

# Cytotaxonomical observations on flowering plants from the Balearic Islands

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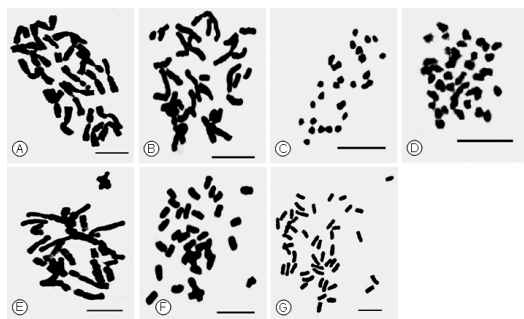
The mitotic chromosome numbers of 40 accessions of 33 species of vascular plants collected in the Balearic Islands are presented. The triploid cytotype of *Allium commutatum* ( $2n = 24$ ), the tetraploid cytotype of *Scilla autumnalis* ( $2n = 28$ ), and the hexaploid ( $2n = 36$ ) and octoploid ( $2n = 48$ ) cytotypes of *Tuberaria guttata* are reported here for the first time in the Balearic archipelago. The tetraploid cytotype of *Dactylis glomerata* subsp. *hispanica* was not previously known from the Western Balearics, where it grows sympatrically with the diploid endemic *D. glomerata* subsp. *ibizensis*. The divergent chromosome number  $2n = 26$  is confirmed for *Micromeria inodora*. A new chromosome number ( $2n = 44$ ) was determined for the restricted endemic *Rubia balearica* subsp. *caespitosa*. The existence of several infraspecific cytotypes bear phylogeographical significance.

Key words: continental islands, cytotaxonomy, Mediterranean flora, polyploidy

## Introduction

The Balearic archipelago shows a diverse insular flora as compared with that of other territories of the Mediterranean basin, both in terms of richness (about 1500 native species in 4992 km<sup>2</sup>) and originality (nearly 100 non-apomictic endemic taxa, and up to 30 apomictic microspecies; J. A. Rosselló unpubl. data). Karyological data has been used for a long time to assess the origin and the relationships of any given flora in general (e.g. Löve & Löve 1956, Küpfer 1974, Favarger 1975,

Favarager *et al.* 1979, Galland & Küpfer 1984, Galland 1988), and to analyze the patterns of plant evolution on islands in particular (e.g. Dalgaard 1994, Carr 1998, Stuessy & Crawford 1998). However, few publications have been devoted to the karyological study of the Balearic flora. The chromosome numbers of many taxa need to be studied, given the paucity of the analyzed taxa (less than 20% of the whole vascular flora) and the few accessions studied from different islands, before a comprehensive synthesis of the karyological evolution of the Balearic flora can be made.



**Fig. 1.** Mitotic metaphase plates of plant vascular species from the Balearic Islands. — **A:** *Allium commutatum* (Minorca, Es Mercadal),  $2n = 24$ . — **B:** *Dactylis glomerata* subsp. *hispanica* (Eivissa, Cala Albarca),  $2n = 28$ . — **C:** *Micromeria inodora* (Eivissa, Port des Torrent),  $2n = 26$ . — **D:** *Rubia balearica* subsp. *caespitosa* (Cabrera, L'Anciola),  $2n = 44$ . — **E:** *Scilla autumnalis* (Minorca, Son Mestre),  $2n = 28$ . — **F:** *Tuberaria guttata* var. *eriocaulon* (Minorca, Marina de Ruma),  $2n = 36$ . — **G:** *Tuberaria guttata* (Minorca, Marina de Ruma),  $2n = 48$ . Scale bars = 10  $\mu\text{m}$ .

In this paper we report the mitotic chromosome numbers of 33 native species collected from four islands of the archipelago.

## Material and methods

Seeds and living material (either whole plants or cuttings) were collected from natural populations across the Balearic Islands (Mallorca, Minorca, Cabrera and Eivissa). Living plants were transferred and cultivated in pots at the Botanical Garden of Valencia University. Seeds were germinated on solid agar in Petri dishes in a constant temperature of 20 °C and 12 hours of white light daily. Voucher specimens are preserved at VAL (Herbarium of the Botanical Garden of Valencia University).

Root tips were pre-treated with 0.002 M 8-hydroxyquinoline solution for 2h at 4 °C and 2h at room temperature, washed with distilled water, fixed in fresh Carnoy I solution (glacial acetic acid: absolute ethanol; 1:3) overnight and stored in 70% ethanol at 4 °C until used. For chromosome counts, the root tips were hydrolysed for 5–10 min in 1 M HCl at 60 °C, washed and stained in aceto-orcein for 4–6 h. Stained meristems were squashed in a drop of 45% acetic

acid and permanent preparations were made by mounting in Canada balsam. Photomicrographs of well-spread metaphases were taken with a digital camera and processed with a computer programme. Chromosome counts were made from 1–5 individuals per population, by direct observation and from the photomicrographs of at least five well-spread metaphases per individual.

## Results and discussion

The sporophytic chromosome number of 40 accessions belonging to 33 flowering plants from the Balearic Islands is reported here. The enumeration of the analysed species and studied accessions, the report of their chromosome numbers, and the indication of previous chromosome counts are shown in Table 1. The chromosome number of 11 species was not previously determined from Balearic accessions (Table 1). Most of the determined chromosome numbers agree with other cytogenetic records reported from non-Balearic accessions. Our results confirm a low level of karyological change in the endemic flora of the Balearic Islands (Castro & Roselló 2006), when compared with that of other archipelagos. However, some of the recorded chromosome numbers have karyological, phyto-geographical, or taxonomic interest, and are discussed below.

### *Allium commutatum*

This species belongs to the *A. ampeloprasum* complex, a group of diploid and polyploid species widely spread in the Mediterranean basin. To date, three cytotypes (diploid,  $2n = 16$ ; triploid,  $2n = 24$ ; tetraploid,  $2n = 32$ ) have been reported for *A. commutatum*, but cytotype distribution does not appear to be geographically structured (Von Bothmer 1982, Guern *et al.* 1991, Marcucci & Tornadore 1997). The plants from the Balearic Islands are triploid (Fig. 1A), and this polyploid level has also been reported from French, Italian and Greek populations (Von Bothmer 1982, Guern *et al.* 1991, Marcucci & Tornadore 1997).

### ***Dactylis glomerata* subsp. *hispanica***

The only reported entity of the *D. glomerata* complex in Eivissa and Formentera islands was the Western Balearic endemic *D. glomerata* subsp. *ibizensis*. Subspecies *ibizensis* is diploid ( $2n = 14$ ) and, although *Dactylis* plants are widespread in Eivissa and Formentera islands, only two accessions of this subspecies have been cytogenetically checked (Stebbins & Zohary 1959, Wetsching 1991). The finding of tetraploid *D. glomerata* plants in the Western Balearic Islands (Fig. 1B) is interesting and requires further karyological work in the area to assess (i) whether diploid and tetraploid cytotypes are geographically structured, and (ii) whether gene flow between the cytotypes occurs.

### ***Micromeria inodora***

Three chromosome counts implying a different basic chromosome number have been previously reported for Balearic accessions of *M. inodora*:  $2n = 26$  (Cardona 1973),  $2n = 30$  (Morales 1990), and  $2n = 48$  (Cardona & Contandriopoulos 1983). However, the  $2n = 26$  and  $2n = 48$  reports have been questioned on the basis of the presence of a basic chromosome number  $x = 10$  in the genus (Morales 1993). In fact, only the  $2n = 30$ ,  $2n = 50$  and  $2n = 60$  have been retained as verified chromosome numbers within *Micromeria* section *Micromeria* (Bräuchler *et al.* 2005), where *M. inodora* belongs. Our results (Fig. 1C) agree with the earlier report of the  $2n = 26$  cytotype (Cardona 1973), and points out to a more complex karyological pattern in this Western Mediterranean species.

Accessory chromosomes have been reported in a single accession of *M. inodora* ( $2n = 30 + 0 - 2B$ ; Morales 1990). It could be argued that the  $2n = 26$  chromosome number is the standard complement of the species, and that higher chromosome numbers ( $2n = 30$ ,  $2n = 31$ ,  $2n = 32$ ) may have originated by accumulation of accessory chromosomes. Although this is possible, it is difficult to explain the origin of the  $2n = 48$  cytotype by a such increase of accessory chromosomes alone. Alternatively,  $2n = 26$  could be

the result of loss of the chromosomes by aneuploidy. Within individual plants, chromosomal instability have been reported in other *Micromeria* species, e.g. *M. filiformis*,  $2n = 30$  (Dahlgren *et al.* 1971, Cardona and Contandriopoulos 1980, Morales 1990) and  $2n = 60$  (Dahlgren *et al.* 1971), but it refers only to euploid changes. The nature of this karyological variation in *M. inodora* could be better assessed by accurate observations of its meiotic behaviour.

### ***Rubia balearica* subsp. *caespitosa***

Our results showing  $2n = 44$  (Fig. 1D), disagree with the earlier reports of Cardona (1984) reporting the hexaploid level ( $2n = 66$ ) for plants endemic to Cabrera island. This is intriguing since both counts have been determined from accessions originating from the same population (L'Anciola). Although it is possible that two separate cytotypes may be present, the L'Anciola population has only few individuals, most of which reproduce asexually by rhizomes. Further, populations of the related *R. balearica* subsp. *balearica* have uniformly had a single cytotype ( $2n = 66$ ), both within and between populations (Castro & Rosselló 2006, and references therein).

### ***Scilla autumnalis***

The *S. autumnalis* species complex comprises a single morphological species showing impressive karyological diversity (Vaughan *et al.* 1997). Up to ten distinct cytological races, implying three levels of ploidy, have been detected and the existence of at least two additional races at some stage of the evolution of the complex have been hypothesized (Vaughan *et al.* 1997). Our counts from Minorcan accessions ( $2n = 28$ ; Fig. 1E) agree with the previous report from Mallorca (Battaglia 1957). Until now, no diploid individuals have been detected in the Balearic Islands, although they have been identified in surrounding territories of the Iberian Peninsula and Sardinia.

**Table 1.** List of investigated species with chromosome numbers and accession details. Previous Balearic chromosome counts are indicated. MA = Mallorca; ME = Minorca; DR = Dragonera; CA = Cabrera; EI = Eivissa.

Taxon	Chrom. number	Accession	Previous Balearic counts	References
<i>Allium commutatum</i>	2n = 24	Minorca, Binidonaire, Es Mercadal, grassy coastal slopes on siliceous soils, 10 m, <i>P. Fraga</i> , 16.VII.2004	–	–
<i>Anagallis arvensis</i>	2n = 40	Cabrera, L'Anciola, rocky places near the sea, <i>M.A. Conesa</i> , <i>A. Molins</i> , <i>M. Mus</i> & <i>J.A. Rosselló</i> , 5.VII.2005	2n = 40 [MA, ME]	Nilsson & Lassen (1971) Dahlgren et al. (1971)
<i>Asphodelus microcarpus</i>	2n = 28	Cabrera, Es Frate, calcareous soils on rocky places, <i>M.A. Conesa</i> , <i>A. Molins</i> , <i>M. Mus</i> & <i>J.A. Rosselló</i> , 5.VII.2005	2n = 28 [MA]	Nilsson & Lassen (1971) Dahlgren et al. (1971)
<i>Astragalus balearicus</i>	2n = 16	Cabrera, Cala Galiota, maritime slopes, <i>M.A. Conesa</i> , <i>A. Molins</i> , <i>M. Mus</i> & <i>J.A. Rosselló</i> , 5.VII.2005	2n = 16 [MA, ME]	Cardona (1978)
	2n = 16	Cabrera, L'Anciola, rocky places near the sea, <i>M.A. Conesa</i> , <i>A. Molins</i> , <i>M. Mus</i> & <i>J.A. Rosselló</i> , 5.VII.2005		Castro & Rosselló (2006) Guinochet & Lefranc (1972)
<i>Cistus creticus</i>	2n = 18	Minorca, Ciutadella de Menorca, Alzinar d'Alforí, established sand dunes, 90 m, <i>P. Fraga</i> , 11.IX.2005	–	–
<i>Cistus monspeliensis</i>	2n = 18	Cabrera, Cala de Santa María, calcareous slopes, <i>M.A. Conesa</i> , <i>A. Molins</i> & <i>J.A. Rosselló</i> , 6.VII.2005	2n = 18 [MA]	Nilsson & Lassen (1971)
	2n = 18	Minorca, Ferreries, Es Calatrat, scrub on calcareous soils, 40 m, <i>P. Fraga</i> , 18.IX.2005		
<i>Cistus salvifolius</i>	2n = 18	Minorca, Ferreries, Es Calatrat, scrub on calcareous soils, 40 m, <i>P. Fraga</i> , 18.IX.2005	2n = 18 [MA]	Nilsson & Lassen (1971) Dahlgren et al. (1971)
<i>Cneorum tricoccon</i>	2n = 36	Cabrera, Port de Cabrera, rocky places near the sea, <i>M.A. Conesa</i> , <i>A. Molins</i> & <i>J.A. Rosselló</i> , 6.VII.2005	2n = 36 [DR]	Dahlgren et al. (1971)
<i>Chelidonium majus</i>	2n = 12	Minorca, Ferreries, Barranc d'Algendar, shady calcareous soils, 50 m, <i>P. Fraga</i> , 12.IX.2005	–	–
<i>Dactylis glomerata</i> subsp. <i>hispanica</i>	2n = 28	Mallorca, Sóller, Serra d'Alfàbia, 900 m, vertical cliffs, <i>M.A. Conesa</i> , <i>P. Fraga</i> & <i>J.A. Rosselló</i> , 2.XII.2004	–	–
	2n = 28	Eivissa, Sant Antoni de Portmany, Cala Albarca, calcareous crevices, 210 m, <i>J.A. Rosselló</i> & <i>N. Torres</i> , 5.XI.2004		
<i>Dorycnium fulgurans</i>	2n = 14	Cabrera, L'Anciola, rocky places near the sea, 5 m, <i>M.A. Conesa</i> , <i>A. Molins</i> , <i>M. Mus</i> & <i>J.A. Rosselló</i> , 5.VII.2005	2n = 14 [MA, ME]	Cardona (1973) Cardona et al. (1983)
<i>Euphorbia exigua</i>	2n = 12	Minorca, Maó, Binicalat Nou, scrub on calcareous soils, 30 m, <i>P. Fraga</i> , 1.IV.2004	2n = 24 [MA]	Nilsson & Lassen (1971) Dahlgren et al. (1971)
<i>Hypericum balearicum</i>	2n = 24	Cabrera, Es Penyal Blanc, northern crevices on calcareous hills, <i>M.A. Conesa</i> , <i>A. Molins</i> & <i>J.A. Rosselló</i> , 6.VII.2005	2n = 24 [MA]	Nilsson & Lassen (1971) Dahlgren et al. (1971)
<i>Lobularia maritima</i>	2n = 24	Cabrera, Es Port, litoral scrub, 10 m, <i>M.A. Conesa</i> , <i>A. Molins</i> & <i>J.A. Rosselló</i> , 6.VII.2005	2n = 24 [MA]	Reynaud (1986)
<i>Micromeria inodora</i>	2n = 26	Eivissa, Sant Josep de sa Talaia, Port des Torrent, litoral scrub, <i>N. Torres</i> & <i>J.A. Rosselló</i> , 17.XI.2004	–	–
			2n = 26 [EI]	Cardona (1973), Morales (1990)
			2n = 30 + 0-2B [EI]	Cardona & Contandriopoulos (1983)
			2n = 48 [EI]	

<i>Misopates orontium</i>	2n = 16	Eivissa, Sant Josep de sa Talaia, near Cala Comte, open places, J.A. Rosselló & N. Torres, 17.XI.2004	–	–	–
<i>Narcissus elegans</i>	2n = 20	Mallorca, Felanitx, Porto Colom, littoral scrub near the road, 50 m, J.A. Rosselló, 12.XI.2005	–	–	–
<i>Narcissus serotinus</i>	2n = 30	Minorca, Maó, Forma, coastal rocky scrub on calcareous soils, 33 m, P. Fraga, 16.X.2005	2n = 30 [MA]	–	Fernandes (1968)
	2n = 30	Eivissa, Sant Josep de sa Talaia, Puig d'En Serra, calcareous crevices near the road, 380 m, N. Torres & J.A. Rosselló, 18.XI.2004	–	–	–
<i>Ononis crispa</i>	2n = 30	Cabrera, Cala de Santa Maria, littoral scrub, 2 m, M.A. Conesa, A. Molins & J. A. Rosselló, 5.VII.2005	2n = 30 [ME]	–	Cardona & Contandriopoulos (1983)
subsp. <i>crispa</i>					
<i>Ornithogalum baeticum</i>	2n = 54	Eivissa, Sant Josep de sa Talaia, Ses Salines, crevices on rocky slopes, 5 m, N. Torres & J.A. Rosselló, 18.XI.2004	–	–	–
<i>Rubia balearica</i>	2n = 44	Cabrera, L'Anciola, crevices near the sea, 5 m, M.A. Conesa, A. Molins, M. Mus & J.A. Rosselló, 5.VII.2005	2n = 66 [CA]	–	Cardona (1984)
subsp. <i>caespitosa</i>					
<i>Scilla autumnalis</i>	2n = 28	Minorca, Es Mercadal, Binimel·là, rocky calcareous soils, 40 m, P. Fraga, 31.VII.2005	2n = 28 [MA]	–	Battaglia (1957)
	2n = 28	Minorca, Ciutadella, Son Mestres, , scrub on calcareous soils, 50 m, P. Fraga, 9.X.2005	–	–	–
	2n = 28	Minorca, Es Migjorn Gran, Muntanya de Ses Fonts Rodones, rock crevices on siliceous rocks, 143 m, P. Fraga, 18.IX.2005	–	–	–
	2n = 8	Minorca, Es Mercadal, Santa Teresa, Sa Cavalleria des Martinells, crevices on calcareous rocks, 40 m, P. Fraga, 16.X.2005	–	–	–
<i>Scilla obtusifolia</i>	2n = 8	Eivissa, Sant Josep de sa Talaia, Port des Torrent, littoral scrub, N. Torres & J.A. Rosselló, 17.XI.2004	–	–	–
	2n = 24	Cabrera, L'Anciola, rocky places near the sea, M.A. Conesa, A. Molins, M. Mus & J.A. Rosselló, 6.VII.2005	2n = 24 [MA]	–	Dahlgren et al. (1971)
<i>Silene secundiflora</i>					
<i>Soleirolia soleirolii</i>	2n = 20	Mallorca, Andratx, Ses Basses, near La Trapa, 150 m, shady places near vertical cliffs, M.A. Conesa & J.A. Rosselló, 27.I.2005	–	–	–
<i>Smilax aspera</i>	2n = 32	Cabrera, L'Anciola, rocky places near the sea, 5 m, M.A. Conesa, A. Molins, M. Mus & J.A. Rosselló, 5.VII.2005	2n = 32 [ME]	–	Cardona & Contandriopoulos (1980)
subsp. <i>balearica</i>					
<i>Succowia balearica</i>	2n = 36	Mallorca, Felanitx, Sant Salvador, rocky places near the hills, 490 m, J.A. Rosselló, 12.XI.2005	–	–	–
	2n = 30	Cabrera, Es Coll Roig, calcareous slopes, M.A. Conesa, A. Molins, M. Mus & J.A. Rosselló, 5.VII.2005	2n = 28, 30, 32 [ME]	–	Valdés-Bermejo (1981)
<i>Teucrium marum</i>					
subsp. <i>marum</i>	2n = 36	Minorca, Es Mercadal, Ses Salines Noves, coastal sandy saline siliceous soils, P. Fraga, 18.X.2005	2n = 36 [MA]	–	Dahlgren et al. (1971)
<i>Triglochin bulbosum</i>					
subsp. <i>barrelieri</i>	2n = 18	Minorca, Ferreries, Son Gornés, sandy wetty siliceous soils, 110 m, P. Fraga, 17.X.2005	–	–	–
<i>Triglochin bulbosum</i>					
subsp. <i>laxiflora</i>	2n = 48	Minorca, Ferreries, Marina de Ruma, sandy siliceous soils, 230 m, P. Fraga, 15.V.2005	2n = ca 24 [MA]	–	Dahlgren et al. (1971)
<i>Tuberaria guttata</i>					
<i>Tuberaria guttata</i>	2n = 36	Minorca, Ferreries, Marina de Ruma, sandy siliceous soils, 230 m, P. Fraga, 15.V.2005	–	–	–
var. <i>ericaulon</i>					
<i>Valantia muralis</i>	2n = 18	Cabrera, Cala de Santa Maria, calcareous crevices, 10 m, M.A. Conesa, A. Molins & J.A. Rosselló, 6.VII.2005	2n = 18 [MA]	–	Nilsson & Lassen (1971) Dahlgren et al. (1971)

## *Tuberaria guttata*

The genus *Tuberaria* is karyologically one of the most diverse within Cistaceae. A polyploid complex has been reported from *Tuberaria* sect. *Scorpioides*, where *T. guttata* s. lato belongs (Gallego & Aparicio 1991), including tetraploid ( $2n = 24$ ), hexaploid ( $2n = 36$ ), and octoploid ( $2n = 48$ ) entities. Chromosome numbers and morphology are poorly correlated, and up to two chromosome numbers have been reported in several taxa (Gallego & Aparicio 1991, Gallego 1993). Some authors (Gallego 1993) have distinguished microspecies within the traditional concept of *T. guttata*, although the presence of frequent interspecific gene flow and hybrid swarms were recognized (Gallego & Aparicio 1991). Our accessions from Minorca belong to hexaploid ( $2n = 36$ ; Fig. 1F) and octoploid ( $2n = 48$ ; Fig. 1G) cytotypes, that are characteristic of *T. guttata* s. stricto (Gallego 1993). However, the plants here ascribed to *T. guttata* var. *ericoaulon* ( $2n = 36$ ) can be distinguished from *T. guttata* ( $2n = 48$ ) on the basis of several morphological characteristics. The indumentum is longer than in *T. guttata* s. stricto, with more stellate hairs throughout the plant, thus giving the plant a silvery aspect. The outer sepals are smaller and narrower and the flowers are typically tricolour, being dark-brown in the centre, reddish purple in the middle and yellow in the outer part of the sepals. This association of characteristics indicates that the taxonomic status of this variety should be reinterpreted, but a revision of material from the whole distribution area is needed. Previously, only the tetraploid cytotype ( $2n = ca. 24$ ; Dahlgren et al. 1971) was known in the Balearic Islands.

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