

蜘蛛抱蛋属植物的核型不对称性分析*

乔琴^{1,2}, 张长芹^{1**}, 马永鹏^{1,2}, 田伟^{1,2}

(1 中国科学院昆明植物研究所, 云南 昆明 650204; 2 中国科学院研究生院, 北京 100049)

摘要: 报道了蜘蛛抱蛋属 (*Aspidistra*) 两种植物的染色体数目和核型, 其中河口蜘蛛抱蛋 (*A. hekouensis*) 的染色体数目 ($2n = 38$) 为首次报道, 四川蜘蛛抱蛋 (*A. sichuanensis*) 染色体数目也为 $2n = 38$, 但其核型与以往的报道有差别。使用染色体内不对称系数 (A_1) 和染色体间不对称系数 (A_2) 对该属 34 种植物核型的不对称性进行了分析, 结果表明该属植物的核型似乎并没有向不对称性增强的方向演化。

关键词: 蜘蛛抱蛋; 核型不对称性; 染色体内不对称系数; 染色体间不对称系数

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Karyotype Asymmetry of *Aspidistra* (Convallarieae, Ruscaceae)

QIAO Qin^{1,2}, ZHANG Chang-Qin^{1**}, MA Yong-Peng^{1,2}, TIAN Wei^{1,2}

(1 Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China;

2 Graduate University of Chinese Academy of Sciences, Beijing 100049, China)

Abstract: Two species of *Aspidistra* from Yunnan, China, were cytologically studied. The chromosome number of *A. hekouensis*, $2n = 38$, is reported for the first time. The chromosome number of *A. sichuanensis*, also $2n = 38$, was consistent with earlier reports, but the karyotype differs from the previous report. Furthermore, we used two numerical parameters, A_1 (intrachromosomal asymmetry index) and A_2 (interchromosomal asymmetry index), to estimate the karyotype asymmetry in 34 species of. The results showed that there is no predominant trend towards an increasing of asymmetry of the karyotype in *Aspidistra*.

Key words: *Aspidistra*; Karyotype asymmetry; Intrachromosomal asymmetry index; Interchromosomal asymmetry index

The genus *Aspidistra*, formerly treated in the Convallariaceae, is now in the family Ruscaceae (Rudall *et al.*, 2000; APGII, 2003). Species in this genus are mostly distributed in South-east Asia. The total number of species in *Aspidistra* is 76 species (Tillich, 2005), 51 of which are known from China. According to cytological data of 38 species, two different basic chromosome numbers appear within the genus: $x = 18$ and $x = 19$, and both of them approximately encompasses 50% species in the published data (Wang *et al.*, 2001). There has been debate about which basic

number is ancestral one, that is, chromosome number has increased or decreased during evolution in *Aspidistra* (Hong *et al.*, 1986; Huang *et al.*, 1997; Wang *et al.*, 2001; Yamashita and Tamura, 2004). Based on the analysis of karyological asymmetry and morphological characters, Wang (2001) proposed that species in $x = 18$ were ancestor and have higher karyotype asymmetry than species in $x = 19$, but their result was lack of quantitative analysis. On the contrary, we consider it would be more reasonable to assume that species in $x = 18$ of *Aspidistra* was derived from an ancestral $x = 19$

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** Author for correspondence; E-mail: zhangchangqin@mail.kib.ac.cn

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作者简介: 乔琴 (1981-) 女, 在读博士研究生, 主要从事开口箭的保护生物学研究。

according to the result of Yamashita and Tamura (2004). Romero (1986) proposed an alternative method for measuring the karyotype asymmetry by using quantification and graphic representation, and two numerical parameters (A_1 = intrachromosomal asymmetry index and A_2 = interchromosomal asymmetry index) were introduced in analyzing patterns of karyotype asymmetry.

In the present study we undertook a cytotoxic analysis of *A. hekouensis* and *A. sichuanensis* in Yunnan, China, and analysed the karyotype asymmetry of *Aspidistra* following Romero's method, and discussed the evolutionary trends of karyotype asymmetry in the genus.

Materials and Methods

Aspidistra sichuanensis and *A. hekouensis* were collected from the Botanical Garden of Kunming Institute of Botany (Table 1). Voucher specimens are deposited in the Herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences (KUN). Cells of root tips were used for chromosome count and karyotype analysis. Root tips were pretreated in 2 mmol/L hydroxyquinoline at room temperature for 4-5 hours, and then fixed in Carnoy solution (ethanol:acetic acid=3:1) at 4 °C for 18 hours. After maceration in 1N hydrochloric acid at 60 °C for 10 minutes, material was stained with Carbol Fuchsin and squashed for observation in 45% acetic acid. Five individuals were investigated for each species. Chromosome measurements were obtained from the photographs of the best 5 mitotic metaphase plates. Terminology for position of centromeres on chromosomes follows Levan *et al.* (1964), and karyotypes following the classification of Stebbins (1971).

The intrachromosomal (A_1) and interchromosomal (A_2) asymmetry indices were calculated according to Romero Zarco (1986). The data of relative chromosome length (including short arms and long arms) were obtained from previous cytological studies (Hong *et al.*, 1986; Huang *et al.*, 1997; Wang *et al.*, 1999; Wang *et al.*, 2000; Wang *et al.*, 2001).

Results

Aspidistra hekouensis

The species *A. hekouensis* was investigated for the first time. The chromosome number is $2n = 38$, with the karyotype formula of $22m + 2sm + 14st$. Pair 9th is sm-type chromosomes, pairs 2nd-8th are st-type, other pairs are m-type. No satellites were observed in our study. The chromosomes showed a trimodal variation in length. Pair 1st is long chromosomes, while the number of pairs of the medium and short chromosomes are 7 and 11 pairs respectively. The ratio of the longest to the shortest chromosomes is 4.63:1, and the karyotype symmetry is 2C (Table 2; Fig. 1: A, C).

Aspidistra sichuanensis

The chromosome number of *A. sichuanensis* is $2n = 38$, with the karyotype formula of $2n = 38 = 21m + 7sm + 12st$. Pairs 9th, 17th and one of pair 4th are sm-type chromosomes, pairs 2nd, 3rd, and 5th-8th are st-type, others are m-type. The chromosomes of the fourth pair differ significantly in their length, which have median centromeres (m-type) and submedian centromeres (sm-type) respectively. No satellites were observed in the present study. The chromosomes showed a trimodal variation in length. Pair 1st is long chromosomes, while the number of pairs of the medium and short chromosomes are 7 and 11 pairs respectively. The ratio of the longest to the shortest chromosomes is 6.13:1, and the karyotype symmetry is 2C (Table 2; Fig. 1: B, D). The karyotype of this species differed from those reported earlier (i.e., $2n = 38 = 24m + 4sm + 10st$ (2SAT) (Wang *et al.*, 2000), and $2n = 38 = 22m + 4sm$ (2SAT) + 12st (Hong *et al.*, 1986). $2n = 38 = 22m + 4sm$ (2SAT) + 12st + 5B, $2n = 38 = 20m + 6sm + 12st$ (2SAT) (Huang *et al.*, 1997).

The intrachromosomal asymmetry index (A_1) and the interchromosomal asymmetry index (A_2) were calculated based on the data available for 34 species (Table 3), including 16 species in $2n = 36$ and 19 species in $2n = 38$. A_1 varied from 0.41 to 0.51 in $2n = 36$ and 0.36 to 0.54 in $2n = 38$; A_2 varied from 0.51 to 0.66 in $2n = 36$ and 0.52 to 0.69 in $2n = 38$. The variance of

Table 1 Species of *Aspidistra* examined, their vouchers and karyotype formula

Species	Collection	Karyotype	Type
<i>Aspidistra hekouensis</i>	Hekou, Yunnan, China, Qiao qin 200702	$2n = 38 = 22m + 2sm$ (2SAT) + 14st	2C
<i>A. sichuanensis</i>	Kunming, Yunnan, China, Qiao qin 200703	$2n = 38 = 20m + 6sm$ (2SAT) + 12st	2C

Vouchers are deposited in the herbarium of the Kunming Institute of Botany (KUN)

Table 2 The parameters of chromosomes of *A. hekouensis* and *A. sichuanensis*

<i>Aspidistra sichuanensis</i>				<i>Aspidistra hekouensis</i>			
No.	Relative length	Ratio	Type	No.	Relative length	Ratio	Type
1	2.94 + 3.37 = 6.31	1.15	m	1	2.77 + 3.44 = 6.21	1.24	m
2	0.74 + 4.02 = 4.76	5.43	st	2	0.67 + 3.37 = 4.04	5.03	st
3	0.98 + 3.68 = 4.66	3.76	st	3	0.67 + 3.3 = 3.97	4.93	st
4	1.28 + 3.14 = 4.42	2.45	sm	4	0.67 + 3.24 = 3.91	4.84	st
5	0.64 + 3.39 = 4.03	5.30	st	5	0.67 + 3.03 = 3.7	4.52	st
6	0.64 + 3.24 = 3.88	5.06	st	6	0.67 + 2.9 = 3.57	4.33	st
7	1.07 + 2.80 = 3.73	2.62	sm	7	0.54 + 3.03 = 3.57	5.6	st
8	1.59 + 2.14 = 3.73	1.34	m	8	0.54 + 2.7 = 3.24	4.99	st
9	0.54 + 2.16 = 2.70	4.00	st	9	0.67 + 1.42 = 2.09	2.12	sm
10	0.59 + 1.42 = 2.01	2.41	sm	10	0.81 + 0.94 = 1.75	1.16	m
11	0.69 + 0.88 = 1.57	1.28	m	11	0.81 + 0.94 = 1.75	1.16	m
12	0.69 + 0.83 = 1.52	1.20	m	12	0.67 + 1.08 = 1.75	1.61	m
13	0.59 + 0.79 = 1.38	1.34	m	13	0.67 + 0.94 = 1.71	1.4	m
14	0.59 + 0.79 = 1.38	1.34	m	14	0.82 + 0.81 = 1.63	1.01	m
15	0.68 + 0.69 = 1.37	1.01	m	15	0.82 + 0.81 = 1.63	1.01	m
16	0.68 + 0.69 = 1.37	1.01	m	16	0.67 + 0.81 = 1.48	1.21	m
17	0.39 + 0.79 = 1.18	2.03	sm	17	0.67 + 0.74 = 1.41	1.1	m
18	0.54 + 0.79 = 1.33	1.46	m	18	0.68 + 0.67 = 1.35	1.01	m
19	0.49 + 0.54 = 1.03	1.10	m	19	0.68 + 0.67 = 1.35	1.01	m

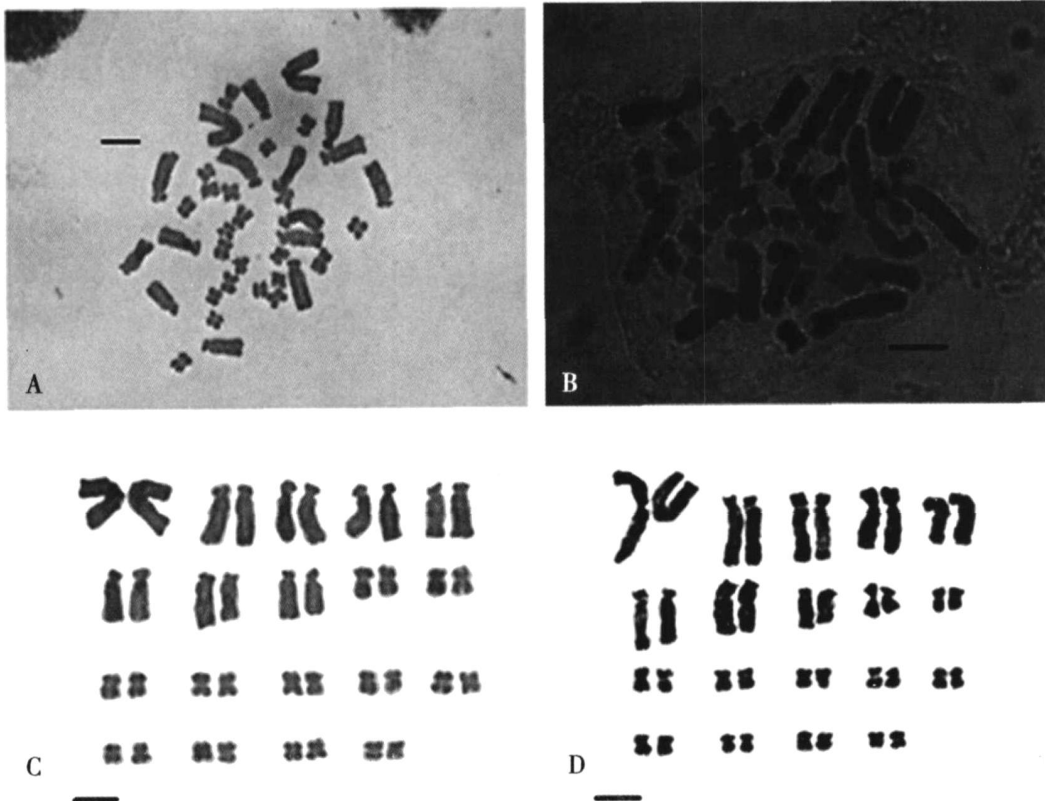


Fig. 1 Somatic metaphase chromosome and ideograms of somatic metaphase chromosome of two species in *Aspidistra*
A, C. *A. hekouensis*; B, D. *A. sichuanensis*. Bar = 5 μ m.

A_1 and A_2 between $2n = 36$ and $2n = 38$ were also analyzed. The results indicated that the mean value of A_1

in $2n = 36$ and $2n = 38$ are 0.46 ± 0.31 and 0.44 ± 0.48 respectively, which does not vary significantly (F

Table 3 Intrachromosomal asymmetry index (A_1) and interchromosomal asymmetry index (A_2) of *Aspidistra* species

Species	Chromosome counts	Stebbins types	A_1	A_2	References *	Species	Chromosome counts	Stebbins types	A_1	A_2	References *
<i>A. fungilliformis</i>	2n = 36	2C	0.48	0.59	Huang et al. 1997	<i>A. longiloba</i>	2n = 38	2C	0.39	0.59	Wang et al. 1999
<i>A. longipedunculata</i>	2n = 36	2C	0.43	0.51	Wang et al. 2000b	<i>A. marginella</i>	2n = 38	2C	0.44	0.64	Wang et al. 2001
<i>A. hainanensis</i>	2n = 36	2C	0.42	0.58	Wang et al. 2001	<i>A. leshanensis</i>	2n = 38	2C	0.45	0.69	Hong et al. 1986
<i>A. claviformis</i>	2n = 36	2C	0.42	0.59	Wang et al. 2000a	<i>A. subrotata</i>	2n = 38	2C	0.43	0.62	Huang et al. 1997
<i>A. yingjiangensis</i>	2n = 36	2C	0.51	0.55	Wang et al. 2001	<i>A. hekouensis</i>	2n = 38	2C	0.40	0.52	the present study
<i>A. triloba</i>	2n = 36	2C	0.47	0.60	Huang et al. 1997	<i>A. patentiloba</i>	2n = 38	2C	0.41	0.61	Wang et al. 2000
<i>A. dolichanthera</i>	2n = 36	2C	0.50	0.60	Huang et al. 1997	<i>A. longanensis</i>	2n = 38	2C	0.43	0.62	Wang et al. 1999
<i>A. ebianensis</i>	2n = 36	2C	0.45	0.62	Wang et al. 2001	<i>A. fimbriata</i>	2n = 38	2C	0.44	0.64	Huang et al. 1997
<i>A. muricata</i>	2n = 36	2C	0.45	0.58	Wang et al. 2001	<i>A. oblanceifolia</i>	2n = 38	2C	0.49	0.63	Hong et al. 1986
<i>A. lurida</i>	2n = 36	2C	0.51	0.61	Wang et al. 2000a	<i>A. zongbaya</i>	2n = 38	2C	0.45	0.66	Hong et al. 1986
<i>A. cavicola</i>	2n = 36	2C	0.47	0.61	Wang et al. 2000a	<i>A. flaviflora</i>	2n = 38	3C	0.54	0.56	Hong et al. 1986
<i>A. retusa</i>	2n = 36	2C	0.48	0.55	Huang et al. 1997	<i>A. omeiensis</i>	2n = 38	2C	0.48	0.60	Hong et al. 1986
<i>A. elator</i>	2n = 36	2C	0.41	0.66	Huang et al. 1997	<i>A. caespitosa</i>	2n = 38	2C	0.49	0.68	Hong et al. 1986
<i>A. tonkinensis</i>	2n = 36	2C	0.48	0.61	Huang et al. 1997	<i>A. luodianensis</i>	2n = 38	2C	0.51	0.66	Wang et al. 2000
<i>A. saxicola</i>	2n = 36	2C	0.47	0.61	Wang et al. 2001	<i>A. minutiflora</i>	2n = 38	2C	0.51	0.61	Huang et al. 1997
<i>A. hexianensis</i>	2n = 36	2C	0.47	0.55	Huang et al. 1997	<i>A. cruciformis</i>	2n = 76	2C	0.36	0.60	Wang et al. 2001
<i>A. sichuanensis</i>	2n = 38	2C	0.42	0.63	Huang et al. 1997	<i>A. xilinensis</i>	2n = 76	2C	0.40	0.65	Wang et al. 2001

* Data used in this paper to calculate A_1 and A_2 obtained from cited cytological studies.

= 1.87, $P = 0.181 > 0.05$) between the two groups. As the scatter diagram shows all of the species grouped closely together along the A_1 axis (Fig. 2). However, it should be noted that the mean value of A_2 in $2n = 36$ and $2n = 38$ are 0.59 ± 0.36 and 0.62 ± 0.40 respectively. The variance are significant ($F = 6.86$, $P = 0.013 < 0.05$) between the two groups, which indicated the karyotypes of $2n = 36$ tend to present lower interchromosomal (A_2) asymmetry than those of $2n = 38$. As scatter diagram shows most of the species in $2n = 38$ distributed higher than species in $2n = 36$ along A_2 axis (Fig. 2).

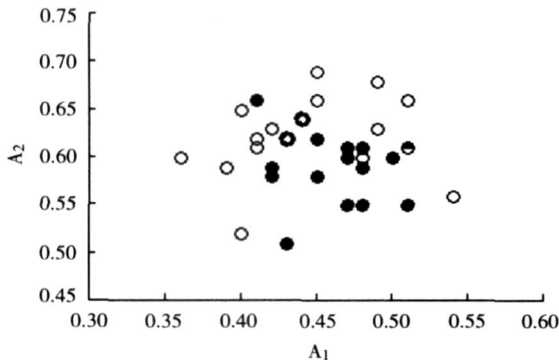


Fig. 2 Scatter diagram showing karyotype asymmetry due to ratio between arm length (A_1) against that due to variation between chromosome total lengths (A_2). The species in group $2n = 38$ in *Aspidistra* (○); the species in $2n = 36$ in *Aspidistra* (●)

Discussion

Two species of *Aspidistra* examined by us exhibited trimodal karyotype as other species reported in *Aspidistra*. In contrast to an earlier report, in which satellites were considered as a stable character in *Aspidistra* (Huang et al., 1997), no satellites were observed in our study. *Aspidistra* is essentially a dibasic genus with $x = 18$ and $x = 19$. Although there has been debate about which basic number is ancestral one, we consider it is reasonable to assume that the $x = 19$ of *Aspidistra* may have been derived from an ancestral $x = 18$ according to the phylogenetic analyses of Yamashita and Tamura (2004).

According to the graphical and quantitative results (Table 3), we can see the variation of intrachromosomal asymmetry index (A_1) was relatively small between the two groups, whereas the interchromosomal asymmetry index (A_2) in $x = 18$ is significantly ($F = 6.86$, $P = 0.013 < 0.05$) lower than $x = 19$. Our results showed that the ancestral group has higher karyotype asymmetry than the derived group. It seems that the tendency for an increase in karyotype asymmetry suggested by Stebbins didn't present in *Aspidistra*. Similar trends of karyotype asymmetry evolution have been reported in gymnosperm and other monocotyledons, such as Podocarpaceae, Cycadaceae (Zheng et al., 2002)

and Orchidaceae (Li *et al.*, 2003). Wang (2001) also considered that evolution trends of karyotype in *Aspidistra* seemly don't agree with the tendency of an increase in karyotype asymmetry, but they got the converse opinion that species in $x = 18$ have higher karyotype asymmetry than species in $x = 19$ based on the frequency of m-type chromosomes without quantifiable analysis. In the present paper, only 34 species out of 76 species were analyzed, more species should be studied to obtain authentic phylogenetic relationships and reasonable chromosome evolution hypothesis in the near future.

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