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# PLOIDY ANALYSIS OF THE TWO MOTILE FORMS OF *CHRYSOCHROMULINA POLYLEPIS* (PRYMNESIOPHYCEAE)[1]

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ABSTRACT

*In some cultures of the flagellate* Chrysochromulina polylepis *Manton et Parke, established from cells isolated from the massive bloom in Skagerrak and Kattegat in 1988, we observed two motile cell types. They were termed authentic and alternate cells and differed with respect to scale morphology. To investigate whether or not the two cell forms were joined in a sexual life cycle, the relative DNA content per cell and relative size of cells of several clonal cultures of* C. polylepis *were determined by flow cytometry. Percentages of authentic and alternate cells in the cultures were estimated by transmission electron microscopy.*

*Pure authentic cultures (α) contained cells with the lowest level of DNA and were termed haploid. Two pure alternate cultures (β) contained cells with double the DNA content of authentic cells and were termed diploid. Other pure alternate cultures contained haploid cells only, or both haploid and diploid cells. Three cell types were observed, each capable of vegetative propagation: authentic haploid, alternate haploid, and alternate diploid cells. Both the haploid and diploid alternate cells were larger than the haploid authentic cells. Cultures containing diploid cells appeared unstable: cell type ratio and ploidy ratio changed during the experiment where this cell type was present, particularly when grown in continuous light. In contrast, cultures with only haploid cells remained unchanged at all growth conditions tested. Light condition may influence cell type ratio and ploidy ratio. Our attempt to induce syngamy by mixing different authentic haploid clones did not result in mating. Assuming that the authentic and alternate cell types are of the same species, the life cycle of* C. polylepis *includes three flagellated scale-covered cell forms. Two of the cell types are haploid and may function as gametes, and the third is diploid, possibly being the result of syngamy.*

*Key index words: alternate cell type;* Chrysochromulina polylepis; *flow cytometry; life cycle; ploidy; Prymnesiophyceae*

Information on life histories in the class Prymnesiophyceae is fragmentary, and the complete life cycle is only known for a few species (for review see Billard 1994). The life cycles described show a remarkable variability. Complex life cycles with several morphologically distinct alternating cell types are known for a number of species. Usually, a flagellated stage alternates with a nonmotile stage. The flagellated holococcolithophorid *Crystallolithus hyalinus* Gaarder et Markali turned out to be a stage in the life cycle of the earlier described nonmotile heterococcolithophorid *Coccolithus pelagicus* (Wallich) Schiller (Parke and Adams 1960). Klaveness (1972) showed that the nonmotile coccolith-bearing cell (C cell) of *Emiliania huxleyi* Hay et Mohler was joined in a life cycle with a scaly flagellated cell (S cell) and a naked nonmotile cell (N cell). The life cycles in *Pleurochrysis* spp. involve coccolith-bearing motile or nonmotile cells (*Hymenomonas* stage), filamentous thalli (*Apistonema* stage), and scaled swarmers (von Stosch 1955, 1967, Leadbeater 1970, Gayral and Fresnel 1983, Fresnel and Billard 1991). *Phaeocystis* spp. may alternate between large colonies of nonmotile cells, free-living nonmotile cells, swarmers, and microzoospores (Kornmann 1955, Rousseau et al. 1994).

Little work has been done on the life cycles in *Chrysochromulina* spp. Parke et al. (1956) observed amoeboid cells on the bottom of a culture flask in old cultures of *Chrysochromulina ephippium* Parke et Manton. The amoeboid cells then formed walled progeny cells, from which motile cells were released. Similar observations were made on *C. alifera, C. brevifilum, C. chiton, C. ericina, C. kappa, C. minor,* and *C. spinifera* (Parke et al. 1955, 1956, 1958, 1959). In cultures of *Chrysochromulina polylepis* Manton et Parke, a "pseudofilament" of four cells was observed once (Manton and Parke 1962).

While all the preceding examples of prymnesiophyte life histories embrace alternation between motile and nonmotile stages, examination of field material of coccolithophorids from Disco Bay, West Greenland, revealed the occurrence of five different types of "combination cells" bearing both hetero- and holococcoliths originally described from two separate flagellated species (Thomsen et al. 1991). Similarly, culture studies on *C. polylepis* indicate that two motile cell types might be joined in a life cycle of this species

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(Edvardsen and Paasche 1992). The occurrence of generations with different ploidy levels within the Prymnesiophyceae has been reported in a few studies (Billard 1994). The relative amount of DNA per cell was analyzed by flow cytometry in *Phaeocystis* spp. (Vaulot et al. 1994) and *E. huxleyi* (Course et al. 1994).

In *Chrysochromulina,* alternation of nuclear phases has not been described. In this paper, we investigate whether or not generations with different ploidy levels occur in *C. polylepis.* In cultures established from cells isolated during the toxic bloom in Skagerrak and Kattegat in 1988 (Paasche et al. 1990, Skjoldal and Dundas 1991), two motile cell types were observed. They were termed authentic and alternate cells and differed with respect to scale morphology (Paasche et al. 1990, Edvardsen and Paasche 1992). Repeated cloning failed to yield pure cultures of the alternate cell type on a permanent basis. Cultures able to produce both cell types were termed $\beta$ cultures, whereas clones consisting exclusively of the authentic cell type were called $\alpha$ clones. The latter have remained pure for 6 years. The argument that both cell types belong to *C. polylepis* relies on dilution series and repeated clonings (Edvardsen and Paasche 1992).

To investigate whether or not the two cell forms may be joined in a sexual life cycle, we determined the relative DNA content per cell and relative size of cells from several clone cultures of *C. polylepis* by flow cytometry. The relative numbers of authentic and alternate cells in the cultures were estimated using a transmission electron microscope in order to examine the relation among ploidy level, cell type, and cell size. The two cell types are indistinguishable in the light microscope. We also examined whether or not certain growth conditions may induce the transition from one cell type or ploidy level to the other.

## MATERIALS AND METHODS

Strains of *Chrysochromulina polylepis* used in this study were isolated during the 1988 bloom from two localities on the Norwegian Skagerrak coast. Isolates obtained by single-cell capillary isolation are termed clones; those obtained by a serial dilution method (Throndsen 1978) are termed strains. The $\alpha$ clones K, S, W, and strain T originate from locality "Ris" (58°30′N, 9°30′E, isolated on 2 June 1988) and the $\beta$ clones B11, B13, and B152j and the $\alpha$ clone B1511 originates from locality "Tor" (59°00′N, 10°45′E, isolated on 29 May 1988). The clones were made bacteria-free by an antibiotic treatment (Droop 1967). Clones K and W, however, contained small amounts of the bacterium *Hyphomicrobium* sp.

*Standard culture conditions.* The algae were grown in borosilicate tubes or flasks with filtered (Whatman GF/C), autoclaved seawater diluted to 25 practical salinity units (PSU). Vitamins, chelated trace metals, and nutrients were added as in IMR 1/2 medium (Eppley et al. 1967), supplemented with 10 nM selenite (Dahl et al. 1989). Cultures were grown at 15° C under white fluorescent light with a quantum flux of 100 $\mu$mol photons·$m^{-2}$·$s^{-1}$.

*Growth experiments.* In the cell type experiment, the relation among cell type, ploidy level, and cell size was examined. Batch cultures of the $\alpha$ clones K and S and the $\beta$ clones B11, B13, and B152j were grown in 1-L flasks, one culture of each clone in continuous light (24:0 cultures) and one on a 12:12 h LD cycle (12:12 cultures). The cell concentration was determined daily with an electronic particle counter (Coulter Electronics Ltd., Industrial Model D), starting 3 days after inoculation (day = 1).

In the photocycle experiment, we examined whether or not different photocycles may induce a switch in cell type and/or ploidy level. Batch cultures of the $\beta$ clone B11 were grown in 50-mL growth tubes in duplicates under three different photocycles: 24:0, 16:8, and 10:14 h LD.

In the salinity and nutrient experiment, we examined whether salinity or nitrogen-to-phosphorus ratio (N/P) may induce a switch in cell type and/or ploidy level. Semicontinuous cultures of the $\alpha$ clone K were grown at different salinities (20, 27, and 34 PSU) and N/P ratios (100/1, 25/1, 10/1, and 5/1 provided by nitrate-to-phosphate concentrations of 25/0.25, 25/1, 25/2.5, and 12.5/2.5 $\mu$M). The cultures were grown in 15-mL growth tubes on a 12:12 h LD cycle.

Because the alternate cell type was first observed after we had mixed two authentic strains (Edvardsen and Paasche 1992), we examined in a mating experiment whether or not authentic cells could mate and give rise to alternate and/or diploid cells. Five different $\alpha$ clones or strains of authentic *C. polylepis* (K, S, T, W, and B1511) were mixed two by two and grown in 15-mL growth tubes on a 12:12 h LD cycle. Experimental conditions and sampling times are shown in Table 1.

*Transmission electron microscopy.* Percentages of authentic and alternate cells in the cultures were determined by transmission electron microscopy as described in Edvardsen and Paasche (1992). Cells were fixed for 1 min by 2% $OsO_4$ vapor and stained for 15 min with 2% uranyl acetate. The cells were then viewed in a JEOL 100CX or a JEOL 1200X transmission electron microscope. Unless indicated otherwise, at least 30 cells were examined for each sample.

*Cell fixation and staining for flow cytometry.* Algal samples were fixed with paraformaldehyde at a final concentration of 0.5% for 10 min, frozen in liquid $N_2$, and stored at −80° C. Prior to flow cytometric analysis, samples were thawed and the detergent Nonidet was added (final concentration 0.1%). In experiments 2–4, the samples were instead mixed with a HEPES buffer (55 mM HEPES, 5 mM EDTA, 30 mM $MgCl_2$, 125 mM sorbitol, 20 mM sodium citrate, 0.1% Triton X-100, and 0.5% $\beta$-mercaptoethanol, pH 7.5, 1:4 volume sample : volume buffer, Sigma Chemicals). The latter protocol resulted in better staining. The cells were stained for DNA using chromomycin A3 (Sigma Chemicals, final concentration 23.5 $\mu g \cdot mL^{-1}$) and incubated at room temperature for 20 min in darkness. Nuclei of calf thymocytes (Fluoro Trol GF; Ortho Diagnostic Systems Inc., Westwood, Massachusetts) were added to the cell suspension at a final concentration of 0.3 $\mu L \cdot mL^{-1}$ prior to staining, to serve as internal standards for DNA fluorescence. Beads (0.95 $\mu$m, Polysciences) were used as internal standards for light scatter.

*Flow cytometric analysis.* All the analyses were performed with an EPICS 541 (Coulter Electronics, Hialeah, Florida) flow cytometer using the 457-nm laser line as the excitation light source, delivering 100 mW. Forward-angle light scatter and right-angle light scatter, here called side light scatter, were used as estimates of relative cell size, and green-orange fluorescence at 580 ± 20 nm as a measure of the relative amount of DNA per cell. About $5–10 \cdot 10^3$ cells were analyzed per sample. Data were analyzed with CYTOPC (Vaulot 1989) and plotted with WinMDI from Joseph Trotter.

## RESULTS

*Percentage alternate cells in cultures.* In the cell type experiment, the cultures of $\alpha$ clones K and S con-

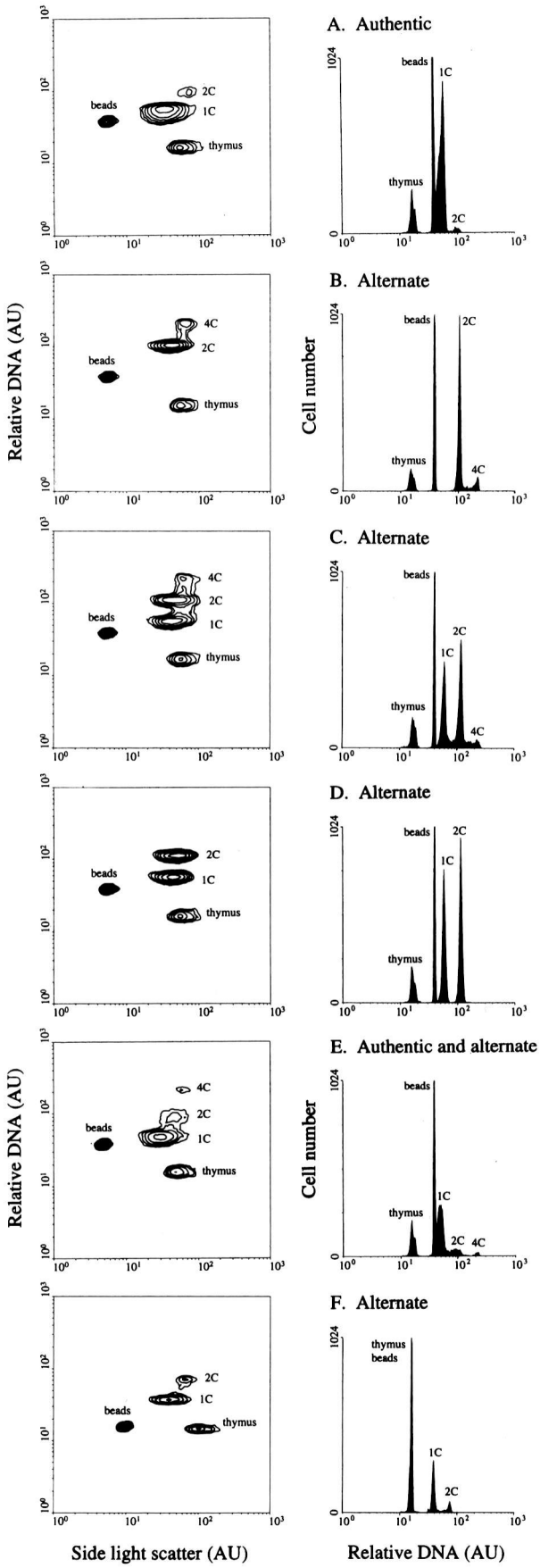
TABLE 1. *Experimental conditions and percentage of alternate cells (n = 30) in cultures of* Chrysochromulina polylepis. *TEM = transmission electron microscopy, FC = flow cytometry. Day 0 = day of inoculation (first three experiments) or mixing (last experiment).*

| Experiment | Culture | Type of strain/clone | LD cycle | Sampling day | | % Alternate cells | |
|---|---|---|---|---|---|---|---|
| | | | | TEM | FC | Beginning of experiment | End of experiment |
| Cell type | B11(24:0) | β | 24:0 | 0, 10 | 2, 3, 4, . . ., 10 | 100 | 100[a] |
| | B11(12:12) | β | 12:12 | 0, 10 | 2, 3, 4, . . ., 10 | 100 | 100[a,b] |
| | B13(24:0) | β | 24:0 | 0, 10 | 2, 3, 4, . . ., 10 | 50 | 0[c] |
| | B13(12:12) | β | 12:12 | 0, 10 | 2, 3, 4, . . ., 10 | 50 | 50 |
| | B152j(24:0) | β | 24:0 | 0, 10 | 2, 3, 4, . . ., 10 | 100 | 97 |
| | B152j(12:12) | β | 12:12 | 0, 10 | 2, 3, 4, . . ., 10 | 100 | 100 |
| | K(24:0) | α | 24:0 | 0, 10 | 2, 3, 4, . . ., 10 | 0 | 0 |
| | K(12:12) | α | 12:12 | 0, 10 | 2, 3, 4, . . ., 10 | 0 | 0 |
| | S(24:0) | α | 24:0 | 0, 10 | 2, 3, 4, . . ., 10 | 0 | 0 |
| | S(12:12) | α | 12:12 | 0, 10 | 2, 3, 4, . . ., 10 | 0 | 0 |
| Photocycle | B11-1 and B11-2 | β | 24:0 | 0, 1, 2, . . ., 6 | 2, 3, 4, 5 | 100 | 100 |
| | B11-3 and B11-4 | β | 16:08 | 0, 1, 2, . . ., 6 | 2, 3, 4, 5 | 100 | 100 |
| | B11-5 and B11-6 | β | 10:14 | 0, 1, 2, . . ., 6 | 2, 3, 4, 5 | 100 | 100 |
| Salinity/nutrient | K | α | 12:12 | 0 | 0, 7, 8, 9 | 0 | 0 |
| Mating | K, S, T, W, B1511, K + S, K + T, K + W, K + B1511, S + T, S + W, S + B1511, T + W, T + B1511, W + B1511 | α | 12:12 | 0, 7, 21, 240 | 0, 14 | 0 | 0 |

[a] A few detached authentic scales were observed.
[b] n = 3.
[c] Detached alternate scales were observed.

tained 100% authentic cells through the whole experiment (Table 1); cultures of these clones always have contained 100% authentic cells during the 6 years in culture. Prior to the cell type experiment, the β clones B11 and B13 were mixtures of the two cell types, but the inoculum of B11 appeared to contain alternate cells only. In cultures B11(24:0) and B11(12:12), only alternate cells were observed, although a few detached body scales from authentic cells were seen at the end of the experiment. In cultures of B13 grown in continuous light, the percentage of alternate cells changed from 50 to 0% during the experiment, but some detached alternate scales were also observed. In cultures of B13 grown on a 12:12 h LD cycle, the percentage of alternate cells was not altered. Clone B152j was isolated by capillary isolation only a few weeks before the experiment. Clone B152j grown on a 12:12 h LD cycle remained 100% alternate throughout the experiment, whereas the same clone grown in continuous light also contained authentic cells at the end of the growth period (day 10). In the photocycle experiment, cultures (B11-1 to B11-6) contained only alternate cells during the entire experimental period. In the salinity/nutrient and mating experiments, cultures contained only authentic cells during the whole experiment (Table 1).

*Relative DNA content per cell.* The DNA content per cell was distributed in three populations or ploidy levels that may be termed 1C, 2C, and 4C levels, indicating the relative amount of DNA in the corresponding three groups of cells (Goff and Coleman 1990). Cultures in continuous light displayed DNA histograms with two (sometimes three) peaks corresponding to $G_1$ and $G_2$ + M phases of the cell cycle (Fig. 1A, B, F). In-between these two peaks were cells in S phase, where DNA synthesis takes place. Pure authentic cultures of *C. polylepis* contained cells with the lowest DNA level for the $G_1$ peak (1C cells, Fig. 1A). In the following, these cells are termed haploid. In two pure alternate cultures (B152j(24:0) and (12:12), the latter not shown), the $G_1$ cells contained twice as much DNA (2C cells) as authentic cells and are termed diploid (Fig. 1B, Table 2). Other apparently pure alternate cultures contained both haploid and diploid cells (B11(24:0) and (12:12), Fig. 1C, D, Table 2). Of the three DNA peaks in culture B11(24:0) (Fig. 1C), the first peak presumably represents haploid cells in $G_1$ (1C), the second peak represents haploid cells in $G_2$ + M and diploid cells in $G_1$ (2C), and the third peak represents diploid cells in $G_2$ + M (4C). Samples from the same clone grown on a 12:12 h LD cycle and taken 5 h after lights went on showed two DNA peaks, and the populations appeared to be somewhat synchronized with a strong dominance of haploid and diploid cells in the $G_1$ phase (Fig. 1D). Cultures with both authentic and alternate cells contained both haploid and diploid cells (Fig. 1E). The pure alternate β cultures B11-1 to B11-6 in the photocycle experiment contained only haploid cells (Fig. 1F, Table 2). In the cell type experiment, the average ratio between 1C




and 2C DNA levels (normalized to thymus) was 1.9 in $\alpha$ cultures and 2.0 in $\beta$ cultures. The average ratio between 1C and 4C DNA levels in $\beta$ cultures was 3.8 (Table 2). In summary, $\alpha$ cultures contained only haploid cells, whereas $\beta$ cultures contained either haploid, diploid, or both haploid and diploid cells.

*Changes in cell type and ploidy level in batch cultures over time.* All cultures grew exponentially between days 1 and 4 and entered stationary phase on day 4 or 5, except B152j(12:12), which reached stationary growth phase on day 9 (Fig. 2).

All $\alpha$ cultures contained exclusively authentic, haploid cells throughout experiment duration, with 78–99% 1C cells and the rest being 2C cells (Fig. 2A). Similarly, all haploid $\beta$ cultures (B11-1 to B11-6) remained pure alternate and haploid at all light : dark cycles tested (Tables 1, 2, Fig. 2E). Whereas haploid cultures of both $\alpha$ and $\beta$ type remained unchanged at all growth conditions tested, $\beta$ cultures containing diploid cells changed in regard to cell type ratio and ploidy ratio when grown in continuous light (Fig. 2B–D, Table 1).

In a $\beta$ culture containing alternate, diploid cells only (B152j(24:0)), authentic cells were observed after 10 days in continuous light (Table 1). Only a few haploid cells were observed on day 1 (0.8% 1C cells, n = 395), but the frequency of haploid cells increased between days 2 and 10 (2.4% 1C cells on day 10, n = 8230, Figs. 2B, 3A). The same clone grown on a 12:12 h LD cycle contained 99–100% alternate diploid cells with a strong dominance of cells in $G_1$ phase, throughout the whole experiment (10 days, Fig. 2B). In a $\beta$ culture with about 50% authentic and 50% alternate cells grown on a 12:12 h LD cycle, the percentage of alternate cells and diploid cells remained constant through the experiment (Fig. 2D). When the same clone was grown in continuous light, haploid cells became dominant. At the end of the experiment, only authentic cells were observed, and all cells were probably haploid because the frequency of 1C cells was about 98% and no 4C cells were registered (Figs. 2D, 3B). These results suggest that light condition may influence cell type ratio and ploidy ratio.

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FIG. 1. Flow cytometric signatures of six *Chrysochromulina polylepis* cultures growing exponentially in continuous light (24:0) or on a 12:12 h LD cycle (12:12). Left) Distributions of relative DNA content per cell (log green fluorescence) versus side light scatter (related to cell size, arbitrary units [AU]). Right) Distributions of relative DNA content per cell (log green fluorescence, AU). 1C cells are assumed to be haploid cells in $G_1$ phase, 2C haploid cells in $G_2$ + M phase and/or diploid cells in $G_1$ phase, and 4C diploid cells in $G_2$ + M phase. Calf thymocyte nuclei and 0.95-$\mu$m beads were used as internal standards. Cultures: A) authentic S(24:0) day 3, B) alternate B152j(24:0) day 4, C) alternate B11(24:0) day 3, D) alternate B11(12:12) day 3, E) authentic and alternate B13(24:0) day 2, F) alternate B11-2 (on 24:0 h LD cycle) day 3.

TABLE 2. *Relative DNA content (±SD) per cell normalized to thymus DNA of 1, 2, and 4C cells of* Chrysochromulina polylepis *cultures. n = number of samples for estimating 1C, 2C, and 4C values.*

| Experiment | Culture | Type of strain/ clone | n | Relative DNA content per cell normalized to thymus DNA | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 1C | 2C | 4C | 2C/1C | 4C/1C |
| Cell type | B11(24:0) | β | 8, 8, 8 | 3.09 ± 0.37 | 6.25 ± 0.84 | 10.73 ± 1.66 | 2.02 | 3.47 |
| | B11(12:12) | β | 8, 8, 8 | 3.71 ± 0.33 | 7.45 ± 0.74 | 12.25 ± 2.23 | 2.00 | 3.30 |
| | B13(24:0) | β | 8, 8, 8 | 3.33 ± 0.31 | 6.22 ± 0.61 | 12.59 ± 1.99 | 1.87 | 3.78 |
| | B13(12:12) | β | 8, 8, 8 | 3.18 ± 0.30 | 6.70 ± 0.47 | 11.93 ± 1.79 | 2.11 | 3.75 |
| | B152j(24:0) | β | 5, 9, 9 | 3.33 ± 1.00 | 7.06 ± 0.65 | 13.94 ± 1.37 | 2.12 | 4.19 |
| | B152j(12:12) | β | 0, 9, 9 | — | 6.78 ± 0.62 | 13.40 ± 1.65 | — | — |
| | K(24:0) | α | 8, 8, 0 | 3.10 ± 0.24 | 5.62 ± 0.35 | — | 1.81 | — |
| | K(12:12) | α | 8, 8, 0 | 3.10 ± 0.22 | 5.77 ± 0.47 | — | 1.86 | — |
| | S(24:0) | α | 8, 8, 0 | 3.10 ± 0.31 | 5.92 ± 0.49 | — | 1.91 | — |
| | S(12:12) | α | 8, 8, 0 | 3.05 ± 0.31 | 5.95 ± 0.47 | — | 1.95 | — |
| | All above β cultures | β | 36, 49, 46 | 3.33 ± 0.50 | 6.75 ± 0.77 | 12.49 ± 2.00 | 2.03 | 3.75 |
| | All above α cultures | α | 32, 32, 0 | 3.09 ± 0.26 | 5.82 ± 0.45 | — | 1.88 | — |
| Photocycle | B11-1 to B11-6 | β | 28, 28, 0 | 2.75 ± 0.05 | 4.64 ± 0.33 | — | 1.69 | — |
| Mating | See Table 1[a] | α | 18, 18, 0 | 2.45 ± 0.04 | 4.68 ± 0.20 | — | 1.91 | — |

[a] All cultures in mating experiment.

*Induction of diploid or alternate cells.* The ploidy level or cell type in originally haploid β cultures was not influenced by different photocycles (24:0, 16:8, and 10:14 h LD, Fig. 2E). Similarly, authentic cells did not change ploidy level or cell type in response to change in growth factors such as salinity (20, 27, and 34 PSU) and N/P ratios (100/1, 25/1, 10/1, and 5/1). Mixing five different authentic, haploid strains or clones two by two did not induce a shift to alternate or diploid cells (Tables 1, 2).

*Cell size.* Prior to statistical analysis, the distributions were tested for normality with the Shapiro-Wilk *W*-test (Zar 1984). In experiment 1, the diploid alternate cells in cultures B152j(24:0) and (12:12) had a significantly larger side light scatter than the authentic cells in cultures K(24:0), K(12:12), S(24:0) and S(12:12) (*t*-test, $P < 0.001$, Zar 1984), suggesting that they had a larger size (Fig. 4). Also, the haploid alternate cells in the photocycle experiment had higher side light scatter than the authentic haploid cells in the salinity/nutrient experiment (*t*-test, $P < 0.001$, Zar 1984, not shown). Scale morphology more than ploidy level appeared to influence cell size.

*Influence of bacteria on DNA content and cell size.* Authentic *C. polylepis* cells can ingest particles by phagocytosis (e.g. Manton and Parke 1962). All cultures in this study were axenic except those of clones K and W, containing small amounts of the bacteria *Hyphomicrobium* sp. and strain T containing bacteria. The DNA content per cell did not differ in cultures of strain T compared to cultures of authentic cells containing no or low levels of bacteria. The light scatter per cell in cultures containing strain T were, however, significantly higher than in other authentic cultures in the mating experiment, indicating larger cell size or possibly higher cell density (not shown).

## DISCUSSION

*Evidence for diploid cells in* Chrysochromulina polylepis. The DNA content in haploid cells of the $G_2$ + M phase in the cell cycle and diploid cells in the $G_1$ phase coincides (both are 2C, Goff and Coleman 1990). In cultures growing in continuous light, all phases of the cell division cycle will be present in amounts relative to the length of each phase. The evidence for diploid cells is the presence of diploid cells in the $G_2$ + M phases containing four times the amount of DNA (4C cells) compared to haploid cells in $G_1$ (1C cells) in an asynchronous culture. In addition, the number of cells with 2C DNA level (2C cells) will be higher in cultures with diploid cells compared to cultures with only haploid cells. During growth in LD cycles, the cell cycle of most species of phytoplankton become phased or synchronized (Chisholm et al. 1984), and cell division in Prymnesiophyceae is most pronounced during the dark period (Chisholm 1981). In such cases, most cells are usually in the $G_1$ phase during the light period. β cultures of *C. polylepis* growing on a LD cycle had, 5 h after the onset of the light period, one or two DNA peaks of similar size and only a few cells with intermediate DNA content (S phase). This leads us to believe that the majority of the cells most probably were synchronized in the $G_1$ phase, the first peak representing the $G_1$ phase of haploid cells, the second the $G_1$ phase of diploid cells. β cultures growing in continuous light had two or three DNA peaks as well as cells in the S phase, which suggests asynchronous growth. The third DNA peak contained on average 3.75 times more DNA than the 1C DNA level, indicating that this represented diploid cells in $G_2$. Thus, both haploid and diploid cells appeared to be present in β cultures of *C. polylepis.*

In a previous study by Edvardsen and Paasche

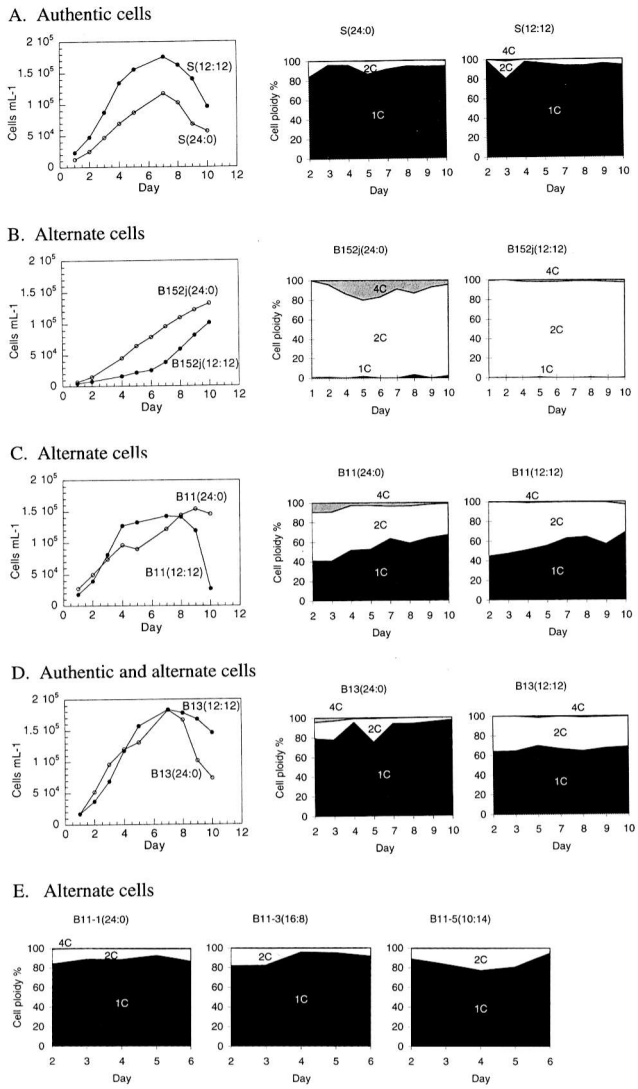


FIG. 2. Cell numbers (left) and percentage cells with DNA content of 1C, 2C, and 4C levels (right) in cultures of *Chrysochromulina polylepis* over time. Cultures: A) authentic S(12:12) and S(24:0); B) alternate on day 0, B152j(24:0) and (12:12); C) alternate on day 0, B11(24:0) and (12:12); D) authentic and alternate B13(24:0) and (12:12); E) alternate B11-1, B11-3, and B11-5.

(1992), preliminary results from flow cytometric analyses of *C. polylepis* indicated that authentic cells in an $\alpha$ culture contained an equal amount of DNA per cell compared to alternate cells in a $\beta$ culture. The result regarding DNA content of the authentic cells in the $\beta$ culture was unclear. Only a limited number of cultures were analyzed at the time, however, and both staining and analysis protocols have been improved since.

*Sexual reproduction in* Chrysochromulina. All $\alpha$ cultures, consisting exclusively of authentic cells, contained haploid cells only on all occasions and in all growth conditions tested. Some of the cultures containing alternate cells ($\beta$ cultures) consisted of haploid cells only, other cultures consisted of diploid cells only, and finally some $\beta$ cultures contained both haploid and diploid cells. Obviously the relationship between scale morphology and ploidy level is not simple. Both the haploid and diploid alternate cells were larger than the authentic cells. The presence of both haploid and diploid cells in cultures of *C. polylepis* is an indication that sexual reproduction does occur in the genus *Chrysochromulina.* Syngamy and meiosis was not, however, directly observed. Sexual reproduction in the class of Prymnesiophyceae has been demonstrated earlier (for review see Billard 1994). By observing fixed and stained preparations under the microscope, both Rayns (1962) and von Stosch (1967) have found that the coccolithophorid *Pleurochrysis carterae* (= *Hymenomonas carterae*) (Braarud et Fagerland) Christensen had a haploid scaly thallus stage (*Apistonema* stage) and a diploid coccolith-bearing stage (*Hymenomonas* stage). Sexuality was also shown to occur in other *Pleurochrysis* species (Gayral and Fresnel 1983). Cariou et al. (1994) and Vaulot et al. (1994) have shown by flow cytometry of *Phaeocystis* spp. that flagellated cells are either haploid or diploid, whereas colonial cells are diploid. In *Emiliania huxleyi,* Paasche and Klaveness (1970) showed by nuclear staining and spectrophotometry that the calcifying C cells and naked N cells have the same amount of DNA. By flow cytometry, Course et al. (1994) found that flagellated S cells of *E. huxleyi* could be haploid and calcifying cells diploid. In *Chrysochromulina,* evidence for generations with different ploidy levels has not previously been reported.

*Transition between cell types and ploidy levels.* Whereas haploid cultures of both $\alpha$ and $\beta$ type remained unchanged in all growth conditions tested, $\beta$ cultures containing diploid cells changed with respect to cell type and ploidy during the experiments, particularly when grown in continuous light. It appears as if alternate diploid cells can give rise to authentic haploid cells when they are grown in continuous light. The observed relative increase of authentic cells could alternatively or partly be due to higher growth rate in continuous light of this cell type compared to the alternate cell type. Our results do, however, suggest that light condition may influence cell type ratio and ploidy ratio. Growth experiments in a previous study showed $\beta$ cultures of *C. polylepis* to be less tolerant to high irradiances than $\alpha$ cultures (Edvardsen and Paasche 1992). The alternate cell type has not yet been found in natural water samples. However, detached scales identical to those of the alternate cell type have been observed in three different water samples from Skagerrak, Kattegat, and off the northwest coast of Norway collected mainly in March and April (Wenche Eikrem, pers. commun., Helge Thomsen, pers. commun.). It remains to be shown whether the alternate cell type occurs in nature under low-light conditions and/or short

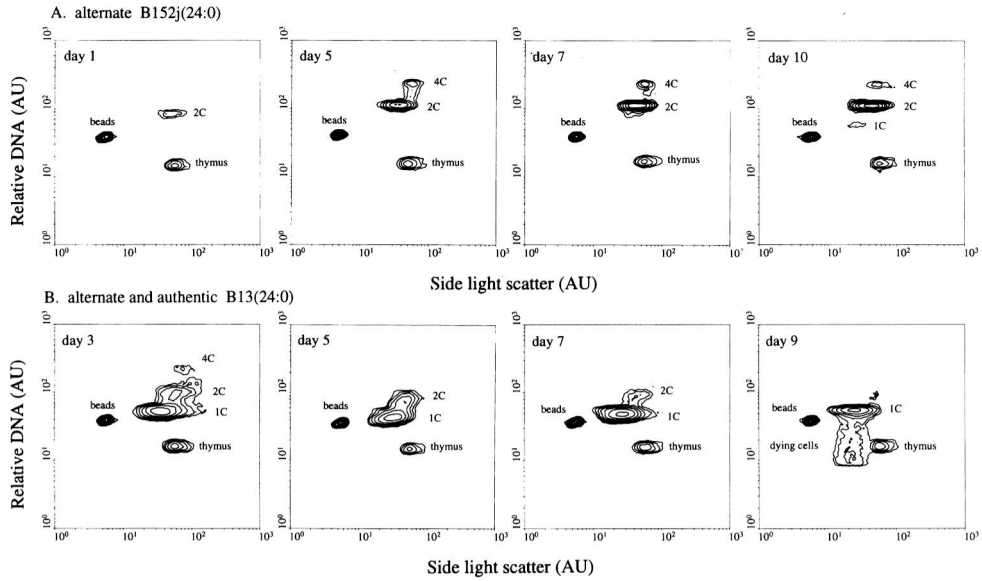


FIG. 3. Flow cytograms of relative DNA content per cell (log green fluorescence) versus side light scatter (related to cell size, AU) in cultures of *Chrysochromulina polylepis* over time. Calf thymocyte nuclei and 0.95G beads were used as internal standards. A) Culture B152j(24:0) with 100% alternate cells on day 0 and 97% on day 10; B) culture B13(24:0) with 50% alternate cells on day 0 and 0% on day 10.

light periods. We were not able to induce the transition from one cell type or ploidy level to the other by altering salinity or the N/P ratio.

The stability in $\alpha$ cultures and variability in $\beta$ cultures is also reflected in the relative DNA content per cell and in cell size (Table 2, Fig. 4). Similarly, the growth rates in two $\beta$ clones grown at different temperatures were highly variable from one experiment to another though almost constant in two $\alpha$ clones (Edvardsen and Paasche 1992). The difference in growth patterns could not be explained by a change in the percentage of alternate cells. The toxicity of $\beta$ clones varied from nondetectable to a level equalling that of pure authentic cultures, and it was not always linearly related to the percentage of alternate cells (Edvardsen 1993). The diploid cell type may differ from the alternate haploid cell type in respects other than DNA content per cell and cell size. Some of this variability of $\beta$ cultures can possibly be explained by the fluctuating percentage of diploid alternate cells in cultures where this cell type is present. In fact, a haploid clone of the alternate cell type (B11) has remained completely alternate for a year, supporting the view that stability is linked to haploidy rather than to scale morphology. In batch cultures of *Phaeocystis globosa,* Jahnke (1989) observed large variabilities in various physiological cell parameters, which were hypothesized to be caused by the varying amounts of different cell types of this species in the culture.

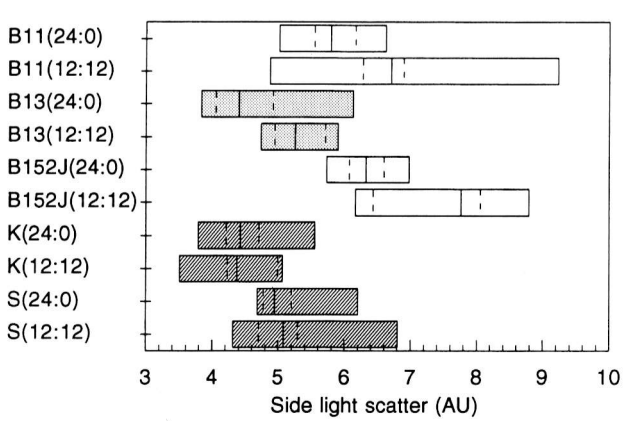


FIG. 4. Side light scatter normalized to 0.95G beads (related to cell size, AU) for 10 cultures of *Chrysochromulina polylepis* days 2–10. The range of the boxes with associated lines indicates quartiles, from left to right: 5, 25, 50 (median), 75, and 95% of the cells with equal or lower light scatter. White = 100% alternate cells, stippled = alternate and authentic cells, hatched = 100% authentic cells.

*Syngamy.* Our attempt to induce sexual reproduction by mixing different authentic haploid clones apparently did not result in mating. Either all authentic haploid cells in our cultures are of the same mating type, or mating may occur under specific growth conditions only, or the authentic haploid cell type is not involved in mating. Further work on elucidating syngamy should include mixing haploid authentic with haploid alternate cells and looking for diploid cells. A diagram illustrates a hypothetical life cycle of *C. polylepis* (Fig. 5). We suggest that *C. polylepis* has a haplo-diploid life cycle that includes three flagellated scaly cell forms: two morphologically distinct haploid cell types (authentic and alternate) and one diploid (alternate) cell type. Transition from alternate to authentic cells occurred in 10

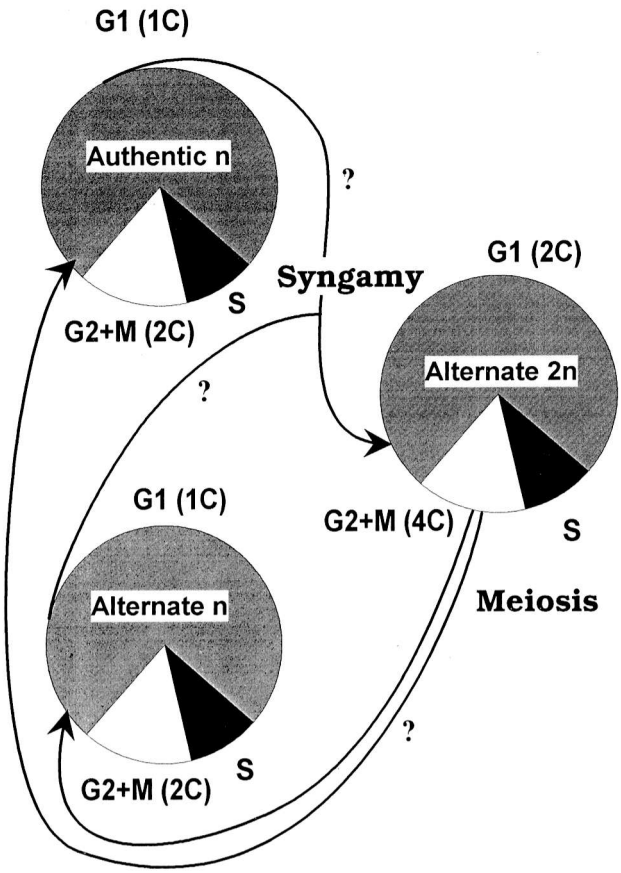


FIG. 5. Hypothetical life cycle of *Chrysochromulina polylepis* (for explanation see text).

clones that were examined regularly by transmission electron microscopy (Edvardsen and Paasche 1992). Transition from authentic to alternate cells was observed only once, when two authentic strains of *C. polylepis* were mixed and the mixture was made bacteria-free by antibiotics (Edvardsen and Paasche 1992). The haploid alternate and authentic cell types may be able to function as sexual stages (gametes) representing different mating types, the diploid alternate cells being the result of syngamy. Exponential growth in pure cultures of all three cell types indicates that they are all capable of vegetative propagation. Because alternate cells seem to be rare in nature, it is likely that *C. polylepis* has a life cycle where the haploid authentic stage dominates.

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