Karyotype and Fluorescence In Situ Hybridization Analysis of 15 *Lilium* Species from China

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ABSTRACT. Karyotype comparison and fluorescence in situ hybridization (FISH) were conducted to analyze the wild *Lilium* species distributed in China. The karyotype results revealed that all species except *Lilium lancifolium* ($2n = 3X = 36$) were diploid and had two pairs of metacentric or submetacentric chromosomes. The karyotypes of all species are similar. FISH analysis revealed that there are 5–12 45S rRNA gene loci dispersed on the chromosomes of the 14 diploid species, and 15 45S rRNA gene loci were detected in the triploid species *L. lancifolium*. Most of the FISH signals were detected on the long arms and the centromeric regions. Three samples of *L. brownii* [Hubei, China (lat. 31°28'N, long. 110°23'E); Liaoning, China (lat. 40°07'N, long. 124°19'E); and Guangxi, China (lat. 25°06'N, long. 107°27'E)] showed very similar chromosome patterns in both the karyotype and the FISH analyses, further demonstrating that these samples belonged to the same species. *L. brownii* is widely distributed in China from latitude 25°06'N to 40°07'N, indicating that it is highly adaptable to the environment.

Lilies (*Lilium* sp.) have substantial ornamental, medicinal, and edible value and are the most important cut flowers worldwide (Long et al., 1999). The genus *Lilium* is native to the Northern Hemisphere, including Asia, Europe, and North America, and it includes ≈100 species (Anderson, 1986; McRae, 1998). These species have been classified by different authors into 5–10 sections or subgenera (Comber, 1949; De Jong, 1974; Endlicher, 1836; Mabberley, 1990). All species of *Lilium* show the same basic chromosome number ($x = 12$), similar karyotype features, and a very large genome size (Zonneveld et al., 2005). Karyotype characteristics for most *Lilium* species have been examined (Stewart, 1947), and two large metacentric chromosome pairs and 10 subtelocentric or telocentric pairs are always present.

China possesses the greatest *Lilium* diversity in the world, with ≈55 species, 36 of which are unique to China (Long et al., 1999). Some of these have been used to breed new cultivars. For example, the ‘Olympic’, ‘Lady Byng’, and ‘Queen Charlotte’, which are still grown today, were all selected from Chinese species (Long et al., 1999). As the

orange flowered member of the *Lilium* genus, *L. henryi* from Hubei, China, has received considerable attention for a long time. Many cultivars, including the famous ‘Black Beauty’, were all selected from the hybrids of *L. henryi* with other species. ‘Enchantment’ and ‘Connecticut King’ are considered descendants part of the ancestry of *L. lancifolium*. *L. regale* is an important breeding *Lilium* species that has been widely grown. *L. pumilum* is famous for its resistance to *Fusarium* and *Botrytis cinerea* (Long et al., 1999). Two American cultivars, ‘Nepera’ and ‘City Lights’, were bred from hybrids of *L. tsingtauense*. Thus, Chinese *Lilium* plants have made an important contribution to global breeding of the genus (Long et al., 1999). To further explore, use, and protect these resources, several major projects have been dedicated to their collection and in vitro preservation (Chen et al., 2007; Zhang et al., 2004) of *Lilium* germplasm in three regions of China (southwest, northwest, and northeast China). Three southwestern provinces, Sichuan, Yunnan, and Tibet, form the region with the broadest population distribution of the genus *Lilium*, containing ≈36 species (Wang and Tang, 1980; Wu et al., 2006). Four northwestern provinces, Shanxi, Gansu, Hubei, and Henan, contain ≈14 species and cultivars (Long et al., 1999). Northeast China comprises three provinces, Heilongjiang, Jilin, and Liaoning, and some studies of *Lilium* germplasm have been conducted in this area (Wang and Tang, 1980).

Based on morphological and physiological characteristics, *Lilium* plants in China have been assigned to the sections

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Table 1. Materials used for karyotype and FISH analysis of *Lilium* species native to China (Comber, 1949).

<i>Lilium</i> species	Location in China
Section <i>Leucolirion</i>	
<i>L. brownii</i> (Hubei)	Shennongjia, Hubei (lat. 31°28'N, long. 110°23'E)
<i>L. brownii</i> (Liaoning)	Dandong, Liaoning (lat. 40°07'N, long. 124°19'E)
<i>L. brownii</i> (Guangxi)	Nandan, Guangxi (lat. 25°06'N, long. 107°27'E)
<i>L. leucanthum</i>	Qianjiang, Chongqing (lat. 29°26'N, long. 108°42'E)
<i>L. longiflorum</i>	Kaili, Guizhou (lat. 24°41'N, long. 107°50'E)
<i>L. regale</i>	Maoxian, Sichuan (lat. 31°51'N, long. 103°37'E)
<i>L. sargentiae</i>	Baoxing, Sichuan (lat. 30°33'N, long. 102°42'E)
<i>L. sulphureum</i>	Baoxing, Sichuan (lat. 30°33'N, long. 102°42'E)
Section <i>Sinomartagon</i>	
<i>L. henryi</i>	Kaili, Guizhou (lat. 26°37'N, long. 107°56'E)
<i>L. jinfushanense</i>	Jinfo Mountain area, Chongqing (lat. 29°06'N, long. 107°11'E)
<i>L. pumilum</i>	Taibai Mountain, Shanxi (lat. 34°07'N, long. 107°53'E)
<i>L. speciosum</i>	Lushan Mountain area, Jiangxi (lat. 29°39'N, long. 115°59'E)
<i>L. lancifolium</i>	Yixing, Jiangsu (lat. 31°21'N, long. 119°46'E)
Section <i>Lophophorum</i>	
<i>L. concolor</i>	Yichun, Heilongjiang (lat. 47°43'N, long. 128°53'E)
Section <i>Martagon</i>	
<i>L. tsingtauense</i>	Haiyang, Shandong (lat. 36°51'N, long. 121°06'E)

Table 2. Types of chromosomes in plants defined based on centromere location and the arm ratio (Li and Chen, 1985).

Arm ratio ^z	Location of centromere	Abbreviation
1	Median point	<i>M</i>
1.01–1.70	Median region	<i>m</i>
1.71–3.00	Submedian region	<i>sm</i>
3.01–7.00	Subterminal region	<i>st</i>
>7.00	Terminal region	<i>t</i>

^zLength of the long arm/length of the short arm.

Table 3. *Lilium* karyotypes defined by the ratio of the length of the longest chromosome to that of the shortest chromosome (*Lt/St*), and the percentage of chromosomes with an arm ratio of greater than 2:1 (Stebbins, 1971).

<i>Lt/St</i>	Chromosomes with an arm ratio >2:1 (%)			
	0	0.01–0.5	0.51–0.99	1
<2:1	1A	2A	3A	4A
2:1 to 4:1	1B	2B	3B	4B
>4:1	1C	2C	3C	4C

Leucolirion, *Sinomartagon*, *Martagon*, and *Lophophorum* (Wang and Tang, 1980). Because of their similar morphological characteristics, especially conserved flower shapes in distantly related species, identification of *Lilium* species using only external morphology is difficult. For example, *L. henryi*, which has previously been classed into section *Sinomartagon* based on its morphological characteristics, matched those of *L. regale* and *L. sulphureum* quite well in a C-band pattern, and its transfer to section *Leucolirion* has been proposed. This is consistent with results from interspecies hybrids between *L. henryi* and *L. regale* (and related species), which are reportedly fertile (Smyth et al., 1989). However, *L. henryi* has always previously been assigned to section *Sinomartagon*.

Cytogenetic studies are useful for plant taxonomy. Cytogenetic investigations of some *Lilium* interspecific hybrids have received considerable attention (Lim et al., 2000, 2001a, 2001b; Noda, 1971), but cytogenetic studies are still not generally performed for Chinese *Lilium* species, and the reports are not always consistent. The karyotype of *L. dauricum* from the Mao'er Mountain region of Heilongjiang Province was 4B in a report by Yang et al. (1996), but it was reported as 3A by Rong et al. (2009). Similarly, the karyotype of *L. davidii* was reported as 3A in a study by Dai et al. (2006) but as 3B in a report by Xie and Wu (1993). Yu et al. (2000) analyzed the karyotypes of seven different *L. sargentiae* populations in China. The results showed that for all seven populations, all karyotypes belonged to Stebbin's type 3B. The karyotypic formulas showed only

a few variations: relative length, arm ratio, and secondary constitution number and distribution. Members of the same species appear to always show similar karyotypes, with minor differences reflecting their distribution.

In addition to karyotypes, FISH is another technique providing chromosomal markers that show the positions of specific genes, thereby permitting chromosomal identification. The use of FISH with 5S and 45S rDNA probes has provided molecular cytogenetic markers for the identification of somatic chromosomes in different *Lilium* species (Lee et al., 2014; Liu et al., 2006; Nguyen et al., 2016; Sultana et al., 2010, 2011). FISH has been used for the verification of *Lilium* hybrids for many years (Lee et al., 2014; Lim et al., 2001a, 2001b; Sultana et al., 2011). However, the FISH analysis of rRNA genes is still rarely used for identifying Chinese *Lilium* species except in a report by Liu et al. (2016). The objective of this work was to compare the karyotypes and the 45S rRNA gene locations among 15 *Lilium* species from China.

Materials and Methods

PLANT MATERIALS. Bulbs of 15 *Lilium* species were collected from different locations, as shown in Table 1 and were preserved vegetatively as clones at Nanjing Forestry University. Twelve of the 15 species were distributed between latitudes 34°07'N and 25°06'N. *L. brownii* was the most widespread species, showing high adaptability in China. Three similar variants were collected from Nandan, Guangxi, China (lat. 25°06'N, long. 107°27'E); Shennongjia, Hubei, China (lat. 31°28'N, long. 110°23'E); and Dandong, Liaoning, China (lat. 40°07'N, long. 124°19'E).

KARYOTYPE ANALYSIS OF MITOTIC CHROMOSOMES. Root tips (1.5–2 cm in length) were pretreated with 0.7 mmol·L⁻¹ cyclohexamide at room temperature for 8 h, washed with distilled water, and fixed in Carnoy's fixative (3:1 ethanol : acetic acid v/v) (Gill et al., 1991) for 24 h at room temperature. For chromosome preparations, the root tips were rinsed thoroughly

and treated with an enzyme mixture (3% pectolyase and 3% cellulase in 150 mM citrate buffer) at 37 °C for 2.5 h. Macerated root tips were placed on the slides, 20 µL of 60% acetic acid was added, and the slides were put on the slide heater (TPJ-A; Huahai, Hangzhou, China) at 55 °C, smeared with a dissecting needle, washed with Carnoy's fixative, and air dried.

Slides used for karyotype analysis were dyed with carbol fuchsin (Carr and Walker, 1961) (30%, 1–3 min) and then at least 10 metaphases nuclei were observed and photographed using a microscope with a 60× objective (BX51; Olympus, Tokyo, Japan). Chromosome length was recorded as the average of five metaphases. The karyotype analysis was performed according to Li and Chen (1985), as shown in Table 2. Karyotyping was performed in accordance with Stebbins (1971), as shown in Table 3.

FISH ANALYSIS. Probe labeling and FISH of the *Lilium* plants were performed according to a previous report (Liu et al., 2016) as follows. The slides were prepared as for the karyotype analysis. The cloned 45S rDNA from rice (*Oryza sativa*) was labeled with fluorescein-12-dUTP by nick translation. To label the probe, 50 µL of labeling mixture, containing 5 µL of 10× buffer, 1.5 µg of 45S rDNA, 5 µL of deoxynucleotide mix (0.4 mM deoxyadenosine triphosphate, 0.4 mM deoxycytidine triphosphate, 0.4 mM deoxyguanosine triphosphate, 0.4 mM deoxythymidine triphosphate), 4 U DNA Polymerase I, 0.002 U DNase I, and 1 nM fluorescein-12-dUTP (Roche, Mannheim, Germany), was reacted at 15 °C for 2 h and held at 4 °C in a PCR instrument (Sultana et al., 2011). Then, 5 µL of ethylenediaminetetraacetic acid (0.5 M, pH 8.0) was added to stop the reaction. The FISH procedure was adopted from Lim et al. (2005). First, the chromosomes were pretreated with 100 µg·mL⁻¹ DNase-free RNase (Qbic S&T, Shanghai, China) dissolved in 2× saline sodium citrate (SSC) for 1 h at 37 °C, treated with pepsin [5 µg·mL⁻¹ (Amresco, Solon, OH)] for 10 min at 37 °C, and fixed in 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO) for 10 min at room temperature. The slides were washed in 2× SSC for 5 min, dehydrated in an ethanol gradient (70%, 95%, 100%), and air-dried. The hybridization mixture consisted of 50% formamide, 10% sodium dextran sulfate, 2× SSC, 0.5 µL of sperm DNA per slide (10 mg·mL⁻¹), and DNA probe (100 ng per slide). The chromosomes were denatured in 70% formamide at 78 °C for 70 s, dehydrated in a prechilled (–20 °C) ethanol gradient (70%, 95%, and 100%), and air-dried. The DNA probes were denatured at 100 °C for 10 min and then cooled on ice. Next, 15 µL of hybridization mix was added to each slide and covered with a cover glass, and the slides were kept in a moist chamber overnight at 37 °C. After hybridization, the slides were washed at 42 °C in 2× SSC, 50% formamide, and 2× SSC for 10 min each, followed by a 5-min wash in

1× phosphate buffered saline (PBS). The slides were then counterstained with 5 mg·mL⁻¹ propidium iodide for 4 min, washed three times with 1× PBS for ≈20 s each time, coated with 10 µL mounting medium (VectaShield; Vector Laboratories, Burlingame, CA) and then covered with 20 × 20-mm glass coverslips. Hybridization signals were observed using a fluorescence microscope (BX51; Olympus). At least 10 metaphase and 10 interphase cells were examined per sample. Selected

Fig. 1. Carbol fuchsin-stained mitotic metaphase chromosomes of 15 *Lilium* species native to China: (A) *L. brownii* (Hubei), (B) *L. brownii* (Liaoning), (C) *L. brownii* (Guangxi), (D) *L. leucanthum*, (E) *L. longiflorum*, (F) *L. regale*, (G) *L. sargentiae*, (H) *L. sulphureum*, (I) *L. henryi*, (J) *L. jinfushanense*, (K) *L. pumilum*, (L) *L. speciosum*, (M) *L. lancifolium*, (N) *L. concolor*, (O) *L. tsingtauense*.

Table 4. Karyotype formula, ratio of the length of the longest chromosome to that of the shortest chromosome (*Lt/St*), and types of 15 *Lilium* species native to China.

<i>Lilium</i> species	Karyotype formula ^a	<i>Lt/St</i>	Type
Section <i>Leucolirion</i>			
<i>L. brownii</i> (Hubei)	$2n = 2x = 24 = 4m + 12st + 8t$	1.9	3A ^y
<i>L. brownii</i> (Liaoning)	$2n = 2x = 24 = 4m + 14st + 6t$	2.01	3B ^x
<i>L. brownii</i> (Guangxi)	$2n = 2x = 24 = 4m + 16st + 4t$	2.01	3B
<i>L. leucanthum</i>	$2n = 2x = 24 = 2m + 2sm + 18st + 2t$	2.15	3B
<i>L. longiflorum</i>	$2n = 2x = 24 = 4m + 12st + 8t$	1.86	3A
<i>L. regale</i>	$2n = 2x = 24 = 4m + 10st + 10t$	2.14	3B
<i>L. sargentiae</i>	$2n = 2x = 24 = 2m + 2sm + 6st + 14t$	2.14	3B
<i>L. sulphureum</i>	$2n = 2x = 24 = 2m + 2sm + 14st + 6t$	1.92	3A
Section <i>Sinomartagon</i>			
<i>L. henryi</i>	$2n = 2x = 24 = 2m + 2sm + 10st + 10t$	2.01	3B
<i>L. jinfushanense</i>	$2n = 2x = 24 = 2m + 2sm + 4st + 16t$	1.8	3A
<i>L. pumilum</i>	$2n = 2x = 24 = 2m + 2sm + 14st + 6t$	1.93	3A
<i>L. speciosum</i>	$2n = 2x = 24 = 2m + 2sm + 10st + 10t$	2.11	3B
<i>L. lancifolium</i>	$2n = 3x = 36 = 3m + 3sm + 18st + 12t$	1.91	3A
Section <i>Lophophorum</i>			
<i>L. concolor</i>	$2n = 2x = 24 = 4m + 20st$	1.80	3A
Section <i>Martagon</i>			
<i>L. tsingtauense</i>	$2n = 2x = 24 = 4m + 8st + 12t$	2.29	3B

^a*m* = chromosome with arm ratio between 1.01 and 1.70, *st* = chromosome with arm ratio between 3.01 and 7.00, *t* = chromosome with arm ratio above 7.00, *sm* = chromosome with arm ratio between 3.01 and 7.00.

^y*Lt/St* < 2:1 (percentage of chromosomes with an arm ratio >2:1 is between 51% and 99%).

^x*Lt/St* between 2:1 and 4:1.

chromosome spreads from metaphase nuclei were photographed with a digital camera (DP72; Olympus).

Results

KARYOTYPE ANALYSIS. In comparison with a previous study of *L. rosthornii* (Liu et al., 2016), we used a chromosome protocol-based on smearing instead of squashing. From the chromosome images, we can see that the new method is more appropriate than the previous method. In particular, smearing was more effective than squashing at protecting chromosome morphology.

All *Lilium* species except *L. lancifolium* were diploid with chromosomes $2n = 2X = 24$ (Fig. 1). As shown in Table 4 and Figs. 1–3, 14 diploid species had 2 large metacentric chromosomes and 10 smaller acrocentric chromosomes. Chromosomes 9 and 10 of the three *L. brownii* samples had secondary constrictions (SCs) on their long arms. The only triploid species, *L. lancifolium*, also had two groups of *m* or *sm*

chromosomes. The karyotypes of the 15 *Lilium* species could be divided into two groups: 3A and 3B. Seven species, three from *Leucolirion*, three from *Sinomartagon*, and one from *Lophophorum*, were 3A, and the other eight species, five from *Leucolirion*, two from *Sinomartagon*, and one from *Martagon*, were 3B. The *Lt/St* (ratio of the length of the longest chromosome to that of the shortest chromosome) was between 1.80 and 2.29 (Table 4), with the average exactly at 2.00, which is the dividing line between karyotypes 3A and 3B. Thus, although the karyotypes of these 15 species were divided into 3A and 3B, they showed little difference from each other.

FISH ANALYSIS. FISH was used to analyze the variability of 45S rRNA gene loci on the somatic metaphase chromosomes of these 15 Chinese *Lilium* species. The result showed that 45S rDNA loci were distributed in all the chromosomes (Figs. 3–5). The numbers of loci differed significantly among the 15 evaluated species. Among the diploid species, *L. regale* (Figs. 4F and 5F) showed the highest number at 12, and others showed 6, 8, or 10 loci. *L. lancifolium* ($2n = 3X = 36$), the only triploid species, showed 15 45S rRNA gene loci (Figs. 3M, 4M, and 5M). In addition to the differences in the numbers of loci, we observed differences in the placement of the gene loci on the chromosomes.

In *L. brownii* (Hubei), *L. brownii* (Liaoning), and *L. brownii* (Guangxi), three pairs of 45S rDNA signals were recorded on the centromeric region of chromosome 3 and the long arms of chromosomes 9 and 10 (Figs. 3A–C, 4A–C, and 5A–C), which were just the secondary construction area (Fig. 2A–C). In *L. leucanthum*, four pairs of signals were observed on the centromeres of chromosomes 1, 2, 6, and 10 (Figs. 3D, 4D, and 5D). In *L. longiflorum*, six 45S rDNA signals were detected on the centromeric regions of chromosomes 3, 4, and 6 (Figs. 3E, 4E, and 5E). In *L. regale*, 10 of the 12 45S rDNA loci were observed on the centromeric regions of chromosomes 1, 2, 8, 9, and 10, and the other two were located on the long arm of chromosome 7 (Figs. 3F, 4F, and 5F). In *L. sargentiae*, eight paired signals were observed on the centromeres of chromosomes 1, 2, 8, and 11 (Figs. 3G, 4G, and 5G). In *L. sulphureum*, six of the eight 45S rDNA loci were present on the centromeric regions of chromosomes 1, 2, and 11, and the other two were located on the long arm of chromosome 4 (Figs. 3H, 4H, and 5H). In *L. henryi*, five signals were detected on the centromeric region of chromosome 1, the long arm of chromosome 8, and the long arm of chromosome 10 (Figs. 3I, 4I, and 5I). In *L. jinfushanense*, six of the eight 45S rDNA signals were detected on the centromeric regions of the homologous chromosomes 1, 3, and 8, and the other two were located on the long arms of chromosome 6 (Figs. 3J, 4J, and 5J). In *L. pumilum*, eight 45S rDNA signals were detected on the centromeric regions of chromosomes 1, 3, 4, and 6 (Figs. 3K, 4K, and 5K). In *L. speciosum*, ten 45S rDNA loci were present on the centromeric regions of chromosomes 1, 5, 8, 11, and 12 (Figs. 3L, 4L, and 5L). In *L. lancifolium*, which was the only triploid species, 15 45S rDNA signals were detected on the short arm near the centromeric regions of chromosomes 1 and 3, the long arms of chromosomes 6 and 11, and the short arm of chromosome 2 (Figs. 3M, 4M, and 5M). In *L. concolor*, 10 45S rDNA were detected on the centromeres of chromosomes 1, 3, 8, and 10 and on the short arm of chromosome 2 (Figs. 3N, 4N, and 5N). In *L. tsingtauense*, eight 45S rDNA

Fig. 2. Karyograms of the 15 *Lilium* species native to China: (A) *L. brownii* (Hubei), (B) *L. brownii* (Liaoning), (C) *L. brownii* (Guangxi), (D) *L. leucanthum*, (E) *L. longiflorum*, (F) *L. regale*, (G) *L. sargentiae*, (H) *L. sulphureum*, (I) *L. henryi*, (J) *L. jinfushanense*, (K) *L. pumilum*, (L) *L. speciosum*, (M) *L. lancifolium*, (N) *L. concolor*, (O) *L. tsingtauense*.

Fig. 3. Chromosome idiogram of 15 *Lilium* species native to China: (A) *L. brownii* (Hubei), (B) *L. brownii* (Liaoning), (C) *L. brownii* (Guangxi), (D) *L. leucanthum*, (E) *L. longiflorum*, (F) *L. regale*, (G) *L. sargentiae*, (H) *L. sulphureum*, (I) *L. henryi*, (J) *L. jinfushanense*, (K) *L. pumilum*, (L) *L. speciosum*, (M) *L. lancifolium*, (N) *L. concolor*, (O) *L. tsingtauense*.

loci were detected on the long arms of chromosomes 3, 6, 8, and 12 (Figs. 3O, 4O, and 5O).

Loci show high polymorphism not only in number but also in their chromosomal locations. Most signals, 66.1% of the total, were located near the centromeric region of the chromosome. Another 28.2% of the loci were located on the long arm. Only seven loci, $\approx 5.6\%$, were detected on the short arm. In 10 species, chromosome 1 showed 45S rDNA loci on their centromeric regions. The other five species without signals on chromosome 1 all showed signals on chromosome 3. The three *L. brownii* materials from the three different provinces showed very similar signal distributions.

Discussion

As one of the most important worldwide cut and potted flower crops, *Lilium* species are facing the threat of genetic erosion. Therefore, increasing attention has been paid to the conservation of *Lilium* resources (Zhou et al., 2012). The classification and identification of these resources are important for plant material preservation and utilization. Karyotype comparison analysis has long been used to describe chromosome patterns and evolution in some closely related groups (Stebbins, 1971).

Although we obtained clear figures for all materials, sometimes the constriction, especially the second constriction, was difficult to determine.

Members of the tribe Liliae are similar in genome size and karyotype idiogram and have the same basic chromosome number (Stewart, 1947). Chinese *Lilium* species also showed similar karyotypes, 3A or 3B, and always only with two pairs of *m* or *sm*. The *Lt/St* values for Chinese *Lilium* species are close to

2.00, which is the dividing line between 3A and 3B. This has troubled some researchers in *L. rosthornii* karyotype separation. Liu et al. (2010) concluded that *L. rosthornii* belonged to 3B, Qing (2011) believed that it belonged to 3A, and Liu et al. (2016) considered it 3B. Although the karyotypes of *Lilium* species can be divided into 3A and 3B, their karyotypes are similar, as demonstrated in Table 1.

In a previous study, FISH mapping using 45S rDNA probes in Korean *Lilium* species demonstrated that the 45S rDNA loci were distributed across most chromosomes except chromosomes 8, 9, and 12 (Sultana et al., 2010). The sites of these loci were more variable in the Chinese *Lilium* species, with all chromosomes showing 45S rDNA signals. Not all the 45S rDNA loci were located in both homologue chromosomes. There were only five signals detected in *L. henryi* (Figs. 3I, 4I, and 5I). This phenomenon has only been observed in one other Chinese *Lilium* species, *L. rosthornii* (Liu et al.,

2016), but Nguyen et al. (2016) also reported this phenomenon in several diploid *L. lancifolium* materials distributed in Korea. It has also been observed in other plant species (Liu et al., 2006). One possible explanation is that the tandem repeats of rDNA clusters permit chromosomal rearrangements that move the nuclear organization regions (NORs) to other chromosomes that did not previously have rDNA sequences. Nguyen et al. (2016) studied diploid and triploid *L. lancifolium* and reported that the 45S rRNA genes were located on chromosomes 1, 2, 4, 6, 7, and 11, which did not conform to our results, particularly the loci located on chromosomes 3, 4, and 7. In our research, we did not find 45S rRNA signals on chromosomes 4 and 7, but we found signals located on chromosome 3, even though three of their materials were collected from the same region as ours. In a different study by Hwang et al. (2011), 45S rDNA was located on chromosomes 1, 2, 6, 7, and 11. However, in that report, they analyzed the chromosomes mainly by short arm length and not by chromosome total length as in our study. Thus, chromosome 7 in their research was stained, but the stained chromosome was chromosome 3 in our research; this is the reason for the disagreement. And we did not observed 45S rDNA loci on chromosome 4 of *L. lancifolium*. Hwang et al. (2011) also did not found 45S rDNA site on chromosome 4 in triploid *L. lancifolium*. But they found 45S rDNA signals on chromosome 4 in diploid *L. lancifolium*. These disagreements may indicate that 45S rDNA site on chromosome 4 was not stable in *L. lancifolium*.

According to the ITS dataset, *L. longiflorum* and two *L. brownii* species had been classified into section *Lilium* Wang and Tang (Gao et al., 2012). In 45S rDNA FISH analysis, all three *L. brownii* materials and one *L. longiflorum* sample showed six signals. Other than *L. tsingtauense*, these are the

Fig. 4. Locations of 45S rDNA on metaphase chromosomes of 15 *Lilium* species native to China: (A) *L. brownii* (Hubei), (B) *L. brownii* (Liaoning), (C) *L. brownii* (Guangxi), (D) *L. leucanthum*, (E) *L. longiflorum*, (F) *L. regale*, (G) *L. sargentiae*, (H) *L. sulphureum*, (I) *L. henryi*, (J) *L. jinfushanense*, (K) *L. pumilum*, (L) *L. speciosum*, (M) *L. lancifolium*, (N) *L. concolor*, (O) *L. tsingtauense*.

Fig. 5. Comparison of 45S rDNA location on the chromosomes from 15 *Lilium* species native to China: (A) *L. brownii* (Hubei), (B) *L. brownii* (Liaoning), (C) *L. brownii* (Guangxi), (D) *L. leucanthum*, (E) *L. longiflorum*, (F) *L. regale*, (G) *L. sargentiae*, (H) *L. sulphureum*, (I) *L. henryi*, (J) *L. jinfushanense*, (K) *L. pumilum*, (L) *L. speciosum*, (M) *L. lancifolium*, (N) *L. concolor*, (O) *L. tsingtauense*.

only four materials that did not show signals on chromosome 1 but did show signals on chromosome 3. This demonstrated that our FISH results were partly consistent with the ITS results.

Interestingly, the three *L. brownii* species had different Chinese names and were obtained from widely distant locations (Table 1). According to Long et al. (1999), the only difference between *L. brownii* (Hubei) and *L. brownii* (Guangxi) was the leaf shape. The leaf shape of *L. brownii* (Hubei) was lanceolate to oval, and the leaf shape of *L. brownii* (Guangxi) was lanceolate or narrow lanceolate to lorate. In the present study, *L. brownii* samples from these locations showed very similar karyotype and FISH results. Gao et al. (2012) studied the karyotypes of 32 species of *Lilium* from China, including 10 of the materials we used. They divided *L. brownii* into a new section, *Lilium* Wang and Tang. This classification had also been proposed by Wang and Tang (1980). This was consistent with our results. We also found two pairs of chromosomes, 9 and 10, with SCs on their long arms where we also found the 45S rRNA gene loci. Noda (1971) concluded that the major difference among species of the genus *Lilium* corresponded to the number and position of SCs and the activity of the NORs. Therefore, this was additional evidence that these three *L. brownii* collections may be the same despite having different Chinese names. They were obtained from widely separated locations in China, Dandong, Liaoning (lat. 40°07'N, long. 124°19'E) to Nandan, Guangxi (lat. 25°06'N, long. 107°27'E). According to the information available online, Dandong, Liaoning, has a cold-temperate climate, and Nandan Guangxi has a mountain plateau climate. The annual average temperatures in these locations are 9 °C (Dandong, Liaoning), 16.9 °C (Nandan, Guangxi), and 12.9 °C (Shennongjia, Hubei). This indicated *L. brownii* can show extensive adaptability.

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