Variation in the sexual behaviour of natural clones of *Achnanthes longipes* (Bacillariophyta)

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Monoecious, bisexual and unisexual clones of *Achnanthes longipes* were isolated from the Black Sea and studied in laboratory culture. Clones differed in their growth characteristics: in monoecious clones the cells formed tufted aggregations while in other clones they were more dispersed. Bisexual and unisexual clones exhibited intraclonal (monoecious) reproduction, but only at a very low frequency and usually within a more restricted size range than in monoecious clones. Interclonal crosses were made in all possible pairwise combinations. Abundant auxosporulation took place in all crosses, except where unisexual clones of the same sex were incubated together. Auxosporulation was more vigorous and occurred over a wider size range in interclonal crosses than during monoecious reproduction. Sexual reproduction is isogamous. In the commonest pattern of auxosporulation, two paired gametangia each produce two gametes, which fuse to give two auxospores. More rarely (9% of pairs), the gametangia produce only one gamete apiece, and hence only one auxospore. In addition, very small cells can enlarge vegetatively, although genetic or cytological damage sometimes compromises their long-term viability.

Key words: *Achnanthes*, auxosporulation, compatibility, diatom, sexual reproduction, vegetative enlargement

Introduction

The widespread benthic diatom *Achnanthes longipes* Agardh is an important component of the marine microphytobenthos (Hendey, 1951; Round, 1971; Proshkina-Lavrenko, 1963; McIntire & Moore, 1977). It is also a convenient organism to study in the laboratory, since it is easy to grow in culture and methods have been developed for manipulating its life cycle and morphogenesis (von Stosch, 1942, 1965). Since 1979, Black Sea populations of *A. longipes* have been investigated at Karadag, Crimea (Ukraine), mainly in clonal culture, and the main features of the life cycle established (Roshchin, 1982, 1984*a*, *b*, 1994*a*, *b*; Roshchin & Chepurnov, 1992; Chepurnov & Roshchin, 1995). Size reduction occurs during the vegetative phase and size restitution takes place via auxospores, as in most diatoms (Round *et al.*, 1990), and auxosporulation is usually associated with allogamous sexual reproduction. In addition, however, clones exhibit abrupt size reduction and vegetative cell enlargement – phenomena that have been reported in several other, unrelated diatoms (e.g. Locker, 1950; von Stosch, 1965; Gallagher, 1983; Kling, 1993).

With respect to its sexuality, *A. longipes* is 'monoecious–dioecious' (Roshchin, 1994*a*). In such diatoms some clones are monoecious, so that sexual reproduction occurs in monoclonal culture, while others are unisexual and must usually be mated with a clone of the opposite sex. Other examples of monoecious–dioecious diatoms include *Nitzschia lanceolata* W. Smith (Roshchin, 1990, 1994*a*), *Tabularia tabulata* (Agardh) Snoeijs (= *Synedra tabulata*: Roshchin, 1987, 1989*a*, 1994*a*) and *Fragilaria delicatissima* Proshkina-Lavrenko (Roshchin, 1994*a*); this last species does not belong to *Fragilaria* Lyngbye *sensu stricto*, as circumscribed by Williams & Round (1987), nor is it the same as *Fragilaria delicatissima* (W. Smith) Lange-Bertalot (Lange-Bertalot, 1980). In *S. tabulata* and *F. delicatissima*, during dioecious reproduction, the copulating cells (gametangia) are differentiated such that one produces two active gametes, the other two passive gametes (termed *cis* physiological anisogamy by Mann, 1982). However, in unisexual clones all the gametangia behave alike, furnishing either the passive gametes or the active ones ('female' and 'male' respectively). In *N. lanceolata*, on the other hand, each gametangium produces one active and one passive gamete (*trans* physiological anisogamy *sensu* Mann, 1982). Unisexual clones of *N. lanceolata* and *A. longipes*, though predominantly dioecious, demonstrate a limited capacity to form auxospores monoeciously.

In our studies of *A. longipes*, monoecious and unisexual clones (of both sexes) have repeatedly been isolated from the Crimean coast of the Black Sea. In addition, among the progeny of inbred crosses made in the laboratory, bisexual clones have been obtained with limited ability to reproduce monoeciously but the capacity to mate vigorously with unisexual clones of either sex (Roshchin,

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1994*b*; Chepurnov & Roshchin, 1995). One bisexual clone was also found among clones of inbred origin in *F. delicatissima* (Roshchin, 1994*a*).

Observations of inbred clones in *A. longipes* have revealed an interesting diversity in sexual behaviour. When inbred unisexual clones are crossed, up to five modes of auxosporulation can be found (Chepurnov & Roshchin, 1995). In three of these, auxospores are produced allogamously. In the first, two gametes are produced per gametangium and fuse isogamously (Geitler's type IC auxosporulation; cf. Geitler, 1973). In the second, fusion is again isogamous but only one gamete is produced by each gametangium (Geitler's type IIA2a, with pairing via the girdle and no copulation tube). Both these types have been reported previously (type IC commonly but type IIA2a only rarely) in clones of *A. longipes* isolated directly from natural populations. The third method of auxosporulation is intermediate between types IC and IIA2a. Here one of two paired gametangia produces two gametes while its partner produces only one (Chepurnov & Roshchin, 1995). Inbred clones also exhibit two other types of behaviour: paedogamous auxosporulation (Geitler's type IIIA), where two gametes fuse within an unpaired gametangium, and haploid parthenogenesis (see also Geitler, 1979; Mann, 1994).

These intriguing observations, which contrast starkly with the apparently uniform sexual behaviour of many species of pennate diatoms (Geitler, 1973), could conceivably be artifacts of long-term culture and inbreeding. We have therefore returned to studies of natural clones (i.e. clones derived directly from natural populations, as opposed to clones isolated following auxosporulation in culture) to ascertain whether these too exhibit the diversity of auxosporulation patterns observed in inbred clones and to establish whether bisexual clones occur in nature. We have also explored further the sexual interactions amongst monoecious, unisexual and bisexual clones.

Materials and methods

Samples of Black Sea microphytobenthos containing *A. longipes* were collected in April and May 1993 from the stony sublittoral near Karadag, S.E. Crimea, Ukraine, at depths of 0·2–0·5 m. Twelve clones of *A. longipes* were isolated by micropipette. Clones 1 and 7 were isolated on 19 April, clones 2 and 11 a day later, and clones 3–6, 8–10 and 12 on 14 May (Table 1). To minimize the possibility that cells isolated from nature were themselves derived from the same clone, cells were isolated from several samples taken from various sites along a 200 m stretch of coastline. With the exception of clone 10, clones were established from cells at or somewhat above the upper size limit for sexual reproduction (Table 1), which we had previously determined to be approximately 50 μm (see references above).

Clones were incubated in 90 mm Petri dishes with 20–30 ml of medium, which was based on sea water (*c.* 16‰) collected off Karadag. The sea water was pasteurized by heating to 70–75 °C on each of three successive days and enriched with KNO_3 (202 mg l^{-1}), $Na_2HPO_4 . 12H_2O$ (17·9 mg l^{-1}), $FeSO_4 . 7H_2O$ (0·287 mg l^{-1}), $MnCl_2 . 4H_2O$ (0·198 mg l^{-1}), $CoCl_2 . 6H_2O$ (0·238 mg l^{-1}), Na_2EDTA (3 mg l^{-1}), $Na_2S_2O_3 . 5H_2O$ (1·2 mg l^{-1}), $Na_2SiO_3 . 9H_2O$ (10 mg l^{-1}), vitamin B_{12} (2 μg l^{-1}). Cultures were maintained at 19 ± 1 °C in diffuse natural light from a north-facing window and reinoculated into fresh medium every 4–6 days.

All pairwise combinations of clones were studied. Mixed cultures were prepared in 50 mm diameter Petri dishes containing 7 ml of medium and examined daily thereafter for signs of sexual reproduction. The parent cultures were always growing exponentially when used to make mixed cultures. Inoculum size was not important. If no crossing was observed for a particular combination, new mixed cultures were prepared up to four times, on different dates (and therefore under different light conditions) and at different stages during the size reduction cycle. These checks were made in order to establish that the absence of crossing was not accidental or due to the cells being outside the size range for sexualization. Where possible, mixed cultures were prepared when the cells of the two cultures differed in size, so that it was easy to distinguish between monoecious reproduction and intercrossing.

Methods for preparing cleaned frustules for identification, and for obtaining abrupt size reduction or vegetative cell enlargement, have been given by Roshchin & Chepurnov (1992). Abrupt size reduction occurs through unequal cell division and probably occurs spontaneously at a very low rate in all cultures of *A. longipes*. However, its frequency can be increased by keeping cultures in stationary phase for 1–2 weeks (during which the cell contents become much darker and the mucilage stalks become long and bent). The small cells that are produced can then be used to initiate new cultures, by isolation into new medium, though it is important to choose cells that appear healthy and which behave normally, e.g. in their ability to form stalks. Vegetative enlargement is spontaneous, but only occurs in very small cells (see below).

Observations of sexual reproduction were made using an MBI-6 photomicroscope (USSR); use of a water immersion lens made it possible to observe cells *in situ* in the mixed cultures. Cell lengths were measured with an ocular micrometer using a Carl Zeiss Jena NF microscope. Because of frequent subculturing, cell length could be characterized adequately within a culture (mean and standard error of the mean) by measuring 10 cells.

Results

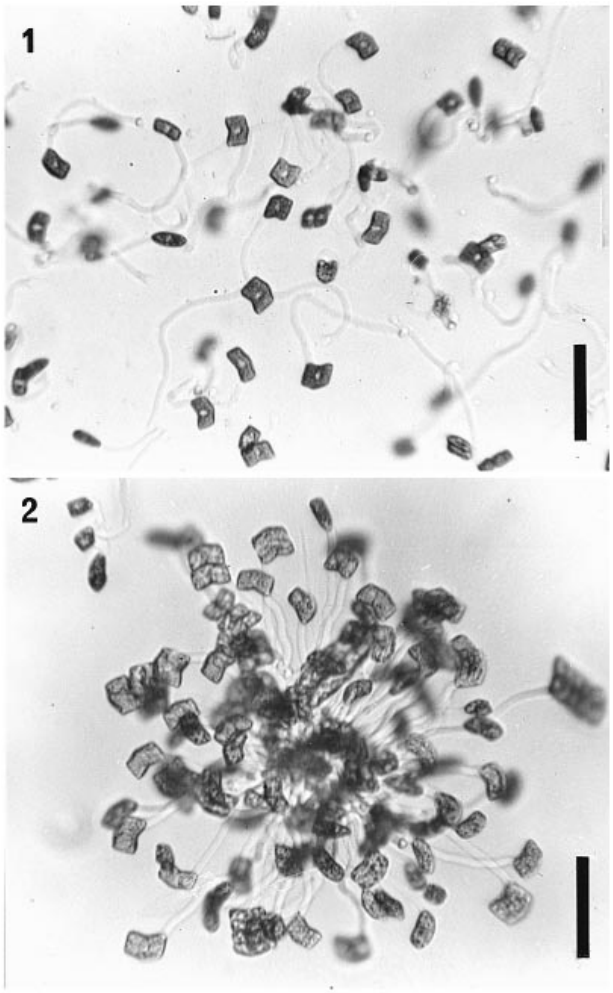
The characteristics of single clones

All the *A. longipes* clones could reproduce monoeciously (i.e. allogamous auxosporulation occurred intraclonally),

Table 1. Characteristics of *Achnanthes longipes* clones, isolated from the Black Sea sublittoral

Clone	Clone type	Date of isolation	Date of first measurement	Initial length (μm)	Maximal observed lengths[a] of cells capable of: Monoecious reproduction (μm)	Maximal observed lengths[a] of cells capable of: Interclonal crossing (μm)
1	Monoecious	19.4.93	5.5.93	90 ± 1	47 ± 1	61 ± 2
2	Monoecious	20.4.93	5.5.93	81 ± 2	56 ± 1	57 ± 1
3	Monoecious	14.5.93	24.5.93	72 ± 1	48 ± 1	55 ± 2
4	Monoecious	14.5.93	24.5.93	79 ± 1	45 ± 2	45 ± 2
5	Monoecious	14.5.93	24.5.93	65 ± 1	46 ± 1	50 ± 1
6	Monoecious	14.5.93	24.5.93	56 ± 2	49 ± 1	49 ± 1
7	Unisexual	19.4.93	5.5.93	84 ± 1	42 ± 2	51 ± 1
8	Unisexual	14.5.93	24.5.93	86 ± 1	25 ± 1	58 ± 2
9	Unisexual	14.5.93	24.5.93	69 ± 1	39 ± 1	50 ± 1
10	Unisexual	14.5.93	24.5.93	134 ± 1	35 ± 2	51 ± 1
11	Bisexual	20.4.93	5.5.93	47 ± 1	25 ± 1	47 ± 1
12	Bisexual	14.5.93	24.5.93	67 ± 2	41 ± 2	40 ± 2

[a] The maximum lengths of cells capable of monoecious reproduction or interclonal crossing refer to measurements (mean ± SE) of cells in the cultures where reproduction was observed, not to measurements of the paired cells themselves.

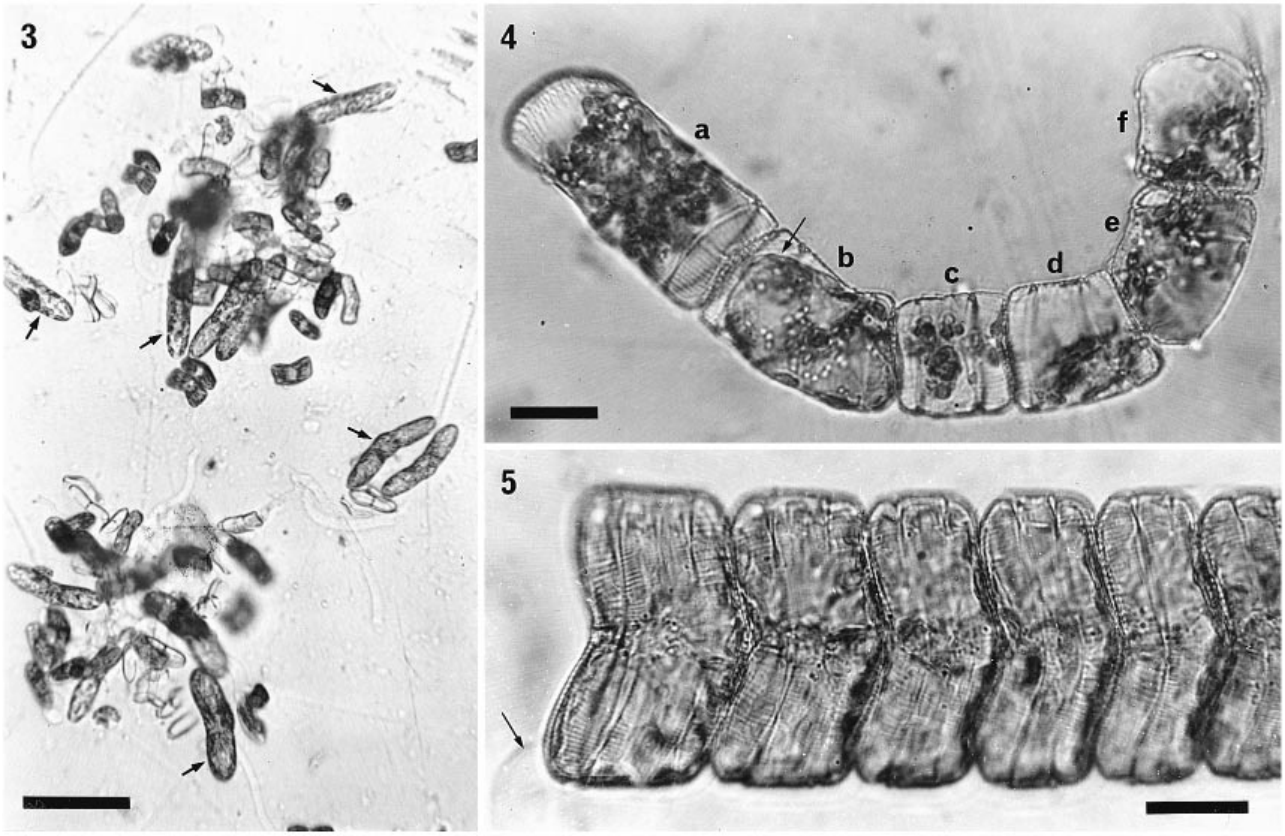


Figs 1, 2. *Achnanthes longipes*. Characteristic patterns of distribution of cells in clonal culture towards the end of exponential growth. Fig. 1. Unisexual clone (clone 7): cells dispersed. Fig. 2. Monoecious clone (clone 3): cells forming dense tufts. Scale bars represent 100 μm.

but the intensity varied. Clones could be divided into two groups. In group A, comprising clones 1–6 (Table 1), monoecious reproduction was relatively frequent, tens of pairs of gametangia being found after each subculturing in each 90 mm diameter Petri dish, towards the end of the exponential phase of growth, once the cells had reached the sexually inducible size range. The most vigorous monoecious reproduction observed was found in clone 6, when cells had decreased in size to 30 ± 1 μm. Here, 2·2% of cells became transformed into gametangia at a culture density of 79 ± 8 cells mm^{-2} of the Petri dish (n = 40). In other group A clones, the frequency of monoecious reproduction was usually less than 1%. The upper size limit for monoecious reproduction varied from 56 ± 1 μm (clone 2) to 45 ± 2 μm (clone 4) (Table 1).

In *A. longipes* there is also a lower limit of size for auxosporulation, i.e. the sexual size range is 'closed' (Roshchin & Chepurnov, 1992), unlike in those diatoms in which even the smallest cells are still capable of auxosporulation, such as *Rhabdonema adriaticum* Kützing (von Stosch, 1958), *Licmophora ehrenbergii* (Kützing) Grunow (Roshchin, 1986), *Surirella ovalis* Brébisson (Roshchin, 1989*b*) and *Nitzschia lanceolata* W. Smith (Roshchin, 1990). In group A clones, the smallest cells capable of sexual reproduction measured 20–25 μm. Below this, cells continued to divide for a short time but then began to enlarge without auxospore formation, as described by Roshchin & Chepurnov (1992). The upper limit for vegetative enlargement was between 15 and 18 μm. Cells that had reduced to 8–10 μm were still able to enlarge vegetatively, producing cells of 23–54 μm, which is within the sexual range.

Clones of group B (clones 7–12, Table 1) were characterized by rare and somewhat sporadic monoecious reproduction. Only at high culture densities towards the



Figs 3–5. *Achnanthes longipes*. Fig. 3. Abundant auxosporulation (arrows) following intercrossing between clones in mixed culture. Fig. 4. Chain of abnormal cells (a–f) produced after vegetative cell enlargement in clone 9. Several cells (especially a, b) are unusually elongate along the pervalvar axis and in some (c, d) the cell contents have degenerated; in cell b the protoplast has contracted away from the frustule (arrow). Note that no stalk is present. Fig. 5. A chain of normal cells with its stalk (arrow). Scale bars represent: Fig. 3, 100 μm; Figs 4 and 5, 20 μm.

end of exponential growth, and even then not at every re-inoculation within the sexual range, did any auxosporulation occur. The lower size limit for monoecious reproduction and the upper limit for vegetative enlargement were the same as in group A clones. However, the upper size limit for monoecious reproduction in group B was always lower than in group A clones. In clone 7, monoecious reproduction occurred when cells reached 42 ± 2 μm, but in clones 8 and 11 the upper limit for monoecious reproduction was 25 μm. Hence, there is a much narrower window for monoecious reproduction during the life cycles of group B clones, and a very narrow window indeed in clones 8 and 11 (between 25 and 20 μm).

There was a further difference between group A clones, which will henceforth be called monoecious clones, and the essentially non-monoecious group B clones. This concerned the distribution of cells in culture. Solitary cells and short, ribbon-like chains of 4–8 cells (Fig. 5) were produced by all clones, either attached to the Petri dish or motile. In non-monoecious clones, attached cells and colonies were dispersed more or less evenly across the substratum (Fig. 1), while in monoecious clones they often formed tufts, in which the mucilage stalks were clustered close together (Fig. 2). Similar aggregations of cells were illustrated without comment by von Stosch (1942, figs 14, 15, 17) and described by Roshchin (1984*a*). Monoecious auxosporulation occurred much more frequently within tufts than between dispersed, solitary cells.

During pairing, in both intraclonal and interclonal crosses, one cell stays attached to its stalk while the other moves to mate with it. Apart from this, the gametangia are not visibly different and copulation between the gametes is strictly isogamous.

Results of interclonal crosses

Mixed cultures of clones within the sexual size range demonstrate that the monoecious clones can mate with any other clone, monoecious or non-monoecious. Panmixis was also exhibited by clones 11 and 12. The other four non-monoecious clones were unable to mate in all combinations (Table 2). Clones 7 and 8 could not mate with each other, nor could 9 mate with 10. These four clones can therefore be regarded as unisexual. Clones 11 and 12, on the other hand, are bisexual, since they can reproduce vigorously with either type of unisexual clone, while their capacity to reproduce monoeciously is very limited.

In mixed cultures, except in the incompatible combinations $7 + 8$ and $9 + 10$, auxosporulation was always much more abundant than in clonal cultures (Fig. 3: the frequency of auxosporulation is clearly much higher than the maximum of 2·2% observed for monoecious re-

Table 2. *Achnanthes longipes*: results of crosses between non-monoecious clones. Bisexual clones 11 and 12 mate with any other clone. Clones 7 and 8 are incompatible, as are clones 9 and 10

		Clone					
Clone	Sexuality	7	8	9	10	11	12
7	Unisexual-1	[trace]					
8	Unisexual-1	0	[trace]				
9	Unisexual-2	+	+	[trace]			
10	Unisexual-2	+	+	0	[trace]		
11	Bisexual	+	+	+	+	[trace]	
12	Bisexual	+	+	+	+	+	[trace]

Unisexual-1, unisexual clone of sex 1; unisexual-2, unisexual clone of sex 2.
[trace], very limited monoecious reproduction in monoclonal culture; +, clones compatible, resulting in abundant auxosporulation in mixed culture (see text); 0, clones incompatible.

production), where monoecious reproduction alone can occur. Not uncommonly, 50% or more of cells had become paired by the end of the exponential phase of growth. Even with monoecious clones, mating is preferentially inter- rather than intraclonal.

Table 1 gives the sizes of the largest cells capable of sexualization for each clone in mixed culture. For clones 1, 4, 5, 8 and 10, we can be confident that these sizes do indeed represent the upper limit for sexualization because larger cells were mixed several times with clones already known to be sexual, but never mated with them. For the other clones, the lengths given are only minima and indicate when sexual reproduction was first observed in any intercross. If we exclude clones 6 and 12 from consideration, since these were already capable of monoecious reproduction when first isolated, the data show that, in our culture conditions, the upper limit for crossing between clones is generally higher than for monoecious reproduction, sometimes considerably so (clones 8 and 11) (Table 1). The only exception is the monoecious clone 4, where both upper limits are the same. The lower size limits for mating between clones are the same as for mating within clones.

Initial cells formed after intercrossing did not differ obviously in length from those produced monoeciously. The ranges were 119–138 μm for monoeciously produced initial cells of clone 6 and 124–138 μm for the progeny of crosses between clones 6 and 10 (10 observations in each case). In earlier work, involving hundreds of measurements, initial cells varied between 100 and 178 μm (Roshchin, 1984*b*, 1994*a*; Roshchin & Chepurnov, 1992; Chepurnov & Roshchin, 1995).

Patterns of reproductive behaviour during intraclonal and interclonal auxosporulation

Reproductive behaviour was studied in 829 pairs of gametangia in 11 mixed cultures and during monoecious reproduction in clone 6 (Table 3). In most cases (91% of pairs), auxosporulation was of the 'normal type' (Geitler, 1973; Drebes, 1977), where both gametangia produce two gametes. The details of auxosporulation conformed to Geitler's type IC (cf. Geitler, 1973). The remaining pairs (9%) exhibited 'reduced type' auxosporulation (Geitler, 1973; Drebes, 1977), in which each gametangium produces only one gamete. The intermediate type of behaviour (where one gametangium produces two gametes and the other only one) and haploid parthenogenesis, both found previously in inbred clones (Chepurnov & Roshchin, 1995), did occur in our cultures but only extremely rarely, and so they were not found among the 829 pairs analysed in Table 3. Paedogamy was never observed.

The 'normal type' of sexual reproduction generally results in the production of two auxospores, through the fusion of two pairs of gametes (Geitler, 1973; Drebes, 1977; Mann, 1993). In our experiments, however, this was true in only about 60% of pairs (Table 3). In the rest each pair produced only one viable auxospore, together with an aborted zygote or two unfused gametes, the latter being less common.

Effects of vegetative enlargement

Vegetative enlargement generally gives rise to viable cells, 23–54 μm long, capable of sexual reproduction or of renewed mitotic division and size reduction. Furthermore, a clone can usually take part in several cycles of vegetative enlargement and subsequent size reduction, without loss of vitality (Roshchin, 1994*a*). However, in some instances the process of vegetative enlargement, or perhaps irregularities in nuclear or cell division in the smallest cells before enlargement, seems to bring about genetic or cytological damage, producing abnormal development. This was observed in clones 5 and 9 (monoecious and unisexual, respectively). Immediately after vegetative enlargement of these clones, new subclones were isolated, in which most of the cells became strongly elongate along the pervalvar axis (Fig. 4; contrast Fig. 5). The cell contents seemed disorganized and no stalks were formed. Following division, daughter cells generally did not separate but formed very long, twisted, ribbon-like colonies. Only a few cells were solitary and motile.

The newly enlarged subclones of clones 5 and 9 (produced following vegetative enlargement) were crossed with other clones with which they were known to be compatible (Table 2) and pairing took place as before. However, in most pairs the gametes failed to fuse and later aborted. In the few instances where plasmogamy occurred, not every auxospore developed and those that did often had abnormal shapes and died. Only once were auxospores formed that appeared to function normally. These were produced in a cross between clones 5 and 10, made immediately after the vegetative enlargement of clone 5, before the cytological abnormalities noted above had

Table 3. *Achnanthes longipes*: patterns of auxosporulation in mixtures of clones (inoculated in pairs) and during the monoecious reproduction of clone 6 in pure culture

				Type of auxosporulation		
				Type IC ('normal')		
Date	Clones mated	Clone types	Cell lengths[a] (μm)	2 expanded auxospores[b]	1 expanded auxospore[b,c]	Type IIA ('reduced')[b]
2.8.93	3	Monoecious	48 ± 2	70	28	3
	6	Monoecious	31 ± 1			
2.8.93	5	Monoecious	40 ± 1	18	25	10
	7	Unisexual-1	42 ± 2			
8.10.93	1	Monoecious	27 ± 1	17	16	16
	10	Unisexual-2	51 ± 1			
8.10.93	4	Monoecious	31 ± 1	44	5	5
	10	Unisexual-2	51 ± 1			
6.12.93	6	Monoecious	24 ± 2	158	43	3
	10	Unisexual-2	37 ± 1			
20.12.93	8	Unisexual-1	40 ± 1	13	13	4
	10	Unisexual-2	35 ± 2			
23.12.93	7	Unisexual-1	37 ± 1	4	11	10
	10	Unisexual-2	35 ± 2			
23.12.93	8	Unisexual-1	40 ± 1	11	51	0
	12	Bisexual	32 ± 1			
23.12.93	7	Unisexual-1	37 ± 1	13	31	4
	12	Bisexual	32 ± 1			
8.10.93	10	Unisexual-2	51 ± 1	8	25	16
	12	Bisexual	24 ± 1			
23.12.93	10	Unisexual-2	35 ± 2	17	25	2
	12	Bisexual	32 ± 1			
7.12.93	6	Monoecious	24 ± 2	92	15	3

[a] Measurements of cell length (mean ± SE) were made within ± 5 days 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 by two non-copulating gametes, thus indicating clearly that the type of auxosporulation was of type I ('normal'), not the reduced type, where each gametangium produces only one gamete.

developed fully. The auxospores gave rise to cells that grew and divided normally until they had reduced in size to 39 μm, when the abnormalities recurred.

After vegetative enlargement, clone 9 never exhibited monoecious reproduction; clone 5 did, but less frequently than hitherto and within a narrower size range (45 ± 1 to 35 ± 5 μm). When the cells had again reduced to below 20 μm, it was found that they had lost the ability to enlarge vegetatively. The protoplasts of a few cells retracted from their frustules and began to emerge, but viable enlarged cells were not produced. Finally, after some further divisions, clones 5 and 9 died.

Vegetatively enlarged cells of *A. longipes* are often of irregular shape and structure (von Stosch, 1965; Roshchin & Chepurnov, 1992) and at first we believed that this might be responsible for the abnormal development and sexual behaviour of clones 5 and 9. This seems unlikely, however, since the vegetatively enlarged cells of these clones were no more curiously shaped initially than those of other clones that behaved quite normally. Furthermore, clone 6 has been taken successfully through six cycles of vegetative enlargement. The irregular shape of its valves does not hinder it from producing viable auxospores and initial cells, and cells with disorganized contents are not found. Thus, the causes of the failure of clones 5 and 9 remain a mystery. Caution may perhaps be urged, however, for those culturing *A. longipes* or other species that exhibit vegetative enlargement. Following enlargement, several subclones should be established, using cells that exhibit the fewest cytological, morphological or growth abnormalities. In *A. longipes*, for example, cells should be chosen that have narrow girdles and can produce mucilage stalks.

Discussion

The data confirm that *A. longipes* is a monoecious–dioecious species and that monoecious, unisexual and bisexual clones all occur in natural populations. Experiments with mixed cultures suggest that outbreeding is favoured, since even in monoecious clones sexual activity is more vigorous between clones than within them. However, as yet there is little information about how clones with different types of sexual behaviour are distributed in nature. We do not know whether *A. longipes* forms mosaics of different clones, interacting only at their edges, or occurs as thoroughly mixed, homogeneous populations that are virtually panmictic. The little in-

formation available to us suggests the mosaic model may be more accurate. First, in culture and perhaps also in nature, monoecious clones tend to grow as small tufts. This will tend to increase the likelihood of inbreeding, especially when cell densities are low. Second, one of us (V.A.C.) has often collected samples from the sublittoral at Karadag and picked out from each several cells of *A. longipes*, all within the sexual size range, to be grown together in rough culture. Sometimes, auxospores have been produced in abundance, as during crosses between clones. In other cases, sexual reproduction occurred infrequently in dense cultures, as during intraclonal auxosporulation. In still other cases no gametangia were seen, even though culture densities, conditions and cell sizes seemed to be appropriate for auxosporulation; here, perhaps all the cells isolated were of the same sex.

The idea that the distributions of different types of clone – monoecious, unisexual, bisexual – are heterogeneous in nature also receives support from observations of dioecious species (Chepurnov, unpublished data). For instance, the araphid pennate diatoms *Licmophora abbreviata* Agardh and *Striatella unipunctata* (Lyngbye) Agardh, both of them dioecious (Chepurnov in Roshchin, 1994*a*), were abundant in the upper sublittoral of the Black Sea near Karadag in spring 1989 and spring 1990, respectively. During these periods, no auxosporulation was ever seen in samples from the upper sublittoral and all clones isolated proved to be of the same sex when they were mated with each other and with test strains. Clones of the opposite sex were only present elsewhere or at other times. Thus, in *S. unipunctata* the opposite sex was found in deeper parts of the sublittoral, at 20 m, where it colonized collectors for *Mytilus* cultivation suspended in the water column. In *L. abbreviata*, both sexes were found together in September 1989, when the species was again abundant in the upper sublittoral.

In the present study, the largest cells of *A. longipes* capable of monoecious reproduction were usually smaller than those capable of intercrossing (Table 1), so that monoecious reproduction is restricted to a shorter period of the life cycle. This should favour outbreeding. In some ways, both monoecious reproduction and vegetative enlargement can be regarded as a form of 'insurance', which will allow populations to persist in circumstances where panmixis is restricted or where sexual reproduction fails altogether, because environmental conditions do not trigger it or because cells are too widely separated and do not find a mate. Other studies of *A. longipes* have shown the maximal size of sexualized cells to be much greater than in our experiments. Roshchin (1984*b*) gives a maximum of 96 μm for monoecious reproduction in the clones he studied, which contrasts markedly with our maximum of 61 μm found in interclonal crosses involving the monoecious clone 1. This difference may reflect genetic variation within Black Sea populations of *A. longipes*, since there clearly are differences between clones regarding the sexual size limits (Table 1), or the effects of undetermined external factors on sexualization.

The most usual method of auxosporulation in *A. longipes* is type IC, defined by Geitler (1973) as 'Gameten $\pm$ willkürlich isogam, umgelagert, abgekugelt, relativ frei in weicher Kopulationsgallerte, daher Auxosporen beliebig ausgerichtet oder ihre Apikalachse untereinander und zu denen der Mutterzellen $\pm$ parallel'. The behaviour is the same whatever the kind of cross – monoecious–monoecious, unisexual–monoecious, unisexual–unisexual, etc. High rates of zygote abortion were found and also malfunction of gametes, which may perhaps reflect the presence of deleterious recessive genes, unmasked by haploidy in the gametes and in the dikaryons of young auxospores, before karyogamy (see also Mann, 1987).

The coexistence of both the 'normal' and the 'reduced' types of auxosporulation has not been reported in any other diatom except *Navicula cryptocephala* Kützing (Geitler, 1958), although in the latter it is possible that the 'normal' and 'reduced' types of auxosporulation occur in genetically distinct races, rather than in the same population as observed in *A. longipes*. In type I ('normal') auxosporulation, both nuclei survive from meiosis I; then cytokinesis takes place and meiosis II, followed by the degeneration of one daughter haploid nucleus in each protoplast (Drebes, 1977; Mann, 1993). For a change to type II ('reduced') auxosporulation, where only one gamete is produced per gametangium, one of the products of meiosis I must be non-functional and cytokinesis must either be suppressed altogether, as appears to happen in *A. longipes*, or one of the daughter cells must degenerate to form a small residual cell, as occurs in species of *Sellaphora* Mereschkowsky (Mann, 1989). In addition, an intermediate type of auxosporulation occurs in *A. longipes*, in which one gametangium produces two gametes, but the other only one. This is especially common during matings between inbred clones (Chepurnov & Roshchin, 1995). Another species that appears to be very plastic in its reproductive behaviour is *Dickieia ulvacea* Berkeley *ex* Kützing (Mann, 1994), but there is no information about sexual differentiation between clones in this species. Thus, *Achnanthes longipes* remains the most suitable species in which to study the control of gametogenesis and hence to gain insights into the evolution of different modes of auxospore formation in pennate diatoms.

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