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Different Size of Total Chromosome Lengths in two Diploid Species of *Artemisia* (Asteraceae) in Japan

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國府方吾郎[1]・近藤勝彦[2]・松本　定[1]：日本産ヨモギ属二倍体種2種における染色体長サイズの違い

The genus *Artemisia* (Asteraceae) is primary distributed in the warm and the temperate regions and consists of about 250 species (Kitamura 1981). Previously some cytological studies have been performed in this genus (*e.g.*, Shimotomai 1946, Kawano *et al.* 1995, Masumori *et al.* 1995). However, infrageneric classifications of the genus *Artemisia* are still under discussion, and require more taxonomical information including cytological data (Belenovskaja 1996).

The aim of the present study is to investigate chromosomal characters of *Artemisia capillaris* Thunb. and *A. keiskeana* Miq. by the conventional aceto-orcein staining squash method.

Materials and Methods

Plant materials

Somatic chromosomes of *Artemisia capillaris* cultivated in the Tsukuba Botanical Garden and those of *A. keiskeana* collected from Yoji-gawa, Iwaki, Fukushima, Japan were investigated in the present cytological study. Taxonomic treatment in the present study followed Koyama (1995). The voucher specimens of the two species were deposited in the herbarium of the National Science Museum, Tokyo (TNS).

Conventional aceto-orcein staining squash method

Root tips of the two species were harvested, pretreated in 2 mM 8-hydroxyquinoline at 15°C for 3 h and fixed in acetic ethanol (1 : 3) at 4°C for approximately 2 h. They were macerated in a mixture of 1N hydrochloric acid and acetic acid (2 : 1) at 60°C for 10 sec, put on glass slides, and then were stained in 2% aceto-orcein at room temperature for 4 h and squashed.

Chromosomes at mitotic metaphase were classified by arm ratio (R = long arm length/short arm length) following Levan *et al.* (1964). Median-centromeric ($1.0 \geq R < 1.7$), submedian-centromeric ($1.8 \geq R < 3.0$), subterminal-centromeric ($3.1 \geq R < 7.0$), and terminal-centromeric chromosomes ($7.1 \geq R$) were abbreviated and symbolized as "**m**", "**sm**", "**st**" and "**t**".

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Results and Discussion

An individual of *Artemisia capillaris* showed the chromosome number of 2n = 18, and the karyotype consisted of fourteen **m** and four **sm** chromosomes (Figs. 1A and 2A). An individual of *A. keiskeana* showed the chromosome number of 2n = 18 and one supernumerary chromosome (B chromosome), and the karyotype consisted of fourteen **m** and four **sm** chromosomes with a B chromosome (Figs. 1B and 2B). Mean of the total length of eighteen chromosomes based on three mitotic complements in *A. capillaris* was 70.3 μm, while that, without the B chromosome, of *A. keiskeana* was 154.1 μm (Table 1).

The chromosome numbers and the karyotypes in *A. capillaris* and *A. keiskeana* were respectively confirmed those reported by Shimotomai (1946) and Kawano *et al.* (1995). Masumori *et al.* (1995)

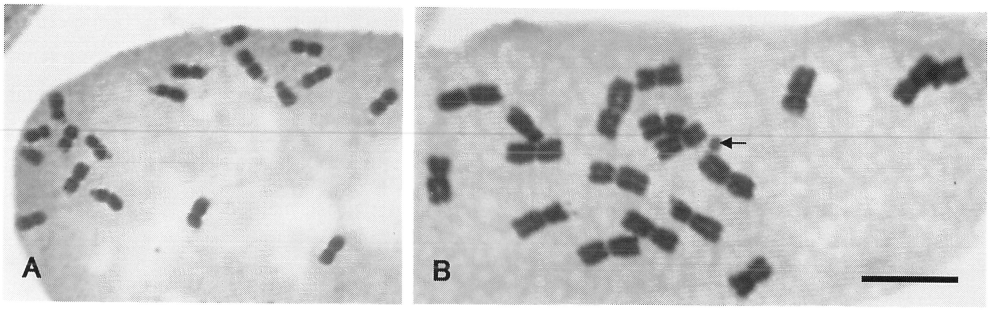

Fig. 1. Orcein-stained chromosomes at mitotic metaphase of two *Artemisia* species. **A**. *A. capillaris*. **B**. *A. keiskeana*. Arrow indicates B chromosome in *A. keiskeana*. Bar shows 10 μm.

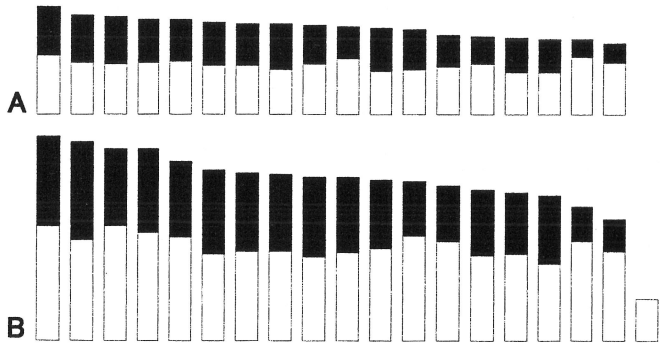

Fig. 2. Ideograms of two *Artemisia* species. **A**. *A. capillaris*. **B**. *A. keiskeana*. Opened areas show long arms and solid areas show short arms.

Table. 1. Chromosomal characters investigated in two *Artemisia* species

Species	2n	Karyotype	Total length of 18 chromosomes (μm)*
A. capillaris	18	14**m** + 4**sm**	70.3 (= (86.1 + 66.7 + 58.3)/3)
A. keiskeana	18	14**m** + 4**sm** + 1B	154.1 (= (163.4 + 152.0 + 146.9)/3)

*Total length of 18 chromosomes is mean based on three chromosome complements at mitotic metaphase; B chromosome was excluded from total length of chromosomes in *A. keiskeana*.

examined cytological investigation on 391 individuals in eleven populations of *A. keiskeana*, and reported that B chromosomes were found in frequency of 21% of the 391 individuals. The present study confirmed the existence of B chromosome in *A. keiskeana* reported by Masumori *et al.* (1995). Although some satellites were unstably visible in the mitotic complements of two *Artemisia* species, it was difficult to detect exact numbers and distribution patterns of too small satellites by the present aceto-orcein squash method. Further study using the FISH method could clarify the number and the distribution patterns of rDNA sites on somatic chromosomes.

Two *Artemisia* speices were diploid cytotype with x = 9 agreed with the previous reports (Shimotomai 1946, Kawano *et al.* 1995, Masumori *et al.* 1995). However, the total lengths of eighteen chromosomes based on three mitotic complements of two *Artemisia* speices were significantly different from each other by the Student's *t*-test ($p< 0.001$; Table 1). In some genera of Asteraceae, infrageneric-different total lengths of a haploid karyotype have previously been reported, for example in *Aster sensu lato* (Tara 1972). According to taxonomic treatment of Koyama (1995) based on morphological characters, *A. capillaris* was classified into the section *Dracunculus*, while *A. keiskeana* was classified into the section *Abrotanum*. It is difficult to explain that the quantitative difference of the total chromosome length is caused by interspecific or inter-sectional factors at present, but this study implicates that the genus *Artemisia* could possess infrageneric polymorphism of nuclear DNA amounts.

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Summary

Somatic chromosomes of *Artemisia capillaris* and *A. keiskeana* were investigated by the standard aceto-orcein squash method. Chromosome number of *A. capillaris* was 2n = 18, and that of *A. keiskeana* was 2n = 18 +1B. Chromosome complement at mitotic metaphase of *A. capillaris* consists of fourteen **m** and four **sm** chromosomes, and that of *A. keiskeana* consists of fourteen **m** and four **sm** chromosomes with a B chromosome. Total length of eighteen chromosomes in *A. capillaris* was significantly shorter than that in *A. keiskeana* ($p<0.001$).

摘　要

日本産ヨモギ属2種，カワラヨモギ（*Artemisia capillaris*），イヌヨモギ（*A. keiskeana*）の体細胞染色体をアセトオルセイン押し潰し法を用いて観察した。観察の結果，カワラヨモギの染色体数は2n = 18で核型は14個の **m** 染色体と4個の **sm** 染色体から構成されていた。一方，イヌヨモギの染色体数は2n = 18 + 1B で核型は14個の **m** 染色体と4個の **sm** 染色体そして1個のB染色体から構成されていた。3つの中期染色体像をもとに2種の全染色体長を比較した結果，カワラヨモギの全染色体長はイヌヨモギの全染色体長よりも有意に短かった（$p<0.001$）。

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