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Cytogenetic Studies on Wild *Chrysanthemum* sensu lato in China. IV. Karyomorphological Characteristics of Three Species of *Ajania*

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中国産野生キク属（広義）の細胞遺伝学的研究. IV. *Ajania* 3種の核形態学的研究

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The chromosome numbers of three species of *Ajania* from Sichuan Province, China, are reported here for the first time; 2n = 18 (diploid) of *A. ramosa* (Chang) Shih, 2n = 36 (tetraploid) of *A. przewalskii* Poljakov, and 2n = 36 (tetraploid) of *A. tenuifolia* (Jacq.) Tzvel. The three species of *Ajania* have the common characteristics of the resting nucleus of the complex chromocenter type and the prophase chromosome of the interstitial type. The diploid *A. ramosa* contains mostly median- and submedian-centromeric chromosomes and two subterminal-centromeric chromosomes, while the two tetraploid species contain mostly median- and submedian-centromeric chromosomes and only one subterminal-centromeric chromosomes. *Ajania ramosa* displays four sat-chromosomes, *A. przewalskii* displays six sat-chromosomes and *A. tenuifolia* displays seven sat-chromosomes.

(Continued from Nakata et al., Chrom. Inf. Serv. **51:** 16–18, 1991)

Chinese *Ajania* consists of approximately 30 species and is centered in Chinghai, Sichuan and Xizang (Tibet) Provinces (Shih and Fu 1983). Chromosome survey in *Ajania* has been documented in the standard references only by Sokolovskaya (1966), although that in the Japanese

Ajania species taxonomically treated as *Chrysanthemum* has been made by various workers (*eg.*, Sugiura 1936, 1937, Nagami 1957, Kitagawa and Nagami 1960, Tanaka and Shimotomai 1961). Thus, karyotype of *Ajania* has been characterized only in one reference using a plant named after *Chrysanthemum rupestre* in Japan (Tanaka and Shimotomai 1961). Karyomorphological characteristics of Chinese *Ajania,* however, are poorly recorded in most standard references.

Materials and Methods

The plants of *Ajania przewalskii* Poljakov, *A. ramosa* (Chang) Shih and *A. tenuifolia* (Jacq.) Tzvel. were collected in Sichuan Province, the People's Republic of China (Table 1). They were cultivated in pots in the experimental garden of Hiroshima University before their root-tips were harvested for the chromosome study. Root-tips were treated in 0.002M hydroxyquinoline at 18°C for 1.5 hours before they were fixed in 45% acetic acid at 4°C for 15 minutes, following which they were hydrolyzed in 2:1 mixture of 1 N-hydrochloric acid and 45% acetic acid at 60°C for 12 seconds, and stained and squashed in 1% aceto-orcein.

Karyomorphological classifications of resting and mitotic prophase chromosomes followed Tanaka (1971). Karyotype formula of each species was based on the data of sizes of the chromosome characters of ten somatic metaphase cells. Position of the primary constriction of chromosome followed Levan et al. (1964): Arm ratio calculated by long arm/short arm, 1.0–1.7 was described as median centromere, 1.8–3.0 submedian centromere, 3.1–7.0 subterminal centromere, and 7.1 or more terminal centromere.

The herbarium specimens of these species were deposited in the Herbarium, Laboratory of Plant Chromosome and Gene Stock, Faculty of Science, Hiroshima University.

Results and Discussion

1. *Ajania ramosa* (Chang) Shih Figs. 1 and 4

Sixteen individuals of this species were collected in rocky cliff, altitude approximately 3100 m above the sea-level, Mt. Zheduo, Sichuan Province, China (Table 1). Those plants were woody, up to 1.5 m high and up to 2.0 cm diameter (see the upper portion of the species in Fig. 1A).

Karyomorphological type of the resting nucleus of this species was of the complex chromocenter type and that of the mitotic prophase chromosome was of the interstitial type (Fig. 1B and C).

All of the plants of this species studied showed the chromosome number of $2n = 18$ (diploid). The metaphase chromosomes were relatively long and graded from the longest chromosome of 8.5 μm to the shortest chromosome of 5.9 μm (Fig. 4A). Fourteen chromosomes (seven pairs) were median-centromeric, two chromosomes (one pair; the 17th and 18th chromosomes) were submedian-centromeric and satellited, and two chromosomes (one pair; the 13th and 14th chromosomes) were subterminal-centromeric and satellited (Fig. 4A).

Ajania ramosa is morphologically and karyomorphologically similar to the Japanese diploid species, *Dendranthema rupestre* (Matsum. et Koidz.) Kitamura (= *Chrysanthemum rupestre* Matsum. et Koidz.). *Dendranthema rupestre* has two subterminal-centromeric chromosomes and four sat-chromosomes of which two are submedian-centromeric and the other two are subterminal-centromeric (Tanaka and Shimotomai 1961). However, the positions of the sat-chromosomes in the metaphase chromosome alignment or the chromosome complement in *A. ramosa* are different from those in *D. rupestre*. *Ajania ramosa* has more median-centromeric chromosomes in number than *D. rupestre*. These phenomena suggest that *A. ramosa* might be more primitive than *D. rupestre*.

Table 1. Localities of three Chinese species of *Ajania* studied.

Species	Locality	Herbarium specimen
A. przewalskii Poljakov	China: Sichuan Province, between Lisian Co. and Marekang Co., near the top of Mt. Zegu, on roadside, alt. ca. 3800 m	Kondo 2328
A. ramosa (Chang) Shih	China: Sichuan Province, ca 20 km from Kangdin Co. toward Mt. Zheduo, on rock cliff on roadside, alt. ca. 3100 m	Kondo 2329
A. tenuifolia (Jacq.) Tzvel.	China: Sichuan Province, Kangdin Co., near the top of Mt. Zheduo, on roadside, alt. ca. 4150 m	Kondo 2330

The specimens were identified by Prof. Chu Shih, Institute of Botany, Academia Sinica.

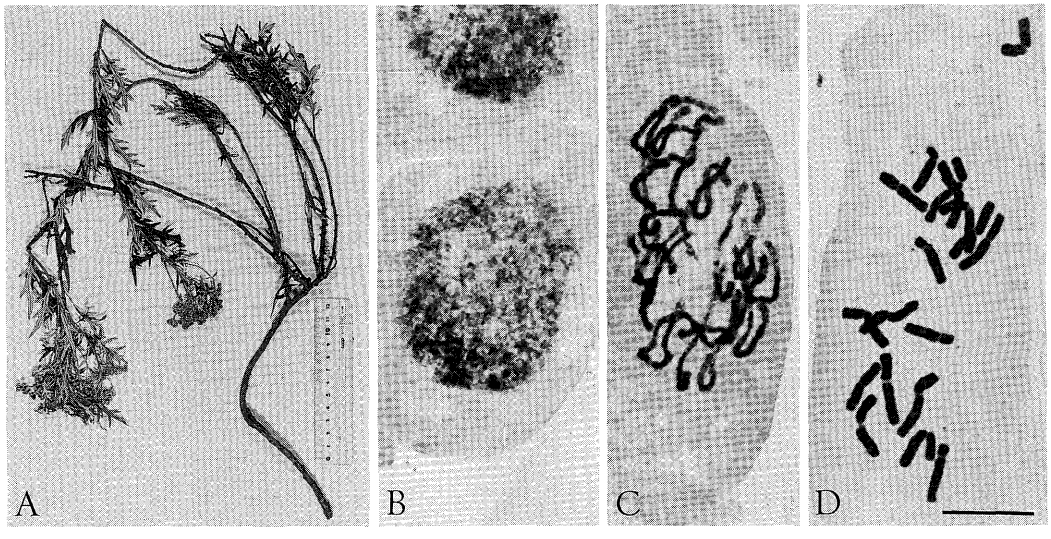

Fig. 1. *Ajania ramosa* (Chang) Shih. A. Voucher specimen. B. The resting nucleus of the complex chromocenter type. C. The prophase chromosomes of the interstitial type. D. Metaphase chromosomes of 2n = 18. Bar = 10 μm for B–D.

2. *Ajania przewalskii* Poljakov Figs. 2 and 4

Twenty-six individuals of this species were collected on roadside, altitude approximately 3800 m above the sea-level, Mt. Zegu, Sichuan Province, China (Table 1). These plants were herbaceous, up to 35 cm high (Fig. 2A).

Karyomorphological type of the resting nucleus of this species was of the complex chromocenter type (Fig. 2B) and that of the mitotic prophase chromosome was of the interstitial type (Fig. 2C).

All of the plants of this species studied showed the chromosome number of 2n = 36 (tetraploid) (Fig. 2D). The metaphase chromosomes graded from the longest chromosome of 4.5 μm to the shortest chromosome of 2.7 μm (Fig. 4B). Twenty-four chromosomes were median-centromeric, 11 chromosomes were submedian-centromeric, and one chromosome was subterminal-centromeric (Fig. 4B). The twenty-fifth median-centromeric chromosome and the 26th, 27th, 28th, 33rd and 34th submedian-centromeric chromosomes were satellited (Fig. 4B).

Tetraploid *Ajania* (2n = 36) has been reported only in *A. pallasiana* (Fisch. *ex* Bess.) Poljakov in

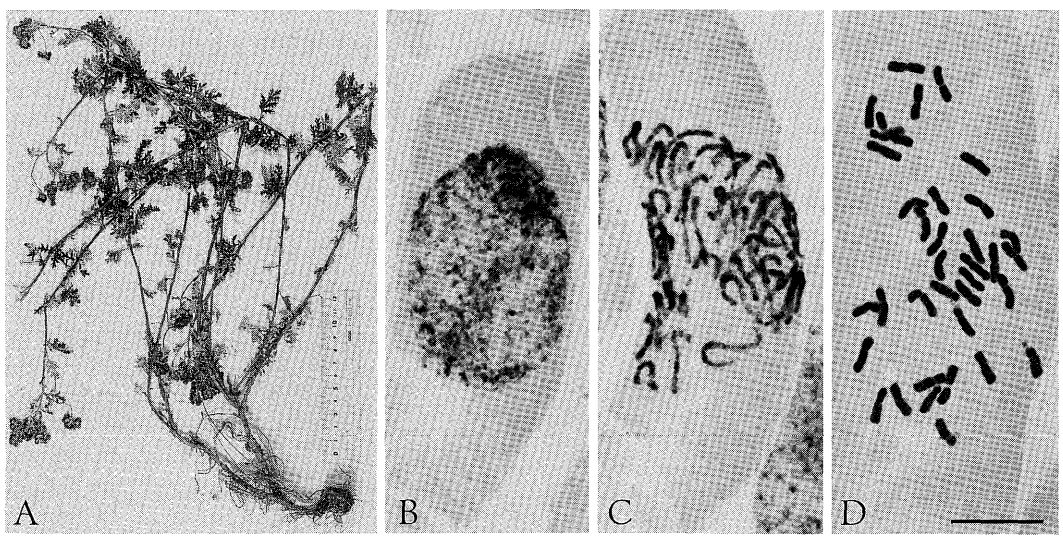

Fig. 2. *Ajania przewalskii* Poljakov. A. Voucher specimen. B. The resting nucleus of the complex chromocenter type. C. The prophase chromosomes of the interstitial type. D. Metaphase chromosomes of 2n = 36. Bar = 10 μm for B–D.

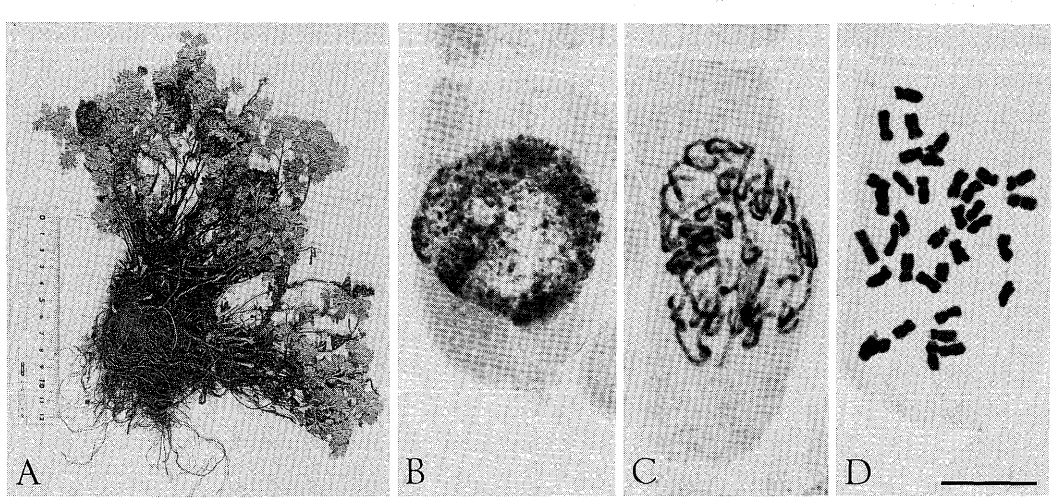

Fig. 3. *Ajania tenuifolia* (Jacq.) Tzvel. A. Voucher specimen. B. The resting nucleus of the complex chromocenter type. C. The prophase chromosomes of the interstitial type. D. Metaphase chromosomes of 2n = 36. Bar = 10 μm for B–D.

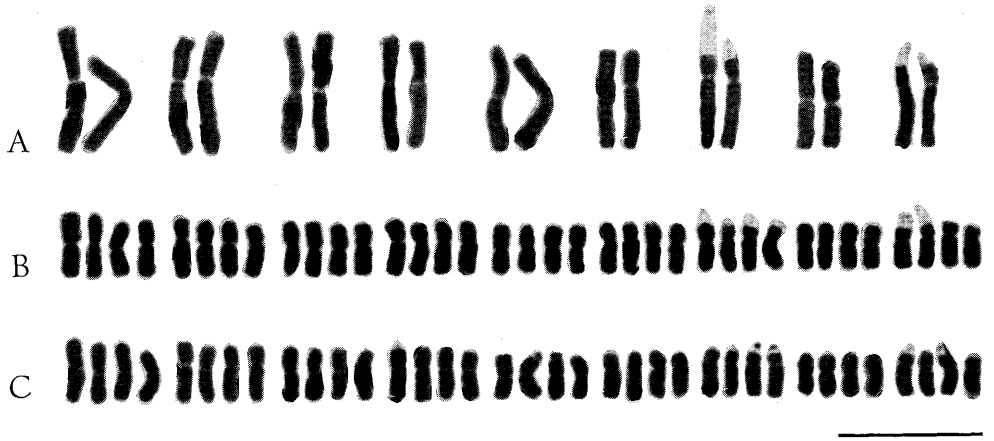

Fig. 4. Chromosome alignments of the chromosome complements of three species of *Ajania* at metaphase. A. *A. ramosa*. B. *A. przewalskii*. C. *A. tenuifolia*. Bar = 10 μm.

Russia by Sokolovskaya (1966), but however, karyomorphological and karyotypical characteristics of this species have not been characterized and reported.

3. *Ajania tenuifolia* (Jacq.) Tzvel. Figs. 3 and 4

Ninety-one individuals were collected in flat area near the nival zone, altitude approximately 4150 m above the sea-level, nearly the top of Mt. Zheduo, Sichuan Province, China (Table 1). The plants were herbaceous and prostrate to form like a mat (Fig. 3A).

Karyomorphological type of the resting nucleus of this species was of the complex chromocenter type (Fig. 3B) and that of the mitotic prophase chromosome was of the interstitial type (Fig. 3C).

All of the plants of this species studied showed the chromosome number of $2n=36$ (tetraploid) (Fig. 3D). The metaphase chromosomes graded from the longest chromosome of 4.4 μm to the shortest chromosome of 2.7 μm (Fig. 4C). Among the members of the chromosome complement, 24 chromosomes were median-centromeric, 11 were submedian-centromeric and one was subterminal-centromeric (Fig. 4C). The 18th and 19th median-centromeric chromosomes and the 13th, 27th, 28th, 33rd and 35th submedian-centromeric chromosomes were satellited (Fig. 4C).

This tetraploid karyotype was quite similar to that of *A. przewalskii,* excepting it had one extra median-centromeric sat-chromosome and some differences in positions of certain chromosomes in the chromosome alignment (Fig. 4B and C). However, plant morphological characters, habits and habitats of the two tetraploid *Ajania* were different from each other, perhaps due to differences in elevations and severe alpine environments.

The chromosome numbers and karyomorphological characteristics of three Chinese species of *Ajania* were, thus, reported here for the first time.

This paper was the first of our series study on *Ajania* in China to improve our concept of the interrelationships among the members of *Chrysanthemum sensu lato*.

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要 旨

中国四川省高山地帯産キク科 *Ajania* 属3種の染色体観察を行った．*Ajania ramosa*（Chang）Shih は 2n＝18（二倍体），*A. przewalskii* Poljakov は 2n＝36（四倍体），*A. tenuifolia*（Jacq.）Tzvel. は 2n＝36（四倍体）であった．染色体の形態は3種共に，体細胞静止期では複雑染色中央粒型，分裂期前期では介在型，分裂期中期では中部型または次中部型が大多数で，次端部型が二倍体種では1対，四倍体種では1個であった．また，*A. ramosa* は4個の付随体染色体，*A. przewalskii* は6個の付随体染色体，*A. tenuifolia* では7個の付随体染色体が観察された．