Genome size and chromosome number of *Micromeria acropolitana* (*Lamiaceae*), a steno-endemic from Greece

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Abstract. The chromosome number 2n = 30, and nuclear DNA amount 2C = 0.79 pg, are determined for the first time for *Micromeria acropolitana*, a rare and endangered species from the Acropolis in Athens, Greece. The plant was considered extinct but rediscovered in 2006, a hundred years later. Its current status in the original habitat is assessed, and proposals for ensuring its survival presented.

Key words: Acropolis, chromosome number, conservation, *Micromeria*, nuclear DNA content

Introduction

Micromeria acropolitana Halácsy belongs to the family *Lamiaceae,* subfam. *Nepetoideae,* tribe *Mentheae* and subtribe *Menthinae* (Bräuchler & al. 2008). Its *locus classicus* is the famed archaeological site of Greece, the Acropolis of Athens where it inhabits both the natural limestone rock of the hill and the quarried marble used for the buildings. It was considered extinct when it had not been sighted for a hundred years. It was rediscovered in 2006, a hundred years later (Tan, Kit & al. 2010). The species is endangered as the population size is small and the habitat in a densely populated area of urban activity. Thousands of visitors invade the site at the height of the tourist season including 11,000 people daily during July 2010 when the new Acropolis Museum was opened. The Acropolis is the great historic monument to Greek civilization and regarded as part of the Greek heritage. In order to protect both the monument and *Micromeria acropolitana*, the one and only endangered endemic plant of the Acropolis, we realised the biology of species should be better known. Various studies were carried out and the chromosome number and genome size determined for the first time in this paper.

Material and methods

Chromosome preparation

Seeds were collected from a natural population on the Acropolis, Athens. They were laid on moist filter paper in Petri dishes and kept at 28 °C in a thermostat germinator. The root tip meristems were removed from germinated seedlings and treated with 8-hydroxy-quinoline solution for 3 h at 16 °C, and subsequently fixed in fresh ethanol:acetic acid (3:1) before storing at 4 °C for 24–48 h. The meristems were stained in Schiff's reagent after hydrolysis in 1 N HCl for 12 min at 60 °C, and squashed in a drop of acetic carmine following Östergren & Heneen's technique (1962). Chromosome counts were made on well-spread metaphase plates from a minimum of 10 seedlings and approximately 10

cells per slide. The slides were analyzed using a Zeiss Axiophot microscope coupled with a highly sensitive CCD camera (RETIGA 2000R; Princeton Instruments, Every, France) and an image analyser (Metavue, Every, France).

Flow cytometry for 2C DNA value

The DNA amount was determined by flow cytometry following the technique of Marie & Brown (1993). The leaves of the *Micromeria* and an internal standard (*Petunia hybrida* (Hook) Vilm. cv. 'PxPC6'; 2C = 2.85 pg) were chopped together using a razor blade in Galbraith buffer (Galbraith & al. 1983) containing 0.1 % (w/v) Triton X-100 with addition of fresh 10 mM sodium metabisulphite and 1 % polyvinylpyrrolidone. Nuclei were stained with 30 µg/ml propidium iodide (Sigma Chemical Co. St. Louis, USA), a DNA intercalating dye used after RNAse treatment (2.5 units/ml; Roche). At least 5 individuals per population and 5000 to 10,000 nuclei per individual were analysed on a flow cytometer (CyFlow SL3, Partec, Munster, Germany) to obtain the mean DNA content. The 2C DNA value was calculated using the linear relationship between the fluorescent signals from stained nuclei of *Micromeria* leaves and the internal standard.

Results and discussion

Due to an excellent 100 % germination success the chromosome number 2n = 30 was determined in ten seedlings. This number was stable in each meristem. The chromosomes are small in size (1–2 µm). One pair with secondary constrictions bearing satellites was observed (Fig. 1A & B, arrows). The observed chromosome number (2n = 30) is the more frequently encountered one in the genus *Micromeria.* The number 2n = 20 has been reported for *M. thymifolia* (Scop.) Fritsch, and *M. dalmatica* Benth. (Papes & Silic 1981) and 2n = 60 for *M. graeca* (L.) Benth. (Luque & Lifante 1991) and *M. fontanesii* Pomel (Vogt & Oberprieler 1994).

The DNA amount of *M. acropolitana* was 2C = 0.79 (± 0.02) pg (Fig. 1C) which represents the very small genome size (1C ≤ 1.40 pg) according to the classification of Leitch & al. (1998).

Despite the size and great economic importance of the Lamiaceae (225 genera and 6170 species according to Wielgorskaya 1995) there is not much data concerning chromosome number or genome size of the member species. In the *Plant DNA C-values Database* of Bennett & Leitch (http://data.kew.org/cvalues/, latest release 5.0, December 2010) the DNA content for the *Lamiaceae* was only presented for 46 species belonging to 19 genera. The genome size (2C DNA) ranges from 0.55 pg for *Nepeta teydea* (Suda & al. 2003) to 12.47 pg for *Stachys grandiflora* (Barow & Meister 2003). Only five out of 221 known species of the genus *Micromeria* have been measured up to now, and their genome size ranges from 0.72 pg for *Micromeria hyssopifolia* Webb & Berthel. to 0.88 pg for *M. glomerata* P. Pérez (Suda & al. 2003). The value of *M. acropolitana* (2C = 0.79) is intermediate. Two other species with 2n = 30 (*M. lachnophylla* Webb & Berthel. and *M. varia* Benth.) presented 2C DNA values of 0.74 and

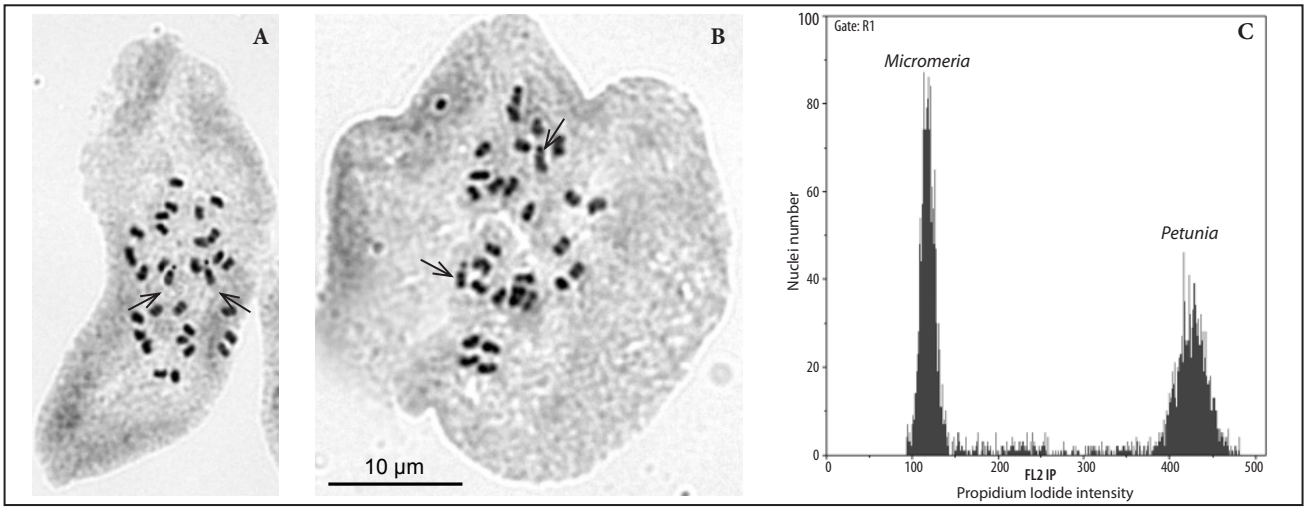

Fig.1. A & B, *Micromeria acropolitana*: Two mitotic metaphases showing 2n = 30 with one pair of satellite chromosomes (arrowed); **C,** Histogram of nuclear DNA content of *M. acropolitana* and internal standard *Petunia hybrida*. Their nuclei form two distinct peaks whose positions indicate relative nuclear DNA content.

0.75 pg respectively (Suda & al. 2003). Unfortunately, no data for the chromosome number and genome size of four closely related species (*M. microphylla* Benth., *M. sphaciotica* Boiss. & Heldr., *M. carpatha* Rech., and *M. hispida* Boiss. & Heldr.) were available for comparison which would be very useful.

Additional plant sites on the Acropolis

Since the publication of the rediscovery several new sites have been located, not only on the natural rock but also in wall crevices and marble blocks of the temples and other monuments. Here, we must make mention of the habitat and substrate. The sacred hill of the Acropolis was never quarried, the limestone for the buildings and walls was cut from several of the adjacent hills or from Piraeus, Mt Hymettos and the island of Aegina. The famous white marble of the buildings came from Mt Pendeli to the northeast. The large limestone blocks forming the walls of the monuments are not natural rock but porolithos. In Fig. 2C, a plant is shown growing between a crack formed by porolithos and marble. Plants thrive in such habitats, in wall crevices and broken marble with only a little soil. Fig. 2A shows a plant growing on the natural rock of the hill of Acropolis which is Late Cretaceous limestone overlying marl and sandstone, and Fig. 2B, a plant on porolithos.

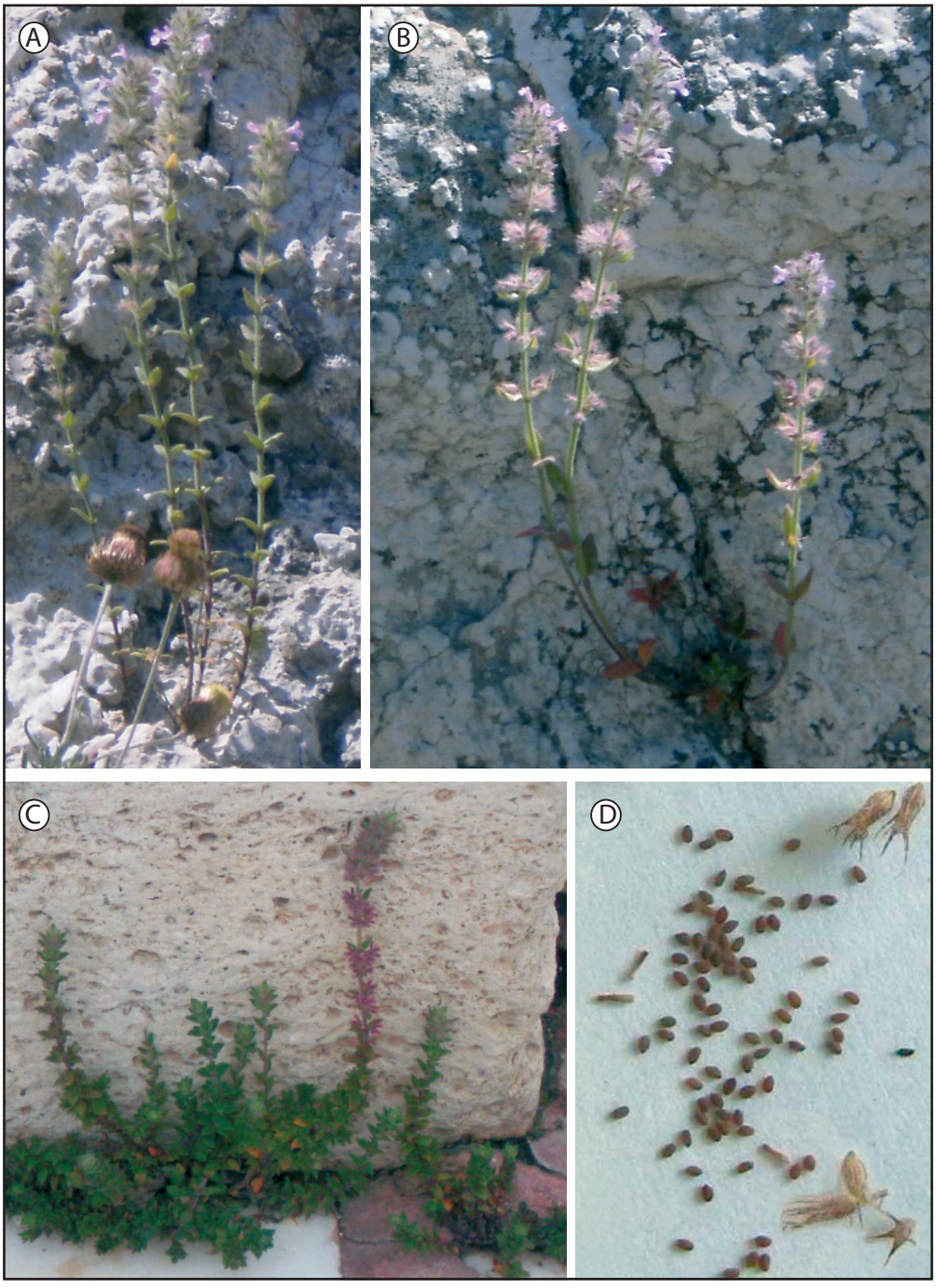

Fig. 2. A, *Micromeria acropolitana* growing in natural rock of the Acropolis; **B**, Plant in crevice of large limestone block (porolithos, not natural rock); **C**, Plant in crack formed by porolithos and Pendelis marble; **D**, ripe seeds.

An increase in size of the populations

In June 2009 we noted c. 150 plants and in July 2010, after plentiful and prolonged winter rains we estimated that the total number of plants on the Acropolis must be at least 400. At the time of sending this paper to press (May 2011) we counted c. 450 plants. The populations have not only remained steady but have shown a slight increase in numbers of individuals. The plant was first discovered in flower on 30 August 1906 by Maire and Petitmengin, two French botanists and explorers. 2011 was an unusual year as the plants flowered in February instead of May and June. From floristic notes kept by resident amateur botanists there is a tendency for species to come into flower earlier and earlier over a period of years, e.g., *Crocus* beginning to flower in late December instead of the usual February or March. Seed set of *M. acropolitana* was good in 2011 despite unsettled weather in the late spring with almost daily "tropical" rain.

Conservation measures taken

Seed from various populations on the natural rock and on the quarried limestone and marble blocks (Fig. 2D) has been collected and stored at the Institute of Biology, University of Copenhagen at a room temperature of 20 °C.

Conservation measures proposed

Clearly, the greatest threat within the archaeological site is human disturbance.

Thus the populations should be periodically monitored.

The areas where the plants are most at risk of being trodden by visitors should be fenced off or surrounded. Bilingual signs in Greek and English should be displayed to prohibit the collection of plants and animals, e.g., butterflies.

Certain areas of the stonework not in the direct eye of the public should undergo less thorough spring-cleaning so as not to eradicate established plants. The presence of plants, soil and loose material help retard water run-off and should not be removed.

A small rock garden with established *Micromeria* populations can be created near the entrance of one of the theatres. In this way all visitors can see the rare endemic plant of the Acropolis without disturbing the wild populations.

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References

Barow, M. & Meister, A. 2003. Endopolyploidy in seed plants is differently correlated to systematics, organ, life strategy and genome size. – Pl. Cell Environm., **26**: 571-584.

Bräuchler, C., Ryding, O. & Heubl G. 2008. The genus *Micromeria* (*Lamiaceae*), a synoptical update. – Willdenowia, **38**: 363-410.

Galbraith, D., Harkins, K., Maddox, J., Ayres, N., Sharma, D. & Firoozabady, E. 1983. Rapid flow cytometric analysis of the cell cycle in intact plant tissues. – Science, **220**: 1049-1051.

Leitch, I.J., Chase, M.W. & Bennett, M.D. 1998. Phylogenetic analysis of DNA C-values provides evidence for a small ancestral genome size in flowering plants. – Ann. Bot., **82** (Suppl. A): 85-94.

Luque, T. & Lifante, Z.D. 1991. Chromosome numbers of plants collected during Iter Mediterraneum I in the SE of Spain. – Bocconea, **1**: 303-364.

Marie, D. & Brown, S.C. 1993. A cytometric exercise in plant DNA histograms with 2C values for 70 species. – Biol. Cell, **78**: 41-51.

Östergren, G. & Heneen, K.W. 1962. A squash technique for chromosome morphological studies. – Hereditas, **48**: 332-341.

Papes, D. & Silic, C. 1981. In: Chromosome number reports LXX. – Taxon, **30**: 70.

Suda, J., Kyncl, T. & Freiova, R. 2003. Nuclear DNA amounts in Macaronesian angiosperms. – Ann. Bot., **92**: 153-164.

Tan, Kit, Tsounis, G. & Tsounis, L. 2010. *Micromeria acropolitana* (*Lamiaceae*) rediscovered in Athens (Greece). – Phytol. Balcan., **16**: 237-242.

Vogt, R. & Oberprieler, C. 1994. Chromosome numbers of North African phanerogams. IV. – Candollea, **49**: 549-570.

Wielgorskaya, T. 1995. Dictionary of generic names of seed plants. Consulting Editor Takhtajan A. Columbia Univ. Press, New York.