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Possible significance of different DNA content ranges of gametophytic and tetrasporophytic nuclei in two species of *Laurencia* (Rhodomelaceae, Rhodophyta)

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In species of the red algal genus *Laurencia* the life cycle is isomorphic, with haploid gametophytic and diploid tetrasporophytic thalli similar or identical in shape. Identical total nuclear DNA contents are postulated in the literature for homologous somatic thallus cells of both generations. However, in the closely related genus *Osmundea* differences in DNA content were observed between some homologous somatic cells of the isomorphic gametophytes and tetrasporophytes. To find out if differences between nuclear gametophytic and tetrasporophytic DNA contents also exist in *Laurencia*, comparative studies were carried out in two species of this genus. Microfluorometric measurements of nuclear DNA contents in the uninucleate cells of the central filaments in distal portions of thalli of *Laurencia majuscula* and *Laurencia* sp. were up to 64C for tetrasporophytes and up to 32C for gametophytes. Most probably nuclei of apical cells in G_2 phase were 128C in tetrasporophytes and 64C in gametophytes. Nuclei of the multinucleate medullary, cortical and epidermal cells were mostly 8C and 4C in tetrasporophytes and gametophytes. Flow charts for nuclear DNA contents starting with apical cells are presented. Average C-levels of gametophytic nuclei were lower than those of homologous tetrasporophytic nuclei, reflecting the respective haploid and diploid states in these isomorphic generations. Because homologous gametophytic and tetrasporophytic cells had identical numbers of nuclei, total nuclear DNA contents per cell were also different. Nuclear DNA content ranges in multinucleate cells were 1C–16C in gametophytes and 2C–32C in tetrasporophytes. So, some tetrasporophytic nuclei had C-values identical to those of some gametophytic nuclei. Tetrasporophytic and gametophytic nuclei with identical C-levels are considered to be responsible for isomorphy of the two generations. We suggest that low C-levels in trichoblasts of gametophytes and high C-levels in cortical and epidermal cells of tetrasporophytes control the development of gametangia and tetrasporangia, respectively. Previous reports that nuclear DNA contents in homologous gametophytic and tetrasporophytic cells are identical in *Laurencia* are not confirmed.

Key words: apoptosis, gametophyte, *Laurencia*, nuclear DNA content, Rhodophyta, tetrasporophyte

Introduction

It is well known from plant breeding that in a particular species higher ploidy levels result in larger nuclei, larger cells and larger plants (giant forms) (Bresch, 1965; Leibenguth, 1982). In spite of this fact, haploid gametophytic and diploid sporophytic phases of the life cycles of many algae are similar in appearance or completely isomorphic, and haploid and diploid cells are equal in size, except for the reproductive structures (Ettl, 1980; Goff & Coleman, 1987). In species of the subclass Florideophycidae of the red algae, life cycles frequently are triphasic, involving haploid sexual gametophytes, diploid carposporophytes developing on the (female) gametophytes, and free-living diploid tetrasporophytes (Bold & Wynne, 1978). If gametophytes and tetrasporophytes are similar or identical in shape, the respective life cycle is called isomorphic. Such life cycles exist in the order Ceramiales (Maggs & Hommersand, 1993). Goff & Coleman (1987) proposed a convincing explanation for the cytological paradox of isomorphic gametophytes and tetrasporophytes in one such species, *Griffithsia pacifica* (Ceramiales, Ceramiaceae), which has multinucleate cells. The somatic diploid nuclei of tetrasporophytes are surrounded by a cytoplasmic domain which is twice as large as the respective domain in haploid gametophytes, so gametophytic

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cells have twice as many nuclei as tetrasporophytic cells, and total DNA contents are identical in homologous cells of both generations (Goff & Coleman, 1987). Total nuclear DNA contents of cells probably determine cell and thallus sizes. However, more or less identical numbers of nuclei occur in homologous multinucleate cells of gametophytes and tetrasporophytes of species of the Rhodomelaceae (Ceramiales) (Goff & Coleman, 1986, 1990; Schnetter *et al.*, 2000). The number of nuclei per cell may depend on the position of the respective cell within the thallus, and DNA contents of individual nuclei in a single cell can differ (Schnetter *et al.*, 2000).

In *Laurencia* Lamouroux and the closely related genus *Osmundea* Stackhouse (Nam *et al.*, 1994; Garbary & Harper, 1998) apical cells, cells of the central filament, trichoblasts, and also, in a few species, epidermal cells, are uninucleate, while medullary, cortical, and, in most species, epidermal cells, are multinucleate (see Schnetter *et al.*, 2000). In *Polysiphonia* and other rhodomelacean species Goff & Coleman (1986, 1990) showed a stepwise reduction of ploidy levels of nuclei during the development of thalli, starting from a highly polyploid apical cell. They postulated ultimately non-polyploid nuclei for *Dasya, Polysiphonia, Bostrychia,* and *Laurencia* (Goff & Coleman, 1990, figs 3–6) and proposed that 'homologous somatic cells of gametophytes and tetrasporophytes have the same amount of DNA and are the same size' (Goff & Coleman, 1990, p. 67). In *Osmundea pinnatifida* similar numbers of mostly polyploid nuclei occur in homologous cells of the fleshy parts of gametophytes and tetrasporophytes, and in some cases (such as in the epidermis) total nuclear DNA content of such cells really is identical (Schnetter *et al.*, 2000). This fact perhaps explains isomorphy, but there remains the question of how the haploid and diploid status of both thallus types is determined. In *Osmundea pinnatifida* total cellular nuclear DNA content of some types of multinucleate somatic cells with identical numbers of nuclei was higher in tetrasporophytes than in gametophytes (Schnetter *et al.*, 2000). These cells in *Osmundea* were from below the zone of formation of reproductive structures (tetrasporangia, spermatangia, carpogonia).

The present study investigated nuclear DNA contents in different cell types of reproductive apical and subapical parts of gametophytic and tetrasporophytic thalli of *Laurencia majuscula* (Harvey) Lucas and *Laurencia* sp. [*Laurencia* cf. *obtusa* (Hudson) Lamouroux]. We tested the hypothesis that 1C-level nuclei are present in somatic cells of gametophytes but not tetrasporophytes. These could be markers for the haploid level, although most nuclei are polyploid. We also determined whether maximum C-levels were greater and mean nuclear DNA contents were higher in tetrasporophytes than in gametophytes.

Materials and methods

Collection sites

All collections were made in Tenerife, Canary Islands, from basaltic rocks in the lower eulittoral zone. Gametophytes and tetrasporophytes of *Laurencia majuscula* were collected in El Prís on 10 February, 1998, and those of *Laurencia* sp. in Punta del Hidalgo on 11 February, 1998.

Vouchers are deposited in the Herbarium of the Universidad de La Laguna (TFC Phyc. Nos: 9929; 9940; 9944; 9945; 9946; 9947).

Preparation of thalli and measurement of nuclear DNA content

Thalli were transported to the Botany laboratory in La Laguna University in a cool box, then fixed in glutaraldehyde (2.5%) and passed stepwise through Karo (light corn syrup) (Schnetter *et al.*, 2000). Later, 20–30 distal thallus pieces of about 5 mm in length or transverse sections were treated with methanol (100%; 15 min; at $-20\,^{\circ}C$), rinsed in distilled water (1 min), and stained for 16 h with a DAPI solution (98 μl of a DAPI stock solution (2 mg DAPI in 10 ml distilled water) and 255 μl DMSO in 5 ml distilled water) in a refrigerator. After staining, the thallus pieces or sections were rinsed for 2 h in flowing tap water. Thereafter, sections were transferred onto slides for microscopy or squash preparations were made from the thallus pieces. During the whole preparation procedure, light was avoided. Relative fluorescence intensities (RFI) were measured using a Leica Diaplan epifluorescence microscope with a 75 W XBO xenon lamp (Osram), a filter block A (Leica) for UV excitation, and a computer supported Hamamatsu C 2400 video camera. A Leica fluorescence standard was used for the calibration of the cytophotometric assembly. Red autofluorescence of plastids was suppressed by a Schott filter BG 18, because the Hamamatsu camera has a high response to red light (Beutlich & Schnetter, 1993; Schnetter *et al.*, 2000). Nuclei for measurement of RFI of DNA content were randomly selected from all studied cell types. To avoid effects of photobleaching, generally, the fluorescence of only 1 nucleus per cell was individually measured, and then the number of nuclei of the respective cell was noted.

In this paper nuclear DNA contents are given either as RFI or C-values. For the identification of the 1C value, the RFI of 346 spermatangial nuclei were measured in each species. The egg-shaped spermatangia with a nucleus in the upper part of the cell could be easily identified. Spermatangia of *Laurencia majuscula* and *Laurencia* sp. corresponded to the descriptions given by Nam & Saito (1995) for *Laurencia tumida* and

by Masuda *et al*. (1998, fig. 23) for *Laurencia intricata*. RFI values of spermatangial nuclei of *Laurencia majuscula* and *Laurencia* sp. had a large amplitude of variation. Maturation of spermatangia obviously correlates with a reduction of nuclear DNA content, starting from 32C. In the interpretation of our data, we followed Goff & Coleman (1990) who considered the DNA content of spermatangial nuclei with the lowest RFI values to correspond to 1C. Based upon these data, DNA contents of other nuclei were assigned to C-levels (1C, 2C, 4C, 8C, 16C, 32C, 64C, 128C), ascertained separately for each species. This procedure was performed on 650 nuclei each of female, male and tetrasporophytic thalli of both species, comprising 50 nuclei of each cell type listed in Tables 1 and 2. RFI were statistically analysed using the one-way ANOVA and the Kruskal-Wallis post test (GraphPad Software, San Diego, CA, USA; SPSS Software, München, Germany).

Results

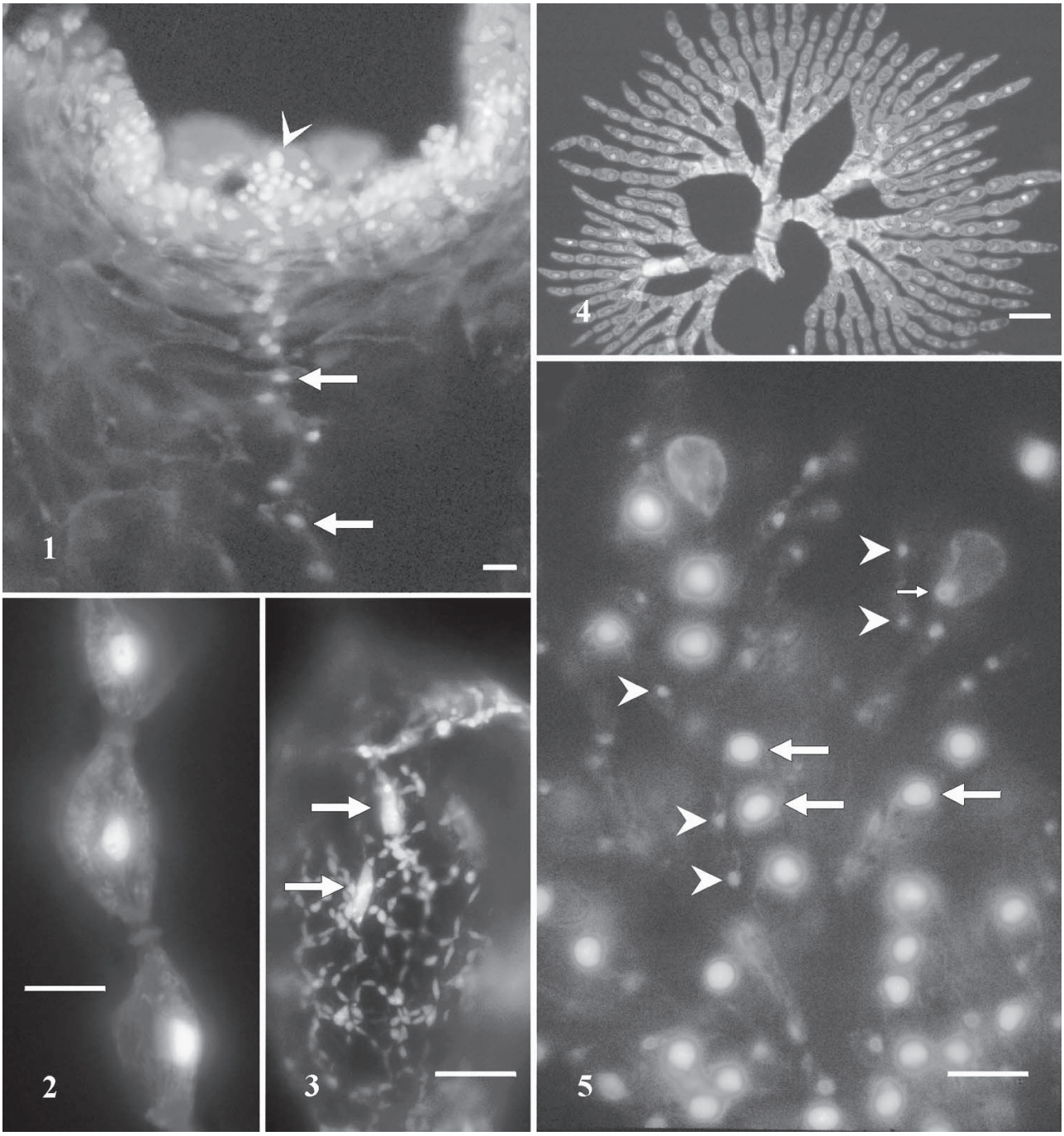
Most homologous cells of gametophytes and tetrasporophytes had identical numbers of nuclei in *Laurencia majuscula* and *Laurencia* sp. Apical cells (Fig. 1), cells of central filaments (Figs 1, 2), trichoblast cells (Fig. 4), very young and undifferentiated cells and spermatangia (Fig. 5) had only 1 nucleus. Epidermal cells had 1–3, mostly 2, nuclei. In pericentral cells 4–5 nuclei were frequently observed, and cells with 1 nucleus or with 8 nuclei were rarely found. In medullary cells there were at least 6 nuclei, mostly 7–8, occasionally 15 nuclei. Cortical cells (Fig. 3) had 1–5, generally 4, nuclei (Table 1). In both species variability of nuclear DNA content was higher in tetrasporophytes than in

Table 1. C-levels often occurring in nuclei of different cell types of tetrasporophytes and gametophytes of *Laurencia majuscula* and *Laurencia*. sp. Frequencies of listed C-levels: $\geqslant 40\%$, (30–39%)

	Tetrasporophytes		Gametophytes	
Types of cells	*L. majuscula*	*Laurencia* sp.	*L. majuscula*	*Laurencia* sp.
Central filament	32C, (64C)	64C	(8C, 16C)	32C, (16C)
Trichoblast	16C	32C	8C	(16C, 32C)
Pericentral	(4C)	(4C)	2C, (4C)	4C
Medulla, 6 nuclei	(4C, 8C)	8C, (16C)	4C	4C, (8C)
Medulla, 7–8 nuclei	4C, 8C	(8C, 16C)	4C	8C, (4C)
Medulla, > 8 nuclei	(4C, 8C)	8C	(2C, 4C)	4C, 8C
Cortex, 1–3 nuclei	8C, (4C)	8C	4C	4C, 8C
Cortex, 4 nuclei	8C	(32C)	4C, (8C)	8C, (4C)
Cortex, 5 nuclei	(4C, 8C)	8C	4C	8C, (4C)
Epidermis, 1 nucleus	(4C, 8C)	16C, (8C)	4C	8C
Epidermis, 2 nuclei	4C	8C	4C	8C
Epidermis, 3 nuclei	4C	8C	2C, 4C	8C, (4C)

Table 2. DNA contents of nuclei in RFI in different homologous cell types of *Laurencia majuscula* and *Laurencia* sp. (mean ± SD, $n = 50$). Undifferentiated cells were uninucleate. Statistical significances of differences between female gametophytic (G ♀) and tetrasporophytic (T) cells, and between tetrasporophytic and male gametophytic (G ♂) cells, respectively: NS = no significance; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

	Laurencia majuscula						*Laurencia* sp.					
Types of cells	G ♀		T		G ♂		G ♀		T		G ♂	
Central filament	142.26 ± 61.85	***	337.78 ± 130.86	***	145.56 ± 39.90		101.40 ± 43.97	***	237.20 ± 52.51	***	123.04 ± 36.77	
Trichoblast	91.30 ± 45.45	***	168.68 ± 73.36	***	104.98 ± 37.86		69.90 ± 30.57	***	103.92 ± 37.65	***	73.78 ± 25.57	
Undifferentiated	169.26 ± 84.80	***	236.26 ± 89.04	*	187.50 ± 60.46		104.94 ± 22.63	***	201.76 ± 71.54	***	115.92 ± 25.23	
Pericentral	28.52 ± 24.25	***	92.30 ± 67.63	***	23.82 ± 15.72		23.06 ± 18.33	***	39.86 ± 26.35	***	20.22 ± 18.65	
Medulla, 6 nuclei	41.44 ± 39.38	***	90.40 ± 40.27	***	53.60 ± 31.86		27.92 ± 14.43	***	56.36 ± 24.93	***	23.06 ± 11.79	
Medulla, 7–8 nuclei	32.74 ± 24.64	***	70.58 ± 28.35	***	51.82 ± 25.40		35.76 ± 16.67	***	58.36 ± 24.71	***	32.94 ± 21.99	
Medulla, > 8 nuclei	32.94 ± 20.21	***	85.78 ± 60.89	***	38.06 ± 30.43		33.64 ± 20.75	**	48.36 ± 22.90	***	30.44 ± 10.00	
Cortex, 1–3 nuclei	42.96 ± 29.08	***	101.82 ± 63.29	***	48.14 ± 33.67		25.52 ± 16.61	***	49.76 ± 26.95	***	28.20 ± 12.57	
Cortex, 4 nuclei	82.86 ± 35.01	NS	84.22 ± 50.38	**	54.38 ± 25.82		27.90 ± 14.33	***	66.80 ± 34.26	***	28.14 ± 15.52	
Cortex, 5 nuclei	59.04 ± 36.61	NS	79.14 ± 44.60	**	48.48 ± 18.47		29.56 ± 8.67	***	44.62 ± 17.52	***	28.86 ± 14.94	
Epidermis, 1 nucleus	64.24 ± 32.77	*	72.92 ± 22.78	***	54.68 ± 15.84		45.18 ± 13.16	NS	53.76 ± 21.09	***	34.52 ± 17.71	
Epidermis, 2 nuclei	54.36 ± 25.02	NS	55.16 ± 18.37	**	44.10 ± 13.00		29.10 ± 12.56	***	40.74 ± 17.78	***	29.03 ± 7.77	
Epidermis, 3 nuclei	37.82 ± 10.84	**	47.72 ± 14.89	*	40.74 ± 15.99		29.26 ± 10.03	**	37.08 ± 10.36	***	27.92 ± 7.08	



Figs 1–5. *Laurencia majuscula* cells, showing nuclei stained with DAPI, viewed under UV illumination. Fig. 1. Longitudinal section of the apical part of a thallus with apical cell (arrowhead) and cells of central filament with nuclei (arrows). Cells of the central filament become larger with age. Fig. 2. Three cells of a central filament below apical region with one nucleus each. Fig. 3. Cortical cell. Two nuclei are marked by arrows. Fig. 4. Trichoblasts. Fig. 5. Spermatangial branch with spermatangia and their nuclei (large arrows), rudimentary nuclei in sterile cells (arrowheads), and terminal vesicular cell with nucleus (small arrow). (Scale bars represent: Figs 1, 4: 20 μm; Figs 2, 3, 5: 10 μm).

female and male gametophytes (Fig. 6, Table 2). This is particularly clear for the nuclei of central filaments, of undifferentiated cells and trichoblast cells (nuclear DNA content in tetrasporophytes is about twice as high as in gametophytes), but also evident in most other cell types (Table 2). High nuclear DNA content in some uninucleate epidermal cells (Fig. 6*A*, *C*–*F*) may indicate onset of mitosis.

Nuclei of developing spermatangia of *Laurencia majuscula* and *Laurencia* sp. had ploidy levels higher than 1C. Pin-like sterile uninucleate cells with RFI below 1C, frequently with 0.5C or less, were found in both species of *Laurencia*. Also, in *Laurencia majuscula* the nuclear DNA contents of cells of axes of ripe spermatangial branches (Fig. 5) were extremely low (below 1C), and therefore were not taken into account.

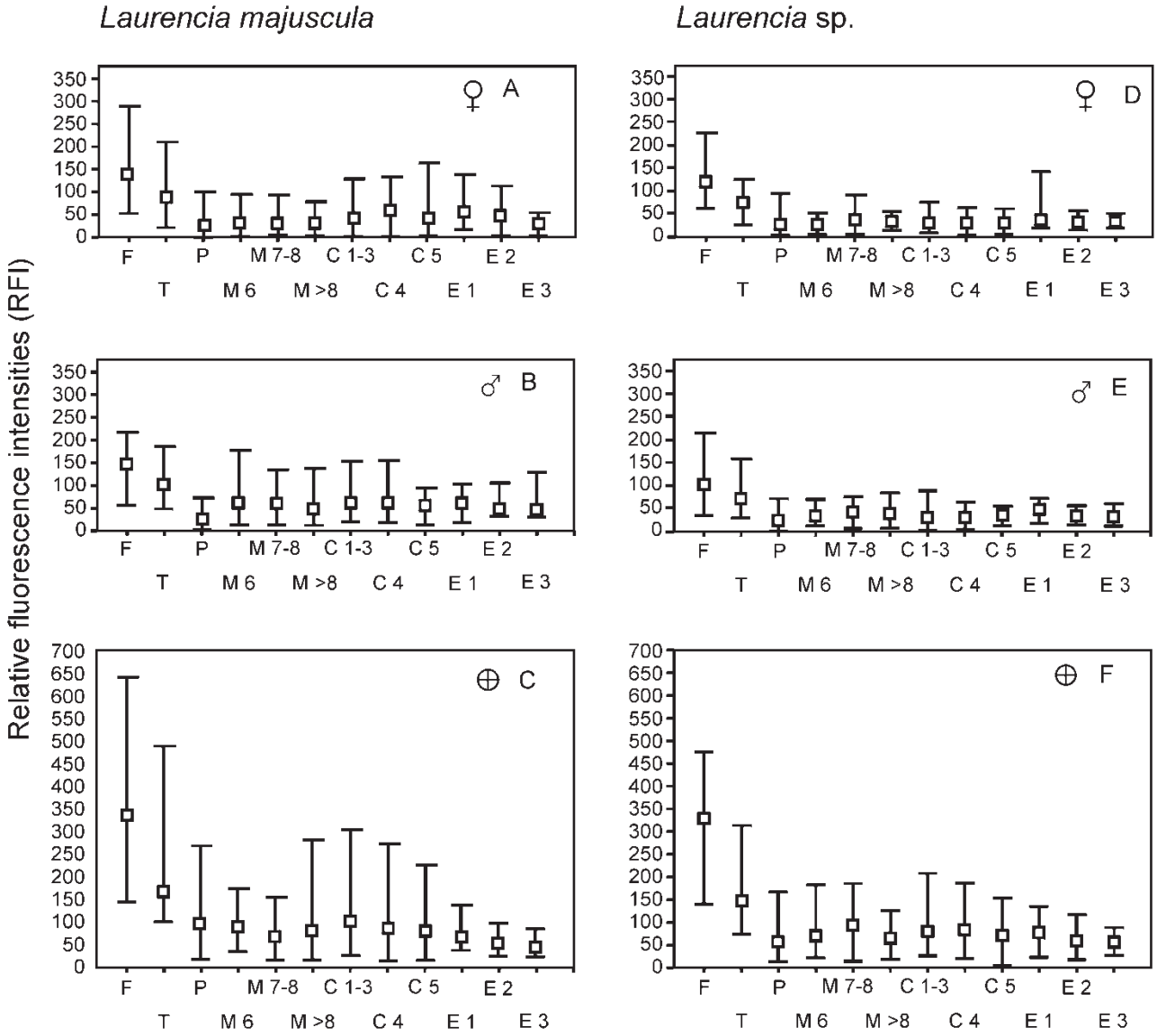


Fig. 6. DNA contents in relative fluorescence intensities (RFI) of individual nuclei in different cell types of gametophytic and tetrasporophytic thalli of *Laurencia majuscula* (graphs A – C) and *Laurencia* sp. (graphs D – F). Cell types: F, cells of central filaments; T, cells of trichoblasts; P, pericentral cells; M 6, medullary cells with 6 nuclei; M 7 – 8, medullary cells with 7 – 8 nuclei; M > 8, medullary cells with more than 8 nuclei; C 1 – 3, cortical cells with 1–3 nuclei; C 4, cortical cells with 4 nuclei; C 5, cortical cells with 5 nuclei; E 1, epidermal cells with 1 nucleus; E 2, epidermal cells with 2 nuclei; E 3, epidermal cells with 3 nuclei. Columns show maxima, means and minima of RFI.

The most frequent ploidy levels of all cell types from distal thallus parts of both *Laurencia* species are shown in Table 1. DNA contents of nuclei from homologous cells of female and male gametophytes do not differ significantly from each other, so they were treated together.

In both species DNA contents of individual nuclei in homologous cells of gametophytes and tetrasporophytes were overlapping (Fig. 6, Table 1). However, in all studied cell types average DNA contents of nuclei of tetrasporophytes were higher than those of female and male gametophytes. With the exceptions of cortical female cells with 4 or 5 nuclei, trinuclear epidermal cells in *Laurencia majuscula* and of the uninucleate epidermal cells in *Laurencia* sp., these differences between gametophytic and tetrasporophytic nuclei are statistically significant (Table 2). The striking decrease in cell size from medulla to epidermis was accompanied by a reduction in numbers of nuclei per cell. However, cell sizes were not measured due to shrinkage caused by the fixation and preservation procedure. Apart from spermatia, 1C nuclei were present only in gametophytic pericentral, medullary and cortical cells of both species (Fig. 7).

Measurements of nuclear DNA content in central thallus portions were very difficult because of very high background noise due to non-specific cell wall staining; only a few acceptable data were obtained. These showed that C-levels of individual

nuclei may increase with cell age, from 32C – 64C in epidermal cells to 64C or more in cortical and medullary cells and 128C in the central filament.

Discussion

Goff & Coleman (1990) postulated that some isomorphic Florideophycidae have the same amount of DNA in homologous somatic cells of tetrasporophytes and gametophytes. In *Griffithsia pacifica,* they found that cells of gametophytic thallus axes have twice as many haploid nuclei as homologous diploid tetrasporophytic cells, resulting in an identical total amount of DNA in both cell types. In the species of *Laurencia* studied in the present work, homologous cells of gametophytes and tetrasporophytes have identical numbers of mostly polyploid nuclei. Further, DNA contents of nuclei of all types of cells clearly reflect the haploid or diploid character of the respective *Laurencia* thalli (Tables 1, 2; Fig. 7). This supports the observation of Schnetter *et al.* (2000) who showed differences between DNA contents of nuclei of tetrasporophytes and gametophytes in multinucleate cells with identical numbers of nuclei in *Osmundea pinnatifida*. Statistically significant differences in nuclear DNA contents between gametophytes and tetrasporophytes exist in all types of cells of both studied species of *Laurencia*, with the exception of three female cell types (cortical with 4 and 5 nuclei, epidermal with 2 nuclei) of *Laurencia majuscula* and one female cell type (epidermal with 1 nucleus) in *Laurencia* sp. (Table 2). Differences in DNA contents of individual nuclei may be high in all multinucleate cells. DNA content of nuclei in tetrasporophytes often is twice as high as the respective nuclear DNA content in gametophytes. For example, in multinucleate medullary cells of *Laurencia majuscula* 8C nuclei prevail while in the gametophyte 4C nuclei are dominant (Table 1). Evident differences exist also between tetrasporophytic and gametophytic nuclei of cells of the central filament and trichoblasts and pericentral cells (Table 1). In *Laurencia* sp. the relations between gametophytes and tetrasporophytes are similar (Table 2). Tetrasporophytic nuclei of medullary cells tend to have higher DNA contents (16C) than those in the gametophyte, whereas, with the exception of cells with 6 nuclei, 4C nuclei appear to be less frequent than 8C nuclei (Table 1). As previously pointed out, homologous cells of gametophytes and tetrasporophytes have identical numbers of nuclei in both species of *Laurencia*. Because nuclei for measurement of DNA contents were chosen randomly, and mean DNA contents of the gametophytic nuclei are (mostly significantly) lower than those of homologous tetrasporophytic nuclei (Table 2), it is evident that total nuclear DNA content of the respective cell types of gametophytes is also lower than that in tetrasporophytes.

Based on the C-levels of the nuclei of the different cell types we present flow charts of the distribution of different nuclear DNA contents in the thalli of *Laurencia obtusa* and *Laurencia* sp. (Fig. 7). The 128C-levels (tetrasporophyte) and 64C-levels (gametophyte) of apical cells were

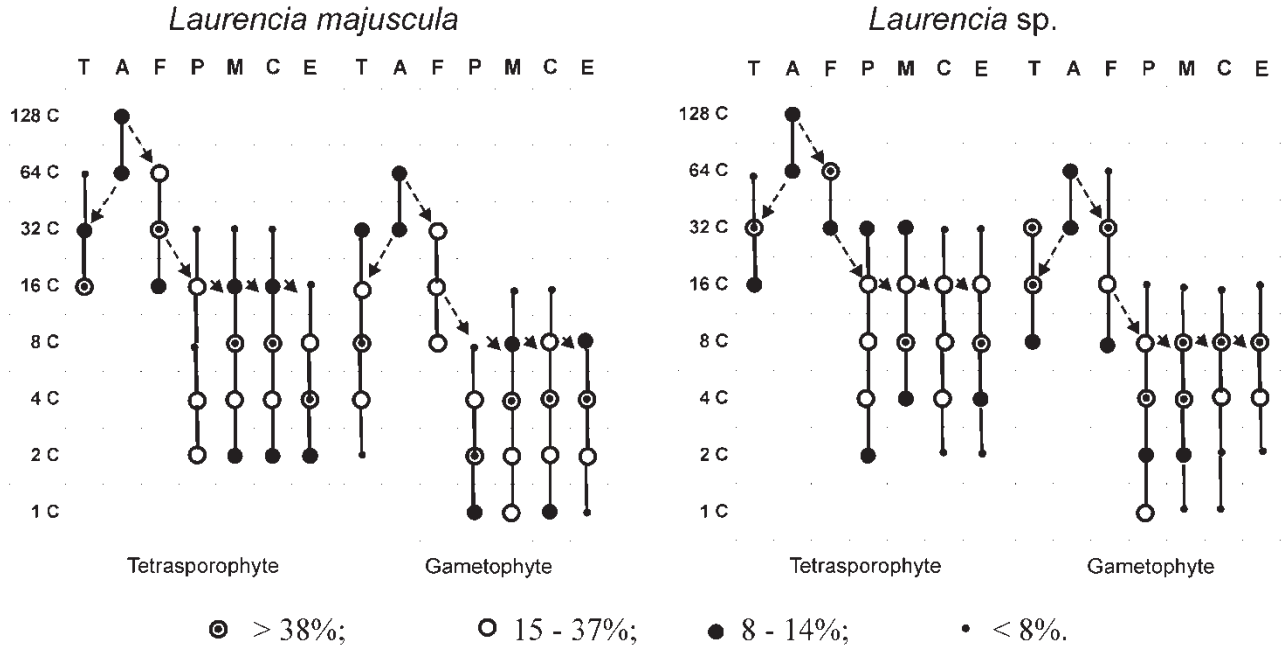


Fig. 7. Flow charts of C levels in tetrasporophytes and gametophytes of *Laurencia majuscula* and *Laurencia* sp. A, apical cells; for abbreviations of other cell types see Fig. 6.

derived hypothetically from the C-levels of the central filaments. These hypothetical values could be confirmed by some measurements in apical cells. As stated above, statistically significant differences between DNA contents of nuclei of gametophytic and tetrasporophytic cells exist (Table 2). However, the correlation of increasing cell sizes determined by simple microscopic observation with increasing numbers of nuclei per cell perhaps supports to a certain degree the hypothesis of a correlation between size and nuclear DNA content of cells (see Goff & Coleman, 1990). As in *Osmundea pinnatifida* (Schnetter *et al*., 2000), in both species of *Laurencia* DNA contents of individual nuclei show high variability, even in single cells.

Nevertheless, total ranges and averages of DNA contents of nuclei of all studied cell types of gametophytes differ from those of tetrasporophytes and reflect the haploid and diploid status of both thallus types in the two studied species of *Laurencia* (Table 2, Fig. 7). In *Laurencia majuscula* as well as in *Laurencia* sp. 32C nuclei are present only in tetrasporophytic pericentral, medullary, cortical and epidermal cells while 16C nuclei represent the highest ploidy levels in the respective gametophytic cells. The number of such nuclei is small. Further, we demonstrate that 1C nuclei are present only in gametophytic pericentral, medullary and cortical cells of both species. In the respective cells of tetrasporophytes, lowest values are 2C (Fig. 7). Nuclei with DNA content level below 1C were found in axes of ripe spermatangial branches (Fig. 5). Sterile cells of those spermatangial branches as well as the pin-like branchlets also observed in spermatangial clusters of *Osmundea pinnatifida* (Schnetter *et al*., 2000) are degenerating structures. The figure presented by Kylin (1956) suggests the occurrence of pin-like branchlets with very small nuclei in *Osmundea truncata* (Kützing) Nam et Maggs (as *Laurencia pinnatifida* in Kylin, 1956, and *O. pinnatifida* in Schnetter *et al*., 2000; see Nam *et al*., 2000). Probably, such structures with low nuclear DNA content indicate apoptosis in the respective cells (see Garbary & Clarke, 2001).

DNA contents of nuclei in the central filaments frequently correspond to 64C and 32C in tetrasporophytes and gametophytes, respectively (Table 1). An extremely high variability of DNA contents could be observed in the not yet differentiated near apical cells (data not shown). We propose that this high variability is a consequence of rapid mitotic and synthetic processes in the respective nuclei. In these nuclei the most frequent C-level corresponds to the nuclear DNA content in the central filament cells.

Similar C-values for most frequent ($> 38\%$) and frequent (15–37%) nuclei in pericentral, medullary, cortical and epidermal cells (Fig. 7) are proposed as a possible reason for isomorphy of tetrasporophytes and gametophytes although total DNA content ranges of both generations are different. Isomorphy develops in spite of considerable differences in the nuclear DNA content of cells of the central filament and other cell types. Taking into account the number of nuclei per cell, the total DNA content per cell of gametophytes vs tetrasporophytes frequently is about 1 : 2 (see Table 2).

Trichoblast structures play an important role in the formation of procarps and antheridia (Nam *et al*., 1991; see also Delivopoulos, 2002). Amounts of nuclear DNA in trichoblasts of gametophytes are significantly lower than in those of tetrasporophytes (Table 2), but 1C nuclei are missing (Fig. 7). It seems that in trichoblasts markers for the haploid or the diploid status are present. Tetrasporangia of *Laurencia* are formed from cortical (and epidermal) or pericentral cells (Nam *et al*., 1991), in which the DNA content ranges of nuclei comprise 2C and 32C in sporophytes of both studied species, while 1C and 16C occur in gametophytes (Fig. 7). Perhaps the lowest values of these ranges (1C, 2C), present in some (but not all) cells of the respective thalli, control their gametophytic or tetrasporophytic behaviour.

However, mixed phase thalli of *Osmundea pinnatifida* form both spermatangia and tetrasporangia. Cortical and medullary nuclear DNA contents indicate the haploid character of these thalli, but some nuclei of epidermal cells attain higher values (nearly 8C) than those of homologous nuclei in tetrasporophytes and normal gametophytes (4C) (Schnetter *et al*., 2000, fig. 10). In this species tetrasporangia develop from the outermost cortical (epidermal) cells (Nam *et al*., 1994). It is likely that the formation of tetrasporangia in gametophytes resulting in mixed phase thalli may be a dysfunction caused by anomalously high C-levels in some nuclei. This might also indicate that high DNA content of polyploid nuclei is a stimulus for tetrasporophytic behaviour.

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