Low incidence of polyploids and high uniformity of karyotypes displayed by Delphinium (Ranunculaceae) in the Hengduan Mountains region of south-west China

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The chromosome numbers and morphology in 92 populations belonging to 49 species and three varieties in the genus Delphinium L. (Ranunculaceae), mostly from the Hengduan Mountains region of south-west China, were studied. Forty seven species and three varieties were diploid, with $2n = 16$, one species was tetraploid, with $2n = 32$, and one species had diploid and tetraploid cytotypes. Three species had B chromosomes, representing the first time the occurrence of B chromosomes has been reported in the genus. The karyotypes of all the diploid species were quite uniform, commonly bimodal, and usually consisted of one pair of large median-centromeric (m), one pair of large submedian-centromeric (sm), five pairs of medium-sized subterminal-centromeric (st), and one pair of smaller sm (rarely st) chromosomes. The low incidence of polyploids in Delphinium from the Hengduan Mountains region indicates that polyploidy has played a minor role in the speciation of this highly diversified genus in the region.


ADDITIONAL KEYWORDS: chromosome numbers – chromosomes – cytotaxonomy – speciation.

INTRODUCTION

Delphinium L. (Ranunculaceae), a large genus of c. 320 species in the world, is widely distributed in the temperate regions of the Northern Hemisphere (Tamura, 1995; Starmüller, 1999; Malyutin, 2001). This genus belongs to the tribe Delphineae, which includes three closely related genera, Aconitum, Delphinium, and Consolida, all characterized by the zygomorphic flower (Tamura, 1995). The genus Delphinium is usually divided into three subgenera, with subgenus Delphinastrum being the largest and consisting of c. 300 perennial species, subgenus Stathisagria including only three biennial species, and subgenus Delphinium including c. 18 species, which are mostly annual, rarely biennial or perennial (Tamura, 1995). China, in which 173 species have been described (Wang & Warnock, 2001), is undoubtedly one of the most important distribution centres of the genus. In particular, most of the species in subgenus Delphinastrum are concentrated in the Hengduan Mountains region of south-west China (Blanché, 1990; Starmüller, 1999), spanning most sections and series and covering most of the morphological range in this subgenus, with both primitive and advanced representatives, including many local endemics (Wang, 1979; Tamura, 1995). All of the species considered to be most primitive morphologically by Wang (1979), such as D. forrestii Diels and D. trichophorum Franch. in section Aconitoides, are only known from this region. It should be noted that the species number of Delphinium in China may be decreasing, as shown by a recent taxonomic revision of the genus from all other regions of China except for the Xinjiang Uygur Autonomous Region (Yuan, 2006). In this revision, 42 species were treated as synonyms, and nearly 80 species, mostly locally endemic, were recorded to occur in the Hengduan Mountains region, indicating that this region is indeed a centre of species diversity and endemism of the genus.

The Hengduan Mountains region, which is located at the end of the eastern Himalayan range in...
south-west China, and is recognized as a biodiversity ‘hot spot’ (Boufford & van Dyck, 1999; Myers et al., 2000), may harbour the richest temperate flora of seed plants in the world (Wu, 1988; Li & Li, 1993). About 8000 species of seed plant have been recorded from the 500 000 km² of terrain (Li & Li, 1993; Wang, 1993, 1994). Floristically, it is also a highly natural region and is one of the areas of the world with a large concentration of endemic species (Wu, 1988; Li & Li, 1993). As stated clearly by Nie et al. (2005), a high rate of polyploidy could be expected in this region because of the unusually high species diversity and endemism, the widespread alpine environment, and the importance of polyploidy in plant evolution. However, Nie et al. (2005) have indicated that polyploidy may have played only a minor role in the evolutionary diversification in the region, based on their statistical analysis of the chromosome numbers of 552 taxa. Although this point of view is somewhat supported by chromosome studies of some highly diversified genera in the region, such as Cremanthodium (Liu et al., 2001) and Ligularia (Liu, 2004), the results obtained from investigations on other groups tell a different story. For instance, Yuan & Yang (2006b) and Chen, Sun & Sun (2007) have found that polyploidy has played an important role in the speciation of Aconitum subgenus Lycocotonum and Buddleja, respectively, from the Hengduan Mountains region. This suggests that it is not easy to evaluate the role played by polyploidy in the speciation of this region with a very high species diversity but a very small proportion of species having available chromosomal data. More plant groups with possibly different evolutionary histories should be studied cytologically to gain a better understanding of the speciation patterns in this region.

A statistical study performed by Simon et al. (1999) showed that the chromosome numbers of c. 140 Delphinium species are known. Nearly 69% of the chromosome counts belong to the diploid level (2n = 16), and c. 31% to the polyploid level (2n = 32, rarely 48). In two species, D. bolosii C.Blanché & J.Molero and D. staphisagria L., the number 2n = 18 was counted (Blanché & Molero, 1983), indicating another basic number, x = 9, in the genus. Sarkar et al. (1982) reported a number of 2n = 20 in D. denudatum Hook. f. & Thomson, although some previous reports have shown that this species has n = 8 (Mehra & Ramanandan, 1972), 2n = 16 (Koul & Gohil, 1973), or 2n = 32 (Al-Kelidar & Richards, 1981). Blanché (1991) considered this number to represent an aneuploid, but Tamura (1995) considered it to represent another basic number in the genus, x = 10.

The Chinese Delphinium species are cytologically not well known, with only c. 24 species having chromosomal data (Table 1). In this paper, 92 populations in 49 species and three varieties of Delphinium from China, mostly from the Hengduan Mountains region, were investigated in order to gain a better understanding of the chromosomal evolution of this genus and of the plant speciation patterns in the Hengduan Mountains region.

**MATERIAL AND METHODS**

All the plants studied were collected in the field from Beijing, Henan, Hunan, Jiangsu, Qinghai, Shaanxi, Sichuan, Yunnan, and Xizang (Tibet), China (Table 1). They were cultivated in pots before their root tips were harvested for cytological study. Actively growing root tips were pretreated in 0.1% colchicine at about 20 °C for 3 h before being fixed in Carnoy I (glacial acetic acid: absolute ethanol, 1:3) at 4 °C for 30 min; they were then macerated in a 1:1 mixture of 1 m HCl and 45% acetic acid at 60 °C for 2 min, and stained and squashed in 1% aceto-orcein. As the plants of *D. erlangshanicum* and *D. sinoscaposum* did not produce roots in the glasshouse, their young leaves were harvested for cytological study using the same methods as applied to the roots.

Karyotypic formulae were based on measurements of mitotic-metaphase chromosomes, in most cases, of at least three cells taken from photographs, only occasionally of one cell (Table 1). The symbols used to describe the karyotypes followed Levan, Fredga & Sandberg (1964): m, median-centromeric chromosome with an arm ratio of 1.0–1.7; sm, submedian-centromeric chromosome with an arm ratio of 1.7–3.0; st, subterminal-centromeric chromosome with an arm ratio of 3.0–7.0. The karyotype asymmetry indices defined by Romero Zarco (1986) were adopted: A₁, intrachromosomal asymmetry index; A₂, interchromosomal asymmetry index.

Voucher specimens were deposited at the herbarium of the Institute of Botany (PE), Chinese Academy of Sciences.

For species treatment, we followed Yuan (2006). For the infrageneric classification system, we followed Wang (1979).

**RESULTS**

Chromosome counts were obtained for 49 species and three varieties (Table 1). All of these are illustrated in Figs 1–54. The chromosome numbers of 31 species and two varieties are reported for the first time. Forty-seven species and three varieties were found to be diploid, with 2n = 16 (Figs 1–3, 5–21, 23–54), one species (*D. chrysotrichum* Finet & Gagnep.) was found to be tetraploid, with 2n = 48 (Fig. 4), and one species (*D. spirocentrum* Hand.-Mazz.) was found to have both diploid (Fig. 21) and tetraploid (Fig. 22).
Table 1. Source of material studied, chromosome numbers, and karyotype formulae

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Provenance</th>
<th>Reference</th>
<th>Voucher</th>
<th>Karyotype formula</th>
<th>$A_1$</th>
<th>$A_2$</th>
<th>TCL/n</th>
<th>NMC</th>
<th>Figures</th>
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<td>D. forrestii var. forrestii</td>
<td>YN: Zhongdian</td>
<td>This study</td>
<td>Yang &amp; Yuan 112</td>
<td>$2n = 2m + 4sm + 10st$</td>
<td>0.64</td>
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<td>1, 55</td>
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<td>Yang (2001)</td>
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<td>$2n = 2m + 4sm + 10st$</td>
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<td>YN: Zhongdian</td>
<td>Yang, Kong (2001)</td>
<td>Yang &amp; Kong 98-329</td>
<td>$2n = 2m(1sat) + 4sm + 10st$</td>
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<td>0.42</td>
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<td>SC: Yajiang</td>
<td>This study</td>
<td>Yang &amp; Yuan 272</td>
<td>$2n = 2m + 4sm + 10st$</td>
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<td>D. kansense</td>
<td>QH: Pingan</td>
<td>Yang (2001)</td>
<td>Yang 95-23</td>
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<td>0.43</td>
<td>20.48</td>
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<td>D. chrysotrichum</td>
<td>YN: Dêqên</td>
<td>This study</td>
<td>Yang &amp; Yuan 089</td>
<td>$2n = 12m + 20sm$</td>
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<td>D. tangkulateae</td>
<td>QH: Madoi</td>
<td>Liu &amp; Ho (1999)</td>
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<td>YN: Zhongdian</td>
<td>This study</td>
<td>Yang &amp; Yuan 026</td>
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<td>0.46</td>
<td>37.00</td>
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<td>4, 57</td>
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<td>YN: Dêqên</td>
<td>This study</td>
<td>Yang &amp; Yuan 087</td>
<td>$2n = 2m(1sat) + 4sm + 10st(1sat)$</td>
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<td>0.46</td>
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<td>D. pylzowii var. trigynum (as D. pylzowii)</td>
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<td>Yang (2001)</td>
<td>Yang 95-31</td>
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<td>D. candelabrum (as D. candelabrum var. monanthum)</td>
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<td>Liu &amp; Ho (1999)</td>
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<td>D. wardii</td>
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<td>This study</td>
<td>Yang &amp; Yuan 516</td>
<td>$2n = 2m + 4sm + 10st$</td>
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<td>Liu &amp; Ho (1999)</td>
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<td>D. trisectum*</td>
<td>HN: Luoshan</td>
<td>This study</td>
<td>Yang &amp; Yuan 238</td>
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<td>D. erlangshanicum</td>
<td>SC: Tianquan</td>
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<td>Yang &amp; Yuan 536</td>
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<td>D. humilius</td>
<td>SC: Yajiang</td>
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<td>Yang &amp; Yuan 282</td>
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<td>26.23</td>
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<td>D. pachycentrum</td>
<td>SC: Dêgê</td>
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<td>Yang &amp; Yuan 451</td>
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<td>0.39</td>
<td>46.90</td>
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This study

\[ 2n = 2m + 4sm + 10st(1sat) \]

0.56 0.48 38.83 5 15

XZ: Gongbo’gyamda

Subsection Pogonantha

D. orthocentrum

SC: Songpan

This study

Yang & Yuan 314

\[ 2n = 2m + 4sm + 10st(2sat) \]

0.62 0.38 37.73 5 16, 61

D. micropetalum

YN: Weixi

This study

Yang & Yuan 060

\[ 2n = 2m + 4sm + 10st \]

0.59 0.40 35.88 5 17

D. delavayi

YX: Liangyang (2001)

This study

Yang & Yuan 390

\[ 2n = 2m + 4sm + 10st(1sat) \]

0.65 0.44 44.65 3 18, 62

D. pogonanthum

YN: Zhongdian

This study

Yang & Yuan 158

\[ 2n = 2m + 4sm + 10st(2sat) \]

0.64 0.43 46.65 3 19

D. delavayi

YN: Zhongdian

Yang 94-93

This study

Yang & Yuan 104

\[ 2n = 2m + 4sm + 10st(1sat) \]

0.66 0.43 43.58 3 21, 63

D. umbrosum

YN: Weixi

Yang (2001)

This study

Yang & Kong 98-184

\[ 2n = 2m + 4sm + 10st \]

0.59 0.46 45.38 3 172–188

D. omeiense

SC: Xide

This study

Yang & Yuan 328

\[ 2n = 2m + 4sm + 10st(1sat) \]

0.62 0.43 42.70 3 20

SC: Yanyuan

This study

Yang & Yuan 355

\[ 2n = 2m + 4sm + 10st(1sat) \]

0.62 0.44 44.35 3 20

SC: Muli

This study

Yang & Yuan 333

\[ 2n = 2m + 4sm + 10st(2sat) \]

0.59 0.51 39.75 3 20, 63

D. spicenthrum

YN: Zhongdian

This study

Yang & Yuan 104

\[ 2n = 2m + 4sm + 10st(2sat) \]

0.63 0.44 49.38 3 20

SC: Yanyuan

This study

Yang & Yuan 055

\[ 2n = 2m + 4sm + 10st(2sat) \]

0.66 0.49 42.95 3 22, 64

SC: Tianquan

This study

Yang & Yuan 234

\[ 2n = 2m + 4sm + 10st(1sat) \]

0.59 0.49 40.06 3 22, 64

D. tongolense

SC: Yajiang

This study

Yang & Yuan 115

\[ 2n = 2m + 4sm + 10st(1sat) \]

0.62 0.46 43.58 3 20

SC: Baoxing

This study

Yang & Yuan 055

\[ 2n = 2m + 4sm + 10st(2sat) \]

0.66 0.49 42.95 3 22, 64

SC: Maoxian

This study

Yang & Yuan 055

\[ 2n = 4m + 8sm + 20st(1sat) \]

0.64 0.49 40.06 3 22, 64

SC: Dujiangyan

This study

Yang & Yuan 205

\[ 2n = 2m + 4sm + 10st(1sat) \]

0.62 0.43 43.00 3 25, 65

D. pseudotongolense

SC: Wenchuan

This study

Yang & Yuan 212

\[ 2n = 2m + 4sm + 10st \]

0.59 0.50 48.40 3 20

D. bulleyanum

YN: Zhongdian

This study

Yang & Yuan 019

\[ 2n = 2m + 4sm + 10st(2sat) \]

0.63 0.47 39.98 3 26

YN: Zhongdian

Yang (2001)

This study

Yang & Yuan 94-94

\[ 2n = 2m + 4sm + 10st(1sat) \]
Table 1. Continued

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Provenance</th>
<th>Reference</th>
<th>Voucher</th>
<th>Karyotype formula</th>
<th>An</th>
<th>Ar</th>
<th>TCL/n</th>
<th>NMC</th>
<th>Figures</th>
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<tr>
<td><em>D. muliense</em></td>
<td>YN: Zhongdian</td>
<td>This study</td>
<td>Yang &amp; Yuan 029</td>
<td>2n = 2m + 4s + 10st</td>
<td>0.61</td>
<td>0.46</td>
<td>43.45</td>
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<td>Yang &amp; Yuan 226</td>
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<td>0.53</td>
<td>40.73</td>
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<td><em>D. campylocentrum</em></td>
<td>SC: Hongyuan</td>
<td>This study</td>
<td>Yang &amp; Yuan 302</td>
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<td>D. giraldis*</td>
<td>SX: Meixian</td>
<td>This study</td>
<td>Yang &amp; Yuan 378</td>
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Section *Delphinastrum*

Subsection *Ceratophora*

| *D. taliense*                | YN: Lijiang | This study        | Yang & Yuan 150 | 2n = 2m + 4s + 10s(2sat)          | 0.65 | 0.41 | 49.88  | 5   |         |
| (as *D. bowvalotii*)         | SC: Huili   | This study        | Yang & Yuan 306 | 2n = 2m + 2s + 12s(3sat)          | 0.62 | 0.37 | 53.23  | 3   | 31, 66  |
| *D. yunnanense*              | YN: Kunming | This study        | Yang & Yuan 187 | 2n = 2m + 2s + 12s(1sat)          | 0.67 | 0.38 | 53.10  | 5   | 32      |
| (as *D. yunnanense*)         | YN: Kunming | Yang (2001)      | Yang 93-22     | 2n = 2m + 2s + 12s + 10s(1sat)    |     |      |        |     |         |
| *D. pseudocampylocentrum*    | YN: Dali    | This study        | Yang & Yuan 180 | 2n = 2m + 4s + 10s(1sat)          | 0.68 | 0.39 | 56.78  | 5   | 33      |
| *D. ceratophorum*            | YN: Lijiang | This study        | Yang & Yuan 141 | 2n = 2m + 2s + 12s(1sat)          | 0.67 | 0.45 | 40.28  | 5   |         |
| YN: Yanyuan                  | SC: Yanyuan | This study        | Yang & Yuan 359 | 2n = 2m + 2s + 12t                | 0.63 | 0.36 | 40.30  | 5   | 34      |
| YN: Weixi                    | SC: Yanyuan | Yang (2001)      | Yang 94-72     | 2n = 2m + 4s + 10st               | 0.62 | 0.38 | 41.13  | 5   | 35      |
| *D. coleopodium*             | YN: Lijiang | This study        | Yang & Yuan 157 | 2n = 2m + 4s + 10s(1sat)          | 0.62 | 0.38 | 41.13  | 5   |         |
| YN: Lijiang                  | SC: Yanyuan | Yang (2001)      | Yang 94-116    | 2n = 2m + 4s + 10t                | 0.57 | 0.46 | 33.95  | 3   | 36      |
| *D. pseudoamatum*            | YN: Weixi   | This study        | Yang & Yuan 356 | 2n = 2m + 4s + 10s(1sat)          | 0.62 | 0.35 | 47.25  | 3   | 37      |
| *D. dolichocentroides*       | SC: Ninglang| This study        | Yang & Yuan 363 | 2n = 2m + 2s + 12t                | 0.62 | 0.35 | 47.25  | 3   | 37      |
| *D. autumnale*               | SC: Ninglang| This study        | Yang & Yuan 339 | 2n = 2m + 2s + 12t                | 0.56 | 0.43 | 37.53  | 3   | 38      |
| *D. yunnanense*              | YN: Zhongdian| This study        | Yang & Yuan 001 | 2n = 2m + 4s + 10t                | 0.64 | 0.44 | 35.45  | 3   |         |
| YN: Zhongdian                | SC: Ninglang| This study        | Yang & Yuan 006 | 2n = 2m + 4s + 10t(1sat)          | 0.61 | 0.45 | 36.08  | 5   | 39      |
| (as *D. tenii*)              | YN: Zhongdian| Yang (2001)      | Yang & Kong 98-159 | 2n = 2m + 4s + 10t(2sat)         | 0.62 | 0.42 | 39.15  | 5   |         |
| YN: Weixi                    | YN: Zhongdian| Yang & Kong 98-159 | 2n = 2m + 4s + 10t(1sat)          | 0.54 | 0.39 | 33.20  | 5   | 40      |
| *D. thibeticum var. thibeticum* | YN: Lijiang | This study        | Yang & Yuan 458 | 2n = 2m + 4s + 10t                | 0.58 | 0.41 | 37.15  | 5   | 41      |
| XZ: Jomda                    | This study  | Yang & Yuan 458   | Yang & Kong 98-325 | 2n = 2m + 4s + 10st               | 0.57 | 0.40 | 32.30  | 4   | 42      |
| *D. souliei*                 | SC: Kangding| This study        | Yang & Yuan 534 | 2n = 2m + 4s + 10t                | 0.61 | 0.39 | 40.70  | 4   |         |
| SC: Kangding                 | SC: Kangding| This study        | Yang & Yuan 532 | 2n = 2m + 4s + 10t                | 0.61 | 0.39 | 40.70  | 4   |         |
| *D. hais* (as *D. smithanum*)| YN: Zhongdian| Yang (2001)      | Yang & Kong 98-325 | 2n = 2m + 4s + 10s(1sat)         |     |      |        |     |         |
| *D. sinocaposum*             | SC: Baxiong | This study        | Yang & Yuan 540 | 1                                |     |      |        |     | 43      |
| YN: Zhongdian                | SC: Baxiong | This study        | Yang & Kong 98-526 | 2n = 2m + 4s + 10t               | 0.54 | 0.39 | 33.20  | 5   | 40      |
Subsection Grandiflora

<table>
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GS, Gansu; HN, Henan; HUN, Hunan; JS, Jiangsu; QH, Qinghai; SC, Sichuan; SX, Shaanxi; XZ, Xizang; YN, Yunnan; A, intrachromosomal asymmetry index; B, interchromosomal asymmetry index; NMC, number of metaphase cells observed; TCL, total length of chromosomes; the species indicated by an asterisk do not occur in the Hengduan Mountains region.
Figures 1-11. Photomicrographs of metaphase chromosomes in Delphinium. Fig. 1. *D. forrestii*, 2n = 16. Fig. 2. Yajiang population of *D. trichophorum* var. *trichophorum* (Yang & Yuan 272), 2n = 16. Fig. 3. *D. trichophorum* var. *subglaberrium*, 2n = 16. Fig. 4. *D. chrysotrichum*, 2n = 32. Fig. 5. Zhongdian population of *D. batangense* (Yang & Yuan 26), 2n = 16. Fig. 6. *D. pylzowii* var. *trigynum*, 2n = 16. Fig. 7. *D. candelabrum*, 2n = 16. Fig. 8. *D. wardii*, 2n = 16. Fig. 9. *D. smithanum*, 2n = 16. Fig. 10. *D. albocoeruleum*, 2n = 16. Fig. 11. *D. trisectum*, 2n = 16.
Figures 12–21. Photomicrographs of metaphase chromosomes in *Delphinium*. Fig. 12. *D. erlangshanicum*, $2n = 16$. Fig. 13. *D. humilis*, $2n = 16$. Fig. 14. *D. pachycentrum*, $2n = 16$. Fig. 15. Gongbo'gyamda population of *D. gyalanum* (Yang & Yuan 509), $2n = 16$. Fig. 16. *D. orthocentrum*, $2n = 16$. Fig. 17. *D. micropetalum*, $2n = 16$. Fig. 18. Ninglang population of *D. delavayi* (Yang & Yuan 360), $2n = 16$. Fig. 19. Lijiang population of *D. pogonanthum* (Yang & Yuan 159), $2n = 16$. Fig. 20. Yanyuan population of *D. omeiense* (Yang & Yuan 352), $2n = 16$. Fig. 21. Weixi population of *D. spirocentrum* (Yang & Yuan 055), $2n = 16$. © 2008 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2008, 158, 172–188
Figures 22–31. Photomicrographs of metaphase chromosomes in Delphinium. Fig. 22. Weixi population of *D. spirocentrum* (Yang & Yuan 055), 2n = 32. Fig. 23. *D. tongolense*, 2n = 16. Fig. 24. Tianquan population of *D. potaninii* (Yang & Yuan 234), 2n = 16. Fig. 25. Dujiangyan population of *D. pseudotongolense* (Yang & Yuan 205), 2n = 16. Fig. 26. *D. bulleyanum*, 2n = 16. Fig. 27. *D. muliense*, 2n = 16. Fig. 28. *D. pseudocampylocentrum*, 2n = 16. Fig. 29. *D. campylocentrum*, 2n = 16. Fig. 30. *D. giraldii*, 2n = 16. Fig. 31. Huili population of *D. taliense* (Yang & Yuan 366), 2n = 16.

Figures 32–43. Photomicrographs of metaphase chromosomes in Delphinium. Fig. 32. D. yunnanense, $2n = 16$. Fig. 33. D. pyenocentrum, $2n = 16$. Fig. 34. Yanyuan population of D. ceratophorum (Yang & Yuan 359), $2n = 16$. Fig. 35. D. coleopodium, $2n = 16$. Fig. 36. D. pseudohamatum, $2n = 16$. Fig. 37. D. dolichocentroides, $2n = 16$. Fig. 38. D. autumnale, $2n = 16$. Fig. 39. Zhongdian population of D. yuanyun (Yang & Yuan 001), $2n = 16$. Fig. 40. D. thibeticum var. thibeticum, $2n = 16$. Fig. 41. D. thibeticum var. laceratilobum, $2n = 16$. Fig. 42. Kangding population of D. souliei (Yang & Yuan 534), $2n = 16$. Fig. 43. D. sinoscaposum, $2n = 16$. 

cytotypes. In one population of each of three species, *D. tatsienense* Franch., *D. mosoyense* Franch., and *D. beesianum* W. W. Smith, one or two small B chromosomes were observed (Figs 45, 49–50).

The karyotypes of some of the species are given in Figs 55–68 to illustrate the chromosome morphology and size. The karyotype formulae of all the Chinese species with available karyotypic data are shown in Table 1. If we disregard the differences in the presence or absence and number of satellites in the chromosome complement, the karyotypes of the diploid species were quite uniform, most often consisting of one pair of large *m*, one pair of large *sm*, five pairs of medium-sized *st*, and one pair of smaller *sm* (rarely *st*) chromosomes. The karyotypes were obviously bimodal, with the first two chromosome pairs distinctly larger than the remaining ones.

The chromosome morphology of the only tetraploid species studied here, *D. chrysotrichum* (Fig. 57), was markedly different from that of the diploid species (Figs 55, 56, 58–63, 65–68) and the tetraploid cytotype (Fig. 64) in *D. spirocentrum*. The chromosomes of *D. chrysotrichum* were smaller and all belonged to *m* or *sm* types. The chromosomes of the tetraploid cytotype in *D. spirocentrum* showed no essential differences from those of the diploid cytotype (Fig. 63) in morphology and size.

**DISCUSSION**

**BASIC NUMBERS IN THE GENUS DELPHINIUM**

All of the *Delphinium* species studied here are diploid (2*n* = 16) or tetraploid (2*n* = 32), based on x = 8, confirming that the most common basic chromosome number in this genus is x = 8. The numbers x = 9 and x = 10, reported by Blanché & Molero (1983) and Sarkar et al. (1982), respectively, are very rare in the genus, as has been noted by Tamura (1995). The number x = 9 has been reported in *D. bolosii* (Blanché & Molero, 1983) and *D. staphisagria* (Bosh, 1999), and the number 2*n* = 20 has been reported in only one population of *D. denuudatum*. In view of the fact that, in *Consolida*, a genus closely related to *Delphinium*, the number x = 9 was also reported for one taxon, *C. scleroclada* (Boiss.) Schröd. var. *rigida* (Freyn & Sint.) Davis (Hong, 1986), this basic number, albeit very rare, may occur in the tribe Delphineae of the Ranunculaceae. From the marked discrepancy of the chromosome numbers (2*n* = 16, 20, 32) reported previously for *D. denuudatum* as mentioned above, the number 2*n* = 20 is quite doubtful and needs to be verified.

**B CHROMOSOMES IN THE GENUS DELPHINIUM**

One or two very small B chromosomes were observed in three species: *D. beesianum*, *D. mosoyense*, and *D. tatsienense*. Although B chromosomes have been reported for at least eight species in *Aconitum* (Simon et al., 1999; Yang, 2001), our finding represents the first report of the presence of B chromosomes in the genus *Delphinium*. The occurrence of B chromosomes does not seem to be constant within species in the genus, with only one population found to have such chromosomes for each of the three species.

**POLYPLOIDY IN THE GENUS DELPHINIUM FROM THE HENGDUAN MOUNTAINS REGION**

As shown in Table 1, the chromosome numbers of 54 Chinese *Delphinium* species are known, 50 of which occur in the Hengduan Mountains region. Of these 50 species, only one, *D. chrysotrichum*, is tetraploid, and one species, *D. spirocentrum*, has diploid and tetraploid cytotypes. Compared with the frequency of c. 31% polyploids reported previously in *Delphinium*, and considering that the species studied span all sections and series and cover most of the morphological range in *Delphinium* from the Hengduan Mountains region, this surprisingly low incidence of polyploids strongly suggests that polyploidy may have played a minor role in the speciation of the genus from this region. This situation is similar to that of *Delphinium* species in California, USA, another area of high endemism and diversification of species (Raven & Axelrod, 1978), where the species are mostly diploid, although a few species have diploid and tetraploid cytotypes (Lewis et al., 1951). Our results seem to provide an example to corroborate the viewpoint proposed by Nie et al. (2005) that polyploidy may have played only a minor role in the evolutionary diversification in the Hengduan Mountains region.

It is noteworthy that, in the genus *Aconitum*, a group closely related to *Delphinium* as mentioned above, the situation is somewhat different. Of the 54 *Aconitum* species which have been studied cytologically from the region (Yang et al., 1993; Yang, 1996, 2001; Luo, 2003; Yuan & Yang, 2006b; Yuan, 2006), 11 species and one variety are tetraploid or have tetraploid cytotypes (Yang et al., 1993; Yuan, 2006; Yuan & Yang, 2006b). These include: *A. chrysotrichum* W.T.Wang, 2*n* = 32; *A. brevicalcaratum* Finet & Gagnep. Diels, 2*n* = 32; *A. brevicalcaratum* var. *parviflorum* Chen & Liu, 2*n* = 32; *A. crassiflorum* Hand.-Mazz., 2*n* = 32; *A. rilonense* Kadota, 2*n* = 32; *A. yunlingense* Q.E.Yang & Z.D.Fang, 2*n* = 32; *A. rockii* Fletcher & Lauener, 2*n* = 16, 32; *A. piepunense* Hand.-Mazz., 2*n* = 16, 32; *A. fengii* W.T.Wang, 2*n* = 16, 32; *A. legendrei* Hand.-Mazz., 2*n* = 16, 32; *A. pseudokongboense* W.T.Wang, 2*n* = 32; *A. huiliense* Hand.-Mazz., 2*n* = 32. In particular, the subgenus *Lycopodium* has long been considered to be characterized by a uniformly diploid chromosome number of 2*n* = 16, but five of the six taxa occurring in
Figures 44–54. Photomicrographs of metaphase chromosomes in *Delphinium*. Fig. 44. Qamdo population of *D. nangchienense* (Yang & Yuan 470), 2n = 16. Fig. 45. Yanyuan population of *D. mosoyinense* (Yang & Yuan 358), 2n = 16 + Bs (arrow indicates B chromosome). Fig. 46. Xiaojin population of *D. kamaonense* (Yang & Yuan 292), 2n = 16. Fig. 47. Kangding population of *D. caeruleum* (Yang & Yuan 259), 2n = 16. Figs 48, 49. *D. beesianum*. Fig. 48. Lijiang population (Yang & Yuan 172), 2n = 16. Fig. 49. Déqên population (Yang & Yuan 076), 2n = 16 + 2Bs (arrow indicates B chromosome). Figs 50, 51. *D. tatsienense*. Fig. 50. Xide population (Yang & Yuan 330), 2n = 16 + 1Bs (arrow indicates B chromosome). Fig. 51. Huaping population (Yang & Yuan 364), 2n = 16. Fig. 52. *D. pumilum*, 2n = 16. Fig. 53. *D. grandiiflorum*, 2n = 16. Fig. 54. Sangzhi population of *D. anthriscifolium* (Yang & Yuan 627), 2n = 16.

the Hengduan Mountains region, including *A. chrysotrachelum*, *A. brevicalcaratum*, *A. brevicalcaratum* var. *parviflorum*, *A. crassiflorum*, and *A. rilongense*, all endemic to the area, are tetraploid, indicating that polyploidy has played an important role in the speciation of this subgenus in the region. Chen et al. (2007) reported a similar result for the genus *Buddleja* in the Hengduan Mountains region, with ten of the 18 species in the region found to be polyploid. Furthermore, it is worth mentioning that, in some widely distributed species, the polyploid cytotype is restricted to the Hengduan Mountains region, whereas the diploid cytotype occurs outside the area. In Ranunculaceae, such species include *Beesia calthifolia* (Maxim.) Ulbr., *Cimicifuga foetida* L., *Anemone davidii* Franch., and *Ranunculus ficariaefolius* Lövl. & Vant. (Yang, 2002; Yuan & Yang, 2006a; Q. Yuan and Q.-E. Yang, unpubl. data; Nie et al., 2007). Although the tetraploid cytotypes are not yet sufficiently well differentiated from the diploid cytotypes in terms of gross morphology to be treated as independent species, the distribution pattern of the two cytotypes in these species strongly suggests that the Hengduan Mountains region may have indeed provided favourable environmental conditions for the polyploidization of at least some, if not all, groups of angiosperms.

It seems that the question of the extent of the role of polyploidy in plant speciation in the Hengduan Mountains region is much more difficult to answer than expected (Nie et al., 2005). The relationship between the ploidy levels of the flora of seed plants in this region and the Sino-Himalayan orogeny may be very complicated, as a low or high incidence of polyploidy in a given flora may not only be associated with the environmental conditions during the course of florogenesis, as stated by Nie et al. (2005), but also

**Figures 55–61.** Karyotypes of seven species of *Delphinium*. Fig. 55. *D. forrestii*, $2n = 16$. Fig. 56. *D. trichophorum* var. *subglaberrimum*, $2n = 16$. Fig. 57. *D. chrysotrachelum*, $2n = 32$. Fig. 58. *D. wardii*, $2n = 16$. Fig. 59. *D. trisectum*, $2n = 16$. Fig. 60. *D. pachycentrum*, $2n = 16$. Fig. 61. *D. orthocentrum*, $2n = 16$. 

strongly affected by the internal factors of the plants comprising the flora, such as the growth habit and breeding system. As noted by Stebbins (1971), floras cannot be dealt with as a whole when compiling chromosomal data for the establishment of correlations between the distribution of polyploids and the environmental conditions, because of the large differences in the frequency of polyploidy in plants with different life cycles and growth habits, and particularly because the proportion of plants with different life cycles and growth habits differs greatly from one habitat to another. Stebbins (1971) considered that significant facts about the distribution of polyploids are much more likely to be obtained from comparisons within groups of related species and chromosomal races than from statistical comparisons of entire floras. Further investigations of groups characteristic of the Hengduan Mountains region, with possibly different evolutionary histories, are needed to evaluate more precisely the role played by polyploidy in the speciation of the area.

**High Uniformity of Karyotypes in the Genus Delphinium from China**

The karyotypes of the diploid Delphinium species studied are highly uniform, usually consisting of one pair of large m, one pair of large sm, five pairs of medium-sized st, and one pair of smaller sm (rarely st) chromosomes. The chromosomes of the tetraploid...
cytotype (Fig. 64) in *D. sprocentrum* show no essential differences from those of the diploid cytotype (Fig. 63) in size and morphology, with the karyotype of the former being a simple doubling of the latter. This, together with the fact that the two cytotypes grow together, indicates that the tetraploid should be an autopolyploid of recent origin. However, the karyotype of *D. chrysotrichum* is an exception. The chromosomes of this tetraploid species are smaller and all belong to sm or m types (Fig. 57), suggesting that the chromosomes of this tetraploid species may have undergone large structural rearrangements and deletions of duplicate fragments during the course of polyploidization, and that the formation of this tetraploid may not be a recent event. A very similar situation is observed in *Aconitum* subgenus *Lycocotonum* from the Hengduan Mountains region, a group related to *Delphinium*. The karyotypes of the tetraploid taxa in this subgenus from the region are also considerably different from those of the diploid taxa in terms of chromosome size and morphology (Yuan & Yang, 2006b). The chromosomes of the diploid taxa are mostly st types, and are larger than those of the tetraploid taxa, which are mostly sm or m types. The 32 chromosomes of *D. chrysotrichum* could be arranged quite well into eight groups of four homologues. In view of this, it is tempting to suggest that the plant may be an autopolyploid, although the type of polyploidy, autopolyploidy vs. allopolyploidy, cannot be confirmed with certainty because no meiotic observation or other experiments were carried out. Recently, López-Pujol et al. (2007) have conducted an allozyme analysis of a western Mediterranean tetraploid species of *Delphinium*, *D. montanum* DC., the chromosomes of which are also shorter than those in diploids, and may have undergone stronger rearrangements and deletions of duplicate fragments. The allozyme data lend support to the autopolyploid condition in this species.

*Delphinium pentagynum* Lam., a species belonging to section *Oligophyllum* in subgenus *Delphinastrum*, and occurring in the Iberian Peninsula and North Africa, was reported to have a karyotype formula of 2n = 2M + 4m + 4sm + 6st (Blanché, 1991), which is markedly different from that of the diploid species studied here by the presence of one pair of M, one pair of m, and one pair of sm chromosomes in the complement. The three chromosome pairs are all medium in size. By contrast, the corresponding chromosome pairs in the Chinese species and some cytologically known species from other countries were usually found to be st or t types (Lewis et al., 1951; Kurita, 1957; Blanché, Molero & Simon, 1990; Liu & Ho, 1999; Yang, 2001; this study). The karyotype of *D. pentagynum* is the most symmetrical so far known in the genus in terms of the presence of a much higher proportion of sm and m chromosomes. In Ranunculaceae, the karyotypes are generally considered to have evolved towards increasing asymmetry (Lewitsky, 1931; Stebbins, 1971), but *D. pentagynum* belongs to a relatively advanced section in the genus *Delphinium* (Tamura, 1995). Therefore, the highest karyotype symmetry reported in this species, if verified, indicates the occasional inconsistency between the trends of karyotypic evolution and gross morphological evolution in the genus.

Although the *Delphinium* species studied represent two distinct subgenera, subgenus *Delphinastrum* and subgenus *Delphinium*, their karyotypes do not exhibit considerably marked differentiation at the subgeneric level. The karyotypes of *D. forrestii* (Fig. 55) and *D. trichophorum* var. *subglaberrimum* Hand.-Mazz. (Fig. 56), two perennial taxa regarded by Wang (1979) as morphologically most primitive, show no obvious differences in terms of the karyotypic constitution from that of *D. anthrisciflorum* (Fig. 68), the only annual and possibly the most advanced representative in China in subgenus *Delphinium* (Wang, 1979), a group which mainly consists of annuals mostly distributed in Mediterranean and Irano-Turanian regions (Blanché, 1990). This is quite different from the situation in the genus *Aconitum*, in which the karyotypes exhibit obvious differentiation at the subgeneric level in chromosome size and morphology, as well as karyotype asymmetry (Schafer & La Cour, 1934; Shang & Lee, 1984; Yang, 1996, 2001). Nevertheless, it can be seen from Table 1 that the A2 value of *D. anthrisciflorum* is larger than that of all the species in subgenus *Delphinastrum*, indicating that the karyotype of this annual species has the highest interchromosomal asymmetry amongst the species studied.

It is noteworthy that the karyotype of *D. anthrisciflorum* is quite different from that of the annuals in the subgenus *Delphinium* from Mediterranean and Irano-Turanian regions in terms of karyotypic constitution. In the karyotypes of the annual species from Mediterranean and Irano-Turanian regions, one pair of chromosomes is markedly larger and submetaacentric, and the remaining seven pairs are shorter and nearly acrocentric (Blanché, 1990), whereas, in the karyotype of *D. anthrisciflorum*, two pairs of chromosomes are markedly larger and metaacentric or submetaacentric, and the remaining six pairs are shorter and nearly acrocentric (Fig. 68). The fact that the karyotype of *D. anthrisciflorum* shows no obvious differences from that of the Chinese perennials, but the karyotypes of the annuals from Mediterranean and Irano-Turanian regions are considerably different from those of the perennials, may imply that this Chinese annual may have an origin different from that of the annuals from Mediterranean and Irano-Turanian regions.
molecular phylogenetic analysis of the genus Delphinium sampled from different biogeographical regions is needed to verify or falsify this hypothesis.

Blanché (1990) noted that there is a progressive decrease in chromosome size from the perennial subgenus Delphinastrum to the mainly annual subgenus Delphinium, which may be a result of DNA loss. Al-Kelidar & Richards (1981) also reported that there is a relationship between chromosome length and the nuclear DNA amount in the genus Delphinium, and that the species with a smaller mean chromosome length and lower nuclear DNA amount can be considered as karyologically derivative. However, this trend, as shown in Table 1, does not seem to be obviously displayed in the Chinese species studied. The nuclear DNA amount of the Chinese Delphinium species should be estimated precisely to gain a better understanding of the variation pattern of genome size and its possible evolutionary significance.

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