

# Cytological studies on the Sino-Himalayan endemic *Anisodus* and four related genera from the tribe Hyoscyameae (Solanaceae) and their systematic and evolutionary implications

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Cytological studies were carried out on eight species of five genera (*Anisodus*, *Atropanthe*, *Hyoscyamus*, *Mandragora* and *Przewalskia*) in the tribe Hyoscyameae (Solanaceae). First chromosome counts were reported in six species: *Anisodus luridus*, *A. acutangulus*, *A. tanguticus*, *A. carniolicoides*, *Atropanthe sinensis* and *Mandragora caulescens*, all with  $2n = 8x = 48$ . Two records, for *Hyoscyamus niger* ( $2n = 2x = 34$ ) and *Przewalskia tangutica* ( $2n = 4x = 44$ ), were also confirmed. All species studied showed the proximal type of mitotic prophase chromosome condensation pattern. Three types of interphase nuclei were recognized: the round prochromosome type for *Anisodus*, *Atropanthe* and *Mandragora*, the rod prochromosome type for *Przewalskia* and the complex chromocentre type for *Hyoscyamus*. The cytological data supported the close relationship of all four species of *Anisodus*. Evidence from the interphase nuclei and chromosome base numbers supported the traditional classification of Hyoscyameae into two groups, i.e. *Physochlaina praealta* + *Hyoscyamus* (complex interphase type and  $x = 7, 14, 17$ ) and *Przewalskia* + *Atropanthe* + *Anisodus* + *Scopolia* + *Atropa* + *Mandragora* (prochromosome type and  $x = 6, 11$ ). Polyploidy is found in most species of the tribe in the Himalayan–Hengduan Mountains, as well as in the Mediterranean region, west–central Asia and eastern Asia. It seems that it probably occurred very early in Hyoscyameae evolution, before the uplift of the Himalayan–Hengduan Mountains. The Himalayan orogeny might have played a minor role in the polyploid evolution of plants in this tribe. © 2005 The Linnean Society of London. *Botanical Journal of the Linnean Society*, 2005, 147, 457–468.

**ADDITIONAL KEYWORDS:** chromosome base number – interphase nuclei – karyotype – prophase chromosome.

## INTRODUCTION

The family Solanaceae is distributed widely in the tropical and temperate zones of the world and is centred in tropical America (Badr *et al.*, 1997). Hyoscyameae is the only tribe of Solanaceae distributed in Eurasia and is centred in the Himalayan–Hengduan Mountains and the adjacent areas of south-west China (Lu & Zhang, 1986; Hoare & Knapp, 1997; Yang *et al.*, 2002a, b). Plants in this tribe are of great economic and medicinal importance (Chen & Chen, 1977;

Lu & Zhang, 1986). The taxonomy of Hyoscyameae has been studied widely (Chen & Chen, 1977; Kuang & Lu, 1978; D'Arcy & Zhang, 1992; Zhang, Lu & D'Arcy 1994; Lu, 1997) and in order to establish a clear phylogeny of this tribe, studies on morphology and geography, leaf epidermis, palynology, embryology, floral organogenesis and development, cladistic analysis and molecular biology for some of the genera and species have been carried out (Zhang & Lu, 1984; Lu & Zhang, 1986; Hala & Olmstead, 1996; Hoare & Knapp, 1997; Lu *et al.*, 1999; Olmstead *et al.*, 1999; Yang, Zhang & Wen, 2000; Yang *et al.*, 2002a, b; Yang, 2002). Although there is enough evidence to support the union of Hyoscyameae, which includes *Anisodus*,

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*Atropanthe*, *Hyoscyamus*, *Archihyoscyamus*, *Physochlaina*, *Przewalskia* and *Scopolia*, into a monophyletic group (Yang, 2002), the delimitation of the tribe and the systematic position of each genus are quite controversial. For example, Hoare & Knapp (1997) thought that *Atropa* and *Mandragora* should be included in Hyoscyameae, whereas Lu & Zhang (1986) had a different interpretation; *Przewalskia* was thought by Lu & Zhang (1986) to stand in an advanced position in Hyoscyameae, while an opposite opinion was held by Hoare & Knapp (1997).

The Sino-Himalayan endemic genus *Anisodus* consists of *A. luridus* Link, *A. acutangulus* C. Y. Wu & C. Chen, *A. tanguticus* (Maxim.) Pascher and *A. mairei* (H. Léveillé) C. Y. Wu & C. Chen in the system of Chen & Chen (1977), while D'Arcy & Zhang (1992) merged *A. luridus* Link and *A. mairei* (H. Léveillé) C. Y. Wu & C. Chen as *A. luridus* Link and transferred *Scopolia carniolicoides* C. Y. Wu & C. Chen to *Anisodus* as *A. carniolicoides* (C. Y. Wu & C. Chen) D'Arcy & Z. Y. Zhang. To solve these different interpretations, new data from other disciplines are needed.

Cytological data are of great importance in the study of plant evolution and diversification (Stebbins, 1971; Hong, 1990) and these data have been widely used in discussing classification and phylogeny of Solanaceae (Philomina, 1980; Olmstead & Palmer, 1992; Badr *et al.*, 1997; Olmstead *et al.*, 1999). Systematic relationships within Hyoscyameae have not been discussed cytologically, although Hoare & Knapp (1997) thought that cytological data 'may be informative' in this tribe. Therefore, we undertook a study to collect members of Hyoscyameae from the Himalayan–Hengduan Mountains – which are not only the distributional centre of this tribe (Lu & Zhang, 1986) but also one of the world's 'hot spots' of biodiversity (Boufford & Van Dijk, 1999; Myers *et al.*, 2000) – and to carry out cytological work on them with the aims of

understanding the taxonomy of *Anisodus*, the evolutionary relationships within this tribe and the chromosome evolution in plants in this interesting region. Chromosome counts and/or karyomorphology of all four species in *Anisodus* and of one each in *Mandragora*, *Atropanthe*, *Hyoscyamus* and *Przewalskia* (see Table 2) are reported.

## MATERIAL AND METHODS

All cytological observations were made from root tip cells. The root tips of *Anisodus*, *Hyoscyamus* and *Przewalskia* were obtained from germinating seeds and those of *Atropanthe* and *Mandragora* were from transplanted plants cultivated in pots in the experimental garden of the Kunming Institute of Botany, Chinese Academy of Sciences. All cytological material studied was collected in the Himalayan–Hengduan Mountains and adjacent regions. Detailed collection data are shown in Table 1. The vouchers of all collections and permanent slides are deposited in the herbarium of Kunming Institute of Botany (KUN). Root tips were pretreated in a 0.002 mol L<sup>-1</sup> 8-hydroxyquinoline solution for 100 min, then fixed with Carnoy's fluid (absolute alcohol : glacial acetic acid, 3 : 1, v/v) at 4 °C for at least 30 min. The fixed roots were hydrolysed in 1 : 1 solution of 1 N HCl : 45% acetic acid at 60 °C for 22 s, stained with 1% aceto-orcein for 1 h and squashed for cytological observation. Permanent slides were made using the standard liquid nitrogen method.

We determined karyotypes of somatic chromosomes at metaphase using at least ten well-spread metaphases of three or more different root tips from each species. The karyomorphological classification of the mitotic interphase nuclei and prophase chromosomes followed Tanaka (1971, 1977), the designation of the centromere position as median (m) and submedian (sm) followed Levan, Fedga & Sandberg (1964)

**Table 1.** Localities, geographical positions, altitudes and voucher numbers of the investigated species of *Anisodus*, *Atropanthe*, *Hyoscyamus*, *Mandragora* and *Przewalskia*. All collections were made from the Himalayan–Hengduan Mountains and the adjacent regions in Yunnan, Sichuan and Chongqing, China

Taxon	Locality	Altitude (m)	Voucher (KUN)
<i>Anisodus luridus</i> Link	Heqing, Yunnan	2390	T. Y. Tu 02101
	Daocheng, Sichuan	3750	T. Y. Tu 0283
<i>A. acutangulus</i> C. Y. Wu & C. Chen	Lijiang, Yunnan	3100	T. Y. Tu 0212
<i>A. tanguticus</i> (Maxim.) Pascher	Daocheng, Sichuan	3750	T. Y. Tu 0280
<i>A. carniolicoides</i> (C. Y. Wu & C. Chen) D'Arcy & Z. Y. Zhang	Zhongdian, Yunnan	3370	T. Y. Tu 0220
<i>Atropanthe sinensis</i> (Hemsley) Pascher	Shizhu, Chongqing	2900	T. Y. Tu 02107
<i>Hyoscyamus niger</i> L.	Ganzi, Sichuan	3400	T. Y. Tu 0276
<i>Mandragora caulescens</i> C. B. Clarke	Zhongdian, Yunnan	3700	T. Y. Tu 0266
<i>Przewalskia tangutica</i> Maxim.	Shiqu, Sichuan	4320	T. Y. Tu 0275

and the asymmetry of the karyotype followed Stebbins (1971).

## RESULTS

### INTERPHASE NUCLEI

The interphase nuclei of eight species from five genera analysed in this paper can be classified into three categories based on the shape and the distribution pattern of chromatin. Those of *Anisodus*, *Atropanthe* and *Mandragora* show some smooth-faced round prochromosomes, which are dispersed evenly throughout the nuclei (Figs 1–3). According to Tanaka (1971, 1977), they can be categorized as the round prochromosome type. The interphase nuclei of *Przewalskia* show many rod-shaped heteropycnotic bodies, which are dispersed evenly throughout the nuclei (Fig. 5). We categorize this pattern as the rod prochromosome type. The interphase nuclei of *Hyoscyamus* belong to the complex chromocentre type, which is characterized by darkly stained chromocentres of irregular shape and lightly stained chromatin threads (Fig. 4).

### PROPHASE CHROMOSOMES

Heterochromatin and euchromatin segments are clearly seen at mitotic prophase in all five genera. The heterochromatin segments are located in the proximal regions that are deeply stained, indicating early condensation, while the euchromatin segments in the distal regions of chromosomes are lightly stained and extended, indicating late condensation (Figs 6–10). According to Tanaka (1971, 1977), the prophase chromosomes of all species in this study are of the proximal type.

### CHROMOSOME COUNTS AND KARYOMORPHOLOGY

Three different somatic chromosome numbers were encountered,  $2n = 48$  for *Anisodus luridus*, *A. acutangulus*, *A. tanguticus*, *A. carniolicoides*, *Atropanthe sinensis* and *Mandragora caulescens*,  $2n = 44$  for *Przewalskia tangutica* and  $2n = 34$  for *Hyoscyamus niger*. Except for *M. caulescens*, which was not analysed karyomorphologically, the other seven species share the same characteristics, with only metacentric (m) and submetacentric (sm) chromosomes being present. One to three satellites attached to different pairs of chromosomes were observed in all species except *A. sinensis*. The karyotype asymmetry pattern of *Przewalskia* and *Hyoscyamus* is type 2A, while that of the other genera is type 2B. The metaphase chromosomes of each species are shown in Figures 11–19. Their detailed parameters and karyotype formulae are listed in Tables 2, 3.

#### *Anisodus luridus* Link

Plants of both populations from Daocheng and Heqing have the same karyotype formula  $2n = 48 = 32m(2SAT) + 16sm$ . The chromosomes with centromeres at the sm position are pairs 2, 3, 11, 17, 18, 19 and 21 from Daocheng and pairs 3, 5, 7, 11, 13, 14 and 17 from Heqing. The ratio of the longest to the shortest chromosome in both populations is 2.5. Their karyotype asymmetry is classified as 2B (Figs 11, 12, 20A, B).

#### *Anisodus acutangulus* C. Y. Wu & C. Chen

The karyotype of this species is  $2n = 48 = 40m(1SAT) + 8sm$ . Of the 48 chromosomes, four pairs are sm in centromere position (pairs 3, 4, 6 and 23) and the other pairs are m. The first chromosome of the fifteenth pair has one satellite at the terminal region of the short arm. The ratio of the longest to the shortest chromosome is 2.4. The asymmetry of the karyotype is classified as 2B (Figs 13, 20C).

#### *Anisodus tanguticus* (Maxim.) Pascher

The karyotype of this species is  $2n = 48 = 40m(2SAT) + 8sm$ . Of the 48 chromosomes, four pairs are sm in centromere position (pairs 6, 17, 21 and 22) and the other pairs are m. Two chromosomes of the fifteenth pair have satellites on the terminal regions of the short arms. The ratio of the longest to the shortest chromosome is 2.1. The asymmetry of the karyotype is classified as 2B (Figs 14, 20D).

#### *Anisodus carniolicoides* (C. Y. Wu & C. Chen) D'Arcy & Z. Y. Zhang

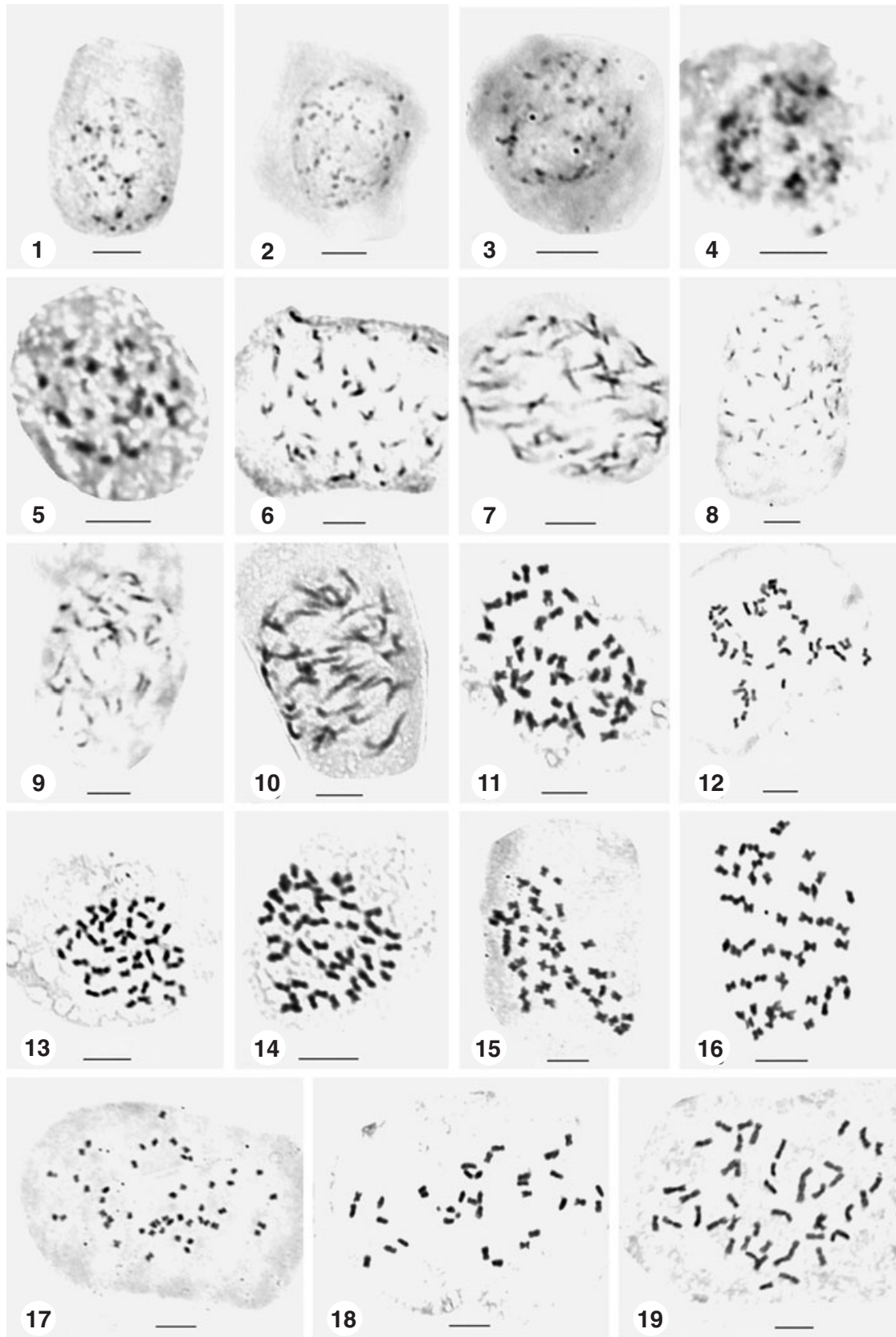
The karyotype of this species is formulated as  $2n = 48 = 40m(3SAT) + 8sm$ . Of the 48 chromosomes, four pairs are sm in centromere position (pairs 6, 11, 15 and 24) and the other pairs are m. The first chromosome of the first pair and two chromosomes of the seventh pair have satellites at the terminal regions of the short arms. The ratio of the longest to the shortest chromosome is 2.1. The asymmetry of the karyotype is classified as 2B (Figs 15, 20E).

#### *Atropanthe sinensis* (Hemsley) Pascher

The karyotype is  $2n = 48 = 46m + 2sm$ . Of the 48 chromosomes, only the last pair is sm in centromere position and the other pairs are m. No satellites have been observed in this species. The ratio of the longest to the shortest chromosome is 2.5. The asymmetry of the karyotype is classified as 2B (Figs 16, 20F).

#### *Przewalskia tangutica* Maxim.

The counts of the mitotic chromosomes investigated here agree with Huang, Shen & Lu (1996). The karyotype of this species is investigated here for the first time. The karyotype formula of this species is



**Figures 1–19.** Cytological features. Scale bars = 5  $\mu\text{m}$ . Figs 1–3. Interphase nuclei of smooth-faced round prochromosomes. Fig. 1. *Anisodus luridus* (Daocheng). Fig. 2. *Atropanthe sinensis*. Fig. 3. *Mandragora caulescens*. Fig. 4. *Hyoscyamus niger*, interphase nucleus of irregular-shaped chromocentres. Fig. 5. *Przewalskia tangutica*, interphase nucleus of rod-shaped prochromosomes. Figs 6–10. Mitotic prophases of proximal type. Fig. 6. *Anisodus luridus* (Daocheng). Fig. 7. *Atropanthe sinensis*. Fig. 8. *Mandragora caulescens*. Fig. 9. *Hyoscyamus niger*. Fig. 10. *Przewalskia tangutica*. Figs 11–19. Mitotic metaphases. Fig. 11. *Anisodus luridus* (Daocheng),  $2n = 48$ . Fig. 12. *Anisodus luridus* (Heqing),  $2n = 48$ . Fig. 13. *Anisodus acutangulus*,  $2n = 48$ . Fig. 14. *Anisodus tanguticus*,  $2n = 48$ . Fig. 15. *Anisodus carniolicoides*,  $2n = 48$ . Fig. 16. *Atropanthe sinensis*,  $2n = 48$ . Fig. 17. *Mandragora caulescens*,  $2n = 48$ . Fig. 18. *Hyoscyamus niger*,  $2n = 34$ . Fig. 19. *Przewalskia tangutica*,  $2n = 44$ .

**Table 2.** Somatic chromosome number ( $2n$ ), chromosome base number and ploidy level, karyotype formula ( $n$ ), locality and data source of Hyoscyameae taxa in this and previous reports

Taxon	$2n$ /base no./ ploidy level	Karyotype formula ( $n$ )	Locality	Data source
<i>Anisodus luridus</i>	48/6/8x	16m(2SAT) + 8sm	Hengduan Mountains (Heqing)	This study
	48/6/8x	16m(2SAT) + 8sm	Hengduan Mountains (Daocheng)	This study
<i>A. acutangulus</i>	48/6/8x	20m(1SAT) + 4sm	Hengduan Mountains	This study
<i>A. tanguticus</i>	48/6/8x	20m(2SAT) + 4sm	Hengduan Mountains	This study
<i>A. carniolicoides</i>	48/6/8x	20m(3SAT) + 4sm	Hengduan Mountains	This study
<i>Atropanthe sinensis</i>	48/6/8x	23m + 1sm	S.W. China	This study
<i>Atropa belladonna</i>	72/6/12x	?	Europe (Germany)	Badr <i>et al.</i> (1997)
	72/6/12x	?	Europe (England)	Wentworth, Bailey & Gornall (1991)
	60/6/10x	?	W. China (transplanted from Europe)	Ma, Qin & Xing (1986)
<i>A. acuminata</i>	72/6/12x	?	Europe (Germany)	Badr <i>et al.</i> (1997)
<i>Hyoscyamus muticus</i>	28/14/2x	6m + 8sm	Africa (Egypt)	Tyagi & Dubey (1989)
<i>H. tenuicaulis</i>	28/14/2x	?	C. Asia (Iran)	Sheidai, Mosallanejad & Khatamsaz (1999)
<i>H. albus</i>	68/17/4x	?	Europe (Germany)	Badr <i>et al.</i> (1997)
<i>H. senecionis</i>	34/17/2x	?	C. Asia (Iran)	Ghaffari (1988)
<i>H. niger</i>	34/17/2x	?	W. Asia (Iran)	Ghaffari (1988)
	34/17/2x	?	Europe (Germany)	Badr <i>et al.</i> (1997)
	34/17/2x	12m + 5sm(2SAT)	Hengduan Mountains	This study
<i>Mandragora caulescens</i>	48/6/8x	?	Hengduan Mountains	This study
<i>M. autumnalis</i>	84/6/14x	?	Mediterranean region	Hawkes (1972)
	96/6/16x	?	Europe (Slovakia)	Murin (1978)
	24/6/4x	?	Europe (Italy)	Lentini, Romano & Raimondo (1988)
<i>Physochlaina praealta</i>	42/7/6x	34m + 8sm	W. Himalayas (China)	Gu <i>et al.</i> (1993)
	41	?	W. Himalayas (China)	Vasudevan (1975)
<i>Przewalskia tangutica</i>	44/11/4x	18m(1SAT) + 4sm	E. Qinghai–Tibetan Plateau	This study
	44/11/4x	?	E. Qinghai–Tibetan Plateau	Huang <i>et al.</i> (1996)
<i>Scopolia carniolica</i>	48/6/8x	?	C. & S.W. Europe	Hawkes (1972)

$2n = 44 = 36m(1SAT) + 8sm$ . Pairs 1, 4, 14 and 19 are sm chromosomes and the others are m chromosomes. The first chromosome of the eleventh pair has one satellite on the short arm. The ratio of the longest to the shortest chromosome is 1.7. The asymmetry of the karyotype is classified as 2A (Figs 17, 20G).

#### *Hyoscyamus niger* L.

The chromosome number of  $2n = 34$  counted in this study confirms previous reports (Table 2). The ratio of the longest to the shortest chromosome is 1.8. The karyotype of this species is  $2n = 34 = 24m + 10sm(2SAT)$ . Pairs 2, 3, 10, 14 and 15 are sm chromo-

**Table 3.** Karyomorphological parameters of species of *Anisodus*, *Atropanthe*, *Hyoscyamus* and *Przewalskia*

Pair no.	RL	AR	CI	Type	RL	AR	CI	Type
	<i>Anisodus luridus</i> (Heqing)				<i>Anisodus luridus</i> (Daocheng)			
1	6.1	1.2	45.4	m	5.7	1.6	38.1	m
2	5.5	1.7	36.9	sm	5.2	1.1	47.9	m
3	5.4	2.2	31.4	sm	5.2	2.0	33.3	sm
4	5.1	1.3	44.4	m	5.1	1.1	46.6	m
5	5.0	1.3	43.2	m	5.0	2.1	32.3	sm
6	4.7	1.6	37.8	m	4.9	1.3	44.0	m
7	4.5	1.3	44.3	m	4.8	2.2	31.6	sm
8	4.4	1.6	38.7	m*	4.7	1.1	47.7	m
9	4.4	1.9	34.6	sm	4.5	1.3	43.1	m
10	4.2	1.3	43.5	m	4.3	1.3	43.8	m
11	4.2	1.9	34.0	sm	4.3	1.8	35.8	sm
12	4.1	1.6	39.2	m	4.2	1.6	38.5	m
13	4.0	1.2	46.4	m	4.2	2.1	31.8	sm
14	3.9	1.0	48.9	m	4.1	1.8	35.5	sm
15	3.9	1.4	41.2	m	4.1	2.0	33.3	sm
16	3.8	1.1	47.4	m	3.9	1.1	47.3	m
17	3.8	1.7	36.8	sm	3.7	2.0	32.8	sm
18	3.8	2.3	30.1	sm	3.7	1.1	48.5	m
19	3.7	1.3	44.3	m	3.4	1.4	42.4	m
20	3.5	1.8	35.5	sm	3.3	1.0	49.6	m*
21	3.4	1.1	48.3	m	3.2	1.4	41.7	m
22	3.2	2.0	33.3	sm	3.1	1.5	40.5	m
23	3.1	1.2	44.5	m	2.8	1.4	41.9	m
24	2.4	1.6	38.8	m	2.3	1.5	40.0	m
	<i>Anisodus acutangulus</i>				<i>Anisodus carniolicoides</i>			
1	5.8	1.2	46.2	m	5.2	1.5	39.6	m*
2	5.6	1.1	48.4	m	4.7	1.4	42.4	m
3	5.3	2.0	33.3	sm	4.7	1.4	41.7	m
4	5.0	1.9	34.8	sm	4.7	1.4	41.7	m
5	4.9	1.4	41.8	m	4.7	1.5	39.6	m
6	4.8	2.0	33.3	sm	4.7	1.9	34.7	sm
7	4.6	1.1	48.4	m	4.5	1.3	43.5	m*
8	4.6	1.4	41.3	m	4.4	1.1	46.7	m
9	4.4	1.4	41.7	m	4.4	1.4	42.1	m
10	4.3	1.5	40.7	m	4.3	1.5	39.4	m
11	4.1	1.2	46.4	m	4.2	1.8	35.9	sm
12	4.0	1.6	39.1	m	4.1	1.5	39.7	m
13	3.9	1.3	44.4	m	4.1	1.4	42.4	m
14	3.9	1.3	43.4	m	4.0	1.3	43.1	m
15	3.9	1.4	41.5	m*	4.0	2.0	33.9	sm
16	3.8	1.0	49.0	m	3.9	1.1	46.7	m
17	3.8	1.5	40.4	m	3.8	1.0	49.6	m
18	3.7	1.4	41.2	m	3.8	1.4	41.4	m
19	3.6	1.5	40.0	m	3.8	1.4	41.4	m
20	3.5	1.4	42.3	m	3.8	1.5	39.7	m
21	3.5	1.5	40.2	m	3.7	1.4	41.1	m
22	3.4	1.2	45.7	m	3.4	1.3	43.3	m
23	3.2	1.7	37.1	sm	3.3	1.5	40.0	m
24	2.4	1.4	41.8	m	2.5	1.9	34.7	sm
	<i>Anisodus tanguticus</i>				<i>Atropanthe sinensis</i>			
1	5.7	1.2	46.2	m	5.9	1.2	45.1	m
2	5.6	1.4	41.2	m	5.8	1.1	48.3	m

Table 3. Continued

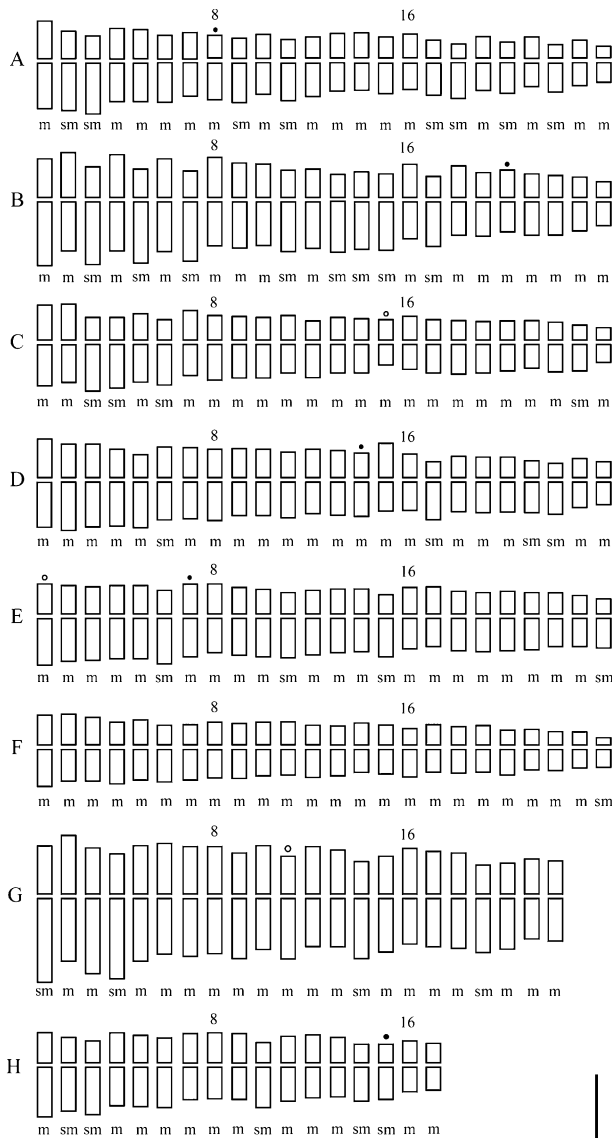
Pair no.	RL	AR	CI	Type	RL	AR	CI	Type
3	5.4	1.3	42.9	m	5.0	1.5	40.0	m
4	5.0	1.5	39.7	m	4.9	1.2	45.2	m
5	4.7	1.2	44.8	m	4.6	1.6	38.4	m
6	4.7	1.9	34.3	sm	4.5	1.1	47.4	m
7	4.6	1.2	45.3	m	4.5	1.2	44.8	m
8	4.6	1.3	42.9	m	4.5	1.3	43.3	m
9	4.3	1.1	46.6	m	4.5	1.5	40.3	m
10	4.3	1.2	46.2	m	4.4	1.1	46.6	m
11	4.2	1.4	41.9	m	4.3	1.1	46.9	m
12	4.1	1.0	49.2	m	4.3	1.4	42.5	m
13	4.1	1.2	45.2	m	4.1	1.4	42.1	m
14	4.1	1.4	41.9	m	4.0	1.0	49.2	m
15	3.8	1.1	47.0	m*	4.0	1.3	43.7	m
16	3.7	1.3	43.9	m	4.0	1.6	38.1	m
17	3.7	2.2	31.0	sm	3.8	1.2	46.4	m
18	3.5	1.3	42.6	m	3.8	1.2	46.4	m
19	3.5	1.5	40.7	m	3.8	1.2	44.6	m
20	3.5	1.4	41.1	m	3.6	1.6	38.0	m
21	3.3	1.7	36.6	sm	3.3	1.3	43.9	m
22	3.3	2.1	32.3	sm	3.2	1.5	40.4	m
23	3.1	1.3	43.2	m	2.9	1.4	41.9	m
24	2.7	1.4	42.2	m	2.4	2.2	31.0	sm
	<i>Przewalskia tangutica</i>				<i>Hyoscyamus niger</i>			
1	6.0	1.7	36.7	sm	7.7	1.6	38.7	m
2	5.5	1.1	48.5	m	6.7	1.7	37.4	sm
3	5.5	1.6	38.4	m	6.7	2.1	32.3	sm
4	5.5	2.0	33.7	sm	6.6	1.3	44.2	m
5	5.1	1.3	44.0	m	6.5	1.4	41.3	m
6	4.9	1.1	47.7	m	6.4	1.6	38.7	m
7	4.8	1.2	45.3	m	6.0	1.1	47.9	m
8	4.7	1.2	46.4	m	5.9	1.0	49.1	m
9	4.6	1.4	41.0	m	5.9	1.1	48.3	m
10	4.6	1.1	48.8	m	5.9	1.9	33.9	sm
11	4.5	1.6	38.9	m*	5.8	1.3	43.9	m
12	4.4	1.0	49.7	m	5.7	1.1	47.7	m
13	4.3	1.1	47.7	m	5.4	1.1	46.7	m
14	4.2	1.8	35.5	sm	5.1	1.8	36.0	sm
15	4.2	1.4	41.7	m	4.9	1.7	37.5	sm*
16	4.2	1.0	50.0	m	4.6	1.1	47.2	m
17	4.2	1.1	46.7	m	4.2	1.2	46.3	m
18	4.2	1.2	45.3	m				
19	3.9	1.8	35.5	sm				
20	3.8	1.6	38.5	m				
21	3.5	1.2	46.4	m				
22	3.5	1.3	44.0	m				

Abbreviations: RL, relative length; AR, arm ratio (L/S); CI, centromeric index (100S/TL); m, median region; sm, submedian region. Chromosome pairs are assigned Arabic numerals. \*Satellited chromosome.

some and the others are m chromosomes. Two satellites were observed, attached to the short arms of the fifteenth pair. The asymmetry of the karyotype is classified as 2A (Figs 18, 20H).

*Mandragora caulescens* C. B. Clarke

We obtained a chromosome number of this species of  $2n = 48$ . Numbers of  $2n = 24$ ,  $2n = 84$  and  $2n = 96$  have been reported for another species in *Mandragora*



**Figure 20.** Karyotype idiograms. Scale bar = 2  $\mu$ m. A, *Anisodus luridus* (Heqing),  $2n = 48$ . B, *A. luridus* (Daocheng),  $2n = 48$ . C, *A. acutangulus*,  $2n = 48$ . D, *A. tanguticus*,  $2n = 48$ . E, *A. carniolicoides*,  $2n = 48$ . F, *Atropanthe sinensis*,  $2n = 48$ . G, *Przewalskia tangutica*,  $2n = 44$ . H, *Hyoscyamus niger*,  $2n = 34$ . m, metacentric; sm, submetacentric. • = two satellites, ○ = one satellite.

(Table 2). We could not obtain the karyotype formula of this species because the chromosomes are too small (Fig. 19).

## DISCUSSION

### TAXONOMY OF *ANISODUS*

All four species of *Anisodus* examined in this study exhibit the same interphase nuclei and prophase chro-

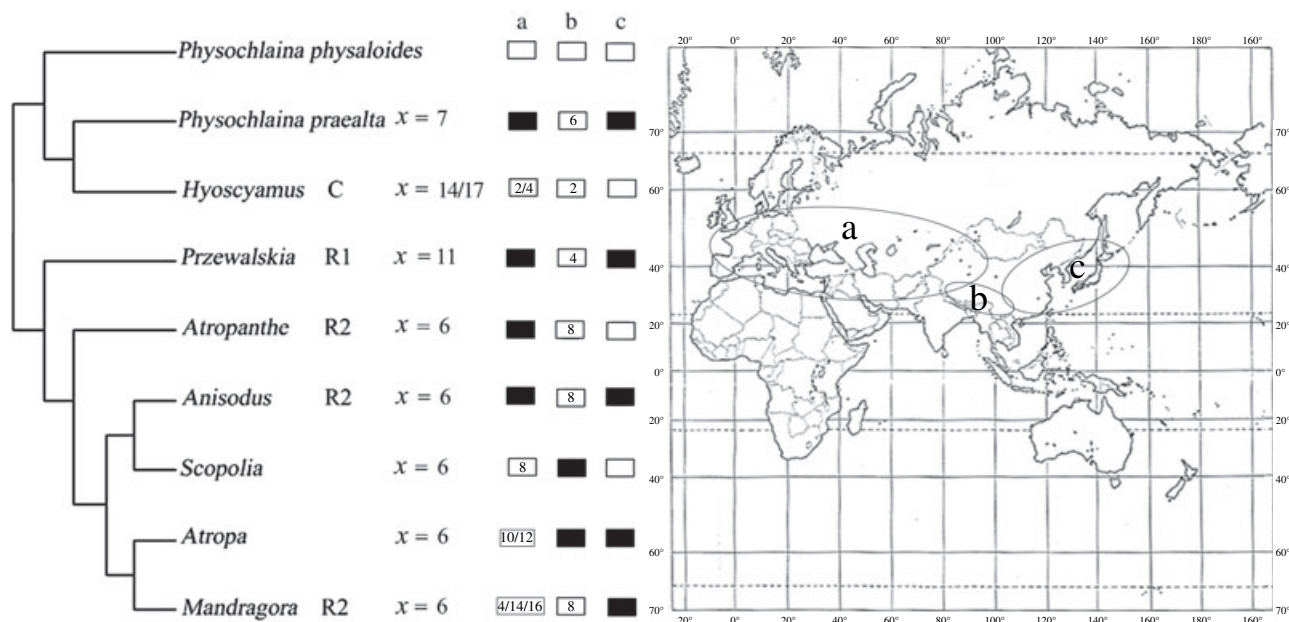
mosome condensation pattern. Their karyotypes and chromosome number of  $2n = 48$  are reported for the first time. They all have m and sm chromosomes and the same type 2B karyotype asymmetry pattern. Despite these similarities, each species has some distinguishable cytological features. For example, *A. luridus* has 32m and 16sm chromosomes, while other three species have 40m and 8sm chromosomes; the satellite number of *A. carniolicoides*, *A. tanguticus* and *A. acutangulus* is three, two and one, respectively.

*Anisodus* and *Scopolia* are closely related and are easily confused (Chen & Chen, 1977; Lu & Zhang, 1986). Chen & Chen (1977) recognized *Anisodus carniolicoides* (C. Y. Wu & C. Chen) D'Arcy & Z. Y. Zhang as *Scopolia carniolicoides* C. Y. Wu & C. Chen, based on its calyx tightly wrapping around the subglobose fruit, the circinal calyx lobes in bud and the slightly lobed corolla. Sandina & Tarasevich (1982) studied the pollen of *Anisodus* (*Whitleya*) and *Scopolia* and considered the differences in the structure of the pores and the pattern of the exine to be of generic significance. The pollen morphology of *A. carniolicoides* (C. Y. Wu & C. Chen) D'Arcy & Z. Y. Zhang is similar to that of other species in *Anisodus*, but different from that of *Scopolia* (Zhang & Lu, 1984; D'Arcy & Zhang, 1992). Based on the geographical distribution and morphology of *Anisodus* and *Scopolia*, D'Arcy & Zhang (1992) transferred this species from *Scopolia* to *Anisodus*. In this study, the cytological features of *A. carniolicoides* are very similar to those of *A. luridus*, *A. acutangulus* and *A. tanguticus* in the mitotic interphase nuclei, prophase chromosomes and karyotype. This study supports the revisions of D'Arcy & Zhang (1992) and Zhang *et al.* (1994).

### SYSTEMATIC RELATIONSHIPS WITHIN HYOSCYAMEAE

Ever since the tribe Hyoscyameae was first proposed by Endlicher (1839), its circumscription and phylogeny have been controversial (Miers, 1850; Wettstein, 1895; Lu & Zhang, 1986; D'Arcy, 1991; Zhang *et al.*, 1994; Yang, 2002). Lu & Zhang (1986) suggested a phylogeny for the whole of Hyoscyameae for the first time and this was followed by Hoare & Knapp (1997). In the system of Lu & Zhang (1986), *Atropa* and *Mandragora* were excluded from this tribe because they possess berries while other genera have capsules. They put *Hyoscyamus* and *Physochlaina* in one branch and *Anisodus*, *Atropanthe*, *Scopolia* and *Przewalskia* in another. In the latter branch, *Scopolia* and *Anisodus* were considered to form a basic group, with *Przewalskia* and *Atropanthe* developing from them. In the cladistic study of Hoare & Knapp (1997), the tribe Hyoscyameae was also divided broadly into two clades, one with *Hyoscyamus* and *Physochlaina* and the other with *Przewalskia* and the rest of the genera.





**Figure 21.** Phylogenetic relationships within Hyoscyameae and its cytological features and distribution. The simplified tree is redrawn from Hoare & Knapp (1997). Abbreviations: C, complex chromosome type; R1, rod prochromosome type; R2, round prochromosome type. The distribution states are represented by the grid of boxes in the middle, where three columns marked by a, b and c correspond to Mediterranean and west-central Asia, Sino-Himalayan region and Sino-Japanese region, respectively. Open boxes indicate the region occupied by the taxa. Black boxes indicate that the taxa are absent from the region. The numbers in the open boxes indicate the ploidy level of taxa for the distribution region.

The former clade was the same as that of Lu & Zhang (1986). *Atropa* and *Mandragora* were also included in the latter clade and *Przewalskia* occurred at the base of the clade and was sister to the rest of the genera including *Atropanthe*, *Anisodus*, *Scopolia*, *Mandragora* and *Atropa*, in which *Atropanthe* formed a clade as the sister group to the *Anisodus* + *Scopolia* and *Mandragora* + *Atropa* clade (Fig. 21).

Cytological studies of every genus in Hyoscyameae except *Archihyoscyamus*, which was upgraded from *Hyoscyamus leptocalyx* by Lu (1997), have been carried out so far (Table 2), so it is possible for us to discuss the taxonomy and systematic relationships within this tribe cytologically.

The interphase nuclei and prophase chromatin condensation pattern in mitotic cells are stable characters within taxa, so they can be used in taxonomy and phylogeny (Tanaka, 1971; Okada & Tamura, 1979). In this study, the chromosomes of all five genera in Hyoscyameae examined at prophase exhibit the same proximal type, while the interphase nuclei show different patterns, the complex chromocentre type in *Hyoscyamus*, rod prochromosome type in *Przewalskia* and round prochromosome type in *Anisodus*, *Atropanthe* and *Mandragora*. The same interphase nuclei pattern found in *Anisodus*, *Atropanthe* and *Mandragora* indicates a close relationship between them and supports

the inclusion of *Mandragora* in this tribe by Hoare & Knapp (1997). The rod prochromosome type in *Przewalskia* and round prochromosome type in *Anisodus*, *Atropanthe* and *Mandragora* are subclasses of the prochromosome type, which differs from the complex chromocentre type in *Hyoscyamus*. Based on these distinguishable cytological features, the five genera can be divided broadly into two groups: one with *Hyoscyamus* and the other with the remaining four genera (Fig. 21). This result confirms the traditional classification of Hyoscyameae (Lu & Zhang, 1986; Hoare & Knapp, 1997).

A characteristic of all four species of *Anisodus* and that of the monotypic genus *Atropanthe* is the presence only of m and sm chromosomes in their karyotypes. The karyotype formula, chromosome size and karyotype asymmetry of *Anisodus* are very similar to those of *Atropanthe*. The slight differences of karyotype between these two genera are that *Anisodus* has four or eight pairs of sm chromosomes, while *Atropanthe* has only one. These similar cytological features indicate that they might not be the basal groups, but have a recent evolutionary history. The position of *Przewalskia* has been open to contention. Wu & Wang (1983) and Lu & Zhang (1986) treated it as a very advanced genus, while Hoare & Knapp (1997) considered it to be basal. The basal position of *Przewalskia*

can also be supported by the karyotype asymmetry in this study because type 2A of *Przewalskia* is often considered a more primitive character than type 2B of *Anisodus* and *Atropanthe* (Stebbins, 1971).

The chromosome base numbers of  $x = 14$  and 17 have been accepted widely for *Hyoscyamus* (Table 2). Badr *et al.* (1997) studied the chromosomal evolution of Solanaceae and suggested that  $x = 14$  and 17 in *Hyoscyamus* might have arisen from  $x = 7$  or 8 in hypothetical ancestors. In light of its reported chromosome numbers of  $2n = 41$  (Vasudevan, 1975) and  $2n = 42$  (Gu *et al.*, 1993) and its basal position to *Hyoscyamus* (Lu & Zhang, 1986; Hoare & Knapp, 1997), we speculate that *Physochlaina praealta* is based on  $x = 7$ . The chromosome number  $2n = 44$  has been consistently recorded for *Przewalskia* in a previous study (Huang *et al.*, 1996) and in this research. We conclude a basic number of  $x = 11$  for it. *Mandragora* was suggested to have  $x = 12$  ( $2x$ ,  $4x$ ,  $7x$  and  $8x$ ) and *Atropa*  $x = 12$  ( $5x$  and  $6x$ ) by former workers (Olmstead & Palmer, 1992; Badr *et al.*, 1997; Olmstead *et al.*, 1999), but because odd-number polyploids are sexually unstable and cannot normally comprise entire natural populations, we suggest the chromosome base number of  $x = 6$  for these taxa, making the *Mandragora* records  $4x$ ,  $6x$ ,  $14x$  and  $16x$  and *Atropa*  $10x$  and  $12x$ . Based on the close relationship between *Mandragora* and *Anisodus*, *Atropanthe* and *Scopolia* (see above), we also suggest  $x = 6$  for the latter three genera. Thus it seems plausible to exclude *Atropa* and *Mandragora* from Hyoscyameae as Badr *et al.* (1997) did only on the basis of the difference in basic number between these two genera ( $x = 12$  in previous works) and *Hyoscyamus* ( $x = 14$  and 17).

#### CHROMOSOME EVOLUTION WITHIN HYOSCYAMEAE

There are three hypotheses for the origin of the Hyoscyameae. Lu & Zhang (1986) concluded that the Hengduan Mountains area is probably the birthplace of this tribe. Symon (1991) considered the distribution of Hyoscyameae to be consistent with the proto-Hyoscyaminae being rafted north on the Indian Plate. On meeting Eurasia, the group evolved in the developing Himalayas and subsequently spread from there. The viewpoint of Hoare & Knapp (1997) approximates to that of Symon (1991). They proposed that Hyoscyameae was a primarily Gondwanan element, because the main areas occupied by genera of this tribe were once part of the Gondwana supercontinent. This group radiated extensively in Eurasia along with the climate change in this area after the collision event between India and Asia.

The known cytological data of Solanaceae show that the basal groups in this family have primitive base numbers of  $x = 7$  and 8 and possess a tropical Ameri-

can distribution (Olmstead *et al.*, 1999), while the Hyoscyameae has developed various basic numbers and is distributed in Eurasia. This variation of basic numbers in Hyoscyameae indicates their possible rise or fall through polyploidy or aneuploid reduction of polyploids. These cyto-geographical features indicate that Hyoscyameae is perhaps advanced in Solanaceae, as suggested by molecular data (Olmstead *et al.*, 1999) and that the tribe might have a Gondwanan origin. As discussed above, genera studied cytologically in Hyoscyameae can be divided into two groups: *Hyoscyamus* + *Physochlaina praealta* and *Przewalskia* + the rest of the genera (Fig. 21). In the former group, *Hyoscyamus muticus* and *H. tenuicaulis* are diploid with  $x = 14$  and are distributed in north Africa across to west Asia. The higher  $x = 17$  has been found frequently in *H. niger* and other species of *Hyoscyamus*, which are distributed widely in Eurasia. The tetraploid species *H. albus*, however, is restricted in southern Europe and north Africa. In the latter group, the basal genus *Przewalskia* is narrowly restricted to the Qinghai–Tibetan Plateau and has developed particular morphological features to cope with the extreme environment conditions there. Its systematic position and distribution indicate it could be a descendant of a Tertiary Tethyan relic. Two species of *Scopolia* are distributed disjunctively in southern Europe and in Japan and Korea, indicating its once wide distribution in Eurasia. This disjunct distribution pattern probably formed because of Tertiary Tethyan aridity in central Asia during the uplift of the Himalayan–Hengduan Mountains. The rather short evolutionary history of *Anisodus* and *Atropanthe* indicates that they could have differentiated during the uplift of the Himalayan–Hengduan Mountains. The other two Tethyan relic genera *Atropa* and *Mandragora* (Wu & Wang, 1983) are distributed widely from western Europe to the Himalayas, of which the octoploid of *M. caulescens* is found in the Hengduan Mountains, while species in western Europe to the Mediterranean region exhibit both a lower level ( $4x$ ) and higher levels ( $12x$ ,  $14x$ ,  $16x$ ) of ploidy (Table 2, Fig. 21). All these characters indicate that the early differentiation of Hyoscyameae might have occurred along the Tethys margin and, following the uplift of Himalayan–Hengduan Mountains and the aridity of central Asia, some young groups developed in south-west China.

Polyploidy is very common in Hyoscyameae and is found in most species of this tribe in the Himalayan–Hengduan Mountains. It appears to be strongly correlated with the uplift of the Mountains and has been considered to play an important role in plant speciation, especially in the harsh conditions of alpine regions (Hanelt, 1966). From cytological data, we have found that both the Sino-Himalayan endemic *Anisodus* and the China endemic *Atropanthe* show octo-

ploidy and no other ploidy level was counted in this study. The European and eastern Asian disjunct genus *Scopolia*, which is closely related to *Anisodus* and *Atropanthe* (Chen & Chen, 1977; Lu & Zhang, 1986), has the same chromosome number. The octoploid level occurs in different genera and various habitats throughout Eurasia, indicating that their polyploidization probably occurred at an early point in their evolution. The palaeopolyploids in *Hyoscyamus* also show an early occurrence of polyploidization, which was followed by diploidization to produce neodiploids with  $x = 14$  and  $x = 17$ . Tetraploids of *Hyoscyamus albus* are distributed in north Africa, southern Europe and west Asia. The higher polyploids ( $14x$ ,  $16x$ ) of this tribe occur in *Mandragora autumnalis* in the Mediterranean region, while octoploid species in this genus occur in the Hengduan Mountains (Table 2, Fig. 21). It cannot be seen that there is any correlation between the uplift of the Himalayan–Hengduan Mountains and the polyploidy in Hyoscyameae. These cytogeographical characters in Hyoscyameae indicate that the polyploidization of this tribe might have taken place mostly along the Tethys margin, before the uplift of the Himalayan–Hengduan Mountains and the strong environment changes that resulted from the uplift of the mountains might not have been an important factor in the polyploidization in this tribe. Some recent cytological studies on other Sino-Himalayan genera came to the same conclusion. For example, most species of *Ligularia*, with its diversity centre in the Sino-Himalayan region, are diploids with high chromosome base number ( $x = 29$  and  $x = 30$ ), except for a few triploid species (Liu, 2004); all eight species of *Solms-laubachia*, an endemic genus to high mountains of the Sino-Himalayan region, are diploids with  $x = 7$  except for one population of *S. retropilosa* that is tetraploid (Yue *et al.*, 2003, 2004); for *Tibetia*, all three species studied share the same number:  $2n = 16$  (Nie, Gu & Sun, 2002). Both this and previous studies indicate that, although the various habitats that resulted from the uplift of the Himalayan–Hengduan Mountains helped to develop many endemic genera and species and make it a centre of plant diversity, plant evolution in this region may not have taken place through polyploidy but through intrachromosomal variation in structure at the same ploidy level. However, to test this hypothesis, further cytological studies are needed.

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