

THE KARYOTYPES AND CHROMOSOME NUMBERS IN NINE SPECIES OF *GENTIANA* FROM ALPINE MOUNTAINS OF WESTERN CHINA

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Abstract

The karyotypes and the chromosome numbers in nine species of the genus *Gentiana* (Tourn.) L. from the alpine mountains of western China are reported in the present paper. All the chromosome data for these species in this paper are recorded for the first time. In *Gentiana yunnanensis* Franch. the karyotype formula and the chromosome complement of relative length are $K(2n) = 14 = 12m + 2M = 8M_2 + 6M_1$; in *G. aristata* Maxim. $K(2n) = 18 = 14m + 4M = 8M_2 + 10M_1$; in *G. haynaldii* Kanitz $K(2n) = 18 = 16m + 2sm = 8M_2 + 10M_1$. The karyotypes of three species all belong to Stebbins' type 1A and are symmetrical or subsymmetrical with low asymmetry indices (A_1 , A_2 and $As. k\%$). In *G. burkii* H. Smith $K(2n) = 32 = 26m + 6sm = 14M_2 + 16M_1 + 2S$ and in *G. vernayi* Marquand $K(2n) = 26 = 24m + 2sm = 2L + 12M_2 + 8M_1 + 4S$. Their karyotypes belong to Stebbins' type 2A or 1B respectively and are asymmetrical with high asymmetry indices (A_1 , A_2 and $As. k\%$). The chromosome numbers are reported for four following species: *G. atuntsiensis* W. W. Smith $2n = 36$, *G. rigescens* Franch. ex Hemsl. $2n = 36$, *G. pseudoaquatica* Kusnez. $2n = 40$, and *G. squarrosa* Ledeb. $2n = 76$. The karyotype evolution is distinctly correlated with geographical distribution progression. The more leaves the distribution centre, the more occur polyploidy species.

Key words: *Gentiana*; chromosome number; karyotype; polyploid; geographical distribution.

The project was supported by the National Natural Sciences Foundation of China.

Received 13 November 1995.

Gentiana, a large genus including about 361 species, is widely distributed all over the world except in Africa. There are 247 species in China. Since last half century, *Gentiana* has been studied cytologically. However, most studies are concentrated on the accumulation of chromosome number counts, and only a few on karyology, such as those by Shigenbu (1984) and Yuan & Kupfer (1993). The karyotypes of five species and the chromosome number counts of four species are recorded for the first time in the present paper.

Materials and Methods

The materials used in this study were seeds of the *Gentians*. The seeds and the voucher specimens were collected at the same places of the field, and the specimens all were conserved in the herbarium of Northwest Plateau Institute of Biology, the Chinese Academy of Sciences (HNWP).

The seeds were germinated in petri dishes on wet filter paper at room temperature. Root tips were pretreated in 0.002 mol/L 8-hydroxyquinoline for 15—20 hours at 4°C and then fixed with carnoy's solution (100% alcohol : glacial acetic acid = 3 : 1) for at least four hours. After washing out the fixer completely with water, the root tips were macerated in 1 mol/L HCL at 60°C for 4—5 minutes, stained and squashed in a dilute solution of Carbol's fuchsing.

The chromosome numbers were counted from examined 50—100 cells for each species. The detailed karyotype analyses were microphotographed and measured from at least five cells. The observations of the chromosomes were made at mitotic metaphase. The terminologies used for centromere positions are those defined by Leven et al. (1964). The calculated method of chromosome complement of relative length (I. R. L.) used by Kuo et al. (1972), the classification of karyotype asymmetry of Stebbins (1971), and the intrachromosomal asymmetry index (A_1) and the interchromosomal asymmetry index (A_2) proposed by Romero Zarco (1986) were followed.

Results

The measurements of the chromosomes in five species of *Gentiana* are listed in Table 1 and the karyotype analyses of these species in Table 2. The somatic chromosomes and karyograms are illustrated in Plate I and II.

The results are described as follows:

1. *Gentiana yunnanensis* Franch. (Sect. *Microsperma* T. N. Ho)

The somatic chromosomes are $2n=14$, $2x$. The karyotype consists of six pairs of metacentric chromosomes and one pair of exact metacentric chromosomes so the karyotype formula is $K(2n)=14=12m+2M$. The chromosome complement of relative length has four pairs of longer chromosomes and three pairs of shorter chromosomes. It can be formulated into $2n=14=8M_2+6M_1$. The length sum of the genome is of $15.22\mu\text{m}$ with

the length range from 1.80 to 2.49 μm . The ratio of the longest chromosome to the shortest chromosome is 1.38 and the proportion of chromosome with arm ratio >2 is

Table 1 Measurements of the chromosomes at mitotic metaphase in 5 species of *Gentiana*

表 1 5种龙胆属植物染色体参数

Taxa 种类	No 编号	Length(μm)长度			R. L. 相对长度	I. R. L. 相对长度系数	C. R. L. 相对长度组分	Arm ratio 臂比	Classif- ications 类型
		Short arm 短臂	Long arm 长臂	Total 总长					
<i>G. yunnanensis</i> 云南龙胆	1	1.20	1.29	2.49	16.36	1.14	M ₂	1.08	m
	2	1.12	1.39	2.48	16.29	1.14	M ₂	1.21	m
	3	1.00	1.25	2.25	14.78	1.03	M ₂	1.25	m
	4	1.12	1.12	2.24	14.72	1.03	M ₂	1.00	M
	5	1.00	1.05	2.05	13.47	0.94	M ₁	1.10	m
	6	0.91	1.00	1.91	12.55	0.88	M ₁	1.10	m
	7	0.85	0.95	1.80	11.83	0.83	M ₁	1.12	m
<i>G. vernayi</i> 露蕊龙胆	1	1.08	1.84	2.92	10.10	1.32	L	1.70	m
	2	0.93	1.71	2.64	9.13	1.19	M ₂	1.83	sm
	3	1.25	1.38	2.63	9.09	1.18	M ₂	1.10	m
	4	1.04	1.58	2.62	9.06	1.18	M ₂	1.52	m
	5	1.13	1.36	2.49	8.61	1.12	M ₂	1.20	m
	6	1.08	1.34	2.42	8.37	1.09	M ₂	1.24	m
	7	1.12	1.19	2.31	7.99	1.04	M ₂	1.06	m
	8	1.04	1.11	2.15	7.43	0.97	M ₁	1.07	m
	9	0.88	1.09	1.97	6.82	0.89	M ₁	1.24	m
	10	0.76	1.12	1.88	6.50	0.85	M ₁	1.47	m
	11	0.72	1.13	1.85	6.40	0.83	M ₁	1.57	m
	12	0.74	0.88	1.62	5.60	0.73	S	1.19	m
	13	0.62	0.80	1.42	4.91	0.64	S	1.29	m
<i>G. aristata</i> 刺芒龙胆	1	1.05	1.43	2.48	12.96	1.17	M ₂	1.36	m
	2	1.00	1.46	2.46	12.85	1.16	M ₂	1.46	m
	3	1.00	1.26	2.26	11.81	1.06	M ₂	1.26	m
	4	0.98	1.27	2.25	11.76	1.06	M ₂	1.30	m
	5	1.00	1.01	2.01	10.50	0.95	M ₁	1.01	m
	6	0.98	1.02	2.00	10.45	0.94	M ₁	1.04	m
	7	0.98	1.00	1.98	10.34	0.93	M ₁	1.02	m
	8	0.96	0.96	1.92	10.03	0.90	M ₁	1.00	M
	9	0.89	0.89	1.78	9.30	0.84	M ₁	1.00	M

Table 1(continued)表1(续)

Taxa 种类	No 编号	Length(μ m)长度			R. L.	I. R. L.	C. R. L.	Arm	Classif- ications 类型
		Short arm 短臂	Long arm 长臂	Total 总长	相对 长度	相对长 度系数	相对长 度组分	臂比	
<i>G. haynaldii</i> 钻叶龙胆	1	0.86	1.70	2.56	14.47	1.30	M ₂	1.98	sm
	2	0.96	1.40	2.36	13.34	1.20	M ₂	1.46	m
	3	0.84	1.24	2.08	11.76	1.06	M ₂	1.48	m
	4	0.92	1.08	2.00	11.31	1.02	M ₂	1.17	m
	5	0.92	0.98	1.90	10.74	0.96	M ₁	1.07	m
	6	0.86	0.94	1.80	10.18	0.91	M ₁	1.12	m
	7	0.86	0.90	1.76	9.95	0.89	M ₁	1.05	m
	8	0.76	0.87	1.63	9.21	0.83	M ₁	1.14	m
	9	0.64	0.96	1.60	9.04	0.81	M ₁	1.50	m
<i>G. burkii</i> 条纹龙胆	1	0.96	1.77	2.73	8.65	1.38	M ₂	1.85	sm
	2	0.95	1.41	2.36	7.48	1.20	M ₂	1.49	m
	3	0.96	1.36	2.32	7.35	1.18	M ₂	1.42	m
	4	0.72	1.55	2.27	7.20	1.15	M ₂	2.16	sm
	5	0.96	1.27	2.23	7.07	1.13	M ₂	1.33	m
	6	0.86	1.32	2.18	6.91	1.11	M ₂	1.53	m
	7	0.82	1.32	2.14	6.78	1.08	M ₂	1.61	m
	8	0.68	1.23	1.91	6.06	0.97	M ₁	1.81	sm
	9	0.91	0.95	1.86	5.89	0.94	M ₁	1.05	m
	10	0.90	0.92	1.82	5.77	0.92	M ₁	1.03	m
	11	0.86	0.91	1.77	5.61	0.90	M ₁	1.06	m
	12	0.82	0.91	1.73	5.48	0.88	M ₁	1.11	m
	13	0.82	0.86	1.68	5.33	0.85	M ₁	1.05	m
	14	0.77	0.82	1.59	5.04	0.81	M ₁	1.07	m
	15	0.64	0.91	1.55	4.91	0.79	M ₁	1.42	m
	16	0.64	0.77	1.41	4.47	0.72	S	1.20	m

R. L. = relative length; I. R. L. = index of relative length C. R. L. = complement of relative length

R. L. = 相对长度 I. R. L. = 相对长度系数 C. R. L. = 相对长度组分

0.00. According to Stebbins' classification, this kind of karyotype belongs to type 1A. The karyotype asymmetry indices are the intrachromosomal asymmetry index $A_1 = 0.0984$, the interchromosomal asymmetry index $A_2 = 0.123$, and the karyotype asymmetry index $As. k\% = 52.7$. Therefore, the karyotype of *G. yunnanensis* is symmetrical with the low asymmetry indices and all chromosomes are very similar. [Plate I: A, I: J]

Table 2 The karyotype analyses of 5 species in *Gentiana*

表 2 5 种龙胆属植物核型分析

Taxa 种类	Karyotypic formula & chromosome complement of relative length	L/S	P>2	Karyotype asymmetry			
				Type	A1	A2	As. k%
<i>G. yunnanensis</i> 云南龙胆	2n=14 =12m+2M =8M ₂ +6M ₁	1.38	0.00	1A	0.098	0.123	52.7
<i>G. vernayia</i> 露蕊龙胆	2n=26 =24m+2sm =2L+12M ₂ +8M ₁ +4S	2.06	0.00	1B	0.235	0.202	57.2
<i>G. aristata</i> 刺芒龙胆	2n=18 =14m+4M =8M ₂ +10M ₁	1.39	0.00	1A	0.121	0.116	53.8
<i>G. haynaldii</i> 钻叶龙胆	2n=18 =16m+2sm =8M ₂ +10M ₁	1.60	0.00	1A	0.214	0.165	56.9
<i>G. burkii</i> 条纹龙胆	2n=32 =26m+6sm =14M ₂ +16M ₁ +2S	1.94	0.19	2A	0.239	0.182	57.9

L=length of the longest chromosome; S=length of the shortest chromosome; P>2=proportion of chromosome with arm ratio>2; A₁=the intrachromosomal asymmetry index defined by Romero Zarco(1986); A₂=the interchromosomal asymmetry index defined by Romero Zarco (1986); As. K%=index of the karyotype asymmetry defined by Hiso Arano (1963).

L, 最长染色体的长度; S, 最短染色体的长度; P>2, 臂比大于2的染色体比例; A₁, 染色体之间的不对称系数; A₂, 染色体之内的不对称系数; AS. K%, 核型不对称系数。

The seed materials were collected from Huadianba of Tali Canshan in Yunnan province, on wet meadow beside the stream, alt. 2950 m, 19 October 1989, T. N. Ho 1369.

2. *Gentiana vernayi* Marquand (Sect. *Microsperma* T. N. Ho)

The somatic chromosomes are 2n=26, 2X. The karyotype consists of twelve pairs of metacentric chromosomes and one pair of submetacentric chromosomes so the karyotype formula is K(2n) = 26 = 24m + 2sm. The chromosome complement of relative length has one pair of long chromosomes, six pairs of longer chromosomes and four pairs of shorter chromosomes and two pairs of short chromosomes. It can be expressed as 2n = 26 = 2L + 12M₂ + 8M₁ + 4S. The length sum of the genome is of 28.92 μm with the length range from 1.42 to 2.92 μm. The ratio of the longest chromosome to the shortest chromosome is 2.06 and the proportion of chromosome with arm ration > 2 is 0.00. Thus, the classification of the karyotype belongs to Stebbins' type 1B. The karyotype asymmetry indices are the intrachromosomal asymmetry index A₁=0.235, the interchromosomal asymmetry index A₂=0.202 and the karyotype asymmetry index As. k%=57.2. Therefore, the karyotype of *G. vernayi* is symmetrical with high asymmetry in-

dices. [Plate I : E, I : N]

The studied materials were collected from Beima snow-mountain of Deqin hsien in Yunnan province, on meadow, alt. 4300 m, 1 October 1989, T. N. Ho 1299.

3. *Gentiana burkii* H. Smith (Sect. Chondrophylla Bge.)

The somatic chromosomes are $2n=32, 4\times$. The karyotype consists of thirteen pairs of metacentric chromosomes and three pairs of submetacentric chromosomes so the karyotype formula is $K(2n) = 32 = 26m + 6sm$. The chromosome complement of relative length has seven pairs of longer chromosomes, eight pairs of shorter chromosomes and one pair of short chromosomes, and it can be formulated into $2n=32=14M_2+16M_1+2S$. The length sum of the genome is $30.83 \mu\text{m}$ with the length range from 1.41 to $2.73 \mu\text{m}$. The ratio of the longest chromosome to the shortest chromosome is 1.94 and the proportion of chromosome with arm ratio > 2 is 0.19 . Thus, classification of the karyotype belongs to Stebbins' type 2A. The karyotype asymmetry indices are the intrachromosomal asymmetry index $A_1=0.239$, the interchromosomal asymmetry index $A_2=0.182$ and the karyotype asymmetry index $As. k\%=57.9$. Therefore the karyotype of *G. burkii* is asymmetrical. [Plate I : D, I : M]

The studied materials were collected from Gucao xien of Tingri hsien in southwestern Xizang, on meadow, alt. 4500 m, 15 September 1992, J. Q. Liu 77.

4. *Gentiana aristata* Maxim. (Sect. Chondrophylla Bge.)

The somatic chromosomes are $2n=18, 2\times$. The karyotype consists of seven pairs of metacentric chromosomes and two pairs of exact metacentric chromosomes so the karyotype formula is $K(2n) = 18 = 14m + 4M$. The chromosome complement of relative length has four pairs of longer chromosomes and five pairs of shorter chromosomes and it can be expressed as $2n=18=8M_2+10M_1$. The length sum of the genome is $19.14 \mu\text{m}$ with the length range from 1.78 to $2.48 \mu\text{m}$. The ratio of the longest chromosome to the shortest chromosome is 1.39 and the proportion of chromosome with arm ratio > 2 is 0.00 . The karyotype classification hence belongs to Stebbins' type 1A. The karyotype asymmetry indices are the intrachromosomal asymmetry index $A_1=0.121$, the interchromosomal asymmetry index $A_2=0.116$ and the karyotype asymmetry index $As. k\%=53.8$. The karyotype of *G. aristata* is symmetrical with low asymmetry indices. [Plate I : B, I : K]

The studied material were collected from Ra'gya xien of Tongde hsien in Qinghai province, alt. 3160 m, 11 August 1990, X. F. Lu 014.

5. *Gentiana haynaldii* Kanitz (Sect. Dolichocarpa T. N. Ho)

The somatic chromosomes are $2n=18, 2\times$. The karyotype consists of eight pairs of metacentric chromosomes and one pair of submetacentric chromosomes so the karyotype

formula is $K(2n) = 18 = 16m + 2sm$. The chromosome complement of relative length has four pairs of longer chromosomes and five pairs of shorter chromosomes. It can be expressed as $2n = 18 = 8M_2 + 10M_1$. The length sum of the genome is $17.69 \mu\text{m}$ with the length range from 1.60 to $2.56 \mu\text{m}$. The ratio of the longest chromosome to the shortest chromosome is 1.60 and the proportion of chromosome with arm ratio > 2 is 0.00 . The karyotype classification hence belongs to Stebbins' type 1A. The karyotype asymmetry indices are the intrachromosomal asymmetry index $A_1 = 0.214$, the interchromosomal asymmetry index $A_2 = 0.165$ and the karyotype asymmetry index $As. k\% = 56.9$. The karyotype of *G. aristata* is subsymmetrical with low asymmetry indices (A_2 and $As. k\%$). [Plate I : C, I : L]

The studied materials were collected from nearby city of Zhongdian hsien in Yunnan province, on meadow, alt. 3300 m , 26 September 1989, T. N. Ho 1233.

The chromosome counts are reported for the following species:

6. *Gentiana atuntsiensis* W. W. Smith (Sect. *Frigida* Kusnez.)

The root tip cells from germinated seeds had the chromosome numbers of $2n = 36, 6 \times$. [Plate I : I]

The studied materials were collected from Beima snow-mountains of Deqin hsien in Yunnan province, among *Rhododendron* shrubs, alt. 4400 m , 1 October 1989, T. N. Ho 1281.

7. *Gentiana rigescens* Franch. ex Hemsl. (Sect. *Monopodiae* T. N. Ho)

The root tip cells from germinated seeds had the chromosome numbers of $2n = 36, 6 \times$. [Plate II : G]

The studied materials were collected from Kunming in Yunnan province, on grassland, alt. 2000 m , 27 October 1989, T. H. Ho 1222.

8. *Gentiana pseudoaquatica* Kusnez. (Sect. *Chondrophylla* Bge.)

The root tip cells from germinated seeds had the chromosome numbers of $2n = 40, 4 \times$. [Plate I : H]

The studied materials were collected from around Kokonor Lake (Qinghai Lake), on sandy place, alt. 3200 m , 19 August 1989, X. F. Lu 129.

9. *Gentiana squarrosa* Ledeb. (Sect. *Chondrophylla* Bge.)

The root tip cells from germinated seeds had the chromosome numbers of $2n = 76, 4 \times$. [Plate I : F]

The studied materials were collected from Ra' gya xien of Tongde hsien in Qinghai province, alt. 3160 m , 7 August 1990, X. F. Lu 016.

Discussion

The karyotype evolution in *Gentiana* is distinctly correlated with geographical distribution progression. It mainly shows on the chromosome polyploid from low to high. From the phytogeographical data, the major distribution centre of the genus *Gentiana* oc-

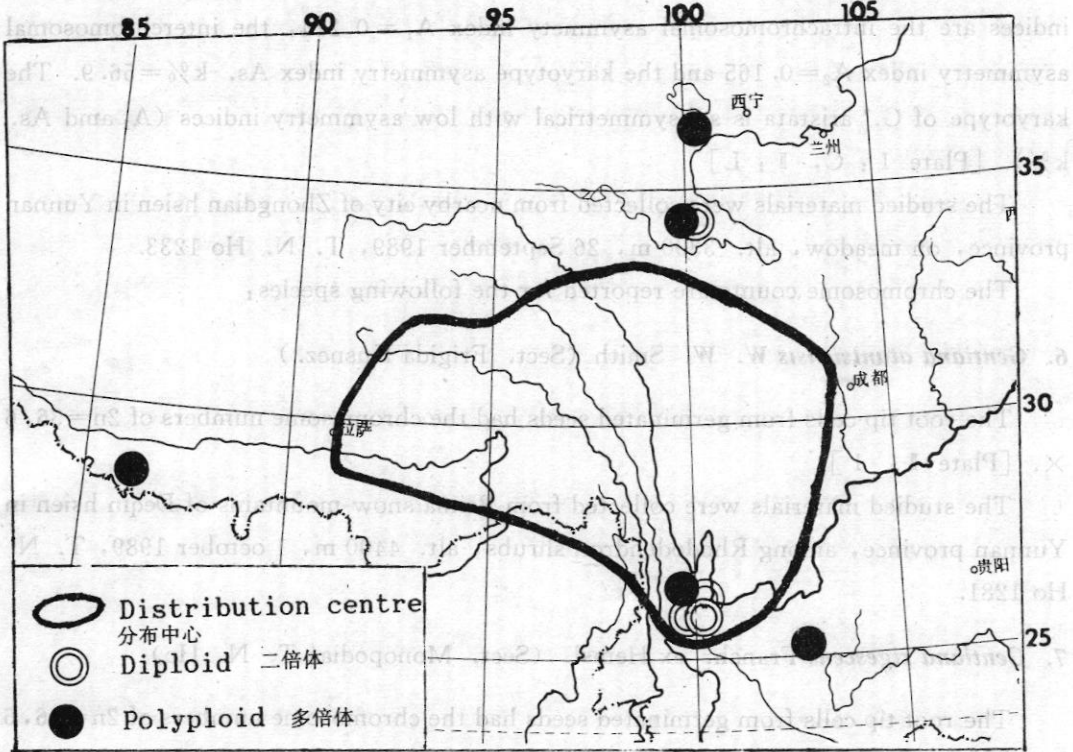


Fig. 1 The chromosome distribution map in 9 species of *Gentiana* shows the correlation between the ploidy level and the geographical distribution progression.

图1 9种龙胆属植物染色体地理分布图，示染色体倍性与地理分布递渐的相关性

curs in the mountains of SW. China (NW. Yunnan, W. Sichuan and SE. Xizang) and in NE. Burma area, the square between 25°—34° N and 91°—105°E. The chromosome ploidy levels of most species in this centre are diploids. However, the more leaves this centre, the more occur polyploids. It can be indicated by the correlation in the present paper. Among four studied species in NW. Yunnan, three of four species (*G. yunnanensis*, *G. vernayi* and *G. haynaldii*) are diploids, and only one (*G. atunsiensis*) is a hexaploid with $2n=36$. Outside this distribution centre, four of five species (*G. pseudoaquatica*, *G. burkii*, *G. squarrosa* and *G. rigescens*) are polyploids and only one (*G. aristata*) is a diploid. Moreover, this hexaploid (*G. atunsiensis*) of distribution centre grows in the high mountain of alt. 4400 m whereas this diploid (*G. aristata*) outside distribution centre in relatively low mountain of alt. 3160 m. [Fig. 1]

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中国西部高山的9种龙胆属植物的 染色体数目和核型*

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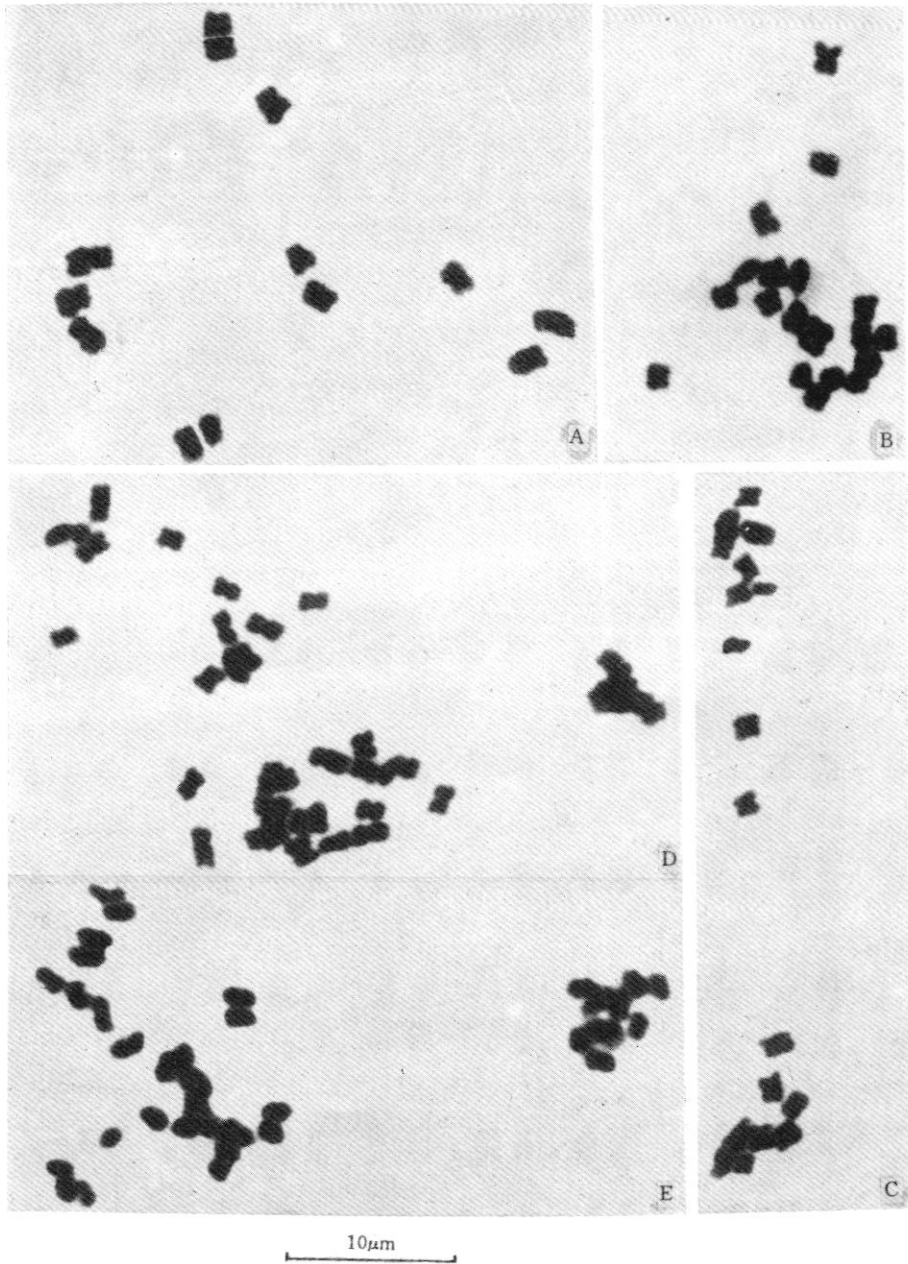
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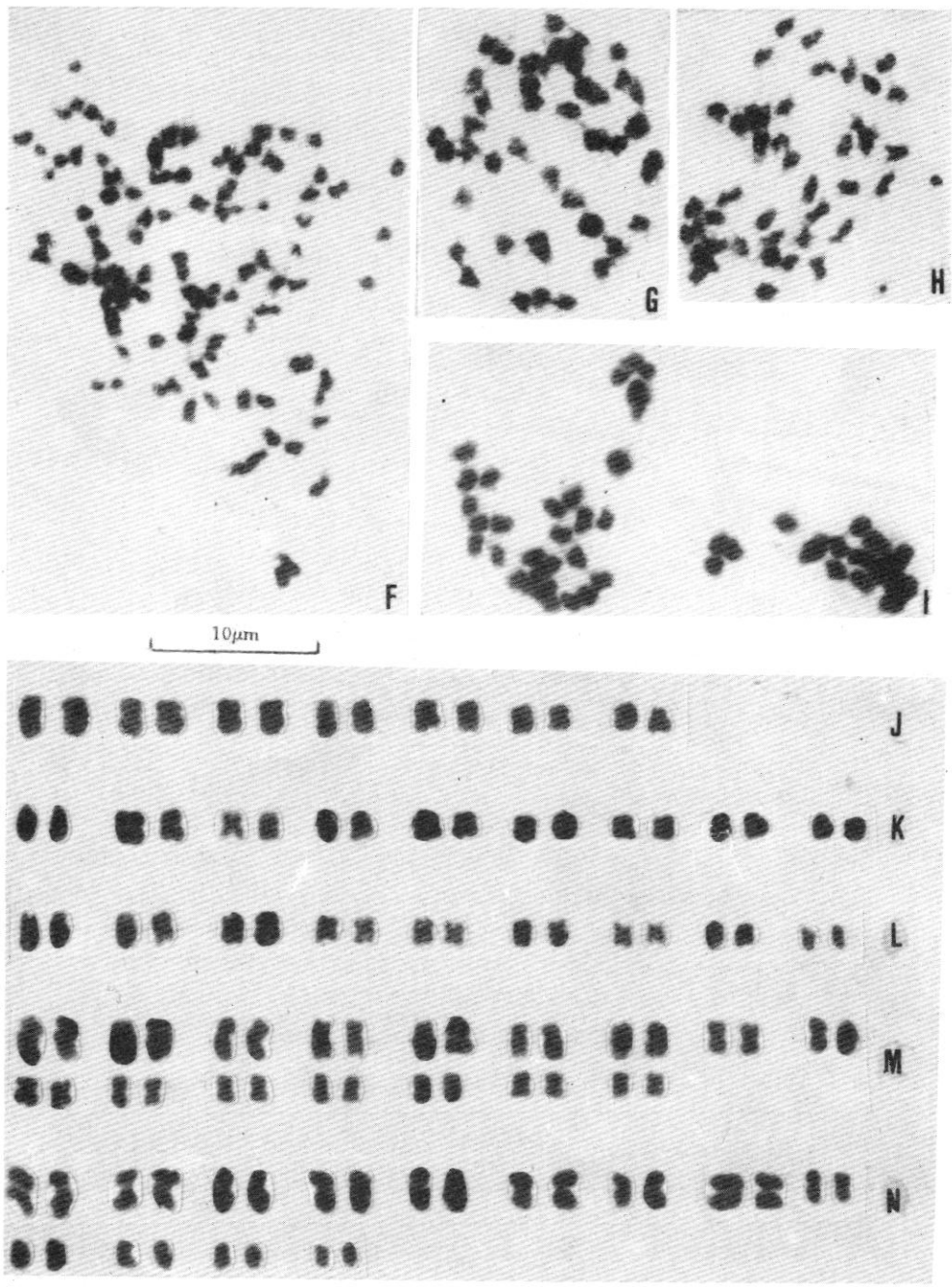
报道了中国西部高山的龙胆属9种植物的核型和染色体数目。这些染色体资料均属首次报道。其中, 云南龙胆 *Gentiana yunnanensis* Franch. 核型公式及相对长度组成为 $K(2n) = 14 = 12m + 2M = 8M_2 + 6M_1$; 刺芒龙胆 *G. aristata* Maxim. $K(2n) = 18 = 14m + 4M = 8M_2 + 10M_1$; 钻叶龙胆 *G. haynaldii* Kanitz $K(2n) = 18 = 16m + 2sm = 8M_2 + 10M_1$; 3种植物的核型均属于 Stebbins (1971) 的 1A 类型, 为对称或近于对称的核型, 具有低的不对称系数 (A_1 , A_2 和 $AS.K\%$)。条纹龙胆 *G. burkii* H. Smith 和露蕊龙胆 *G. vernayi* Marq. 与上面3种植物的核型差异较大。前者的核型与相对长度组成为 $K(2n) = 32 = 26m + 6sm = 14M_2 + 16M_1 + 2S$, 后者为 $K(2n) = 26 = 24m + 2sm = 2L + 12M_2 + 8M_1 + 4s$, 它们的核型分别属于 Stebbins (1971) 的 2A 或 1B 类型, 不对称系数 (A_1 , A_2 和 $AS.K\%$) 较高, 为不对称性核型。下述4个种的染色体数目: *G. atunsiensis* W. W. Smith, $2n = 36$; *G. rigescens* Franch. ex Hemsl., $2n = 36$; *G. pseudoaquatica* Kusnez., $2n = 40$; *G. squarrosa* Ledeb., $2n = 76$ 。龙胆属的核型进化明显与地理分布的递渐成正相关, 即愈离开地理分布中心, 愈出现多倍体种类。

关键词: 龙胆属; 染色体数目; 核型; 多倍体; 地理分布

* 国家自然科学基金资助项目。
本文1995年11月13日收到。



5 种龙胆属植物染色体中期分裂相. A. 云南龙胆; B. 刺芒龙胆; C. 钻叶龙胆; D. 条纹龙胆; E. 露蕊龙胆.
Mitotic metaphase chromosomes in 5 species of *Gentiana*. A. *Gentiana yunnanensis*, B. *G. aristata*, C. *G. haynaldii*, D. *G. burkii*, E. *G. vernayi*.



9种龙胆属植物体细胞染色体. F—I, 示以下植物的染色体数目: F. 鳞叶龙胆; G. 滇龙胆草; H. 假水生龙胆; I. 阿墩子龙胆; J—N, 示核型: J. 云南龙胆; K. 刺芒龙胆; L. 钻叶龙胆; M. 条纹龙胆; N. 露蕊龙胆.
Somatic chromosome of 9 species in *Gentiana*. F—I, showing the chromosome numbers; F. *G. squarrosa*, G. *G. rigescens*, H. *G. pseudoaquitica*, I. *G. atuntsiensis*; J—N, showing karyograms; J. *G. yunnanensis*, K. *G. aristata*, L. *G. haynaldii*, M. *G. burkii*, N. *G. vernayi*.