Breeding systems and cytology in Greek populations of five Limonium species (Plumbaginaceae)

Abstract


Information is provided about the reproductive mechanisms and the chromosome numbers of Limonium palmare, L. roridum, L. graecum, L. virgatum and L. narbonense, notes on their taxonomy are given and the correlation between their breeding systems and cytology is discussed. L. palmare and L. roridum are facultatively apomictic with 2n = 4x = 34 and 2n = 5x = 43, respectively. L. graecum is apomictic and its chromosome number of 2n = 6x = 52 is reported for the first time, L. virgatum is also apomictic with 2n = 3x = 27 and L. narbonense is sexual with 2n = 6x = 54 and 2n = 8x = 72. The latter number is a new record and the highest number reported so far for the genus Limonium.

Key words: angiosperms, sea lavender, Greece, chromosome number, sexual reproduction, apomictic reproduction.

Introduction

The genus Limonium comprises about 350 (Kubitzki 1993) or even 400 species (Palacios & Gonzales-Candelas 1997), most of them concentrated in the Mediterranean region, which constitutes the centre of its diversity. Due to its reproductive behaviour, i.e. both sexual and apomictic reproduction, as well as the frequent occurrence of polyploidy, Limonium is one of the most interesting and taxonomically difficult plant groups.

Limonium is characterized by an incompatibility system linked with pollen and stigma dimorphism. According to Baker (1948, 1953b-c) and Erben (1978, 1979) the following four pollen and stigma combinations occur: (A) cob-like stigmata and coarsely reticulate pollen grain surface; (B) papillate stigmata and finely reticulate pollen grain surface; (C) cob-like stigmata and finely reticulate pollen grain surface, (D) papillate stigmata and coarsely reticulate pollen grains. Combinations A and B are self-incompatible (plants self-sterile), while C and D are

* The present paper is dedicated to Prof. Werner Greuter on the occasion of his 68th birthday.
self-compatible (plants self-fertile). Species presenting both combinations A and B in each population are sexually reproduced (cross-fertilized), while those with only A or B are apomictic. Species with the combinations C or D are self-fertilized.


In this work reproductive mechanism and cytology of Greek populations of *Limonium palmarum* (Sm.) Rech. f., which is endemic to the Aegean area, *L. roridum* (Sibth. & Sm.) Brullo & Guarino and *L. graecum* (Poir.) Rech. f., are studied, notes on their taxonomy are given and the correlation between their reproductive mechanisms and cytology is discussed.

**Material and methods**

This study is based on plants collected by the authors as well as on herbarium specimens used for comparison. Voucher specimens of our collections are kept in UPA.

41 populations of *Limonium palmarum* from Kiklades (Tinos, Siros, Paros, Antiparos and Naxos), 25 of *L. roridum* from Kiklades (Tinos, Siros, Dilos, Serifos, Paros and Naxos), Elafonisos island (SW of Peloponnissos) and N Kriti, one of *L. graecum*, 14 of *L. virgatum* (Willd.) Fourn. and *L. narbonense* Mill. aggr., which have a wider Mediterranean distribution, are studied, notes on their taxonomy are given and the correlation between their reproductive mechanisms and cytology is discussed.

**Results and discussion**

**Notes on the taxonomy of the material studied**

*Limonium palmarum*, *L. roridum* and *L. graecum* are very closely related taxa and subject of considerable taxonomic and nomenclatural confusion. *L. roridum* has been treated by many authors as a synonym of *L. graecum* (Halácsy 1904, Hayek 1928, Rechinger 1943, Pignatti 1972, Bokhari & Edmondson 1982, Mayer 1995, Raus 1996, Artelari & Georgiou 2002, Chilton & Turland 2004), while *L. palmarum* was included either in *L. graecum* (Halácsy 1904, Rechinger 1943) or *L. roridum* (Boissier 1879), or was considered synonymous with *L. graecum* (Hayek 1928). Greuter & al. (1989) include all three taxa in the *L. palmarum* group, considering *L. roridum* as synonym of *L. graecum*, and *L. palmarum* as a distinct species. Brullo & Guarino (2000) distinguish *L. roridum* as a separate species, while Artelari & Georgiou (2002) follow the majority of the authors and place it under *L. graecum*.

The populations from Kiklades, Elafonisos and N Kriti used in the present paper belong to the *Limonium palmarum* group and present differentiation in a number of morphological characters correlated with different chromosome numbers. After consultation with Dr Erben (pers. comm.), we adopt the recognition of three separate species, *L. palmarum*, *L. roridum* and *L. graecum*.

Thus, we place under *Limonium palmarum* plants with branches ± straight, tuberculate-sca-brous, leaves abruptly tapering into a petiole longer than the rotundate to obcordate lamina, inner bract glabrous, middle bract oblong, calyx sparsely hairy to subglabrous with limb about equal to
the tube and lobes acute up to 0.8 mm long; they have a chromosome number of $2n = 34$.

Plants having the same habitus with the iconotype of Statice rorida, characterized by strongly and densely tuberculate, zig-zag articulate segments forming mostly wide angles, leaves gradually tapering into petiole about equal to the lamina which is usually oblong, inner bract hairy at the upper part, middle bract rotundate, calyx usually densely hairy with limb shorter than the tube and lobes obtuse up to 0.3 mm long, are placed under L. roridum; they have a chromosome number of $2n = 43$.

Finally, plants with stems forming acute angles, finely tuberculate, with less sterile branches, leaves gradually tapering into petiole longer than the lamina which is oblong, longer spikes, inner bract glabrous and middle bract oblong, are placed under L. graecum; they have a chromosome number of $2n = 52$ (Table 1).

Based on the above data, material treated in previous papers as Limonium graecum (Artelari 1989a, Artelari & Georgiou 2002) is considered now either as L. palmare or L. roridum (see further comments under “Reproductive mechanism” and “Cytology”).

Given that Limonium palmare and L. roridum coexist on some of the Kiklades islands (Tinos, Siros, Paros and Naxos), intermediates with unstable characters are found. On Siros, L. palmare is limited to the west coast, L. roridum occurs on the south and southeast coast and intermediate populations are found between these areas, while on the other islands the intermediates are scattered. On Naxos, besides the above two taxa, L. graecum was also found only in a locality on the northeast coast (Lionas bay) opposite to the Donoussa island, which is the “locus classicus” of this taxon.

Limonium virgatum is a common taxon in Greece, often growing together with other Limonium taxa and forming intermediates with them (Pignatti 1972, Artelari 1984, Erben 1993). The populations studied here are stable and present the typical morphological characters of the species given in detail by Artelari & Georgiou (2002).

For Limonium narbonense we use here the term “aggregate”, because the Greek plants are very polymorphic and not well distinguished from the closely related L. brevipetiolatum Artelari & Erben. L. brevipetiolatum has been described from the Ionian islands and W Greece by Artelari & Erben (1986) and according to them, it mainly differs from L. narbonense Mill. [= L. angustifolium (Tausch) Turril] in having shorter petioles and bracts, calyces and petals, and a different chromosome number ($2n = 54$ for L. brevipetiolatum and $2n = 36$ for L. narbonense). Having studied in the past years numerous populations and herbarium specimens from many Greek localities and found $2n = 36$ also in plants with the characters of L. brevipetiolatum, it became obvious that the morphological and cytological distinctions of these two taxa cannot be held up and that this group needs a detailed revision.

Reproductive mechanism

The populations of Limonium palmare were found to be dimorphic or monomorphic in respect to their pollen and stigma combination. In each dimorphic population both self-incompatible combinations A and B occur, while in each monomorphic population only one combination A or B is present. All populations have extremely ranging pollen stainability (0-99 %) and good seed production. The above data reveal that L. palmare is facultatively apomictic. Monomorphic populations with the combinations A (Naxos, Phitos 16380) and B (Milos, Artelari 435) treated in Artelari (1989a) as L. graecum has been revised as L. palmare according to the morphological and cytological differentiation given above of these closely related taxa.

The populations of Limonium roridum were also found to be monomorphic (A or B) or dimorphic. All populations have extremely ranging pollen stainability (0-86 %) and good seed production. The above data indicate that L. roridum is facultatively apomictic, too. Additionally, the fact that populations from Serifos (Tiniakou 76, 90, 283), Milos (Artelari 429, 431, 438, 446), Amorgos
and Kithira (Artelari & Georgiou 1505, 1526), cited in Artelari (1989a) and Artelari & Georgiou (2002) as *L. graecum* and considered now as *L. roridum*, were either monomorphic or dimorphic, supports the facultatively apomictic behaviour of this taxon. Of great interest is the finding of a monomorphic population of *L. roridum* with the self-compatible combination D on the island of Dilos. According to Baker (1953b), combination D occurs in the majority of the “secondarily monomorphic species, which are not apomictic, but self-compatible”. However, the evidence from the above population suggests that it is probably apomictic and not self-fertilized. The pollen grains present the picture that characterizes apomictic taxa. They are variable in size, conspicuously large or conspicuously small, many of them have a bowl-like shape and the pollen stainability is low, 1-18(-28) %. The stained grains are always some of the larger ones, well-shaped and 4-5-colpate, but none of them has been observed germinating on the stigmata. These pollen grains according to Erben (1979) can be considered containing unreduced gametes. The probable apomictic behaviour of the above population is also supported by its pentaploid chromosome number of 2n = 43. In an analogous case the Italian *L. avei* (De Not.) Brullo & Erben (= *L. longispicatum* Erben), which has the self-compatible combination C (‘B’ pollen and ‘Cob’ stigma), low pollen fertility (7-18 %) and the chromosome number 2n = 27 (Erben 1978), is considered by Ingrouille (1984) “probably agamospermic” because it is triploid. A breeding system of similar complexity has been found in the heteromorphous triploid (2n = 27) Spanish *L. viciosoi* (Pau) Erben, in which dimorphic populations with the combinations A and B, monomorphic populations with the combination A or B, as well as monomorphic populations with the combination D have been observed (Erben 1979).

The population of *Limonium graecum* was found to be monomorphic with the self-incompatible combination B, low pollen stainability (0-4 %) and good seed production. The above data show that *L. graecum* is apomictic. Previous reports of *L. graecum* as facultatively apomictic (Artelari 1989a, Artelari & Georgiou 2002) actually refer to *L. roridum* (see above).

The populations of *Limonium virgatum* were found to be monomorphic with the self-incompatible combination B, low pollen stainability (0-35 %) and good seed production. These data confirm the apomictic behaviour of the species already reported by D’Amato (1949), Dulberger (1975), Erben (1979), Artelari (1984, 1989a) and Artelari & Georgiou (2002). Noticeable is that all the typical Greek populations of *L. virgatum* studied so far have the combination B (Artelari 1984, 1989a, Artelari & Georgiou 2002), while some intermediate populations of this taxon with other *Limonium* species present the combination A (Artelari 1984). Combination B is also reported for *L. virgatum* from Israel (Dulberger 1975), Mallorca (Llorens 1985) and Spain (Soler & Rosselo 1997). On the contrary, Baker (1953b) mentions *L. virgatum* as a dimorphic and sexual taxon, but states that “some varieties of it may not be dimorphic”.

All the populations of *Limonium narbonense* studied here were found to be dimorphic with both self-incompatible combinations A and B and high pollen stainability (50-96 %). Also the populations found in many other Greek localities were dimorphic with the same combinations A and B, e.g., in Evvia, the East Aegean islands, Peloponnissos, etc. (Artelari & Georgiou, unpublished data). The above data suggest that *L. narbonense* is sexually reproduced.

**Cytology**

*Limonium palmarum* was found to be tetraploid with 2n = 4x = 34 (Table 1, Fig. 1A). The karyotype possesses two long metacentric (m) ‘marker’ chromosomes, which, according to Erben (1978, 1979), are characteristic for the karyotypes of the taxa of *L. subg. Limonium* with the basic number x = 8 and are missing in the taxa with x = 9. Thus, 2n = 34 has derived from the combination of 2 × 8 + 2 × 9 genomes and indicates the hybrid origin of *L. palmarum*. The above number has already been reported for material from Naxos (Phitos 16380) and Milos (Artelari 435), which, as mentioned above, was referred to as “*L. graecum*” Artelari (1989a) but is placed now...
Limonium roridum was found to be pentaploid with \(2n = 5x = 43\) (Table 1, Fig. 1B). In its karyotype also one pair of long metacentric (m) ‘marker’ chromosomes is present, showing that the above number has been derived from the combination of \(2 \times 8 + 3 \times 9\) genomes and that L. roridum also originates from hybridization. The number \(2n = 43\) is known from material from Andros (Artelari & al. 451, 454), Milos (Artelari 429, 431), Serifos (Tiniakou 76, 283) and Kithira (Artelari & Georgiou 1505, 1526) referred to by Artelari (1989a) and Artelari & Georgiou (2002) as L. graecum and placed now under L. roridum.

Limonium graecum was found to be hexaploid with \(2n = 6x = 52\) (Table 1, Fig. 1C). The number of \(2n = 52\) is counted for the first time for this taxon. The karyotype possesses two long metacentric ‘marker’ chromosomes, indicating that it has been derived from the combination of \(2 \times 8 + 4 \times 9\) genomes and that it has an hybrid origin, too. Previous reports of \(2n = 34\) and 43 for L. graecum (Artelari 1989a, Artelari & Georgiou 2002) actually refer to L. palmare and L. roridum, respectively (see above).

Limonium virgatum was found to be triploid with \(2n = 3x = 27\) (Table 1, Fig. 1D). As is shown in Fig. 1D and has also been reported previously (Erben 1978, Artelari 1984, 1989a, 1992, Artelari & Georgiou 2002) the karyotype lacks the characteristic ‘marker’ chromosomes. The above chromosome number is also known from other Greek localities (Artelari 1984, 1989a, 1992, Artelari & Georgiou 2002) as well as from the Mediterranean region in general (D’Amato 1949, Erben 1978, 1979, 1993, Chichiricco & Tammaro 1980, Brullo & Pavone 1981, Arrigoni & Diana 1993). Dolcher & Pignatti (1971) state this taxon to be both triploid with the number range \(2n = 24-27\) and tetraploid with the number “c. 32”.

### Table 1. Chromosome numbers and ploidy levels of Limonium palmare, L. roridum, L. graecum, L. virgatum and L. narbonense from the Aegean area (collectors: AC = Artelari & Chondropoulos, AG = Artelari & Georgiou, GM = Georgakopoulou & Manousou).

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Locality</th>
<th>Chromosome number</th>
<th>Ploidy level</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. palmare</td>
<td>Paros, Kolibithres (AC 1643)</td>
<td>(2n = 34)</td>
<td>4x</td>
</tr>
<tr>
<td></td>
<td>Paros, Pounta (AC 1628)</td>
<td>(2n = 34)</td>
<td>4x</td>
</tr>
<tr>
<td></td>
<td>Paros, Ag. Fokas (AC 1649)</td>
<td>(2n = 34)</td>
<td>4x</td>
</tr>
<tr>
<td></td>
<td>Antiparos, Sifneikos Gialos (AC 1664)</td>
<td>(2n = 34)</td>
<td>4x</td>
</tr>
<tr>
<td></td>
<td>Naxos, Moni Chrisostomou bay (AC 1677)</td>
<td>(2n = 34)</td>
<td>4x</td>
</tr>
<tr>
<td>L. roridum</td>
<td>Dilos (AC 1609)</td>
<td>(2n = 43)</td>
<td>5x</td>
</tr>
<tr>
<td></td>
<td>Siros, Fabrika (AC 1765)</td>
<td>(2n = 43)</td>
<td>5x</td>
</tr>
<tr>
<td></td>
<td>S iros, Lazareto (AC 1794)</td>
<td>(2n = 43)</td>
<td>5x</td>
</tr>
<tr>
<td></td>
<td>Elafonissos, Panagitsa bay (AG 1741)</td>
<td>(2n = 43)</td>
<td>5x</td>
</tr>
<tr>
<td>L. graecum</td>
<td>Naxos, Lionas (AC 1698)</td>
<td>(2n = 52)</td>
<td>6x</td>
</tr>
<tr>
<td>L. virgatum</td>
<td>Pref. Magnisia, Mamidakis bay (GM 215)</td>
<td>(2n = 27)</td>
<td>3x</td>
</tr>
<tr>
<td></td>
<td>Pref. Magnisia, Afissos bay (GM 205)</td>
<td>(2n = 27)</td>
<td>3x</td>
</tr>
<tr>
<td></td>
<td>Pref. Magnisia, Kalamos (GM 208)</td>
<td>(2n = 27)</td>
<td>3x</td>
</tr>
<tr>
<td>L. narbonense</td>
<td>Pref. Magnisia, Mamidakis bay (GM 216)</td>
<td>(2n = 54)</td>
<td>6x</td>
</tr>
<tr>
<td></td>
<td>Pref. Magnisia, Chrisi Akti Panagias (GM 214α)</td>
<td>(2n = 54)</td>
<td>6x</td>
</tr>
<tr>
<td></td>
<td>Pref. Magnisia, Chrisi Akti Panagias (GM 214)</td>
<td>(2n = 72)</td>
<td>8x</td>
</tr>
<tr>
<td></td>
<td>Pref. Magnisia, Alikes bay (GM 219)</td>
<td>(2n = 72)</td>
<td>8x</td>
</tr>
</tbody>
</table>
Fig. 1. Microphotographs of mitotic metaphase plates – A: *Limonium palmare* with $2n = 4x = 34$, material from Paros, Kolibithres (Artelari & Chondropoulos 1643); B: *L. roridum* with $2n = 5x = 43$, material from Dilos (Artelari & Chondropoulos 1609); C: *L. graecum* with $2n = 6x = 52$, material from Naxos, Lionas (Artelari & Chondropoulos 1698); D: *L. virgatum* with $2n = 3x = 27$, material from Magnisia, Kalamos (Georgakopoulou & Manousou 208). – Scale bars = 10 µm.
The populations of *Limonium narbonense* were found to be hexaploid with \(2n = 6x = 54\) and octoploid with \(2n = 8x = 72\) (Table 1, Fig. 2). The chromosome number \(2n = 6x = 54\) has also been counted for the closely related Greek endemic *L. brevipetiolatum* (Artelari & Erben 1986) as well as for the European relative *L. humile* Mill. (Erben 1979). *L. narbonense* was known so far as tetraploid with \(2n = 36\) from Greece (Artelari 1992) and as diploid with \(2n = 18\) and as tetraploid from the wider Mediterranean region (Baker 1953c, Erben 1978, 1979, 1993, Brullo & Pavone 1981, Palacios & al. 2000). Both hexaploid and octoploid karyotypes lack the ‘marker’ chromosomes (Fig. 2) and this is also the case in the karyotype with \(2n = 36\) (Erben 1978, Artelari 1992).

As is shown in Table 1, at the locality “Chrisi Akti Panagias” both chromosome numbers \(2n = 54\) and \(72\) were counted. In this place, individuals with \(2n = 72\) were found in the exterior side of the bay, exposed to wind and waves and it seems that their high ploidy level is a response to the extreme ecological conditions. These plants are more robust and advanced in flowering than those with \(2n = 54\), but the detailed study of their morphological characters did not show any other significant difference. The octaploid number \(2n = 72\) is the highest reported so far for the genus *Limonium*.

The pentaploid chromosome number of *Limonium roridum*, the hexaploid number of *L. graecum*, as well as the hexaploid and octaploid numbers of *L. narbonense* give support to the view expressed by Artelari & Georgiou (2002), that the Aegean region has a concentration of *Limonium* taxa with higher ploidy levels compared to the western and central Mediterranean.

**Correlations between breeding system and cytology**

Baker (1953a) first suggested that in *Limonium* apomixis arose through hybridization. Erben (1978, 1979) revealed the importance of cytological studies for the taxonomy of the genus and demonstrated the correlation between chromosome numbers and reproductive mechanisms. Thus, according to Erben’s hypothesis, the taxa of *L. subg. Limonium* with \(2n = 25, 26, 34, 35, 42, 43\) (derived from different combinations of the basic numbers \(x = 8\) and \(x = 9\)), as well as the triploid taxa with \(2n = 27\) are apomictic hybrids. On the contrary, diploid and polyploid taxa with chromosome numbers derived from the basic number \(x = 9\) are sexual.
Our results are in agreement with Erben’s hypothesis (1978, 1979). The facultatively apomorphic behaviour of *Limonium palmare* and *L. roridum* is correlated with the presence of both dimorphic and monomorphic populations and the chromosome numbers $2n = 34 (2 \times 8 + 2 \times 9)$, $2n = 43 (2 \times 8 + 3 \times 9)$ respectively. The apomorphic behaviour of *L. graecum* is correlated with the chromosome number $2n = 52 (2 \times 8 + 4 \times 9)$, the apomorphic behaviour of *L. virgatum* with the triploid number $2n = 27$. The sexual reproduction of *L. narbonense* is correlated with the chromosome numbers $2n = 18, 36, 54$ and 72.

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Address of the authors:
Argyro Georgakopoulou, Sofia Manousou, Assoc. Prof. Rea Artelari, Ass. Prof. Ourania Georgiou, Botanical Institute, Section of Plant Biology, Department of Biology, University of Patras, GR 265 00 Patras, Greece; e-mail: artelari@upatras.gr, ogeorgio@upatras.gr