

## Intra- and Interspecific Variation in DNA Content in *Cistus* (Cistaceae)

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Flow cytometry, using propidium iodide and 4',6-diamidano-2-phenylindole staining, was used to estimate the nuclear DNA content (2C) and the proportion of A–T base pairs in 16 species of the Mediterranean genus *Cistus*. Genome sizes were shown to be constant within species, since no significant intraspecific variation in 2C DNA content was detected. At the genus level, up to about 1.5-fold differences in absolute DNA amounts were observed, ranging from 3.92 pg in *C. crispus* to 5.88 pg in *C. monspeliensis*. The (AT) : (GC) ratio was close to 1, and was similar for all species examined, ranging from 47.87 % A–T content in *C. clusii*, to 50.67 % in *C. populifolius*. Pink-flowered species (subgenus *Cistus*) had lower DNA amounts than white-flowered species (subgenera *Leucocistus* and *Halimioides*). However, the distribution of DNA amounts in *Cistus* appeared to be continuous and did not permit a clear separation of infra-generic ranks in the genus.

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**Key words:** Flow cytometry, nuclear DNA content, genome size, A–T content, *Cistus*, Cistaceae.

### INTRODUCTION

The Cistaceae are a small family (approx. 175 taxa; Mabberly, 1997) that have a mostly holarctic distribution. Shrubby species are not uncommon within the family, and many of them play a prominent role in the configuration of Old World Mediterranean xeric landscapes.

*Cistus* L., one of the seven genera into which the family has traditionally been divided (Grosser, 1903), is relatively small (19 Mediterranean and Macaronesic species are currently recognized; Dansereau, 1939; Greuter *et al.*, 1984), but shows a noteworthy morphological diversification. Vegetative (presence or absence of petiole, leaf shape, hair morphology) and reproductive features (inflorescence architecture, sepal number, petal colour, pollen morphology, style length, type of capsule dehiscence and number of capsule valves) are so variable that several authors have proposed infra-generic classifications to depict taxonomic relatedness better. However, there are conflicting views concerning the circumscription and systematic ranks of the segregates (Spach, 1836; Willkomm, 1856; Grosser, 1903). The most extreme classification (Spach, 1836) divided *Cistus* into five genera, but this view has not been followed by later authors, who included all the variants at the subgeneric (*Cistus*, *Leucocistus*, *Halimioides*) and sectional (*Cistus*, *Macrostyliia*, *Ledonella*, *Ledonia*, *Stephanocarpus*, *Ladanium*, *Halimioides*) ranks within *Cistus* (Table 1).

Taxonomic relationships among *Cistus* species are based largely on morphological characteristics. However, the extent to which these distinguishing features truly indicate natural relationships, or whether they have originated by convergent evolution, is not currently known. Karyological

stability of chromosome number has been repeatedly noted (Markova, 1975, and references therein) and precludes further systematic refinement.

Genome size, being fairly constant within a species, is especially important for taxa delimitation, can be correlated with evolutionary processes within taxonomic groups and can be used as additional evidence in plant systematics (Bennett and Leitch, 1995). Previous reports have shown the usefulness of standardized DNA C-values to define infra-generic boundaries (see Ohri, 1998, for references). There is just one previous report of nuclear DNA amounts in Cistaceae, on a *Helianthemum* species (Bennett and Smith, 1991). This is not surprising since it was estimated that only 1 % of angiosperm species had been analysed up to 1997 (Bennett *et al.*, 1997), although it is expected that data will be available for an additional 1 % within the next few years (Hanson *et al.*, 2001).

We report the use of flow cytometry to evaluate the nuclear 2C DNA content of a representative sample of *Cistus* species, to determine patterns of genome size variation and the relative proportion of A–T base pairs within the genus and to determine possible correlations with the existing infra-generic classifications. As genome size is a key biodiversity character (Hanson *et al.*, 2001) and has special relevance for evolutionary studies (Leitch *et al.*, 1998), determination of the variation in nuclear DNA content in *Cistus* may significantly improve our knowledge of this poorly studied family.

### MATERIALS AND METHODS

#### *Plant material*

This study included representatives of all of the subgenera of the genus *Cistus*. With the exception of *Cistus*

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TABLE 1. *Taxa, code numbers and origin of the accessions of Cistus analysed*

Taxa	Code number	Origin
Subgenus <i>Cistus</i>		
Sect. <i>Macrostyliya</i> Willk.		
<i>C. osbeckiaefolius</i> Webb ex Pitard & Proust	20025	Canary Islands, Spain
<i>C. symphytifolius</i> Lam.	20113	Canary Islands, Spain
Sect. <i>Cistus</i>		
<i>C. albidus</i> L.	20116	Gaucin, Spain
	20066	Ibiza, Balearic Islands
	20030	Sorede, Spain
	20067	Valencia, Spain
<i>C. crispus</i> L.	20093	Badajoz, Spain
	20070	Barig, Spain
	20220	La Murta, Spain
<i>C. creticus</i> L.	20099	Grossetto, Italy
	20022	Florence, Italy
	20090	Valencia, Spain
<i>C. heterophyllus</i> Desf. subsp. <i>Carthaginensis</i> (Pau) Crespo & Mateo	20011	Cartagena, Spain
Sect. <i>Ledonella</i> (Spach) Willk.		
<i>C. parviflorus</i> Lam.	20021	Bot. Garden Valencia
Subgenus <i>Leucocistus</i> Willk.		
Sect. <i>Ledonia</i> Dunal		
<i>C. populifolius</i> L.	20020	Castelló, Spain
	20045	Bot. Garden Coimbra
<i>C. psilosepalus</i> Sweet	20026	Pontevedra, Spain
	20111	Abrantes, Portugal
<i>C. salviifolius</i> L.	20038	Hvar Islands, Croatia
	20066	Valencia, Spain
<i>C. albanicus</i> E.F. Warburg ex Heywood	20027	Bot. Garden Jena
Sect. <i>Stephanocarpus</i> (Spach) Willk.		
<i>C. monspeliensis</i> L.	20014	Ibiza, Balearic Islands
	20080	Valencia, Spain
Sect. <i>Ladanium</i> (Spach) Gren.		
<i>C. ladanifer</i> L.	20010	Bot. Garden Valencia
	20224	Sierra Morena, Spain
<i>C. laurifolius</i> L.	20079	Valencia, Spain
	20027	Granada, Spain
Subgenus <i>Halimioides</i> (Willk.) Demoly & P. Monts		
Sect. <i>Halimioides</i> Willk.		
<i>C. clusii</i> Dunal	20110	Cofrentes, Spain
	20025	Granada, Spain
<i>C. libanotis</i> L.	20228	Cadiz, Spain

*chinamadensis* A. Bañares & P. Romero (sect. *Macrostyliya*), *C. varius* Pourret (sect. *Stephanocarpoidea*) and *C. munbyi* Pomel (sect. *Halimioides*), for which no living material was available, all currently recognized species of *Cistus* were studied. Usually, plant specimens were also grown from seeds of wild origin. However, in a few cases, seeds of plants cultivated in botanical gardens were also used. The list of accessions and the origin of the plant material is shown in Table 1. Voucher specimens are deposited in the herbarium VAL.

#### 2C DNA content measurements

Three individuals from each accession were analysed by flow cytometry. A small amount of fresh leaf material, usually less than 100 mg, was chopped with a sharp razor blade in a nuclei isolation buffer. The samples were stained with propidium iodide (PI), which intercalates into double-stranded DNA, or with 4',6-diamidino-2-phenylindole (DAPI), which binds at AT-rich regions of DNA. For PI

staining, the two-step procedure originally used for DAPI staining by Otto (1990), modified by Doležel and Göhde (1995) and subsequently adapted to intercalating dyes, ethidium bromide (Baranyi and Greilhuber, 1996) and PI (Greilhuber and Obermayer, 1997), was used. Leaves were chopped in a glass Petri dish in 1 ml ice-cold Otto buffer I, containing 0.1 M citric acid and 0.5 % (v/v) Tween 20. The material was filtered through a 40-µm nylon mesh and centrifuged at 150 g for 8 min. The pellet was resuspended in 100 µl fresh Otto buffer I and incubated at room temperature for 60 min. Subsequently, 1 ml of Otto buffer II, consisting of 0.4 M Na<sub>2</sub>HPO<sub>4</sub>, was added to each sample. Nuclei were stained by adding a concentrated, RNase A-containing PI solution to give final concentrations of 50 µg ml<sup>-1</sup> PI and 50 µg ml<sup>-1</sup> RNase A. Finally, after 15 min at room temperature, nuclei suspensions were analysed in the flow cytometer. For DAPI staining, leaf material was chopped at room temperature in 0.4 ml nuclei isolation buffer ('high resolution DNA kit', solution A: nuclei isolation; Partec GmbH, Münster, Germany) and incubated

for 2 h at room temperature after adding 0.4 ml fresh isolation buffer containing 1 % (w/v) cellulase (Onozuka), 2 % (w/v) polyvinylpyrrolidone and 15 mM 2-mercaptoethanol. Finally, the suspension was filtered through a 40- $\mu$ m nylon mesh and mixed with 2 ml of staining buffer ('high resolution DNA kit', solution B: DAPI staining; Partec) for a minimum of 5 min. For each sample, at least 10 000 nuclei were analysed and the ratio of G<sub>1</sub> peak means of the sample and the internal standard was calculated. Various standards recommended for plant DNA flow cytometry (*Raphanus sativus*, *Allium cepa*, *Zea mays*, *Glycine max* and *Vicia faba*), kindly provided by Dr J. Doležel (Institute of Experimental Botany, Olomouc, Czech Republic), were tested in preliminary experiments; *Raphanus sativus* 'Saxa' (2C = 1.1 pg; Doležel *et al.*, 1992) was chosen as an optimal internal standard. Measurements of DNA content were performed with a PA-II flow cytometer (Partec). A 20-mW argon ion laser light source (488 nm wavelength) with an RG 590 longpass filter was used for PI-stained nuclei. The 100 W Osram HBO 100/2 mercury arc lamp, TK420 dichroic mirror and GG 435 longpass filter of the equipment were used in the case of DAPI staining.

The percentage of A–T base pairs was calculated for each sample analysed as described by Ali *et al.* (2000), using the formula of Godelle *et al.* (1993):

$$\% (A + T) (\text{sample}) = \% (A + T) (\text{ref.}) \times \left[ \frac{\text{peak position (sample)/peak position (ref.)}}{\text{DNA content (sample)/DNA content (ref.)}} \right]^{1/5}$$

where % (A + T) (ref.) is the AT content of the internal standard, *Raphanus sativus* (46.99 %; Doležel *et al.*, 1992), peak ratios are calculated from DAPI histograms, and DNA content refers to the 2C values of each sample, previously determined by PI staining, and the internal standard.

ANOVA was performed using the SPSS program to evaluate differences in 2C DNA content and A–T values at several hierarchical levels.

## RESULTS

Following PI or DAPI staining, nuclei suspensions suitable for flow cytometric analysis of nuclear DNA amounts proved to be rather difficult to prepare from the taxa included in this study. The use of standard protocols, such as those established by Doležel *et al.* (1989) or by Pfosser *et al.* (1995), resulted in very high backgrounds and, at best, DNA content histograms of very low, unacceptable resolution. The two-step procedure described in Materials and Methods was finally chosen for PI staining; this produced sharp DNA peaks, with relatively low coefficients of variation (CV) (range 3.4–6.4 %, mean CV = 4.8) for nuclei isolated from young leaves of *Cistus* plants. Simplified versions of this protocol were also tested, as suggested by J. Doležel (<http://www.ueb.cas.cz/Olomouc1/lcgcm/index.htm>), but did not give satisfactory results. DAPI-stained nuclei, prepared

following the procedure recommended by the fluorometer's manufacturer (Partec), modified to include an enzymatic digestion of cell walls, also produced sharp peaks in the DNA histograms. However, they showed higher backgrounds and slightly higher coefficients of variation (range 4.9–6.9 %, mean CV = 5.7) than the PI samples. Omission of the cellulase treatment generally resulted in much broader DNA peaks and even higher CVs.

The values of nuclear DNA content (2C) measured by PI staining are summarized in Table 2, and Fig. 1 shows representative histograms. Amounts of nuclear DNA were remarkably uniform within species. Thus, for a given species, differences among individuals and among accessions were not statistically significant, indicating the stability of genome size in *Cistus*. Mean 2C DNA content in *Cistus* was 4.75  $\pm$  0.55 pg. Nuclear DNA content varied 1.52-fold among accessions, ranging from 3.88 pg (*C. crispus*) to 5.88 pg (*C. monspeliensis*). These two species also showed the lowest (3.92 pg) and highest (5.77 pg) mean 2C DNA amounts (Table 2). Mean DNA contents were significantly different among subgenera (ANOVA,  $F = 18.1$ ,  $P < 0.0001$ ), sections ( $F = 22.75$ ,  $P < 0.0001$ ) and species ( $F = 114.23$ ,  $P < 0.0001$ ). Subgenus *Cistus* showed the lowest mean genome size (2C DNA amount 4.48  $\pm$  0.39 pg), ranging from 3.92 pg in *C. crispus* (sect. *Cistus*) to 4.96 pg in *C. parviflorus* (sect. *Ledonella*). Subgenus *Leucocistus* showed higher mean values (4.88  $\pm$  0.56 pg), ranging from 4.45 pg in *C. ladanifer* (sect. *Ladanium*) to 5.88 pg in *C. monspeliensis* (sect. *Stephanocarpus*), whereas subgenus *Halimiodes* showed the highest mean 2C DNA amounts (5.45  $\pm$  0.26 pg), 5.28 pg in *C. clusii* and 5.77 pg in *C. libanotis*. The (AT) : (GC) ratio was close to 1 and was similar for all species examined (Table 3). The calculated mean of A–T base pairs fraction in *Cistus* was 48.91  $\pm$  0.73 %, ranging from 47.87 % in *C. clusii* (sect. *Halimiodes*) to 50.67 % in *C. populifolius* (sect. *Ledonia*).

## DISCUSSION

Previously, there was only a single report of DNA C-values in Cistaceae: 8.90 pg (4C) in *Helianthemum nummularium* (Bennett and Smith, 1991). This is a diploid perennial species ( $2n = 20$ ) belonging to tribe Cistineae, which also includes *Cistus*. This value is very close to the mean DNA amount of *Cistus* (4.75 pg/2C) and falls well within the ranges detected in this study. However, it is premature to anticipate overall levels of genome size variation within Cistaceae, since the taxa surveyed (now covering about 10 % of the species) show identical life-forms and have similar ecological requirements, two factors that have been correlated with DNA content variation in some plant groups (e.g. Bennett, 1976; Nandini *et al.*, 1997; Naranjo *et al.*, 1998; Dimitrova and Greilhuber, 2000). In fact, the data available for Cistaceae are in the lower range of the C-values reported for the higher group, eurosids (range 0.2–33 pg/2C, mean 17 pg/2C; Leitch *et al.*, 1998), in which it is phylogenetically included (Savolainen *et al.*, 2000).

Genome size and A–T content appear to be fairly stable within *Cistus*, showing little variation within and among

TABLE 2. Nuclear 2C DNA content, in pg (mean  $\pm$  s.d., n = 3), determined by flow cytometry with PI staining, of accessions, species, sections and subgenera of *Cistus*

Taxon	Subgenus	Section	Species	(Ac. No.)	Accession
Subgenus <i>Cistus</i>	4.48 $\pm$ 0.39 <sup>a</sup>				
Sect. <i>Macrostyliia</i>		4.52 $\pm$ 0.43 <sup>a</sup>			
<i>C. osbeckiaefolius</i>			4.13 $\pm$ 0.06 <sup>a,b</sup>	(20025)	4.13 $\pm$ 0.06 <sup>a,b,c,d</sup>
<i>C. symphytifolius</i>			4.91 $\pm$ 0.10 <sup>d</sup>	(20113)	4.91 $\pm$ 0.10 <sup>g,h,i</sup>
Sect. <i>Cistus</i>	4.43 $\pm$ 0.38 <sup>a</sup>				
<i>C. albidus</i>			4.78 $\pm$ 0.08 <sup>d</sup>	(20116)	4.79 $\pm$ 0.09 <sup>e,f,g,h</sup>
				(20066)	4.78 $\pm$ 0.15 <sup>e,f,g,h</sup>
				(20030)	4.76 $\pm$ 0.05 <sup>e,f,g,h</sup>
				(20067)	4.82 $\pm$ 0.02 <sup>f,g,h,i</sup>
<i>C. crispus</i>			3.92 $\pm$ 0.09 <sup>a</sup>	(20093)	3.98 $\pm$ 0.08 <sup>a,b,c</sup>
				(20070)	3.88 $\pm$ 0.13 <sup>a</sup>
				(20220)	3.90 $\pm$ 0.01 <sup>a,b</sup>
<i>C. creticus</i>			4.34 $\pm$ 0.15 <sup>b,c</sup>	(20099)	4.24 $\pm$ 0.12 <sup>b,c,d</sup>
				(20022)	4.38 $\pm$ 0.16 <sup>d,e</sup>
				(20090)	4.40 $\pm$ 0.18 <sup>d,e,f</sup>
<i>C. heterophyllus</i> subsp. <i>Carthaginensis</i>			4.82 $\pm$ 0.02 <sup>d</sup>	(20011)	4.82 $\pm$ 0.02 <sup>f,g,h,i</sup>
Sect. <i>Ledonella</i>		4.96 $\pm$ 0.11 <sup>a</sup>			
<i>C. parviflorus</i>			4.96 $\pm$ 0.11 <sup>d</sup>	(20021)	4.96 $\pm$ 0.11 <sup>h,i,j</sup>
Subgenus <i>Leucocistus</i>	4.88 $\pm$ 0.56 <sup>a</sup>				
Sect. <i>Ledonia</i>		4.84 $\pm$ 0.43 <sup>a</sup>			
<i>C. populifolius</i>			4.29 $\pm$ 0.11 <sup>b,c</sup>	(20020)	4.27 $\pm$ 0.17 <sup>c,d</sup>
				(20045)	4.31 $\pm$ 0.04 <sup>c,d</sup>
<i>C. psilosepalus</i>			5.22 $\pm$ 0.15 <sup>c</sup>	(20026)	5.26 $\pm$ 0.22 <sup>j,k</sup>
				(20111)	5.18 $\pm$ 0.06 <sup>i,j,k</sup>
<i>C. salvifolius</i>			4.76 $\pm$ 0.12 <sup>d</sup>	(20038)	4.77 $\pm$ 0.11 <sup>e,f,g,h</sup>
				(20066)	4.74 $\pm$ 0.15 <sup>e,f,g,h</sup>
<i>C. albanicus</i>			5.34 $\pm$ 0.07 <sup>c</sup>	(20027)	5.34 $\pm$ 0.07 <sup>k</sup>
Sect. <i>Stephanocarpus</i>		5.88 $\pm$ 0.1 <sup>b</sup>			
<i>C. monspeliensis</i>			5.88 $\pm$ 0.14 <sup>f</sup>	(20014)	5.88 $\pm$ 0.17 <sup>l</sup>
				(20080)	5.88 $\pm$ 0.14 <sup>l</sup>
Sect. <i>Ladanium</i>		4.46 $\pm$ 0.15 <sup>a</sup>			
<i>C. ladanifer</i>			4.45 $\pm$ 0.17 <sup>c</sup>	(20010)	4.46 $\pm$ 0.16 <sup>d,e,f</sup>
				(20224)	4.44 $\pm$ 0.22 <sup>d,e,f</sup>
<i>C. laurifolius</i>			4.46 $\pm$ 0.15 <sup>c</sup>	(20079)	4.41 $\pm$ 0.08 <sup>d,e,f</sup>
				(20027)	4.52 $\pm$ 0.20 <sup>d,e,f,g</sup>
Subgenus <i>Halimioides</i>	5.45 $\pm$ 0.26 <sup>b</sup>				
Sect. <i>Halimioides</i>		5.45 $\pm$ 0.26 <sup>b</sup>			
<i>C. clusii</i>			5.28 $\pm$ 0.09 <sup>e</sup>	(20110)	5.30 $\pm$ 0.02 <sup>j,k</sup>
				(20025)	5.27 $\pm$ 0.14 <sup>j,k</sup>
<i>C. libanotis</i>			5.77 $\pm$ 0.14 <sup>f</sup>	(20228)	5.77 $\pm$ 0.14 <sup>l</sup>

Within each column, means followed by the same superscript indicate no significant differences according to the Student–Newman–Keuls method ( $P < 0.01$ ).

accessions belonging to the same species. This is a prerequisite for using DNA C-values as an additional taxonomic character. However, the calculated values of DNA content and A–T percentage and the nature of the variation among species limit its use in *Cistus*. These findings are in accord with the stability of the ploidy level of the genus (all species investigated to date are diploid) and the relative homogeneity of the karyotypes among species (Markova, 1975). At low taxonomic levels, the most promising use of genome size could be as a useful marker for the detection of hybrids. Hybridization has been reported to be an active process in *Cistus* and many hybrid combinations within and among pink- or white-flowered species have been recorded in the field, based on intermediate morphological characters (Grosser, 1903). Furthermore, extensive artificial hybridizations have

shown the lack of crossing barriers between most *Cistus* species (Gard, 1910, 1912, 1914). However, the extent to which reticulation has played a significant role in the diversification of the genus has not been assessed. Two species have been suggested to be of hybrid origin. *Cistus albanicus*, a narrow endemic plant from south-east Europe (Warburg, 1968), was originally described as a hybrid between *C. monspeliensis* and *C. salvifolius* (*C. × florentinus* Lamark nm. *adriaticus* Margraf), although it was not growing with either. Some studies have reported that diploid interspecific hybrids have a DNA content intermediate between values of the parents involved (Buitendijk *et al.*, 1997). The mean 2C DNA content of *C. albanicus* (5.34 pg) is very close to the expected value (5.3 pg) of the F<sub>1</sub> hybrid between *C. monspeliensis* and *C. salvifolius*. On this basis, its hybrid origin should be

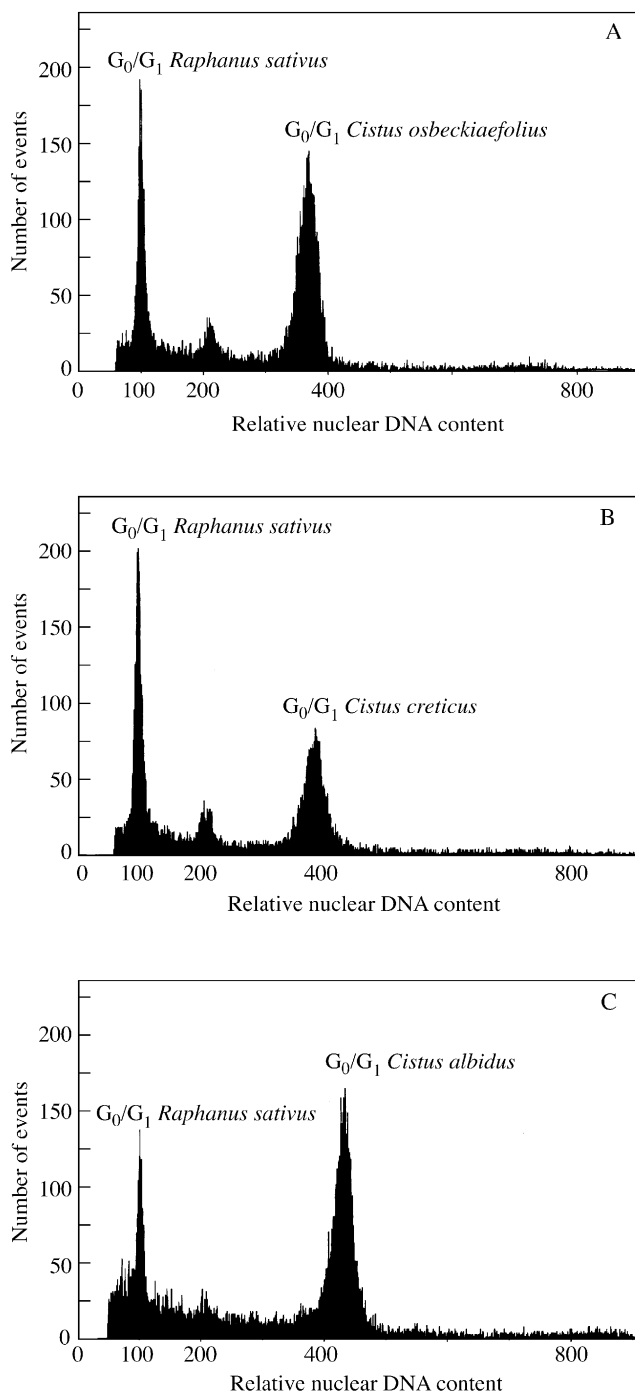


FIG. 1. Histograms of relative nuclear DNA content obtained after simultaneous flow-cytometric analysis of propidium iodide-stained nuclei of *Raphanus sativus* (internal standard) and *Cistus osbeckiaefolius* (A), *C. creticus* (B) or *C. albidus* (C).

reconsidered and further studied using discrete and more powerful molecular markers. In either case, the DNA content of *C. albanicus* is significantly different ( $P < 0.001$ ) from that of *C. monspeliensis* and *C. salvifolius*, the only two species in subgenus *Leucocistus* growing in south-east

Europe. *Cistus varius* is a narrow endemic Mediterranean species, and is the only representative of sect. *Stephanocarpoidea* (Dansereau, 1939). It has been suggested that it is also of hybrid origin, having *C. crispus* and *C. monspeliensis* (which belong to different subgenera) as putative parents (Grosser, 1903). Unfortunately, no living accession of this rare species was available for study. Determination of its nuclear DNA content should assist in the clarification of its taxonomic status, due to the genome size differences found between the proposed parents. Thus, it is hypothesized that if *C. varius* is an  $F_1$  hybrid between *C. crispus* and *C. monspeliensis*, its  $2C$  DNA content should be close to 4.8 pg.

Characteristic genome sizes or skewed base pair compositions could not be associated with currently recognized infra-generic units within *Cistus* in this study. In fact, variation in DNA content is mainly continuous, with overlapping values among sectional and even subgeneric taxa. This, together with the fact that the nuclear DNA amount in *Cistus* is relatively constant, with a variation of only 1.5-fold, limits its use in delimiting infra-generic divisions. In *Cistus*, patterns of variation in DNA amount do not parallel those of morphological variation. On this basis, few suggestions concerning the delimitation and rearrangement of its infra-generic divisions could be made. Even one of the earlier and most followed divisions of the genus, the splitting of *Cistus* into pink- and white-flowered species (Spach, 1836; Willkomm, 1856), is only loosely correlated with the DNA content or the proportion of A–T base pairs. Certainly, our results indicate that pink-flowered species (subgenus *Cistus*) have significantly lower mean DNA content than white-flowered ones (subgenus *Leucocistus* and *Halimioides*). However, the ranges are, to a large extent, overlapping. This makes flower colour an uncertain predictor of the DNA content in the genus. The critical systematic position of members of subgenus *Halimioides* has been reported several times (Dansereau, 1939; Démoly and Montserrat, 1991). These species (*C. clusii*, *C. libanotis* and *C. munbyi*) are closely related morphologically to taxa of sect. *Commutata* of the genus *Halimium*. Some authors have suggested the merging of *Cistus* and *Halimium* into a single genus (Löve and Kjellqvist, 1964), due to the existence of these intermediates and the sharing of a unique basic chromosome number ( $x = 9$ ) in the family. DNA amounts in subgenus *Halimioides* range among the highest reported values in *Cistus*. Nevertheless, their genome size is very similar to that of *C. monspeliensis*, a species unanimously included within subgenus *Leucocistus* and with which no apparent taxonomic relatedness or unifying morphological features have been postulated. Interestingly, preliminary results on DNA content in two subspecies of *Halimium umbellatum* (sect. *Commutata*), closely related to *C. clusii* and *C. libanotis*, have shown significant lower mean  $C$ -values (3.76–3.92 pg/ $2C$ ) than those reported for species of *Cistus* subgenus *Halimioides* (5.28–5.77 pg/ $2C$ ) (M. Boscaiu, P. Ellul and O. Vicente, unpubl. res.). Unfortunately, at present, there is no established morphological or molecular-based phylogeny of Cistaceae and it is, therefore, difficult to interpret the variation detected in genome size from an evolutionary perspective.

TABLE 3. Percentage of A–T content (mean  $\pm$  s.d.) of species, sections and subgenera of *Cistus*

Taxon	Subgenus	Section	Species
Subgenus <i>Cistus</i>	48.87 $\pm$ 0.49 <sup>a</sup>		
Sect. <i>Macrostyli</i>		48.65 $\pm$ 0.66 <sup>a,b</sup>	
<i>C. osbeckiaefolius</i>			48.82 $\pm$ 0.99 <sup>a,b</sup>
<i>C. symphytifolius</i>			48.47 $\pm$ 0.20 <sup>a,b</sup>
Sect. <i>Cistus</i>		48.96 $\pm$ 0.46 <sup>a,b</sup>	
<i>C. albidus</i>			48.88 $\pm$ 0.27 <sup>a,b</sup>
<i>C. crispus</i>			48.65 $\pm$ 0.41 <sup>a,b</sup>
<i>C. creticus</i>			49.39 $\pm$ 0.54 <sup>b,c</sup>
<i>C. heterophyllus</i> subsp. <i>carthaginensis</i>			48.62 $\pm$ 0.31 <sup>a,b</sup>
Sect. <i>Ledonella</i>		48.73 $\pm$ 0.10 <sup>a,b</sup>	
<i>C. parviflorus</i>			48.73 $\pm$ 0.10 <sup>a,b</sup>
Subgenus <i>Leucocistus</i>	49.18 $\pm$ 0.83 <sup>a</sup>		
Sect. <i>Ledonia</i>		49.08 $\pm$ 0.87 <sup>a,b</sup>	
<i>C. populifolius</i>			50.67 $\pm$ 0.15 <sup>d</sup>
<i>C. psilosepalus</i>			49.00 $\pm$ 0.33 <sup>a,b</sup>
<i>C. salvifolius</i>			48.89 $\pm$ 0.33 <sup>a,b</sup>
<i>C. albanicus</i>			48.02 $\pm$ 0.52 <sup>a</sup>
Sect. <i>Stephanocarpus</i>		49.11 $\pm$ 0.19 <sup>a,b</sup>	
<i>C. monspeliensis</i>			49.11 $\pm$ 0.19 <sup>a,b</sup>
Sect. <i>Ladanium</i>		49.51 $\pm$ 0.91 <sup>b</sup>	
<i>C. ladanifer</i>			48.81 $\pm$ 0.36 <sup>a,b</sup>
<i>C. laurifolius</i>			50.21 $\pm$ 0.68 <sup>c,d</sup>
Subgenus <i>Halimioides</i>	47.92 $\pm$ 0.32 <sup>b</sup>		
Sect. <i>Halimioides</i>		47.92 $\pm$ 0.32 <sup>a</sup>	
<i>C. clusii</i>			47.87 $\pm$ 0.25 <sup>a</sup>
<i>C. libanotis</i>			47.97 $\pm$ 0.44 <sup>a</sup>

Within each column, means followed by the same superscript indicate no significant differences according to the Student–Newman–Keuls method ( $P < 0.01$ ).

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