

Karyological notes on another eight species of *Achillea* (Asteraceae) from Turkey

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Abstract: The chromosome number and morphology in eight species of the sections Ptarmica (Mill.) W. D. J. Koch, Anthemoideae (DC.) Heimerl, Arthrolepis Boiss., Santolinoideae (DC.) Heimerl and Achillea of the genus *Achillea* L. (Asteraceae) were investigated using karyological techniques. Sample plants and seeds of *A. biserrata* M. Bieb., *A. fraasii* var. *troiana* Aschers. & Heimerl, *A. multifida* (DC.) Boiss., *A. brachyphylla* Boiss. & Hausskn., *A. pseudaleppica* Hub.-Mor., *A. cretica* L., *A. latiloba* Ledeb. ex Nordm., and *A. kotschy* Boiss. subsp. *kotschy* were collected from natural habitats in 2003 and 2004. The chromosome number found in seven species was $2n = 18$, while only *A. kotschy* had $2n = 36$. All chromosomes had median point (M), median region (m), and submedian (sm) centromeres. In addition, only *A. biserrata* species had one subterminal (st) chromosome. An increase in asymmetry was not observed in the karyotypes of the species studied. None of the studied species had any B chromosomes.

Key words: *Achillea*; chromosome number; karyotypes; mitosis; Turkey

Introduction

The genus *Achillea* L. has approximately 130 species (Saukel et al. 2004; Guo et al. 2004). Most of the species are distributed in Eurasia, some in North Africa, and a few can be found in North America and in the Southern Hemisphere (Post 1933; Bremer & Humphries 1993; Bremer 1994; Zheng-Yi & Raven 1994). The genus is also widespread in Anatolia, with 44 species (including 50 taxa) belonging to six sections, out of which 22 species are endemic to Turkey (Huber-Morath 1975; Valant-Vetchera & Kästner 1998; Demirkus 1999; Duman 2000).

The basic chromosome number in *Achillea* is $x = 9$, with polyploidy occurring frequently. The karyology of the genus has been studied by several researchers and reported chromosome numbers include $2n = 18, 36, 54$, and 72 (Lawrence 1947; Contandriopoulos & Martin 1967; Löve 1972, 1973; Oświecimska 1974; Tutin et al. 1980; Androschchuk & Kostinenko 1981; Dąbrowska 1989, 1992; Maffei et al. 1993; Danihelka & Rotreklová 2002, 2001; Constantinidis & Kalpoutzakis 2005). Only a few cytological studies/papers have been published on the genus in Turkey. One study was conducted on *A. multifida*, *A. setacea*, and *A. spinulifolia* (Martin-Noguét 1969) and another one on *A. sipikorensis* and *A. sintenisii* (Turkoglu & Akpulat 2004), and the third one on *Achillea cucullata*, *A. vermicularis*, *A. monocephala*, *A. boissieri*, *A. santolina*, *A. gypsicola*, *A.*

goniocephala, and *A. spinulifolia* (Sahin et al. 2006). These indicated that the chromosome numbers and chromosome morphology were unknown for many of the *Achillea* species in Turkey. The aim of this study was to determine the chromosome number and morphology in further *Achillea* species present in Turkey.

Material and methods

Plant samples and their seeds were collected from natural habitats in 2003 and 2004, and herbarium specimens were deposited at the Inonu University Herbarium. The localities are presented in the results according to the grid system adopted for the Flora of Turkey by Davis (1975), geographical position, altitude and voucher number of the investigated samples. Seeds were germinated at 25°C on moist filter paper in Petri dishes. Actively-growing root tips, 1 cm in length, were excised from the germinating seeds and pretreated with aqueous colchicine (0.05%) for 3–3.5 h at room temperature. They were fixed with Carnoy (1:3 glacial acetic acid/absolute ethanol) for at least 24 h at 4°C, hydrolysed in 1N HCl at 60°C for 15 min and then rinsed in tap water for a minimum of 3–5 min. Staining was carried out in Feulgen for 1 h and squash preparations were made with 45% acetic acid (Elci 1982). Microphotographs of good quality metaphase plates of each specimen (normally at least three) were taken using an Olympus BX51 microscope and were recorded with an Olympus Camedia C-4000 digital camera. Karyotypes were obtained from well-spread metaphase plates. Long arm, short arm and total length of each chromosome were measured and relative lengths,

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Table 1. Somatic chromosome number, ploidy level, karyotype formula, ranges of chromosome lengths, total karyotype length (TKL), asymmetry index (A1, A2; Romero Zarco 1986) and symmetry classes (SC; Stebbins 1971) for the *Achillea* species investigated.

Taxon	2n	Ploidy level	Karyotype formula	Chromosome length range (μm)	TKL (μm)	A1	A2
<i>A. biserrata</i>	18	2X	2M+5m+1sm+1st	3.38–4.07	33.18	0.24	0.05
<i>A. fraasii</i> var. <i>troiana</i>	18	2X	2M+6m+1sm	3.22–4.83	35.22	0.24	0.13
<i>A. multifida</i>	18	2X	2M+5m+2sm	3.05–4.27	32.83	0.27	0.11
<i>A. brachyphylla</i>	18	2X	3M+6m	4.83–7.55	55.62	0.14	0.16
<i>A. pseudaleppica</i>	18	2X	2M+7m	4.38–6.00	46.65	0.14	0.10
<i>A. cretica</i>	18	2X	1M+4m+4sm	2.94–4.61	32.89	0.29	0.15
<i>A. latiloba</i>	18	2X	1M+7m+1sm	2.77–3.99	30.66	0.28	0.11
<i>A. kotschy</i> subsp. <i>kotschy</i>	36	4X	2M+15m+1sm	2.07–2.98	46.02	0.26	0.10

arm ratios and centromeric indices were determined. The chromosome nomenclature followed Levan et al. (1964). The intra-chromosomal asymmetry index (A_1) was calculated according to the formula proposed by Romero Zarco (1986), while the inter-chromosomal asymmetry index (A_2) was measured as the ratio of chromosome length/mean chromosome length. The karyotype symmetry nomenclature followed Stebbins (1971).

Results

Achillea sect. *Ptarmica*

Achillea biserrata M. Bieb. – $2n = 18$

A7 Trabzon: from Gumushane to Trabzon, 41 km, Zigana pass ($40^\circ 41' \text{ N}$, $39^\circ 30' \text{ E}$), 1 600 m a.s.l., 31.vii.2003, T. Arabaci 1605.

The species is Euxine element. Its chromosome number is $2n = 18$ and this is the first report on the chromosome number and morphology of this species. The ratio of the longest chromosomes to the shortest ones is 1.2:1 and karyotype symmetry is 2A. It consists of two median point (M), five median region (m), one submedian (sm), and one subterminal (st) chromosomes. Satellites were not observed in karyotype of this species (Tables 1, 2, Figs 1, 9A).

Achillea sect. *Anthemoideae*

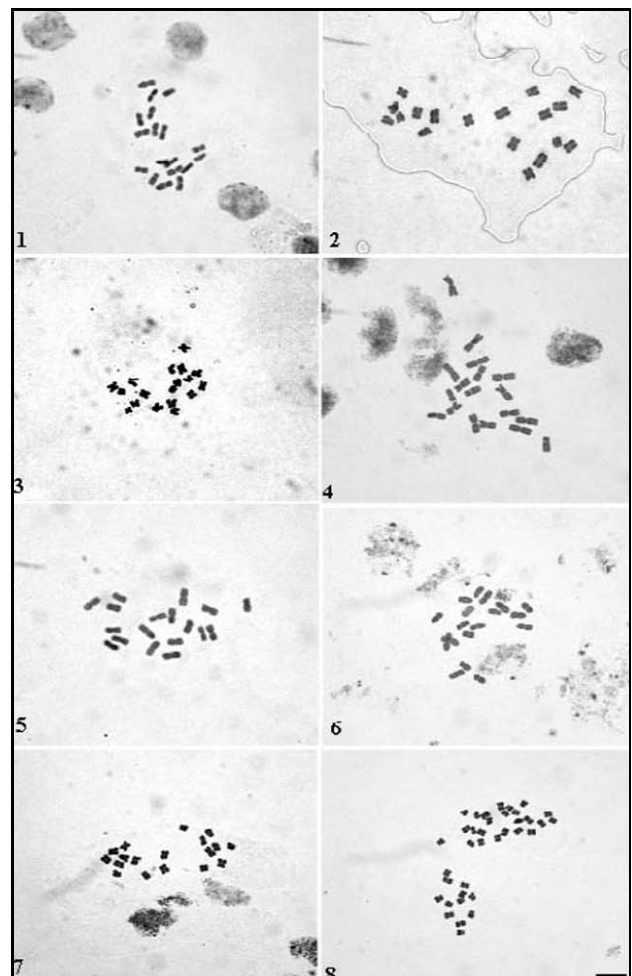
Achillea fraasii var. *troiana* Aschers. & Heimerl – $2n = 18$

B1 Balıkesir, Edremit, Kaz Mountain., Sarikiz Hill ($39^\circ 42' \text{ N}$, $26^\circ 52' \text{ E}$), 1 700 m a.s.l., calcareous substrate 26.vi.2002, B. Yildiz 15282.

The species is endemic to the Eastern Mediterranean element of Turkey. Its chromosome number is $2n = 18$ and this is the first report on the chromosome number and morphology of this species. The ratio of the longest chromosome to the shortest one is 1.5:1 and karyotype symmetry is 2A. It consists of two median point (M), six median region (m), and one submedian (sm) chromosomes. Satellites were not observed in the karyotype of this species (Tables 1, 2, Figs 2, 9B).

Achillea multifida (DC.) Boiss. – $2n = 18$

A2(A) Bursa, Uludag surroundings of hotels ($40^\circ 07' \text{ N}$,



Figs 1–8. Metaphase chromosomes of *Achillea* species. 1 – *A. biserrata* ($2n = 18$), 2 – *A. fraasii* var. *troiana* ($2n = 18$), 3 – *A. multifida* ($2n = 18$), 4 – *A. brachyphylla* ($2n = 18$), 5 – *A. pseudaleppica* ($2n = 18$), 6 – *A. cretica* ($2n = 18$), 7 – *A. latiloba* ($2n = 18$), 8 – *A. kotschy* subsp. *kotschy* ($2n = 36$). Scale bar = 10 μm .

$29^\circ 08' \text{ E}$), 1 800–1 900 m a.s.l., 04.viii. 2004, B. Yildiz 15889.

The species is endemic to the Uludag Mts. in Turkey. Its chromosome number is $2n = 18$. The ratio of the longest chromosome to the shortest one is 1.4:1 and karyotype symmetry is 1A. It consists of two median point (M), five median region (m) and two sub-

Table 2. Karyomorphological parameters of the *Achillea* species investigated. Abbreviations: RL, relative length; AR, arm ratio (L/S); CI, centromeric index (100S/TL); m, median region; sm, submedian region; M, median point. Chromosome pairs are assigned Roman numerals.

Pair No.	RL	AR	CI	Type	Pair No.	RL	AR	CI	Type
<i>A. biserrata</i>					<i>A. fraasii</i> var. <i>troaina</i>				
I	12.28	1.16	46.32	m	I	13.70	1.29	43.72	m
II	11.20	1.03	49.24	m	II	12.26	1.29	43.71	m
III	11.52	1.30	43.42	m	III	11.98	1.00	50.00	M
IV	11.37	1.00	50.00	M	IV	11.66	1.24	44.64	m
V	10.03	1.00	50.00	M	V	10.71	1.00	50.00	M
VI	10.03	1.20	45.36	m	VI	10.71	1.34	42.67	m
VII	10.69	3.27	23.40	st	VII	10.08	2.04	32.82	sm
VIII	10.69	2.55	28.20	sm	VIII	9.76	1.58	38.70	m
IX	10.20	1.35	42.56	m	IX	9.13	1.64	37.93	m
<i>A. multifida</i>					<i>A. brachyphylla</i>				
I	13.02	1.48	40.25	m	I	13.58	1.00	50.00	M
II	12.85	1.38	42.10	m	II	13.27	1.15	46.61	m
III	11.83	1.33	42.83	m	III	12.27	1.24	44.70	m
IV	11.14	1.54	39.38	m	IV	12.18	1.00	50.00	M
V	11.81	1.78	35.96	sm	V	10.78	1.00	50.00	M
VI	11.80	1.00	50.00	M	VI	10.69	1.23	44.87	m
VII	10.14	1.61	38.34	m	VII	9.78	1.33	42.89	m
VIII	10.13	1.00	50.00	M	VIII	8.79	1.44	40.93	m
IX	9.28	1.74	36.43	sm	IX	8.68	1.23	44.89	m
<i>A. pseudaleppica</i>					<i>A. cretica</i>				
I	12.85	1.16	46.30	m	I	14.01	1.18	45.88	m
II	12.36	1.00	50.00	M	II	12.65	1.27	43.99	m
III	11.77	1.20	45.42	m	III	12.14	1.25	44.41	m
IV	11.42	1.18	45.81	m	IV	11.29	1.92	34.29	sm
V	11.06	1.27	44.12	m	V	11.13	1.00	50.00	M
VI	10.93	1.00	50.00	M	VI	10.62	1.74	36.55	sm
VII	10.23	1.15	46.54	m	VII	9.79	1.90	34.47	sm
VIII	9.98	1.27	44.02	m	VIII	9.45	1.80	35.73	sm
IX	9.39	1.26	44.29	m	IX	8.93	1.28	43.81	m
<i>A. latiloba</i>									
I	13.01	1.25	44.36	m					
II	12.31	1.43	41.17	m					
III	11.59	1.56	39.12	m					
IV	11.57	1.00	50.00	M					
V	11.22	1.96	33.82	sm					
VI	10.84	1.31	43.33	m					
VII	10.48	1.32	43.15	m					
VIII	9.94	1.39	41.79	m					
IX	9.05	1.62	38.10	m					

median (sm) chromosomes. Satellites were not observed in the karyotype of this species (Tables 1, 2, Figs 3, 9C).

Achillea sect. *Arthrolepis*

Achillea brachyphylla Boiss. & Hausskn. – $2n = 18$
C7 Sanliurfa: from Sanliurfa to Viransehir, Tek Tek Mountain, Coban Bogazi pass, calcareous rocks (37°14' N, 35°21' E), 500 m a.s.l., 24.v.2003, T. Arabaci 1512.

The species is endemic to the Irano-Turanian element of Turkey. Its chromosome number is $2n = 18$ and this is the first report on the chromosome number and morphology of this species. The ratio of the longest chromosome to the shortest one is 1.5:1 and karyotype symmetry is 1A. It consists of three median point (M) and six median region (m) chromosomes. Satellites were

not observed in the karyotype of this species (Tables 1, 2, Figs 4, 9D).

Achillea sect. *Santolinoideae*

Achillea pseudaleppica Hub.-Mor. – $2n = 18$
C8 Mardin: from Mardin to Kiziltepe, 2 km from slopes (37°19' N, 40°46' E), 700 m a.s.l., 07. vi. 2002, B. Yildiz & T. Arabaci 1389.

The species is endemic to the Irano-Turanian element of Turkey. Its chromosome numbers is $2n = 18$ and this is the first report on the chromosome number and morphology of this species. The ratio of the longest chromosome to the shortest one is 1.3:1 and karyotype symmetry is 1A. It consists of two median point (M) and seven median region (m) chromosomes. Satellites

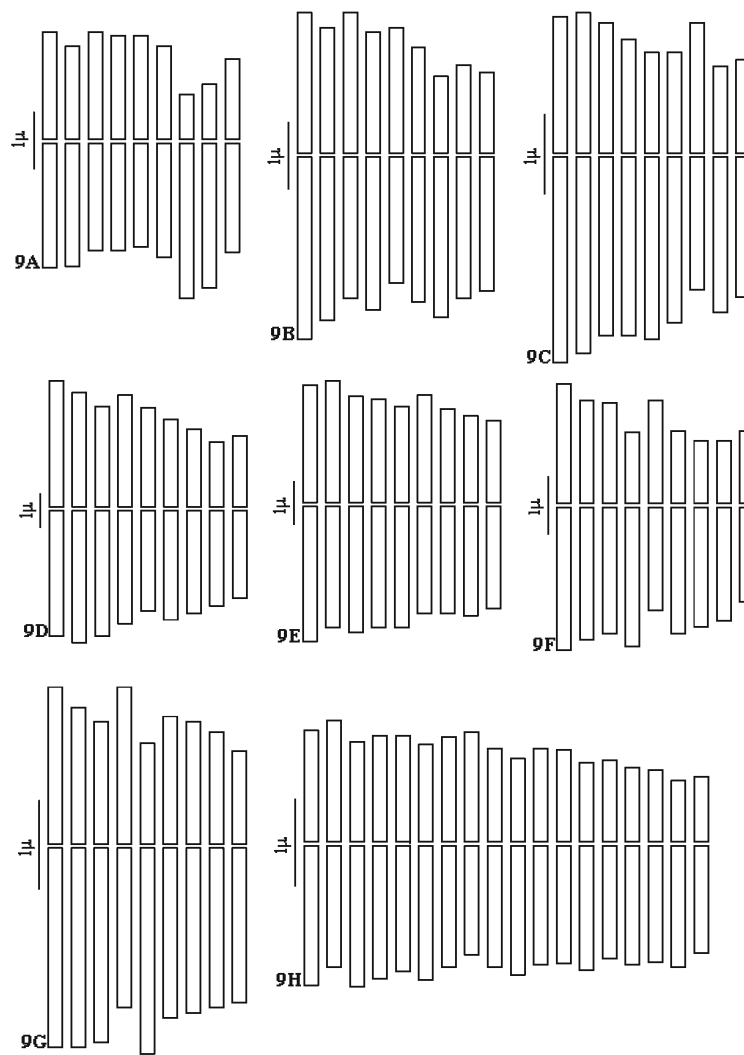


Fig. 9. Haploid idiograms. A – *Achillea biserrata*, B – *A. fraasii* var. *troiana*, C – *A. multifida*, D – *A. brachyphylla*, E – *A. pseudaleppica*, F – *A. cretica*, G – *A. latiloba*, H – *A. kotschy* subsp. *kotschy*.

were not observed in the karyotype of this species (Tables 1, 2, Figs 5, 9E).

Achillea cretica L. – $2n = 18$

C2 Mugla: Datca, Knidos ($36^{\circ}43' N$, $27^{\circ}34' E$), in ancient ruins 0–520 m a.s.l., 19. vi. 2004, B. Yildiz 15634.

The species is Eastern Mediterranean element. Its chromosome number is $2n = 18$ and this is the first report on the chromosome number and morphology of this species. The ratio of the longest chromosome to the shortest one is 1.5:1, and karyotype symmetry is 1A. It consists of one median point (M), four median region (m), and four submedian (sm) chromosomes. Satellites were not observed in the karyotype of this species (Tables 1, 2, Figs 6, 9F).

Achillea sect. *Achillea*

Achillea latiloba Ledeb. ex Nordm. – $2n = 18$

A8 Rize: İkizdere, Cimil plateau, Baskoy, ($40^{\circ}45' N$, $45^{\circ}40' E$), shrubberies, 2 200 m a.s.l., 01. viii. 2003, T. Arabaci 1612.

The species is Euxine element. Its chromosome numbers is $2n = 18$ and this is the first report on the chromosome number and morphology of this species. The ratio of the longest chromosome to the shortest one is 1.4:1 and karyotype symmetry is 1A. It consists of one median point (M), seven median region (m), and one submedian (sm) chromosomes. No satellites were observed in the karyotype of this species (Tables 1, 2, Figs 7, 9G).

Achillea kotschy Boiss. subsp. *kotschy* – $2n = 36$

B6 Kahramanmaraş: Yesilkent, Binboga Mountain, Dayioluk ($38^{\circ}15' N$, $36^{\circ}35' E$), 2 100–2 600 m a.s.l., 22. vii. 2004, T. Arabaci 1950.

Its chromosome number is $2n = 36$ and this is the first report on the chromosome number and morphology of this species. The ratio of the longest chromosome to the shortest one is 1.4:1 and karyotype symmetry is 1A. It consists of two median point (M), fifteen median region (m), and one submedian (sm) chromosomes. Satellites were not observed in the karyotype of this species (Tables 1, 3, Figs 8, 9H).

Table 3. Karyomorphological parameters of *Achillea kotschyi* subsp. *kotschyi*: RL, relative length; AR, arm ratio (L/S); CI, centromeric index (100S/TL); m, median region; sm, submedian region; M, median point. Chromosome pairs are assigned Roman numerals.

Pair No	RL	AR	CI	Type
<i>A. kotschyi</i> subsp. <i>kotschyi</i>				
I	6.48	1.26	44.30	M
II	6.26	1.00	50.00	M
III	6.21	1.40	41.61	M
IV	6.19	1.25	44.38	M
V	6.00	1.18	45.77	M
VI	5.97	1.38	41.99	M
VII	5.82	1.16	46.27	M
VIII	5.66	1.00	50.00	M
IX	5.56	1.31	43.36	M
X	5.49	1.56	39.05	M
XI	5.47	1.29	43.58	M
XII	5.42	1.29	43.58	M
XIII	5.29	1.56	39.12	M
XIV	5.06	1.38	41.98	M
XV	5.01	1.60	38.49	M
XVI	4.87	1.63	38.10	M
XVII	4.76	1.96	33.79	Sm
XVIII	4.49	1.67	37.42	M

Discussion

In this study, the karyology of eight species of *Achillea* in Turkey, four of which are endemic to examined region. No previous studies have been made on the chromosomes of these species, apart from one on *A. multifida* (Martin-Noguét 1969), which reported $2n = 18$ in agreement with our results. In all of the examined species the basic chromosome number was $x = 9$, with *A. biserrata*, *A. fraasii* var. *troiana*, *A. multifida*, *A. brachyphylla*, *A. pseudaleppica*, *A. cretica*, and *A. latiloba* being diploid and *A. kotschyi* subsp. *kotschyi* tetraploid. These findings are in good agreement with the basic number and chromosome numbers given for *Achillea* species in previous studies (Lawrence 1947; Contandriopoulos & Martin 1967; Martin-Noguét 1969; Löve 1972, 1973; Oświecimska 1974; Tutin et al. 1980; Androschchuk & Kostinenko 1981; Dąbrowska 1989, 1992; Maffei et al. 1993; Danihelka & Rotreklová 2001, 2002; Turkoglu & Akpulat 2004; Constantinidis & Kalpoutzakakis 2005; Sahin et al. 2006). Samples of *Achillea* species with chromosomes number of $2n = 54$ and $2n = 72$ were reported by Danihelka & Rotreklová (2001) and Sahin et al. (2006), but this number was not observed in the species examined in the present study. B chromosomes, which are quite common within the *A. millefolium* group (Ehrendorfer 1959), further reported in *A. pratensis* by Danihelka & Rotreklová (2001) and in *A. spinulifolia* by Sahin et al. (2006), were not found in any of the *Achillea* species examined in the present study. In general, there was no obvious difference of karyotype asymmetry among the studied species, all having a symmetrical karyotype structure, as most chromosomes had median and submedian cen-

tromeres (Romero Zarco 1986; Stebbins 1971). *Achillea kotschyi*, believed to be a tetraploid derivative of the cross between *A. nobilis* and a member of the *A. millefolium* group (due to its morphology), was shown to be tetraploid, which is the chromosome number found also in its Dinaric counterpart *A. virescens* (Guo et al. 2004). Including the counts presented here, only diploids (with minor exceptions; Guo et al. 2004) have been found in the sections Anthemoideae and Ptarmica. The diploid count in *A. brachyphylla* is the first count ever done in a member of sect. Arthrolepis. Even though the two species of sect. Santolinoideae investigated here were diploid, the counts presented elsewhere (Sahin et al. 2006) suggest that polyploidization was an important evolutionary pathway not only within sect. *Achillea* (Ehrendorfer 1959) but probably also within sect. Santolinoideae.

The present study is only a part of a planned larger study on the karyological characteristics of all *Achillea* species in Turkey, so a comprehensive analysis of chromosomal similarities and differences among them will be done when our survey is completed.

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