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Chromosome counts and karyomorphology of some species of *Artemisia* (Asteraceae) from Turkey

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Abstract: Chromosome numbers and karyotypes of some species belonging to the genus *Artemisia* L. (Asteraceae) were investigated, and chromosome counts of 12 species, 1 of which was counted for the first time, are presented with a thorough compilation of previously published data. All of the species studied belong to the subgenus *Artemisia* Chromosome numbers of 6 taxa confirmed the previous data. Additionally, karyotypes of 8 species were reported. Karyotype analyses of 6 *Artemisia* species are reported here for the first time. The basic chromosome numbers in the species studied were x = 8 and x = 9, with ploidy levels ranging from 2x to 4x and 6x. B chromosomes were also present in *Artemisia incana* (L.) Druce as 2n = 2x = 16 + 2B.

Key words: *Artemisia*, chromosome numbers, karyotype, dysploidy, cytotaxonomy

Türkiye'den bazı *Artemisia* (Asteraceae) türlerinin kromozom sayıları ve karyomorfolojisi

Özet: *Artemisia* L. cinsine ait bazı türlerin kromozom sayısı ve karyotipleri araştırılmış ve birisi ilk kez olmak üzere 12 türün kromozom sayısı önceden basılmış olan verilerin tam bir derlemesi ile sunulmuştur. Çalışılan türlerin hepsi *Artemisia* altcinsine aittir. Altı taksonun kromozom sayısı önceki verilerle onaylanmıştır. Ayrıca sekiz türün karyotipleri belirlenmiştir. Bu çalışmada altı *Artemisia* türünün karyotip analizleri ilk kez rapor edilmiştir. Çalışılan türlerin temel kromozom sayıları x = 8 ve x = 9 olup, 2x'den 4x ve 6x'e değişen ploidi seviyeleri tespit edilmiştir. Ayrıca diploid bir tür olan *Artemisia incana* (L.) Druce taksonunda iki adet B kromozomunun (2n = 2x = 16 + 2B) varlığı saptanmıştır.

Anahtar sözcükler: *Artemisia*, kromozom sayısı, karyotip, disploidi, sitotaksonomi

Introduction

Artemisia L. is one of the largest genera of the family Asteraceae. The genus comprises more than 500 species, depending on the opinions of the authors who have studied the genus (Ling, 1991a, 1991b; Vallès & Šiljak-Yakovlev, 1997; Funk et al., 2009). It

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is distributed throughout the northern hemisphere, with very few representatives (not more than 10 species) in the southern hemisphere. Western and central Asia are 2 important speciation centres of the genus (Vallès et al., 2005). Most species in the genus are perennial; only approximately 10 species are annual or biannual (Vallès et al., 2003). Many species of the genus have great economic importance, including usages as medication, foodstuff, ornamentals, or soil stabilisers in disturbed habitats; some taxa are lethal or allergenic and some others are insidious weeds that can negatively impact harvests (Chehregani et al., 2010; Hayat et al., 2010). The genus is currently divided into 5 main groups [*Artemisia*, *Absinthium* (Mill.) Less., *Dracunculus* (Besser) Rydb., *Seriphidium* Besser, and *Tridentatae* (Rydb.) McArthur], but subgeneric classification is subject to rearrangements in light of recent molecular studies (Vallès et al., 2003).

Many karyological surveys have been made of the genus *Artemisia*, enhancing the available cytogenetic and karyological data (Kawatani & Ohno, 1964; Vallès, 1987; Gabriellian & Vallès, 1996; Torrell et al., 1999; Torrell & Vallès, 2001; Torrell et al., 2001; Vallès & McArthur, 2001; Vallès et al., 2001a, 2001b; Hoshi et al., 2003; Kreitschitz & Vallès, 2003; Inceer & Hayirlioglu-Ayaz, 2007; Pellicer et al., 2007a, 2007b, 2008; Chehregani & Hajisadeghian, 2009; Nazırzadeh et al., 2009; Chehregani et al., 2010). Nevertheless, the chromosome numbers of some species of the genus remain unknown or doubtful. The chromosome number in diploids is most often 2n = 18 or 16, the highest chromosome number known is 144 (Pellicer et al., 2007b), and aneuploidy is frequent. The most common basic chromosome numbers in *Artemisia* are x = 8 and 9, although x = 7, 10, 13, and 17 have also been reported by some researchers (Wiens & Richter, 1966; Vallès et al., 2005; Chehregani & Hajisadeghian, 2009; Matoba & Uchiyama, 2009; Park, 2009).

A high percentage of *Artemisia* species are polyploid. This phenomenon is present in all of the major groups into which the genus is divided. Both basic chromosome numbers (x = 8 and x = 9) show polyploidy, with levels up to 12x for x = 9 and 6x for x = 8 (Vallès & McArthur, 2001). Clearly, dysploid-polyploid complexes have played a major role in the karyological evolution of *Artemisia*. In *Anthemideae*, and particularly in *Artemisia* and its relatives, both changes in the basic chromosome number and polyploidy seem to occur mostly in the later stages of their evolutionary history (Vallès & Šiljak-Yakovlev, 1997; Vallès & McArthur, 2001). Dysploidy is the change in the chromosomal base number through a rearrangement of chromatin and the loss or gain of a centromere without necessarily changing the amount of chromatin in the karyotype. In Asteraceae, dysploidy decreases are common or very common in some clades, while increases appear to be rare or very rare depending upon how the higher x numbers are interpreted (Semple & Watanabe, 2009). For these reasons, new karyological studies are still necessary to unravel the conflicts. Karyological studies including karyotype analysis are welcome as a possible contribution to the systematics of large and difficult genera such as *Artemisia*.

The aim of the present study was to newly discover the chromosome numbers of several representatives of *Artemisia* from different Anatolian localities and to identify or verify chromosome numbers and karyological characters of the subgenus *Artemisia* in light of the new cytogenetic data.

Materials and methods

Ripe achenes from adult plants were collected from wild populations of each species. An indication of the provenance of the species studied is shown in Table 1. Root-tip meristems were obtained from wild-collected achenes germinated on wet filter paper in petri dishes in an incubator at 20 ± 1 °C for several days. The germinated achenes (1-1.5 mm) were pretreated with saturated paradichlorobenzene for 4 h at room temperature. Materials were fixed in a solution of ethanol and glacial acetic acid (3:1) overnight at room temperature and stored at 4 °C in 70% ethanol until used. They were then hydrolysed in 1 N HCl for 15-18 min at 60 °C, stained by the Feulgen method, and squashed onto slides in 45% acetic acid (Sharma & Gupta, 1982). The best metaphase plates were photographed (100×) with a digital camera (Olympus C-5060) mounted on an Olympus CX41 microscope. Thereafter, chromosome morphologies and karyograms were constructed. The system of Levan et al. (1964) was used in determining centromere locations.

Table 1. Provenance of the populations of the subgenus *Artemisia* studied.

Taxa	Localities, collector(s), and data
A. vulgaris	Turkey, B8 Muş: Toprakbaba Park, 1300 m a.s.l., 38°43′692″N, 41°34′806″E, 23.11.2007, *Ş.Civelek* & *M.Kurşat* 1108.
A. abrotanum	Turkey, B8 Muş: Centre, near Meslek Yüksek Okulu, 1266 m a.s.l., 38°47′423″N, 41°29′795″E, 23.11.2007, *Ş.Civelek* & *M.Kurşat* 1107.
A. austriaca	Turkey, B9 Van: Gürpınar, between Hamurkesen and Işıkpınar village, 1975 m a.s.l., 38°20′442″N, 43°37′321″E, 20.09.2007, *Ş.Civelek* & *M.Kurşat* 1054.
A. incana	Turkey, B9 Muş: Malazgirt, between Aktuzla anf Karıncalı village, 1550 m a.s.l., 39°21′474″N, 42°15′551″E, 26.11.2007, *M.Kurşat* 1120.
A. armeniaca	Turkey, B10 Ağrı: Doğubeyazıt-Iğdır road, south hillsides of Kori Mountain, north hillsides of Karabulak, Bardaklı village, 2565 m a.s.l., 39°43′601″N, 44°03′501″E, 23.09.2007, *Ş.Civelek* & *M.Kurşat* 1068.
A. chamaemelifolia	Turkey, B10 Ağrı: Doğubeyazıt-Iğdır road, south hillsides of Kori Mountain, north hillsides of Karabulak, Bardaklı village, 2565 m a.s.l., 39°43′601″N, 44°03′501″E, 23.09.2007, *Ş.Civelek* & *M.Kurşat* 1069.
A. tournefortiana	Turkey, B9 Van: Gürpınar, Hamurkesen village, 1953 m a.s.l., 38°20′774″N, 43°37′377″E, 24.11.2007, *Ş.Civelek* & *M.Kurşat* 1113.
A. absinthium	Turkey, B9 Muş: Malazgirt, between Aktuzla and Karıncalı village, 1550 m a.s.l, 39°21′474″N, 42°15′551″E, 24.11.2007, Ş. Civekek & *M.Kurşat* 1080.
A. arborescens	Turkey, C5 Hatay: Samandağı, Musa Mountain, Çevlik, 53 m a.s.l., 36°07′211″N, 38°55′695″E, 12.09.2007, *Ş.Civelek* & *M.Kurşat* 1040.
A. splendens	Turkey, B9 Van: Gürpınar, Sopakonak village, 2692 m a.s.l., 38°12′533″N, 43°37′055″E, 25.07.2008, *M.Kurşat* 1153.
A. caucasica	Turkey, C5 Niğde: Çamardı, vicinity of Demirkazık village, 1560 m a.s.l., 37°51′472″N, 35°04′534″E, 03.09.2007, *M.Kurşat* 1019.
A. haussknechtii	Turkey, C9 Hakkari: Hakkari-Van highway, after 20 km, Kırıkdağ village, Kırbaş Castle, 31.10.2008, *M.Kurşat* 1183.

*Vouchers deposited in the herbarium of the Faculty of Arts and Sciences, University of Fırat (FUH).

Average chromosome measurements were calculated on 10 metaphase plates. The quantitative values were obtained from chromosome character measurements. These were chromosome number, total length (C), long-arm length (L), short-arm length (S), arm ratio (r = L/S), centromeric index (I = 100 × S/C), relative length (RL = total length (C)/total haploid length × 100), and chromosome type. Karyotype asymmetry was estimated using the mean centromeric index, the ratio of the shortest-to-longest pairs, and the intrachromosomal asymmetry (A_1) and interchromosomal asymmetry (A_2) indices (Romero, 1986). To assess the existence of previously published chromosome counts in the species studied, we used the most common indexes of plant chromosome numbers (Vallès et al., 2001a, 2001b), previous publications (Vallès et al., 2005), and online chromosome number databases (Goldblatt & Johnson, 1979; Watanabe, 2010).

Results and discussion

The systematics of Asteraceae are very dynamic; every year, several new genera are described and many more are resurrected or moved into synonymy (Bremer, 1994; Vural et al., 2010; Hamzaoğlu et al., 2011). For the Turkish flora, one of the most recently resurrected genera is *Artemisia* (Kurşat et al., 2011a, 2011b). The infrageneric classification of *Artemisia* is an unresolved problem. Some authors have proposed different series of sections and subsections in *Artemisia* (Ling, 1991a, 1991b, 1995a, 1995b; Shishkin, 1995), but a global treatment of the entire genus at these levels has not yet been achieved. Many species of *Artemisia*, a parafiletic genus, are largely similar (Bremer, 1994). These similarities make it difficult to taxonomically classify the genus. In the present study, we have followed the nomenclature of *Flora of the USSR* as adopted by Shishkin (1995).

Chromosome numbers, ploidy levels, and basic numbers of all taxa studied are presented in Table 2. Eight of these taxa are given together with the karyotype characteristics, A_1 index, and A_2 index. Karyotypic characters of 4 taxa could not be obtained due to difficulties in their germination (*Artemisia chamaemelifolia* Vill., *A. absinthium* L., *A. arborescens* L., and *A. splendens* Willd.). The somatic metaphase chromosomes of 12 taxa are displayed in Figure 1. In addition, karyograms of 8 species are presented in Figure 2. Both the chromosome number and karyotype characters of *A. haussknechtii* are new reports for the species. Nine of the chromosome counts reported here confirm previous reports, and 2 counts (for *A. armeniaca* and *A. caucasica*) are the second reports for those species. Six species were diploid with 2n = 18, 3 species were diploid with 2n = 16, 1 species was tetraploid with 2n = 4x = 32, and 1 species was hexaploid with 2n = 6x = 48. In 1 species, the presence of B chromosomes was detected as 2n = 16 + 2B (*A. incana*). The chromosomes were all median (m) or submedian (sm) according to the classification system of Levan et al. (1964).

Table 2. Karyological data of the *Artemisia* species studied. [1]Total chromosome length [long-arm length (L) + short-arm length (S)], [2]arm ratio (r = L/S), [3]relative length, [4]chromosomal formula according to Levan et al. (1964); A_1: intrachromosomal asymmetry index, A2: interchromosomal asymmetry index, *new chromosome number record, **new karyotype formula record.

Taxa	2n	x	Ploidy level	C (μ)[1]	AR (μ)[2]	RL[3]	Karyotype formula[4]	A_1	A_2
A. vulgaris	16	8	2x	1.22-2.02	1.22-1.76	4.88-8.08	14m + 2sm	0.25	0.16
A. abrotanum	18	9	2x	1.56-2.42	1.18-1.64	4.49-6.97	18m**	0.26	0.14
A. austriaca	48	8	6x	1.09-2.63	1.10-1.71	1.40-3.38	46m + 2sm**	0.26	0.20
A. incana	16 + 2B	8	2x	2.14-4.07	1.09-1.51	4.88-9.28	16m + 2B**	0.24	0.21
A. armeniaca	18	9	2x	1.55-2.48	1.26-1.73	4.38-7.00	16m + 2sm**	0.30	0.14
A. chamaemelifolia	18	9	2x	-	-	-	-		
A. tournefortiana	18	9	2x	1.93-3.04	1.08-1.48	4.25-6.69	16m + $2m^{Sat}$	0.16	0.13
A. absinthium	18	9	2x	-	-	-	-		
A. arborescens	18	9	2x	-	-	-	-		
A. splendens	32	8	4x	-	-	-	-		
A. caucasica	16	8	2x	1.61-2.62	1.22-1.72	5.13-8.35	12m + 4sm**	0.28	0.15
A. haussknechtii	16*	8	2x	1.88-3.15	1.17-1.75	4.83-8.09	14m + 2sm**	0.24	0.16

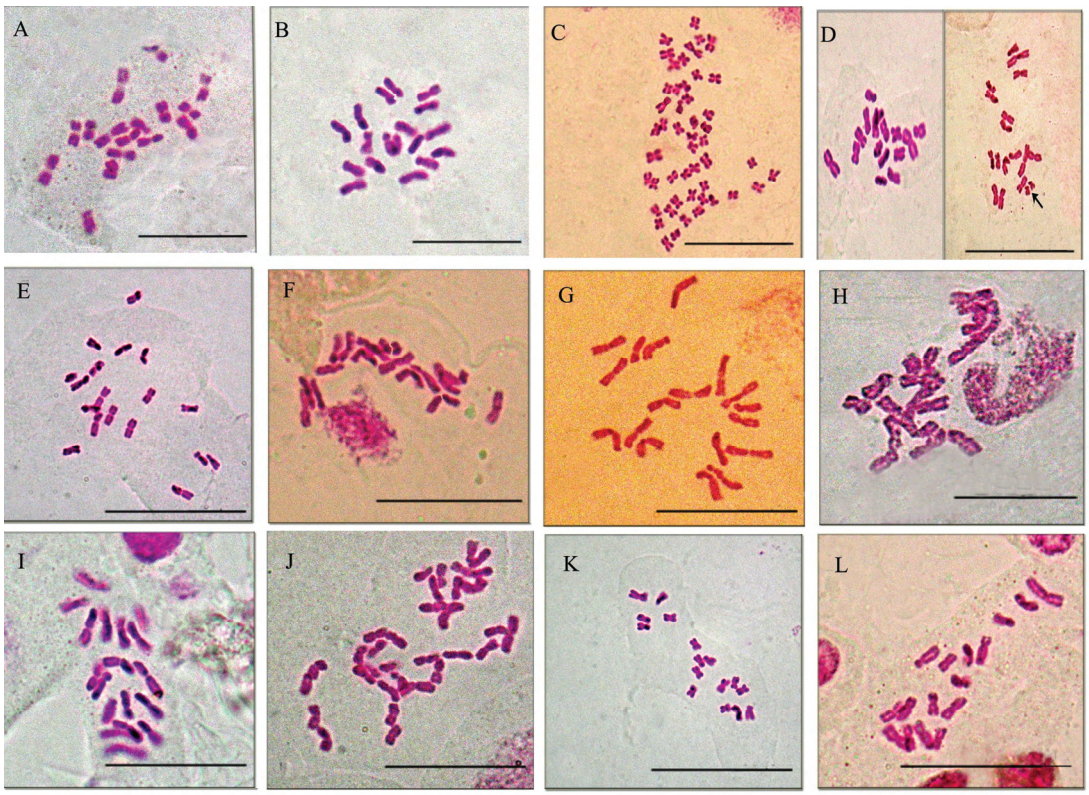

Figure 1. Micrographs of the somatic metaphase chromosomes of 12 taxa of the genus *Artemisia*: A- *A. vulgaris* (2n = 2x = 16), B- *A. abrotanum* (2n = 2x = 18), C- *A. austriaca* (2n = 6x = 48), D- *A. incana* (2n = 2x = 16) with arrow indicating the B chromosomes, E- *A. armeniaca* (2n = 2x = 18), F- *A. chamaemelifolia* (2n = 2x = 18), G- *A. tournefortiana* (2n = 2x = 18), H- *A. absinthium* (2n = 2x = 18), I- *A. arborescens* (2n = 2x = 18), J- *A. splendens* (2n = 4x = 32), K- *A. caucasica* (2n = 2x = 16), L- *A. haussknechtii* (2n = 2x = 16). Karyotypic characters of some taxa (F, H, I, and J- could not be obtained due to difficulties in their germination. Scale bars = 10 μm.

***Artemisia vulgaris* L. (2n = 2x = 16) 14m + 2sm (Figures 1 and 2)**

The most common ploidy of *A. vulgaris* in Canada and the United States is 2n = 16 (Radford et al., 1968; Gleason & Cronquist, 1991). The European genotypes are dibasic at n = 8 and 2n = 16 from Germany, while specimens from the high Himalayas, which underwent a period of glaciation, have been found to be exclusively n = 9 and 2n = 18 (Koul, 1964). At different elevations in the same region, Koul (1964) also found examples of tetraploid (2n = 36) and hexaploid (2n = 54) specimens. Karyological analysis has been performed on diploid European biotypes of *A. vulgaris*. Oliva and Vallès (1994) found the basic number to be n = 8 for all populations investigated, which is the accepted basic chromosome number. However, biotypes from other regions of the world have been reported to have chromosome numbers of 2n = 16, 18, 24, 36, and 45 (Vallès & Šiljak-Yakovlev, 1997).

According to our data, the chromosome number of this species is 2n = 2x = 16; this count was also confirmed by numerous other authors (Gabriellian & Vallès, 1996; Hoshi et al., 2003; Vallès et al., 2005; Pellicer et al., 2008). The chromosome numbers of this species were determined as 2n = 16 and 2n = 34 by Torrell and Vallès (2001), Vallès and McArthur (2001), and Bennett and Leitch (2005). However, data related to the karyotypes of the species are still scarce. Our karyological data agree entirely with those of Hoshi et al. (2003) (14m + 2sm), but differ from those of Pellicer et al. (2008) (14m + 2sm^{Sat}). The emergence of these karyological differences may result from habitat and climatic conditions.

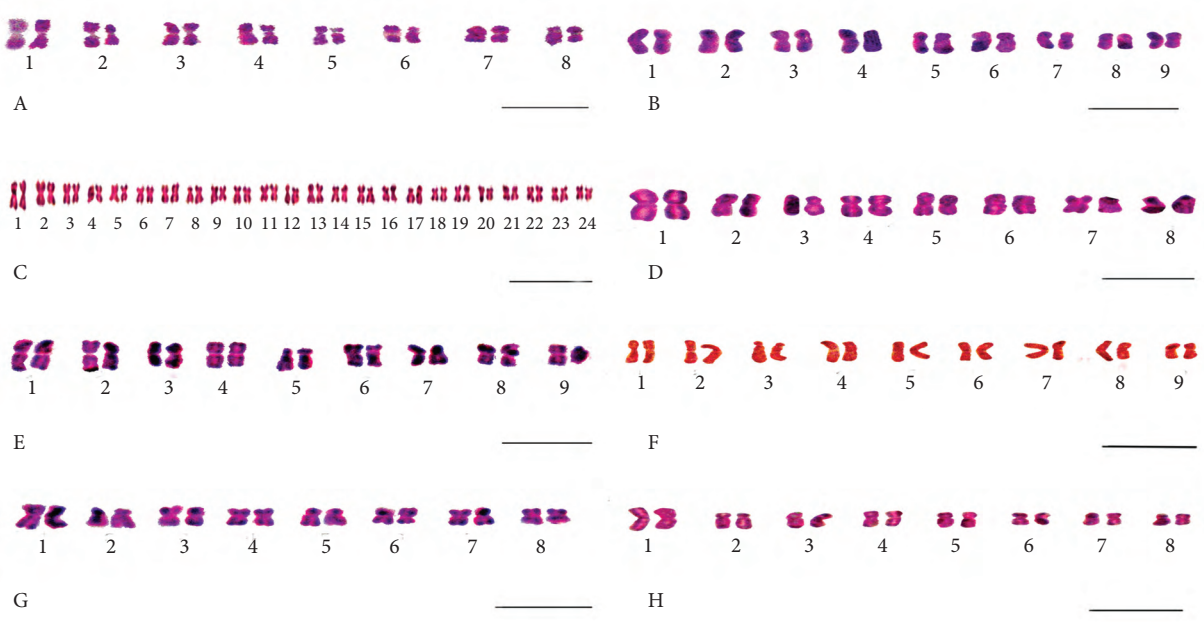

Figure 2. Karyomorphology of some *Artemisia* taxa observed: A- *A. vulgaris* (14m + 2sm), B- *A. abrotanum* (18m), C- *A. austriaca* (46m + 2sm), D- *A. incana* (16m + 2B) with arrow indicating the B chromosomes, E- *A. armeniaca* (16m + 2sm), F- *A. tournefortiana* (16m + $2m^{Sat}$), G- *A. caucasica* (12m + 4sm), H- *A. haussknechtii* (14m + 2sm). Karyotypic characters of *A. chamaemelifolia*, *A. absinthium*, *A. arborescens*, and *A. splendens* could not be obtained due to difficulties in their germination. Scale bars = 10 µm.

***Artemisia abrotanum* L. (2n = 2x = 18) 18m (Figures 1-2)**

Our report on the chromosome numbers of this species agrees with the 2 previous studies carried out by Johnson and Brandham (1997) and Zohary and Heywood (1997). However, Kreitschitz and Vallès (2003) found a record of the tetraploid level with 2n = 36, although they reported a diploid population (2n = 18). No data on any karyological characters have been reported until now. The data presented here are the first report of the karyological features of this species.

***Artemisia austriaca* Jacq. (2n = 6x = 48) 46m + 2sm (Figures 1-2)**

A. austriaca is a critical species from the karyotaxonomical point of view. Numerous authors have reported the existence of different chromosome numbers and ploidy levels for this species, including 2n = 16 (Inceer & Hayirlioglu-Ayaz, 2007), 2n = 32 (Lavrenko & Serditov, 1991), 2n = 36 (Kawatani & Ohno, 1964), 2n = 48 (Torrell et al., 2001), and 2n = 54 (Tavassoli & Derakhshandeh-Peikar, 1993).

According to our data, this hexaploid level is the first report for this species for Turkish material. There are no detailed data related to the karyological features of the species. The karyotype of *A. austriaca* is reported for the first time in this study.

***Artemisia incana* (L.) Druce (2n = 2x = 16, 2n = 16 + 2B) 16m + 2B (Figures 1-2)**

The chromosome number of the species was identified as 2n = 2x = 16. This count is in accordance with a prior report about an Iranian population in West Azerbaijan (Saedi et al., 2005). Torrell et al. (2001) reported that the species was 2n = 16, and their study was the third record for the species. The first record came from plants from Moscow studied by Kawatani and Ohno (1964). Moreover, these researchers reported that the chromosome number of this species was recorded as 2n = 6 by Fedorov (1969), but they argued that this was probably a typographical error (6 instead of 16) in the original publication. Indeed, among the present cytogenetic knowledge of the genus *Artemisia*, a basic number of x = 3 is not known. The chromosome number

of the species was reported as 2n = 2x = 18 by Chehregani and Mehanfar (2008). Chehregani et al. (2010) agreed that the basic chromosome number was x = 9 for *A. incana*, but in some populations, chromosome-sticking was a common phenomenon that caused a decrease in chromosome number from 2n = 2x = 18 to 2n = 2x = 16. The existence of 2 basic numbers (dysploidy), x = 8 and x = 9, appears in 3 of the main groups in the genus [*Artemisia* L., *Absinthium* (Tourn.) DC., and *Dracunculus* (Bess.) Rydb.] (Vallès & McArthur, 2001). Vallès and Šiljak-Yakovlev (1997) found evidence that the dysploidy in the genus *Artemisia* descends from x = 9 to x = 8 as a result of a chromosomal fusion. They suggested that the production of a very long metacentric chromosome pair in the x = 8 taxa was derived from this chromosomal fusion. Hence, Torrell et al. (2001) asserted that *A. incana* was a dysploid species. This long metacentric chromosome pair in *A. incana* was also seen in our study (Figure 1). However, the existence of 2 small acrocentric chromosomes is evidence that the species is not dysploid (Figure 1). We believe that this species contains 2 B chromosomes. It was also reported that the chromosome number for *A. incana* was 2n = 3x = 24 in a population collected from Jolfa, Iran (Chehregani et al., 2010). This situation supports the argument that the basic chromosome number of the species is x = 8 and that *A. incana* is not actually dysploid. The apparent presence of B chromosomes in the species may be caused by a kind of illusion. B chromosomes have been frequently observed in other subgenera of *Artemisia*, such as *Absinthium* (Pellicer et al., 2007b) or *Tridentatae* (Garcia et al., 2007). A variable number of B chromosomes (1-5) in *A. chamaemelifolia* belonging to subgenera *Artemisia* was reported (Pellicer et al., 2008). B or supernumerary chromosomes are extra chromosomes found in some, but not all, individuals within a species, and they have been described in many plants and animals; their function, composition, and origin are not completely known. The number of B chromosomes is also variable, from 1 to 3, when present (Trivers et al., 2004; Jones et al., 2008). Different kinds of B chromosomes might be found in plants and animals linked to their origin. That is, they may be acrocentric, metacentric, or acentric fragments (Vallès & Šiljak-Yakovlev, 1997; Houben et al., 1999; Garcia et al., 2007; Pellicer et al., 2007a, 2008). Frequently, the occurrence of this type of chromosome in *Artemisia* is a matter of debate (McArthur & Sanderson, 1999; Garcia et al., 2007).

There are no existing literature data on the karyological features of the species. The karyotype of this species is reported for the first time in the present work.

***Artemisia armeniaca* Lam. (2n = 2x = 18) 16m + 2sm (Figures 1-2)**

According to our data, the chromosome number of this species is 2n = 2x = 18 and its karyotype formula is 16m + 2sm. *A. armeniaca* is a very polymorphic species and it has a wide distribution (Gabriellian & Vallès, 1996). However, the only previous study on the chromosome number of this species as reported by Torrell et al. (2001) and our count are in agreement. To our knowledge, this count is the second record and the first karyotype analysis for this taxon.

***Artemisia chamaemelifolia* Vill. (2n = 2x = 18) (Figure 1)**

The chromosome number of the species was determined as 2n = 2x = 18. In our literature review, much information on the number of chromosomes of the species was found. The most commonly reported chromosome number is 2n = 18 (Vallès, 1987; Johnson & Brandham, 1997; Torrell et al., 2001; Bennett & Leitch, 2005; Pellicer et al., 2008). It was reported that the chromosome numbers of the species were 2n = 18 + 0-1B in an Iranian population and 2n = 36 in an Armenian population (Torrell et al., 2001). These researchers determined that the polyploids did not display significant morphological differences from the diploid populations. These researchers also reported that the diploid population of the species has B chromosomes. Pellicer et al. (2008) suggested that the species is 2n = 18, carrying 1-5 B chromosomes, and its karyotype formula is $10m + 2m^{Sat} + 4sm + 2sm^{Sat}$.

***Artemisia tournefortiana* Rchb. (2n = 2x = 18) 16m + $2m^{Sat}$ (Figures 1-2)**

The found chromosome number of the species (2n = 2x = 18) is in agreement with many other counts in surveys of published chromosome numbers (Vallès, 1987; Torrell & Vallès, 2001; Vallès et al., 2001b;

Bennett & Leitch, 2005). However, karyological information on the species is scarce. Our findings related to the karyological characteristics of the species are consistent with those of Vallès (1987).

***Artemisia absinthium* L. (2n = 2x = 18) (Figure 1)**

According to our data, this count confirms some previous ones from different populations (Zohary & Heywood, 1997; Torrell & Vallès, 2001, Vallès et al., 2001a; Bennett & Leitch, 2005; Inceer & Hayirlioglu-Ayaz, 2007). Kreitschitz and Vallès (2003) suggested that the chromosome number of *A. absinthium* was 2n = 18 in most of the individuals of the population studied and 2n = 36 in 5 meristems from 4 individuals. These researchers reported that 2n = 36 was the first record of the tetraploid level of this species. The karyotype formula of the species was determined as 12m + 2sm+ 4sm^{Sat} (Kokubugata et al., 2002), 12m + 2m^{Sat} + 4sm (Pellicer et al., 2008), and 14m + 2m^{Sat} + 2st (Nazırzadeh et al., 2009) in several recent reports. However, in the present work, the karyotypic characters of the species could not be determined.

***Artemisia arborescens* L. (2n = 2x = 18) (Figure 1)**

Information on the chromosome numbers and karyological data on *A. arborescens* is quite limited. We recognised its chromosome number as 2n = 2x = 18. The chromosome number found confirms the limited previous reports (Vallès et al., 2005; Abd El-Twab et al., 2008). The karyotype formula of the species was determined as 14m + 4sm (Abd El-Twab et al., 2008).

***Artemisia splendens* Willd. (2n = 4x = 32) (Figure 1)**

According to our data, the somatic chromosome number of this species is 2n = 4x = 32 and its basic number is also x = 8. This finding agrees with those of Torrell and Vallès (2001), Torrell et al. (2001), and Bennett and Leitch (2005), but differs from that of Kawatani and Ohno (1964) (2n = 18, x = 9). In a phylogenetic study by Torrell et al. (1999), this species was classified together with other species with the basic chromosome number x = 8. As mentioned above (*A. incana*), the existence of 2 long metacentric chromosomes may be evidence of descending dysploidy in the genus. No karyological data on this species have been reported to date.

***Artemisia caucasica* Willd. (2n = 2x = 16) 12m + 4sm (Figures 1-2)**

A. caucasica has a high economic value for ornamentation (Vallès & McArthur, 2001). To our knowledge, this is the second report of the chromosome number of the species. Our count confirms the only previous study, by Johnson and Brandham (1997). There are no literature data related to the karyological features of the species. The karyotype of this species is reported for the first time in the present work.

***Artemisia haussknechtii* Boiss. (2n = 2x = 16) 14m + 2sm (Figures 1-2)**

The chromosome number of this species was found to be 2n = 2x = 16, and its karyotype formula is 14m + 2sm. No data related to the chromosome number or karyological features of the species have been reported so far. This work is the first report of both the chromosome number and the karyological features of this species.

In general, the results obtained confirm the existence of 2 basic chromosome numbers in the genus. Six of the *Artemisia* species studied have the basic number, x = 9, predominant in the entire family Asteraceae (Oliva & Vallès, 1994). The other 6 *Artemisia* species have the less common number x = 8. Descending dysploidy resulting from chromosome fusion was confirmed in 1 species studied (*A. splendens*) according to our results. It has been supposed that the dysploidy may take place in *Artemisia* during the spread and evolution of this genus, which probably contributes to its adaptation to different ecological factors (Zhao et al., 2009). This phenomenon is common in many genera of the family Asteraceae (Vallès & Šiljak-Yakovlev, 1997; Torrell et al., 2001; Vallès et al., 2001a, 2001b).

Polyploidy is another relevant evolutionary mechanism in some groups of Asteraceae (Vallès et al., 2001a, 2001b). Polyploidy has been ubiquitous in plant evolution and is thought to be an important engine of biodiversity that facilitates speciation, adaptation, and range expansion. Polyploid species can exhibit higher ecological tolerance than their progenitor species. For allotetraploid species, this higher tolerance is often attributed to the existence of heterosis, resulting from entire genome duplication.

However, multiple origins of allopolyploid species may further promote their ecological success by providing genetic variability in ecological traits underlying local adaptation and range expansion (Meimberg et al., 2009). Only 2 species studied in this research are polyploid (1 tetraploid and 1 hexaploid). The chromosome number of *A. austriaca* is the first report of the hexaploid level (2n = 4x = 48) for this species. B chromosomes, common in other subgenera of *Artemisia* (Pellicer et al., 2007b; Garcia et al., 2007), were observed in 1 species studied (*A. incana*, 2n = 2x = 16 + 2B).

In the genus *Artemisia*, due to the interspecific and intraspecific similarity of chromosome conformation, it is difficult to clarify and justify the systematic relationships using karyotypes (Hoshi et al., 2003). The results obtained from this research have allowed us to compare the karyotype formula of some species of *Artemisia*. In the present work, we report chromosome numbers for 12 taxa of the genus *Artemisia*, and 1 of these chromosome numbers was counted here for the first time (*A. haussknechtii*). The chromosome complements of 6 of 10 taxa with unknown karyological features to date were presented for the first time in this study. All of the studied species exhibited very similar karyotypes, with chromosome pairs barely distinguishable from one another. The chromosomes of all of the species studied were median (m) or submedian (sm). The longest chromosome complement was found in *A. incana* (2.14-4.07 μm) while the shortest was observed in *A. vulgaris* (1.22-2.02 μm). *A. tournefortiana* had a satellite connecting to the short arm of chromosome 3 (Table 2).

In the present work, we have provided additional karyomorphological parameters using A_1 and A_2 indices, which do not depend on chromosome number or chromosome size (Romero, 1986). *A. tournefortiana* had the lowest A_1 value (0.16), while *A. armeniaca* had the highest A_1 value (0.30) of all the species. The differences in A_1 values among the other species were not significant (0.24-0.28). *A. tournefortiana* showed the lowest A_2 value (0.13), and *A. abrotanum* and *A. armeniaca* had relatively small A_2 values (0.14). None of the other species were significantly different from each other (0.15-0.21). The results for A_1 and A_2 values are shown in Table 2 and plotted in a scatter diagram in Figure 3.

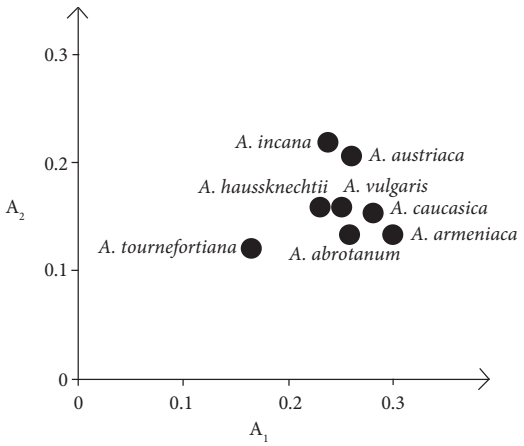

Figure 3. Scatter diagram of intrachromosomal (A_1) and interchromosomal (A_2) asymmetry of *Artemisia* taxa investigated.

In the subgenus *Artemisia*, the basic chromosome numbers are difficult to distinguish in the A_1 index. Species with x = 8 showed intermediate A_1 values (0.24-0.28), while species with x = 9 showed higher A_1 values (0.26-0.30), except for *A. tournefortiana* (0.16). On the other hand, the A_2 index indicates a difference between 2 basic chromosome numbers of *Artemisia*. Species with x = 9 had relatively small A_2 values (0.13-0.14), while species with x = 8 had higher A_2 values (0.15-0.21). Each species seems to be more affected by A_1 values than by A_2 values. Stebbins (1971) suggested that asymmetrical karyotypes are more advanced than symmetrical ones in relation to phylogeny and evolutionary processes. Matoba et al. (2007) reported that x = 9 was the ancestral basic chromosome number of the genus *Artemisia* while x = 8 was advanced, and species based on x = 17 in this section were plotted between species with x = 9 and x = 8. Our asymmetry index based on A_1 values shows that the tendency of chromosomes in the *Artemisia* species studied here evolved, and *A. armeniaca* is presumably more advanced than other species in relation to chromosomal evolutionary processes (Figure 3). Therefore, it seems that the A_1 parameter is useful for estimation of evolutionary relationships among some subgenera or sections of genus *Artemisia*.

Concluding remarks

The abundance of polyploids and the existence of species with the 2 basic chromosome numbers in the

genus *Artemisia* may lead to misunderstandings of the relationships between species. Therefore, many more investigations in karyomorphological analyses, molecular cytogenetics, and molecular phylogenetics are necessary to clarify and justify the *Artemisia* species' taxonomical relationships, evolution, and polyploidisation mechanisms. We believe that such comprehensive studies will contribute to future revisionist, monographic, and floristic studies. Karyological (from chromosome counts to molecular cytogenetics) and molecular (combining different techniques in as many taxa as possible) studies in *Artemisia* and the subtribe to which it belongs (Artemisiinae) will also be effective tools in better systematic definition.

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