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Cytological study of *Tibetia* (Fabaceae) in the Hengduan Mountains region, China

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Abstract The Hengduan Mountains comprise one of the world's most important hot spots of biodiversity. *Tibetia* (Ali) H.P. Tsui (Fabaceae), which has four or five species in two sections, is one of the genera endemic to the region. This paper describes for the first time the karyotype of three of those species. The chromosome counts of all three are $2n = 16$. The karyotypes of the species examined contain chromosomes of variable karyotypic symmetry with centromeres at median and submedian positions that correlate with the morphological characteristics of the species. Karyotypic variation at the diploid level appears to be the predominant feature of chromosome evolution in the genus and may provide a clue to the study of evolutionary patterns of plants in this region.

Key words Chromosome evolution · Fabaceae · Hengduan Mountains · Karyotype · *Tibetia*

Introduction

The Hengduan Mountains lie at the eastern end of the Himalayan range and extend from western Sichuan and northern Yunnan provinces to eastern Tibet in China and into northernmost Myanmar (Fig. 1). The area comprises a series of spectacular north–south trending ridges alternating with deep valleys. Altitudes range from 2000 to 6000 m above sea level. The region contains more than 9000 species of plants and is especially rich in endemic species and genera (Ying and Zhang 1984; Ying et al. 1993; Li 1993, 1994; Hao 1997; Boufford and Van Dyck 2000; Wang 2000), which belong to three main historic-phytogeographic components (i.e., Laurasian, Gondwanan, Tethyan) (Wu 1988; Li 1993). It was first referred to as the eastern Himalayan "hot spot" (Myers 1988; Wilson 1992; Myers et al. 2000) and more recently as the Hengduan Mountains hot spot by Boufford and Van Dyck (2000). Because of its location in a region of recent and spectacular geological activity and because of the many unusual plants and animals found in the region, it is a focus for the study of relations between florogenesis and plate tectonics and the origin of the North temperate flora including that of eastern Asia, North America, and Europe, and about the evolution of seed plants in general (Wu 1988; Wu and Wu 1998).

On the basis of prior work on the flora of this region (Wang et al. 1993), it was clear that much was unknown and that further studies just to obtain basic information were needed. Cytological data are essential to the study of plant evolution and diversification (Stebbins 1950, 1971; Hong 1990; Stace 2000). However, cytological information on plants from the Hengduan Mountains are scanty (Hong 1984; Tang et al. 1984; Mu and Shue 1985; Gu and Na 1986; Hong and Zhang 1990; Kondo et al. 1992; Wang 2001). We therefore chose the endemic genus *Tibetia* (Ali) H.P. Tsui (Fabaceae) to begin our cytological studies and the study of chromosome evolution in the plants in this area.

Tibetia is a herbaceous perennial comprising about four species in two sections (Tsui 1998). It was distinguished from *Gueldenstaedtia* by Tsui (1979) as a small genus endemic to the mountains and semiarid meadows of the Hengduan Mountains above 2000 m. One species, *Tibetia himalaica*, extends to Bhutan and Nepal.

This paper reports the karyotypes of three species of *Tibetia* from the Hengduan Mountains, compares and discusses the cytology and morphology of the species, and considers chromosome evolution in the region.

Materials and methods

All observations were made from root tip cells at mitotic metaphase. The root tips were obtained from germinating seeds collected in the Hengduan Mountains (Fig. 1). Detailed collection data are shown in Table 1. Vouchers of all

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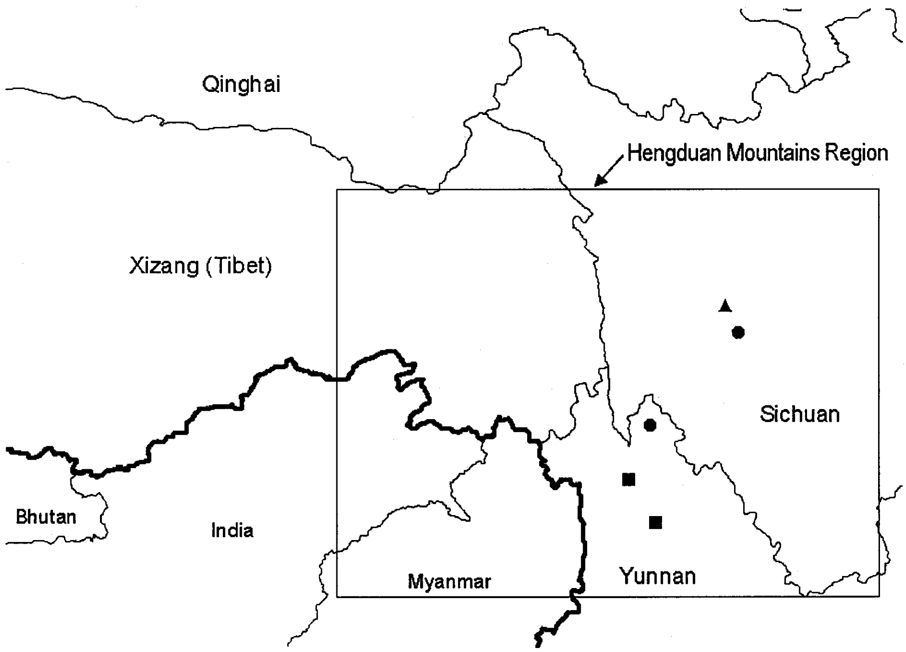


Fig. 1. Approximate range of Hengduan Mountains and location of collection sites of *Tibetia* used in this study. *Squares*, *T. yunnanensis*; *triangles*, *T. himalaica*; *circles*, *T. tongolensis*

Table 1. Species, localities, and vouchers of plants of *Tibetia* collected in the Hengduan Mountains, China

Species	Locality	Voucher
T. yunnanensis (Franch.) H.P.Tsui	Yunnan, Lijiang	*Nie 200007*
	Yunnan, Zhongdian	*Nie 200016*
T. tongolensis (Ulber.) H.P.Tsui	Yunnan, Zhongdian	*Sun 200001*
	Sichuan, Daocheng	*Nie 200032*
T. himalaica (Baker) H.P.Tsui	Sichuan, Daocheng	*Nie 200022*

All vouchers are deposited in the herbarium of the Kunming Institute of Botany (KUN)

collections will be deposited in the herbarium of the Kunming Institute of Botany (KUN). Root tips were pretreated in a 0.002 M 8-hydroxyquinoline solution for 1.5–2.0 h, then fixed with Carnoy fluid (1 : 3 glacial acetic acid/absolute alcohol) at about 3°C for 30 min. The fixed roots were hydrolyzed in 1 N HCl at 60°C for 30 S, stained with 1% acetoorcein, and then squashed for cytological observation. Permanent slides of these squashed specimens remain in the authors' possession. Measurements were done in 10 well-spread metaphases of five or more plants of each species. The cytological classification of the resting and mitotic prophase chromosomes follows Tanaka's categories (1971, 1977). The symbols for the description of chromosomes follow Levan et al. (1964). The symmetry of karyotypes is classified according to Stebbins (1971).

Results

The three species, two of which were from two populations, had basically similar chromosome morphology. The interphase nucleus (Fig. 2A) exhibited many darkly stained chromocenters with an irregularly protruding rough surface that gradually transformed into diffuse chromatin. We categorized this pattern as the complex chromocenter type (Tanaka 1971, 1977). At mitotic prophase (Fig. 2B), hetero- and euchromatic segments were distinguishable, with the heterochromatic segments distributed in the interstitial and proximal regions. The prophase chromosomes therefore were of the interstitial type (Tanaka 1977).

Metaphase chromosomes of each species are shown in Figs. 2 and 3, with detailed parameters listed in Table 2. Brief descriptions of the cytological features of each species are as follows.

Section Tibetia

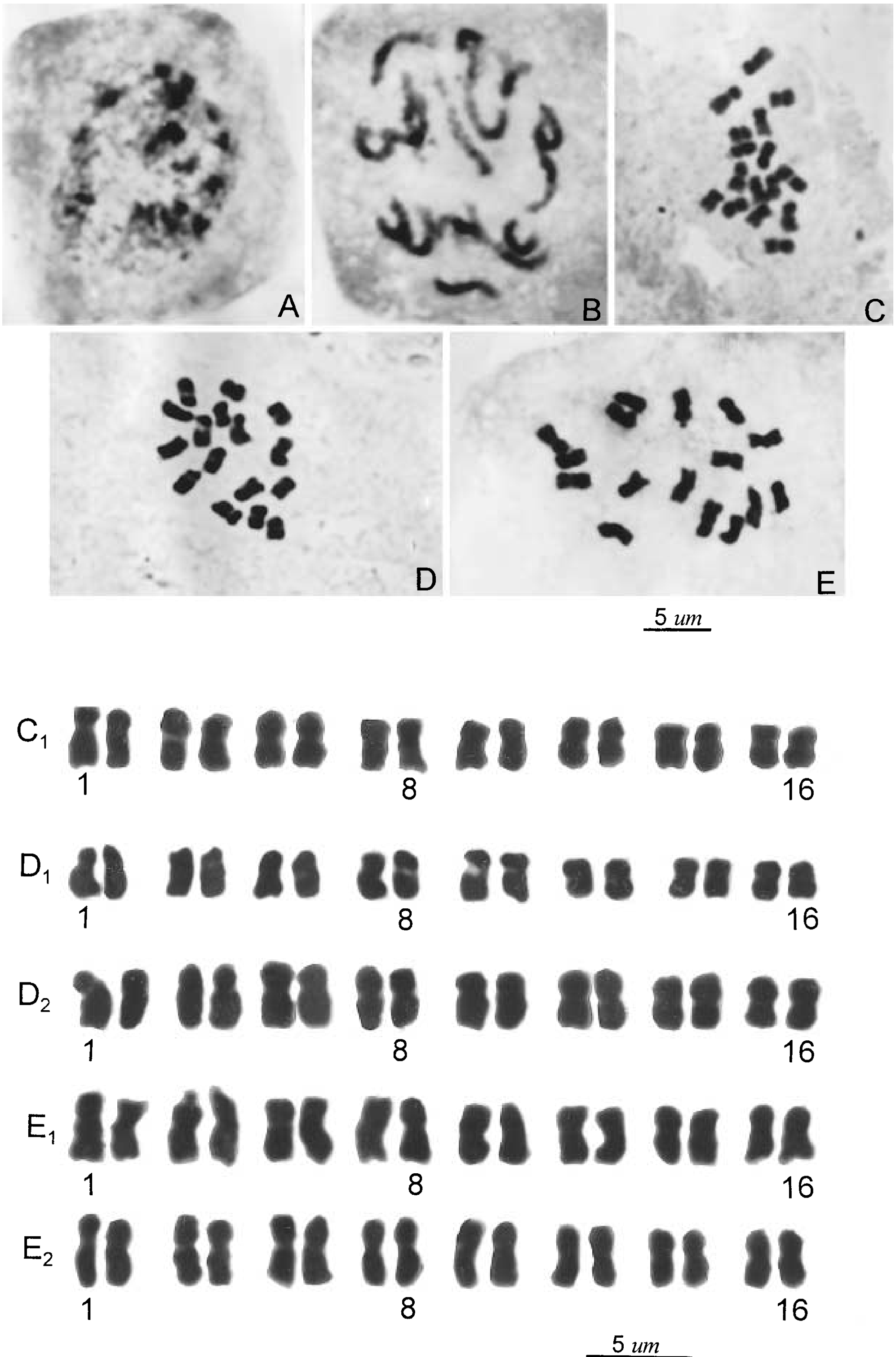
Tibetia yunnanensis *(Franch.) H.P.Tsui*

Tibetia yunnanensis has the karyotype formula $2n = 16 = 16m$. The ratio of the longest to the shortest chromosome was 1.22, and no chromosome had an arm ratio of more than 2.00. The asymmetry of the karyotype is categorized as type 1A. The population from Lijiang had satellites attached to the short arm of the second pair of chromosomes (Fig. 2E).

Tibetia himalaica *(Baker) H.P.Tsui*

Tibetia himalaica is similar to *T. yunnanensis*. The karyotype formula was $2n = 16 = 14m + 2sm$. The first pair of chromosomes has centromeres at a submedian position (Fig. 2C). The ratio of the longest to the shortest chromosome was 1.35, and 12.5% chromosomes have an arm ratio of more than 2.00. The asymmetry of the karyotype is categorized as type 2A.

Fig. 2A–E. Micrographs of chromosomes of *Tibetia*. **A**, **B**, **E**, **E**$_1$ *T. yunnanensis* from Lijiang. **E**$_2$ *T. yunnanensis* from Zhongdian. **C**, **C**$_1$ *T. himalaica*. from Daocheng. **D**, **D**$_1$ *T. tongolensis* from Zhongdian. **D**$_2$ *T. tongolensis* from Daocheng. **A** Resting nucleus. **B** Prophase chromosomes. **C–E** Metaphase chromosomes. **C**$_1$**–E**$_2$ Karyotype



Section Glabrae

Tibetia tongolensis *(Ulber.) H.P.Tsui*

The two populations of *Tibetia tongolensis* (from Zhongdian and Daocheng) had similar karyotypes, with the formulation $2n = 16 = 10m + 6sm$. The chromosomes with centromeres at submedian position are the first, second, and fifth pairs in the population from both Zhongdian (Fig. 2D$_1$) and Daocheng (Fig. 2D$_2$). The karyotype of the plants in section Glabrae differs from that of section Tibetia, with 25% of chromosomes having an arm ratio exceeding 2.00

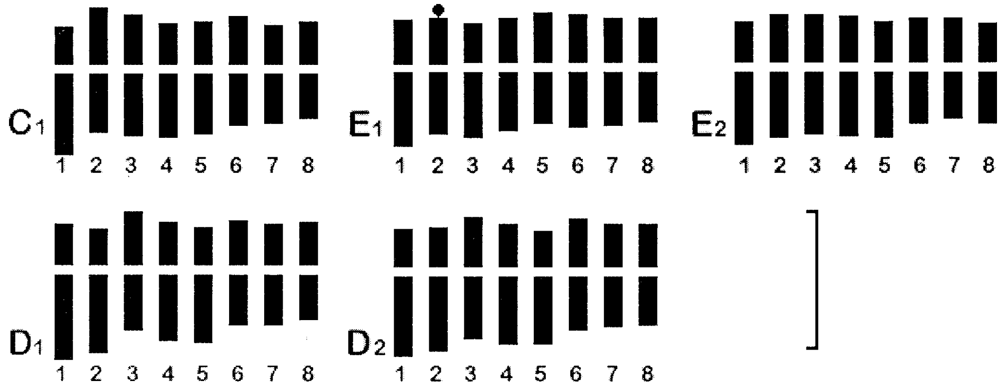


Fig. 3. Idiograms of somatic metaphase chromosomes of *Tibetia*. $\mathbf{C_1}$ *T. himalaica* from Daocheng. $\mathbf{E_1}$ *T. yunnanensis* from Lijiang. $\mathbf{E_2}$ *T. yunnanensis* from Zhongdian. $\mathbf{D_1}$ *T. tongolensis* from Zhongdian. $\mathbf{D_2}$ *T. tongolensis* from Daocheng. Scale 5 μm

and three pairs of chromosomes with centromeres at a submedian position. The ratio of the longest to the shortest chromosome was 1.30–1.40. The asymmetry of the karyotype is categorized as type 2A.

Discussion

The three species of *Tibetia* examined in this study have a consistent somatic chromosome number of $2n = 16$ and exhibit the same interphase nuclear morphology and prophase chromosomes. The ratio of the longest to the shortest chromosome in all three species did not exceed 2.00, indicating that the species we studied have a relatively low interchromosomal asymmetry and a monomodal karyotype. The karyotypes showed a predominance of chromosomes with centromeres in a median position and uniform chromosome size (Table 2). Despite this basic pattern, variation in chromosome symmetry can be used to distinguish the species and to indicate the possible evolutionary line in *Tibetia*.

In section Tibetia, *T. yunnanensis* and *T. himalaica* have similar karyotypes, except that the arm ratio of the first pair of chromosomes in the latter is more than 2.00 (Table 2). As shown in Table 3, *T. yunnanensis* and *T. himalaica* share common morphological features, although the latter has some features (e.g., more and hairy leaflets) that may adapt it to the severe environment of high mountains. Within the species, populations of *T. yunnanensis* in the area extending from Lijiang to Zhongdian with an increase in leaflet number, show a similar karyotype, although a pair of satellites were observed attached to the second pair of chromosomes in the former (Fig. $2E_1$).

In section Glabrae, *T. tongolensis* exhibits much more variation in chromosome asymmetry, with three pairs of chromosomes having centromeres at a submedian position compared with the section Tibetia, which has only one. In regard to morphological features, the ovary and pollen of *T. tongolensis* also differ from the two species of section Tibetia (Table 3). Populations of *T. tongolensis* from Zhongdian and Daocheng have similar karyotypes and similar morphology.

As discussed above, both the morphological and cytological information supports the traditional classification

Table 2. Parameters of mitotic metaphase chromosomes of three species in *Tibetia* from the Hengduan Mountains, China

Relative length	Arm Ratio	Type[a]
T. yunnanensis from Lijiang		
4.4 + 2.6 = 7.0	1.7	m
3.7 + 2.8 = 6.5	1.3	m[b]
3.9 + 2.4 = 6.3	1.6	m
3.5 + 2.7 = 6.2	1.3	m
3.1 + 3.1 = 6.2	1.0	m
3.3 + 2.9 = 6.2	1.1	m
3.2 + 2.7 = 5.9	1.2	m
3.0 + 2.7 = 5.7	1.1	m
T. yunnanensis from Zhongdian		
4.3 + 2.5 = 6.8	1.7	m
3.9 + 2.9 = 6.8	1.3	m
3.7 + 2.9 = 6.6	1.3	m
3.7 + 2.8 = 6.5	1.3	m
3.9 + 2.5 = 6.4	1.6	m
3.1 + 2.7 = 5.8	1.1	m
2.8 + 2.7 = 5.5	1.0	m
3.1 + 2.4 = 5.5	1.3	m
T. himalacica from Daocheng		
4.8 + 2.2 = 7.0	2.2	sm
3.5 + 3.4 = 6.9	1.0	m
3.7 + 3.1 = 6.8	1.2	m
3.8 + 2.5 = 6.3	1.5	m
3.6 + 2.6 = 6.2	1.4	m
3.1 + 2.9 = 6.0	1.1	m
3.0 + 2.4 = 5.4	1.3	m
2.6 + 2.6 = 5.2	1.0	m
T. tongolensis from Zhongdian		
5.0 + 2.5 = 7.5	2.0	sm
4.6 + 2.2 = 6.8	2.1	sm
3.3 + 3.2 = 6.5	1.0	m
3.8 + 2.6 = 6.4	1.5	m
4.1 + 2.3 = 6.4	1.8	sm
3.0 + 2.7 = 5.7	1.1	m
3.0 + 2.5 = 5.5	1.2	m
2.7 + 2.6 = 5.3	1.0	m
T. tongolensis from Daocheng		
4.7 + 2.3 = 7.0	2.0	sm
4.4 + 2.4 = 6.8	1.8	sm
3.8 + 3.0 = 6.8	1.3	m
4.0 + 2.6 = 6.6	1.5	m
4.0 + 2.2 = 6.2	1.8	sm
3.2 + 3.0 = 6.2	1.1	m
3.0 + 2.6 = 5.6	1.2	m
2.9 + 2.6 = 5.5	1.1	m

m, chromosomes with arm ratio of 1.0 : 1.7, having centromeres at median position; sm, chromosomes with arm ratio of 1.7 : 3.0, having centromeres at the submedian position
[a] Type of chromosomes estimated according to Levan et al. (1964)
[b] Chromosomes with satellites

Table 3. Comparison of the morphology of *Tibetia yunnanensis*, *T. himalacia*, and *T. tongolensis*

Feature	*T. yunnanensis*	*T. himalaica*	*T. tongolensis*
Ovary	Hairy	Hairy	Glabrous
Pollen	3-Colpate	3-Colpate	4-Colpate
Stipule	Small; apex acute	Small; apex acute	Large; apex rounded
Leaflets	3–7	9–12, hairy	5–7

and suggests an increase in karyotype variation (from section Tibetia with one pair of chromosomes having centromeres at a submedian position to section Glabrae with three pairs) concomitant with somewhat advanced morphological characteristics (Table 3).

Based on molecular data from the nuclear ribosomal ITS region (Sanderson and Wojciechowski 1996), *Chesneya* appears to be the closest relative of *Tibetia* and *Gueldenstaedtia* followed by *Caragana*, *Oxytropis*, and *Astragalus*. Based on cytological data, the karyotype of *Chesneya nubigena* (D. Don) Ali is $2n = 16 = 10m + 6sm$ (Gu et al. 1993; Gu and Sun 1996), simliar to the karyotype of *Tibetia*. Among other relatives, *Caragana* has a similar karyotype, containing only chromosomes with centromeres at median and submedian positions based on $x = 8$ (Löve 1978, 1979; Gu et al. 1993), as does *Oxytropis* (Gurzenkov and Pavlova 1985; Wang et al. 1994). Additionally, some 95% of the species of *Astragalus*, the most speciose relative of *Tibetia*, surveyed from Eurasia have the same chromosome number based on $x = 8$ (Goldblatt 1981; Liu 1984; Ma et al. 1984; Gurzenkov and Pavlova 1985; Podlech 1986; Ashraf and Gohil 1988, 1989; Chen and Zhu 1990; Wang et al. 1994). Despite some species of *Astragalus* having a karyotype simliar that of *Tibetia* and its relatives, there are many species of *Astragalus* with chromosomes having centromeres at a subterminal position (Wang et al. 1994). It seems that the intrachromosomal variation in the structure of *Tibetia* and its relatives represents a major evolutionary line at the diploid level in the Old World, which is perhaps one of the main cytological patterns of evolution in plants in the Hengduan Mountains region. Our study of *Tibetia* is only a start toward understanding the relation between ecological specialization, morphological divergence, speciation, and chromosome variation in the plants of this region. Further cytological studies of the plants in the Hengduan hot spot are needed.

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