

中国西南地区鹿药属 4 种 15 居群核型研究

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摘要: 对产于中国西南部的鹿药属 (*Maianthemum*) 4 种植物进行了细胞学研究, 包括染色体数目, 多倍化, 非整倍性和随体染色体, 以及核型不对称性和核型进化。结果表明: 1) 除了在云南丽江采集的 *Maianthemum tatsienensis* 染色体数目为 $2n=72$ 之外, 其余的居群全为 $2n=36$; 2) 核型在居群间存在变异, 特别是在具中部染色体和近中部染色体的数目以及随体染色体的数目和位置上。此外, *M. nanchuanense* 和 *M. szechuanicum* 的核型是首次报道, B 染色体也是首次在该属中发现。我们推测鹿药属的进化方式包括频繁的染色体畸变以及不同水平上的多倍化, 而中国西南部是该属的分化中心。

关键词: 染色体; 进化; 核型; 鹿药属

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Karyotypes of Fifteen Populations of Four Species in *Maianthemum* (Liliaceae) from Southwestern China

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Abstract: Fifteen populations of four species of *Maianthemum* from southwestern China were analyzed in terms of base number, polyploidy, dysploidy, chromosome size, chromosome morphology, satellited chromosomes, structural heteromorphism, and karyotype asymmetry and karyotype evolution. The results show that 1) chromosomal number of all the species examined is $2n=36$, with *M. tatsienensis* (Population Lijiang, Yunnan) as an exceptional case of which the chromosomal number is $2n=72$; 2) karyotypes show difference among populations, especially in the numbers of metacentric, submetacentric, and acrocentric chromosomes as well as satellites. The karyotypes of *M. nanchuanense* and *M. szechuanicum* are documented the first time. B-chromosome is discovered in this genus the first time, too. It is inferred that the evolutionary mechanisms in the genus *Maianthemum* include the frequent somatic chromosome aberration and the polyploidy at various levels, and that the southwestern China is one of the differentiation centers of this genus.

Key words: Chromosome; Evolution; Karyotype; *Maianthemum*

Maianthemum Wiggers *sensu lato* (including *Smilacina*), a genus of the tribe Polygonatae of Ruscaceae (Rudall *et al.*, 2000; APGII 2003), comprises about 35 species distributed in East Asia and North America, with only one species *M. bifolium* extending

to Europe (Chen and Kawano, 2000). This genus used to be represented by 19 species, which occurred mainly in the Hengduan Mountains, southwestern China (Li, 1990). Karyologists have paid much attention to this genus and a lot of cytological research has been done (Ku-

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mar, 1959; Kurosawa, 1966; Sharma, 1970; Mehra and Sachdeva, 1976; Hong and Zhu, 1987, 1990; Wang *et al.*, 1990; Wang *et al.*, 1993; Gu and Sun, 1998; Meng *et al.*, 2005). 18 of the 35 species have been studied with respect to chromosome features and interspecific variation in karyotype of somatic chromosomes (Meng *et al.*, 2005). The origin and evolution of *Maianthemum* have been discussed with evidences from cytological studies (Hong and Zhu, 1987, 1990).

This paper provides results of our study on karyotype analyses of somatic chromosomes in 15 populations of four species of *Maianthemum* from southwestern China, including 2 species not studied before, and discusses the karyotype evolution according to both our and previous research data.

1 Materials and methods

The species names and populations, along with their origins, are list in Table 1. Voucher specimens are deposited in the herbarium of Sichuan University (SZ). Plant individuals were collected from native habitats, and cultivated in pots for cytological study. Root tips were collected in the morning from each individual, pretreated with a solution of mixed liquor (0.1% aqueous colchicines: saturated dichlorobenzene = 1:1) for 6-7hr at 20-21°C, fixed in 3:1 ethanol-acetic acid for at least one day, and stored in 70% ethanol at 4 ± 2°C. The root tips were macerated in a mixture of 1 mol/L HCl for 10 min at 60°C, stained by 1% acetic orcein for 2-3 hr, and squashed on a glass slide to observe the cells. One to five individual plants from each population and several roots per plant were studied. The chromosomes of at least 30 metaphase plates from each root were studied.

Number, size and shape of chromosomes were observed, and karyotype asymmetry was evaluated. Satellites were not taken into calculation of chromosome size and the highest number of satellited chromosomes in each plate was recorded, followed Lifante (1996). Nomenclatures for the centromeric positions of chromosome introduced by Levan, Fredga and Sandberg (1964) were followed: m = median centromeric chromosome with arm ratio of 1.0-1.7; sm = submedian centromeric chromosome with arm ratio of 1.7-3.0; st = subterminal centromeric chromosome with arm ratio of 3.0-7.0. Karyotype asymmetry was described according to Stebbins (1971), who established these by recognizing three degrees of difference (A-C) between the largest and smallest Chromosome of the complement, and four degrees (1-4) with respect to the proportion of chromosomes which are metacentric with an arm ratio of less than 2:1. So there are 12 types. The index of asymmetry (As K%) also adopted Arano (1963), i.e. the ratio of the sum of long arm length to the total haploid length of the chromosome complement.

2 Results and Discussion

The karyotype structures, classifications and asymmetry indexes of all populations of the species analyzed are list in Table 2. Diploid was found in all taxa studied, while tetraploid found only in *Maianthemum tatsienensis*, and dysploidy ($2n = 35$) was found in a Yunnan population of *M. henryi*. Most taxa have a karyotype asymmetry of type 2C according to Stebbins (1971), except *M. tatsienensis* has a 2B type. The indexes of asymmetry (As K%) of these species are high, which ranges from 64.75% - 66.82%. The bimodal karyotype was obvious.

Table 1 Origins, altitude and voucher numbers of the *Maianthemum* species and populations examined

Taxon	Locality	Altitude (m)	Collector & Voucher
<i>M. tatsienensis</i> (Franchet) LaFrankie	Emei Jinding, Sichuan	3070	Z.H. Tang, SZTM-017
	Emei, Leidongping, Sichuan	2430	Z.H. Tang, SZTM-158
	Emei, Chudian, Sichuan	1740	Z.H. Tang, SZTM-206
	Jinshanzhuang, Chongqing	1800	Z.H. Tang, SZ TJ-06
	Guofodong, JinshoShan, Chongqing	2000	Z.H. Tang, SZ TJ-11
	Wanyuan, Dazhou, Sichuan	1850	Z.H. Tang, SZ TW-01
	Lijiang, Yunnan	2800	Q. Wang, SZ WQ-006
<i>M. henryi</i> (Baker) LaFrankie	Dali, Yunnan	2930	Q. Wang, SZ WQ-003
	Emei Chudian, Sichuan	1740	Z.H. Tang, SZ TM-11
	Kangding, Sichuan	2880	Q. Wang, SZ WQ-0027
	Wolong, Sichuan	3400	Q. Wang, SZ WQ-0026
<i>M. szechuanicum</i> (F.T. Wang & Tang) H. Li	Emei Leidongping, Sichuan	2430	Z.H. Tang, SZ TM-008
<i>M. atropurpureum</i> (Franchet) LaFrankie	Emei Luohanpo, Sichuan	2300	Z.H. Tang, SZ TM-040
	Guofodong, JinshoShan, Chongqing	2000	Z.H. Tang, SZ TJ-01
<i>M. nanchuanense</i> H. Li & J. L. Huang	Jinshanzhuang, Chongqing	1800	Z.H. Tang, SZ TJ-05

Table 2 Karyotype structures of populations and species of *Maianthemum*

Taxon	Collection number	Karyotype formula	As K%	Type
<i>M. tatsienensis</i> (Franchet) LaFrankie	SZ TM-017	$2n = 36 = 14m + 14sm + 8st$	65.80	2B
	SZ TM-158	$2n = 36 = 18m(1sat) + 12sm(2sat) + 6st$	66.05	2B
	SZ TM-206	$2n = 36 = 20m + 12sm + 4st$	65.00	2B
	SZ TJ-06	$2n = 36 = 14m + 18sm + 4st$	64.73	2B
	SZ TJ-11	$2n = 36 = 12m + 18sm + 6st$	65.85	2B
	SZ TW-01	$2n = 36 = 12m + 20sm + 4st (1sat)$	65.39	2B
	SZ WQ-006	$2n = 72 = 32m(1sat) + 28sm + 12st$	64.75	2B
<i>M. henryi</i> (Baker) LaFrankie	SZ WQ-003	$2n = 36 = 14m + 14sm(1sat) + 8st$	66.15	2C
	SZ TM-11	$2n = 36 = 18m + 12sm + 6st$	66.04	2C
	SZ WQ-0027	$2n = 36 = 18m + 10sm + 8st$	66.28	2C
	SZ WQ-0026	$2n = 36 = 20m + 6sm + 10st$	66.48	2C
<i>M. szechuanicum</i> (F.T. Wang & Tang) H. Li	SZ TM-008	$2n = 36 = 14m + 14sm + 8st$	66.80	2C
<i>M. atropurpureum</i> (Franchet) LaFrankie	SZ TM-040	$2n = 36 = 20m + 8sm(1sat) + 8st$	65.37	2C
	SZ TJ-01	$2n = 36 = 16m + 16sm + 4st$	65.59	2C
<i>M. nanchuanense</i> H. Li & J. L. Huang	SZ TJ-05	$2n = 36 = 22m(2sat) + 2sm + 12st + B$	66.28	2C

2.1 Base number

The chromosome number of $2n = 35, 36, 72$ has been found in our study. Kumar (1959), Kurosawa (1966), and Sharma (1970) reported the chromosome number $2n = 28, 36, 66, 72$. Mehra and Sachdeva (1976) reported the chromosome number $2n = 36$ of *S. purpurea*. The chromosome number of *S. japonica* and *S. henryi* collected in Taibai mountain of China is $2n = 36$. Hong and Zhu (1987), Wang *et al.* (1990) reported the chromosome number $2n = 36$ of *S. tatsienensis* collected from Sichuan province. Wang *et al.* (1993) reported the chromosome number $2n = 36$ of some species of *Smilacina* from Yunnan province. Gu and Sun (1998) reported the chromosome number $2n = 54$ of *S. fusa* from Motuo Xizang (Tibet). It could be inferred that most species of *Maianthemum* has a chromosomal number of $2n = 36$, occasionally $2n = 54$ or 72 . All the studied species in *Maianthemum* have chromosome number of $2n = 36$, which is currently considered as diploid. Our research confirmed this hypothesis, because we didn't find any individual has a chromosome number below 36 (except a dysploidy with $2n = 35$). The base number $2n = 18$ was very high, and it was against with Stebbins (1971) hypothesis that all genera and families should have a base number equal or below $x = 12$. So this group might be an ancient polyploidy. Further studies need to confirm this hypothesis.

2.2 Polyploidy and dysploidy

Hong and Zhu (1987) have shown an incidence

of polyploidy in *Maianthemum*, and this is confirmed in our study. By putting these data together with information reported in earlier studies, the diploid level has been found in all taxa studied, and the tetraploid level found in *M. tatsienensis* and *M. atropurpureum*. *M. fuscum* with $2n = 54$ may be an ancient hexaploid with base number $x = 9$, as discussed above.

The number $2n = 35$ was only found in a Yunnan population of *M. henryi*, which makes up 6% of the total cells examined (Figs.9 - 10). The largest chromosome is a metacentric of type M, with double centromeres, which could have resulted from a Robertsonian translocation between two sm chromosomes and the loss of the short arms (Yu and Huang, 1994). The ratio of those cells with $2n = 35$ was low, further studies are needed to determine its role.

2.3 Polymorphism of karyotype

There are significant inter-population differences in karyotype, particularly in B chromosomes, as well as the number and location of satellites and karyotype asymmetry. Moreover, our karyotype data are often different from those reported in the earlier studies.

2.3.1 B chromosome B chromosome is found in *Maianthemum* the first time, and only present in *M. nanchuanense*, and B chromosome was observed only in one individual among the three individuals studied by us. According to the ninth pair chromosome (Figs.25 - 26), we think the possible mechanism is chromosomal structure heteromorphism in response to environmental

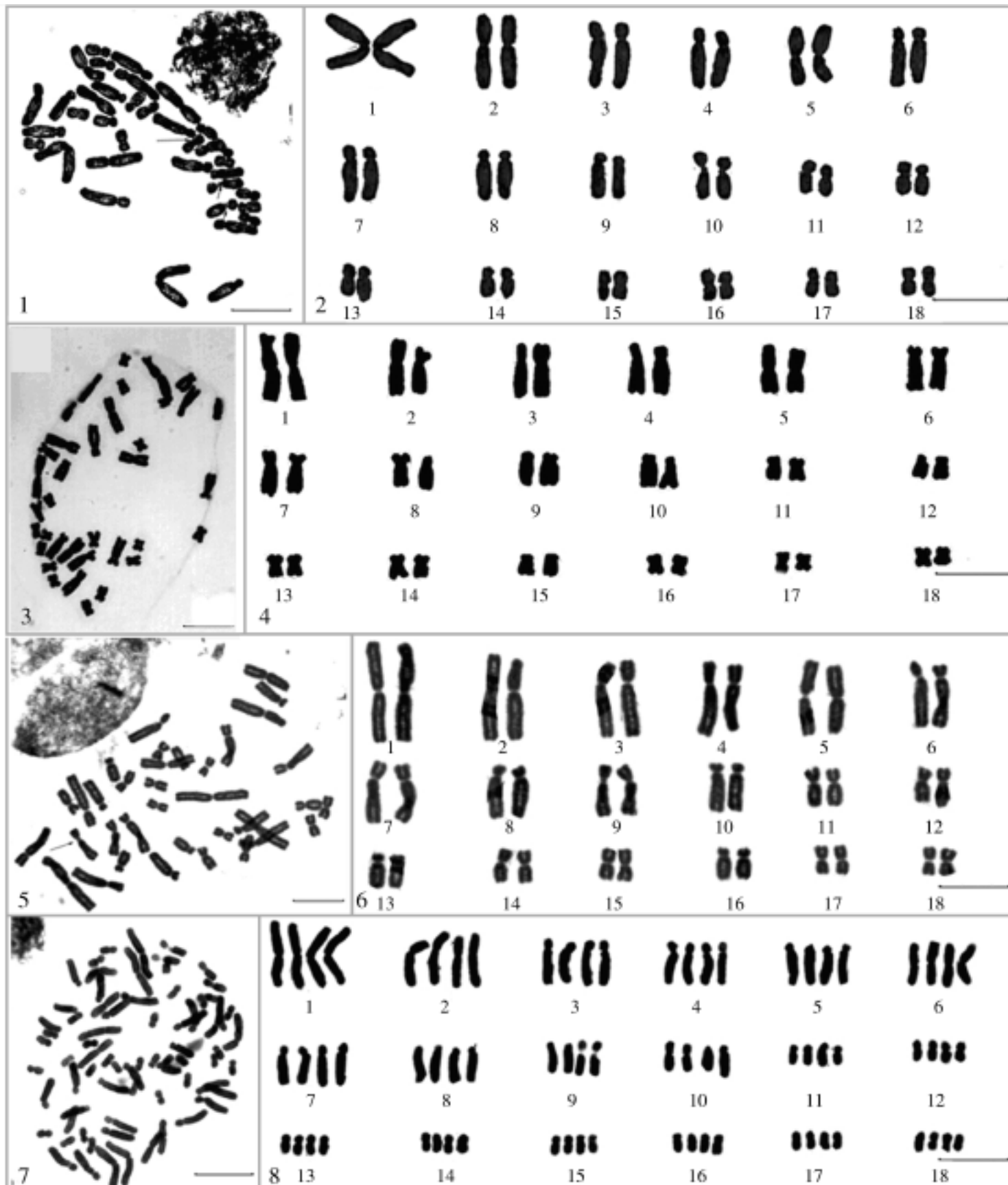
stresses . It seems the B chromosome has some relativity with morphogenesis, such as the size of rhizome, stem and inflorescence; whether those are just individual variation or intrinsic relation need further experiment to work it out, as well as the influences of the fertility .

2.3.2 Bimodality The karyotypes of 15 populations studied are all bimodal . Ten pairs of chromosomes in the karyotype are significantly larger than the rest, and the number of the macrochromosomes is almost equal to that of microchromosome . We agree with Levitzky

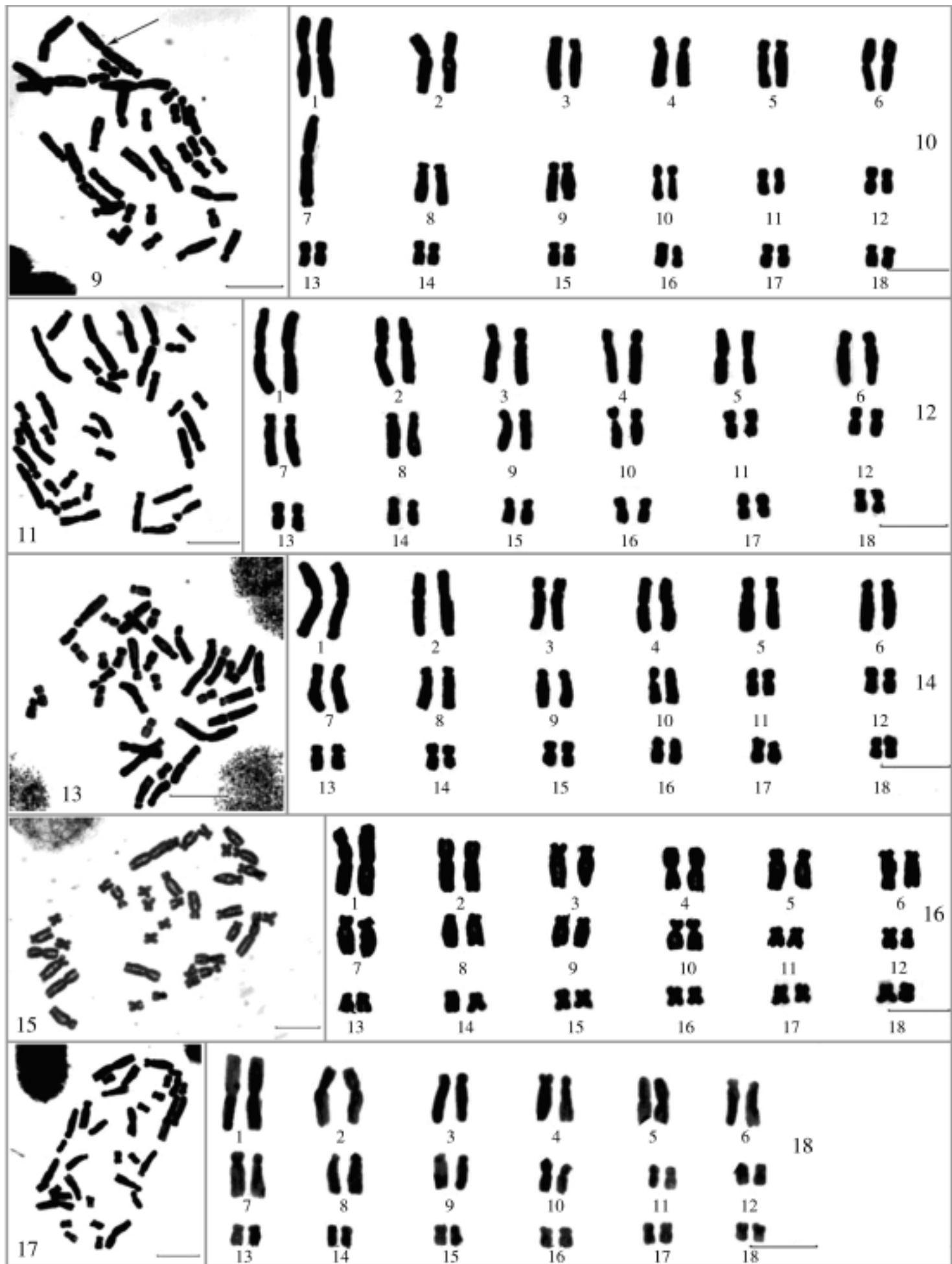
(1931) that bimodal karyotypes come from unequal translocation . Besides, centric fusions could have played an important role in the evolution of the karyotype of this genus . Bimodality is considered as an extremely specialized form of asymmetry (Stebbins, 1971), so the genus *Maianthemum* is likely to be an advanced group in the tribe Polygonatae of Ruscaceae .

2.3.3 Chromosomal structural heteromorphism

There is a high level of structural heteromorphism present in both satellited and non-satellited chromosomes



Figs . 1 - 8 . Photomicrographs of metaphase chromosomes and their karyotypes in four populations of *M. tatsienensis* . 1 - 2 . Population Emei, Leidongping, Sichuan, $2n = 36$; 3 - 4 . Population Gufdong, Chongqing, $2n = 36$; 5 - 6 . Population Wang yuan Sichuan, $2n = 36$; 7 - 8 . Population Li jiang Yunnan, $2n = 72$. ((Arrows indicating satellited chromosome . Scale bars = 100 μm)



Figs . 9-18 . Photomicrographs of metaphase chromosomes and their karyotypes in four populations of *M. henryi* . 9-10 . Population Dali, Yunnan, $2n = 35$; 11-12 . Population Dali, Yunnan $2n = 36$; 13-14 . Population Emei, Chudian, Sichuan, $2n = 36$; 15-16 . Population Kangding, Sichuan, $2n = 36$; 17-18 . Population Wolong, Sichuan, $2n = 36$. (Arrows indicating satellited chromosome . Scale bars = 100 μm)

in almost all species . Chromosomal structural heteromorphism occurs frequently in *M. henryi* with double centromeres, while *M. tatsienensis* has the lowest level . Chromosomal heteromorphism is obvious, which indicates that a karyotypic differentiation does exist among the populations . We have reason to believe that

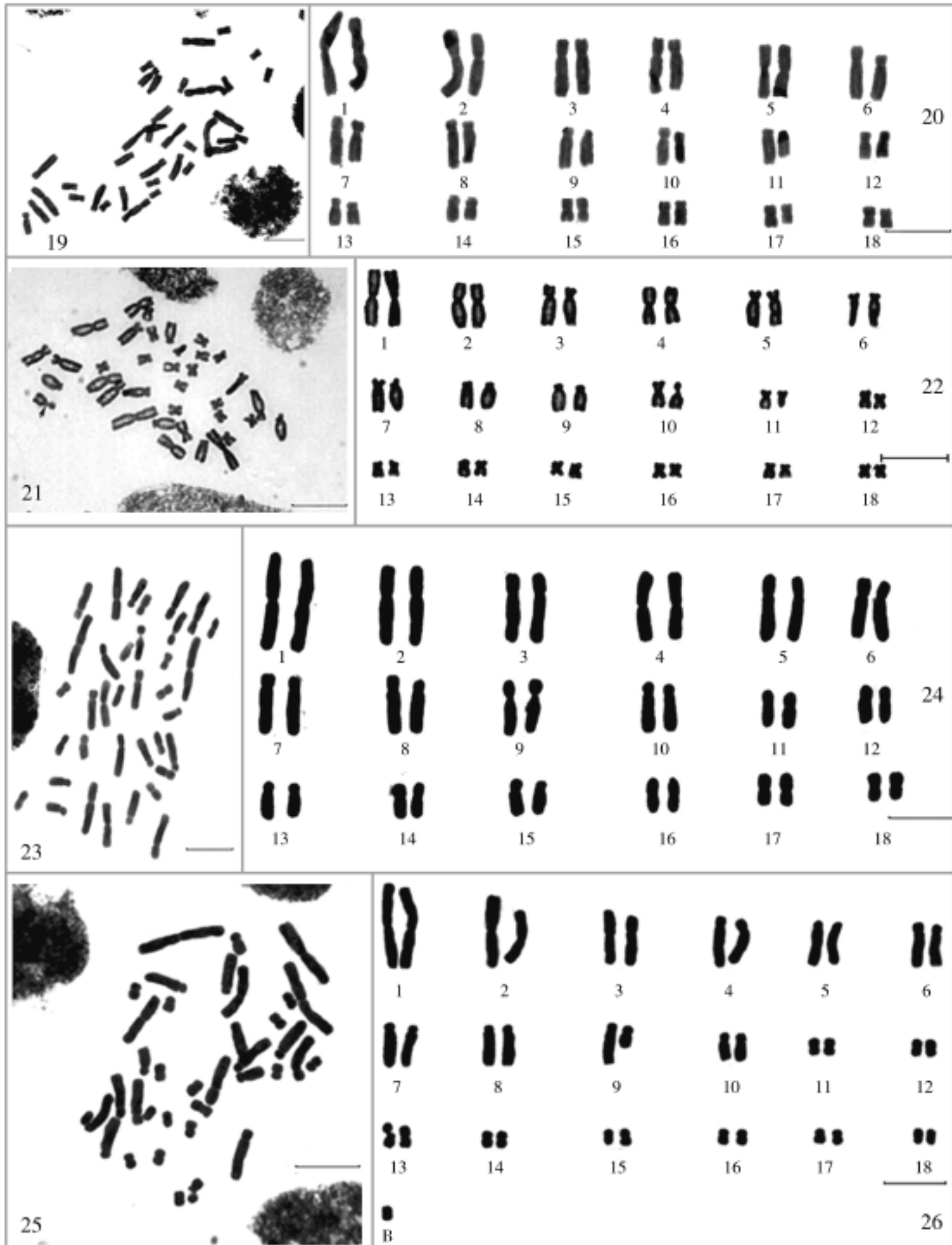
pericentric inversion and the Robertsonian or unequal reciprocal translocations also exist . Hence the karyotype is somewhat polymorphic at the intraspecific level . We believe that the diversified habitats might have induced the chromosomal structural heteromorphism, and that the facultative apomictic reproduction and

polyploidy might help to surmount the obstacle of reduced fertility and thus maintain chromosomal heteromorphism among populations (Yang *et al.*, 1992).

3 Differentiation center

Firstly, in all the 19 species of *Maianthemum* in

China, eight are concentrated in Hengduan Mountains, with others distribute sparsely in the rest of East Asia and mostly have a distribution in southwestern China. This distribution pattern shows that Hengduan Mountains, located in the centre of southwestern China, have a core status in the distribution of *Maianthemum*.



Figs . 19 - 26 . Photomicrographs of metaphase chromosomes and their karyotypes in three species of *Maianthemum* . 19-20 . *M. szechuanicum*, $2n = 36$; 21 - 24 . *M. atropurpureum*, $2n = 36$. 21 - 22 . Population Luohanpo, Sichuan, $2n = 36$; 23 - 24 . Population Jinfoshanzhuang, Chongqing, $2n = 36$; 25 - 26 . *M. nanchuanense* . (Arrows indicating satellited chromosome . Scale bars = 100 μm)

Secondly, as discussed above, the karyotype evolution of *Maianthemum* includes polyploidy, structural reorganizations as well as dysploidy. Structural reorganizations can produce new supergene, so that it will be given a higher fitness to occupy a new niche, while the structural reorganizations can be fixed through asexual reproduction. Those variations in present work indicate that this genus are young and are undergoing an intense differentiation progress.

On the other hand, according to previous studies (Kumar, 1959; Kurosawa, 1966; Sharma, 1970; Mehra and Sachdeva, 1976; Gu and Sun, 1998) and the present work, it is noteworthy that all the polyploid taxa are concentrated in the Hengduan Mountains region, which is recognized as a biodiversity 'hot spot' (Myers *et al.*, 2000), and hold the richest temperate flora of seed plants in the world (Wu, 1988; Li and Li, 1993). Floristically, it is also a highly natural region and is one of the areas of the world with a high concentration of endemic species (Wu, 1988; Li and Li, 1993). So the origin and the evolvement of ploidy level are probably due to the geological events which created different habitats.

In a word, the correlation of modern distribution pattern and the polymorphism of karyotype observed in present study, together with the particularity of Hengduan Mountains discussed above show that southwestern China is probably the diversity and differentiation center of genus *Maianthemum*.

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