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. pycnocephala* and *A. sossowskii* 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 andacentric 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 1C-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* (Rydbr.) 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 pheno- typic 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.rbgkew.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 Dracunculus, 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 Vil. ‘PxPc6’ (2C=2.85 pg) and Pismum 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 contents 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 Cientificotècnics’ 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. czekeanowskiana Trautv. (Fig. 1), A. globosa Krusch. (Fig. 7), A. ledebouriana Besser (Fig. 8), A. lithophila Turcz. ex DC. (Figs. 4a, b), A. macilenta Maxim. (Krasch.) (Fig. 9), A. pyenornhiza Ledebs. (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 Whitting 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 Siplivinski (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

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Chromosome number (ploidy level)</th>
<th>Localities</th>
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<tbody>
<tr>
<td><strong>Subgenus Artemisia</strong></td>
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<td><strong>Subgenus Dracunculus Besser</strong></td>
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<tr>
<td>A. bargasinensis Spreng.</td>
<td>36 (4x)</td>
<td>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).</td>
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<tr>
<td>A. oxycephala Kitag.</td>
<td>18 (2x)</td>
<td>Mongolia, Tuv (Central) aimag: Mungunmort sum, 10 km S of the sum. 7-IX-2004. Leg. et det. A.A. Korobkov (LE 04-115).</td>
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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.
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. cze-kanowskiana* ($2n=10x=89, 90$) and *A. macrantha* ($2n=12x=106, 108$). Previous reports in *A. sericea* Web. ex Stchem. (Kawatani and Ohno 1964; Krogulevich and...
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 similar behaviour has been reported in *A. dracunculus* (2n=87, 88, 89, 90; Kreitschitz and Vallés 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; Coudere *et al.* 1980). Somatic metaphase plates belonging to the same and different individuals of *A. macrantha* 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).

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 Vallés 2001; García *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 Vallés 2001; García *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*, García *et al.* 2005) and in previous studies of *Artemisia* (Torrell and Vallés 2001; García *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.
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 1Cx value lower than tetraploids, with each increasing ploidy level (Table 4), as generally is observed that nuclear DNA content (1Cx) decreases or be related to the possible recent origin of this hexaploid cytotype is an exception; even though its mean 2C-value (pg) is 15.58±0.41, its 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 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 (mean1Cx: diploid: 2.60 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 hexaploid, 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. changaica Kitag., A. pycnorrhiza Ledeb., A. sabiniana Kitag. * and A. subdigitata Mattf. *, we 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. changaica Kitag., A. pycnorrhiza Ledeb., A. sabiniana Kitag. *, A. subdigitata Mattf. *, and 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 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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