HELGOLÄNDER MEERESUNTERSUCHUNGEN
Helgoländer Meeresunters. 45, 273–285 (1991)

Karyology and nuclear DNA quantification of four species of *Chaetomorpha* (Cladophorales, Chlorophyta) from the western Atlantic

Todd K. Hinson & Donald F. Kapraun

Department of Biological Sciences, University of North Carolina; Wilmington, North Carolina 28403, USA

ABSTRACT: Chromosome numbers are given for four species of *Chaetomorpha* from the warm temperate and tropical western Atlantic. The basic chromosome number is six, with three median and three submedian chromosomes. *Chaetomorpha* species represent a polyploid series, with numbers of 12, 18 and 24 found in the present study. Microspectrophotometry data for each species were quantified by reference to standards with known DNA contents. Results indicate similar 2X =1C = 12 genome sizes for *C. aerea* (0.20 pg) and *C. brachygona* (0.26 pg), and for *C. antennina* (0.53 pg) and *C. melagonium* (0.58 pg). These findings are compared with karyological features of *Cladophora* species to characterize the karyology of the cladophoralean genome.

INTRODUCTION

Members of the genus *Chaetomorpha* (Chlorophyta, Cladophorales), with their relatively large chromosomes and synchronous nuclear divisions, have been the subject of numerous cytogenetic studies (Sarma, 1983). Chromosome numbers reported for various taxa range from N = 12 to N = 36, with 2X = 12 to 4X = 24 being most common (Sinha, 1967; Sarma, 1983). Species apparently represent a polyploid series in which the basic chromosome complement is X = 6.

In the closely related genus *Cladophora* (Olsen-Stojkovich et al., 1986; Kapraun & Breden, 1988), species show an unusual uniformity of chromosome numbers, with differences in chromosome size resulting in the karyotype complement of one species appearing to be a reduced or enlarged version of that of another (Kapraun & Gargiulo, 1987a, 1987b). These six basic chromosomes include three with median centromeres and three with sub-median ones (Wik-Sjöstedt, 1970). Analysis of seven *Cladophora* species indicated correlations between karyotype pattern/chromosome (genome) size and plant morphology, habitat specificity and phytogeography (Kapraun & Gargiulo, 1987a, 1987b). Furthermore, estimates of the basic genome (1X) for these *Cladophora* species indicated large-scale, discontinuous variation in their nuclear DNA content.

It is not known to what extent these generalizations apply to species of *Chaetomorpha*, as published karyological reports are limited to chromosome numbers. Consequently, the present cytogenetic investigation was initiated on four *Chaetomorpha* species from the warm temperate and tropical western Atlantic to provide details on their

chromosome sizes and karyotypes. In addition, cytophotometry was used to estimate the haploid (2X = 1C = 12) genome size of these species to determine patterns of interspecific DNA variation.

MATERIALS AND METHODS

Source of specimens

Two *Chaetomorpha* species were collected from North Carolina sites: *C. aerea* (Dillw.) Kütz. from Kure Beach, and *C. melagonium* (Weber et Mohr) Kütz. from Ft. Macon. *Chaetomorpha brachygona* Harvey specimens were obtained from Ft. Pierce, Florida, and *C. antennina* (Bory) Kütz. was collected from South Point, Barbados.

Fixation and karyotype analysis

Specimens were fixed in the laboratory at 24:00 in 3:1 absolute ethanol-glacial acetic acid, left overnight, and transferred to 70 % ethanol for storage (Kapraun & Martin, 1987). Aceto-orcein staining procedures used for the karyological study have been previously described (Kapraun & Gargiulo, 1987a). Documentation by photomicrographs was provided by an Olympus BH2-RFK fluorescence microscope. Karyotypes were prepared by viewing 35 mm Kodak Plus-X negatives with a 48X microfiche reader and tracing the projected images (Kapraun & Freshwater, 1987).

Determination of nuclear DNA-content

Fixed material for measurement of nuclear DNA was prepared as follows. *Chaetomorpha* filaments were transferred from alcohol and soaked in distilled water for 1–3 h to soften material. Filaments were then macerated using ground glass slides to disrupt cells and liberate nuclei from the cytoplasm. Ground material was rinsed onto coverslips coated with Subbing solution (0.1 g gelatin, 0.01 g chrome alum in 100 ml water), and allowed to dry at 40° C to evaporate all residual alcohol. Coverslips were soaked in phosphate buffer solution (PBS) for 1 h, stained with hydroethidine for 1–2 mins, and allowed to destain in PBS overnight (Kapraun & Bailey, 1989).

Data were standardized to the fluorescence values (I_f) of the hydroethidine-stained angiosperm *Antirrhinum majus* L. with a 2C nuclear DNA content of 3.2 pg (Bennett & Smith, 1976) and *Cladophora albida* (Huds.) Kütz. (Cladophorales, Chlorophyta) with 2C = 0.8 pg DNA (Bot et al. 1989a; Kapraun & Dutcher, 1991). Angiosperm seeds were germinated in Petri dishes lined with filter paper moistened with distilled water. Root tips 1 cm long with abundant root hairs were collected, fixed and stained. Chromosome counts from root tip meristems were made to confirm the ploidy level of the cultivar (Bennett & Smith, 1976). Zoospores of *C. albida* released in culture readily settled on coverslips, facilitating fixation and staining.

Observations and photomicrographic documentation were made with brightfield and epi (incident) UV illumination using the above microscope and exciter filter BP-545, dichroic mirror DM-580 and barrier filter O-590 which are specific for hydroethidine emissions (Kapraun et al., 1988). A microphotometer (Kinetek Photometry Systems) equipped with a rotating nosepiece housing an array of perforated diaphragms permitted

selection of pinhole apertures corresponding to the diameter of the nucleus being viewed. Consequently, error from cytoplasmic (extranuclear) fluorescence was greatly reduced (Kapraun & Shipley, 1990). Fluorescence data were analysed and presented in histograms (Goff & Coleman, 1984) to demonstrate the I_f peaks associated with 2C and 4C nuclei.

RESULTS

Identification of specimens

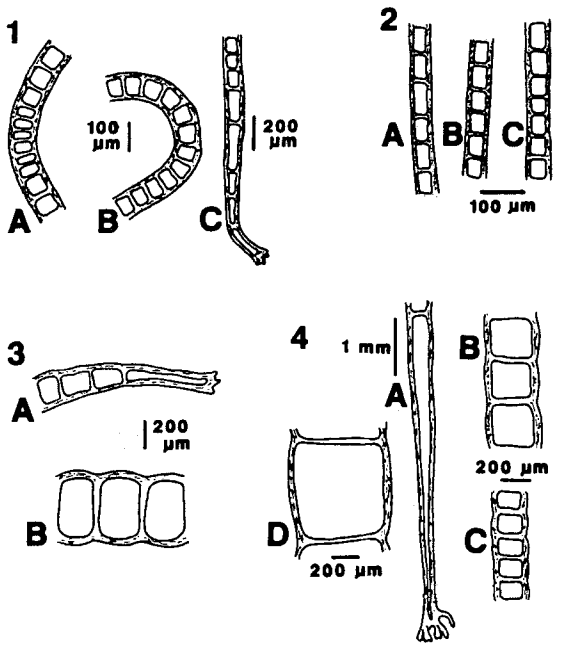
Comparative cytogenetic investigations are of limited value unless accurate determinations can be made for the included taxa. *Chaetomorpha* species pose formidable systematics problems due to the morphological plasticity of the genus and the small number of characters considered to have taxonomic significance. Determinations for this investigation follow Blair's (1983) monographic treatment for NE America and are based primarily on cell diameter and length/width ratios, growth habit, and basal cell size (Table 1). Two pairs of superficially similar *Chaetomorpha* species were included in this study.

Table 1. Cell dimensions in four species of *Chaetomorpha*

	Cell diameter (μm)		Filament cell L:W		Basal cell L:W	
	Reported*	Observed	Reported*	Observed	Reported*	Observed
C. brachygona	80–150	55	1.0–1.3	1.3	–	–
C. aerea	125–400	80–90	1.5–2.5	0.9–2.4	3–8	6–7
C. melagonium	350–750	440	1.0–2.0	0.7	8–14	9
C. antennina	450–550	420–540	2.0–4.0	1.5–2.5	8–12	7

* Taylor (1960), Blair (1983)

C. brachygona Harvey and *C. aerea* (Dillw.) Kütz. are both relatively small, delicate plants (Figs 1 and 2). *C. brachygona* filaments are typically 80–150 μm diam, and grow in unattached, entangled masses with few basal cells. *C. aerea* filaments are thicker (Table 1), and grow in erect tufts attached to the substratum by small basal cells (Blair, 1983). *C. melagonium* (Weber et Mohr) Kütz. and *C. antennina* (Bory) Kütz. (= *C. media* [C. ag.] Kütz, Wynne 1986) are both large, coarse plants (Figs 3 and 4), with filament diameters of 350–750 μm and 450–550 μm, respectively (Table 1). *C. melagonium* has plate-like cells (L:W = 1–2) while *C. antennina* has subquadrate cells (Figs 3 and 4). In culture, *C. melagonium* produced multicellular, uniseriate filaments (Fig. 5) within 10–14 days of zoospore attachment, while *C. antennina* zoospores germinated into multinucleate, clavate germlings (Fig. 6) which remained unicellular for up to 8 weeks.

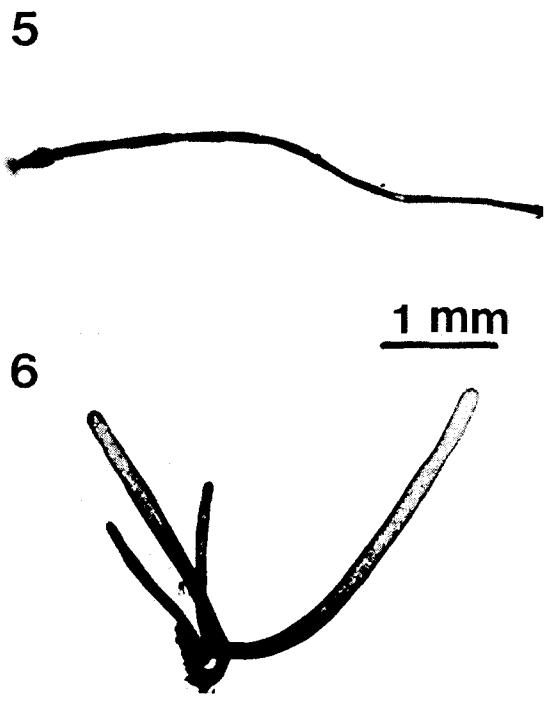


Figs 1–4. Morphology of *Chaetomorpha* specimens collected in nature
Fig. 1. *Chaetomorpha aerea*. A: erect filament with intercalary divisions; B: subquadrate cells in apical region of erect filament; C: basal region of filament and basal cell
Fig. 2. *C. brachygona*. A–C: filaments showing length:width (L:W) variation in vegetative cells
Fig. 3. *C. melagonium*. A: basal region of filament and basal cell; B: characteristic platelike vegetative cells
Fig. 4. *C. antennina*. A: basal cell; B & C: filaments showing L:W variation of vegetative cells; D: characteristic subquadrate vegetative cells

Karyology

In *Chaetomorpha antennina* and *C. melagonium*, interphase nuclei had similar diameters of 4–6 µm. *Chaetomorpha brachygona* and *C. aerea* had substantially smaller interphase nuclei (3–5 µm diam). The presence of highly stained chromocenters associated with nuclei in both of the former species (Fig. 7), and their absence in the latter (Fig. 8), suggests a positive correlation between heterochromatin and genome size (Sarma, 1983; Wik-Sjöstedt & Nordquist, 1970; Kapraun & Gargiulo, 1987a).

Nuclear divisions in the *Chaetomorpha* species investigated were synchronous, and often large numbers of contiguous cells were found with nuclei in some stage of division (Fig. 9). Chromosome numbers for the four *Chaetomorpha* species in the present study are given in Table 2. Apparently, this is the first published report of karyological data for *C. brachygona*. Despite their similar filament diameters, *C. aerea* and *C. brachygona* can be readily distinguished by their characteristic karyotypes. *Chaetomorpha aerea* has relatively small chromosomes (0.5–1.0 µm), with little distinction in size between the largest and smallest (Fig. 10). *Chaetomorpha brachygona* chromosomes range from

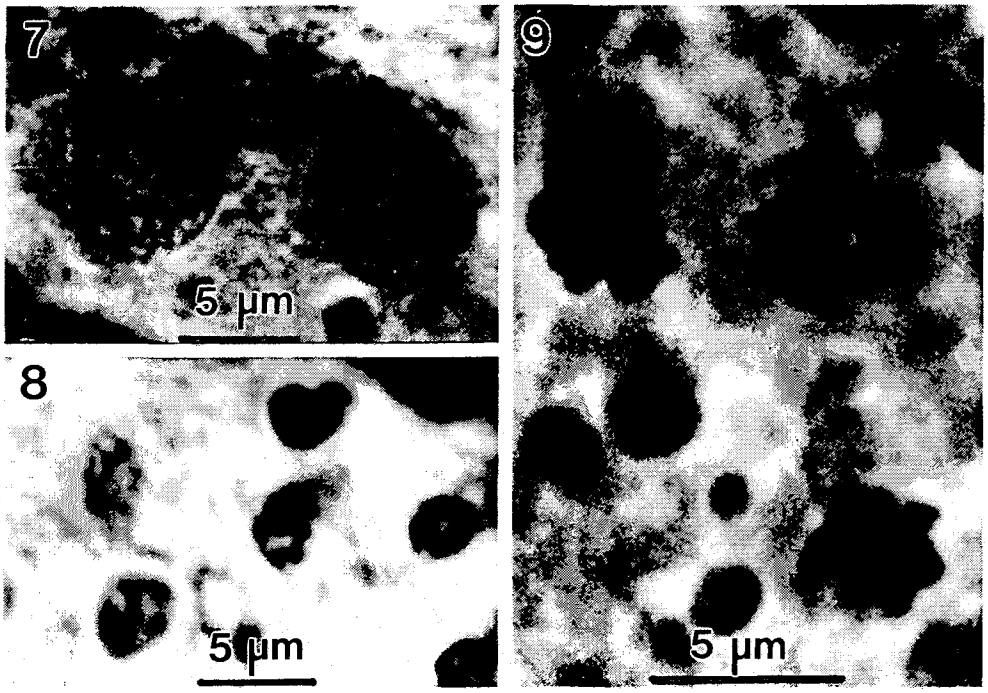


Figs 5 and 6. Germling development in culture
Fig. 5. *Chaetomorpha melagonium* 10-day old germlings
Fig. 6. *C. antennina* 8-week old germlings

Table 2. List of chromosome numbers reported in *Chaetomorpha*

Species	Chromosome number 2X	3X	4X	
Chaetomorpha aerea (Dillw.) Kütz.	12		24	Patel (1971)
C. aerea (Dillw.) Kütz.		18		Sinha (1958)
C. aerea (Dillw.) Kütz.	10		20	Hartmann (1929)
C. aerea (Dillw.) Kütz.	12			Kornmann (1968)
C. aerea (Dillw.) Kütz.	12		24	Present study
C. antennina (Bory) Kütz.		18	24	Present study
C. antennina (Bory) Kütz.		17–18		Bodenbender & Schnetter (1990)
C. brachygona Harvey	12		24	Present study
C. melagonium (Web. & Mohr.) Kütz.	12		24	Patel (1971)
C. melagonium (Web. & Mohr.) Kütz.	12		24	Bodenbender & Schnetter (1990)
C. melagonium (Web. & Mohr.) Kütz.		18		Sinha (1958)
C. melagonium (Web. & Mohr.) Kütz.		18		Present study

0.6–1.5 μm long (Fig. 11). Although centromeric regions could not be distinguished with certainty, presence of straight and curved chromosomes suggests submedian and median centromeres, respectively (Kapraun & Gargiulo, 1987a).



Figs 7–13. *Chaetomorpha* nuclei after aceto-orcein staining
Fig. 7. *C. antennina* nuclei with extensive dense heterochromatic regions
Fig. 8. *C. aerea* with diffuse heterochromatic regions
Fig. 9. *C. aerea* with synchronous mitotic divisions in 4X = 24 nuclei

The species pair characterized by large-diameter filaments was found to have similar karyotypes. Both *C. antennina* and *C. melagonium* have chromosomes ranging from 0.8–1.8 µm long (Figs 12 and 13), with the longest showing clear evidence of submedian centromeric regions. Presence of equal numbers of straight and curved chromosomes suggests an equal distribution of submedian and median centromeres in both of these species.

DNA cytofluorometry

Microspectrophotometry with DNA-localizing fluorochromes has been used previously for quantification of nuclear DNA in coenocytic algae (Kapraun et al., 1988; Calderón-Saenz & Schnetter, 1989). In the present study, hydroethidine staining for periods as brief as 1 min followed by 12–24 h destaining at 4° C resulted in intense nuclear fluorescence.

I_f values for the angiosperm *Antirrhinum majus* and the marine alga *Cladophora albida* (Fig. 14) were plotted against their known DNA contents to derive a standard line (Kapraun & Shipley, 1990). In a typical series of observations, the ratio of their 2C I_f values (62:18 = 3.4) was found to closely approximate the ratio of their reported 2C DNA (pg) contents (3.2:0.8 = 4.0) (Fig. 15).

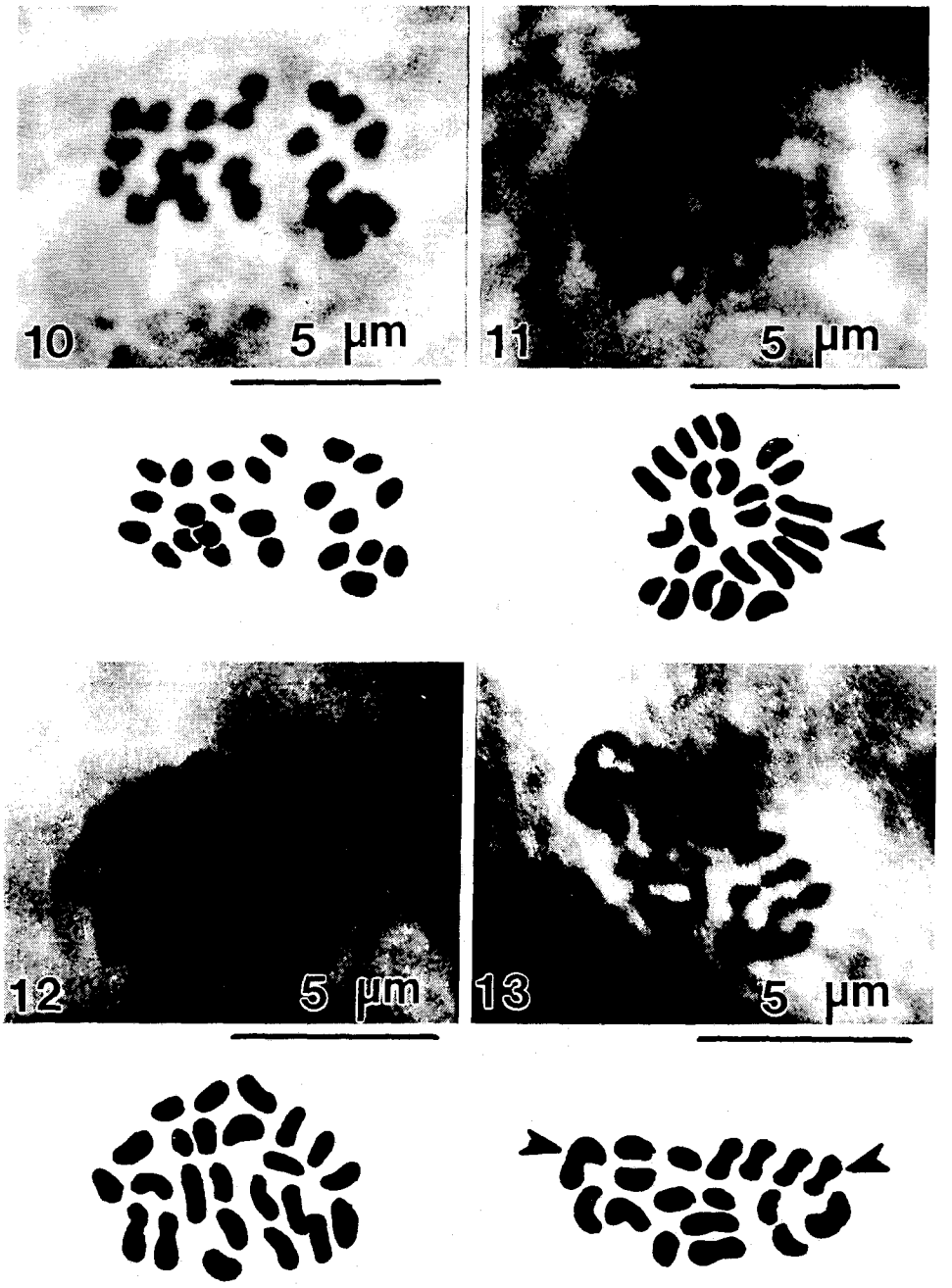


Fig. 10. *C. aerea* late prophase mitotic nucleus with 4X = 24 chromosomes
Fig. 11. *C. brachygona* late prophase mitotic nucleus with 4X = 24 chromosomes. Arrow indicates pairing of homologous chromosomes
Fig. 12. *C. antennina* late prophase mitotic nucleus with 4X = 24 chromosomes
Fig. 13. *C. melagonium* late prophase mitotic nucleus with 3X = 18 chromosomes. Arrows indicate submedian centromeric regions

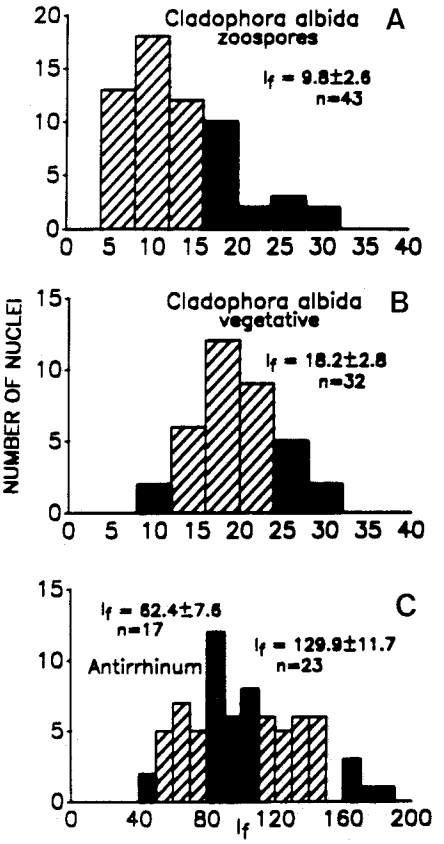


Fig. 14. Comparison of frequency distributions of relative DNA values for nuclei after hydroethidine staining in (A) *Cladophora albida* zoospores and (B) vegetative cells, and (C) *Antirrhinum majus*. n = number of nuclei, indicated by cross-hatching, used to calculate C-levels (Kapraun & Shipley, 1990); I_f = fluorescence intensity mean ± SD

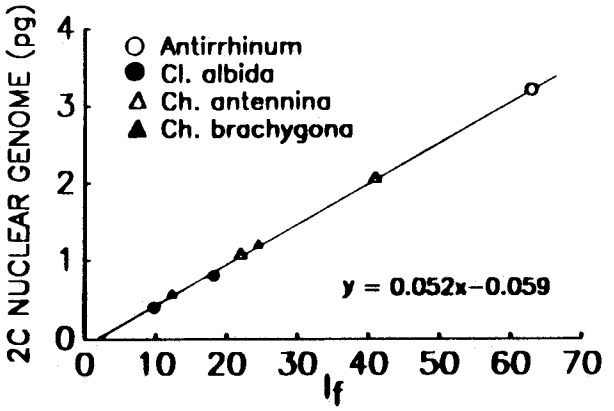


Fig. 15. I_f values for 1C and 2C nuclei in *Cladophora albida* and 2C nuclei in *Antirrhinum majus* plotted against their known DNA contents (Bot et al., 1989b; Bennett & Smith, 1976) to derive a standard line. DNA contents for *Chaetomorpha antennina* and *C. brachygona* 1C and 2C nuclei are extrapolated from their I_f values. Data standardized to mean I_f values of 2C nuclei in *A. majus*

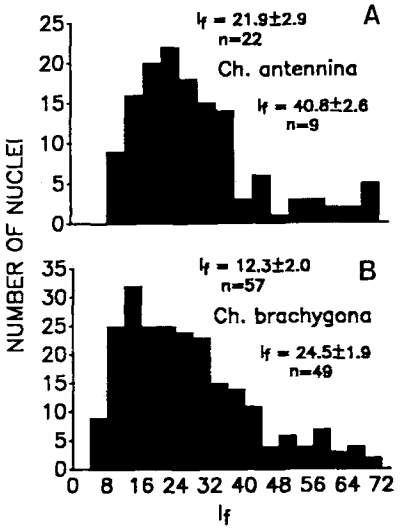


Fig. 16. Comparison of typical frequency distributions of relative DNA values for 2C and 4C nuclei (4X = 24) in *Chaetomorpha antennina* and *C. brachygona* after hydroethidine staining. See Fig. 14 legend symbols for explanation

Table 3. DNA contents determined for four species of *Chaetomorpha* and their estimated 2-C level (2X = 2N = 12) genomes (pg)

	DNA contents (pg) determined for isolates 3X		4X		Extrapolated DNA contents (pg) for 2C-level genomes 2X = 2N = 12
	2C	4C	2C	4C	
C. aerea			0.33		0.17
n = 24			0.36		0.18
			0.37		0.19
				0.93	0.23
				0.75	0.19
				0.83	0.21
					X = 0.20 ± 0.02
C. antennina			0.98		0.49
n = 24			1.08		0.54
				2.16	0.54
				2.07	0.52
					X = 0.53 ± 0.02
C. brachygona			0.44		0.22
n = 24			0.58		0.29
				0.86	0.22
				1.21	0.30
					X = 0.26 ± 0.04
C. melagonium	0.93				0.62
n = 18	0.79				0.53
		1.84			0.61
		1.62			0.54
					X = 0.58 ± 0.04

Comparison of I_f values for *Chaetomorpha* species (Fig. 16) permitted extrapolation of their DNA contents. As specimens examined included both 3X and 4X chromosome complements, nuclear DNA contents were standardized to the 2C level of haploid (2X = 2N = 12) genomes (Table 3). Results indicate similar values of 0.20 and 0.26 pg DNA for *C. aerea* and *C. brachygona*, respectively, and 0.53 and 0.58 pg for *C. antennina* and *C. melagonium*, respectively (Table 3). Thus, haploid nuclear genomes in the latter species contain approximately twice the nuclear DNA of the former species. Derived nuclear DNA contents for the *Chaetomorpha* species in the present study are within the range of values reported for related multinucleate green algae: *Cladophora sericea* (Huds.) Kütz. = 0.31 pg and *C. rupestris* (L.) Kütz. = 0.32 pg (Bot et al., 1989a), *C. albida* isolates = 0.8 and 0.7 pg (Bot et al., 1989b), and *Dictyosphaeria cavernosa* (Forssk.) Børg. = 1.79 pg (Olsen et al., 1986).

DISCUSSION

Results of the cytophotometric investigation of four *Chaetomorpha* species indicate a correlation between plant habit (cell dimensions) and genome size. Both *C. brachygona* and *C. aerea*, with small diameter filaments (< 100 μm) have haploid genomes (2X = 2N = 12) approximately half as large as those of *C. antennina* and *C. melagonium* with filament diameters > 400 μm. A similar correlation between genome size and plant habit (cell dimensions) has been reported in *Cladophora* (Kapraun & Gargiulo, 1987a, 1987b).

This relationship is of particular interest in multinucleate algae because of their potential to maintain an optimal ratio between genome content per cell and cell size by an increase in either the number of nuclei per cell or nuclear genome size (Goff & Coleman, 1984, 1986). Although no attempt was made at quantification in the present study, microscopic examination of whole cell preparations following aceto-orcein staining suggested similar numbers of nuclei per cell in the four species studied. Consequently, the well-documented correlation in vascular plants between nuclear volume (genome size) and cell parameters including cell size and duration of mitosis and meiosis (Price & Bachmann, 1975; Bennett, 1976; Bachmann et al., 1985) seems to apply equally to these multinucleate green algae.

It is tempting to speculate that in the Cladophorales, increased numbers of small nuclei would not provide a genome dose per cell equivalent to fewer large nuclei because of the qualitative difference between large and small genomes in related taxa. Specifically, large nuclear genomes are associated with proportionately greater amounts of repetitive DNA and/or heterochromatin (Rees & Jones, 1972; Narayan & Rees, 1976; Narayan, 1983; Ohri & Khoshoo, 1986). In the present study, conspicuous heterochromatin contents were indicated for the two species with the largest cell dimensions, *C. antennina* and *C. melagonium*. Similarly, reassociation kinetics have demonstrated a significant repetitive component (64–75 %) in two species of *Cladophora* with relatively large nuclear genomes (Bot et al., 1989a, 1989b).

In the present study, chromosome numbers of 12, 18 and 24 in four species of *Chaetomorpha* provide additional evidence of a polyploid series in this genus (Sarma, 1983). Although small chromosome size often prevented a precise determination of centromere positions, homologous pairing of straight and curved chromosomes suggests the presence of equal numbers of median and submedian centromeres (Figs 11 and 13). In addition, estimates of nuclear DNA indicate a significant difference in genome sizes

between species with small and large filament diameters. Thus, *Chaetomorpha*, and the closely related genus *Cladophora* (Wik-Sjöstedt, 1970; Kapraun & Gargiulo, 1987a, 1987b), appear to share several karyological features:

(1) a uniformity of chromosome numbers consisting of a polyploid series with a basic genome complement of X = 6,

(2) a constant karyotype with equal numbers of median and submedian centromeres,

(3) differences in chromosome size resulting in the karyotype complement of one species appearing to be a reduced or enlarged version of that of another, and

(4) large-scale, discontinuous interspecific variation in nuclear DNA content.

Data from comparative studies of cytology, life history, cytokinesis and cell wall composition have been cited as evidence that the Siphonocladales and Cladophorales should be merged into one order (Hoek, 1981, 1984; Hoek et al., 1988), with Cladophorales Haeckel having priority (Papenfuss & Chihara, 1975). In addition, immunological distance estimates suggest that the genus *Cladophora* is paraphyletic (Olsen-Stojkovich et al., 1986; Hoek et al., 1988) with different species emerging in distant portions of the proposed phylogenetic tree. Such a scheme would seem to require that the unique set of karyological features enumerated above evolved independently following several divergence events.

Karyological studies on the Cladophorales are in closer agreement with results of a cladistic analysis of rRNA sequence data for these multinucleate green algae (Zechman et al., 1990). Phylogenetic distance (most parsimonious branch length) estimates segregated cladophoralean algae into several closely related groups: (1) *Chaetomorpha* and *Cladophora* (Cladophorales); (2) *Anadyomene* and *Microdictyon*; (3) *Cladophoropsis*, and (4) *Dictyosphaeria*. It seems significant that these groups apparently have different basic chromosome complements (Kapraun & Breden, 1988).

Acknowledgements. The authors gratefully acknowledge financial support for this research from the National Science Foundation (BSR-RUI 88-17846). This paper is contribution No. 20 to the Center for Marine Science Research, UNC-Wilmington.

LITERATURE CITED

Bachmann, K., Chambers, K. L. & Price, H. J., 1985. Genome size and natural selection, observations and experiments in plants. In: The evolution of genome size. Ed. by T. Cavalier-Smith. Wiley, New York, 267–276.

Bennett, M. D., 1976. DNA, latitude, and crop plant distribution. – Environ. exp. Bot. *16*, 92–108.

Bennett, M. D. & Smith, J. B., 1976. Nuclear DNA amounts in angiosperms. – Phil. Trans. R. Soc. Lond. *274*, 227–274.

Blair, S. M., 1983. Taxonomic treatment of the *Chaetomorpha* and *Rhizoclonium* species (Cladophorales; Chlorophyta) in New England. – Rhodora *85*, 175–211.

Bodenbender, S. & Schnetter, R. 1990. Nuclear behavior during the life cycles of *Chaetomorpha, Ernodesmis* and *Struvea* (Ulvophyceae, Chlorophyta) under culture conditions. – Crypt. Bot. *1*, 340–354.

Bot, P. V. M., Holton, R. W., Stam, W. T. & Hoek, C. van den, 1989a. Molecular divergence between North Atlantic and Indo-West Pacific *Cladophora albida* (Cladophorales, Chlorophyta) isolates as indicated by DNA-DNA hybridization. – Mar. Biol. *102*, 307–313.

Bot, P. V. M., Stam, W. T., Boele-Bos, S. A., Hoek, C. van den & Selden, W. van, 1989b. Biogeographic and phylogenetic studies in three North Atlantic species of *Cladophora* (Cladophorales, Chlorophyta) using DNA-DNA hybridization. – Phycologia *28*, 159–168.

Calderón-Sáenz, E. & Schnetter, R., 1989. Life cycle and morphology of *Bryopsidella ostreobiformis* spec. nov. (Bryopsidaceae, Chlorophyta) from the Mediterranean, under culture conditions, with comments on the phylogeny of the *Bryopsis/Derbesia* complex. – Botanica Acta *102*, 249–260.

Goff, L. J. & Coleman, A. W., 1984. Elucidation of fertilization and development in a red alga by quantitative DNA microspectrofluorometry. – Devl Biol. *102*, 173–194.

Goff, L. J. & Coleman, A. W., 1986. A novel pattern of apical cell polyploidy, sequential polyploidy reduction and intercellular nuclear transfer in the red alga *Polysiphonia*. – Am. J. Bot. *73*, 1109–1130.

Hartman, M., 1929. Untersuchungen über die Sexualität und den Generationswechsel von *Chaetomorpha* und *Enteromorpha*. – Ber. ital. bot. Ges. *47*, 485–494.

Hoek, C. van den, 1981. Chlorophyta, morphology and classification. In: The biology of seaweeds. Ed. by C. S. Lobban & M. J. Wynne. Blackwell, Oxford, 86–132.

Hoek, C. van den, 1984. The systematics of the Cladophorales. In: The systematics of the green algae. Ed. by D. E. G. Irvine & D. M. John. Acad. Press, New York, 157–178.

Hoek, C. van den, Stam, W. T. & Olsen, J. L., 1988. The emergence of a new chlorophytan system, and Dr. Kornmann's contribution thereto. – Helgoländer Meeresunters. *42*, 339–383.

Kapraun, D. F. & Bailey, J. C., 1989. Karyology and nuclear DNA content of *Gelidium pusillum* (Gelidiales, Rhodophyta) from North Carolina, USA. – Jap. J. Phycol. *37*, 201–207.

Kapraun, D. F. & Breden, P. C., 1988. Karyological studies of *Cladophoropsis* (Siphonocladales, Chlorophyta) from Bermuda. – Botanica mar. *31*, 515–520.

Kapraun, D. F. & Dutcher, J. A., 1991. Cytophotometric estimation of inter- and intraspecific nuclear DNA content variation in *Gracilaria* and *Gracilariopsis* (Gracilariales, Rhodophyta). – Botanica mar. *34*, 139–144.

Kapraun, D. F. & Freshwater, D. W., 1987. Karyological studies of five species in the genus *Porphyra* (Bangiales, Rhodophyta) from the North Atlantic and Mediterranean. – Phycologia *26*, 82–87.

Kapraun, D. F. & Gargiulo, G. M., 1987a. Karyological studies of four species of *Cladophora* (Cladophorales, Chlorophyta) from coastal North Carolina. – Ital. J. Bot. *121*, 1–26.

Kapraun, D. F. & Gargiulo, G. M., 1987b. Karyological studies of three species of *Cladophora* (Cladophorales, Chlorophyta) from Bermuda. – Ital. J. Bot. *121*, 165–176.

Kapraun, D. F. & Martin, D. J., 1987. Karyological studies of three species of *Codium* (Codiales, Chlorophyta) from coastal North Carolina. – Phycologia *26*, 228–234.

Kapraun, D. F. & Shipley, M. J., 1990. Karyology and nuclear DNA quantification in *Bryopsis* (Codiales, Chlorophyta) from North Carolina, USA. – Phycologia *29*, 443–453.

Kapraun, D. F., Gargiulo, G.M. & Tripodi, G., 1988. Nuclear DNA and karyotype variation in species of *Codium* (Codiales, Chlorophyta) from the North Atlantic. – Phycologia *27*, 273–282.

Kornmann, P., 1968. Das Wachstum einer *Chaetomorpha*-Art von List/Sylt. – Helgoländer Meeresunters. *18*, 194–207.

Narayan, R. K. J., 1983. Chromosome changes in the evolution of *Lathyrus* species. In: Kew chromosome conference II. Ed. by P. E. Brandham & M. D. Bennett. Allen & Unwin, London, 243–250.

Narayan, R. K. J. & Rees, H., 1976. Nuclear variation in *Lathyrus*. – Chromosoma *54*, 141–154.

Ohri, D. & Khoshoo, T. N., 1986. Plant DNA, contents and systematics. In: DNA systematics. Ed. by S. K. Dutta. CRC Press, Boca Raton, Fla., 2–19.

Olsen-Stojkovich, J., West, J. A. & Lowenstein, J. M., 1986. Phylogenetics and biogeography in the Cladophorales complex (Chlorophyta). Some insights from immunological distance data. – Botanica mar. *29*, 239–249.

Papenfuss, G. F. & Chihara, M., 1975. The morphology and systematic position of the green algae *Ernodesmis* and *Apjohnia*. – Phycologia *14*, 309–316.

Patel, R., 1971. Cytotaxonomical studies on *Chaetomorpha* I. *Chaetomorpha linum* (Muell) Kütz. and *C. aerea* (Dillw.) Kütz. – Phykos *11*, 17–22.

Price, H. J. & Bachman, K., 1975. DNA content and evolution in the Microseridinae. – Am. J. Bot. *62*, 262–267.

Rees, H. & Jones, R. N., 1972. The origin of the wide species variation in nuclear DNA content. – Int. Rev. Cytol. *32*, 53–92.

Sarma, Y. S. R. K., 1983. Algal karyology and evolutionary trends. In: Chromosomes in evolution of eukaryotic groups. Ed. by A. K. Sharma & A. Sharma. CRC Press, Boca Raton, Fla., 177–224.

Sinha, J. P., 1958. Chromosome numbers and life cycles in members of Cladophorales. – Br. phycol. J. *1*, 24–27.

Sinha, J. P., 1967. Cytotaxonomical studies on *Cladophora glomerata*, four freshwater forms. – Cytologia *32*, 507–518.

Taylor, W. R., 1960. Marine algae of the eastern tropical and subtropical coasts of the Americas. University of Michigan Press, Ann Arbor, 870 pp.

Wik-Sjöstedt, A., 1970. Cytogenetic investigations in *Cladophora*. – Hereditas *66*, 233–262.

Wik-Sjöstedt, A. & Nordquist, T., 1970. Preliminary observations on the ultrastructure of some *Cladophora* and *Spongomorpha* species. – Botanica mar. *13*, 6–12.

Wynne, M. J., 1986. A checklist of benthic marine algae of the tropical and subtropical western Atlantic. – Can. J. Bot. *64*, 2239–2281.

Zechman, F. W., Theriot, E. C., Zimmer, E. A. & Chapman, R. L., 1990. Phylogeny of the Ulvophyceae (Chlorophyta): Cladistic analysis of nuclear-encoded rRNA sequence data. – J. Phycol. *26*, 700–710.