

Chromosome numbers and morphology of eighteen Anthemideae (Asteraceae) taxa from China and their systematic implications

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Abstract — The chromosome number and morphology of eighteen taxa belonging to seven genera in Anthemideae endemic to China were investigated using karyological technique. Chromosome numbers of six species i.e. *Ajania kbartensis* (Dunn) C. Shih ($2n=4x=36$), *A. potaninii* (Krasch.) Poljakov ($2n=2x=18$), *A. remotipinna* (Hand.-Mazz.) Y. Ling ex C. Shih ($2n=2x=18$), *Phaeostigma variifolium* (Chang) Muldashev ($2n=2x=18$), *Achillea acuminata* (Ledeb.) Sch. Bip ($2n=2x=18$) and *A. wilsoniana* Heimerl ex Hand.-Mazz. ($2n=4x=36$) are firstly reported, and new ploidy level of *A. fastigiata* (C.Winkl.) Poljakov ($2n=4x=36$) and *Hippolytia alashanensis* (Y. Ling) C. Shih ($2n=2x=18$) is reported for the first time. The further karyological studies show that most of the studied taxa have more symmetrical karyotype 2A (that of *N. pectinata* is 1A), and only Jingyuan (JY) population of *A. przewalskii* and *A. remotipinna* have more asymmetrical karyotype 2B. The correlations among the ploidy, geographic distribution and morphology are further discussed.

Key words: Anthemideae, chromosome morphology, chromosome number.

INTRODUCTION

Anthemideae (Asteraceae) is a medium-sized tribe consisting about 109 genera and 1740 species including many useful species such as chrysanthemums, daisies, chamomiles, tarragons, as well as the widespread sagebrushes, which dominate most cold and many warm deserts in the Northern Hemisphere (BREMER and HUMPHRIES 1993; WATSON *et al.* 2000; 2002). Nearly 30 genera (belonging to 7 subtribes) endemic to eastern Asia particularly in China and 8 genera (belonging to 4 subtribes) are introduced to cultivation. Subtribe Artemisiinae, the most important subtribe of Anthemideae, in which 15 genera are distributed to eastern Asia and 8 of 15 genera are endemic to the region. Taxa in Artemisiinae can be classified into two large groups i.e. the *Artemisia* L. group and the radiate group in term of the morphological characteristics (BREMER and HUMPHRIES 1993). However, phylogenetic analysis based on the ITS sequences doesn't support the classification based

on morphology (WATSON *et al.* 2002; VALLÈS *et al.* 2003; OBERPRIELER *et al.* 2007). Moreover, intergeneric hybridizations between these genera are readily obtainable (OHISHI *et al.* 1996; ABD EL-TWAB *et al.* 1999a; ABD EL-TWAB and KONDO 1999b; 2001; KONDO *et al.* 2002; 2003; YIN 2005; LI 2006; ZHAO *et al.* 2007), further suggesting the complicated phylogenetic relationship between genera in this subtribe. The systematic relationships of genera within the subtribe remain complex, which should be further investigated and illustrated.

The attempt to reveal the relevance of karyological information and systematic knowledge of genera in this tribe, particularly those discretely distributed and eastern Asian endemic taxa, is of great value. Karyological information of a large number of Chinese taxa are available (LI *et al.* 1983; QIAO, YAN and ZHANG, 1990; WANG *et al.* 1991; 1993; KONDO *et al.* 1992; 1995; 1998; 1999; CHEN *et al.* 1996; ZHOU *et al.* 1996; ZHOU and WANG 1997; LI and ZHAO 1998; SUZUKI *et al.* 2001; CHEN *et al.* 2003; OHASHI and YONEKURA 2004; GUO *et al.* 2005; VALLÈS *et al.* 2005; SÁNCHEZ-JIMÉNEZ *et al.* 2009). However, ploidy levels varied upon species, even in different populations of the same species. For example, different ploidies

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from diploid ($2x$) to decaploid ($10x$) exists in *Ajania* Poljakov and *Chrysanthemum* L., even up to hexaidecaploid ($16x$) in *Artemisia* (PELLICER *et al.* 2007). Therefore, chromosomal data on Chinese taxa in this tribe is still worthy to be investigated. In present study, the taxa (mainly *Ajania*, *Chrysanthemum* and their allied taxa) of Anthemideae collected mainly from the northwest of China are employed for karyotype analysis. We here provide novel cytological information for some genera of the tribe. The systematics and evolution in Anthemideae endemic to eastern Asia is discussed on the basis of the combination of new cytological data, the biogeography and the morphology data as well.

MATERIALS AND METHODS

Plant materials - Eighteen taxa belonging to seven genera in Anthemideae endemic to China were included in the analysis (Table 1). Plant samples

or their seeds were collected from natural habitats in 2004 to 2006, and specimens were deposited at China Chrysanthemum Preserving Center, Nanjing Agricultural University.

The classification of Anthemideae follows OBERPRIELER *et al.* (2006), BREMER and HUMPHRIES (1993) and SHIH and FU (1983).

Chromosome analysis - Seedlings were raised from seeds or cuttings. Vigorous root tips (1-3 cm in length) were excised from the seedlings and pre-treated with ice water for 20-24 h at 4 °C temperature. Then the roots were fixed with Carnoy fixed solution (1:3 glacial acetic acid-absolute ethanol) for at least 24 h at 4 °C, and then squashed with a drop of 45% acetic acid, and observed with an Olympus BX41 phasecontrast microscope (Olympus, Japan). Photos were recorded by an Olympus Camedia C-5060 wide zoom digital camera.

Karyotypes were obtained from well-spread metaphase plates. Karyotype analysis was made using the software *ikaros* and followed LI and CHEN (1985). The long arm, short arm, and total

Table 1 — The source, chromosome number and ploidy level in Anthemideae from China. *YTS pop.: the material from Yuntaishan mountain, Henan; LS pop.: the material from Laoshan mountain, Shandong; MRK pop.: the material from Maerkang, Sichuan; JY pop.: Jingyuan, Gansu.

Taxon	Source of materials	Source of roots	$2n$	Ploidy level
<i>Chrysanthemum</i> L.				
<i>C. oreastrum</i> Hance	TBS	Seedling	36	$4x$
<i>C. indicum</i> L. (YTS pop. *)	YTS	Cutting plantlet	18	$2x$
<i>C. indicum</i> L. (LS pop. *)	LS	Cutting plantlet	36	$4x$
<i>Ajania</i> Poljakov				
<i>A. fastigiata</i> (C. Winkl.) Poljakov	ML	Cutting plantlet	36	$4x$
<i>A. kbartensis</i> (Dunn) C. Shih	KD	Cutting plantlet	36	$4x$
<i>A. myriantha</i> (Franch.) Y. Ling ex C. Shih	KD	Cutting plantlet	36	$4x$
<i>A. potaninii</i> (H. Kraschen.) Poljakov	JC	Cutting plantlet	18	$2x$
<i>A. przewalskii</i> Poljakov (MRK pop. *)	MEK	Cutting plantlet	36	$4x$
<i>A. przewalskii</i> Poljakov (JY pop. *)	JY	Cutting plantlet	18	$2x$
<i>A. remotipinna</i> (Hand.-Mazz.) Y. Ling ex C. Shih	TBS	Cutting plantlet	18	$2x$
<i>A. tenuifolia</i> (Jacq.) Tzvelev	HY	Cutting plantlet	36	$4x$
<i>Phaeostigma</i> Muldashev				
<i>P. salicifolium</i> (Mattf.) Muldashev	JY	Seedling	18	$2x$
<i>P. variifolium</i> (Chang) Muldashev	TBS	Seedling	18	$2x$
<i>Neopallasia</i> Poljakov				
<i>N. pectinata</i> (Pall.) Poljakov	HS	Seedling	18	$2x$
<i>Opisthopappus</i> C. Shih				
<i>O. taihangensis</i> (Y. Ling) C. Shih	YTS	Cutting plantlet	18	$2x$
<i>Hippolytia</i> Poljakov				
<i>H. alashanensis</i> (Y. Ling) C. Shih	HLS	Seedling	18	$2x$
<i>Achillea</i> L.				
<i>A. acuminata</i> (Ledeb.) Sch. Bip.	TBS	Seedling	18	$2x$
<i>A. wilsoniana</i> Heimerl ex Hand.-Mazz.	TBS	Seedling	36	$4x$

lengths of each chromosome were measured, from which the relative lengths, arm ratios, centromeric indices, the percentage of chromosome with arm ratio beyond 2 were calculated. The sum of chromosomes arms (number fundamental, N.F) were determined as well, for metacentric and submetacentric chromosomes, the arm No. was assigned as 2, while for acrocentric chromosomes, the arm No. was 1 (LI and CHEN; 1985). The chromosome nomenclature followed LEVAN *et al.* (1964). The intrachromosomal asymmetry index (A_1) and the interchromosomal asymmetry index (A_2) were calculated according to the formula proposed by ROMERO ZARCO (1986). The ratio of length of all long arms in chromosome set to total chromosome length in set (AsK%) was calculated according to ARANO (1963). The nomenclature of karyotype asymmetry type followed STEBBINS (1971).

RESULTS

The somatic chromosome numbers and the ploidy levels are presented in Table 1, and the karyotype formula, ranges of chromosome relative length (RL), the ratio of the longest chromosome to shortest chromosome (LC/SC), the percentage of chromosome with arm ratio beyond 2 (P.C.A), number fundamental (N.F), the intrachromosomal asymmetry index (A_1) and the interchromosomal asymmetry index (A_2), the ratio of length of all long arms in chromosome set to total chromosome length in set AsK%, and Karyotype asymmetry type were summarized in Table 2. Somatic chromosome metaphases of the eighteen taxa were presented in Figures 1-18 and the idiograms of each taxon in Figures 19-36.

Subtribe Artemisiinae Less. emend. Bremer and Humphries

Genus *Chrysanthemum* L.

Chrysanthemum oreastrum Hance. Shanxi, Taibaishan mountain (TBS): Fangyangshi, stone lacunes, 2950 m, Zhao H.-B. and Ren G.-B., Zhao. TB06-04, $2n = 4x = 36$ (Figs 1, 19).

This species is endemic to Far East, Korea and China (Hebei, Hunan, Shaanxi, Shanxi and Jilin province) and has a discontinuous distribution from central and western China to Far East. It is mainly distributed in high-altitude (1800 – 3200 m) stone lacunes, grassy slopes and alpine meadows. The karyotype asymmetry type is 2A and includes 16 median region (m), 16 submedian region (sm) and four subterminal region (st) centromere-location chromosomes. ZHOU and

WANG (1997) reported two chromosome counts on this species respectively from Jishou (Hunan province, China) ($2n = 36 = 24m + 8sm + 4t$) and Xiaowutaishan mountain (Hebei province, China) ($2n = 18 = 12m + 6sm$), and the karyotype asymmetry type of both taxa are all 2A. This species shows either a diploid or a tetraploid in two discontinuously distributed regions.

Chrysanthemum indicum L. Henan, Yuntaishan mountain (YTS), roadsides, streamsides and grassy slopes, 300-500 m, Zhao H.-B. and Wang Y.-F., Zhao.HN06-01, $2n = 2x = 18$ (Figs 2, 21).

Shandong, Qingdao: Laoshan mountain (LS), roadsides and stone lacune, 800 m, Zhao H.-B., Zhao.SD04-01, $2n = 4x = 36$ (Figs 3, 22).

Chrysanthemum indicum L. is a wide-distributed species in Eastern Asia and has rich variations in morphology. Many varieties in this species were reported in China (LI *et al.* 1983) and Japan (OHASHI and YONEKURA 2004). It is commonly tetraploid (LI *et al.* 1983; WANG *et al.* 1993; CHEN *et al.* 1996; ZHOU and WANG 1997; SUZUKI *et al.* 2001; VALLÈS *et al.* 2005) and rare diploid (KIM *et al.* 2003) and hexaploid (OHASHI and YONEKURA 2004). *Chrysanthemum indicum* in Yuntaishan mountain (Henan province) population (YTS pop.) is diploid, and the karyotype asymmetry type is 2A including 14 median region (m) and four submedian region (sm) centromere-location chromosomes. Laoshan mountain (Shandong province) population (LS pop.) is tetraploid, and the karyotype asymmetry type is 2A including 24 median region (m), 11 submedian region (sm) with centromere-location chromosomes and one subterminal region (st) centromere-location chromosomes. The YTS population (diploid) bears unique morphology such as bipinnatifid leaves and long filate bracts (> 2 cm in length). Whereas the LS pop. shows the smooth and coriaceous leaves, which are morphologically different from that of protospecies, suggesting that these two populations may be two new subspecies or varieties.

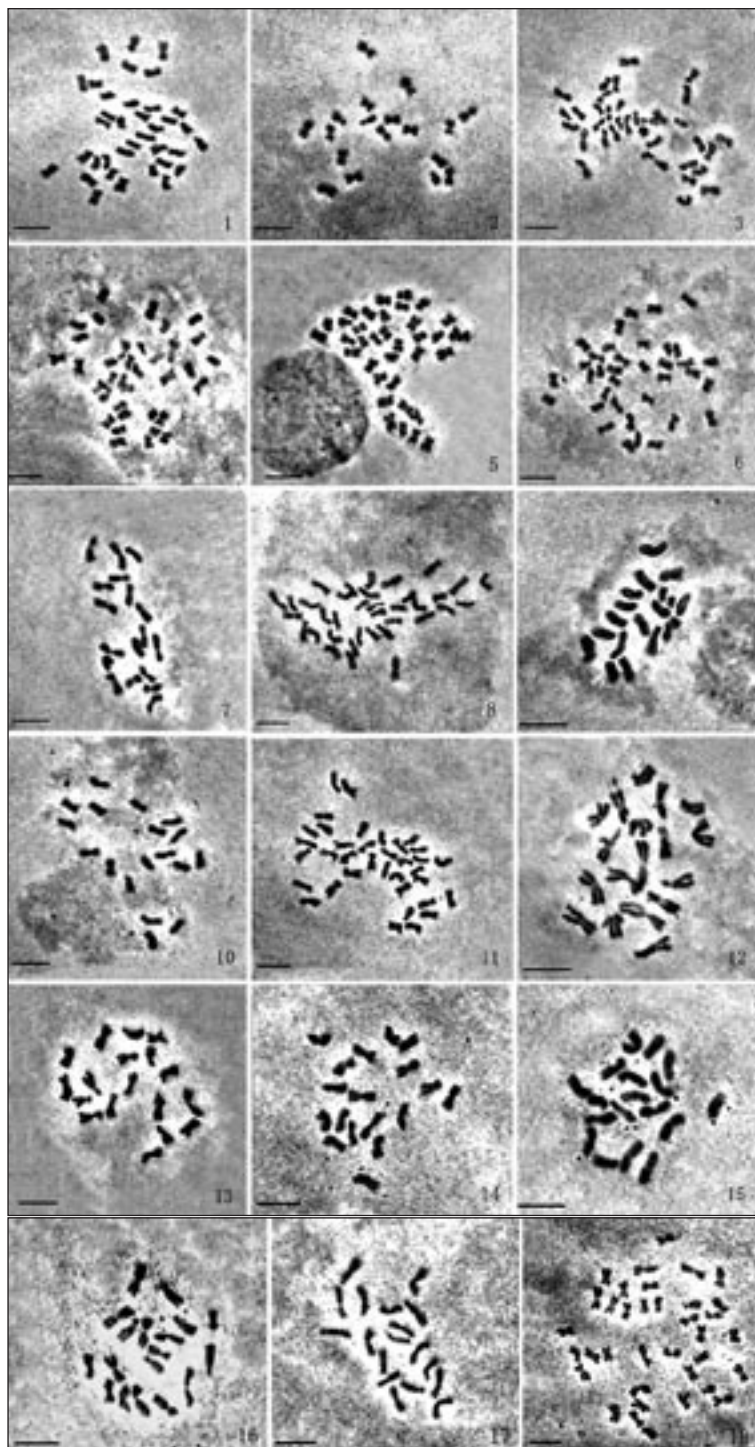
Genus *Ajania* Poljakov

Ajania fastigiata (C.Winkl.) Poljakov. Xinjiang, Mulei (ML), grassy slopes, 1220 m, Zhao H.-B., Zhao.XJ06-07, $2n = 4x = 36$ (Figs 4, 20).

This taxa is endemic to Tianshan mountain (Xinjiang province) and mainly distributes in grassy slopes and sandy steppes. The taxa is a tetraploid while MALTZEVA (1969) reported the diploid. The karyotype asymmetry type of present population from Mulei (Xinjiang province) is 2A and includes one median point (M), 17 median region (m), 16 submedian region (sm) and two

Table 2 — Karyotype formula, ranges of chromosome relative length (RL), the ratio of the longest chromosome to shortest chromosome (LC/SC), the percentage of chromosome with arm ratio beyond 2 (P.C.A), number fundamental (N.F.), the intrachromosomal asymmetry index (A₁) and the interchromosomal asymmetry index (A₂) of ROMERO ZARCO (1986), the ratio of length of all long arms in chromosome set to total chromosome length in set (AsK%) of ARANO (1963), and karyotype asymmetry type of STEBBINS (1971).

Taxon	Karyotype Formula	Range of RL	LC/SC	P.C.A (%)	N.F.	A ₁	A ₂	As K%	KaryotypeAsymmetry Type
<i>C. oreastrum</i>	16m + 16sm + 4st	2.15-3.54	1.65	41.67	68	0.26	0.10	64.65	2A
<i>C. indicum</i> (YTS pop.)	14m + 4sm	4.59-6.56	1.43	16.67	36	0.71	0.11	60.49	2A
<i>C. indicum</i> (LS pop.)	24m + 11sm + 1st	2.05-3.76	1.83	19.44	71	0.39	0.13	60.70	2A
<i>A. fastigiata</i>	1M + 7m + 16sm + 2st	2.01-3.60	1.79	22.22	70	0.34	0.12	63.37	2A
<i>A. khartensis</i>	15m + 21sm	2.41-3.21	1.33	19.44	72	0.35	0.08	63.39	2A
<i>A. myriantha</i>	22m + 11sm + 3st	2.21-3.32	1.50	25.00	69	0.34	0.12	63.04	2A
<i>A. potaninii</i>	9m + 8sm + 1st	4.37-6.99	1.60	27.78	35	0.66	0.14	63.76	2A
<i>A. przewalskii</i> (MRK pop.)	19m + 16sm + 1st	2.14-3.40	1.59	33.33	71	0.33	0.11	63.24	2A
<i>A. przewalskii</i> (JY pop.)	8m + 8sm + 2st	3.05-7.11	2.33	38.89	34	0.58	0.20	64.63	2B
<i>A. remotipinna</i>	8m + 10sm	3.57-7.97	2.23	38.89	36	0.63	0.22	64.15	2B
<i>A. tenuifolia</i>	18m + 15sm + 3st	2.34-3.30	1.41	33.33	69	0.30	0.11	63.90	2A
<i>P. salicifolium</i>	12m + 5sm + 1st	4.18-7.84	1.88	27.78	35	0.66	0.20	62.23	2A
<i>P. varifolium</i>	10m + 7sm + 1st	4.52-7.04	1.56	22.22	35	0.67	0.12	62.81	2A
<i>N. pectinata</i>	15m + 3sm	4.79-6.79	1.46	0.00	36	0.74	0.10	58.19	1A
<i>O. tathangensis</i>	11m + 7sm	4.15-7.09	1.71	11.11	36	0.69	0.14	61.64	2A
<i>H. alabanensis</i>	10m + 6sm + 2st	4.60-6.52	1.42	33.33	34	0.63	0.11	64.71	2A
<i>Ac. acuminata</i>	12m + 4sm + 2st	4.67-6.74	1.44	11.11	34	0.68	0.11	61.41	2A
<i>Ac. wilsoniana</i>	23m + 10sm + 3st	2.15-3.89	1.81	22.22	69	0.35	0.14	61.44	2A



Figures 1-15 — Somatic metaphase chromosomes.

Figures 16-18 — Somatic metaphase chromosomes.

Figures 1-18 — Somatic metaphase chromosomes. Fig. 1. *C. oreastrum* ($2n=36$). Fig. 2. *C. indicum* (YTS pop.) ($2n=18$). Fig. 3. *C. indicum* (LS pop.) ($2n=36$). Fig. 4. *A. fastigiata* ($2n=36$). Fig. 5. *A. kbartensis* ($2n=36$). Fig. 6. *A. myriantha* ($2n=36$). Fig. 7. *A. potaninii* ($2n=18$). Fig. 8. *A. przewalskii* (MRK pop.) ($2n=36$). Fig. 9. *A. przewalskii* (JY pop.) ($2n=18$). Fig. 10. *A. remotipinna* ($2n=18$). Fig. 11. *A. tenuifolia* ($2n=36$). Fig. 12. *P. salicifolium* ($2n=18$). Fig. 13. *P. variifolium* ($2n=18$). Fig. 14. *N. pectinata* ($2n=18$). Fig. 15. *O. taibangensis* ($2n=18$). Fig. 16. *H. alashanensis* ($2n=18$). Fig. 17. *A. acuminata* ($2n=18$). Fig. 18. *A. wilsoniana* ($2n=36$). Bar: 10 μm .

subterminal region (st) centromere-location chromosomes.

Ajania kbartensis (Dunn) C. Shih. Sichuan, Kangding (KD): Zheduoshan mountain, rocky mountain slopes, 3750-4200 m, Zhao H.-B. and Yang Z.-Q., Zhao.SC06-01, $2n = 4x = 36$ (Figs 5, 24).

To our knowledge, this is the first chromosome count of tetraploid taxa from Zheduoshan mountain (Kangding, west of Sichuan province). This species is endemic to Sichuan, Qinghai, Gansu, Yunnan and Tibet province and mainly distributes in rocky slopes and hills at altitude 2500 to 5300 m. The karyotype asymmetry type of the taxa is 2A and includes 15 median region (m) and 21 submedian region (sm) centromere-location chromosomes. Other information on karyotype of this species was hexaploid ($2n = 31m + 9sm + 14st$) from Qinghai province (KONDO *et al.* 1998).

Ajania myriantha (Franch.) Y. Ling ex C. Shih. Sichuan, Kangding and Jinchuan (JY), roadsides, streamsides and boscajes, Zhao H.-B. and Yang Z.-Q., 2150-2600 m, Zhao.SC06-09, $2n = 4x = 36$ (Figs 6, 23). This species is mainly endemic to north of Yunnan, west and north-west of Sichuan, southeast of Gansu, south of Qinghai and southeast of Tibet. Different populations varied in morphology such as the indumentum, leaf dissection, life form and habit. KONDO *et al.* (1995) reported two populations from Sichuan are respectively diploid ($2n = 14m + 4st$) and tetraploid ($2n = 30(m + sm) + 6st$), and five populations from Qinghai are all diploid ($2n = 10m + 4sm + 4st$) (KONDO *et al.* 1998). However, the taxa from Sichuan population in present study is tetraploid, and its karyotype asymmetry type is 2A including 22 median region (m), 11 submedian region (sm) and three subterminal region (st) centromere-location chromosomes.

Ajania potaninii (Krasch.) Poljakov. Sichuan, Danba and Jinchuan, roadside, stone lacunes and stony slopes, Zhao H.-B. and Yang Z.-Q., 1700-2750 m, Zhao.SC06-06, $2n = 2x = 18$ (Figs 7, 26).

This taxa is endemic to southwest of Shaanxi, central and north of Sichuan and southeast of Gansu. There are many different forms or varieties of the species which are rich in variation of leaf morphology among different geographic distributions. Here we firstly report the chromosome number of this species. The karyotype asymmetry type of the population from Jinchuan population (Sichuan province) is 2A and includes nine median region (m), eight submedian region (sm) and one subterminal region (st) centromere-location chromosomes.

Ajania przewalskii Poljakov. Sichuan, Hongyuan (HY): Chazhiliangzi, roadsides and stony slopes, 3700 m, Zhao H.-B. and Yang Z.-Q., Zhao.SC06-14, $2n = 4x = 36$ (Figs 8, 28); Gansu, Jingyuan: Maxiaoshan mountain, grassy slopes and roadsides, 2800 m, Zhao H.-B. and Ren G.-B., Zhao.GS06-01, $2n = 2x = 18$ (Figs 9, 25).

This species is mainly endemic to Sichuan, Qinghai and Gansu province. The karyotype asymmetry type of the population from Sichuan is 2B including 19 median region (m), 16 submedian region (sm) and one subterminal region (st) centromere-location chromosomes, while the karyotype asymmetry type of the population from Gansu is 2B and includes eight median region (m), eight submedian region (sm) and two subterminal region (st) centromere-location chromosomes. Our findings are agreed with the previous reports that there are diploid ($2n = 14(m + sm) + 4st$) and tetraploid ($2n = 24m + 11sm + 1st$ and $2n = 28(m + sm) + 8st$) in different populations (KONDO *et al.* 1992, 1995).

Ajania remotipinna (Hand.-Mazz.) Y. Ling ex C. Shih. Shaanxi, Taibaishan mountain, grassy slopes, stone lacune and boscajes, 2800-2950 m, Zhao H.-B. and Ren G.-B., Zhao.TB06-01, $2n = 2x = 18$ (Figs 10, 27).

We here firstly reported the chromosome number of this species which is endemic to Shaanxi, Sichuan, Gansu and Tibet provinces. The karyotype asymmetry type of the population from Taibaishan mountain (Gansu province) is 2B and includes 8 median region (m) and 10 submedian region (sm) centromere-location chromosomes.

Ajania tenuifolia (Jacq.) Tzvelev. Sichuan, Hongyuan: Shuajinshi, roadsides and meadows, 3200 m, Zhao H.-B. and Yang Z.-Q., Zhao.SC06-13, $2n = 4x = 36$ (Figs 11, 29).

This species is endemic to Gansu, Sichuan, Qinghai and Tibet province. KONDO *et al.* (1992) reported tetraploid of the species (KONDO *et al.* 1992). Our results confirmed Kondo's finding in ploidy level of the taxa. Here, the karyotype asymmetry type of the population from Sichuan is 2A and includes 18 median region (m), 15 submedian region (sm) and three subterminal region (st) centromere-location chromosomes.

Genus *Phaeostigma* Muldashev

Phaeostigma salicifolium (Mattf.) Muldashev Gansu, Jingyuan: Maxiaoshan mountain, grassy slopes and boscajes, 2800 m, Zhao H.-B. and Ren G.-B., Zhao.GS06-02, $2n = 2x = 18$ (Figs 12, 30).

This species is endemic to China (Gansu, Qinghai, Sichuan and Shaanxi province) and dis-

tributes in grassy slopes, stony slopes and boscages, altitude 2600 to 4600 m. We found that the karyotype asymmetry type of Maxiaoshan mountain population (Gansu province) is 2A including 12 median region (m), five submedian region (sm) and one subterminal region (st) centromere-location chromosomes. KONDO *et al.* (1995, 1998) reported the chromosome number and karyotype asymmetry type of five populations from Gansu and Qinghai province. All populations are diploid with different number of satellites at different chromosomes. Moreover, the chromosomes of this species are largest in length within all taxa of *Ajanina*, *Chrysanthemum* and *Phaeostigma* studied so far.

Phaeostigma variifolium (Chang) Muldashev. Shaanxi, Taibaishan mountain, grassy slopes, alpine meadows and boscages, Zhao H.-B. and Ren G.-B., 3400-3500 m, Zhao.TB06-02, $2n = 2x = 18$ (Figs 13, 31).

This is the first report on the chromosome number of this Chinese species endemic to Hubei, Shanxi and Heilongjiang province. It distributes in rocky slopes and subalpine meadows, altitude 1200 to 3500m. The karyotype asymmetry type of this species from Taibaishan mountain (Shaanxi province) is 2A and includes 10 median region (m), seven submedian region (sm) and one subterminal region (st) centromere-location chromosomes.

Genus *Neopallasia* Poljakov

Neopallasia pectinata (Pall.) Poljakov. Xinjiang, Heshuo (HS): Hongshan, stony slopes near the river and sandy steppes, 1700 m, Zhao H.-B., Zhao.XJ06-05, $2n = 2x = 18$ (Figs 14, 32).

This species is endemic to Central Asia, Southern Siberia, Mongolia and China. The taxa from Xinjiang province are diploid and the karyotype asymmetry type is 1A including 15 median region (m) and three submedian region (sm) centromere-location chromosomes. The results agreed with the most previous reports of diploid level (SUZUKA 1952; KAWATANI and OHNO 1964; QIAO *et al.* 1990; GARCIA *et al.* 2006; SÁNCHEZ-JIMÉNEZ *et al.* 2009). At the same time, VALLÈS *et al.* (2005) reported a tetraploid population of this species from southern slope of Eastern Tianshan mountain (Xinjiang province).

Subtribe Tanacetinae Bremer and Humphries

Genus *Opisthopappus* C. Shih

This genus including two species is endemic to China with a restricted distribution in Taihangshan mountain.

Opisthopappus taihangensis (Y. Ling) C. Shih. Henan, Yuntaishan: Zhuyufeng, stone lacunes, 900 m, Zhao H.-B. and Wang Y.-F., Zhao.HN06-03, $2n = 2x = 18$ (Figs 15, 33).

Opisthopappus taihangensis (Y. Ling) C. Shih is endemic to southeast of Taihangshan mountain and distributes in rocky slopes and stone lacunes, altitude 800 to 1000 m. The materials from Yuntaishan mountain are diploid (LI *et al.* 2008) and the karyotype asymmetry type is 2A including 11 median region (m) and seven submedian region (sm) centromere-location chromosomes.

Genus *Hippolytia* Poljakov

Hippolytia alashanensis (Y. Ling) C. Shih Ningxia, east of Helanshan mountain (HLS): Suyukou, stone lacunes, 2000 m, Zhao H.-B. and Ren G.-B., Zhao.HL06-02, $2n = 2x = 18$ (Figs 16, 34).

Here, we firstly report on new ploidy level (diploid) of this species. The previous report for this species was given to be a tetraploid cytotype ($2n=36$) in a population from southwest slopes of Helanshan mountain (Tonguan, Alxa, Inner Mongolia, China) by SÁNCHEZ-JIMÉNEZ *et al.* (2009). It inferred that the polyploidization may take place to its adaptation to different ecological factors and habitat conditions in different distributed regions even different parts of the same regions of this species. The species usually distributes in stony slopes, stone lacunes and sandy steppes in Ningxia, central Gansu and west of Inner Mongolia provinces. BREMER and HUMPHRIES (1993) placed it to *H. kaschgarica* Poljakov as the synonym. The taxa from east of Helanshan mountain are diploid and the karyotype asymmetry type is 2A including 10 median region (m), six submedian region (sm) and two subterminal region (st) centromere-location chromosomes. Somatic chromosome number and ploidy level are in consistent with previous reports on *H. darvasica* (C. Winkl.) Poljakov from the Pamir mountains (ASTANOVA, 1989) and *H. megacephala* (Rupr.) Poljakov from Zhambulskaia oblast, Kazakhstan (VALLÈS and TORRELL 2001).

Subtribe Achilleinae Bremer and Humphries

Genus *Achillea* L.

Achillea acuminata (Ledeb.) Sch. Bip Shaanxi, Western Taibaishan: Jiangjiawen, grassy slopes, 1860 m, Zhao H.-B. and Ren G.-B., Zhao.TB06-08, $2n = 2x = 18$ (Figs 17, 35).

This species is endemic to Eastern Siberia, Far East, Mongolia, Japan and China. It is the first report on the chromosome number of the species. The karyotype asymmetry type of this species

from Taibaishan mountain is 2A and includes 12 median region (m), four submedian region (sm) and two subterminal region (st) centromere-location chromosomes.

Achillea wilsoniana Heimerl ex Hand.-Mazz. Shaanxi, Western Taibaishan: Jiangjiawen, grassy slopes, 1860 m, Zhao H.-B. and Ren G.-B., Zhao. TB06-09, $2n = 4x = 36$ (Figs 18, 36).

The species is endemic to Yunnan, Sichuan, Guizhou, northwest of Hunan, west of Hubei, northwest of Henan, south of Shanxi, central and south of Shaanxi and east of Gansu. It is the first report on the chromosome number of this species. The karyotype asymmetry type of this species from Taibaishan mountain is 2A and includes 23 median region (m), 10 submedian region (sm) and three subterminal region (st) centromere-location chromosomes.

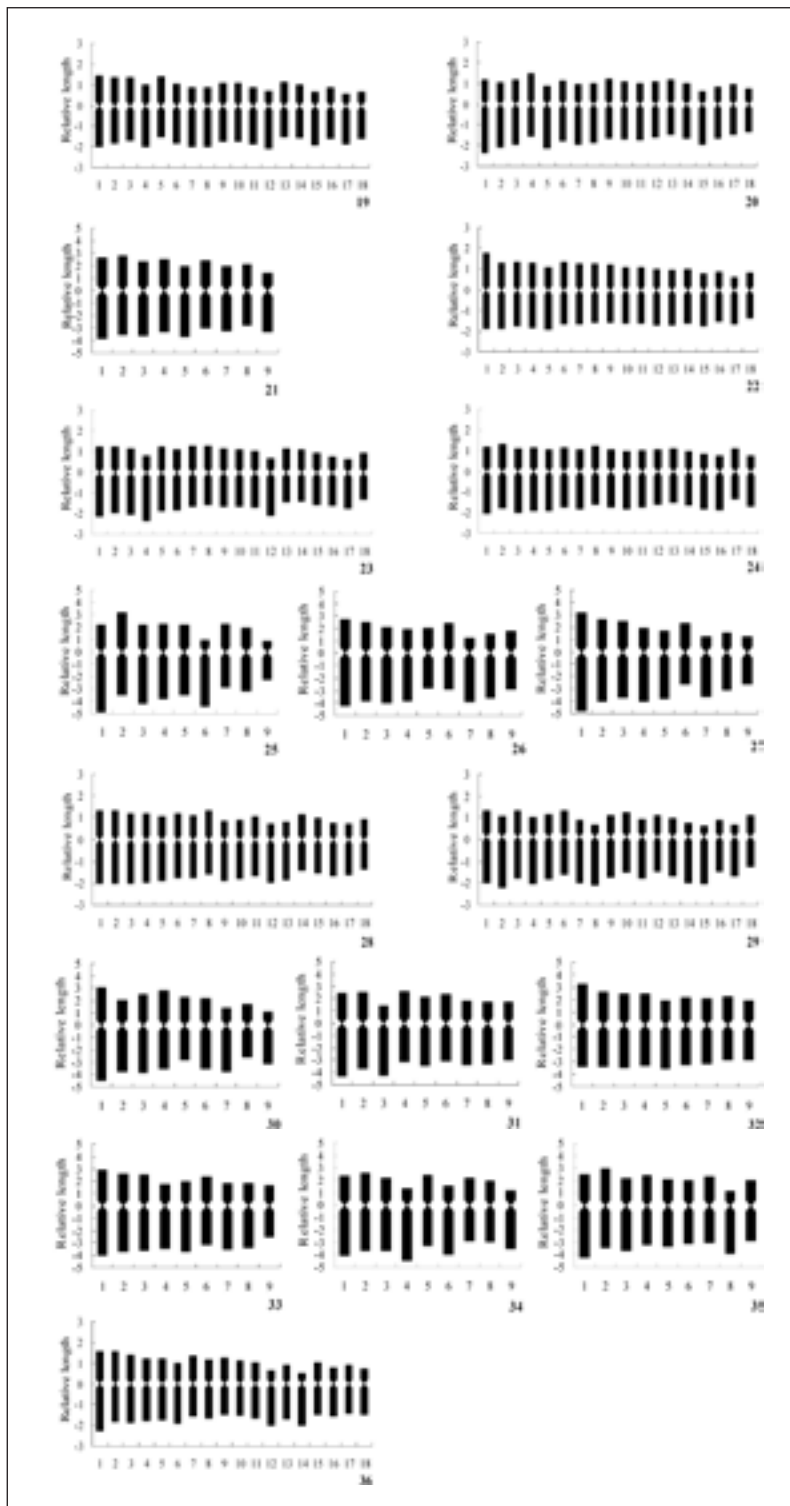
DISCUSSION

Basic chromosome number and dysploidy - All of the examined taxa have the same basic chromosome number $x = 9$, the most common basic number in tribe Anthemideae and the family Asteraceae (LAWRENCE 1947; ARANO 1963; 1970; SHETTY 1964; MORTON 1981; KAUL and BAKSHI 1984; KRASNIKOV and LOMONOSOVA 1990; KONDO *et al.* 1992; 1995; 1998; 1999; ZHOU and WANG; 1997; VALLÈS *et al.* 2001a; 2001b; 2005; GARCIA *et al.* 2006; INCEER *et al.* 2007). However, basic chromosome number $x = 7, 8$ and 9 were ever reported in *Artemisia* although $x=9$ is largely dominant (MCARTHUR *et al.* 1981; STAHEVITCH and WOJTAS 1988; QIAO *et al.* 1990; BREMER and HUMPHRIES 1993; OLIVA and VALLÈS 1994; VALLÈS *et al.* 2001a; 2001b). In tribe Anthemideae, most of monotypic, small genera (including 2 - 10 species) such as *Opisthopappus*, *Crossostephium* (LI *et al.* 2008) and *Hippolytia* (ASTANOVA 1989; VALLÈS and TORRELL 2001; SÁNCHEZ-JIMÉNEZ *et al.* 2009) and medium genera (11 - 50 species) such as *Chrysanthemum* (LI *et al.* 1983; WANG *et al.* 1993; CHEN *et al.* 1996; ZHOU and WANG 1997; SUZUKI *et al.* 2001; KIM *et al.* 2003; VALLÈS *et al.* 2005) and *Ajania* (MALTZEVA 1969; KONDO *et al.* 1992, 1995; 1998), show basic chromosome number of $x = 9$. These genera are distributed in relatively narrow, restricted or local regions. However *Artemisia* has multi basic chromosome number, widely distributed in mid- to high-latitude and dominate most cold and many warm deserts (LING 1992; BREMER and HUMPHRIES 1993; TORRELL *et al.* 1999; VALLÈS *et al.* 2001a; 2001b; 2003; WATSON *et al.* 2002). It

was 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. This phenomenon is common in many genera of Asteraceae (GARCIA-JACAS *et al.* 1996; VALLÈS *et al.* 2001a; 2001b).

Polyploidy - Polyploidy is another remarkable evolutionary mechanism in plants (SOLTIS and SOLTIS 1999; SOLTIS *et al.* 2004; OTTO and WHITTON 2000; GUO *et al.* 2005). In Anthemideae, polyploidy is very common, particularly in genera such as *Chrysanthemum* (LI *et al.* 1983; NAKATA *et al.* 1987; 1991a; 1991b; 1992; WANG *et al.* 1991; 1993; KONDO *et al.* 1995; 1998; 1999; CHEN *et al.* 1996; ZHOU *et al.* 1996; ZHOU and WANG 1997; LI and ZHAO 1998; SUZUKI *et al.* 2001; KIM *et al.* 2003; CHEN *et al.* 2003; OHASHI and YONEKURA 2004), *Ajania* (MALTZEVA 1969; KONDO *et al.* 1992; 1995; 1998; SUZUKI *et al.* 2001; GARCIA *et al.* 2006), *Artemisia* (MCARTHUR *et al.* 1981; STAHEVITCH and WOJTAS 1988; QIAO *et al.* 1990; OLIVA and VALLÈS 1994; WANG *et al.* 1998; VALLÈS *et al.* 2001a; 2001b) and *Achillea* (LAWRENCE 1947; SHETTY 1964; ARANO 1970; MORTON 1981; KRASNIKOV and LOMONOSOVA 1990; LAVRENKO and SERDITOV 1991; HOLLINGSWORTH *et al.* 1992; SAHIN *et al.* 2006; PELLICER *et al.* 2007). Ploidy levels range from diploid ($2n = 2x = 18$) to octaploid ($2n = 8x = 72$) or decaploid ($2n = 10x = 90$) or even up to hexaidecaploid ($2n = 16x = 144$). Additionally, a euploid series with different ploidy exist in different populations of the same species, such as diploid and tetraploid in *C. oreastrum*, *C. indicum*, *A. fastigiata*, *A. przewalskii* and *N. pectinata* and tetraploid and hexaploid in *A. khartensis*. The co-existence of different ploidy suggests that the high ploidy cytotype may be of auto-polyploidization origin, single or multiple duplication and addition of the whole chromosome sets. In the same way, the polyploidization is also responding to the ecological tolerances.

Karyotype symmetry - Most of the studied taxa have more symmetrical karyotype 2A except that of *N. pectinata* is 1A and only JY population of *A. przewalskii* and *A. remotipinna* have more asymmetrical karyotype 2B. The asymmetry of karyotype is one of the important standards evaluating the evolutionary relationship (STEBBINS 1971; LI and CHEN 1985; PASZKO 2006). There is a general assumption that, within the angiosperms, asymmetrical karyotypes are derived from symmetrical ones (STEBBINS 1971; STACE 1989). However, in this case, the change in ploidy levels and karyotype asymmetry (A_1 , A_2 and $AsK\%$) is not necessarily



Figures 19-32 — Haploid ideograms.

Figures 33-36 — Haploid ideograms.

Figures 19-36 — Haploid ideograms. Fig. 19. *C. oreastrum*. Fig. 20. *A. fastigiata*. Fig. 21. *C. indicum* (YTS pop.). Fig. 22. *C. indicum* (LS pop.). Fig. 23. *A. myriantha*. Fig. 24. *A. khartensis*. Fig. 25. *A. przewalskii* (JY pop.). Fig. 26. *A. potaninii*. Fig. 27. *A. remotipinna*. Fig. 28. *A. przewalskii* (MRK pop.). Fig. 29. *A. tenuifolia*. Fig. 30. *P. salicifolium*. Fig. 31. *P. variifolium*. Fig. 32. *N. pectinata*. Fig. 33. *O. taibangensis*. Fig. 34. *H. alashanensis*. Fig. 35. *A. acuminata*. Fig. 36. *A. wilsoniana*.

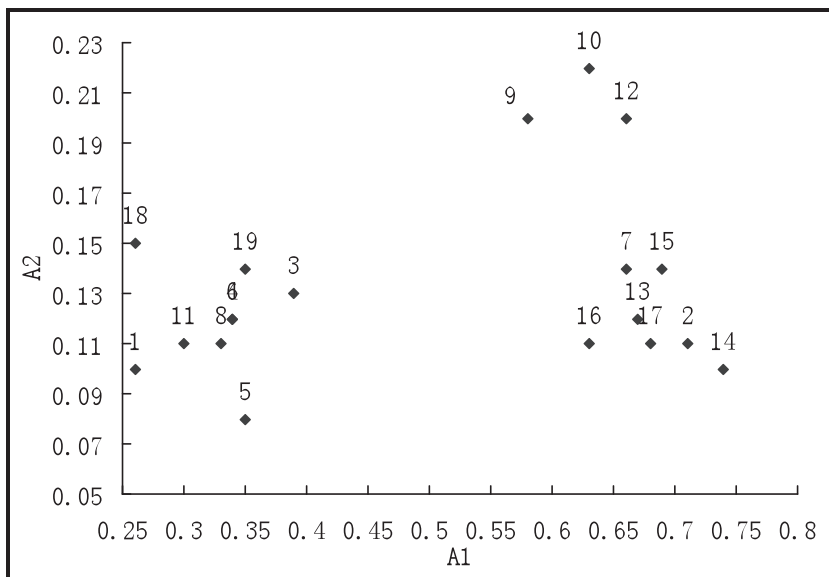


Figure 37 — Scattergram of intrachromosomal (A₁) and interchromosomal (A₂) asymmetry of all taxa. Taxa are numbered as in Fig. 1-18.

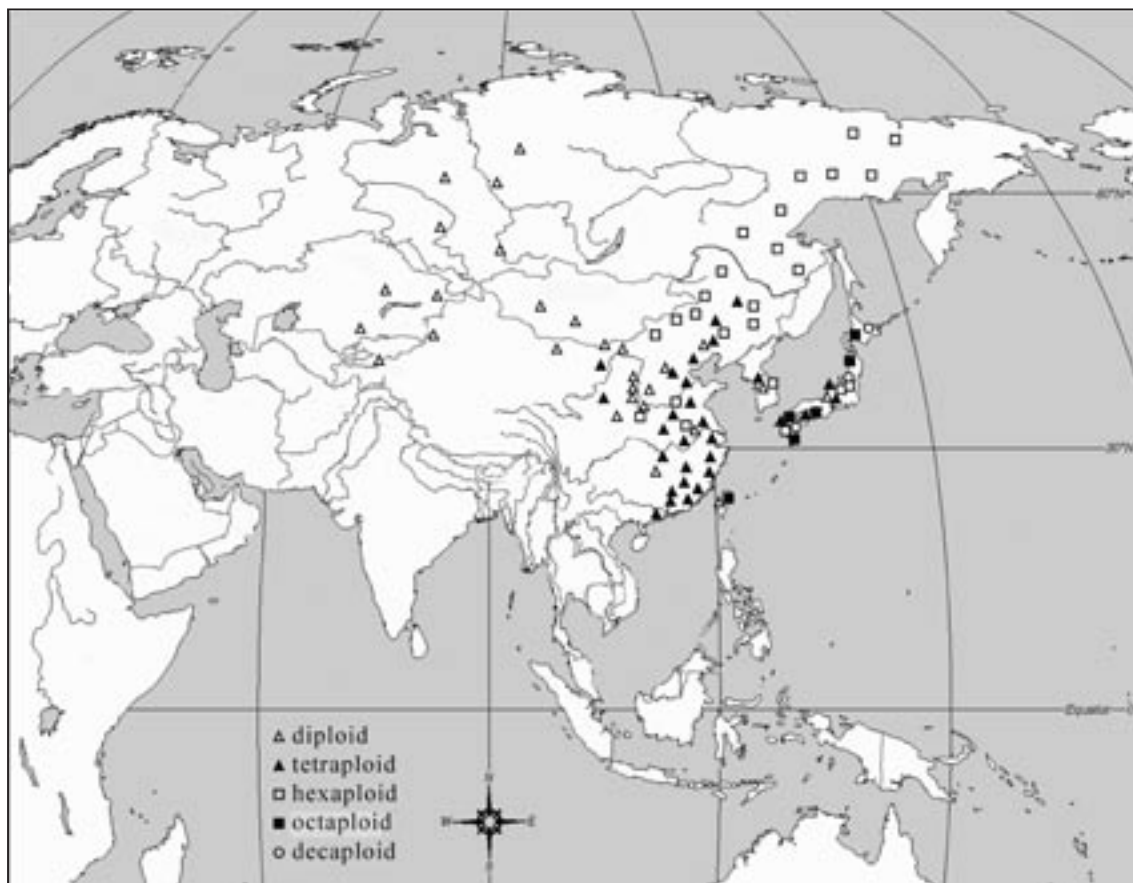


Figure 38 — Distribution of different ploidy levels in *Chrysanthemum* L..



Figure 39 — Distribution of different ploidy levels in *Ajania*. Poljakov.

coincident (Fig. 37). It suggests that not only the polyploidization plays an important role during the karyotype evolution and the speciation, but also ecological selection should be an alternative important factor for karyotype asymmetry.

Ploidy level, geographic distribution and morphology - The correlations among the ploidy, geographic distribution and morphology can be found in *Chrysanthemum* and *Ajania*. Species in these two genera have different ploidy levels: 1) diploid species such as *Chrysanthemum nankingense* Hand.-Mazz., *C. naktongense* Nakai, *C. lavandulifolium* (Fisch. ex Trautv.) Makino, *C. potentilloides* Hand.-Mazz., *C. arisanense* Hayata and *C. mongolicum* Y. Ling; *Ajania achilloidea* (Turcz.) Poljakov ex Grubov, *A. fruticulosa* (Ledeb.) Poljakov, *A. potaninii* (H. Kraschen.) Poljakov, *A. remotipinna* (Hand.-Mazz.) Y. Ling and C. Shih and *A. rupestris* (Matsum. ex Koidz.) Muldashev (MALTZEVA 1969; LI *et al.* 1983; NAKATA *et al.* 1991a; 1991b; WANG *et al.* 1991; 1993; KONDO *et al.* 1992; 1995;

1998; ZHOU *et al.* 1996; ZHOU and WANG 1997; LI and ZHAO 1998; SUZUKI *et al.* 2001; KIM *et al.* 2003; OHASHI and YONEKURA 2004; GARCIA *et al.* 2006), 2) tetraploid species such as *C. indicum*, *C. okiense* Kitam., *C. yoshinaganthum* Makino ex Kitam. and *C. coreanum* Nakai; *A. fastigiata* (C. Winkl.) Poljakov, *A. khartensis* (Dunn) C. Shih, *A. latifolia* C. Shih, *A. myriantha* (Franch.) Y. Ling ex C. Shih and *A. tenuifolia* (Jacq.) Tzvelev (NAKATA *et al.* 1987; KONDO *et al.* 1992; 1995; 1998; ZHOU and WANG 1997; VALLÈS *et al.* 2005), 3) hexaploid such as *C. japonense* Nakai, *C. weyrichii* (Maxim.) Miyabe, *C. aphrodite* Kitam., *C. vestitum* (Hemsley) Stapf and *C. argyrophyllum* Y. Ling; *A. khartensis* (Dunn) C. Shih and *A. pallassiana* (Fischer ex Besser) Poljakov (NAKATA *et al.* 1987; WANG *et al.* 1991; IWATSUKI *et al.* 1997; ZHOU and WANG 1997; KONDO *et al.* 1998), 4) octaploid such as *C. ornatum* Hemsley and *C. morii* Hayata; *A. shiwogiku* (Kitam.) Bremer and Humphries and *A. nematoloba* (Hand.-Mazz.)

Y. Ling ex C. Shih (NAKATA *et al.* 1987; HOTTA *et al.* 1996; KONDO *et al.* 1998; 1999) and decaploid such as *C. crassum* (Kitam.) Kitam. and *C. yezoense* Maekawa; *A. pacifica* (Nakai) K. Bremer and Humphries (NAKATA *et al.* 1987; NISHIKAWA and KOBAYASHI 1989; HOTTA *et al.* 1996). These taxa are continuously distributed in Central Asia, China, Siberia, Korea, Japan and Far East (Figs 38, 39). From west to east in Eurasia, the distribution of taxa of different ploidy level is of a geographically staircase rule. In *Chrysanthemum*, the diploid taxa mainly distribute in Central Asia, northwest of China, west of Siberia and Mongolia; the tetraploid and hexaploid taxa chiefly distribute in east and south of China, east of Siberia and Far East; while Japan and Taiwan of China are the distribution area of the octaploid and decaploid taxa (Fig. 38). The distribution of *Ajania* is of the same trends as the *Chrysanthemum* (Fig. 39). Interestingly, this treppe distribution of the taxa is associated with the variation in their morphology. Most of taxa endemic to Central Asia, northwest of China, west of Siberia and Mongolia sometimes have thin or procumbent stem and less, dissected, pinnatipartite or pinnatisect leaves with tomentum on both surfaces. However, the taxa endemic to east of China and Japan have erect stem and relatively large, lobed or sinuose leaves that are rarely tomentose or with tomentum only on the lower surface. Thus, the conjunction of chromosome data and the distribution can be used as additional evidences to support the morphology classification. The correlation of ploidy level and the geographic distribution revealed here further confirm the evolutionary route, i.e. from western or central Asia gradually to eastern Asia, eastern Siberia and the Far East, of Anthemideae endemic to eastern Asia (ZHAO *et al.* 2009 in press).

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