植物研究雑誌
J. Jpn. Bot.
70: 85–94 (1995)

Cytogenetic Studies on Wild *Chrysanthemum* sensu lato in China V. A Chromosome Study of Three Species of *Ajania, Cancrinia maximowiczii* and *Dendranthema lavandulifolium* in the Chrysantheminae, the Anthemideae, the Compositae in Chinese Highlands

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(Received on July 8, 1994)

Ajania myriantha (Franch.) Ling ex Shih from Sichuan Province, China, and *A. przewalskii* Poljakov, *A. salicifolia* (Mattf.) Poljakov, *Cancrinia maximowiczii* C. Winkl. and *Dendranthema lavandulifolium* (Fisch. ex Trautv.). Ling et Shih from Gansu Province, China, show the common karyomorphology of the resting nucleus of the complex chromocenter type, the prophase chromosome of the interstitial type. *Ajania myriantha* has the intraspecific polyploid with 2n=18 (diploid karyotype) of 14 median- or submedian-centromeric and four subterminal-centromeric chromosomes and 2n=36 (tetraploid karyotype) of 30 median- or submedian-centromeric and six subterminal-centromeric chromosomes. The majority of *A. przewalskii* has 2n=36 (tetraploid karyotype), while a population shows 2n=18 (diploid karyotype) with 14 median- or submedian-centromeric and four subterminal-centromeric chromosomes, as well as 2n=36. *Ajania salicifolia, Cancrinia maximowiczii*, and *Dendranthema lavandulifolium* display commonly 2n=18 (diploid karyotype) of 14 median- or submedian-centromeric and four subterminal-centromeric chromosomes. It seems that the endemic species of *Ajania* localized and restricted to certain small areas has 2n=18, while some cosmopolitic species of the genus have both 2n=18 and 2n=36. (Continued from Kondo et al., J. Jpn. Bot. **67**: 324–329, 1992)

The Chinese Compositae, the tribe Anthemideae, the subtribe Chrysantheminae consists of the genera of *Argyranthemum, Chrysanthemum, Leucanthemella, Leucanthemum, Brachanthemum, Dendranthema, Matricaria, Tripleurospermum, Pyrethrum, Opisthopappus, Coleostephus, Tanacetum, Formania, Waldheimia, Hippolytia, Stilpnolepis, Elachanthemum, Cancrinia, Ajania, Ajaniopsis, Filifolium, Kaschgaria, Neopallasia, Crossostephium, Centipeda, Cotula* and *Soliva* (Ling and Shih 1983). They may be closely related to each other and may even make cross-hybrids with each other and would sometimes perform introgressive hybridization in the nature if the long-term experiments and observations of

Shimotomai and Tanaka and their associates in the Japanese *Chrysanthemum* sensu lato are adapted (e.g., Tanaka and Shimotomai 1978). Thus, individual plants and herbarium specimens are sometimes difficult to identify from the published descriptions. Since those genera are closely related, they should be examined for possible chromosomal evidence which would contribute to satisfactory taxonomic treatment. Then, these chromosome evidences should also be compared with those of the Japanese species to justify and clarify our cytogeographical and evolutionary concepts.

Extensive chromosome investigations in Chinese, wild *Dendranthema* began in 1989 as a Japan-China joint research (e.g., Nakata et al. 1991a, 1991b, 1992, Tanaka 1992). However, chromosome survey in the other Chinese genera of the subtribe Chrysantheminae, the tribe Anthemideae, the Compositae is very much lacking in most standard references. The first paper on the chromosome study in the Chinese *Ajania* was made from *A. ramosa*, *A. przewalskii* and *A. tenuifolia* collected in the western part of Sichuan Province by Kondo et al. (1992). Moreover, the world-first chromosome count in *Ajania* of the world was reported by Sokolovskaya (1966), although the chromosome document in the Japanese *Ajania* species formerly, taxonomically-treated as *Chrysanthemum* has been made by various workers (e.g., Sugiura 1936, 1937, Nagami 1957, Kitagawa and Nagami 1960, Tanaka and Shimotomai 1961). Among the members of *Cancrinia* of the world, only the Mongolian *C. discoidea* (Ledeb.) Poljakov showed the chromosome number of 2n=14 for the first time (Mesicek and Sojak 1969). However, karyomorphology of Chinese *Cancrinia* has been poorly recorded.

This paper is the second of our series in *Ajania* chromosomes and the first of our series in *Cancrinia* chromosomes.

Materials and Methods

The plants of *Ajania myriantha* (Franch.) Ling ex Shih, *A. przewalskii* Poljakov, *A. salicifolia* (Mattf.) Poljakov, *Cancrinia maximowiczii* C. Winkl. and *Dendranthema lavandulifolium* (Fisch. ex Trautv.) Ling et Shih studied were collected in Gansu and Sichuan Provinces, the People's Republic of China (Table 1).

They were cultivated in pots in the experimental garden of Laboratory of Plant Chromosome and Gene Stock, Faculty of Science, Hiroshima University before their root-tips were harvested and pretreated in 0.002M hydroxyquinoline at 18°C for 1.5 hours. They were fixed in 45% acetic acid at 4°C for 15 minutes, following which they were hydrolyzed in 2:1 mixture of 1N-hydrochloric acid and 45% acetic acid at 60°C for 12 seconds, 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 grouped as median centromere, 1.8–3.0 submedian centromere, 3.1–7.0 subterminal centromere, and 7.1 or more terminal centromere.

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

Results and Discussion

The karyomorphological results in the three species of *Ajania*, one species of *Cancrinia* and one species of *Dendranthema* are shown in Figs. 1–6.

High karyomorphological stability and similarity were found in those species of *Ajania* and its closely related *Cancrinia* and *Dendranthema* in Chinese highlands: The resting nucleus of all the species was of the

Table 1. Localities and chromosome numbers of three species of *Ajania*, one species of *Cancrinia* and one species of *Dendranthema* studied

Species	Chromosome number (2n)	Locality
Ajania		
A. myriantha (Franch.) Ling ex Shih	18, 36	Sichuan Province: Wenchuan Co., along the road between Taopin and Tonghua, alt. 1,400 m
A. przewalskii Poljakov	18, 36	Gansu Province: Lingtao Co., Majiashan, Shangguancun, alt. 2,170 m
A. salicifolia (Mattf.) Poljakov	18	Gansu Province: Hezuo Co., Bola, alt. 2,850 m (small-sized form 5-10 cm tall)
	18	Gansu Province: Watershed between Weiyuan Co. and Zhang Co., alt. 2,880 m (large-sized form 50–100 cm tall)
Cancrinia maximowiczii C. Winkl.	18	Gansu Province: Yongdeng Co., Tulugousenlingongyuan, alt. 2,270 m
Dendranthema lavandulifolium (Fisch. ex Trautv.) Ling et Shih	18	Gansu Province: Nanping Co., Longzhaishaba, alt. 1,890 m

complex chromocenter type and the mitotic prophase chromosomes were of the interstitial type. These results supported those of *A. ramosa* (Chang) Shih, *A. przewalskii* of Sichuan and *A. tenuifolia* (Jacq.) Tzvel. (Kondo et al. 1992). Those species have an intergeneric polyploid series with the basic chromosome number of X=9; they consist of diploid (2n=18) and tetraploid (2n=36). The species of *Ajania* rather widely distributed, such as *A. myriantha* (Fig. 1) and *A. przewalskii* (Fig. 2), formed an intraspecific polyploidy with 2n=18 and 36 plants which grew sympatrically, while the species localized and restricted to certain small area such as *A. salicifolia* (Figs. 3 and 4) were diploid (2n=18). Another cosmopolitic *Ajania przewalskii* and *A. tenuifolia* previously reported (Kondo et al. 1992) were tetraploid, while the localized *A. ramosa* reported was diploid. All the diploid plants of *Ajania* studied showed the common karyotype of 14 median- or submedian- and four subterminal-centromeric chromosomes. However, they have 2–6 different sat-chromosomes. The diploid *A. myriantha* showed satellites on the 17th and the 18th chromosomes (Fig. 1), while *A. przewalskii* showed no satellite (Fig. 2). In contrast, the large-sized form of *A. salicifolia* had satellites on the 3rd, the 6th and the 9th pairs of chromosomes (5th, 6th, 11th, 12th, 17th and 18th chromosomes) (Fig. 4), while the small-sized form had no satellites (Fig. 3). *Ajania ramosa* previously reported displayed satellites on the 7th and 9th pairs of chromosomes (13th, 14th, 17th and 18th chromo-

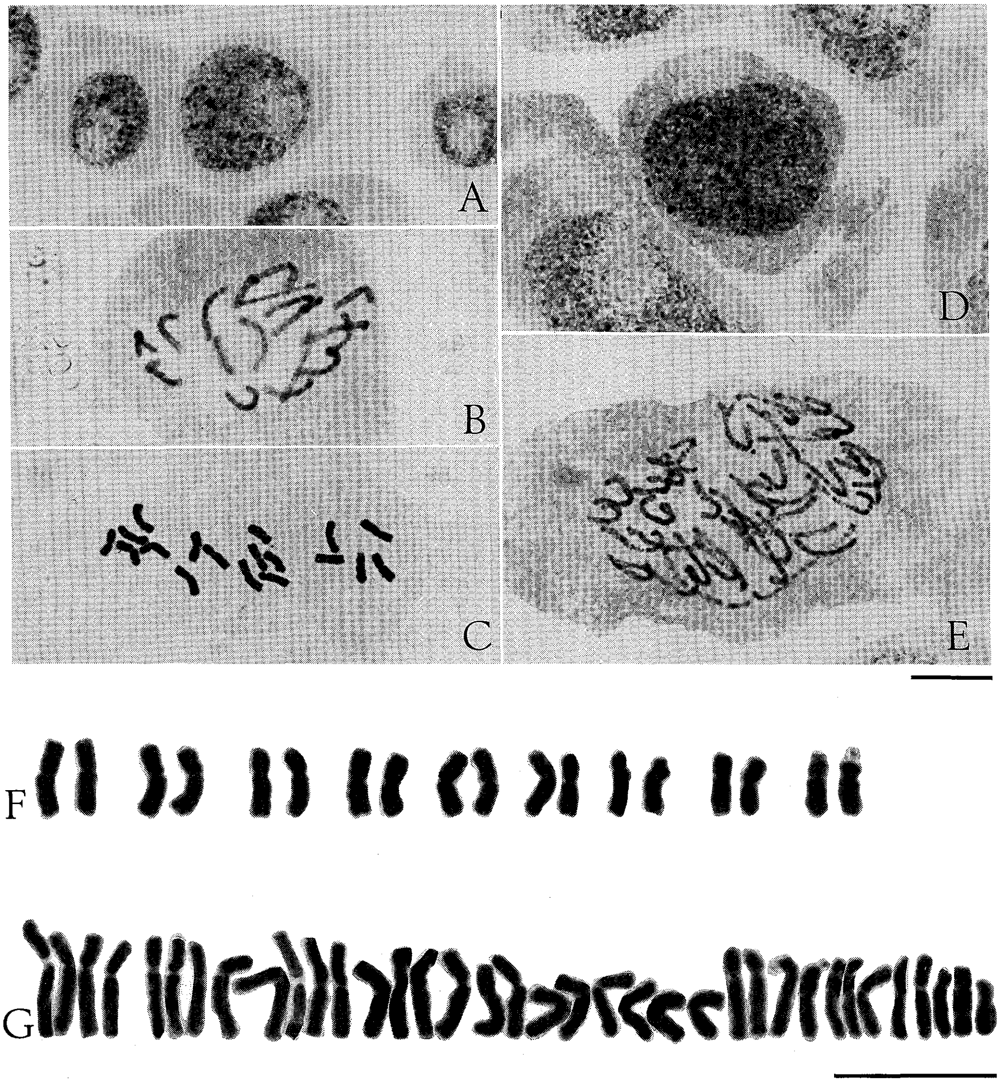

Fig. 1. Karyomorphology of *Ajania myriantha* (Franch.) Ling ex Shih, 2n=18 and 36. A–C and F. 2n=18 (diploid) karyomorphology. D, E and G. 2n=36 (tetraploid) karyomorphology. A and D. The resting nucleus of the complex chromocenter type. B and E. The mitotic prophase chromosomes of the interstitial type. C. 2n=18 at mitotic metaphase. F. The diploid karyotype with 14 median- and 4 subterminal-centromeric chromosomes. G. The tetraploid karyotype with 30 median- or submedian- and 6 subterminal-centromeric chromosomes. Bar=10 μm.

somes) (Kondo et al. 1992). *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* had two subterminal-centromeric chromosomes and four sat-chromosomes of which two were submedian-centromeric and the other two were subterminal-centromeric (Tanaka and Shimotomai 1961). Those sat-chromosomes were placed at the 4th and the 8th pairs in the complement (7th, 8th, 15th and 16th chromosomes) (Tanaka and Shimotomai 1961).

The tetraploid plant of *A. myriantha* showed 30 median- or submedian- and 6 subterminal-centro-

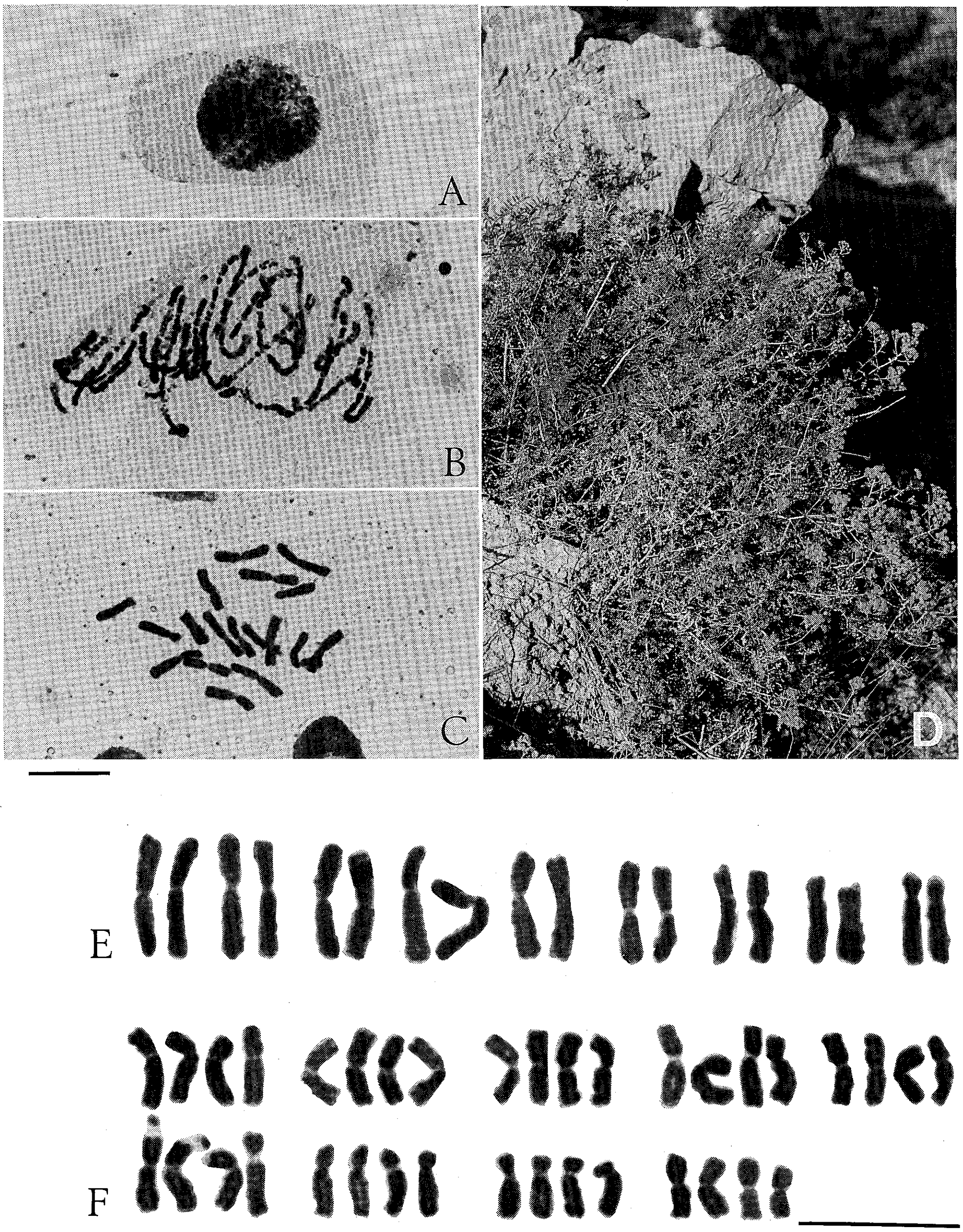

Fig. 2. Karyomorphology of *Ajania przewalskii* Poljakov, 2n=18 and 36. A–C and E. 2n=18 (diploid) karyomorphology. A. The resting nucleus of the complex chromocenter type. B. Mitotic prophase chromosomes of the interstitial type. C. 2n=18 at mitotic metaphase. E. The diploid karyotype with 14 median- or submedian- and 4 subterminal-centromeric chromosomes. D. A diploid plant. F. The tetraploid karyotype with 28 median- or submedian- and 8 subterminal-centromeric chromosomes. Bar=10 μm.

meric chromosomes (Fig. 1G). Satellites were shown on the 27th and the 28th chromosomes. The tetraploid plant of *A. przewalskii* had 28 median- or submedian- and 8 subterminal-centromeric chromosomes (Fig. 2F). Satellites were shown on the 21st and the 22nd chromosomes. These results in the tetraploid *A.*

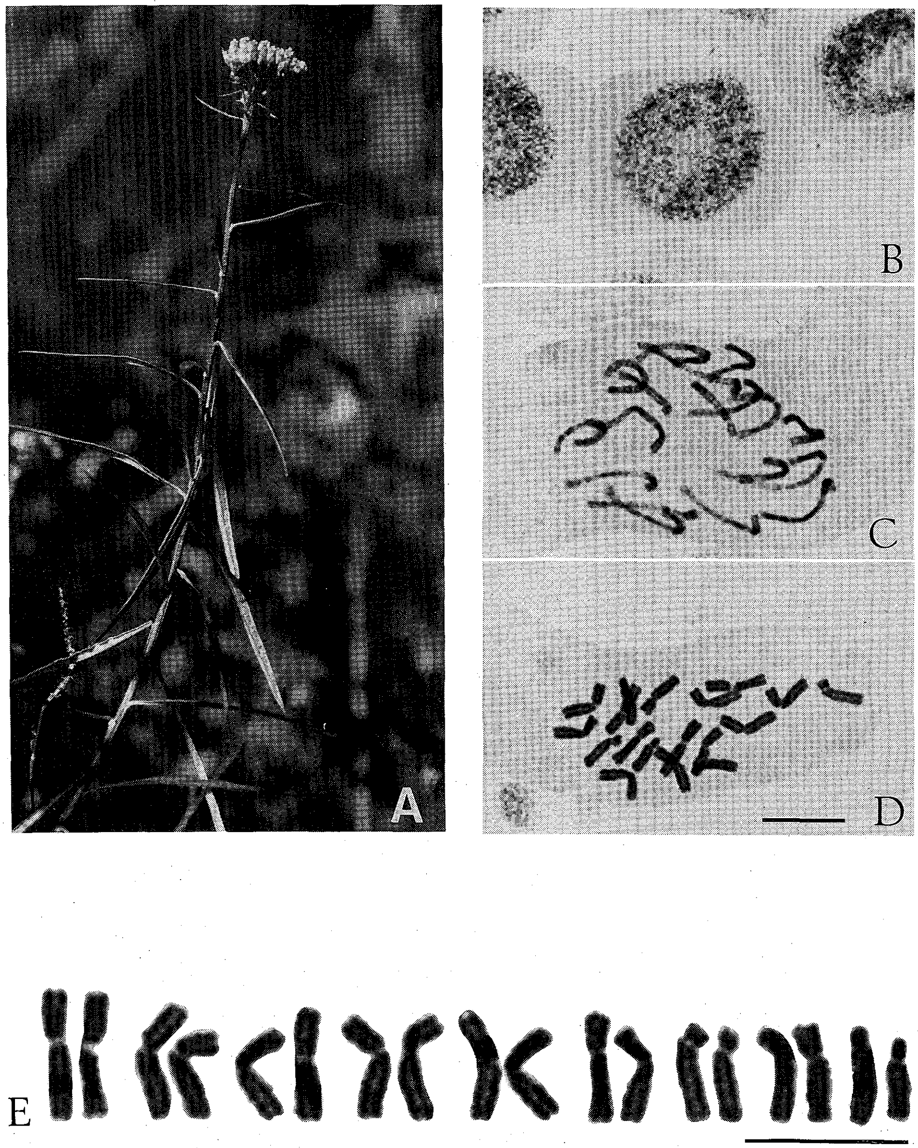

Fig. 3. Karyomorphology of *Ajania salicifolia* (Mattf.) Poljakov, a small-sized form 5–10 cm tall at the flowering stage, 2n=18. A. A flowering plant. B. The resting nucleus of the complex chromocenter type. C. The mitotic prophase chromosomes of the interstitial type. D. 2n=18 at mitotic metaphase. E. The diploid karyotype with 14 median- or submedian- and 4 subterminal-centromeric chromosomes. Bar=10 μm.

przewalskii were different from those of the previous document (Kondo et al. 1992); in which 24 chromosomes were median-centromeric, 11 were submedian-centromeric, and one was subterminal-centromeric. The twenty-fifth, the 26th, the 27th, the 28th, the 33rd and the 34th chromosomes were satellited (Kondo et al. 1992).

Another disc-flowered only *Cancrinia maximowiczii* displayed 14 median- or submedian- and four subterminal-centromeric chromosomes which were same to the diploid *Ajania* and exhibited two sat-chromosomes (the 10th and the 11th chromosomes)

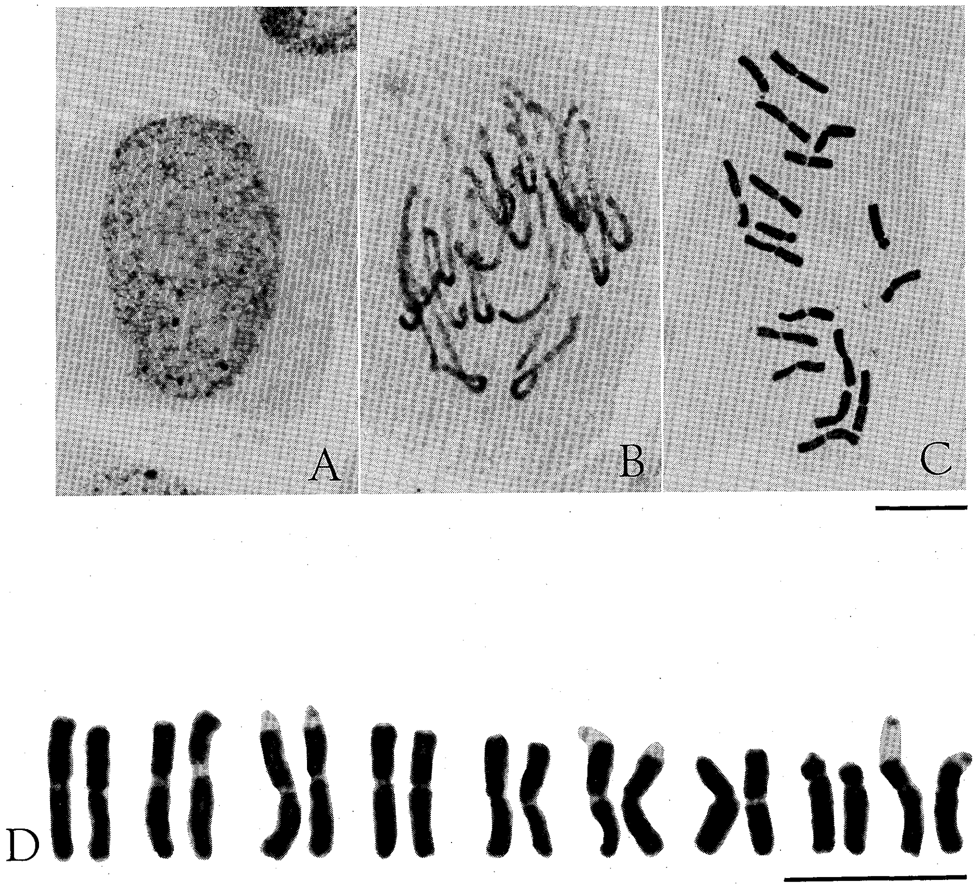

Fig. 4. Karyomorphology of *Ajania salicifolia* (Mattf.) Poljakov, a large-sized form 50–100 cm tall at the flowering stage, 2n=18. A. The resting nucleus of the complex chromocenter type. B. The mitotic prophase chromosomes of the interstitial type. C. 2n=18 at mitotic metaphase. D. The diploid karyotype with 14 median- or submedian- and 4 subterminal-centromeric chromosomes. Bar=10 μm.

(Fig. 5D). *Dendranthema lavandulifolium*, which has one of the widest distribution among the members of the genus throughout China from low as 50 m up to 1,900 m above the sea-level, showed stable diploid karyotype of 14 median- or submedian-centromeric and four subterminal-centromeric chromosomes same as those of the previous report (Tanaka and Taniguchi 1987, Taniguchi and Tanaka 1987). No karyotypic characteristics were correlated with high elevation. The species had either four or five sat-chromosomes (Fig. 6G–H).

These karyomorphological similarities among the three genera in the subtribe Chrysantheminae, the tribe Anthemideae, the Compositae strongly suggested that they were closely related with each other and could be possibly placed in same genus. Further experiments such as crossability, fluorescent-banding comparison, in situ hybridization (GISH) will be expected to clarify these intergeneric relationships.

We thank Prof. Chu Shih, Institute of Botany, Chinese Academy of Sciences, Beijing, for identifying the specimens. This study was financially supported by the Grant-in-Aid for the Monbusho In-

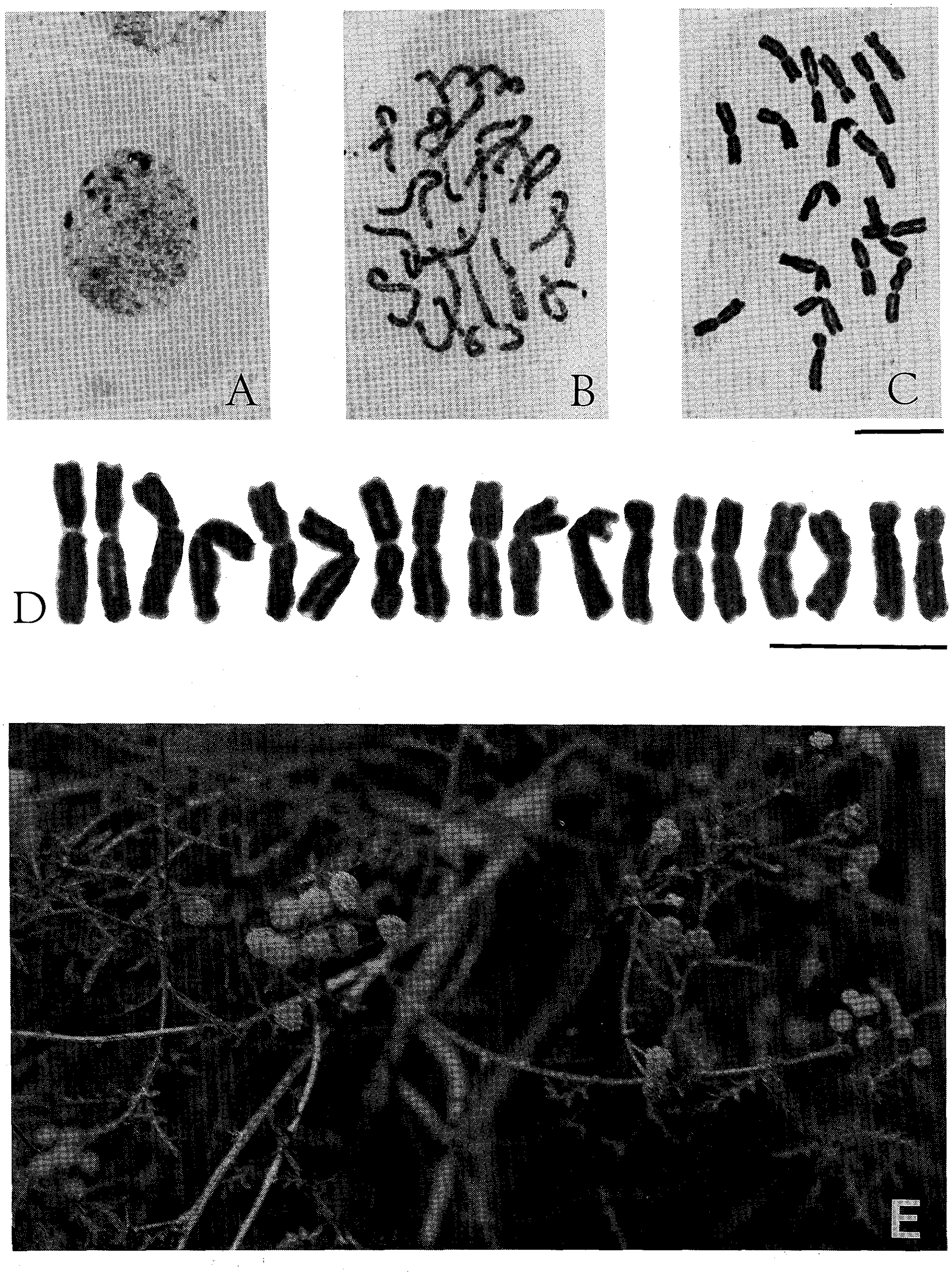

Fig. 5. Karyomorphology of *Cancrinia maximowiczii* C. Winkl., 2n=18. A. The resting nucleus of the complex chromocenter type. B. The mitotic prophase chromosomes of the interstitial type. C. 2n=18 at mitotic metaphase. D. The diploid karyotype with 14 median- or submedian- and 4 subterminal-centromeric chromosomes. Bar=10 μm. E. A flowering, woody nature of a plant.

ternational Research Program–Joint Research No. 04044128 for 1992–1993 (the representative: Ryuso Tanaka) of the Ministry of Education, Science and Culture of Japan. This paper was contributed from Laboratory of Plant Chromosome and Gene Stock, Faculty of Science, Hiroshima University (Contribu-

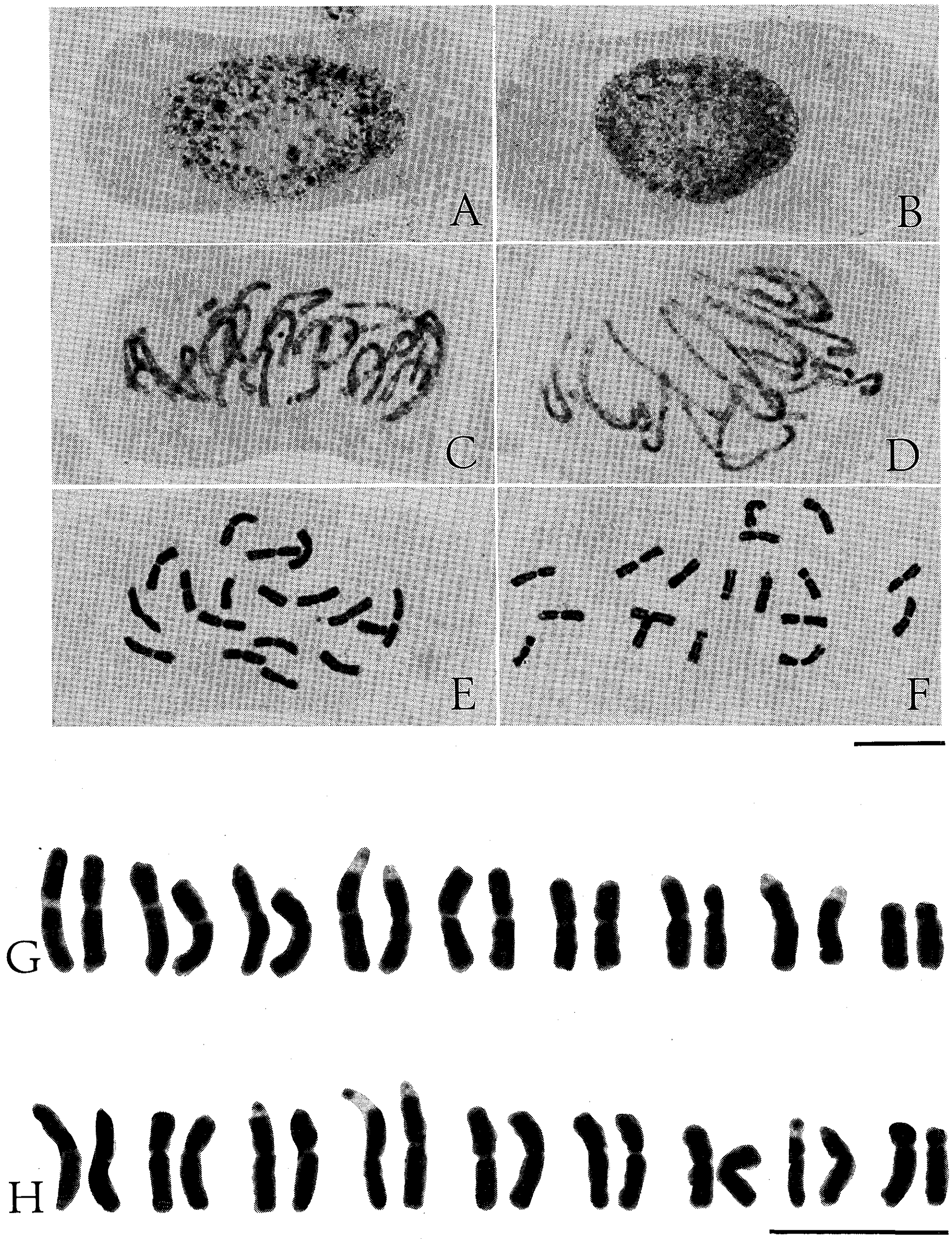

Fig. 6. Karyomorphology of *Dendranthema lavandulifolium* (Fisch. ex Trautv.) Ling et Shih, two different individuals, 2n=18. A and B. The resting nucleus of the complex chromocenter type. C and D. The mitotic prophase chromosomes of the interstitial type. E and F. 2n=18 at mitotic metaphase. G and H. The diploid karyotype with 14 median- or submedian- and 4 subterminal-centromeric chromosomes. Bar=10 μm.

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近藤勝彦，田中隆荘，洪　徳元，日詰雅博，楊　親二，中田政司：**中国産野生キク属（広義）の細胞遺伝学的研究 V. 中国高地産 *Ajania* 3種とその近縁種 *Cancrinia maximowiczii* 及び *Dendranthema lavandulifolium* の染色体の研究**

中国高地産 *Ajania* 3種，*Cancrinia maximowiczii* 及び *Dendranthema lavandulifolium* は，共通して複雑染色中央粒型静止期核，介在型分裂期前期染色体の核形態を示した．*Ajania myriantha* と *A. prezewalskii* は，染色体数が2n＝18（二倍体）の個体と，2n＝36（四倍体）の個体があった．そのほかの種は，すべて2n＝18（二倍体）であった．分裂期中期核型は，全二倍体種に共通して，14個の中部または次中部動原体型染色体と4個の次端部動原体型染色体からなっていた．四倍体核型は，共通して，30個の中部または次中部動原体型染色体と6個の次端部動原体型染色体からなっていた．*Ajania* 属において，狭い地域に固有している種は二倍体であり，汎存種に種内倍数性がみられる傾向があるように思われた．