Katsuyuki YOSHIMURA*: **Studies on the chromosome number and karyotype of *Pinguicula ramosa* Miyoshi (Lentibulariaceae)****

吉村克之*: コウシンソウの染色体数と核型に関する研究**

Two species of the genus *Pinguicula* occur in Japan. One is *Pinguicula macroceras* Link, which is distributed not only in Japan, but also widely in the western part of North America. The other is *Pinguicula ramosa* Miyoshi, which is distributed only in Tochigi prefecture, the northern part of the Kanto district. *P. ramosa* was for the first time, found out on Mt. Kōshin in Tochigi prefecture, and afterward, it was discovered in two other neighboring places, on Mt. Nantai and Mt. Nyohō in Nikko. *P. ramosa* is a carnivorous plant endemic to Japan and found only in the limited area, but it is not clear whether this plant is epibiotic- or neo-endemic species. Cytological investigations on *P. ramosa* were made in order to clear the systematic relationships between *P. ramosa* and other species of the genus *Pinguicula*.

Materials and methods All of the materials used in this study were collected by the author on Mt. Nyohō (ca. 1,500–1,600 m above the sea-level), in Nikko. The observations of the somatic cell division were made on root tip cells, young anther cells and young glandular hair cells of scapes. Meanwhile the observations of the meiosis were taken in pollen mother cells. The materials except PMCs were pretreated in 0.002 M solution of 8-oxyquinoline for about 2 hours before fixation. All of the materials were fixed in Carnoy's solution. Preparations utilized in these observations were made by the aceto-carmine squash method.

Karyotype was mostly studied in the stage of metaphase and prometaphase of the somatic cell division of young glandular hair cells. Karyotype was expressed in terms of aggregate length and position of

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centromere in each chromosome.

Results and discussion Of the materials used in observing the somatic chromosomes, the young glandular hair cells gave the best results. Until now, the chromosome number of *P. ramosa* has not been known, but in this observation, the somatic chromosome number of *P. ramosa* is recognized 2n=18 (Fig. 1). The somatic chromosome numbers of the genus *Pinguicula* so far reported, are 2n=12, 16, 22, 32, 44, 64 (Table 1). To these numbers, the new somatic chromosome number, 2n=18, is now added.

The karyotype formula of *P. ramosa* is as follows: (Figs. 2 & 3)
$K(2n)=18=4L^{st}+4M^{st}+2M^{sm}+2M^{m}+2S^{sm}+4S^{m}$

There are 18 chromosomes in 9 pairs. These 9 pairs of the chromosomes can be divided into three groups according to their lengths—two pairs of large chromosomes, four pairs of medium-sized ones and three pairs of small ones. The two pairs of the large chromosomes were approximately 3.6 μ to 4.0 μ in length, with subterminal centromere. The existence of the two pairs of the large chromosomes makes the kapyotype of *P. ramosa* characteristic and unique. The four pairs of medium-sized chromosomes were 2.4 μ to 2.8 μ in length. Out of them, two pairs have subterminal and one pair has submedian and the other pair has median centromere. The three pairs of the small chromosomes measured 1.8 μ to 2.1 μ. Two pairs of them have median and the other one pair has submedian centromere.

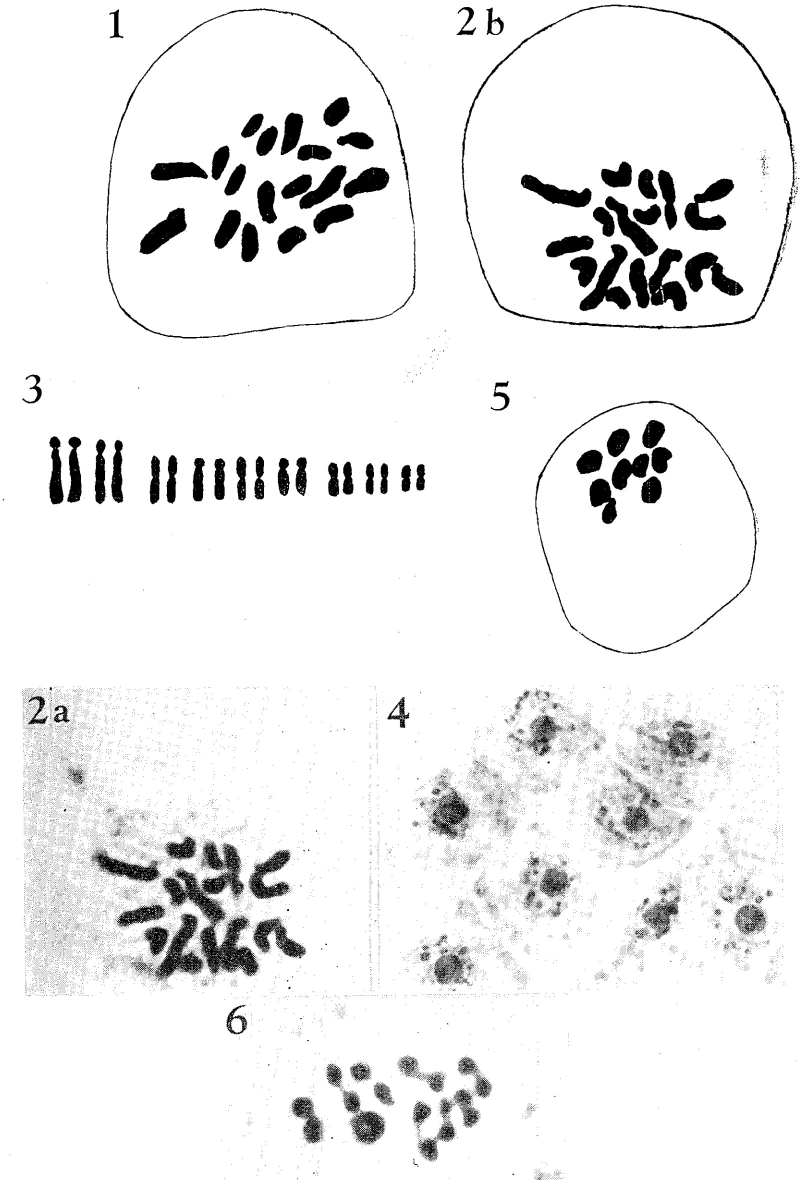
Therefore, the length of the large chromosomes was on an average about twice the length of the small chromosomes. The karyotype of *P. ramosa* mentioned above shows that shorter chromosomes were symmetric while longer chromosomes were asymmetric in general.

Moreover, a nucleus of each cell has apparently one large nucleolus in the same way as in *Pinguicula alpina* by Casper (1963) (Fig. 4).

Then, 9 bivalent chromosomes were observed clearly at metaphase I and late diakinesis stage of meiosis in PMCs (Fig. 5 & 6). Each bivalent chromosome is normal, and consists of two elements of the equal size. One of the 9 bivalent chromosomes was ring-shaped, conjugated at both ends, and its size was large. The other 8 bivalent chromosomes were cocoon-shaped, conjugated end to end. Until now, 6, 8 and 11 have been known as basic chromosome numbers of the genus *Pinguicula* (Table 1).

Table 1. List of chromosome numbers of the genus *Pinguicula*

Species name	n	2n	Authors
Subgen. Pinguicula			
Sect. Orcheosanthus			
P. colimensis		22	Kondo, 1968
P. moranensis		44	Casper, 1963
P. gypsicola		22	Casper, 1963; Kondo, 1968
P. caudata		44	Wood & Godfrey, 1957; Casper, 1962; Kondo, 1968
		22	Kondo, 1968
Sect. Pinguicula			
P. longifolia		32	Doulat, 1947; Casper, 1960
P. corsica		16	Casper, 1963
P. leptoceras		32	Casper, 1960
P. grandiflora		32	Löve & Löve, 1944; Doulat, 1947; Casper, 1963
		64	Wood & Godfrey, 1957
P. vulgaris		64	Löve & Löve, 1944, 1956; Doulat, 1947; Zurzycki 1953; Westergaard, 1958; Skalinska 1959; Sokolovskaja & Strelkova, 1960; Casper, 1962
	32		Zurzycki, 1953; Casper, 1960
Sect. Nana			
P. villosa		16	Knaben, 1950; Doulat, 1947; Wood & Godfrey, 1957; Casper, 1963
Subgen. Isoloba			
Sect. Isoloba			
P. lusitanica		12	Casper, 1963; Kondo, 1968
P. caerulea		32	Godfrey & Stripling, 1961; Casper, 1963; Kondo, 1968
	16		Godfrey & Stripling, 1961
P. lutea		32	Godfrey & Stripling, 1961; Casper, 1963; Kondo, 1968
P. planifolia		32	Godfrey & Stripling, 1961; Casper, 1963
P. primuliflora		32	Godfrey & Stripling, 1961; Casper, 1963
P. ionantha		22	Godfrey & Stripling, 1961; Casper, 1963
P. pumila		22	Godfrey & Stripling, 1961; Casper, 1963
Sect. Cardiophyllum			
P. hirtiflora		16	Honsell, 1959
	8		Honsell, 1959; Casper, 1960
Subgen. Micranthus			
Sect. Temnoceras			
P. crenatiloba		16	Casper, 1963
Sect. Micranthus			
P. alpina		32	Löve & Löve, 1944; Doulat, 1947; Zurzycki, 1953; Wood & Godfrey, 1957; Casper, 1960 Sokolovskaja & Strelkova, 1960 etc.
	16		Casper, 1960


1
2b
3
5
2a
4
6

However, the chromosome number of *P. ramosa* is 2n=18, and as its karyotype shows distinctly, it does not prove a triploid of the lowest basic number 6.

The author would like to discuss the relationships between *P. ramosa* and other species of the genus *Pinguicula* on their karyotypes. Together with other two species, *P. alpina* and *P. variegata*, *P. ramosa* belongs to the same group of formal-semblance as the section *Micranthus* according to Casper (1963). However, this investigation showed that the chromosome number of *P. alpina* was 2n=32, and it differed from that of *P. ramosa* completely. In addition, in *P. alpnia*, such large chromosomes as in *P. ramosa* were not found. In the meantime, in *P. variegata* its chromosome number has not been yet known, so that the karyological relationship between both species is not elucidated.

Accordingly, setting aside a point of morphological view, it seems to the author that there is no close relationship between *P. alpina* and *P. ramosa* karyologically. Besides, there are remarkable differences in external forms between both species, namely only *P. ramosa* has ramose scape, squashy formal corolla, deep laciniated labium, etc.

To sum up, at this stage the author would like to consider that *P. ramosa* is isolated from other species of the genus *Pinguicula*. Therefore the author wants to emphasize the possibility that *P. ramosa* is an epibiotic-endemic species. But, in order to solve these problems, it is quite necessary to carry on karyological studies more widely and deeply.

In consequence, the author infers that a progenitor of *P. ramosa* was one ancient species, which had the chromosome number of 2n=22 like some species of the subgenus *Isoloba*. Because, if fusions took place between two pairs respectively in any four pairs of chromosomes out of eleven pairs in all, that is, in the chromosome number 2n=22, two pairs of nearly twice the length of the former would newly be made, so that the new chromosome number would decrease to 2n=18 from 2n=22. Consequently, such a

Fig. 1. Chromosomes of *Pinguicula ramosa*. 1. Somatic chromosomes in metaphase stage, (2n=18). ca. ×2,000. 2a. Photomicrograph of somatic chromosomes in prometaphase, (2n=18). ca. ×1,800. 2b. Somatic chromosomes in glandular hair cells in prometaphase, (2n=18), ca. ×2,000. 3. Schematic representation of the karyotype. ca. ×2,000. 4. Photomicrograph of somatic cells with resting nucleus in young scape. ca. ×850. 5. Bivalent chromosomes of metaphase I in PMCs (n=9). ca. ×1,250. 6. Photomicrograph of bivalent chromosomes of late diakinesis stage in PMCs. ca. ×1,500.

karyotype may fundamentally accord with the above-mentioned karyotype of *P. ramosa*.

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本邦固有の食虫植物コウシンソウ（タヌキモ科ムシトリスミレ属）の染色体数および核型の研究を行なった。従来，本種の染色体に関する研究はなかったが，筆者は体細胞染色体数が，2n=18 であることを確めた。この染色体数は，これまで知られたムシトリスミレ属の染色体数 2n=12, 16, 22, 32, 44, 64のいずれでもなく，ここに新たな染色体数が加わったことになる。

次に本種の核型は，$K(2n)=18=4L^{st}+4M^{st}+2M^{sm}+2M^{m}+2S^{sm}+4S^{m}$ の核型式で示される。上記の核型の中で，2 対の長い染色体の存在が特徴的で，これらの各染色体の長さの平均は，短かい染色体のそれのおよそ 2 倍になっている。こうした特徴のある本種の核型は，同属他種との類縁関係を究明する上で，重要な手がかりになるものと思われる。さらに，花粉母細胞による減数分裂の観察から，n=9 であることが知られた。かつ，9 個の 2 価染色体の対合は正常であった。

ところで，ムシトリスミレ属植物の研究者キャスパー氏（Casper, 1963）が，コウシンソウと同一の類似形態群の中に含めている *Pinguicula alpina* の染色体数は，2n=32 であることが確められている。したがって，これら両種の間には少なくとも核学的には直接の類縁関係は認められないことになる。こうした点で，コウシンソウは比較的古い時期に分化し，現存の同属他種からは孤立している遺存固有種と推定される。

なお，コウシンソウの染色体数 2n=18 の由来についての筆者の推論を，本文中に記しておいた。