A karyological study of six species of *Silene* L. (Caryophyllaceae) from the Hengduan Mountains, SW China

DONG LUO[1,2], DAN LIU[1], BO XU[1,2], ZE-LONG NIE[2], HANG SUN[2] and ZHI-MIN LI[1*]

[1]Life Sciences school of Yunnan Normal University, Kunming 650092, Yunnan, China.
[2]Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, China.

Abstract — Karyology of six *Silene* (Caryophyllaceae) species from the Hengduan Mountains of SW China was analyzed for the first time. The results indicate that the basic chromosome number for the six species is x=12, with karyotypic formula as follows: *S. orientalimongolica* (2n=2x=24=24m); *S. nepalensis* (2n=2x=24=16m+8sm); *S. trachyphylla* (2n=2x=24=8m+6sm+10st); *S. davidii* (2n=4x=48=48m); *S. nigrescens* (2n=4x=48=40m+8sm); *S. yetii* (2n=6x=72=64m+8sm). The karyotype asymmetry of *S. orientalimongolica* and *S. davidii* are 1A and *S. trachyphylla* is 3A, distinct from the other three species with 2A type. The significance of the cytological evolution of *Silene* from the Hengduan Mountains is briefly discussed.

Key words: chromosome number, Hengduan Mountains, karyotype, *Silene*.

INTRODUCTION

The genus *Silene* L. belongs to the Caryophyllaceae and consists of approximately 600 species distributed mainly in the Northern Hemisphere, but also in Africa and South American. About 110 species (67 endemic) occur in China (ZHOU *et al.* 2001), with 49 species and 2 varieties in the Hengduan Mountains of SW China (WU 1993).

The Hengduan Mountains is one of the biodiversity hotspots (BOUFFORD *et al.* 2004); there are at least 7954 species occurring in the region (LI and LI 1993). However, the chromosome data from the region are very poor; up to now only 522 taxa have been reported for cytological data (NIE *et al.* 2005). Although 49 species and 2 varieties of *Silene* occur in the Hengduan Mountains, so far only one species has been reported the chromosome number (LI and HE 2007). Therefore, it is necessary to add new data for the cytological studies of the genus in the region.

As part of our effort to accumulate cytological data from this area, in this study we present the karyomorphological data of six species of *Silene*, which grow in harsh alpine habitats at altitudes between 3700 and 4800m (TABLE 1).

MATERIAL AND METHODS

A list of the species studied, collection localities and accession numbers is given in (TABLE 1). Voucher specimens are deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN). All cytological observations were made from root tips. Seeds germinated on wet filter papers in Petri dishes. Root tips approximately 1cm long were cut and pretreated in 0.003 mol/L 8-hydroxyquinoline solution at room temperature in darkness for 3-6h, then fixed with absolute alcohol: glacial acetic acid (3:1) at approximately 4°C for 30 minutes, then rinsed in distilled water twice for approximately 20 minutes. Hydrolysed in 1 mol/L HCl at 60°C for 8-16 minutes, stained with 1% aceto-orcein overnight, and squashed for cytological observation. Permanent slides were made following the standard liquid nitrogen method.

*Corresponding author: Phone/Fax: +86-871-5215002; e-mail: lizhimin_vip@163.com

Observations were made of somatic mitotic interphase, prophase and metaphase. Karyomorphological classification of the mitotic interphase nuclei and prophase chromosomes was following TANAKA (1971; 1977). The designation of the centromeric position as median (m) or submedian (sm) was following LEVAN *et al.* (1964). Karyotype asymmetry (KA) was classified according to STEBBINS (1971).

RESULTS

1. *Interphase nuclei and prophase chromosomes* - The interphase nuclei of *Silene yetii* is stained lightly and protruded rough surface which gradually transforms into a diffused chromatin (Fig. 1: A). According to the system of TANAKA (1971; 1977), it can be categorized as simple chromocenter type. However, the other five species are stained darkly and have an almost homogeneous distribution throughout the nucleus, forming a fibrous network of the chromonemata (Fig. 1: B). They can be categorized as diffuse type.

The chromosomes at mitotic prophase of all six species stain darkly and homogeneously, showing several small elastic constrictions (Fig. 1: C). There are no conspicuous heterochromatic or euchromatic segments in the species investigated. According to the system of TANAKA (1971; 1977), the prophase chromosomes of the genus are classified as the interstitial type.

2. *Chromosome counts and karyomorphology* - Metaphase chromosomes of the six species of *Silene* are displayed in Fig. 1. Descriptions of the cytological features of each species are shown below.

Silene orientalimongolica Kozhevnikov - The sample from Anjiula Shan Pass (Baxoi) has a formula of 2n=2x=24=24m. The ratio of the longest to the shortest chromosome is 1.51 and the KA is 1A type (Fig. 1: D, D').

Silene nepalensis Majumdar - The karyotype formula is 2n=2x=24=16m+8sm, with pairs 8, 10, 11 and 12 being submetacentrics. The ratio of the longest to the shortest chromosome is 1.49, and the KA is 2A type (Fig. 1: E, E').

Silene trachyphylla Franchet - The karyotype formula is 2n=2x=24=8m+6sm+10st, with the 5th, 6th and 10th pairs being submetacentrics. Moreover, pairs 7, 8, 9, 11 and 12 of chromosomes are subterminal type. The ratio of the longest to the shortest chromosome is 1.52 and the KA is 3A type (Fig. 1: F, F').

Silene davidii (Franchet) Oxelman & Lidén - The population was collected from Zheduo Shan Pass (Kangding) is a tetraploid with a formula of 2n=4x=48=48m. The ratio of the longest to the shortest chromosome is 1.73, and the KA is 1A type (Fig. 1: G, G').

Silene nigrescens (Edgeworth) Majumdar - The chromosome number for this species is 48 and has a formula of 2n=4x=48=40m+8sm, which is also tetraploid with pairs 8, 10, 21 and 23 being submetacentrics. The ratio of the longest to the shortest chromosome is 1.70, and the KA is 2A type (Fig. 1: H, H').

Silene yetii Bocquet - The karyotype formula for this species is 2n=6x=72=64m+8sm, which is hexaploid with pairs 11, 19, 23 and 33 being submetacentrics. The ratio of the longest to the shortest chromosome is 1.87, and the KA is 2A type (Fig. 1: I, I').

TABLE 1 — Collection and accession information of *Silene* species studied.

Taxon	Locality	Altitude	Position	Voucher (KUN)
Silene davidii (Franchet) Oxelman & Lidén	Zheduo Shan Pass, Kangding, Sichuan	4700m	30°04'11.3"N 101°48'17.1"E	Xu Bo-371
Silene nepalensis Majumdar	Rawu, Baxoi, Xizang	3800m	29°55'53.0"N 96°39'58.0"E	SunH-07ZX-1603
Silene nigrescens (Edgeworth) Majumdar	Anjiula Shan Pass, Baxoi, Xizang	4800m	29°39'36.7"N 96°42'55.1"E	SunH-07ZX-1422
Silene orientalimongolica Kozhevnikov	Anjiula Shan Pass, Baxoi, Xizang	4800m	29°39'36.7"N 96°42'55.1"E	SunH-07ZX-1421
Silene trachyphylla Franchet	Make Cun, Zogong, Xizang	3700m	29°31'34.3"N 97°56'11.1"E	SunH-07ZX-2086
Silene yetii Bocquet	Luoni Xiang, Markam, Xizang	4000m	29°57'16.9"N 98°32'15.0"E	ZhangDC-07ZX-1300

DISCUSSION

Silene orientalimongolica, *S. nepalensis* and *S. trachyphylla* in this study are documented as diploids (2n=24), with different karyotype asymmetry of 1A, 2A, and 3A, respectively. *Silene davidii* and *S. nigrescens* are found to be tetraploids with KA categorized as 1A and 2A type, respectively. *Silene yetii* is found to be hexaploid with 2A type. Previous cytological studies also indicated a high level of variation of chromosome number in the genus, which has been reported as 2n=20,

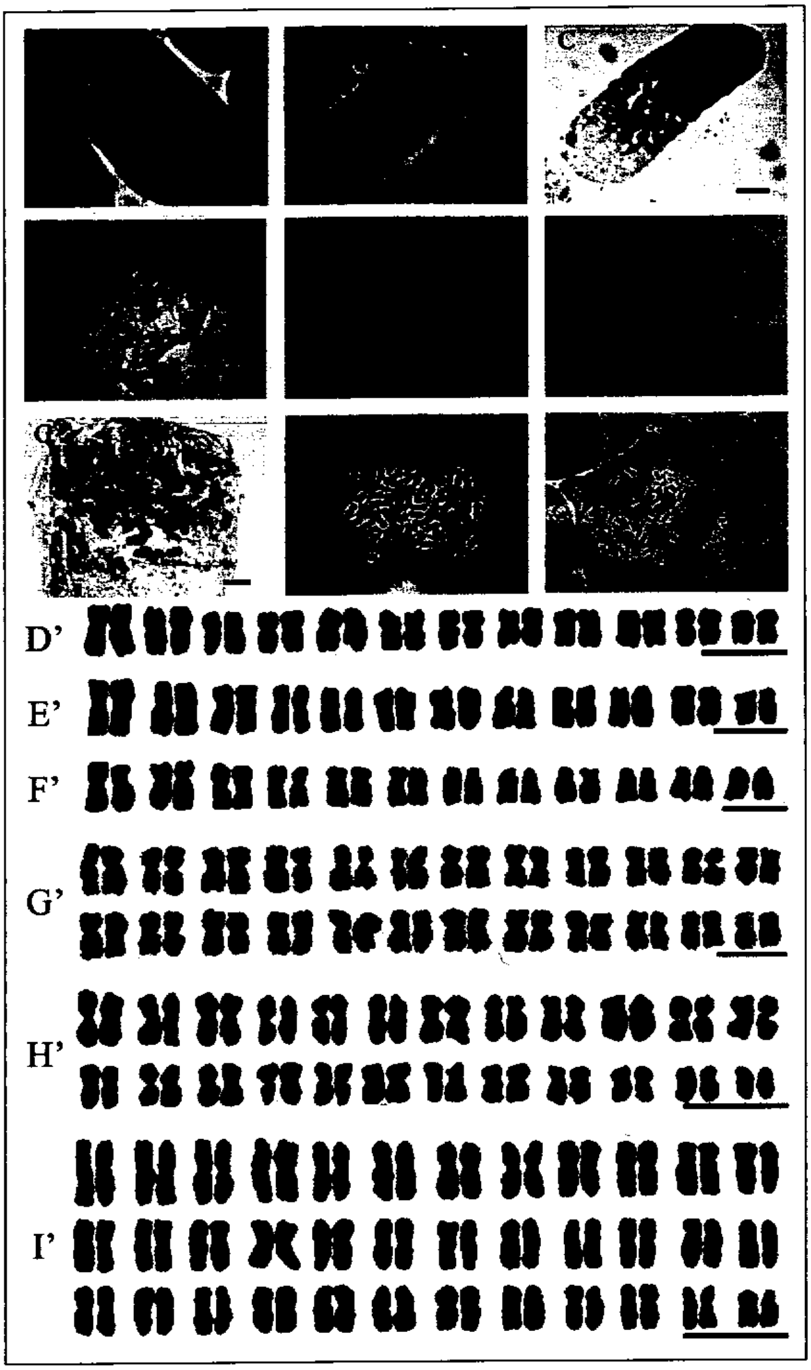

Fig. 1. — Mitotic nuclei, metaphasic chromosomes and karyotype of six species of *Silene*. A-B, Mitotic interphase nuclei. C, Mitotic prophase. D-I, Mitotic metaphase. D'-I', Karyotype. D, D', *S. orientalimongolica*, 2n=24. E, E', *S. nepalensis* 2n=24. F, F', *S. trachyphylla* 2n=24. G, G', *S. davidii* 2n=48. H, H', *S. nigrescens* 2n=48. I, I', *S. yetii* 2n=72. Scale bars=5 μm.

24, 30, 48, 72 (HEASLIP 1951; BARI 1973; ZHANG 1994; WANG 2004; LI and HE 2007; MINARECI *et al.* 2009; YILDIZ *et al.* 2009).

Half of our reported species are polyploids. However, of the 322 *Silene* species with chromosome counts, about 77% are diploids as 2n=24 and only about 15% of the reported species are polyploids (WANG 2004). It seems that *Silene* keeps a chromosomal evolution on a diploid level mainly based on the chromosome number of 2n=24.

Most species of *Silene* with high endemism grow in harsh alpine habitats of the Hengduan Mountains which is one of the centre of the genus distribution and differentiation (WU 1993; ZHOU *et al.* 2001). Because polyploidy is common in plants of cold climates with harsh and stressful environments (LÖVE and LÖVE 1949; 1967; BROCHMANN *et al.* 2004), we would expect a relatively high frequency of polyploidy in the genus in this region. However, so far only *S. gracilicaulis* (2n=24) has been studied (LI and HE 2007). Including this study, there are only 7 available cytological data for the genus *Silene* from Hengduan Mountains, of which 4 species are diploids. Therefore it is uncertain whether polyploidy plays a significant role in the genus evolution from the harsh alpine habitat of the region. Karyomorphological studies on more species of the genus from the Hengduan Mountains are needed in order to better understand the chromosomal evolution of the genus in this biodiversity hotspot.

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