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Intra- and inter-specific differences in gametangial and initial cell size in diatoms

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Abstract

Most diatom species that have been carefully studied undergo sexual reproduction as part of their diplontic life cycle to restore the cell line to its maximum size. The life history of diatoms is characterized by two 'cardinal' or turning points marking the beginning and end of sexual reproduction: the size of the gametangial cells and the post-auxospore size of initial cells, respectively. The precise controls of auxospore growth have not been fully elucidated; however, auxospore expansion may be regulated by combinations of gametangial size, environmental, and genetic factors. The relation between gametangial size and initial cell size was investigated for several freshwater centric and pennate taxa by examining natural and cultured populations of *Cymbella*, *Cocconeis*, *Gomphonema*, *Aulacoseira*, and *Stephanodiscus* undergoing allogamy, oogamy, and parthenogenetic reproduction. Gametangial size was found to be correlated with initial cell size in most, but not all, investigated taxa. Cardinal points often represented a very wide range of cell sizes and differed among populations of the same taxon indicating that these ranges and differences may be critical in diatom systematics and taxonomy for differentiating species- and population-level differences.

Introduction

Since the mid-1800's it has been accepted that size reduction accompanies each vegetative mitotic division during the diploid phase of the diatom life cycle (MacDonald 1869, Pfitzer 1869). Of the two progeny cells resulting from a normal mitotic division, one will be identical in size to the parent cell and the other will be slightly smaller. This results in a population of cells that has first, a decreasing mean cell size as measured along the common taxonomic morphometrics (valve diameter or length), and second, an increasing variance of cell sizes over time. With natural losses and losses explained by the more recent suggestion that valves have determinate life spans (Mann 1988, Jewson 1992b), diatom taxa must possess mechanisms to regain their larger cell size. For most taxa, size restoration involves episodic sexuality (Edlund & Stoermer 1997); however, other vegetative and parthenogenetic life history

1927; von Stosch 1965, 1967; Gallagher 1983; Nagai *et al.* 1995).

Sexuality and size restitution can occur in diatoms only after several conditions have been met (reviewed in Edlund & Stoermer 1997). First, vegetative cells must reach an inducible size range for gametogenesis. Second, the proper environmental conditions must occur (Schmid 1995). Third, it has been suggested that cells must be in the proper phase of their mitotic cell cycle for gametogenesis to begin (Armbrust *et al.* 1990, Jewson 1992b, Davidovich 1998). Only when these conditions have been met can sexual reproduction occur. Size restitution occurs following syngamy with the production of one or two auxospores-specialized cells capable of growth or expansion. The growth of the auxospore may be controlled by various siliceous structures, which include the properizonium and epizonium in centric diatom and the perizonium in pennates. Following expansion and at least two acytokinetic mitoses, two silicified valves are formed within the auxospore; this stage in the diatom life history is known as the initial cell. The initial cell has many of the characteristics of the normal vegetative cell; however, its valves tend to be rounded and/or possess aberrant structure (Mann 1984, 1994; Edlund & Stoermer 1991).

The gametangial cell size and the size of the initial cells represent the two cardinal points in the diatom life history (Geitler 1932). These points have classically been considered to be species-specific, reflecting the full range of sizes that a taxon can achieve during size reduction. Differences in cardinal points among populations of related taxa may be indicative of species-level differences or the identification of races of diatoms (Geitler 1932, Mann 1984). Whereas Geitler (1932) suggested that cardinal points represent discrete and narrow size intervals, some workers have presented data indicating that pre- and post-sexual cells often have a wide size range both within and among species populations (Bethge 1925; Nipkow 1927; Skabitschewsky 1929; von Stosch & Drebes 1964; Migita 1967; Drebes 1974; Stoermer & Ladewski 1982; Gallagher 1983; Mann 1984, 1988; Kociolek & Stoermer 1989; Edlund & Stoermer 1991, 1996, 1997; Armbrust & Chisholm 1992; Jewson 1992a, b; Waite & Harrison 1992; Davidovich 1994, 1998; Schmid 1995; Nagai *et al.* 1995; Nagai & Imai 1997). Confounding these reports is our poor understanding of the process of auxospore expansion. At the cytological level, the period of auxospore expansion appears to be accompanied by a breakdown of the cytoskeleton, which later reorganizes after expansion is complete (Schmid 1984). Auxospore growth is also thought to be largely an expression of vacuolar expansion, suggesting that osmotic relations may be more important than the photosynthetic capacity of the auxospore (Hoops & Floyd 1979, Davidovich 1994). The completion of auxospore expansion and silicification in darkness and/or under nutrient-poor conditions bears this out (Waite & Harrison 1992, Davidovich 1994), but also identifies the parental reserves as critical to completing auxospore expansion (Davidovich 1998).

An additional factor that may influence auxospore expansion and subsequent initial cell size is gametangial or parental cell size. Authors have noted positive linear relationships between parental and initial cells following auxosporulation in *Melosira moniliformis* (O. Müller) Agardh, *Skeletonema costatum* (Greville) Cleve (Migita 1967), *Aulacoseira subarctica* (O. Müller) Haworth (Jewson 1992b), *Nitzschia*

lanceolata W. Smith, *Licmophora ehrenbergii* (Kützing) Grunow, and *Synedra tabulata* (C. Agardh) Kützing (Davidovich 1994, 1998), and following pseudoauxosporulation in *Coscinodiscus wailesii* Gran *et* Angst (Nagai *et al.* 1995, Nagai & Imai 1999). Yet among the several hundreds of diatom species whose sexuality has been studied, this relationship has been shown only in these few. Furthermore, the size and/or range of sizes of both gametangial and initial cells may have autecological consequences related to fitness (Mann 1993), plankton ecology (Round 1982, Davey 1986, Jewson 1992b, Nagai & Imai 1997), nutrient uptake dynamics (Grover 1989), and potential losses to the population (Jewson 1992b, Edlund & Francis 1999).

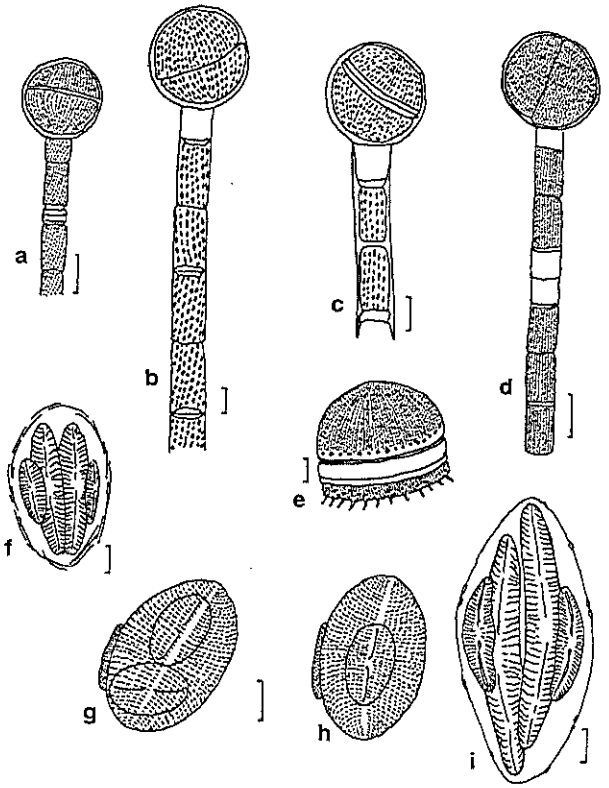

Fig. 1. Physical relationship of gametangial and initial cells for the taxa examined in this study. Scale bars = 10 μm. **Fig. 1a.** *Aulacoseira ambigua*, oogamy (modified from Bethge 1925). **Fig. 1b.** *A. granulata*, oogamy (modified from Canter-Lund & Lund 1995). **Fig. 1c.** *A. baicalensis*, oogamy (modified from Skabitschewsky 1929). **Fig. 1d.** *A. islandica* ssp. *helvetica*, oogamy (modified from Bethge 1925). **Fig. 1e.** *Stephanodiscus niagarae*, oogamy with free auxospores (modified from Edlund & Stoermer 1991). **Fig. 1f.** *Gomphonema parvulum*, Type I (modified from Edlund & Stoermer 1997). **Fig. 1g.** *Cocconeis placentula*, Type IIb (original). **Fig. 1h.** *Cocconeis placentula*, parthenogenesis (original). **Fig. 1i.** *Cymbella cistula*, Type I (modified from Edlund & Stoermer 1997).

Recent work by Nagai & Imai (1997) has furthered our understanding of parental and environmental controls of auxospore expansion. *Coscinodiscus wailesii* undergoes size restoration by either oogamy or pseudoauxosporulation. Pseudoauxosporulation is a vegetative process that involves parent cell expansion, protoplast release, auxospore expansion, and eventual silicification of an initial cell via the normal cellular mechanism used following sexual reproduction. The size of pseudoauxospore-produced initial cells of *C. wailesii* was positively correlated not only with the asexual parent cell size (Nagai *et al.* 1995), but also with total daily irradiance levels present during pseudoauxospore expansion (Nagai & Imai 1997). Pseudoauxospore size of *C. wailesii* was also related in part to salinity (Nagai & Imai 1999). In contrast, Davidovich (1994, 1998) found a correlation between parental gametangia and initial cell size in *Nitzschia lanceolata*, *Synedra tabulata*, and *Haslea subagnita* (Proschkina-Lavrenko) Makarova *et* Karayeva, but no correlation between light dosage and initial cell size.

In this paper, we present data on gametangial and initial cell size in natural and cultured populations of *Stephanodiscus*, *Gomphonema*, *Cymbella*, and *Cocconeis* undergoing oogamy, allomixis, and parthenogenesis. Additionally, data from Bethge (1925) and Skabitschewsky (1929) on *Aulacoseira* are reanalyzed.

Material and methods

Material used in this study came from several sources. Sexual reproduction and initial cell sizes in two populations of *Stephanodiscus niagarae* Ehrenberg have been previously reported (Edlund & Stoermer 1991). A third population of *S. niagarae* (M. B. Edlund personal collection MBE 205) was taken with a 35-µm mesh plankton net from the Barton Pond impoundment on the Huron River, Washtenaw County, Michigan on 24 March 1990. Sexual material of *Gomphonema parvulum* (Kützing) Kützing was collected (MBE 157) from epilithic material in the Huron River below the Barton Dam, Washtenaw County, Michigan on 06 June 1991. Two sexual populations of *Cymbella cistula* (Ehrenberg *in* Hemprich *et* Ehrenberg) Kirchner *in* Cohn were also studied. One population came from epiphytic material in Hell Creek, Livingston County, Michigan collected on 16 March 1992 (MBE 463). The other collection was taken on 12 June 1996 from gelatinous, nearshore periphyton in Lake Hovsgol, Hatgal, Mongolia (MBE M114). A population of *Cocconeis placentula* Ehrenberg undergoing anisogamous allomixis (Geitler's Type IIb) and parthenogenesis [Geitler (1973) Type IVb] was collected by Dr. E. F. Stoermer in the summer of 1957 on colonized microscope slides incubated in the nearshore area of West Okoboji Lake off the Iowa Lakeside Laboratory property, Dickinson County, Iowa. The colonized slide was dehydrated, stained with iron-hematoxylin and mounted in Hyrax, and is deposited in the Diatom Herbarium at the University of Michigan (E. F. Stoermer, curator). All other collections were preserved in either 5% formaldehyde or paraformaldehyde-formaldehyde solution (Lazinsky & Sicko-Goad 1979). Material and/or slides are available from the senior author.

Subsamples of wet, preserved material were mounted on semi-permanent microscope slides and observed using brightfield optics capable of X1250 and N.A of 1.32. An ocular micrometer calibrated against a Bausch and Lomb #370 stage micrometer was used to measure gametangial and initial cells in sexual material. Diameter of *Stephanodiscus* initial valves was measured (three populations, n = 50, 34, and 50). In pennate material, valve lengths of gametangial cells and initial cells were recorded. For allogamous pairings of pennate diatoms, the lengths of the two parent gametangial cells were taken separately and later combined for analysis (*Gomphonema*, two *Cymbella* populations, and *Cocconeis*; n = 27,27,27,31, respectively). In Type I pennate allogamy (*Gomphonema* and *Cymbella*), the two progeny initial cell lengths were measured separately and also combined for later analysis; only pairings that successfully produced two silicified initial cells were measured. The designation of cell 1 or 2 (gametangia or initial) within mating pairs was randomly assigned; however, in the measurements, initial cells were assigned the same number (1 or 2) as their associated passive gametangia (see Fig. 13 in Edlund & Stoermer 1997). The lengths of the single parent cell and single initial cell were measured for parthenogenetic reproduction (*Cocconeis placentula*, n=22). Data on oogonial cell diameter and initial cell diameter of *Aulacoseira baicalensis* (K.I. Meyer) Simonsen (n=200), *A. granulata* (Ehrenberg) Simonsen (n=14), *A. ambigua* (Grunow) Simonsen (n=50), and *A. islandica* ssp. *helvetica* (O. Müller) Simonsen (n=55 and 28 from two dates in Plöner See, n=77 from the Havel) were taken from the literature (Bethge 1925, Skabitschewsky 1929).

Differences of means of gametangial or initial cell size among populations were investigated using paired *t*-tests. Significance of linear relationships between gametangial cell sizes and initial cell sizes within populations was tested with one-way ANOVA. All statistical analyses were performed with SYSTAT software (Wilkinson *et al.* 1992) with $\alpha = 0.05$.

Results

RELATION OF GAMETANGIAL SIZE TO INITIAL CELL SIZE, SINGLE POPULATIONS

Aulacoseira ambigua, A. granulata, A. baicalensis

In 1925, Bethge published a monograph on the freshwater *Melosira*. Included in his widely contested taxonomic treatment was an abundance of data on auxosporulation in several taxa now included in the genus *Aulacoseira*. One *Aulacoseira* has been shown to undergo oogamous sexual reproduction (*A. subarctica*; Jewson 1992b), but others may use asexual or parthenogenetic mechanisms of size restoration. Regardless, in most species the initial cell remains attached to the maternal filament for a short period of time, allowing the relationship between maternal cell size and initial cell size to be evaluated (Figs 1a-d).

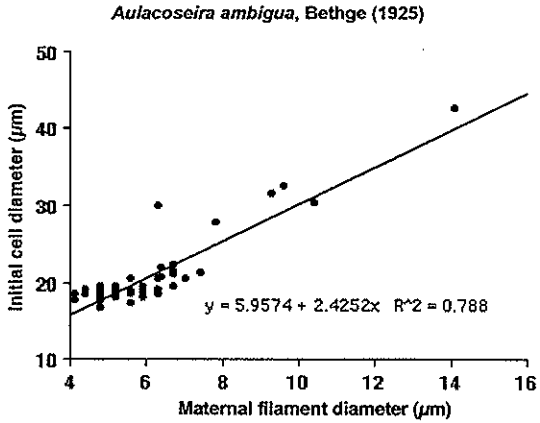

Fig. 2. Relation between gametangial and initial cell diameter in *Aulacoseira ambigua* from Müggelsee (n = 50). Data from Bethge (1925).

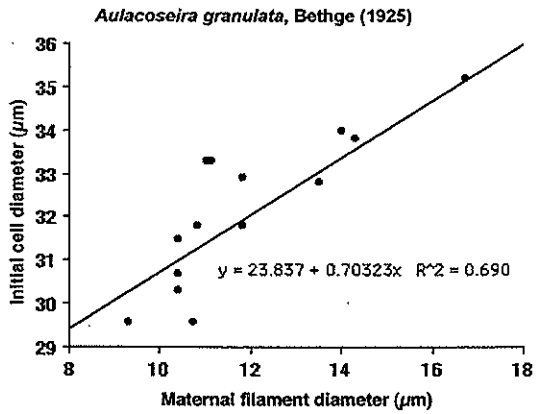

Fig. 3. Relation between gametangial and initial cell diameter in *Aulacoseira granulata* from Nil (n = 14). Data from Bethge (1925).

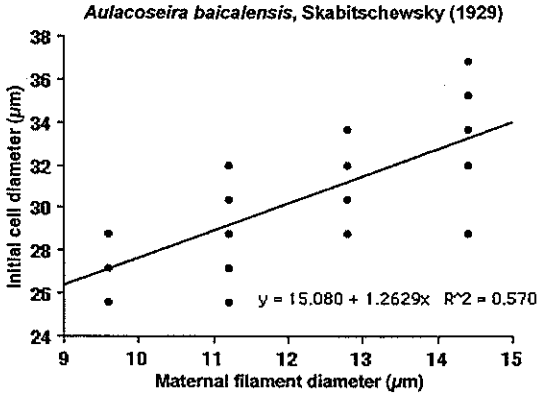

Fig. 4. Relation between gametangial and initial cell diameter in *Aulacoseira baicalensis* from Lake Baikal (n = 200; each point may represent more than one datum). Data from Skabitschewsky (1929).

Bethge reported initial cell diameter and maternal filament diameter in *A. ambigua* (Figs 1a, 2, n=50, maternal diameter range 4.1-14.1 µm, mean 6.1 µm, S.D.=1.76; initial cell range 16.7-42.6 µm, mean 20.8 µm, S.D.=4.81) and *A. granulata* (Figs 1b, 3, n=14, maternal diameter range 9.3-16.7 µm, mean 11.9 µm, S.D.=2.03; initial cell range 29.6-35.2 µm, mean 32.2 µm, S.D.=1.71) populations in Müggelsee and Nil, respectively. A significant positive linear relationship existed between the diameter of the maternal filament and the diameter of the initial cell in both *A. ambigua* (r^2=0.788, F=179.0, p<0.001) and *A. granulata* (r^2=0.690, *F*=26.7, p<0.001). Skabitschewsky's (1929) data on *A. baicalensis* provide an additional example of a significant positive correlation (Figs 1c, 4, r^2=0.57, *F*=263.0, p<0.001) between maternal filament diameter (n=200, range 9.6-14.4 µm, mean 12.1 µm, S.D.=1.16) and auxospore diameter (n=200, range 25.6-36.8 µm, mean 30.4 µm, S.D.=1.94).

Gomphonema parvulum

Gomphonema parvulum in the Huron River, Michigan, utilized Type IA sexual reproduction (Geitler 1932, Hohn 1959). Two parent cells were paired within a copulation mucilage and each parent produced two gametes. Following gamete exchange, plasmogamy, and auxospore expansion, two initial cells are produced.

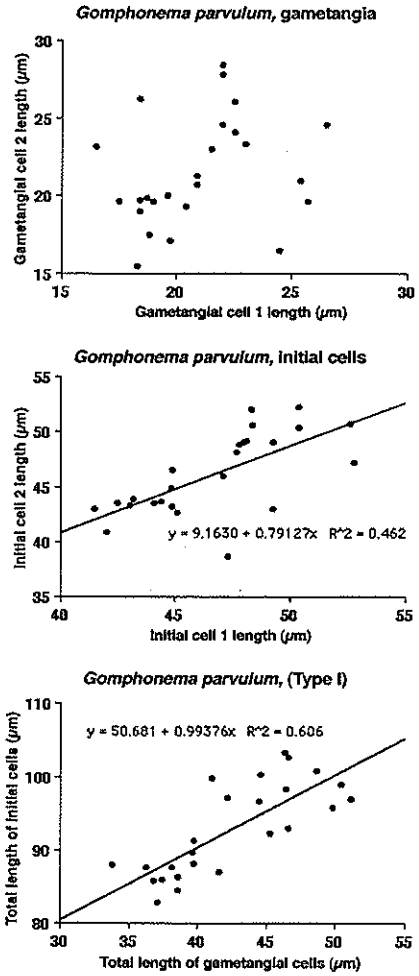

Fig. 5. *Gomphonema parvulum* undergoing Type I sexual reproduction. **Fig. 5a.** Comparison of gametangial pairs indicates random mating among gametangial cells. **Fig. 5b.** Comparison of two initial cells from Type I reproductive pairings shows resultant similar-sized initial cells. **Fig. 5c.** Linear relation of sum lengths of gametangial cells to sum lengths of initial cells.

The paired gametangial cells remained within the copulation mucilage until initial cell silicification was complete (Fig. 1f), which allowed the sizes of the gametangia (n=54, length 15.5-28.4 µm, mean 21.2 µm, S.D.=3.10) and initial cells (n=54, length 38.6-52.8 µm, mean 46.4 µm, S.D.=3.42) to be measured and compared. Twenty-seven mating pairs were analyzed and these data provided several interesting observations on *Gomphonema* sexuality. The range of gametangial cell sizes was large and pairing of gametangial cells appeared random (Fig. 5a); however, in many of the pairings, the two initial cells produced were similar-sized (Fig. 5b). When lengths of paired parent cells and paired initial cells were each summed, a significant, positive linear relationship is apparent (Fig. 5c, n=27, r^2=0.606, F=35.4, p<0.001) suggesting that combined gametangial contribution is a major factor in final initial cell size.

RELATIONSHIP OF GAMETANGIAL SIZE TO INITIAL CELL SIZE IN ONE POPULATION UNDERGOING TWO REPRODUCTIVE STRATEGIES

Cocconeis placentula

Cocconeis placentula undergoes Geitler's (1932) Type IIb sexual reproduction. In this mating strategy, two parent cells each produce single gametes which combine to form a single auxospore (Fig. 1g). A common modification of this strategy in *Cocconeis* is parthenogenesis (Fig. 1h, Type IVb), in which a single parent cell produces a single auxospore via apomixis (Geitler 1927, 1932, 1973; von Stosch 1967; Mann 1993). In Type IIb reproduction, the single auxospore was underlain on the slide by two raphid gametangial valves (Fig. 1g), whereas in Type IVb parthenogenesis, the single auxospore was underlain by a single raphid gametangial valve (Fig. 1h). It should be clarified that, because the investigated samples were not specially prepared for nuclear analysis and were well past the stage of gametogenesis, it was not possible to absolutely determine whether the Type IV initial cells were parthenogenetic or automictic (*sensu* Geitler 1973). In both cases, it was clear that the gametangial and the initial cells were *C. placentula* var. *placentula* (*sensu* Patrick & Reimer 1966) and not one of the common varieties of this taxon.

Gametangial and initial cell lengths were measured and compared, and Type IIb parent lengths were combined for regression analysis. Type IIb parent cells (n=62, range 14.9-23.2 µm, mean 18.1 µm, S.D.=1.87) and parthenogenetic parent cells (n=22, range 14.6-21.7, mean 17.0 µm, S.D.=1.71) showed a range of sizes. No significant difference was found between gametangial cell sizes regardless of reproductive strategy (Fig. 6a, t=-1.793, p=0.087); however, some gametangial mating size selectivity was possible for Type IIb pairings as indicated by similar-sized gametangial pairings and may represent sister cell pairings in *Cocconeis* (Fig. 6b). Comparison of initial cell sizes (Type IIb, n=31, range 32.9-50.6 µm, mean 39.5 µm, S.D.=5.74; parthenogenesis, n=22, range 32.6-50.0 µm, mean 37.1 µm, S.D.=4.11) showed no significant difference between the two reproductive strategies (t=-0.825, p=0.419). Furthermore, regardless of reproductive strategy, there was a significant linear relationship between parent cell length(s) and initial cell length (Fig. 6c) in both Type

IIb reproduction (r^2=0.458, F=24.5, p<0.001) and parthenogenesis (r^2=0.448, F=16.173, p<0.001).

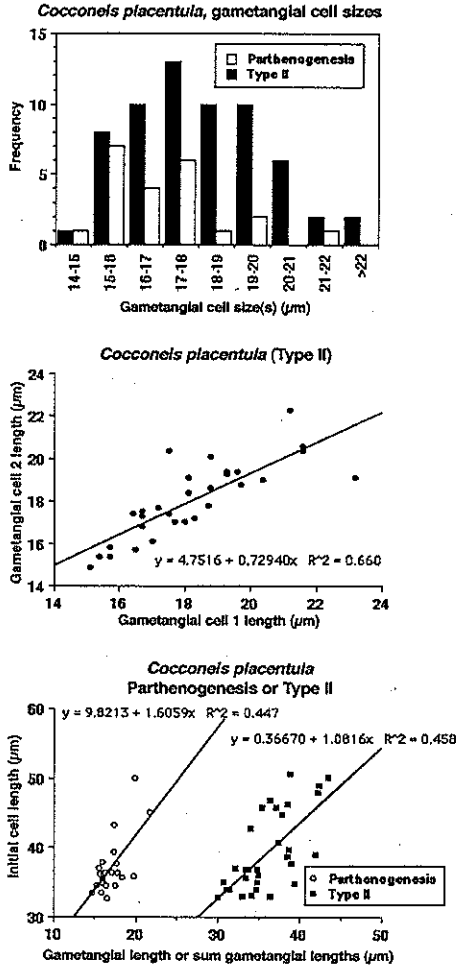

Fig. 6. *Cocconeis placentula* undergoing two mating strategies: Type IIb and parthenogenesis. **Fig. 6a.** Frequency histogram of *C. placentula* gametangial cells undergoing two mating strategies. **Fig. 6b.** Comparison of gametangial pairs indicates non-random mating among gametangial pairs for Type II reproduction. **Fig. 6c.** Linear relation of length of gametangial cell (parthenogenesis) or sum lengths of gametangial cells (Type IIb) to length of single initial cell produced following sexual reproduction of *C. placentula*.

DIFFERENCES AMONG DIATOM POPULATIONS IN GAMETANGIAL AND INITIAL CELL SIZE

Stephanodiscus niagarae

Edlund & Stoermer (1991) reported on oogamous reproduction and initial cell sizes in two temporally segregated populations of *Stephanodiscus niagarae* that were collected from the North American Great Lakes. *Stephanodiscus* produces free auxospores (Nipkow 1927, Edlund & Stoermer 1991, Jewson 1992a), and hence the relationship between maternal and progeny cells cannot be easily quantified (Fig. 1e). Earlier, Edlund & Stoermer (1991) reported significant differences in initial cell size distribution (t=16.682, p<0.0001) in two temporally segregated populations of *Stephanodiscus niagarae* that were collected from the North American Great Lakes: a sexual clonal population collected and cultured in 1990 (culture ME184, n=50, range 45.1-82.3 µm, mean 61.7 µm, S.D.=7.3) and a population collected in 1878 (Smith 1878, n=36, range 70.6-105.8 µm, mean 91.9 µm, S.D.=7.98) from within the same drainage system. We compared the distribution of *S. niagarae* initial cell sizes in Edlund & Stoermer (1991) with new data from a natural *S. niagarae* population (Barton Pond, n=50, range 54.1-86.2 µm, mean 65.6 µm, S.D.=6.51) undergoing auxosporulation. A frequency histogram (Fig. 7) and paired t-tests of all three populations indicated that the mean initial cell size produced in Barton Pond differed significantly from H. L. Smith's (1878) collection (t=12.308, p<0.001), and from the cultured population (ME184) reported in Edlund & Stoermer (1991) although to a lesser degree of significance (t=-2.765, p=0.008).

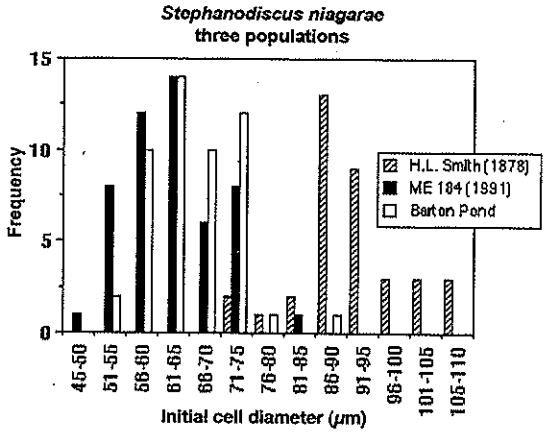

Fig. 7. Frequency histogram of *Stephanodiscus niagarae* initial cells from three populations.

Aulacoseira islandica ssp. *helvetica*

Bethge (1925) presented data on maternal filament diameter and initial cell diameter in *Aulacoseira islandica* ssp. *helvetica* (Fig. 1d) from two European localities: Plöner See and Havel. The Plöner See population was collected undergoing sexual reproduction

on two sampling dates (10.2.1919, 22.1.1920) and the Havel population on 17.7.1923. In all populations, wide ranges of maternal filament and initial cell diameters were noted (Fig. 8) during the several years that samples were taken. In Plöner See on 10.2.1919, maternal cells (n=52) had diameters from 7.0 µm to 11.8 µm (mean 9.7 µm, S.D.=1.25) and initial cells (n=52) ranged from 18.5 µm to 30.3 µm (mean 23.8 µm, S.D.=3.42). In Plöner See on 22.1.1920, maternal cells (n=28) had diameters from 7.4 µm to 12.2 µm (mean 9.8 µm, S.D.=1.20) and initial cells (n=28) ranged from 18.5 µm to 30.7 µm (mean 23.4 µm, S.D.=2.69). In Havel on 17.7.1923, maternal cells (n=77) had diameters from 4.8 µm to 10.8 µm (mean 7.2 µm, S.D.=1.32) and initial cells (n=77) ranged from 14.1 µm to 30.4 µm (mean 22.3 µm, S.D.=4.05).

The relation between *Aulacoseira islandica* ssp. *helvetica* maternal cell diameter and initial cell diameter (Fig. 8) was tested and found to have a weakly significant linear relationship in Plöner See on 10.2.1919 (r=0.321, F=5.760, p=0.020), nearly significant linear relationship in Plöner See on 22.1.1920 (r=0.370, F=4.136, p=0.052), and a significant linear relationship in Havel on 17.7.1923 (r=0.839, F=178.775, p=0.000). Paired comparisons among populations' maternal cell diameters showed that the means of the two Plöner See populations were not significantly different (t=0.443, p=0.066), whereas the maternal cell diameter means of both the Plöner See 10.2.1919 population (t=-12.250, p=0.000) and the Plöner See 22.1.1920 (t=-7.567, p=0.000) were significantly different than the Havel population. Paired comparisons among populations' initial cell diameters showed that the means of the initial cells in two Plöner See populations were not significantly different (t=0.845, p=0.406); however, the mean initial cell diameters of both the Plöner See 10.2.1919 population (t=-2.672, p=0.010) and the Plöner See 22.1.1920 (t=-2.400, p=0.024) were significantly different than the Havel population.

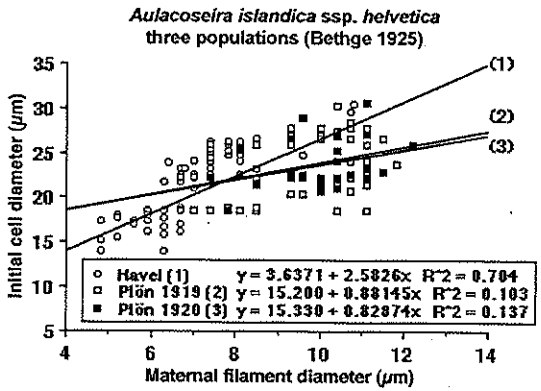

Fig. 8. Relation between maternal filament diameter and initial cell diameter of *Aulacoseira islandica* ssp. *helvetica* in three collections from two localities. Data from Bethge (1925).

Cymbella cistula

Two populations identified as *Cymbella cistula* and undergoing Geitler's Type IA sexual reproduction were studied (Fig. 1i).

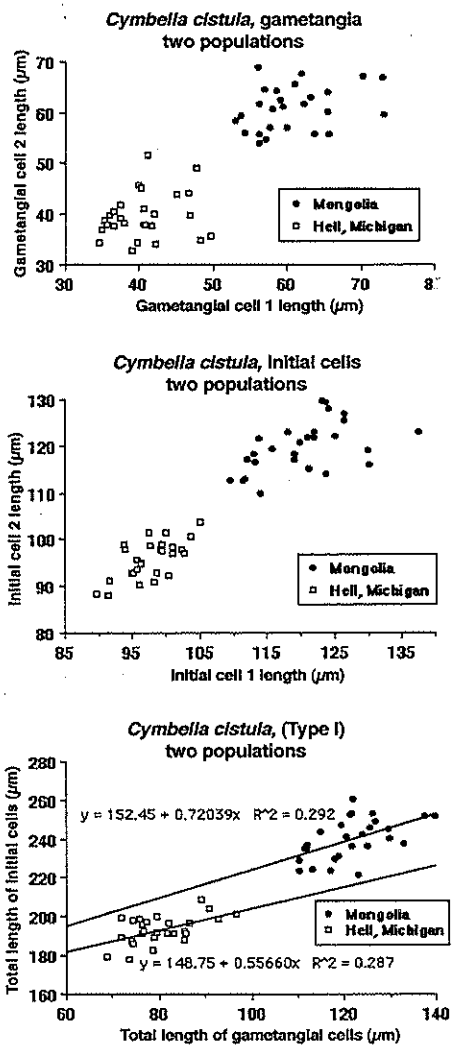

Fig. 9. Two sexual populations (Hell, Michigan and Lake Hovsgol, Mongolia) of *Cymbella cistula* undergoing Type I sexual reproduction. **Fig. 9a.** Comparison of gametangial pairs indicates random mating among gametangial cells in two populations. **Fig. 9b.** Comparison of two initial cells from each Type I reproductive pairing shows two similar-sized resultant initial cells. **Fig. 9c.** Linear relation of sum lengths of gametangial cells to sum lengths of initial cells in two populations of *Cymbella cistula*.

Both populations were springtime periphyton; one was from Hell Creek, Michigan (Edlund & Stoermer 1997) and the other from Lake Hovsgol, Hatgal, Mongolia. Three aspects were noted in each population. First, there was a wide range of gametangial cell lengths in each population, from 32.9 to 51.7 µm (mean 40.2 µm, S.D.=4.47) in Hell and 53.1 to 73.1 µm (mean 60.1 µm, S.D.=4.89) in Mongolia, and there was no apparent specificity in sizes of mating pairs (Fig. 9a). Second, there was variability in the size of the initial cells among mating pairs (Hell, n=54, range 88.0 to 105.1 µm, mean 96.7 µm, S.D.=4.03; Mongolia, n=54, range 32.9 to 50.6 µm, mean 39.5 µm, S.D.=6.02) although in many, but not all pairings, the two resultant initial cells were similar-sized (Fig. 9b). Third, there was a significant linear relationship (Fig. 9c) between the summed gametangial lengths and the summed initial cell lengths from each mating pair within the Hell population (r^2=0.287 F=10.083, p=0.004) and the Mongolian population (r^2=0.292, F=10.292, p=0.004). A comparison of the gametangial cell lengths indicated a significant difference between the means of the population from Hell Creek and Mongolia (t=-22.945, p<0.0001). A significant difference was also found between the means of the initial cell lengths between these two populations (t=-23.642, p<0.0001).

Discussion

DIFFERENCES IN SIZE AMONG VEGETATIVE POPULATIONS

A confounding issue throughout the study of diatoms has been the difference in diatom sizes among populations, but within a species (Lewis 1865). Differences in size and shape have long been weighed as potential species- or varietal-level differences versus simply species-level variability (Hufford & Collins 1972). Confusing the matter further is the natural variation within a clonal line or population that is simply a reflection of the normal size, shape, and ornamentation changes associated with the vegetative portion of the diatom life cycle (e.g., Geitler 1932, Tropper 1975, Stoermer & Ladewski 1982, Krammer 1997, among others).

Size differences of diatom cells have autecological significance. In planktonic populations, size is one factor determining sinking rates (Round 1982, Davey 1986), and in benthic or sedimented planktonic populations, size selective herbivory contributes to loss rates (Jewson 1992b, Edlund & Francis 1999). Nutrient uptake capacity was shown by Grover (1989) to change with cell size; centric diatoms were better able to compete for phosphorus at smaller cell sizes. Factors implicated in causing cell size differences among populations include salinity and trophic level changes. Euryhaline taxa that have invaded fresh waters have been noted for their smaller cell sizes compared to marine counterparts (Lowe & Busch 1975, Edlund *et al.* 2000). Turkia & Lepistõ (1999) showed that cell biovolumes of *Aulacoseira ambigua* and *A. distans* (Ehrenberg) Simonsen were lower in more nutrient-rich waters. Temporally separated populations that show changes in cell size have been used as further evidence of trophic change. Burckle & McLaughlin (1977) studied ocean sediment cores and, noting size changes in *Coscinodiscus nodulifer* A. Schmidt, felt that the appearance of a bimodal size distribution with larger cells was an indication of

warmer climate conditions. Vegetative populations of *Stephanodiscus niagarae* and *Gomphoneis herculeana* (Ehrenberg) Cleve from the North American Great Lakes were demonstrably larger before European settlement, nutrient enrichment, and landscape disturbance (Stoermer & Ladewski 1982, Stoermer *et al.* 1989). Conditions in Lake Ontario have now changed to such a degree that *Stephanodiscus niagarae* has been extirpated possibly due to its inability to undergo sexual reproduction (Julius *et al.* 1998).

Genetic differences among populations of a diatom taxon have also been shown to be significant. Lewis *et al.* (1997) found less within-population variation than among-population variation in a latitudinal study of RAPD-DNA fragments from North American reservoir populations of *Fragilaria capucina* Desmazières. Differences that were measured by allozyme frequency also identified greater among-population differences than within-population differences in clones of *Asterionella formosa* Hassall (Soudek & Robinson 1983). Genetic differences likely explain the interclonal differences exhibited in physiological and life history parameters within diatom species (e.g., Rodhe 1948, Wiedling 1948, French & Hargraves 1986).

WITHIN-POPULATION VARIATION IN GAMETANGIAL AND INITIAL CELL SIZES

Similarly, differences and variability within and among populations are being identified in life history parameters of diatom species. Geitler's (1932) cardinal point hypothesis identified the cell sizes initiating sexual reproduction and immediately post-sexual reproduction as the two critical points in the diatom life history. However, in contrast to the classical opinions that these points represent discrete, narrow size classes within species (or races), a growing collection of evidence suggests differently. First, the smaller cardinal point, or gametangial cell size, has been shown to vary across a wide range of cell sizes within a taxon (Hohn 1959, von Stosch 1967, Drebes 1974, Hargraves & French 1983, Mann 1988, Armbrust *et al.* 1990, Waite & Harrison 1992, Davidovich 1994, 1998). Schmid (1995) illustrated this very elegantly in cultured *Coscinodiscus granii* Gough, showing that the sexually inducible size range for gametangial cells actually overlapped the size of initial cells. Waite & Harrison (1992) were even able to induce gametogenesis in immediate post-sexual vegetative cell lines of *Ditylum brightwellii* (T. West) Grunow *in* Van Heurck. Second, post-sexual initial cells have also been shown to have a wide size range within and between species populations of many marine and freshwater diatoms (Bethge 1925; Nipkow 1927; Skabitschewsky 1929; Hohn 1959; Holmes 1967; Migita 1967; Drebes 1974; Gallagher 1983; Mann 1984, 1988; Kociolek & Stoermer 1989; Edlund & Stoermer 1991; Jewson 1992a, 1992b; Jewson *et al.* 1993; Schmid 1995; Davidovich 1994, 1998).

The data we have presented here, through analysis of numerous mating pairs and multiple populations, corroborate this revised view of the cardinal point hypothesis (see also Davidovich 1998). For example, in *Gomphonema parvulum*, if we compare the smallest gametangial cell (15.5 µm length) to the largest initial cell (52.8 µm length), we find a full size range in this population is 37.3 µm, excluding 'Kümmer-formen'. However, the range of gametangial (15.5-28.4 µm) and initial cells (38.6-52.8

µm) can each account for 34.6% and 38.1%, respectively, of the whole size range of the Huron River population. Similar comparisons of the other data we have presented show that the gametangial cell size range contributes from 16.6% to 28.6% of the full size range for each taxon, whereas the initial cell size range even more dramatically contributes between 21.6% and 67.3% of the normal cell size range within each population. Rather than representing a narrow size range, the gametangial and initial cells clearly show great variability within a population. This observation confounds the traditional predictability associated with the diatom life history (Geitler 1932, Lewis 1984, Jewson 1992b), but may represent a mechanism of maintaining a sexually ready population for a longer time period and/or present a wider range of cell sizes post-sexuality to minimize size-selective pressures (Round 1982, Davey 1986, Jewson 1992b, Edlund & Stoermer 1997, Edlund & Francis 1999).

AMONG-POPULATION DIFFERENCES IN GAMETANGIAL AND INITIAL CELL SIZES

Less frequently reported are differences among populations in gametangial and initial cell size. Mann (1984) tabulated evidence from geographically disjunct *Rhoicosphenia curvata* (Kützing) Grunow populations, from his data and data of Cholnoky (1927) and Geitler (1952), that differed in gametangial and initial cell size and suggested that these differences may be indicative of species-level differences or the identification of races of diatoms. In the North American Great Lakes, temporally separated populations of *Stephanodiscus niagarae* and *Gomphoneis herculeana* had significant differences in initial cell size (Stoermer & Ladewski 1982, Edlund & Stoermer 1991) that were attributed to major changes in trophy of this system following European settlement. Armbrust & Chisholm (1992) showed that, among many isolates of *Thalassiosira weissflogii* (Grunow) Fryxell *et* Hasle, the maximum size of initial cells (and rate of vegetative size decrease) varied. Reanalysis of data from Bethge (1925) on three samplings of *Aulacoseira islandica* ssp. *helvetica* from two localities indicated that small but significant differences in maternal and initial cell sizes were present between sampling locations, but not within a location (Plöner See) sampled on two different dates.

The two *Cymbella cistula* populations investigated provide further evidence of inter-population variability. Significant differences were found in geographically separated populations (Mongolia and Michigan, USA) in both gametangial and initial cell size. The smaller-sized population (Hell, Michigan) was from a meso-eutrophic stream, whereas the larger-sized population (Mongolia) was from the wave-zone of an oligotrophic large lake. These differences may reflect size selectivity to trophic levels, perhaps a similar response as seen in temporally separated populations of *Gomphoneis herculeana* from the North American Great Lakes (Stoermer & Ladewski 1982) and temporally and trophically separated populations of *Stephanodiscus niagarae* (Edlund & Stoermer 1991, this study). Exceptionally large-sized variant diatom populations have been termed gigantism and noted in ancient and pristine lake systems such as Lake Baikal [Meyer 1922, *Navicula bacillum* Ehrenberg; Stoermer *et al.* 1986, *Didymosphenia geminata* (Lyngbye) M. Schmidt]. Pristine conditions and lengthy existence as lake systems may promote selection of 'giant' cell lines or perhaps the

large cell lines are true species resulting from evolution within large lake systems (Kociolek & Stoermer 1988). Evidence shown for *C. cistula* suggests that increases in gametangial and initial cell size are a primary outcome of selection under lengthy pristine conditions, such as found in ultraoligotrophic and ancient Lake Hovsgol, Mongolia. Similar slopes of size increase among the *C. cistula* populations, as shown by no significant differences in slopes between populations (Fig. 9c), suggest that the relations between gametangial contribution and initial cell size is a constant attribute among these populations, whereas the size differences of these special cells is more likely a product of genetic selection, possibly in response to trophic differences between Hell Creek and Lake Hovsgol. This hypothesis remains to be tested in the laboratory. Alternatively, the differences in gametangial and initial cell sizes between these populations could be interpreted as species-level differences.

CONTROLS ON INITIAL CELL SIZE

Auxospore expansion is probably influenced by several factors: gametangial contribution, genetic differences and determination, and environmental controls. Separating the influence of each of these factors will require extensive sampling of natural populations and laboratory studies testing environmental factors that may control auxospore expansion among clones (Nagai & Imai 1997, 1999). The persistent difficulty of controlling sexual reproduction in culture hinders progress in this area, as well as our continued poor understanding of the physiology and cellular mechanics of auxospore expansion.

Previous workers have shown a significant linear relation between gametangial cell size and initial cell size in *Melosira moniliformis*, *Skeletonema costatum* (Migita 1967), *Aulacoseira subarctica* (Jewson 1992b), *Nitzschia lanceolata*, *Licmophora ehrenbergii*, and *Synedra tabulata* (Davidovich 1994, 1998), and following pseudoauxosporulation in *Coscinodiscus wailesii* (Nagai *et al.* 1995). Our new data and reanalysis of earlier work have similarly confirmed a significant linear relationship between the gametangial cells and the initial cells in *A. ambigua*, A. *granulata*, *A. islandica* ssp. *helvetica* (Bethge 1925), *A. baicalensis* (Skabitschewsky 1929), *Gomphonema parvulum*, two populations of *Cymbella cistula*, and in *Cocconeis placentula* undergoing two types of auxospore production. Only in one population of *A. islandica* ssp. *helvetica* (Plöner See, 22.1.1920) was this relationship found to be not significant. The positive linear relationship of maternal filament diameter to initial cell diameter in the four *Aulacoseira* taxa is most probably related to maternal filament contribution rather than differential expansion related to environmental variability. Bethge's (1925) and Skabitschewsky's (1929) sexual samples of *Aulacoseira* were gathered on single sampling dates and in all probability the resultant initial cell sizes in each collection represent a narrow range of environmental conditions. Similarly, our data on *G. parvulum*, *Cocconeis* and the two populations of *Cymbella cistula* also came from single sampling dates and lend support that a significant relation exists between gametangial cell size and initial cell size in most taxa when environmental conditions are narrow. Gametangial size is obviously a factor in eventual initial cell size (Davidovich 1994, 1998) in most taxa; however, a few cases exist where this relation

did not hold. For example, Armbrust & Chisholm (1992) reported that 'there was no consistent relationship between the minimum average cell size and the maximum size... populations underwent anywhere from a 1.2- to a 3.5-fold increase in mean cell size' in clonal isolates of *Thalassiosira weissflogii*, and Schmid (1995) showed that initial cell size was independent of parental size in *Coscinodiscus granii*.

Clonal- or population-level differences in gametangial or initial cell size appear to be most strongly defined by genetic differences. Evidence presented by Armbrust & Chisholm (1992) supports this; they found differences in initial cell sizes following multiple auxosporulation events under similar environmental conditions within clones of *Thalassiosira weissflogii* that originated from earlier auxosporulation events from a single clone. Differences in auxospore size were suspected to represent novel gene combinations resulting from auxosporulations. Smaller- or larger-sized cell lines have also been shown to be correlated to trophic changes (Stoermer & Ladewski 1982; Edlund & Stoermer 1991, 1997; Turkia & Lepistö 1999), suggesting that within single water bodies, productivity changes may drive selection for population cell size. This may be related to greater nutrient uptake capacity of smaller cells (Grover 1989), but we can also infer that small-sized populations will have smaller gametangial and initial cell sizes. Data that we have shown and that of Nagai & Imai (1997), indicate that within some species, different populations and different environmental treatments tend to show similar slopes of size increase between gametangial and initial valve sizes [e.g. *Cymbella cistula* (Fig. 9c), *Coscinodiscus wailesii* (Nagai & Imai 1997)]. It appears that selective pressures encourage genetic differences that are manifested in cardinal point differences, especially in gametangial cell size. Although a significant relation has been shown between gametangial and initial cell size in a majority of species, evidence from *Cocconeis placentula* (Fig. 6c) undergoing two modes of sexuality indicates that there is also a probable genetic determinant in initial cell size. Both modes of sexuality began with similar-sized gametangial cells and presumably took place under similar environmental conditions during auxospore enlargement and silicification. However, in spite of different gametangial contribution (one vs. two gametes), the resultant initial cells were not significantly different in size. Thus, in the case of *Cocconeis placentula*, the resultant initial cell size appears to be a combined reflection of genetic determination and gametangial contribution.

In the few cases studied in culture, there is some evidence that environmental factors may be, in part, responsible for differential auxospore expansion. Nagai & Imai (1997, 1999) studied the process of pseudoauxosporulation in *Coscinodiscus wailesii* and showed that final auxospore expansion was not only related to parental cell size, but could also be correlated to salinity and higher irradiance levels and longer photoperiods. In contrast, Davidovich (1994, 1998) was unable to find significant differences in initial cell size with various photoperiod and light intensity treatments. While not explicitly investigated in our study, environmental controls of auxospore expansion may explain some of the variance in the data sets. Differences among populations appear to be more likely a result of genetic differences; however, we did not identify any taxa where different populations had similar-sized gametangial cells but the initial cells were different-sized, or vice versa.

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THE FUTURE OF CARDINAL POINTS

What is clear is that cardinal points are related to one another; a significant relationship between gametangial contribution and initial cell size has now been documented in most investigated taxa. Furthermore, cardinal points often represent a wide range of cell sizes relative to the asexual portion of the diatom life cycle. Temporally and geographically separated populations within taxa often exhibit significant differences in gametangial and initial cell size; however, the cardinal points remain significantly related to one another within populations. Environmental factors appear to play a smaller, but significant, role in this relationship (Nagai & Imai 1997, 1999); they are also in need of further analyses (Davidovich 1994).

Future use of cardinal points in diatom taxonomy and systematics must be considered. Different size classes of initial valves within populations have supported separation and description of new species from among related taxa (Kociolek & Stoermer 1988). Differences in cardinal points among populations have been suggested to be simply an expression of the variability within a species, species-level differences, or the identification of races or varieties of diatoms (Geitler 1932, Burckle & McLaughlin 1977, Mann 1984, Edlund & Stoermer 1991). But what do these differences among populations, especially in initial cell size, actually reflect beyond gametangial contribution? The answer is probably unavailable to us at this time with so few taxa studied, but initial results indicate that cardinal points may provide critical new evidence for deciphering the diatom species. For example, subtle size and ornamentation differences within sympatric demes have been clearly shown to corroborate biological species differences (Mayr 1970; Mann 1989, 1999); however, inclusion of gametangia size, or initial cell size, or the relation between the two has not been considered. Genetic differences in closely related taxa have been considered both definitive of species-level differences (Medlin *et al.* 1991), or simply ambiguous (Zechman *et al.* 1994), but genetic studies rarely consider life history parameters (mating compatibility, cardinal points, etc.) in relation to the results. Because the differences in cardinal points among populations are probably strongly genetic in origin (Armbrust & Chisholm 1992), and in light of the need for studies of mating compatibility among geographically separated clones (but see Chepurnov & Mann 1999, Mann 1999, Mann *et al.* 1999), it is clear that morphological, biological, genetic, and cardinal point (sexuality) evidence will be necessary to elucidate species-level differences among the diatoms.

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