New or rarely reported chromosome numbers in taxa of subtribe Artemisiinae (Anthemideae, Asteraceae) from Mongolia

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Short Titles:

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CHROMOSOME NUMBERS IN MONGOLIAN ARTEMISIINAE

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This study encompasses 25 chromosome counts of 18 species in the subtribe Artemisiinae (tribe Anthemideae) of the family Asteraceae, coming from Mongolian populations. Most of them (15 species) belong to the biggest genus of the subtribe, *Artemisia*, while the others come from two genera very closely related to it: *Ajania* (two species) and *Neopallasia* (one species). Twelve counts are new reports, two are not consistent with the previous, and the remnants confirm the scanty previous information. The majority of species have x=9 as basic chromosome number, but there are some x=8 taxa. Ploidy levels range from 2x to 6x. The presence of B-chromosomes has been detected in one species, *Ajania fruticulosa*.

ADDITIONAL KEYWORDS: *Ajania* - *Artemisia* – *Artemisiinae* - B-chromosomes – cytotaxonomy - Compositae - chromosome numbers - dysploidy - karyology - *Neopallasia* - polyploidy.

INTRODUCTION

The *Artemisiinae*, the largest subtribe in the *Anthemideae* (Asteraceae) comprise a group of worldwide distributed genera, which embrace many useful species, and countless karyological surveys have been made that focus on them (Watanabe, 2002, and references therein). The present paper is centred in the genus *Artemisia*, one of the largest in the family Asteraceae (circa 500 taxa) and, to a lesser extent, two small genera phylogenetically close to it, *Ajania* and *Neopallasia* (so much so that some have been previously classified as members of *Artemisia*; see the synonymy of some of the taxa studied given below). Since the earliest chromosome studies of the genus *Artemisia*, developed in the first half of the 20th century (Weinedel-Liebau, 1928) many others have followed enhancing the available cytogenetic and karyological data, not only on this genus, but also on its allies (Eherendorfer, 1964; Kawatani & Ohno, 1964; Korobkov, 1972; McArthur *et al.*, 1981; Ouyahya &Viano, 1981, 1988; Vallès & Siljak-Yakovlev, 1997; Vallès *et al.*, 2005, and references therein). However, chromosomal data are still scarce or non-existent for numerous *Artemisiinae* taxa. Additionally, chromosome reports on Mongolian populations (some of them endemic) of these taxa are also limited, and since this region is regarded as one of the most outstanding speciation and diversification points of the subtribe, the interest on these data is even increased. Many of the counts here reported are new ones; some others confirm unique or very scarce reports and only two differ from those cited earlier on. Besides, some evolutionary and systematic traits of these genera are also discussed considering these new cytogenetic data.

MATERIAL AND METHODS

Root-tip meristems were obtained from wild-collected achenes by germinating them on wet filter paper in Petri dishes at room temperature. They were pretreated with 0.05% aqueous colchicine at room temperature for 2 h 30 min. The material was fixed in absolute ethanol and glacial acetic acid (3:1) for 2-4 h at room temperature and then stored in the fixative at 4°C. Samples were hydrolyzed in 1N HCl for 2 minutes at 60°C, stained in 1% aqueous aceto-orcein for 2-12 hours at room temperature, and squashed and mounted in a drop of 45% acetic acid-glycerol (9:1). The best metaphase plates were photographed on a Zeiss Axioplan microscope with a digital camera AxioCam MRc 5 and adquired with the software AxioVision AC v. 4.2. (Carl Zeiss Vision, GmbH). Vouchers of the species studied are deposited in the herbarium of the Centre de Documentació de Biodiversitat Vegetal de la Universitat de Barcelona (BCN).

To assess the existence of published chromosome counts in the species studied we used the most common indexes of plant chromosome numbers (cited in Vallès, Torrell & Garcia-Jacas, 2001a), as well as the chromosome number databases Index to Plant Chromosome Numbers (Missouri Botanical Garden, http://mobot.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; Watanabe, 2004).

RESULTS AND DISCUSSION

We are presenting the data and comments of the different taxa grouped by genera in alphabetical order. In *Artemisia* we have considered the main sections (treated by several authors as subgenera) in which the genus is divided. The localities are given with the indication of Mongolian administrative divisions, aimag (province, written aimak in Russian language works) and sum (village, written somon in Russian language works).

Genus *Ajania* Poljakov

Ajania achilleoides (Turcz.) Poljakov [*Artemisia achilleoides* Turcz., *Chrysanthemum achilleoides* (Turcz.) Hand.-Mazz., *Hippolytia achilleoides* (Turcz.) Poljakov]

Mongolia, Dund (Central) Gobi aimag: Erdene-Dalai sum, 16 km NE of the sum, dry step hills, *Sh. Dariimaa, Sh. Tsooj & J. Vallès*, 5.ix.2004 (BCN 23793). 2n=18 (Fig. 1).

Mongolia, Dund (Central) Gobi aimag: Erdene-Dalai sum, 46 km NE of the sum, dry step hills, *Sh. Dariimaa, Sh. Tsooj & J. Vallès*, 5.ix.2004 (BCN 23794). 2n=18.

According to our data this is the first count on this species, endemic to Mongolia and China, confirmed in two very close populations. It is a diploid based on x=9. This count confirms the basic number in the genus *Ajania*. The diploid level was reported on *Ajania fastigiata* (2n=18, Maltzeva, 1969). Other works on the relatively large (about 40 species) genus *Ajania* deal with non-Central Asian species (Sokolovskaya, 1966; Probatova & Sokolovskaya, 1990; Kondo *et al.*, 1992; Abd El-Twab *et al.*, 1998, 1999) and report different ploidy levels up to decaploid.

Ajania fruticulosa (Ledeb.) Poljakov [*Tanacetum fruticulosum* Ledeb. *Chrysanthemum fruticulosum* (Ledeb.) B. Fedtsch.]

Mongolia, Umnu (South) Gobi aimag: Mandal Oboo sum, 20 km SE of the sum, desert step with *Anabasis*, *Sh. Dariimaa, Sh. Tsooj & J. Vallès*, 4.ix.2004 (BCN 23795). 2n=36(+0-2-4B) (Fig. 2).

This is the second report on chromosome number for this species native from Central Asia, and the first in a Mongolian population. It confirms the former, which was obtained from a Chinese population by Kondo *et al.* (1998). In our case, the presence of B-chromosomes has been detected in some cells, from two to four (see chromosomes marked with arrows in fig. 2). The presence of B-chromosomes in the subtribe is not unusual (Vallès & Garnatje, 2005). Tetraploid is a common level in the genus, since many other counts of 36 chromosomes have been reported (e.g.: *A. przewalskii*, Kondo *et al.*, 1992; A. *latifolia*, Kondo *et al.*, 1998).

Genus *Artemisia* L.

Section *Artemisia*

Artemisia adamsii Besser

Mongolia, Tuv (Central) aimag: Bayan Undjuul sum, 20 km N of the sum, dry step, *Sh. Dariimaa, Sh., Tsooj & J. Vallès*, 5.ix.2004 (BCN 23815). 2n=18 (Fig. 3).

This count confirms the only previous one, from a population collected in northeast China by Wang *et al.* (1999), on plant material from Mongolia. It represents a diploid level of x=9, the most common basic chromosome number in the genus (Vallès *et al.*, 2005, and references therein).

Artemisia leucophylla (Turcz. ex Besser) C. B. Clarke (*Artemisia vulgaris* L. var. *leucophylla* Turcz. ex Besser)

Mongolia, Arkhangai aimag: Taryat sum, Khorgo-Terkh national park, near lake Terkhen Sagan nur, *Sh. Dariimaa, Sh. Tsooj & J. Vallès*, 27.viii.2004 (BCN 23796). 2n=16 (Fig. 4).

Mongolia, Tuv (Central) aimag: Mungunmort sum, 10 km NW of the sum, path margins, step, *Sh. Dariimaa, Sh. Tsooj, J. Vallès & E. Yatamsuren*, 7.ix.2004 (BCN 23797). 2n=16.

In this case, our counts, assessed in two well separated populations, do not coincide with the only previous one, corresponding to a Russian population (Krogulevich, 1978) from Eastern Sayana (Siberia), with 2n=18. Many species of the genus, especially those belonging to sections *Artemisia* and *Absinthium* (Torrell *et al.*, 1999), display the basic chromosome number x=8, less common than the dominant x=9 (Torrell *et al.*, 2001). Evidence is given in several studies (Vallès & Siljak-Yakovlev, 1997; Torrell *et al.*, 2001) that this less usual basic chromosome number comes from a descending dysploidy process from the former x=9, as a result of a chromosomal fusion, responsible for the existence of a long metacentric chromosome pair in the x=8 taxa (such as in *A. splendens*, Torrell *et al.*, 2001). This long metacentric chromosome pair with certain centromeric fragility, also confirmed in other species with the same phenomenon (Torrell *et al.*, 2001; Torrell and Vallès, unpublished), could explain the former count of 2n=18 in this species. *Artemisia leucophylla* is closely related to *A. vulgaris* (up to the point that it has previously been classified as a variety of this species, see the synonymy), *A. mongolica* and *A. obscura* (see below for this species), three taxa also with 2n=16 (Watanabe, 2002, and references therein).

Artemisia medioxima Krasch. ex Poljakov

Mongolia, Tuv (Central) aimag: Mungunmort sum, 10 km NW of the sum, path margins, step, *Sh. Dariimaa, Sh. Tsooj, J. Vallès & E. Yatamsuren*, 7.ix.2004 (BCN 23792). 2n=36 (Fig. 5).

On the authority of our data, this is the first count carried out in this Mongolian and Russian endemic species. It is a tetraploid based on x=9, the most common basic chromosome number in *Artemisia*, confirming the relevance of polyploidy in the genus (Vallès & Garnatje, 2005; Vallès *et al.*, 2005, and references therein).

Artemisia messerschmidtiana Besser

Mongolia, Tuv (Central) aimag: Bayan Undjuul sum, Ikh Khairkhan mountains, *Sh. Dariimaa, Sh. Tsooj & J. Vallès*, 5.ix.2004 (BCN 23798). 2n=36 (Fig. 6).

According to our bibliographical research, this is the first report of the chromosome number in this species, endemic to Siberia, Mongolia and the North of China.

Artemisia obscura Pamp.

Mongolia, Umnu (South) Gobi aimag: Bulgan sum, E Gurvan Saikhan mountains, canyon, *Sh. Dariimaa, Sh. Tsooj, J. Vallès & E. Yatamsuren*, 2.ix.2004 (BCN 23799). 2n=16 (Fig. 7).

This is the first count reported for this species, with a distribution restricted to Mongolia and China, and together with *Artemisia leucophylla* and many other members from the *A. vulgaris* group (Vallès & Garnatje, 2005, and references therein) confirms the existence of the basic chromosome number x=8 in this complex of the genus (discussed for *Artemisia leucophylla*, see above).

Artemisia transbaicalensis Leonova

Mongolia, Uvur-Khangai aimag: Kharkhorin sum, 2 km W of the sum, margin of *Betula* and *Larix* forest, 1,900 m, step, *Sh. Dariimaa, Sh. Tsooj & J. Vallès*, 26.viii.2004 (BCN 23800). 2n=36 (Fig. 8).

Again, this is the first report on the chromosome number of this species, which grows in the Baikal Lake basin (Siberia and Mongolia) and whose basic chromosome number (x=9) and ploidy level are usual in the genus.

Artemisia umbrosa Turcz. ex DC.

Mongolia, Selenge aimag: Shaamar sum, Tujiin Nars, 5 km E of the sum, *Sh. Dariimaa, Sh. Tsooj, J. Vallès & E. Yatamsuren*, 9.ix.2004 (BCN 23789). 2n=54 (Fig. 9).

New count for this species, according to our bibliographical research. The existence of this hexaploid cytoype is an evidence of a high polyploidization activity as an evolutionary mechanism of the genus in this area. The species belongs, likewise *A. obscura* and *A. leucophylla*, to the *A. vulgaris* group, but in this case the basic chromosome number is x=9, the original in the genus (Vallès & Garnatje, 2005).

Section *Absinthium* DC.

Artemisia anethoides Matff.

Mongolia, Selenge aimag: Shaamar sum, 3 km W of the sum, Buureg Tolgoi hills, near river Orkhon, 700 m, *Sh. Dariimaa, Sh. Tsooj, J. Vallès & E. Yatamsuren*, 7.ix.2004 (BCN 23790). 2n=16 (Fig. 10).

To our knowledge, this is the second count on this species, an endemism of China and Mongolia, and agrees with the previous one, done in plants from Northeast China by Wang *et al.* (1998). It confirms the existence of the basic chromosome number x=8 in section *Absinthium*.

Artemisia macrocephala Jacquem. ex Besser

Mongolia, Arkhangai aimag: Tsenkher sum, near river Urd-tamir, *Sh. Dariimaa, Sh. Tsooj & J. Vallès*, 26.viii.2004 (BCN 23801). 2n=18 (Fig. 11).

Mongolia, Uvur Khangai aimag: Arvaykheer city, ruderal in streets, *Sh. Dariimaa, Sh. Tsooj & J. Vallès*, 30.viii.2004 (BCN 23802). 2n=18.

Mongolia, Dund (Central) Gobi aimag: Erdene-Dalai sum, 6 km NE of the sum, step, *Sh. Dariimaa, Sh. Tsooj & J. Vallès*, 4.ix.2004 (BCN 23803). 2n=18.

Verified in three well separated populations, the present one confirms the two previous counts, one from a Siberian population (Republic of Tuva, Russia, Krogulevich & Rostovtseva, 1984) and the other from Tadzhikistan (Astanova, 1989). These are the first counts in Mongolian populations of this species, which has a large distribution area throughout Central and Eastern Asia.

Section *Dracunculus* Besser

Artemisia depauperata Krasch.

Mongolia, Arkhangai aimag: Tsetserleg city, Sagaan-Davaa pass, 2200 m, *Sh. Dariimaa, Sh. Tsooj & J. Vallès*, 26.viii.2004 (BCN 23804). 2n=36 (Fig. 12).

Our report coincides with the only previous one, and confirms the tetraploid cytotype of this species reported by Krogulevich (1978), from a Russian population (Eastern Sayana, Siberia). It is the first count in a Mongolian population of the species, endemic to Mongolia and Russia.

Artemisia dolosa Krasch.

Mongolia, Uvur Khangai aimag: Khujirt sum, 20 km S of the sum, meadow step, *Sh. Dariimaa, Sh. Tsooj & J. Vallès*, 30.viii.2004 (BCN 23805). 2n=18 (Fig. 13).

Mongolia, Tuv (Central) aimag: Mungunmort sum, 20 km W of the sum, W slope of Mungun mountain, *Sh. Dariimaa, Sh. Tsooj, J. Vallès & E. Yatamsuren*, 7.ix.2004 (BCN 23791). 2n=36 (Fig. 14).

These are the two first counts reported in this species, endemic to Mongolia and the Altai mountains of Russia, one population being diploid and the other tetraploid. Both the basic chromosome number x=9 and the two ploidy levels are habitual in the genus. Also, the existence of both cytotypes, diploid and tetraploid, has been confirmed separately by flow cytometry (Garcia *et al.*, unpublished data). The occurrence of a high diversification activity (with a relevant role played by polyploidy) in the studied area is also supported by this finding.

Artemisia giraldii Pamp.

Mongolia, Bulgan aimag: Sansar sum, NE slope of Khugunkhaan mountain, step near *Betula* and *Pinus* forest, 2,000 m, *Sh. Dariimaa, Sh. Tsooj & J. Vallès*, 25.viii.2004 (BCN 23806). 2n=18 (Fig. 15).

According to our data, this is the first report of the chromosome number in this endemic species from China and Mongolia. This is a taxon very closely related to *A. dracunculus* L., which presents a poylploid series based on x=9, starting for the same diploid level than *A. giraldii* and reaching the decaploid level (Vallès *et al.*, 2001a, and references therein).

Artemisia klementzae Krasch.

Mongolia, Bulgan aimag: Sansar sum, Khugunkhaan mountain, sandy step, *Sh. Dariimaa, Sh. Tsooj & J. Vallès*, 25.viii.2004 (BCN 23807). 2n=36 (Fig 16).

Again, we present a new chromosome count from a Mongolian endemic species.

Artemisia monostachya Bge. ex Maxim. [*A. pubescens* Ledeb. var. *monostachya* (Bge. ex Maxim.) Y.R. Ling]

Mongolia, Arkhangai aimag: Ikh Tamir sum, 30 km NW of the sum, Khoer Davaa pass, *Sh. Dariimaa, Sh. Tsooj & J. Vallès*, 26.viii.2004 (BCN 23808). 2n=36 (Fig. 17).

Mongolia, Arkhangai aimag: Taryat sum, Khorgo-Terkh national park, rocky mountain slope near lake Terkhen Sagan nur, *Sh. Dariimaa, Sh. Tsooj & J. Vallès*, 27.viii.2004 (BCN 23809). 2n=36.

To our knowledge, the chromosome number of this taxon had never been reported before, so this is the first count, confirmed in two close populations, of this species endemic to Russia, China and Mongolia.

Artemisia xanthochroa Krasch.

Mongolia, Uvur-Khangai aimag: Tugrug sum, 40 km S of the sum, desert step with *Caragana*, *Sh. Dariimaa, Sh. Tsooj & J. Vallès*, 31.viii.2004 (BCN 23810). 2n=36 (Fig. 18).

This is the first chromosome count for this endemic species to China and Mongolia, according to our data.

Genus *Neopallasia* Poljakov

Neopallasia pectinata (Pall.) Poljakov (*Artemisia pectinata* Pall.)

Mongolia, Umnu (South) Gobi aimag: Bulgan sum, 10 km S of the sum, *Sh. Dariimaa, D. Samjid, Sh. Tsooj & J. Vallès*, 26.viii.2004 (BCN 23811). 2n=18 (Fig. 19).

Mongolia: Ulaanbaatar, ruderal in the city, *Sh. Dariimaa, Sh. Tsooj & J. Vallès*, 7.ix.2004 (BCN 23812). 2n=18.

Confirmed in two separated Mongolian populations, the present record validates one of the previous counts on this Central Asian species, reported under its original name *Artemisia pectinata* (Suzuka, 1952; Kawatani & Ohno, 1964; Qiao *et al.*, 1990). A tetraploid population of this taxon has also been reported (Vallès *et al.*, 2005) from Chinese material.

CONCLUDING REMARKS

The results obtained prove again the existence of two basic chromosome numbers in *Artemisia* (Vallès & Garnatje, 2005). All the studied taxa but three have x=9, the most common basic chromosome number in the genus, subtribe, tribe and family (Solbrig, 1977; Schweizer & Ehrendorfer, 1983, Oliva & Vallès, 1994; Vallès & Siljak-Yakovlev, 1997). Of all the taxa considered, three present x=8 as basic chromosome number, evidence for the descending dysploidy -resulting from chromosome fusion- occurring in the genus (Vallès & Siljak-Yakovlev, 1997; Torrell *et al.*, 2001, Vallès & Garnatje, 2005). This fact has also been reported in many genera of the Asteraceae (Fernández Casas & Susanna, 1986; Garcia-Jacas, Susanna & Ilarslan, 1996; Siljak-Yakovlev, 1996; Vallès & Siljak-Yakovlev, 1997; Torrell *et al.*, 2001; Vallès *et al.*, 2001a, 2001b), and considered an important evolutionary mechanism. Another remarkable and well known evolutionary mechanism in plants is polyploidy (Bretagnolle *et al.*, 1998; Soltis & Soltis, 1999; Soltis *et al.*, 2004), and the set studied in this paper is a good example of its relevance in the *Artemisiinae*. In our sample 10 out of the 19 taxa analyzed are polyploid, nine being tetraploid and one hexaploid. In the genus *Artemisia*, many of the species colonizing extremely arid lands are frequently polyploids, supporting the hypothesis that there is a connection between ecological tolerance and polyploidization in many plant groups (Otto & Whitton, 2000). Additionally, many authors support that polyploids have radiated and expanded more than diploids and our data seem to confirm it, because the species on this study were randomly chosen (among those who had never or scarcely been karyologically studied), and more than half of them are polyploids. This agrees with the premise that polyploids usually cover larger territories than related diploids (Ehrendorfer, 1980) and the present data seem to support this hypothesis, though assuming many exceptions to this rule (Bretagnolle *et al.*, 1998).

Finally, the abundance of polyploids and the existence of species with the two basic chromosome numbers found in the genus *Artemisia*, together with the high number of species from this genus and the subtribe which inhabit the Mongolian area confirm that this country is as a speciation and diversification centre both for the genus and for the subtribe Artemisiinae.

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Figure captions

Figures 1-13. Somatic metaphases. Fig. 1. *Ajania achilleoides* (2n=18). Fig. 2. *Ajania fruticulosa* (2n=36 + 4B); arrows indicate the B chromosomes. Fig. 3. *Artemisia adamsii* (2n=18). Fig. 4. *Artemisia leucophylla* (2n=16); arrows indicate the long metacentric chromosome pair. Fig. 5. *Artemisia medioxima* (2n=36). Fig. 6. *Artemisia messerschmidtiana* (2n=36). Fig 7. *Artemisia obscura* (2n=16); arrows indicate the long metacentric chromosome pair. Fig. 8. *Artemisia transbaicalensis* (2n=36). Fig 9. *Artemisia umbrosa* (2n=54). Fig 10. *Artemisia anethoides* (2n=16). Fig. 11. *Artemisia macrocephala* (2n=18). Fig. 12. *Artemisia depauperata* (2n=36). Fig. 13. *Artemisia dolosa* (2n=18).

Figures 14-19. Somatic metaphases. Fig 14. *Artemisia dolosa* (2n=36). Fig. 15. *Artemisia giraldii* (2n=18). Fig. 16. *Artemisia klementzae* (2n=36). Fig. 17. *Artemisia monostachya* (2n=36). Fig. 18. *Artemisia xanthocrhoa* (2n=36). Fig. 19. *Neopallasia pectinata* (2n=18).