

Chromosome Number and Karyotype of *Agatea violaris* (Violaceae) from New Caledonia

by

Goro KOKUBUGATA* and Tamotsu HASHIMOTO*

國府方吾郎*・橋本 保* : ニューカレドニア産 *Agatea violaris* (スミレ科) の
染色体数と核型

Agatea violaris A. Gray (Violaceae) distributed in New Guinea, Solomons, New Caledonia and Fiji, and is probably a monotypic species (Jacobs and Moore 1971). *Agatea violaris*, which is a woody climber unlike most of *Viola*, is an interested key taxon for phylogenetic study in the family. Until now, however, only one cytological result was published by Carr and McPherson (1986). They reported that the chromosome number is $n=8$ at meiotic metaphase without showing arm ratio. In this study, chromosome number and karyotype of somatic chromosomes of the juvenile leaf of *A. violaris* were investigated with the standard aceto-orcein staining method.

Material and Methods

The material was collected at the park area of 'Les Chutes de la Madleine', New Caledonia by T. Hashimoto in 1996, and was conserved in the Tsukuba Botanical Garden, National Science Museum. The voucher specimen for this investigation is preserved in the herbarium of the Tsukuba Botanical Garden, National Science Museum. The plant is a compact shrubby type which reaches at about 1 m high in 'Maquis' vegetation, but elongates its shoot, and becomes to a climber in our green-house.

The juvenile leaf from a living plant was harvested and pretreated in 2 mM 8-hydroxyquinoline at 20°C for two hours. It was fixed in acetic ethanol (1:3) at 4°C for forty hours, and was macerated in a 2:1 mixture of 1N-HCl and 45% HOAc at 60°C for five seconds. The macerated juvenile leaf was placed on a glass slide, and was stained with 2% aceto-orcein at 23°C for four hours. Then, it was prepared by the squash method for standard karyotype analysis.

Morphological classification of chromosomes at interphase was followed Tanaka (1977). Somatic chromosome at mitotic metaphase was classified according to centromeric positions defined with arm ratio (Levan *et al.* 1964). Chromosome length longer than $0.4\mu\text{m}$ was designated as "long" and that smaller than $0.4\mu\text{m}$ was designated as "short" in the present study.

Results and Discussion

The chromosomes at interphase showed the morphological characteristic of the simple

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

chromocenter type (Fig. 1A). At mitotic metaphase, the chromosome number of *A. violaris* was $2n=12$ (Fig. 1B), and these chromosomes varied in length from $0.2\ \mu\text{m}$ to $0.7\ \mu\text{m}$ (Table 1). The chromosome complement of this species was composed of four long subterminal-, two long median-, two short submedian-, and four short median-centromeric-chromosomes (Fig. 1C and Table 1). Two long subterminal- and two long median-centromeric chromosomes of them indicated long distance which may be secondary constriction between the long arm and the short arm (Fig. 1C arrows).

On the other hand, Carr and McPherson (1986) reported that *Agatea* sp. from Riveiere Blue Valley, New Caledonia showed a chromosome number of $n=8$ at meiotic metaphase. However, this genus *Agatea* was considered as a monotypic, but a polymorphic taxon by Jacobs and Moore (1971). If their opinioin is agreeable, the authors can say that *A. violaris* might show polymorphic chromosome number, or Carr and McPherson (1986) might count four chromosomes by mistake for a long

Table 1. Arm ratios and chromosome length in the complement at mitotic metaphase in *Agatea violaris* studied

| Chromosome in order | Long arm (μm) | Short arm (μm) | Chromosome length (μm) | Arm ratio | Karyotype |
|---------------------|----------------------------|-----------------------------|-------------------------------------|-----------|-----------|
| 1 | 0.52 | 0.16 | 0.68 | 3.3 | st |
| 2 | 0.45 | 0.14 | 0.59 | 3.2 | st |
| 3 | 0.38 | 0.25 | 0.63 | 1.5 | m |
| 4 | 0.32 | 0.22 | 0.54 | 1.5 | m |
| 5 | 0.42 | 0.10 | 0.52 | 4.2 | st |
| 6 | 0.33 | 0.08 | 0.41 | 4.1 | st |
| 7 | 0.18 | 0.18 | 0.36 | 1.0 | m |
| 8 | 0.16 | 0.14 | 0.30 | 1.1 | m |
| 9 | 0.18 | 0.09 | 0.28 | 2.0 | sm |
| 10 | 0.18 | 0.10 | 0.28 | 1.8 | sm |
| 11 | 0.16 | 0.12 | 0.28 | 1.3 | m |
| 12 | 0.12 | 0.08 | 0.20 | 1.5 | m |

m = median-centromeric chromosome, sm = submedian-centromeric chromosome, st = subterminal-centromeric chromosome and t = terminal-centromeric chromosome.

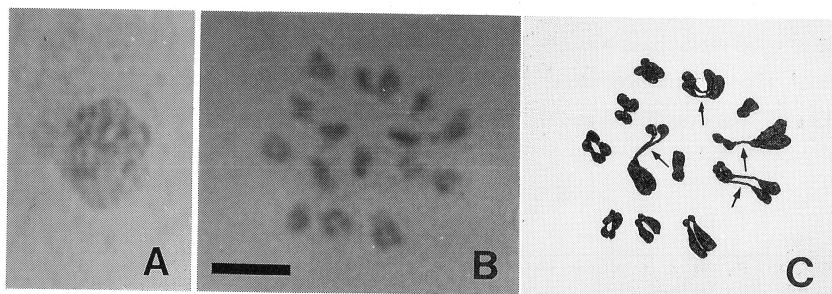


Fig. 1. Somatic chromosomes of *Agatea violaris*. A: Photomicrograph of interphase nuclear; B: Photomicrograph of chromosome at mitotic metaphase; C: Explanatory drawings of A. Bar shows $1\ \mu\text{m}$. Arrows point the long distance between the long arm and the short arm.

submedian- and a median-centromeric chromosomes indicated the long distance between the long arm and the short arm in the present study.

It is apparent that *Viola* is the most advanced genus within the family Violaceae by morphological characters as Hashimoto (1961) had described. Miyaji (1929) reported that *Viola* diverged from an ancestral taxon having a chromosome basic number of $X=6$ by polyploidization. The result of our investigation indicates that *A. violaris* is cyto-phylogenetically close to an ancestral taxon of *Viola*.

Acknowledgements

This study was partially supported by International Scientific Research Program, No. 0841165, Ministry of Education, Science, Sports and Culture, Japan.

Summary

Somatic chromosomes of *Agatea violaris* collected from New Caledonia is investigated with the standard aceto-orcein staining method. *Agatea violaris* studied shows the simple chromocenter type at interphase, the chromosome number of $2n=12$ and chromosome complement composed of four long subterminal-, two long median-, two short submedian-, and four short median-centromeric chromosomes at mitotic metaphase. The results are suggested that *A. violaris* might be cyto-phylogenetically close to an ancestral taxon of *Viola*.

摘 要

ニューカレドニア産 *Agatea violaris* の体細胞間期核及び有糸分裂中期染色体がアセトオルセイン染色法により観察された。その結果、間期核は単純染色中央粒型に分類された。また、染色体数は $2n=12$ であり、その構成は 4 個の長い端部動原体型染色体、2 個の長い中部動原体型染色体、2 個の短い次中部動原体型染色体、4 個の短い中部動原体型染色体からなっていた。この結果は Carr and McPherson (1986) の報告とは異なり、本研究に用いた本種の基本数は $X=6$ であった。スミレ科の外部形態による系統考察と同様に、核型分析においても *Agatea* 属はスミレ属の祖先分類群と近縁であることが示唆された。

References

- Carr, D. and G. McPherson, 1986. Notes chromosome numbers of New Caledonian plants. *Ann. Missouri Bot. Gard.* **73**: 486-489.
- Hashimoto, T., 1961. The violet of Japan. 1-206, i-xxi, Seibundo-shinko-sha, Tokyo (in Japanese).
- Jacobs, M. and D. M. Moore, 1971. Violaceae. 179-212. *In*: Vansteenis, C.G.G.J. (ed.), *Malesiana Ser.1 Vol.7 Part.1.*, Noordhoff International Publishing, Leyden.
- Levan A., K. Fredga and A. A. Sandberg, 1964. Nomenclature centromeric position on chromosomes. *Hereditas* **52**: 201-220.
- Miyaji, Y., 1929. Studien über die Zahlenverhältnisse der Chromosomen bei der Gattung *Viola*. *Cytologia* **1**: 28-58.
- Tanaka, R., 1977. Recent karyotype studies, 293-326. *In*: Ogawa, K., K. Kurosumi, S. Koike and S. Sato (eds.), *Plant Cytology*, Asakura Book Co., Ltd., Tokyo (in Japanese).