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DNA base composition heterogeneity in some agarophytes (Gracilariales, Rhodophyta) from Mexico and the Philippines

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Abstract

Estimates of nuclear DNA base composition by determination of thermal denaturation temperatures (T_m) indicate guanine + cytosine (G + C) levels of 35.4–46.8% for ten species of the Gracilariaceae, representing the genera *Gracilaria* and *Hydropuntia*. T_m values were found to be reproducible with variation among most samples and replicates of less than 1 °C and 2 mol%. Interspecific variation in G + C values was less than 11.4% among *Gracilaria* species. Calculation of intragenomic base pair composition distribution based on mid-resolution thermal denaturation (Δ 1 °C/min with 4s interval ΔH and dT logging) indicated an inverse relationship between maximum similarity values and taxonomic rank. Intraspecific (population level) maximum similarity (homology) values were estimated to range from 79–90% in *Gracilaria tikvahiae* (4 isolates). Interspecific values of 46–69% were found in 13 species of *Gracilaria*. Nucleotide distribution similarity values for the Gracilariaceae are compared with previous information for genome organization and complexity, genome size and karyotype patterns.

Introduction

Members of the family Gracilariaceae, especially species of *Gracilaria* and *Gracilariopsis*, are among the world's most valuable agarophytes (Santelices & Doty, 1989). Considerable research has been directed toward understanding the phylogenetic relationships of these taxa, and in developing a systematics scheme that reconciles morphological (phenotype) and biological (genotype) species concepts (Guiry, 1992; Bird, 1995). A review of current information suggests a paradox in that the genetic discontinuities in these seaweeds which are exaggerated at both the inter- and intraspecific levels are not reflected by fixed diagnostic features, primarily as a result of the plastic development of their relatively simple morphologies (Santelices & Varela, 1993).

Genetic discontinuities are reflected by intraspecific (population level) infertility which may be as high as 40% in some species (Richard et al., 1993). Molecular genetic investigations of the 18S rDNA sequences, which are typically highly conserved, indicate intergeneric differences of as much as 6.8% in the Gracilariaceae (Bird et al., 1992, 1994). This amount is an order of magnitude greater than values reported in brown seaweeds in the order Laminariales (Saunders et al., 1993). Similarily, comparison of intragenomic base pair composition distributions of DNA indicate intraspecific (population level) maximum nuclear genome similarity values of 78–89% in three populations of *Gracilaria tikvahiae* McLachlan. Interspecific values have even wider ranges: 53–62% in five species of *Gracilaria*, and 73–81% in three species of *Gracilariopsis* (Kapraun et al., 1993a). Reassociation kinetics data confirm the presence of a wide range of repetitive DNA sequence amounts (13–95%) as well in species of both *Gracilaria* and *Gracilariopsis* (Dutcher et al., 1990a; Kapraun et al., 1993b; Lopez-Bautista & Kapraun, 1995). Thus, the Gracilariaceae present profound systematics problems as intraspecific genetic

differences can be as great as interspecific differences reported in vascular plants, while morphological simplicity and plasticity mask the phenotypic discontinuities which taxonomists rely on to characterize species (Guiry, 1992).

The present investigation was initiated to expand our knowledge of nuclear genome profiles in the Gracilariaceae by providing data for additional taxa from the Philippines and the east coast of Mexico. Specifically, we have analyzed nine more species of *Gracilaria* as well as a species of *Hydropuntia*. The generic name *Hydropuntia* is retained as proposed by Wynne (1986) even though the relationship of this entity to *Gracilaria* remains uncertain (Bird et al., 1992; Bird, 1995). This information can be used to estimate maximum possible genome homology between genetic populations and, thus, predict the proportion of shared (ancestral) genome at population, species and genus taxonomic levels (King & Ingrouille, 1987a, 1987b). It is hoped that these studies will be useful at the applied level by aiding in the selection of appropriate genetic engineering procedures for cultivar development (Law, 1983; Kapraun et al., 1993b) as well as at the theoretical level by promoting a better understanding of the relationship between the shared nuclear genome common to all members of a species, and its phenotypic range and reproductive limits.

Materials and methods

Source of specimens

Ten species of Gracilariaceae were used for extraction of nuclear DNA in the present study (Table 1). Specific collection sites for specimens are listed elsewhere (Kapraun et al., 1996; Lopez-Bautista & Kapraun, 1995). The samples were quick-dried in silica gel and then stored at −20 oC.

DNA isolation and G + C determination

Detailed procedures for isolation and purification of nuclear DNA for algal samples have been specified previously (Dutcher et al., 1990b; Kapraun et al., 1992). Algal DNA samples, with an *E. coli* DNA standard, were heated to 100 °C, 1°C/min, in a closed thermostatically controlled cuvette of a Gilford model 2600 spectrophotometer equipped with a thermoprogrammer. Absorbance and temperature signals were logged at 4 s intervals. Formulation (Dutcher et al., 1990b) and linearity of the standard equation (Freshwater et al., 1990) are available elsewhere. Thermal denaturation temperatures (T_m's) were determined from the hyperchromatic shift following thermal denaturation. Multiple replicates of each sample (Table 2) were used to calculate G + C mol% means ± SD with the derived standard equation (Dutcher et al., 1990a).

Table 1. Source of specimens.

Species	
Gracilaria caudata J. Agardh	Punta Piedras (Laguna Madre) MEX
Gracilaria cervicornis (Turner) J. Agardh	Ciudad Madero, MEX
Gracilaria divaricata Harvey	Punta Piedras (Laguna Madre) MEX
Gracilaria eucheumoides Harvey	Bolinao (Pangasinan) RP
Gracilaria firma Zhang et Xia	Bolinao (Pangasinan) RP
Gracilaria salicornia (C. Agardh) Dawson	Mactan Is. (Cebu) RP
Gracilaria tikvahiae McLachlan	Punta Piedras (Laguna Madre) MEX
Gracilaria sp. 1	Baguey, Cagayan (Luzon) RP
Gracilaria sp. 2	Sorsogon RP
Hydropuntia fastigiata (Zhang et Xia) Wynne	Bolinao (Pangasinan) RP

MEX = Mexico; RP = Republic of the Philippines.

Nucleotide compositional profile determination

The base pair compositional %G + C distribution was derived from the thermal denaturation curve of DNA, with the absorbance plotted versus temperature (DeLey, 1969). This value can be expressed as ΔT (°C) or extrapolated to ΔG + C (within the range 22–63%) (Table 2) by determining mean G + C mol% at the temperature coinciding with 15.9 and 84.1% hyperchromicity, respectively. The asymmetric nature of denaturation curves resulting from the non-Gausian distribution of %G + C in the genome has been discussed previously (Kapraun et al., 1993a). The degree of asymmetry is given by the ratio O_l/O_r which expresses the standard deviation for the AT-rich and GC-rich genome components, respectively (Figure 1). Thus, the greater the O_l/O_r ratio values, the more heterogenous is the compositional %G + C distribution (Table 2).

Table 2. Compositional G + C mol% distributions of Gracilariales nuclear genomes. Midpoint of denaturation (T_m) and G + C mol% mean ± SD ($\overline{GC}$) values are averages of all samples. Other data were derived from a single representative denaturation profile. ΔT (°C) values represent a range of temperatures over which 17–83% of hyperchromicity occurred, $\overline{G+C}$ = G + C mol% mean ± SD, $\Delta G+C = [(O_r+G+C)-(\overline{G+C})]+[(\overline{G+C})-(O_lG+C)]$, $O_l/O_r = O_l\overline{G+C}/O_r\overline{G+C}$.

Species	Number of samples	T_m ± SD (°C)	ΔT (°C)	$\overline{G+C}$	ΔG + C	O_l/O_r (%G + C)
Gracilaria						
G. caudata	2	85.7±0.7	15.2	41.7±0.6	34.6	2.2
G. cervicornis	3	86.0±0.1	9.7	42.3±1.1	22.1	0.6
G. divaricata	3	86.4±0.3	10.8	43.2±0.6	24.6	1.7
G. eucheumoides	2	85.5±0.7	6.8	41.2±1.6	15.5	4.2
G. firma	2	84.2±0.4	11.7	38.2±0.9	26.7	1.4
G. salicornia	5	84.3±0.0	9.4	38.4±0.6	21.4	0.6
G. tikvahiae	3	87.6±0.4	9.5	46.0±0.8	21.6	1.5
G. sp. 1	3	87.9±0.6	8.2	46.8±1.3	18.7	1.5
G. sp. 2	2	82.9±0.1	11.0	35.4±0.1	25.1	1.3
Hydropuntia						
H. fastigiata	3	86.2±0.1	11.7	42.8±0.3	26.7	2.1

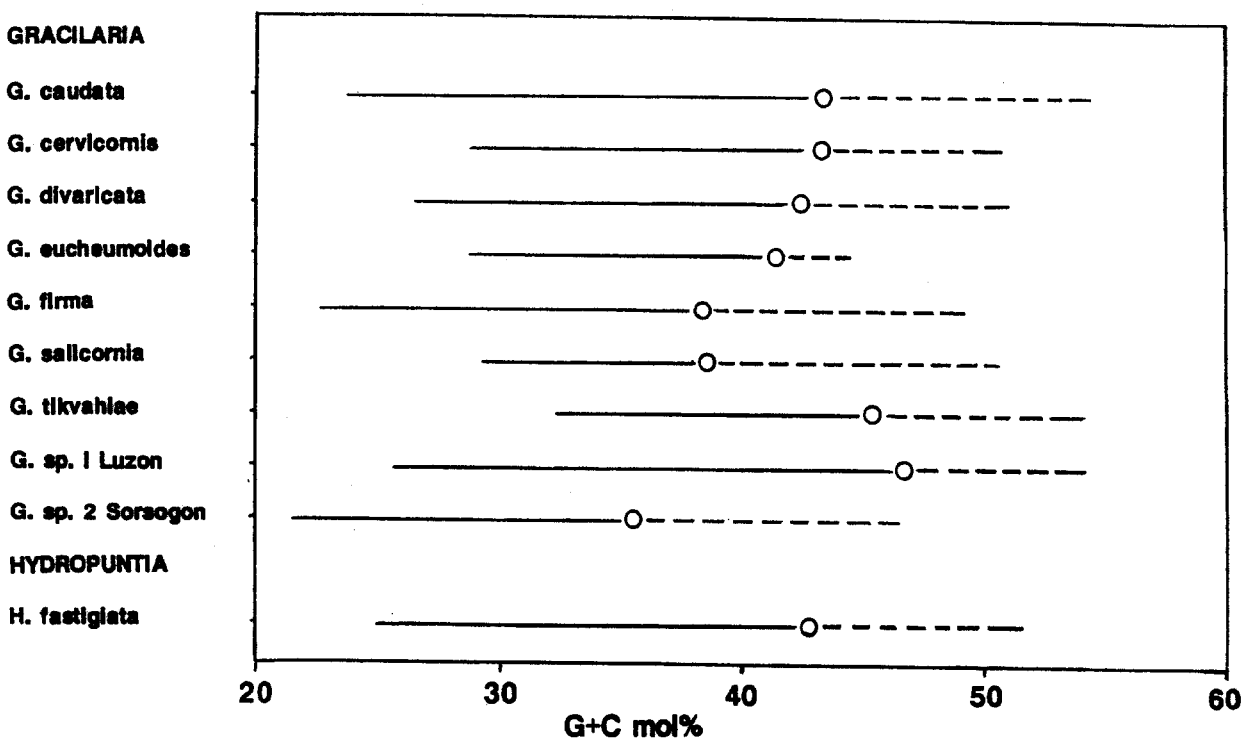

Figure 1. Thermal denaturation midpoint (T_m) mean and standard deviation (SD) ranges for O_l AT-rich (solid line) and O_r GC-rich (dashed line) genome components. Calculations are based on a single representative denaturation profile.

Table 3. Similarity of DNA base composition distribution derivative profiles between 16 species and populations of *Gracilaria* and the composite *Gracilaria* genome.

Species	% similarity
G. caudata	52
G. cervicornis	46
G. divaricata	59
G. eucheumoides	69
G. firma	59
G. salicornia	61
G. tikvahiae	80
Gracilaria sp. 1	66
Gracilaria sp. 2	54

An accurate representation of the proportions of sequences differing in base composition was produced by adjusting first derivative thermal denaturation profiles (Figure 2) to account for the relative contribution to hyperchromicity of the dissociation of A + T and G + C base pairs at 260 nm (King & Ingrouille, 1987a, 1987b; Szécsi & Dobrovolszky, 1985). Data for these profiles provide an estimate of maximum possible nucleotide base pair composition homology (Szécsi & Dobrovolszky, 1985). Theoretical maximum intra- and interspecific homology for algal samples was determined by comparing individual distribution areas with composites of the appropriate taxa (Figure 3). Calculated maximum theoretical homologies (Figure 4) include previously published data for three species of *Gracilariopsis: G. bailinae* Zhang et Xia (as *G. heteroclada* Zhang et Xia), *G. tenuifrons* (Bird et Oliveira) Fredericq et Hommersand, and North Carolina specimens of a seaweed referred to *G. lemaneiformis* (Bory) Dawson, Acleto et Foldvik which 18S rDNA data have subsequently shown probably represents an undescribed species (Bird et al., 1994).

Results

UV absorption data indicated yields of approximately 100 μg mL^{-1} DNA from 30 g algal sample. Hyperchromicity values excluded the presence of significant UV impurities or chloroplast and mitochondrial contamination (Kapraun et al., 1993a). Nuclear DNA base pair composition estimates from thermal denaturation temperatures (T_m) range from 35.4–48.6 mol% guanine-cytosine (G + C) in these red algae (Table 2). Most T_m values had variations of less than 0.6 °C among samples resulting in G + C mol% estimate variations of less than 1.4% among most replicates.

An estimate of intragenomic base composition heterogeneity was obtained from the temperature interval (ΔT) between 17 and 83% of the absorbance rise (Figure 2). Most of the ten species of the Gracilariaceae investigated were characterized by one of two denaturation profiles (Figure 3): 1) highly asymmetric (plurimodal) and skewed toward the A–T rich (O_l) side (e.g. *Gracilaria eucheumoides*), or 2) symmetric with equivalent O_l and O_r sides (e.g. *Gracilaria* sp. 2 from Sorsogon). Only *Gracilaria salicornia* had an asymmetric profile skewed toward the GC-rich (O_r) side, Comparison at AT side and GC side heterogeneity (Table 2) with the ratio of O_l/O_r indicates substantial (>2.0) AT side skewing in only 3 of the ten species investigated (e.g. *Gracilaria eucheumoides*).

Estimates of intragenomic base composition distributions are summarized in Figure 4. Intraspecific comparison of the maximum similarity values for nucleotide base pair sequences in *Gracilaria tikvahiae* from Mexico with previous data for three *G. tikvahiae* isolates revealed a range of 79–90%. It should be noted that the four similarity values approximate the range extremes rather than variations around the mean. Data for the nine species of *Gracilaria* in the present study and the five species previously investigated (Kapraun et al., 1993a) indicate an interspecific range of 46–69%. Previously determined variations for *Gracilariopsis* (Kapraun et al., 1993a, 1993b) are included for comparison.

Discussion

Results of the present study widen slightly the range of reported G + C values reported in the Gracilariaceae from 9.5 to 11.4 mol%. The mean G + C mol% values for *Gracilaria* (n = 17) of 42.6, *Gracilariopsis* (n = 3) of 44.3 and *Hydropuntia* (n = 1) of 42.8 appear to have little values as exclusionary taxonomic criteria. As narrower ranges and lower mean G + C values are associated with phylogenetic advancement (Stanier et al., 1986; Sueoka, 1961), the relatively wide range of values and high mean G + C% estimates for the Gracilariaceae ($\bar{X}$ = 42.9) and all Rhodophyta ($\bar{X}$ = 41.3) are not surprising (Kapraun et al., 1993a).

Previously reported intraspecific variation in G + C values of less than 3% among three geographic populations of *Gracilaria tikvahiae* was not altered by the

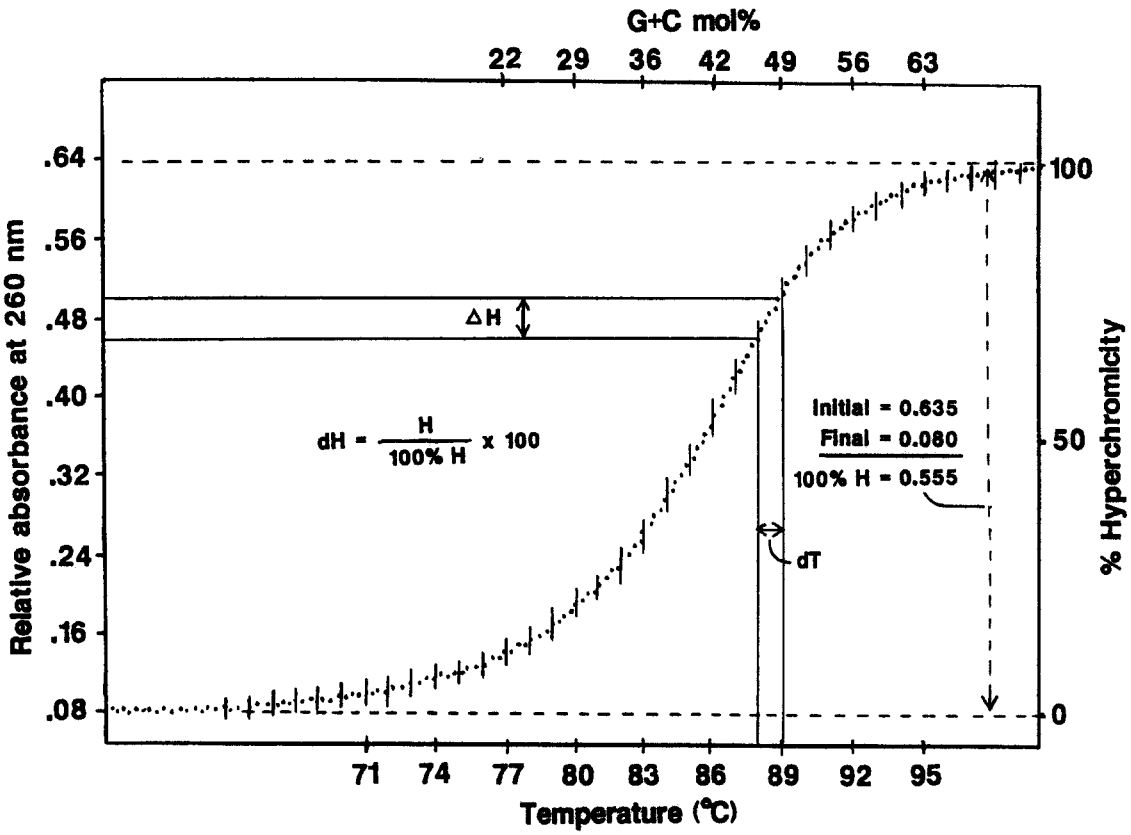

Figure 2. Melting curve of *Gracilaria divaricata* illustrating procedures for quantifying denaturation profiles. dH (% of total hyperchromicity) is calculated from the total hyperchromicity (H) or change in relative absorbance (ΔH) at 1 °C intervals (ΔT). In this example, the dT interval 88–89 °C corresponds to a ΔH of 0.035 relative absorbance, and H = 0.635–0.080 or 0.555. Thus, dH = $\frac{0.035}{0.555} \times 100 = 6.3$.

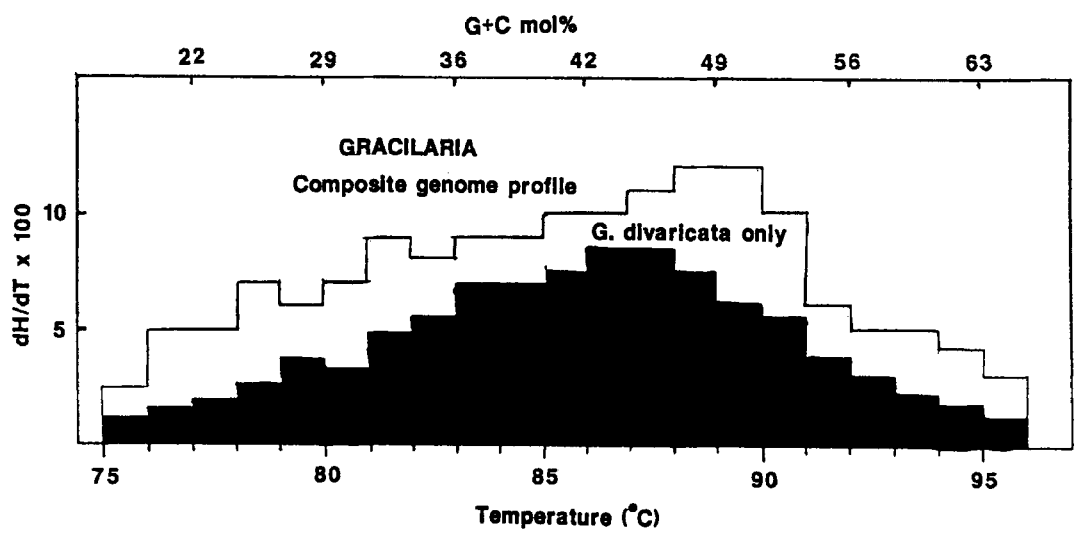

Figure 3. Superimposed denaturation profiles for *Gracilaria divaricata* (black) and composite *Gracilaria* (white) representing 16 species and populations of *Gracilaria*. Thus, the outlined area corresponds to the maximum DNA base composition distribution for the genus. In this example, similarity of derivative profiles between *G. divaricata* and the total *Gracilaria* genome = $\frac{182 \text{ squares}}{311 \text{ squares}} \times 100 = 59\%$.

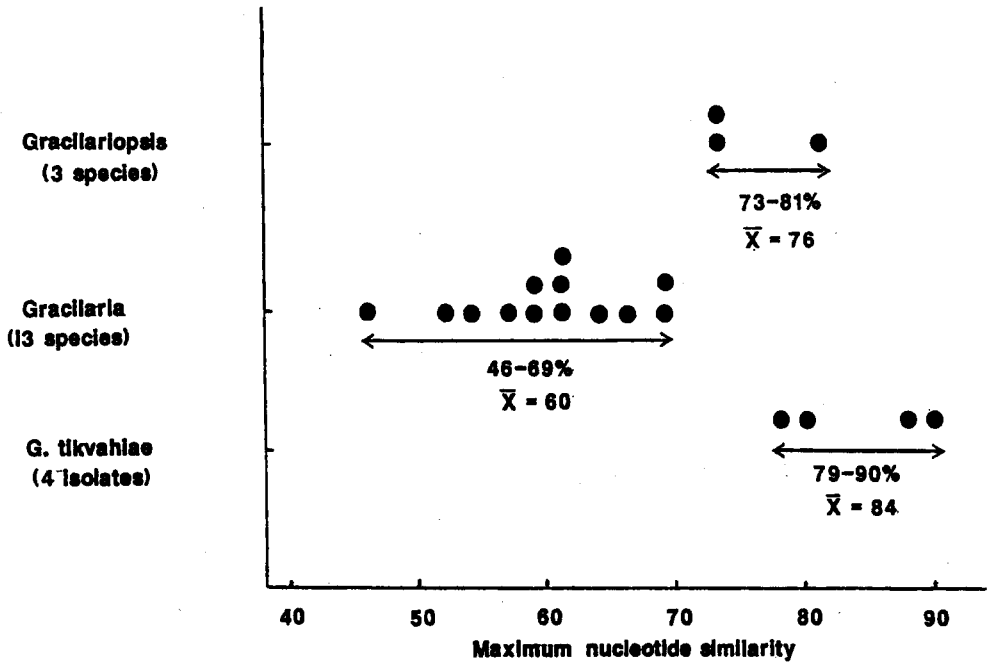

Figure 4. Intra- and interspecific range of maximum similarity values for nucleotide base pair sequences in some Gracilariales. Data for *Gracilaria tikvahiae* include four geographic populations: Nova Scotia, North Carolina, Florida (Kapraun et al., 1993a) and Mexico (Lopez-Bautista & Kapraun, 1995); *Gracilaria* includes nine species from the present study as well as seven additional species and populations (Kapraun et al., 1993b).

addition of data for a *G. tikvahiae* isolate from Mexico. Similarly, the 12.7 mol% G + C range found in five species of *Gracilaria* was unchanged by inclusion of data for the nine species in the present study.

Nucleotide sequences of all genomes are subject to compositional constraints and are the result of environmental pressures (see Bernardi & Bernardi, 1986 for a discussion of theoretical implications). Consequently, nucleotide base pair compositions which significantly vary from an ancestral value must have resulted from substantial selective pressure. In the Gracilariaceae, *Gracilaria pacifica* (37.0 mol%) (Kapraun et al., 1993a) and *Gracilaria* sp. 2 (35.4 mol%) show the greatest deviation from the G + C mol% mean of 42.6 in *Gracilaria*. It is not readily apparent how these presumptive AT increases reflect environmental parameters: the *G. pacifica* specimens originated from the temperate coast of Santa Barbara, California while the *Gracilaria* sp. 2 was collected in a tropical Philippine lagoon. As previously noted (Kapraun et al., 1993a), factors in addition to environmental temperature constraints must influence directional change in nucleotide sequence composition.

Conclusions

The present paper concludes a series of investigations on members of the commercially important red algal family Gracilariaceae. It has been our intent to develop nuclear genome profiles by publishing information on nuclear genome size, chromosome numbers and karyotype symmetry (Kapraun & Dutcher, 1991; Kapraun, 1993; Lopez-Bautista & Kapraun, 1995; Kapraun et al., 1996) as well as genome organization and complexity (Dutcher et al., 1990a, 1990b; Kapraun et al., 1993a, 1993b; Lopez-Bautista & Kapraun, 1995). We now have basic information for 24 species of *Gracilaria*, three species of *Gracilariopsis* and three species of *Hydropuntia*, representing about one-fourth of the total species currently recognized in these three genera (Wynne, 1986; Oliveira & Plastino, 1994; Bird, 1995). Although sample size is limited, some generalizations seem warranted:

1. Species of *Gracilaria, Gracilariopsis* and *Hydropuntia* are characterized by both a constant chromosome complement and a narrow range of genome sizes. *Gracilaria* and *Hydropuntia* species, with few exceptions (Godin et al., 1993), have a haploid

chromosome number of 1N = 24, while *Gracilariopsis* species have a probable chromosome number of 1N = 32 (28–32). Members of all three genera have a small, constant genome size in the range of 0.33–0.47 pg (X = 0.41 pg).

2. In contrast to the monotony of cytogenetic details, genome organization and complexity show extreme variation with repeated sequences ranging from 13–95%, suggesting that the proportion of unique or unshared genome for some species can be quite large.
3. Thermal denaturation temperature (T_m) profiles indicate maximum similarity (homology) values at the intraspecific level of 79–90% in *Gracilaria tikvahiae* populations, and at the interspecific level of 46–69% for *Gracilaria* species, and 73–81% for *Gracilariopsis* species.
4. Results of our studies seem to be in general agreement with investigations of the nuclear-encoded small-subunit (18S) rRNA genes which indicate relatively large divergence within the family (Bird et al., 1992, 1994; Ragan et al., 1994; Bird, 1995).
5. Finally, in the Gracilariaceae, as in vascular plants (Flavell, 1980), it can be assumed that a relatively small proportion (5–10%) of the DNA is sequence-specific or genic, while the remainder of the genome represents diverged repeated sequences (fossil repeats). Using the conversion expression 0.956×10^9 bp = 1 pg (Britten & Davidson, 1971) and accepting a mean genome size of 0.41 pg and minimum shared genome of 46% in *Gracilaria* results in an estimate of 0.19 pg (0.18×10^9 bp). A genic component of 5–10% of the total nuclear genome would approximate 0.02 pg (0.2×10^8 bp). This amount seems quite large in comparison with the angiosperm *Arabidopsis thaliana* (Linnaeus) Heynbold with a 2 C DNA content of 0.5 pg (Bennett & Smith, 1976) and genic component as small as 0.05 pg.

These conclusions concerning genome profiles can be used at the applied level to evaluate the relative chances of success represented by specific biotechnology procedures currently available for the development of new genotypes for mariculture:

Polyploidy. Polyploids contain an even number multiple of the basic genome and can develop spontaneously from a failure of cytokinesis in tetrasporangia after meiosis (van der Meer, 1977). In *Gracilaria* and *Gracilariopsis*, the apparent absence of naturally occurring polyploid chromosome complements suggests that their genomes do not tolerate a polyploid condition, and are not amenable to polyploid construction. Assessment of polyploid cultivars in controlled conditions has confirmed their generally poor performance (van der Meer & Patwary, 1983; Patwary & van der Meer, 1984).

Aneuploidy. Aneuploids contain less than a complete complement of additional chromosomes. In *Gracilaria* and *Gracilariopsis* (probably), chromosome numbers appear to be highly conserved, suggesting that the genome does not tolerate an aneuploid condition either. Attempts to produce aneuploids by crossing N = 24 × N = 16 strains have produced stunted tetrasporophytes (Godin et al., 1993).

Hybridization and heterosis. Although many *Gracilaria* species are sympatric, there is no evidence that hybrids between parents of different species are viable (Bird & Rice, 1990; Rice & Bird, 1990). Consequently, attempts to produce new genotypes through hybridization are unlikely to succeed. Heterosis or hybrid vigor results from the combination (inter- or intraspecific) of different but compatible parental genomes. No evidence for heterozygote superiority has yet been demonstrated in *Gracilaria* (Patwary & van der Meer, 1983).

Foreign gene introduction. The potential use of biotechnology for genetic manipulation of macroalgae has been summarized previously (Barclay & McIntosh, 1986; Cheney, 1988). Recently, a specific procedure for the introduction of foreign genes with plasmids into the red seaweed *Porphyra* has been demonstrated (Kübler et al., 1994). A review of additional techniques for the introduction of exogenous genetic material into algae includes particle bombardment, vortexing with glass beads, polyethyleneglycol treatment of protoplasts, microinjection and electroporation (Kübler et al., 1994). Results of our investigations which reveal a wide range of values for the ratio of unique to repetitive nucleotide segments in the Gracilariaceae (Dutcher et al., 1990a) suggest that these taxa should be amenable to gene introduction. This is especially true as these procedures do not substantially alter genome size or chromosome complements.

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