The Solution to the Cytological Paradox of Isomorphy

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Abstract. Cells with polyploid nuclei are generally larger than cells of the same organism or species with nonpolyploid nuclei. However, no such change of cell size with ploidy level is observed in those red algae which alternate isomorphic haploid with diploid generations. The results of this investigation reveal the explanation.

Nuclear DNA content and other parameters were measured in cells of the filamentous red alga *Griffithsia pacifica*. Nuclei of the diploid generation contain twice the DNA content of those of the haploid generation. However, all cells except newly formed reproductive cells are multinucleate. The nuclei are arranged in a nearly perfect hexagonal array just beneath the cell surface. When homologous cells of the two generations are compared, although the cell size is nearly identical, each nucleus of the diploid cell is surrounded by a region of cytoplasm (a "domain") nearly twice that surrounding a haploid nucleus. Cytoplasmic domains associated with a diploid nucleus contain twice the number of plastids, and consequently twice the amount of plastid DNA, than is associated with the domain of a haploid nucleus. Thus, doubling of ploidy is reflected in doubling of the size and organelle content of the domain associated with each nucleus. However, cell size does not differ between homologous cells of the two generations, because total nuclear DNA (sum of the DNA in all nuclei in a cell) per cell does not differ. This is the solution to the cytological paradox of isomorphy.

In both prokaryotic and eukaryotic cells, strong correlations have been reported between genome size and cell volume (Jacobi, 1925; Commoner, 1964; Cavalier-Smith, 1978, 1985; Watanabe and Tanaka, 1982; Shuter et al., 1983; Brodsky and Uryvaena, 1985; Lewis, 1985). Thus, cells of larger size have more nuclear DNA and, in eukaryotes, larger nuclei. Not only is this correlation apparent in organisms which undergo polyploidy (i.e., yeast [Gunge and Nakotomi, 1972; Shuter et al., 1983], higher plant cells [Rees, 1972; Bennett, 1972], and mammalian liver cells [Epstein, 1967; Epstein and Gatens, 1967; Sweeney et al., 1979]), it has also been observed in interspecific comparisons (i.e., prokaryotes [Shuter et al., 1983], phytoplankton [Holm-Hansen, 1969], ciliates [Soldo et al., 1981; Shuter et al., 1983], angiosperm meristem cells [Price et al., 1973; and Shuter et al., 1983], and fish and amphibian erythrocytes [Pedersen, 1971; Oeldorf et al., 1978; Olmo and Morescalchi, 1975; Shuter, 1983]).

An apparent paradox emerges however upon the consideration of organisms that have isomorphic life histories. In several lines of multicellular protists such as the red, green, and brown algae, there are taxa that undergo a regular "alternation of generations" between haploid and diploid individuals that, except for reproductive stages, are morphologically indistinguishable. Homologous cells in the two generations should theoretically differ twofold in their DNA content and consequently, their cell volume. Yet, no such differences have been reported.

In the present study we examine this apparent paradox by determining the cell size and amount of nuclear DNA of functionally homologous cells of the diploid and haploid generations of the red alga *Griffithsia pacifica*. Relative DNA content of nuclei was measured by microfluorometry after fixation and staining with the DNA fluorochrome 4',6 diamidino-2-phenylindole (DAPI).[1] As we will demonstrate, nuclear DNA content is correlated with cell size even in organisms with isomorphic life histories; the resolution of the paradox lies in the existence of polygenomy. In addition, the amount of plastid DNA (ptDNA) is directly correlated with the level of nuclear DNA in both generations.

Methods and Materials

Haploid and diploid *Griffithsia pacifica* cultures were established from tetraspores (1N) and carpospores (2N) released from plants collected at Stillwater Cove (Monterey County), California, April 1982. Cultures were maintained in modified Provasoli's Enriched Seawater (PES/4) (McLachlan, 1973), incubated in a 12-h light/12-h dark cycle, and illuminated by cool white fluorescent lamps providing ∼20–25 $\mu E\ m^{-2}\ s^{-1}$ irradiance. Haploid and diploid plants to be compared were established by isolating apical tips (apical cell and two subtending cells) from 1N or 2N plants. Each tip was grown separately under identical culture conditions for 1 mo before use.

Tissue Preparation

The standard cytological fixative 3:1 (3 parts 95% EtOH and 1 part glacial

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[1] *Abbreviations used in this paper*: DAPI, 4',6 diamidino-2-phenylindole; OP, optical path; OPD, optical path difference; ptDNA, plastid DNA.

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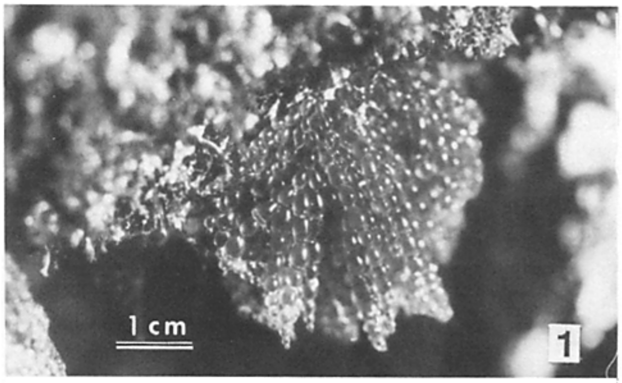
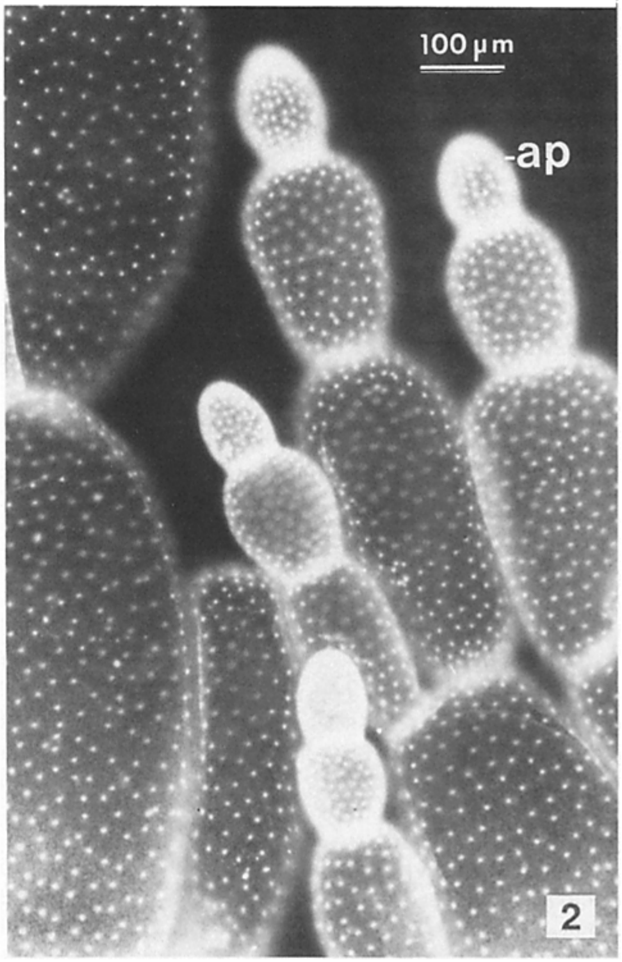




Figures 1 and 2. (*Fig. 1*) *Griffithsia monilis* (1N, male); intertidal habitat at Robe, South Australia. (*Fig. 2*) *Griffithsia globulifera* (2N); staining with DAPI reveals the regular distribution of nuclei in all cells, including the apical cell (*ap*). Microwave-fixed in 0.5 µg/ml DAPI in seawater.

acetic acid) used successfully for fluorometry of many algae (Goff and Coleman, 1984, 1986) could not be used for *Griffithsia* as this fixative caused extensive cell shrinkage and cytoplasmic rearrangement in these extremely large, highly vacuolate cells. However, during the course of this study we found that extremely rapid heat fixation of cells, using microwaves (Login, 1978), gave excellent cell preservation and no size or shape distortion. Consistent quantitative staining of nuclear DNA and ptDNA could be obtained by microwaving the cells directly in DAPI.

Griffithsia cells were placed in a 35- × 10-mm plastic petri dish containing 3 ml of 0.5 µg/ml DAPI in seawater. The dish was placed in the middle of the oven (GE dual wave 625 W) and heated at maximum power for 10-11 s. At the point of fixation, the cells change in color from red to green. After fixation and staining, the cells were mounted on a slide in the fluorochrome, a coverslip was applied, and the edges were sealed after removing the excess stain. This process also softens the cells and collapses the large central vacuole; in properly prepared slides, the cells flatten so that one layer of cytoplasm from the optical top of the cell is directly over the layer on the other side of the cell. Each plane may be clearly resolved optically, but microspectrophotometric and density measurements were made only from the top plane; this minimized the path length, and hence the self-absorption of the excitation and emission epifluorescence illumination.

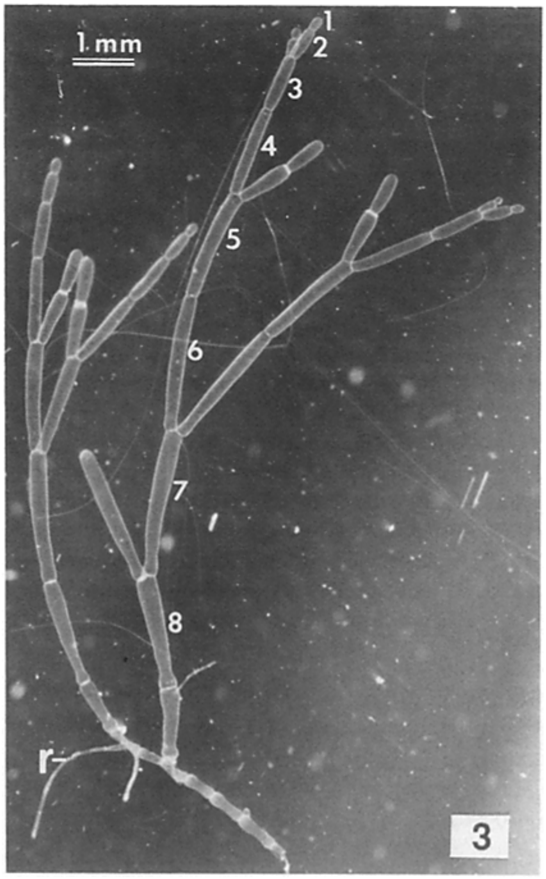


Figure 3. Griffithsia pacifica (1N, male); 21-d-old plant grown from a single isolated intercalary cell. The smaller rhizoid (*r*) producing basal cells of the prostrate system are evident, subtending the dichotomously branched filaments of the erect system. Cell numbers correspond to those indicated in Fig. 4.

Microspectrophotometry

Cells were examined and their nuclear DNA and ptDNA measured using a Leitz Orthoplan microscope equipped with an epiillumination system and Zeiss plan neofluor phase objectives, and interfaced to a Leitz MPV-3 microspectrofluorometer. A 100 W mercury lamp (voltage stabilized) provided the excitation energy. For DAPI fluorescence, a Leitz filter system A cube (No. 513410, UV excitation range, exciting filter BP 340-380, beam-splitting mirror RKP 400, suppression filter LP430) was used. To suppress autofluorescence from phycobilin and other pigments not removed by the fixation process, a Zeiss 46 79 60 (KP 500) short pass interference filter was placed in the emission beam path. The filter effectively blocked all emission above 500 nm and reduced emission intensity by ~40%.

The field of excitation was restricted with an illumination diaphragm which limited the specimen field of illumination to about three times the area of the object to be measured. A turret of measuring diaphragms provided appropriate sized "pinholes" to further narrow the field of emission light transmitted to the photometer. The fluorescence from a single excited nucleus was measured using the 40× oil immersion objective, by positioning the nucleus in the center of the measuring diaphragm using a Stahl automatic X Y fine stage (0.1 µm step resolution) controlled by a Stahl 517 MF

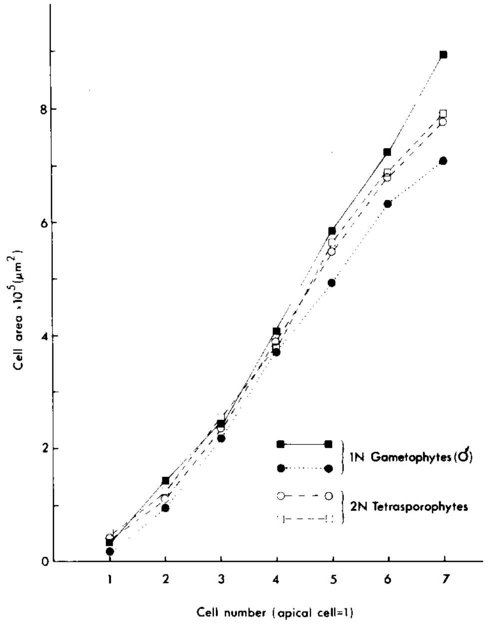


Figure 4. Cell area increase in *G. pacifica*. Each set of symbols corresponds to a single branch from either a 1N (male) or 2N plant. As indicated in Fig. 3, cell 1 is the dividing apical cell and cell 7 is the seventh cell in the axis, and is the most basal in the erect system. Each plant was grown from a single intercalary cell isolated 21 d previously. All plants were grown under identical conditions. Areas must be doubled to approximate true cytoplasmic area in these flattened cells, a correction not incorporated here.

tracking ball driven microposition controller. This controller, interfaced via an RS232 port to an HP-85 microcomputer permitted X Y coordinates for any point to be stored and recalled. For each point, an adjacent nonnuclear area was measured and subtracted as background. Fading of DAPI fluorescence was minimal under the conditions used. ptDNA per plastid was measured in DAPI-stained preparations in the same fashion, using a 63× oil immersion objective.

The size of the measuring diaphragm (0.15–1.2 mm) was selected to just circumscribe the object to be measured. Thus, nuclei which differed in size significantly were frequently measured using different diaphragms. By measuring individual nuclei with different size diaphragms and subtracting background readings made with that same size pinhole, it was determined that there was no significant difference in the amount of fluorescence measured using different pinholes. However, with the larger pinhole, more variation was encountered due to the increased variation in the background reading.

A Leitz fluorescent uranyl standard was used to measure instrument stability, which varied by <2–3%. In addition, *Griffithsia* sperm cells and/or chick red blood cells (Coleman et al., 1981) fixed by identical experimental procedures served as "internal standards" as described by Goff and Coleman (1984). All measurements were made with the same high voltage and gain settings on the photomultiplier.

Readings were recorded and processed directly with an HP-85 computer interfaced to the MPV-3, using software written for this system and an HP statistics program. For each histogram, the total number of points (n) and peak means ($\bar{x}$) are included. Variation is expressed as percentage variation (i.e., coefficient of variation = [standard deviation/mean] × 100] so that peaks of different numbers of points can be compared directly. Photographs were made using Ilford XP-1 film and exposure time of 5–15 s.

Image Processing

To measure cell size and determine the density of nuclei within multinucleate cells, a Unicomp image processing system (Southern Micro Instruments, Inc., Atlanta, GA) was used. In this system, a Dage 650 high resolution television camera was used on an Olympus Vanox microscope equipped with an epifluorescence high pressure mercury lamp (200-W lamp) illumination system and fluorescence and phase optics. The television image was transferred to an Apple IIe microcomputer interfaced to a Houston Hi-Pad digitizer. The interfaced eqiupment was controlled by Unicomp image processing software.

Cell and plastid sizes were measured directly as area on uniformly flattened preparations. Distances between the centers of nuclei in *Griffithsia* cells were measured using a point–point distance program, and the density of nuclei and plastids in *Griffithsia* cells was determined by first establishing an area (~10,000–15,000 μm² for nuclei and 1,000–2,000 μm² for plastids) within the cell in which point counts were to be made (this area was chosen to exclude any artifacts introduced by the curvature of cell ends and sides) and then counting the number of nuclei or plastids within that area. Comparative measurements of cell size and the density of nuclei in cells of haploid and diploid individuals were always made in homologous cells (i.e., same size, age, and position from apical cells). For each cell measured, the ploidy level of the nuclei contained therein was determined using microspectrofluorometry.

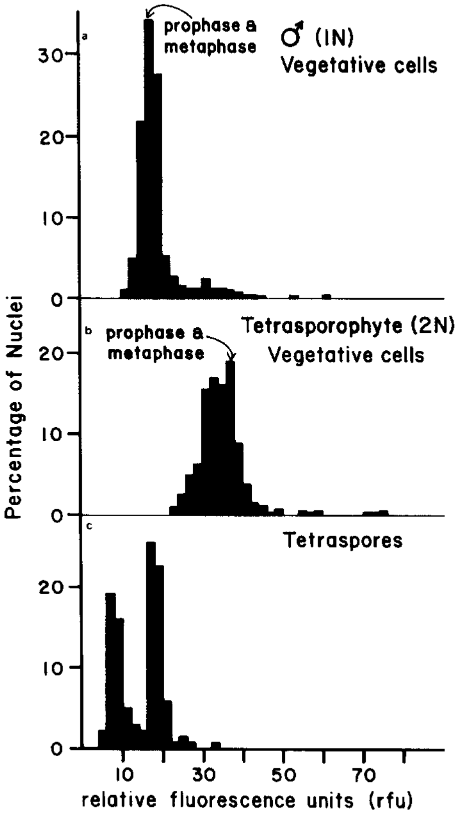
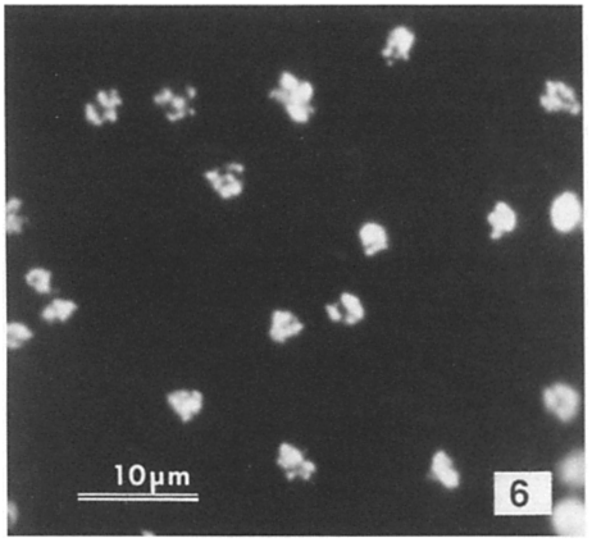


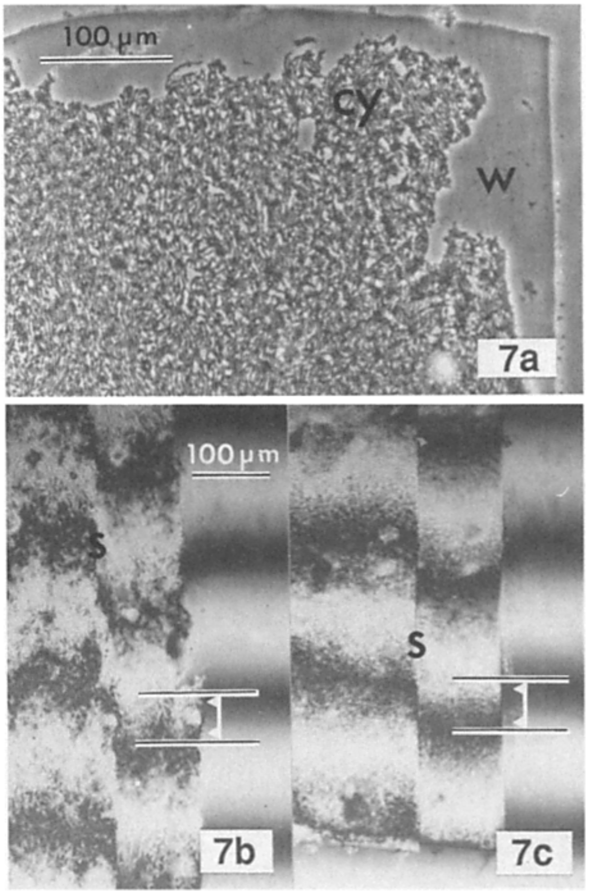
Figure 5. Histogram comparing the relative fluorescence units of DAPI-stained nuclei of 1N (*a*) and 2N (*b*) *G. pacifica* vegetative cells with those of tetraspores (meiospores) (*c*). In *a* (n = 736 readings), the primary peak (x = 16.53 rfu) corresponds to the 2C level of DNA. Observed prophase and metaphase figures fall within this peak. A small percentage of nuclei are polyploid (4–8C). In *b* (n = 450), the mean of the major peak is 32.81 rfu, corresponding to the 4C level of DNA. Some 8C(+) nuclei are present. In *c*, tetraspores (before release) were measured (n = 137) to provide a 1–2C standard. The peak of lower values ($\bar{x}$ = 8.48 rfu) was from tetraspores in G_1 and they represent the 1C level of DNA. The higher peak ($\bar{x}$ = 18.14 rfu) is the 2C (G_2) DNA level of the mature tetraspore.

Interference Microscopy

The relative thickness of the peripheral cytoplasmic layer in haploid and diploid cells was determined using an aus Jena Peraval Interphako interference microscope (courtesy of Dr. Paul Green, Stanford University, CA).

Homologous haploid and diploid *Griffithsia* cells (same age and position from apical cell) were fixed in modified Karnofsky fixative (Goff, 1981). Using microsurgical tools, these cells were cut open and a central portion of the cell (~200 × 200 μm²), consisting of one wall layer and a single





Figures 6 and 7. (*Fig. 6*) Dividing nuclei from *G. pacifica* 1N (male) plant (third cell in filament). All nuclei from this central third of the cell were in prophase or early metaphase. Approximately 5–8 chromosomes are seen in haploid (2C) nuclei. (*Fig. 7*) *G. pacifica* 1N (female). *a* (phase-contrast microscopy) is a region of an intercalary cell fixed in glutaraldehyde from which interferometry readings (Table III) were obtained. The underlying wall (*w*) is evident as is the cytoplasmic layer (*cy*). This section is mounted in 0.5 μg/ml DAPI so that nuclei could be visualized using epifluorescence microscopy; (*b*) Interference pattern generated when green (549 nm) light is passed through a region of cytoplasm and wall from a *G. pacifica* (1N) cell. The sheared image (*S*) is indicated. By measuring the shift (in arbitrary units) between the background (mounting medium) and sheared image (difference between bars), the actual fraction of a wavelength shift that results from this interference can be calculated. (*c*) Interference pattern from a 2N *G. pacifica* cell.

layer of cytoplasm, was mounted on a glass slide with the wall contiguous to the slide surface. The tissue was mounted in 0.5 μg/ml DAPI in seawater and examined with the interference microscope. For each piece of tissue 50–100 measurements were made of the optical path difference (OPD) between a path of light passing through and being interfered with the specimen and that of a path which passes through the background only. As discussed in the Appendix, this OPD is proportional to the mass and relative thickness of the tissue piece. OPD values were determined both for the wall only, and the wall plus the adjacent cytoplasmic layer. The difference gave the OPD of the cytoplasm. The distribution of nuclei in the cytoplasm of the cell pieces measured was also determined, using the image analysis system, and found to be comparable to that observed in the preparations used for microspectrofluorometry.

Results

Griffithsia is an intertidal marine red alga of worldwide distribution. As in most other florideophycean red algae, *Griffithsia* undergoes an isomorphic alternation of diploid generations. Meiosis occurs at the production of tetraspores by the tetrasporophyte (diploid) generation.

Griffithsia is morphologically simple, composed of uniseriate, sparingly branched filaments of cells. A characteristic feature of this genus is the size of the somatic cells which in some species may exceed 2 mm in both length and width (Fig. 1). All cells of the upright thallus are derived from a dividing apical cell, and like the apical cell, are highly multinucleate (Fig. 2). After being cut off from the apex, the cells of the filaments elongate markedly (Fig. 3). In both haploid and diploid plants, the surface area of cells increases linearly along the axis (Fig. 4). These changes in cell size, as well as the growth rate, are the same in haploid and diploid filaments, permitting comparison of homologous cells (i.e., same age and position from apical cell) between generations.

Because each somatic cell in *Griffithsia* is highly multinucleate, the total nuclear DNA per cell is a function of the ploidy of each individual nucleus and the total number of nuclei per cell. Therefore, to compare the total amount of nuclear DNA in haploid and diploid cells, both parameters must be measured in homologous haploid and diploid cells.

Nuclear Ploidy Comparisons in Haploid and Diploid Cells

The relative ploidies of nuclei in cells of both the haploid and diploid generations are reflected in the relative nuclear DNA levels and chromosome numbers. Microspectrofluorometry of nuclei in nonapical cells indicates that the majority of haploid and diploid nuclei are 2C and 4C, respectively (i.e., both reside at G_2) (Fig. 5). The 1C value can be determined from nuclear DNA fluorescence values of the developing tetraspores, which, as products of meiosis, have nuclei with DNA levels either at 1C (G_1) or 2C (G_2) (Goff and Coleman, 1984). This interpretation is consistent with measurements of telophase nuclei, prophase nuclei, and metaphase plates in dividing gametophytic nuclei, which measure 1C, 2C, and 2C, respectively (Fig. 5). In both the haploid and diploid vegetative cells, a small population of nuclei appears to undergo an additional cycle of DNA synthesis and as a result is polyploid (i.e., 4C nuclei in the gametophyte and 8C nuclei on the sporophyte). Spontaneous polyploidy occurs

Table I. Relative Nuclear DNA Levels and Chromosome Numbers

Cell type	Nuclear DNA fluorescence	C value	Chromosome numbers*
	rfu		
Vegetative Cell (1N) (male genophyte)	16.5 ± 1.9	2C	5–8 (*n* = 45)
	34.8 ± 3.1	4C	10–16 (*n* = 10)
	70.1 ± 5.0	8C	No counts
Vegetative Cell (1N) (female genophyte)	16.4 ± 0.9	2C	5–8 (*n* = 21)
	35.3 ± 2.1	4C	10–16 (*n* = 4)
Vegetative Cell (2N) (tetrasporophyte)	32.8 ± 2.7	4C	10–16 (*n* = 25)
	70.0 ± 4.6	8C	≥20 (*n* = 2)

* Chromosome counts were made from nuclei of dividing (late prophase) vegetative cells; *n* is the number of counts made. C values were determined by direct microspectrophotometry measurements.

relatively rarely among the nuclei of a haploid or diploid cell; the percentage of polyploid nuclei rarely exceeds 0.5% of all nuclei in a cell. Chromosome numbers (Fig. 6) also clearly reveal differences in diploid and haploid nuclei and indicate that in cases of polyploid nuclei, chromosomes increase in number proportionately to the increase in C level (Table I).

Comparison of Nuclear Number in Haploid and Diploid Cells

The number of nuclei in cells of *G. pacifica* ranges from ~100 in newly divided apical cells to several thousand in larger cells. Because of the large numbers of nuclei and the cell geometry, the total number of nuclei per cell could not be accurately or readily determined. Instead, a comparison of the relative numbers of nuclei in haploid and diploid cells was made by determining the number of nuclei per cytoplasmic area. Cytoplasmic area per nucleus was then calculated. In addition, the distance between adjacent nuclei was measured in both cell types (Table II). Comparisons between homologous cells of the two generations are possible because the nuclei in all cells are distributed uniformly in the thin peripheral layer of cytoplasm surrounding the central vacuole and no cyclosis occurs in these cells. Furthermore, quantitative interference microscopy (interferometry) revealed that the thickness of the peripheral cytoplasmic layer (Fig. 7 *a*) does not differ between haploid and diploid cells (Table III) (Fig. 7, *b* and *c*).

*Table II. Internuclear Distance**

		n	$\bar{x}$	$S_{\bar{x}}$	Ratio: 2× ploidy / × ploidy
Haploid	N (2C)	689	18.11	2.88	–
Diploid	2N (4C)	1,193	24.87	3.65	1.37
Tetraploid	4N (8C)	451	34.57	4.11	1.39
Octoploid	8N (16C)	63	48.39	4.84	1.40

* µm, in cell No.'s 3–8.

Differences in total nuclear numbers, nuclear density, internuclear distance, and nuclear size (and fluorescence intensity) in haploid and diploid cells are clearly seen when comparison is made of homologous cells of the two generations (Fig. 8, *a* and *b*). Differences in nuclear size and spacing associated with the occasional tetraploid or octaploid nucleus are even more obvious (Figs. 9 and 10). The density of nuclei in haploid cells is nearly twice as great as that in diploid cells and accordingly, each diploid nucleus is associated with nearly twice the area of cytoplasm associated with each haploid nucleus (Fig. 11).

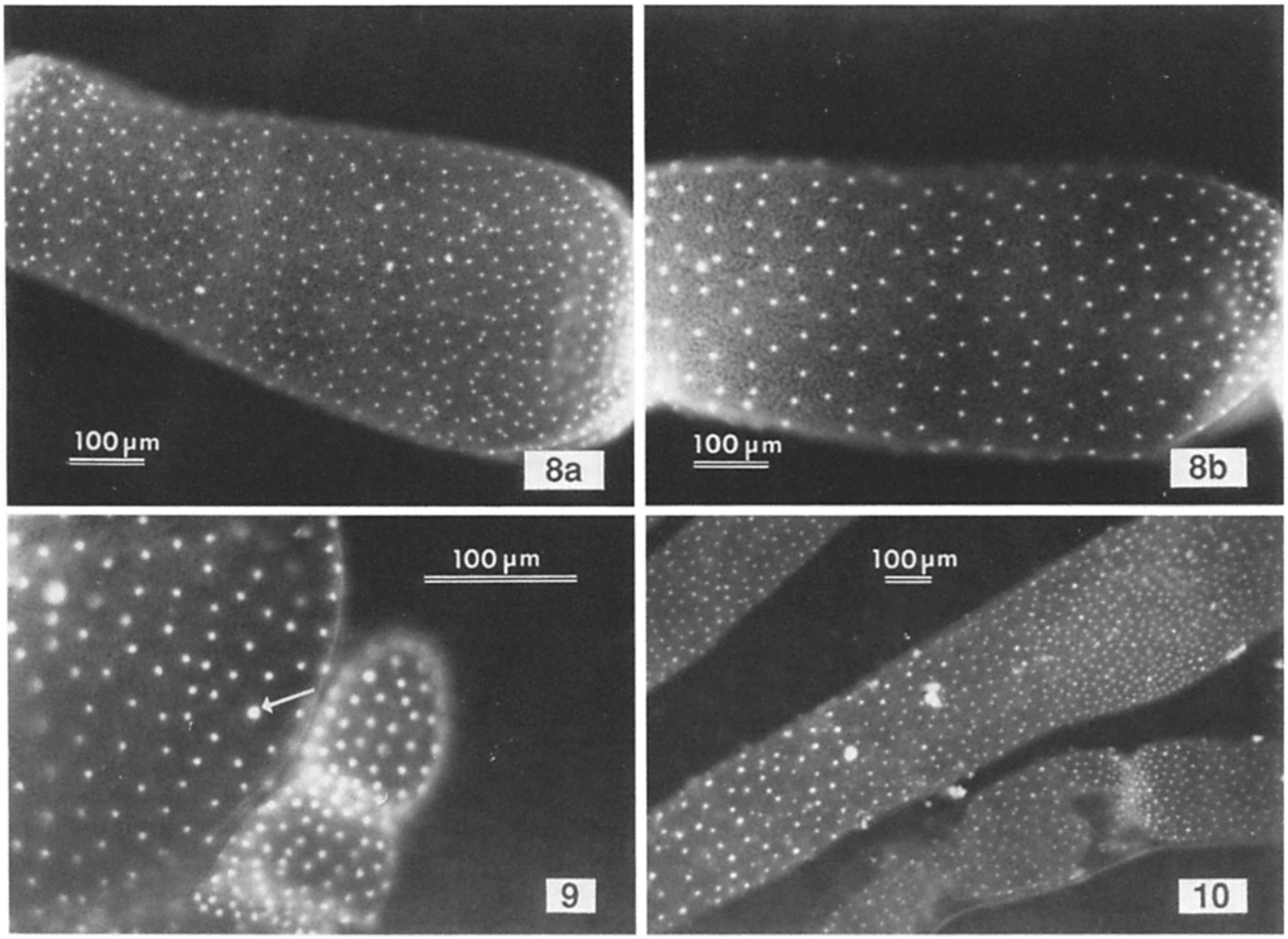
Table III. Griffithsia pacifica: Relative Cytoplasmic Thickness and Nuclear Density As Determined by Interferometry and Microspectrofluorometry

OPD of wall			OPD wall + cytoplasm			OPD cytoplasm	OPD cytoplasm	cyt area/ nucleus
	au		*au*	*au*	*au*	*au*	*au*	*(µm²)*
n	$\bar{x}$	$S_{\bar{x}}$	n	$\bar{x}$	$S_{\bar{x}}$			
Gametophyte (1N)								
10	11.64	2.81	10	32.61	1.58	20.97	107.0	377.4
27	11.94	2.45	18	31.74	1.36	19.80	101.0	380.1
18	11.13	2.06	20	31.62	3.11	20.49	104.5	318.2
30	11.70	1.80	30	32.03	2.10	20.33	103.7	389.3
25	10.90	1.92	25	31.34	1.80	20.44	104.2	356.4
							x = 104.08 ± 2.1	x = 364.3 ± 28.4
Tetrasporophyte (2N)								
25	11.01	1.12	25	32.64	1.84	21.63	110.3	620.7
25	10.95	1.64	29	20.91	2.14	19.96	101.8	681.2
25	11.15	1.09	25	31.55	1.91	20.40	104.8	600.0
20	12.09	2.06	20	31.96	2.11	19.87	101.3	586.7
12	13.0	1.92	21	32.62	2.12	19.62	100.1	606.4
							x = 103.66 ± 4.10	x = 619.0 ± 36.86

All measurements made from fifth cell in filament.

OPD measurements of cytoplasm represent the difference between the OPD (au) of wall plus cytoplasm and the OPD of the wall only.

OPD of the cytoplasm in nm may be calculated since the number of arbitrary units (au) per wavelength of light used (green = 549 nm) is known (107.6 au/549 nm).



Figures 8–10. (*Fig. 8*) *G. pacifica* (1N, male) showing nuclear size and distribution in the fourth cell in the filament (*a*). (*b*) *G. pacifica* (2N). This cell is homologous (same position and age) as that in Fig. 10. Notice the larger nuclei and increased internuclear distance. (*Fig. 9*) *G. pacifica* 2N cells. The arrow indicates a polyploid (8C) nucleus. Most of the other nuclei are 4C. Notice the greater area of surrounding cytoplasm associated with the 8C nucleus. (*Fig. 10*) *G. pacifica* 2N. The nuclei of this unusual cell have undergone endopolyploidy (8–32C) at one end of the cell, and have increased in number at the other end of the cell. Proportionately larger cytoplasmic area (volume) is associated with the higher ploidy nuclei; the larger, irregularly shaped fluorescent objects are aggregates of bacteria on the cell surface; DAPI-stained, epifluorescence microscopy.

Nuclei in *Griffithsia* cells are arranged in a nearly perfect hexagonal array (Figs. 8, *a* and *b*, and 9) which must be maintained as the cells grow. Nuclear division in *Griffithsia* proceeds as a wave, passing from the tip of the apical cell back through the rapidly elongating cells (Waaland and Waaland, 1975). During these processes, the precise relative positioning of nuclei is maintained and is effected by the orientation of the metaphase plates and the distance the telophase pair of nuclei move from one another. Colchicine-treated cells, with nuclei arrested in metaphase, clearly show the varying orientation of metaphase plates with respect to one another and the long axis of the cell (Fig. 12). When colchicine is removed from these cells, the telophase pairs move apart to a distance characteristic of the nuclear ploidy level.

Nuclear–Plastid Interactions in Haploid and Diploid Cells

The highly organized arrangement of the nuclei in the *Griffithsia* cell gives the appearance of there being a domain of cytoplasm of particular size that is associated with each nucleus; and the extent of this domain appears to be a function of the ploidy level of the nucleus.

The most conspicuous organelles occupying the "cytoplasmic domain" of each nucleus are the plastids. In nearly all cells of both haploid and diploid plants (the exception being cell 2 or 3 where plastids are frequently enlarged, highly lobed, and active in budding and binary fission [Fig. 13 *c*], the plastids are very similar in size (Fig. 13, *a* and *b*). These are distributed in a single layer in the thin peripheral cytoplasm and occupy most of the cytoplasm between adjacent nuclei (Fig. 14). The multiple nucleoids of DNA within plastids can easily be seen when stained with DAPI; for photography, their clarity is greater in filaments depleted of phycobilins by low nitrogen media (Fig. 15). In nearly mature cells, compared in this study, the average number of plastids in the cytoplasmic domain surrounding a haploid nucleus is half that for a diploid one (Fig. 16), and there are half as many around a diploid nucleus as a tetraploid nucleus (Table IV).

There is no significant difference in the total amount of plastid DNA per plastid (measured as the relative fluorescence of all DAPI-stained nucleoids of a single plastid) in the haploid and diploid cells (Fig. 17), nor do the plastids differ in average size. Therefore in *Griffithsia*, there is a constant ratio between the amount of chloroplast DNA and nuclear DNA in each cytoplasmic domain; diploid (4C) nuclei are associated with approximately twice as much ptDNA as are haploid (2C) nuclei (Table IV).

Discussion and Conclusions

Each giant cell of *Griffithsia*, including the apical cell, is in effect multicellular in the sense that each is composed of hundreds or thousands of cytoplasmic domains. Each cytoplasmic domain may contain numerous chloroplasts, mitochondria, and other organelles, and may be "controlled" by a single haploid or diploid nucleus. The cytoplasmic domains lack any surrounding plasmalemma and wall which would otherwise delineate them as cells. As suggested further by the obviously greater area of the domain associated with a polyploid nucleus (e.g., Fig. 9), the size of a domain is directly correlated with the DNA content of its nucleus. The "packing" of the domains within the cell is not random, but rather hexagonal. It is this observation, perhaps, more than any other which supports the possibility that each nucleus effectively has a field of cytoplasm and organelles surrounding it, a domain within which the mutual requirements of nucleus and organelles can be satisfied.

Most prior studies of other organisms which sought to detect whether nuclear DNA doubling increased cell volume or any other parameter proportionately, dealt with geometrically similar cells which were spheres (Epstein, 1967). In *Griffithsia*, a nuclear "domain" encompasses a short (4–5 μm) thick cylinder (which approaches a hexagon in cross section) of cytoplasm. The thickness is the distance from the vacuole to the plasmalemma and the base of the cylinder has a radius equal to half the internuclear distance. The volume of each domain appears to be governed by the ploidy of its nucleus and may directly reflect the maximum distances over which gene products (nuclear, plastid, and mitochondrial) might be transported (diffused?) effectively in the absence of cyclosis.

Since the thickness of the cytoplasm (height of the cylinder) is the same for haploid and diploid domains (as determined by interferometry), a domain which is double in volume is changed only in the dimensions of the base of the cylinder. Volume differences of twofold would generate differences in comparable linear measurements such as internuclear distances of $2^{1/2} = 1.4142$. Almost exactly this factor characterizes the difference in internuclear distances measured between haploid and diploid cells (Table II).

The number of plastids occupying the domain of a diploid nucleus is approximately twice that of a haploid nucleus (Table IV). Since plastid size and DNA content per plastid are equivalent in haploid and diploid cells, this result signifies a doubling of the amount of ptDNA per diploid nucleus over that associated with the domain of a haploid nucleus. This in turn suggests that the ratio of nuclear DNA to organelle DNA is a component directly affected by ploidy doubling, and one which may have functional significance. However, little is yet known of the control of the interactions between nuclear and organelle genomes, and there are almost no other data on the correlation of ptDNA quantity (much less mitochondrial DNA quantity) with nuclear DNA level. The exception is the biochemical determination of ptDNA per cell in haploids compared to vegetative diploids of *Chlamydomonas reinhardtii*; the clones differed by twofold (Whiteway and Lee, 1977).

The finding of proportionate increase in nuclear domain size and in plastid number per nucleus associated with doubling the nuclear DNA can be compared to other such studies. Fantes et al. (1975) and others have discussed the relationship of cell size to the initiation of mitosis in multiplying eukaryote cells. With respect to differentiating cells, nuclear spacing in haploid and diploid fungi is proportional to ploidy level (Clutterbuck, 1969). Epstein and Gatens (1967) found liver cells with doubled DNA levels to increase their nuclear volume by twofold, and Epstein (1967) found an approximate doubling of cytoplasmic volume, although he also cites studies where animal cell volume does not double with ploidy level. Plant cell volume increases with ploidy level, but seems rarely to double precisely (Butterfass, 1973). Reviewing an extensive literature, Butterfass (1973) concluded that increase in plastid number, rather than cell area or cell volume, was more directly proportional to increase in nuclear DNA content; in different cell types and different species, plastid numbers increased from 20 to 120% with doubling of ploidy, with the mean for all species being a 70% (or 1.7-fold) increase in plastid number. Quantitative data for the plastid sizes and particularly for ptDNA content in these studies is lacking.

The remarkable similarity in size and other characteristics of vegetative haploid and diploid plants of isomorphic species can be attributed to the fact that there are similar amounts of total DNA in homologous cells. Current studies (Goff and Coleman, manuscript in preparation) of other iso-

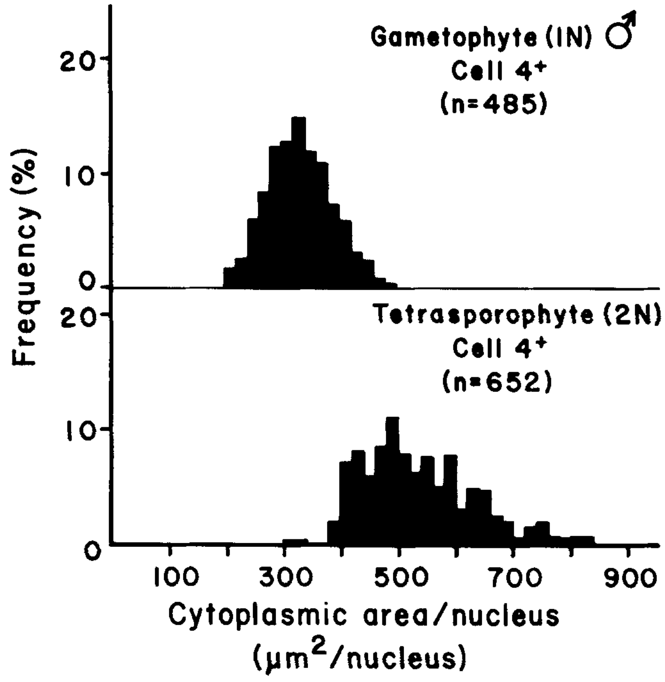
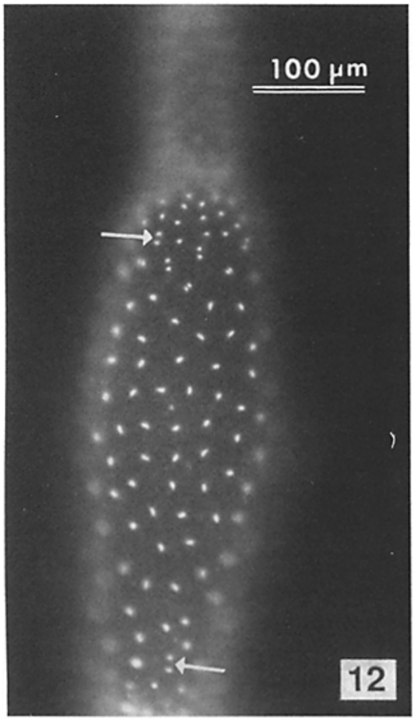
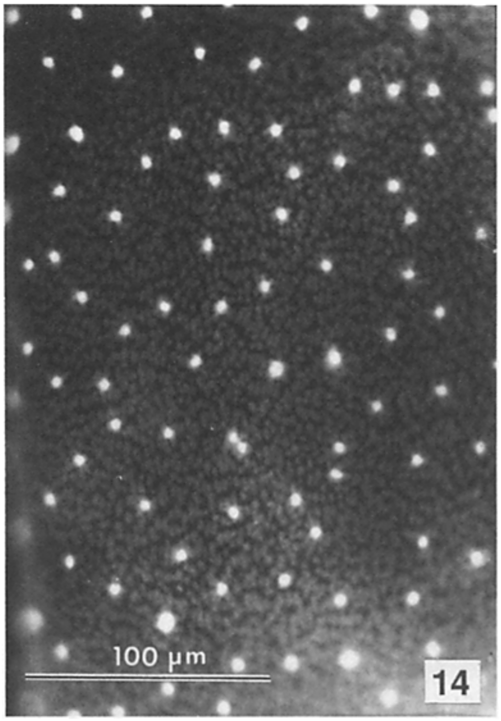
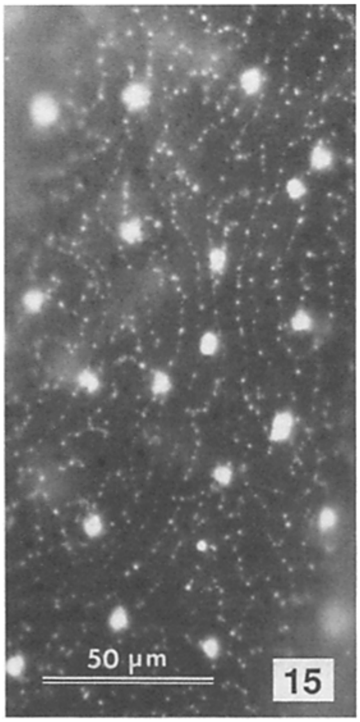
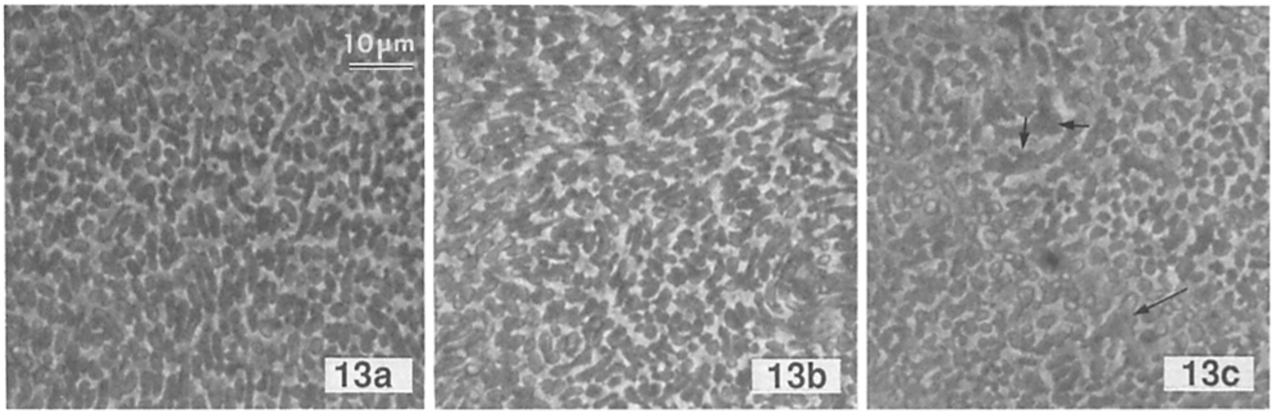


Figure 11. Cytoplasmic area (μm²) associated with nuclei from 1N and 2N *G. pacifica*. All measurements were made from homologous cells (cell 4) from plants of the same age. The mean value from 1N cells is 329.14 ± 60.6 ($n = 483$) and that from 2N cells is 533.9 ± 97.4 ($n = 648$).









Figures 12–15. (*Fig. 12*) *G. pacifica* (2N), third cell. After exposure to 2.5 mM colchicine in seawater for 24 h, most nuclei are arrested at metaphase. Those at the ends of the cells (*arrows*) have been blocked at telophase. At this level of colchicine, cells washed free of the drug resumed normal mitoses within 8–12 h. DAPI-stained, epifluorescence microscopy. (*Fig. 13*) *G. pacifica* chloroplast development. Compare the similar sizes of plastids from *G. pacifica* (2N) (fifth cell in filament) (*a*) to those from a homologous 1N cell (*b*). Numerous dividing and budding plastids are evident in the third cell (2N) of a filament (*c*). (*Fig. 14*) Numerous plastids occur between the regularly positioned nuclei of this 2N *G. pacifica* cell (cell 5). DAPI-stained nuclei; the phycobiliproteins of the plastids autofluorescence pink. (*Fig. 15*) Numerous ptDNA nucleoids are evident within the plastids that are distributed between the 2N nuclei. This *G. pacifica* cell was from a nitrogen-starved individual. Lowering the nitrogen causes a reduction of phycobilins, and thus permits more optimal observation of ct-DNA nucleoids.

morphic red algae, including those in which cells are uninucleate, also demonstrate the strict correlation of nuclear DNA levels and cell size. As in the case of *Griffithsia*, the nuclear genome of uninucleate forms exists in multiple copies; instead of being distributed in numerous nuclei, these copies are contained in a single nucleus which reaches increasing levels of polyploidy as the cell enlarges. The nuclei of homologous haploid and diploid cells have the same number of genome copies, which is directly proportional to cell size.

This phenomenon is interesting not only as an explanation of similarity in morphology in organisms which undergo isomorphic life histories and as an additional example of the general observation of cell size dependency on nuclear DNA content, but also for the evolutionary implications arising from the existence of constitutive nuclear polygenomy in an organism. One of the presumed advantages of the diploid over the haploid state is the possibility present in the diploid for heterozygosity which, at the very least, might protect a cell from a lethal mutation at a crucial allele. The polygenomic character of isomorphic red algae incorporates such protection in most cells of both the haploid and diploid stages. Whether nuclear polygenomy characterizes other organisms such as the numerous brown and green algae which have isomorphic life histories is currently under investigation in our laboratories.

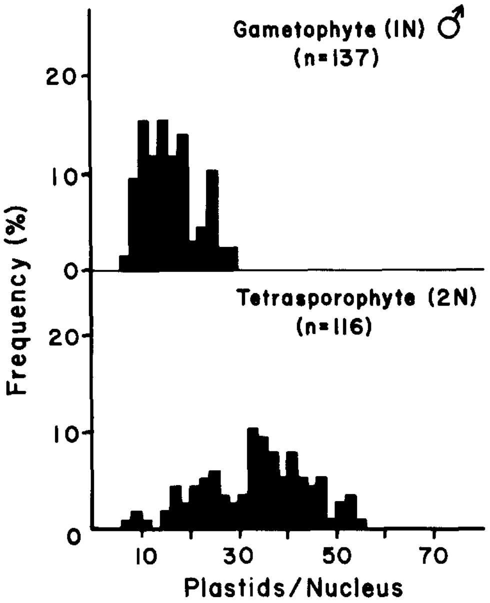


Figure 16. Number of plastids per nucleus in 1N and 2N *G. pacifica* cells (cells 4–6). 1N: $\bar{x}$ = 16.41 ± 5.48; 2N: $\bar{x}$ = 33.82 ± 10.90.

In summary, homologous haploid and diploid cells of *Griffithsia* (i.e., same size and developmental stage) have the same amount of nuclear DNA. Somatic cells of *Griffithsia* are multinucleate and each cell may contain hundreds or thousands of haploid, or diploid, nuclei. The nuclei are positioned in the cell periphery in a near-perfect hexagonal array. The area (and volume) of cytoplasm associated with each nucleus is a function of nuclear ploidy. The amount of ptDNA associated with each nucleus is a function of nuclear ploidy. *Griffithsia* cells are functionally "multicellular." Each cell is composed of numerous cytoplasmic compartments, each under the control of a single nucleus. The domain of cytoplasm associated with each nucleus represents the "zone of influence" of the nucleus. The size of the zone is determined by the ploidy of the nucleus and may represent the distance over which nuclear gene products diffuse.

Table IV. Major Quantitative Features of Griffithsia pacifica*

	Haploid	Diploid	Ratio dip/hap	Predicted Value
C values	2C	4C	2.0	2.0
Nuclear DNA (rfu)	16.5	32.8	1.9	2.0
Chromosome No.	5–8 (2C)	10–16 (4C)	2.0	2.0
Internuclear distance (μm)	18.11	24.9	1.37	$2^{1/2}=1.41$
Cytoplasmic area/nucleus (μm^2)	329.1	533.9	1.6	$2^{2/2}=2$
Relative cytoplasmic thickness (OPD in nm)	104.1	103.7	–	–
Plastids per nucleus	16.41	33.82	2.06	$2^{2/2}=2$
ptDNA per plastid (rfu/plastid)	34.4	37.6	1.1	1.0
ptDNA per nucleus (Σ plastid rfu/nuc)	564.5	1270.3	2.25	2.0

* From figures 5, 11, 16, 17, and Tables I–III.

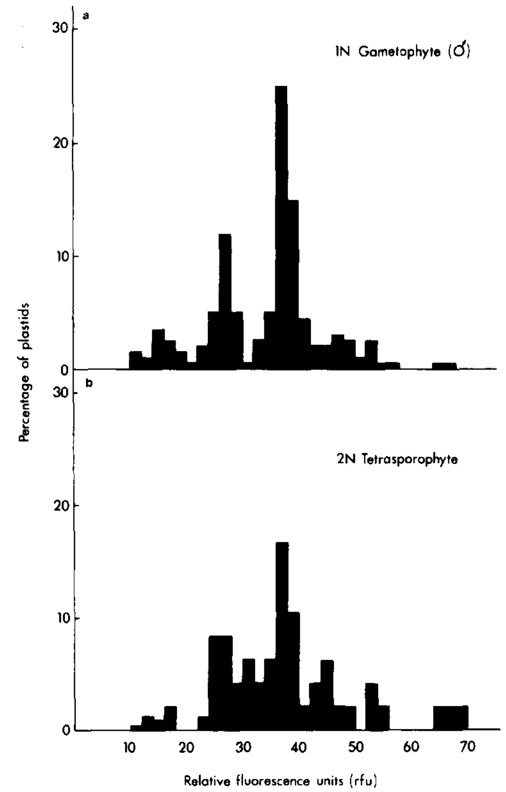


Figure 17. ptDNA fluorescence (rfu) per plastid in haploid (*a*) and diploid (*b*) *G. pacifica* cells (cells 4–6). The mean value for haploid cells is $\bar{x}$ = 34.40 (n = 205) whereas the mean for diploid cells is 37.56 (n = 241).

Appendix

The aus Jena Peraval Interphako X interfereroscope generates interference patterns by optically shearing ("Shearing Method") the microscopic image into a primary focused image and a secondary image. These are positioned side by side or just overlapping in the field of view (see discussions by Davies, 1958; Green, 1960; Gertel and Green, 1977; Berlyn and Miksche, 1976; Spencer, 1982).

One then optically measures the difference in optical path (OP) or the impeded light which passes through the object (primary image of the shear), and that which passes only through the medium (background). Each OP is a product of the linear distance through the object (t = thickness) and its refractive index (n): OP = nt. The optical path difference (OPD) is the difference between the two OP's (through the object and background): OPD = $(n_1\ t_1 - n_2t_2)$, or OPD = $(n_s - n_u)t$, where t = thickness, n_s = refractive index of sample, and n_u = refractive index of medium. The aus Jena Interphako microscope measures the OPD by measuring the shift in the interference band that passes through the object relative to the unimpeded background band. The shift (measured in arbitrary units [au]) represents a fraction of the wavelength of light used to generate the interference pattern. Thus, arbitrary units can be converted to fractions of wavelength by measuring the units between two interference bands. Using monochromatic green light (540 nm) and 160× magnification, there are 107.6 au/1 band or 107.6 au/549 nm (n = 20, S_x = 2.19 x = 107.6). Therefore, 1 au of shift equals 5.1 nm and OPD = 5.1 nm (au).

Since the OPD is a function of both the thickness and refractive index, OPD may be used to compare thickness between different objects only if their refractive indices (and that of the mounting medium) are the same. Since the cytoplasm of homologous haploid and diploid *Griffithsia* cells contain similar numbers of similarly sized materials (primarily plastids), each presumably has the same refractive index. Therefore, $(n_s - n_m)$ = constant = c; OPD = $(n_s - n_m)t$; OPD = (c)t; and the OPD is directly proportional to the thickness of the object.

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Note Added in Proof. Although previous attempts to disrupt the regular hexagonal patterning of nuclei in *G. pacifica* with cytoskeletal poisons proved to be unsuccessful, we recently determined that the microtubule-destabilizing drug griseofulvin totally disrupts nuclear positioning but has no effect on plastid arrangement. The effect of 1, 5, and 10 μM griseofulvin was partially reversible and at these concentrations the drug was not toxic. We acknowledge A. W. Sylvester of the University of Washington who suggested that we test this drug.

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