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Karyomorphology of *Incarvillea* (Bignoniaceae) and its implications in distribution and taxonomy

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The karyomorphology of 11 species of the genus *Incarvillea* Juss. is reported. The chromosome numbers of all species studied are $2n = 22$. The interphase nuclei and prophase chromosomes were found to be of the simple chromocentre type and the interstitial type, respectively. The asymmetry of the karyotype of *I. arguta* (two populations) in subgenus *Amphicome* is type 2A. The karyotypes of *Incarvillea sinensis* var. *sinensis*, *I. sinensis* var. *przewalskii,* and *I. olgae* in subgenus *Incarvillea* are of asymmetry type 3A. The remaining nine species and one variety in subgenus *Pteroscleris* are also of asymmetry 3A. Data on three species and one variety studied are first reports. This study indicates that karyotype variation at the diploid level appears to be the predominant feature of chromosome evolution in the genus *Incarvillea*. According to this study of karyomorphology, morphological characteristics and geographical distribution, it seems that the three subgenera should be regarded as three independent genera. The geography of the genus is discussed.

ADDITIONAL KEYWORDS: China – chromosome – diploid.

INTRODUCTION

Incarvillea Juss. is a small genus in Bignoniaceae. It is composed of 15 species distributed mainly in Eastern and Central Asia. There has been some confusion in the taxonomy of the genus, the key point being the systematic position of subgenera *Amphicome* (Royle) R. Br. apud Royle and *Niedzwedzkia* (B. Fedtsch.) Grierson. Grierson published his revision of this genus (Grierson, 1961), recognizing four subgenera, which are *Amphicome* (Royle) R. Br. apud Royle, *Incarvillea*, *Pteroscleris* Bailon, and *Niedzwedzkia* (B. Fedtsch.) Grierson. Subgenus *Amphicome* includes *I. arguta* (Royle) Royle and *I. emodi* (Lindl.) Chatterjee. Subgenus *Incarvillea* includes *I. olgae* Regel, *I. potaninii* Batalin, *I. sinensis* Lam. with two subspecies: ssp. *sinensis* and ssp. *variabilis* (Batalin) Grierson. In *Flora Reipublicae Sinicae* (Wang Went-Sai *et al.*, 1990), *I. sinensis* Lam. was divided into *I. sinensis* Lam. var. *sinensis* and *I. sinensis* Lam. var. *przewalskii* (Batalin) C. Y. Wu & W. C. Yin. The subgenus *Pteroscleris* includes *I. lutea* Bur. & Franch., which has two subspecies: ssp. *lutea* and ssp. *longiracemosa* (Sprague) Grierson, *I. beresowskii* Batalin, *I. altissima* G. Forrest, *I. forrestii* Fletcher, *I. compacta* Maxim., *I. younghusbandii* Sprague, *I. delavayi* Bur. & Franch. and *I. mairei* (Lévl.) Grierson, which is divided into two varieties, var. *mairei* and var. *grandiflora* (Wehrhahn) Grierson. In Wang *et al*. (1990), a third variety is described, var. *multifoliolata* (C. Y. Wu & W. C. Yin) C. Y. Wu & W. C. Yin. The subgenus *Niedzewdzkia* has only one species, *I. semiretschenskia* Grierson. One point that should be mentioned here is that the subgenus *Niedzwedzkia* is described as an independent genus named *Niedzwedzkia* B. Fedtsch. in *Flora Unionis Rerumpublicarum Socialisticarum Sovietcarum* (Vassilczenko, 1958). Differences between them are obvious and great. Subgenera *Amphicome* and *Incarvillea* are distributed in Eastern and Central Asia. Subgenus *Pteroscleris* is endemic to Eastern Asia and is distributed mainly in the Himalaya–Hengduan Mountain region. The subgenus *Niedzwedzkia* is endemic to Central Asia.

The differences between the four subgenera are obvious in their stamens, capsule texture and seeds

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(see Table 3), so there is doubt as to their taxonomic rank and the relationships between them. In view of this and their interesting distribution pattern, karyotypes of 11 species were studied including two populations of one species and two varieties of another, and the geography, systematic position and evaluation of the genus based on a combination of its karyomorphological and morphological characters are discussed.

MATERIAL AND METHODS

All the seeds used for the present study were collected from natural habitats, except those of *I. olgae* which were obtained from the München-Nymphenburg Botanic Garden (Germany) through seed exchange. The material studied was as follows.

Subgenus *Amphicome*: *I. arguta* (Royke) Royle. Two populations of this species were studied. Specimens from one population, which has comparatively large leaflets and puberulous stems, were collected from Barkam, Sichuan Province. Material from another population, which has relatively small leaflets and glossy stems, was collected from Zhongdian, Yunnan Province.

Subgenus *Incarvillea*: *I. olgae*, *I. sinensis* var. *sinensis* and *I. sinensis* var. *przewalskii* (Wang *et al.*, 1990).

Subgenus *Pteroscleris*: *I. lutea*, *I. beresowskii*, *I. compacta*, *I. mairei* var. mairei and *I. mairei* var. *grandiflora* (Grierson, 1961), *I. younghusbandii*, *I. delavayi*, *I. zhongdianensis* (Grey-Wilson, 1998), and *I. dissectifoliola* (Zhao, 1988).

The locations of the studied material and voucher numbers are given in Table 1. All voucher specimens are housed in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Science (KUN).

Root tips from germinated seeds were used for all of the karyological studies. They were pretreated in 0.002 M aqueous 8-hydroxyquinoline for 4 h, then fixed in Carnoy's fluid (1 : 3 glacial acetic acid/absolute alcohol) at about 4 °C for 30 min. The fixed roots were hydrolysed in 1 N HCl at 60 °C for 8–10 min, then stained with 1% aceto-orcein, and then squashed for cytological observation. Permanent slides of these squashed specimens remain in the Kunming Institute of Botany. Each observation was made on five well-spread metaphases. The cytological classification of the interphase and mitotic prophase chromosomes follows the categories of Tanaka (1971; 1977). The symbols for the description of metaphase chromosomes follow Leván, Fredga & Sandberg (1964). The asymmetry of the karyotype is classified according to Stebbins (1971).

RESULTS

The interphase nuclei (Fig. 1) and prophase chromosomes (Fig. 2) of all these species are categorized as the simple chromocentre type and the interstitial type, respectively.

It can be seen that chromosomes of the subgenus *Pteroscleris* are larger than those of the other two subgenera studied. The parameters are listed in Table 2.

The main cytological characters of each species are as follows.

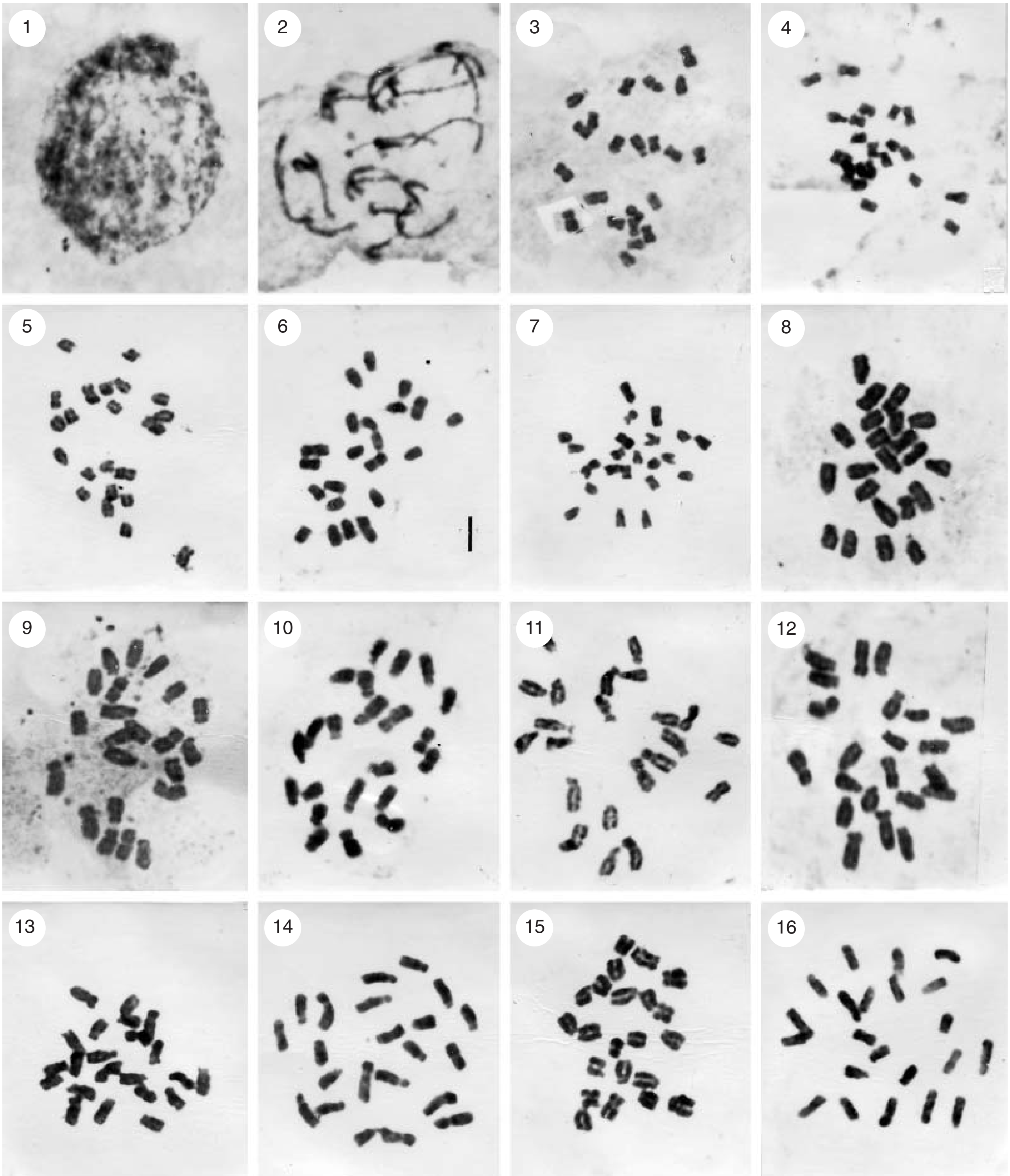
SUBGENUS *AMPHICOME*

Incarvillea arguta (the population from Barkam, Sichuan Province)

The karyotype formula is $2n = 22 = 18m + 4sm$. The ratio of the longest to the shortest chromosome is 1.49,

Table 1. Species studies, their locations and vouchers used for studies

Species	Location	Voucher
I. arguta (Yunnan pop.)	Yunnan, Zhongdian	*Chen ST200103*
I. arguta (Sichuan pop.)	Sichuan, Markang	*Chen ST200104*
I. beresowskii	Sichuan, Barkam	*Chen ST200109*
I. compacta	Sichuan, Shiqu	*Chen ST200111*
I. delavayi	Yunnan, Lijang	*Chen ST200108*
I. dissectifoliola	Sichuan, Yanyuan	*Chen ST200107*
I. lutea	Sichuan, Daocheng	*Chen ST200110*
I. mairei var. *mairei*	Yunnan, Dali	*Chen ST200101*
I. mairei var. *grandiflora*	Yunnan, Lijang	*Chen ST200105*
I. olgae	Kunming Botanical Garden	*Chen ST200112*
I. sinensis var. *sinensis*	Sichuan, Markang	*Chen ST200106*
I. sinensis var. *przewalskii*	Kunming Botanical Garden	*Chen ST200113*
I. younghusbandii	Xizhang, Nielamu	*Chen ST200114*
I. zhongdianensis	Yunnan, Zhongdian	*Chen ST200102*



Figures 1–16. Fig. 1. Interphase chromosomes of *Incarvillea arguta*. Fig. 2. Prophase chromosomes of *I. arguta*. Figs 3–16. Metaphase chromosomes, all $2n = 22$. Fig. 3. *I. arguta* (Sichuan). Fig. 4. *I. arguta* (Yunnan). Fig. 5. *I. olgae*. Fig. 6. *I. sinensis* var. *sinensis*. Fig. 7. *I. sinensis* var. *przewalskii*. Fig. 8. *I. lutea*. Fig. 9. *I. beresowskii*. Fig. 10. *I. compacta*. Fig. 11. *I. zhongdianensis*. Fig. 12. *I. delavayi*. Fig. 13. *I. dissectifoliola*. Fig. 14. *I. younghusbandii*. Fig. 15. *I. mairei* var. *mairei*. Fig. 16. *I. mairei* var. *grandiflora*. Scale bar = 5 μm.

Table 2. Parameters of mitotic metaphase chromosomes of 11 species. A.R. = arm ratio; T = type of chromosomes classified following Leván *et al*., 1964); m = chromosome with arm ratio of *c.* 1–1.7; sm = chromosome with arm ratio of *c.* 1.7–3.0; st = chromosome with arm ratio of *c.* 3.0–7.0

	Relative length of arms of chromosome 1–11	A.R.	T
I. arguta (Sichuan)	8.46 + 3.11 = 11.57	2.72	sm
	5.35 + 5.18 = 10.53	1.03	m
	6.74 + 3.63 = 10.37	1.86	sm
	5.35 + 3.45 = 8.80	1.55	m
	5.35 + 3.45 = 8.80	1.55	m
	5.18 + 3.45 = 8.63	1.50	m
	5.18 + 3.45 = 8.63	1.50	m
	5.01 + 3.45 = 8.46	1.45	m
	4.84 + 3.45 = 8.29	1.40	m
	4.49 + 3.63 = 8.12	1.24	m
	4.66 + 3.11 = 7.77	1.50	m
I. arguta (Yunnan)	9.48 + 3.37 = 12.85	2.81	sm
	5.90 + 4.85 = 10.75	1.22	m
	7.06 + 3.58 = 10.64	1.97	sm
	5.27 + 4.21 = 9.48	1.25	m
	5.37 + 3.48 = 8.85	1.54	m
	4.43 + 4.21 = 8.64	1.05	m
	4.43 + 4.21 = 8.64	1.05	m
	4.21 + 4.00 = 8.21	1.05	m
	4.00 + 3.79 = 7.79	1.06	m
	3.79 + 3.58 = 7.37	1.06	m
	4.00 + 2.74 = 6.74	1.46	m
I. olgae	8.21 + 3.90 = 12.11	2.11	sm
	7.80 + 3.08 = 10.88	2.53	sm
	6.37 + 4.52 = 10.89	1.41	m
	8.01 + 2.05 = 10.06	3.91	st
	4.93 + 4.52 = 9.45	1.09	m
	7.39 + 1.64 = 9.03	4.51	st
	6.57 + 1.64 = 8.21	4.01	st
	5.75 + 2.26 = 8.01	2.54	sm
	4.11 + 3.70 = 7.81	1.11	m
	5.34 + 2.05 = 7.39	2.60	sm
	3.70 + 2.46 = 6.16	1.50	m
I. sinensis var. *sinensis*	7.11 + 5.20 = 12.31	1.37	m
	6.07 + 5.20 = 11.27	1.17	m
	7.98 + 2.08 = 10.06	3.84	st
	7.11 + 2.78 = 9.89	2.56	sm
	6.07 + 2.95 = 9.02	2.06	sm
	5.98 + 2.95 = 8.93	2.03	sm
	5.20 + 3.47 = 8.67	1.50	m
	5.55 + 2.78 = 8.33	2.00	sm
	5.55 + 1.73 = 7.28	3.21	st
	5.20 + 2.08 = 7.28	2.50	sm
	5.38 + 1.56 = 6.94	3.45	st
I. sinensis var. *przewalskii**	7.13 + 3.98 = 11.11	1.79	sm
	6.50 + 4.19 = 10.69	1.55	m
	7.13 + 2.73 = 9.86	2.61	sm
	6.29 + 3.35 = 9.64	1.88	sm
	6.71 + 2.73 = 9.44	2.46	sm
	6.29 + 2.52 = 8.81	2.50	sm

Table 2. *Continued*

	Relative length of arms of chromosome 1–11	A.R.	T
I. delavayi	8.69 + 2.68 = 11.37	3.24	st
	6.44 + 4.29 = 10.73	1.50	m
	7.08 + 3.33 = 10.41	2.13	sm
	7.73 + 2.15 = 9.88	3.60	st
	6.97 + 1.93 = 8.90	3.61	st
	4.61 + 4.29 = 8.90	1.07	m
	6.55 + 2.04 = 8.59	3.21	st
	6.33 + 2.25 = 8.58	2.81	sm
	5.47 + 2.25 = 7.72	2.43	sm
	5.79 + 1.82 = 7.61	3.18	st
	5.36 + 1.93 = 7.29	2.78	sm
*I. dissectifoliola**	7.91 + 2.62 = 10.53	3.02	st
	5.53 + 4.61 = 10.14	1.20	m
	6.85 + 2.63 = 9.48	2.60	sm
	8.03 + 1.32 = 9.35	6.08	st
	7.90 + 1.32 = 9.22	5.98	st
	6.58 + 2.63 = 9.21	2.50	sm
	6.58 + 2.63 = 9.21	2.50	sm
	6.58 + 2.17 = 8.75	3.03	st
	6.19 + 1.97 = 8.16	3.14	st
	5.53 + 2.50 = 8.03	2.21	sm
	4.08 + 3.82 = 7.90	1.07	m
*I. younghusbandii**	8.25 + 3.13 = 11.38	2.64	sm
	6.69 + 4.08 = 10.77	1.64	m
	8.25 + 2.30 = 10.55	3.59	st
	7.52 + 2.51 = 10.03	3.00	sm
	6.27 + 3.24 = 9.51	1.94	sm
	6.79 + 1.88 = 8.67	3.61	st
	6.48 + 1.88 = 8.36	3.45	st
	4.18 + 3.97 = 8.15	1.05	m
	6.06 + 1.88 = 7.94	3.22	st
	6.27 + 1.15 = 7.42	5.45	st
I. mairei var. *mairei*	9.46 + 2.08 = 11.54	4.55	st
	7.37 + 3.53 = 10.90	2.09	sm
	7.21 + 3.21 = 10.42	2.25	sm
	7.85 + 1.92 = 9.77	4.09	st
	6.41 + 2.72 = 9.13	2.36	sm
	7.21 + 1.76 = 8.97	4.10	st
	4.81 + 3.37 = 8.18	1.43	m
	6.09 + 1.76 = 7.85	3.46	st
	4.17 + 3.69 = 7.86	1.13	m
	6.09 + 1.60 = 7.69	3.81	st
	4.49 + 3.21 = 7.70	1.40	m
I. mairei var. *grandiflora*	8.68 + 3.60 = 12.28	2.41	sm
	8.44 + 2.85 = 11.29	2.96	sm
	5.96 + 3.97 = 9.93	1.50	m
	7.44 + 2.36 = 9.80	3.15	st
	4.84 + 4.71 = 9.55	1.03	m
	6.58 + 2.11 = 8.69	3.12	st
	6.08 + 2.61 = 8.69	2.33	sm
	6.20 + 1.99 = 8.19	3.12	st
	6.20 + 1.61 = 7.81	3.85	st
	4.22 + 2.73 = 6.95	1.55	m
	5.21 + 1.61 = 6.82	3.24	st

Table 2. *Continued*

	Relative length of arms of chromosome 1–11	A.R.	T
	7.34 + 1.26 = 8.60	5.83	st
	7.13 + 1.47 = 8.60	4.85	st
	4.82 + 3.14 = 7.96	1.54	m
	4.40 + 3.35 = 7.75	1.31	m
	6.29 + 1.26 = 7.55	4.99	st
I. lutea	7.38 + 3.63 = 11.01	2.03	sm
	7.26 + 3.51 = 10.77	2.07	sm
	7.51 + 2.30 = 9.81	3.27	st
	7.38 + 2.30 = 9.68	3.21	st
	5.45 + 4.24 = 9.69	1.29	m
	6.66 + 2.18 = 8.84	3.06	st
	6.30 + 2.42 = 8.72	2.60	sm
	6.05 + 2.54 = 8.59	2.38	sm
	6.05 + 2.42 = 8.47	2.50	sm
	5.08 + 2.18 = 7.26	2.33	sm
	3.63 + 3.51 = 7.14	1.03	m
*I. beresowskii**	7.73 + 3.20 = 10.93	2.42	sm
	8.28 + 2.21 = 10.49	3.75	st
	5.52 + 4.42 = 9.94	1.25	m
	6.41 + 3.31 = 9.72	1.94	sm
	7.51 + 1.77 = 9.28	4.24	st
	6.74 + 2.21 = 8.95	3.05	st
	4.64 + 4.31 = 8.95	1.08	m
	6.52 + 2.26 = 8.78	2.88	sm
	5.63 + 2.32 = 7.95	2.43	sm
	5.52 + 2.21 = 7.73	2.50	sm
	5.52 + 1.77 = 7.29	3.12	st
I. compacta	8.25 + 4.44 = 12.69	1.86	sm
	9.14 + 2.54 = 11.68	3.60	st
	6.47 + 3.17 = 9.64	2.04	sm
	7.61 + 1.52 = 9.13	5.01	st
	6.47 + 2.54 = 9.01	2.55	sm
	6.35 + 2.66 = 9.01	2.39	sm
	4.95 + 3.93 = 8.88	1.26	m
	6.35 + 2.28 = 8.63	2.79	sm
	6.22 + 1.52 = 7.74	4.09	st
	5.71 + 1.27 = 6.98	4.50	st
	4.28 + 2.32 = 6.60	1.84	sm
I. zhongdianensis	8.45 + 2.14 = 10.59	3.95	st
	7.49 + 3.10 = 10.59	2.42	sm
	6.42 + 4.06 = 10.48	1.58	m
	6.42 + 3.21 = 9.63	2.00	sm
	7.70 + 1.50 = 9.20	5.13	st
	6.52 + 2.14 = 8.66	3.05	st
	6.42 + 2.14 = 8.56	3.00	sm
	6.31 + 2.14 = 8.45	2.95	sm
	4.39 + 4.06 = 8.45	1.08	m
	5.88 + 2.46 = 8.34	2.39	sm
	5.56 + 1.50 = 7.06	3.71	st

*First reports.

and 9.1% of the chromosomes have an arm ratio of more than 2.00. The asymmetry of the karyotype is type 2A (Fig. 3).

Incarvillea arguta (population from Zhongdian, Yunnan Province)
This population has a karyotype formula similar to the above, $2n = 22 = 18m + 4sm$. However, the incidence of chromosomes with an arm ratio of more than 2.00 and the ratio of the longest to the shortest chromosome are 9.1% and 1.91, respectively, which are different from those of the population from Sichuan. The asymmetry of the karyotype is classified as type 2A (Fig. 4).

SUBGENUS *INCARVILLEA*

Incarvillea olgae
The karyotype formula is $2n = 22 = 8m + 8sm + 6st$. 63.6% of the chromosomes have an arm ratio of more than 2.00. The ratio of the longest to the shortest chromosome is 1.96. The asymmetry of the karyotype is categorized as type 3A (Fig. 5).

Incarvillea sinensis var. sinensis
The karyotype formula is $2n = 22 = 6m + 10sm + 6st$. The ratio of the longest to the shortest chromosome is 1.77, and 63.6% of the chromosomes have an arm ratio of more than 2.00. The asymmetry of the karyotype is type 3A (Fig. 6).

Incarvillea sinensis var. przewalskii
The karyotype formula is $2n = 22 = 6m + 10sm + 6st$. The ratio of the longest to the shortest chromosome is 1.47, and 54.5% of the chromosomes have an arm ratio of more than 2.00. The asymmetry of the karyotype is categorized as type 3A (Fig. 7).

SUBGENUS *PTEROSCLERIS*

Incarvillea beresowskii
The karyotype formula is $2n = 22 = 4m + 10sm + 8st$. The ratio of the longest to the shortest chromosome is 1.50, and 72.7% of the chromosomes have an arm ratio of more than 2.00. The asymmetry of the karyotype is type 3A (Fig. 8).

Incarvillea lutea
The karyotype formula is $2n = 22 = 4m + 12sm + 6st$. 81.8% of the chromosomes have an arm ratio of more than 2.00, and the ratio of the longest to the shortest chromosome is 1.54. The asymmetry of the karyotype is categorized as type 3A (Fig. 9).

Incarvillea compacta
The karyotype formula is $2n = 22 = 2m + 12sm + 8st$. The number of chromosomes with an arm ratio of more than 2.00 is 72.7% and the ratio of the longest to the shortest chromosome is 1.92. The asymmetry of the karyotype is classified as type 3A (Fig. 10).

Incarvillea zhongdianensis
The karyotype formula is $2n = 22 = 4m + 10sm + 8st$, which is the same as that of *I. beresowkii*. The ratio of the longest to the shortest chromosome is 1.50 and 72.7% of the chromosomes have an arm ratio of more than 2.00. The asymmetry of the karyotype is type 3A (Fig. 11).

Incarvillea delavayi
The karyotype formula is $2n = 22 = 4m + 8sm + 10st$. 81.8% of the chromosomes have an arm ratio of more than 2.00 and the ratio of the longest to the shortest chromosome is 1.56. The asymmetry of the karyotype is categorized as type 3A (Fig. 12).

Incarvillea dissectifoliola
The karyotype formula is $2n = 22 = 4m + 8sm + 10st$ which is like that of *I. delavayi.* The number of chromosomes with an arm ratio of more than 2.00 is 81.8% and the ratio of the longest to the shortest chromosome is 1.33. The asymmetry of the karyotype is classified as type 3A (Fig. 13).

Incarvillea younghusbandii
The karyotype formula is $2n = 22 = 4m + 6sm + 12st$. The ratio of the longest to the shortest chromosome is 1.59 and 72.7% of the chromosomes have an arm ratio of more than 2.00. The asymmetry of the karyotype is type 3A (Fig. 14).

Incarvillea mairei var. mairei
The karyotype formula is $2n = 22 = 6m + 8sm + 8st$. The ratio of the longest to the shortest chromosome is 1.50 and 72.7% chromosomes have an arm ratio of more than 2.00. The asymmetry of the karyotype is categorized as type 3A (Fig. 15).

Incarvillea mairei var. grandiflora
The karyotype formula $2n = 22 = 6m + 6sm + 10st$. The number of chromosomes with an arm ratio of more than 2.00 is 72.7% and the ratio of the longest to the shortest chromosome is 1.80. The asymmetry of the karyotype is classified as type 3A (Fig. 16).

DISCUSSION

The number of diploid chromosomes in all species studied is $2n = 22$. The karyomorphology and chromosome numbers of $2n = 22$ for six species of *Incarvillea* were reported by Xiao *et al.* (2002). In the family *Bignoniaceae*, n = 20 predominates, but $x = 7$ may be ancestral for the family (Goldblatt & Gentry, 1979).

The genus *Incarvillea* is far from the uniformity predominating in *Bignoniniaceae*. The investigation of karyomorphology shows that karyotypic variation of the diploid is the main character of the chromosomes of this genus in its chromosome evolution. In general, the evolutionary tendency of this genus seems to be an increase in the asymmetry of the karyotype.

The chromosomes of the subgenera *Amphicome* and *Incarvillea* are smaller than those of subgenus *Pteroscleris* (Figs 2–16). All chromosomes of the subgenus *Amphicome* and *Incarvillea* are less than 4 µm long, while in the subgenus *Pteroscleris* all species have chromosomes that are more than 4 µm. Lima-De-Faria (1980) divided chromosomes of eukaryotic organisms into four grades: (I) length less than 1 µm; (II) length between 1 and 4 µm; (III) length between 4 and 12 µm; and (IV) length more than 12 µm. Chromosomes of the first and the fourth grades are not advantageous for plant evolution. Chromosomes of the second grade have normal centromeres and telomeres, but the genes affect each other greatly. Most plants have chromosomes in the third grade, whose fields work well (Lima-De-Faria, 1980). Thus, the subgenus *Pteroscleris* has advanced more actively than the other two subgenera. This is also shown by the fact that it comprises ten species, while subgenera *Amphicome* and *Incarvillea* have only two and three species, respectively.

The karyotypic asymmetry of subgenus *Amphicome* is categorized as type 2A, while the other two subgenera *Incarvillea* and *Pteroscleris* are type 3A. The asymmetry of the karyotype of subgenus *Pteroscleris* is greater than that of subgenus *Incarvillea*. The morphological difference among these three subgenera is obvious in stamen, texture of capsule and seed characters (Table 3). The swollen bases of the calyx teeth make subgenus *Incarvillea* easily distinguishable from subgenus *Pteroscleris*.

Relationships of some species can be deduced in some degree by karyotypic studies. Some taxa have similar karyotype formulae, but their karyomorphology is different (Table 2).

In subgenus *Incarvillea*, *I. olgae* has a karyotypic formula $2n = 22 = 8m + 8sm + 6st$, while the formula of two varieties of *I. sinensis* is $2n = 22 = 6m + 10sm + 6st$. All the natural populations of *I. olgae* are perennial, while some wild populations of *I. sinensis* are annual. The close affinity is indicated by the similarity between species.

In subgenus *Pteroscleris*, the karyotypic formulae of *I. lutea* and *I. beresowskii* are similar, $2n = 22 = 4m + 10sm + 8st$ and $2n = 22 = 4m + 12sm + 6st$, respectively. They are also similar morphologically, both of them having erect stems. *Incarvillea dissectifoliola*, *I. delavayi*, *I. zhongdianensis*, *I. younghusbandii* and *I. mairei* are acaulous groups. Our research results indicate that *I. zhongdianensis* has the same karyotypic formula as that of *I. beresowkii*, which is $2n = 22 = 4m + 10sm + 8st$. *Incarvillea delavayi* and *I. dissectifoliola* have the same karyotypic formula of $2n = 22 = 4m + 8sm + 10st$. The divided leaflets of *I. dissectifoliola* are the main morphological difference between them. These two species and *I. zhongdianensis* show a close affinity through their similar karyotypic formula, while *I. younghusbandii*, which occurs at the highest altitude of all species in the genus, has a karyotype formula of $2n = 22 = 4m + 6sm + 12st$. It is probably the most advanced species in the genus.

Based on the difference among three subgenera mentioned above, we suggest the subgenera should be separated as independent genera. This idea seems to be supported by their distribution pattern.

The subgenera *Amphicome* and *Incarvillea* are distributed in eastern and Central Asia. The subgenus *Pteroscleris* is endemic to eastern Asia and distributed mainly in the Himalaya–Hengduan mountain region (Fig. 17). Subgenus *Niedzwedzkia* is endemic to Central Asia. The subgenus *Amphicome* has two species: *Incarvillea arguta* and *I. emodi*. *Incarvillea arguta* is distributed in Yunnan, Guizhou, Sichuan, Ganshu, Tibet, Nepal and India at an elevation of 1585–3505 m. *Incarvillea emodi* is distributed in Afghanistan, the west of Pakistan, Kashmir and Nepal, at 607–2740 m. Subgenus *Incarvillea* has three species: *Incarvillea sinesis*, *I. olgae* and *I. potaninii*. *Incarvillea sinensis* is distributed in a wide area from north-east China to south-west China, at 500–3800 m. The distribution area of *I. olgae* includes Turkestan, Bokhara and Afghanistan. *Incarvillea potaninii* is distributed in Mongolia. The subgenus *Pteroscleris* is

Table 3. Comparisons of morphology and karyotypic asymmetry of three subgenera

Subgenera	Stamen	Seed	Capsule	Karyotypic asymmetry	Plant
Amphicome	Hairy	Coma of whitish hairs	Fibrous	2A	Woody at base
Incarvillea	Glabrous	Hyaline wing	Coriaceous	3A	Woody at base
Pteroscleris	Glabrous	Opaque wing	Ligno-coriaceous	3A	Not woody

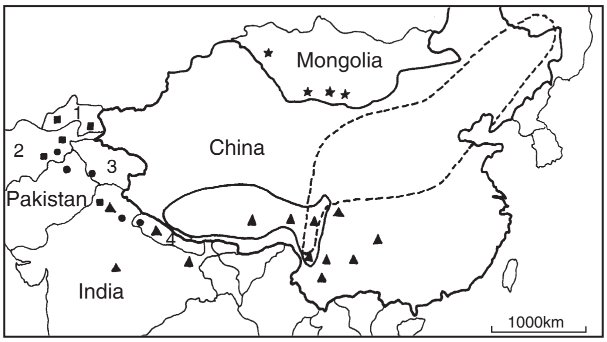


Figure 17. Distribution of three subgenera of *Incarvillea*. 1. Kirghizia. 2. Afghanistan. 3. Kashmir. 4. Nepal. ■ *I. olgae*. ▲ *I. arguta*. ● *I. emodi*. ★ *I. potaninii*. Area limited by broken line = distribution of *I. sinensis*. Area limited by continuous line = distribution of species of the subgenus *Pteroscleris*.

endemic to the Himalaya–Hengduan Mountain region, and its altitude, from 2200 to 4500 m, is the highest of the four subgenera,

In general, the relatively primitive genera *Amphicome* and *Incarvillea* are distributed in the surroundings and margin of the Himalaya–Hengduan mountains (Fig. 17). They have relatively small chromosomes and the asymmetry of their karyotype is low. In contrast, the genus *Pteroscleris* is distributed in the Himalaya–Hengduan mountains, and its species have comparatively large chromosomes with an asymmetry of the karyotype higher than the other two subgenera studied. During the Pleistocene the advancing ice sheets destroyed the northern distribution and bignoniaceous genera in Asia were restricted to suitable refugia in the Pamirs, Himalayas and south-west China, as well as to tropical regions in the south, and the distribution of *Incarvillea* is still centred around such refuge areas or, extending from them, has spread along the fringe of the monsoon area (Grierson, 1961). Our study indicates that the spread of *Incarvillea*, especially the subgenus *Pteroscleris*, is related to the raising of the Himalaya and Henduan mountains. In this limited refuge, ancestors of modern groups developed rapidly in pace with the growth of these two mountain ranges and has produced modern groups, especially the recent subgenus *Pteroscleris*. The Himalayan and Hengduan mountains became the diversity centre of the genus. Most species can be found in this area.

As discussed above, we suggest that the three subgenera investigated should perhaps be regarded as three genera, as supported by their morphology, karyomorphology and geographical distribution. This karyomorphological study has indicated the affinity of some species.

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REFERENCES

Goldblatt P, Gentry AH. 1979. Cytology of Bignoniaceae. *Botaniska Notiser* **132:** 475–482.

Grey-Wilson C. 1998. A new look on the subgenus *Pteroscleris*. *New Plantsman* **6:** 76–98.

Grierson AJC. 1961. A revison of the genus *Incarvillea*. *Notes from the Royal Botanical Garden Edinburgh* **23:** 303–354.

Leván A, Fredga K, Sandberg AA. 1964. Nomenclature for the centromeric position on chromosomes. *Hereditas* **52:** 201–220.

Lima-De-Faria A. 1980. Classification of genes, rearrangements and chromosomes according to the chromosome field. *Hereditas* **93:** 1–46.

Stebbins GL. 1971. *Chromosomal evolution in higher plants*. London: Edward Arnold, 87–90.

Tanaka R. 1971. Types of resting nuclei in Orchidaceae. *Botanical Magazine.* **84:** 118–122.

Tanaka R. 1977. Recent karyotype studies. In: Ogawa, K. *et al.*, eds. Plant cytology. Tokyo: Asakura Shoten, 293–326.

Vassilczenko. 1958. Niedzwedzkia. *Flora Unionis Rerumpublicarum Socialisticarum Sovietcarum* **23:** 5.

Wang W, Pan K, Zhang Z, Li Z. 1990. *Incarvillea* Juss. *Flora Reipublicae Popularis Sinicae.* **69:** 34–49.

Xiao H, Zhou Q-X, Gu Z-J, Guan K-Y. 2002. Karyomorphology of six *Incarvillea* species. *Acta Botanica Yunnanica.* **24:** 87–93.

Zhao Q-S. 1988. A new species of *Incarvillea* in Sichuan (Bignoniaceae). *Acta Phytotaxonomica Sinica.* **26:** 78–79.