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

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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 Niedzwedzia (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 Niedzwedzia (B. Fedtsch.) Grierson. Subgenus Amphicome includes I. arguta (Royle) Royle and I. emodi (Lindl.) Chatterjee. Subgenus Incarvillea includes I. olgae Regel, I. potaninii Bailawa & Franch, and I. multifoliolata (C. Y. Wu & W. C. Yin). The subgenus Pteroscleris includes I. lutea Bur. & Franch., has two subspecies: ssp. lutea and ssp. longiracemosa (Sprague) Grierson, I. beresowskii Bailawa & Franch. and I. altissima G. Forrest. One point that should be mentioned here is that the subgenus Niedzewdzia is described as an independent genus named Niedzewdzia B. Fedtsch. in Flora Siberica (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 Niedzewdzia is endemic to Central Asia.

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

| Species, Location, Voucher | Yunnan, Zhongdian | Sichuan, Markang | Sichuan, Barkam | Sichuan, Shiqu | Yunnan, Lijang | Sichuan, Yanyuan | Sichuan, Daelong | Yunnan, Dali | Yunnan, Lijang | Kunming Botanical Garden | Kunming Botanical Garden | Xizhang, Nielamu | Yunnan, Zhongdian |
|----------------------------|-------------------|------------------|-----------------|----------------|---------------|-----------------|-----------------|-------------|---------------|---------------------|---------------------|-----------------|-----------------|
| *I. arguta* (Yunnan pop.)  |                   |                  |                 |                |               |                 |                 |             |               |                     |                     |                 |                  |
| *I. arguta* (Sichuan pop.) |                   |                  |                 |                |               |                 |                 |             |               |                     |                     |                 |                  |
| *I. beresowskii*           |                   |                  |                 |                |               |                 |                 |             |               |                     |                     |                 |                  |
| *I. compacta*              |                   |                  |                 |                |               |                 |                 |             |               |                     |                     |                 |                  |
| *I. delavayi*              |                   |                  |                 |                |               |                 |                 |             |               |                     |                     |                 |                  |
| *I. dissectifoliola*       |                   |                  |                 |                |               |                 |                 |             |               |                     |                     |                 |                  |
| *I. lutea*                 |                   |                  |                 |                |               |                 |                 |             |               |                     |                     |                 |                  |
| *I. mairei* var. *mairei*  | Yunnan, Dali       |                 |                 |                |               |                 |                 |             |               |                     |                     |                 |                  |
| *I. mairei* var. *grandiflora* | Yunnan, Lijang     |                 |                 |                |               |                 |                 |             |               |                     |                     |                 |                  |
| *I. olgae*                 | Kunming Botanical Garden |         |                 |                |               |                 |                 |             |               |                     |                     |                 |                  |
| *I. sinensis* var. *sinensis* | Sichuan, Markang |                 |                 |                |               |                 |                 |             |               |                     |                     |                 |                  |
| *I. sinensis* var. *przewalskii* | Kunming Botanical Garden |         |                 |                |               |                 |                 |             |               |                     |                     |                 |                  |
| *I. younghusbandii*        | Xizhang, Nielamu   |                 |                 |                |               |                 |                 |             |               |                     |                     |                 |                  |
| *I. zhongdianensis*        | Yunnan, Zhongdian  |                 |                 |                |               |                 |                 |             |               |                     |                     |                 |                  |
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. dissectifolola*. 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

| Species          | 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 + 3.56 = 7.35 | 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 + 3.05 = 10.85 | 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 |

*First reports.
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  |

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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 |
| 7.38 + 3.63 = 11.01                     | 2.03 | sm |
| **I. lutea**                             |      |    |
| 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 |

*aFirst 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, $2n = 22 = 18m + 4s$. 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 3A (Fig. 4).

**Incarvillea olgae**

The karyotype formula is $2n = 22 = 8m + 8s + 6s$. 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 $2n = 22 = 6m + 10s + 6s$. 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 $2n = 22 = 6m + 10s + 6s$. 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 INCARVILLEA**

**Incarvillea compacta**

The karyotype formula is $2n = 22 = 2m + 12s + 8s$. 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 $2n = 22 = 4m + 10s + 8s$, which is the same as that of *I. beresoukii*. 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 $2n = 22 = 4m + 8s + 10s$. 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 dissectifolia**

The karyotype formula is $2n = 22 = 4m + 8s + 10s$, 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 $2n = 22 = 4m + 6s + 12s$. 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 $2n = 22 = 6m + 8s + 8s$. 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 $2n = 22 = 6m + 6s + 10s$. 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*

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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 Bignoniaceae. 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 \( \mu \)m long, while in the subgenus Pteroscleris all species have chromosomes that are more than 4 \( \mu \)m. Lima-De-Faria (1980) divided chromosomes of eukaryotic organisms into four grades: (I) length less than 1 \( \mu \)m; (II) length between 1 and 4 \( \mu \)m; (III) length between 4 and 12 \( \mu \)m; and (IV) length more than 12 \( \mu \)m. 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 \( 2n = 22 = 8m + 8m + 6st \), 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 \( I. \) beresowskii, 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, Gansu, 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 sinensis, \( I. \) olgae and \( I. \) potaninii. Incarvillea sinensis is distributed in a wide area from northeast 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     |
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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 *Amphi-come* 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 Hengduan 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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