Cryptogamie, Algologie, 2011, 32 (2): 205-219

Nuclear content estimates suggest a synapomorphy between *Dictyota* and six other genera of the Dictyotales (Phaeophyceae)

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(Received 15 July 2010, Accepted 18 October 2010)

Abstract – The DNA-localizing fluorochrome DAPI (4', 6-diamidino-2-phenylindole) and chicken erythrocytes standard (RBC) were used with image analysis and static microspectrophotometry to estimate nuclear DNA contents in 14 species and varieties of Dictyotales from the Atlantic Ocean (Spain and USA) and the Mediterranean Sea (Spain). Negligible differences were found between specimens fixed in Carnoy's solution (EtOH) and methanol-Carnoy's (methacarn). Present and previously published nuclear DNA content estimates expand our database to include 17 species and varieties representing seven genera with a 2C range of 0.7 – 1.7 pg. Intraplant variation (endopolyploidy) was observed in most isolates and 8C nuclei were quantified in five species. In four species, fluorescence intensity (I_f) levels in 2C gametophyte nuclei were found to closely approximate 50% of 4C values in vegetative cells of mature sporophytes, consistent with meiosis and a sexual life history in diplobiontic algae. Availability of consensus higher-level phylogenetic trees for Dictyotales has opened the way for determining evolutionary trends in DNA amounts. Both estimated genome sizes and published chromosome numbers for Dictyotales suggest that evolution in the order was accompanied by multiple, discrete polyploidy events which are largely obscured by subsequent small scale loss or gain of chromosomes (aneuploidy). Members of the genus *Dictyota* are characterized by a narrow range of 2C genome sizes (0.7-0.9 pg) relative to other Dictyotales investigated (1.0-1.7 pg).

Dictyotales / DNA C-values / nuclear genome size / Phaeophyceae

Résumé – **Les contenus nucléaires suggèrent une synapomorphie entre *Dictyota* et six autres genres de Dictyotales (Phéophycées).** Nous avons estimé les teneurs d'ADN nucléaire de 14 taxons de Dictyotales de l'Atlantique (Espagne et États-Unis) et de la Méditerranée (Espagne) par microspectrophotométrie et analyse d'images, en utilisant DAPI (4', 6-diamidino-2-phenilindole) comme fluorochrome et les érythrocytes de poulet comme standard (RBC). Les différences observées entre les spécimens fixés en Carnoy (EtOH) et en méthanol-Carnoy (methacarn) ont été négligeables. Les données des quantités d'ADN apportées dans ce travail donnent, avec celles déjà publiées, un total de 17 taxons correspondant à set genres de Dictyotales, avec un rang de 2C de 0,7-1,7 pg. Les

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Communicating editor: Frederik Leliaert

variations dans un même individu sont fréquentes (endopolyploïdie) et des valeurs de 8C ont été observées dans cinq espèces. Dans quatre espèces les valeurs de l'intensité de fluorescence (I_f) pour les noyaux 2C des gamétophytes étaient très proches au 50 % des valeurs 4C des cellules végétatives des sporophytes, ce qui concorde avec la méiose et un cycle de vie diplobiontique. La disponibilité d'arbres phylogénétiques de haut consensus pour les Dictyotales a permis de déterminer les traits de l'évolution des quantités d'ADN dans ce groupe. Les estimations de la taille du génome ainsi que les nombres chromosomiques publiés pour les Dictyotales suggèrent que l'évolution dans ce groupe a été accompagnée par de multiples phénomènes de polyploïdie masquée par de postérieures petites pertes ou gains de chromosomes (aneuploïdie). Les espèces du genre *Dictyota* se caractérisent par un étroit rang des valeurs 2C (0,7-0,9 pg) en comparaison avec les autres genres de Dictyotales étudiés (1,0-1,7 pg).

Dictyotales / valeurs C d'ADN / quantité d'ADN nucléaire / Phaeophyceae

INTRODUCTION

In the last decade, DNA sequence data have shown that classic brown algal phylogenies based on a sequence of simple/primitive to complex/advanced were more apparent than real (Rousseau & Reviers, 1999a; Phillips *et al*., 2008). As DNA sequence data begin to develop a comprehensive phylogeny of the Phaeophyceae, several monophyletic early lineages can be resolved, while most other brown algae form two groups: Group I including Dictyotales and Sphacelariales, among others (Lee & Bae, 2002; Kraft *et al*., 2004; De Clerck *et al*., 2006) and Group II, a crown group with the remaining brown algae (Rousseau & Reviers, 1999a, 1999b; Rousseau *et al*., 1997, 2001; Phillips *et al*., 2008; Silberfeld *et al.,* 2010). Orders in Group I share apical growth, cells with numerous plastids lacking pyrenoids, and polystichous construction (Phillips *et al*., 2008).

Dictyotales includes approximately 20 genera and 1000 species (Kraft *et al*., 2004; De Clerk *et al*., 2006). Traditional taxonomic treatments recognize three families: Dictyotopsidaceae Allender (1980), Scoresbyellaceae Womersley (1987) and Dictyotaceae Lamouroux *ex* Dumortier (1822), with the later divided into two tribes: Dictyoteae Greville (1830) and Zonarieae De Toni (1895). Contemporary molecular phylogenetic trees for Dictyotales (Kraft *et al*., 2004; Lee & Bae, 2002; Phillips *et al*., 2008) support the monophyly of this group (Bittner *et al*., 2008; Ni-Ni-Win *et al*., 2010). However, recent molecular investigations suggest several genera do not resolve as monophyletic clades and contemporary generic delineations, based almost exclusively on vegetative anatomical characters, appear to be completely irreconcilable with contemporary molecular data (De Clerk *et al*., 2006; Bittner *et al*., 2008). Dictyotales probably include a single family, the Dictyotaceae, and subdivision into two tribes is probably unwarranted (Bittner *et al*., 2008).

New availability of both DNA genome size databases (Kapraun, 2005; Gregory *et al*., 2007) and trees derived from a consensus of several different studies (Lee & Bae, 2002; Hoshina *et al.,* 2004; Kraft *et al*., 2004; Hwang *et al*., 2005) have opened the way for determining evolutionary trends in DNA amounts in algae (Kapraun, 2005, 2007). However, nuclear DNA estimates have been published for only six species of Dictyotales (Kapraun, 2005) despite their importance as conspicuous, dominant components of tropical and temperate marine algal floras (De Clerck *et al*., 2006). Hörnig *et al.* (1992) also gave data

about nuclear DNA content of nine *Dictyota* species but only in arbitrary units, which makes them not comparable to other data. The present investigation was initiated to provide nuclear DNA content estimates for additional taxa of Dictyotales, to determine the extent of inter- and intraspecific nuclear DNA content variation, to correlate genome sizes with emerging patterns of evolution and phylogeny, to determine if DNA contents are diagnostic and represent synapomorphies, as well as to corroborate an alternation of haploid and diploid nuclear DNA contents in gametophyte and sporophyte phases in these isomorphic taxa.

MATERIALS AND METHODS

Source of specimens

Fourteen taxa of Dictyotales were collected along the Atlantic coast of North America [Wrightsville Beach (North Carolina)] and along Spanish coasts [Mediterranean: Calella, Palamós and Cadaqués (Girona), and Atlantic: A Coruña (Galicia), Ondarreta and Zumaya (Guipúzcoa)]. Of these fourteen taxa, five corresponded to the Dictyoteae tribe (genus *Dictyota*) and nine to the Zonarieae tribe (genera *Dictyopteris*, *Lobophora*, *Padina*, *Spatoglossum, Taonia* and *Zonaria*). The specimens studied are housed at the WNC herbarium (Biological Sciences Department, University of North Carolina, Wilmington) and the BCN-Phyc herbarium (Centre de Documentació de Biodiversitat Vegetal, University of Barcelona) (Table 1).

Assignment of ploidy level

Assignment of estimated nuclear DNA contents to specific C-values in the present study is presumptive in that no karyological investigations were conducted on the algal samples used for nuclear DNA content estimates. The C-values correspond to the basic amount of DNA in the no replicated haploid chromosome complement (Goff & Coleman, 1990). The specimens were identified as gametophytes (haploid with 2C nuclei), tetrasporophytes (diploid with 4C nuclei), and infertile plants (either haploid or diploid). The 2C and 4C values refer to a replicated haploid and diploid chromosome complement, respectively. According to Goff & Coleman (1987), in red algae the major portion of their cell cycle is in G_2, at least two copies of the nuclear genome are present during most of the interphase. In other groups of algae, Fucales (Gómez Garreta *et al.,* 2010) or Phaeophyceae and Clorophyceae (Kapraun, 2005), the dominance of the G_2 phase is observed, 2C values corresponding to the haploid cells and 4C values to the diploid cells.

Nuclear DNA content estimates

For each species, one or two specimens were studied and from each one, several samples were analyzed. Vegetative cells were examined from tetrasporophytes, gametophytes or from infertile plants. Algal material was fixed in Carnoy's solution (Kapraun, 2005) and in methacarn (methanol-Carnoy) to avoid

Table 1. Nuclear DNA contents of Dictyotales taxa. Data standardized to the DNA level of chicken erythrocytes (RBC) = 2.4 pg.

Taxa	*Collection location*	*Fixation*	*No. slides*	*No. nuclei*	*Nuclear Genome Size (pg)*		
					2C	*4C*	*8C*
	voucher number						
Dictyotales							
Dictyota ciliolata Kützing	Wrightsville Beach WNC 2006-011	Me	3	106		1.4 ± 0.3	
	Wrightsville Beach WNC 2006-011	Me	3	65		1.3 ± 0.2	
Dictyota dichotoma var. *intricata* (C. Agardh) Greville	Zumaya BCN-Phyc 5651	Et	1	29		1.7 ± 0.4	
	Zumaya BCN-Phyc 5651	Me	4	198		1.7 ± 0.4	
Dictyota linearis (C. Agardh) Greville	Cadaqués BCN-Phyc 5966	Et	3	79		1.3 ± 0.2	
	Cadaqués BCN-Phyc 5966	Me	3	130	0.7± 0.1		
Dictyota menstrualis (Hoyt) Schnetter, Hörning *et* Weber-Peukert	Wrightsville Beach WNC 2006-025	Me	2	26		1.6 ± 0.3	
	Wrightsville Beach WNC 2006-025	Me	3	101			2.7 ± 0.3
	Wrightsville Beach WNC 2006-025	Et	3	100			2.7 ± 0.4
Dictyota spiralis Montagne	Cadaqués BCN-Phyc 5967	Et	3	73		1.3 ± 0.3	
	Cadaqués BCN-Phyc 5967	Me	2	50		1.3 ± 0.3	
Dictyota spiralis	Ondarreta BCN-Phyc 5968	Me	2	68	0.7 ± 0.1		
	Ondarreta BCN-Phyc 5968	Et	2	45	0.7 ± 0.1		
Dictyopteris delicatula Lamouroux	Wrightsville Beach WNC 2006-037	Me	2	40	1.5 ± 0.3		
	Wrightsville Beach WNC 2006-037	Me	3	80			4.3 ± 0.8
Dictyopteris hoytii Taylor	Wrightsville Beach WNC 2006-006	Me	4	211		2.3 ± 0.4	
	Wrightsville Beach WNC 2006-006	Et	3	105		2.0 ± 0.4	
	Wrightsville Beach WNC 2006-006	Me	2	38		2.0 ± 0.3	

Table 1. Nuclear DNA contents of Dictyotales taxa. Data standardized to the DNA level of chicken erythrocytes (RBC) = 2.4 pg. *(continued)*

Taxa	*Collection location*	*Fixation*	*No. slides*	*No. nuclei*	*Nuclear Genome Size (pg)*		
					2C	*4C*	*8C*
	voucher number						
Dictyopteris polypodioides (DeCandolle) Lamouroux	Wrightsville Beach WNC 2006-024	Me	3	68		2.54 ± 1.4	
	Wrightsville Beach WNC 2006-024	Me	2	65			5.5 ± 1.2
	Wrightsville Beach WNC 2006-024	Me	4	90		3.1 ± 0.6	
	Wrightsville Beach WNC 2006-024	Me	1	32			4.3 ± 1.1
	Wrightsville Beach WNC 2006-024	Et	3	76			4.5 ± 0.8
Dictyopteris polypodioides	Cadaqués BCN-Phyc 5969	Et	1	20			3.8 ± 0.7
	Cadaqués BCN-Phyc 5969	Et	3	77		2.55 ± 0.6	
	Cadaqués BCN-Phyc 5969	Me	4	100			3.6 ± 0.7
Lobophora variegata (Lamouroux) Womersley	Wrightsville Beach WNC 2008-019	Et	3	94		2.8 ± 0.4	
	Wrightsville Beach WNC 2008-019	Me	1	25		2.4 ± 0.6	
Padina gymnospora (Kützing) Sonder	Wrightsville Beach WNC 2006-026	Me	4	120			4.9 ± 0.8
	Wrightsville Beach WNC 2006-026	Et	3	60			4.1 ± 0.8
	Wrightsville Beach WNC 2006-026	Me	4	82			4.3 ± 0.6
	Wrightsville Beach WNC 2006-026	Et	3	76			4.0 ± 0.6
	Wrightsville Beach WNC 2006-026	Me	3	76			4.1 ± 1.0
Padina pavonica (Linnaeus) Thivy *in* W.R. Taylor	Calella de Palafrugell BCN-Phyc 5970	Et	3	93		3.1 ± 1.0	
	Calella de Palafrugell BCN-Phyc 5970	Et	2	55			7.1 ± 1.1
	Calella de Palafrugell BCN-Phyc 5970	Et	2	60		3.3 ± 0.6	
	Calella de Palafrugel lBCN-Phyc 5970	Me	2	33	1.7 ± 0.3		

Table 1. Nuclear DNA contents of Dictyotales taxa. Data standardized to the DNA level of chicken erythrocytes (RBC) = 2.4 pg. *(continued)*

Taxa	*Collection location*	*Fixation*	*No. slides*	*No. nuclei*	*Nuclear Genome Size (pg)*		
					2C	*4C*	*8C*
	voucher number						
Spatoglossum solierii (Chauvin *ex* Montagne) Kützing	Palamós BCN-Phyc 5648	Et	2	40	1.1 ± 0.2		
Taonia atomaria J.G. Agardh	Palamós BCN-Phyc 5646	Et	2	40	1.1 ± 0.2		
	Palamós BCN-Phyc 5646	Me	2	44	1.0 ± 0.1		
Zonaria tournefortii (Lamouroux) Montagne	Wrightsville Beach WNC 2006-001	Me	4	164		2.1 ± 0.3	
	Wrightsville Beach WNC 2006-001	Me	3	124		1.9± 0.3	

reported staining inhibition associated with intracellular phenolic compounds (Puchtler *et al*., 1970). Samples were stored in 70% ethanol at 4ºC, rehydrated in water and softened in 5% w/v EDTA (Goff & Coleman, 1990) for 12-48 h. Algal specimens were transferred to cover slips treated with subbing solution and then air dried and stained with 0.5µg/mL 4', 6-diamidino-2-phenylindole (DAPI, Sigma Chemical Co., St. Louis, MO 63178) as previously described (Goff & Coleman, 1990; Kapraun & Nguyen, 1994). Nuclear DNA content estimates based on microspectrophotometry with DAPI followed procedures specified previously (Kapraun & Nguyen, 1994; Kapraun, 1994) using a protocol modified after Goff & Coleman (1990).

Nuclear DNA contents of Dictyotales specimens were estimated by comparing their fluorescence intensity (I_f) values with those of chicken erythrocytes (Kapraun, 1994; Kapraun & Dunwoody, 2002) as a DNA standard. The 2C DNA content of *Gallus gallus* was reported to be within the range of 2.33 (Galbraith *et al*., 1983) to 2.39 pg (Clowes *et al*., 1983), with 2.4 pg being the accepted value for our laboratory (Kapraun, 2005). DAPI binds by a non-intercalative mechanism to adenine and thymine rich regions of DNA which contain at least four A-T base pairs (Portugal & Waring, 1988). Consequently, chicken erythrocytes can be used directly as standards for determining amounts of DNA only when the A-T contents of both standard and experimental DNA are equivalent (Coleman *et al*., 1981). Chicken erythrocytes have a nuclear DNA base composition of 42-43 mol % G + C (Marmur & Doty, 1962). Limited published data for the Phaeophyceae indicate values in the range of 38-43 mol % G + C (Olsen *et al*., 1987; Stam *et al*., 1988; Le Gall *et al*., 1993). Members of the Phaeophyceae investigated in this study are assumed to have a similar range of base pair compositions, and the linearity is accepted between DAPI-DNA binding in both RBC and algal samples (Le Gall *et al*., 1993). The number of algal nuclei examined in each sample and the nuclear genome size estimates (pg) ± SD are recorded in Table 1.

Nuclear DNA content data for these and other brown algae are incorporated into a database of plant genome sizes (Kapraun, 2005; Kapraun *et al.,* 2004; Gregory *et al*., 2007) hosted by the Royal Botanic Gardens Kew web page (http://www.rbgKew.org.uk/cval/homepage.html).

RESULTS

DAPI staining yielded reproducible, stable nuclear fluorescence with little apparent interference from autofluorescence, non-specific binding or other cellular material. Algal material fixed in Carnoy's solution and methacarn resulted in similar I_f values. Estimated nuclear DNA content variation between samples with different fixations and within samples with the same fixation was typically less than 10% (Table 1).

Comparison of I_f values for the Dictyotales species to I_f values for chicken erythrocytes (RBC) permitted estimation of nuclear DNA contents for taxa investigated in this study. Nuclear genome size estimates for seven genera of Dictyotales from the Atlantic and Mediterranean coasts of Spain and Atlantic coasts of the USA (Table 1) increase our database to 17 for this order. Our results reveal that members of the Dictyotales are characterized by discrete ranges of 2C nuclear genome sizes: species of *Dictyota* have a range of 0.7-0.9 pg while other genera in the Dictyotales have a range of 1.0-1.7 pg (Fig. 1).

In four species investigated, 2C nuclear DNA levels in haploid gametophytes were found to closely approximate 50% of the 4C values in diploid sporophyte vegetative cells (Table 2). Polyploid nuclei were observed in most samples of Dictyotales investigated and 8C nuclei were quantified in vegetative cells of *Dictyota menstrualis*, *Dictyopteris delicatula, Dictyopteris polypodioides, Padina gymnospora* and *Padina pavonica* (Table 1).

DISCUSSION

EtOH versus Methanol

Brown algae are generally polysaccharide and polyphenol rich, making DNA extraction and quantification problematic (Lewis *et al*., 1993; Phillips *et al*., 2001). The methacarn fixative (Puchter *et al*., 1970), a substitution for Carnoy's, is recommended to enhance DNA-localizing fluorochrome performance. However, in the present study, similar DNA content estimates were obtained from samples following both fixation protocols, as observed in the order Fucales (Gómez Garreta *et al*., 2010).

DNA content and phylogeny

The 2C nuclear genome size range in the Dictyotales of 0.6-1.6 pg (Fig. 1) is within the range of 0.2-1.7 pg previously reported in the Phaeophyceae (Kapraun, 2005). Mb values of 686-1666 can be derived for species of Dictyotales using the

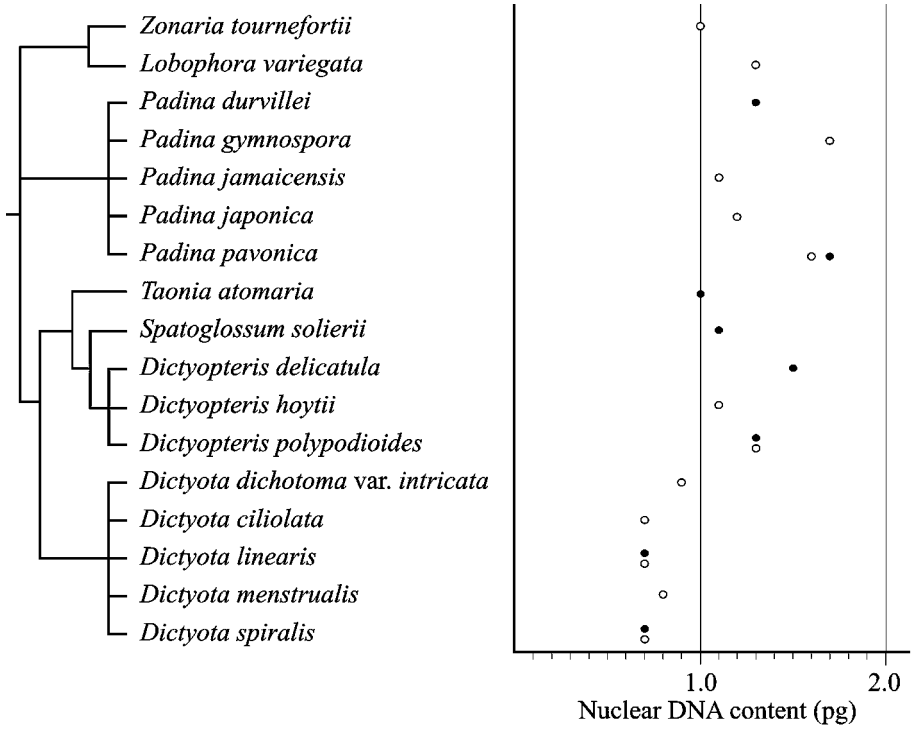

Fig. 1. 2C DNA contents for Dictyotales superimposed on a consensus molecular phylogenetic tree (Lee & Bae, 2002; Hoshina *et al.*, 2004; Hwang *et al.*, 2005; Cho *et al.*, 2006; De Clerk *et al.*, 2006; Phillips *et al.*, 2008; Bittner *et al.*, 2008). (●) 2C nuclear DNA contents estimated from I_f values of replicated haploid nuclei; (○) 2C nuclear DNA contents extrapolated from 50% of the 4C values in diploid nuclei.

expression 1 pg = 980 Mb (Cavalier-Smith, 1985; Bennett *et al.*, 2000) or Mb values of 685-1663 can be derived using the more recently published value of 1 pg = 978 Mb (Dolezel *et al.,* 2003). The relatively large sizes of these 2C algal genomes are best appreciated when compared with the minimum amount of DNA estimated for specifying the mRNA sequences required for angiosperm development. Specifically, the genomes of *Genlisea margaretae* Hutchinson and *Arabidopsis thaliana* (L.) Heynhold, with 2C = 126 and 157 Mb respectively (Riechmann *et al.,* 2000; Bennett *et al.,* 2003; Greilhuber *et al.*, 2006), are among the smallest found in angiosperms (Bennett & Smith, 1976), but they still have 1.5-2x the estimated 15,000 genes per haploid genome required for development (Flavell, 1980). Assuming a greater complexity of morphology and development in angiosperms than in brown algae (Bell, 1997), the large genome sizes in the Dictyotales are consistent with a high level of genomic redundancy (Bennetzen, 2002; Kapraun, 2005).

Species of *Dictyota* are characterized by a narrow range of 2C nuclear genome sizes (0.7-0.9 pg) which distinguish them from other taxa of Dictyotales that have larger genome sizes of 1.0-1.7 pg (Fig. 1). In *Dictyota*, the narrow, discrete range of DNA contents appears to be diagnostic and may represent a synapomorphy. However, as present results are based on about 10% of the ~ 80 reported species of *Dictyota* (Bittner *et al.*, 2008) this distinction may prove to be more apparent than real once additional taxa are investigated.

Table 2. Mean values of nuclear DNA content estimates (pg) for Dictyotales. 1 Data from Kapraun (2005)

Taxa	*2C (Mean I_f)*	*2C (50% of 4C)*	*4C (Mean I_f)*
Dictyota ciliolata		0.7	1.3
Dictyota dichotoma var. *intricata*		0.9	1.7
Dictyota linearis	0.7	0.7	1.3
Dictyota menstrualis		0.8	1.6
Dictyota spiralis	0.7	0.7	1.3
Dictyopteris delicatula	1.5		
Dictyopteris hoytii		1.1	2.2
Dictyopteris polypodioides[1]	1.3		
Dictyopteris polypodioides		1.3	2.6
Lobophora variegata		1.3	2.6
Padina durvillei[1]	1.3		
Padina gymnospora[1]		1.7	3.5
Padina jamaicensis[1]		1.1	2.2
Padina japonica[1]		1.2	2.4
Padina pavonica	1.7	1.6	3.2
Spatoglossum solierii	1.1		
Taonia atomaria	1.0		
Zonaria tournefortii		1.0	2.0

DNA content and morphotype

Although little correlation generally exists between nuclear genome size and an organism's complexity (the C-value enigma) (Gregory, 2005), there is substantial evidence that the nucleotype affects the phenotype in a non-genic manner in response to environmental demands (Cavalier-Smith, 1985). For example, in both plants and animals (Price, 1988) genome size and cell size extend their influence to ecological selection types. Significantly, larger genome size is associated with K-selection that favors slower development, delayed reproduction, and larger body size. Smaller genome size is associated with r-selection that favors rapid development, high population growth rate, early reproduction, and small body size (Cavalier-Smith, 1978, 1985; Begon *et al*., 1990). Observed correlations between morphotype and nucleotype in red and green algae (Kapraun, 2005, 2007; Kapraun *et al*., 2007) imply a significant but poorly understood role for the nucleotype in gene expression (Gregory, 2001).

In brown algae, it has been noted that taxa characterized by oogamy and large female gametes, including the Dictyotales, Fucales, Laminariales, and Sphacelariales, have the largest genomes in the Phaeophyceae (Kapraun, 2005; Gómez Garreta *et al*., 2010). Evidence is emerging that genome size is highly correlated with both cell and spore size (Ngan & Price, 1979; Kapraun & Dunwoody, 2002). Consequently, it is not surprising that members of these orders have the largest nuclear genomes in brown algae (Kapraun, 2005). It is probable that their large genome size and large spore size are related to ecological specialization rather than to relative phylogenetic position in the Phaeophyceae. Although

nuclear genome size is highly correlated with many cellular and ecological parameters (Kapraun & Dunwoody, 2002), 'correlation' and 'causation' are far from interchangeable (Gregory, 2005). The many complex causal factors behind our observations of large genome sizes in the Dictyotales remain obscure.

DNA content and life history

Microspectrophotometry has been used successfully to demonstrate life cycle-associated DNA content variation in brown algae (Motomura, 1995; Gómez Garreta *et al*., 2010). Members of Dictyotales are characterized by an alternation of isomorphic haploid gametophytes and diploid sporophytes (Phillips, 1997; Bell, 1997). In four taxa, in which the two generations were available, 2C nuclear DNA levels in gametophytes were found to closely approximate 50% of the 4C values in sporophytes (Table 2), consistent with gametic meiosis in a diplobiontic life history (Bell, 1997). Endopolyploid nuclei were observed in most samples of Dictyotales investigated and 8C nuclei were quantified in vegetative cells of five species (Table 1).

Chromosome number and DNA content

Polyploidy has been reported widely in the Phaeophyceae, especially in the Laminariales (Lewis *et al*., 1993; Lewis, 1996; Gall *et al.,* 1996; Garbary & Clarke, 2002) and Fucales (Kapraun, 2005; Gómez Garreta *et al*., 2010). In the Ectocarpales, development of polyploid populations is well documented, with haploid, diploid and tetraploid plants connected with each other in a complex system of meiosis, heteroblasty and a spontaneous increase in chromosome numbers (Müller, 1967, 1970; Coelho *et al*., 2007).

Chromosome numbers for the Dictyotales, including six taxa in the present investigation (Fig. 2), confirm polyploidy in this order (Cole, 1967; Lewis, 1996). For example, tetraploid chromosome numbers (2n = 32) have been reported in western Pacific specimens of *Pachydictyon coriaceum* (Holmes) Okamura (Kumagae, 1975), *Dictyopteris prolifera* (Okamura) Okamura (Kumagae, 1970) and *Dictyota dichotoma* (Hudson) Lamouroux (Kumagae & Inoh, 1960; Kumagae *et al*., 1960). It is noteworthy that tetraploid chromosome numbers have not been reported in European specimens of *D. dichotoma* (Lewis, 1996), which may not be conspecific with western Pacific populations.

Reported 1*n* chromosome numbers in the Dictyotales range from 9 to 32, but the lower numbers are primarily from the early literature and almost certainly are incorrect (Lewis, 1996). The basic ancestral chromosome number has been reported to be 4 (Hörnig *et al*., 1992), 8 or 12 (Lewis, 1996). Published chromosome numbers for the Dictyotales (Lewis, 1996) suggest two separate doubling sequences $x = 12 \rightarrow 2x = 24$ and $x = 16 \rightarrow 2x = 32$ (Fig. 2). These doubling sequences do not appear to be genus-specific as both sequences are apparent in species of *Dictyota*.

Despite chromosome number evidence of ancestral polyploidy in the Dictyotales, the present study suggests a poor correlation between chromosome number and DNA contents (Fig. 3). By example, *Dictyota menstrualis,* with n = 24, has a 2C DNA content of 0.7 pg while *Padina pavonica,* with n = 12, has a DNA content of 1.7 pg. It is unclear why species of *Dictyota* has consistently small genome sizes but the widest range of chromosome complements. The most parsimonious explanation is to assume that proposed polyploidy events occurred

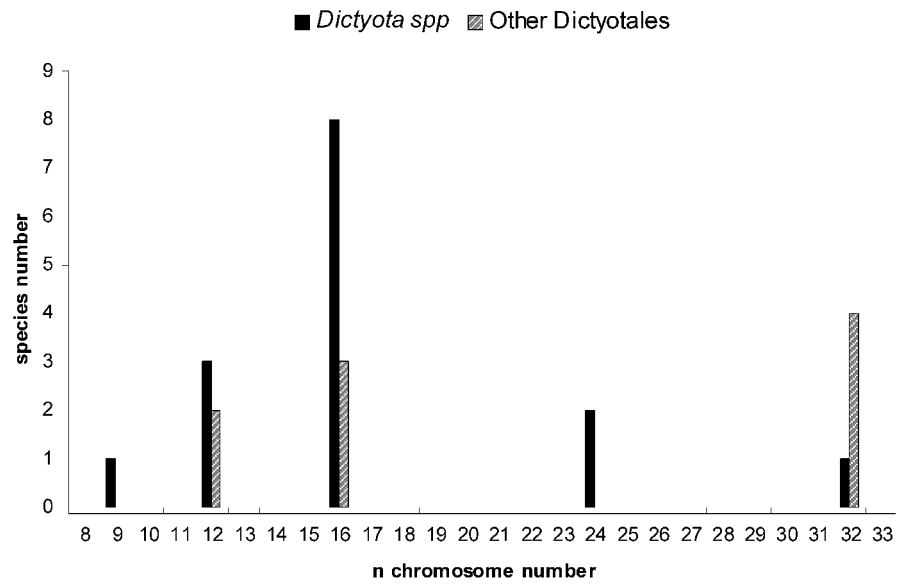

Fig. 2. Frequency distribution of chromosome numbers reported in species of Dictyotales (Cole, 1967; Lewis, 1996).

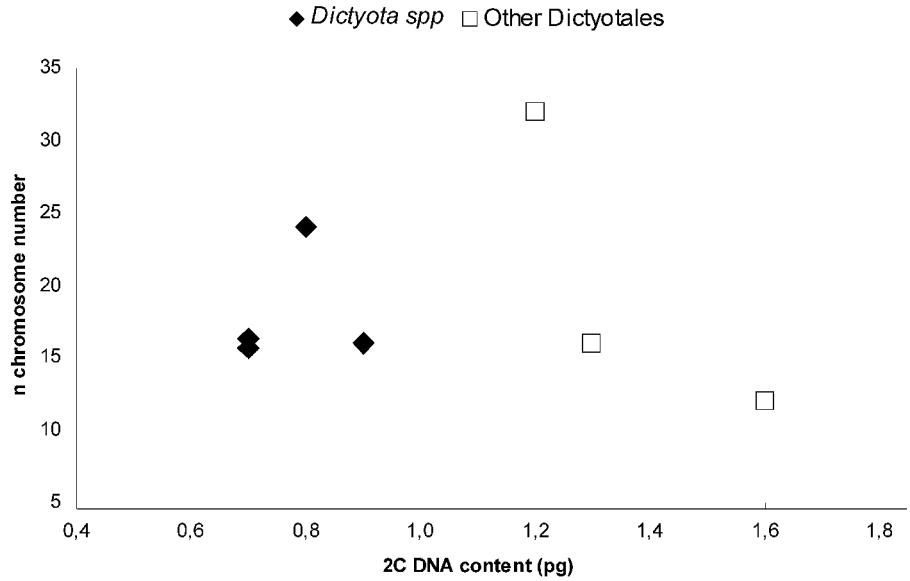

Fig. 3. Comparison of 2C nuclear DNA contents from the present study and Kapraun (2005) and 1*n* chromosome numbers (Lewis, 1996) for 7 species of Dictyotales. (●) Values for species of *Dictyota*; (○) Values for species of all other genera. Nuclear DNA contents estimated from I_f values of haploid 2C nuclei or extrapolated from 50% of the 4C values in diploid nuclei (Table 2).

broadly across the Dictyotales, but are obscured by subsequent, sequential loss of genome in species of *Dictyota*. Differential loss or gain of replicated elements or 'fossil repeats' (Kapraun *et al*., 1993a) often derived from transposable element amplification (Bennetzen, 2002) can produce downstream species characterized by polyploid chromosome complements and 'aneuploid' DNA-content genomes (Kapraun, 2005, 2007). Polyploidization has been studied in land plants and is now recognized as an important process in plant speciation and evolution (Otto & Whitton, 2000; Adams & Wendel, 2005; Leitch & Leitch, 2008). Abundant transposable elements and polyploidization can work in tandem to rapidly resize and restructure genomes (Soltis *et al*., 2004; Chen, 2007). These processes have been identified previously in both rhodophytes (Dutcher *et al*., 1990; Kapraun *et al*., 1993b; Kapraun *et al*., 1996) and chlorophytes (Kapraun, 1993), including the Codiales (Kapraun *et al*., 1988), Dasycladales (Kapraun & Buratti, 1998) and Ulvales (Kapraun & Bailey, 1992).

Acknowledgements. We are grateful to Ignacio Barbara, Coro Casares and Angela Noguerol for providing material from the Atlantic coast of Spain, Tanya Grace Johnson for reviewing the English text and Raquel García and Manel Bosch for their technical assistance with image analysis. This study was supported by the projects CGL2005-02330/BOS and CGL2009-09589/BOS of the Spanish Government.

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Mise en page et impression
bialec, nancy (France)
Dépôt légal n° 75868 - 30 mai 2011