

## Chromosome numbers in some *Artemisia* (Asteraceae, Anthemideae) species and genome size variation in its subgenus *Dracunculus*: Karyological, systematic and phylogenetic implications

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**ABSTRACT.** Chromosome counts in 12 *Artemisia* species from Russia are presented in this paper. Chromosome numbers of *A. czekanowskiana*, *A. globosa*, *A. ledebouriana*, *A. lithophila*, *A. macilenta*, *A. pycnorhiza* and *A. sosnovskyi* are reported for the first time. The chromosome counts carried out in *A. czekanowskiana* ( $2n=10x=90$ ) and *A. macrantha* ( $2n=12x=108$ ) indicate cases of aneusomy. The presence of a dicentric chromosome and acentric fragments or a B-chromosome is reported for one species. Besides these, genome size in 21 populations of 18 species of *Artemisia* belonging to the subgenus *Dracunculus*, mainly from Russia and Mongolia, has been assessed by flow cytometry. The nuclear DNA content ranges from  $2C=4.21$  to  $2C=24.58$  pg, and the nuclear DNA content per basic chromosome set (1Cx) from 2.06 to 3.00 pg. The constancy of genome size has been evaluated concluding that there exists a nuclear DNA loss (at the 1Cx-value level) within ascending ploidy levels. Possible correlations between genome size, morphological traits and the phylogenetic position of species have been tested.

**KEYWORDS:** Acentric fragments, Aneusomy, B-chromosomes, Chromosome numbers, C-value, Dicentric chromosomes, Karyology, Nuclear DNA content, Polyploidy

The genus *Artemisia* L. is one of the largest of the Asteraceae, with more than 500 species according to different authors (Mabberley 1990; Ling 1991a,b, 1995a,b; Bremer and Humphries 1993; Vallès and Garnatje 2005). After various taxonomic rearrangements, the genus was divided into five large groups which have been considered at sectional or subgeneric level; *Absinthium* DC., *Artemisia* (= *Abrotanum* Besser), *Dracunculus* Besser, *Seriphidium* Besser and *Tridentatae* (Rydb.) McArthur (Torrell *et al.* 1999, and references therein). Even so, this classification is not accepted by all authors. A general agreement exists concerning the idea that this infrageneric division does not represent natural groups (Persson 1974; McArthur *et al.* 1981; Vallès and McArthur 2001; Vallès and Garnatje 2005). This confusion is particularly problematic in the case of subgenus *Dracunculus*, because the demarcation of the group is variable depending on the authors consulted (Shishkin and Bobrov 1995; Ling *et al.* 2006). The subgenus is spread across Eastern Europe and Asia, where the genus is native from (Wang 2004), and reaches North Africa and North America. Cassini (1817) treated this subgenus as a new genus, *Oligosporus* Cass., which was later returned to *Artemisia* (Besser 1829, 1832, 1834, 1835; Candolle 1837). The inclusion of this group within *Artemisia* has been confirmed by molecular phylogenetic data (Torrell *et al.*

1999; Watson *et al.* 2002; Vallès *et al.* 2003). The genus has two basic chromosome numbers;  $x=9$ , and the less extended  $x=8$ , with polyploid series up to  $16x$  for  $x=9$  and hexaploid for  $x=8$  (Ehrendorfer 1964, 1980; Estes 1969; Persson 1974; McArthur and Pope 1979; Oliva and Vallès 1994; McArthur and Sanderson 1999; Vallès and Garnatje 2005; Pellicer *et al.* in press and references therein).

Genome size has been investigated in a large number of *Artemisia* species (Garcia *et al.* 2004, and references therein) obtaining a great number of  $2C$  values. The C-value term was coined by Swift (1950) to refer to the amount of DNA of an unreplicated nuclear genome, which is considered constant within a species. It is also correlated with many biological characters, such as cell and nuclear volume, chromosome size, and developmental parameters like minimum generation time or duration of male meiosis, among others (Price *et al.* 1981; Bennett 1987). Many other relationships have been described, e.g. with reproductive biology, ecology and plant distribution (Bennett 1998, Knight and Ackerley 2002; Knight *et al.* 2005 and references therein). All these correlations make C-value data an interesting tool to predict different phenotypic and ecologic traits at multiple levels (Underbrink and Pond 1976; Chung *et al.* 1998; Suda *et al.* 2003). Thus, systematics, taxonomy and molecular biology,

physiology and development of plants can all be better understood when C-value data are considered. Available data regarding genome size are still scarce in angiosperms; wherefore, there is a need for additional DNA C-values estimation in different plants (Bennett and Leitch 1995; Hanson *et al.* 2001a,b). This fact has promoted the compilation of different data on DNA amounts obtained since 1976, creating the Plant DNA C-values Database (<http://www.rbgekew.org.uk/cval/> homepage.html; Bennett and Leitch 2004).

The principal aims of the present study are: i) to enlarge the data on chromosome numbers for the genus, ii) to increase the knowledge of C-values for the subgenus *Dracuncululus*, with special attention to the variation in polyploid taxa, and iii) to test the existence of possible relationships between genome size and biological parameters.

## MATERIALS AND METHODS

**Plant materials** Table 1 shows the species studied, grouped at subgeneric level, with their origin and herbarium information. All the specimens analysed come from achenes collected in the field. Plants have been grown in the Laboratori de Botànica of the Facultat de Farmàcia, Universitat de Barcelona and in the Institut Botànic de Barcelona. As internal standards, *Petunia hybrida* Vilm. 'PxPc6' (2C=2.85 pg) and *Pisum sativum* L. 'Express Long' (2C=8.37 pg) (Marie and Brown 1993) were used. Seeds of standards were provided by the Institut des Sciences du Végétal, Gif-sur-Yvette (France). Vouchers of most species are deposited in the herbarium of the Centre de Documentació de Biodiversitat Vegetal, Universitat de Barcelona (BCN) and the remaining ones are in the herbarium of the Botanical Institute 'V.L. Komarov', Sankt Peterburg (LE-Korobkov).

**Chromosome counts** The chromosome counts were carried out following the methodology described in Pellicer *et al.* (in press). The best metaphase plates were photographed with a digital camera (AxioCam MRc5 Zeiss) mounted on a Zeiss Axioplan microscope, and images were analysed with Axio Vision Ac software version 4.2.

To assess the existence of previously-published chromosome counts in the studied species we used the most common indexes of plant chromosome numbers (cited in Torrell *et al.* 2001), previous publications (Vallès *et al.* 2005; Garcia *et al.* 2006a and references therein) as well as the chromosome number databases, Index to Plant Chromosome Numbers (Missouri Botanical Garden, <http://mobot.org/W3T/Search/ipcn.html>) and Index to Chromosome Numbers in the Asteraceae (Watanabe 2002, <http://www-asteraceae.cla.kobe-u.ac.jp/index.html>).

**Nuclear DNA amount measurement** Nuclear DNA con-

tent estimations were developed by flow cytometry following the procedure described in Garcia *et al.* (2004). Prior to making measurements, standards were tested alone to check their suitability and the calibration of the flow cytometer. Assessments were developed at 'Serveis Científicotècnics' of the Universitat de Barcelona using an Epics XL flow cytometer (Coulter Corporation, Hialeah, USA).

**Statistics** Statistical analyses were carried out to evaluate the relationships between the studied variables. All the analyses were performed with the Statgraphics Plus 5.0 program (Statistical Graphics Corp., Rockville, Md.).

## RESULTS AND DISCUSSION

**Chromosome numbers** The chromosome counts carried out in *A. czekanowskiana* Trautv. (Fig. 1), *A. globosa* Krasch. (Fig. 7), *A. ledebouriana* Besser (Fig. 8), *A. lithophila* Turcz. ex DC. (Figs. 4a, b), *A. macilenta* Maxim. (Krasch.) (Fig. 9), *A. pycnorhiza* Ledeb. (Fig. 11) and *A. sosnovskyi* Krasch. (Fig. 12) are all new; for the remainder, only one or few previous reports have been published. We also present the second count for *A. monostachya* Bunge ex Maxim. (Fig. 10), but the first for a Russian population; a previous count for this species was carried out by Garcia *et al.* (2006a) in Mongolian material, reporting, as does the present one, a tetraploid population.

**Relevance of polyploidy** Only  $x=9$ -based species have been found, confirming  $x=8$  as less common basic chromosome number in the genus. Different ploidy levels have been found, ranging from diploid ( $2x$ , e.g. *A. jacutica* Drob. and *A. lithophila*, Figs. 2, 4a, b) to dodecaploid ( $12x$ , *A. macrantha* Ledeb., Fig. 5) species.

In the genus *Artemisia*, many of the species that colonize extremely arid landscapes are polyploid, supporting the hypothesis of a connection between ecological tolerance and polyploidization in many plant groups (Otto and Whitton 2000). This fact shows the important role that this factor plays in the speciation of the genus, and is also consistent with the results obtained in previous works (Vallès *et al.* 2001; Garcia *et al.* 2006b; Pellicer *et al.* 2007) where the proportion of polyploid species found lead us to see this phenomenon as an active ongoing evolutionary force.

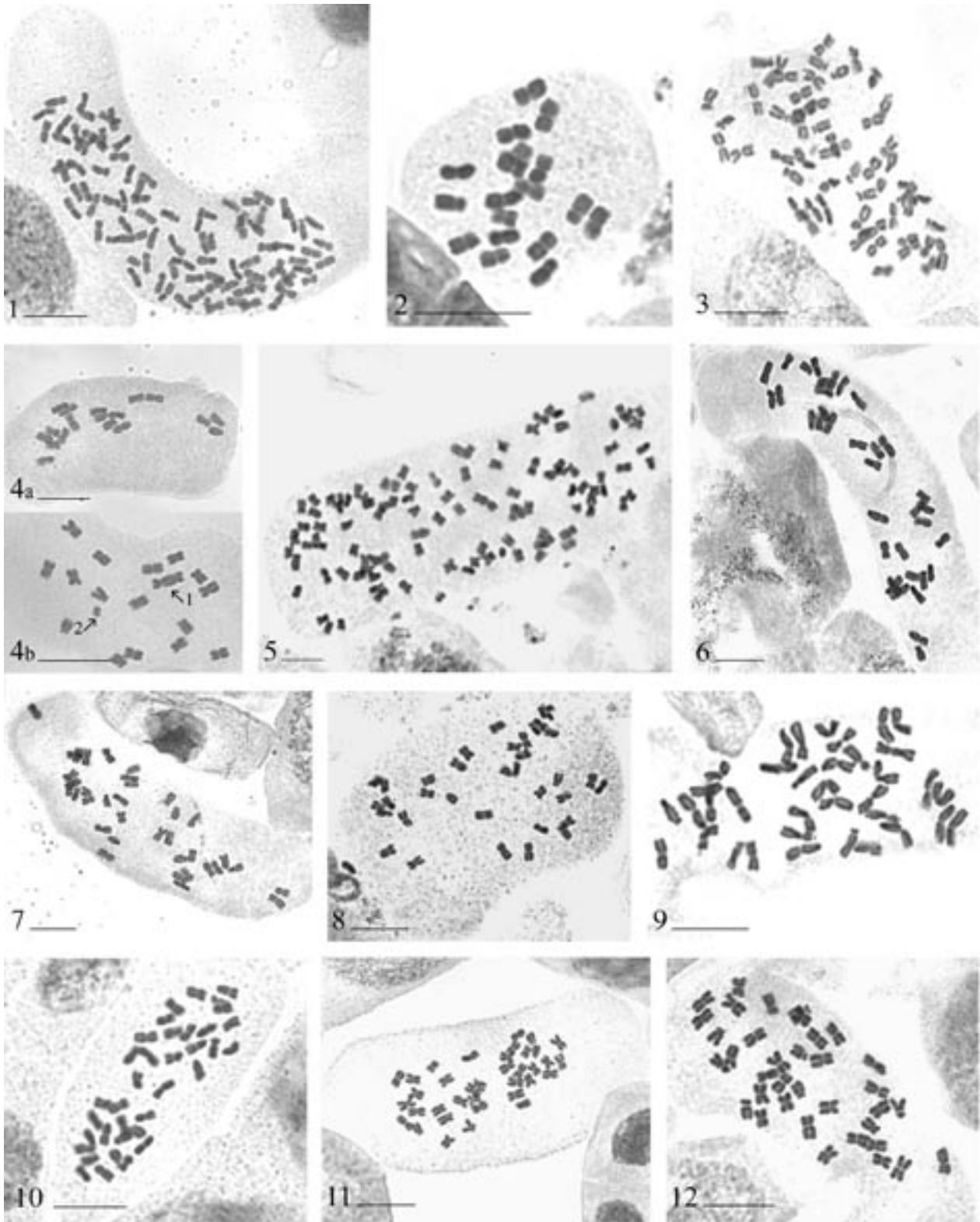
The chromosome count carried out in *A. lagocephala* (Fischer ex Besser) DC. (Fig. 3) reports a high ploidy level for this species ( $2n=6x=54$ ). In two previous works (Kawatani and Ohno 1964, Vallès *et al.* 2005) diploid populations from Russia were counted, whereas Korobkov (1981) already counted  $2n=54$  in several northern Russian populations. Belyaeva and Siplivinskii (1977) also reported a diploid Russian population, but Korobkov and Kotseruba (2003) emended this count as a typo-

Table 1. Chromosome number and localities of the species studied

Taxa	Chromosome number (ploidy level)	Localities
Subgenus <i>Absinthium</i> DC.		
<i>A. czeakanowskiana</i> Trautv. ( <i>A. sericea</i> Weber ex Stechm.) *	89, 90 (10x)	Russia, Krasnoyarsk krai. Southeastern Taimyr, rocks in Medvezhya river. 14-VIII-2005. Leg. I. N. Pospelov, E. B. Pospelova, det. A. A. Korobkov (LE 06-39).
<i>A. jacutica</i> Drob. *	18 (2x)	Russia, Sakha Republic (Yakutya). Ust-Aldans camp, near the village of Oner, ruderal. 10-IX-2005. Leg. V. N. Zakharova, det. A. A. Korobkov (LE 06-31).
<i>A. lagocephala</i> (Fischer ex Besser) DC.	54 (6x)	Russia, Sakha Republic (Yakutya), Aldan raion. Ugoyan, near the mouth of Tommozh river, forest. 26-VIII-2005. Leg. V. N. Zakharova, det. A. A. Korobkov (LE 06-29).
<i>A. lithophila</i> Turcz. ex DC. *	18 (2x)	Russia, Buryat Republic, Okinsk raion. Northern Sayan, upper left side of Zun-Kholba river, rocky blocs. 3-IX-2005. Leg. N. K. Badmaeva, det. A. A. Korobkov (LE 06-28).
Subgenus <i>Artemisia</i>		
<i>A. macrantha</i> Ledeb. *	106, 108 (12x)	Russia, Sakha Republic (Yakutya). Ust-Aldans camp, near the village of Oner, forest. 10-IX-2005. Leg. V.N. Zakharova, det. A. A. Korobkov (LE 06-32).
<i>A. tanacetifolia</i> L.	36 (4x)	Russia, Chita oblast, Kyra raion. Reserve of Sokhodin, path to Enda, mountain pass of Agutsa river, forest. 28-VIII-2005. Leg. et det. A. A. Korobkov (LE 06-18).
Subgenus <i>Dracunculus</i> Besser		
<i>A. arenaria</i> DC.	36 (4x)	Russia, Volgograd oblast. Silicic sands. Hill slopes, <i>Artemisia</i> , Poaceae and grass steppe among <i>Betula</i> . 11-X-2000. Leg. et det. A. A. Korobkov (LE-Korobkov 00-41).
<i>A. bargusinensis</i> Spreng.	36 (4x)	Russia, Tyva Republic, Pi-Khem raion. 60 km N-NE of Turan, slope grasslands with steppe. 11-VIII-2002. Leg. V. Nikitin, V. Byalt and A. Sytin, det. A. A. Korobkov (LE-Korobkov).
<i>A. changaica</i> Krasch.	36 (4x)	Mongolia, Arkhangai aimag. Taryat sum, Khorgo-Terkh National Park, <i>Larix sibirica</i> forest above lake Terkhen Sagan nur. 27-VIII-2004. Leg. Sh. Dariimaa, Sh. Tsooj and J. Vallès (BCN 34487).
<i>A. depauperata</i> Krasch.	36 (4x)	Russia, Tyva Republic, Erzin raion. Right riverside of Tes-Khem river, beneath calcareous mountains, deposit of pebbles. 18-IX-2003. Leg. et det. A. A. Korobkov (LE-Korobkov).
<i>A. desertorum</i> Spreng.	36 (4x)	Russia, Primorie krai, Nadezhda raion. Near the town of Terekhovk, abrupt rocky slope on the right side of the coast, meadows in a <i>Quercus</i> forest. 10-X-2004. Leg. et det. A. A. Korobkov (LE-Korobkov).
<i>A. dracunculus</i> L.	18 (2x)	Russia, Chita oblast, Kyra raion. Near the village of Kyra, northern slope South of the village, rich steppe with herbs and bushes. 1-IX-2005. Leg. et det. A. A. Korobkov (LE 06-04).
<i>A. dracunculus</i> L.	36 (4x)	Russia, Volgograd oblast. Left shore of Khoper river, between gypseous slopes, meadows. 15-X-2000. Leg. et det. A. A. Korobkov (LE 00-40).
<i>A. dracunculus</i> L.	54 (6x)	Kazakhstan, Chimkent oblast. Chokpak ornithological station, railroad edges near Chokpak railway station, 500 m, A. A. Ivaschenko, A. Susanna S-2211 and J. Vallès, 1-IX-2000 (BCF 50688).
<i>A. dracunculus</i> L.	90 (10x)	Poland, Lower Silesia, Wrocław (Fabryczna), in the embankment. 8-VIII-2001. Leg. A. Kreitschitz, det. A. Wąsowicz. (Herbarium A. Kreitschitz).
<i>A. dracunculoides</i> Pursh	54 (6x)	USA, Arizona. Globe, Pinal mountains, margins of a path. 16-XII-1995. Leg. J. Peñuelas (BCN 13323).
<i>A. giraldii</i> Pamp.	36 (4x)	Mongolia, Bulgan aimag. Sansar sum, north-east slope of Khugunkhaan mountain, steppe near <i>Betula</i> and <i>Pinus</i> forest, 2000 m, Sh. Dariimaa, Sh. Tsooj and J. Vallès, 25-VIII-2004 (BCN 23806).
<i>A. glauca</i> Pall. ex Willd.	36 (4x)	Russia, Tyva Republic. Near the city of Kyzyl, summits of hills, groupments of <i>Artemisia</i> and Poaceae. 12-IX-2003. Leg. et det. A. A. Korobkov. LE.
<i>A. globosa</i> Krasch. *	36 (4x)	Russia, Tyva Republic, Erzin raion. Northern shore of the lake Tere-Khol, sandy area of Tsuguer-Ellis. 13-IX-1003. Leg. et det. A. A. Korobkov (LE 04-116).
<i>A. ledebouriana</i> Besser *	36 (4x)	Russia, Buryat Republic, Pribaikal raion. Shore of the lake Baikal, at 159-160 km on road from the village of Turku, sand dunes. 16-IX-2005. Leg. et det. A.A. Korobkov (LE 06-06).
<i>A. macilenta</i> (Maxim.) Krasch. *	36 (4x)	Russia, Chita oblast, Kyra raion. Northern Onon-Baldzhin mountain system, southern slope, deposits of sand and stones, steppe. 8-IX-2005. Leg. Et det. A. A. Korobkov (LE 06-02).
<i>A. marschalliana</i> Spreng.	18 (2x)	Russia, Volgograd oblast. Silicic sands of small hills, steppes of <i>Artemisia</i> , Poaceae and grass among <i>Betula</i> . 11-X-2000. Leg. et det. A. A. Korobkov (LE 00-37).
<i>A. monostachya</i> Bunge ex Maxim. [ <i>A. pubescens</i> Ledeb. var. <i>monostachya</i> (Bunge ex Maxim.) Y. R. Ling] *	36 (4x)	Russia, Chita oblast, Kyra raion. Near Kyra, southern rocky slope in the left Kyra river shore, mountain steppe among <i>Prunus armeniaca</i> . 9-IX-2005. Leg. et det. A. A. Korobkov (LE 06-07).
<i>A. oxycephala</i> Kitag.	18 (2x)	Mongolia, Tuv (Central) aimag: Mungunmort sum, 10 km S of the sum. 7-IX-2004. Leg. Sh. Dariimaa, Sh. Tsooj, J. Vallès and E. Yatamsuren.
<i>A. pycnorhiza</i> Ledeb. *	36 (4x)	Russia, Tyva Republic, Erzin raion. Left shore of Tes-Khem river, 20 km NW of the city of Erzin, base of Izvestkyakov mountains, rocks. 18-IX-2003. Leg. et det. A. A. Korobkov (LE 04-115).
<i>A. sosnovskiyi</i> Krasch. *	36 (4x)	Russia, Daguestan Republic, Tsumand raion. Near the village of Asvali, rocky dry slopes of eastern exposition. 28-X-2005. Leg. R. N. Murtazaliev, det. A. A. Korobkov (LE 06-34).
<i>A. subdigitata</i> Mattf.	36 (4x)	Mongolia, Umnu (South) Gobi aimag. Bulgan sum, E Gurvan Saikhan mountains, canyon near Brigat, rocky slopes. 1-IX-2004. Sh. Dariimaa, Sh. Tsooj and J. Vallès (BCN 34846).

The species with chromosome number reported for the first time in the present work are marked with an asterisk (\*). The localities are given with the use of Russian ("krai", region, territory; "oblast", province; "raion", district) and Mongolian ("aimag", province, written "aimak" in Russian language works; "sum", village, written "somon" in Russian language works) administrative divisions





Figs. 1-12. Somatic metaphases. 1. *Artemisia czekanowskiana* ( $2n=90$ ). 2. *A. jacutica* ( $2n=18$ ). 3. *A. lagocephala* ( $2n=54$ ). 4a. *A. lithophila* ( $2n=18$ ). 4b. Arrows show a dicentric chromosome and a chromatin body (acentric fragment or B-chromosome). 5. *A. macrantha* ( $2n=108$ ). 6. *A. tanacetifolia* ( $2n=36$ ). 7. *A. globosa* ( $2n=36$ ). 8. *A. ledebouriana* ( $2n=36$ ). 9. *A. macilenta* ( $2n=36$ ). 10. *A. monostachya* ( $2n=36$ ). 11. *A. pycnorhiza* ( $2n=36$ ). 12. *A. sosnovskyi* ( $2n=36$ ). Scale bars = 10  $\mu\text{m}$ .

graphic error, based on a herbarium specimen of this population collected by Belyaeva and annotated by herself with  $2n=54$ . To sum up, *A. lagocephala* seems to have a clear dominance of hexaploids. High ploidy levels

have also been observed in species such as *A. czekanowskiana* ( $2n=10x=89, 90$ ) and *A. macrantha* ( $2n=12x=106, 108$ ). Previous reports in *A. sericea* Web. ex Stchem. (Kawatani and Ohno 1964; Krogulevich and

Rostovtseva 1984; Stepanov 1994; Pellicer *et al.* in press), of which *A. czezanowskiana* has been considered a synonym (Shishkin and Bobrov 1995), detected different chromosome numbers, such as  $2n=18, 36, 88,$  and  $90$ . The present count contributes to enlarge the list. It is not strange that high polyploids show a variable chromosome number for the same ploidy level. Duncan (1945) labelled this phenomenon under aneusomaty, referring to an intraindividual aneuploidy. Many cases of aneusomaty have been described in *Artemisia* before, e.g. *A. verlotiorum* Lamotte ( $2n=48-52$ ; Martinoli and Ogliotti 1970, Vallès 1987), *A. laciniata* Willd. ( $2n=56-60$ ; Krasnikova *et al.* 1983) or *A. dracunculus* L. ( $2n=87, 88, 89, 90$ ; Kreitschitz and Vallès 2003). Chromosome number variations at populational and individual level are frequent in high polyploids, especially in plants with an active vegetative reproduction (Duncan 1945; Lewis 1970; Persson 1974; Couderc *et al.* 1980). Somatic metaphase plates belonging to the same and different individuals of *A. marcrantha* have also shown a variable chromosome number,  $2n=106, 108$ . The case of *A. tanacetifolia* L. (Fig. 6) ( $2n=4x=36$ ) is another good example of polyploidization in the genus; a previous count exists (Wang *et al.* 1999) in a diploid Chinese population, and the tetraploid (one population) and the hexaploid (two populations) levels were reported from Russia by Korobkov and Kotseruba (2003).

*Presence of dicentric and accessory chromosomes* Other interesting peculiarities have been found in one metaphase plate of *A. lithophila*. The arrows in Fig. 4b show a dicentric chromosome (1) and a chromatin body that can account for a B-chromosome or for two acentric fragments together (2). Dicentric chromosomes (chromosomes with two centromeres) appear as a consequence of dysfunctional telomeres. A key function of telomeres is to prevent the natural ends of chromosomes from fusing to each other (McKnight 2004). These dysfunctional telomeres, however, are recognized as DNA double-strand breaks (DSBs), and when recognized as such they are subject to DSB repair activities (Bertuch 2002), which try to fuse these to other chromosome ends, forming end-to-end associations that give rise to dicentric chromosomes. An important consequence of this chromosomal aberration can occur at anaphase, when the two centromeres on the same chromatid are pulled in opposite directions; in this case, the chromatid will form a bridge between the daughter cells and will break again between the centromeres. Then, the just broken daughter chromosomes can fuse again to form more dicentric chromosomes, resulting in a breakage-fusion-bridge cycle (BFBC) that can be repeated indefinitely (Sumner 2003), a phenomenon first described by McClintock (1938). This BFBC can induce genomic instability which might be phenotypically reflected in, for example, the increase in the occurrence of variegation (Ramanna *et al.*

1985; Lukaszewski 1995).

Moreover, when a dicentric chromosome is formed, it is possible that acentric fragments, coming from the broken ends of the fused chromosomes, are also present in the cell (Sumner 2003). The chromatin body of Fig. 4 could account for one acentric fragment, with the chromatids lying parallel through their length. However, another acentric fragment should also be visible -the one corresponding to the broken chromosome end of the other fused chromosome- and in this case it is not. Hence, another explanation to this chromatin body would be that it is a B-chromosome. The presence of B-chromosomes has been previously reported in many species of the genus *Artemisia* (Vallès and Garnatje 2005 and references therein). B-chromosomes are extra chromosomes, not needed for the survival of the species, and smaller than the usual A-chromosomes. They can be present in some individuals, though not necessarily in every single cell, neither in the same number in every cell of the organism. They have been frequently found in many plants and animals. The origin and function of B's are not well known (Palestis *et al.* 2004), though their presence does not necessarily damage the viability of the species. In this case, however, given that the presence of dicentric chromosomes is not a current finding, we think that this chromatin body is best explained as an acentric fragment.

*Nuclear DNA assessments* According to the existing data in the plant C-value database and previous studies consulted (Geber and Hasibeder 1980; Greilhuber 1988; Torrell and Vallès 2001; Garcia *et al.* 2004; Pellicer *et al.* unpublished), this is the first study focused on species of the subgenus *Dracunculus*. Almost all (17 out of 18) taxa included have not yet been studied from this standpoint (Table 2). For statistical analyses, data from previous works carried out by our team on *Artemisia* have been used (Torrell and Vallès 2001; Garcia *et al.* 2004).

*Relationship with karyological characters* A statistically significant difference has been found between 2C values and ploidy levels (Table 3) (mean 2C,  $p=0.000$ , of diploids= $5.33$  pg; mean of tetraploids  $2C=10.07$  pg; for hexaploids  $2C=15.63$  pg and mean 2C for decaploids= $23.90$  pg). A similar behaviour has been reported in other genera (*Achillea*, Dąbrowska 1992; *Tripleurospermum*, Garcia *et al.* 2005) and in previous studies of *Artemisia* (Torrell and Vallès 2001; Garcia *et al.* 2004). These clear differences among different ploidy levels have promoted this method to establish ploidy levels in groups in which at least the nuclear DNA amount of diploids is known (Vilhar *et al.* 2002). Nowadays, it is known that species belonging to the same genus but with different ploidy levels can show a nearly identical nuclear DNA content (Suda *et al.* 2006), wherefore, before inferring ploidy levels from a cytometric analysis, it is essential to count the chromosome number.

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

Subgen. <i>Dracunculus</i>	Chromosome number (P.L.) <sup>a</sup>	2C-value (pg) <sup>b</sup>	2C-value (Mbp) <sup>c</sup>	1Cx-value (pg) <sup>d</sup>	Leaves <sup>e</sup>	Standard <sup>f</sup>
<i>A. arenaria</i> DC.*	36(4x)	10.40±0.09	10171.20	2.60	D	<i>Pisum</i>
<i>A. bargusinensis</i> Spreng.*	36(4x)	8.70±0.10	8508.60	2.17	D	<i>Petunia</i>
<i>A. changaica</i> Krasch.*	36(4x)	11.70±0.20	11442.60	2.92	E/D	<i>Pisum</i>
<i>A. depauperata</i> Krasch.*	36(4x)	8.82±0.18	8625.96	2.20	D	<i>Petunia</i>
<i>A. desertorum</i> Spreng.*	36(4x)	8.27±0.31	8088.06	2.06	D	<i>Petunia</i>
<i>A. dracunculus</i> L.	18(2x)	5.94±0.10	5809.32	2.97	E/D	<i>Petunia</i>
<i>A. dracunculus</i> L.	36(4x)	11.82±0.10	11559.96	2.95	E/D	<i>Pisum</i>
<i>A. dracunculus</i> L.	54(6x)	15.71±0.10	15364.38	2.62	E/D	<i>Pisum</i>
<i>A. dracunculus</i> L.	90(10x)	24.58±0.29	24039.24	2.44	E/D	<i>Pisum</i>
<i>A. dracunculoides</i> Pursh*	54(6x)	15.58±0.41	15237.24	2.60	E/D	<i>Pisum</i>
<i>A. giraldii</i> Pamp.*	18(2x)	6.00±0.04	5868.00	3.00	E/D	<i>Pisum</i>
<i>A. glauca</i> Pall. ex Willd.*	36(4x)	11.97±0.17	11706.66	2.99	E/D	<i>Pisum</i>
<i>A. globosa</i> Krasch.*	36(4x)	9.27±0.14	9066.06	2.32	D	<i>Petunia</i>
<i>A. ledebouriana</i> Besser*	36(4x)	8.75±0.10	8557.50	2.19	D	<i>Petunia</i>
<i>A. macilenta</i> (Maxim.) Krasch.*	36(4x)	9.05±0.08	8850.90	2.26	D	<i>Petunia</i>
<i>A. marschalliana</i> Spreng.	18(2x)	5.37±0.06	5251.86	2.68	D	<i>Pisum</i>
<i>A. monostachya</i> Bunge ex Maxim. [ <i>A. pubescens</i> Ledeb. var. <i>monostachya</i> (Bunge ex Maxim.) Y. R. Ling]*	36(4x)	8.78±0.15	8586.84	2.20	D	<i>Petunia</i>
<i>A. oxycephala</i> Kitag.*	18(2x)	4.21±0.04	4117.38	2.10	D	<i>Petunia</i>
<i>A. pycnorhiza</i> Ledeb.*	36(4x)	9.02±0.29	8821.56	2.26	D	<i>Petunia</i>
<i>A. sosnovskyi</i> Krasch.*	36(4x)	10.56±0.10	10327.68	2.64	D	<i>Pisum</i>
<i>A. subdigitata</i> Matff.*	36(4x)	11.69±0.19	11432.82	2.92	E/D	<i>Pisum</i>

The taxa with genome size estimated for the first time are marked with an asterisk (\*). <sup>a</sup>Chromosome numbers: somatic chromosome number (ploidy level). <sup>b</sup>2C nuclear DNA content (mean value ± standard deviation). <sup>c</sup>1 pg = 978 Mpb (Doležel *et al.* 2003). <sup>d</sup>1Cx nuclear DNA content per basic chromosome set. <sup>e</sup>Lowermost leaf morphology: D: divided; E/D: mainly entire but with some divided. <sup>f</sup>Standard: internal standard used in each case for flow cytometric measurements (see text for details about *Petunia* and *Pisum*)

Table 3. Mean nuclear DNA amount (2C and 1Cx values) for the subgenus *Dracunculus*

Ploidy level	Mean 2C (pg)	Mean 1Cx (pg)
2x	5.33	2.66
4x	10.07	2.51
6x	15.63	2.60
10x	23.90	2.38

The analysis of the variation of the 1Cx values in the subgenus (Table 3) indicates a relative constancy of this parameter among ascending ploidy levels with non-significant differences ( $p=0.71$ ), although we have detected a decrease of genome size per basic chromosome set from diploids to decaploids. In all cases, diploids have a higher nuclear DNA content per monoploid genome (mean 1Cx; diploid: 2.66 pg; tetraploid: 2.51 pg; hexaploid: 2.60 pg and decaploid: 2.38 pg). The case of the hexaploid cytotype is an exception; even though its mean 1Cx value is lower than diploids, we have observed an increase when compared with tetraploids. This fact is most likely explained by a non-representative sampling of the subgenus at this ploidy level but could also reflect or be related to the possible recent origin of this hexa-

Table 4. Nuclear DNA loss with increasing ploidy level of the species closely related to *A. dracunculus*

Ploidy level	Mean 1Cx (pg)	DNA loss (%)
2x	2.98	-
4x	2.94	1.34
6x	2.60	12.75
10x	2.38	20.13

ploid, as it has been observed that older polyploids tend to have still less monoploid genome size than newly-formed ones. However, when we compare 1Cx-values of the species having a phylogenetic position close to *A. dracunculus*, such as *A. dracunculoides* Pursh, *A. glauca* Pall. ex Willd., *A. giraldii* Pamp., *A. subdigitata* Matff. or *A. changaica* Krasch. (Pellicer *et al.*, unpublished), it is observed that nuclear DNA content (1Cx) decreases with each increasing ploidy level (Table 4), as generally happens in plants. This phenomenon is intensified when plants attain high ploidy levels. Table 4 shows the rate of nuclear DNA loss of the polyploid species with respect to diploid cytotypes. While tetraploids do not exhibit a great loss (1.34% less nuclear DNA content than diploids), the effects of polyploidization in hexaploids and decaploids



are more apparent, about 12.75% and 20.13% DNA loss respectively. Polyploidy is a well known parameter which influences directly in genome size changes (Bennett and Leitch 2004). At the generic level in *Artemisia*, a gain of nuclear DNA content in ascending ploidy levels coupled with a decrease of this amount per haploid genome has been noted (Garcia *et al.* 2004; Pellicer *et al.* unpublished). A nuclear DNA loss per basic chromosome set in polyploids has been frequently reported in plants (Bennett and Leitch 2004 and references therein). Changes at chromosome and DNA sequence level (Wendel *et al.* 1995; Leitch and Bennett 1997), as well as amplification, reassortment or elimination of highly repetitive sequences (Hanson *et al.* 1998) and low-copy DNA sequences (Feldman *et al.* 1997; Ozkan *et al.* 2001) might influence in this direction. In cases of newly formed allopolyploids (Ozkan *et al.* 2001), this non-random sequence elimination has been linked to a stabilizing mechanism for the union of the two parental genomes in the nucleus.

**Interspecific variability** We have noted that subgenus *Dracunculus* is quite homogeneous in terms of C-values in spite of the variations induced by polyploidy. The ratio between maximum and minimum nuclear DNA amount at the same ploidy level observed (ratio 2C,  $2x=1.42$  pg; ratio 2C,  $4x=1.44$  pg) and nuclear DNA amount per basic chromosome set (ratio 1Cx=1.45 pg, including all ploidy levels found) is quite low. Comparing these results with those obtained for the remaining subgenera of *Artemisia*, *Dracunculus* appears as the most homogeneous (Garcia *et al.* 2004), and this fact is also reflected in the phylogeny of the subgenus (Pellicer *et al.* unpublished).

**Phylogenetic approach, morphological traits and life cycle** Correlations between C-value and many biological and ecological traits have been noted long ago (Bennett 1987, 1998; Knight *et al.* 2005). Thus, species that belong to neighbouring phylogenetic groups present similar genome sizes for the same ploidy level. In the subgenus *Artemisia*, the unresolved position of some species is reflected in the phylogenies of the genus (Torrell *et al.* 1999; Watson *et al.* 2002; Vallès *et al.* 2003), and genome size data become more heterogeneous (Garcia *et al.* 2004). The case of *Dracunculus* seems to be the opposite. A preliminary phylogeny of the subgenus, based on the analysis of nuclear DNA regions (ITS, ETS) reveals the existence of different groups within the subgenus (Pellicer *et al.* unpublished), and the analysis of the nuclear DNA content for the species also points in this direction. This fact could support C-value data as being an important tool which can help in elucidating phylogenetic positions of controversial taxa. These groups seem to reflect the different pattern of leaf morphology yet described (Shishkin and Bobrov 1995, Ling *et al.* 2006), that is, species with all lowermost leaves divided are

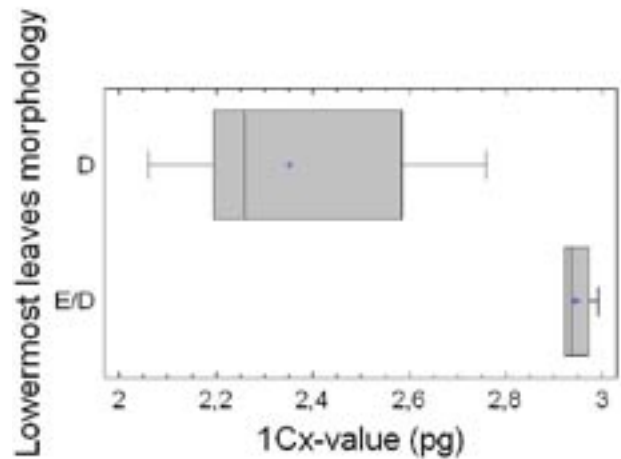


Fig. 13. Box and whisker plot of the 2C value of tetraploid species with lowermost leaf divided (D) or mainly entire but with some divided (E/D)

grouped together, separated from those with entire or few divided leaves, which belong to another clade. When analyzing genome sizes (1Cx) of tetraploid species bearing in mind leaf morphology, these two groups present a statistically significant difference (Fig. 13;  $p=0.0002$ ).

Variations in the C-value of closely related plants but with a different life cycle have been detected in genera such as *Echinops* or *Tripleurospermum* (Garnatje *et al.* 2004; Garcia *et al.* 2005). These differences, depending on the annual or perennial character of some species, could be related to oscillations in the duration of the cell cycle (Nagl and Ehrendorfer 1974; Rees and Narayan 1981; Bennett and Leitch 2003). The present study does not shed light in this respect because all species studied are perennials, although in annual or biennial taxa of *Artemisia* genome sizes that differ substantially from their perennial relatives have been reported (Torrell *et al.* 2001; Garcia *et al.* 2004; Pellicer *et al.* unpublished).

## CONCLUDING REMARKS

The present study reflects on the one hand the great incidence of polyploidy in the genus *Artemisia*, and on the other hand, the effect that polyploidy exerts in the dynamics of genome size. This is a work mostly centred on the subgenus *Dracunculus*, and it is an approach toward a better understanding of what kind of processes are taking place at subgeneric and, consequently, at generic level. Thus, further research in the genus and in this area will be developed from this standpoint.

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