

Ryuso TANAKA* & Hisashi YOSHIOKA*: **Karyomorphological studies on *Viola brevistipulata* and its allied species**

田中隆莊*・吉岡 寿*: オオバキスミレおよびその近縁種における核形態学的研究

Viola brevistipulata (Fr. et Sav.) W. Becker placed in sect. *Chamaemelianum* (Becker 1925; Maekawa & Hashimoto 1963) is endemic to Japan, and occurs mainly in high mountain regions of the Japan Sea side of Honshu and Hokkaido. Since this species shows some divergence in the size of external morphology, especially in plant height and leaf, this species is often subdivided into two subspecies, four varieties and five forms (Maekawa & Hashimoto 1963). The majority of these taxa can be found restrictedly in a certain different localities (Maekawa & Hashimoto 1963); e.g., *V. brevistipulata* subsp. *brevistipulata* var. *kishidai* is localized in alpine zones of mountain regions of the central part of Honshu and *V. brevistipulata* subsp. *minor* is localized in subalpine zones of mountain regions of the western part of Honshu. On the other hand, since morphological variation among these taxa is considered to be slight, the taxa are sometimes reduced to only two varieties under the same species (Ohwi 1965).

Morphological analyses of the chromosomes including chromosome numbers can often clarify intra- and inter-specific relationships of plants. According to chromosome studies in the taxa placed in sect. *Chamaemelianum* of Japan, only the chromosome numbers have been reported to be the same to each other, $n=6$ or $2n=12$ (Miyaji 1913, 1929; Sokolovskaya 1966; Lee 1969). However, chromosomal relationships among them have been poorly characterized.

This paper deals with karyotype analysis in three taxa of *V. brevistipulata* and its allied two species within sect. *Chamaemelianum*.

Materials and methods The materials collected from various natural localities and grown in pots in the experimental garden, Botanical Institute, Faculty of Science, Hiroshima University, are listed in Table 1. The nomenclature follows that of Maekawa & Hashimoto (1963, 1968). Observations of mitotic

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Table 1. Localities and chromosome numbers of *Viola brevistipulata* and its allied species observed

Taxa	Localities	Chromosome number (2n)	No. of plants observed
<i>Viola brevistipulata</i>			
subsp. <i>brevistipulata</i>			
var. <i>brevistipulata</i>			
	Mt. Iwate, Iwate Pref.	12	2
	Mt. Komagatake, Akita Pref.	12	2
	Mt. Mikuni, Gunma Pref.	12	1
	Mt. Mikuni, Shiga Pref.	12	2
	var. <i>kishidai</i>		
	Mt. Naeba, Niigata Pref.	12	4
	subsp. <i>minor</i>		
	Mt. Daisen, Tottori Pref.	12	3
	Mt. Tateeboshi, Hiroshima Pref.	12	6
<i>V. yubariana</i>	Mt. Yubaridake, Hokkaido Dist.	12	4
<i>V. orientalis</i>	Mt. Tsurumidake, Oita Pref.	12	2
	Mt. Yufudake, Oita Pref.	12	1
	Mt. Aso, Kumamoto Pref.	12	1

chromosomes were made from the meristematic tissues of root tips. The root tips were pretreated with 0.002 M 8-hydroxyquinoline for four hours at 20°C before they were fixed in acetic-alcohol (99% ethanol: glacial acetic acid=3:1) for two hours at 10°C. Then, they were macerated in 1:2 mixture of 45% acetic acid and 1N hydrochloric acid at 60°C for 20 seconds, and then stained and squashed in 2% aceto-orcein.

Observations Results of chromosome countings in the five taxa used were shown in Table 1. Morphology of the chromosomes in the five taxa was as follows:

1) *Viola brevistipulata* (Fr. et Sav.) W. Becker subsp. *brevistipulata* var. *brevistipulata*. $2n=12$ (Figs. 1A and 2A).

Chromosomes were counted to be $2n=12$ which verified the previous reports (Miyaji 1913, 1929). The chromosomes varied gradually in length from the longest chromosome, approximately 2.2 μm , to the shortest chromosome, approximately 1.4 μm (Fig. 2A). Thus, the gradual alignment of the chromosomes of a complement was categorized to be the homogeneous karyotype (cf. Tanaka 1980). The longest chromosome (No. 1 in Fig. 2A) of the complement had

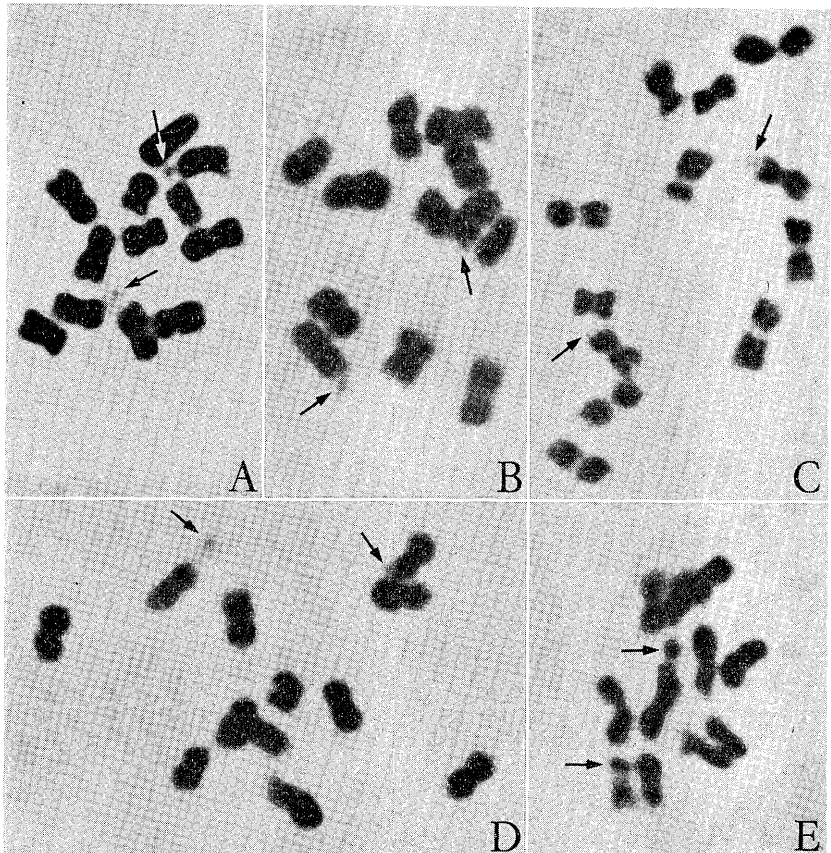


Fig. 1. Photomicrographs of chromosomes at mitotic metaphase in five taxa of *Viola*. A: *V. brevistipulata* subsp. *brevistipulata* var. *brevistipulata*, $2n=12$. B: *V. brevistipulata* subsp. *brevistipulata* var. *kishidai*, $2n=12$. C: *V. brevistipulata* subsp. *minor*, $2n=12$. D: *V. yubariana*, $2n=12$. E: *V. orientalis*, $2n=12$. Arrows indicate satellites. $\times 4000$.

the centromere located in the median position. The 9th and 10th chromosomes (Fig. 2A) had the centromere located in the submedian position. The other chromosomes had the centromere located in the median position. The arm ratio of the chromosomes of a complement was categorized to be the symmetric karyotype (cf. Tanaka 1980).

Satellites which were minute and faintly stained were found in the distal end of the short arms in the 7th and 8th chromosomes, with median centro-

mere (Fig. 2A).

2) *Viola brevistipulata* (Fr. et Sav.) W. Becker subsp. *brevistipulata* var. *kishidai* (Nakai) F. Maekawa et Hashimoto. $2n=12$ (Figs. 1B and 2B).

The chromosome number of $2n=12$ was observed verifying the previous Miyaji's report (1929). Miyaji's chromosome count of $2n=12$ was reported in *V. kishidai*, which is the basionym of *V. brevistipulata* subsp. *brevistipulata*

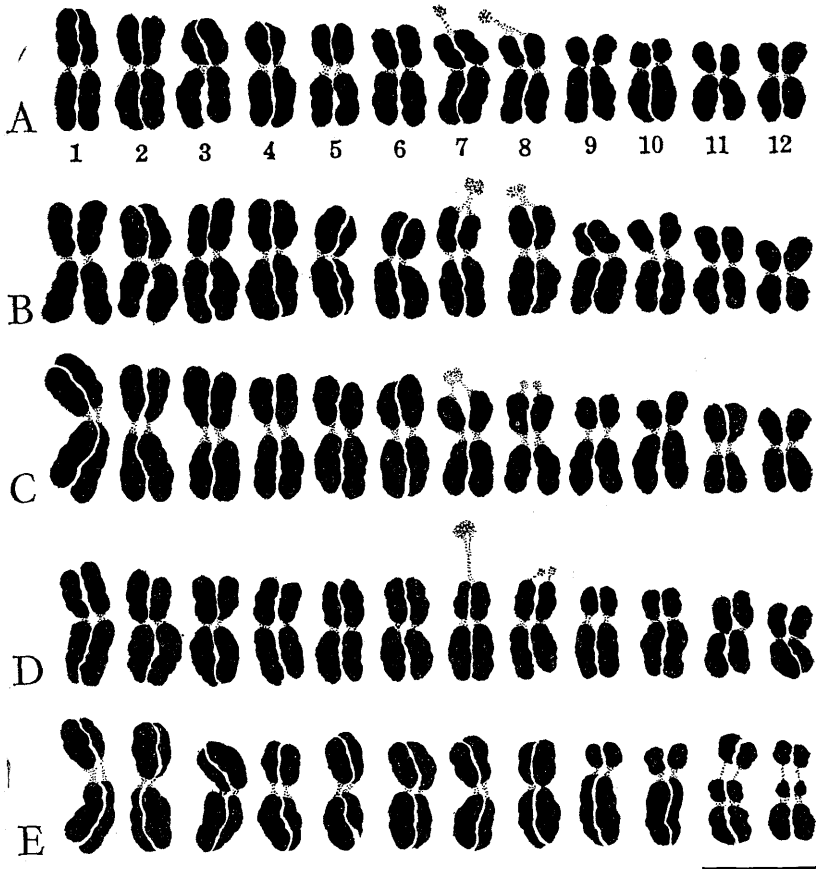


Fig. 2. Mitotic metaphase chromosomes in five taxa of *Viola*. A: *V. brevistipulata* subsp. *brevistipulata* var. *brevistipulata*, $2n=12$. B: *V. brevistipulata* subsp. *brevistipulata* var. *kishidai*, $2n=12$. C: *V. brevistipulata* subsp. *minor*, $2n=12$. D: *V. yubariana*, $2n=12$. E: *V. orientalis*, $2n=12$. Bar shows 2 μ m.

var. *kishidai* by Maekawa & Hashimoto (1963, 1968).

3) *Viola brevistipulata* (Fr. et Sav.) W. Becker subsp. *minor* (Nakai) F. Maekawa et Hashimoto. $2n=12$ (Figs. 1C and 2C).

The chromosome number of $2n=12$ is a new report for this taxon.

4) *Viola yubariana* Nakai. $2n=12$ (Figs. 1D and 2D).

The chromosome number of $2n=12$ is a new report for this taxon.

Morphology of the somatic chromosomes in *V. brevistipulata* subsp. *brevistipulata* var. *kishidai*, *V. brevistipulata* subsp. *minor* and *V. yubariana* described above was found to be similar to that of *V. brevistipulata* subsp. *brevistipulata* var. *brevistipulata* described above.

5) *Viola orientalis* (Maxim.) W. Becker. $2n=12$ (Figs. 1E and 2E).

Chromosomes were counted to be $2n=12$ which verified the previous reports (Sokolovskaya 1966; Lee 1969).

Of the $2n=12$ chromosomes ten were found to be morphologically similar to those of the above four taxa, while the remaining two chromosomes, 9th and 10th chromosomes (Fig. 2E), were found to differ in the subterminally situated centromere in contrast to the submedianly situated centromere in the latter. Satellites which were found in the short arms of the 11th and 12th chromosomes (Fig. 2E) were much larger than those of the above four taxa.

Discussion Five taxa studied were found to have the same chromosome number of $2n=12$. Their metaphase chromosome complements were similar to each other with respect to homogeneous variation of size and symmetric arm ratio. *Viola brevistipulata* subsp. *brevistipulata* var. *brevistipulata*, *V. brevistipulata* subsp. *brevistipulata* var. *kishidai*, *V. brevistipulata* subsp. *minor* and *V. yubariana* showed two common features in karyotype as follows: 1) satellites which were minute and faintly stained were found in the distal ends of the short arms of the 7th and 8th chromosomes, both were medium sized chromosomes, and 2) the 9th and 10th chromosomes had the centromere located in the submedian position and the other chromosomes had the centromere located in the median position. In contrast, *Viola orientalis* showed much larger satellites than those of the above four taxa which were found in the short arms of the 11th and 12th chromosomes, the shortest chromosome, with median centromere. Both chromosomes had the secondary constriction located in the interstitial position of their short arms. Moreover, this species contained the 9th and 10th chromosomes with their centromere located in the subterminal

position.

Viola brevistipulata subsp. *brevistipulata* var. *kishidai* and *V. brevistipulata* subsp. *minor* are treated as a synonym of *V. brevistipulata* by Ohwi (1965). Since these two taxa have some distinct morphological characters such as short plant bodies and reddish petioles and stems (Hashimoto 1973), they were treated to be different from *V. brevistipulata* subsp. *brevistipulata* var. *brevistipulata* according to Maekawa & Hashimoto (1963, 1968). No difference was observed among the metaphase karyotypes in these three taxa.

Viola yubariana is endemic to serpentine area of Mt. Yubaridake in Hokkaido District. This species is distinct from *V. brevistipulata* in having the following morphological characters: the stem is four-angular (Watanabe 1961; Ohwi 1965), the leaf is thick (Watanabe 1961; Ohwi 1965; Hashimoto 1973) and lustrous (Hashimoto 1973). The present metaphase chromosome observations indicated no difference between *V. yubariana* and the three taxa of *V. brevistipulata* discussed above. Thus, the results of the present karyomorphological studies suggest that these four taxa can be considered to be closely related to each other.

Viola orientalis is widely distributed in Ussuri, Manchuria, Korea and Japan. In Japan, this species grows in open grasslands in mountains or highlands in Kyushu, Shikoku and Tokai Districts, being found mainly in Aso Volcano areas in Kyushu. This species is distinct from the other four taxa according to specific morphological characters as follows: the rhizome is short and usually erect, the appendage of sepal is ovate, and the root is thick and spreading horizontally as described by Ohwi (1965).

Since some differences were observed in the metaphase karyomorphology in *V. orientalis* and the other four taxa, *V. orientalis* does not seem to be closely related to the other four taxa.

Résumé

Karyomorphological studies were carried out in a taxonomically complexed group: *Viola brevistipulata* subsp. *brevistipulata* var. *brevistipulata* and its allied taxa, *V. brevistipulata* subsp. *brevistipulata* var. *kishidai*, *V. brevistipulata* subsp. *minor*, *V. yubariana* and *V. orientalis*. The five taxa were found to have the same chromosome number, $2n=12$, and similar karyotypes with the exception of *V. orientalis* which showed minor difference in its subterminally

located centromeres in two chromosomes and its large satellites. The results of karyotype analysis suggested that *V. orientalis* was distantly related to the other four taxa which were closely related to each other.

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Viola brevistipulata subsp. *brevistipulata* var. *brevistipulata* オオバキスミレ, *V. brevistipulata* subsp. *brevistipulata* var. *kishidai* ナエバキスミレ, *V. brevistipulata* subsp. *minor* ダイセンキスミレ, *V. yubariana* シツバキスミレと *V. orientalis* キスミレの 5 taxa において核形態学的研究を行った。5 taxa とも $2n=12$ であり、ともに均等的な染色体長および対称的腕比であった。オオバキスミレ、ナエバキスミレ、ダイセンキスミレとシツバキスミレの 4 taxa はともに $2n=12$ の中に 2 個の次中部動原体的染色体をもち、また、付随体は小形で淡染し、2 個の中位大の染色体の短腕端部に存在していた。これに対して、キスミレは $2n=12$ の中に 2 個の次端部動原体的染色体をもち、また、付随体は 2 個の小形の染色体に存在し、大形で濃染し、介在部

型二次狭窄を形成していた。核形態学的には、前述の 4 taxa は互いに高い類似性を示していたが、キスマレはこれら 4 taxa とは異った核型をもっており、核形態学的には類縁性が低いことがわかった。

○クロガネモチの新品種 (新 敏夫) Toshio SHIN: A new forma of *Ilex rotunda* Thunb.

最近クロガネモチの果実の黄色のものが園芸品として、九州方面に出まわりはじめている。初島住彦氏の「日本の樹木」には「黄実のものはキミノクロガネモチとして区別されることがある」と出ているが、まだ学名はつけられていないので、初島氏とも相談の上、次の学名をつけることにする。本品種の野生のものがあるか否かは不明である。

Ilex rotunda Thunb. f. *xanthocarpa* Shin, f. nov.

Fructus flavus.

Loc. Kokubu city, Pref. Kagoshima, Kyushu. (Feb. 28, 1981. Leg. T. Shin et S. Sako) Type in KAG.

Jap. name: Kimino-kuroganemochi.

(鹿児島大学 教養部)

○ナガバノタチツボスミレの一品 (中馬千鶴) Chidzu CHUMA: A form of *Viola ovato-oblonga* (Miq.) Makino

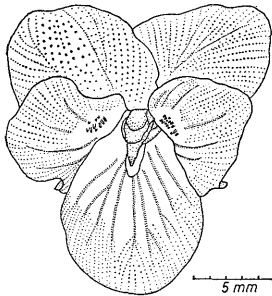


図 1. ナガバノタチツボスミレ

伊勢地方で側弁が有毛のナガバノタチツボスミレを採集した。花は 16~18 mm, 花の色は赤紫色, 花弁は卵円形で互いに重なる。側弁は基部が白色, 中心部から先端にかけて紅紫色, 脈は濃紫色である。側弁の毛は基部近くの上半, 白色部に限り出現する。毛は単細胞か二細胞, 長さ (42 μ)-300-(910 μ) \times 幅 (21 μ)-32-(105 μ), 先端部はほこ型となる。葉は, 根出葉は腎形, 上部の葉は長三角形で明らかにナガバノタチツボスミレである。神宮宮域林の内宮から神路川に沿って約 3 km にわたり 14 地点で調査した結果, 175 株中, 151 株が側弁有毛であり残りの 24 株は側弁に毛は見られなかった。(皇学館高等学校)