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SHORT COMMUNICATION

A cyto-evolutional study of *Campanumoea* Blume (*Campanulaceae*) and a possible pathway for secondary karyotype formation

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Abstract *Campanumoea* is a small genus in the family *Campanulaceae*, with species divided into sections *Campanumoea* and *Cyclocodon*. Sixteen accessions from *Campanumoea* and related genera native to China were used to study their karyotype. The results showed that chromosome characteristics were different between the two sections. For *Campanumoea*, the karyotypic formula was $2n = 2X = 2m + 12sm + 2st = 16,3A$ and for *Cyclocodon* it was $2n = 2X = 6m + 12sm = 18,3B$. These data, combined with chromosomal length characteristics, support the restoration of section *Cyclocodon* as a genus. However, the incorporation of section *Campanumoea* into *Codonopsis* requires more evidence. Comparison of chromosomal length and haploid set length revealed that chromosomal segment rearrangements occurred within sections of *Campanumoea* and between genera, with the difference within sections being greater than that between genera. Therefore, chromosomal segment rearrangements are present in *Campanulaceae*, implying that chromosomal segment rearrangement plays an important role in the evolution of diversity in *Campanulaceae*. By comparing the chromosomal characteristic in section *Campanumoea* and the genus *Adenophora*, we concluded that the secondary chromosome type such as $n = 17, 18$ would be derived by autopolyploidization of $n = 9$, and by chromosome fusion.

Keywords *Campanumoea* Blume (*Campanulaceae*) · Karyotypic formula · Chromosomal segment rearrangement · Autopolyploidization

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Introduction

As a nearly cosmopolitan angiosperm family, the *Campanulaceae* comprise latex-bearing, primarily perennial herbs or occasional subshrubs that typically have alternate leaves, sympetalous corollas, and mostly inferior ovaries (De Candolle 1830; Schönland 1889; Fedorov 1972; Takhtajan 1987; Kovanda 1978; Kolakovsky 1987). Worldwide, there are 600–950 (1,045 according to Lammers 2007) species in this family in the strict sense (s.s.), i.e., when not merged with the *Lobeliaceae*, in 35–55 genera (Kovanda 1978; Takhtajan 1987; Lammers 2007). There are 17 genera and 150 species native to China, most of them distributed in the southwest of the country (Hong and Lian 1983). Based on 26 characters, Hong (1995) considered that a small area of SW China (E and N Yunnan, SW Sichuan, and SW Guizhou) was the most concentrated region of genera in the family and the center of primitive forms (mostly west of the Tanaka–Kaiyong Line; also see Eddie et al. 2003).

Campanumoea is a small genus in the family *Campanulaceae*, with five species distributed in tropical and subtropical areas of eastern Asia. All five species can be found in southern China (Hong and Lian 1983). Due to

differences in their morphology and distribution, the species in this genus were divided into two sections: *Campanumoea* and *Cyclocodon* (Clarke 1881; Tsoong 1935; Hong and Lian 1983; Shimizu 1993). *Campanumoea* has baccate fruits and the ovary is often six-locular with a six-lobed stigma, whereas in *Codonopsis* s.s. it is usually five-locular. It also lacks the distinctive foxy odor, and its distribution extends farther south to tropical India, Indochina, the Philippines, Malaysia, Indonesia, and New Guinea (Moeliono and Tuyn 1960). Also, *Campanumoea* is a vigorous climbing plant of warm-temperate and subtropical to tropical forest edges, generally at lower elevations than *Codonopsis* (Eddie et al. 2003).

Several authors have incorporated *Campanumoea* into *Codonopsis* (Moeliono and Tuyn 1960; Van Thuan 1969; Grey-Wilson 1990; Lammers 1992; Morris and Lammers 1997). Murthy (1983) discovered that the pollen of section *Campanumoea* was identical to that reported for *Codonopsis*: suboblate, 5–7-colpate, with spinules ca. 1 μm long. In contrast, in species of section *Cyclocodon*, the pollen grains were oblate spheroidal and 3-colporate, with spinules ca. 3 μm long. According to pollen characteristics, Murthy proposed that the species of section *Campanumoea* be incorporated into *Codonopsis* while section *Cyclocodon* should be treated as a distinct genus. Murthy retained the name *Campanumoea* for his residual genus, but the rules of nomenclature require that the correct name for the genus thus constituted would be *Cyclocodon* Griff. (Greuter et al. 1994); based on this viewpoint, *Campanumoea* would then become a synonym of *Codonopsis*. More over, Morris and Lammers (1997) confirmed the primary finding of Murthy (1983) that pollen of section *Campanumoea* is essentially indistinguishable from that of *Codonopsis*, while pollen of section *Cyclocodon* is quite distinct. These results add additional support to Murthy's suggestion that section *Cyclocodon* be recognized as a distinct genus while section *Campanumoea* should be subsumed into *Codonopsis*. Meanwhile, they cautioned against accepting the revision of generic boundaries proposed by Murthy and Grey-Wilson (1990) (also see Eddie et al. 2003). They drew attention to the fact that simple incorporation of *Campanumoea* into *Codonopsis* is complicated by the results of palynological analysis, which revealed more variation among the species of *Codonopsis* than was indicated by Murthy. All species known to Murthy were members of subg. *Codonopsis*, whereas samples of subg. *Pseudocodonopsis* (Kom.) and subg. *Obconicapsula* Hong examined in their study differed.

As a large family, *Campanulaceae* appears to be prone to considerable phenotypic plasticity as well as ontogenetic variation (Eddie 1997; Eddie and Ingrouille 1999), and therefore more detailed information is needed for generic circumscription for *Campanumoea*. Recently, Zhang et al. (2005) found that flavonoids, such as 5-hydroxy-4,7-dimethoxyflavone, were present in *Campanumoea javanica* subsp. *javanica* Blume, but this flavone was not present in any species of *Codonopsis*. In contrast, the exclusive metabolic intermediate for *Codonopsis*, tangshenoside I, was found in *Campanumoea* (Zhang et al. 2005), which suggests that secondary metabolite differences exist between *Codonopsis* and *Campanumoea*. Flavonoids are abundant plant secondary metabolites and have been reported even from primitive taxa such as liverworts (*Conocephalum conicum*) (Feld et al. 2003) and horsetails (*Equisetum arvense*) (Oh et al. 2004). The ability to accumulate a large number of secondary compounds, which play unique roles in the interaction between plants and the environment, seems to be a general characteristic of plants (Xiaoyun et al. 2001). Products synthesized by the early steps of the flavonoid pathway are found in bryophytes and ferns, whereas gymnosperms and angiosperms accumulate late additional classes of flavonoids, suggesting that plants need to recruit more genes for biosynthesis of novel flavonoid compounds to survive during evolution (Xiaoyun et al. 2001). Therefore, from a genetics viewpoint, differences in secondary metabolite synthesis between *Codonopsis* and *Campanumoea* should exist.

Large-scale genome sequencing provides a comprehensive view of the complex evolutionary forces that have shaped the structure of eukaryotic chromosomes. To date, genome sequencing has been limited to model plants (e.g., *Arabidopsis*, Yogeeswaran et al. 2005). Sequence comparison could be a main way of understanding chromosome evolution for most plants. Cytological studies of *Campanulaceae* began with the seminal investigations of Gadella (1964). Later, Damboldt (1976) vastly increased our knowledge of intrageneric relationships, particularly of the genus *Campanula* L. (for review, see Eddie et al. 2003). The most common chromosome number in the *Campanulaceae* is $n = 17$, which appears to have evolved independently several times in relatively unrelated genera (e.g., in *Campanula*, *Nesocodon* Thulin (1975), *Ostrowskia* Regel, and *Canarina* L.). Forty-two percent of the published chromosome counts of the family *Campanulaceae* s.l. have this number (Lammers 1992). The base number in the family was suggested as $X = 8$ (Böcher 1964; Contandriopoulos 1984), but Raven (1975) thought that $X = 7$ was the ancestral number. An ancestral base number of $X = 7$ is supported by counts for *Cyananthus* Wall. ex Benth (Kumar and Chauhan 1975; Hong and Ma 1991). Until now, cytological information about *Campanumoea* was lacking. The aim of this study is to offer further evidence from cytology to confirm the relationship between the two sections in *Campanumoea*, to discuss their relationship with other species in the family *Campanulaceae*, and to suggest a possible pathway for secondary karyotype formation.

Materials and methods

All seeds were provided by the Chinese South-West Wild Germplasm Source Bank, Kunming Institute of Botany, Chinese Academic of Science (Table 1). Eleven accessions of three species in two sections of the genus *Campanumoea* were used in this experiment. Five accessions (SCSB-A-000172, SCSB-A-000360, SCSB-B-000261, SCSB-A-000303, and SCSB-B-000589) belonged to *C. javanica* subsp. *javanica*, three (SCSB-HN-0804, SCSB-HN-1179, and SCSB-HN-1093) belonged to *C. javanica* subsp. *japonica* (Makino) D. Y. Hong, two (SCSB-B-000612 and SCSB-B-000618) belonged to *C. lancifolia* (Roxb.) Merrill, and one (SCSB-B-000566) belonged to *C. parviflora* (Wall. ex A.DC.) Benth. ex C. B. Clarke. Seeds of five species in related Chinese genera were also sown in this experiment (Table 1).

Seed germination was conducted in a growth chamber held at 25°C. Seeds were soaked with deionized (DI) water in beakers for 15 min before they were placed in Petri dishes on two layers of Whatman no. 1 filter paper thoroughly wetted with deionized (DI) water. Germinating seeds with 1–5-mm radicles were treated with 0.003 M 8-hydroxyquinoline solution for 2 h in small vials at room temperature. Root tips from germinating seeds were used for chromosome preparation. After treatment with 8-hydroxyquinoline, the seeds were placed in freshly prepared Carnoy's fixative reagent [glacial acetic acid:ethanol (v/v) 1:3] and stored in a refrigerator for at least 24 h before use (Lei et al. 2007). After treatment with dissociated solution [1 N HCl:45% acetic acid (v/v) 1:1] at 60 min for 10 s, the root tips were separated from seed, then washed with DI water, and finally dyed with orcein for 30 min.

The chromosome samples were prepared using the squash method. For each accession, ten metaphases were digitized by using a digital microcamera (Optronics) attached to an Olympus BX 60 microscope, for counting and measurements. The length of the short or long arm of each chromosome was obtained using Sigma Scan Pro v.1.0 software. The following values were calculated: chromosome number (CN), total length of chromosome TL (TL = short arm length + long arm length), haploid set length (HSL = total chromosome length of set), arm ratio

Table 1 Seed sources and origins used in chromosome evaluation

Species	Accession no.	Date collected	Seed origin	Altitude (m)	Environment
Campanumoea parviflora	SCSB-B-000566	2007-11-10	Lűchun, Yunan	1,823	Edge of monsoon evergreen broadleaved forest
Campanumoea lancifolia	SCSB-B-000612	2007-12-9	Malipo, Yunnan	1,219	Among evergreen broadleaved forest
Campanumoea lancifolia	SCSB-B-000618	2007-12-10	Maguan, Yunnan	1,553	Roadside of hillside
Campanumoea javanica subsp. *javanica*	SCSB-B-000589	2007-11-13	Jingpin, Yunnan	2,183	Edge of evergreen broadleaved forest
Campanumoea javanica subsp. *javanica*	SCSB-A-000172	2006-9-23	Malong, Yunnan	2,406	Stock of China root greenbrier in the riverside
Campanumoea javanica subsp. *javanica*	SCSB-A-000360	2006-12-7	Fumin, Yunnan	1,770	Secondary forest on the riverside
Campanumoea javanica subsp. *javanica*	SCSB-B-000261	2006-11-20	Jinghong, Yunnan	1,033	Edge of tropical rainforest
Campanumoea javanica subsp. *javanica*	SCSB-A-000303	2006-10-25	Tengchong, Yunnan	1,531	Bushes of roadside
Campanumoea javanica subsp. *japonica*	SCSB-HN-0804	2007-9-25	Yizhang, Hunan	1,302	Bushes
Campanumoea javanica subsp. *japonica*	SCSB-HN-1179	2007-10-2	Laifeng, Hubei	855	Bushes
Campanumoea javanica subsp. *japonica*	SCSB-HN-1093	2007-10-5	Lipin, Guizhou	942	Bushes
Codonopsis clematidea	SHI-A2007465	2007-9-7	Nileke, Xinjiang	1,620	Grassland
Cyananthus inflatus	SCSB-A-000412	2006-11-5	Jingdong, Yunnan	2,450	Weed of moor
Platycodon grandiflorus	SCSB-JS0317	2007-10-11	Jurong, Jiangsu	113	Flat land
Adenophora trachelioides	SCSB-JS0302	2007-10-11	Jurong, Jiangsu	113	Flat land
Asyneuma chinense	SCSB-B-000591	2007-11-13	Jingpin, Yunnan	2,183	Edge of evergreen broadleaved forest

(AR = long arm length/short arm length), and relative length of chromosome RL (RL = TL/HSL). Chromosomes were classified on the basis of arm ratio according to Levan et al. (1964).

Results

Chromosome number and karyotype in *Campanumoea*

The number of chromosomes for *C. javanica* subsp. *javanica* and *C. javanica* subsp. *japonica* was $2n = 2X = 16$ (Table 2). In contrast, the chromosome number for *C. parviflora* and *Campanumoea lancifolia* was $2n = 2X = 18$ (Table 2). Typical mitotic metaphase cell plates and idiogram for each species are shown in Fig. 1. On comparison of karyotypic data, intraspecific variation was found in five accessions of *C. javanica* subsp. *javanica* (Table 3). Similar variation also existed in three accessions of *C. javanica* subsp. *japonica* (data not shown). The karyotypic formula for SCSB-B-000589, SCSB-A-000360, SCSB-A-000303, SCSB-B-000261, and SCSB-A-000172 in *C. javanica* subsp. *javanica* was $2n = 2X = 16$sm, $2n = 2X = 2$m + 12sm + 2st, $2n = 2X = 2$m + 12sm + 2st, $2n = 2X = 2$m + 12sm + 2st, and $2n = 2X = 6$m + 10sm, respectively. All asymmetry types were 3A, except for SCSB-A-000172 for which it was 2A (Table 3).

When the karyotypic formula was arranged alongside altitude biotopes in *Campanumoea*, we found that the karyotypic formulae correlated with different biotopes. From high to low elevation, the karyotypic formula was $2n = 2X = 6$m + 10sm, $2n = 2X = 16$sm, $2n = 2X = 2$m + 12sm + 2st, $2n = 2X = 2$m + 12sm + 2st, and $2n = 2X = 2$m + 12sm + 2st. In two chromosome samples of *Campanumoea* from higher elevations, the HSL (total chromosome length of set) was smaller than in one from low elevation (Table 3). We thought that the difference was derived from differential identification of centromere position in the smaller HSL (see "Discussion"). Therefore, we selected the accessions with larger SHL and coincident karyotype as a model, and considered the karyotype formula of *C. javanica* subsp. *javanica* as $2n = 2X = 2$m + 12sm + 2st, with asymmetric type 3A. For the same reason, the karyotype formula of *C. javanica* subsp. *japonica* was recognized as $2n = 2X = 4$m + 12sm. Unfortunately, we were unable to obtain a sharp image of *C. lancifolia*, so we could not calculate its karyotype formula. The karyotypic formula for *C. parviflora* was $2n = 2X = 6$m + 12sm, with asymmetric type 3B.

Chromosome length and HSL variation in *C. javanica*

Chromosomal data of the five accessions of *Campanumoea javanica* subsp. *javanica* are listed in Table 3, while typical mitotic metaphase cell plates and ideograms are shown in Fig. 2. By comparison of their karyotypic data, we found that intraspecific variation existed in these five accessions (Table 3). The longest chromosome was 7.09, 7.12, 7.39, 8.53, and 7.38 in relative length (average for ten metaphases) for SCSB-B-000589, SCSB-A-000172, SCSB-A-000303, SCSB-A-000360, and SCSB-B-000261, respectively. The shortest chromosome was 5.22, 5.54, 4.85, 5.26, and 4.85 in relative length for SCSB-B-000589, SCSB-A-000172, SCSB-A-000303, SCSB-A-000360, and SCSB-B-000261, respectively (Table 3).

For analysis of chromosome length and karyotype polymorphism, clustering methods using SPSS software

Table 2 Karyotype formula and symmetry type of some investigated *Campanulaceae* species

Species	Accession no.	Chromosome number	Karyotype formula	Type of symmetry
Campanumoea parviflora	SCSB-B-000566	$2n = 18$	$2n = 2X = 6$m + 12sm	3B
Campanumoea lancifolia	SCSB-B-000612	$2n = 18$	**	b
Campanumoea lancifolia	SCSB-B-000618	$2n = 18$	**	b
Campanumoea javanica subsp. *javanica*	SCSB-B-000589	$2n = 16$	$2n = 2X = 16$sm	3A
Campanumoea javanica subsp. *javanica*	SCSB-A-000360	$2n = 16$	$2n = 2X = 2$m + 12sm + 2st	3A
Campanumoea javanica subsp. *javanica*	SCSB-A-000303	$2n = 16$	$2n = 2X = 2$m + 12sm + 2st	3A
Campanumoea javanica subsp. *javanica*	SCSB-B-000261	$2n = 16$	$2n = 2X = 2$m + 12sm + 2st	3A
Campanumoea javanica subsp. *javanica*	SCSB-A-000172	$2n = 16$	$2n = 2X = 6$m + 10sm	2A
Campanumoea javanica subsp. *japonica*	SCSB-HN-0804	$2n = 16$	$2n = 2X = 4$m + 12sm	3A
Campanumoea javanica subsp. *japonica*	SCSB-HN-1179	$2n = 16$	$2n = 2X = 2$m + 12sm + 2st	3A
Campanumoea javanica subsp. *japonica*	SCSB-HN-1093	$2n = 16$	$2n = 22X = 2$m + 12sm + 2st	3A

Chromosome number was counted based on about ten cells

** Space indicates no result in the experiment

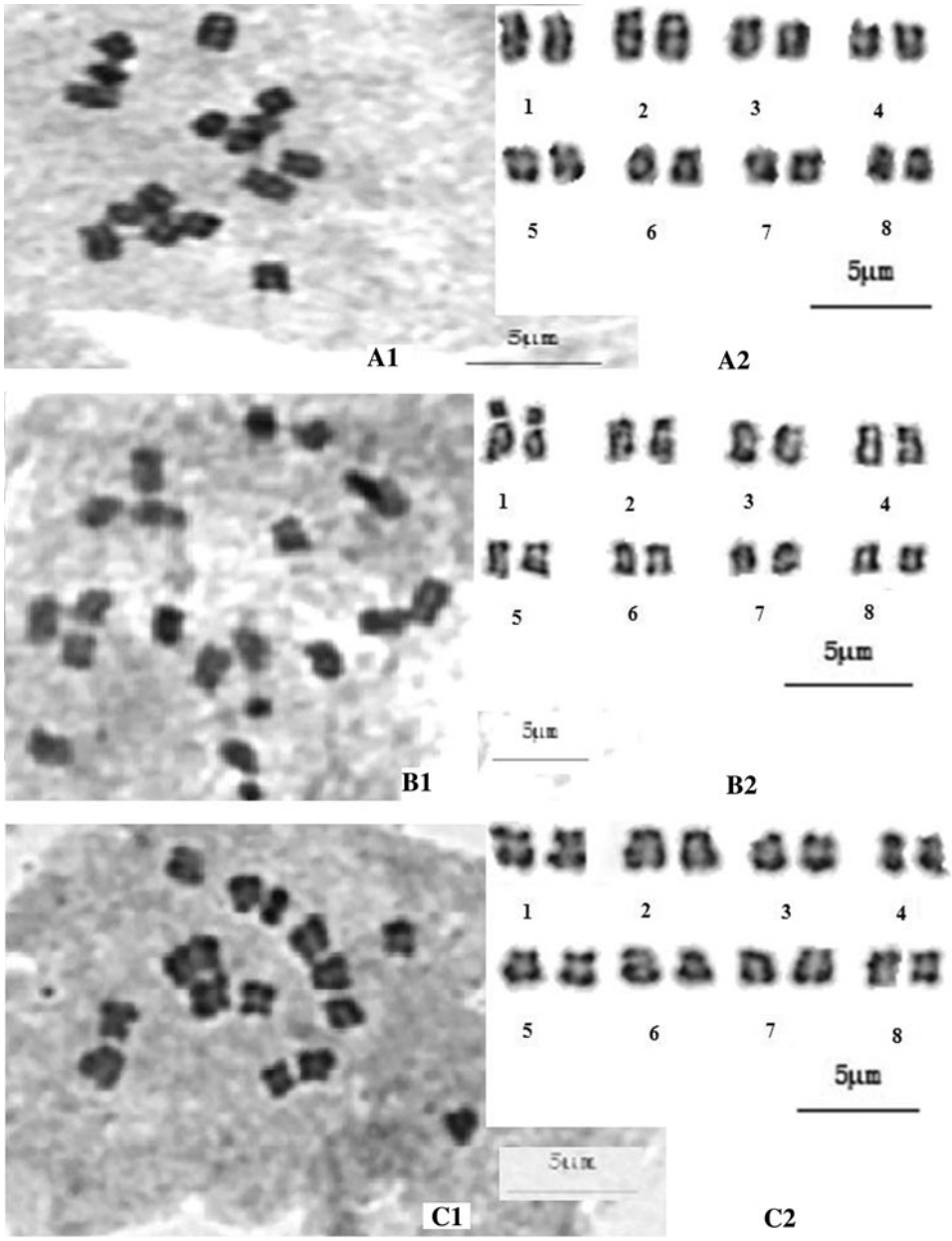

Fig. 1 Metaphase chromosomes and idiograms for three species in *Campanumoea*. *A1*, *B1*, and *C1* show metaphase chromosome for *C. parviflora*, *C. javanica* subsp. *javanica*, and *C. javanica* subsp. *japonica*, respectively. *A2*, *B2*, and *C2* show ideogram for *C. parviflora*, *C. javanica* subsp. *javanica*, and *C. javanica* subsp. *japonica*, respectively. All at same magnification; bar indicates 5 μm

were used to analyze their correlations based on chromosomal relative total length and arm ratio. The results (Fig. 3) revealed a high correlation with their altitude biotope: SCSB-A-000303 and SCSB-B-000261 clustered in one branch, whereas SCSB-B-000589, SCSB-A-000303, and SCSB-A-000360 clustered in another branch. We found that the degree of chromosome coiling could affect the length of chromosome. However, different accessions (or species) which have unique biotopes also have special cell cycles and chromosomal morphological characters. In the five accessions, which were distributed at altitudes from 1,033 to 2,406 m, there were ten metaphases with shorter chromosomes that came from species living at higher altitudes, thus indicating that chromosome length could be considered as a phenotypic variation during the cell cycle, especially in metaphase. Although we did not measure their cell cycles, we calculated the mitotic index (MI) in five accessions (Table 4); the data showed that the index decreased as altitude increased. Furthermore, regression analysis with SPSS software was used to estimate the correlation between altitude, HSL, and MI (Table 5). The correlation coefficient was 0.8416, 0.9321, and 0.6517 for HSL and MD, MD and altitude, and HSL and altitude, respectively, which suggested that MD and HSL are affected by altitude. MD and HSL had strong positive correlation, suggesting that the five accessions had great diversity in their cell cycle, while HSL had negative correlation with altitude.

Table 3 Average chromosome features for five accessions in *C. javanica* subsp. *javanica*

SCSB-B-000589 $2n = 2X =$ 16sm 2183						SCSB-A-000172 $2n = 2X =$ 6m + 10sm 2406					SCSB-A-000303 $2n = 2X =$ 2m + 6sm + 2st 1531				
CL	TL (μm)[a]	RL	AR	Type	New TL (μm)[b]	TL (μm)[a]	RL	AR	Type	New TL (μm)[b]	TL (μm)[a]	RL	AR	Type	New TL (μm)[b]
I	1.81 (±0.2)	7.09	2.49	Sm	1.81	1.81 (±0.1)	7.12	1.38	m		2.17 (±0.2)	7.39	1.73	m	1.88
II	1.71(±0.1)	6.72	1.90	Sm	1.71	1.71 (±0.2)	6.74	1.87	m		2.11 (±0.3)	7.17	1.83	sm	1.83
III	1.71 (±0.2)	6.72	2.60	sm	1.71	1.62 (±0.2)	6.37	1.83	sm		1.86 (±0.1)	6.33	3.38	st	1.61
IV	1.62 (±0.2)	6.34	1.83	sm	1.62	1.62 (±0.1)	6.37	1.83	sm		1.86 (±0.2)	6.33	1.65	sm	1.61
V	1.52 (±0.3)	5.97	2.20	sm	1.52	1.52 (±0.2)	5.99	2.67	m		1.86 (±0.2)	6.33	1.77	sm	1.61
VI	1.52 (±0.1)	5.97	2.20	sm	1.52	1.52 (±0.1)	5.99	3.00	sm		1.74 (±0.2)	5.91	2.50	sm	1.51
VIII	1.52 (±0.2)	5.97	1.92	sm	1.52	1.52 (±0.3)	5.99	2.20	sm		1.68 (±0.1)	5.70	2.38	sm	1.45
VIII	1.33 (±0.2)	5.22	1.80	sm	1.33	1.41 (±0.1)	5.54	2.42	sm		1.43 (±0.2)	4.85	2.34	sm	1.24
HSL	12.76 (±0.52)				12.73	12.73 (±0.58)					14.70 (±0.63)				12.3

SCSB-A-000360 $2n = 2X =$ 2m + 12sm + 2st 1770						SCSB-B-000261 $2n = 2X =$ 2m + 6sm + 2st 1033				
CL	TL (μm)[a]	RL	AR	Type	New TL (μm)[b]	TL (μm)[a]	RL	AR	Type	New TL (μm)[b]
I	2.24 (±0.3)	8.53	1.60	m	2.17	2.21 (±0.2)	7.38	1.59	m	1.87
II	1.94 (±0.2)	7.36	2.17	sm	1.88	2.16 (± 0.2)	7.17	1.83	sm	1.83
III	1.73 (±0.1)	6.59	3.96	st	1.67	1.91 (±0.2)	6.33	3.68	st	1.62
IV	1.53 (±0.2)	5.81	2.00	sm	1.48	1.91 (±0.3)	6.33	2.50	sm	1.62
V	1.48 (±0.1)	5.62	1.90	sm	1.43	1.91 (±0.1)	6.33	2.40	sm	1.62
VI	1.43 (±0.1)	5.43	2.50	sm	1.38	1.78 (±0.2)	5.91	2.50	sm	1.51
VIII	1.43 (±0.2)	5.43	2.50	sm	1.38	1.72 (±0.2)	5.70	2.38	sm	1.46
VIII	1.38 (±0.1)	5.26	2.03	sm	1.34	1.46 (±0.1)	4.85	2.34	sm	1.24
HSL	13.15 (±0.55)				12.73	15.04 (±0.68)				12.73

CL chromosome, *TL* total chromosome length, *RL* relative length, *HSL* haploid set length, *AR* arm ratio, *m* metacentric, *sm* submetacentric, *st* subtelocentric

[a] Data are the average of ten cells, with data in brackets indicating standard error

[b] Data were calculated from TL using the TL of SCSB-A-000172 as standard, meaning division of each TL by its HSL/12.73

Discussion

Circumscription of the two sections in *Campanumoea*

In our experiment, the basic chromosome number in *Campanumoea javanica* subsp. *javanica* in five accessions was $X = 8$, and diploid, karyotype asymmetry type was 3A (Table 2), except for one accession (SCSB-A-000172) for which it was 2A (Table 2). This exception may have arisen due to incorrect arrangement of the centromere in the minor chromosome of the high-altitude biotope accessions. In general, the karyotype asymmetry of *Campanumoea javanica* subsp. *javanica* could be considered as 3A, a higher asymmetry type, and its chromosomal formula as $2n = 2X =$ 6m + 10sm. Chromosome numbers of *Campanumoea javanica* subsp. *japonica* were $2n = 2X = 16$, the karyotype formula was considered as $2n = 2X =$ 2m + 14sm, and the asymmetry type was 3A.

In section *Cyclocodon*, the diploid chromosome number of *Campanumoea parviflora* (accession SCSB-A-000566) is $2n = 18$, karyotype formula was $2n = 2X =$ 6m + 12sm = 18, and asymmetry type was 3B (Table 3). The diploid chromosome number of *Campanumoea lancifolia* (SCSB-B-000618) was $2n = 18$, but we did not obtain the karyotype and chromosome formula. On the basis of data from *Campanumoea parviflora*, we infer the basic number in section *Cyclocodon* to be $X = 9$, $2n = 2X = 18$, and the karyotype asymmetry to be 3B (Table 2). The main chromosomes were the middle and submiddle chromosomes, and the karyotype asymmetry was no higher than that of *Campanumoea javanica*.

From the data presented in Tables 2 and 3, we found that the basic chromosome number in two sections of *Campanumoea* was different. According to Stebbins's (1971) theory, the direction of the karyotype in higher plants is from symmetry to asymmetry. The asymmetry level in section *Campanumoea* was higher than that of section *Cyclocodon*. Therefore, species in section *Campanumoea* may be derived from *Cyclocodon*. Based on our results, section *Cyclocodon* should be considered as a

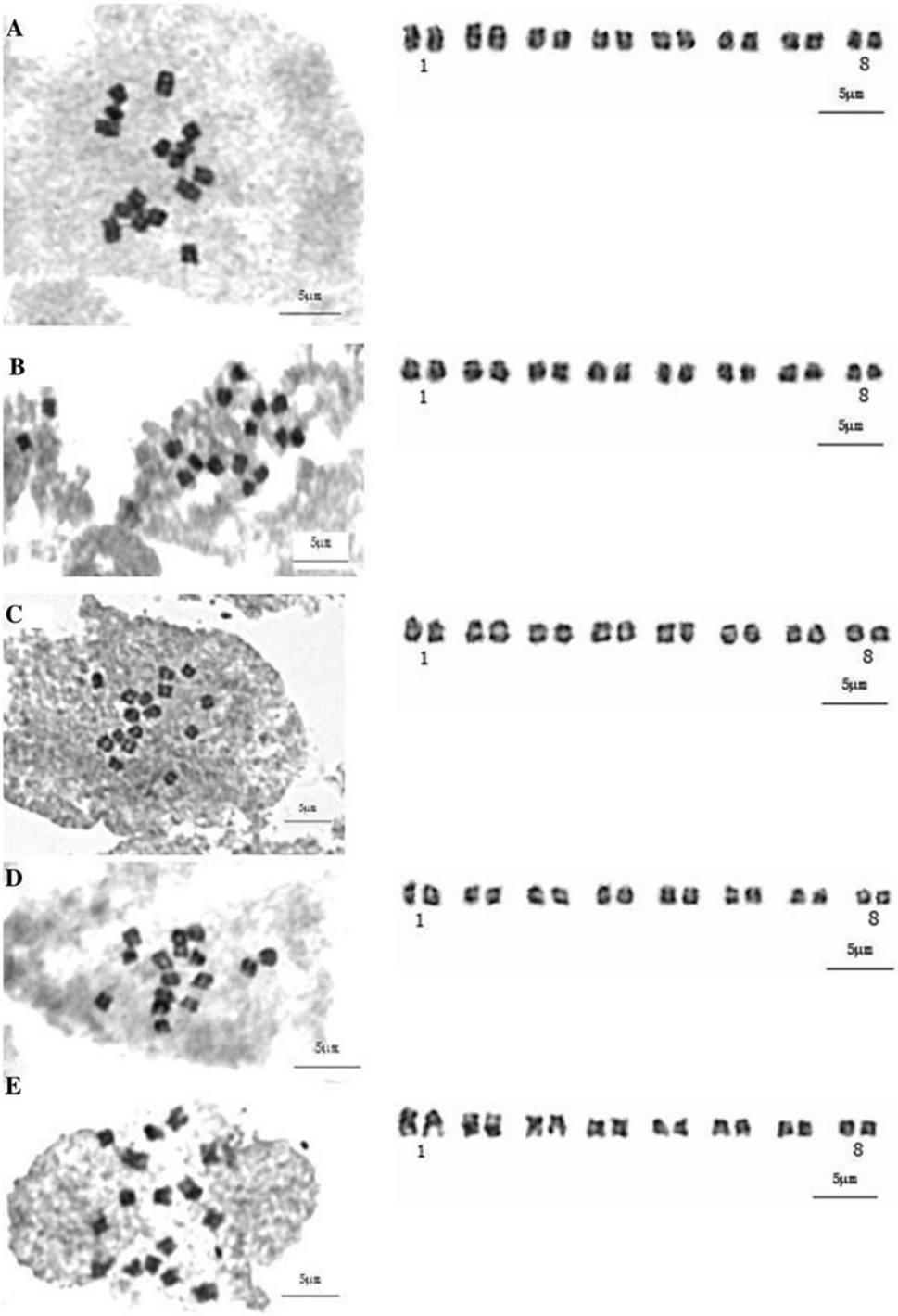

Fig. 2 Metaphase chromosomes and karyotype in five accessions in *C. javanica* subsp. *javanica*. *A*, *B*, *C*, *D*, and *E* show metaphase chromosome and idiograms for SCSB-B-000589 (SCSB-A-000172), SCSB-A-000172 (SCSB-B-000589), SCSB-A-000360 (SCSB-A-000303), SCSB-B-000261 (SCSB-A-000360), and SCSB-A-000303 (SCSB-B-000261), respectively. All at the same magnification; bar indicates 5 μm

separate genus, as supported by data from pollen morphologies (Dunbar and Wallentinus 1976; Lammers 1992; Qiu and Hong 1993).

It is necessary to discuss the relation between section *Campanumoea* and genus *Codonopsis*. In *Codonopsis clematidea* (Schrenk) C. B. Clarke, the basic number was $X = 8$, $2n = 2X = 6\text{m} + 10\text{sm} = 16$, and its karyotype asymmetry was 2A in our experiment (Table 2). Many karyotypes have been reported for *Codonopsis*: $2n = 2X = 12\text{m} + 4\text{sm} = 16$ for *Codonopsis pilosula* (Franch) Nannf. (Tian and Zhao 2007), and $2n = 2X = 12\text{m} + 4\text{sm} = 16$ for *Codonopsis lanceolata* (Siebold & Zucc.) Trautv. Their asymmetry type was 2A (Li and Li 2005). Based on chromosome number and karyotype, we found that the asymmetry type of *Campanumoea* was more advanced than that of *Codonopsis*; in addition the flavonoid compounds,

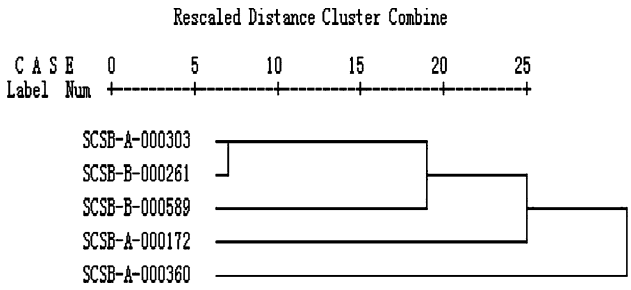

Fig. 3 Total chromosome cluster based on chromosomal relative length and arm ratio among five accessions in *Campanumoea javanica* subsp. *javanica*. SCSB-A-000303 and SCSB-B-000261 first cluster into one branch, then SCSB-B-000589, SCSB-A-000303, and SCSB-A-000360 clustered to the branch ordinal, respectively. Among them, SCSB-A-000303 and SCSB-B-000261 had similar chromosomal data. However, for SCSB-B-000589, there was great disparity in HSL and karyotype from SCSB-A-000303 and SCSB-B-000261. On the other hand, SCSB-A-000360 was closer to them, although their distance was than that of SCSB-B-000589. The distance represents the degree of approximation

which are never found in *Codonopsis*, were found in *Campanumoea* (Zhang et al. 2005).

Two ways to form secondary chromosome-type species in *Campanulaceae*

The specialized chromosome class group of *Campanula* L. was regarded as paleopolyploid (Gadella 1964; Böcher 1964). By diploidization of its ancestor's genome, the secondary karyotypes with $n = 17/18$ could be derived from $X = 8$ or $X = 9$ (Gadella 1964; Böcher 1964; Hong and Ma 1991). Two hypothesis have been proposed to account for this: (1) doubling of $X = 9$, to form $X = 18$, then loss of one chromosome or fusion of two telechromomeres to form $X = 17$; and (2) hybridization between $X = 8$ and $X = 9$ to form $X = 17$ (allopolyploid), in this case chromosome breakage in the paleopolyploid could then result in $X = 18$ (Gadella 1964; Böcher 1964; Hong and Ma 1991). If the first hypothesis is correct for the phylogenesis of *Campanulaceae*, the level of asymmetry for karyotype $X = 18$ would be the same as or similar to $X = 9$, and the HSL of $X = 18$ would be double or almost double that of $X = 9$. If the second hypothesis is correct, the level of asymmetry of $X = 18$ would be more advanced or complex than that of $X = 9$ or $X = 8$, while the asymmetry of $X = 18$ would be the same as or similar to that of $X = 17$. In previous studies, the asymmetry type was low in many species of *Adenophora* Fisch. ($2n = 2X = 36$) (Wang and Song 1998; Qiu and Hong 1987). Using T.C. (centromeric terminalization value, T.C.%: long arm length/total chromosome length × 100) to repress the level of asymmetry of the karyotype, Wang and Song (1998) found that the T.C. was 58.4%, 59.3%, 59.3%, and 59.4% for *A. borealis* (D. Y. Hong & Y. Z. Zhao), *A. wulingshanica* (D. Y. Hong), *A. petiolata* (Pax & K. Hoffm.), and *A. potaninii* (Korsh), respectively. The only exception was *A. lobophylla*, in which T.C. was 62.0%. Therefore, $n = 17$ or $n = 18$ in *Campanulaceae* is derived mainly by doubling of $n = 9$, by autopolyploidy, in accordance with the first hypothesis mentioned above.

On further comparison of chromosomal data of *Campanumoea parviflora* (SCSB-B-000566, $2n = 2X = 6m + 12sm = 18$) and *Adenophora trachelioides* Maxim. [=*Adenophora remotiflora* (Siebold & Zucc.) Miq.] (SCSB-JS0302, $2n = 2X = 18m + 18sm = 36$), we found that the HSL of *Campanumoea parviflora* was less than double that of *Adenophora trachelioides* (Table 6). We also found that 16 out of 18 chromosomes in *Adenophora trachelioides* were paired lengthwise and were the same or almost the same in length. When we recalculated the TL of *Adenophora trachelioides* SCSBJS0302, and divided each TL by 1.57 (49.61/2 × 1580), the average new TL of each pair was very close to that of *Campanumoea parviflora* (SCSB-B-000566). These data offer partial evidence for our hypothesis as mentioned above regarding the

Table 5 Partial correlation coefficients between altitude, HSL, and MD

Control varibles	MD	HSL	Altitude
MD	1.0000	0.8416**	0.9321*
HSL	0.8416**	1.0000	0.6517
Altitude	0.9321*	0.6517	1.0000

* Correlation is significant at 0.05 level (two-tailed)

** Correlation is significant at 0.02 level (two-tailed)

Table 4 Mitotic index of five accessions in *Campanumoea javanica* subsp. *javanica*

Species	Accession no.	Intermitotic cell	Division	Total cell	Mitotic index (%)	Altitude (m)	MI ratio
Campanumoea javanica subsp. *javanica*	SCSB-B-000589	1,146	57	1,203	4.74	2,183	1.045
Campanumoea javanica subsp. *javanica*	SCSB-A-000172	1,075	51	1,126	4.53	2,406	1.000
Campanumoea javanica subsp. *javanica*	SCSB-A-000360	1,096	62	1,158	5.35	1,770	1.181
Campanumoea javanica subsp. *javanica*	SCSB-B-000261	998	65	1,063	6.11	1,033	1.349
Campanumoea javanica subsp. *javanica*	SCSB-A-000303	1,076	60	1,136	5.28	1,531	1.166

Table 6 Average values for chromosome features of *Campanumoea parviflora* and *Adenophora trachelioides*

Campanumoea parviflora SCSB-B-000566 $2n = 2X = 6m + 12sm = 18$					*Adenophora trachelioides* SCSB-JS0302 $2n = 2X = 18m + 18sm = 36$					
CL	TL (μm)[a]	RL	AR	Type	CL	TL (μm)[a]	RL	AR	Type	New TL (μm)[b]
I	2.51 (±0.06)	7.94	2.57	sm	I	3.58 (±0.11)	3.78	1.55	m	2.28
II	1.91 (±0.02)	6.03	2.17	sm	II	3.48 (±0.09)	3.30	1.48	m	2.22
III	1.91 (±0.03)	6.03	1.72	m	III	3.04 (±0.07)	3.30	1.18	m	1.94
IV	1.80 (±0.02)	5.71	2.09	sm	IV	3.04 (±0.10)	3.14	1.46	m	1.94
V	1.71 (±0.01)	5.40	1.13	m	V	2.89 (±0.07)	3.04	1.85	sm	1.84
VI	1.61 (±0.02)	5.08	2.20	sm	VI	2.80 (±0.05)	2.99	1.16	m	1.78
VIII	1.50 (±0.02)	4.76	2.25	sm	VIII	2.70 (±0.04)	2.93	1.65	m	1.72
VIII	1.50 (±0.03)	4.76	1.50	m	VIII	2.55 (±0.04)	2.77	1.36	m	1.62
IX	1.36 (±0.02)	4.29	2.03	sm	IX	2.55 (±0.03)	2.77	2.71	sm	1.62
HSL	15.80 (±0.12)				X	2.49 (±0.02)	2.71	2.01	sm	1.59
					XI	2.50 (±0.06)	2.72	2.01	sm	1.59
					XII	2.45 (±0.04)	2.66	1.96	sm	1.56
					XIII	2.36 (±0.03)	2.56	1.67	m	1.50
					XIV	2.31 (±0.02)	2.51	1.35	m	1.47
					XV	2.31 (±0.03)	2.51	1.94	sm	1.47
					XVI	2.15 (±0.02)	2.34	1.96	sm	1.37
					XVIII	1.96 (±0.04)	2.13	2.34	sm	1.25
					XVIII	1.72 (±0.05)	1.87	2.22	sm	1.10
					HSL	49.61 (±0.36)				30.18

Symbols are the same as in Table 3

[a] The data are averages for ten cells, with standard error in parenthesis

[b] Data was calculated from TL using the TL of SCSB-B-000566 as standard, meaning division of each TL by its HSL/15.80

chromosome number change in *Campanulaceae*. Further experiments using chromosomal banding or in situ hybridization could possibly offer more evidence for our hypothesis.

Most eukaryotic genomes have undergone whole genome duplications during their evolutionary history, with angiosperms having a particular enrichment in this process (Vision et al. 2000; Blanc and Wolfe (2004; Blanc et al. 2003; Wolfe and Shields 1997). Recent studies have shown that the function of these duplicated genes can diverge from the ancestral gene via neo- or subfunctionalization within single genotypes (Kliebenstein 2008). An additional possibility is that gene duplicates may also undergo partitioning of function among different genotypes of a species, leading to genetic differentiation (Kliebenstein 2008).

Finally, the ability of gene duplicates to diverge may be limited by their biological function (Kliebenstein 2008). In *Arabidopsis*, some analyses have estimated that over 60% of genes appear to have been rearranged since the last polyploidization occurred in this lineage (Vision et al. 2000). In comparisons with rice and sorghum, two highly diploidized species that diverged from a common ancestor 50–70 mya (Grass Phylogeny Working Group 2001), it appears that approximately 20% of genes have been rearranged (Bennetzen and Ma 2003). In addition to whole genome events, small local events can generate tandem duplicated genes, which are often considered younger than segmental duplicated genes obtained from whole genome duplication (Vision et al. 2000; Rizzon et al. 2006). Following either tandem or segmental duplication events, one of the duplicated genes can be rendered nonfunctional via the accumulation of deleterious mutations. This would be the reason why the HSL of *Adenophora trachelioides* was larger than double that of *Campanumoea parviflora*. From the karyotypic changes we found that chromosomal rearrangements have occurred in *Campanulaceae*, and tandem duplication could occur because of chromosomal length polymorphism. Other experiments indicate that extensive chloroplast rearrangements are also present wildly in *Campanulaceae* (see Cosner et al. 2004).

Chromosome rearrangement in *Campanulaceae*

As mentioned above, karyotypic variation existed among accessions, and the variation could be correlated with altitude. We choose two accessions in *Campanumoea* from similar altitude (SCSB-A-000303 and SCSB-HN-0804, for *C. javanica* subsp. *javanica* and *C. javanica*

subsp. *japonica*, respectively) to analyze their karyotype characteristics (Table 7). The karyotypic features of the two species were also quite similar. Content of DNA was assessed by measuring the HSL and chromosome RL. Average values for haploid set length were 13.15 μm in *C. javanica* subsp. *javanica* and 13.16 μm in *C. javanica* subsp. *japonica*. The largest chromosome (chromosome I) of *C. javanica* subsp. *javanica* represented 17.03% of the total genome, and the shortest (chromosome) represented 10.49%. The same values in *C. javanica* subsp. *japonica*, were 15.50% and 10.49%. This matching was observed for the other eight chromosome pairs, with all of them demonstrating very similar total length (Table 7). In *C. javanica* subsp. *javanica*, the exceptions were 2.24, 1.53 and 1.48 μm, respectively; in *C. javanica* subsp. *japonica* the exceptions were 2.04, 1.65, and 1.65 μm, respectively.

Considering the results presented in Table 7 for HSL and RL, these differences in chromosome morphology can be explained by rearrangement of segments within the chromosome, without gain or loss of genetic material, because the two species had the same karyotype. Also for the effect of altitude on chromosome length, we chose SCSB-A-000360 (*Campanumoea javanica* subsp. *javanica*, altitude 1,770 m) and SHI-A2007465 (*Codonopsis clematidea*, altitude 1,620 m) to study the correlation of HSL and single chromosome length. The SHL for SCSB-A-000360 and SHI-A2007465 was 13.15 and 33.25, respectively. If we calculated the data as above, we found that the new HSL of SHI-A2007465 was 2.528 times that for SCSB-A-000360. We divided the TL of SHI-A2007465 by 2.528, and obtained a new TL of SHI-A2007465 of 2.06, 1.93, 1.75, 1.67, 1.59, 1.43, 1.43, and 1.3 by chromosome (Table 7). These data were very close to those of SCSB-A-000360 (Table 7) but different from those of SCSB-A-000360 (Table 7). Though we consider *Campanumoea* to be a valid genus, it has a very close relationship with *Codonopsis* (as shown by pollen studies). Rearrangement of chromosome segments exists within *Campanulaceae*, and it is implied that chromosome segments rearrangement occurred in the evolution of diversity in *Campanulaceae* (Eddie et al. 2003).

Cytogenetic studies have indicated that major chromosomal rearrangements could mark specific lineages of plants and other eukaryotes (Kellogg and Bennetzen 2004). Though they appear to be relatively rare in most plant lineages, chromosomal rearrangements could have dramatic biological effects, particularly in terms of fertility of inversion and translocation heterozygotes. For instance, only four major chromosome rearrangements differentiate the rice and foxtail millet lineages, despite more than 50 million years of independent evolutionary descent (Devos et al. 1998). On the other hand, Rieseberg (2001) and Rieseberg et al. (2003) have shown that genome rearrangements can occur immediately upon hybridization, even at the diploid level. In contrast to the rarity of large chromosomal rearrangements, small chromosomal rearrangements are incredibly abundant in plants. From our experiment, we also found that chromosome rearrangement would be the main factor in species diversity, which is why the intraspecific differences between *C. javanica* subsp. *japonica* and *C. javanica* subsp. *japonica* would be greater than intergeneric differences between *Campanumoea* and *Codonopsis*. Further cytogenetic investigations (including chromosome banding, in situ hybridization or genome comparison) of these two subspecies of *Campanumoea javanica*, plus species in *Codonopsis*, would provide the information necessary to elucidate the evolutionary and taxonomic relationships within this group of platycodonoids (Eddie et al. 2003).

Table 7 Average values for chromosome features of *C. javanica* subsp. *javanica*, *C. javanica* subsp. *japonica*, and *Codonopsis clematidea*

CL	*C. javanica* subsp. *javanica*				*C. javanica* subsp. *japonica*[a]				*Codonopsis clematidea*				
	TL (μm)[a]	RL	AR	Type	TL (μm)	RL	AR	Type	New TL (μm)[b]	RL	AR	Type	New TL (μm)[b]
I	2.24	8.50	1.60	m	2.04	7.58	2.07	sm	5.21	7.84	1.36	m	2.06
II	1.94	7.36	2.17	sm	1.92	7.32	2.50	sm	4.88	7.34	2.18	sm	1.93
III	1.73	6.59	3.96	st	1.75	6.65	2.33	sm	4.42	6.64	1.21	m	1.75
IV	1.53	5.81	2.00	sm	1.65	6.27	1.79	sm	4.22	6.34	2.72	m	1.67
V	1.48	5.62	1.90	sm	1.65	6.27	2.29	sm	4.02	6.04	2.33	sm	1.59
VI	1.43	5.43	2.50	sm	1.46	5.57	2.20	sm	3.61	5.43	2.86	sm	1.43
VIII	1.43	5.43	2.50	sm	1.38	5.23	2.75	sm	3.61	5.43	1.70	sm	1.43
VIII	1.38	5.26	2.03	sm	1.31	5.10	1.77	sm	3.28	4.93	1.89	sm	1.30
HSL	13.15				13.16				33.25				18.07

Symbols in the table are the same as in Table 3

[a] Data are the averages of ten cells

[b] Data were calculated from TL using the TL of *C. javanica* subsp. *javanica* as standard, meaning division of each TL by its HSL/13.16 = 2.53

Effect of biotope on chromosome length and HSL variation

Flowering plants have an impressive range of nuclear genome size, including some species with less than 50 Mb of DNA per haploid nucleus and others with more than 85,000 Mb (Bennett 2003). Greilhuber (2005) mentions that such genome size variation would be multifaceted, depending on, e.g., instrumental–technical, preparative, material-dependent, and taxonomical aspects. He considered that any fault in methods would cause variation rather than constancy of measurement results. Therefore the finding of variation should instantly provoke the question of whether this is real variation in DNA amount or simply an artifact. This has been shown clearly in studies on endogenous staining inhibitors (Greilhuber 1986, 1988; Noirot et al. 2000, 2005), for example, in *Dasypyrum villosum* (L.) (syn. *Haynaldia villosa*; $2n = 14$), which exhibited a remarkable individual fruit-color polymorphism from yellow to almost black. Innocenti and Bitonti (1983) reported a longer mitotic cycle in seedlings with pale rather than dark caryopses, while Cremonini et al. (1994) found 20–24% more DNA in early prophase nuclei in seedlings with pale caryopses compared with dark ones. Quantitative modulation of a certain near-telomeric sequence was found in chromosomes of germinating seedlings from the two types of caryopses, indicating the existence of a variable element in the genome of *D. villosum* (Frediani et al. 1994). Later, Greilhuber (2005) found that there is no genome size plasticity associated with fruit color in *D. villosum*.

Arachis duranensis (Krapov. & W.C. Gregory) ($2n = 20$, $2X$) is morphologically variable and exhibits more molecular variation than *A. hypogaea* L. (Stalker et al. 1995). Singh et al. (1996) reported 1.11-fold variation in genome size in 28 accessions. In 11 of these, for which data on the altitude of the collection site were given, strong negative correlation between C-values and altitude was found. In a reinvestigation of this case, covering 20 accessions, Temsch and Greilhuber (2001) also found some variation. At 1.07-fold, it was not as high as that reported by Singh et al. (1996), but there was also a negative correlation with altitude of the sampling sites as given by ICRISAT.

In our experiment, chromosome length and HSL variation was found in accessions in both sections of *Campanumoea*. One reason, as mentioned above, could be a measurement mistake. In fact, in our experiment, all chromosome data were calculated from ten cells, and the data were not significantly different on analysis of variance (ANOVA, Analysis of variance) (data not shown). Therefore, we thought the variation was caused by the difference in chromosome coiling density and genetic background. Many genes or gene families are involved in cell cycle and chromosome condensation. It is also known that several *AGO* (ARGONAUTE) family members mediate heterochromatin assembly (Hall et al. 2002; Ken-Ichi Nonomura et al. 2007; Reinhart and Bartel 2002; Volpe et al. 2002, 2003; White and Allshire 2009). For example, in fission yeast, deletion of *AGO1* disrupts heterochromatin-mediated silencing, which correlates with loss of *H3K9* methylation and *SWI6/HP1* association with heterochromatic loci (Volpe et al. 2002; Hall et al. 2002). Indeed, determination of whether the genes concerned with heterochromatin assembly are different between accessions distributed in dissimilar biotopes needs further evidence. In the chromosome samples from different species at different minor stages of mitotic metaphase, the chromosome coiling was different, which caused differences in chromosome length. In the five accessions, the shortest HSL was 12.73, the largest HSL was 14.70, and the difference was 15.48%. In pollen grain mitosis of *Scilla sibirica* L., Fivawo and Rees (1985) found that the duration of the mitotic cycle affects the length of chromosomes at metaphase. In grains which divide early, the chromosomes at metaphase are twice the length of chromosomes in grains dividing late. They suggest that the increase in the mitotic cycle is due to more intensive coiling, which in turn is explained by lengthening of G2 and of the prophase, which could explain why the chromosome at mitotic metaphase was shorter in polyploids than in their diploid ancestors.

Although we did not determine the differences in mitotic cycle between different populations, few metaphase cells were found in the samples from populations that grew at higher altitude, and the mitotic index decreased as altitude increased (Table 3). Using SPSS software, we found partial correlation coefficients of 0.9321 and 0.6517 of altitude with mitotic index (MI) and HSL, respectively (Table 5). The partial correlation coefficient was 0.8416 between MI and HSL, suggesting a high level of correlation between MI and HSL.

Plants native to higher altitude could have an adaptive mechanism against metabolic perturbations, which would act to lower reactive oxygen species and also provide metabolic homeostasis in plants of higher altitude (HA) (Narinder et al. 2007). In our experiment, we found that chromosome length increased as altitude decreased (Table 3). In experiments for karyotype comparisons in related species, it is more useful to choose population from similar altitudes in order to reduce the degree of error.

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References

Bennett MD, Leitch IJ (2003) Plant DNA C-values database (release 2.0). http://www.rbgkew.org.uk/cval/homepage.html. Accessed 20 October 2003

Bennetzen JL, Ma J (2003) The genetic colinearity of rice and other cereals based on genomic sequence analysis. Curr Opin Plant Biol 6:128–133

Blanc G, Wolfe KH (2004) Widespread paleopolyploidy in model plant species inferred from age distributions of duplicate genes. Plant Cell 16:1667–1678

Blanc G, Hokamp K, Wolfe KH (2003) A recent polyploidy superimposed on older large-scale duplications in the *Arabidopsis* genome. Genome Res 13:137–144

Böcher T (1964) Chromosome connections and aberrations in the *Campanula persicifolia* group. Svensk Bot Tidskr 58:1–18

Clark CB (1881) *Campanulaceae*. In: Hooker JD (ed) Fl Brit Ind, vol 3. L Reeve Co., London, pp 348–356

Contandriopoulos J (1984) Differentiation and evolution of the genus *Campanula* in the Mediterranean Region. In: Plant biosystematics. Academic, Canada, pp 141–158

Cosner ME, Raubeson LA et al (2004) Chloroplast DNA rearrangements in *Campanulaceae*: phylogenetic utility of highly rearranged genomes. BMC Evol Biol 4(27):1471–2148

Cremonini R, Colonna N, Stefani A, Galasso I, Pignone D (1994) Nuclear DNA content. Chromatin organization and chromosome banding in brown and yellow seeds of *Dasypyrum villosum* (L.) P. Candargy. Heredity 72:365–373

Damboldt J (1976) Materials for a flora of Turkey 32: *Campanulaceae*. Notes Roy Bot Gard Edinburgh 35:39–52

De Candolle A (1830) Monographie des Campanulêes. Paris

Devos KM, Wang ZM, Beales J, Sasaki T, Gale MD (1998) Comparative genetic maps of foxtail millet (*Setaria italica*) and rice (*Oryza sativa*). Theor Appl Genet 96:63–68

Dunbar A, Wallentinus H-G (1976) On pollen of *Campanulaceae* III. A numerical taxonomic investigation. Bot Not 129:69–72

Eddie WMM (1997) A global reassessment of the generic relationships in the bellflower Family (*Campanulaceae*). Ph.D. thesis, University of Edinburgh, Scotland

Eddie WMM, Ingrouille MJ (1999) Polymorphism in the Aegean—five-loculed—species of the genus *Campanula*, sect. Quinquel-oculares (Campanulaceae). Nordic J Bot 19:153–169

Eddie WMM, Shulkina T, Gaskin J, Haberle RC, Jansen RK (2003) Phylogeny of Campanulaceae s. str. Inferred from ITS sequences of nuclear ribosomal DNA. Ann Missouri Bot Gard 90:554–575

Fedorov AA (1972) *Campanulaceae*. In: Flora of the U.S.S.R. Shishkin BK (ed) Akademia Nauk SSSR, Moskva-Lenin grad, pp 92–324

Feld H, Zapp J, Becker H (2003) Secondary metabolites from the liverwort *Tylimanthus renifolius*. Phytochemistry 64:1335–1340

Fivawo NC, Rees H (1985) Chromosome size variation during pollen grain development in *Scilla sibirica*. Theor Appl Genet 70(4):417–421

Frediani M, Colonna N, Cremonini R, De Pace C, Delre V, Caccia R, Cionini PG (1994) Redundancy modulation of nuclear DNA sequences in *Dasypyrum villosum*. Theor Appl Genet 88:167–174

Gadella TWJ (1964) Cytotaxonomic studies in the genus *Campanula*. Wentia 11:1–104.8

Greilhuber J (1986) Severely distorted Feulgen DNA amounts in Pinus (Coniferophytina) after nonadditive fixations as a result of meristematic self-tanning with vacuole contents. Can J Gen Cytol 28:409–415

Greilhuber J (1988) 'Self-tanning'—a new and important source of stoichiometric error in cytophotometric determination of nuclear DNA content in plants. Plant Syst Evol 158:87–96

Greilhuber J (2005) Intraspecific variation in genome size in Angiosperms: identifying its existence. Ann Bot 95:91–98

Greuter W, Barrie FR, Burdet HM et al (1994) International code of botanical nomenclature (Tokyo code) adopted by the Fifteen International Botanical Congress, Yokohama, August–September 1993. Regnum Veg., vol 131, pp 1–389

Grey-Wilson C (1990) A survey of *Codonopsis* in cultivation. Plantsman 12:65–99

Hall IM, Shankaranarayana GD, Noma K, Ayoub N, Cohen A et al (2002) Establishment and maintenance of a heterochromatin domain. Science 297:2232–2237

Hong D-Y (1995) The geography of the *Campanulaceae*: on the distribution centres. Acta Phytotax Sin 33(6):521–536

Hong D-Y, Lian YS (1983) Flora of China. Edited by: Editorial Board of the Flora of China, vol 73(2). Science Publishing Company, Beijing, pp 69–74

Hong D-Y, Ma L-M (1991) Systematics of the genus *Cyananthus* Wall ex Royle. Acta Phytotax Sin 29:25–51

Innocenti AM, Bitonti MB (1983) Different duration of the mitotic cycle in seedlings from brown and black caryopses of *Haynaldia villosa* Schur. Caryologia 36:27–32

Kellogg EA, Bennetzen JL (2004) The evolution of nuclear genome structure in seed plants. Am J Bot 91:1709–1725

Kliebenstein DJ (2008) A role for gene duplication and natural variation of gene expression in the evolution of metabolism. PLoS ONE 1 3(3):e1838. http://www.plosone.org

Kolakovsky AA (1987) System of the *Campanulaceae* family from the old world. Bot Zhurnal 72:1572–1579

Kovanda M (1978) *Campanulaceae*. In: Heywood VH (ed) Flowering plants of the world. Mayflower Books, New York, pp 254–256

Kumar V, Chauhan KPS (1975) Cytology of *Cyananthus linifolius* Wall. *Cat. Proc. Indian Sci. Congr. Assoc.* 62, Group B, vol 130

Lammers TG (1992) Systematics and biogeography of the *Campanulaceae* of Taiwan. In: Peng C-I (ed) Phytogeography and botanical inventory of Taiwan. Inst. Bot. Acad. Sin. Monogr. Ser. No. 12, Taipei, pp 41–61

Lammers TG (2007) World Checklist and Bibliography of the Campanulaceae. Richmond. Royal Botanic Gardens, Kew, Surrey

Lei Z, Ding K-Y, Xu L et al (2007) A cytological study on *Homocodon brevipes* (*Campanulaceae*), a species endemic to China. Acta Bot Yunnan 29(3):323–326

Levan A, Fredga K, Sandberg AA (1964) Nomenclature for centromeric position on chromosomes. Hereditas 52:201–220

Li GT, Li M (2005) The nucleus type analysis of the chromosome of *Codonopsis lanceolata*. Forest By-Product Speciality China 01:58–61

Moeliono B, Tuyn P (1960) Campanulaceae. In: van Steenis CGGJ (ed) Flora Malesiana Ser., vol 6(1). Noordhoff-Kolff, Djakarta, pp 107–141

Morris KE, Lammers TG (1997) Circumscription of *Codonopsis* and the allied genera *Campanumoea* and *Leptocodon* (*Campanulaceae*: *Campanuloideae*). Palynolodical data. Bot Bull Acad Sin 38:277–284

Murthy GVS (1983) Pollen morphology of Indian *Campanumoe* Bl.—a revision of the Genus. J Palynol 18:55–59

Narinder K, Vyasa D, Sanjay K (2007) Plants at high altitude exhibit higher component of alternative respiration. J Plant Physiol 164(1):31–38

Noirot M, Barre P, Louarn J, Duperray C, Hamon S (2000) Nucleus-cytosol interactions—a source of stoichiometric error in flow cytometric estimation of nuclear DNA content in plants. Ann Bot 86:309–316

Noirot M, Barre P, Duperray C, Hamon S, De Kochko A (2005) Investigation on the origins of stoichiometric error in genome

size estimation using heat experiments. Consequences on data interpretation. Ann Bot 95:111–118

Nonomura Ken-Ichi, Morohoshi Akane, Nakano Mutsuko et al (2007) A germ cell–specific gene of the ARGONAUTE family is essential for the progression of premeiotic mitosis and meiosis during sporogenesis in rice. Plant Cell 19(8):2583–2594

Oh H, Kim DH, Cho JH, Kim YC (2004) Hepatoprotective and free radical scavenging activities of phenolic petrosins, flavonoids isolated from *Equisetum arvense*. J Ethnopharmacol 95:421–424

Qiu JZ, Hong D-Y (1987) A cytogeographical study on *Adenophora gmelinii* complex (*Campanulaceae*). In: Hong D-Y (ed) Plant chromosome research. Proc. Sino—Jpn. symposium, P1. Chromos. Nishiki Print, Hiroshima, pp 69–73

Qiu JZ, Hong DY (1993) A biosystematic study in *Adenophora* gmelinii complex (*Campanulaceae*). Acta Phytotax. Sin. 31(1):17–41

Raven PH (1975) The bases of angiosperm phylogeny: cytology. Ann Missouri Bot Gard 62:724–764

Reinhart BJ, Bartel DP (2002) Small RNAs correspond to centromere heterochromatic repeats. Science 297:1831

Rieseberg LH (2001) Chromosomal rearrangements and speciation. Trends Ecol Evol 16:351–358

Rieseberg LH, Raymond O, Rosenthal DM, Lai Z, Livingstone K, Nakazato J, Durphy JL, Schwarzbach AE, Donovan LA, Lexer C (2003) Major ecological transitions in wild sunflowers facilitated by hybridization. Science 301:1211–1216

Rizzon C, Ponger L, Gaut BS (2006) Striking similarities in the genomic distribution of tandemly arrayed genes in *Arabidopsis* and rice. Plos Computat Biol 2:989–1000

Schönland S (1889) *Campanulaceae*. In: Engler A, Prantl K (eds) Die natürlichen Pflanzenfamilien IV, vol 5, pp 40–70. W. Engelmann, Leipzig

Shimizu T (1993) *Campanulaceae*. In: Iwatsuki K et al (eds) Flora of Japan, vol 3a. Kodansha, Tokyo, pp 405–418

Singh KP, Raina SN, Singh AK (1996) Variation in chromosomal DNA associated with the evolution of *Arachis* species. Genome 39:890–897

Stalker HT, Dhesi JS, Kochert G (1995) Genetic diversity within the species *Arachis duranensis* Krapov. & W.C

Stebbins GL (1971) Chromosomal evolution in higher plant. Edward Arnold, London, pp 85–104

Takhtajan AL (1987) Systema Magnoliophytorum. Nauka, Leningrad

Temsch EM, Greilhuber J (2001) Genome size in *Arachis duranensis*: a critical study. Genome 44:826–830

Thulin M (1975) The genus *Wahlenbergia* s. lat. (Campanulaceae) in tropical Africa and Madagascar. Symb Bot Upsal 21:1–223

Tian YS, Zhao XM (2007) The karyotype analysis of chromosomes of *Codonopsis pilosula* (Franch.) Nannf. Chin Wild Plant Resour 2:105–110

Tsoong PC (1935) Preliminary study on Chinese *Campanulaceae*. Contr Inst Bot Natl Acad Peiping 3:61–118

Vision TJ, Brown DG, Tanksley SD (2000) The origins of genomic duplications in *Arabidopsis*. Science 290:2114–2117

Volpe TA, Kidner C, Hall IM, Teng G, Grewal SI et al (2002) Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. Science 297:1833–1837

Volpe T, Schramke V, Hamilton GL, White SA, Teng G et al (2003) RNA interference is required for normal centromere function in fission yeast. Chromosome Res 11:137–146

Wang K-Q, Song GE (1998) A karyotype study on five species of *Adenophora*. Acta Botanica Yunnanica 20(1):58–62

White SA, Allshire RC (2009) RNAi-mediate chromatin silencing in fission yeast. Curr Top Microbiol Immunol 329:157–183

Wolfe KH, Shields DC (1997) Molecular evidence for an ancient duplication of the entire yeast genome. Nature 387:708–713

Xiaoyun D, Braun EL, Grotewold E (2001) Functional conservation of plant secondary metabolic enzymes revealed by complementation of *Arabidopsis* flavonoid mutants with maize genes. Plant Physiol 127:46–57

Yogeeswaran Krithika, Frary Amy, York Thomas L et al (2005) Comparative genome analyses of *Arabidopsis* spp.: inferring chromosomal rearrangement events in the evolutionary history of *A. thaliana*. Genome Res 15:505–515

Zhang Z-J, Yang xiao-sheng, Zhu wen-shi et al (2005) The primary study on chemical composite in *Campanumoea javanica* Bl. Chinese Tradit Herbal Drugs 36(8):1144–1146