*Journal of Applied Phycology* **4**: 129–137, 1992.



# Nuclear genome characterization of the carrageenophyte *Agardhiella subulata* (Rhodophyta)

Donald F. Kapraun, Julie A. Dutcher & Juan Lopez-Bautista
*Center for Marine Science Research, University of North Carolina-Wilmington, 7205 Wrightsville Avenue, Wilmington, North Carolina 28403, U.S.A.*

Received 22 February 1992; revised 7 April 1992; accepted 8 April 1992

*Key words: Agardhiella*, carrageenophyte, cytophotometry, Gigartinales, nuclear genome, reassociation kinetics

## Abstract

DNA reassociation kinetics were used to determine nuclear genome organization and complexity in *Agardhiella subulata* (Gigartinales, Rhodophyta). Results indicate the presence of three second-order components corresponding to fast (22%), intermediate (68%) and slow (10%) fractions. Thus, the genome consists of 90% repetitive sequences. Microspectrophotometry with the DNA-localizing fluorochrome DAPI was used to confirm ploidy level differences in the gametophytic and tetrasporophytic phases. Results indicate that meiosis occurs during tetrasporogenesis. Comparison of mean nuclear DNA ($I_f$) values to chicken erythrocytes (RBC) resulted in an estimate of 0.9 pg/2C genome for *Agardhiella*. Karyological studies using aceto-orcein revealed a chromosome complement of $2N = 44$ in carposporangia and the presence of 22 bivalents during diakinesis of tetraspore mother cells.

## Introduction

Members of the Gigartinales provide the basis for a significant carrageenan manufacture industry (Craigie, 1990). Until the 1970's, the supply of seaweed biomass was obtained primarly from the harvest of natural populations of *Chondrus crispus* and *Gigartina stellata* from the North Atlantic, and *Iridea* from Chile (McHugh, 1984). More recently, the industry has come to rely on mariculture, with *Eucheuma* cultivation in the Philippines representing more than half of the world's supply (Abbott & Cheney, 1982; McHugh, 1984). Domestication of the 'cottonii types' of *Eucheuma* (i.e. *K. alvarezii* (Doty) Doty), which produce the hydrocolloid kappa-carrageenan, is generally cited as one of the most significant developments leading to large-scale phycoculture (Doty & Alvarez, 1975; Glenn & Doty, 1981; Doty, 1985).

Strain improvement of these carrageenophytes has been restricted to phenotypic selection of vigorous clones (for a recent review, see van der Meer, 1990). Surprisingly, no attempt has been made to characterize or quantify the nuclear genomes of these taxa, although such information could provide a basis for genetic improvement programs (Kapraun & Dutcher, 1991) such as development of somatic hybrids (e.g. Cheney 1985, 1988, 1990).

In contrast, information is now available for genome size, organization and complexity in several species of *Gracilaria* and *Gracilariopsis* (Gracilariales) (Dutcher *et al.*, 1990; Kapraun & Dutcher, 1991) which are sources of the commer-

cial gels agar and agarose (Dawes, 1987). Recently, we initiated a research program to adapt these cytophotometric and DNA reassociation kinetics techniques to the carrageenophytes from the warm temperate western Atlantic. The present paper concerns results with *Agardhiella subulata* (C. Agardh) Kraft and Wynne which is considered to have potential industrial value (Chopin *et al.*, 1990).

## Materials and methods

### *Source of specimens*

*Agardhiella subulata* specimens for the molecular biology procedures were obtained from shallow bays near Kure Beach, North Carolina (for location map and habitat descriptions see Kapraun, 1980) during February and March 1990. Material for cytophotometry was collected in the lower Chesapeake Bay near Gloucester Point, Virginia (for habitat descriptions see Humm, 1979) during October 1991.

### *Fixation and karyotype analysis*

Specimens were fixed at 24:00 h in 3:1 absolute ethanol: glacial acetic acid (Carnoy's) for 24 h, and transferred to 70% v/v ethanol for storage at 4 °C (Kapraun & Bailey, 1989). Branch tips with developing tetrasporangia were identified with a dissecting microscope, removed and transferred to 5% w/v EDTA for 1–2 h to soften tissue (Goff & Coleman, 1990). Small sections of cortical tissue containing tetrasporangia were separated from medulla, squashed on coverslips treated with Subbing solution (0.1 g gelatin, 0.01 g chromium potassium sulfate in 100 ml water) and stained with 2% w/v aceto-orcein (Kapraun & Bailey, 1989). Cystocarps were dissected from medullary tissue, transferred to EDTA and squashed on coverslips as above, and stained with DAPI as described for cytophotometry. Chromosome numbers were determined from late prophase-early metaphase meiotic figures in tetraspore mother cells, and from mitotic carposporangia. Instrumentation for photomicrographic documentation has been described previously (Kapraun & Shipley, 1990).

### *DNA Cytophotometry*

Algal material for cytophotometry was fixed and prepared for examination on subbed slides as described above. Chicken erythrocytes (RBC) used as the internal standard were collected from 18-day-old embryos in a solution of 19 mg sodium citrate in 0.5 ml distilled water, centrifuged, and supernatant removed. Cells were fixed in 9:1 methanol: formaldehyde (Gold & Price 1985) for 20 min, centrifuged, and the supernatant removed. The resulting pellet was rinsed in distilled water and centrifuged three times to remove fixative, and transferred to 70% v/v ethanol for storage at 4 °C (Gold & Price, 1985). The fixed RBC were smeared on a glass coverslip and air dried. Immediately upon drying, coverslips with algal material and RBC were rehydrated in 200 mM KCl (2.98 g KCl in 200 ml water) for 30 min (Goff & Coleman, 1984, 1985), stained in the dark for 30 min with 0.5 μg/ml 4′-6 diamidino-2-phenylindole (DAPI) (Sigma Chemical Co., St. Louis, Mo) in McIlvane's pH 4.1 buffer (Goff & Coleman, 1985, 1990; Gonzalez & Goff, 1989) and the slide sealed with nail polish (Goff & Coleman, 1984, 1985). Prepared slides were stored in a refrigerator overnight prior to examination.

Cytophotometric measurements are made with an Olympus BH2-RFK fluorescence microscope equipped with a high pressure mercury vapor lamp (HBO, 100W), and a 340-nm excitation filter and a 420-nm suppression filter which are specific for DAPI-bound emissions (Shihira-Ishikawa, 1984; Shihira-Ishikawa & Kuroiwa, 1984).

Microspectrophotometric data for 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). Comparison of frequency distributions ($I_f$) for 2C nuclear values obtained for gametophyte and tet-

rasporophyte tissue served as an internal standard. Nuclear DNA contents for *Agardhiella* specimens were estimated by comparing the $I_f$ values for G1- and G2-phase nuclei to the $I_f$ value of RBC standard (Johnson *et al.*, 1987; Tiersch *et al.*, 1989; Kapraun *et al.*, 1991): RBC $I_f$/*Agardhiella* $I_f = 2.4$ pg/$X$ pg. The number of algal nuclei examined in each sample and the number of samples (each including 3–5 plants) for each isolate were recorded (Table 1).

Proportionality of DAPI-staining between the RBC standard and algal nuclei was previously evaluated by comparison of $I_f$ values obtained with red algae having known nuclear DNA contents (Kapraun & Dutcher, 1991; Kapraun *et al.*, 1991).

*DNA isolation and purification*

Nuclear DNA was isolated from algal samples using a modification of a technique previously described for bacteria and vascular plants (Marmur, 1961; Bachmann & Price, 1977). Thirty grams of freshly thawed sample were homogenized in 5–10 ml of TES buffer (0.15 M tris hydroxymethylaminomethane hydrochloride (Tris), 0.05 M ethylenediaminetetra-acetic (EDTA), 0.1 M NaCl, and adjusted to pH 8.0) with a virtis tissue homogenizer. Aliquots of the homogenate were stored frozen (−90 °C) until use. Frozen samples were thawed, diluted with 20–30 ml TES buffer, and sodium dodecyl sulfate added to a final concentration of 1% w/v. The mixture was heated to 60 °C then frozen and thawed to lyse cells. Sodium perchlorate (5 M, pH 8.0) was added to the cell suspension to a final concentration of 1%. Chloroform-isoamyl alcohol (24:1) was added to an equal volume of cell suspension, gently mixed until homogeneous and centrifuged 14000 × **g**/10 min. The resulting supernatent was decanted and treated again with chloroform-isoamyl alcohol as above. The aqueous solution was further purified of polysaccharides with hy-

*Table 1.* Genome size (pg) for *Agardhiella subulata* nuclei after DAPI staining. Data standardized to the DNA level of chicken erythrocytes (RBC = 2.4 pg).

| | Number of slides | Number of nuclei | Nuclear genome size (pg) | | |
|---|---|---|---|---|---|
| | | | 2C | 4C | 8C |
| *Gametophyte* (♀) | | | | | |
| Spherical medulla | 7 | 343 | 0.9 ± 0.0 | | |
| Filamentous medulla | 6 | 283 | 0.9 ± 0.0 | | |
| Telophase nuclei | 1 | 28 | 0.8 ± 0.1 | | |
| *Carposporophyte* | | | | | |
| Carpospores | 5 | 232 | | 1.8 ± 0.1 | |
| *Tetrasporophyte* | | | | | |
| Spherical medulla | 4 | 190 | 1.0 ± 0.1 | | |
| Filamentous medulla | 1 | 4 | | 2.0 ± 0.0 | |
| Telophase nuclei | 1 | 5 | | 2.1 ± 0.2 | |
| *Tetrasporangia* | | | | | |
| Uninucleate | 2 | 21 | | | 3.8 ± 0.1 |
| Binucleate | 2 | 9 | | 1.9 ± 0.2 | |
| *Tetraspores* | 1 | 8 | 1.0 ± 0.1 | | |
| | | Mean ($\bar{X}$) | 0.9 ± 0.0 | 1.9 ± 0.1 | 3.8 ± 0.1 |

droxylapatite (Johnson, 1981). Nucleic acids in the aqueous phase are bound by mixing with hydroxylapatite under low salt conditions in 0.10 M sodium phosphate buffer (equimolar amounts of $Na_2HPO_4$ and $NaH_2PO_4$, pH 8.0). Addition of 0.5 M sodium phosphate buffer selectively elutes double stranded DNA. The resulting solution was mixed with 0.10 mg/ml DNase-free RNase (Sigma) (2 mg/ml on 0.15 M NaCl at pH 5.0, boiled 10 min, stored frozen) and allowed to digest for 1 h at 37 °C. Purified DNA was precipitated at room temperature with 0.1 volume of 2 M sodium acetate and 0.54 volumes of isopropyl alcohol. The precipitate was dried and dissolved in 0.12 M phosphate buffer.

*Determination of $C_o t$ curves*

Dialyzed algal and *E. coli* (standard) DNA were sheared by several passages through an 18 gauge needle. DNA samples, 50–60 $\mu$g/ml, were placed in stoppered cuvettes, and sealed with a thin layer of mineral oil to prevent evaporation. DNA was heat denatured to 100 °C, 1 °C/min in closed thermostatically controlled cuvettes of a Gilford Model 2600 spectrophotometer with thermoprogrammer. Thermal denaturation temperature ($T_m$) was determined from the hyperchromatic shift.

Denatured DNA was reassociated in 0.12 M phosphate buffer (0.18 M $Na^+$) at ($T_m$–25 °C) by perfusing the cell housing with ice water. The optical density after the temperature drop was allowed to stabilize and was taken to represent 0% renaturation. Renaturation was monitored continuously for 24 h or until the optical density reading stabilized. 100% renaturation corresponded to the optical density of DNA before the hyperchromatic shift.

Renatured DNA was plotted log $C_o t$ versus percent reassociation (Britten & Kohne, 1968). Reassociation data were analysed with a computer program generating a best fit non-linear regression representing three second order components. DNA of *E. coli* ($4.2 \times 10^6$ bp, $C_o t$ 1/2 pure 3.23) was used as a standard to convert $C_o t$ values into nucleotide base pairs or complexity of each component (Lewin, 1990).

## Observations and discussion

*Karylogy*

Aceto-orcein staining revealed the presence of 22 bivalents in several late prophase-early metaphase (diakinesis) meiotic nuclei in *Agardhiella subulata* tetrasporocytes (Fig.1). The haploid chromosome complement of $1N = 22$ includes 20 dot-like chromosomes as well as two which are conspicuously larger (Fig. 1). Mitotic nuclei in carposporangia visualized following DAPI staining had chromosome complements of $2N = 44$ (Fig. 2). Speculation concerning a basic chromosome complement in the Soliariaceae would be premature as pub-

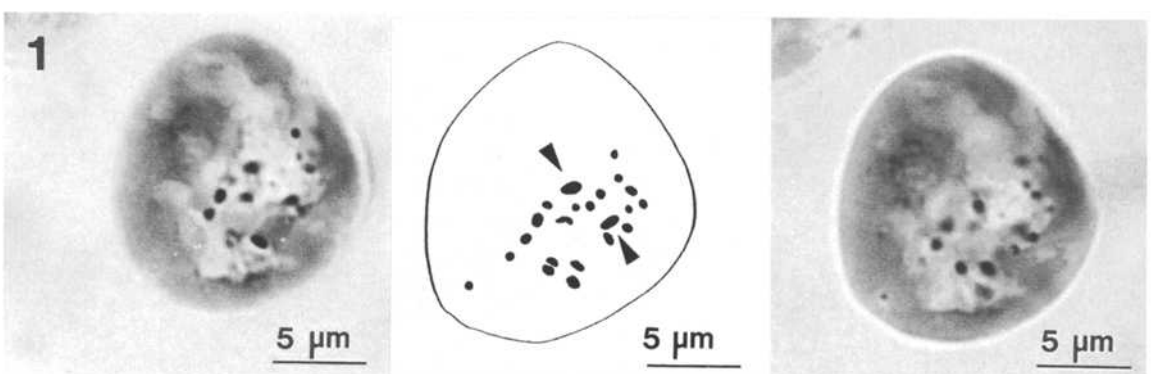


*Fig. 1.* Optical sections and composite drawing of early metaphase (diakinesis) meiotic nucleus following aceto-orcein staining showing 22 bivalents. Arrows indicate presence of two larger chromosomes.

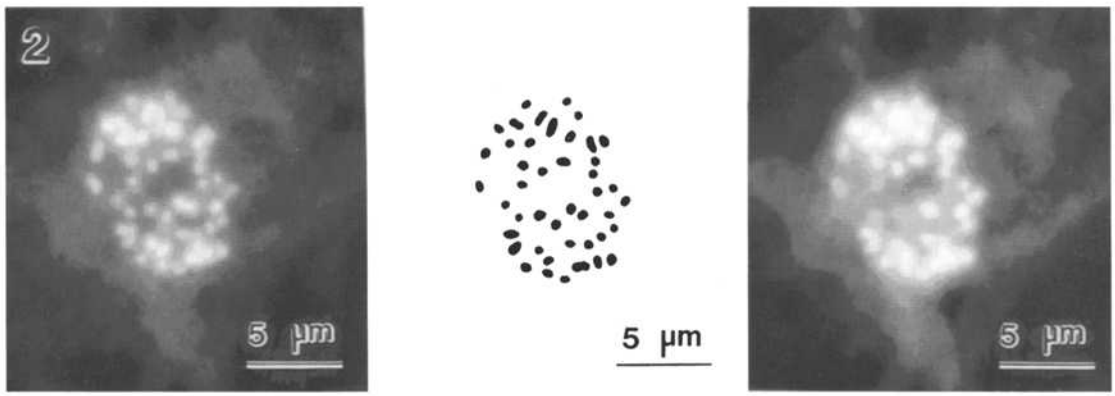


*Fig. 2.* Optical sections and composite drawing of mitotic carposporangial nucleus following DAPI staining and visualization with UV illumination showing $2N = 44$.

lished reports are restricted to *Anatheca montagnei* Schmitz, $N = 7–8$ (Bodard, 1966) and *Turnerella pennyi* (Harv.) Schmitz, $N = 18–20$ (South *et al.*, 1972).

*Microspectrophotometry*

Microspectrophotometry with DNA-localizing fluorochromes has been used previously to estimate the relative DNA contents of marine red algae (for recent review see Goff & Coleman, 1990). These techiques can be used for DNA quantification as well, providing that specific conditions are met (Kapraun & Shipley, 1990; Kapraun & Dutcher, 1991). In the present study, DAPI staining with the protocol developed by Goff & Coleman (1984, 1985, 1990) resulted in reproducible, stable nuclear fluorescence with only slight cytoplasmic interference (Fig. 3).

Variations in nuclear DNA levels associated with ploidy level differences in gametophytic and tetrasporophytic phases of red algae have been demonstrated with microspectrophotometry (Goff & Coleman, 1990; Kapraun & Dutcher, 1991). In the present study, mean fluorescence ($I_f$) values for telophase (replicated) G2-phase nuclei in female gametopytes closely approximate 50% of the values for telophase (4 C) G2-phase nuclei in tetrasporophytes (Fig. 4).

Extrapolated nuclear DNA contents reveal a 2 C genome size of 0.9 pg for *A. subulata* (Table 1). Mean DNA values for gametophyte medullary cells indicate that these nuclei have 2 C DNA contents corresponding to G2-phase haploid levels. While tetrasporophyte medullary filaments have the expected 4 C DNA contents indicative of G2-phase diploid nuclei (Kapraun & Bailey, 1989), spherical medullary cells typically have 2 C DNA contents corresponding to G1-phase (unreplicated) diploid levels (Table 1).

Reduction in nuclear DNA levels in tetraspore

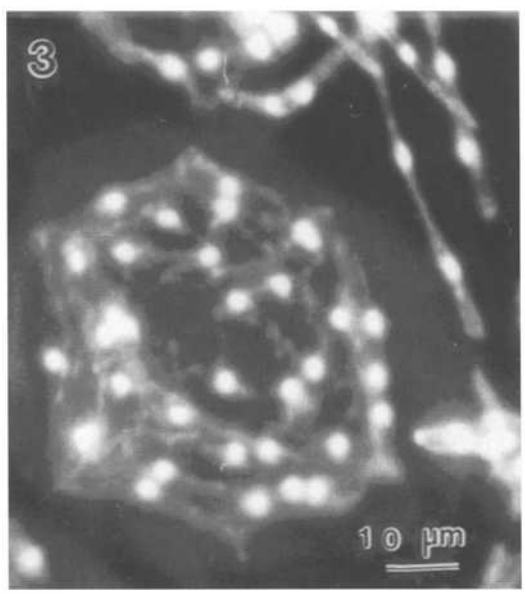


*Fig. 3.* Haploid interphase nuclei in female gametophyte filamentous and spherical medullary cells following DAPI staining and visualised with UV illumination.

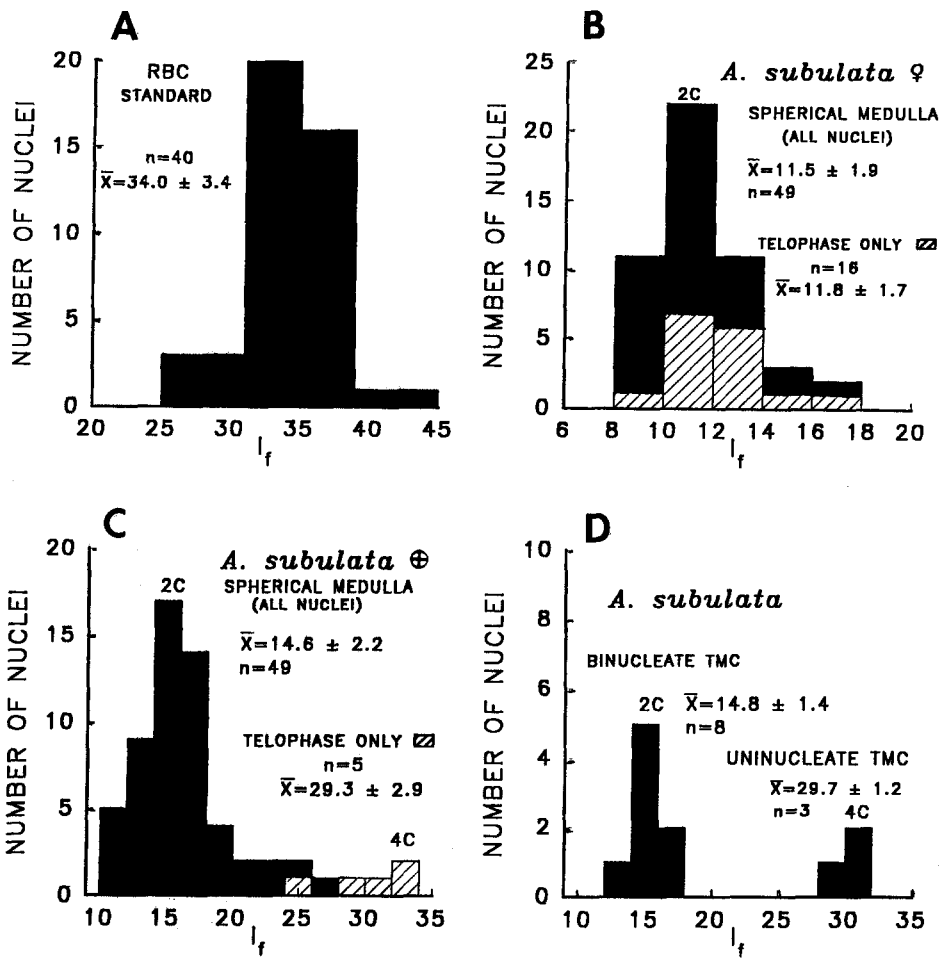


*Fig. 4.* Comparison of frequency distributions of relative DNA values for nuclei after DAPI staining. $n$ = number of nuclei observed, $I_f$ = fluorescence intensity mean ± SD. (A) Chicken erythrocytes (RBC) standard, (B) 2C level nuclei in *Agardhiella subulata* female gametophyte. $I_f$ values for all nuclei observed and telophase nuclei only indicated, (C) 2C and 4C level nuclei in *Agardhiella subulata* tetrasporophyte. $I_f$ values for all nuclei observed and telophase nuclei only indicated, (D) $I_f$ values for unicucleate ($2N$) tetrasporocytes (tetraspore mother cells) and binucleate (meiosis II) nuclei in *Agardhiella subulata.*

mother cells (tetrasporocytes) associated with meiosis has previously been demonstrated red algae (Goff & Coleman, 1984, 1985, 1990; Gonzalez & Goff, 1989). In these species, replicated tetrasporocyte nuclei were found to have DNA contents corresponding to 4 C levels. In *Agardhiella subulata*, DNA contents of uninucleate tetrasporophyte medullary cells indicate that these pre-meiotic nuclei have 8 C DNA levels (Table 1). Since tetrasporocytes in diakinesis have 22 bivalents (Fig. 1) and mitotic nuclei in gametophytic medullary filaments were observed to have 18–22 chromosomes, it appears that the elevated DNA level in tetrasporocytes is not associated with increased numbers of chromosomes (polyploidy) as reported in some red algal apical cells (Goff & Coleman, 1990).

### *Reassociation kinetics*

DNA Reassociation operates on the principle that separated complementary strands of purified DNA recognize each other, and under appropriate conditions, specifically reassociate. This phenomenon provides a useful tool for determining

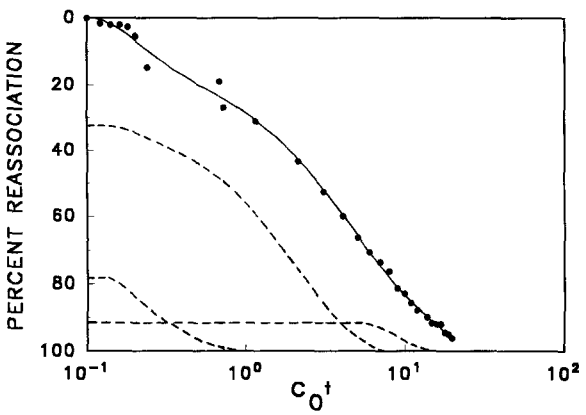


*Fig. 5.* Reassociation kinetics of whole cell DNA from *Agardhiella subulata*. $C_0t$ (moles of nucleotides per litre × s), DNA concentrations were 50–60 μg DNA $ml^{-1}$ in 18 M $Na^+$ at 60 °C. Solid lines: best non-linear regression analysis of data. Dashes lines: predicted second order kinetic components, correlation coefficient 0.997.

the presence and complexity of repetitive sequences (Britten & Kohne, 1970). $C_0t$ 1/2, when reassociation is half complete, is proportional to the complexity of the DNA sequences present. DNA reassociation follows the time course of a single second order reaction. If repetitive sequences are present, up to three second order components could be present in one reassociation curve. The rapidly reassociating fraction represents the highly repetitive sequences, 1000–10000 copies. The intermediate fraction represents the mid-repetitive sequences, 10–1000 copies. The final fraction represents the unique sequences or single copy.

Requirements for efficient, reproducible reassociation reactions have been specified previously (Marmur *et al.*, 1963; Dutcher *et al.*, 1990). In the present study, reassociation kinetics for *Agardhiella subulata* were carried out under moderately stringent conditions (Tm–25 °C, 0.18 M $Na^+$) Computer analysis of DNA reassociation revealed the presence of three second order components (correlation coefficient.997), a fast fraction representing highly repetitive sequences (22%), an intermediate fraction of mid-repetitive sequences (68%) and a slow fraction of unique or single copy sequences (10%) (Fig. 5). Complexities and copy numbers for each fraction are summarized in Table 2. Although the repetitive fraction may contain some chloroplast DNA as whole cell DNA was used, significant chloroplast contamination is not indicated as double melt profiles were not observed during DNA denaturation.

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 component $C_0t$ 1/2 (Dutcher *et al.*, 1990). Consequently, genome size estimates from cytophotometry were used to extrapolate a complexity of $8.2 \times 10^8$ bp using the $0.913 \times 10^9$ bp/pg (Britten & Davidson, 1971). The magnitude of the *A. subulata* genome becomes apparent when compared to the angiosperm *Arabidopsis thaliana* (L.) Heynhold with $7 \times 10^7$ bp (Leutwiler *et al.*, 1984).

Reassociation kinetics have been used successfully with a wide variety of vascular plants to examine their genome organization (Bachmann & Price, 1977; Leutwiler *et al.*, 1984). Only recently have these techniques been applied to seaweeds. Published results indicate that both green (Olsen *et al.*, 1987; Bot *et al.*, 1989) and brown algae (Stam *et al.*, 1988) have genomes with unique and

*Table 2.* Kinetic analysis of *Agardhiella subulata* DNA reassociation.

| Isolate & kinetic component | Fraction | Complexity (base pairs) | Repetition frequency | Genome size (pg) |
|---|---|---|---|---|
| *Agardhiella subulata* | | | | 0.90 |
| Fast | 0.22 | $3.2 \times 10^5$ | 570 | |
| Intermediate | 0.68 | $1.7 \times 10^6$ | 330 | |
| Slow | 0.10 | $8.2 \times 10^7$ | 1 | |

repetitive segments. The only red algae investigated previously, *Gracilaria* and *Gracilariopsis* (Gracilariales), demonstrated significant inter- and intra-specific variation of genome organization and complexities with repeated sequences comprising 13–95% (Dutcher *et al.*, 1990). In these Gracilariales, a positive correlation was suggested between nuclear genome profiles characterized by large repetitive components and biosynthesis of commercial quality gels. Specifically, it was hypothesized that these taxa have an abundance of (repetitive) gene loci for enzymes associated with polymerization of galactan subunits.

*Agardhiella subulata*, like other species of Soliariaceae investigated, contains kappa family carrageens (Asare, 1980; Cheney *et al.*, 1987; Chopin *et al.*, 1990). It would be of great interest to obtain comparative genome organization and complexity information for these taxa, but especially for *Kappaphycus alvarezii* (Doty) Doty (= *Euchuema alvarezii* Doty; *E.* 'cottonii-types') which most closely approaches the idealized kappa carrageenan structure (Craigie, 1990).

In summary, the present investigation of the carrageenophyte *Agardhiella subulata* demonstrates that cytogenetic, cytophotometric and molecular biology techniques used successfully with the Gracilariales can be applied, with little modification, to the commercially important members of the Solieriaceae (Gigartinales). It is suggested that information for characterization and quantification of nuclear genomes provides an invaluable basis for genetic improvement programs.

## Acknowledgements

The authors gratefully acknowledge financial support for this research from the National Science Foundation (NSF-RUI-88-17846) and the North Carolina Center for Biotechnology (NCBC 9010-IDG-1001). This paper represents contribution number 50 from the Center for Marine Science Research, UNC-Wilmington.

## References

Abbott IA, Cheney DP (1982) Commercial uses of algal products: introduction and bibliography. In: Rosowski JR, Parker BC (eds), Selected Papers in Phycology II. Phycol. Soc. Amer., Lawrence, Kansas, 779–787.

Asare SD (1980) Seasonal changes in sulphate and 3,6-anhydrogalactose in phycocolloids from two red algae. Bot. mar. 23: 595–598.

Bachmann K, Price HJ (1977) Repetitive DNA in Cichorieae (Compositae). Chromosoma (Berl.) 61: 267–275.

Bodard M (1966) Sur le développement des tétrasporocystes d'*Anatheca montagnei* Schmitz (Soliériacees, Gigartinales). Bull. Inst. Fond Afrique Noire 28: 867–894.

Bot PVM, Stam WT, Boele-Bos SA, Hoek C van den, Delden W van (1989) Biogeographic and phylogenetic studies in three North Atlantic species of *Cladophora* (Cladophorales, Chlorophyta) using DNA-DNA hybridization. Phycologia 28: 159–168.

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

Britten RJ, Kohne DE (1968) Repeated sequences in DNA. Science 161: 529–540.

Britten RJ, Kohne DE (1970) Repeated segments of DNA. Sci. Am. 222: 24–31.

Cheney DP (1988) Genetic engineering in seaweeds: applications and current status. Nova Hedwegia 83: 22–29.

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 DF (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. Connecticut Sea Grant College Program, CT-SG-89-07, Groton, CT, 15–26.

Cheney DP, Luistro AH, Bradley PM (1987) Carrageenan analysis of tissue cultures and whole plants of *Agardhiella subulata*. Hydrobiologia 151/152: 161–166.

Chopin T, Hanisak MD, Koehn FE, Mollion S (1990) Studies on carrageenans and effects of seawater phosphorus concentration on carrageenan content and growth of *Agardhiella subulata* (C. Agardh) Kraft et Wynne (Rhodophyceae, Solieriaceae). J. Applied Phycology 2: 3–16.

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

Craigie JS (1990) Cell Walls. In: Cole KM, South RG (eds), Cambridge University Press, New York, 211–157.

Dawes CJ (1987) The biology of commercially important tropical marine algae. In: Bird KT, Benson PH (eds), Sea-

weed Cultivation for Renewable Resources. Developments in Aquaculture and Fisheries Science, 16. Elsevier, Amsterdam, 155–190.

Doty MS (1985) *Eucheuma alvarezii* sp. nov. (Gigartinales, Rhodophyta) from Malaysia. In: Abbott IA, Norris JN (eds), Taxonomy of Economic Seaweeds with Reference to some Pacific and Caribbean Species. California Sea Grant College Program, T-CSGDP-011, San Diego, CA, 37–45.

Doty MS, Alvarez VB (1975) Status, problems, advances and economics of *Eucheuma* farms. Mar. tech. Soc. J. 9: 30–35.

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

Glenn EP, Doty MS (1981) Photosynthesis and respiration of the tropical red seaweeds, *Eucheuma striatum* (Tambalang and Elkhorn varieties) and *E. denticulatum*. Aquatic Bot. 10: 353–364.

Goff LJ, Coleman AW (1984) Elucidation of fertilization and development in a red alga by quantitative DNA microspectrofluorometry. Developmental Biol. 102: 173–194.

Goff LJ, Coleman AW (1985) The role of secondary pit connections in red algal parasitism. J. Phycol. 21: 483–508.

Goff LJ, Coleman AW (1990) DNA: microspectrofluorometric studies. In: Cole KM, Sheath RG (eds), Biology of the Red Algae. Cambridge University Press, New York, 43–72.

Gold MA, Price HJ (1985) Genome size variation among North American minnows (Cyprinidae). I. Distribution of the variation in five species. Heredity 54: 297–305.

Gonzalez MA, Goff LJ (1989) The red algal epiphytes *Microcladia coulteri* and *M. californica* (Rhodophyceae, Ceramiaceae). I. Taxonomy, life history and phenology. J. Phycol. 25: 545–558.

Humm HJ (1979) The Marine Algae of Virginia. Charlottesville, Va, University of Virginia Press, 263.

Johnson JL (1981) Genetic Characterization. In: Gerhardt P (ed.), Manual of Methods for General Bacteriology. Amer. Soc. Microbiol., Washington, DC, 450–472.

Johnson OW, Utter FM, Rabinovitch PS (1987) Inter-species differences in salmonid cellular DNA identified by flow cytometry. Copeia 4: 1001–1009.

Kapraun DF (1980) An Illustrated Guide to the Marine Algae of Coastal North Carolina I. Rhodophyta. University of North Carolina Press, Chapel Hill, 206.

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

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, Shipley MJ (1990) Karyology and nuclear DNA quantification in *Bryopsis* (Codiales, Chlorophyta) from North Carolina, USA. Phyclogia 29: 443–453.

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.

Leutwiler LS, Hough-Evans BR, Meyerowitz EM (1984) The DNA of *Arabidopsis thaliana*. Mol. Gen. Genet. 194: 15–23.

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

Marmur J (1961) A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J. Mol. Biol. 3: 208–218.

Marmur J, Falkow S, Mandel M (1963) New approaches to bacterial taxonomy. Annu. Rev. Microbiol. 17: 329–372.

McHugh DJ (1984) Marine phycoculture and its impact on the seaweed colloid industry. Hydrobiologia 116/117: 351–354.

Olsen JL, Stam WT, Bot PVM, Hoek C van den (1987) scDNA-DNA hybridization studies in Pacific and Caribbean isolates of *Dictyosphaeria cavernosa* (Chlorophyta) indicate a long divergence. Helgoländer Meeresunters. 41: 377–383.

Shihira-Ishikawa I (1984) Chromosome behavior in the primary nucleus of *Acetabularia calyculus* as revealed by epifluorescence microscopy. Protoplasma 12: 27–34.

Shihira-Ishikawa, I Kuroiwa T (1984) Morphological transition of the nucleus during the whole life cycle of *Acetabularia calyculus* Quoy et Gaimard. Jap. J. Phycol. 32: 147-157.

South GR, Hooper RG, Irvine LM (1972) The life history of *Turnerella penneyi* (Harv.) Schmitz. Br. phycol. J. 7: 221–233.

Stam WT, Bot PVM, Boele-Bos SA, Rooij JM, van den Hoek C (1988) Single copy DNA-DNA hybridizations among five species of *Laminaria* (Phaeophyceae): phylogenetic and biogeographic implications. Helgoländer Meeresunters. 42: 251–267.

Tiersch TR, Chandler RW, Kallman KD, Wachtel SS (1989) Estimation of nuclear DNA content by flow cytometry in fishes of the genus *Xiphophorus*. Comp. Biochem. Physiol. 94B: 465–468.

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