

Karyology of *Limonium* (Plumbaginaceae) species from the Balearic Islands and the western Iberian Peninsula

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Received September 2006; accepted for publication June 2007

Somatic chromosome numbers, conventional karyotype features and idiograms are reported for 27 *Limonium* species inhabiting the Western Mediterranean basin (Iberian Peninsula and the Balearic Islands). The chromosome numbers of *Limonium barceloi* ($2n = 36$), *L. ejulabilis* ($2n = 24$), *L. inexpectans* ($2n = 26$), *L. interjectum* ($2n = 24$), and *L. scopulorum* ($2n = 25$) were determined for the first time. In addition, new aneuploid and/or polyploid cytotypes are reported in *L. alcudianum* ($2n = 26$), *L. bonafei* ($2n = 26$), *L. camposanum* ($2n = 26$), *L. companyonis* ($2n = 26$), *L. dufourii* ($2n = 26$), *L. geronense* ($2n = 36$), *L. marisolii* ($2n = 54$), *L. migjornense* ($2n = 50$), and *L. pseudodictyocladon* ($2n = 16$). A group of polyploid species showed karyotypes comprising homologous chromosomes in groups of three (*L. antonii-llorensii*, *L. ejulabilis*, *L. interjectum*, *L. virgatum*, and *L. wiedmanii*), four (*L. geronense*), or six (*L. marisolii*), which suggests an autopolyploid origin. Other polyploid species were characterized by the presence of two different chromosome sets ($x = 8$ and $x = 9$) in the genome. The species *L. alcudianum*, *L. bonafei*, *L. camposanum*, *L. companyonis*, *L. dufourii*, *L. gibertii*, *L. girardianum*, *L. inexpectans*, *L. leonardi-llorensii*, *L. magallufianum*, *L. migjornense*, *L. minoricense*, and *L. scopulorum* showed various combinations of paired and unpaired $x = 8$ and $x = 9$ chromosome sets, suggesting that they are allopolyploids. © 2007 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2007, 155, 257–272.

ADDITIONAL KEYWORDS: apomict – evolution – Mediterranean flora – polyploidy – sea lavender – triploidy.

INTRODUCTION

Patterns of karyological evolution have been documented in several biodiversity-rich oceanic archipelagos, including the Canary Islands (Borgen, 1979), Madeira (Dalgaard, 1994), Hawaii (Carr, 1998), and the Juan Fernandez Islands (Stuessy & Crawford, 1998). A sound karyological basis is required to interpret and integrate data from various sources correctly if we aim to elucidate evolution in insular scenarios. Thus, cytogenetic knowledge has been important in identifying patterns and processes of interspecific hybridization further (Borgen, Leitch & Santos-Guerra, 2003), to understand the phylogenetic behaviour of polyploid species when using multicopy DNA

sequences (Ballard & Sytsma, 2000), and to assess patterns of genome size evolution (Suda, Kyncl & Freiová, 2003).

The Balearic Islands comprise a continental archipelago for which the basic karyological knowledge of the sexually reproducing endemic flora has been revised recently (Castro & Rosselló, 2005, 2006, 2007). Among the genera containing agamospermous species, *Limonium* Mill. (sea lavender), is the only one featuring Balearic endemics. *Limonium* is a taxonomically intricate group, comprising mainly sexual and apomictic perennial herbs and subshrubs distributed worldwide, but showing a centre of diversification in the Mediterranean basin, with several hundreds of species endemic to this region.

About 44 species of *Limonium* have been reported as native to the Balearic Islands by Erben, 1993, who recognized five sexual (diploid) and 26 apomictic

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(polyploid) species that are putative Balearic endemics. Other authors have defended a more synthetic alternative taxonomic scheme. In particular, a broader species concept is applied to sexual taxa, based on the argument that environmental changes are responsible for a number of phenotypic differences, which do not deserve taxonomic recognition (e.g. Llorens, 1986). Under this view, no sexual species endemic to the Balearic archipelago are recognized and only 21 apomictic microspecies differing by very few morphological features are considered to be endemic to this archipelago (Sáez, 2005).

Several published chromosome counts in Balearic species of *Limonium* are available (see Erben, 1993; for a recent summary). However, conflicting results have been reported for some species and a detailed study of the karyotypes of Balearic *Limonium* is not available. Knowledge of the chromosome number, ploidy level, and karyotype description in this genus is imperative to assess the patterns of microevolution and species diversification in the Mediterranean basin (Erben, 1978, 1979).

In this work, the somatic chromosome numbers of 21 Balearic species of *Limonium* are provided, together with a karyotype description for 18 of them. In addition, six species from the Iberian Peninsula are also analysed for comparative purposes.

MATERIAL AND METHODS

PLANT MATERIAL

Living material (either whole plants or seeds) was collected from natural populations across the Balearic Islands and the Western Iberian Peninsula (Table 1). Living plants were cultivated in pots at the Botanical Garden of Valencia University. Seeds were germinated on solid agar in Petri dishes at a constant temperature of 20 °C and with 12 h of white light daily. Voucher specimens are preserved at VAL.

CHROMOSOME PREPARATION AND KARYOTYPE ANALYSIS

Root tips were pretreated with 0.002 M 8-hydroxyquinoline solution for 2 h at 4 °C and 2 h at room temperature, washed with distilled water, fixed in fresh Carnoy I solution (absolute ethanol: glacial acetic acid; 3:1) overnight and stored in 70% ethanol at 4 °C until used. For chromosome counts and karyotype determination, the root tips were hydrolysed for 5 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. Chromosome counts were made from 1 to 5

individuals per population, by direct observation and from the photomicrographs of at least five well-spread metaphases per individual. The photomicrographs were taken with an Olympus Camedia C-2000-Z digital camera and processed using Adobe Photoshop v.7.0. Chromosome measurements were made on digital images using the processing image software IMAGETOOL v.5.0 and the freeware application MICROMEASURE v.3.3 (available at <http://www.colostate.edu/depts/biology/micromeasure>). Idiograms of several taxa showing sufficient chromosomal resolution were obtained from chromosome measurements of five well-spread metaphase plates from several plants. For each metaphase plate, the lengths of the short (S) and long (L.) arms of chromosomes were expressed in relative values (haploid chromosome set = 100%). For centromere position, the nomenclature of Levan, Fredga & Sandberg (1964) was followed. The intrachromosomal asymmetry index ($A_1 = 1 - \Sigma[(b/B)/n]$) and interchromosomal asymmetry index ($A_2 = s/x$) were determined, where b and B are the mean lengths of the short and long arms of each pair of homologues, respectively, n is the number of homologues, s is the standard deviation, and x is the mean chromosome length (Romero Zarco, 1986).

RESULTS

The sporophytic chromosome numbers of 27 Western Mediterranean species of *Limonium*, their ploidy level, karyotype formula, and asymmetry indexes are shown in Tables 2 and 3. The low numbers of metaphase plates and their quality did not provide sufficient resolution to construct idiograms for *L. grosii*. The chromosome numbers of *L. barceloi* ($2n = 36$; Figs 2, 17C), *L. inexpectans* ($2n = 26$; Figs 10, 20B), *L. interjectum* ($2n = 24$; Figs 11, 20C) and *L. scopulorum* ($2n = 25$; Figs 16, 22C) were determined for the first time. New cytotypes were found in *L. alcludianum* ($2n = 26$; Figs 1, 17A), *L. bonafei* ($2n = 26$; Figs 3, 17D), *L. camposanum* ($2n = 26$; Figs 4, 18A), *L. companyonis* ($2n = 26$; Figs 5, 18B), *L. dufourii* ($2n = 26$; Figs 6, 18D), *L. geronense* ($2n = 36$; Figs 8, 19C), *L. marisolii* ($2n = 54$; Figs 12, 21B), *L. migjornense* ($2n = 50$; Figs 13, 21C), *L. minoricense* ($2n = 25$; Figures 14 and 21D), and *L. pseudodictyocladon* ($2n = 16$; Figs 15, 22B). Lastly, an accurate chromosome count was obtained for *L. eju-labilis* ($2n = 24$; Figs 7, 19A), confirming the triploid level suggested previously on the basis of an approximate report by Rosselló, Mus and Soler (1994).

Our results have confirmed previous chromosome number reports (Table 2) for *L. antonii-llorensi* ($2n = 27$; Fig. 17B), *L. biflorum* ($2n = 25$), *L. cossonianum*

Table 1. List of investigated *Limonium* species, including their distribution, details of sampled populations, and voucher specimens

| Species | Distribution | Accession | Voucher |
|---|--|---|------------|
| <i>L. alcuadianum</i> Erben | Balearic Islands | Mallorca, Albufera de Alcudia, on sandy soil near the saltmarsh, 1 m, 15.x.2005, <i>M.A. Conesa et al.</i> | VAL 156868 |
| <i>L. antoni-llorensi</i> L. Llorens | Balearic Islands | Mallorca, Colonia de Sant Jordi, on sandy soil near the sea, 1 m, 14.x.2005, <i>M.A. Conesa et al.</i> | VAL 156873 |
| <i>L. barceloi</i> Gil & L. Llorens | Balearic Islands | Mallorca, Ses Fontanelles, on saltmarsh soil near the sea, 1 m, 13.x.2005, <i>M.A. Conesa et al.</i> | VAL 176068 |
| <i>L. biflorum</i> (Pignatti) Pignatti | Balearic Islands | Mallorca, Illetes, on sandy soil near the sea, 2 m, 14.x.2005, <i>M.A. Conesa et al.</i> | VAL 176092 |
| <i>L. bonafei</i> Erben | Balearic Islands | Mallorca, Felanitx, S'Algar, rocky places, 5 m, 12.x.2005, <i>J.A. Rosselló</i> | VAL 156872 |
| <i>L. camposanum</i> Erben | Balearic Islands | Mallorca, Calvià, Magalluf, Sa Porrassa, in clearings of disturbed saltmarsh, 2 m, 12.x.2005, <i>M.A. Conesa et al.</i> | VAL 156869 |
| <i>L. companyonis</i> (Gren. & Billot) Kuntze | Balearic Islands | Mallorca, Soller, Ses Tres Puntes, rocky soil near the sea, 1 m, 13.x.2005, <i>M.A. Conesa et al.</i> | VAL 156870 |
| <i>L. cossonianum</i> Kuntze | Iberian Peninsula and Western Balearic Islands | Formentera, Punta Pedrera, 10 m, rocky coast, vii.1996, <i>J.A. Rosselló & N. Torres</i> | VAL 156871 |
| <i>L. dufourii</i> (Girard) Kuntze | Iberian Peninsula | Spain, Valencia, Sagunto, Marjal del Moro, brackish soil in clearings of the saltmarsh, 5 m, vii.1996, <i>J.A. Rosselló</i> | VAL 176069 |
| <i>L. ejulabilis</i> Rosselló, Mus & Soler | Balearic Islands | Mallorca, Calvià, Magalluf, Sa Porrassa, in clearings of disturbed saltmarsh, 2 m, 12.x.2005, <i>M.A. Conesa et al.</i> | VAL 176070 |
| <i>L. furfuraceum</i> (Lag.) Kuntze | Iberian Peninsula | Spain, Alicante, Cap dels Horts, littoral slopes, 1 m, ix.1995, <i>J.A. Rosselló</i> | VAL 176071 |
| <i>L. geronense</i> Erben | Iberian Peninsula | Spain, Girona, Port Lligat, rocky coast, x.1995, <i>J.A. Rosselló & L. Sáez</i> | VAL 176072 |
| <i>L. gibertii</i> (Sennen) Sennen | Iberian Peninsula and Balearic Islands | Mallorca, Calvià, Cala Major, coastal slopes, 12.x.2005, <i>M.A. Conesa et al.</i> | VAL 176073 |
| | | Mallorca, Es Coll d'en Rebassa, Es Carnatge, coastal slopes, x.1995, <i>J.A. Rosselló</i> | No voucher |
| | | Mallorca, Palma de Mallorca, Porto Pi, coastal slopes, x.1995, <i>J.A. Rosselló</i> | No voucher |
| | | Eivissa, Santa Eulàlia, Cala Llonga, 1 m, rocky coast, x.1995, <i>J.A. Rosselló & N. Torres</i> | No voucher |
| | | Eivissa, Sant Francesc, 1 m, sandy soil, x.1995, <i>J.A. Rosselló & N. Torres</i> | No voucher |
| | | Formentera, Ses Illetes, 5 m, sandy soil, x.1995, <i>J.A. Rosselló & N. Torres</i> | No voucher |
| | | Tarragona, Punta de la Mora, rocky coast, without date, <i>L. Sáez</i> | No voucher |
| <i>L. girardianum</i> (Guss.) Fourr. | South France and Iberian Peninsula | Spain, Valencia, El Saler, clearings of a saltmarsh, x.1995, <i>J.A. Rosselló</i> | VAL 176074 |
| | | Spain, Castelló, Torreblanca, clearings of a saltmarsh, x.1995, <i>J.A. Rosselló</i> | No voucher |
| | | Spain, Alicante, Santa Pola, clearings of a saltmarsh, x.1995, <i>A. Carvalho & J.A. Rosselló</i> | No voucher |
| | | Spain, Tarragona, Delta de l'Ebre, clearings of a saltmarsh, x.1995, <i>J.A. Rosselló</i> | No voucher |
| <i>L. grosii</i> L. Llorens | Balearic Islands | Formentera, Estany des Peix, sandy soil, 1 m, x.1993, <i>J.A. Rosselló</i> | VAL 176075 |

Table 1. Continued

| Species | Distribution | Accession | Voucher |
|---|---|---|------------|
| <i>L. inexpectans</i> L. Sáez & Rosselló | Balearic Islands | Mallorca, Magalluf, Son Matias 12.x.2005, <i>M.A. Conesa et al.</i> | VAL 176076 |
| | | Mallorca, Magalluf, Sa Porrassa, x.1995, <i>J.A. Rosselló</i> | VAL 176077 |
| <i>L. interjectum</i> Soler & Rosselló | Iberian Peninsula | Spain, Alicante, Xàbea, rocky coast, x.2005, <i>J.A. Rosselló</i> | VAL 176078 |
| <i>L. leonardi-llorensii</i> L. Sáez, Carvalho & Rosselló | Balearic Islands | Mallorca, Calvià, Cala Major, coastal slopes, 12.x.2005, <i>M.A. Conesa et al.</i> | VAL 176079 |
| <i>L. magallufianum</i> L. Llorens | Balearic Islands | Mallorca, Magalluf, Sa Porrassa, clearings of saltmarshes, 12.x.2005, <i>M.A. Conesa et al.</i> | VAL 176080 |
| <i>L. marisolii</i> L. Llorens | Balearic Islands | Mallorca, Lluçmajor, Cap Blanc, 12.x.2005, <i>M.A. Conesa et al.</i> | VAL 176081 |
| <i>L. migjornense</i> L. Llorens | Balearic Islands | Mallorca, Campos, Salines de Sa Colònia, brackish soil, 12.x.2005, <i>M.A. Conesa et al.</i> | VAL 176082 |
| <i>L. minoricense</i> Erben | Balearic Islands | Minorca, Cala de Sant Esteban, rocky coast, x.1995, <i>J.A. Rosselló</i> | VAL 176083 |
| <i>L. minutum</i> (L.) Chaz. | South France, NE Iberian Peninsula and Balearic Islands | Mallorca, Felanitx, Porto Colom, 50 m, 10.x.2005, <i>J.A. Rosselló</i> | VAL 176084 |
| | | Mallorca, Pollensa, Puig Gros de Ternelles, litoral slopes, 300 m, <i>M.A. Conesa et al.</i> | VAL 176085 |
| | | Cabrera, Cala Santa Maria, sandy soil, 5.vii.2005, <i>M.A. Conesa et al.</i> | VAL 176086 |
| | | Formentera, Punta Pedrera, 10 m, rocky coast, vii.1996, <i>J.A. Rosselló</i> | VAL 176087 |
| <i>L. pseudodictyocladon</i> L. Llorens | Balearic Islands | Mallorca, Capdepera, Es Carregador, 1 m, 14.x.2005, <i>M.A. Conesa et al.</i> | VAL 176088 |
| <i>L. scopulorum</i> M.B. Crespo & M.D. Lledó | Iberian Peninsula | Spain, Alicante, Denia, Cova Tallada, rocky coast, x.1999, <i>J.A. Rosselló</i> | VAL 176089 |
| <i>L. virgatum</i> (Willd.) Fourr. | Mediterranean basin | Mallorca, Colonia de Sant Jordi, on sandy soils near the sea, 1 m, 14.x.2005, <i>M.A. Conesa et al.</i> | VAL 176090 |
| <i>L. wiedmannii</i> Erben | Balearic Islands | Formentera, Ses Illetes, sandy soil, 1 m, x.1995, <i>J.A. Rosselló</i> | VAL 176091 |

($2n = 16$; Fig. 18C), *L. furfuraceum* ($2n = 18$; Fig. 19B), *L. gibertii* ($2n = 26$; Fig. 9, 19D), *L. girardianum* ($2n = 26$; Fig. 20A), *L. grosii* ($2n = 36$), *L. leonardi-llorensii* ($2n = 26$; Fig. 20D), *L. magallufianum* ($2n = 26$; Fig. 21A), *L. minutum* ($2n = 18$; Fig. 22A), *L. virgatum* ($2n = 27$; Fig. 22D) and *L. wiedmannii* ($2n = 24$; Fig. 22E).

According to karyotype morphology, the polyploid taxa analysed fall within two well-defined groups. The first includes those species showing karyotypes comprising homologous chromosomes in groups of three (*L. antonii-llorensii*, *L. ejulabilis*, *L. interjectum*, *L. virgatum* and *L. wiedmannii*), four (*L. barceloi* and *L. geronense*), or six (*L. marisolii*). This probably suggests an autopolyploid origin, although an allopolyploid origin from closely related species

showing the same karyotype cannot be ruled out. The second group includes the remaining polyploids characterized by the presence of two different chromosome sets ($x = 8$ and $x = 9$) in the genome. The species showed various combinations of paired and unpaired $x = 8$ and $x = 9$ chromosome sets as follows: *L. alcedianum*, *L. bonafei*, *L. camposanum*, *L. companyonis*, *L. dufourii*, *L. gibertii*, *L. girardianum*, *L. inexpectans*, *L. leonardi-llorensii*, *L. magallufianum* (all $8 + 9 + 9$), *L. migjornense* ($4 \times 8 + 2 \times 9$), *L. minoricense* and *L. scopulorum* (both $8 + 8 + 9$), suggesting that they are allopolyploids.

Chromosomes with secondary constrictions or satellites were not observed in any of the analysed species. The presence of a variable number of a conspicuous metacentric chromosome (the longest of the

Table 2. Chromosome numbers and ploidy level of studied *Limonium* species from the Iberian Peninsula and the Balearic Islands, including previous references. IP = Iberian Peninsula; MA = Mallorca; ME = Minorca; EI = Eivissa FO = Formentera; CA = Cabrera. *Some aneuploid cells were rarely observed within individuals

| | 2n | Ploidy level | Location | Population | Reference |
|----------------------------|-------|--------------|----------|------------------------|---------------------------------------|
| <i>L. alcuadianum</i> | 26 | 3x | MA | Albufera d'Alcudia | This work |
| | 27 | 3x | MA | Albufera d'Alcudia | Erben (1989) |
| <i>L. antoni-llorensii</i> | 27 | 3x | MA | Colonia Sant Jordi | Erben (1988) |
| | 27 | 3x | MA | Es Trenc | This work |
| <i>L. barceloi</i> | 36 | 4x | MA | Ses Fontanelles | This work |
| <i>L. biflorum</i> | 25 | 3x | MA | Ca'n Simoneta | Erben (1988) |
| | 25 | 3x | MA | Illetes | This work |
| <i>L. bonafei</i> | 26 | 3x | MA | Porto Colom | This work |
| | 27 | 3x | MA | Not reported | Erben (1993) |
| <i>L. camposanum</i> | 26 | 3x | MA | Prat de Magalluf | This work |
| | 27 | 3x | MA | Colònia Sant Jordi | Erben (1989) |
| <i>L. companyonis</i> | 26 | 3x | MA | Sóller | This work |
| | 27 | 3x | EI | Platja d'en Bossa | Erben (1981) |
| | 27 | 3x | MA | Carregador Capdepera | Erben (1989), sub <i>L. connivens</i> |
| | 27 | 3x | MA | Not reported | Erben (1989), sub <i>L. bianorii</i> |
| | 27 | 3x | MA | Not reported | Erben (1993), sub <i>L. orellii</i> |
| <i>L. cossonianum</i> | 27 | 3x | MA | Prat Magalluf | Erben (1989), sub. <i>L. validum</i> |
| | 16 | 2x | IP | Huercal Overa | Erben (1978) |
| | 16 | 2x | IP | Cabo Gata | Erben (1978) |
| | 16 | 2x | IP | Mojacar and Carboneras | Erben (1978) |
| | 16 | 2x | IP | Almeria | Erben (1978) |
| | 16 | 2x | IP | Cuevas Almanzora | Erben (1978) |
| | 16 | 2x | IP | Garrucha | Erben (1978) |
| | 16 | 2x | IP | La Unión | Erben (1978) |
| | 16 | 2x | IP | Portman | Erben (1978) |
| | 16 | 2x | IP | Cabo Palos | Erben (1978) |
| | 16 | 2x | IP | Mazarrón | Erben (1978) |
| | 16 | 2x | IP | Cartagena | Erben (1978) |
| | 16 | 2x | IP | Cabo Roig | Erben (1978) |
| | 16 | 2x | IP | Torrelamata | Erben (1978) |
| | 16 | 2x | IP | Hellín | Erben (1978) |
| | 16 | 2x | FO | Punta Pedrera | Mayol <i>et al.</i> (1998) |
| | 16 | 2x | FO | Punta Pedrera | This work |
| <i>L. dufourii</i> | 26 | 3x | IP | El Saler | This work |
| | 27 | 3x | IP | El Saler | Erben (1978) |
| <i>L. ejulabilis</i> | 24 | 3x | MA | Prat de Magalluf | This work |
| | c. 25 | 3x | MA | Prat de Magalluf | Rosselló <i>et al.</i> (1994) |
| <i>L. furfuraceum</i> | 18 | 2x | IP | South Alicante | Erben (1978) |
| | 18 | 2x | IP | El Altet | Erben (1978) |
| | 18 | 2x | IP | Torreveja | Erben (1978) |
| | 18 | 2x | IP | Jijona | Erben (1978) |
| | 18 | 2x | IP | Campello | Erben (1978) |
| <i>L. geronense</i> | 18 | 2x | IP | Cap dels Horts | This work |
| | 35 | 4x | IP | Cadaqués | Erben (1978) |
| | 35 | 4x | IP | Cadaqués | Erben (1978) |
| <i>L. gibertii</i> | 36 | 4x | IP | Port Lligat | This work |
| | 26 | 3x | IP | Punta de la Mora | This work |
| | 26 | 3x | IP | Tarragona | Erben (1978) |
| | 26 | 3x | IP | Ametlla del Mar | Erben (1978) |
| | 26 | 3x | IP | Tamarit | Erben (1978) |

Table 2. Continued

| | <i>2n</i> | Ploidy level | Location | Population | Reference |
|------------------------------|-----------|--------------|----------|----------------------|---|
| | 26 | 3x | MA | Cala Major | This work |
| | 26 | 3x | MA | Es Carnatge | This work |
| | 26 | 3x | MA | Porto Pi | This work |
| | 26 | 3x | EI | Cala Llonga | This work |
| | 26 | 3x | EI | Sant Francesc | This work |
| | 26 | 3x | FO | Ses Illetes | This work |
| | 27 | 3x | MA | Coll d'en Rebassa | Erben (1989), sub <i>L. bonnetii</i> |
| <i>L. girardianum</i> | 26 | 3x | IP | El Saler | This work |
| | 26 | 3x | IP | Torreblanca | This work |
| | 26 | 3x | IP | Santa Pola | This work |
| | 26 | 3x | IP | Delta de l'Ebre | This work |
| <i>L. grosii</i> | 36 | 4x | FO | Not reported | Erben (1993) |
| | 36 | 4x | FO | Estany des Peix | This work |
| <i>L. inexpectans</i> | 26 | 3x | MA | Son Maties | This work |
| | 26 | 3x | MA | Sa Porrassa | This work |
| <i>L. interjectum</i> | 24 | 3x | IP | Xàbea | This work |
| <i>L. leonardi-llorensii</i> | 26* | 3x | MA | Cala Major | Sáez <i>et al.</i> (1998a) |
| | 26 | 3x | MA | Cala Major | This work |
| | 26 | 3x | MA | Punta Negra | Sáez <i>et al.</i> (1998a) |
| <i>L. magallufianum</i> | 26 | 3x | MA | Prat de Magalluf | Erben (1988) |
| | 26 | 3x | MA | Prat de Magalluf | This work |
| <i>L. marisoliai</i> | 25 | 3x | MA | Cap Blanc | Erben (1988) |
| | 27 | 3x | MA | Not reported | Erben (1993) |
| | 27* | 3x | MA | Cap Blanc | Sáez <i>et al.</i> (1998a) |
| | 27 | 3x | MA | Pas de sa Senyora | Sáez <i>et al.</i> (1998a) |
| | 54 | 6x | MA | Cap Blanc | This work |
| <i>L. migjornense</i> | 25 | 3x | MA | Colònia Sant Jordi | Erben (1988) |
| | 50 | 6x | MA | Colònia Sant Jordi | This work |
| <i>L. minoricense</i> | 25 | 3x | ME | Cala de Sant Esteban | This work |
| | 27 | 3x | ME | Not reported | Erben (1993) |
| | 27 | 3x | ME | Not reported | Erben (1993), sub <i>L. artruchium</i> |
| <i>L. minutum</i> | 18 | 2x | IP | L'Estartit | Erben (1978), sub <i>L. revolutum</i> |
| | 18 | 2x | – | Not reported | Erben (1989) |
| | 18 | 2x | MA | Not reported | Erben (1993), sub <i>L. balearicum</i> |
| | 18 | 2x | CA | Estells | Erben (1988), sub <i>L. caprariense</i> |
| | 18 | 2x | CA | Cala Sta Maria | This work |
| | 18 | 2x | MA | Porto Colom | This work |
| | 18 | 2x | MA | Ternelles | This work |
| | 18 | 2x | MA | Cap Blanc | Erben (1989), sub <i>L. pseudibusitanum</i> |
| | 18 | 2x | MA | Cala Sant Vicenç | Erben (1989), sub <i>L. tenuicaule</i> |
| | 18 | 2x | CA | Estells | Erben (1989), sub <i>L. pseudibusitanum</i> |
| | | | | | Erben (1988), sub <i>L. caprariense</i> |
| | 18 | 2x | ME | Cala Escorxada | Erben (1989), sub <i>L. pseudibusitanum</i> |
| | 18 | 2x | EI | Cap Nonó | Erben (1988), sub <i>L. ebusitanum</i> |
| | 18 | 2x | FO | Not reported | Erben (1989), sub <i>L. scorpioides</i> |
| | 18 | 2x | FO | Punta Pedrera | Mayol <i>et al.</i> (1998) |
| | 27 | 3x | MA | Cala Sant Vicenç | Erben (1988), sub <i>L. balearicum</i> |
| | 27 | 3x | CA | Not reported | Erben (1989), sub <i>L. caprariense</i> |
| | 27 | 3x | – | Not reported | Erben (1993), sub <i>L. caprariense</i> |

Table 2. Continued

| | 2n | Ploidy level | Location | Population | Reference | |
|------------------------------|----------------------|--------------|----------|----------------------|---|--------------|
| <i>L. pseudodictyocladon</i> | 16 | 2x | MA | Carregador Capdepera | This work | |
| | 27 | 3x | MA | Not reported | Erben (1993) | |
| | 27 | 3x | MA | Carregador Capdepera | Erben (1989), sub <i>L. pseudoarticulatum</i> | |
| <i>L. scopulorum</i> | 25 | 3x | IP | Cova Tallada | This work | |
| <i>L. virgatum</i> | 27 | 3x | MA | Es Trenc | This work | |
| | 27 | 3x | IP | Cádiz | Erben (1978) | |
| | 27 | 3x | IP | Calpe | Erben (1978) | |
| | 27 | 3x | IP | Playa del Puig | Erben (1978) | |
| | 27 | 3x | IP | Benicarló | Erben (1978) | |
| | 27 | 3x | IP | Peñíscola | Erben (1978) | |
| | 27 | 3x | IP | Castelló | Erben (1978) | |
| | 27 | 3x | IP | Amposta | Erben (1978) | |
| | 27 | 3x | IP | Tamarit | Erben (1978) | |
| | 27 | 3x | IP | Delta Ebro | Erben (1978) | |
| | 36 | 4x | MA | Ca's Català | Nilsson & Lassen (1971) | |
| | <i>L. wiedmannii</i> | 24 | 3x | FO | Salines Marroig | Erben (1986) |
| | | 24 | 3x | FO | Ses Illetes | This work |

complement and assumed to be the marker chromosome reported by Erben, 1978) was observed in most species showing a partial or complete $x = 8$ genome. Thus, one of these was observed in *L. alcudianum*, *L. bonafei*, *L. camposanum*, *L. gibertii*, *L. girardianum*, *L. inexpectans*, *L. leonardi-llorensii*, *L. magallufianum* (all $2n = 26$) and *L. grosii* ($2n = 36$), two in *L. cossonianum* ($2n = 16$) and *L. scopulorum* ($2n = 25$) and three in *L. ejulabilis*, *L. interjectum* and *L. wiedmannii* (all $2n = 24$). The only exception was *L. migjornense*, in which no long metacentric chromosomes could be observed, despite the fact that four copies of the $x = 8$ genome were observed in its karyotype.

The $x = 8$ and $x = 9$ chromosome sets showed similar and statistically nonsignificant mean intra-chromosomal (A_1) asymmetry values (0.377 ± 0.07 and 0.373 ± 0.06 , respectively; $P = 0.555$). Moreover, the $x = 8$ chromosome sets were more asymmetrical (0.383 ± 0.12) than the $x = 9$ ones (0.336 ± 0.05) concerning the mean interchromosomal A_2 index, although the averages were not statistically significant ($P = 0.165$). Overall, it was not possible to discern both chromosome sets clearly when the two asymmetry indexes were used (Table 3, Fig. 23).

DISCUSSION

CHROMOSOME NUMBERS AND PLOIDY LEVEL

Chromosome number diversity is represented unevenly in infrageneric groups of Mediterranean

Limonium. Thus, karyologically checked taxa belonging to sections *Pteroclados*, *Ctenostachys*, *Pruinosa*, *Hyalolepidae*, *Polyarthion*, *Siphonanta* and *Myriolopis* are uniformly diploid, whereas section *Schizhy-menium* comprises a diploid taxon (*L. echioides*) and a closely related triploid one (*L. avei*). In contrast, section *Limonium* shows two basic chromosome numbers ($x = 8$ and 9) and displays great cytological diversity, including diploid ($2n = 16, 18$), triploid ($2n = 24, 25, 26, 27$), tetraploid ($2n = 32, 35, 36$), pentaploid ($2n = 43$) and hexaploid ($2n = 51, 54$) cytotypes. All of the Balearic and Iberian *Limonium* species analysed in this paper belong to section *Limonium* and the chromosome numbers reported here fall within the known ranges of variation within the section. Despite this, our data have revealed new chromosome numbers for 10 species, of which three (*L. marisoliai*, *L. migjornense* and *L. pseudodictyocladon*) represent changes in ploidy level. In other species-rich apomictic genera (e.g. *Hieracium*), infraspecific polymorphism concerning ploidy level has been reported previously for geographically widespread species, and different cytotypes are even reported to coexist within populations of a species (Chrték, 1994; Rotreková, 2004). However, this situation is exceptional in *Limonium*, where a single chromosome number and ploidy level usually characterize each species. The origin of such divergent cytotypes will be discussed below. The triploid level has been documented in 84.6% of the cytologically studied species in the Balearic Islands, whereas the presence of diploid (7.7%) and tetraploid (7.7%) taxa is mar-

Table 3. Karyotype descriptions, number of long metacentric chromosomes and asymmetry values (A_1 , A_2) of the basic $x = 8$ and $x = 9$ genomes present in Western Mediterranean species of *Limonium*. The number of metacentric markers, as predicted by Erben (1979), is as expected (*) or not as expected (†)

| Species | $2n$ | No. of metacentric marker chromosomes | Karyotype (no. of sets present) | | A_1 | | A_2 | |
|------------------------------|------|---------------------------------------|---------------------------------|--------------------|---------|---------|---------|---------|
| | | | $x = 8$ | $x = 9$ | $x = 8$ | $x = 9$ | $x = 8$ | $x = 9$ |
| <i>L. alcedianum</i> | 26 | 1* | 5m + 3sm (1) | 4m + 5sm (2) | 0.23 | 0.35 | 0.40 | 0.32 |
| <i>L. antoni-llorensi</i> | 27 | 0* | -(0) | 4m + 5sm (3) | - | 0.39 | - | 0.41 |
| <i>L. barceloi</i> | 36 | 0* | -(0) | 3m + 5sm + 1st (4) | - | 0.48 | - | 0.35 |
| <i>L. bonafei</i> | 26 | 1* | 3m + 3sm + 2st (1) | 4m + 5sm (2) | 0.51 | 0.37 | 0.37 | 0.35 |
| <i>L. camposanum</i> | 26 | 1* | 2m + 5sm + 1st (1) | 8m + 1sm (2) | 0.51 | 0.27 | 0.68 | 0.31 |
| <i>L. companyonis</i> | 26 | 0† | 3m + 3sm + 2st (1) | 4m + 5sm (2) | 0.42 | 0.36 | 0.37 | 0.36 |
| <i>L. cossonianum</i> | 16 | 2* | 3m + 5sm (2) | -(0) | 0.42 | - | 0.35 | - |
| <i>L. dufourii</i> | 26 | 0† | 4m + 3sm + 1st (1) | 6m + 3sm (2) | 0.43 | 0.25 | 0.30 | 0.29 |
| <i>L. ejulabilis</i> | 24 | 3* | 5m + 3sm (3) | -(0) | 0.33 | - | 0.37 | - |
| <i>L. furfuraceum</i> | 18 | 0* | -(0) | 5m + 4sm (2) | - | 0.37 | - | 0.33 |
| <i>L. geronense</i> | 36 | 0* | -(0) | 5m + 4sm (4) | - | 0.35 | - | 0.35 |
| <i>L. gibertii</i> | 26 | 1* | 4m + 2sm + 2st (1) | 5m + 2sm + 2st (2) | 0.41 | 0.42 | 0.37 | 0.34 |
| <i>L. girardianum</i> | 26 | 1* | 5m + 2sm + 1st (1) | 6m + 3sm (2) | 0.36 | 0.30 | 0.29 | 0.37 |
| <i>L. inexpectans</i> | 26 | 1* | 6m + 2sm (1) | 6m + 3sm (2) | 0.29 | 0.33 | 0.48 | 0.27 |
| <i>L. interjectum</i> | 24 | 3* | 5m + 2sm + 1st (3) | -(0) | 0.35 | - | 0.36 | - |
| <i>L. leonardi-llorensi</i> | 26 | 2† | 2m + 6sm (1) | 4m + 5sm (2) | 0.47 | 0.41 | 0.25 | 0.47 |
| <i>L. magallufianum</i> | 26 | 1* | 4m + 2sm + 2st (1) | 4m + 2sm + 3st (2) | 0.41 | 0.47 | 0.62 | 0.39 |
| <i>L. marisoli</i> | 54 | 0* | -(0) | 7m + 2sm (6) | - | 0.28 | - | 0.22 |
| <i>L. migjornense</i> | 50 | 0† | 5m + 3sm (4) | 3m + 6sm (2) | 0.32 | 0.42 | 0.20 | 0.34 |
| <i>L. minoricense</i> | 25 | 0† | 4m + 4sm (2) | 4m + 4sm + 1st (1) | 0.35 | 0.44 | 0.27 | 0.26 |
| <i>L. minutum</i> | 18 | 0* | -(0) | 4m + 5sm (2) | - | 0.40 | - | 0.29 |
| <i>L. pseudodictyoctadon</i> | 16 | 0† | 5m + 3sm (2) | -(0) | 0.32 | - | 0.25 | - |
| <i>L. scopulorum</i> | 25 | 2* | 5m + 3sm (2) | 5m + 2sm + 2st (1) | 0.33 | 0.37 | 0.41 | 0.36 |
| <i>L. virgatum</i> | 27 | 0* | -(0) | 3m + 5sm + 1st (3) | - | 0.44 | - | 0.34 |
| <i>L. wiedmannii</i> | 24 | 3* | 5m + 3sm (3) | -(0) | 0.34 | - | 0.56 | - |



Figures 1–8. Mitotic metaphase plates of *Limonium* species. Fig. 1. *L. alcudianum* (Mallorca, Albufera d'Alcudia), $2n = 26$. Fig. 2. *L. barceloi* (Mallorca, Ses Fontanelles), $2n = 36$. Fig. 3. *L. bonafei* (Mallorca, Porto Colom), $2n = 26$. Fig. 4. *L. camposanum* (Mallorca, Prat de Magalluf), $2n = 26$. Fig. 5. *L. companyonis* (Mallorca, Sóller), $2n = 26$. Fig. 6. *L. dufourii* (Iberian Peninsula, El Saler), $2n = 26$. Fig. 7. *L. ejulabilis* (Mallorca, Prat de Magalluf), $2n = 24$. Fig. 8. *L. geronense* (Iberian Peninsula, Port Lligat), $2n = 36$. Scale bars = 5 μm .

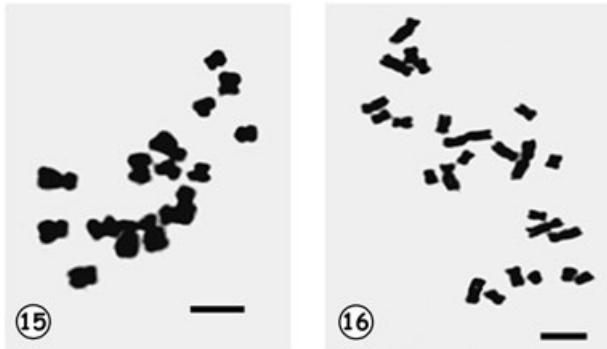
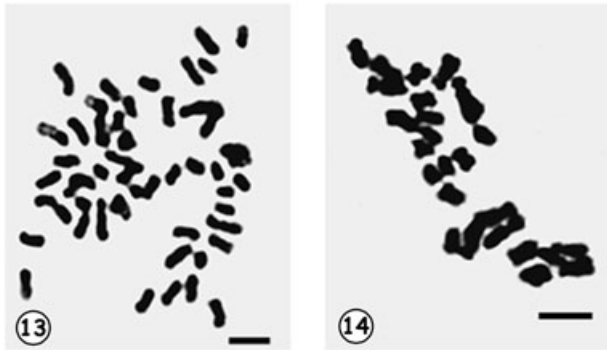
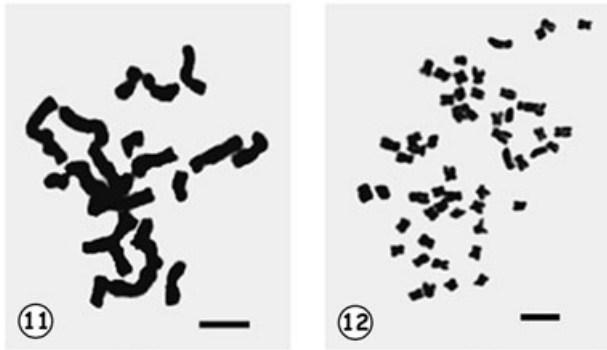
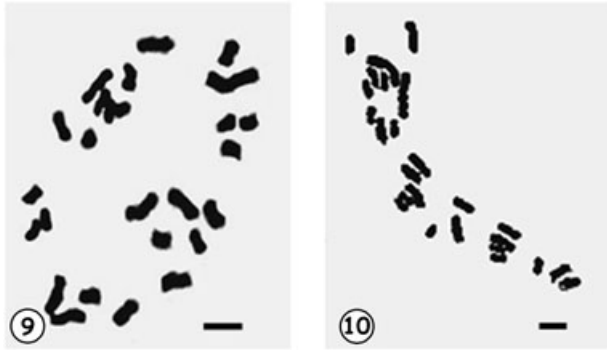
ginal. These results support previous data (Erben, 1993) indicating that triploids are by far the predominant *Limonium* cytotypes in the Iberian Peninsula and the Balearic Islands. From a cyto-biogeographical point of view, the Western Mediterranean area is remarkable, because (i) most of the polyploid cytotypes in the genus are reported to be present there, and (ii) species showing the two basic chromosome numbers of section *Limonium* ($x = 8$ and 9) and growing in sympatry are restricted to a very restricted area comprising south-east Spain and the western Balearic Islands.

INFRASPECIFIC KARYOLOGICAL VARIATION

Diploid species of *Limonium* are apparently stable karyologically, because accessory chromosomes have not been reported and, with the exception of *L. minutum* (Table 2), every studied taxon is characterized by a single chromosome number. However, conflicting results have been reported previously in polyploid species. Some authors have found different chromosome numbers, not only in the same species, but also in the same population, or even in the same individual, rendering a precise count virtually impossible (Dolcher & Pignatti, 1967, 1971; Arrigoni & Diana, 1993; Rosselló, Sáez & Carvalho, 1998).

The most extreme pattern of variation has been detected in the restricted Corsican endemic *L. bonifaciense* Arrigoni & Diana (Diana, 1995). Nearly 50 seeds collected from 18 field individuals of this species were germinated and studied cytologically. In only 46.9% of the seedlings was a single chromosome number found, either $2n = 27$ (34.7%), $2n = 36$ (10.2%) or $2n = 18$ (2%). In the other seedlings, mixoploidy was present and plants combining several of the chromosome numbers $2n = 9, 15, 18, 21, 24, 27, 30, 32,$ and 36 were found (Diana, 1995).

Aneuploidy has also been reported for *L. carvalhoi* Rosselló & L. Sáez, a narrowly distributed species from the Balearic Islands, in which three chromosome numbers $2n = 24, 25$ and 26 were found (Rosselló *et al.*, 1998). The $2n = 25$ cytotype was present in all



Figures 9–16. Mitotic metaphase plates of *Limonium* species. Fig. 9. *L. gibertii* (Mallorca, Cala Major), $2n = 26$. Fig. 10. *L. inexpectans* (Mallorca, Prat de Magalluf), $2n = 26$. Fig. 11. *L. interjectum* (Iberian Peninsula, Xàbea), $2n = 24$. Fig. 12. *L. marisoliai* (Mallorca, Cap Blanc), $2n = 54$. Fig. 13. *L. migjornense* (Mallorca, Colònia Sant Jordi) $2n = 50$. Fig. 14. *L. minoricense* (Minorca, Cala San Esteban) $2n = 25$. Fig. 15. *L. pseudodictyocladon* (Mallorca, Carregador Capdepera) $2n = 16$. Fig. 16. *L. scopolorum* (Iberian Peninsula, Cova Tallada) $2n = 25$. Scale bars = 5 μm .

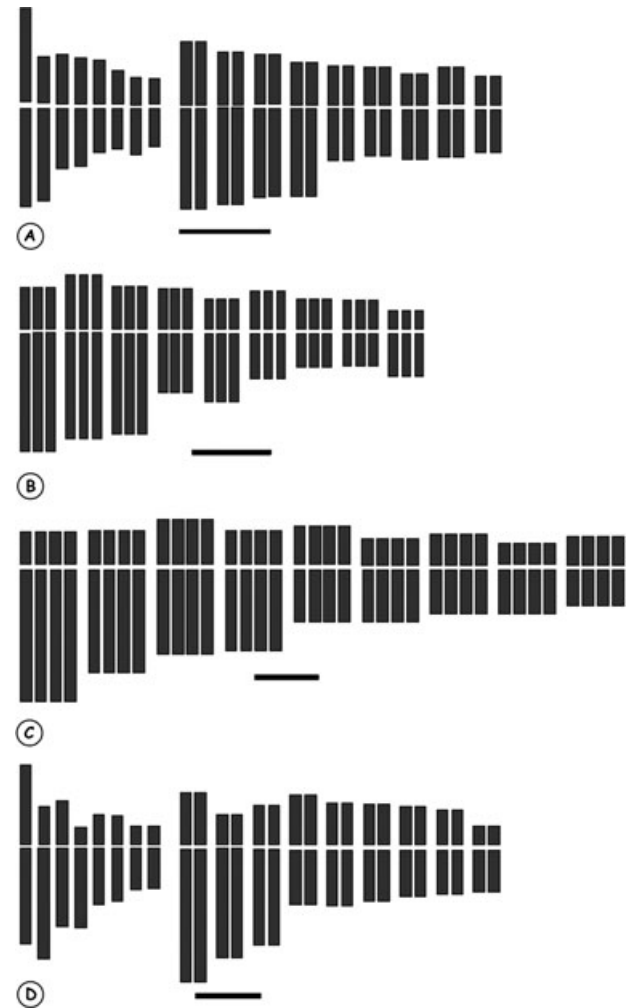


Figure 17. Idiograms of *Limonium* species. A, *L. alcludianum*, $2n = 26$ (8 + 9 + 9). B, *L. antoni-llorensi*, $2n = 27$ (9 + 9 + 9). C, *L. barceloi*, $2n = 36$ (4 × 9). D, *L. bonafei*, $2n = 26$ (8 + 9 + 9). Scale bars = 2.5 μm .

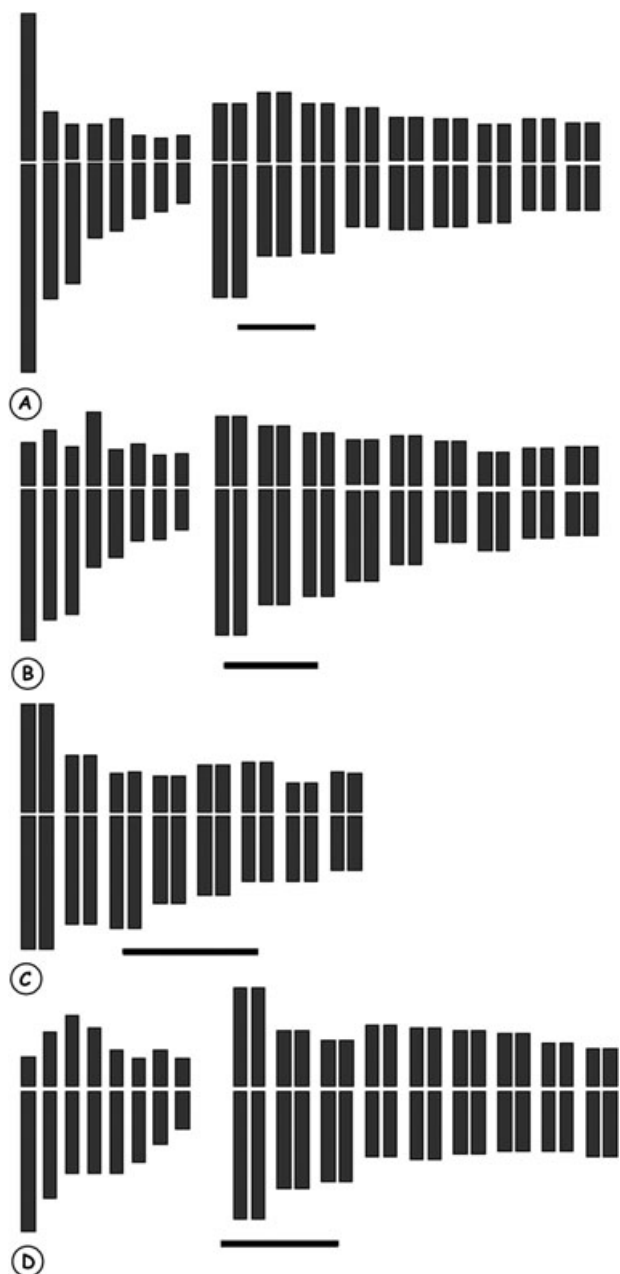


Figure 18. Idiograms of *Limonium* species. A, *L. camposanum*, $2n = 26$ (8 + 9 + 9). Scale bar = 2.5 μm . B, *L. companyonis*, $2n = 26$ (8 + 9 + 9). Scale bar = 2.5 μm . C, *L. cossonianum*, $2n = 16$ (8 + 8). Scale bar = 1.5 μm . D, *L. dufourii*, $2n = 26$ (8 + 9 + 9). Scale bar = 1.5 μm .

examined plants and was by far the most common (70.3% of all examined cells). In contrast, three out of nine individuals had $2n = 24$ (21.6% of cells) and the $2n = 26$ cytotype was detected in only a single plant (in 2.7% of cells). Overall, mixoploidy was detected in a third of the studied seedlings. The karyotypes of the three cytotypes did not differ by the presence or

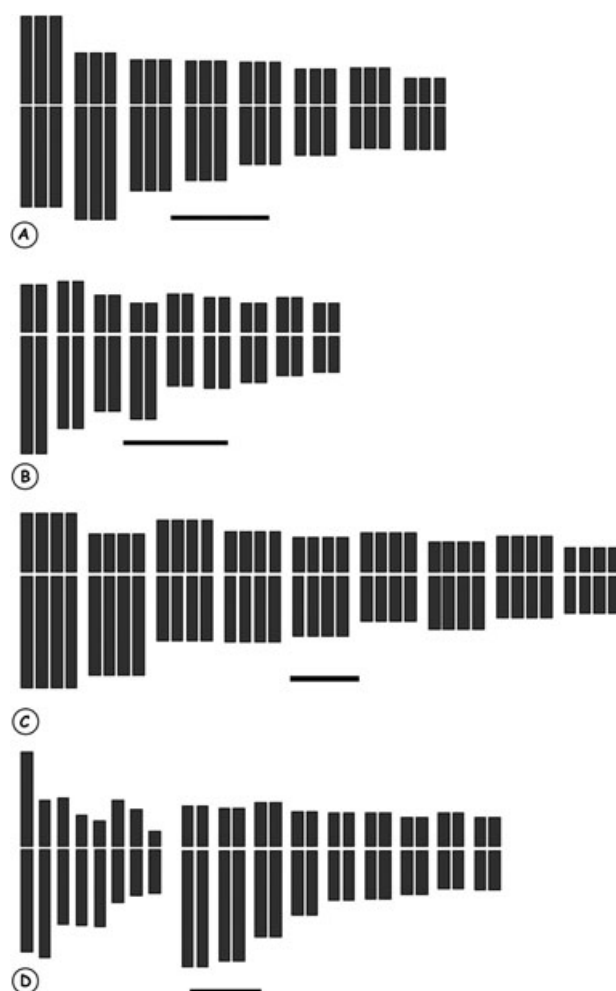


Figure 19. Idiograms of *Limonium* species. A, *L. ejulabilis*, $2n = 24$ (8 + 8 + 8). Scale bar = 2.5 μm . B, *L. furfuraceum*, $2n = 18$ (9 + 9). Scale bar = 5 μm . C, *L. geronense*, $2n = 36$ (9 + 9 + 9 + 9). Scale bar = 2.5 μm . D, *L. gibertii*, $2n = 26$ (8 + 9 + 9). Scale bar = 2.5 μm .

absence of specific chromosomes, but by changes in the morphology of most chromosomes, suggesting that drastic rearrangements of the karyotype were involved in their origin (Rosselló *et al.*, 1998).

Limited karyological variation has also been detected previously in *L. leonardi-llorensii* and *L. marisoliai*, in each of which aneusomatic cells with descending aneuploid karyotypes were observed (Sáez, Carvalho & Rosselló, 1998a). In *L. marisoliai*, a very narrowly distributed species, this cytological variation is further expanded by the report of two chromosome numbers ($2n = 25$ and 27 ; Erben, 1988, 1993).

In sharp contrast to these data, no such infraspecific karyological variation has been reported in any other Mediterranean triploid or tetraploid species of

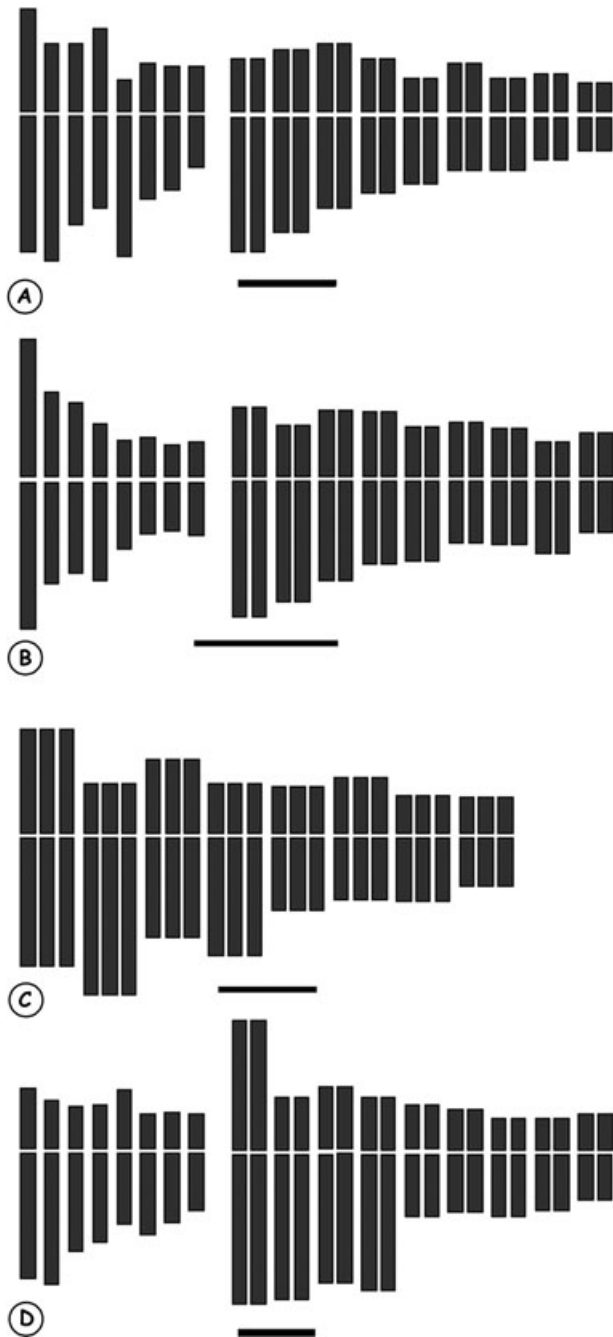


Figure 20. Idiograms of *Limonium* species. A, *L. girardianum*, $2n = 26$ (8 + 9 + 9). Scale bar = 2.5 μm . B, *L. inexpectans*, $2n = 26$ (8 + 9 + 9). Scale bar = 5 μm . C, *L. interjectum*, $2n = 24$ (8 + 8 + 8). Scale bar = 2.5 μm . D, *L. leonardi-llorensi*, $2n = 26$ (8 + 9 + 9). Scale bar = 2.5 μm .

Limonium (Erben, 1978, 1979, 1988; Brullo & Pavone, 1981; Artelari, 1989a, 1989b, 1992).

Our data, providing new chromosome numbers for about 45% of the analysed polyploid species, suggest

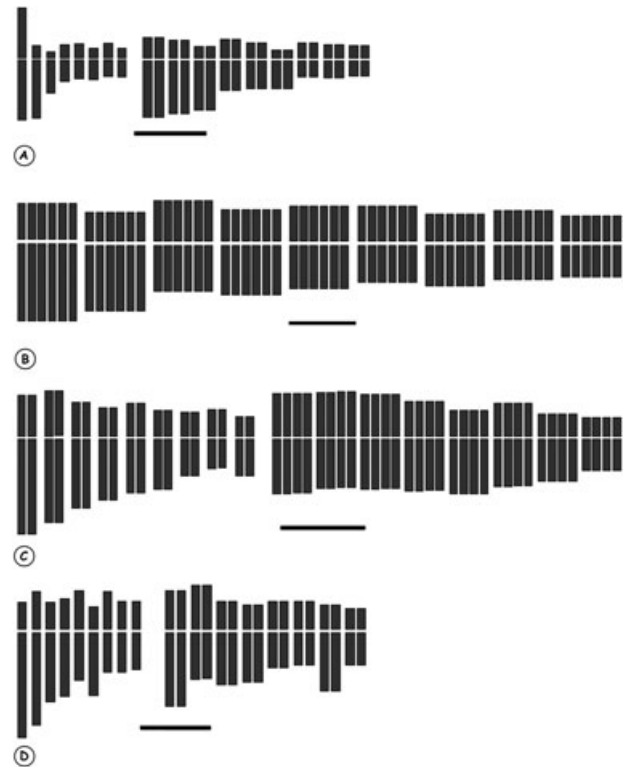


Figure 21. Idiograms of *Limonium* species. A, *L. magalufianum*, $2n = 26$ (8 + 9 + 9). Scale bar = 5 μm . B, *L. marisoli*, $2n = 54$ (9 + 9 + 9 + 9 + 9 + 9). Scale bar = 1.5 μm . C, *L. migjornense*, $2n = 50$ (8 + 8 + 8 + 8 + 9 + 9). Scale bar = 2.5 μm . D, *L. minoricense*, $2n = 25$ (8 + 8 + 9). Scale bar = 2.5 μm .

that karyological polymorphisms in polyploid *Limonium* are more widespread than was thought previously. Why this karyological variation has been reported so rarely is intriguing. It might be speculated that divergent chromosome numbers in addition to the expected ones are widespread, but that they could have been rejected simply on the assumption that they were technical artefacts. Karyological polymorphisms in some polyploid *Limonium* species from the British Isles have also been pointed out (Dawson, 1990), although technical artefacts were suggested as explanation for the eight cytotypes found in populations of *L. humile* Mill. from UK ($2n = 36, 38, 48, 49, 50, 51, 52, 54$). Excessive pressure during squashing, mixing of the contents of two adjacent cells and the size of the smallest chromosomes of the complement were regarded as plausible reasons to explain this chromosomal variability (Dawson, 1990).

Technical reasons could be put forward to explain some of the divergent results presented here and those reported previously by other authors (Table 2). However, it seems unrealistic that the whole range of

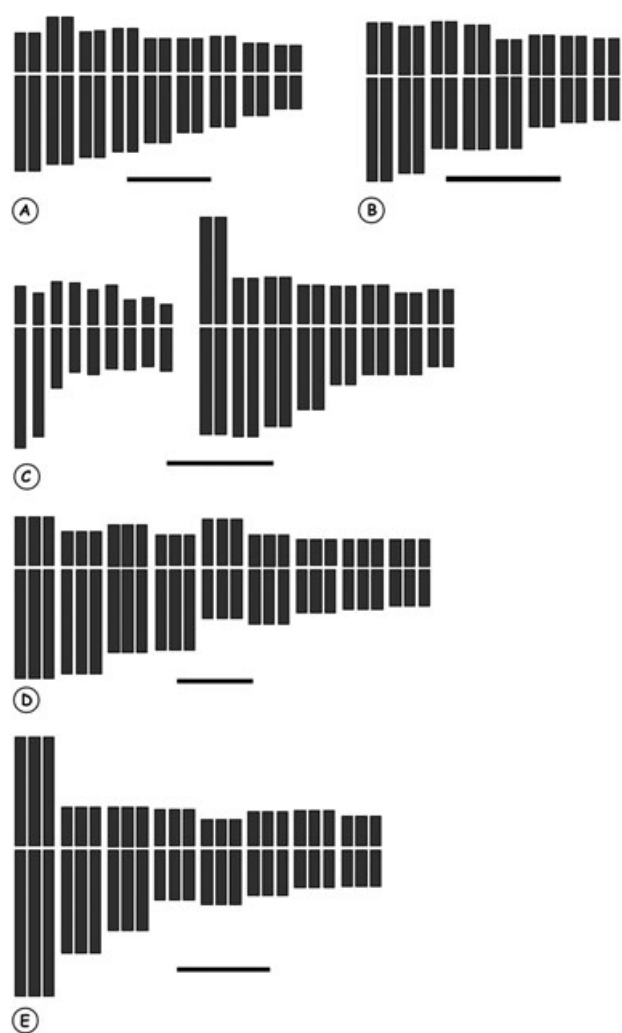


Figure 22. Idiograms of *Limonium* species. A, *L. minutum*, $2n = 18$ (9 + 9). Scale bar = 2.5 μm . B, *L. pseudodictyocladon*, $2n = 16$ (8 + 8). Scale bar = 2.5 μm . C, *L. scopolorum*, $2n = 25$ (8 + 8 + 9). Scale bar = 2.5 μm . D, *L. virgatum*, $2n = 27$ (9 + 9 + 9). Scale bar = 2.5 μm . E, *L. wiedmanii*, $2n = 24$ (8 + 8 + 8). Scale bar = 5 μm .

cytological variation shown here and by other authors is due entirely to artefacts. Rather, biological reasons could explain better why *Limonium* polyploids are prone to cytogenetic variability:

1. they are of hybrid origin (but see below);
2. they are self-sterile due to very low levels of pollen fertility and the presence of a sporophytic, heteromorphic, self-incompatible breeding system;
3. they could show facultative female meiosis, forming reduced gametes (up to 20% of the analysed embryo sacs in a member of the *L. virgatum* complex (D'Amato, 1940b), together with unreduced ones;

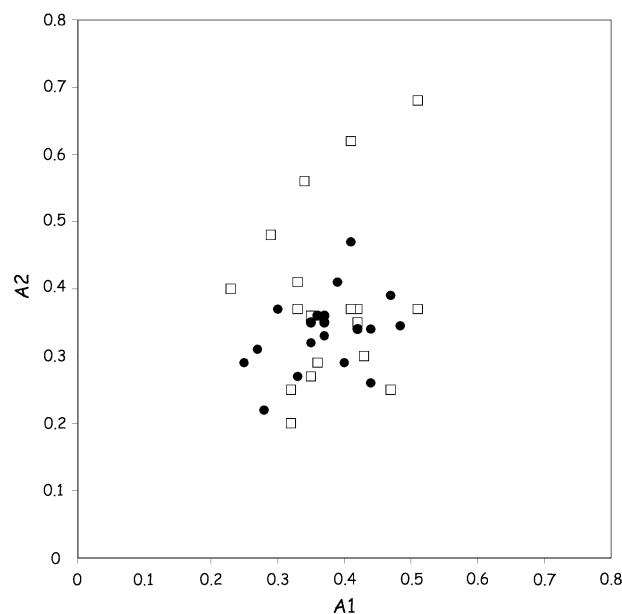


Figure 23. Ordination of the $x = 8$ (squares) and $x = 9$ (circles) chromosome sets of the analysed *Limonium* species on the basis of A_1 and A_2 asymmetry indexes.

4. they hold higher residual male fertility than suggested earlier (0–17 (–28)%; Erben, 1978, 1979), reaching fertility values up to 86% (Ingrouille & Stace, 1985; Artelari, 1989a); and
5. they show gametophytic apomixis, involving apospory coupled with autonomous embryo and endosperm formation (D'Amato, 1940a, 1940b, 1949).

From this it follows that most polyploids may not yet be genetically stabilized and that somatic mutations and auto-segregation, karyotype rearrangements and other asexual processes, such as those suggested to play a determinant role in other apomictic plant genera (Manning & Dickson, 1986; King & Schaal, 1990; Richards, 1996), could be operating fully in *Limonium*. Thus, the variation detected within *L. carvalhoi*, *L. leonardi-llorensi*, *L. marisolii*, *L. bonifaciense* and in the seven aneuploid cytotypes reported here could be due to these asexual processes.

In addition, facultative apomictic polyploids, acting as seed parents, can generate polyploid progeny and are capable as pollen donors to give rise to new chromosome combinations that, if viable, can be perpetuated by apomictic reproduction. This is the likely origin of the hexaploid cytotypes of *L. marisolii* ($2n = 54$) and *L. migjornense* ($2n = 50$), and the diploid cytotype of *L. pseudodictyocladon* ($2n = 16$) from triploid ancestors through sexual processes. In fact, recovery of diploid cytotypes from crosses between

facultative polyploid apomicts followed by later genome segregation has been documented in *Hieracium* (Chapman & Bicknell, 2000; Morgan-Richards *et al.*, 2004).

Unfortunately, most karyological data available for *Limonium* have been obtained exclusively from mitotic cells of root apices. Meiotic studies, which are extremely rare in the genus thus far (D'amato, 1940a, 1940b, 1949; Choudhuri, 1942; Ingrouille, 1982), should be attempted in order to assess fully the nature of infraspecific variation in the polyploid species.

CHROMOSOME MORPHOLOGY AND THE ORIGIN OF POLYPLOID TAXA

Chromosomal landmarks are rare in section *Limonium*, as a consequence of the non-detection of satellite chromosomes and secondary constrictions and to the gradient in sizes and the similar chromosome morphology in all species. However, Erben (1978, 1979) reported the presence of a pair of long metacentric chromosomes in species with $2n = 16$ that were absent from those with $2n = 18$. He hypothesized that these marker chromosomes originated from the fusion of a pair of telocentric chromosomes present in the $2n = 18$ species (Erben, 1979). Further, he suggested that their variable presence (0, 1, 2 and 3) in polyploid taxa was indicative of the number of $x = 8$ genomes involved in their genesis. Thus, he hypothesized that polyploid species originated from interspecific hybridization of the $x = 8$ and $x = 9$ genomes through the fusion of reduced and unreduced gametes. Nuclear ribosomal ITS sequences have supported the hybrid origin of some polyploid taxa (Palacios, Rosselló & González-Candelas, 2000). Two relatively divergent ITS types were present at the intraindividual level in the triploid *L. delicatulum* and each type was related to a different group of diploid species, one with a base haploid chromosome number $n = 8$ (represented by *L. cossonianum*) and the other with $n = 9$ (represented by *L. minutum*).

The correlation between chromosome numbers and metacentric marker chromosomes was phylogenetically informative and was used by Erben (1978, 1979) to infer the numbers of $x = 8$ and $x = 9$ genomes present in triploid ($2n = 24$, $8 + 8 + 8$, with three chromosome markers; $2n = 25$, $8 + 8 + 9$, with two chromosome markers; $2n = 26$, $8 + 9 + 9$, with one chromosome marker; $2n = 27$, $9 + 9 + 9$, with no chromosome markers), tetraploid ($2n = 32$, $8 + 8 + 8 + 8$, with four chromosome markers; $2n = 34$, $8 + 8 + 9 + 9$, with two chromosome markers; $2n = 35$, $8 + 9 + 9 + 9$, with one chromosome marker; $2n = 36$, $9 + 9 + 9 + 9$, with no chromosome markers), pentaploid ($2n = 43$, $8 + 8 + 9 + 9 + 9$, with two chromosome

markers), and hexaploid species ($2n = 51$, $8 + 8 + 8 + 9 + 9 + 9$, with three chromosome markers; $2n = 54$, $9 + 9 + 9 + 9 + 9 + 9$, with no chromosome markers). However, unexpected results concerning chromosome number and number of marker metacentric chromosomes have also been reported. For example, a long marker chromosome was reported in the karyotypes of *L. bonifaciense* and in *L. vigoi* ($2n = 36$), both with chromosome numbers based only on $x = 9$ (Diana, 1995; Sáez, Curcó & Rosselló, 1998b), although none should have been expected according to the Erben (1979) hypothesis. Likewise, four metacentric chromosomes were identified in *L. silvestrei*, a triploid species with $2n = 26$ (Aparicio, 2005), although only one should be present if this species originated from a direct hybridization between $n = 8$ and $n = 18$ gametes, as predicted by Erben (1979). In this work, unexpected instances of metacentric chromosomes have been found in several species (Table 3), with their occurrence and numbers confirming Erben (1979) in 19 cases, but not so in six cases (Figs 18B, 18D, 20D, 21C, 21D, 22B).

These results suggest that other karyological processes could also be operating in *Limonium* and that the predictive value of this chromosome marker in the $x = 8$ genome should be used with caution.

One important question that should be considered in *Limonium* karyology is the homology of the marker chromosomes. Ingrouille (1984) and Ingrouille and Stace (1985) have hypothesized that distinct evolutionary pathways other than hybridization (such as structural karyotype rearrangements, including somatic mutations and auto-segregation), have been operating in *Limonium* to explain its karyological diversity. Such phenomena may be responsible for some of the above-mentioned unexpected results, because it is unlikely that the metacentric marker chromosome has not been involved in karyotype rearrangements in polyploid *Limonium*. Alternatively, this chromosome could have appeared more than once in the evolution of section *Limonium* and thus is not homologous in some pairs of species. Chromosomal morphology alone could be misleading with regard to species relationships and the hypothesis that the longest metacentric chromosomes of the polyploid taxa are homologous to those of the diploid $2n = 16$ cytotypes should be assessed by means of more powerful cytological techniques such as FISH (Fluorescent *in situ* hybridization), or chromosomal banding.

The analysis of the chromosome complements of polyploid *Limonium* taxa has yielded convincing evidence supporting the nature of their polyploidy. The presence of two sets of paired and one set of unpaired chromosomes in the Mediterranean endemics *L. leonardi-llorensii*, *L. carvalhoi* (Rosselló *et al.*, 1998; Sáez *et al.*, 1998a), *L. contortirameum* (Mabille)

Erben, *L. florentinum* Arrigoni & Diana, *L. tarcoense* Arrigoni & Diana and *L. dubium* (Andr. ex Guss.) Lit. (Arrigoni & Diana, 1993) suggests that they are allopolyploids or, alternatively, are derived from allopolyploid ancestors. On the other hand, autopolyploidy has been inferred in the tetraploid *L. patrimonien* ($2n = 36$), because four sets of paired chromosomes were observed in its karyotype (Arrigoni & Diana, 1993).

The idiograms of *L. antonii-llorensii*, *L. barceloi*, *L. ejulabilis*, *L. geronense*, *L. nterjectum*, *L. virgatum* and *L. wiedmannii* suggest, but do not prove, that they are likely to be autopolyploids. The similarity of the karyotypes of the diploid *L. cossonianum* and the triploids *L. ejulabilis* and *L. wiedmannii* suggests that the former could be involved in the origin of the latter two species. If this is further corroborated, then autopolyploidy would have been demonstrated both in $x = 8$ and $x = 9$ species.

On the other hand, the presence of two morphologically divergent chromosome sets in various combinations strongly suggests that the restricted endemic species *L. alcludianum*, *L. bonafei*, *L. camposanum*, *L. companyonis*, *L. dufourii*, *L. gibertii*, *L. girardianum*, *L. inexpectans*, *L. leonardi-llorensii*, *L. magalufianum*, *L. migjornense*, *L. minoricense* and *L. scopulorum* probably originated through allopolyploidy between $x = 8$ and $x = 9$ ancestors. However, no putative parent species could be suggested for these species, due to the limited karyological data so far available for the Western Mediterranean taxa having $x = 9$.

ACKNOWLEDGEMENTS

We thank our colleagues M.A. Conesa, M. Mus and N. Torres for their generous help with the field sampling. Initial drafts of the manuscript were greatly improved by the comments and ideas shared with G. Nieto-Feliner. This work has been partly supported by funds of the project MMA 034/2002.

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