Matthias Baltisberger

Cytological investigations of some Greek plants

Abstract


The chromosome numbers of 35 species from Greece are presented (Tab. 1). The numbers of Rumex crispatus (2n = 80) and Stachys acutifolia (2n = 30) were previously unknown. In Achillea holosericea (2n = 4x = 36) and Onosma heterophyllum (2n = 2x = 14, 2n = 3x = 21) new ploidy levels are reported. The numbers of Centaurea pindicola (2n = 40), Genus asphodeloides (2n = 24) and Malabalia aurea (2n = 20) differ (at least in part) from the indications in literature. The detailed karyotype of Crepis rubra is given.

Introduction

Several excursions were made in the Balkan Peninsula during 1977-1989 to collect plant material for biosystematic investigations. The plants were cultivated in the garden of our institute, and vouchers are deposited in the herbarium ZT (abbreviation according to Holmgren & al. 1981). Some chromosome numbers of Greek plants have been published earlier (Baltisberger 1980, 1981, 1986, 1988a, 1989, Baltisberger & Aeschimann 1988, Baltisberger & Lenzerr 1984a, Baltisberger & Huber 1987a, 1987b). The series is continued here with plants collected on an excursion by the author with U. Meili in 1987.


Methods

All cytological investigations were performed on root tips. These were pretreated for 1/2 to 2 hours with colchicine (0.05%), fixed in ethanol : acetic acid (3 : 1) and stained and squashed in lacto-propionic orcein (Dyer 1963). For the determination of the chromosome number, 5-10 metaphases were counted out of each individual (for the number of individuals investigated see Tab. 1).

In Crepis rubra the karyotype was investigated in detail; 10 metaphases were measured. Based on the positions of the centromere, Levan & al. (1964) distinguish 4 groups of chromosomes which are characterized by the arm ratio (long arm/short arm):
Table 1. Alphabetical list of the investigated species. Collected material: K = detailed karyotype given; L = living plants; S = seeds; * first record; Δ new ploidy level; + deviating from literature.

<table>
<thead>
<tr>
<th>Species</th>
<th>Herbarium specimen</th>
<th>Collected material</th>
<th>Individuals investigated</th>
<th>2n</th>
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<tr>
<td>Achillea holosericea</td>
<td>11867</td>
<td>L</td>
<td>3</td>
<td>36Δ</td>
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<td>Alyssum alyssoides</td>
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<td>36</td>
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<td>34</td>
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<td>9</td>
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<td>11</td>
<td>80*</td>
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<td>Rumex pulcher</td>
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<td>4</td>
<td>14</td>
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<td>12028</td>
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<td>Smyrnium rotundifolium</td>
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<td>28</td>
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<td>8</td>
<td>22</td>
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<td>11244</td>
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<td>11</td>
<td>30*</td>
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<tr>
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<td>11</td>
<td>14</td>
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<tr>
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<td>11735</td>
<td>S</td>
<td>11</td>
<td>14</td>
</tr>
</tbody>
</table>
— metacentric (m) arm ratio 1.0-1.7
— submetacentric (sm) arm ratio 1.7-3.0
— subtelocentric (st) arm ratio 3.0-7.0
— acrocentric (t) arm ratio more than 7.0

Chromosomes with about the same arm ratio should be combined in one group if the difference is not more than 10% in total length (Patau 1960, 1965) as was the practice in previous works (Baltisberger & Müller 1981, Baltisberger 1984, Müller & Baltisberger 1984, Baltisberger & al. 1990).

Pollen was investigated in Achillea holosericea and Onosma heterophyllum. Pollen grains were stained in carmine acetic acid. The pollen fertility (percentage of well developed pollen) was established for 100-200 grains per plant and the dimensions of 20 pollen grains per plant were noted.

Results

Boraginaceae


Dry, open vegetation in culture of Prunus dulcis (Miller) D. A. Webb, S of Manthirea, 15 km S of Tripolis, Peloponnisos, 750 m, 16.6.1987, (cult. nr. 11675).

Onosma heterophyllum Griseb. (det. H. Teppner, Graz). — 2n = 14, 21 (Fig. 1)

Roadside on the road to Mount Kajmakcalan, Voras Oros, 25 km NW of Edhessa, 900 m, 25.6.1987, nr. 11424 [cult. nr. 11691 (2n = 14), 11692 (2n = 21)].

Three of the four plants investigated showed 2n = 2x = 14 chromosomes, and one plant was triploid with 2n = 21 chromosomes. In one (and only one) root tip of a diploid plant all metaphases had 2n = 28 chromosomes whereas the other root tips of the same plant always showed 2n = 14 chromosomes. Endomitosis, as observed here, is not rare in Onosma (Teppner in lit.).

The chromosome numbers of O. heterophyllum indicated in literature are not homogenous: Grau (1971), Strid (1971), Markova & Ivanova (in Löve 1973) and Strid & Franzén (in Löve 1983) give 2n = 26, whereas Teppner (1971) found 2n = 26 and 2n = 28, and Baltisberger (1988b) indicates 2n = 27 chromosomes (one hypotetraploid plant from Albania). As Teppner communicated (in lit.) diploid (2n = 14) and tetraploid (2n = 28) races exist in O. heterophyllum but the diploid number has not been published till now.

Pollen fertility was investigated. 7 plants from the natural site (nr. 11424) as well as the 3 diploid plants in culture (nr. 11691) had a pollen fertility of nearly 100%. The triploid plant (nr. 11692) showed more than 90% well developed pollen grains. It seems that unpair ploidy level (3x) or aneuploidy (Baltisberger 1988b) have no effect on the formation of the pollen in Onosma. But whether or not these well developed pollen grains in aneuploid and triploid plants are really fertile cannot be said.
Figs. 1-2. 1: Somatic metaphase of *Onosma heterophyllum*. A: diploid with \(2n = 14\). B: triploid with \(2n = 21\); 2: Somatic metaphase of *Achillea holosericea*. 
Compositae

Achillea holosericea Sibth. & Sm. — 2n = 36 (Fig. 2)

NNE side of Mount Karava, 30 km W of Karditsa, 1900-2000 m, 20.6.1987, (cult. nr. 11867).

In literature only the diploid chromosome number 2n = 18 is indicated (Contandriopoulos & Martin 1967, Strid & Franzén in Löve 1981, Baltisberger 1984). But the 3 plants from Karava showed 2n = 4x = 36 chromosomes. This is the first record of tetraploid A. holosericea. The karyotype of the tetraploid plants consists of 28 metacentric and 8 submetacentric to subtelocentric chromosomes, the latter with satellites. This corresponds (except for the ploidy level) with the karyotype of diploid A. holosericea (Baltisberger 1984). Here, too, the pollen was investigated. The 3 tetraploid plants showed a pollen fertility of about 43%. The diameter of the pollen was 30 μm with a deviation of s = ±3.17. Additionally, as a comparison between tetraploid plants and diploids, 3 diploid plants from Mali i Snoi, E of Tirana, Albania (nr. 82/1617, cult. nr. 83/1003, Baltisberger & Lenherr 1984b, Baltisberger & Lippert 1987) were investigated. They had more than 95% well developed pollen grains. The diameter of the pollen of these diploid plants was 24.2 μm, and there was less variation in the pollen size (s = ±1.60). Similar results were obtained in Ranunculus kuepferi Greuter & Burdet by Küpfer (1974) and Huber (1984, 1988). Diploid plants of R. kuepferi had a fertility of 80-100% while the tetraploids showed less than 50% well developed pollen grains. The diameter of the pollen in diploids was about 28 μm, in tetraploids 32-38 μm (Huber 1988). The increase of pollen diameter from diploid to tetraploid plants in R. kuepferi is in about the same range (14-36%) as in A. holosericea (24%). The great variation in size and the low fertility within tetraploids indicate that meiosis may be irregular and chromosome contents may vary within individual pollen grains. Based on the 3 tetraploid plants the following can be said about morphology: the leaves of tetraploids are less divided, the cauline leaves are larger and the capitula somewhat bigger than in diploids. But it seems that there is no significant difference in morphological characters between diploid and tetraploid A. holosericea.

Anthemis carpathica Willd. — 2n = 36

Alpine meadow, SE side of Mount Kajmakcalan, Voras Oros, 25 km NW of Edhessa, 2100 m, 25.6.1987, nr. 11415 (cult. nr. 11688).

Bellis perennis L. — 2n = 18

Roadside, W side of Mount Tsoumerka, 40 km SE of Ioannina, 1000 m, 15.6.1987, nr. 11228 (cult. nr. 11669).

Centaurea pindicola (Griseb.) Griseb. ex Boiss. (det. G. Wagenitz, Göttingen). — 2n = 40 (Fig. 3)

Rocky place near Refuge A, E side of Mount Olympus 2000 m, 23.6.1987, nr.
Figs. 3-4. 3: Somatic metaphase of *Centaurea pindicola*; 4: Somatic metaphase of *Crepis rubra*.
All plants investigated showed 2n = 40 chromosomes. The only indication in literature (Strid & Franzén in Löve 1981) gives 2n = ca. 44. *C. pindicola* belongs to *C. subg. Cyanus* (Miller) Hayek (Dostál 1976).

Within this subgenus different chromosome numbers are known: 2n = 20, 22, 24, 44. In *C. subg. Cyanus* two systematically difficult complexes exist with different basic chromosome numbers: *C. triumfetti* All. s.l. with 2n = 22, 44 (see Van Loon 1987) and *C. napulifera* Rochel s.l. with 2n = 20 (*C. napulifera*: Strid & Andersson 1985; *C. tuberosa* Vis.: Siljak-Yakovlev in Löve 1985). In view of the two different chromosome numbers indicated for *C. pindicola* (2n = 40 resp. ca. 44) it cannot be said whether this species is more closely related to *C. napulifera* s.l. (with the basic chromosome number x = 10) or *C. triumfetti* s.l. (with x = 11).

**Crepis rubra** L. — 2n = 10 (Figs. 4, 5)

Dry open vegetation in culture of *Prunus dulcis* (Miller) D. A. Webb, S of Manthira, 15 km S of Tripolis, Peloponnisos, 750 m, 16.6.1987, (cult. nr. 11673).

The chromosome number of 2n = 2x = 10 corresponds with most of the indications in literature. The karyotype consists of 2 pairs of large submetacentric, 1 pair of medium sized telocentric, 1 pair of small metacentric and 1 pair of small subtelocentric chromosomes (Fig. 5, Tab. 2).

The telocentric chromosomes bear small satellites, the subtelocentric chromosomes have satellites which are much larger than the short arms of the chromosomes. A similar karyotype and idiogram for *C. rubra* is given by Ferrer & Lacadena (1977), but they indicate satellites only for the subtelocentric chromosomes.

*C. rubra*, along with *C. alpina* L. and *C. foetida* L., belongs to *C. sect. Barkhausia* (Moench) Gaudin (Sell 1976). All species of this section have the same chromosome number 2n = 10 (Babcock 1947, Sell 1976) but the karyotypes are different. *C. foetida* has the chromosome formula 2n = 2x = 10 = 4m + 4sm + 2st*sat* [Bartolo & al. 1978, Brullo & al. 1979, Baltisberger (unpublished)]. A somewhat different karyotype (2m + 6sm + 2st*sat*) is indicated by Nazarova (1968). The formula of *C. rubra* is 2n = 2x = 10 = 2m + 4sm + 2st*sat* + 2st*sat*. The karyotype of *C. alpina* is not known in detail.

<table>
<thead>
<tr>
<th>Chromosome number</th>
<th>Long arm</th>
<th>Short arm</th>
<th>Satellites</th>
<th>Arm ratio</th>
<th>Chromosome group</th>
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</thead>
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<tr>
<td></td>
<td>x</td>
<td>s</td>
<td>x</td>
<td>s</td>
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</tr>
<tr>
<td>1-4</td>
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<td>1.54</td>
<td>0.13</td>
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<tr>
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<td>0.15</td>
<td>0.39</td>
<td>0.08</td>
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<td>0.11</td>
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</tr>
<tr>
<td>9+10</td>
<td>1.66</td>
<td>0.11</td>
<td>0.36</td>
<td>0.08</td>
<td>4.61 st</td>
</tr>
</tbody>
</table>

Table 2. Chromosome values (in μm) measured in 10 metaphase plates of *Crepis rubra*.
**Doronicum columnae** Ten. — *2n = 60*

Summit area of Mount Karava, 30 km W of Karditsa, 2150-2184 m, 20.6.1987, nr. 11301 (cult. nr. 11676).

**Ptilostemon chamaepeuce** (L.) Less. — *2n = 32*

Rocky places, peninsula of Monemvasia, 65 km SE of Sparta, Peloponnisos, 20-50 m, 17.6.1987, nr. 11254.

![Diagram](image)

**Fig. 5. Idiogram of Crepis rubra.**

**Cruciferae**

**Alyssum alyssoides** (L.) L. (= *A. calycinum* L.). — *2n = 32*

W side of Mount Tsoumerka, 40 km SE of Ioannina, 1400-1600 m, 15.6.1987, nr. 11216 (cult. nr. 11723).
Draba lasiocarpa Rochel. — $2n = 16$

NNE side of Mount Karava, 30 km W of Karditsa, 1900-2000 m, 20.6.1987, nr. 11313 (cult. nr. 11868).

Dipsacaceae

*Tremostelma palaestinum* (L.) Janchen. — $2n = 14$

Dry, open vegetation in culture of *Prunus dulcis* (Miller) D. A. Webb, S of Manthirea, 15 km S of Tripolis, Peloponnisos, 750 m, 16.6.1987, (cult. nr. 11674). Roadside NE of Gythion, 35 km SSE of Sparta, Peloponnisos, 20 m, 17.6.1987, (cult. nr. 11735).

All plants examined from both localities had $2n = 2x = 14$ chromosomes. However, life strategy and behaviour of plants from the two populations in the greenhouse were quite different. For this study plants were kept in the same chamber of the greenhouse in all stages (from seed to adult plant). The seeds were sown in September 1987. They germinated within the following 4 weeks, and then the seedlings formed leaf rosettes. The plants grown from seeds from Manthirea remained in this stage and did not flower until the next summer (1988), as with typical biennials. The plants had stems up to 30 cm long with the few lateral branches rising above the central stem, and with mostly 5 capitula. The plants of seeds from Gythion did not remain in the rosette stage, but flowered immediately between January and March 1988 and behaved like true annuals. Those plants were much larger (up to 1 m) with numerous lateral branches not rising above the central stem, and with a greater number of capitula (9-25).

The differences found between plants from the two sites in the greenhouse correspond with the specific ecology in their native habitats. In Manthirea the canopy was not closed (probably because of the poor, very stony soil), and most co-occurring plants were not higher than 30 cm. Therefore enough room and light is available for the rosettes of *Tremostelma* to survive until flowering the following year (biennial). In Gythion, however, the lush vegetation was nearly closed, and many plants of other species reached a size of 1 m or more. Under these conditions rosettes of *Tremostelma* could not survive. Plants are forced to grow up immediately as high as the co-occurring species to be able to flower and produce seeds (annual). This obvious ecological adaptation of plants from the two sites may be the first step towards an evolutionary separation into different races (and further on, different species).

Geraniaceae

*Geranium asphodeloides* Burm. fil. (confirm. P. F. Yeo, Cambridge). — $2n = 24$

Wet place in *Pinus* wood S of Kataphyigion, Pieria Ori, 30 km E of Kozani, 1500 m, 24.6.1987, nr. 11392 (cult. nr. 11680). The chromosome number of $2n = 24$ corresponds with the indications of Van Loon & Oudemans (in Löve 1982) and Van Loon (1984) but other numbers have also been communicated (see Yeo 1984): $2n = 26$ (Guittonneau 1975), $2n = 28$ (Warburg 1938) and $2n = 30$ (Strid & Franzén in Löve 1981).
Figs. 6-7. 6: Somatic metaphase of *Stackys acutifolia*; 7: Somatic metaphase of *Rumex cristatus*.
**Geranium macrorrhizum** L. (confirm. P. F. Yeo, Cambridge). — 2n = 46

Wet roadside in open wood, on the road from Kataphygon to Velvendos, Pieria Ori, 30 km E of Kozani, 1250 m, 24.6.1987, nr. 11411 (cult. nr. 11871).

**Geranium pyrenaicum** Burm. fil. (confirm. P. F. Yeo, Cambridge). — 2n = 26

Open wood near Kastanea, 40 km WNW of Trikala, 800 m, 22.6.1987, nr. 11349 (cult. nr. 11677).

**Geranium subcaulescens** L'Hér. [= *G. cinereum* Cav. subsp. *subcaulescens* (L'Hér. ex DC.) Hayek]. — 2n = 28

Summit area of Mount Karava, 30 km W of Karditsa, 2150-2184 m, 20.6.1987, nr. 11298 (cult. nr. 11732). (conf. P. F. Yeo, Cambridge)
Alpine meadow, SE side of Mount Kajmakcalan, Voras Oros, 25 km NW of Edhessa; 2100 m; 25.6.1987; (cult. nr. 11686).

**Gramineae**

**Anthoxanthum odoratum** L. — 2n = 10


**Labiatae**

**Lamium garganicum** L. — 2n = 18

Open wood near Kastanea, 40 km WNW of Trikala, 800 m, 22.6.1987, (cult. nr. 11870).


Open wood S of Kataphygon, Pieria Ori, 30 km E of Kozani, 1500 m, 24.6.1987, nr. 11395.

**Marrubium velutinum** Sibth. & Sm. — 2n = 34

W side of Mount Tsoumerka, 40 km SE of Ioannina, 1400-1600 m, 15.6.1987, nr. 11220 (cult. nr. 11694).
*Nepeta nuda* L. — *2n = 18*


*Prasium majus* L. — *2n = 34*

Rocky place near Gythion, 35 km SSE of Sparta, Peloponnisos, 20 m, 17.6.1987, (cult. nr. 11842).

*Salvia triloba* L. fil. — *2n = 14*

Rocky place near Gythion, 35 km SSE of Sparta, Peloponnisos, 20 m, 17.6.1987, (cult. nr. 12028).

*Stachys acutifolia* Bory & Chaub. — *2n = 30* (Fig. 6)

Dry, open vegetation in culture of *Prunus dulcis* (Miller) D.A. Webb, S of Manthirea, 15 km S of Tripolis, Peloponnisos; 750 m; 16.6.1987; nr. 11244 (cult. nr. 11672 & 11861).

*S. acutifolia* is related to *S. germanica* L. s.l. (Ball 1972). The chromosome number of *S. acutifolia* was not known up to now. With its 30 chromosomes it corresponds with the indications for all other species of this group.

**Papilionaceae**

*Medicago minima* (L.) Bartal. — *2n = 16*

Open wood near Kastanea, 40 km WNW of Trikala, 700 m, 22.6.1987, nr. 11363 (cult. nr. 11654).

**Polygonaceae**

*Rumex crispatus* DC. (= *R. graecus* Boiss. & Heldr.). — *2n = 80* (Fig. 7)

Roadside near Kalanos, on the road from Patras to Kalavrita, Erimanthos Oros, Peloponnisos, 650 m, 16.6.1987, nr. 11243.

The chromosome number of *R. crispatus* was unknown up to now. *R. crispatus* is closely related to *R. kernerii* Borbas and *R. olympicus* Boiss. (Rechinger 1933, Akeroyd 1986). With its chromosome number of *2n = 80*, *R. crispatus* corresponds with the indications for these two related species (for *R. kernerii* see Van Loon 1987; *R. olympicus*...
see Baltisberger 1990).

**Rumex nebroides** Campd. (= *R. gussonei* Arcangeli see Rechinger 1971). — female
2n = 14, male 2n = 15


In the recently published "Mountain Flora of Greece" (Strid 1986) the name *R. nebroides* is used instead of the name *R. gussonei* as in "Flora Europaea".

**Rumex pulcher** L. — 2n = 20

Dry, open vegetation in culture of *Prunus dulcis* (Miller) D. A. Webb, S of Mantheira, 15 km S of Tripolis, Peloponnisis, 750 m, 16.6.1987, nr. 11249.

**Rosaceae**

**Aremonia agrimonioides** (L.) DC. — 2n = 42

Open wood S of Kataphygion, Pieria Ori, 30 km E of Kozani, 1500 m, 24.6.1987, nr. 11399 (cult. nr. 11682).

**Geum coccineum** Sibth. & Sm. — 2n = 42

Wet place in *Pinus* wood S of Kataphygion, Pieria Ori, 30 km E of Kozani, 1500 m, 24.6.1987, (cult. nr. 11681).
Wet place, SE side of Mount Kajmakcalan, Voras Oros, 25 km NW of Edhessa, 1850 m, 25.6.1987, (cult. nr. 11872).

**Geum montanum** L. — 2n = 42

Alpine meadow, SE side of Mount Kajmakcalan, Voras Oros, 25 km NW of Edhessa, 2220 m, 25.6.1987, nr. 11417 (cult. nr. 11689).

*G. montanum* has its main distribution in the Alps (Gajewski 1968). Only a few stations are known in the northernmost part of Greece (Persson 1986), one of them is Mount Kajmakcalan (Strid 1978).

**Saxifragaceae**

**Saxifraga bulbifera** L. — 2n = 28

Open place in *Pinus* wood S of Kataphygion, Pieria Ori, 30 km E of Kozani, 1500
m, 24.6.1987, nr. 11407 (cult. nr. 11684).

_Umbelliferae_

*Lagoecia cuminoides* L. — *2n = 16*

Rocky place, S side of Ori Lidorikiou, near the road from Amfissa to Lidorikion, 650-700 m, 19.6.1987, nr. 11290 (cult. nr. 11651).

_Malabaila aurea* (Sibth. & Sm.) Boiss. — *2n = 20* (Fig. 8)

Roadside near Kalanos, on the road from Patras to Kalavrita, Erimanthos Oros, Peloponnisos, 650 m, 16.6.1987, nr. 11241 (cult. nr. 11724).

The chromosome number of *2n = 20* does not correspond with the indications in literature where *2n = 22* is given [Moore in Tutin 1968 (see Moore 1982), Van Loon & Snelders in Løve 1979].

![10 μm](image)

Fig. 8. Somatic metaphase of *Malabaila aurea*.

_Smyrnium rotundifolium* Miller. — *2n = 22*

Roadside near Kalanos, on the road from Patras to Kalavrita, Erimanthos Oros, Peloponnisos, 650 m, 16.6.1987, nr. 11238 (cult. nr. 11860).

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