

## A note on the karyotype of *Farfugium japonicum* (Asteraceae)

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## Hajime Okuno<sup>1</sup>, Masashi Nakata<sup>2</sup>, Masahiro Mii<sup>3</sup> and Toshiaki Shiuchi<sup>2</sup>: A note on the karyotype of *Farfugium japonicum* (Asteraceae)

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*Farfugium japonicum* (L.f.) Kitam. (Asteraceae) is an evergreen perennial herb distributed in Japan, South Korea and China (Taiwan and the middle part of Mainland China) (Koyama 1968 ; Kitamura 1981). In Japan it grows mainly on rocky cliffs near seashores in Honshu (south-western part), Shikoku, Kyushu, Ryukyu and the Ogasawara Isls. (Koyama 1968, 1995). This species has long been cultivated for ornamental purpose since the Edo Era, and a number of cultivars are known (Kitamura et al. 1994).

Chromosome number of *F. japonicum* has been reported to be  $n=30$  (Ishikawa 1916, as *Ligularia tussilaginea*), and  $2n=60$  for the plants from Honshu (Arano 1962), Ryukyu (Miyagi 1971), Taiwan (Hsu 1970) and China (Su and Liu 1995, cultivated in the botanical garden, Wuhan Institute of Botany). The karyotypes of the species have also been presented by Arano (1962) as  $K=60=42\ st+16\ sm+2\ sm(cs)$  and by Su and Liu (1995) as  $2n=60=12\ st+46\ sm+2\ m$  with no satellite chromosomes. In a part of studies on *F. japonicum* as a traditional garden plant of Ja-

pan, we report here the presence of four telocentric chromosomes in the chromosome complement of wild *F. japonicum*, which is different from those reported by Arano (1962) and Su and Liu (1995).

### Materials and methods

A strain cultivated in the Botanic Gardens of Toyama, which was originally collected in Kodakara-jima, Tokara Isls., Kagoshima Pref. (accession number 44120), was used for karyotype analysis. Additional observations were made on four individuals collected in Saga, Hyogo, Shizuoka and Ishikawa Prefectures. Their localities are listed in Table 1 and the voucher specimens of these plants are stored in TYM.

Fresh root tips of 5 mm length were excised from potted plants, pretreated with 2 mM 8-hydroxyquinoline aqueous solution at 15°C for 8 h, and fixed with Farmer's fixative (99.5% ethanol : glacial acetic acid = 3 : 1) at 5°C for 20 h. The fixed root tips were macerated in a mixture of

Table 1. Localities and voucher data of *Farfugium japonicum* observed in this study

Locality	Voucher specimen
Kodakara-jima, Tokara Isls., Kagoshima Pref.	BGT 44120
Nishinohama-machi, Karatsu City, Saga Pref.	Okuno 4102
Okinoura, Kasumi-ku, Kami Cho, Hyogo Pref.	Okuno 2803
Omaezaki, Omaezaki City, Shizuoka Pref.	Okuno 2203
Kurosaki-machi, Kaga City, Ishikawa Pref.	Okuno 1901

1 N hydrochloric acid and 45% acetic acid (2:1, v/v) at 60°C for 15 sec, and immediately rinsed with water. Then the meristematic regions of 1 mm length were cut off from the root tips and stained with 1% acetic orcein (synthetic; Kanto Kagaku, Co.) at room temperature for 10 min, covered with a cover glass, and then slightly heated by an alcohol lamp for a few seconds before squashing. Description of chromosome morphology followed that employed by Tanaka (1977, 1980).

### Results and discussion

Somatic chromosomes at interphase formed nucleus of 15–25  $\mu\text{m}$  in diameter or in major axis (Fig. 1 A). Chromomeric granules and small heterochromatic bodies were observed throughout the nucleus with several aggregated regions. Thus, the type of the interphase nucleus was considered to be intermediate between the diffuse type and the simple chromocenter type (Tanaka 1977). One to four nucleoli were frequently observed in the nucleus. The mitotic prophase chromosomes had early condensed regions at the proximal position, which gradually diffused into chromosome ends (Fig. 1 B). Thus, they were classified into the gradual type (Tanaka 1977).

The chromosome number  $2n=60$  was counted in both prophase and metaphase (Figs. 1 B and C), confirming the previous counts of Ishikawa (1916), Arano (1962), Miyagi (1971), Hsu (1970) and Su and Liu (1995). The size of the 60 chromosomes showed gradual variations from 1.8 to 4.1  $\mu\text{m}$  in length (Fig. 1 D). Long arm of the longest chromosome in the figure was considered to be elongated accidentally by squashing procedure. The chromosome complement was composed of 4 telocentric, 50 subtelocentric or submetacentric and 6 metacentric chromosomes. The chromosome end of the centromeric side of the four telocentric chromosomes had weakly stained chromosome structure (see arrowheads in Fig. 1 D) named “seta”.

Arano (1962) reported the karyotype of *F. japonicum* as  $K=60=42\text{ st}+16\text{ sm}+2\text{ sm}(\text{cs})$ , while Su and Liu (1995) described it as  $2n=60=12\text{ st}+46\text{ sm}+2\text{ m}$  with no satellite chromosome. The present result differs from those in respect to the

presence of four telocentric chromosomes. The ratio of metacentric, submetacentric and subtelocentric chromosomes in the  $2n=60$  chromosome complement slightly differed among cells or roots within the same individual because of the change in the form of chromosomes by pretreatment condition. However, the form of the four telocentric chromosomes was stable and distinguishable from the other 56 chromosomes. The four telocentric chromosomes were also found in the plants collected in Saga, Hyogo, Shizuoka and Ishikawa Prefectures (Figs. 2 A–D). Thus, the presence of the telocentric chromosomes might be common to *F. japonicum*.

The genus *Farfugium* contains two species and five varieties distributed in southeastern China, southeastern Korea, Taiwan, Ryukyu, Kyushu, Shikoku and western Honshu, while the genus *Ligularia*, which is considered to be closely related to *Farfugium*, contains about 100 species distributing mainly from mountain to alpine regions in East Asia (Koyama 1968). According to the recent report (Gong et al. 2001) and our unpublished data, nine species of *Ligularia* in China and Japan have all the same chromosome number  $2n=58$  with highly symmetric karyotype, mainly composed of metacentric and submetacentric chromosomes without telocentric chromosomes. The mean chromosome length varies 2.8–3.9  $\mu\text{m}$  among four *Ligularia* species of Japan (unpublished data), thus the chromosome size of the genus *Ligularia* is as large as that of *Farfugium japonicum*. Judging from its distribution and habitat, i.e., rocky cliff near seashore in East Asia, *F. japonicum* might be more derivative than *Ligularia* species. From the karyomorphological point of view, the four telocentric chromosomes in *F. japonicum* might have derived from two metacentric chromosomes by the Robertsonian type structural change in chromosome (centric fission). To confirm this hypothesis, it is necessary to make a comparative karyotype analysis in *Farfugium* and *Ligularia*, especially devoting an attention to chromosome banding.

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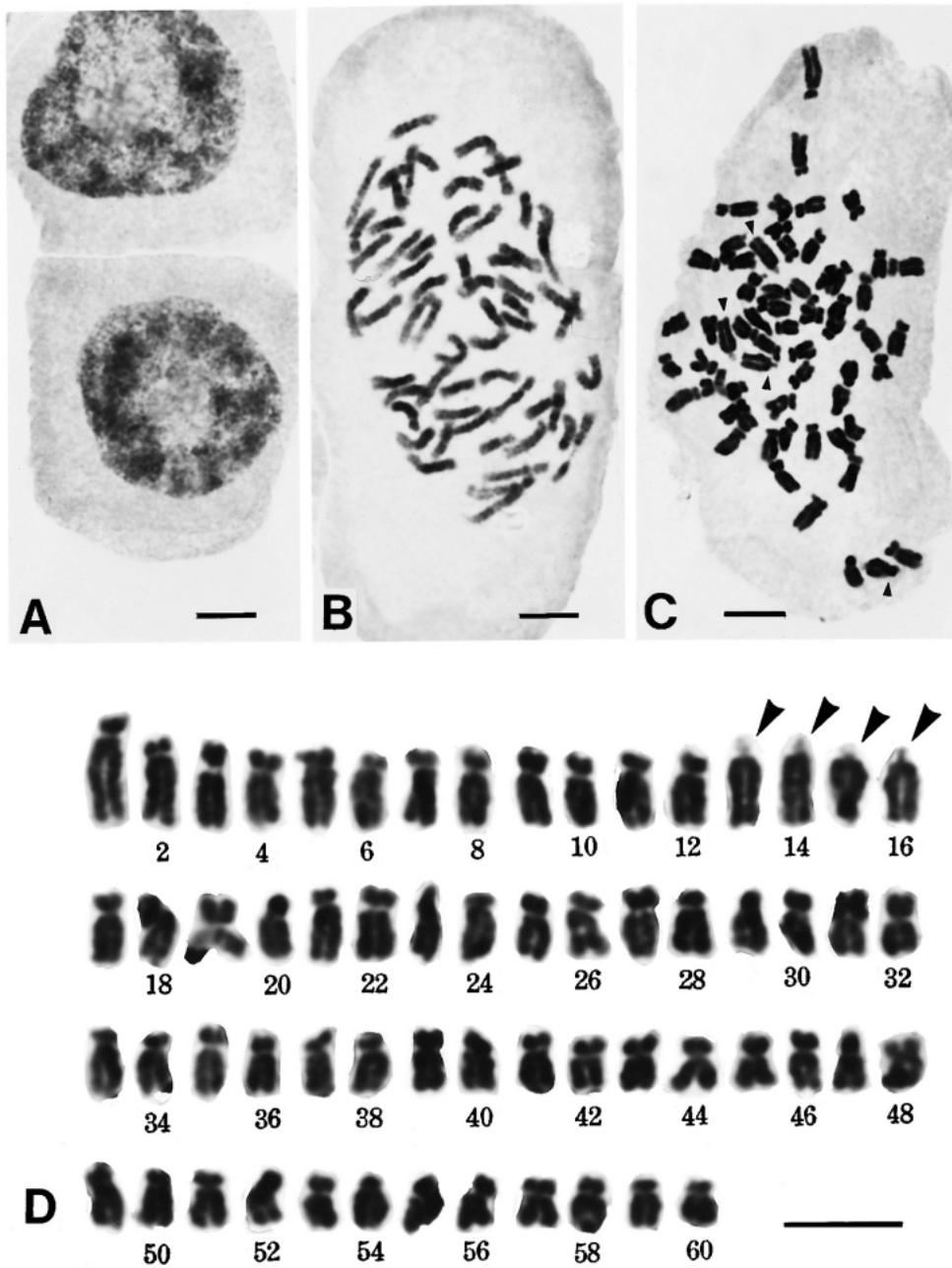


Fig. 1. Somatic chromosomes of *Farfugium japonicum*. A: Interphase nuclei. B: Mitotic prophase chromosomes ( $2n=60$ ). C: Mitotic metaphase chromosomes ( $2n=60$ ). D: Individual chromosomes of the figure 1 C. Arrow-heads indicate four telocentric chromosomes. Scale bars indicate 5  $\mu$ m.

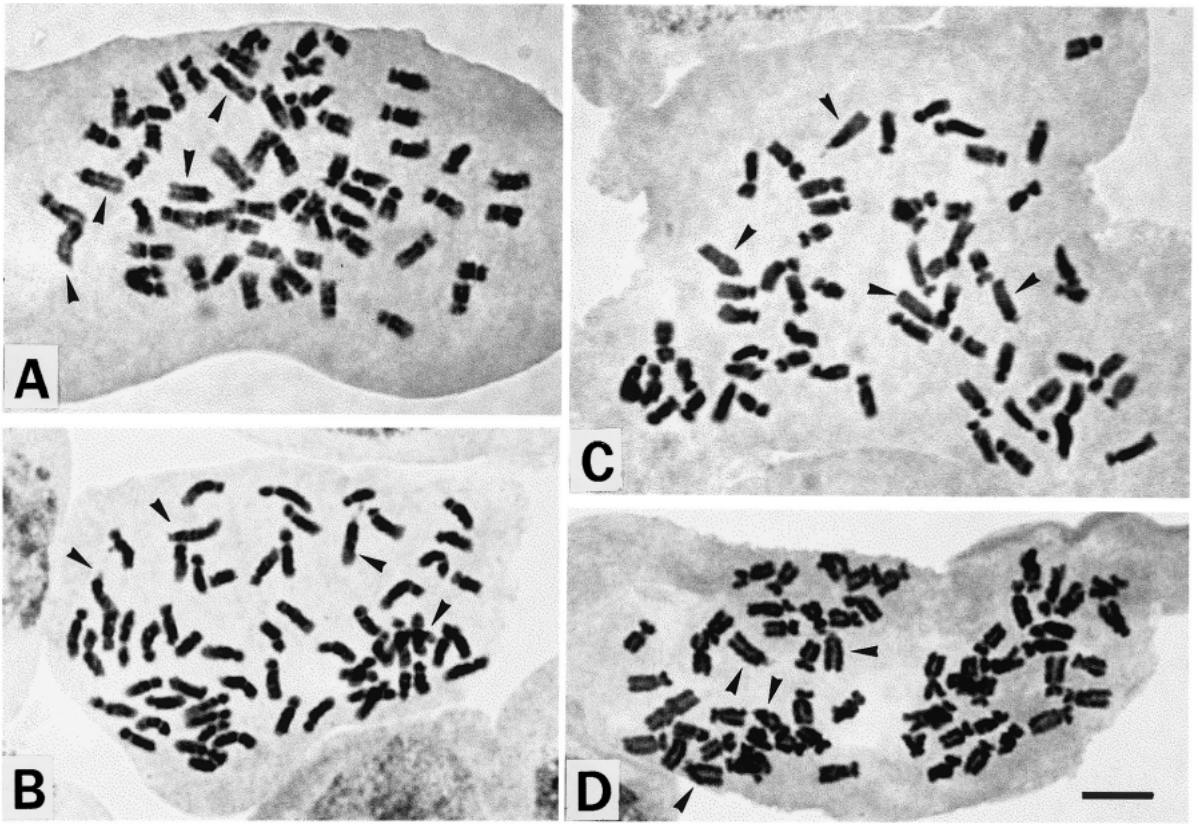


Fig. 2. Somatic metaphase chromosomes,  $2n=60$ , of *Farfugium japonicum* collected in Saga (A), Hyogo (B), Shizuoka (C) and Ishikawa (D) Prefectures. Arrowheads indicate four telocentric chromosomes. Scale bar indicates 5  $\mu$ m.

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奥野 哉<sup>1</sup>・中田政司<sup>2</sup>・三位正洋<sup>3</sup>・志内利明<sup>2</sup>：ツワブキの核型に関する新知見

ツワブキ *Farfugium japonicum* (L. f.) Kitam. は、日本(中部～琉球)、朝鮮南部、中国(台湾、中部)の主に海岸に分布し、日本では江戸時代から栽培されて多くの園芸品種が知られている。本種の染色体数は日本、台湾、中国の材料で  $2n=60$  が算定されており、核型も  $2n=60=42\text{ st}+16\text{ sm}+2\text{ sm}$

(cs) (荒野 1962),  $2n=60=12\text{ st}+46\text{ sm}+2\text{ m}$  (Su and Liu 1995) の報告がある。今回、トカラ列島のツワブキについて観察したところ、染色体数は  $2n=60$  で、6個の中部動原体型染色体、50個の次端部または次中部動原体型染色体の他に、端部に角状の染色質と動原体を持つ特徴的な染色体が4本認められた。この4本の末端動原体型染色体は、佐賀、兵庫、静岡、石川の各県産ツワブキにも観察されたことから、ツワブキに共通する特徴と考えられる。ツワブキ属と近縁とされるメタカラコウ属の染色体数は、日本と中国の種では  $2n=58$  で、中部動原体型染色体が22～30本、次中部動原体型染色体が26～34本と対称性が高く、末端動原体型染色体は存在しない (Gong et al. 2001; 未発表データ)。メタカラコウ属の分布の中心は中国にあり約100種が東アジアの山地～高山に分布するのに対し、ツワブキ属は2種5変種が東アジアの海岸域に分布しており (Koyama 1968)、分布域や生育地から、より派生的と考えられる。ツワブキの2対の末端動原体型染色体はメタカラコウ属の1対の中部動原体型染色体のロバートソン型切断に由来する可能性がある。

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