Karyomorphological comparison in two taxa of *Chelidonium* (Papaveraceae) in Altai and Japan*

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アルタイ産と日本産のクサノオウ属（ケシ科）の核形態学的比較 *

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Abstract. The karyotype of *Chelidonium majus* L. var. *majus* in Altai area was compared with the karyotype of *C. majus* L. var. *asiaticum* (Hara) Ohwi in Japan. The chromosome number of *C. majus* var. *majus* in Altai area was $2n$=12, and the chromosome number of *C. majus* var. *asiaticum* in Japan was $2n$=10. *C. majus* var. *majus* in Altai area showed a homogeneous and symmetric karyotype, while *C. majus* var. *asiaticum* in Japan showed a low homogeneous and low symmetric karyotype.

Introduction

Genus *Chelidonium* L., the family Papaveraceae, consists of the single species. *Chelidonium majus* L. is a perennial herbaceous plant, and distributes widely in Europe, North Africa and East Asia. The group distributed in Europe and North Africa is considered to be an elementary species (*C. majus* L.), and the group distributed in East Asia is treated as a subspecies (*C. majus* L. subsp. *asiaticum* Hara) or a variety [*C. majus* L. var. *asiaticum* (Hara) Ohwi]. Thus, it is treated as a variety.

Chelidonium majus L. var. *majus* in Altai District and Altai Republic, South Siberia, Russia is found around a roadside and a house. The growth environments of *C. majus* L. var. *majus* in Altai area are approximately similar to those of *C. majus* L. var. *asiaticum* (Hara) Ohwi in Japan.

A capsule of *C. majus* var. *majus* grows in linear column form of 3-4 cm in length obediently and produces many seeds, while a capsule of *C. majus* var. *asiaticum* does not lengthen obediently, because it produces some immaturity ovules. Previous cytological work showed that the maturity of pollen of *C. majus* var. *asiaticum* in Japan was low and 70% of them were sterile (Shimizu, 1977).

The chromosome number of *C. majus* var. *majus* was reported in $2n$ = 12 (Hill, 1989. Krahulcova, 1982. Strid and Franzen, 1983) and *C. majus* var. *asiaticum* was counted in $2n$ = 10 (Nishikawa, 1985. Probatova and Sokolovskaya, 1986).

In this report, the karyotype of *C. majus* var. *majus* in Altai area was studied in detail and was compared with the karyotype of *C. majus* var. *asiaticum* in Japan.

Material and Methods

The seeds of *C. majus* L. var. *majus* collected at the roadside in Altai area were sown in September 2008 and the seedlings were used as materials for chromosome observations.

Chelidonium majus L. var. *asiaticum* (Hara) Ohwi which was used for chromosome observations was collected in Hiroshima prefecture and was cultivated repeatedly at the Hiroshima Botanical Garden.

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Somatic chromosomes were observed by staining the meristematic cells from root tips according to the aceto-orcein squash method by Tanaka and Kamemoto (1960), with a slight modification. Growing root tips were cut into small pieces in about 2-3 mm thickness. The pieces were immersed in 0.002M 8-hydroxyquinoline for 4 h at 18°C, fixed in 45% acetic acid for 10 min at 5°C, hydrolyzed (treated) in a mixture of 1N hydrochloric acid and 45% acetic acid (2:1) for 30 sec at 60°C, and then, they were stained and squashed in 1% aceto-orcein solution.

The chromosomes at a resting stage were morphologically studied by observing their condensed figures and classified into the types as defined and proposed by Tanaka (1971, 1980).

The chromosomes at a mitotic metaphase were arranged in descending order in length and numbered starting from 1, thus grading the chromosomes from the longest to the shortest. Long and short arm lengths for each chromosome were measured and the ratios of the long- to the short-arm lengths were calculated. According to Levan *et al.* (1964), the arm ratios from 1.0 to 1.7 are median, those from 1.8 to 3.0 are submedian, and those from 3.1 to 7.0 are subterminal. The karyotype formulas were based on the chromosome lengths and positions of centromeres according to Tanaka (1980).

Results

The chromosomes at a resting stage, a prophase and a metaphase of two taxa are shown in Fig. 1. The properties of a mitotic metaphase chromosome after measuring and analyzing are shown in Table 1 and 2. The chromosomes at metaphase arranged in long order are shown in Fig. 2.

C. majus L. var. *majus*

The chromosome number of *C. majus* var. *majus* in Altai area at a mitotic metaphase was $2n$=12, which confirmed the previous reports (Hill, 1989. Krahulcova, 1982. Strid and Franzen, 1983).

This nucleus at a resting stage was 10 μm in diameter and the chromosomes were observed as chromomeric granules, fibrous threads and chromatin blocks scattered throughout the nucleus. The chromosome features at a resting stage were of the complex chromocenter type according to Tanaka's classification (1971). In contrast, the chromosomes at a mitotic prophase shaped several early condensed segments located in the interstitial, proximal and terminal regions of both arms.

The $2n$=12 chromosomes during a mitotic metaphase showed a gradual decrease in length from the longest (5.3 μm) to the shortest (4.0 μm).

Among the 12 chromosomes, eight chromosomes (Nos. 1-4, 7, 8, 11, 12) had the arm ratios from 1.2-1.6 and had centromeres at the median regions, whereas four chromosomes (Nos. 5, 6, 9, 10) had the ratios from 1.9-2.8 and their centromeres at the submedian regions. Four chromosomes (Nos. 5-8) were accompanied with a satellite at the distal end of the short arm.

C. majus L. var. *asiaticum* (Hara) Ohwi

The chromosome number of *C. majus* var. *asiaticum* at mitotic metaphase was $2n$=10, which confirmed the previous reports (Nishikawa, 1985. Probatova and Sokolovskaya, 1986).

The chromosomes at a resting stage and a mitotic prophase of *C. majus* var. *asiaticum* were morphologically similar to those of *C. majus* var. *majus* described above.

The $2n$=10 chromosomes during a mitotic metaphase showed a gradual decrease in length from the longest (6.1 μm) to the shortest (4.1 μm).

Among the ten chromosomes, two chromosomes (Nos. 3, 4) had the arm ratios from 1.2-1.6 and had centromeres at the median regions, whereas six chromosomes (Nos. 1, 2, 5-8) had the ratios from 1.8-2.3 and their centromeres at the submedian regions. Two chromosomes (Nos. 9, 10) gave the ratio of 3. 1 having the centromeres at the subterminal

regions. Four chromosomes (Nos. 5-8) were accompanied with a satellite at the distal end of the short arm.

Discussion

12 chromosomes of *C. majus* var. *majus* were constructed in $2n$=12 (8m+4sm), and each of six pairs of chromosomes showed homology.

Thus, this variety showed a homogeneous and symmetric karyotype.

In contrast, ten chromosomes of *C. majus* var. *asiaticum* were constructed in $2n$=10 (2m+6sm+2st), and there were not two small size chromosomes which existed in *C. majus* var. *majus*.

Among five pairs of chromosomes of *C. majus* var. *asiaticum*, three pairs (Nos. 1-2, 3-4, 5-6) showed not homology.

Thus, this variety showed a low homogeneous and low symmetric karyotype.

Four chromosomes (Nos. 5-8) were accompanied with a satellite in the distal end of the short arm in both taxa. And the satellites appeared with Nos. 5-6 chromosomes clearly with two taxa, but a tendency to be lacking in the vividness was seen in the satellite of Nos. 7-8 chromosomes.

Thus, the observation for karyomorphology supports the phenomena with a lot of unripe ovules and the low pollen fertility in *C. majus* var. *asiaticum*.

The karyotypic comparison of two taxa indicated that *C. majus* var. *asiaticum* might be more progressive than *C. majus* var. *majus*, because a symmetry of the chromosome length of the long arm and the short arm of *C. majus* var. *majus* was higher than that of *C. majus* var. *asiaticum*.

Further investigation of the karyotype of the plants in different localities is necessary to clarify a reason of heterogeneous syndesis of *C. majus* var. *asiaticum*, and to clarify relationship between *C. majus* var. *majus* and *C. majus* var. *asiaticum*.

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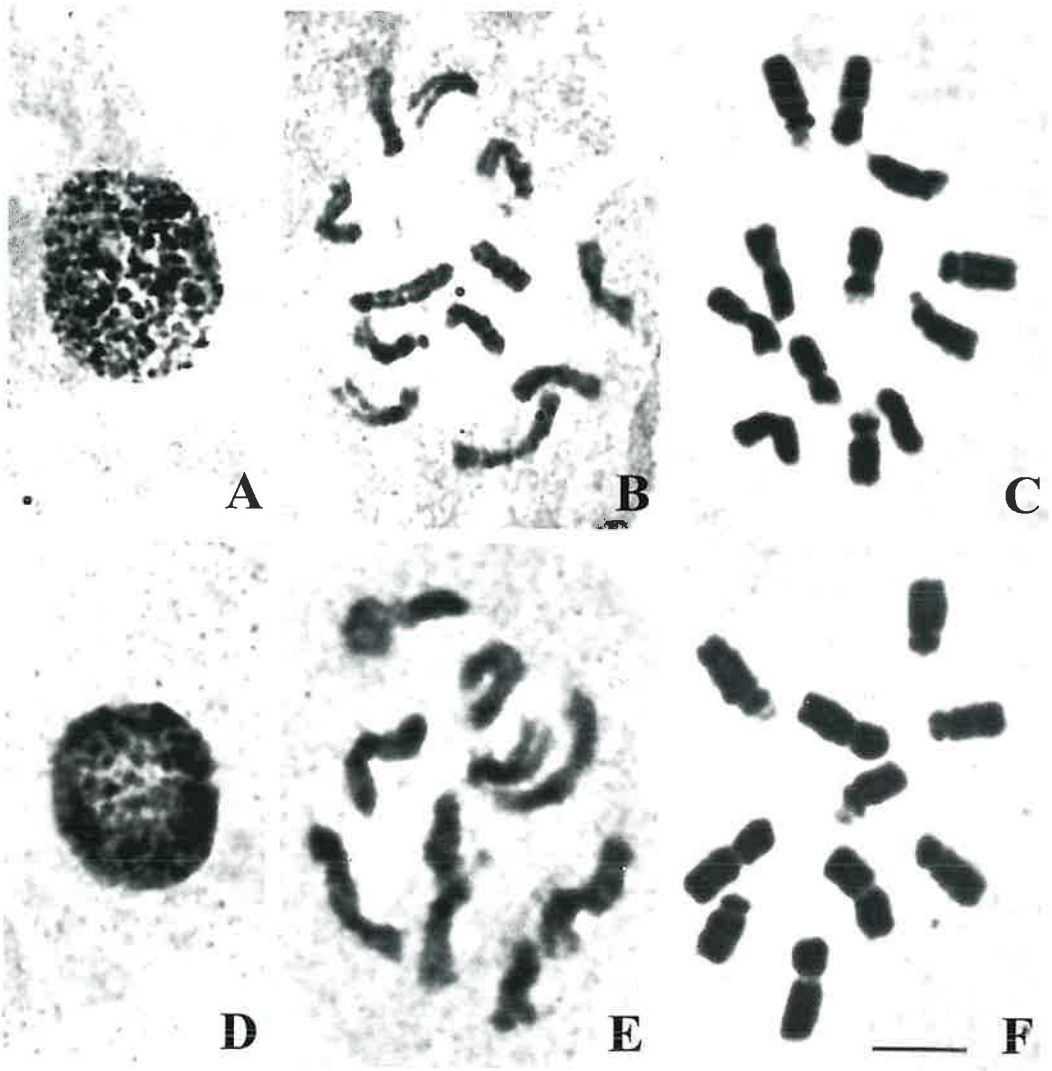

Fig. 1. Somatic chromosomes of two taxa of *Chelidonium*.
A-C. *C. majus* var. *majus*, 2*n*=12. D-F. *C. majus* var. *asiaticum*, 2*n*=10. A and D. resting stage. B and E. mitotic prophase. C and F. mitotic metaphase. Bar indicates 5μm.

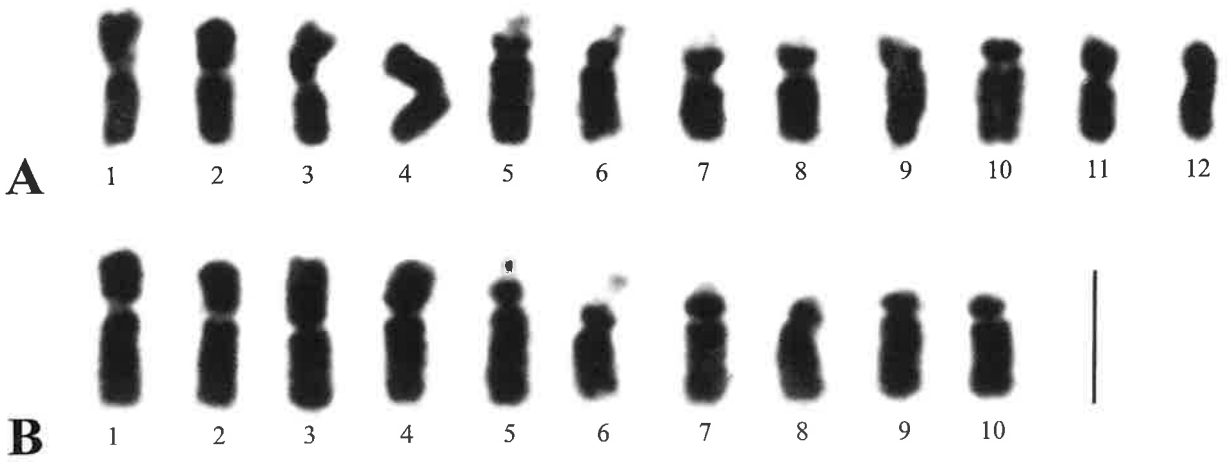

Fig. 2. Metaphase chromosomes alignments of two taxa of *Chelidonium*.
A. *C. majus* var. *majus*, 2*n*=12. B. *C. majus* var. *asiaticum*, 2*n*=10. Bar indicates 5μm.

Table 1. Measurements of somatic chromosomes of *Chelidonium majus* var. *majus* at mitotic metaphase, 2*n*=12

Number of arranged chromosome order	Chromosome length (μm)				Relative length	Arm ratio	Form
	satellite	short arm	long arm	total length			
1		2.3	3.0	5.3	7.1	1.3	m
2		2.3	3.0	5.3	7.1	1.3	m
3		2.1	2.6	4.7	6.3	1.2	m
4		2.1	2.6	4.7	6.3	1.2	m
5*	0.7	0.9	3.1	4.7	6.3	1.9	sm
6*	0.7	0.9	3.1	4.7	6.3	1.9	sm
7*	0.5	1.1	2.6	4.2	5.6	1.6	m
8*	0.5	1.1	2.6	4.2	5.6	1.6	m
9		1.1	3.1	4.2	5.6	2.8	sm
10		1.1	3.1	4.2	5.6	2.8	sm
11		1.6	2.4	4.0	5.3	1.5	m
12		1.6	2.4	4.0	5.3	1.5	m

*: Chromosome with secondary constriction

Table 2. Measurements of somatic chromosomes of *Chelidonium majus* var. *asiaticum* at mitotic metaphase, $2n=10$

Number of arranged chromosome order	Chromosome length (μm)				Relative length	Arm ratio	Form
	satellite	short arm	long arm	total length			
1		2.1	4.0	6.1	8.7	1.9	sm
2		2.1	3.7	5.8	8.3	1.8	sm
3		2.6	3.1	5.7	8.2	1.2	m
4		2.1	3.4	5.5	7.9	1.6	m
5*	0.7	0.9	3.7	5.3	7.6	2.3	sm
6*	0.7	0.9	3.1	4.7	6.7	1.9	sm
7*	0.5	1.0	3.1	4.6	6.6	2.1	sm
8*	0.5	1.0	3.1	4.6	6.6	2.1	sm
9		1.0	3.1	4.1	5.9	3.1	st
10		1.0	3.1	4.1	5.9	3.1	st

*: Chromosome with secondary constriction