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Sexual and vegetative phases in the planktonic diatom *Pseudo-nitzschia multistriata*

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ABSTRACT

The planktonic diatom *Pseudo-nitzschia multistriata* (Takano) Takano is known to produce the toxin domoic acid and it is recorded during late summer and autumn in the Gulf of Naples (Tyrrhenian Sea, Italy). We describe the sexual cycle of this species and report information on the variability of growth and cell size reduction rates at different experimental conditions. We induced sexual reproduction by crossing monoclonal cultures of opposite mating type. *P. multistriata* has a heterothallic life cycle that follows the general pattern reported for other congeneric species. Sexual stages were detected in cultures with an average apical length between 55 and 39 μm. The size of the initial cells produced at the end of the sexual phase was comprised between 72 and 82 μm and the lower cell size detected in culture was 26 μm. Sexual reproduction was thus recorded within a size window corresponding to 39–71% of the maximum cell apical length. Both growth performances and cell size reduction rates depend on cell size. The largest cells showed slower growth rates and larger size reduction rates at each division, while the relationship was opposite for cells smaller then 60% of the maximum size.

1. Introduction

A wide cell size range – mainly cell width in centrics and cell length in pennates – is a typical feature of diatom species (Hasle and Syvertsen, 1997). This variability in cell size is due to the peculiar structure of the rigid siliceous frustule and to the unique division mode of these microalgae. The frustule is constituted by two, slightly unequal thecae fitting together as the lid on a box. This 'siliceous fortress' does not open even when cells undergo vegetative division. In fact, following mitosis and cytokinesis, each sibling cell inherits one maternal theca, which becomes the larger epitheca (the lid), and synthesizes *ex novo* the smaller hypotheca (the box) (Round et al., 1990). The synthesis of the new hypothecae takes place within the maternal frustule and it follows that the cell inheriting the larger maternal epitheca will keep the size of the mother while the sibling inheriting the smaller maternal hypotheca will be smaller (Crawford, 1980). The implications of this peculiar cell division mode are described by the McDonald–Pfitzer rule: vegetative growth – i.e. the progression of cell divisions – causes a reduction of the average cell size and the contemporary increase of its standard deviation in a population (MacDonald, 1869; Pfitzer, 1869; Round et al., 1990). This reduction of cell size can be by-passed by the onset of sexual reproduction and – in several centric diatoms and a few pennates – by vegetative cell enlargement (Gallagher, 1983; Nagai and Imai, 1999; Chepurnov and Mann, 1997). At a defined, species-specific, threshold size vegetative cells might turn into gametangia, undergo meiosis and produce gametes, which conjugate resuming the diploid state. Oogamy is widespread among centric diatoms, while anisogamy and isogamy have been mainly reported for pennate species (Chepurnov et al., 2004). The zygote lacks the rigid siliceous wall and is free to expand forming the auxospore. Within this specialised structure, a cell of the largest species-specific size – the initial cell – is produced. On the other hand, the formation of an auxospore-like structure bypassing the sexual phase has been reported in some species. This process is called vegetative cell enlargement and also produces a large size cell (Chepurnov et al., 2004).

Pseudo-nitzschia H. Peragallo is an important genus of planktonic pennate diatoms present in coastal and open waters (e.g. Dortch et al., 1997; Hasle, 2002; Kaczmarska et al., 2005; Fehling et al., 2006). Species within this genus are heterothallic (i.e. sexual reproduction occurs only between clones belonging to a different mating type) and sexual reproduction can occur over a relatively wide cell size window (Davidovich and Bates, 1998; Kaczmarska et al., 2000; Amato et al., 2005; Chepurnov et al., 2005). In *Pseudo-nitzschia* species – as in the majority of pennate diatoms – cell size reduction mainly involves the reduction of the apical axis, whereas

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the trans-apical and pervalvar axes do not change significantly (Amato et al., 2005; Chepurnov et al., 2005). *Pseudo-nitzschia multistriata* has been described from Japanese waters (Takano, 1993; Takano, 1995) and it has been reported from different locations (e.g. Rhodes et al., 2000; Akallal et al., 2002; Cho et al., 2002) including the Mediterranean Sea (Orsini et al., 2002; Zingone et al., 2006), where it has been shown to produce domoic acid (Orsini et al., 2002). In the Gulf of Naples, this species regularly blooms in late summer–autumn but it can be recorded at lower concentrations up to late winter (Ribera d'Alcalà et al., 2004; Zingone et al., 2006; Sarno, unpublished data).

We describe the sexual cycle of *P. multistriata* (Takano) Takano assessing cell size range for gametogenesis and size of initial cells by carrying out multiple crosses. We also estimated growth rates and cell size reduction per generation at different experimental conditions and discuss their implications for setting the timing of sexual events in the life cycle of this species.

2. Materials and methods

2.1. Strains

Strains of *P. multistriata* were obtained by the isolation of single cells or short chains from surface net samples collected at the long-term station MareChiara (MC) (Ribera d'Alcalà et al., 2004) in the Gulf of Naples (Tyrrhenian Sea, Mediterranean Sea) (Table 1). Strains were maintained in K-medium, with the addition of silicate (Keller et al., 1987) at 15 °C, 10 μmol photons m^{-2} s^{-1} and 12:12 h L:D photocycle.

2.2. Life cycle

Mating experiments were carried out inoculating into tissue-culture plate wells filled with 5 ml of *k*/2 medium pairs of exponentially growing strains (Table 1) at a final concentration of 1000–2000 cell ml^{-1}. Plates were incubated at 20 °C, 40 μmol photons m^{-2} s^{-1} and 12:12 h L:D photocycle. Before performing the experiments, at least 20 cells of each strain were measured at 400× magnification using a Zeiss Axiophot light microscope equipped with an ocular micrometer. Strain plates were inspected daily to check for the presence of sexual stages. The different life stages were observed and measured using a Zeiss Axiovert and/or Axiophot microscope and photographed using a Zeiss Axiocam digital system. Strains containing sexual stages were prepared for scanning electron microscopy (SEM) as described in Amato et al. (2005) and observed using a SEM JEOL JSM-6500F electron microscope. Based on the fact that adjacent cells in a chain represent the product of vegetative division, we estimated cell size reduction at each cell division on selected chains of strains DD5 and DD20. The length of the apical axis of the epivalve of adjacent cells in girdle view was measured in SEM at 3000× magnification. The difference in cell length represents the decrease in cell size at each mitotic division, expressed as μm $division^{-1}$.

Table 1
Strains of *P. multistriata* used for: mating experiments (*), the assessment of cell size reduction within neighboring cells in a chain (#), and for experiments 1 (1) and 2 (2)

Strain code	Isolation date	Apical axis (μm)	Experiment
Pm1	18 January 2005	53.80 ± 1.14	*
Pm4	18 January 2005	39.63 ± 1.30	*
Pm17	18 January 2005	54.84 ± 1.24	*
Pm18	18 January 2005	44.87 ± 0.77	*
Pm25	18 January 2005	39.33 ± 1.20	*
DD2	30 August 2005	41.20 ± 0.66	*, 2
DD4	30 August 2005	41.48 ± 1.20	*, 2
DD5	30 August 2005	40.58 ± 0.87	#, 2
DD11	6 October 2005	39.13 ± 1.31	*
DD12	6 October 2005	39.98 ± 1.34	*
DD20	4 January 2006	70.50 ± 1.92	#
AA2	20 September 2006	37.50 ± 4.44	1
AA4	20 September 2006	43.20 ± 1.47	1
AA5	20 September 2006	37.05 ± 1.18	1
AA6	20 September 2006	52.72 ± 0.63	1
AA10	20 September 2006	38.67 ± 0.80	1
AA11	20 September 2006	47.23 ± 2.62	1
AA21	7 November 2006	55.15 ± 0.49	1
AA22	7 November 2006	47.20 ± 0.41	1
AA28	7 November 2006	56.59 ± 0.61	1
Pm4 × Pm25-1	3 October 2005	82.00	1
Pm4 × Pm25-2	3 October 2005	80.00	1
Pm4 × Pm25-3	3 October 2005	80.00	1

For each strain, the isolation date and the length of the apical axis at the moment in which the experiment was carried out are reported.

2.3. Growth rates at high irradiance and temperature conditions (experiment 1)

Nine strains of *P. multistriata* with average cell size spanning from 37 to 57 μm in length (Table 1) were grown at a temperature of 25 °C and photoperiod of 12L:12D h corresponding to late summer—early autumn conditions in the Gulf of Naples when the species is recorded at the highest concentrations. Experiments were carried out at an irradiance of 150 μmol photons m^{-2} s^{-1} corresponding to an average value of irradiance in the first 20 m of the water column. Strains were acclimated to the experimental conditions for 2 weeks, keeping them in exponential growth. Strains were then inoculated in 30 ml glass tubes filled with 15 ml of K + Si medium prepared with Sigma© Artificial Sea Water at an initial concentration ranging between 500 and 1000 cells ml^{-1}. Strains were kept in exponential growth by semi-continuous dilutions (MacIntyre and Cullen, 2005) and growth was monitored by measuring the *in vivo* fluorescence with a Turner Designs Fluorometer. The linear relationship between cell biomass, expressed as relative fluorescence units, and cell concentration over the exponential growth phase was previously assessed. Fluorescence measurements were always taken after 2 h from the onset of the light phase. After the first period of exponential growth, strains were diluted to the initial concentration and this procedure was repeated till full acclimation was achieved. This was inferred by the stabilization of the growth rate, after 3–5 dilution cycles. Growth rate, expressed as number of divisions day^{-1}, was calculated for each of the exponential intervals applying the equation:

$$k_2 = \frac{\mathrm{Ln}(f_u^{\mathrm{end}} / f_u^{\mathrm{start}})}{(t^{\mathrm{end}} - t^{\mathrm{start}})0.6931} \quad (1)$$

where f_u^{end} and f_u^{start} correspond to the initial and final fluorescence values and t^{start} and t^{end} to the initial and final day. For each strain, no less than 3 values were used to calculate the average growth. We also estimated growth rates for three initial cells from cross Pm4 × Pm25 (Table 1). Each initial cell was placed into a well of tissue culture plate and incubated at 25 °C, 12–12 L:D h, and 150 μmol photons m^{-2} s^{-1}. Cells were grown for 10 days; cell concentration was estimated using a Sedgwick–Rafter chamber and k_2 was calculated applying Eq. (1) where fluorescence values were substituted with cell concentrations.

2.4. Growth rates at low irradiance and temperature conditions (experiment 2)

Three replicates of strains DD4 and DD5 were inoculated at a concentration between 500 and 1000 cells ml^{-1} in 30 ml-glass tubes filled with 15 ml of K + Si medium prepared in artificial sea

water and incubated at 15 °C, a light:dark cycle of 12L:12D h, and an irradiance of 10 μmol photons m^{-2} s^{-1}. These conditions simulated those recorded in the deeper layers of the water column in the Gulf of Naples (Ribera d'Alcalà et al., 2004). Strains were monitored for growth and subsequently diluted to the initial concentration once a month due to the very low growth rates. Algal growth was determined by measuring *in vivo* fluorescence with a Turner Designs Fluorometer, 2 h after the onset of the light phase. The growth rate k_2 was calculated as illustrated above.

2.5. Cell size reduction rates

The cell apical length of 30 cells for each strain was estimated at the beginning (when strains were inoculated in the glass tubes) and at the end of experiment 1. The number of divisions (G) performed over each time interval comprised between two successive dilutions was calculated as

$$G = k_2(t^{\text{end}} - t^{\text{start}}) \quad (2)$$

The total number of divisions (G_{tot}) was the sum of the divisions over all the dilution intervals. Cell size reduction per generation (R) expressed as μm division^{-1} was estimated dividing the decrement in cell size estimated over the experiment by the number of cell divisions (G_{tot}). In experiment 2 the cell apical length of 30 cells for each strain was estimated at the beginning and at the end of the experiment. Cell size reduction per generation (R) was estimated dividing the decrement in average cell size estimated over the duration of the experiment by the number of cell divisions performed (G_{tot}) given by the sum of growth rate values estimated at each month.

We also measured cell size reduction rates for the three initial cells isolated from cross Pm4 × Pm25. Cell size reduction was determined as the difference between the size of the initial cell and the average size of the population after 10 days. The size reduction rate (R) was calculated as described above.

3. Results

3.1. Cell morphology

Cells of *P. multistriata* were either solitary or joined in chains. In girdle view, they showed the peculiar sigmoid shape while they appeared lanceolate in valve view (Fig. 1a–c). Following mitotic division, the two daughter cells were first aligned parallel to each other (Fig. 1b) and then glided towards opposite directions. The ascending apex of the first cell was the base on which the descending apex of the sibling cell was attached (Fig. 1d and e). Cells in a chain were joined at the distal portion of their adjacent

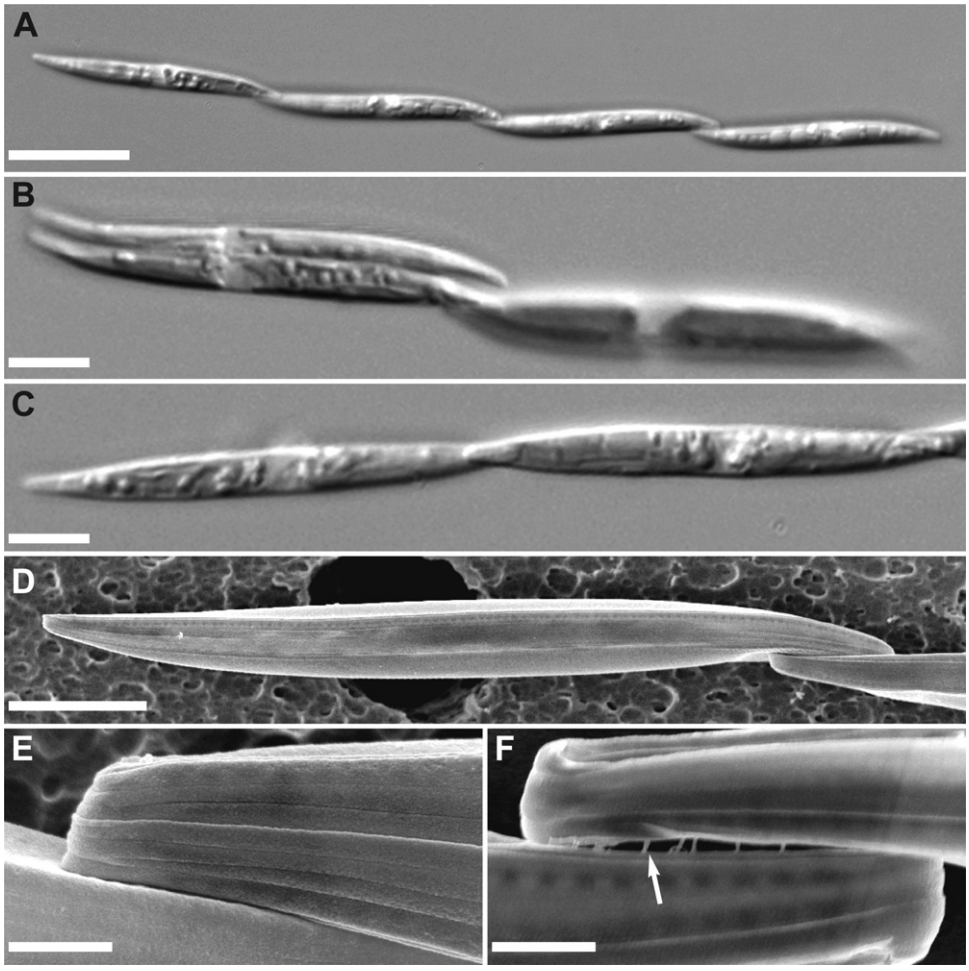

Fig. 1. *Pseudo-nitzschia multistriata* cell morphology. (A) Colony in girdle view (LM, scale bar = 30 μm); (B) neighboring cells in girdle view (LM, scale bar = 10 μm); (C) neighboring cells in valve view (LM, scale bar = 10 μm); (D) colonial cell in girdle view (SEM scale bar = 10 μm); (E) detail of the cingular bands at cell apex (SEM, scale bar = 1 μm); (F) detail of the junction between two cells in a chain, the arrow indicates mucous threads (SEM, scale bar = 1 μm).

valves by the extrusion of polysaccharide threads at the level of the poroids (Fig. 1f).

3.2. Sexual phase

P. multistriata is heterothallic since sexual reproduction occurred only after mixing strains of different mating type and was never observed in monoclonal strains. All strains decreased in size over time and we can thus exclude that vegetative enlargement occurs in this species. Strains were crossed on different occasions when the average size of their apical axes was comprised between 39 and 55 μm (Table 1), and the formation of sexual stages was always observed. At the tested experimental conditions, sexual reproduction started about 72 h (3 days) after the inoculum with the pairing between gametangia. Pairing was followed by protoplasm re-arrangement and contraction and two morphologically identical gametes per gametangium were produced (Fig. 2a). Two spherical zygotes adhered to the frustule of the "minus" mating type gametangium ($P\,Nm^{-1}$ following the notation suggested by Chepurnov et al., 2005) (Fig. 2b) via mucous threads (Fig. 2c). Auxospores (Fig. 2d), generally connected to parental frustule during the whole elongation process (Fig. 2e), grew by bi-polar enlargement (Fig. 2f). The auxospore presented one cap at each end (Fig. 2g) and was surrounded by a transversal perizonium constituted by fairly ornamented bands (Fig. 2h). The initial cell was produced within the auxospore and escaped by rupturing the distal end of the perizonium. Initial cell showed two central chloroplasts close to the epitheca and two marginal ones close to the hypotheca (up-ward and down-ward arrows in Fig. 2h, respectively). The initial cell did not have the sigmoid shape typical of vegetative cells but a straight outline in both girdle and valvar view, which is kept for the first mitotic divisions (Fig. 2i). The apical length of initial cells was comprised between 72 and 82 μm (76.8 ± 2.4 μm, $n = 40$). The fraction of gametangia within each crossing experiment was very low (around 1–2%, or even lower in some crosses). The smallest cell ever detected in our cultures was 26-μm long. Nuclear fragmentation was observed in strains with an average cell size around 30 μm (data not shown).

3.3. Growth and cell size reduction rates

3.3.1. Experiment 1: high irradiance and temperature conditions

The growth rates of *P. multistriata* strains at high light and temperature conditions spanned from 1.27 to 2.40 divisions day^{-1} (Fig. 3). Large sized cells – i.e. the strains established from initial cells – grew at a rate ranging between 1.27 and 1.45 divisions day^{-1}, while growth rates were higher for shorter cells (apical axis between 40 and 60 μm). A slight decrease in

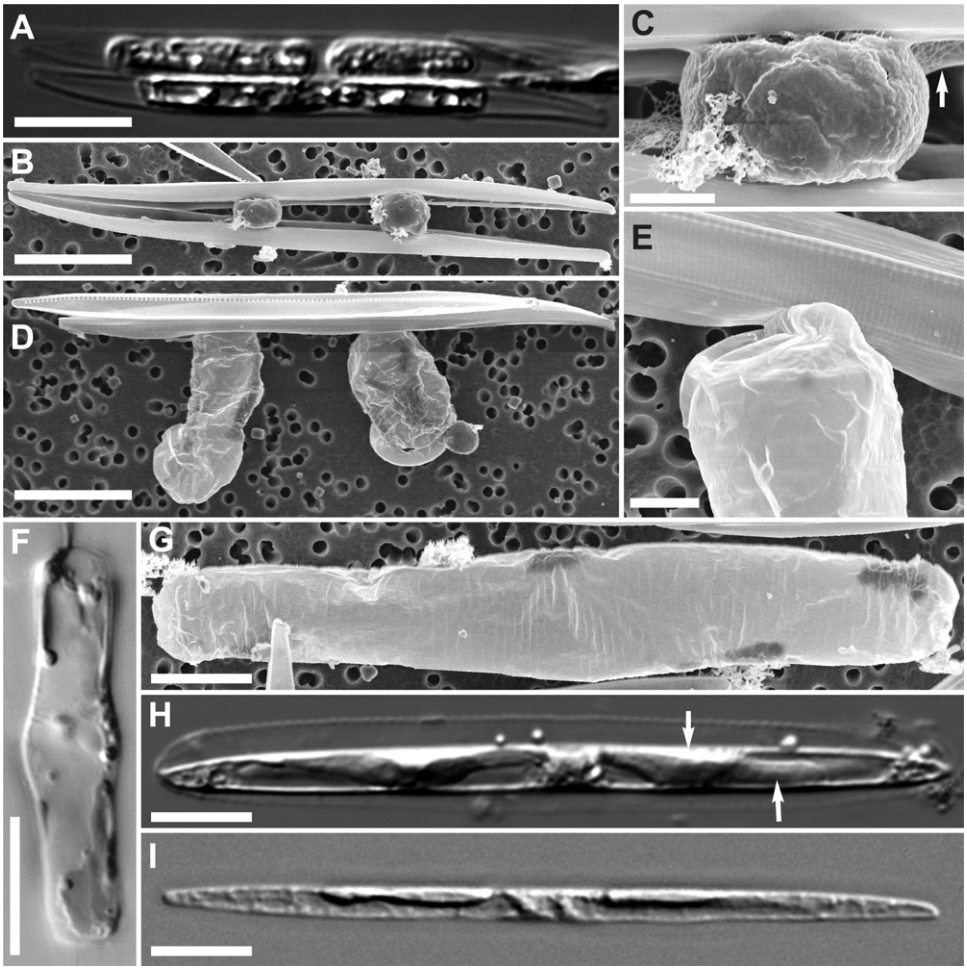

Fig. 2. *Pseudo-nitzschia multistriata* sexual stages. (A) gametangia (LM, scale bar = 10 μm); (B) zygotes (SEM, scale bar = 10 μm); (C) zygote, the arrow indicates the mucous threads connecting the zygote to the parental valve (SEM scale bar = 1 μm); (D) early auxospore connected to the parental valve (SEM, scale bar = 10 μm); (E) detail of the connection between auxospore and parental valve (SEM, scale bar = 1 μm); (F) expanding auxospore (LM, scale bar = 10 μm) (G) full grown auxospore (SEM, scale bar = 10 μm); (H) initial cell still embedded within the perizonium, the arrows indicate two chloroplasts (LM, scale bar = 10 μm); (I) large sized cell, a few divisions after auxosporulation (SEM, scale bar = 10 μm).

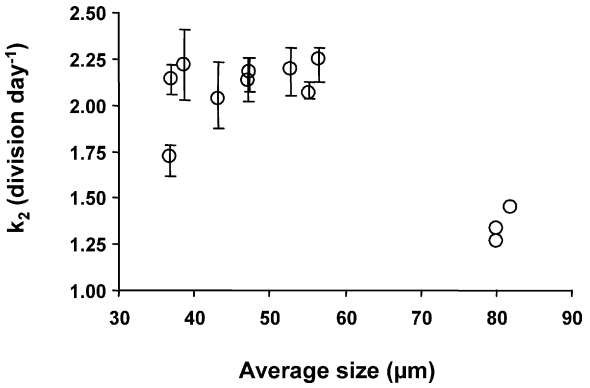

Fig. 3. Growth rate (divisions day^{-1}) vs. cell size for *P. multistriata* strains grown at high irradiance and temperature conditions.

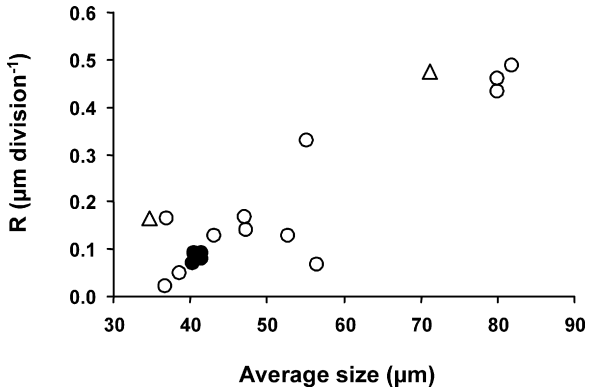

Fig. 4. Cell size reduction rate (μm division^{-1}) vs. cell size in *P. multistriata*. (○) = data gathered at high irradiance and temperature conditions; (●) = data gathered at low irradiance and temperature conditions; (△) = data gathered measuring difference in size between neighboring cells in a chain.

growth rates was observed for cells in the lower size range (apical axis between 35 and 45 μm). The cell size reduction per generation in medium and small cells ranged from 0.02 (strain AA2, cell size = 37.50 ± 4.44 μm) to 0.33 μm division^{-1} (strain AA21, cell size = 55.15 ± 0.49 μm) (Table 2, Fig. 4). The strains established from large initial cells showed a cell size decrease per generation comprised between 0.43 and 0.49 μm division^{-1} (Table 2, Fig. 4).

3.3.2. Experiment 2: low irradiance and temperature conditions

The growth rates of strains DD4 and DD5 at low temperature and light conditions were comprised between 0.26 and 0.29 division day^{-1}. The total number of divisions over the experiment (G_{tot}) was comprised between 43 and 44 in strain DD4 and between 39 and 41 in DD5 (Table 2). The cell size reduction per generation approached 0.1 μm division^{-1} in all replicates of the two strains (Table 2). In one replicate of strain DD4, some smaller cells (25–26 μm in length) were observed at the end of the experiment. The appearing of this cohort of small cells could be associated to the abrupt size reduction.

The average cell size decrease in neighboring cells in a chain was 0.48 μm division^{-1} (n = 22) in strain DD20 (cell size between 73.4 and 67.4 μm) and 0.17 μm division^{-1} (n = 31) in strain DD5 (cell size ranging between 29.4 and 36.9 μm). Abrupt size reduction was detected in both strains under examination. In DD20 it accounted up to 2.32 μm division^{-1} (1 value); in DD5, up to 0.51 μm division^{-1} (1 value). These out-of-average values were not included into the calculation of the average values reported above. Estimates of cell size reduction per generation obtained with different approaches for strains of different size are summarized in Fig. 4. Cell size decrease is higher for larger cells, and decreases as cell size reduces.

4. Discussion

4.1. Sexual phase

P. multistriata has a heterothallic life cycle and sexual reproduction is induced only when strains belonging to different mating types are mixed together. The general pattern of the sexual process was similar to that described for other *Pseudo-nitzschia* species: *P. multiseries* (Hasle) Hasle (Davidovich and Bates, 1998; Kaczmarska et al., 2000), *P. pseudodelicatissima* (Hasle) Hasle (Davidovich and Bates, 1998), *P. pungens* (Gunov ex Cleve) Hasle (Chepurnov et al., 2005), *P. calliantha* Hasle and Lundholm (Davidovich and Bates, 1998, as *P. pseudodelicatissima*) and *P. delicatissima* (Cleve) Heiden (Amato et al., 2005). All these life

Table 2
Cell size reduction rates for *P. multistriata* strains at different irradiance and temperature conditions: average cell size ± S.D. at the beginning (size start) and at the end (size end) of the experiment, length of the experiment (time), average cell size reduction over the experimental period (r), number of divisions over the experimental period (G_{tot}), and average cell size decrease at each division (R)

Strains	Size start (μm)	Size end (μm)	Time (day)	r (μm)	Gtot (division)	R (μm/division)
Experiment 1: high irradiance, high temperature						
AA5	37.05 ± 1.18	32.45 ± 3.19	21	4.60	27.85	0.16
AA2	37.50 ± 4.44	36.82 ± 1.45	13	0.68	21.68	0.02
AA10	38.67 ± 0.80	37.73 ± 0.64	14	0.93	19.67	0.05
AA4	43.20 ± 1.47	40.30 ± 1.66	14	2.90	22.81	0.13
AA22	47.20 ± 0.41	44.80 ± 0.77	18	2.40	14.31	0.17
AA11	47.23 ± 2.62	44.25 ± 0.77	17	2.98	21.29	0.14
AA6	52.72 ± 0.63	49.10 ± 2.24	21	3.62	28.08	0.13
AA21	55.15 ± 0.49	46.40 ± 1.39	17	8.75	26.61	0.33
AA28	56.59 ± 0.61	54.97 ± 1.10	13	1.63	24.38	0.07
Pm4 × Pm25-1	82.00	74.95 ± 1.01	10	7.05	14.50	0.49
Pm4 × Pm25-2	80.00	75.25 ± 0.98	10	5.75	13.32	0.43
Pm4 × Pm25-3	80.00	74.15 ± 0.53	10	5.85	12.70	0.46
Experiment 2: low irradiance, low temperature						
DD4-1	41.48 ± 1.20	37.35 ± 1.49	152	4.12	44.60	0.09
DD4-2	41.49 ± 1.18	38.15 ± 1.29	152	3.80	44.40	0.09
DD4-3	41.45 ± 1.43	38.18 ± 3.83	152	3.27	43.10	0.08
DD5-1	40.58 ± 0.87	36.93 ± 1.47	152	3.65	41.10	0.09
DD5-2	40.35 ± 0.87	37.75 ± 0.81	152	2.60	39.25	0.07
DD5-3	40.75 ± 0.74	37.18 ± 1.17	152	3.20	39.25	0.08

cycles are characterized by behavioral anisogamy – the gametes produced in the two gametangia behave differently – and by a '*cis*-type' gamete fusion i.e. the two active gametes (+) glide towards the two sessile (−) ones (Chepurnov et al., 2004). Auxospore development occurs by bi-polar expansion through the deposition of transversal perizonial bands of the zygote attached to the (−) gametangium. In *P. multistriata*, sexual reproduction was observed in strains with an average cell size range spanning from 39% to 71% to the maximum size, i.e. from 39 to 55 μm of average cell apical length. We did not attempt crossing strains of larger or smaller cell size, so we cannot exclude that the size window for sexualization in this species might be even broader. A wide cell size range for sexual reproduction has been reported in experimental conditions also for *P. multiseries* (23–70% of the maximum size, Hiltz et al., 2000) and *P. delicatissima* (20–85% of the maximum size, Amato et al., 2005). A narrower size range of gametangia (10–31 μm corresponding to about 11–34% of the initial cell size) was instead reported for the phylogenetically closely related biraphid diatom *Fragilariopsis kerguelensis* (O'Meara) Hustedt from observations carried out on natural populations (Assmy et al., 2006).

We observed sexual reproduction only when mixing exponentially growing strains, as was reported for *P. multiseries* (Davidovich and Bates, 1998) and *P. delicatissima* (Amato et al., 2005). This suggests that in the natural environment sexual reproduction might occur when species are blooming, i.e. when the highest growth rates are detected. Massive production of sexual stages has been reported during a bloom of the planktonic centric diatom *Corethron pennatum* (Grunow) Ostenfeld (as *C. cryophylum* in Crawford, 1995) and auxospores were detected during a bloom of *Fragilariopsis kerguelensis* (Assmy et al., 2006). Nevertheless, other environmental factors might regulate the occurrence of the sexual phase. A positive correlation between daylength and population fecundity, expressed as the ratio between the number of gametes and the number of vegetative cells was reported for *P. multiseries*, where the highest number of gametes was observed at 16 h of light (Hiltz et al., 2000). However, the highest production of initial cells was recorded when strains were exposed at lower daylength (10 h L), suggesting that sexual reproduction was more successful when daylength corresponded to that recorded during the fall bloom in the studied area. Also in the epiphytic diatom *Cocconeis scutellum* Ehrenberg the highest production of auxospores occurred at short daylength conditions (10:14 L:D) corresponding to the post-bloom period for this species at sea (Mizuno and Okuda, 1985).

4.2. Vegetative phase: growth rates

We recorded an increase in growth rate along with a decrease in cell size in *P. multistriata*. A similar trend was reported for *P. delicatissima*, where larger cells showed lower growth, the maximum values were reached when the population reached about 60% of the maximum cell size, and a slight decrease of growth rates was detected again for cells in the smaller size (Amato et al., 2005). Mizuno (1991) also described an inverse relationship between growth rate and cell size in the benthic *Cocconeis scutellum*. A sudden increase of growth rates towards the medium cell size range and a slight decrease towards small cells have been reported also for *Ditylum brightwellii* (T. West) Grunow, *Licmophora hyalina* (Kützing) Grunow (Paasche, 1973), *Coscinodiscus pavillardii* Forti (Findlay, 1972), *Thalassiosira nordenskioeldii* Cleve (Durbin, 1977) and *Thalassiosira weissflogii* (Grunow) G. Fryxell and Hasle (von Dassow et al., 2006). This trend might be related to a higher energy requirement for doubling biomass of large post-initial cells and to an optimal surface area/volume and nucleus/cytoplasm ratios in medium sized cells, which then becomes sub-optimal when cell size reaches the lowest values. However, no significant variation in growth rate versus cell size has been reported for the centric diatom *Thalassiosira decipiens* (Grunow) E.G. Jørgensen (Paasche, 1973), and even an increase in growth rate after cell size restoration was reported in *T. weissflogii* (Chisholm and Costello, 1980; Costello and Chisholm, 1981) and *Skeletonema costatum* (Greville) Cleve (Davis et al., 1973). For the latter two species, no information was provided on the cell size of the population at which sexual reproduction was induced. One possible explanation for the considerable increase in growth rate coincident with appearance of larger cells is that strains were at the lowermost cell size range, where growth rate is particularly low.

4.3. Vegetative phase: size reduction

We derived cell size reduction rates in *P. multistriata* using different approaches and obtained consistent values. Size reduction rates derived from experiments 1 and 2 were similar to values obtained by measuring neighbor cells in a chain at high magnification (Fig. 4). The cell size reduction process in *P. multistriata* showed a pattern similar to what reported for *P. delicatissima*, where a decreasing rate of size reduction per generation was detected as cell size decreased (Amato et al., 2005). The same trend was found by Jewson (1992) for *Aulacoseira subarctica* (O.F. Müller) E.Y. Haworth, where the largest reduction in size was detected in broader cells and a gradual decrease was recorded as cells became smaller.

We sporadically detected an abrupt decrease in cell size of *P. multistriata*. Abrupt size reduction – i.e. the formation of a daughter valve much more reduced in size – has been reported in strains of *P. pungens* along the whole size range (Chepurnov et al., 2005). In *Aulacoseira subarctica* an abrupt reduction (up to 1 μm per division) was detected in natural populations at low nutrient concentrations or low irradiance (Jewson, 1992). We observed a sudden decrease of cell size only in one out of six cultures incubated at low light conditions, which does not support a direct link between light limitation and abrupt size reduction. Although factors inducing this peculiar way of division are still obscure, this might represent a mechanism through which a large variability in the size range of natural populations is produced so to allow a fraction of the population to reach faster the size threshold for sexualization.

4.4. In situ life cycle

The appearing of large-sized cells in natural populations provides evidence for recent sexual reproduction events in species that rely on sex to restore their size (Mann, 1988). Moreover, a marked reduction of the average cell size should be detected in periods of sustained growth, due to the fact that cell size reduction in a population is related to the number of divisions performed. Experimental data gathered for *P. multistriata* thus help to model life cycle patterns of this species in nature. We can predict the number of division needed to reach the threshold size for sexual reproduction and to go from the large initial cell to the smallest size. Fig. 5 illustrates the relationship between cell size and number of division derived from our experimental data. An initial 80 μm-long cell will have to go through 50 divisions to give rise to a population with a modal size of 65 μm (Fig. 5, point a) and 50 more divisions to reach the size threshold for sexualization (55 μm, Fig. 5, point b), considering that cell size reduction for this size interval is lower. A higher number of divisions are further required to reach smaller size (e.g. 45 and 35 μm, Fig. 5, points c and d, respectively). Considering that sexual reproduction only involves a small fraction of the whole vegetative population and that large-sized cells have a faster size reduction rate, we can

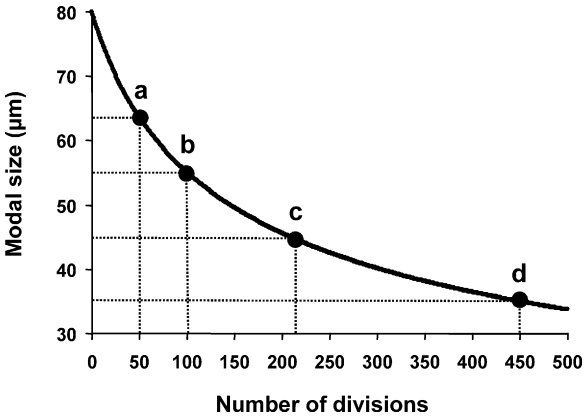

Fig. 5. Variation of the modal cell size in dependence of the number of divisions in a clonal population of *P. multistriata*.

expect them to be very rare in natural populations, as shown by the few available experimental data (Jewson, 1992; Assmy et al., 2006). On the contrary, medium and smaller cells persist for a longer time, because of their slower reduction rates. If we assume that *P. multistriata* grows on average at one division per day (not considering here the variability in growth rates related to cell size), an initial cell will take 100 days (a little bit more than 3 months) to reach the threshold for sexualization, while it will take 450 days to reach the lowest modal size at which cells start dying. If we assume that growth rates are higher in bloom periods – up to 2 divisions per day, as indicated by our experiments – the time required to reach the abovementioned modal size steps halves. Under this scenario, *P. multistriata* might theoretically undergo sexual reproduction every 3–4 months. However, we know that growth rate is not constant along the cell size interval. Low growth rates in post-initial cell and in very small cells might retard the decrease in size thus retarding the occurrence of sex and cell death at small cell sizes.

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