crosses between MI clones and the MU1 clones, using a chi-square analysis. For example, the outcome of monoecious reproduction in clone MI(1A) (Chepurnov & Mann, 1999, table 2) can be compared with the outcomes of the MI(1A) × MU1(1A) and MI(1A) × MU1(2A) crosses (Table 7). In general, more intense inbreeding, as during monoecy in the MI clones, produces significantly lower proportions of type I ('normal') auxosporulation, and correspondingly higher proportions of the 'intermediate' and 'reduced' types, than in interclonal crosses. A similar trend, again correlated with a higher degree of inbreeding, can be detected in able 4 by comparing crosses that involve natural clones and those involving inbred clones of the same sexuality. In particular, comparisons are possible between MU1 × 4 crosses and MU1 × MI crosses, since clone 4 and the MI clones are all monoecious; thus, chi-square comparisons can be made of the frequencies of different types of auxosporulation between MU1(1A) × 4 and MU1(1A) × MI(1A), between MU1(2A) × 4 and MU1(2A) × MI(1A), and so on (Table 7). In almost all cases, a significant excess of the 'intermediate' and 'reduced' patterns of auxosporulation can be found in the MU1 × MI crosses, relative to the MU1 × 4 crosses.

At present we cannot determine the extent to which the change from 'normal' to 'reduced' auxosporulation reflects the general effects of inbreeding, through exposure of deleterious recessive alleles, loss of heterosis, etc. (see Chepurnov & Mann, 1999), or specific disruption of the mechanisms that regulate sex determination, meiosis and gametogenesis. In other diatoms there is evidence for both general and specific effects. Thus, for example, in *Licmophora abbreviata* C. A. Agardh, sibling clones (obtained from the two auxospores produced by a single pair of gametangia) are of opposite sex and can be mated, but although sexual reproduction is normal, the progeny are not viable: the inbred F_2 lineages die, either im-

Table 7. *Achnanthes longipes*: comparisons between crosses showing different degrees of inbreeding, with respect to the frequencies of different types of auxosporulation. Data from this paper (Table 4) and Chepurnov & Mann (1999).

	Type of auxosporulation			
	Type IC ('normal')			
Crosses compared	Two fully developed auxospores[a]	One fully developed auxospore[b]	Type IIA ('reduced')[a]	chi-square[c]
MI(1A) × MI(1A) (monoecious)[d]	13	25	21	12.34**
MI(1A) × MU1(1A)	21	20	4	
MI(1A) × MI(1A) (monoecious)[d]	13	25	21	5.11
MI(1A) × MU1(2A)	11	26	7	
MI(1A) × MI(1A) (monoecious)[d]	13	25	21	17.15***
MI(1A) × MU1(2B)	51	29	14	
MI(3A) × MI(3A) (monoecious)[d]	11	20	13	10.63**
MI(3A) × MU1(4B)	39	24	9	
MU1 (1A) × 4	88	30	7	8.16*
MU1(1A) × MI(1A)	21	20	4	
MU1 (1A) × 4	88	30	7	18.32***
MU1 (1A) × MI(2A)	5	14	3	
MU1 (2A) × 4	15	7	3	8.57***
MU1 (2A) × MI(1A)	11	26	7	
MU1 (2A) × 4	15	7	3	4.20
MU1(2A) × MI(2A)	37	38	23	
MU1 (2B) × 4	153	9	5	49.22***
MU1(2B) × MI(1A)	51	29	14	
MU1(2B) × 4	153	9	5	77.49***
MU1(2B) × MI(2A)	15	20	12	
MU1(2B) × 4	153	9	5	41.13***
MU1(2B) × MI(4A)	15	12	5	
MU1(3A) × 4	85	26	9	25.41***
MU1(3A) × MI(4A)	30	33	20	
MU1(4B) × 4	90	13	3	20.69***
MU1(4B) × MI(3A)	39	24	9	

[a] Counts as in the equivalent columns in Table 4 and in Chepurnov & Mann (1999).

[b] Counts combine cases where both auxospores expanded but then one degenerated, and those where only one auxospore expanded; data from Table 4 and Chepurnov & Mann (1999).

[c] Significance:* $p < 0.05$, ** $p < 0.001$, *** $p < 0001$. In some contingency tables, one cell (and in one case, two cells) had an expected frequency < 5; significance levels were not altered by combining columns to make all expected frequencies > 5.

[d] The cases of monoecious reproduction chosen from table 2 of Chepurnov & Mann (1999) were those with the largest counts; these were the 21.4.94 observations for MI(1A) and the 22.2.94 observations for MI(3A).

mediately or after a few divisions of the initial cells (Chepurnov in Roshchin, 1994*a*). In *Tabularia tabulata* (C. A. Agardh) Snoeijs and *Nitzschia lanceolata* W. Smith, on the other hand, the effects of inbreeding are more precise: inbreeding regularly leads to the abortion of one of the two auxospores produced by each pair of gametangia (Roshchin, 1989, 1990, and 1994*a*, fig. 38, v), as in *A. longipes* during intraclonal reproduction of monoecious clone 6 (Chepurnov & Mann, 1999) or during the production of the MU2 generation (present study). Abortion of one auxospore is the predominant mode of behaviour in inbred *N. lanceolata* and is apparently obligate in *T. tabulata*. In *Haslea subagnita* (Proshkina-Lavrenko) Makarova & Karayeva and *Nitzschia longissima* (Brébisson) Ralfs, sibling clones are incompatible, although crossing experiments, using clones of known sexuality, show that they are of opposite sex (Roshchin, 1991, 1994*a*; Chepurnov in Roshchin, 1994*a*) and so could be expected to mate. In contrast to these species, *Fragilaria delicatissima* Proshkina-Lavrenko did not show any sign of inbreeding depression during five successive inbred generations (Roshchin, 1994*a*).

As with our previous paper (Chepurnov & Mann, 1999), more questions are raised than answered by our data, which reveal a highly complex mating system. Nevertheless, we commend *Achnanthes longipes* as a model organism for future studies of breeding systems in diatoms. *A. longipes* is cosmopolitan, it is easily cultured, and it can be manipulated to shorten or extend the life cycle through abrupt size reduction and vegetative enlargement (von Stosch, 1965; Roshchin & Chepurnov, 1992; Roshchin, 1994*a*; Chepurnov & Mann, 1997); the latter makes it possible to maintain clones indefinitely. The capacity for haploid parthenogenesis (Chepurnov &

Roshchin, 1995) creates further opportunities for experiments on sex determination.

Acknowledgements

We are very grateful to Dr A. M. Roshchin for initiating this work and encouraging one of us (V.A.Ch.) for many years. Our collaboration has been supported by INTAS grants 93-3605 and 93-3605-ext.

References

Chepurnov, V.A. & Mann, D.G. (1997). Variation in the sexual behaviour of natural clones of *Achnanthes longipes* (Bacillariophyta). *Eur. J. Phycol.*, **32**: 147–154.

Chepurnov, V.A. & Mann, D.G. (1999). Variation in the sexual behaviour of *Achnanthes longipes* (Bacillariophyta). II. Inbred monoecious lineages. *Eur. J. Phycol.*, **34**: 1–11.

Chepurnov, V.A. & Roshchin, A.M. (1995). Inbreeding influence on sexual reproduction of *Achnanthes longipes* (Bacillariophyta). *Diatom Res.*, **10**: 21–29.

Davidovich, N.A. & Bates, S.S. (1998). Sexual reproduction in the pennate diatoms *Pseudo-nitzschia multiseries* and *P. pseudodelicatissima* (Bacillariophyceae). *J. Phycol.*, **34**: 126–137.

Drebes, G. (1977). Sexuality. In *The Biology of Diatoms* (Werner, D., editor), Botanical Monographs, **13**: 250–283. Blackwell Scientific, Oxford.

Geitler, L. (1932). Der Formwechsel der pennaten Diatomeen. *Arch. Protistenk.*, **78**: 1–226.

Geitler, L. (1973). Auxosporenbildung und Systematik bei pennaten Diatomeen und die Cytologie von *Cocconeis*-Sippen. *Öst. Bot. Z.*, **122**: 299–321.

Hustedt, F. (1930). Bacillariophyta (Diatomeae). In *Die Süsswasser-Flora Mitteleuropas* (Pascher, A., editor), vol. 10, 2nd edn. G. Fischer, Jena.

Mann, D.G. (1993). Patterns of sexual reproduction in diatoms. *Hydrobiologia* **269/270**: 11–20.

Mann, D.G., Chepurnov, V.A. & Droop, S.J.M. (1999). Sexuality, incompatibility, size variation, and preferential polyandry in natural populations and clones of *Sellaphora pupula* (Bacillariophyceae). *J. Phycol.*, **35**: 152–170.

Roshchin, A.M. (1984). Zhiznennye tsikly bentosnoj diatomovoj vodorosli *Achnanthes longipes* Ag. *Biol. Nauki* (*Mosc.*), 1984 (11): 71–78.

Roshchin, A.M. (1989). Proyavlenie intsukhta u diatomovoj vodorosli *Synedra tabulata*. *Zh. Obshch. Biol.*, **50**: 412–416.

Roshchin, A.M. (1990). Sochetanie odnodomnosti i dvudomnosti u diatomovoj vodorosli *Nitzschia lanceolata* W. Sm. *Zh. Obshch. Biol.*, **51**: 699–708.

Roshchin, A.M. (1991). Dvudomnaya diatomovaya vodorosl' iz semejstva navikulovykh. *Deponirovana v VINITI* [deposited paper, VINITI library] *13.03.91, No. 1094–B91*, Moscow. 7 pp.

Roshchin, A.M. (1994*a*). *Zhiznennye tsikly diatomovykh vodoroslej*. Naukova Dumka, Kiev.

Roshchin, A.M. (1994*b*). Dvudomnoe vosproizvedenie *Achnanthes longipes* Ag. (Bacillariophyta). *Algologia*, **4**(1): 22–29.

Roshchin, A.M. & Chepurnov, V.A. (1992). Vegetativnoe ukrupnenie kletok v zhiznennykh tsiklakh *Achnanthes longipes* Ag. (Bacillariophyta). *Algologia*, **2**(3): 26–32.

Roshchin, A.M. & Chepurnov, V.A. (1999). Dioecy and monoecy in the pennate diatoms (with reference to the centric taxa). In *Proceedings of the 14th International Diatom Symposium* (Mayama, S., Idei, M. & Koizumi, I., editors). Koeltz Scientific Books, Koenigstein.

Stosch, H.A. von (1965). Manipulierung der Zellgrösse von Diatomeen im Experiment. *Phycologia*, **5**: 21–44.

Wiese, L. (1969). Algae. In *Fertilization, Comparative Morphology, Biochemistry and Immunology* (Metz, C.B. & Monroy, A., editors), **2**: 135–188. Academic Press, London and New York.