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Cell cycle studies of the dinoflagellates *Gonyaulax polyedra* Stein and *Gyrodinium uncatenum* Hulburt during asexual and sexual reproduction

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Abstract: A microscope-photometer system was used to quantify the relative DNA content of individual cells of the dinoflagellates *Gonyaulax polyedra* Stein and *Gyrodinium uncatenum* Hulburt during asexual reproduction and, for the latter species, during sexuality as well. The cell cycles of these two dinoflagellates are distinctly different although both include discrete periods of DNA synthesis. In *G. polyedra*, DNA synthesis and cell division are tightly phased to restricted segments of the photocycle, separated in time by distinct gap phases. All cells that replicate their DNA and enter G_2 divide during the next division interval. G_1 is the stage occupied by the cells most of the time. In *G. uncatenum*, cell division is more broadly distributed and occurs throughout half the photocycle. Cell division is thus phased only to the extent that it is restricted to the same half of the photocycle each day. G_1 is very brief as DNA synthesis begins immediately after cell division. G_2 is the dominant stage through time since it includes cells about to divide as well as those that just divided. *G. uncatenum* is the first free-living photosynthetic dinoflagellate to exhibit a pattern of DNA synthesis immediately after mitosis. The tight phasing of *G. polyedra* division and the restriction of G_2 to cells that must divide at the end of the dark period should make it possible to calculate the growth rate of this species in mixed natural assemblages using these techniques. It should also be possible to distinguish sexual stages (zygotes) from vegetative cells. Since *G. uncatenum* vegetative cells often have two complements of DNA, as do zygotes following gamete fusion, these stages could not be distinguished from each other on the basis of DNA measurements alone. However, these data in combination with cell counts were used to define the onset of sexuality and to show that the gametes of this species result from two rapid divisions.

Key words: Cell cycle; Dinoflagellate; Dinoflagellate reproduction; DNA synthesis; Mitosis

Introduction

Cell division in dinoflagellates is often phased to a restricted period in a 24-h L : D cycle (reviewed in Chisholm, 1981), so it is reasonable to expect that DNA synthesis would also be a discrete event. However, various investigations utilizing labeled DNA precursors, total DNA measurements and visual observations have yielded variable results. DNA synthesis is reported to be a discrete event for some species (Franker, 1971; Lobelich, 1976; Allen et al., 1975; Spector et al., 1981; Sweeney, 1982) or a

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continous process through the cell cycle in others (Dodge, 1966; Filfilan & Sigee, 1977; Galleron & Durrand, 1979). Part of the variability may reflect the manner in which the uptake of labelled precursors is interpreted as a marker for DNA synthesis since some workers consider the low level uptake of label after a maximum period to mean continuous synthesis (Filfilan & Sigee, 1977) while others infer a discrete synthesis phase (Galleron & Durrand, 1979).

A less ambiguous way to address this issue and to describe a species' cell cycle is to measure the DNA content of individual cells at closely spaced intervals over a 24-h photocycle. Homma (1987) measured DNA in *Gonyaulax polyedra* Stein at 4-h intervals using flow cytometry. Cheng & Carpenter (1988) used a microscope-image processor to quantify DNA and analyze the cell-cycle phases in *Heterocapsa triquetra*. Both investigations suggested a discrete phase of DNA synthesis.

In our study, we used a microscope-photometer system to monitor the time course of DNA content of individual cells of the dinoflagellates *G. polyedra* and *Gyrodinium uncatenum* Hulburt. Data collection is slower than with flow cytometry or image processing but the photometer is less expensive to purchase and maintain and simpler to operate. Unlike flow cytometry, the operator can choose the cells on which measurements are to be made from the field of view of the microscope, so cells in specific life-cycle stages can be identified or individual species of interest selected from mixed field assemblages. In addition, few cells $\cdot$ sample^{-1} are needed – an important consideration for large dinoflagellate species which often do not attain high cell concentrations.

Two experiments were conducted. The first was designed to describe the DNA content over time during exponential vegetative growth of the two dinoflagellate species. In the second, we induced sexuality in *G. uncatenum* and attempted to detect sexual stages on the basis of changes in DNA content. Such discrimination would be possible only if DNA synthesis and cell division occurred during discrete periods of the cell cycle. There might then be a time during which all vegetative cells would have the same complement of DNA (either 1C or 2C), allowing the identification and quantitation of either zygotes (2C) or gametes (1C) but not both. Our results document significant differences in the cell cycles of the two species, both of which have discrete rather than continuous DNA synthesis. The cell-cycle pattern of one of the species (*G. polyedra*) we examined would allow this photometric method to be used by itself to identify asexual and sexual stages.

Materials and Methods

Cultures and Experimental Design

Cultures of *G. polyedra* (isolate GpPP8) and *G. uncatenum* (isolate GUPP4) were grown in 25-ml culture tubes or 250-ml flasks in K medium (Keller et al., 1987) at 20 °C on a 14 : 10 L : D photoperiod at $\approx 250\ \mu E \cdot m^{-2} \cdot s^{-1}$. For sexual induction, nitrate was

omitted from the growth medium and ammonium was added at half the usual concentration. Cells were kept in early to mid-exponential growth through several transfers before experiments were initiated. Sets of culture tubes for experiments were inoculated with measured volumes from single-parent cultures. Two separate sets of tubes were inoculated for each 24-h experiment for each species.

The "vegetative growth experiments" were carried out by sampling a different culture tube from each set every hour during mid-exponential growth. Counts of single and dividing cells were made either immediately on live cells in a flat capillary tube with a dissecting microscope or later on samples preserved in Utermöhl's fixative; 500–1000 cells were counted in each sample. Duplicate 10-ml vols. were preserved for DNA measurements. Each sample is denoted by the number of hours into the light (L) or dark (D) period (e.g., 4L = 4 h after the lights went on).

In the "sexual induction experiment", measured volumes from 250-ml flask cultures were removed once each day until the stationary phase was established. The time chosen for daily sampling was just prior to the time when dividing cells first appeared, determined from the vegetative-growth experiments. Samples were preserved for cell density and DNA measurements. The presence or absence of planozygotes was assessed using live cell suspensions mixed 1 : 1 with 1% polyethylene oxide in filtered seawater on a microscope slide (Spoon et al., 1977) and observed under phase contrast. In this solution, dinoflagellates swim slowly and it is possible to detect planozygotes by the presence of their two longitudinal flagella (Coats et al., 1984).

STAINING

Samples for DNA measurements were fixed in 2% formaldehyde (*G. polyedra*) or 5% Bouin's fixative (*G. uncatenum*) for 10 min, centrifuged, resuspended in cold methanol and stored refrigerated. Methanol extraction for at least 8 h was necessary to remove pigments that interfere with DNA measurements (Olson et al., 1986b). Initial formalin or Bouin's fixation is necessary because dinoflagellates do not preserve well when placed directly in methanol. However, we found formalin concentration and storage time in formalin to be inversely proportional to DNA staining intensity; long-term storage (days to weeks) in formalin is not desirable.

Cells were stained with 3.2 mM Hoechst 33342 (1.8 $mg \cdot ml^{-1}$) in filtered seawater for 10 min, rinsed three times in filtered seawater and once in methanol to remove excess stain, and resuspended in filtered seawater for measurements (which were completed in <8 h of staining). Both the mean fluorescence intensity and the coefficient of variation (CV) increased for *G. polyedra* held in the refrigerator several days after staining. At Days 0, 3, 4 and 5 after staining, $\bar{x}$ and CV values for relative fluorescence of the same sample were 33.6 (8.9%), 50.3 (12.7%), 55.2 (16.5%) and 68.3 (15.8%), respectively, for $n = 35$ cells. Ratios of these means to dansyl chloride-stained bead standards (Flow Cytometry Standards) were 1.8, 2.2, 2.4 and 2.7.

Calf thymus nuclei were stained under the same conditions and their fluorescence measured as a reference for the variance of DNA measurements.

DNA MEASUREMENTS

DNA-Hoechst fluorescence measurements were made using a Zeiss IM35 inverted epifluorescent microscope (Zeiss filter set 48 77 02, excitation 365 nm, barrier at 420 nm, plus red attenuating filter BG 38) with a 50 W Hg lamp. The microscope was equipped with a Nikon P-1 photometer system interfaced with a computer. Stained cells suspended in filtered seawater were mounted on glass slides under coverslips which were supported by extra-thin coverslips on two sides or in Palmer–Maloney counting slides. Samples were scanned with phase contrast at 250 × and individual cells selected for measurement were aligned in the center of the field. The light path to the photomultiplier was restricted by a pinhole only large enough to admit light from the selected cell or nucleus. A switch-activated electronic shuttering system blocked the W light source and opened the Hg light path and the photometer light path for 800 ms. The photomultiplier recorded total light received and the computer software (Phoscan, Nikon) recorded the maximum emission as relative fluorescent units. The instrument was stable within and between measuring sessions after warm-up (60 min), checked against permanently mounted dansyl chloride-stained plastic beads (Flow Cytometry Standards).

HISTOGRAM ANALYSIS

The percentages of cells in each cell cycle stage were estimated from DNA vs. frequency histograms. Several methods were evaluated. A curve-fitting method commonly used for flow-cytometry data (Dean & Jett, 1974; Olson et al., 1986b) gave inconsistent results because it assumes asynchronous cultures and the presence of at least two peaks in every sample. Graphical methods similar to that used by Olson et al. (1983) produced unrealistic results when peaks were skewed by large numbers of cells in early or late S phase. We thus developed a graphical method based on peak widths of G_1 or G_2 in samples with no cells in S. In general, for experimental data from a given species, if either the G_1 or G_2 peak was present in all samples and there was at least one sample with no cells in S, the width of the G_1 or G_2 peak in that sample was used to define that peak in other samples. This is equivalent to assuming that pure G_1 and G_2 peaks have a constant SD. In order to use a constant width measured in relative units to define a peak, the peak must be centered at the same value in each sample. This is usually true for a set of samples analysed in a consistent manner but, if not, data can be adjusted by multiplying each value by a constant before plotting the histogram. The relative unit values on the abscissa of a histogram can be treated as fluorescence "channels". The number of channels spanned by the reference peak in samples that are clearly free of cells in S can then be used to define the peak in other samples. Channel counting was begun at either the lowest (G_1) or highest (G_2) channel that contained at least one cell and that was not separated from the next channel containing a cell by more than one empty channel. For *G. polyedra*, the mean number of channels in isolated G_1 peaks centered at 35–40 relative units was 18. For *G. uncatenum*, the mean number of

channels in isolated G_2 peaks centered at 65–70 was 17. In this manner, a constant number of channels was objectively chosen to define S. This crude method probably underestimates the proportion of cells in early and late S. However, it does produce consistent results that are more realistic than the models referenced above and is adequate for description of the DNA cell cycle.

RESULTS

MEASUREMENT PROTOCOL

Several unexpected factors can contribute to variability in fluorescence data collected with a microscope-photometer system. Glare, presumably caused by reflection from an interface, can cause anomalously high readings from cells close to the edges of the coverslip. Also, variable degrees of compression of a cell between slide and coverslip due to evaporation of the sample can change values obtained from that cell up to 10%. Both of these problems can be eliminated by using Palmer–Maloney cells instead of glass slides.

We also investigated the contribution of other sources of variation in the DNA measurements: fading of fluorescence due to exposure during prior measurement of nearby cells, orientation of the U-shaped *Gonyaulax* nucleus, and focusing of the nucleus (Table I). To test for fading, an initial measurement was made on a cell, a dummy measurement was then made with the cell just out of the field of view and a final measurement made on the original cell within 60 s of the initial measurement. For other

TABLE I

Effects of fading, nucleus orientation and focus on DNA quantitation. Data are $\bar{x} \pm$ SD (n = number of cells).

	Fading[a]		Orientation[b]	Focus[c]
	Case 1	Case 2		
G. uncatenum	0.96 ± .02 (n = 10)	0.99 ± .01 (n = 10)		0.98 ± .02 (n = 10)
	0.99 ± .01 (n = 10)	0.98 ± .02 (n = 10)		
G. polyedra	0.97 ± .02 (n = 10)	0.97 ± .03 (n = 10)	0.96 ± .05 (n = 20)	0.97 ± .02 (n = 20)

[a] Ratios of second to first measurements taken on same cell (Case 1) or of measurements after and before a measurement in a nearby field (Case 2).
[b] Ratio of second to first measurements taken on same cell after reorientation of the nucleus.
[c] Ratio of second to first measurements taken after realigning and refocusing same cell.

cells, the dummy measurement was omitted. If fading occurred during measurement of cells in nearby fields, the ratio of final to initial fluorescence in the first case should be less than in the second. For both species, there was no difference between the ratios, indicating that the error associated with fading due to measurements in nearby fields was negligible.

To test the effect of focus, a second measurement was made after recentering and refocusing previously measured individual cells. The effect of orientation of the *G. polyedra* nucleus was tested by rolling cells to a new orientation for each measurement by lightly tapping a skewed coverslip. In both cases, there was no difference between the means. Changing orientation increased the SD of the $\bar{x}$ value but not the $\bar{x}$ value itself.

THYMOCYTES

The CV of the $\bar{x}$ values of DNA fluorescence measured from calf thymus nuclei from five separate sample preparations were 4.2% (n = 20), 4.3% (n = 25), 5.0% (n = 25), 5.6% (n = 26) and 5.7% (n = 20). This is comparable to CV for the same batch of thymocytes stained with propidium iodide (Olson et al., 1986a) and analysed with an EPICS V flow cytometer (Coulter Electronics) (R. Olson, pers. comm.).

VEGETATIVE GROWTH EXPERIMENT

Gonyaulax polyedra

Representative DNA vs. frequency histograms during the 24-h experiment are shown in Fig. 1. We interpret these to show that at 5L (Fig. 1A) all cells were in G_1 and had one complement of DNA. At 4D (Fig. 1B), some cells were in G_1 with one complement of DNA, some in G_2 with two complements and some in S with intermediate amounts of DNA. At 9D (Fig. 1C), cells had either one or two full complements of DNA. The CV for the G_1 peak at 5L is 8.7% and for the G_1 and G_2 peaks at 9D is 9.1 and 7.7%, respectively. These are about twice the lowest CV values obtained with thymocytes.

The percentages of the cell population in each cell cycle stage throughout the vegetative growth experiment are shown in Fig. 2. Replicate culture sets (A,B) exhibited similar patterns. At the beginning of the dark period, $\approx$83% of the cells had one complement (1C) of DNA and were in G_1. DNA synthesis began early in the dark period. The percentage of cells in S increased to maximum values of 22–30% at 4D and 2D in Sets A and B, respectively. The percentage in G_2 subsequently increased until $\approx$5D and then stabilized for 5 h. The $\bar{x}$ and SD of the percentages of cells in G_2 during these 5 h were 25.3 and 2.9 for Set A and 31.9 and 3.8 for Set B. Cell division began at the start of the light period and was very tightly phased, occurring entirely between 0L and 4L. Dividing cells were $<$0.5% of the total at times other than between 0L and 4L. The maximum in Set A was 6.3% at 1.5L and 4.3% in Set B at 1L. By 4L, most cells were in G_1 and remained there until late in the light period.

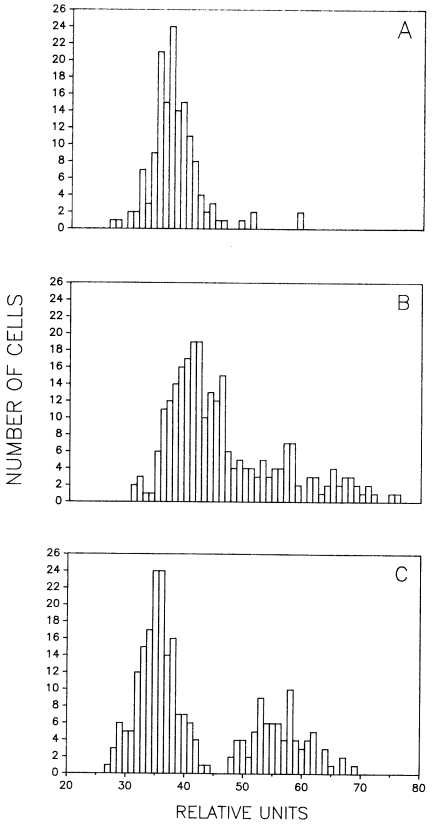

Fig. 1. Representative frequency vs. DNA fluorescence histograms for vegetative growth experiment with *G. polyedra*. Samples at (A) 5L, (B) 4D and (C) 9D.

The majority of the *G. polyedra* cells remained in G_1 throughout the entire photocycle. All cells that divided had replicated their DNA during the dark period and all cells that replicated their DNA during the dark period divided early in the subsequent light period. Therefore, the minimum duration of G_1 for an individual *G. polyedra* cell would be ≈ 8 h (from the completion of mitosis early in the light period until the initiation of DNA synthesis near the light to dark transition). This requires two divisions in 24 h; otherwise, G_1 must last at least 32 h. The duration of S for the population was 6 h (from the time cells began entering S until cells stopped entering G_2). The time one individual cell takes to complete S would be less. Cells that entered G_2 remained there for at least 5 h but not > 10 h. Since the maximum percentage of dividing cells observed at any one time (6.3%) was much lower than the percentage of the population that eventually divided, the duration of mitosis for individual cells must be < 4 h.

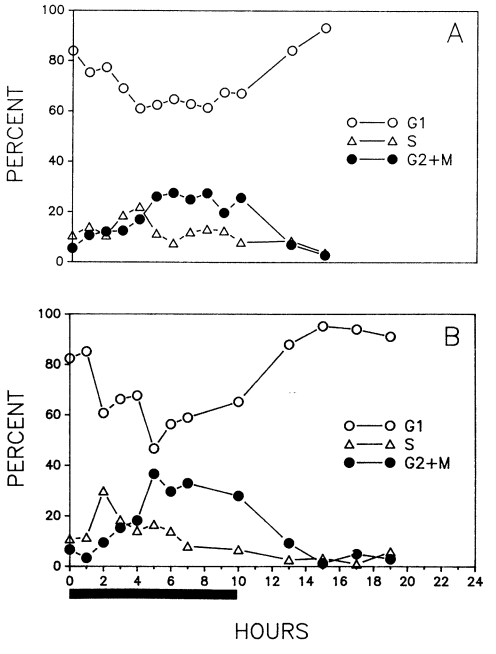

Fig. 2. Percentages of cells in each cell cycle phase (G_1, S, G_2 + M) in vegetative growth experiment with *G. polyedra*. A and B, results from replicate sets; black bar on abscissa, dark period.

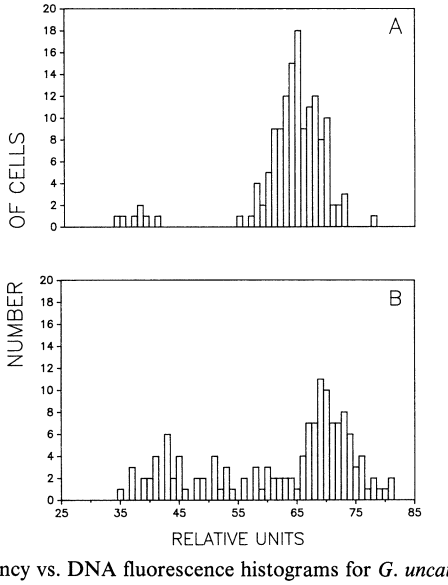

Fig. 3. Representative frequency vs. DNA fluorescence histograms for *G. uncatenum* in vegetative growth experiment from samples at 10D (= 0L) (A) and 13L (B) (h0 = 6L).

Gyrodinium uncatenum

Fig. 3 shows two representative DNA vs. cell frequency histograms for this species. Fig. 3A is the DNA distribution at the dark to light transition (10D) which is representative of the population over the range from ≈6D to 7L. Almost all cells had 2C of DNA and were in G_2. Fig. 3B shows the distribution at 13L. During that time, cells were dividing and synthesizing DNA. Most cells were in G_2 but there were some in G_1 and S as well. The CV for the G_2 peak at 10D was 5.7% (n = 132).

The percentage of cells in each cell cycle stage and percentage of dividing cells for replicate Sets A and B are given in Fig. 4. Mitosis occurred throughout approximately

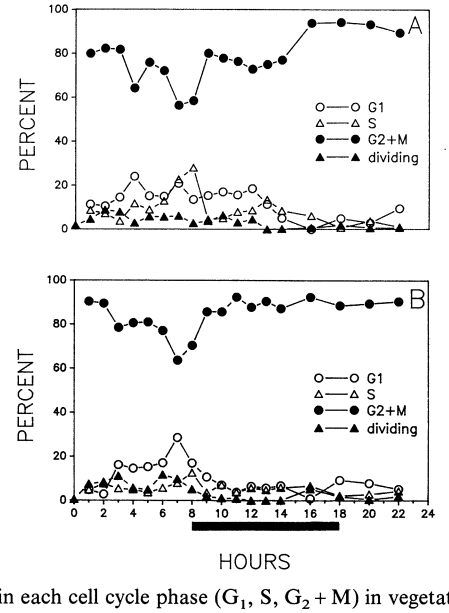

Fig. 4. Percentages of cells in each cell cycle phase (G_1, S, G_2 + M) in vegetative growth experiment with *G. uncatenum*. A and B, results from replicate sets; black bar on abscissa, dark period.

half of the 24-h photocycle – during the latter half of the light and first half of the dark periods, indicated by the increase in G_1 cells. The percentage of dividing cells also fluctuated during that time, reaching maximum values of 8.8 and 11.7% in Sets A and B, respectively. This irregular pattern was verified with flask cultures of the same isolate at a later date to eliminate the possibility that the fluctuations were an artifact of tube to tube differences.

The percentage of cells in G_1 fluctuated but was generally greater and achieved maximum values during the latter half of the light and early part of the dark periods when dividing cells were present (24.1% at 10L in Set A, 28.6% at 13L in Set B). The maximum proportion of cells in S in both culture sets (28.0% in Set A, 12.6% in Set B) occurred at 14L, a few hours after the maximum in G_1. In Set A, the proportion of cells

in G_1 remained high into the early dark period; in set B, it decreased at the beginning of the dark period. From mid-dark to -light, $>85\%$ of the cells in each set were in G_2. G_2 cells began disappearing 1–2 h after dividing cells appeared.

SEXUALITY EXPERIMENTS

Sexuality was not detected in any of several cultures of *G. polyedra* grown in nutrient-deficient medium, so DNA measurements were not made in these experiments.

Cultures of *G. uncatenum* in nutrient-deficient medium were sampled on Days 5, 8, 11, 12, 13 and 15 after inoculation. The sampling time was chosen as the middle of the light period, just prior to the time dividing cells first appeared in the vegetative growth experiments; most cells had 2C of DNA at that time. Cells with two longitudinal flagella were observed on Days 13 and 15, and cysts thereafter. Results of DNA analyses on each sampling day are shown in Fig. 5 along with the cell-count data. The bar plots

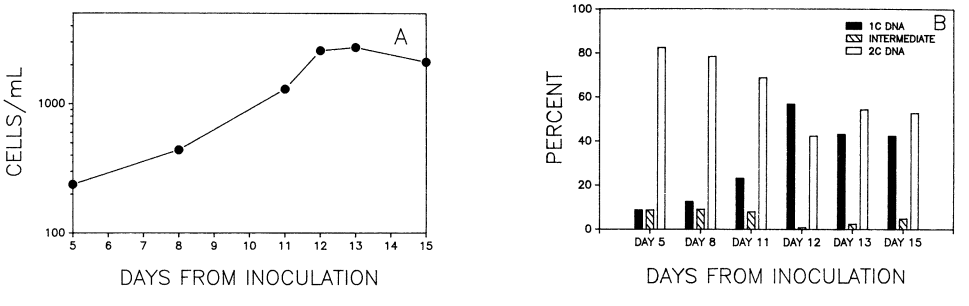

Fig. 5. Results of sexuality experiment with *G. uncatenum*: (A) Cell concentration vs. days time. (B) Histograms showing percentage of cells with 1C, intermediate and 2C DNA, and proportion of 2C cells which were binucleate on each sampling day.

depict the proportion of cells with one, two and intermediate complements of DNA. In the vegetative growth experiment, these were identified as G_1, G_2 and S cells, respectively, but these terms are not necessarily appropriate for sexual stages. The first two samples taken on Days 5 and 8 were similar in composition to samples in the vegetative growth experiment; 82.4% (Day 5) and 78.4% (Day 8) of the cells had 2C DNA and were presumably typical G_2 cells. The rest were evenly distributed between G_1 and S. By Day 11, there were more cells with 1C DNA (23.2%), fewer with 2C, and about the same proportion intermediate as before. On Day 12, 56.8% of the population had only 1C DNA, 42.4% had 2C and $<1\%$ were intermediate. 73.5% of the cells with 2C DNA had two distinct nuclei; these had to be formed by fusion of gametes since dividing cells of this species undergo nuclear fission only at the latest stage of division and are easily distinguished from zygotes (Coats et al., 1984; this study).

DISCUSSION

ASEXUAL REPRODUCTION

Data from a microscope-photometer system were used to demonstrate that the DNA cell cycles of *G. polyedra* and *G. uncatenum* both include discrete periods of DNA synthesis but are otherwise distinctly different. In *G. polyedra*, DNA synthesis and cell division are tightly phased to restricted segments of the photocycle, separated in time by distinct gap phases. G_1 has a minimum duration of ≈ 8 h but can be as long as 32 h plus 24 h multiples. DNA synthesis commits a cell to mitosis; all cells that replicate their DNA and enter G_2 divide during the next division interval. G_2 is a distinct phase lasting 5–10 h; G_1 can be considered the phase *G. polyedra* cells occupy most of the time. In *G. uncatenum*, cell division is more broadly distributed and occurs throughout approximately half the photocycle, fluctuating considerably during that time. Cell division is phased only to the extent that it is restricted to the same half of the photocycle each day. G_1 is very brief as DNA synthesis begins immediately or within several hours after cell division is completed. Cells spend most of the photocycle in G_2 if they do divide and all of it in G_2 if they do not. For *G. uncatenum*, G_2 is the stage occupied most of the time.

The tight phasing of cell division in *G. polyedra* was first observed by Sweeney & Hastings (1958) and has since been more extensively studied by Homma (1987) and Homma & Hastings (1988) who found that, even under a variety of photocycles (LD 18 : 6 to LD 9 : 15), the cell division peak was always <4 h in duration and occurred at or near the dark to light transition. This is entirely consistent with our results using different methods. It is generally agreed that the cell cycle in *G. polyedra* is controlled by a circadian clock mechanism.

Most dinoflagellates that have been studied undergo cell division during the dark to late dark/early light period (Chisholm, 1981). The number of dividing cells usually occurs as a peak with a single maximum. Cell division in *G. polyedra* followed this "typical" dinoflagellate pattern whereas *G. uncatenum* showed a less common pattern, observed in *Scrippsiella sweeneyi* and *Prorocentrum micans* (Hastings & Sweeney, 1964; Sweeney & Hastings, 1964) where division was initiated during the light period.

Our study shows that DNA synthesis occurs in a discrete time period for both *G. uncatenum* and *G. polyedra*. In *G. polyedra*, synthesis is phased to a specific section of the photoperiod whereas in *G. uncatenum*, synthesis is discrete (but not tightly phased) and occurs immediately after cell division. Only *Crypthecodinium cohnii* (Allen et al., 1975) and the zooxanthellae from *Anthopleura elegantissima* (Franker, 1971) have exhibited this pattern of synthesizing DNA shortly after the completion of mitosis. Karentz (1983) hypothesized that the occurrence of this unusual pattern in these two species is a reflection of one being symbiotic and the other heterotrophic. *G. uncatenum* is the first free-living photosynthetic marine dinoflagellate to exhibit a pattern of DNA synthesis immediately after mitosis.

Previous studies have suggested that DNA synthesis is continuous in some dinoflagellates (Dodge, 1966; Karentz, 1983) and phased to a discrete period in others (Franker, 1971, Franker et al., 1974; Loeblich, 1976; Spector et al., 1981; Sweeney, 1982; Karentz, 1983; Homma, 1987; Cheng & Carpenter, 1988). Two species have exhibited both continuous low level synthesis and a period of more rapid synthesis (Filfilan & Sigee, 1977; Galleron & Durrand, 1979). Where Triemer & Fritz (1984) suggest that such variability between species is reasonable in the dinoflagellates given their procaryotic and eucaryotic affinities, Rizzo (1987) argues that what appears to be continuous synthesis may actually be a reflection of incomplete cell synchrony in the experiments or the difficulty in interpreting labelled precursor uptake studies. Our data seem to support the argument for lack of synchrony since measurements of the DNA content of individual cells always showed a few of those cells in S for both species at all times, despite the clear phasing of the bulk of the DNA synthesis to discrete intervals. If these same cultures had been examined with ^{3}H-thymidine or other labelled precursors, a short interval of rapid uptake superimposed on a continuous low level of uptake would have been observed.

In *G. polyedra*, DNA synthesis appears to be phased to an interval of $\approx$6 h. The 1–3-h difference in time between the peak number of cells in S and the maximum in G_2 is an indication of the duration of S for this species (Heller, 1977). Since G_1 is short or absent in *G. uncatenum* and mitosis occurs throughout a 12-h period, the cells are necessarily synthesizing DNA throughout that 12-h period also. The cell division pattern was erratic in our experiments but the disappearance of dividing cells preceded the increase of cells in G_2 to maximum levels by 2–4 h. This gives an approximate estimate of the duration of S for individual *G. uncatenum* cells.

The duration of DNA synthesis for other dinoflagellates is 6–9 h for *Amphidinium carteri* (Galleron & Durrand, 1979) and 7 h for *Cachonina niei* (Loeblich, 1977). Homma (1987) estimated 1.7 h for *G. polyedra* based on flow-cytometric DNA measurements made every 4 h and a mathematical model. Cheng & Carpenter (1988) estimated the duration of S for individual *Heterocapsa triquetra* cells to be 2.8–4.4 h in a population where S occurred over 12–14 h. Olson et al. (1986a) obtained a 2.4-h DNA synthesis for individual *A. carteri* and concluded that although G_1 and G_2 have durations that vary according to growth conditions, the duration of S remains constant.

One potential benefit of the tightly phased cell cycle of *G. polyedra* is that it would allow growth rate calculations for this species in complex natural assemblages. Since this dinoflagellate has the unique characteristic that all of the cells that will divide within a given day are identifiable as cells in G_2 at the end of the dark period, the fraction of the population (f) in G_2 just prior to the initiation of cell division can be used to estimate the growth rate according to $\mu = (1/1 \text{ day}) (\ln (1 + f))$ (McDuff & Chisholm, 1982). Cheng & Carpenter (1988) have developed a more general application of this method that can be used with species not as tightly phased as *G. polyedra*.

SEXUALITY

The dinoflagellate life cycle often includes both asexual and sexual reproduction (reviewed in Pfiester & Anderson, 1987). During sexuality, vegetative cells produce gametes that fuse to form planozygotes which in most cases mature into nonmotile benthic cysts or hypnozygotes. Gametes and zygotes are often difficult to distinguish from vegetative cells by microscopic observation except with laborious staining techniques like protargol silver (Coats et al., 1984).

Both cell cycles in this study lend themselves to the potential detection of sexual stages using our procedures. This would be the easiest for *G. polyedra* since the vast majority of cells are in G_1 at the same time, making planozygotes with two complements of DNA detectable. Unfortunately, *G. polyedra* did not bloom at our field site during this study and our cultures had lost the ability to form cysts, so we were unable to test these methods on this species.

We were able to monitor patterns of sexuality in the DNA histograms of nutrient-deficient cultures of *G. uncatenum*. Our data show that *G. uncatenum* gametes have 1C DNA and newly formed planozygotes have 2C. This agrees with the only other direct measurements of planozygote DNA content in a dinoflagellate (Cembella & Taylor, 1985). However, since vegetative cells spend most of their time in G_2 with 2C DNA, it is not possible to distinguish planozygotes using DNA measurements alone. Gametes have 1C DNA, and vegetative cells do as well, but for only a short portion of their cell cycle. Shifts in successive DNA histograms towards 1C cells would thus be an indication of sexual induction in *G. uncatenum*. Combined with cell-count information, much can be learned about the dynamics of sexuality for this species.

For example, we contend that gametes were formed by a series of rapid divisions at rates far exceeding those for normal vegetative division. During the interval when gametes were formed in large numbers (Days 11–12), the population growth rate appeared to be 1 division $\cdot$ day^{-1}. However, since all binucleate cells present on Day 12 were formed by the fusion of two gametes, twice as many gametes must have been produced as there were binucleate cells present; the actual growth rate must, therefore, have been >1 division $\cdot$ day^{-1}. Assuming that only the 2C DNA cells present on Day 11 (910 or 69% of the total) were potential dividers, the cell density on Day 12 should have been 2191 cells $\cdot$ ml^{-1}. On Day 12, the actual cell concentration was 2578 cells $\cdot$ ml^{-1}, equivalent to 3382 cells $\cdot$ ml^{-1} if binucleate cells are counted twice as representing fusion products. Furthermore, we expect that some of the cells on Day 12 with one nucleus but 2C DNA (42% of the total) were planozygotes, also the result of fusion of two gametes, so our estimate of 3382 cells $\cdot$ ml^{-1} is necessarily low. Our data thus suggest that virtually the entire population became sexual at the same time and that gametes were formed by two rapid divisions in $\leqslant 24$ h as was suggested for this species by Coats et al. (1984) using protargol staining. Following the process further, we see that the cell density between Days 12 and 13 did not change appreciably, presumably because most of the 1C cells on Day 12 were gametes. Thereafter, the proportion of 1C

cells decreased and 2C cells increased, presumably due to gamete fusion. By Day 15, cell density had decreased, as had the percentage of binucleate cells, indicating the completion of nuclear fusion in the planozygotes.

In this study, we have: (a) presented a microscope-photometric method that can be used to describe the DNA cell cycle of dinoflagellates from cultures or mixed field samples; (b) described the DNA cell cycle of *G. polyedra* and *G. uncatenum*, the latter being atypical for free-living photosynthetic dinoflagellates; (c) demonstrated that DNA synthesis is discrete and not continuous in these two species; (d) demonstrated that DNA histogram data may be useful in estimating growth rate in natural populations of *G. polyedra*; and (e) shown that information relating to the timing of sexuality can be obtained for *G. uncatenum* using DNA measurements although those measurements alone cannot be used to group cells into asexual and sexual categories. Cell counts or supplementary morphological or cytological data are also needed. The *G. polyedra* cell cycle makes it theoretically possible to detect and quantify zygotes without these additional types of data.

It may thus be possible to document the detailed asexual and sexual dynamics of at least one dinoflagellate species in a mixed natural assemblage. In this way, it should be possible to separate and quantify these two reproductive processes as they affect the bloom dynamics of cyst-forming dinoflagellates.

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