

Comparison of Karyotypes among three *Heloniopsis* Species (Liliaceae) from Ryukyu Archipelago and Taiwan

Goro KOKUBUGATA¹, Ching-I. PENG² and Masatsugu YOKOTA³

國府方吾郎¹・彭 鏡毅²・横田昌嗣³ : 琉球列島産及び
台湾産ショウジョウバカマ属の染色体比較

The genus *Heloniopsis* (Liliaceae) consists of nine species, and eight of them occur in Asia and the remainder occurs in the North America (Tanaka 1998). In the Ryukyu Archipelago (the Ryukyus) and Taiwan being adjacent to each other, the following three species are occurred: *H. kawanoi* (Koidz.) Honda, *H. leucantha* (Koidz.) Honda in the Ryukyus and *H. umbellata* Baker in Taiwan. Some cytological studies have previously been performed, and confirmed that they commonly had the chromosome number of $2n = 34$ (Tanaka 1998). However, the karyotypes have never been investigated in the three species. In the present study, chromosome number and karyotype of three *Heloniopsis* species in the Ryukyus and Taiwan are observed by the aceto-orcein squash method, and compared to each other.

Materials and Methods

Plant materials were collected in each localities by the authors (Table 1). They were transplanted to and cultivated in the experimental greenhouse of the Tsukuba Botanical Garden for the present cytological observations. Voucher specimens were deposited in the Herbarium of the National Science Museum, Tokyo (TNS).

Root tips were harvested, pretreated in 2 mM 8-hydroxyquinoline at 20°C for 2 h, and fixed in acetic ethanol (1 : 3) at 4°C for 2 h. They were macerated in 2 : 1 mixture of 1N hydrochloric acid and 45% acetic acid at 60°C for 10 sec, and stained in 2% aceto-orcein at 20°C for 2 h. The aceto-orcein squash method that spreaded chromosomes at mitotic metaphase were used for the present cytological study. Chromosomes at mitotic metaphase were classified by arm ratio ($R = \text{long arm length} / \text{short arm length}$) following Levan *et al.* (1964). Median- ($1.0 \leq R < 1.7$), submedian- ($1.8 \leq R < 3.0$), subterminal- ($3.1 \leq R < 7.0$), and terminal- ($7.1 \leq R$) centromeric chromosomes were designated and symbolized as “m”, “sm”, “st” and “t”.

Results and Discussion

Three *Heloniopsis* species, *H. kawanoi*, *H. leucantha* and *H. umbellata* commonly showed the

¹Tsukuba Botanical Garden, National Science Museum, Tsukuba, 305-0005. 国立科学博物館 筑波研究資料センター 筑波実験植物園.

²Institute of Botany, Academia Sinica, Nankang, Taipei 115, Taiwan.

³Laboratory of Ecology and Systematics, Faculty of Science, University of the Ryukyus, Senbaru 1, Nishihara, Nakagami, Okinawa 903-0213, Japan. 〒903-0213 中頭郡西原千原 1, 琉球大学理学部海洋自然科学科.

Table. 1. Three *Heloniopsis* species used as plant materials

Species	Locality*	2n	Karyotype**
<i>H. kawanoi</i>	Japan, Ryukyus: Urauchi-gawa River, Iriomote Is., Okinawa (GK 1492).	34	2sat- sm + 2sat- m + 4 sm + 26 m
<i>H. leucantha</i>	Japan, Ryukyus: Miyara-gawa River, Ishigaki Is., Okinawa (GK 4133).	34	2sat- sm + 2sat- m + 4 sm + 26 m
<i>H. umbellata</i>	Taiwan, Hsinchu: Hsiayulao, side of way, Hsiayulao, Chienshih Hsiang (GK 1461).	34	2sat- sm + 2sat- m + 4 sm + 26 m

*Parenthesized numbers are personal specimen numbers of Goro Kokubugata (TNS).

sat = satellite chromosome; **st = subterminal-centromeric chromosome; **sm** = submedian-centromeric chromosome; **m** = median-centromeric chromosome.

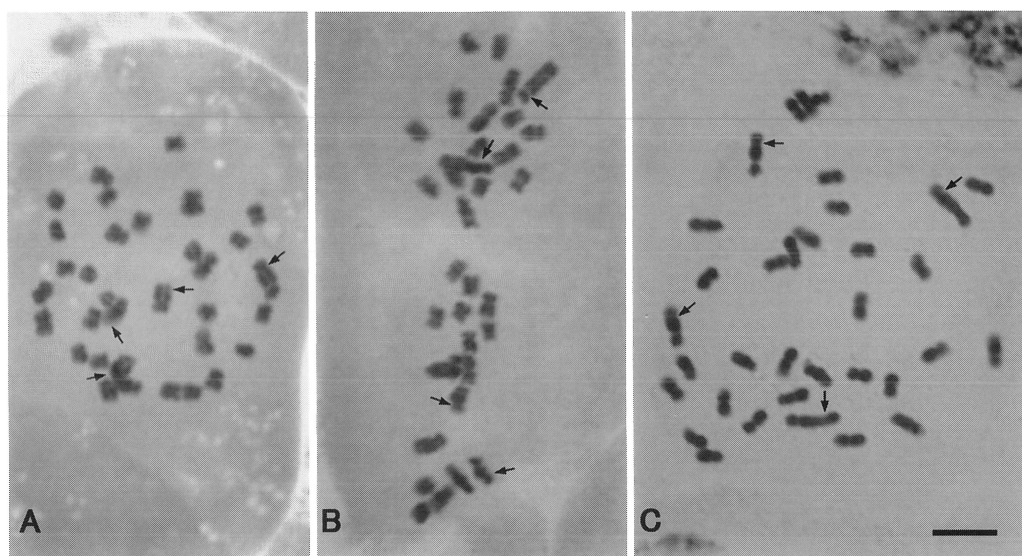


Fig. 1. Somatic chromosomes of three *Heloniopsis* species. **A.** *H. kawanoi*. **B.** *H. leucantha*. **C.** *H. umbellata*. Arrows indicate clearly visible secondary constriction. Bar shows 10 μ m.

chromosome number of $2n = 34$ (Fig. 1). The chromosome number of the three species agreed with those reported by Tanaka (1998). The three species showed the chromosome complement consists of 28 **m** and 6 **sm** chromosomes (Fig. 2). A secondary constriction was observed at the long arm of 2 **m** and 2 **sm** chromosomes in the three species (arrows in Fig. 1).

In molecular phylogeny, Fuse and Tamura (2000) reported that *H. kawanoi*, *H. leucantha* and *H. umbellata* made up a clade, and then the clade joined to that of *H. orientalis* members as a sister group. Nakamura (1967) cytologically reported *Heloniopsis orientalis* Thunb. distributing in Honshu, Shikoku and Kyushu, Japan had four sat-chromosomes in the chromosome complement at mitotic metaphase. The present study confirmed that the three *Heloniopsis* species also had the four sat-chromosomes as same as the most related species, *H. orientalis*.

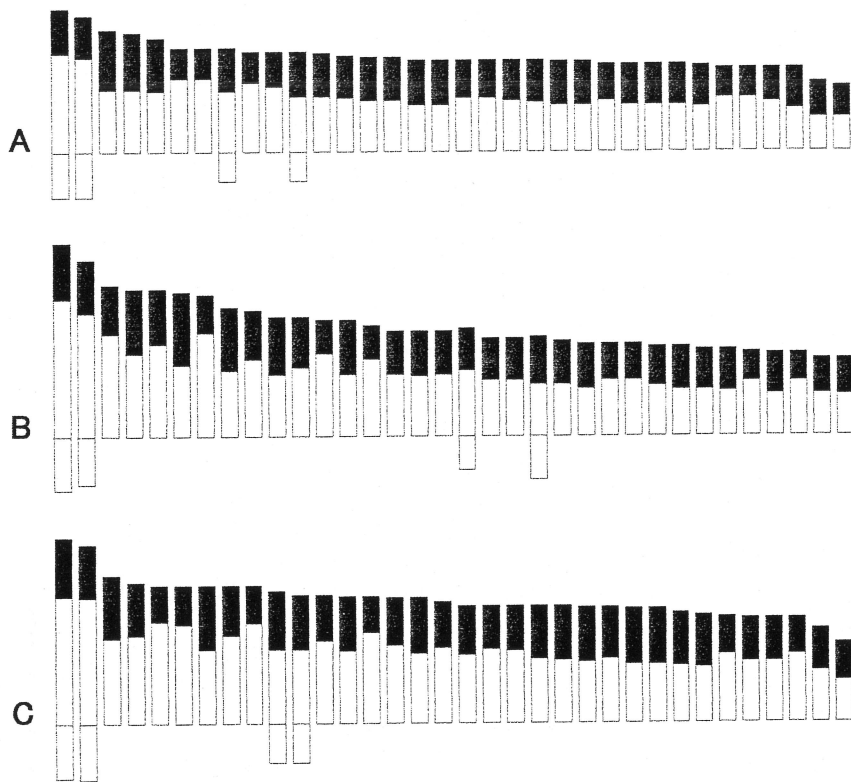


Fig. 2. Ideograms of three *Heloniopsis* species. A. *H. kawanoi*. B. *H. leucantha*. C. *H. umbellata*. Opened areas show long arm, grayish areas show short arm and solid areas show satellite.

Acknowledgement

This study was partly conducted in connection with the projects: "Natural History Researches of the Island Arcs in the Western Pacific" and "Flora studies in the Ryukyus" operated by the National Science Museum, Tokyo and the Grant-in-Aid for the Scientific Program A (No. 15201050; Representative to T. Konishi) of Japanese Society for the Promotion of Science (GK); the grants from the Council of Agriculture and Academia Sinica, Taipei (CP); and the Grant-in-Aid for the Scientific Program C (No. 14540648) of Japanese Society for the Promotion of Science (MY) in part respectively.

Summary

Somatic chromosomes of *Heloniopsis kawanoi* in Iriomote Is., *H. leucantha* in Ishigaki Is. and *H. umbellata* in Hsinchu were investigated by the standard aceto-orcin squash method. The three species showed the chromosome number of $2n = 34$ and the chromosome complement consists of 26 **m**, 2 sat-**m**, 4 **sm** and 2 sat-**m** chromosomes. The karyotypes of the three species were similar to those of *H. orientalis*.

摘 要

ショウジョウバカマ属 (*Heloniopsis*: ユリ科) の 3 種, コショウジョウバカマ (*H. kawanoi*), オオシロショウジョウバカマ (*H. leucantha*), および *H. umbellata*, の 3 種の体細胞染色体をアセトオルセイン押しつぶし法を用いて観察した。3 種ともに, 染色体数は $2n=34$ で, 核型は 26 個の **m** 染色体, 2 個の **sat-m** 染色体, 4 個の **sm** 染色体そして 2 個の **sat-m** 染色体の染色体から構成されていた。これら 3 種の核型は日本本土に分布するショウジョウバカマ (*H. orientalis*) の核型と類似していた。

References

- Fuse, S. and N.M. Tamura, 2000. A phylogenetic analysis of the plastid *makK* gene with emphasis on Melanthiaceae *sensu lato*. *Plant Biol.* **2**: 415-427.
- Levan, A., K. Fredga and A.A. Sandberg, 1964. Nomenclature for centromeric position on chromosome. *Hereditas* **52**: 201-220.
- Nakamura, T., 1967. Cytological studies in family Liliaceae of Japan II. The karyotype analysis in genus *Heloniopsis*. *La Kromosomo* **71**: 2316-2321.
- Tanaka, N., 1998. Phylogenetic and taxonomic studies on *Helonias*, *Ypsilandra* and *Heloniopsis* III. Taxonomic revision. *J. Jpn. Bot.* **73**: 102-115.