The LINNEAN SOCIETY of London — BOTANICAL Journal of the Linnean Society

Botanical Journal of the Linnean Society, 2011, **165**, 378–387. With 1 figure

Extensive chromosome number variation in *Aster ageratoides* var. *pendulus* (Asteraceae)

WEI-PING LI[1]*, MING TANG[1], GEN-SHEN YIN[2], YING YIN[1], FU-SHENG YANG[3] and SAN-MAO CHEN[1]

[1]*College of Life Sciences, Hunan Normal University, Changsha 410081, China*
[2]*Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China*
[3]*State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China*

Received 16 November 2010; revision 21 December 2010; accepted for publication 10 January 2011

Aster ageratoides var. *pendulus*, a recently described taxon, is endemic to Mt Hupingshan of north-western Hunan, China. Field observations and collections were made from the only known population. Root-tip squashes were used to determine the chromosome numbers of 96 plants and 61 seedlings from the achenes of eight sample plants. The results show that var. *pedulus* is a swarm of 30 cytotypes with nearly continuous chromosome numbers from $2n = 60$ to $2n = 92$. Chromosome numbers of 61 seedlings vary from $2n = 61$ to $2n = 91$, belonging to 18 cytotypes. The chromosome number variation of var. *pendulus* is highly unusual not only in the *A. ageratoides* polyploid complex but also in angiosperms. Such an enormous continuous variation of chromosome numbers could have arisen by the combined effect of hybridization, recent origin and high levels of polyploidy. © 2011 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2011, **165**, 378–387.

ADDITIONAL KEYWORDS: aneuploidy – high polyploidy – hybridization – Mt Hupinshan – natural population – neopolyploid.

INTRODUCTION

Aster ageratoides Turcz., a perennial herb of Asteraceae, is widely distributed in the Sino-Japanese Floristic Region and is considered to be one of the indicators of this region (Ling & Chen, 1985; Soejima, Wu & Iwatsuki, 1999; Li, 2002; Soejima *et al*., 2005). This species is characterized by thick rhizomes, triplinerved leaves, winged petioles, and 3 (–5)-seriate imbricate phyllaries (Ling & Chen, 1985), but none is a unique diagnostic character for the species. Morphological variation of *A. ageratoides* is so complicated that it has a long and complex taxonomic history (Ling & Chen, 1985; Ito, Soejima & Nishino, 1994; Ito & Soejima, 1995; Li, 2002). Some studies have suggested that the Japanese *A. ageratoides* Turcz. is phylogenetically distinct from the rest of the species complex, and therefore some of the varieties and subspecies should be removed, and that many species, subspecies and varieties should be regarded as synonyms of *A. ageratoides* and its five varieties (Ito *et al*., 1994; Ito & Soejima, 1995). In the Chinese *A. ageratoides*, 11 varieties have been recognized (Ling & Chen, 1985), but the definition of the intraspecific taxa is problematic and the intraspecific relationships are still confusing (Ling & Chen, 1985; Soejima *et al*., 1999; Li, 2002). These observations have led to the question 'what are the evolutionary mechanisms underlying the complexity of the species?' and various studies have suggested that polyploidy and hybridization may have contributed to its diversity and taxonomic confusion (Huziwara, 1957; Ling & Chen, 1985; Ito *et al*., 1994; Ito & Soejima, 1995; Soejima & Peng, 1998; Soejima *et al*., 1999; Li, 2002, 2006).

*Corresponding author. E-mail: lwp@hunnu.edu.cn

Polyploidy (often referred to as whole genome duplication) is a highly dynamic and ubiquitous process and has long been recognized as a major force in plant evolution (Soltis *et al*., 2010). Polyploidy is certainly one of the most important factors contributing to the taxonomic confusion of *A. ageratoides* (Soejima & Peng, 1998; Soejima *et al*., 1999; Li, 2002) and cytological studies have helped to clarify the chaos (Ito *et al*., 1994; Li & Liu, 2005*a*; Li, 2006). These have shown that *A. ageratoides* is a polyploid complex with three main ploidal levels ($2x$, $4x$ and $6x$; $x = 9$) and four rare ploidal levels ($3x$, $5x$, $8x$ and $9x$) (Huziwara, 1957; Matsuda & Suyama, 1980; Yoshida, Kawakami & Tanaka, 1986; Chen *et al*., 1992; Soejima, 1992; Ito *et al*., 1994; Ito & Soejima, 1995; Soejima & Peng, 1998; Soejima *et al*., 1999, 2005; Li, 2002, 2004, 2005, 2006; Li & Liu, 2005a, b). There is only one cytotype in some varieties, whereas others possess two or more cytotypes. For example, var. *micranthus* Maxim. is a diploid and var. *gerlachii* Chang a tetraploid (Li & Liu, 2005a), whereas var. *laticorymbus* Hand.-Mazz. contains a euploid series of diploids, tetraploids and hexaploids (Chen *et al*., 1992; Li, 2002, 2005) and var. *ageratoides* has a ploidal series ranging from $2x$ to $6x$ (Ito *et al*., 1994; Ito & Soejima, 1995).

In the complex, hexaploids are the most common higher ploidy and they are restricted to var. *laticorymbus* in China (Chen *et al*., 1992; Li, 2002, 2005) and var. *ageratoides* in Japan (Ito *et al*., 1994; Ito & Soejima, 1995). Ploidal levels higher than hexaploids are rare. Li (2002) detected an octaploid cytotype in western Hunan Province of China and a natural nonaploid was found in Japanese var. *ageratoides* (formerly subsp. *leiophyllus* Porter) (Yoshida *et al*., 1986).

Although aneuploidy is common in many plants (Henry, Dilkes & Comai, 2007) it appears rare in the complex. Only 12 aneuploid plants with $2n = 4x$-1, $4x + 1$, $6x$-2, $6x$-1, $6x + 1$ and $9x + 1$ were found in 1382 plants of var. *ageratoides* (formerly subsp. *leiophyllus*) in the Kanto District and surrounding areas in Japan (Matsuda & Suyama, 1980; Matsuda & Shinohara, 1985; Matsuda & Shishido, 1988; Matsuda & Shimohara, 1992).

Hybridization is viewed as another key mechanism generating the diversity observed in this taxonomically challenging complex and cytological studies have also played an important role in providing evidence of hybridization. For example, karyotypic analyses have shown there is hybridization between the complex and closely related species such as *A. ovatus* (Franch. & Sav.) Mot. Ito & Soejima (formerly *A. ageratoides* subsp. *ovatus* Kitam.) (Tara, 1973; Matsuda & Suyama, 1980; Matsuda & Shinohara, 1985; Matsuda, Shinohara & Suyma-Tanaka, 1985; Matsuda & Shimohara, 1992; Matsuda & Inomata, 1993; Tara, 1996) and *A. indicus* L. [= *Kalimeris indica* (L.) Sch.Bip.] (Li, 2006). Moreover, cytological studies have shown that hybridization within the complex may happen when different cytotypes coexist. In Mt Huangshan, East China, *A. ageratoides* var. *laticorymbus* ($2n = 6x$) has crossed with var. *scaberulus* (Miq.) Ling ($2n = 4x$), generating pentaploid plants ($2n = 5x$) (Chen *et al*., 1992; Li, 2002). Based on cytological and morphological studies, *A. ageratoides* var. *intermedius* (Soejima) Mot. Ito & Soejima was recognized as an amphidiploid derived from hybridization between two diploid cytotypes of different varieties (Ito *et al*., 1994; Ito & Soejima, 1995). Nevertheless, cytological studies have also shown that there is reproductive isolation between var. *laticorymbus* ($2n = 4x$, $6x$) and var. *lasiocladus* (Hayata) Hand.-Mazz. ($2n = 2x$), even although these two varieties are frequently sympatric over the vast area from eastern Guizhou province (108°54′E) to eastern Zhejiang province (121°34′E), China (Li, 2002, 2004).

No cytological data have been reported for *A. ageratoides* var. *pendulus* W.P.Li & G.X.Chen, a recently described taxon endemic to Mt Hupingshan of northwestern Hunan, China (Li & Chen, 2006). The variety is similar to var. *laticorymbus*, although it differs markedly by having pendent stems, purple abaxial surfaces of basal leaves and lower stem leaves, linear stem leaves with short bristles above, broader phyllaries and fewer capitula (Li & Chen, 2006). Recently, we investigated the variety cytologically and were surprised to find that its chromosome number was not only high but also enormously variable.

This paper investigates chromosome number in the only known natural population of *A. ageratoides* var. *pendulus* and studies dynamic changes of chromosome number between mother plants and their progeny in order to provide an example of extensive chromosome number variation and to explore the relationship among chromosome number variation and hybridization, high polyploidy and neopolyploidy.

MATERIAL AND METHODS

FIELD OBSERVATION AND COLLECTION

Since *A. ageratoides* var. *pendulus* was first described, only one population of the species has been found in Mt Huping, Hunan Province of China, where it ranges in altitudes from 300 to 400 m above sea level (Li & Chen, 2006). This endemic and endangered taxon is distributed along the mountain road that was built in the 1970s. In 2004, 2007, 2008 and 2009 we collected a ramet from each sampled clone, instead of a whole plant, and efforts were also made to avoid collecting repeatedly from the same clone. The rhizome of each ramet was placed in a pot in the

Botanical Garden of Hunan Normal University for cytological investigation and other parts were used to make vouchers which were deposited in the Herbarium of Hunan Normal University (HNNU).

In the field, achenes were collected from eight sampled plants and the achenes from each were cultured in the laboratory to obtain seedlings for further cytological observation.

Somatic chromosome counts

Actively growing root tips were cut for chromosome observations from the transplanted plants and the seedlings from the achenes. The root tips were pretreated with 0.1% colchicine at 8–12°C for 4 h and then fixed in Carnoy I (3:1 95% ethanol:glacial acetic acid) at 20 ± 5°C for 12–24 h. They were then macerated in 1 M hydrochloric acid at 60 °C for 8 min, stained in 5% ammonium iron(III) sulphate [$NH_4Fe(SO_4)_2 \cdot 12H_2O$] for 3–4 h and 0.75% hematoxylin for 2–3 h at 20 ± 5°C, washed in distilled water for 30 min and finally depigmented and squashed in 45% acetic acid.

The chromosome number of each plant was determined from at least 20 cells at mitotic metaphase. Supernumerary (possibly B) chromosomes were identified according to their size because they are notably smaller than A chromosomes in *A. ageratoides* (Matsuda & Suyama, 1980; Chen *et al*., 1992; Matsuda & Inomata, 1993; Li, 2002). As aneuploids were surprisingly frequent and the chromosome number was highly variable, numerous cytological preparations were made for each sample to ensure the accuracy of the results.

RESULTS

Apart from several samples that died before analysis, 96 transplanted plants of *A. ageratoides* var. *pendulus* were investigated cytologically. The somatic chromosome number was found to be highly variable, with nearly continuous variation from $2n = 60$ to $2n = 92$ (Table 1, Fig. 1) corresponding to 30 cytotypes, if supernumerary chromosomes that were found in 17 plants (Table 1, Fig. 1) are ignored. The somatic chromosome numbers of 61 seedlings grown from eight maternal plants were also shown to vary from $2n = 61$ to $2n = 91$, belonging to 18 cytotypes (Table 2). Of the 61 progeny, ten had one or two supernumerary chromosomes. As the basic number in Eurasian *Aster* L. is $x = 9$, cytotypes with $2n = 63$, 72, 81 and 90 are likely to correspond to heptaploids ($7x$), octoploids ($8x$), nonaploids ($9x$) and decaploids ($10x$), respectively. However, we could not rule out the possibility that some of them are pseudo-euploids (i.e. chromosomally unbalanced but numerically compensated sporophytes). As three maternal plants with $2n = 90$ produced progeny with $2n = 71$, 72, 75, 77, 77, 78, 79 and 80 and so on (Table 2), it implies that chromosome segregation to the gametes is rather irregular and that the maternal plants might be pseudo-decaploid. For the same reason, we cannot be sure if $2n = 61$ is naturally $7x$-2, or whether $2n = 65$ is naturally $7x + 2$, and so on.

Field observations showed that there were several hundred plants of var. *pendulus* in the population. They were found to be growing in gaps or on the thin surface soil of cliffs, a habitat that limited their clonal growth, and hence their rhizomes were short. Consequently, reproduction in the variety is generally sexual through achenes rather than asexual via rhizomes. The plants occurred in various micro-environments of the cliff, including dry and barren soil in rock crevices, damp soil and wet soil near a small waterfall (Table 1).

On Mt Huping, the variety is sympatric with *A. ageratoides* var. *laticorymbus*, a hexaploid (Li, 2002). The latter is distributed from the base (200 m alt.) to the summit (1840 m alt.) of the mountain and grows in half shade and damp soil of the flat roadside rather than on the cliffs. Consequently, *A. ageratoides* var. *laticorymbus* was not usually found growing with var. *pendulus* because the roadside under the plants of var. *pendulus* has too little soil for var. *laticorymbus* to survive. Nevertheless, although the two varieties grow on different parts of the mountain road and do not mix with each other, they were found to be in contact at one site where the smallest distance between the two varieties was just a few metres. *Aster ageratoides* var. *lasiocladus* (Hayata) Hand.-Mazz., a diploid ($2n = 2x$), is another variety growing in the vicinity of var. *pendulus*. However, var. *lasiocladus* is at least 1 km away from the var. *pendulus* and molecular data for internal transcribed spacer (ITS), external transcribed spacer (ETS) and *trnH* shows there is no hybridization between var. *pendulus* and var. *lasiocladus* (W. P. Li, unpubl. data).

DISCUSSION

Unusually high chromosome number diversity in the variety

It is highly unusual to find such chromosome number diversity as was found in the small population of *A. ageratoides* var. *pendulus*, where 30 cytotypes, forming a nearly continuous series from $2n = 60$ to $2n = 92$, were found. Only three cytotypes were missing from the series ($2n = 68$, 82 and 91) and it seems possible that, if we had collected more samples, the absent cytotypes could have been found, especially given that three viable individuals from

Table 1. Somatic chromosome numbers and sample vouchers of *Aster ageratoides* var. *pendulus*

Chromosome number	Sample number	Voucher and soil water content in habitat		
		Wet	Damp	Dry
$2n = 60$	1	lwp0912309		
$2n = 61$	1	lwp0912301		
$2n = 62$	2	lwp0711030; lwp0912303		
$2n = 63$	4	lwp0711028; lwp0912306	lwp0810036	lwp0912007
$2n = 64$	1			lwp0912004
$2n = 65$	2	lwp0912107	lwp0912108	
$2n = 66$	2	lwp0810007		lwp0801017
$2n = 67$	1			lwp0912003
$2n = 69 + 0\text{-}1B$	2	lwp0912114*		lwp0912001
$2n = 70 + 1B$	1			lwp0912102*
$2n = 71 + 0\text{-}1B$	2	lwp0912103*		lwp0810021
$2n = 72$	8	lwp0410009; lwp0410012	lwp0711011; lwp0912109; lwp0912110; lwp0912112	lwp0912009; lwp0912011
$2n = 73$	5	lwp0711006	lwp0711015; lwp0912101; lwp0912111; lwp0912113	
$2n = 74 + 1B$	2	lwp0711031*	lwp0711020*	
$2n = 75$	1		lwp0711005	
$2n = 76$	1			lwp0912901
$2n = 77$	2		lwp0810001; lwp0912106	
$2n = 78 + 1B$	1	lwp0912409*		
$2n = 79$	1	lwp0912407		
$2n = 80$	8	lwp0711002; lwp0711007; lwp0912405; lwp0912451	lwp0711001; lwp0711009; lwp0711012; lwp0810039	
$2n = 81$	5	lwp0711035	lwp0711024; lwp0810011; lwp0912104	lwp0912902
$2n = 83$	4	lwp0912155	lwp0810013; lwp0912105	lwp0912906
$2n = 84 + 0\text{-}1B$	4	lwp0912401*	lwp0810033; lwp0810038	lwp0912904
$2n = 85 + 0\text{-}1B$	5	lwp0810010; lwp0912412	lwp0810003*; lwp0912450	lwp0912012*
$2n = 86 + 0\text{-}1B$	3	lwp0912403*		lwp0912903; lwp0912008*
$2n = 87 + 0\text{-}1B$	6	lwp0810004; lwp0810006; lwp0912410*	lwp0711018*; lwp0810015; lwp0810028*	
$2n = 88 + 0\text{-}1B$	6	lwp0810023; lwp0912404; lwp0912408	lwp0810025*; lwp0810030*	lwp0912905
$2n = 89$	7	lwp0912402; lwp0912411;	lwp0810009; lwp0810016; lwp0810018; lwp0810032	lwp0711016
$2n = 90 + 0\text{-}1B$	7	lwp0810026	lwp0810012*; lwp0810019; lwp0810024; lwp0810031; lwp0810037	lwp0912006
$2n = 92$	1		lwp0810008	
Total	96	35	42	19

*With one supernumerary chromosome.

achenes of two mother plants had $2n = 91$ (Table 2), i.e. one of the missing cytotypes. Although the *A. ageratoides* complex has been cytologically studied for a long time and clearly shows complex patterns of polyploidy (Huziwara, 1957; Matsuda & Suyama, 1980; Yoshida *et al*., 1986; Chen *et al*., 1992; Soejima, 1992; Ito *et al*., 1994; Ito & Soejima, 1995; Soejima & Peng, 1998; Soejima *et al*., 1999, 2005; Li, 2002, 2004, 2005, 2006; Li & Liu, 2005a, b), the chromosome number variation of var. *pendulus* differs from all previous reports in the literature. First, the highest chromosome number in the complex was previously reported to be $2n = 81$ ($2n = 9x$) (Yoshida *et al*., 1986), whereas in var. *pedulus* nine cytotypes with $2n > 81$ were found with the highest being $2n = 92$. Secondly, variation in chromosome number has previously been reported mainly among different varieties and between populations, whereas here the extensive

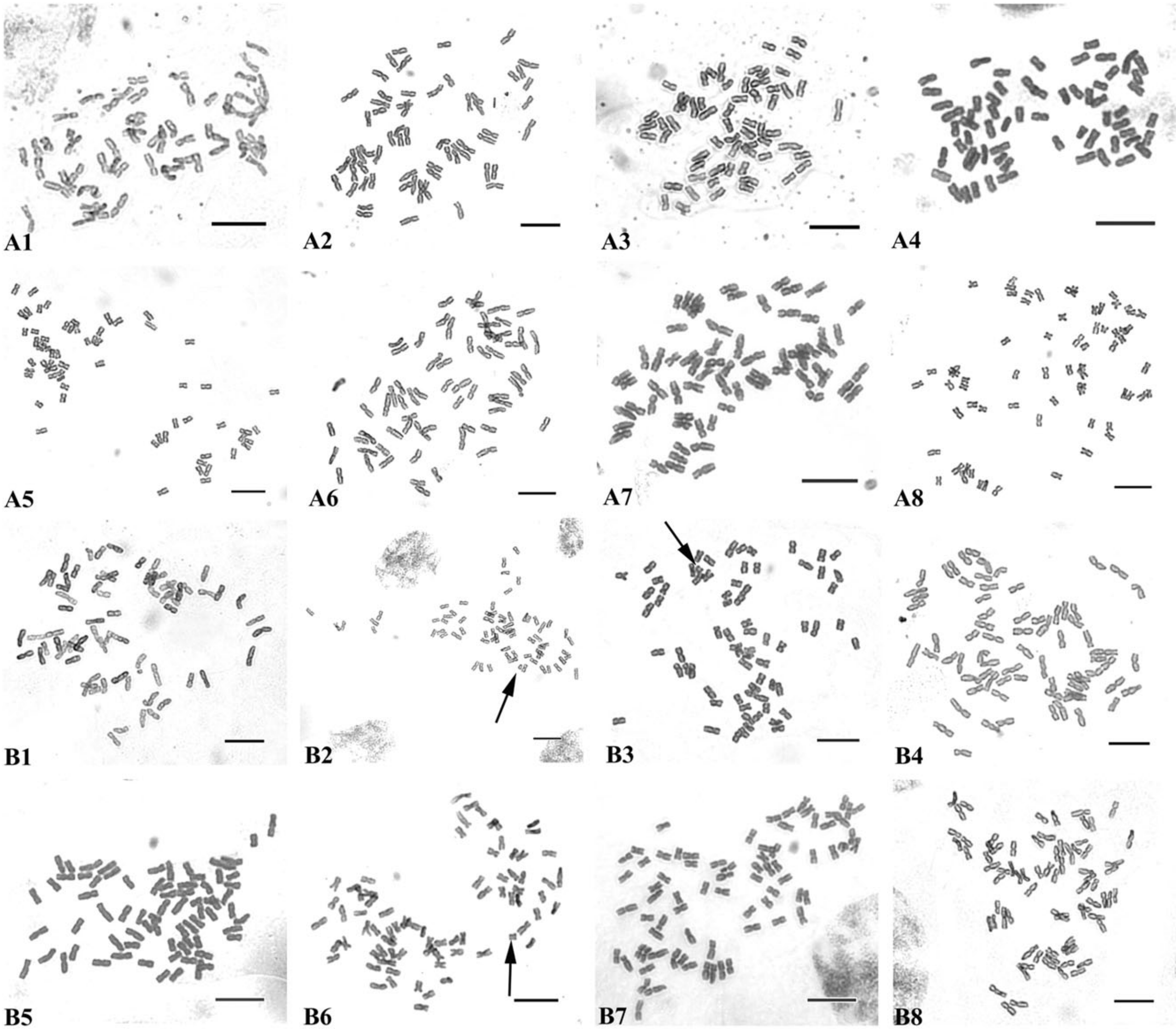

Figure 1. Mitotic metaphase chromosomes of 30 cytotypes of *Aster ageratoides* var. *pendulus*. A1, $2n = 60$ (voucher no. lwp0912309). A2, $2n = 61$ (lwp0912301). A3, $2n = 62$ (lwp0912303). A4, $2n = 63$ (lwp0810036). A5, $2n = 64$ (lwp0912004). A6, $2n = 65$ (lwp0912108). A7, $2n = 66$ (lwp0810007). A8, $2n = 67$ (lwp0912003). B1, $2n = 69$ (lwp0912001). B2, $2n = 70 + 1B$ (lwp0912102). B3, $2n = 71 + 1B$ (lwp0912103). B4, $2n = 72$ (lwp0912109). B5, $2n = 73$ (lwp0711015). B6, $2n = 74 + 1B$ (lwp0711020). B7, $2n = 75$ (lwp0711005). B8, $2n = 76$ (lwp0912901). C1, $2n = 77$ (lwp0912106). C2, $2n = 78 + 1B$ (lwp0912409). C3, $2n = 79$ (lwp0912407). C4, $2n = 80$ (lwp0912451). C5, $2n = 81$ (lwp0912104). C6, $2n = 83$ (lwp0912155). C7, $2n = 84$ (lwp0912904). C8, $2n = 85$ (lwp0810010). D1, $2n = 86 + 1B$ (lwp0912008). D2, $2n = 87$ (lwp0810015). D3, $2n = 88$ (lwp0912905). D4, $2n = 89$ (lwp0912402). D5, $2n = 90$ (lwp0810026). D6, $2n = 92$ (lwp0810008). Scale bar, 10 μm; Arrows indicate supernumerary chromosomes.

variation is found within a population. Finally, the percentage of aneuploids in var. *pendulus* can reach 75%, even if there were no pseudo-euploids in the population. This is much higher than the frequency of 0.87% aneuploids reported in var. *ageratoides* in the Kanto District and surrounding areas of Japan (Matsuda & Suyama, 1980; Matsuda & Shinohara, 1985; Matsuda & Shishido, 1988; Matsuda & Shimohara, 1992). Of course, it is possible that there is more extensive variation in chromosome number in other varieties of *A. ageratoides*, but this has not been revealed because of the limited cytological data available.

Across angiosperms, *A. ageratoides* var. *pendulus* is also highly unusual in the extent of intra-population chromosome number variation. Ramsey & Schemske

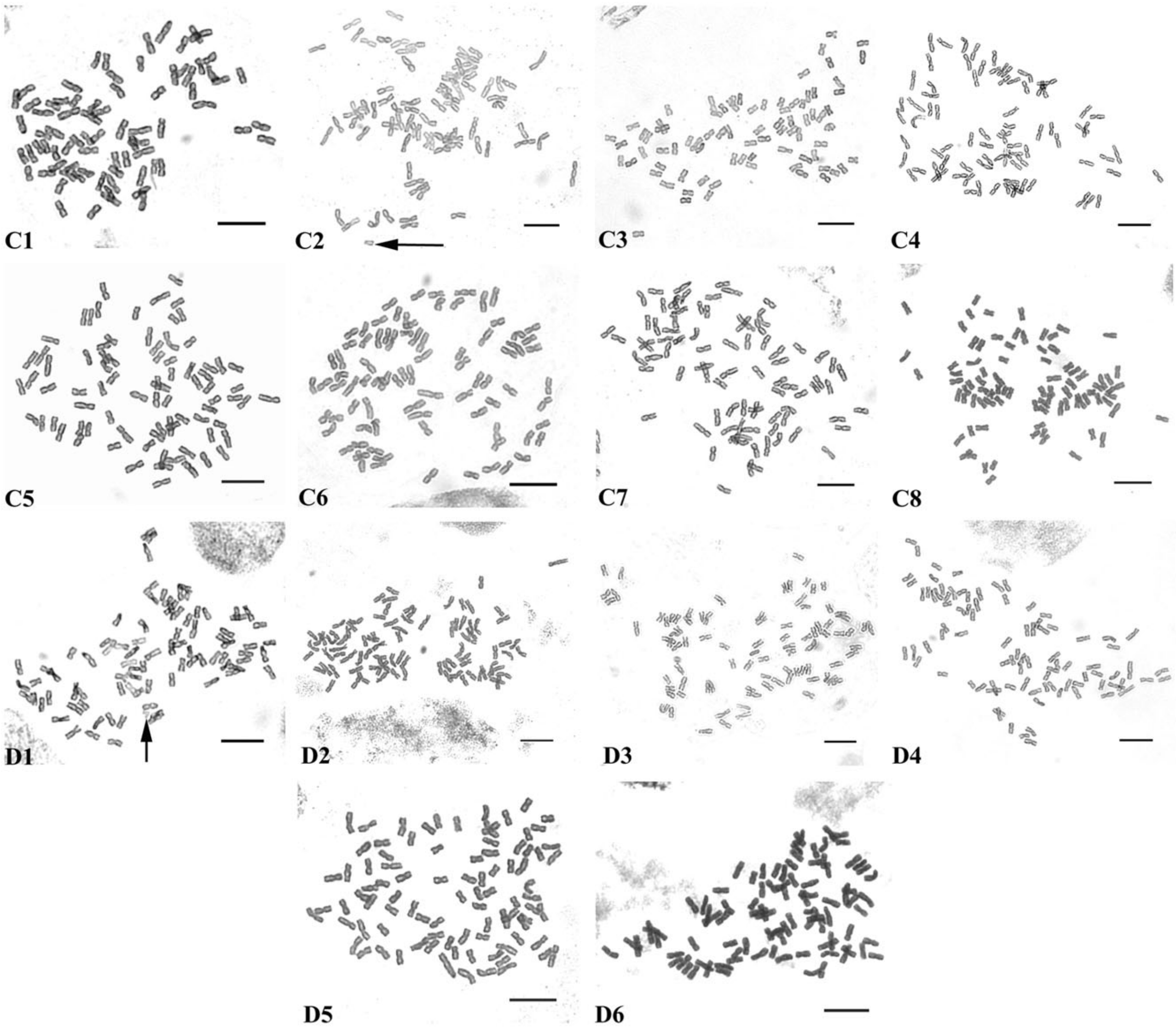

Figure 1. *Continued*

(2002) reviewed the diversity and frequency of aneuploids produced by neopolyploids, but did not cite any examples with such a high diversity as found in *A. ageratoides* var. *pendulus*. Modern sugarcane cultivars (*Saccharum* L. spp.) are high polyploids and aneuploid interspecific hybrids with chromosome numbers ranging from 100 to 130 have been reported (Grivet *et al*., 2004; Raboin *et al*., 2006), but this diversity is at the interpopulation level and reproduction is predominatly vegetative compared with *A. ageratoides* var. *pendulus*, in which the diversity is at the intra-population level and reproduction is predominantly sexual. *Saccharum spontaneum* L., one of the parents of sugarcane, has widely scattered chromosome numbers ranging discontinuously from 40 to 128, but no or few mixed cytotype populations have been reported (Panje & Babu, 1960; Nair & Praneetha, 2005; Praneetha & Nair, 2005; Mary *et al*., 2006). Again, this is different from *A. ageratoides* var. *pendulus*. Another high polyploid species is *Spartina anglica* C.E.Hubbard, which is considered to be allododecaploid, but it has only three chromosome races ($2n = 120$, 122 and 124) (Marchant, 1968; Ainouche, Baumel & Salmon, 2004). Lewis, Oliver & Suda (1967) investigated > 1000 individuals of *Claytonia virginica* L. (Portulacaceae) throughout its range and found 49 different cytotypes ($2n = 12$–191). Although the chromosome number variation occurred mainly among different populations, even within a small population (i.e. 75 m × 20 m) there is also extensive chromosome number diversity, including diploid, triploid, tetraploid, pentaploid, aneutriploids and

Table 2. Eight plants of var. *pendulus* and the chromosome numbers of their offspring

Chromosome numbers of offspring	Number of offspring from eight maternal plants							
	lwp0810017 ($2n = 66$)	lwp0912011 ($2n = 72$)	lwp0810004 ($2n = 87$)	lwp0810023 ($2n = 88$)	lwp0810009 ($2n = 89$)	lwp0810026 ($2n = 90$)	lwp0810031 ($2n = 90$)	lwp0810012 ($2n = 90 + 1B$)
$2n = 61$	1							
$2n = 65$		1						
$2n = 71$				1				1
$2n = 72$				1	1		1	
$2n = 74$	1							
$2n = 75$						1		
$2n = 77$				1		1		
$2n = 78 + 0\text{-}1B$	1						1*	
$2n = 79 + 0\text{-}1B$				1				1 + 2*
$2n = 80 + 0\text{-}2B$			1					1* + 1†
$2n = 81 + 0\text{-}1B$					2 + 2*			
$2n = 84 + 0\text{-}1B$					1	1*		
$2n = 85$			1		2	1		
$2n = 87$				1	1			
$2n = 88 + 0\text{-}1B$				2	2	2 + 1*		1
$2n = 89 + 0\text{-}1B$				2	4	1	1 + 1*	
$2n = 90$					7	2		
$2n = 91$				1		2		
Total (61)	3	1	2	10	22	12	4	7

*With one supernumerary chromosome.
†With two supernumerary chromosomes.

other aneuploids (Lewis & Suda, 1976), which is somewhat similar to *A. ageratoides* var. *pendulus* except for the absence of higher polyploidy. The Arctic is one of the most polyploid-rich areas and includes many species that are recently evolved and that have reached high ploidy levels (Brochmann *et al*., 2004; Brysting *et al*., 2007), but aneuploidy is not common there. For example, the *Cerastium alpinum* L.–*C. arcticum* Lange polyploid complex (a typical representative of the Arctic flora) has been shaped through extensive migration, hybridization and polyploidization (Abbott & Brochmann, 2003) and consists of high polyploids with octoploids ($2n = 72$) and dodecaploids ($2n = 108$) dominating, but aneuploidy is quite rare as only one possible aneuploid chromosome number ($2n = 128$–130) was counted (Brysting, 2000).

Possible causes of the chromosome number variation

It is possible that a combination of hybridization, recent origin and high polyploidy has contributed to the unusual diversity of chromosome numbers found in *A. ageratoides* var. *pendulus*. As discussed in the Introduction, hybridization is considered to be one of the main factors responsible for the taxonomic confusion observed in the *A. ageratoides* complex (Ito *et al*., 1994; Soejima *et al*., 1999; Li, 2002, 2006) and it could also be important in generating the chromosome diversity encountered in *A. ageratoides* var. *pendulus*. In Mt Huping, var. *pendulus* and var. *laticorymbus* are sympatric. Although ecological differentiation leads to spatial separation of the two varieties, the two varieties can be found growing within a few metres of each other, a distance which could be easily overcome by pollinators. Thus, hybridization between var. *pendulus* and var. *laticorymbus* ($2n = 6x$) cannot be ruled out and could be an important cause of the chromosome number diversity of var. *pendulus.* In addition, intra-population hybridization among cytotypes could be an even more important source of chromosome number variation. Of the 61 seedlings, 15 offspring were quite different in chromosome number from their mother plants and the difference was > 9, i.e. more than one haploid set of chromosomes (Table 2), which implies that crosses among cytotypes with different ploidal levels could happen frequently.

The recent origin of *A. ageratoides* var. *pendulus* might also contribute to its chromosome number variation. The variety which grows only on steep rock walls is likely to have arisen in the 1970s following the construction of the mountain road. It is well documented that newly formed polyploids can exhibit multisomic inheritance, which often leads to the production of unbalanced gametes, which in turn generate chromosomally unbalanced aneuploid sporophytes (Ramsey & Schemske, 2002).

The high chromosome numbers encountered in *A. ageratoides* var. *pendulus* ($2n = 60$–92) suggests it is at least a hexaploid ($2n = 6x = 54$) and this high polyploidy level might also contribute to the extensive and continuous variation in chromosome numbers via aneuploidy. Polyploidy can enhance the ability of plants to tolerate gene dosage imbalance caused by aneuploidy because there is less effect of extra or missing chromosomes when the remainder of the genome is increased in copy number (Birchler & Veitia, 2007). Indeed, aneuploidy is reported to be frequent in some polyploid populations (Ramsey & Schemske, 1998, 2002). Clausen, Keck & Hiesey (1945) suggested that the balance of chromosomes and genes in polyploids is relatively flexible, 'permitting survival of plants that deviate slightly from the hexaploid level'. It is quite possible that the higher levels of polyploidy observed in var. *pendulus* buffer it effectively against unbalanced numbers of chromosomes and genes, permitting survival and fertility of plants that deviate from euploidy.

ACKNOWLEDGEMENTS

We thank the Management Department of Hupingshan National Nature Reserve for field assistance and two anonymous reviewers for their helpful advice and comments on the manuscript. This work was financially supported by the National Natural Science Foundation of China (grant no. 30470131) and by the Scientific Research Fund of Hunan Provincial Education Department (grant no. 08A046).

REFERENCES

Abbott RJ, Brochmann C. 2003. History and evolution of the arctic flora: in the footsteps of Eric Hultén. *Molecular Ecology* **12:** 299–313.

Ainouche ML, Baumel A, Salmon A. 2004. *Spartina anglica* C.E.Hubbard: a natural model system for analysing early evolutionary changes that affect allopolyploid genomes. *Biological Journal of the Linnean Society* **82:** 475–484.

Birchler JA, Veitia RA. 2007. The gene balance hypothesis: from classical genetics to modern genomics. *Plant Cell* **19:** 395–402.

Brochmann C, Brysting AK, Alsos I, Borgen L, Grundt HH, Scheen AC, Elven R. 2004. Polyploidy in arctic plants. *Biological Journal of the Linnean Society* **82:** 521–536.

Brysting AK. 2000. Chromosome number variation in the polyploid *Cerastium alpinum–C. arcticum* complex (Caryophyllaceae). *Nordic Journal of Botany* **20:** 149–156.

Brysting AK, Oxelman B, Huber KT, Moulton V, Brochmann C. 2007. Untangling complex histories of genome mergings in high polyploids. *Systematic Biology* **56:** 467–476.

Chen RY, Irifune K, Song WQ, Li XL, Taniguchi K, Tanaka R. 1992. Report on the cytogeographic investigation on Chinese *Aster ageratoides*. In: Ryuso T, ed. *Cytogenetics on plant correlating between Japan and China*. Hiroshima: Hiroshima University Press, 89–112.

Clausen J, Keck DD, Hiesey WM. 1945. *Experimental studies on the nature of species. II. Plant evolution through amphiploidy and autoploidy, with examples from the Madiinae*. Washington, DC: Carnegie Institution of Washington Publication, 564.

Grivet L, Daniels C, Glaszmann JC, D'Hont A. 2004. A review of recent molecular genetics evidence for sugarcane evolution and domestication. *Ethnobotany Research and Applications* **2:** 9–17.

Henry IM, Dilkes BP, Comai L. 2007. Genetic basis for dosage sensitivity in *Arabidopsis thaliana*. *PLoS Genetics* **3:** e70. doi:10.1371/journal.pgen.0030070.

Huziwara Y. 1957. Karyotype analysis in some genera of Compositae. III. The karyotype of the *Aster ageratoides* group. *American Journal of Botany* **44:** 783–790.

Ito M, Soejima A. 1995. *Aster*. In: Iwatsuki K, Yamazaki T, Boufford DE, Ohba H, eds. *Flora of Japan Vol. Iiib*. Tokyo: Kodansha Ltd, 59–73.

Ito M, Soejima A, Nishino T. 1994. Phylogeny and speciation of Asian *Aster*. *Korean Journal of Plant Taxonomy* **24:** 133–143.

Lewis WH, Suda Y. 1976. Diploids and polyploids from a single species population: temporal adaptations. *Journal of Heredity* **67:** 391–393.

Lewis WH, Oliver RL, Suda Y. 1967. Cytogeography of *Claytonia virginica* and its allies. *Annals of the Missouri Botanical Garden* **54:** 153–171.

Li WP. 2002. The biosystematic studies on the *Aster ageratoides* (Asteraceae) polyploid complex. DPhil Thesis, Hunan Agricultural University, China.

Li WP. 2004. Two varieties of the *Aster ageratoides* complex in the Yuelu Mountain, China: karyotype and reproductive isolation. In: Dorbromir E, ed. *Compilation of scientific papers 'natural sciences 2004'*. Shumen: University Press of Konstantin Preslavsky University, 233–240.

Li WP. 2005. The cytogeography of *Aster ageratoides* var. *laticorymbus* (Asteraceae), a polyploid complex endemic to China. *Botanical Bulletin of Academia Sinica* **46:** 355–361.

Li WP. 2006. Natural hybridization between *Aster ageratoides* var. *scaberulus* and *Kalimeris indica* (Asteraceae): evidences from morphology, karyotype and ITS sequences. *Botanical Studies* **47:** 191–197.

Li WP, Chen GX. 2006. *Aster ageratoides* var. *pendulus* W.P.Li & G.X.Chen, a new variety of *Aster* (Asteraceae) from Hunan, China. *Acta Phytotaxonomica Sinica* **43:** 348–350.

Li WP, Liu SX. 2005a. Differentiation between *Aster ageratoides* var. *micranthus* and var. *gerlachii*: evidence from morphology and cytology. *Acta Phytotaxonomica Sinica* **43:** 31–36.

Li WP, Liu SX. 2005b. Cytogeography of *Aster ageratoides* var. *scabulus*. *Life Science Research* **9:** 68–72.

Ling Y, Chen YL. 1985. *Flora reipublicae popularis sinicae, vol. 74*. Beijing: Science Press, 159–166.

Marchant CJ. 1968. Evolution in *Spartina* (Gramineae). II. Chromosomes, basic relationships and the problem of *Spartina townsendii* agg. *Botanical Journal of the Linnean Society* **60:** 381–409.

Mary S, Nair NV, Chaturvedi PK, Selvi A. 2006. Analysis of genetic diversity among *Saccharum spontaneum* L. from four geographical regions of India, using molecular markers. *Genetic Resources and Crop Evolution* **53:** 1221–1231.

Matsuda T, Inomata N. 1993. Karyomorphological study of *Aster ageratoides* subsp. *leiophyllus* group in the Hakone Mountain range and adjacent area. *Reports of the Manazuru Marine Laboratory for Science Education, Faculty of Education, Yokohama University* **9:** 33–47.

Matsuda T, Shimohara O. 1992. Polyploids and distribution of *Aster ageratoides* subsp. *leiophyllus* group in the Kanto District and surrounding area. *Science Reports of the Yokohama National University Section* **39:** 29–40.

Matsuda T, Shinohara N. 1985. Polyploids and distribution of *Aster ageratoides* subsp. *leiophyllus* group in the Kanto District and surrounding area. *Science Reports of the Yokohama National University Section* **32:** 11–26.

Matsuda T, Shishido O. 1988. Polyploids and distribution of *Aster ageratoides* subsp. *leiophyllus* group in the Kanto district and surrounding area. *Science Reports of the Yokohama National University Section* **35:** 23–31.

Matsuda T, Suyama K. 1980. Polyploids and distribution of *Aster ageratoides* supsp. *leiophyllus* group in the Kanto district and surrounding area. *Science Reports of the Yokohama National University Section* **27:** 7–18.

Matsuda T, Shinohara N, Suyma-Tanaka K. 1985. Karyomorphological studies on natural hybrids between hexaploid form of *Aster ageratoides* subsp. *leiophyllus* var. *leiophyllus* ($2n = 54$) and *A. ageratoides* subsp. *ovatus* ($2n = 36$). *Memoirs of the Institute of Field Education, Yokohama National University* **3:** 1–9.

Nair NV, Praneetha M. 2005. Cytomorphological studies on a *Saccharum spontaneum* L. clone from the Car-Nicobar Island, India. *Cytologia (Tokyo)* **70:** 213–216.

Panje R, Babu C. 1960. Studies in *Saccharum spontaneum*, distribution and geographical association of chromosome numbers. *Cytologia (Tokyo)* **25:** 152–172.

Praneetha M, Nair NV. 2005. Cytological studies on *Saccharum spontaneum* L. accessions from Kerala, India. *Cytologia* **70:** 407–413.

Raboin L, Oliveira KM, Lecunff L, Telismart H, Roques D, Butterfield M, Hoarau JY, D'Hont A. 2006. Genetic mapping in sugarcane, a high polyploid, using bi-parental progeny: identification of a gene controlling stalk colour and a new rust resistance gene. *Theoretical and Applied Genetics* **112:** 1382–1391.

Ramsey J, Schemske DW. 1998. Pathways, mechanisms and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* **29:** 467–501.

Ramsey J, Schemske DW. 2002. Neopolyploidy in flowering plants. *Annual Review of Ecology and Systematics* **33:** 589–639.

Soejima A. 1992. Taxonomical study of *Aster leiophyllus* complex (Compositae) in Kanto district, Japan, with special reference to ploidy level. *Botanical Magazine Tokyo* **105:** 13–28.

Soejima A, Peng CI. 1998. Cytological features of the *Aster ageratoides* complex (Asteraceae) in Taiwan. *Botanical Bulletin of Academia Sinica* **39:** 299–302.

Soejima A, Wu SG, Iwatsuki K. 1999. The *Aster ageratoides* complex (Asteraceae) in north-eastern Yunan, China: cytological and morphological features. *Acta Phytotaxonomica Geobotanica* **50:** 179–185.

Soejima A, Pak JH, Morita T, Ito M. 2005. Cytogeography of the *Aster ageratoides* complex (Asteraceae) in Korea. *Acta Phytotaxonomica Geobotanica* **56:** 97–104.

Soltis DE, Buggs RJA, Doyle JJ, Soltis PS. 2010. What we still don't know about polyploidy. *Taxon* **59:** 1387–1403.

Tara M. 1973. Cytogenetic studies on natural intergeneric hybridization in *Aster* alliances. III. Natural hybrid *Aster ageratoides* subsp. *leiophyllus* ($2n = 36$) × *A. ageratoides* subsp. *ovatus* ($2n = 36$). *Journal of Science of the Hiroshima University, Series B, Division 2* **14:** 141–164.

Tara M. 1996. The structure of natural hybrid swarm between *Aster* and *Kalimeris* in Chizu-cha, Yazu-gun, Tottori Pref. *Bulletin of School of Education, Okayama University* **102:** 89–114.

Yoshida M, Kawakami S, Tanaka R. 1986. A natural nonaploid of *Aster ageratoides* subsp. *leiophyllus*. *Chromosome Information Service* **41:** 3–4.