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Cytogenetic characterisation of *Artemisia absinthium* (Asteraceae, Anthemideae) and its Polish endemic var. *calcigena*

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This paper presents a karyological and cytogenetic characterisation of *Artemisia absinthium* var. *absinthium* and *A. absinthium* var. *calcigena* (Asteraceae, Anthemideae). Genome size (assessed as nuclear DNA amount), chromosome number, karyotype parameters, fluorochrome banding and fluorescent *in situ* hybridisation (FISH) of 18S-5.8S-26S and 5S rDNA were studied in Polish specimens of both varieties whose phylogenetic relationships were also established based on their molecular structure. The present study is the first with a focus on *A. absinthium* var. *calcigena*. The results basically agree with those previously reported from other *A. absinthium* populations and show no essential genome organization differences between the varieties.

Key words: Asteraceae, cytogenetics, endemic taxa, FISH, genome size

Introduction

Belonging to the genus *Artemisia*, one of the largest in the family Asteraceae (Vallès & Garnatje 2005), the wormwood (*A. absinthium*) is a perennial plant, common in the northern hemisphere, growing as a chamaephyte and occurring in a wide range of habitats, often in ruderal places. It prefers full light and mineral-humic, dry to fresh, moderately acidic or neutral soils (Zarzycki *et al.* 2002). Wormwood naturally grows in Eurasia and northern Africa and was introduced to North, Central and South America, and to New Zealand, where it is nowadays naturalized (Maw *et al.* 1985, Gams 1987). From ancient times it was used as an antihelminthic and digestive drug, being already mentioned in the Ebers papyrus, ca. 1550 BC (Wright 2002). Some potential allelopathic and insecticide activity has been suggested that may have implications in agriculture (Maw *et al.* 1985, Tan *et al.* 1998, Nikolova & Veličnović 2007) and

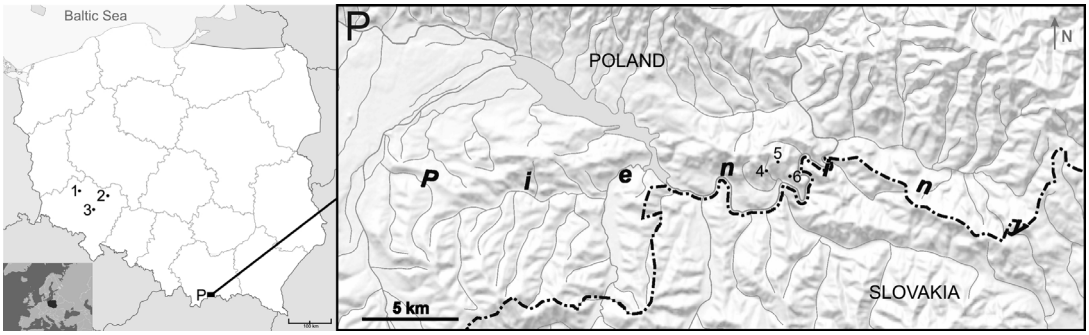

Fig. 1. Geographical distribution of the populations studied. *Artemisia absinthium* var. *absinthium*. 1: Chełmek Wołowski; 2: Czeszów; 3: Gałów. *Artemisia absinthium* var. *calcigena*. 4: Podskalnia Góra; 5: Trzy Korony; 6: Grabczychy.

potentially important medicinal uses have been reported, such as antitumor activity (*in vitro* and in mice and rats), hepatoprotective, antipyretic and antimicrobial (Tan *et al*. 1998, Dülger *et al*. 1999). It is also used as a main ingredient for making various liqueurs such as absinthe and vermouth (Lanier 2004, Lachenmeier *et al*. 2006).

A variety of wormwood, *A. absinthium* var. *calcigena*, was described in the second half of the 19th century as endemic to the Polish part of the Pieniny Mountains, which are located in Poland and Slovakia, and are part of the Western Carpathian Mountains (Rehmann 1868). The description was based on some morphological differences from the type variety: leaves with more acute lobules, panicle with fewer capitula and pedicels longer than the capitulum. The varietal epithet alludes to the limestone soils characteristic of the Pieniny Mountains. This variety was treated by many Polish authors (Żukowski 1971, Zarzycki 1976, 1981, 1982, Szafer *et al*. 1988, Kaźmierczakowa & Perzanowska 2001), all of them confirming its geographical distribution being restricted to the Pieniny Mts. Żukowski (1971) noted that this taxon would need further studies, and Zarzycki (1976) claimed that the only wormwood ecotype growing in the Pieniny Mountains was var. *calcigena*. In the earlier editions of the *Red list of the vascular plants in Poland* (Zarzycki 1986, Zarzycki & Szeląg 1992), it was placed in the "R" category (rare taxa with small populations that are not at present endangered or vulnerable but are at risk), but it is excluded from the most recent list (Zarzycki & Szeląg 2006). It appears also as "rare" in the *Red List of Polish Carpathian Mountains* (Mirek & Piękoś-Mirkowa 1992). However, the taxon was excluded by the same authors from their list of endemic taxa in Polish Carpathians because of its questionable taxonomic status (Piękoś-Mirkowa & Mirek 2003). No karyological or cytogenetic studies have been undertaken to date on this variety.

Karyological and cytogenetic data have revealed to be a powerful tool in plant systematics and evolution studies, and this approach has been intensively applied to *Artemisia* (Garcia *et al*. 2007, 2008, and references therein). The aim of this paper is to characterise, for the first time from the cytogenetic point of view, the Polish endemic *A. absinthium* var. *calcigena*, and to compare its features with those of the type variety (in Polish populations) and also with the previous data on the species.

Material and methods

Plant material

The studied plant material included three populations of *A. absinthium* var. *calcigena*, collected in the Pieniny Mountains, and three populations of *A. absinthium* var. *absinthium* collected from different habitats and locations in Lower Silesia, Poland (Table 1 and Fig. 1). For *A. absinthium* var. *calcigena*, a major part of the known populations was prospected (three out of the four known, I. Wróbel pers. comm.). The dis-

tances between the populations, especially those of the two varieties, are big enough to exclude mixing. Herbarium vouchers are deposited in the Centre de Documentació de Biodiversitat Vegetal, Universitat de Barcelona (BCN), and in Kamil Konowalik's collection.

Chromosome counting and karyotype elaboration

Root tip meristems were obtained from achenes germinating on wet filter paper in Petri dishes placed in dark and room temperature. They were

Table 1. Provenance of populations studied and Genbank accession numbers of the materials sequenced. BCN: Herbarium of the Centre de Documentació de Biodiversitat Vegetal, Universitat de Barcelona.

Taxon	Location and collectors	Herbarium voucher	GenBank accession number
A. absinthium var. *absinthium*	Poland, Lower Silesia, Chełmek Wołowski; 51°27´18´´N, 16°21´43´´E; 107 m; roadside near railway. Herbarium voucher collection: 21.IX.2008 by Kamil Konowalik. Seeds collection: 1.XII.2007 by Kamil Konowalik	BCN 56573	FJ979873, FJ979878
A. absinthium var. *absinthium*	Poland, Lower Silesia, Czeszów; 51°22´39´´N, 17°15´8´´E; 125 m; sands, dry meadow. Herbarium voucher collection: 27.VIII.2008 by Agnieszka Kreitschitz. Seeds collection: 5.XII.2007 by Agnieszka Kreitschitz	BCN 56574	FJ979874, FJ979879
A. absinthium var. *absinthium*	Poland, Lower Silesia, Gałów; 51°6´55´´N, 16°49´30´´E; 130 m; path between forest and cornfield. Herbarium voucher collection: 19.IX.2008 by Kamil Konowalik and Monika Sabat. Seeds collection: 24.XI.2007 by Kamil Konowalik	BCN 56575	FJ979871, FJ979876
A. absinthium var. *absinthium*	Spain, Catalonia, Girona: Setcases. Material collection: 14.IX.1996 by Montserrat Torrell	BCN 12313	FJ979870, FJ979875
A. absinthium var. *calcigena*	Poland, Pieniny Mts., Podskalnia Góra Mt.; 49°24´36´´N, 20°24´20´´E; 650 m; scree, meadow. Herbarium voucher collection: 22.IX.2008 by Iwona Wróbel and Kamil Konowalik. Seeds collection: 22.X.2008 by Iwona Wróbel	BCN 56576	–
A. absinthium var. *calcigena*	Poland, Pieniny Mts., Trzy Korony Mt.; 49°24´49´´N, 20°24´49´´E; 860 m; calcareous rocks, scree, sward. Seeds collection: 21.X.2008 by Iwona Wróbel	BCN-S 1668	–
A. absinthium var. *calcigena*	Poland, Pieniny Mts., Grabczychy Rocks; 49°24´24´´N, 20°25´19´´E; 570 m; calcareous rocks near river, scree. Seeds collection: 7.X.2006 by Iwona Wróbel	BCN-S 1667	FJ979872, FJ979877

pretreated with 0.05% aqueous colchicine for 2.25 hours at room temperature, fixed in a mixture of absolute ethanol and glacial acetic acid (3:1), hydrolyzed in 1 M hydrochloric acid for 2 minutes at 60 °C, stained in 1% aceto-orcein and squashed in a drop of 45% acetic acid and glycerol (9:1), following the protocols described in Pellicer *et al*. (2007b). Observations were carried out under an optical microscope (Axioplan, Carl Zeiss, Germany). Photographs were taken with an AxioCam MRc 5 (Zeiss) and the AxioVision programme (ver. 4.6.3.0). To elaborate the karyotype of each population, at least five metaphase plates from different individuals were used. Chromosome measurements were made using the freeware computer application MicroMeasure ver. 3.3 (http://www.colostate.edu/Depts/Biology/MicroMeasure).

Flow cytometry measurements

To estimate 2C values (holoploid genome size) we used flow cytometry. For measurements we used leaves of plants germinated from seeds in greenhouse, and we had *Petunia hybrida* cv. PxPc6 as an internal standard (2C = 2.85 pg; Marie & Brown 1993). Measurements were made at the 'Serveis Cientificotècnics', Universitat de Barcelona, using an Epics XL flow cytometer (Coulter Corporation, Hialeah, USA), following the techniques described in Garcia *et al*. (2006, 2008). Mean values and standard deviations were calculated based on the results for five individuals (two independent samples per specimen), except for the population from Podskalnia Góra, from which we used three individuals (six measurements) due to scarcity of material.

Chromomycin A_3 banding

Root tip meristems from seedlings were pretreated and fixed as explained above. Fluorochrome banding followed the instructions of Vallès and Siljak-Yakovlev (1997), increasing the time of incubation in chromomycin to 90 minutes. Observations were carried out under a Zeiss Axioplan epifluorescence microscope using a 50 W lamp.

Fluorescence *in situ* hybridisation (FISH)

FISH was employed to reveal 18S-5.8S-26S and 5S rDNA loci, according to the protocols of Torrell *et al*. (2003) and Garcia *et al*. (2007), on plates previously used for chromomycin banding, after distaining with absolute alcohol. Counterstain was done with Vectashield, a mounting medium containing DAPI. Preparations were stored overnight in the dark and then observations were carried out under a Zeiss Axioplan epifluorescence microscope using a 100 W lamp. In both banding and *in situ* experiments photographs were taken with a digital camera AxioCam MRc5 (Zeiss) and the images were analyzed with the Axio Vision Ac software ver. 4.6.3.0.

ITS and ETS sequencing and phylogenetic analyses

We sequenced nrDNA ITS and ETS regions in samples from three *A. absinthium* var. *absinthium* populations and one *A. absinthium* var. *calcigena* population (Table 1), and the sequences were analysed as a part of the matrix of the most updated *Artemisia* molecular phylogeny (Sanz *et al*. 2008), which already contained one *A. absinthium* var. *absinthium* accession. DNA was extracted from plants grown from achenes. DNA extraction, ITS and ETS amplification and sequencing procedures were performed using the methods of Sanz *et al*. (2008). Nucleotide sequences were obtained at the 'Serveis Cientificotècnics', Universitat de Barcelona, using an ABI Prism 3730 DNA Analyzer (Applied Biosystems/Hitachi). They were edited with Chromas 1.56 (Technelysium Pty. Ltd.) and then aligned visually by sequential pairwise comparison in the BioEdit Sequence Alignment Editor 7.0.9.0 (Hall 1999). Outgroup species were designed following the phylogeny of Sanz *et al*. (2008). Data sets were analysed using Mr Modeltest 2.2 (Nylander 2004) to determine the sequence evolution model that best described the present data. This resulted in the acceptance of GTR + Γ + *I* model, as indicated by the Akaike Information Criterion (AIC) and hierarchical likelihood ratio test (hLRT). A Bayesian analysis

with MrBayes 3.1.1 (Huelsenbeck & Ronquist 2001) was performed. Four Markov chains were run simultaneously for 10^6 generations, and these were sampled every 100 generations. Data from the first 1000 generations were discarded as the 'burn-in' period, after confirming that likelihood values had stabilized prior to the 1000th generation. The 50% majority rule consensus phylogeny and posterior probability (PP) of nodes were calculated from the remaining sample.

Results and discussion

Molecular phylogenetic location of *A. absinthium* var. *calcigena*

The position of *A. absinthium* in the molecular phylogeny is well established, constituting a well-supported subclade (PP = 1.00, Fig. 2), known as the *A. absinthium* complex, in a clade formed by representatives of subgenera *Absinthium* and *Artemisia* (PP = 1.00, Fig. 2; Sanz *et al.* 2008, and the references therein). The analyses performed here (Fig. 2) include four *A. absinthium* var. *absinthium* populations and one *A. absinthium* var. *calcigena* population (the previously published ones contained only one population of the type variety). The varieties form distinct groups. Sequence divergence was calculated for the combined ITS and ETS regions using the distance matrix option available in PAUP. Sequence divergence varied within the populations of *A. absinthium* var. *absinthium* from 0% to 1.25%, and percentage of sequence divergence between the populations of *A. absinthium* var. *calcigena* and var. *absinthium* ranged between 0% and 0.63%. As expected, the intraspecific divergence is very low, also between the two varieties.

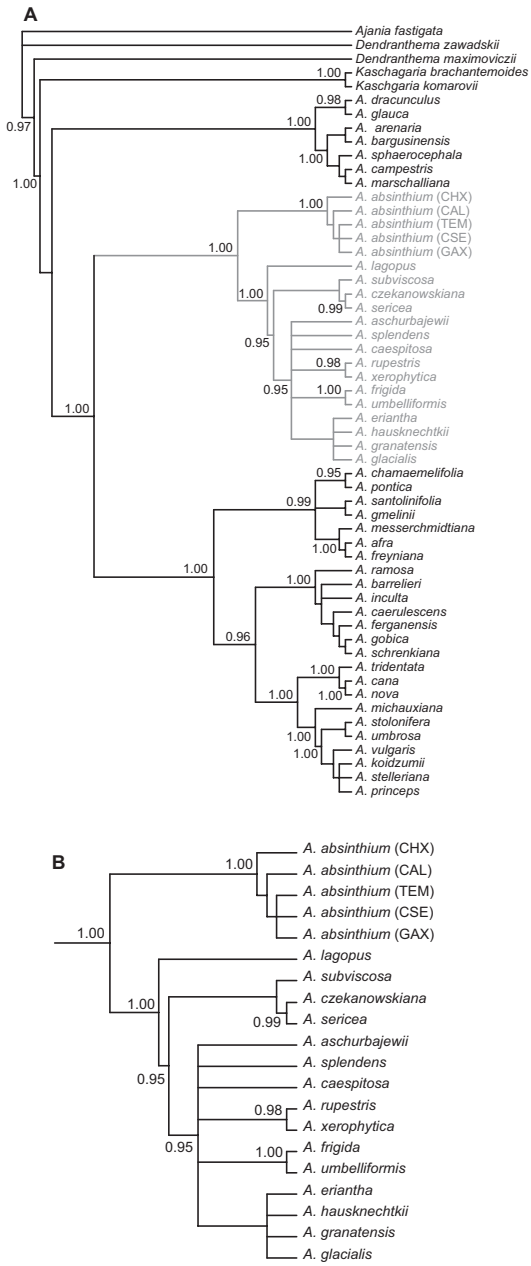

Fig. 2. Phylogenetic tree based on the analyses of nrDNA ETS and ITS sequences, showing the location of the studied taxa in the frame of the genus *Artemisia*. — **A**: General view. — **B**: Detail of the clade including *Artemisia absinthium* (grey in **A**). CAL: *A. absinthium* var. *calcigena*, Grabczychy; GAX: *A. absinthium* var. *absinthium*, Gałów; CHX: *A. absinthium* var. *absinthium*, Chełmek Wołowski; CSE: *A. absinthium* var. *absinthium*, Czeszów; TEM: *A. absinthium* var. *absinthium*, Setcases (the last *A. absinthium* population as well as the remaining taxa, from Sanz *et al.* 2008). The numbers are posterior probability (PP) values.

Chromosome number

All of the studied populations are diploid, with 2n = 18 chromosomes (Table 2 and Fig. 3). No previous reports for *A. absinthium* var. *calcigena* are available, so that this first report on the variety agrees with the chromosome number commonly recorded for the species (Weinedel-

Table 2. Summary of the cytogenetic results. Standard deviations are given in parentheses.

Taxon (population)	2n	Ploidy level	Chromosomal formula[1]	MCL (SD)[2]	CLR[3]	TKL (SD)[4]	CI[5]	*R*[6]	A1[7]	A2[8]	Stebbins class[9]	2C (SD) (pg)[10]	2C (Mbp)[11]
A. absinthium var. *absinthium* (Chełmek Wołowski)	18	2x	7m + 1sm + 1st	4.23 (0.72)	2.72–5.47	76.10 (4.84)	40.20	1.64	0.30	0.17	2A	8.62 (0.26)	8431.01
A. absinthium var. *absinthium* (Czeszów)	18	2x	7m + 2sm	4.97 (0.74)	3.66–6.28	89.45 (3.33)	39.97	1.56	0.32	0.15	2A	8.67 (0.28)	8475.95
A. absinthium var. *absinthium* (Gałów)	18	2x	7m + 2sm	4.32 (0.60)	3.13–5.28	77.83 (4.66)	40.51	1.55	0.30	0.14	2A	8.59 (0.16)	8405.23
A. absinthium var. *calcigena* (Podskalnia Góra)	18	2x	7m + 2sm	4.05 (0.53)	3.02–5.21	72.92 (5.86)	41.22	1.51	0.28	0.13	2A	9.24 (0.09)	9032.32
A. absinthium var. *calcigena* (Trzy Korony)	18	2x	8m + 1st	4.15 (0.52)	3.10–5.08	74.77 (7.08)	40.08	1.63	0.31	0.12	2A	8.90 (0.16)	8703.57
A. absinthium var. *calcigena* (Grabczychy)	18	2x	7m + 1sm + 1st	4.03 (0.49)	3.18–4.97	72.50 (5.27)	40.81	1.57	0.28	0.12	2A	8.47 (0.10)	8286.75

[1] Chromosomal formula according to Levan *et al.* (1964); [2] MCL = mean chromosome length (μm); [3] CLR = chromosome length range; [4] TKL = total karyotype length; [5] CI = centromeric index (mean, I or index in Levan *et al.* 1964); [6] *R* = length ratio of long and short chromosome arms (Levan *et al.* 1964); [7] A1 = intrachromosomal asymmetry index (Romero 1986); [8] A2 = interchromosomal asymmetry index (Romero 1986); [9] Symmetry class according to Stebbins (1971); [10] 2C = nuclear DNA content (pg); [11] 1 pg = 978 Mbp (Doležel *et al.* 2003).

Liebau 1928, Urbańska 1959, Kawatani & Ohno 1964, Bhat *et al*. 1974, McArthur 1977, Popova & Kuzmanov 1986, Májovský & Murín 1987, Malakhova & Markova 1994, Vallès & Torrell 1995, Torrell & Vallès 2001, Kreitschitz & Vallès 2003, Pellicer *et al*. 2008). This confirms x = 9 as the basic number and the diploid level as the usual one for this taxon. Previously, the tetraploid level has only been reported in some individuals from the Czeszów population (Kreitschitz & Vallès 2003), but we did not find tetraploids in the present research.

Dicentric chromosomes were scarcely observed in plants of one population of each variety (Czeszów and Grabczychy). Within the genus *Artemisia*, Pellicer *et al*. (2007a) reported the presence of chromosomes with two centromeres (and the corresponding acentric fragments) in *A. litophila*, and the same infrequent phenomenon has been detected in Polish populations of *A. absinthium* and *A. eriantha* (A. Kreitschitz unpubl. data.).

Genome size

The results obtained from flow cytometry (Table 2) agree with the diploid level indicated by the chromosome counts, and show small genome-size differences within *A. absinthium*, including var. *calcigena*. The 2C values range from 8.47 (Grabczychy) to 9.24 (Podskalnia Góra) pg, both corresponding to var. *calcigena*. The difference between the smallest and the highest quantity is 0.77 pg (8.33%). The difference in the populations studied of var. *absinthium* is only 0.92% (2C values from 8.59 to 8.67 pg). Our results agree with previous results reporting, for populations of *A. absinthium* from different parts of the world, 2C values ranging from 8.29 to 9.06 pg (Torrell & Vallès 2001, Garcia *et al*. 2004, 2006). These results do not show a very high variation (2C values from 8.29 to 9.24 pg, 10.28%) among all of the *A. absinthium* populations studied to date, which cover a wide geographical area. Nagl and Ehrendorfer (1974) reported 7.30 pg for this species, which would suppose a 20.99% difference when all populations are considered. This was the only population studied by microdensitometry after

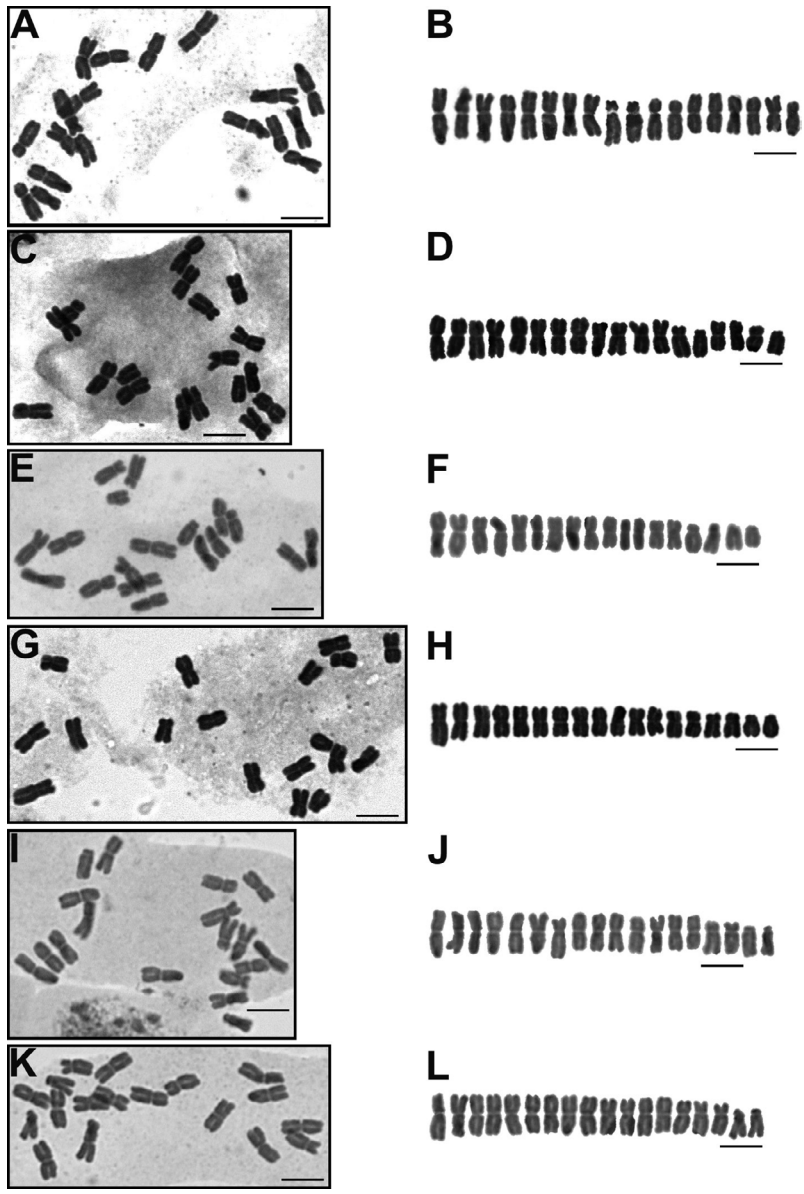

Fig. 3. Metaphase plates and karyograms of the six populations studied. — **A** and **B**: *Artemisia absinthium* var. *absinthium*, Czeszów. — **C** and **D**: *Artemisia absinthium* var. *absinthium*, Gałów. — **E** and **F**: *Artemisia absinthium* var. *absinthium*, Chełmek Wołowski. — **G** and **H**: *Artemisia absinthium* var. *calcigena*, Grabczychy. — **I** and **J**: *Artemisia absinthium* var. *calcigena*, Trzy Korony. — **K** and **L**: *Artemisia absinthium* var. *calcigena*, Podskalnia Góra. Scale bars = 5 μm.

Feulgen staining (all the others having been processed by flow cytometry), in which some aspects, such as the fixation used before Feulgen staining, can cause variations, as reported by Greilhuber (1997, 1998). Hence we believe that the actual differences within this species are around 10%, comparable to the results on the intraspecific variation found in other *Artemisia* (*A. arborescens* [Garcia *et al*. 2006], *A. crithmifolia* [Pellicer *et al*. 2009]) or even in other Asteraceae species (Garnatje *et al*. (2009), on *Cheirolophus intybaceus*, Cardueae). A very low half peak coefficient of variation (1.41%) allows us to discard the influence of the cytometer or of secondary metabolites and to regard the variation as genuine rather than an artifact.

Contrary to expectations, DNA content variation did not correspond to the total karyotype length (TKL, Table 2). Lowest and highest nuclear DNA amounts are found in two var. *calcigena* populations, whereas the populations of this variety show the smallest TKL. That fact

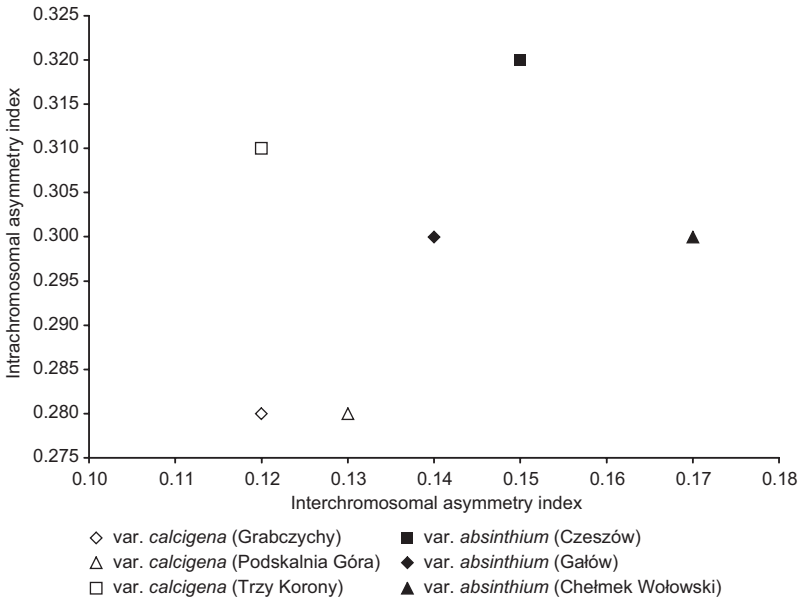

Fig. 4. Representation of the asymmetry indexes (Romero 1986) of the *A. absinthium* populations studied.

proves again that TKL is only a rough indicator of genome size, being highly affected by the pretreatment conditions (time, temperature, antimitotic agent used) and therefore causing differences in the degree of chromosomal condensation (Vallès & Siljak-Yakovlev 1997, and the references therein).

Several authors have reported genome size increases as probable adaptations to different ecological conditions, such as altitude (Bennett 1976, Gregory & Hebert 1999, Garcia *et al*. 2008, Hidalgo *et al*. 2008, and the references therein). We did not find any evidence for that: the highest but also the lowest values were found in var. *calcigena*. On the other hand, although var. *calcigena* is limited to a mountain area, this is not at all a high-mountain zone (for which the adaptive genome size increase has been postulated) and the altitudinal differences are not so great (Table 1).

Karyotype morphometry

Mean chromosome length (MCL) varies from 4.03 to 4.97 μm (Table 2). The populations of var. *calcigena* have the smallest MCL, from 4.03 to 4.15 μm, while the type variety has an MCL from 4.23 to 4.97 μm, but the differences were small. Also the total karyotype length (TKL) is lower in var. *calcigena*, ranging from 72.5 to 74.77 μm, while the other plants have a TKL from 76.1 to 89.45 μm (Table 2), but as above no significant differences were found. All centromeric indexes are close to each other, varying from 39.97 to 41.22 (the closer the value to 50.00, the more centric the centromere; these values fall in the "median region", meaning that the centromere is very close to the median point of the chromosome; Levan *et al*. 1964; Table 2). The same may be said about the ratio between the length of long and short chromosome arms, with values ranging from 1.51 to 1.64 (Table 2). All these data indicate rather symmetrical karyotypes, as expressed by chromosomal formulas in which metacentric and submetacentric chromosomes are largely dominant, as they were in the previously studied population of the species (Pellicer *et al*. 2008). Romero's (1986) asymmetry indexes are low and also very close to each other. Two groups can be observed (Table 2 and Fig. 4; higher values indicate higher asymmetries), corresponding to var. *absinthium* and var. *calcigena*, but the difference in interchromosomal asymmetry index (A2) between the closest populations of the two taxa is very small. The karyotypes of the different populations are also rather homogeneous (Fig. 3), all of them belonging to Stebbins' (1971) class 2A (the second most symmetric) and with very simi-

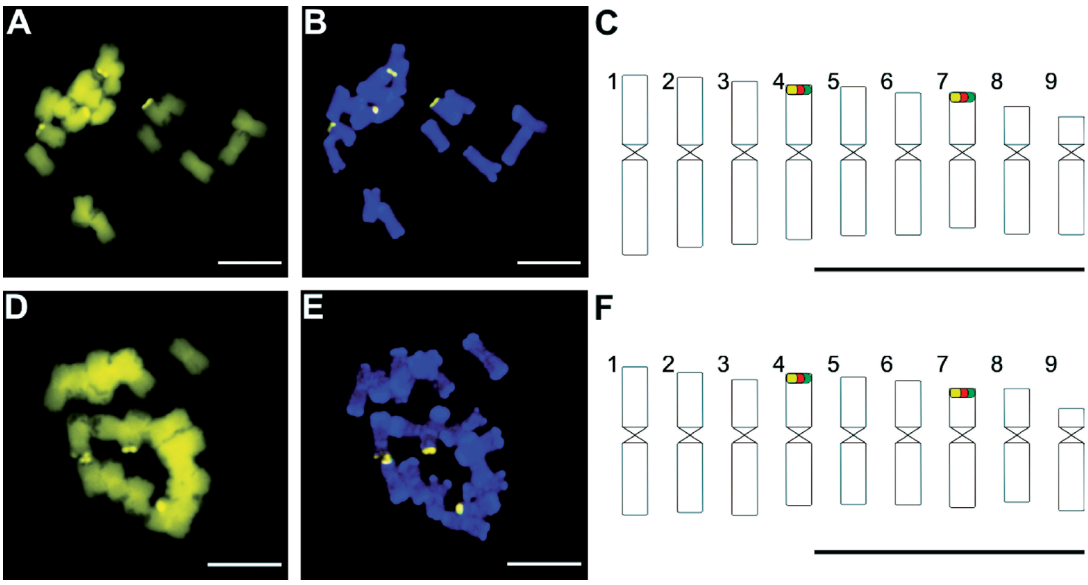

Fig. 5. Metaphase plates and idiograms with chromomycin and FISH signals in the two taxa studied. — **A–C**: *Artemisia absinthium* var. *absinthium*, Czeszów. — **D–F**: *Artemisia absinthium* var. *calcigena*, Trzy Korony. ■ Chromomycin. ■ 18S-5.8S-26S rDNA loci. ■ 5S rDNA loci. Scale bar = 10 μm.

lar centromeric indexes and chromosomal arms length ratio values (Levan *et al*. 1964) (Table 2). In general, the first chromosome pair is larger than the others, this difference being clearer in the Czeszów population. Also the last pair is often very distinguishable, being much smaller than the rest.

Fluorochrome banding and fluorescent *in situ* hybridisation patterns

As for all the other parameters, this is the first characterisation of *A. absinthium* var. *calcigena* using fluorochrome banding and FISH. To date only two populations of the species (belonging to the type variety) had been studied from this point of view (Pellicer *et al*. 2008, Garcia *et al*. 2009). Both varieties show the same pattern (Table 3 and Fig. 5), consisting of four 18S-5.8S-26S telomeric marks (two chromosome pairs), colocalised with the same number of 5S loci and coincidental with all GC-rich regions marked by chromomycin. This model agrees with the previous data on *A. absinthium* (Pellicer *et al*. 2008) and confirms once again the collocation of both ribosomal genes, which is a feature frequently reported for *Artemisia* and the related genera, but it is quite restricted in angiosperms in general (Torrell *et al*. 2003, Abd El-Twab & Kondo 2006, Hoshi *et al*. 2006, Garcia *et al*. 2007, 2009, Pellicer *et al*. 2008). Also in agreement with the precedent *A. absinthium* study (Pellicer *et al*. 2008), a large amount of DAPI-rich bands

Table 3. Number and distribution of GC-rich chromosome regions and of 18S-5.8S-26S and 5S rDNA sites. The superscripts indicate: [1] total number of chromomycin A_3-positive (GC-rich) regions; [2] number of telomeric 18S-5.8S-26S rDNA loci; [3] number of telomeric 5S rDNA loci. All marks are telomeric and colocalized.

Taxon (population)	Chromomycin[1]	18S-5.8S-26S t[2]	5S t[3]
A. absinthium var. *absinthium* (Chełmek Wołowski)	4	4	4
A. absinthium var. *absinthium* (Czeszów)	4	4	4
A. absinthium var. *calcigena* (Trzy Korony)	4	4	4

has been detected in both varieties, mostly in telomeric position (rarely subtelomeric), and basically in both chromosome arms, with the exception of those corresponding to rDNA loci, where the DAPI bands were very weak or absent. The DAPI-positive bands are coincidental with chromomycin-negative ones and *vice versa*.

Concluding remarks

The present results constitute the first molecular study of the endemic *A. absinthium* var. *calcigena* and also a characterization, from this viewpoint, of the type variety and, in general, of the economically interesting plant *A. absinthium*. This is a widely distributed species in which only very few varieties have been described (such as the North American var. *insipida*), and no research on them, apart from that concerning their description, had to date been conducted. It is evident that *A. absinthium* is a rather uniform species morphologically, although some variability exists within it, probably due to its high geographical and ecological spectrum.

Our observations lead to the conclusion that there are no significant differences between the studied varieties, either regarding molecular phylogeny or at the cytological level. Besides, preliminary morphological and anatomical comparative studies between the taxa (K. Konowalik & A. Kreitschitz unpubl. data.) do not show noticeable differences. As described in the introduction, the morphological differences used to establish var. *calcigena* are minor. Practically all molecular and cytogenetic features are also common to all *A. absinthium* specimens and the variation detected may be just a consequence of specific conditions (i.e. environmental) rather than an infraspecific taxonomic character. Different nuclear DNA amount values, chromosomal formulas and lengths of particular chromosomes (especially pairs I and IX) do not reflect a varietal level and may occur throughout in *A. absinthium s. lato*. This homogeneity is not surprising taking into account that "variety" is a very low taxonomic level, implying only slight differences between the taxa. *Artemisia absinthium* var. *calcigena* is probably a particular ecotype of the species, adapted to calcium-rich soils in the Pieniny Mountains, so that it is plausible that no significant genome organization differences appear.

This notwithstanding, a deeper morphological research on *A. absinthium* var. *calcigena* and other possible infraspecific taxa of this species would be needed to assess its variability as comprehensively as possible. Lachenmeier *et al.* (2006) showed that some *A. absinthium* ecotypes vary in their thujone content, so that a deeper consideration of all wormwood variability would not be interesting only from the systematic point of view, but could have commercial implications as well.

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