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Agar analysis, nuclear genome quantification and characterization of four agarophytes *(Gracilaria)* from the Mexican Gulf Coast

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Abstract

DNA reassociation kinetics were used to determine nuclear genome organization and complexity in four species of *Gracilaria* (Gracilariales, Rhodophyta). In *Gracilaria tikvahiae, G. caudata, G. cervicornis* and *G. divaricata*, results indicate the presence of three second order components corresponding to fast, intermediate and slow fractions. Repetitive sequences varied from 13–46% and unique DNA ranged from 45–78%. Thermal denaturation (T_m) indicated guanine + cytosine (G + C) levels of 41.9–46.0 mol % G + C. Microspectrophotometry with the DNA-localizing fluorochrome DAPI was used to quantify nuclear DNA content. Comparisons of mean nuclear DNA (I_f) values to chicken erythrocytes (RBC) resulted in an estimate of 0.37–0.40 pg/2C genomes for the four *Gracilaria* species. Total agar content following alkaline pretreatment ranged from 7–15% dry weight. Gel strengths were generally below commercial levels, ranging from 40–260 g cm^{-2}. Nuclear genome profiles developed from information for genome size, organization and complexity are compared with data for agar quantity and quality. Gel quality and quantity do not appear to be correlated with either large repetitive fraction DNA or a high degree of genome complexity as previously speculated.

Introduction

Members of the Gracilariales (Fredericq & Hommersand, 1989a, 1989b) are among the most economically important agarophytes, supporting a significant industry based on both cultivated and wild plants (Santelices & Doty, 1989). Species of *Gracilaria* are widely distributed in tropical waters, but their economic potential is largely unknown (Dawes, 1987). Although strain improvement of agarophytes generally has emphasized phenotypic selection of vigorous clones (van der Meer, 1990), it has been suggested that basic information for nuclear genome characterization and quantification could provide an invaluable basis for genetic improvement programs (Kapraun & Dutcher, 1991; Kapraun *et al.*, 1993b). Possible correlations between agar production and specific genome organization could be exploited by appropriate genetic manipulation techniques such as somatic hybridization (Cheney, 1988, 1990), polyploidization (Patwary & van der Meer, 1984) and biolistic gene injection (Kapraun, 1993).

Recently a program was initiated to quantify and characterize nuclear genomes in agarophytes from the warm temperate western Atlantic (Kapraun & Dutcher, 1991; Dutcher *et al.*, 1990b, Kapraun, 1993; Kapraun *et al.*, 1993a, 1993b), and to relate the observed genome profiles to agar quality and quantity (Dutcher *et al.*, 1990b; Kapraun *et al.*, 1993b). This program has now been expanded to include representative tropical agarophytes from both the Atlantic-Caribbean and Pacific Oceans. The present paper summarizes information from microspectrophotometry, nuclear DNA base pair composition, reassociation kinetics and agar analysis for additional species of *Gracilaria* from the eastern coast of Mexico (Gulf of Mexico).

Table 1. Source of specimens

Species	Location
Gracilaria caudata J. Agardh	Punta Piedras, Lagune Madre
Gracilaria cervicornis (Turner) J. Agardh	Miramar Beach, Ciudad Madero
Gracilaria divaricata Harvey	Punta Piedras, Laguna Madre
Gracilaria tikvahiae McLachlan	Punta Piedras, Laguna Madre

Materials and methods

Sources and preservation of specimens

Collection sites for four species of *Gracilaria* included in the present study are listed in Table 1. All specimens were collected by the authors and identified by Dr S. Fredericq. Voucher specimens are deposited in the herbariums of the University of North Carolina at Wilmington (WNC) and Instituto Tecnologico de Cd. Victoria, Mexico (ITCV). Algal material for microspectrophotometry was fixed in Carnoy's solution (Kapraun *et al.*, 1992) and stored in 70% ethanol. DNA was isolated from samples quick-dried in silica gel and then stored frozen (−70°C) (Kapraun *et al.*, 1993b). Algal samples for agar analysis were air-dried at collection sites and subsequently dried to constant weight in an warm-air (38°C) herbarium cabinet).

Microspectrophotometry

Detailed procedures for microspectrophotometry with the DNA-localizing fluorochrome DAPI and requirements for reproducible staining have been specified previously (Kapraun *et al.*, 1992). Microspectrophotometric data for chicken erythrocytes (RBC) with a DNA content of 2.4 pg (Clowes *et al.*, 1983) was used to quantify mean fluorescence intensity (I_f) values for algal specimens (Kapraun *et al.*, 1991).

DNA isolation and characterization

Detailed procedures for nuclear DNA isolation and purification and requirements for efficient, reproducible genome characterization have been specified previously (Dutcher *et al.*, 1990a, b). Algal DNA samples, with an *Eschericia coli* DNA standard, were heated to 100°C, 1°C min^{-1}, in a closed thermostatically controlled cuvette of a Gilford model 2600 spectrophotometer equipped with a thermoprogrammer. Thermal denaturation temperatures (T_m's) were determined from the hyperchromatic shift for multiple replicates of each sample using a standard equation: G + C = $T_m(2.280) - 153.7$ (Dutcher *et al.*, 1990a). DNA reassociation kinetics were carried out under moderately stringent conditions (T_m - 25°C, 0.18 M Na^+). Reassociation data were analyzed with a computer program generating a best-fit non-linear regression representing three second-order components (Kapraun *et al.*, 1992). DNA from *E. coli* (4.2×10^6 bp, C_0t 1/2 pure 2.93) was used as a standard to convert C_0t values into nucleotide base pairs or complexity of each component for algal samples (Lewin, 1990; Kapraun *et al.*, 1993b).

Agar extraction and analysis

Detailed procedures for agar extraction following alkaline pretreatment in 1.0 N NaOH have been specified previously (Bird & Hinson, 1992). All extractions were performed using triplicate samples. Agar yield quality determinations included gel strength, gelling and melting temperature (Lemus *et al.*, 1991; Bird & Hinson, 1992). Gel strength was determined from 1.5% gels allowed to cure overnight at room temperature. The gels were covered to prevent drying. A Marine Colloids (Rockland ME 04841) gelometer with a 1 cm^{-2} plunger was used to determine gel strengths. Melting temperature was determined by slowly raising the temperature of 1.5% gels in test tubes held in a hot water bath and determining the temperature at which a small bead sank through the agar. Dynamic gelling temperature was determined by gradually cooling agar solutions and noting when the withdrawal of a thermometer left a permanent deformation of the meniscus. Gel strengths, gelling and melting temperatures were determined in triplicate.

Results

Microspectrophotometry

DAPI protocols from Kapraun *et al.* (1992, 1994) and Kapraun (1993) resulted in reproducible, stable fluorescence of nuclei with only slight cytoplasmic interference (Fig. 1). Comparison of I_f values of RBC erythrocytes and algal samples permitted extrapolation of algal DNA contents (Table 2).

Extrapolated data indicate a DNA content range for 2C genomes of 0.37–0.40 pg for four species of *Gracilaria: G. caudata* J. Agardh, *G. cervicornis* (Turner) J. Agardh, *G. tikvahiae* McLachlan and *G.*

Table 2. Genome size (pg) for *Gracilaria* species nuclei after DAPI staining. Data standarized to the DNA level of chicken erythrocytes (RBC = 2.4 pg)

Species	Number of slides	Number of nuclei	Nuclear genome size (pg)	
			1C	2C
G. caudata	6	243	0.24±0.004	
	6	159		0.40±0.02
G. cervicornis	7	238	0.24±0.02	
	3	97		0.40±0.01
G. divaricata	3	154	0.20±0.05	
				0.40*
G. tikvahiae	6	197	0.18±0.05	
	4	159		0.37±0.01

* Extrapolated from 1C value

Table 3. Mol % G + C values estimated from experimentally determined denaturation temperatures (T_m)

Species	Number of samples	T_m (°C)	Mol % G + C
G. caudata	2	85.7±0.7	41.7±0.6
G. cervicornis	3	86.0±0.5	42.3±1.1
G. divaricata	3	86.4±0.3	43.2±0.6
G. tikvahiae	3	87.6±0.4	46.0±0.8

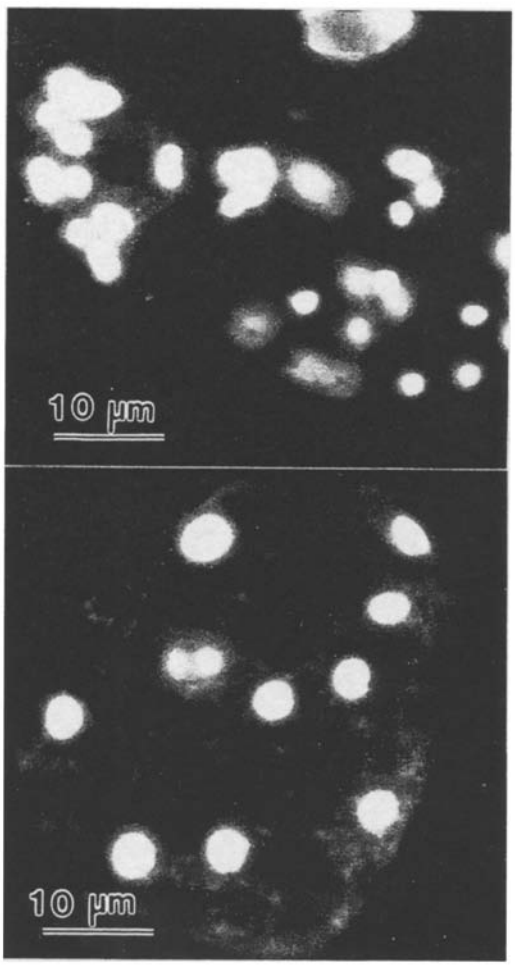

Fig. 1. Gracilaria cervicornis nuclei following DAPI staining and visualized with episcopic (UV) illumination. Gametophyte medullary cells showing 1 N nuclei with 1 C (upper photo) and 2 C (lower photo) DNA contents.

divaricata Harvey (Table 2). Mean fluorescence (I_f) values for telophase nuclei (G_1-phase) in gametophytes closely approximates 50% of the values for telophase nuclei (G_1-phase) in tetrasporophytes (e.g. see data for *Gracilaria tikvahiae* in Table 2).

Nuclear DNA base pair composition

Use of melting point procedures for the Rhodophyta as detailed in Kapraun (1993) resulted in reproducible melting curves for all species of *Gracilaria* tested in this study. Absence of double melting curves in whole DNA melting profiles (Fig. 2) excludes the possibility of significant contribution from organelle DNA contamination. Nuclear DNA base pair composition estimates from thermal denaturation temperatures (T_m) revealed a guanine + cytosine (G + C) range among the algal samples of 41.9–46.0 mol % (Table 3).

Reassociation kinetics

Computer analysis of DNA reassociation kinetics for Mexican Gracilariales revealed the presence of three second order components: a fast fraction representing highly repetitive sequences, an intermediate fraction of mid-repetitive sequences, and a slow fraction of unique or single copy sequences (Fig. 3). Fractions, complexities and repetition frequencies for each species are summarized in Table 4.

Reassociation kinetics can provide an estimate of genome size if reassociation of the slow component is complete (Lewin, 1990). In the present study, experimental parameters precluded determination of slow

Table 4. Kinetic analysis of DNA reassociation in four species of *Gracilaria*

Species	Component	Fraction (%)	Complexity (bp)	Repetition frequency	Genome size (pg)
G. caudata	Fast	1	--	--	0.40
	Intermediate	45	23.79×10^6	7	
	Slow	54	2.09×10^8	1	
G. cervicornis	Fast	1	--	--	0.40
	Intermediate	45	15.38×10^6	11	
	Slow	54	2.80×10^8	1	
G. divaricata	Fast	3	--	--	0.40
	Intermediate	13	12.00×10^6	4	
	Slow	84	3.24×10^8	1	
G. tikvahiae	Fast	2	--	--	0.37
	Intermediate	11	3.58×10^6	10	
	Slow	87	3.10×10^8	1	

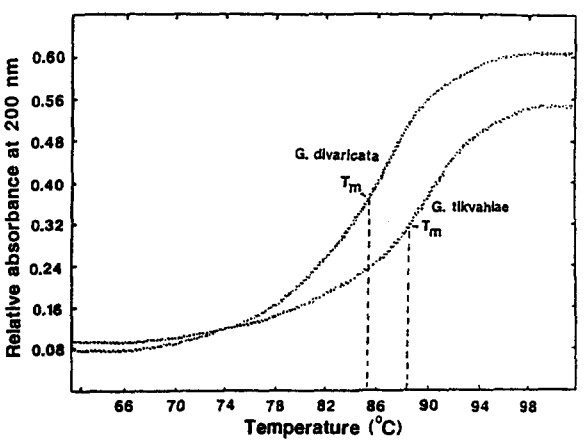

Fig. 2. Melting curve of *Gracilaria divaricata* and *G. tikvahiae*. Temperature at the midpoint of the absorbance rise (T_m) is indicated for both samples.

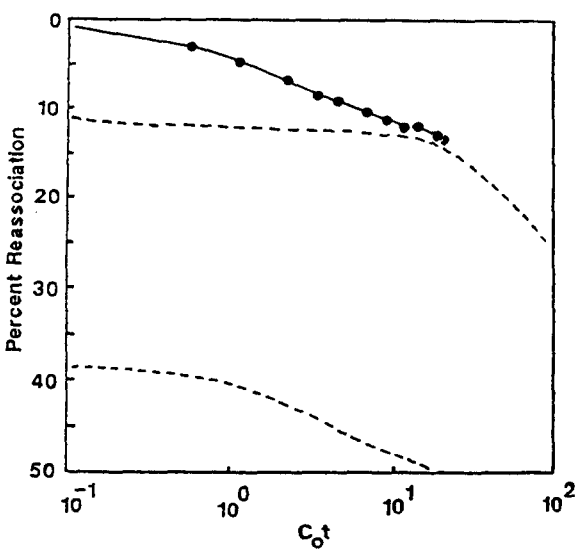

Fig. 3. Reassociation kinetics of whole cell DNA from *Gracilaria tikvahiae*. C_0t (moles of nucleotides per liter × S), DNA concentrations were 50–60 μg DNA ml^{-1} in 18 M Na^+ at 60°C. Solid line: best linear regression analysis of data. Dashed line: predicted second order kinetic components, correlation coefficient > 0.995.

component C_0t 1/2 (Dutcher *et al.*, 1990b). Consequently, genome size estimates from cytophotometry were used to extrapolate a complexity of 0.38×10^9 bp for *G. caudata, G. cervicornis* and *G. divaricata*, and 0.35×10^9 bp for the genome of *G. tikvahiae*, using the conversion expression 0.965×10^9 bp = 1 pg (Britten & Davidson, 1971).

Agar characterization

The total agar content following alkaline treatment for species of *Gracilaria* ranged from 7–15% of dry weight for algal samples (Table 5). Agar yield, gelling and melting temperatures, and gel strength for *G. tikvahiae, G. caudata, G. divaricata* and *G. cervicornis* are summarized in Table 5. Gel strengths ranged from 40 to 260 g cm^{-2}.

Table 5. Characterization of 1 N NaOH alkali modified agars from species of *Gracilaria*. Gel strengths, gelling and melting temperatures were determined using 1.5% w/v agar samples. All determinations were made in triplicate. *Gracilaria tikvahiae, G. divaricata* and *G. cervicarnis* samples were collected in July; *G. caudata* samples were collected in December

Species	Agar yield (%)	Gelling temp. (°C)	Melting temp. (°C)	Gel strength ($g\ cm^{-2}$)
G. caudata	15	30	61	48
G. cervicornis	7	36	75	208
G. divaricata	7	36	67	40
G. tikvahiae	7	42	89	260

Discussion

Microspectrophotometry

Nuclear genome quantifications for *G. caudata, G. cervicornis, G. tikvahiae* and *G. divaricata* indicate similar values of 0.37–0.40 pg which are in the range reported for other Gracilariales (0.33–0.47 pg), and of similar magnitude to Gigartinales and Gelidiales (Kapraun, 1993; Kapraun *et al.*, 1993c). Apparently, geographical isolates of *Gracilaria tikvahiae* from Mexico (Gulf of Mexico), Florida, North Carolina and Nova Scotia have similar 2C genome sizes (Kapraun & Dutcher, 1991; Kapraun, 1993).

Present data for Mexican Gracilariales support the generalization that the Gracilariales are characterized by a narrow range of genome sizes. Previously, chromosome numbers, and karyotype symmetry were shown to be highly conserved in these taxa (Kapraun, 1993).

DNA base pair composition

Results of the present study indicate a similar range of G + C values for Mexican isolates compared with other species of *Gracilaria* (37–49.7 mol %) (Dutcher *et al.*, 1990a; Kapraun *et al.*, 1993b). *Gracilaria tikvahiae* reveals no differences in base pair composition among isolates from Mexican (this study) and other localities from Florida, North Carolina and Nova Scotia (Dutcher *et al.*, 1990a).

Reassociation kinetics

Gracilaria species in this study revealed a range of 13–46% repetitive DNA sequences and 45–87% unique DNA (Table 4). *Gracilaria caudata* and *G. cervicornis* have similar genome organizations consisting of 45.5–46% of repeated sequences and 54–54.5% unique. Similarly, *G. tikvahiae* and *G. divaricata* have 13 and 16% repeated sequences, and 87–84% unique DNA, respectively.

Genome organization in *G. tikvahiae* is characterized by lowest values for repeated segments (13%) and highest values for unique DNA (87%). Similar profiles were obtained with isolates of this species from Nova Scotia, North Carolina and Florida (Dutcher *et al.*, 1990b). Wide variations in unique sequences among species of *Gracilaria* as shown here have been reported in other species of Gracilariales (Dutcher *et al.*, 1990b).

Comparison of the percentage of unique sequences to repetitive sequences (U/R ratio) (Dutcher *et al.*, 1990b) reveals wide variations among species: *G. caudata* and *G. cervicornis* both with 1.10, *G. divaricata*, 5.25 and *G. tikvahiae*, 6.69. Since genome size is small and constant among Gracilariales (Kapraun, 1993), speciation may have been accompanied by changes in the ratio of unique to reptitive segments rather than by segment multiplication which results in genome size increase (Wenzel & Hemleben, 1982; Dutcher *et al.*, 1990b).

Reassociation kinetics show that the complexities of the unique component for *Gracilaria tikvahiae* studied here (3.1×10^8 bp) is similar to other isolates of this species from Nova Scotia (3.03×10^8), North Carolina (3.78×10^8), and Florida (3.24×10^8) (Dutcher *et al.*, 1990b). *G. divaricata* appears to be in the same range (3.24×10^8). Meanwhile *G. cervicornis* revealed an intermediate value (2.8×10^8) and *G. caudata* the lowest value (2.08×10^8) among *Gracilaria* species studied. These and other complexity data (Dutcher *et al.*, 1990b) suggest that evolution in Gracilariales could have involved different accumulation/deletion rates for repetitive sequences.

Agar characterization

Samples included in this study demonstrated a wide range for agar yield % and gel qualities. The lower yields were probably due to water temperatures at the time of collection. The three species with the lowest agar yields were collected during July, when temperatures can exceed 30°C. *Gracilaria caudata* was collected in December, when temperatures are much cooler. Bird (1988) noted that higher water temperatures led to lower agar yields in *Gracilaria* sp. G–16. Among

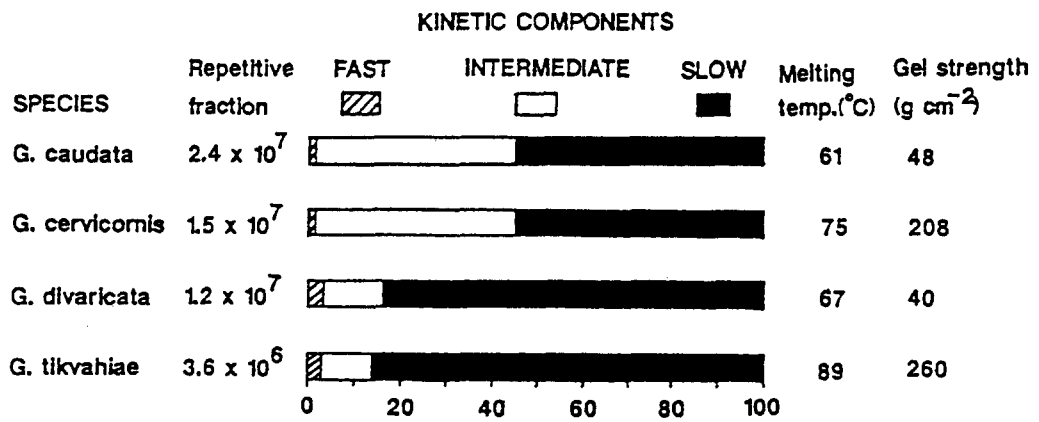

Fig. 4. Estimated percent fast, intermediate and slow kinetic components for four species of *Gracilaria* compared to agar quality following alkaline treatment.

our samples only *G. tikvahiae* and *G. cervicornis* have both high gel strength and high melting temperatures (Table 5), but they still are low when compared with commercial agarophytes (Bird & Hinson, 1992).

Previous comparisons of genome organization and complexity data with information for gel quality suggested a possible correlation between genome profiles and synthesis of commercial-grade agars in species of the Gracilariales (Dutcher *et al.*, 1990; Kapraun *et al.*, 1993b). Specifically, taxa with large portions of repetitive fraction DNA (>85%) and a high degree of complexity in these segmens (>1 × 10^8 bp) were found to produce agars with high gel strength (700–1290 g cm^{-2}) and high melting temperatures (99°C), characteristic of commercial agars (Kapraun *et al.*, 1993b). In the present study, two species with poor gel quality (i.e., *G. caudata* and *G. divaricata*) have both large repetitive fraction DNA and a high degree of complexity in these segments (Fig. 4). In a parallel study of Philippine agarophytes, large repetitive fraction DNA and a high degree of complexity were associated with *Gracilaria* species having both poor (<200 g cm^{-2}) and good (>700 g cm^{-2}) gel quality (unpublished data). Consequently, our initial speculation that genomes with large repetitive fractions have an abundance of gene loci for enzymes associated with polymerization of galactan subunits (Dutcher *et al.*, 1990) seems overly simplistic.

Recently, investigations of DNA base composition heterogeneity in some agarophytes suggest that their unique sequences may represent fossil repeats or extensively diverged repeated sequences (Kapraun *et al.*, 1993c). Furthermore, the DNA which is sequence-specific or genic is probably characterized by compositional compartmentalization into isochores. Therefore, it seems unlikely that target gene loci associated with enzymes for polymerization of galactan subunits are restricted to particular DNA kinetic components.

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References

Bird KT (1988) Agar production and quality from *Gracilaria* sp. strain G-16: Effects of environmental factors. Bot. mar. 31: 33–39.

Bird KT, Hinson TK (1992) Seasonal variations in agar yield and quality from North Carolina agarophytes. Bot. mar. 35: 291–295.

Britten RJ, Davidson EH (1971) Repetitive and nonrepetitive DNA sequences and a speculation on the evolutionary novelty. Q. Rev. Biol. 46: 11–133.

Cheney DP (1988) Genetic engineering and biotechnology of economically important seaweeds. In Sparks AK (ed.), New and Innovative Advances in Biology/Engineering with Potential for Use in Aquaculture. Proc. 14th US-Japan Meeting on Aquaculture, Woods Hole, Mass: 27–28.

Cheney DP (1990) Genetic improvement of seaweeds through protoplast fusion. In: Yarish C, Penniman CA, van Patten P (eds),

Economically Important Marine Plants of the Atlantic: Their Biology and Cultivation. Conneticut Sea Grant College Program, CT-SG-89-07, Groton, CT: 15–26.

Clowes AW, Reidy MA, Clowes MM (1983) Kinetics of cellular proliferation after arterial injury. I. Smooth muscle growth in absence of endothellium. Lab. Investigations 49: 327–333.

Dawes CJ (1987) The biology of commercially important tropical marine algae. In: Bird KT, Benson PH (eds), Seaweed Cultivation for Renewable Resources. Developments in Aquaculture and Fisheries Sciences, 16. Elsevier, Amsterdam: 155–190.

Dutcher JA, Kapraun DF, Sizemore RK (1990b) Inter- and intraspecific variation of nuclear DNA reassociation kinetics in the Gracilariales (Rhodophyta). J. appl. Phycol. 2: 259–267.

Dutcher JA, Sizemore RK, Kapraun DF (1990a) Variation in nuclear DNA base composition (Mol % G + C) in three genera of Gigartinales (Rhodophyta). J. crypto. Bot. 1: 390–395.

Fredericq S, Hommersand MH (1989a) Comparative morphology and taxonomic status of *Gracilariopsis* (Gracilariales, Rhodophyta). J. Phycol. 25: 228–241.

Fredericq S, Hommersand MH (1989b) Proposal of the Gracilariales *ord. nov.* (Rhodophyta) based on an analysis of the reproductive development of *Gracilaria verrucosa*. J. Phycol. 25: 213–227.

Kapraun DF (1993) Karyology and cytophotometric estimation of nuclear DNA content variation in *Gracilaria, Gracilariopsis* and *Hydropuntia* (Gracilariales, Rhodophyta). Eur. J. Phycol. 28: 253–260.

Kapraun DF, Bailey JC, Dutcher JA 91994) Nuclear genome characterization of the carrageenophyte *Hypnea musciformis* (Rhodophyta). J. appl. Phycol. 6: 7–12.

Kapraun DF, Dutcher JA (1991) Cytophotometric estimation of inter- and intraspecific nuclear DNA content variation in *Gracilaria* and *Gracilariopsis* (Gracilariales, Rhodophyta). Bot. mar. 34: 139–144.

Kapraun DF, Dutcher JA, Bird KT, Capecchi MF (1993a) Nuclear genome organization and carrageenan analysis of *Gymnogongrus griffithsiae* (Rhodophyta) from North Carolina. J. appl. Phycol. 5: 99–107.

Kapraun DF, Dutcher JA, Freshwater WD (1993b) Quantification and characterization of nuclear genomes in commercial red seaweeds: Gracilariales and Gelidiales. Hydrobiologia 260/261 (Dev. Hydrobiol. 85): 679–688.

Kapraun DF, Dutcher JA, Freshwater WD (1993c) DNA base composition heterogeneity in some Rhodophyta. J. crypto. Bot. 4: 97–106.

Kapraun DF, Dutcher JA, Lopez-Bautista J (1992) Nuclear genome characterization of the carrageenophyte *Agardhiella subulata* (Rhodophyta). J. appl. Phycol. 4: 1–9.

Kapraun DF, Hinson TK, Lemus AJ (1991) Karyology and cytophotometric estimation of inter- and intraspecific nuclear DNA variation in four species of *Porphyra* (Rhodophyta). Phycologia 30: 458–466.

Lemus A, Bird K, Kapraun DF, Koehn F (1991) Agar yield, quality and standing crop biomass of *Gelidium serrulatum, Gelidium floridanum* and *Pterocladia capillacea* in Venezuela. Food Hydrocolloids 5: 469–479.

Lewin B (1990) A continuum of sequences includes structural genes. In: Genes IV. Oxford University Press, New York, 857 pp.

Patwary MU, van der Meer JP (1984) Growth experiments on autopolyploids of *Gracilaria tikvahiae* (Rhodophyceae). Phycologia 23: 21–27.

Santelices B, Doty MS (1989) A review of *Gracilaria* framing. Aquaculture 78: 95–133.

van der Meer J (1990) Genetics. In: Cole KM, Sheath RG (eds), Biology of the Red Algae. Cambridge University Press, New York: 103–121.

Wenzel W, Hemleben V (1982) A comparative study of genomes of angiosperms. Plant Syst. Evol. 139: 209–227.