Variation of DNA amount in 47 populations of the subtribe Artemisiinae and related taxa (Asteraceae, Anthemideae): karyological, ecological, and systematic implications

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Abstract: Genome size has been estimated by flow cytometry in 47 populations of 40 species of the tribe Anthemideae (Asteraceae), mainly from *Artemisia* and other genera of the subtribe Artemisiinae and related taxa. A range of 2C values from 3.54 to 21.22 pg was found. DNA amount per basic chromosome set ranged from 1.77 to 7.70 pg. First genome size estimates are provided for one subtribe, 10 genera, 32 species, and two subspecies. Nuclear DNA amount correlated well with some karyological, physiological and environmental characters, and has been demonstrated as a useful tool in the interpretation of evolutionary relationships within *Artemisia* and its close relatives.

Key words: Artemisia, C value, ecology, evolution, flow cytometry, genome size, nuclear DNA amount variation, phylogeny, polyploidy, systematics.

Résumé : La taille du génome de 47 populations de 40 espèces de la tribu Anthemideae (Asteraceae), principalement du genre *Artemisia* et d'autres représentants de la sous-tribu Artemisiinae ou de groupes proches à celle-ci, a été estimée par cytométrie en flux. Les valeurs 2C sont comprises entre 3,54 et 21,22 pg. La quantité d'ADN par dotation chromosomique de base est comprise entre 1,77 et 7,70 pg. La taille du génome a été déterminée pour la première fois dans une sous-tribu, 10 genres, 32 espèces et deux sous-espèces. La quantité d'ADN nucléaire est en très bonne corrélation avec des caractères caryologiques, physiologiques et écologiques ; elle s'est avérée aussi utile pour l'interprétation des relations évolutives chez *Artemisia* et ses taxons apparentés.

Mots clés : Artemisia, cytométrie en flux, écologie, évolution, phylogénie, polyploïdie, systématique, taille du génome, valeur C, variation de la quantité d'ADN nucléaire.

Introduction

The amount of nuclear DNA (C value) is a fundamental biodiversity character, directly or indirectly related to many phenotypic traits and other important factors such as reproductive biology, ecology, and plant distribution (Bennett 1998). More than 100 positive or negative correlations with nuclear DNA amount have been documented. Measurements of the amount of nuclear DNA, which initially focused on cytogenetics, physiology, and ecology, have recently become more important in systematic and phylogenetic research (Kellogg 1998; Leitch et al. 1998). With the growing recognition of its relevance, there is a need for additional DNA C-value assessments in plants (Bennett and Leitch 1995; Bennett 1998; Hanson et al. 2001*a*, 2001*b*). Bennett and colleagues have assembled six reference lists of nuclear DNA amounts since 1976; these data are available through an internet database (http://www.rbgkew.org.uk/cval/homepage.html; Bennett and Leitch 2003), which facilitates comparative studies and other data-based research. Nevertheless, the

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existing data scarcely represent the global angiosperm flora (Bennett and Leitch 1995): fewer than 2% of angiosperm species have a known C value and more than 50% of angiosperm families lack even a single estimate of nuclear DNA amount for any species.

Artemisia (Asteraceae, Anthemideae), the principal focus of the present study, is the largest of the tribe Anthemideae and among the largest genera in the family Asteraceae. It comprises from 200 to more than 500 taxa at the specific or subspecific level, according to various authors (see Vallès and McArthur 2001 and references therein). Five large groups (*Absinthium*, *Artemisia*, *Dracunculus*, *Seriphidium*, and *Tridentatae*) are classically considered within *Artemisia*, at sectional or subgeneric levels (Torrell et al. 1999). However, the present infrageneric classification does not represent natural groups (Persson 1974; McArthur et al. 1981; Vallès and McArthur 2001) and there is still no agreement about the global treatment of the genus. Several genera have been segregated from *Artemisia* (Vallès et al. 2003 and references therein): big ones, such as *Seriphidium*, and small, often monotypic ones, such as *Mausolea.* In addition, other genera, such as *Ajania*, are systematically close to *Artemisia* or in taxonomic conflict with it. They are the basis for the subtribe Artemisiinae (Bremer and Humphries 1993), and have close relationships with genera belonging to the subtribes Handeliinae, Tanacetinae, Leucantheminae and Chrysantheminae. Molecular biology can shed light on the real structure of this pool of genera and studies based on DNA sequences have been and are being carried out to clarify its systematics (Watson et al. 2002; Vallès et al. 2003), which indicate the need of rearrangements to achieve a phylogenetically based organization of the Artemisiinae.

Artemisia is a widely distributed genus in the northern hemisphere, mainly in temperate areas, (Bremer 1994) and is rather scarce in the southern hemisphere. It is better represented in Eurasia than in North America. Central Asia constitutes its main centre of speciation and diversification (McArthur and Plummer 1978; Vallès and McArthur 2001). The species of the genus can be found from sea level to high mountains, frequently colonizing semiarid environments. Some *Artemisia* species occur in isolation, but more commonly they form extensive, landscape-dominant populations. Most of the species are perennial, only around 15 are annual or biennial. Polyploidy is a frequent phenomenon in the genus, which also has dysploidy, with two basic chromosome numbers (x = 8 and x = 9). Many *Artemisia* species have a high economic value, in that thay have medical, food, forage, or ornamental uses; on the other hand, some taxa are invasive weeds that can adversely affect agronomic harvests (Vallès and McArthur 2001 and references therein; Wright 2002).

The present study also includes six additional Artemisiinae and four genera belonging to three other Anthemideae subtribes, as detailed in the Materials and methods. These taxa, particularly those belonging to the Artemisiinae, are phylogenetically close to *Artemisia*, up to the point that some of them had been previously classified as members of *Artemisia* (the proximity to this genus can be deduced from the complex synonymy of many of these species presented in Table 1), but alternatively these taxa are placed in other genera.

Materials and methods

Plant material

Table 1 shows the 47 populations studied, grouped by subtribe, genus, and subgenus, along with their site of origin and herbarium voucher information. The study material includes 27 species and four subspecies from the genus *Artemisia*, four from *Dendranthema* (including one subspecies and one variety), and one each from *Brachanthemum*, *Filifolium*, *Kaschgaria*, *Mausolea*, and *Neopallasia* (all from subtribe Artemisiinae). Other Anthemideae taxa represented in the study include one species each of *Lepidolopsis* (Handeliinae), *Nipponanthemum* (Leucantheminae), *Hippolytia*, and *Tanacetopsis* (both, Tanacetinae). The chosen populations represent, apart from different taxonomic groups, distinct geographic areas, life forms, ploidy levels, and chromosome numbers. Vouchers for most materials are deposited in the herbarium of the Centre de Documentació de Biodiversitat Vegetal de la Universitat de Barcelona (BCN). Other vouchers are in the herbaria of the Shrub Sciences Laboratory, Provo, Utah (SSLP), the Botanical Institute V.L. Komarov of the Russian Academy of Sciences, Saint Petersburg (LE), and A. Kreitschitz, Wrocław. Some species have been obtained from botanical gardens through Index Seminum (Sapporo Botanic Garden, Hokkaido University, Japan; and Vladivostok Botanical Institute, Russian Academy of Science, Russia), with known original location.

Young leaves used for flow cytometry assays were taken from plants cultivated in pots. The achenes or adult plants were collected in natural populations. Seeds of *Pisum sativum* L. 'Express Long' and an adult *Petunia hybrida* Vilm. 'PxPc6', both used as internal standards for flow cytometric measurements, were obtained from the Institut des Sciences du Végétal (CNRS, Gif-sur-Yvette, France).

Flow cytometry measurements

DNA 2C values of the tested species were estimated using flow cytometry. *Pisum sativum* L. 'Express Long' and *Petunia hybrida* Vilm. 'PxPc6' (2C = 8.37 and 2.85 pg, respectively; Marie and Brown 1993) were used as internal standards to cover the range of 2C values found. In one case, when the peak of the unknown *Artemisia absinthium* and the internal standard *Pisum sativum* overlapped, an *Artemisia* species (*A. abrotanum*, 2C = 11.41 pg) that had previously been assessed with *Pisum* for nuclear DNA amount estimation, was used as internal standard (Torrell and Vallès 2001); this exceptional case was due to the lack of *Petunia hybrida* at that moment. Young healthy leaf tissues from the species to be studied and a calibration standard were placed together in a plastic Petri dish and chopped with a razor blade in Galbraith's isolation buffer (Galbraith et al. 1983). The amount of target species leaf (about 25 mm^2) was approximately twice that of the internal standard. The suspension of nuclei in the isolation buffer was filtered through a nylon mesh with a pore size of 70 µm and stained for 20 min with propidium iodide (Sigma-Aldrich Química, Alcobendas, Madrid, 60 µg/mL), the chosen fluorochrome standard (Johnston et al. 1999); tubes were kept on ice during staining and then left at room temperature until measurement. For each population, five individuals were analyzed; two

samples of each individual were extracted and measured independently. Measurements were made at the Serveis Cientificotècnics generals de la Universitat de Barcelona using an Epics XL flow cytometer (Coulter Corporation, Hialeah, Fla.). The instrument was set up with the standard configuration: excitation of the sample was done using a standard 488-nm air-cooled argon-ion laser at 15 mW power. Forward scatter (FSC), side scatter (SSC), and red (620 nm) fluorescence for propidium iodide were acquired. Optical alignment was based on optimized signal from 10-nm fluorescent beads (Immunocheck, Epics Division, Coulter Corporation). Time was used as a control of the stability of the instrument. Red fluorescence was projected on 1024 monoparametrical histograms. Gating single cells by their area versus peak fluorescence signal excluded aggregates. Acquisition was automatically stopped at 8000 nuclei. The total nuclear DNA content was calculated by multiplying the known DNA content in *Pisum* or *Petunia* by the quotient between the 2C peak positions of the target species and the chosen internal standard in the histogram of fluorescence intensities for the 10 runs, based on the assumption that there is a linear correlation between the fluorescence signals from stained nuclei of the unknown specimen and the known internal standard and the DNA amount. Mean values and standard deviations were calculated based on the results for the five individuals.

Statistical analyses (analysis of variance, means comparison by least significant difference test) were carried out to evaluate the relationships between the studied variables (DNA content, DNA per basic chromosome set, altitude, and life cycle, among others). All the analyses were performed with the program Statgraphics Plus 5.0 (Statistical Graphics Corp., Rockville, Md.). In addition to the data obtained in the present study (Table 2), those from a previous paper on *Artemisia* genome size (Torrell and Vallès 2001) were also used for the statistical analyses of the present work.

Results and discussion

The results of flow cytometric assessment of the nuclear DNA content of 47 populations of 40 species belonging to the tribe Anthemideae are presented in Table 2, together with genome size data in megabase pairs (1 pg = 978 Mbp, Doležel et al. 2003), other karyological characters, and information on life cycle and on the internal standard used for each estimation. The analyses were of good quality (mean half peak coefficient of variation (HPCV) = 3.06%). This second study of *Artemisia* DNA by flow cytometry also includes some related genera. It expands the flow cytometry database by a factor of three — the earlier work reported 21 *Artemisia* species (Torrell and Vallès 2001). In addition to the flow cytometry work, nuclear DNA content had been estimated for only seven *Artemisia* species by cytodensitometry after Feulgen staining (Nagl and Ehrendorfer 1974; Geber and Hasibeder 1980; Greilhuber 1988; Bennett and Smith 1991; S.R. Band, personal communication; Dąbrowska 1992).

For the genera *Brachanthemum*, *Dendranthema*, *Filifolium*, *Hippolytia*, *Kaschgaria*, *Lepidolopsis*, *Mausolea*, *Neopallasia*, *Nipponanthemum*, and *Tanacetopsis* the DNA content values presented in this paper are the first estimates (Bennett and Leitch 2003). Likewise, the DNA content assessment reported here is the first record for the subtribe Handeliinae, 32 species (13 of the 10 above-cited genera and 19 of *Artemisia*) and three *Artemisia* subspecies (Table 2).

When all the *Artemisia* species with available genome size data — those from this paper, those reported by Torrell and Vallès 2001, and those from the papers cited in the first paragraph of this section, noted in the Bennett and Leitch (2003) database — are taken into account, variations are, respectively, 7.33- and 4.40-fold for DNA amount and DNA amount per basic chromosome set. The variation is 3.04 fold for 2C value and 3.53 fold for DNA per basic chromosome set in the other genera studied.

Relationships with karyological characters

As might be expected, 2C value means are significantly different ($p < 0.005$) for chromosome number and ploidy level. Both minor and major differences are even found between $2n = 16$ and $2n = 18$ taxa (Table 2 and Torrell and Vallès 2001). However, there are exceptions to this positive relationship; diploid *A. abrotanum* has only 5.78 pg of nuclear DNA amount with 18 chromosomes, whereas *A. leucodes* has 15.39 pg with the same chromosome number and, surprisingly, *A.* × *wurzellii*, with 34 chromosomes, has 8.60 pg. Similar results have been seen in other groups of Asteraceae e.g., *Siebera pungens* with a 2C value of 16.98 pg and 20 chromosomes and *Amphoricarpus neumayeri* with 1.73 pg and 24 chromosomes (Garnatje et al. 2004). Nevertheless, the general trend is an increase of nuclear DNA amount with the increase of chromosome number (*Papaver*, Srivastava and Lavania 1991; *Achillea*, Dąbrowska 1992).

Although genome size and ploidy level are highly correlated, nuclear DNA amount per basic chromosome set decreases with polyploidy. Analysis of variance (ANOVA) shows a significant difference ($p < 0.05$) in nuclear DNA amount mean values between diploids and tetraploids, the latter having less nuclear DNA amount per basic chromosome set than the former; we did not perform analyses with other ploidy levels, because we had only minimal representation of each one. This supports the Grant's (1969) hypothesis that there is a decrease in nuclear DNA amount in polyploids associated to an adaptive response for the stabilization of the higher polyploids (dodecaploids) in *Betula*. Nuclear DNA loss per basic chromosome set in polyploids has also been reported in many other taxa (Bennett 1972; Murray et al. 1992; Ohri 1996; Dimitrova and Greilhuber 2000; Friedlender et al. 2002).

Aneusomaty may be another source of genome size variation. Some of the highest standard deviation values in the plants studied, such as those of *Artemisia campestris* subsp. *sericea* (Table 2) or *A. dracunculus* (Torrell and Vallès 2001) correspond to aneusomatic populations (Kreitschitz 2003; Kreitschitz and Vallès 2003). Similar variations have been reported in aneusomatic *Helianthus annuus* (Cavallini and Cremonini 1985; Michaelson et al. 1991).

Systematic implications: intraspecific and interspecific variation

Nuclear DNA amount can be useful in the interpretation of evolutionary relationships. C value may increase or decrease with evolution and comparisons between the different

Table 1. Provenance of the populations of Anthemideae studied.

Taxa	Origin of materials	Herbarium voucher
Subtribe Artemisiinae		
Genus *Artemisia*		
Subgenus *Absinthium*		
A. absinthium L.	Czeszów, Lower Silesia, Poland	Hb. A. Kreitschitz
A. aschurbajewii C. Winkler	Asku-Zhabagli nature reserve, Zhambul district, Kazakhstan	BCN 11693
A. austriaca Jacq.	Swieta Katarzyna, Lower Silesia, Poland	Hb. A. Kreitschitz
A. frigida Willd.	Almond, Colo.	BCN 11568
A. glacialis L.	Valmontey, Aosta valley, Italy	BCN 11566
A. lagocephala (Fischer ex Besser) DC.	Snezhnaya mountain,USSR	I. S. Vladivostok N53, BCN S-805
A. persica Boiss.	Asku-Zhablagli nature reserve, Zhambul district, Kazakhstan	BCN 11696
A. sieversiana Ehrh. in Willd.	Khanatalap, Almaty district, Kazakhstan	BCN 11692
Subgenus *Artemisia*		
A. abrotanum L.	Wrocław (Tarnogaj), Lower Silesia, Poland	Hb. A. Kreitschitz
A. abrotanum L.	Wrocław (Kozanów), Lower Silesia, Poland	Hb. A. Kreitschitz
A. afra Jacq.	Transwaal, Makapan, South Africa	BCN 11570
A. princeps Pampan.	Nopporo forest park, Ebetsu, Sapporo, Japan	BCN S-812
A. santolinifolia Turcz ex H. Kraschen.	Asku-Zhabagli nature reserve, Zhambul district, Kazakhstan	BCN 11694
A. stelleriana Besser	Glazkovka, USSR	I. S. Vladivostok N55, BCN S-813
A. tournefortiana Reichenb.	Karapalpakstan, Uzbekistan	BCN 11630
A. vulgaris L.	Chrząstawa Mała, Lower Silesia, Poland	Hb. A. Kreitschitz
A. vulgaris L.	Staniszów, Lower Silesia, Poland	Hb. A. Kreitschitz.
A. vulgaris L.	Lhasa, Tibet, People's Republic of China	BCN 11590
A. × *wurzellii* C. M. James & Stace in C. M. James, Wurzell & Stace	Northumberland park, London, UK	BCN 11670
Subgenus *Dracunculus*		
A. arenaria DC.	Volgograd, USSR	LE (Korobkov)
A. campestris L.	Wayne County, Utah	SSLP (McArthur 2777)
A. campestris L.	Konotop, Wielkopolska Region, Poland	Hb. A. Kreitschitz
A. campestris L.	Zagan, Ziemia Lubuska Region, Poland	Hb. A. Kreitschitz
A. campestris L. subsp. *sericea* (Fr.) Leuwke & Rothm.	Hel, Helska sandbank, Poland	Hb. A. Kreitschitz
A. filifolia Torrey	Mohave County, Ariz.	SSLP (McArthur 2784)
A. scoparia Waldst. & Kit.	Sultanuizdag Mountains, Karkalpakstan, Uzbekistan	BCN 11628
Subgenus *Seriphidium*		
A. leucodes Schrenk	Dgizak, Uzbekistan	BCN 11631
Subgenus *Tridentatae*		
A. arbuscula Nutt.	Millard County, Utah	SSLP (McArthur 2779)
A. bigelovii A. Gray	Emery County, Utah	SSLP (McArthur 2778)
A. cana Pursh. subsp. *viscidula* (Osterhout) Beetle	Wasatch County, Utah	SSLP (McArthur 2775)
A. nova Nelson	Nye County, Nev.	SSLP (McArthur 2739)
A. pygmaea A. Gray	Emery County, Utah	SSLP (McArthur 2780)
A. tridentata Nutt. subsp. *tridentata*	Juab County, Utah	SSLP (McArthur U-79)
A. tridentata Nutt. subsp. *vaseyana* (Rydb.) Beetle	Juab County, Utah	SSLP (McArthur 2507)
Genus *Brachanthemum*		
Brachanthemum titovii H. Kraschen.	Aktogai, Almaty district, Kazakhstan	BCN 11690

Table 1 (*concluded*).

Taxa	Origin of materials	Herbarium voucher
Genus *Dendranthema*		
D. arcticum Tzvelev subsp. *maekawanum* (Kitam.) H. Koyama	Sapporo, Japan	I.S. Sapporo, BCN S-815
D. indica Des Moul. var. *coreanum* Levl. & Van.	Sapporo, Japan	I.S. Sapporo, BCN S-809
D. maximowiczii (Komarov) Tzvelev	Glazkovka, USSR	I.S. Vladivostok N70, BCN S-810
D. zawadskii (Herbich) Tzvelev	Sapporo, Japan	I.S. Sapporo, BCN S-814
Genus *Filifolium*		
F. sibiricum (L.) Kitam. (*Artemisia sibirica*(L.) Maxim., *Tanacetum sibiricum* L.)	Oktyabrsky, USSR	I.S. Vladivostok N76, BCN S-806
Genus *Kaschgaria*		
K. brachanthemoides (C. Winkl.) Poljakov, *Artemisia brachanthemoides* C. Winkl., *Tanacetum brachanthemoides* (C. Winkl.) H. Kraschen.)	Kurtagai canyon, Almaty district, Kazakhstan	BCN 11691
Genus *Mausolea*		
M. eriocarpa (Bunge) Poljakov *(Artemisia eriocarpa* Bunge)	Gazli, Bukhara, Uzbekista.	BCN 11629
Genus *Neopallasia*		
N. pectinata (Pall.) Poljakov (*Artemisia pectinata* Pall.)	Southern slope of eastern Tien-Shan, Republic of Xingjan-Uigur, People's Republic of China	LE
Subtribe Handeliinae		
Genus *Lepidolopsis*		
L. turkestanica (Regel & Schmalh.) Poljakov (*Crossostephium turkestanicum* Regel & Schmahl., *Artemisia turkestanica* (Regel & Schmalh.) Franch., *Tanacetum turkestanicum* (Regel & Schmalh) Poljakov)	Sostube, Chimkent district, Kazakhstan	BCN S-807
Subtribe Leucantheminae		
Genus *Nipponanthemum*		
N. nipponicum (Franchet ex Maxim.) S. Kitamura (*Chrysanthemum nipponicum* (Franchet ex Maxim.) Sprenger, *Ch. nipponicum* Matsum., *Leucanthemum nipponicum* Franchet ex Maxim.)	Higashi-Hiroshima, Japan	BCN S-811
Subtribe Tanacetinae		
Genus *Tanacetopsis*		
T. goloskokovii (Poljakov) Karmysch.	Sogeti Mountains, Almaty district, Kazakhstan	BCN S-808
Genus *Hippolytia*		
H. megacephala (Rupr.) Poljakov *(Artemisia megacephala* Rupr.)	Asku-Zhabagli nature reserve, Zhambul district, Kazakhstan	BCN 11695

Note: Most of the vouchers are deposited in the herbarium of the Centre de Documentació de Biodiversitat Vegetal, Universitat de Barcelona (BCN). Some others are in the herbarium of the Rocky Mountain Research Station, Provo, Utah (SSLP), in the herbarium of the Botanical Institute V.L. Komarov of the Russian Academy of Sciences, Saint Petersburg (LE), or in the herbarium of A. Kreitschitz (Wrocław). I.S. indicates that the achenes have been obtained through an Index Seminum.

Table 2. Nuclear DNA content and other karyological characters of the populations studied.

Taxa	Life cycle[a]	2C ± SD (pg)[b]	2C (Mbp)[c]	2n[d]	Ploidy level	DNA per basic chromosome set	Standard[e]
Subtribe Artemisiinae							
Genus *Artemisia*							
Subgenus *Absinthium*							
A. absinthium	P	9.06±0.07	8860.7	18(1)	2x	4.53	*A. abrotanum*
*A. aschurbajewii**	P	10.36±0.29	10132.1	36(2)	4x	2.59	*Petunia*
*A. austriaca**	P	5.95±0.15	5819.1	16(3)	2x	2.98	*Pisum*
*A. frigida**	P	5.25±0.06	5134.5	18(4)	2x	2.63	*Pisum*
*A. glacialis**	P	8.52±0.15	8332.6	16(4)	2x	4.26	*Petunia*
*A. lagocephala**	P	6.75±0.06	6601.5	18(4)	2x	3.38	*Petunia*
*A. persica**	P	6.55±0.02	6405.9	18(2)	2x	3.28	*Pisum*
A. sieversiana	A	6.17±0.07	6034.3	18(2)	2x	3.09	*Petunia*
Subgenus *Artemisia*							
*A. abrotanum** (Tarnogaj)	P	11.41±0.11	11159.0	36(1)	4x	2.85	*Pisum*
*A. abrotanum** (Kozánow)	P	5.78±0.07	5652.8	18(1)	2x	2.89	*Pisum*
*A. afra**	P	6.31±0.34	6171.2	18(4)	2x	3.16	*Pisum*
*A. princeps**	P	14.60±0.24	14278.8	52(4)	6x	2.43	*Pisum*
*A. santolinifolia**	P	4.62±0.07	4518.4	18(4)	2x	2.31	*Pisum*
*A. stelleriana**	P	6.10±0.07	5965.8	18(4)	2x	3.05	*Petunia*
A. tournefortiana	A/B	7.06±0.07	6904.7	18(5)	2x	3.53	*Pisum*
A. vulgaris (Tibet)	P	12.15±0.52	11882.7	36(4)	4x	3.04	*Pisum*
A. vulgaris (Mala)	P	6.23±0.04	6092.9	16(3)	2x	3.12	*Pisum*
A. vulgaris (Staniszów)	P	6.49±0.32	6347.2	16(3)	2x	3.25	*Pisum*
A. × *wurzellii**	P	8.60±0.22	8410.8	34(4)	4x	2.15	*Petunia*
Subgenus *Dracunculus*							
*A. arenaria**	P	10.29±0.15	10063.6	36(4)	4x	2.57	*Petunia*
A. campestris (Utah)	P	6.38±0.05	6239.6	18(4)	2x	3.19	*Petunia*
A. campestris (Konotop)	P	9.78±0.13	9564.8	36(3)	4x	2.45	*Pisum*
A. campestris (Zagan)	P	9.92±0.18	9701.8	36(3)	4x	2.48	*Pisum*
A. campestris ssp. *sericea**	P	10.61±0.45	10376.6	36(1)	4x	2.65	*Pisum*
*A. filifolia**	P	7.14±0.18	6982.9	18(4)	2x	3.57	*Petunia*
*A. scoparia**	A	3.54±0.05	3462.1	16(5)	2x	1.77	*Petunia*
Subgenus *Seriphidium*							
*A. leucodes**	A	15.39±0.43	15051.4	18(5)	2x	7.70	*Pisum*
Subgenus *Tridentatae*							
*A. arbuscula**	P	9.22±0.11	9017.2	18(5)	2x	4.61	*Petunia*
*A. bigelovii**	P	15.49±0.10	15149.2	36(6)	4x	3.87	*Pisum*
A. cana ssp. *viscidula**	P	8.54±0.09	8352.1	18(6)	2x	4.27	*Petunia*
*A. nova**	P	6.37±0.14	6229.9	18(6)	2x	3.19	*Petunia*
*A. pygmaea**	P	11.54±0.18	11286.1	18(6)	2x	5.77	*Pisum*
A. tridentata ssp. *tridentata**	P	8.17±0.08	7990.3	18(6)	2x	4.09	*Petunia*
A. tridentata ssp. *vaseyana**	P	8.66±0.07	8469.5	18(6)	2x	4.33	*Petunia*
Genus *Brachanthemum**							
*B. titovii**	P	6.98±0.08	6826.4	18(2)	2x	3.49	*Pisum*
Genus *Dendranthema**							
D. arcticum ssp. *maekawanum**	P	20.03±1.30	19589.3	72(4)	8x	2.50	*Petunia*
D. indica var. *coreanum**	P	12.14±0.11	11872.9	36(4)	4x	3.04	*Pisum*
*D. maximowiczii**	P	15.78±0.17	15432.8	54(4)	6x	2.63	*Pisum*
*D. zawadskii**	P	21.22±0.52	20753.2	72(4)	8x	2.65	*Pisum*
Genus *Filifolium**							
*F. sibiricum**	P	9.44±0.31	9232.3	18(4)	2x	4.72	*Petunia*
Genus *Kaschgaria**							
*K. brachanthemoides**	P	14.09±0.31	13780.0	18(2)	2x	7.05	*Pisum*
Genus *Mausolea**							
*M. eriocarpa**	P	13.79±0.13	13486.6	36(5)	2x	3.45	*Pisum*
Genus *Neopallasia**							
*N. pectinata**	A	10.56±0.21	10327.7	36(4)	2x	2.64	*Pisum*

Table 2 (*concluded*).

Taxa	Life cycle[a]	2C ± SD (pg)[b]	2C (Mbp)[c]	2*n*[d]	Ploidy level	DNA per basic chromosome set	Standard[e]
Subtribe Handeliinae*							
Genus *Lepidolopsis**							
*L. turkestanica**	P	11.14±0.34	10894.9	$18^{(2)}$	2*x*	5.57	*Petunia*
Subtribe Leucantheminae							
Genus *Nipponanthemum**							
*N. nipponicum**	P	11.87±0.17	11608.9	$18^{(4)}$	2*x*	5.94	*Pisum*
Subtribe Tanacetinae							
Genus *Tanacetopsis**							
*T. goloskokovii**	P	9.73±0.27	9515.9	$18^{(4)}$	2*x*	4.87	*Petunia*
Genus *Hippolytia**							
*H. megacephala**	P	12.47±0.19	12195.7	$18^{(2)}$	2*x*	6.24	*Pisum*

Note: The taxa for which genome size has been estimated for the first time are marked with an asterisk (*).
[a]Life cycle: A, annual; B, biennial; P, perennial).
[b]2C nuclear DNA content (mean value ± standard deviation of 10 samples).
[c]1 pg = 978 Mbp (Doležel et al. 2003).
[d]Somatic chromosome number. (1) Kreitschitz and Vallès (2003); (2) Vallès et al. (2001*b*); (3) Kreitschitz (2003); (4) unpublished counts performed by the present authors; (5) Vallès et al. (2001*a*); (6) McArthur and Sanderson (1999). All counts have been carried out in the populations studied in the present paper.
[e]Internal standard used in each case (see text for details about *Pisum* and *Petunia*; for *A. absinthium*, the standard used was another *Artemisia*, *A. abrotanum*, previously measured (2C = 11.41 pg, Torrell and Vallès 2001) against *Pisum*).

genome sizes provide a natural explanation of phylogenetic relationships and systematics of many taxonomic groups (Ohri 1998). Our nuclear DNA results agree with the molecular phylogeny of *Artemisia* and other genera of Artemisiinae (Torrell and Vallès 2001; Vallès et al. 2003), as in other Asteraceae groups (Godelle et al. 1993; Zoldos et al. 1998; Cerbah et al. 1999).

Highly significant statistical differences ($p < 0.005$) have been detected in DNA amount per basic chromosome set in the five subgenera of *Artemisia*, particularly between *Seriphidium* and *Dracunculus*, and between *Tridentatae* on the one hand and *Artemisia* and *Dracunculus* on the other (Table 3). Moreover, subgenus *Tridentatae* is endemic to North America and also forms a well supported clade in the molecular phylogeny based on ITS analysis (Vallès et al. 2003). These data support standing of the subgenus *Tridentatae* as an independent group rather than its inclusion in *Serphidium*.

An important taxonomic character in subtribe Artemisiinae is pollen grain exine ornamentation. Genera belonging to subtribe Artemisiinae (Bremer and Humphries 1993) can be separated, on the basis of exine ornamentation, in two groups: one with *Artemisia* pollen type (with small spines) and another with *Anthemis* pollen type (with longer spines) (Martín et al. 2001, 2003). The genera *Brachanthemum* and *Dendranthema* and other phylogenetically close genera from other subtribes (*Hippolytia*, *Lepidolopsis*) present the *Anthemis* pollen type, while members of *Artemisia* and other Artemisiinae genera such as *Filifolium*, *Kaschgaria*, *Mausolea* and *Neopallasia* present the *Artemisia* pollen type. Pollen morphology is an indicator that the traditional classification of subtribe Artemisiinae is unnatural (Martín et al. 2001, 2003). Genome size data also support separation of the groups by pollen type: species with *Artemisia* pollen type have significantly ($p < 0.01$) less nuclear DNA than species with *Anthemis* pollen type. Genome size variation supports the established correlation between pollen grain ornamentation and the ITS phylogeny (Vallès et al. 2003).

Of the traditional subgeneric classification, the subgenus *Artemisia* is less supported by molecular phylogeny than are the subgenera *Dracunculus*, *Seriphidium* and *Tridentatae*. Its species are dispersed in the phylogenetic tree (Vallès et al. 2003). Furthermore, subgenus *Artemisia* is the most heterogeneous in terms of morphological, chemical, ecological, and karyological data (Ehrendorfer 1964; Torrell et al. 1999). Additionally, in the phylogenetic analysis of Vallès et al. (2003) 5 out of the 10 taxa that were not included in any clade belong to subgenus *Artemisia*, and members from this subgenus appear distributed in four of the eight clades, confirming again that the present infrageneric classification does not represent natural groups (Persson 1974; Vallès and McArthur 2001). Nuclear DNA amount analysis is thus quite useful in support of molecular phylogeny and pollen data. Further support of the heterogeneous nature of the subgenus *Artemisia* is that it has the highest ratio between maximum and minimum nuclear DNA amount per basic chromosome set (Table 4). Conversely, subgenus *Dracunculus*, the most homogeneous according to the molecular phylogeny (Vallès et al. 2003), is the one that presents the lowest genome size variability (the lowest ratio of all subgenera). Nuclear DNA amount per basic chromosome set of *Artemisia leucodes* (7.70 pg) is markedly different from the mean value of the subgenus to which this species belongs, *Seriphidium* (3.89 pg). Similarly, Torrell and Vallès (2001) found a nuclear DNA amount per basic chromosome set for *Artemisia judaica* of 5.76 pg, far different from the mean value of its subgenus, *Artemisia* (2.96 pg). In both cases, these taxa are placed out of their respective traditional subgenera by ITS phylogeny (Vallès et al. 2003). This confirms the value of nuclear DNA content as a systematic marker and agrees with the striking interspecific variation in genome size that occurs

Table 3. Comparison of means of DNA amount per basic chromosome set in the subgenera of *Artemisia*.

Subgenus	Mean (pg)	Homogeneous groups
Dracunculus	2.668	a
Artemisia	3.050	ab
Absinthium	3.563	bc
Seriphidium	3.892	bc
Tridentatae	4.088	c

Table 4. Maximum, minimum, and ratio (maximum/ minimum) of nuclear DNA amount per basic chromosome set (pg) in the subgenera of *Artemisia*.

Subgenus	Maximum	Minimum	Ratio max/min
Absinthium	4.53	2.59	1.75
Artemisia	5.76	1.44	3.27
Dracunculus	2.93	1.77	1.66
Seriphidium	7.69	2.67	2.88
Tridentatae	5.79	3.21	1.80

in many, though not all, major taxonomic groups (Hanson et al. 2001*a*, 2001*b*).

Amount of nuclear DNA per basic chromosome set statistically differs ($p < 0.005$) between *Artemisia* and its related genera from other subtribes (Anthemideae not Artemisiinae). Nuclear DNA amount per basic chromosome set of Artemisiinae (*Artemisia* excluded) also differs ($p < 0.05$) from those genera belonging to subtribes Tanacetinae, Leucantheminae and Handeliinae. On the other hand, there is no statistically significant difference in nuclear DNA amount per basic chromosome set between genus *Artemisia* and the other Artemisiinae analysed. In fact, many of the non-*Artemisia* Artemisiinae studied here had been previously included in *Artemisia*, and subsequently separated in different genera, often new and with only one or two species. DNA sequence analysis of these plants (Vallès et al. 2003) demonstrate most of these genera tightly embedded in the *Artemisia* clade; this could be interpreted to support the elimination of these new genera, and their species returned again to *Artemisia*. The absence of statistically significant difference in nuclear DNA amount per basic chromosome set between these groups also supports this hypothesis. In summary, all these results indicate that nuclear DNA amount is an important tool in the analysis of phylogenetic relationships.

Within the studied *Artemisia* taxa, in the present paper and an earlier one (Torrell and Vallès 2001), different populations have been analysed for some species. The differences detected in nuclear DNA amount give a low degree of variability in most of these species. This can be illustrated by the comparison between the very similar 2C values obtained in the present study and in Torrell and Vallès (2001) for *A. absinthium* (9.06 in the present study / 8.52 in Torrell and Vallès 2001), *A. vulgaris* (6.23, 6.49 / 6.08), *A. campestris* (diploid: 6.38 / 5.87; tetraploid: 9.78, 9.92 / 11.00), and different subspecies of *A. tridentata* (8.17, 8.86 / 8.18). *Artemisia abrotanum* also constitutes a case of nuclear DNA amount constancy: although the analysed populations have different ploidy levels (diploid and tetraploid), nuclear DNA amount per basic chromosome set of both species only differs in 1.40%. This fact is also interesting because polyploids ordinarily have significantly less nuclear DNA per basic chromosome set than corresponding diploids. However, in this case both specimens show quite a similar nuclear DNA amount per basic chromosome set and both *A. abrotanum* specimens came from the same geographic area (Wrocław, Poland), a circumstance that could partially explain this homogeneity. Another possibility could be an autopolyploid origin of the tetraploid population, which has almost exactly double DNA amount of the diploid. Further cytogenetic studies on *A. abrotanum* are necessary to confirm this hypothesis. Additionally, tetraploid *A. campestris* could have the same origin (2*x*, 5.87 pg; 4*x*, 11.0 pg; Torrell and Vallès 2001). Our results support that although nuclear DNA amount or C value is considered constant within a species, it is almost sure that a certain degree of genuine intraspecific variation exists; the processes or mechanisms that are usually able to cause it are duplications, deletions, chromosomal polymorphisms, the existence of B chromosomes, or the presence of transposable elements or repetitive sequences (Greilhuber 1998; Małuszyńska 1999).

Ecology and life cycle

No statistically significant relationship exists between life cycle and C value among the species studied. However, the taxon with the lowest nuclear DNA amount, *A. scoparia*, is annual, and those with the highest C values are perennial, as was the case in a previous report in other *Artemisia* species (Torrell and Vallès 2001) or in other genera, including some Anthemideae (Bennett 1972; Nagl and Ehrendrofer 1974; Rees and Narayan 1981). It is generally assumed that a low C-value correlates with a high rate of development; in other words: if less nuclear DNA is duplicated, the cell cycle is faster, and the developmental rhythm, consequently, is more intense. This is specially useful for annual or ephemeral plants, which have only limited time to carry out their life cycle. The studied *A. scoparia* population inhabited an intermittently dry river bed, and its low C value (3.54 pg, the lowest of the present study) promotes a fast life cycle that is rapidly completed before the seasonal summer or fall floods. This case supports the premise that annual species have a smaller amount of nuclear DNA than perennials. In contrast, however, *Artemisia leucodes*, another annual species, has one of the biggest genomes (2C = 15.39 pg) of all the diploid species studied; its karyotype is made up of large chromosomes (Vallès et al. 2001*a*) and its high C value, despite its annual life cycle, is supported by the Nagl and Ehrendrofer (1974) explanation that large chromosomes could have a higher metabolic rate that facilitates an increase in RNA synthesis. This would increase the synthesis of the necessary proteins to permit a faster life cycle. Although many authors have found a positive correlation between genome size and life cycle duration, numerous exceptions suggest that it is not so clear as initially thought. Some authors have reported even a negative relationship between those parameters (e.g., *Pennisetum*, Martel et al. 1997), whereas others have found no relationship (Grime and Mowforth 1982).

The statistical analysis carried out on the species of this study did not reveal any significant difference between the

studied populations of higher or lower altitudes. The tetraploid *A. vulgaris* studied in this paper has a 24.7% difference with the one studied by Torrell and Vallès (2001) even though both populations are tetraploid. These populations grow in geographically and ecologically distinct conditions. The population with the higher nuclear DNA amount is a Tibetan population growing at 3650 m. An adaptation to altitude could at least partly explain the difference. Many studies on this subject have reported that species inhabiting arctic or high mountain areas tend to present larger genomes, and are most frequently polyploids (Gregory and Hebert 1999, and references therein). Some authors have concluded that natural selection favours the modulation of nuclear DNA content under certain weather conditions, mainly linked with altitude or latitude (Bennett 1976). The high taxonomic complexity of the *A. vulgaris* group may also contribute to an explanation of this difference. Similarly, *A. glacialis*, found in Italy at an altitude of 2300 m has a larger C value than the mean of the analysed diploid species of the genus, conforming to what other authors have stated about the positive correlation between DNA amount and altitude (Caceres et al. 1998). Nevertheless, similar studies have found a negative correlation or even no relationship between altitude and C value, (Creber et al. 1994; Reeves et al. 1998; Vilhar et al. 2002), similar to the relationship between life cycle and genome size, suggesting again that the link between the two is not clear.

It seems likely then that genome size variation in *Artemisia* species does not depend on altitude. Nonetheless, it appears to be a response to other kinds of selective pressures, such as adaptation to arid environments. Sanderson et al. (1989) and McArthur and Sanderson (1999) found a better adaptation to arid habitats in polyploid rather than in diploid *Artemisia* and *Atriplex* in North American semi-desert habitats, and Vallès et al. (2001*a*, 2001*b*) detected that tetraploid species of *Artemisia* were more widely distributed in arid lands than the related diploids. Both studies support the hypothesis that nuclear DNA amount increases — especially by means of polyploidy — in plants adapted to extreme environments. Our results agree with this idea, and show that there can be a DNA amount increase even in diploids. *Artemisia leucodes* and *A. pygmaea*, the two diploid taxa with the highest DNA amount per basic chromosome set of the *Artemisia* species analysed, inhabit desert or semi-desert regions of central Asia and North America, and are well adapted to the extreme conditions of high temperature and drought that characterize these environments. Moreover, diploid *A. filifolia*, another colonizing plant of sandy North American deserts, shows the highest nuclear DNA amount per basic chromosome set of its subgenus, *Dracunculus*.

Artemisia absinthium is a nitrophilous species usually grown in ruderal zones. As the presence of high concentrations of nitrogen in the soil can also be considered as a difficult, if not extreme, environmental condition, it is interesting to observe that our *A. absinthium* population shows a higher nuclear DNA amount per basic chromosome set than the mean for the genus. Torrell and Vallès (2001) reported the same relationship in another ruderal *Artemisia* species, *A. thuscula*, taxonomically related to *A. absinthium*. In both cases, the high nuclear DNA amount could be interpreted as a response to the presence of nitrogen; this would support Evans (1968), who detected a 10% increase in nuclear DNA amount in varieties of *Linum usitatissimum* growing in strongly nitrogenated soils and at high temperatures.

Concluding statement

C values in the subtribe Artemisiinae, including the large genus *Artemisia* and related taxa, are a useful adjunct in parallel or in correlation to other kinds of data, e.g., chromosome number, life form, pollen grain exine patterns, systematic placement, and ecology, in determining evolutionary relationships within this group of plants.

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