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Karyology and cytophotometric estimation of nuclear DNA variation in seven species of Ulvales (Chlorophyta)

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Chromosome complements of 1 N=8–12 are reported for seven species of Ulvales (Chlorophyta) from coastal North Carolina. Microspectrophotometry with the DNA-localizing fluorochrome hydroethidine is used to estimate nuclear genome sizes. Incorporation of fluorescence values for the angiosperm *Antirrhinum majus* L. and the greeen alga *Cladophora albida* (Hudson) Kuetzing with known 2 C DNA contents permitted quantification of I_f values for the Ulvales. In the seven species examined, 2 C DNA contents range from 0.6–1.0 pg. The absence of a positive correlation between estimated genome size and 1 N chromosome complement excludes the possibility that speciation has resulted exclusively from loss or gain of chromosomes and associated genome. Evidence in support of fusion/translocation processes is discussed.

Key Index Words: Cytogenetics—DNA quantification—microspectrophotometry—Ulvales.

Comparative studies of ultrastructural features in both vegetative and motile cells have shown the order Ulvales, as circumscribed by Bliding (1963, 1968), to be a heterogeneous assemblage (Hori 1973, Swanson and Floyd 1978, O'Kelly and Floyd 1983, Stuessy *et al.* 1983). Consequently, several genera have been transfered to the Ulotrichales *sensu* Kornmann (1964) or to the Ulvacean family Kornmanniaceae (Hori 1973, O'Kelly *et al.* 1984, Golden and Cole 1986). *Blidingia, Enteromorpha* and *Ulva* have been retained in the Ulvaceae on the basis of a large number of shared ultrastructural features (Golden and Cole 1986).

Chromosome numbers for species in the Ulvaceae suggest a basic haploid complement of N=10, with numbers of N=5-13 reported (Yabu and Tokida 1960, Gayral 1967, Kapraun 1970, Rhyne 1973, Sarma 1970, 1982, 1983). This aneuploid series could have resulted from non-disjunction and subsequent chromosome loss or gain (Freshwater *et al.* 1990). However, reported variations in chromosome size and karyotype asymmetry in species of *Ulva* and *Enteromorpha* (Niizeki 1957, Sarma and Chaudhary 1975) are consistent with an alternate explanation. Specifically, centric fusion and/or fission as well as translocations could have produced these changes in chromosome numbers. Whereas non-disjunction would be accompanied by nuclear genome size changes, centric fusion/fission could have occurred with little or no change in the total amount of nuclear DNA. Unfortunately, no quantitative data are available for nuclear genome sizes in any species of the Ulvaceae. Consequently, the karyological processes which have accompanied speciation in these green algae remain speculative.

The present investigation was initiated to obtain karyotype information for species of *Blidingia, Enteromorpha* and *Ulva* representative of the Ulvaceae in coastal North Carolina (Kapraun 1984). In addition, estimates of nuclear genome sizes were obtained by cytophotometry to elucidate karyological processes which may have accompanied speciation in these entities.

Materials and Methods

Source of Specimens

Seven species of Ulvaceae were collected from southeastern North Carolina, January through March, 1990: *Blindingia marginata* (J. Ag.) P. Dangeard, *Blidingia minima* (Naeg. *ex* Kuetz.) Bliding, *Enteromorpha clathrata* (Roth) Greville, *Enteromorpha linza* (L.) J. Agardh, *Enteromorpha prolifera* (O. F. Muell.) J. Agardh, *Ulva curvata* (Kuetz.) DeToni and *Ulva fasciata* Delile. Habitat descriptions and location map are available elsewhere (Kapraun 1984).

Fixation and Karyotype analysis

Field collected material was cleaned and placed in enriched seawater medium (Freshwater and Kapraun 1986). Fronds with discolored distal portions indicative of sporulation (Kapraun 1970) were transferred to petri dishes lined with glass coverslips onto which biflagellate swarmers readily settled (Hinson and Kapraun 1991). In the Ulvaceae, both gametes and zoospores can be biflagellate (Bliding 1963, 1968, Kapraun 1970). Consequently, the neutral term "swarmers" is used in this communication for biflagellate motile reproductive cells. Reproductive fronds were fixed at 24:00 and swarmers within 4 hr after attachment to coverslips, in 3:1 absolute ethanol-glacial acetic acid and stored in 70% ETOH. Fixed material for karyotype analysis was transfered to distilled water for 15 min, soaked in 1 N HCL for 4–5 min, and then stained with aceto-orcein as previously described (Kapraun and Martin 1987), or with hydroethidine as detailed below. Karyotypes were prepared by viewing 35 mm Kodak Plus-X film with a 48X microfiche reader and tracing the projected images (Kapraun and Freshwater 1987).

Fixed material for measurement of nuclear DNA was treated as follows: 1) coverslips with attached swarmers were emersed in distilled water for 15 min, 2) soaked in phosphate buffered saline (Polysciences # 8828) for 15 min, 3) stained 1-2 min in hydroethidine (Polysciences, Warrington, PA), and 4) returned to PBS and refrigerated for 24 hr prior to examination.

Determination of nuclear DNA

Microspectrophotometric data for an angiosperm, snapdragon (*Antirrhinum majus* L.) with a 2 C DNA content of 3.2 picograms (Bennet and Smith 1976, Leutwiler *et al.* 1984) and the green seaweed *Cladophora albida* (Huds.) Kuetzing with a 2 C DNA content of 0.8 pg (Bot *et al.* 1989a, Kapraun and Dutcher 1991) were used to quantify fluorescence intensity (I_f) values for specimens of the Ulvaceae. Proportionality of nuclear staining between the angiosperm and *Cladophora* specimens has been demonstrated (Kapraun and Dutcher 1991). Angiosperm seeds were germinated and root tips fixed and stained with hydroethidine as previously described (Kapraun and Dutcher 1991). Released swarmers from *C. albida* were allowed to settle on glass coverslips and then placed in fixative as described above. *Cladophora* material following aceto-orcein staining revealed a haploid chromosome complement of 1 N = 12 (Kapraun and Gargiulo 1987). Consequently, it was assumed that swarmers had DNA contents corresponding to 1 C and 2 C haploid nuclei (Hinson and Kapraun 1991).

Fluorescence intensity (I_f) values of 1 C nuclei of *C. albida* and 2 C nuclei of *Antirrhinum majus* were determined (Fig. 11) and plotted against their known DNA contents (Kapraun *et al.* 1991) to derive a standard line (Fig. 12). DNA contents for isolates of the Ulvaceae were extrapolated by plotting their I_f values of G1- and G2-phase nuclei along this I_f /DNA slope.

Instrumentation and methodology for cytophotometry and I_f data analysis have been described previously (Kapraun and Shipley 1990, Kapraun *et al.* 1991).

Observations and Discussion

Identification of Specimens

Members of the Ulvaceae are characterized by poorly marked interspecific distinctions and numerous intermediate forms. Much of

the taxonomic confusion in this group is probably the result of reliance on criteria such as frond size and color, and degree and pattern of branching which are too variable to be reliable (Bliding 1963, 1968, Kapraun 1984, Koeman 1985). In the present study, identifications were based on features considered to have taxonomic significance including pyrenoid number, cell size and dimension in surface and section views.

Karyology

Use of DNA-localizing fluorochromes and epifluorescence for karyological studies can pose special photomicrographic difficulties. Lightly stained material fluoresces too faintly to produce a clear photographic image, while over-stained specimens result in a fluorescent blush, distorting the actual size of chromosomes. Consequently, absolute size comparisons between specimens stained at different times should be avoided. Despite these potential problems, chromosomes visualized following hydroethidine staining typically appear less condensed than with aceto-orcein or aceto-carmine (Kapraun *et al.* 1988, Kapraun and Shipley 1990). Thus, constrictions indicative of centromeric regions can often be identified, and relative chromosome sizes in individual preparations more accurately determined with DNA-localizing fluorochromes.

Chromosome numbers for the seven species examined in this investigation are listed in Table 1. Apparently, this is the first published report of karyological data for *Blidingia marginata* and *B. minima.* Present results for *Enteromorpha* and *Ulva* species are in general agreement with previously published data (Kapraun 1970, Rhyne 1973, Sarma and Chaudhary 1975). However, chromosome numbers differing from these have been reported for Japanese populations of *Enteromorpha linza* (Niizeki 1957) and *Ulva fasciata* (Migita and Fujita 1987).

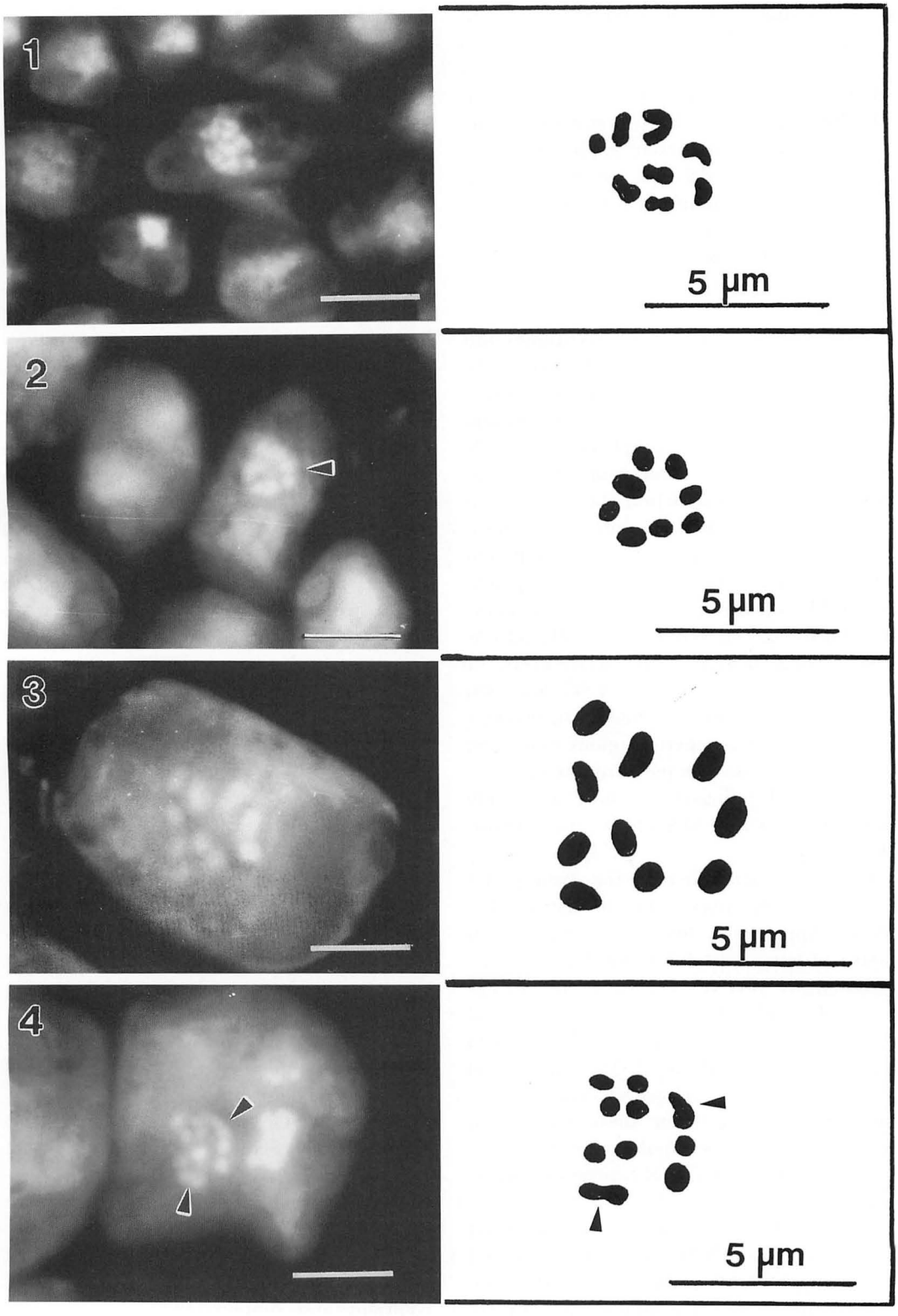
Both species of *Blidingia* are characterized by 1 N=8 (Figs. 1 & 2), with early metaphase chromosomes arranged in a circle (Fig. 1). No size difference was apparent among these highly condensed chromosomes. The three species of *Enteromorpha* have haploid chromosome complements of 1 N=10 (Fig. 8). In *E. clathrata* (Fig. 3) and *E. prolifera* (Fig. 5), karyotypes show a gradual decrease from large to small chromosomes (Fig. 8). In *E. linza* (Fig. 4), the two larger chromosomes with strongly constricted centromeric regions (Fig. 8), can become dissociated in thinly squashed preparations, suggesting the presence of twelve chromosomes. In *U. fasciata*, the haploid chromosome complement of 1 N=10 (Fig. 7) is characterized by four larger, submetacentric chromosomes and an asymmetric karyotype (Fig. 8).

Thus, the karyotypes of both *Blidingia* species as well as those of *Enteromorpha clathrata* and *E. prolifera*, show a uniform gradation from large to small chromosomes (Fig. 8), and can be characterized as asymmetric and unspecialized (Jackson 1971). In contrast, the karyotypes of *Enteromorpha linza* and *Ulva fasciata* with their marked difference between large and small chromosomes and the presence of submetacentrics (Fig. 8), can be characterized as asymmetric and specialized (Jackson 1971).

Chromosome numbers in the Ulvaceae suggest a basic haploid complement of N=10 (Fig. 9). However, reports of a haploid chromosome complement of N=5 (Gayral 1967), haploid meiosis (Hoxmark and Norby 1974) and pairing of homologous chromosomes during mitosis in haploid nuclei (Fig. 7) provide evidence that N=5 may be the basic chromosome number in the Ulvaceae, and that most extant species have a polyploid origin.

DNA cytofluorometry

Microspectrophotometry has been used previously with the marine green algae to estimate relative DNA contents (Eckhart and Schnetter 1984, Schnetter *et al.* 1984, Eckhart and Schnetter 1986, Kapraun *et al.* 1988, Calderón-Saenz and Schnetter 1989, Beutlich *et al.* 1990, Bodenbender and Schnetter 1990) and to quantify their nuclear genomes (Spring *et al.* 1978, Schnetter *et al.* 1981, Kapraun and Shipley 1990, Hinson and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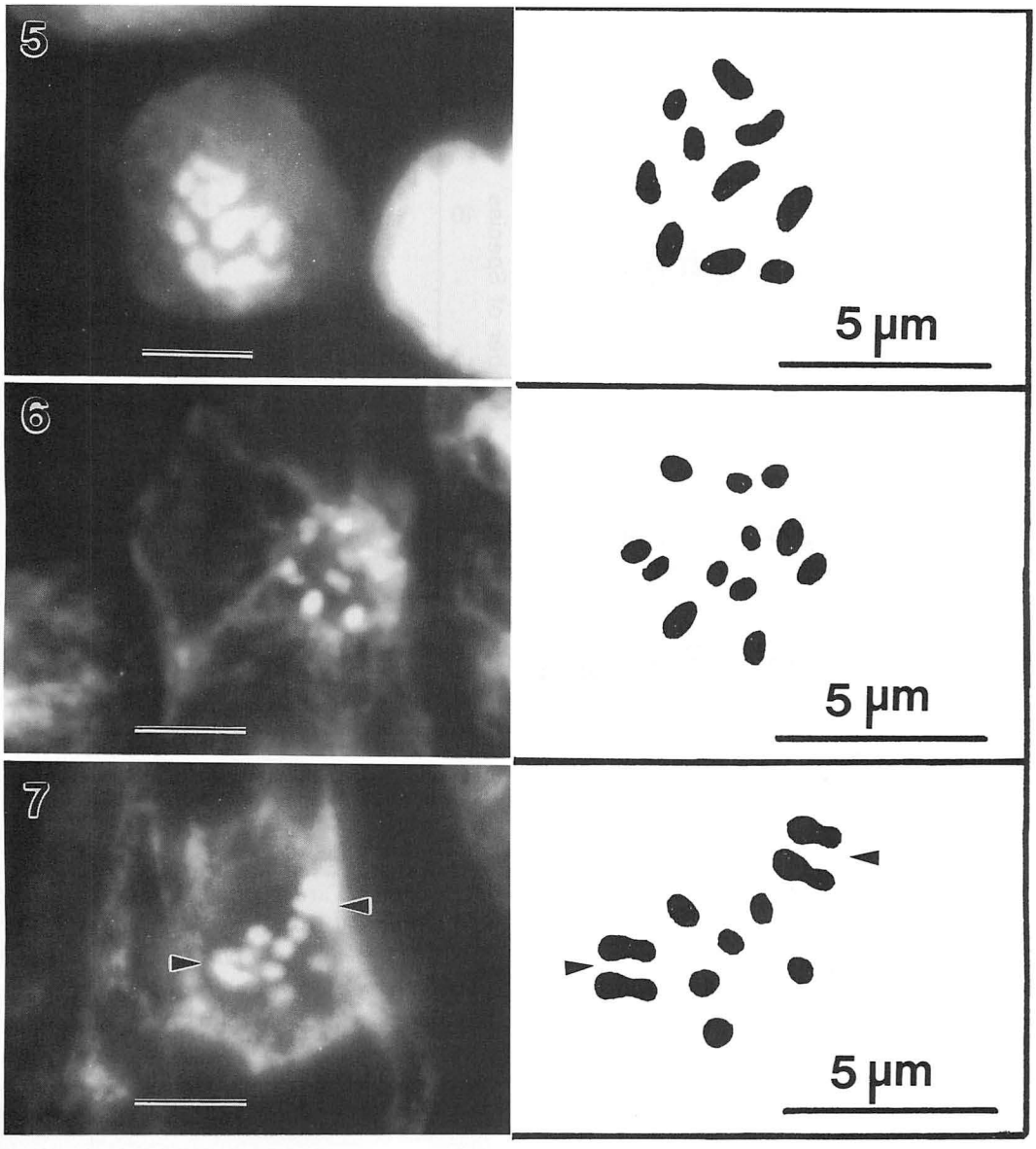

Figs. 1–7. Haploid mitotic nuclei in seven species of Ulvaceae following hydroethidine staining. Scale bar on photographs = 5 µm.

1. *Blidingia marginata* with 1 N = 8.
2. *Blidingia minima* with 1 N = 8. Note late prophase circle of chromosomes in top nucleus.
3. *Enteromorpha clathrata* with 1 N = 10.
4. *Enteromorpha linza* after initiation of sporulation. Note presence of two chromosomes with pronounced centromeric regions (arrows) in nucleus on left with 1 N = 10.
5. *Enteromorpha prolifera* with 1 N = 10.
6. *Ulva curvata* with 1 N = 12.
7. *Ulva fasciata* with 1 N = 10. Note presumed homologous pairing (arrows).

Kapraun 1991).

In the present study, initial observations made with vegetative cells following hydroethidine staining indicated unacceptable levels of fluorescence from extra-nuclear DNA, especially that associated with the

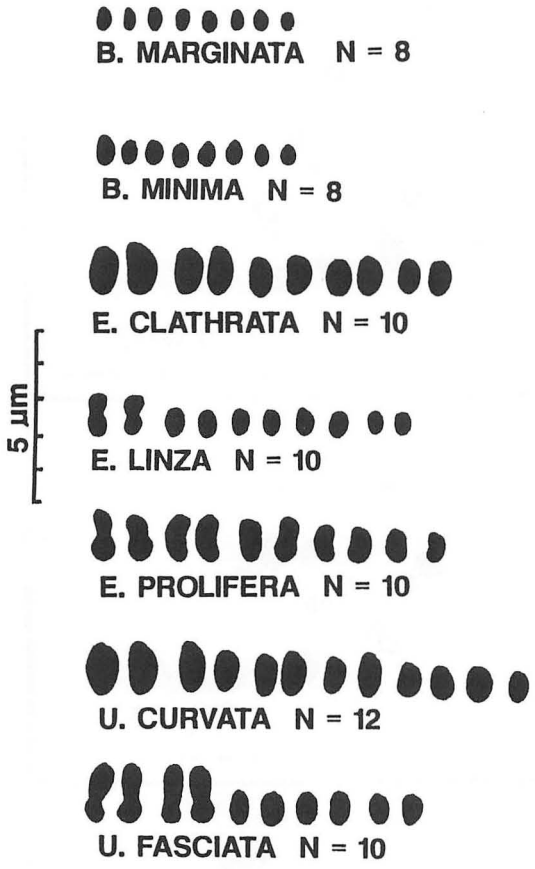

Fig. 8. Typical karyotypes for seven species of Ulvaceae representing chromosomes in haploid mitotic nuclei.

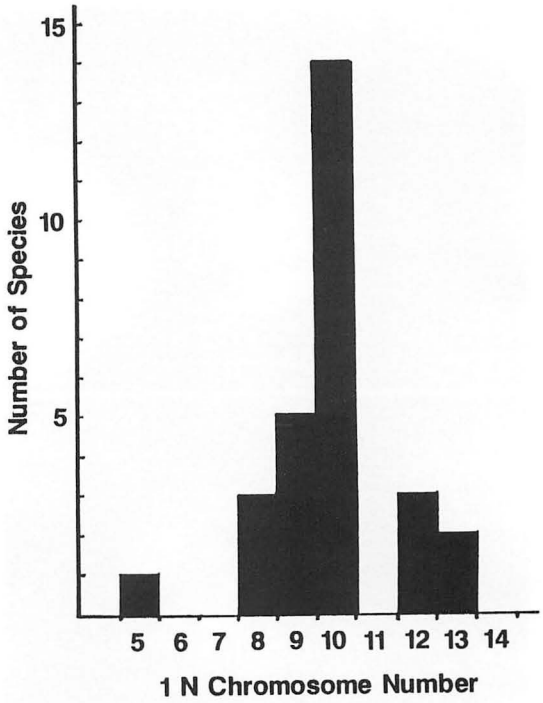

Fig. 9. Haploid chromosome complements reported in species of Ulvaceae: *Blidingia, Enteromorpha* and *Ulva*. Data from present study, Yabu and Tokida 1960, Yabu and Park 1968, Kapraun 1970, Rhyne 1973, Hoxmark and Nordby 1974, Sarma 1982.

large, cup-shaped chloroplast, which obscured the parietal nucleus. Use of swarmers which have a central nucleus distinct from the small chloroplast (Fig. 10) was found to greatly reduce extra-nuclear fluorescence. However, significant variation of I_f peaks among periodic fixations of *Enteromorpha linza* and *Ulva curvata* swarmers suggested that different stages in the S-phase (DNA synthesis) of the nuclear cycle were being observed. Subsequently, I_f observations were restricted to swarmers fixed within 4 hr of release which consistantly had estimated DNA values closely approximating half the G2-phase (2 C DNA levels) obtained from vegetative cells (Table 2).

Conditions necessary for use of microspectrophotometry (I_f data) to estimate nuclear DNA contents are detailed elsewhere (Bennet and Smith 1976, Kapraun and Shipley 1990). Previously, *Antirrhinum majus* and *Cladophora albida* were found to give proportionality between observed I_f values and

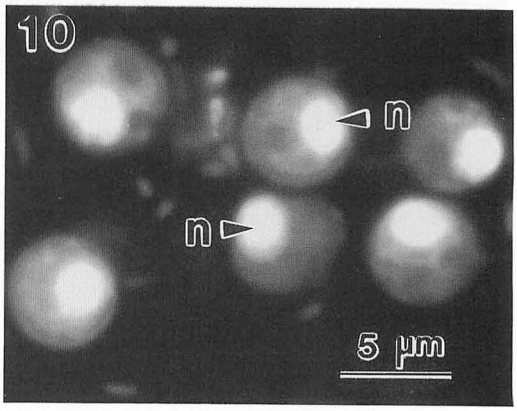

Fig. 10. Settled swarmers of *Ulva fasciata* following hydroethidine staining. n=nucleus.

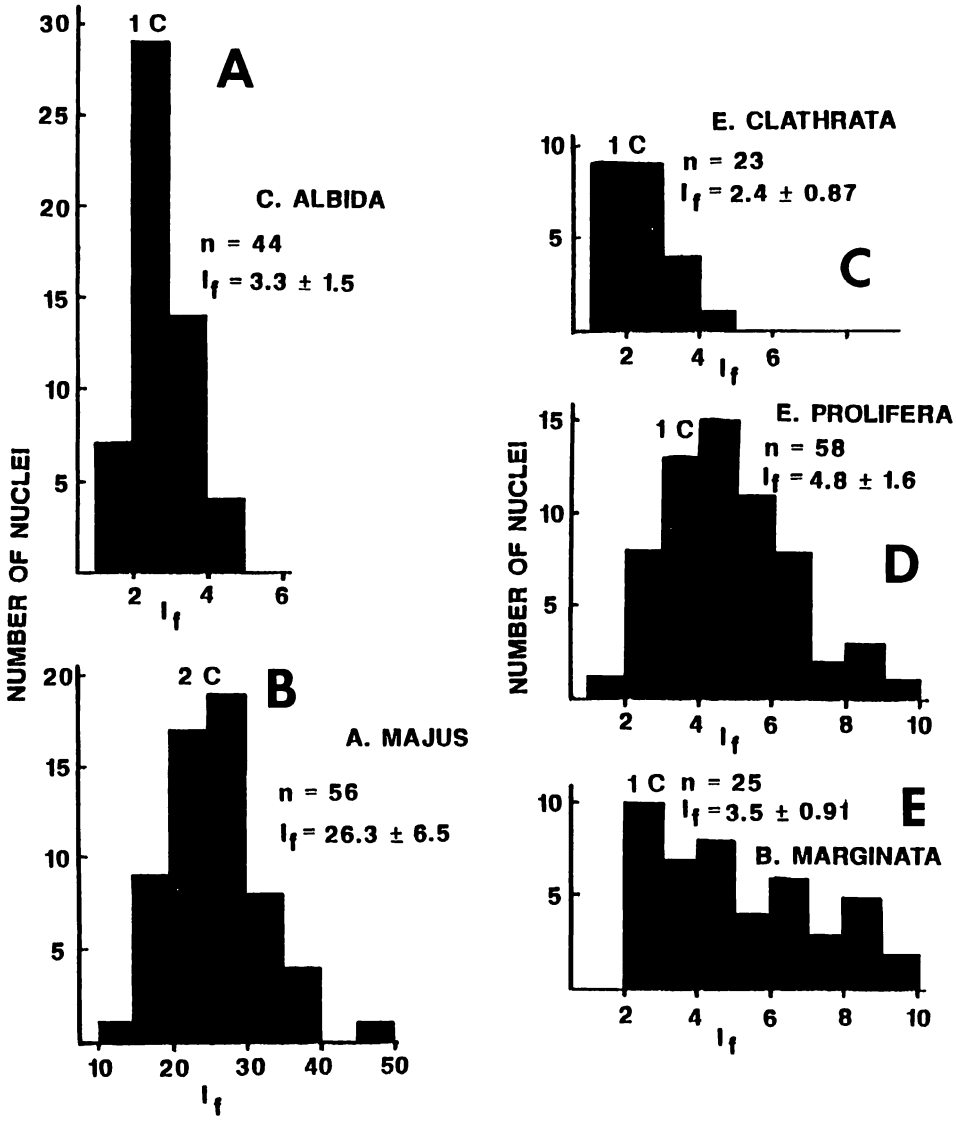

Fig. 11. Comparison of frequency distributions of relative DNA values for nuclei after hydroethidine staining. n=number of nuclei used to calculate C levels (Kapraun and Shipley 1990), I_f=fluorescence intensity mean±SD. *Cladophora albida* (A) and *Antirrhinum majus* (B) were used as standards for *E. clathrata* (C), *E. prolifera* (D) and *B. marginata* (E).

known DNA contents (Kapraun and Shipley 1990, Kapraun and Dutcher 1991), and were used in the present investigation to derive a standard line (Fig. 12). The *Antirrhinum* served as a standard for DNA quantification, and the North Carolina isolate of *Cladophora albida* was used to confirm the proportionality of staining between the angiosperm and green algal samples.

Comparison of I_f values for the Ulvaceae species (Fig. 11) permitted extrapolation of their DNA contents (Fig. 12). Genome size (pg) estimates for 1 C and 2 C nuclei from all observations are listed in Table 2 and summarized in Table 1. The standard deviation (SD) for most observations was less than 0.1 pg, and seldom exceeded 0.2 pg. Mean 2 C nuclear DNA levels for all experiments

Table 1. Comparison of haploid chromosome numbers and genome sizes for seven species of Ulvaceae. Genome size estimates are based on observed I_f values for 1 C and 2 C nuclei and on calculation of the mean 2 C genome size from these I_f data $\left(2\,C\text{ genome}=\frac{2(1\,C)+2\,C}{2}\right)$. Mean chromosome size was calculated from genome size (pg)/1 N chromosome number.

Species	Chromosome number (1 N)	Genome size estimates (pg)			Mean chromosome size (pg/chromosome)
		Observed		Calculated	
		1 C	2 C	2 C	
Blidingia marginata	8	0.4	0.8	0.8	0.10
Blidingia minima	8	0.4	0.9	0.8	0.10
Enteromorpha clathrata	10	0.3	—	0.6	0.06
Entermorpha linza	10	0.3	0.6	0.6	0.06
Enteromorpha prolifera	10	0.5	0.9	1.0	0.10
Ulva curvata	12	0.4	0.7	0.7	0.05
Ulva fasciata	10	0.3	0.6	0.6	0.06

(Table 1) indicate a genome size of 0.6-1.0 pg for the seven species of Ulvaceae observed. These derived nuclear DNA contents are within the range of values reported for multinucleate green algae: *Cladophora*=0.3-0.8 pg (Bot *et al.* 1989a, 1989b), *Chaetomorpha*=0.2-0.6 pg (Hinson and Kapraun 1991), *Bryopsis*=0.7-1.0 pg (Kapraun and Shipley 1990) and *Acetabularia*=1.8 pg (Spring *et al.* 1978) and slightly larger than nuclear genome sizes for unicellular (uninucleate) green algae: *Chlamydomonas*=0.3 pg and

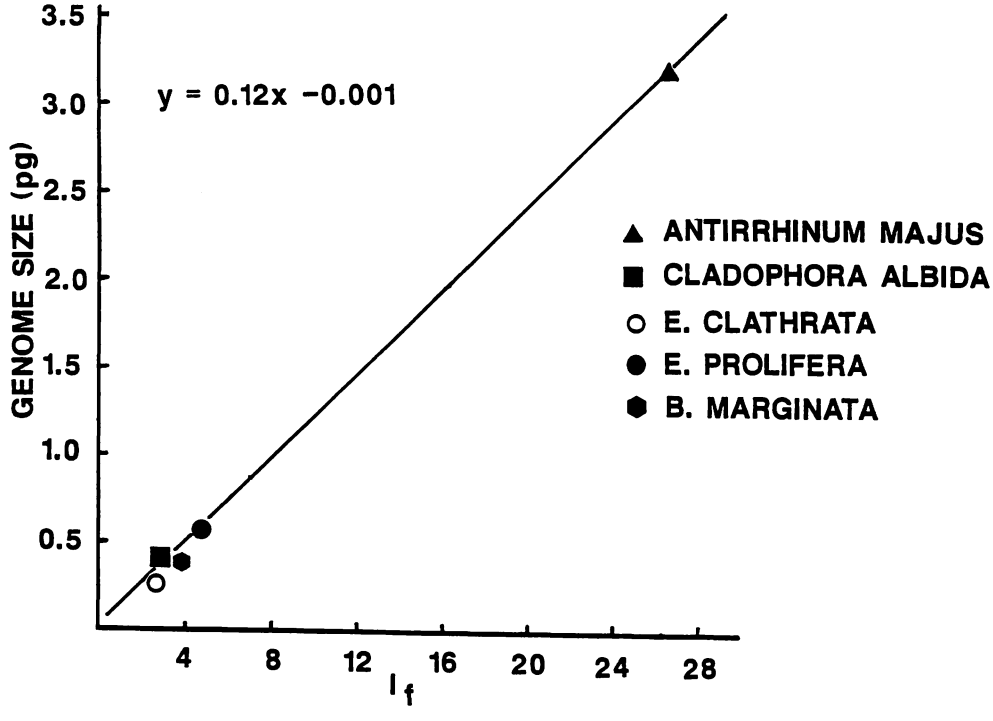

Fig. 12. Fluorescence intensity (I_f) values for *Antirrhinum* 2 C nuclei (▲) and *Cladophora* (■) 1 C nuclei plotted against their known DNA contents (Bennett and Smith 1976, Leutwiler *et al.* 1984, Bot *et al.* 1989, Kapraun and Dutcher 1991) to derive a standard line. DNA contents for 1 C nuclei in *E. clathrata* (○), *E. prolifera* (●) and *B. marginata* (⬢) are extrapolated from their I_f values.

Table 2. Genome size (pg) for 1 C (G1-phase) and 2 C (G2-phase) nuclei for seven species of Ulvaceae. Data standardized to the 2 C DNA level of *Antirrhinum majus* (3.2 pg). Mean ± standard deviation is given for each sample. n = number of nuclei observed in each sample.

Species	n	1 C	2 C
Blidingia	31	0.48±.07	
marginata	37	0.44±.07	
	38	0.33±.12	
	6		0.72±.04
	31	0.27±.05	
	13		0.78±.10
	25	0.43±.10	
		$\bar{X}$=0.39	$\bar{X}$=0.75
Blidingia	17		0.87±.10
minima	11	0.48±0.0	
	39	0.44±.10	
	32	0.43±.10	
		$\bar{X}$=0.43	$\bar{X}$=0.87
Enteromorpha	23	0.29±.10	
clathrata	10	0.30±.02	
	35	0.33±.26	
		$\bar{X}$=0.31	
Enteromorpha	58	0.29±.12	
linza	50	0.29±.08	
	49	0.26±.07	
	50	0.31±.09	
	50	0.33±.13	
	50	0.33±.13	
	26		0.58±.11
	26	0.26±.08	
		$\bar{X}$=0.30	$\bar{X}$=0.58
Enteromorpha	42	0.58±.15	
prolifera	11		0.18±1.3
	47	0.40±.11	
	59	0.43±.06	
	18		1.10±.06
	35	0.36±.06	
	58	0.58±.20	
		$\bar{X}$=0.47	$\bar{X}$=1.09
Ulva curvata	30	0.42±.06	
	22		0.76±.05
	25	0.39±.07	
	10		0.73±.06
	35	0.28±.06	
	43	0.37±.08	
	47	0.36±.08	
	50		0.70±.12
		$\bar{X}$=0.36	$\bar{X}$=0.73
Ulva fasciata	50		0.57±0.1
	37	0.25±0.0	
		0.25	0.57

Polytoma = 0.2 pg (Spring *et al.* 1978).

The three genera represented in this study do not appear to be characterized by a unique genome size (Table 1). It is perhaps noteworthy that the intraspecific range of genome sizes observed in *Enteromorpha* (0.6-1.0 pg) exceeds the intergeneric range for *Ulva* and *Blidingia*. Results of the present study indicate no correlation between nuclear DNA contents and 1 N chromosome numbers (Fig. 13). For example, the three species with 1 N = 10 exhibit a greater range of genome sizes than found between species with N = 8 and N = 12. These data appear to exclude the possibility that speciation has been accompanied exclusively by loss or gain of chromosomes (and associated genome). In such cases, a positive correlation is predicted between chromosome number and genome size (Stucky and Jackson 1975, Ohri *et al.* 1981). In the present study, linear regression analysis of pg/chromosome (mean chromosome size) implies an inverse relationship between genome size and chromosome complement (Fig. 14). These observations are consistent with currently understood mechanisms such as translocations and centric fusion and/or fission which produce independent changes in chromosome numbers and nuclear DNA contents (Pichersky 1990).

A recent investigation of nuclear DNA base pair compositions (mol % G+C) of nuclear genomes in some Ulvales (Freshwater *et al.* 1990) provides indirect evidence of fusion/fission events in the evolution of these algae. Results indicate G+C values of 36-54% for nine species of *Blidingia, Enteromorpha* and *Ulva*. This relatively wide range of base pair compositions as well as the range of chromosome numbers reported for these genera (Fig. 9) are consistent with large-scale translocation events and differential loss or gain of centromere associated genome (Freshwater *et al.* 1990). It is perhaps noteworthy that chromosome numbers less than 10 are nearly twice as common as numbers greater than 10, suggesting that chromosome fusions are more easily tolerated than chromosome fissions.

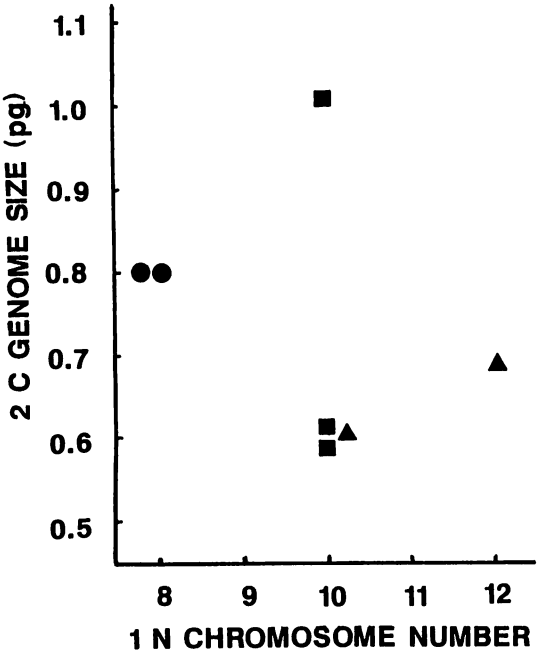

Fig. 13. Comparison of 2 C DNA contents and 1 N chromosome numbers for seven species of Ulvaceae. *Blidingia* (●), *Enteromorpha* (■) and *Ulva* (▲).

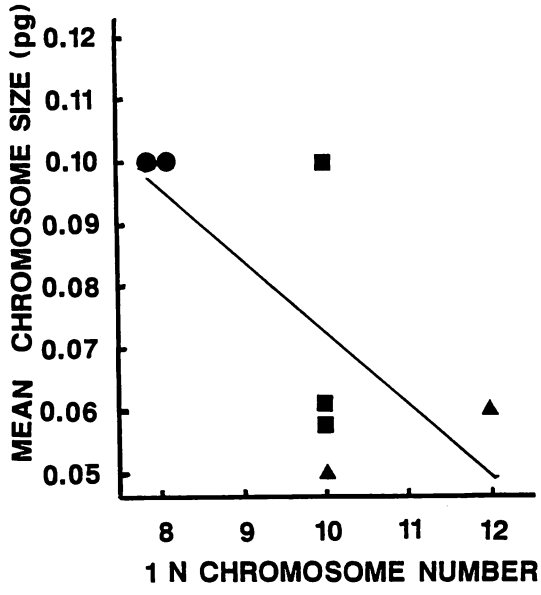

Fig. 14. Relationship of 1 N chromosome number to the mean size of chromosomes (2 C DNA content (pg)/chromosome number) for seven species of Ulvaceae. $Y=-0.0129X+0.201$. *Blidingia* (●), *Enteromorpha* (■) and *Ulva* (▲).

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Donald F. Kapraun・J. Craig Bailey：アサオ目（緑色植物門）の7種における核型分析と顕微測光による核 DNA の変動

ノースカロライナの海岸から採集したアオサ目（緑色植物門）の7種は，染色体数が N＝8–12 であった。DNA に特異的に結合する蛍光物質フロロクローム　ハイドロエチヂンを用いた顕微分光測定法により，核のゲノムの大きさを測った。被子植物 *Antirrhinum majus* L.（キンギョソウ）と緑藻 *Cladophora albida* (Hudson) Kuetzing の既知の 2 C DNA 量に対して取り込まれた蛍光量 (I_f) から，アオサ目で測定した I_f 値を DNA 量に換算した。調べた7種において，2 C DNA 量は 0.6–1.0 pg であった。測定したゲノムの大きさと 1 N 染色体数との間には正の相関が無く，これはアオサ目の種分化が染色体の消失や獲得および結合によって生じた可能性を否定する。染色体の融合と転移の過程によるものであることを指示する証拠を示し考察を行なった。(Center for Marine Science Research, University of North Carolina, 7205 Wrightsville Avenue, Wilmington, North Carolina 28403 U.S.A.)