Cytological studies of 14 Chinese species of *Parnassia* L. (Parnassiaceae) and its phylogenetic implications

Wu Ding, Hong Wang, De Zhu Li* and Jin Mei Lu

Laboratory of Plant Biodiversity and Biogeography, Kunming Institute of Botany, the Chinese Academy of Sciences, Kunming, Yunnan 650204, P. R. China

Abstract — The chromosome numbers and karyomorphology of 14 Chinese species of the genus *Parnassia* were investigated. The chromosome numbers of 11 species were reported for the first time. The report of 2n=48 for *P. monochrofolia* Franch was new to the Chinese species of the genus. There are about 30 species cytologically studied up to now. From the available data, the species of *Parnassia* with the basic chromosome numbers of x=7, 8 and 9 were found in China and the Himalayas, in which the more species with x=9, as well as mainly in western N America, while x=8 was only found in eastern N. America. Polyploidization was common in the genus, more extensively in Northern America. Based on our results and the previously reported data, the basic chromosome numbers and polyploidization are discussed. Combined with gross morphology and geographical distribution of *Parnassia*, the phylogenetic implications of the cytological data are also discussed. It is suggested that eastern Asia and western N America are probably the modern divergent centers of the genus.

Key words: China, Chromosome numbers, Karyomorphology, Parnassia, Phylogenetic Implications

INTRODUCTION

Parnassia L. was treated in a family of its own, Parnassiaceae (GRAY 1821), which was not commonly recognized until HUTCHINSON (1969), Dahlgren (1983) and Takhtajan (1969, 1997). As a subfamily, it had been belonging to a member of Saxifragaceae for a long time (DANDY 1927; Engler 1930; Thorne 1976; Dahlgren 1980; Cronquist 1981; Ku 1987; Gu and Hultgård 2001). In Parnassioideae, Parnassia was also included in Droseraceae (PACE 1912; SCHOENAGEL 1931), and closely related to Hypericaceae (Arber 1913, 1915; Jay 1971), or even Crassulaceae (Ben-SEL and PALSER 1975). Recent molecular systematic studies revealed that Parnassiaceae, including Parnassia and Lepturopetalon Ell. was a sister group of Celastraceae (CHASE et al. 1995; Apg 1998; Soltis et al. 2000; Apg-II 2003).

The genus *Parnassia*, comprising about 70 species, occurred in the North Hemisphere, in which

most species are growing in China and the Himalayas, with approximately 10 species in the North America (Ku 1987; Gu and Hultgård 2001; Simmons 2004). The center of distribution of *Parnassia* is in China and the Himalayan regions, to which most species are endemic (Ku 1987; Wu *et al.* 2003).

Previous cytological studies of Parnassia have investigated over 20 species in North America, Europe and the Himalayas (ERLANDSSON 1942; Hamel 1953; Packer 1964; Zhukova 1966; HEDBERG 1967; LÖVE and RITCHIE 1966; GAS-TONY and SOLTIS 1977; BYE and SOLTIS 1979; Löve and Löve 1980, 1982; Funamoto 1986). Although as many as 63 species are distributed in China (Gu and Hultgård 2001), the chromosome counts have been only reported from about nine species (Löve 1954; Krogulevich 1978; Murin et al. 1980; Malla et al. 1979; 1981; Funamoto et al. 1994; 1996; 1997; 1998; 2001). Cytological studies may contribute to discussions on evolutionary tends through chromosome changes (Guerra 1990). As part of an integrated study on Chinese Parnassia, the present paper reported the chromosome numbers and karyomorphology of 14 species from China, in which 8 species are endemic to China. The chro-

^{*} Corresponding author: phone +86-871-5223503; fax +86-871-5217791; e-mail: dzl@mail.kib.ac.cn.

mosome numbers of 11 species are reported for the fist time.

MATERIALS AND METHODS

The materials studied with voucher specimens were showed in Table 1. All species studied were collected from fields of natural populations in Yunnan, Sichuan and Xinjiang, some species collected from the fields then cultivated for six to twelve months in the Botanical Garden at Kunming Institute of Botany, the Chinese Academy of Sciences.

Table 1 — Species of *Parnassia* investigated.

Species	Localities	Vouchers			
P. bifolia	Tianshan, Xinjang	Ding Wu 03019			
P. brevistyla	Wenzhang, Sichuan	Ding Wu 02014			
P. chinensis	Dali, Yunnan	Ding Wu 02003			
P. delavayi	Lijiang, Yunnan	Ding Wu 02004			
P. epunctulata	Qiaojia, Yunnan	Ding Wu 03890			
P. esquirolii	Yiliang, Yunnan	Ding Wu 03016			
P. faberi	Emeishan, Sichuan	Ding Wu 02017			
P. monochorifolia	Qiaojia, Yunnan	Ding Wu 03022			
P. mysorensis	Lijiang, Yunnan	Ding Wu 02010			
P. pusilla	Zhongdian, Yunnan	Ding Wu 03028			
P. subscaposa	Zhongdian, Yunnan	Ding Wu 03012			
P. tenella	Lijiang, Yunnan	Ding Wu 02002			
P. trinervis	Degin, Yunnan	Ding Wu 03064			
P. venusta	Gongshan, Yunnan	Ding Wu 03022			

Root tips were pretreated in 2 mM hydroxy-quinolone for 4-6 hr at room temperate, and then fixed in carnoy's liquid (3 ethanol: 1 glacial acetic acid) at 4 °C for 6-8 hr, finally kept in 70% ethanol. After maceration in 1N hydrochloric acid and 45% acetic acid (v/v) for 40-50 seconds, materials were stained with Carbol Fuchsin and squashed for microscopic observation. Permanent slides were made by using the standard liquid nitrogen method.

Five individuals were investigated for each species. Chromosome measurements were obtained from the photographs of the best mitotic metaphase plates of each species. The karyomorphological classification of the interphase and prophase chromosomes followed Tanaka (1971; 1977). The symbols for the description of karyotypes followed Leven *et al.* (1964). The symmetry of karyotypes classified according to the Stebbins (1971). Taxonomical treatment followed Gu and Hultgård (2001).

RESULTS

All fourteen species investigated shared the same simple chromocenter type of the resting chromosomes and the proximal type of mitotic prophase chromosome (Figs 1A, 1B). They had a common gradual chromosome decrease in length from the longest to the shortest chromosomes at mitotic metaphase chromosomes. The parameters of chromosomes in the 14 species of *Parnassia* are listed in Table 2.

Sect. Saxifragstrum Drude - Parnassia esquirolii Level. The karyotype formula is 2n=18=16m +2sm. The chromosome number was reported for the first time. Metaphase chromosome ranging from 2.8 μm to 1.6 μm, the ratio of the longest to the shortest chromosome is ca. 1.8, and belonging to Stebinns'2A type (Figs 1G, 2E). This species is endemic to China and distributed in northeastern Yunnan and western Guizhou.

Parnassia tenella Hook f. et Thomas. The karyotype formula is 2n=18=16m+2sm. The chromosome number was reported for the first time. Metaphase chromosome ranging from 1.6 μ m to 1.3 μ m, the ratio of the longest to the shortest chromosome is ca. 1.2, and belonging to Stebinns'2A type (Figs 1E, 2C). It is distributed in southwestern China, extending to Nepal and Sikkim.

Sect. Cladoparnassia Engl. - Parnassia faberi Oliv. The karyotype formula is 2n=18=16m+2sm. The chromosome number was reported for the first time. Metaphase chromosome ranging from 2.4 μm to 1.9 μm, the ratio of the longest to the shortest chromosome is ca.1.3, and belonging to Stebinns'2A type (Figs 1F, 2D). This species is endemic to China and distributed in central Sichuan, northeastern Yunnan.

Sect. Nectarobilobos Ku - Parnassia bifolia Nekress. The karyotype formula is 2n=36=24m+12sm. The chromosome number was reported for the first time. Metaphase chromosome ranging from 2.6 μ m to 1.0 μ m, the ratio of the longest to the shortest chromosome is ca. 2.6, and belonging to Stebinns'2B type (Figs 1O, 2M). Distributed in Xinjiang, Hazakhistan.

Sect. Nectarotrilobos Drude - Subsect. Xiphosandra Franch. - Parnassia brevistyla Hand.-Mazz. The karyotype formula is 2n=14=12m +2sm. The chromosome number corresponded with the counts made by Funamoto, et al. (2001).

Table 2 — Chromosome comparison in the fourteen investigated species of *Parnassia* (RLR: relative length ratio; P: percentage of chromosomes with arm ratio over 2:1; M: median region; SM: submedian; X: chromosome basic number).

Species	Chromosome numbers (2n)	Chromosome sizes (µm)	RLR	P (%)	Symmetry class	M	SM	X
Sect. Saxifragastrum								
P. esquirolii	18	1.6-2.8	1.8	33.3	2A	16	2	9
P. tennella	18	1.3-1.6	1.2	16.7	2A	16	2	9
Sect. Cladoparnassia								
P. faberi	18	1.9-2.4	1.3	11.1	2A	16	2	9
Sect. Nectarotrilobos								9
P. brevistyla	14	2.7-4.2	1.5	28.7	2A	12	2	7
P. delavayi	14	4.1-5.8	1.4	28.7	2A	12	2	7
P. chinensis	18	1.6-2.4	1.5	33.3	2A	14	4	9
P. epunctulata	18	2.0-3.1	1.5	44.4	2A	16	2	9
P. mysorensis	18	1.7-3.1	1.8	22.2	2A	14	2	9
P. pusilla	18	3.9-4.5	1.2	11.1	2A	12	2	9
P. subscaposa	18	2.1-3.5	1.7	22.2	2A	14	4	9
P. trinervis	18	1.9-3.0	1.6	11.1	2A	14	4	9
P. venusta	18	2.1-2.6	1.2	11.1	2A	16	2	9
Sect. Nectarobilobos								
P. bifolia	36	1.0-2.6	2.6	29.1	2B	24	12	9
Sect. Allolobos								
P. monochorifolia	48	0.8-1.8	2.3	45.8	2B	30	18	8

Metaphase chromosome ranging from 4.1 μ m to 2.7 μ m, the ratio of the longest to the shortest chromosome is ca.1.5, and belonging to Stebbins'2A type (Figs 1C, 2A). This species is endemic to China and distributed in southwestern and northwestern China.

Parnassia delavayi Franch. The karyotype formula is 2n=14=12m+2sm. The chromosome number agrees with the counts made by Funamoto *et al.* (1998, 2001). Metaphase chromosome ranging from 5.8 μm to 4.1 μm, the ratio of the longest to the shortest chromosome is ca.1.4, and falling into Stebinns'2A type (Figs 1D, 2B). The species is distributed in southwestern, northwestern and central China and Bhutan.

Sect. Nectarotrilobos Drude - Subsect. Nectarotrilobos Ku - Parnassia chinensis French. The karyotype formula is 2n=18=14m+4sm. The chromosome number was reported for the first time. Metaphase chromosome ranging from 2.4 μm to 1.6 μm, the ratio of the longest to the shortest chromosome is c. 1.5, and belonging to Stebbins'2A type (Figs 1H, 2F). It is distributed in southwestern China, extending to Bhutan, northern Myanmar, Nepal and Sikkim.

Parnassia epunctala J. T. Pan The karyotype formula is 2n=18=16m+2sm. The chromosome number was reported for the first time. Metaphase chromosome ranging from 3.1 μm to 2.0 μm, the ratio of the longest to the shortest chro-

mosome is c. 1.5, and belonging to Stebbins'2A type (Figs 1K, 2I). This species is endemic to China and distributed in northwestern Yunnan.

Parnassia mysorensis Heyhe. The karyotype formula is 2n=18=14m+4sm. The chromosome number was reported for the first time. Metaphase chromosome ranging from 3.1 μm to 1.7 μm, the ratio of the longest to the shortest chromosome is c. 1.8, and belonging to Stebinns'2A type (Figs 1J, 2H). Distributed in southwestern China, northern India, Sikkim.

Parnassia pusilla Wall. ex Arn. The karyotype formula is 2n=18=14m+4sm. Metaphase chromosome ranging from 4.5 μm to 3.9 μm, the ratio of the longest to the shortest chromosome is c. 1.2, and belonging to Stebinns'2A type (Figs 1N, 2L). The species is distributed in southern Xizang, Bhutan, northern India, Nepal, and Sikkim.

Parnassia subscaposa C. Y. Wu ex Ku The karyotype formula is 2n=18=14m+4sm. The chromosome number was reported for the first time. Metaphase chromosome ranging from 3.5 μm to 2.1 μm, the ratio of the longest to the shortest chromosome is c.1.7, and belonging to Stebinns'2A type (Figs 1I, 2G). This species is endemic to China and distributed in northwestern Yunnan.

Parnassia trinervis Drude: The karyotype formula is 2n=18=14m+4sm. The chromosome number agrees with counts made by Funamoto, *et al.* (1996). Metaphase chromosome ranging

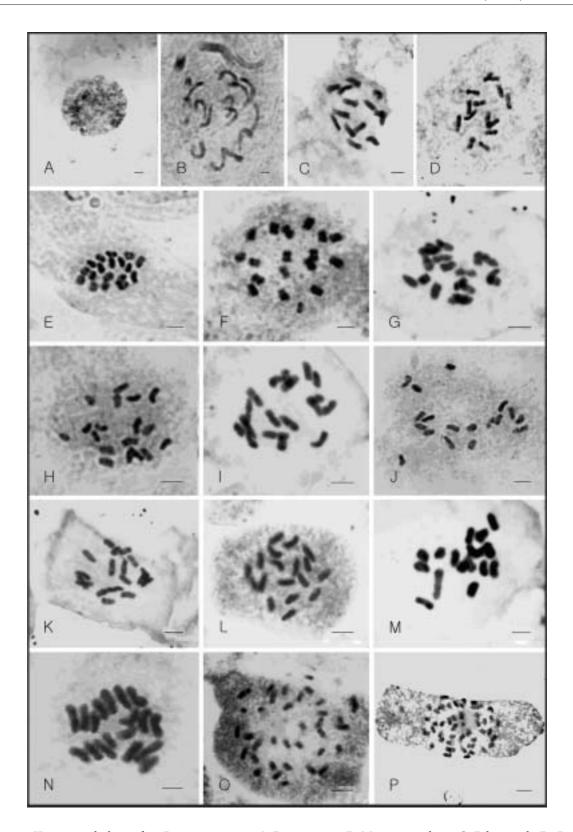


Fig. 1 — Karyomorphology of 14 *Parnassia* species. A: Resting state, B: Mitotic prophase, C: *P brevistyla*, D: *P. delavayi*, E: *P. tenella*, F: *P. faberi*, G: *P. esquirolii*, H: *P. chinensis*, I: *P. subscaposa*, J: *P. mysorensis*, K: *P. epunctulata*, L: *P. venusta*, M: *P. trinervis*, N: *P. pusilla*, O: *P. bifolia*, P: *P. monchorifolia*. All bar=3µm.

from 3.0 μ m to 1.9 μ m, the ratio of the longest to the shortest chromosome is c. 1.6, and belonging to Stebbins'2A type (Figs 1M, 2K). This species is endemic to China and distributed in southwestern and northwestern China.

Parnassia venusta Jien. The karyotype formula is 2n=18=16m+2sm. The chromosome number was reported for the first time. Metaphase chromosome ranging from 2.6 μm to 2.1 μm, the ratio of the longest to the shortest chromosome is c. 1.2, and belonging to Stebbins'2A type (Figs 1L, 2J). This species is endemic to China and distributed in northwestern Yunnan.

Sect. *Allolobos* **Ku** - *Parnassia monochorifolia* **French.** The karyotype formula is 2n=48=30m +18sm. The hexaploid chromosome number was reported for the first time in China. Metaphase chromosome ranging from 1.8 μm to 0.7 μm, the ratio of the longest to the shortest chromosome is ca.2.3, and belonging to Stebinns'2B type (Figs 1P, 2N). This species is endemic to China and distributed in northeastern Yunnan.

DISCUSSIONS

1. Basic Chromosome Number Variation - The basic chromosome number is of importance to determine the systematic position of a taxon at higher taxonomic level (RAVEN 1975). As showed in Table 3, together with previously studied species, the chromosome numbers of about 30 species in the genus *Parnassia* were presently known, representing about 41% of the total number of species in the genus. Based on previous reports and our own studies, there are three basic chromosome numbers (*i.e.* x=7,8, and 9) in *Parnassia* (Funamoto *et al.* 1998; Simmons 2004).

The genus Parnassia might be divided into three groups, based on the chromosome base number. The first group with the basic chromosome number of x=9 was reported from about 20 species, accounted for more than 70% of species studied, and including type species of *Parnassia*, i.e. P. palustris. There were obvious differences in morphology among species, especially in the floral characteristics. These species distributed in N America had most highly elaborated and branched staminodia, while those in E Asia were with staminodia unlobbed or, when deeply lobbed, rarely more than half their length (Ku 1987; Wu et al. 2003). The second group included five species with the basic chromosome number x=8, led from 2n=32 and mainly distributed in N

America. Two species with the same basic chromosome numbers were distributed in China. Parnassia yunnunensis var. longistipitata, which was of low and small, 3 minutely lobed staminodia. (Funamoto et al. 1998), and P. monochorifolia being with flat five-lobed staminodia. The third group with the basic chromosome number x=7 included *P. brevistyla* and *P. delavayi*, both from the Himalayas and SW China. Their fertile stamens were with the anther connection elongated beyond the anther sacs. Based on this character state, Frenchet (1897) proposed an alternative intrageneric classification, by dividing the genus into two sections, one of which consisted of P. delavayi and P. brevistyla. However, the odd difference of the chromosome base number in an Asian species P. wightiana (2n=36, MALLA et al. 1981; 2n=14, Funamoto et al. 1998) suggested that P. wightiana needed to be reinvestigated by using material from different localities.

The center of diversity for Parnassia is in E Asia, especially in China and the Himalayas where the majorities of the species occur (Phillips 1982; Ku 1987; 1995; Wu et al. 2003). Most species with the basic chromosome number of x=9 were distributed in East Asia. Two species in North America had the same chromosome number, one being P. palustris which is the most widespread species of the genus, with populations in North America, Europe, and Asia, and south to Morocco (Korta 1972), the other being *P. kotzebuei*, which is distributed across Siberia and western North America. The chromosome numbers of *P*. glauca is very interesting, with two basic chromosome numbers x=8 (Gastony and Soltis 1977), and 9 (Löve and Löve 1977).

A second center of diversity is in N America where 10 species occur (Phillips 1982; Ku 1987 1995; Wu et al. 2003). Parnassia townsedii is the southernmost species and is endemic to the Sierra Madre Occidental of Mexico (Bye and Soltis 1979). The basic chromosome number of the western North America species is x=9 (Packer 1964; Bye and Soltis 1979; Jorgenson et al. 1977; Löve and Ritchie 1966; Funamoto et al. 1998), while the eastern N America species have a basic chromosome number of x=8 (Gastony and Soltis 1977; Löve and Ritchie 1966). It was suggested that the eastern species were more highly divergent from the western N America and Asian species (Phillips 1982).

Based on the distribution of the three basic chromosome numbers, together with the morphological evidence, it is suggested here that X=9 might be original basic chromosome number in

Table 3 — Chromosome numbers and geographical distributions of *Parnassia* species

Species	Chromosome Numbers (2n)	Distributions	References		
Sect. Saxifragstrum.					
P. esquirolii	2n=18	NE Yunnan, W Guizhou	The present study		
P. tenella	2n=18	SW China; Nepel, Sikkim	The present study		
P. yunnanensis var. longistipitata	2n=32	SW Yunnan, W Sichuan	Funamoto <i>et al.</i> 1997		
Sect. Cladoparnassia					
P. faberi	2n=18	C Sichuan, NE Yunnan	The present study		
Sect. Nectarobilobos					
P. bifolia	2n=36	Xijiang; Kazakhstan	The present study		
Sect. Nectarotrilobos					
P. brevistyla	2n=14	SW, NW China	The present study		
P. acuminum	2n=18	W Sichuan, S Qinghai	Funamoto <i>et al.</i> 1998		
P. chinensis	2n=18	SW China; Bhutan, N Myanmar, Nepal, Sikkim	The present study		
P. delavayi	2n=14	SW, NW, C China; Bhutan	The present study		
P. epuenctulata	2n=18	NW Yunnan	The present study		
P. laxianii	2n=18, 36	C Xinjiang; Kazakhstan, Mongolia, Si-beria	Krogulevich 1978		
P. mysornsis	2n=18	SW China; N India, Sikkim	The present study		
P. nubicola	2n=18	SW China; Afghanistan, Bhutan, India, Kashmir, Nepal, Pakistan	Hamel 1953		
P. oreophila	2n=18, 36	NW, N China	Funamoto et al. 1994; 1996		
P. pusilla	2n=18	S Xizang; Bhutan, N India, Nepal, Sikkim	The present study		
P. scaposa var. yushuensis	2n=18	W Sichuan, S Xizang, S Qinghai	Funamoto <i>et al.</i> 1996		
P. subscaposa	2n=18	NW Yunnan	The present study		
P. trinervis	2n=18	NW Yunnan, Sichuan, Xizang, Qinghai, Gansu	The present study		
P. venusta	2n=18	NW Yunnan	The present study		
P. viridifolia	2n=18,36	SW, NW China	Funamoto <i>et al.</i> 1998; 2001		
Sect. Allolobs					
P. monochorifolia	2n=48	NE Yunnan	The present study		
P. wightiana	2n=36	SW, NW, C China; SE Asia	Malla <i>et al.</i> 1981		
	2n=14		Funamoto et al. 1998		
Sect. Parnassia					
P. asarifolia	2n=32	Eastern N America	Löve and Ritchie 1966 Gastony and Soltis 1977		
P. caroliniana	2n=32	Eastern N America	Gastony and Soltis 1977		
P. fimbriata	2n=36	Western N America	Packer 1964; Taylor		
			and Brochman 1966		
P. glauca	2n=32 2n=36	Eastern N America	Gastony and Slotis 1977 Löve and Löve 1977		
P. grandifolia	2n=32	Eastern N America	Löve and Ritchie 1966		
P. kotzebuei	2n=18, 36	Western N America; Si-beria	Jorgenson <i>et al.</i> 1958; Johnson and Packer 1968		
P. palustris	2n=18,27, 36,45,54	N America; Europe; C Asia	Funamoto <i>et al.</i> , 1998		
P. townsedii	2n=36	Western N America	ByE and Soltis 1979		

Parnassia, and x=8 and x=7 were derived from it by means of chromosome reorganization (such as chromosome fission, fusion and dysploidy). It is noted that the basic chromosome number x=7 is only reported in China. There is a tendency the chromosome number decrease (in evolution line) in the genus. However, further evidence is needed to test this hypothesis.

2. Polyploidization - Polyploidization has occurred extensively during the evolution of angiosperms (Stebbins 1971; Grant 1981; Masterson 1994; Ku *et al.* 2000). The importance of polyploidy as an active process was manifested by the estimated number of speciation events, as many as 2-4% (Otto and Whiffon 2000) and historical success of polyploidy in different floras

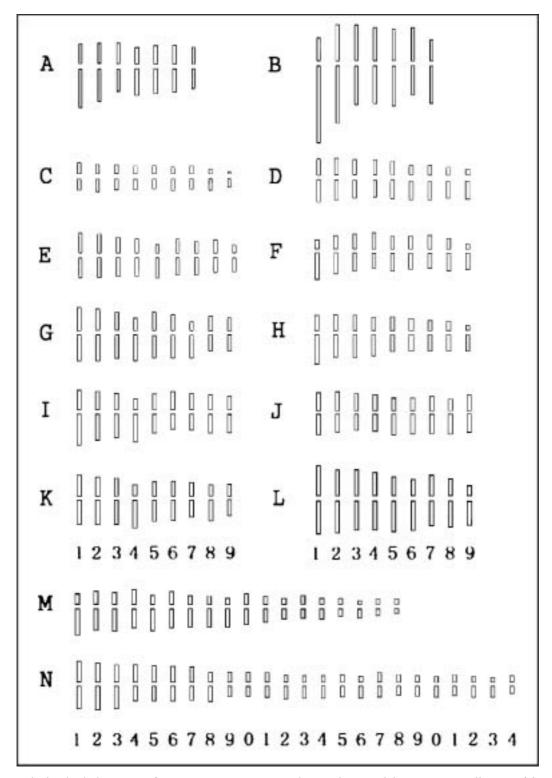


Fig. 2 — The haploid ideograms of 14 Parnassia species. A: P. brevistyla, B: P. delavayi C: P. tenella, D: P. faberi, E: P. esquirolii, F: P. chinensis, G: P. subscaposa, H: P. mysorensis, I: P. epunctulata, J: P. venusta, K: P. trinervis, L: P. pusilla, M: P. bifolia, N: P. monchorifolia.

(Stebbins 1971). In *Parnassia*, as showed in Table 3, polyploidization appeared frequently, as many as 50 % of the observed species were polyploids.

Parnassia palustris is with wide geographical distribution throughout most of the Holarctic region. It includes diploid, triploid, tetraploid, pen-

taploid, and hexaploid individuals (2n=18, 27, 36, 45. and 54) (Funamoto et al. 1998 and references cited therein). Other species with both diploids and tetraploids are P. kotzebuei from Siberia and N America (2n=18 and 36) (Jorgenson et al. 1958; Löve and Ritchie 1966), Parnassia laxmanii from northern Asia extending to Xinjiang (2n= 18 and 36) (Krogulevich 1978; Murin et al. 1980), Parnassia oreophyla from China (2n=18 and 36) (Funamoto et al. 1994; 1996), Parnassia viridifolia from China (Funamoto et al. 1998; 2001). The tetraploids have been considered to be autoploids (e.g., P. oreophyla, Funamoto et al. 1996; P. palustris, Wentworth and Gornull 1996) or intraspecific hybrid polyploidy (e.g. P. palustris, Hultgärd 1987). Furthermore, the only difference in gross-morphology between diploid and tetraploid populations of *P. palustris* are the generally larger pollen grains and seeds in tetraploids (Hultgärd 1987). Rodriguez (1996) pointed out that demographic stochastic and iteroparity of a polyploidy can enhance its chance of successful establishment in a diploid population. This difference in colonizing ability between cytotypes might have helped autotetraploids to escape minority cytotype exclusion in diploid parental populations after the last glaciation. For instance, in Britain and Scandinavia, diploid populations of P. palustris have a southern distribution, whereas tetraploid populations have a more northern distribution (Hultgärd 1987; Wentworth and GORNALL 1996). The tetraploids of *P. palustris* appear to be autopolyploids with no significant reduction in fertility (WENTWORTH and GORNALL 1996), which were better than the diploids to spread north as the glaciers receded.

However, the present study revealed that most of the 14 studied species were diploids, mostly 2n=18, except for *P. brevistyla* and *P. delavayi* with 2n=14. In this report, two polyploids were *P. bifo*lia from Xinjiang and Kazakhstan (2n=4x=36), and P. monochorifolia from northeastern Yunnan (2n=6x=48). The only other tetraploids was P. yunnanensis var. longistiptata with 2n=32 from southwestern Yunnan and western Sichuan (Fu-NAMOTO et al. 1997). Obviously, fewer polyploids were reported in China, especially in southwestern China. Out of the 10 species distributed in N America, eight species including the Holarctic *P*. palustris were all polyploids (Table 3). Although most species distributed in Asia, only 6 species out of the 20 studied species were polyploidy (*P. lax*manii 2n=18, 36, P. oreophyla 2n=18, 36, P. viridifolia 2n=18, 36; P. yunnanensis var. longistiptata 2n=32; P. bifolia 2n=36 and P. monochorifolia

2n=48). At present, only 20 out of the 63 of the Chinese species were studied, but it seems that the Asian species of *Parnassia* have been adopted a different evolutionary strategy from those of the North American species. As mentioned earlier, all three basic chromosome numbers were reported in Asia, while only x=9 and 8 were reported in N America. The polyploidization of the genus needs further study.

The fact that the tetraploid species were mostly distributed in N America, whereas diploids were mostly distributed in Asia, especially in the mountains of SW China implied that the mountains of SW China is the center of diversification. This area was considered to be one of the world's "hot spots" of biodiversity (MYERS 1988; BOUFFORD and VAN DIJK 1999; MYERS *et al.* 2000). More than 60% species of the genus were distributed in these mountains, accounted for about 80% species in China (Ku 1987; 1995).

3. Karyotype Evolution - Parnassia grows in temperate to arctic region, preferentially in open, moist habitats, from shore meadows to mountainsides. It is easy distinguished by the basal rosette of levels, generally with long peduncles, single-flowered inflorescences, penta-merous flowers, commissural stigmas, and the presence of staminodia opposite to petals. The classification of the genus heavily based on the shape of staminodia and the petals (DRUDE 1875; ENGLER 1930; HANDEL-MAZZETTI 1941; KU 1987; 1995; WU et al. 2003).

Firstly, karvomorphology in *Parnassia* is more or less symmetric. Most studied species were with karyotype of Stebbins' 2A type. This is similar to that of P. accuminum (Funamoto et al. 2001), which had median-centromeric chromosomes of the chromosome complements in centromeric position at mitotic metaphase, while the other species had median- and submedian- centromeric chromosome. Obviously, the karyomorphology of P. accuminum was symmetric. According to STEB-BINS (1971), it is postulated that the karyotypes of Parnassia may have evolved towards the decrease of symmetry by the increase of median-centromeric chromosome in the chromosome complements. Second, it seems that the basic chromosome number x=7 in P. brevistyla and P. delavayi (both of the Sect. Nectarotrilobos) were caused by chromosome fusion. Both species had relatively larger chromosomes (see Table 2). For instance, the chromosomes of *P. brevistyla* are about twice larger than those of *P. chinensis* of the same section. Parnassia delavayi showed the chromosome

size twice larger than those of *P. viridiflora* (FUNAMOTO *et al.* 1998), also the same section. The other seven species in Sect. *Nectarotrilobos*, all with smaller chromosomes, were with the basic chromosome number x=9.

4. Karyotype and Classification - Several different classifications were proposed for Parnassia (Drude 1875; Franchet 1897; Engler 1930; Handel-Mazzetti 1941; Ku 1987; 1995; Wu et al. 2003). Drude (1875) recognized four sections based on characteristic of the staminodes, ovary position, and carpel numbers. Ku (1987; 1995) recognized nine sections for the Chinese species. Philips (1982) recognized two sections for the N American species. In spite of the accumulating molecular data, chromosome information continues to be important in assessing phylogenetic relationships (CARR et al. 1999). Although the cytological data on *Parnassia* is not complete, it was concluded that the basic chromosome numbers for the genus are x=7, 8 and 9. Members of section Parnassia are primarily distributed in N America. Although P. kotzebei also extends into Siberia and *P. palustris* occurs throughout much of N America, Europe, and Asia. It was considered that Sect. Parnassia could be divided into two sections according to the basic chromosome numbers and geographical distributions (Phillips

In addition, *Parnassia delavay*i and *P. brevistyla* possessed the feature of 3-lobed staminodia, were placed in Sect. *Nectarotriolbos* (DRUDE 1875; ENGLER 1930; HANDEL-MAZZETTI 1941; KU 1987; 1995). However, the basic chromosome number for *P. brevistyla* (2n=14) and *P. delavayi* (2n=14) is x=7. These species are restricted to China and Himalayas. The other characteristics were that their anther connectives elongated beyond the anther sacs. It was suggested that the both should be erected to a section of their own, as proposed by Franchet (1897) and Wu *et al.* (2003).

Acknowledgements — The authors are indebted to Mr. Jipei Yue and Mr. Jie Cai of the Kunming Institute of Botany for their valuable help. This study was supported by a Keynote Project of the Knowledge Innovation Program, the Chinese Academy of Sciences (KSCX2-1-106B) and a National Program for R & D Infrastructure and Facility Development (2004DKA30430).

REFERENCES

Apg, 1998 — An ordinal classification for the families of flowering plants. Annals of the Missouri Botanical Garden, 85(4): 531-553.

- Apg-II, 2003 An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants. Botanical Journal of the Linnean Society, 141: 399-436.
- Arber A., 1913 On the structure of the androeceum in Parnassia and its bearing on the affinities of the genus. Annals of Botany, 27: 491-510.
- —, 1915 The anatomy of the stamens in certain Indian species of Parnassia. Annals of Botany, 29: 159-160.
- Bennsel C.R., Palser B.F., 1975 Floral anatomy in the Saxifragaceae sensu lato. IV. Baueroideae and conclusions. American Journal of Botany, 62: 688-694
- BOUFFORD D.E. and VAN DIJK P.P., 1999 South-central China. In: MITTERMEIER R.A., MYERS N., GIL P.R. and C.M MITTERMEIER (Eds), "Hotspots: earth's biologically richest and most endangered terrestrial ecoregions". Mexico City: CEMEX, 339-350.
- Bye R. A. Jr. and Soltis D. E., 1979 *Parnassia townsendii* (Saxifragaceae), a *Mexican endemic*. Southw. Naturalist, 24: 209-222.
- CARR G..D., KING R.M., POWELL A.M. and ROBINSON H., 1999 *Chromosome numbers in Compositae*. XVIII. American of Journal of Botany, 86(7): 1003-1013.
- Chase M.W., Soltis D.E., Olmstead R.G., Norgan D., Les D.H., Mishler B.D., Duvall M.R., Price R.A., Hills H.G., Qiu Y.-L., Kron K.A., Rettig J.H., Conti E., Palmer J.D., Manhaet H.R., Sytsma K.J., Michaels H.J., Kress W. J., Karol K.G., Cark W.D., Hedren M., Gaut B.S., Jansen R.K., Kim K.-J., Wimpee C.F., Smith J.F., Furnier G.R., Strauss S.H., Xiang Q.-Y., Plunkett G.M., Soltis P.S., Swensen S.M., Williams S.E., Gadek P.A., Quinn C.J., Eguiarte L.E., Golinber G.E., Learn JR G.H., G rahams.W., Barrett S.C., Dayanandan S.C., Dayanandan S. and Albert V.A., 1993 Phylogenetics of seed plants: An analysis of nucleotide sequences from the plastid gence rbc L. Annals of the Missouri of Botanical Garden, 89: 528-580
- Cronquist A., 1981 An Integrated System of Classification of Flowering Plants. Columbia University Press, New York.
- Dahlgren R. 1980 *A revised system of classification of the angiosperms*. Botanical Journal of the Linnean Society, 80: 91-124.
- —, 1983 General aspects of angiosperm evolution and macrosystematics. Nordic Journal of Botany, 3: 119-149.
- Dandy J.E., 1927 *The genera of Saxifragaceae*. In: Hutchinson J. (Eds), "Contributions towards a phylogenetic classification of flowering plants". VI. Bull. Misc. Inform. 1927:100-118.
- Drude O., 1875 Ueber die Blüthengestaltun und die verwandtschaftsverhältnisse des Genus Parnassia, nebst nebst einer systematischen Revision seiner Arten. Linnaea, 39: 239-324.

Engler A., 1930 — *Saxifragaceae*. In: Engler A. (Ed), "Die Naturlichen Pflanzenfamilien", 18a: 74-225. Wilhelm Engelman, Leipztig.

- Erlandsson S., 1942 Cytologiskt-vaxtgeografiska rasstudier I Nordens Parnassia palustris-population. Acta Horticulture Bergiani, 13: 117-148.
- Franchet A.R., 1897 Les Parnassia de l'Asie orientale. Bulletin de la Société Botanique de France, 44: 244-263.
- Funamoto T., 1986 Karyomorphological studies of the genus Parnassia in Japan. Res. Inst. Evolut. Biol. Sci. Rep. 3:72-81.
- Funamoto T., Konodo K., Hong D.Y., Yang Q.E., Ge S., Hizume M. and Shimada T., 1996 *Karyomorphological studies in Chinese Parnassia* (II) *Three species in Qinghai Province*. La Kromosoma, II-82: 2845-2854.
- Funamoto T., Konodo K., Hong D.Y., Zhou S.L. and Oguro H., 2001 *Chromosomes in four species of Parnssia (Saxifragaceae) in the northern part of Sichuan Provience, China.* Chromosome Science, I: 19-25.
- Funamoto T., Konodo K., Hong D.Y., Zhou S.L. and Shimada T., 1997 Karyomorphological studies in Prnassia yunnanensis var. longistipitata in Sichuan Province, China. Chromosome Science, I: 21-24.
- Funamoto T., Konodo K.. Hong D.Y., Zhou S.L. and Deguchi H., 1998 A chromosome study of three species collected in the Qin Ling Moutains, Shaanxi Provience, China. Chromosome Science 2: 111-115.
- Funamoto T., Tanaka R., Hong D.Y., Luo Y.B., and Nakata M., 1994 *Karyomorphological studies in China region*. La Kromosomo. II-74: 2576-2582.
- GASTONY G.J. and SOLTIS D. E., 1977 Chromosome studies of Parnassia and Lepuropetalon (Saxifragaceae) from the eastern United States: A new base number for Parnassia. Rhodota, 79: 573-578.
- Grant V., 1981 Plant Speciation. Columbia University Press, New York.
- GRAY S.F., 1821 *Parnassieae*. A Natural Arrangement of British Plants, 2:623. New York: Academic Press
- Gu T. and Haltgärd U.M., 2001 *Parnassia*. In Wu C.Y. and Raven, P.H. (Eds), Flora of China, 8, p.358-379, Science Press, Beijing.
- Guerra M., 1990 A situação da citotaxonomia de angiospermas nos tròpicose, em particular, no Brasil. Acta Botanica Brasilica, 4(2):75-86.
- HAMEL J.L., 1953 Contribution a l'etude cytotaxinomique des Saxifragaceaees. Revue de Cytologie et de Biologie Végétales, 14: 113-314.
- HANDEL-MAZZETTI H., 1941 Die chinesischen Parnassia-Arten. Österreichische Botanische Zeitschrift, 90: 127-136.
- Hedberg O., 1967 Chromosomme numbers of vascular plants from arctic and subarctic North America. Arkiv För Botanik, II-6: 309-326.
- HULTGÄRD U.M., 1987 Parnassia palustris L. in Scandinavia. Symbolae Botanicae Upsalienses, 28: 1-128.

- Hutchinson J., 1969 Evolution and phylogeny of flowering plants. Academic Press, New York.
- JAY M., 1971 Quelques problemes taxonomiques et phylogénétiques des Saxigragacées vus à la lumière de la biochemie foavonique. Bulletin du Muséum national d'Histoire naturelle, II-42: 754-775.
- JOHNSON A.W., PACKER J.G., 1968 Chromosome numbers in the Flora of Ogotruk Creek, N.W. Alaska. Botaniska Notiser, 121: 403-456.
- JORGENSEN C.A., SORENSEN and WESTERGAARD T., 1958
 The flowering plants of Greenland: A taxonomical and cytological survey. Biol. Skr., 9: 1-172.
- KORTA J., 1972 Anatomical analysis of Parnassia palustris L. Acta Biol. Cracov., 15: 31-37.
- Krogulevich R. E., 1978 Karyological analysis of the species of the flora of eastern Sayan. In: Malyshev L. I. and Pesbaikal G.A. (Eds), Flora of the Prebaikal, p. 19-48. Novosibrisk.
- Ku H.M., Vision T., Liu J. and Tankaley S. D., 2000 Comparing sequenced segments of the tomato and Arabidopsis genormes; Large-scale duplication followed by selective gene loss creates a network of synteny. Proceedings of the National Academy of Sciences USA, 97: 9121-9126.
- Ku T., 1987 A revision of the genus Parnassia (Saxi-fragaceae) in China. Bulletin of Botanical Research, 7: 1-59.
- Ku T., 1995 Parnassia L. In: Lu L. and HWANG S. (Eds.) "Saxifragaceae", Flora Reipulicae Popularis Sinicae Tomus 35(1), p. 12-66. Science Press, Beiiing.
- LEVAN A., FREDGA K. and SANDLBERG A.A., 1964 Nomenclature of centromeric position on chromosomes. Hereditas: 201-220.
- Löve A., 1954 Cytotaxonomical evalutation of corresponding taxa. Vegetario 5-6: 214-224.
- Löve A. and Löve D., 1980 In: *Chromosome number reports LXIX*. Taxon, 29: 707-709.
- —, 1982 In: IOPB Chromosome number reports LXXV. Taxon, 31: 344-360.
- Löve A. and Ritchie J.C., 1966 *Chromosome numbers from central Canada*. Canadian Journal of Botany, 44: 429-439.
- Malla S.B., Bhattrarai S., Gorkhali M., Salju H. and Kayastha M., 1979 In: *IOPB chromosome number reports LXV*. Taxon, 28: 627-628.
- —, 1981 In: IOPB chromosome number reports LXX. Taxon, 30: 75.
- Masterson J., 1994 Stomatal size in fossil plants: Evidence for polyploidy in majority of angiosperms. Science, 264:421-423.
- Murin A., Haberova I., Zamsran C., 1980 Karyological studies of some species of the Mongolian flora. Folia Geobotanica et Phyto-taxonomica Bohemoslovaca, 15: 395-405.
- Myers N., 1998 Threatened biotas: "hot spots" in tropical forests. Environmentalist, 8: 187-208.
- Myers N., Mittermeier R.A., Mittermeier C.G., Dafonseca G.A.B. and Kent J., 2000 *Biodiversity hotspots for conservation priorities*. Nature, 403: 853-858.

- Otto S.P. and Whitton J., 2000 *Polyploid incidence* and evolution. Annual Review of Genetics, 34: 401-437.
- PACEL L., 1912 *Parnassia and some allied genera*. Botanical Gazette, 54: 306-329.
- PACKER J. G., 1964 Chromosome numbers and taxonomic notes on western Canadian and Arctic plants. Canadian Journal of Botany, 42: 437-494.
- PACKER J. G. and MCPHERSON G. D., 1974 Chromosome numbers in some vascular plants from northern Alaska. Canada Journal of Botany, 52: 1095-1099.
- PHILLIPS R.B., 1982 Systematics of Parnassia (Parnassiaceae): Generic Overview and Revision of North American Taxa. Ph. D. Dissertation, University of California, Berkerley.
- RAVEN P. H., 1975 The bases of angiosperm phylogeny: cytology. Annals of the Missouri Botanical Garden, 62: 724-764.
- Rodriguez D.J., 1996 A model for the establishment of polyploidy in plants: viable but infertile hybrids, iteroparity, and demographic stochasticity. J. Theor. Biol., 180: 189-196.
- Schoenagel E., 1931 Chromosomenzahl und Phylogenie der Saxifragaceen. Botanische Jahrbücher für Systematik, 64: 266-308.
- SIMMONS M. P., 2004 *Parnassiaceae*. In: K. Kubitzki (Ed), "The families and genera of vascular plants", n. 6, p. 291-296, Springer, Berlin.
- Soltis D.E., Soltis P.S., Chase M.W., Mort M.E., Albach D.C., Zanis M., Savolaine N.V., Hahn W.H., Hoot S.B., Fay M.F., Axtell M., Swensen S.M., Nixon K.C. and Farris J.S., 2000 *An*-

- giosperm phylogeny inferred from a combined data set of 18S rDNA, rbcL, and atpB sequences. Botanical Journal of the Linnean Society, 133: 381-461.
- Stebbins G. L., 1971 Chromosomal Evolution in Higher Plants. Edward Arnold, London.
- Takhtajan A., 1969 Flowering Plants: origin and dispersal. Oliver and Boyd, Edinburgh.
- —, 1997 Diversity and Classification of Flowering Plants. Columbia University Press, New York.
- TANAKA R., 1971 Types of resting nuclei in Orchidaceae. Botanical Magazine Tokyo, 84: 118-122.
- —, 1977 Recent Karyotype Studies. In: OGAWA K. et al. (Eds), Plant Cytology, p. 293-326, Asakura Book, Tokyo.
- TAYLOR R.L., BROCKMAN R.P., 1966 Chromosome numbers in some western Canadian plants. Canadian Journal of Botany, 44: 1093-1103.
- THORNE R.F., 1976 A phylogenetic classification of the Angiospermae. Evolution Biology, 9: 35-106.
- Wentwoth J, E. and Gornall R.J., 1996 Cytogenetic evidence for autopolyploidy in Parnassia palustris. New Phytologist, 134: 641-648.
- Wu C.Y., Lu A. M., Tang Y. C., Chen Z. D. and Li D. Z., 2003 The family and genera of angiosperms in China: a comprehensive analysis. Science Press, Beijing.
- ZHUKOVA P.G., 1966 Chromosome numbers in some species of plants of the northeastern part of the U.S.S.R., Botanicheskii Zhurnal, 51: 1511-1516.

Received 30.4.2004; accepted 30.1.2005