

A karyomorphological study on *Selenipedium aequinoctiale* Garay, Orchidaceae*

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ラン科、*Selenipedium aequinoctiale* Garay における核形態学的研究*

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Introduction

The genus *Selenipedium* Rchb.f., belonging to the subfamily Cypripedioideae, consists of about six species and rarely distributes in the tropical Americas. They are terrestrial in wet mountain forest. The transplantation of plant in the genus is very difficult, suggesting a high reliance on mycorrhizae in the habitat.

Among the four genera belonging to this subfamily, on three genera of *Cypripedium*, *Paphiopedilum* and *Phragmipedium* (including the genus *Mexipedium*), the detailed cytological studies were done by many researchers and summarized karyomorphological differences among the three genera, i.e. chromosome features at resting stage, basic chromosome numbers and chromosome sizes (Aoyama and Karasawa 1999). However, as for the genus *Selenipedium*, the cytological research is not accomplished at all.

In this paper, the karyotype of *Selenipedium aequinoctiale* is dealt with and the cytotaxonomical review is compared with other three genera of the subfamily.

Material and methods

A plant of *Selenipedium aequinoctiale* Garay, distributed in northwestern Ecuador, was collected and introduced to Japan in 2002. It was planted in pot for two years and bloomed flowers on the top of two stems in 2003 (Fig. 1A).

The growing root tip cells of the plant were used for the observation of somatic chromosomes made by the aceto-orcein squash method as follows: Growing root tips in 1.0 mm long were pretreated in 2mM 8-hydroxyquinoline at 18° C for four hours and fixed in a mixture of acetic acid and ethanol (1:3) for one day in refrigerator. The fixed tips were replaced in 45% acetic acid at 5° C for about ten minutes. They were macerated in a mixture of 1N hydrochloric acid and 45% acetic acid (2:1) at 60° C for 20 seconds, and finally stained in 1% aceto-orcein and squashed. The description of chromosomes was followed Levan *et al.* (1964) and Tanaka (1971, 1977).

Observation

Resting chromosomes of *Selenipedium aequinoctiale* formed numerous chromomeric granules and many chromocentral blocks which were irregular in size and shape. These chromocentral blocks of 0.5-2.0 μm in diameter varied in number from 15 to 24 per nucleus (Fig. 1B). The chromosome features at resting stage were of the simple chromocenter type of Tanaka (1971).

The chromosomes at mitotic prophase condensed heterogeneously. Early condensed segments were located at the proximal and interstitial regions of both arms of the chromosomes, and late condensed segments were situated at the distal region (Fig. 1C).

The chromosome number of $2n=20$ was counted in ten cells at mitotic metaphase (Fig. 1D). The measurements of

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metaphase chromosomes are shown in Table 1.

The 20 chromosomes varied gradually from 2.7 μm to 1.4 μm in length (Fig. 1E). Among them, eight (nos. 5-6, 11-12, 15-18) were median-centromeric with the arm ratio from 1.0-1.7, eight (nos. 3-4, 7-8, 13-14, 19-20) submedian-centromeric with the arm ratio from 2.0-2.8, and the other four chromosomes (nos. 1-2, 9-10) subterminal-centromeric with the arm ratio from 4.0-6.0. Two chromosomes (nos. 11-12) had the secondary constrictions at the interstitial region of short arms, and the satellites were 0.4 μm and 0.5 μm in length.

Thus, the karyotype of this species was characterized to be homogeneous in length, and composed of both symmetrical and asymmetrical chromosomes in arm ratio.

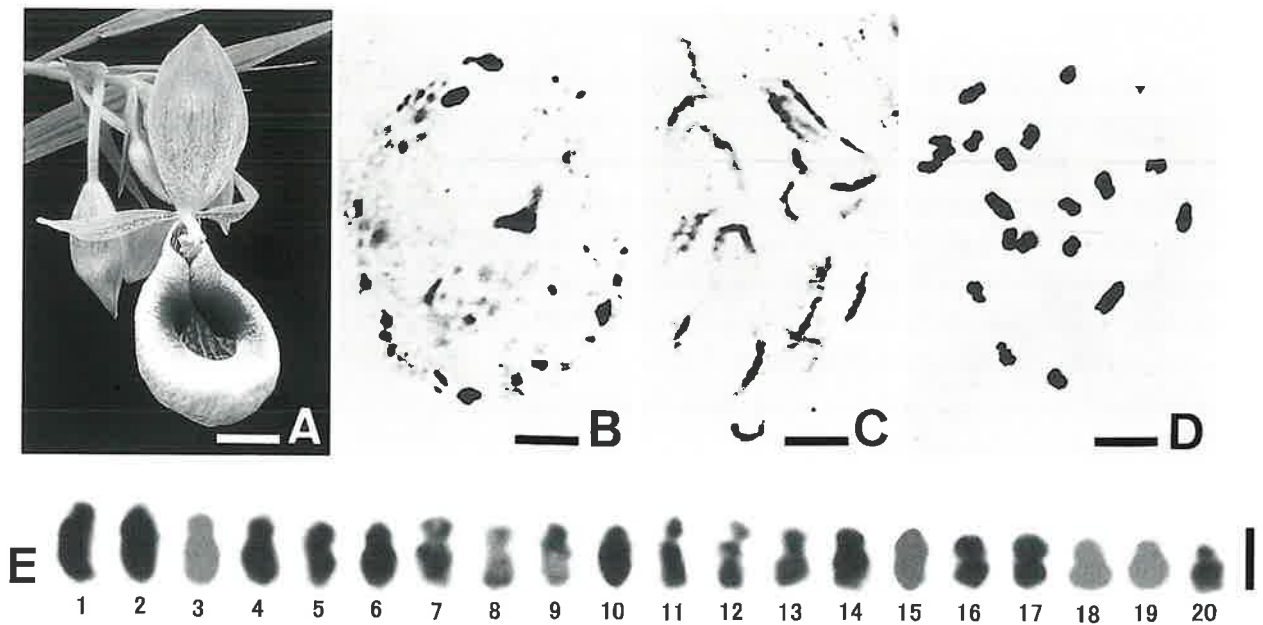


Fig. 1. Photomicrographs of somatic chromosomes of *Selenipedium aequinoctiale*, $2n=20$. A, flower. B, chromosomes at resting stage. C, chromosomes at mitotic prophase. D and E, chromosomes at mitotic metaphase. Bars indicate 1cm in A, 5 μm in B-D and 2 μm in E.

Discussion

In present species and the genus *Cypripedium* (except for *Cyp. debile* categorized to be the complex chromocenter type), the chromosomes at resting stage were commonly categorized to be the simple chromocenter type. Though, the chromomeric granules of this species were stained paler than those of *Cypripedium*. The resting chromosomes of *Paphiopedilum* and *Phragmipedium* were both classified into the complex chromocenter type.

The chromosome feature of present species at mitotic prophase was also similar to that of *Phragmipedium*, while the early condensed segments of *Paphiopedilum* and *Cypripedium* at mitotic prophase were diffused continuously in both arms.

The chromosome number of $2n=20$ in this species was equal to that of *Cypripedium* and some species of *Phragmipedium*. Though the $2n=20$ in *Phragmipedium* was derived from the basic number of $2n=18$ by the centric fission of chromosomes.

The total chromosome length of this species was 40 μm and was clearly shorter than those of other genera; the average of total chromosome length of 283 μm in 15 taxa of *Cypripedium*, 202 μm in 73 taxa of *Paphiopedilum* and 86 μm in 16 taxa of *Phragmipedium*.

Table 1. Measurements of somatic chromosomes of *Selenipedium aequinoctiale*, $2n=20$ at metaphase.

No	Chromosome length(μm)	Relative length	Arm ratio	Form
1	0.4+2.4=2.8	7.0	6.0	st
2	0.4+2.2=2.6	6.5	5.5	st
3	0.6+1.7=2.3	5.7	2.8	sm
4	0.6+1.6=2.2	5.5	2.7	sm
5	0.8+1.4=2.2	5.5	1.7	m
6	0.8+1.4=2.2	5.5	1.7	m
7	0.7+1.4=2.1	5.2	2.0	sm
8	0.6+1.5=2.1	5.2	2.5	sm
9	0.4+1.6=2.0	5.0	4.0	st
10	0.4+1.6=2.0	5.0	4.0	st
11	0.5+0.4+1.1=2.0*	5.0	1.2	m
12	0.4+0.4+1.1=1.9*	4.7	1.4	m
13	0.5+1.4=1.9	4.7	2.8	sm
14	0.5+1.4=1.9	4.7	2.8	sm
15	0.9+1.0=1.9	4.7	1.1	m
16	0.9+0.9=1.8	4.5	1.0	m
17	0.8+0.9=1.7	4.2	1.1	m
18	0.7+1.0=1.7	4.2	1.4	m
19	0.4+1.1=1.5	3.7	2.8	sm
20	0.4+1.0=1.4	3.5	2.5	sm

* chromosome with secondary constriction

The position of the secondary constriction in this species was situated on the interstitial region of short arm. It was different from other genera; on the proximal region of short arm in *Cypripedium*, on the distal region of short arm in *Paphiopedilum* and on the distal region of long arm in *Phragmipedium*.

As mentioned above, this species was clearly different from other genera in karyomorphological characteristics; karyotype of resting chromosome, length of metaphase chromosomes and position of the secondary constriction (Table 2). Even if the observation on one species, the genus *Selenipedium* was distinguishable from other three genera of Cypridioideae by the cytological features as well as the external and anatomical features.

Summary

Karyomorphological observation was carried out in *Selenipedium aequinoctiale*, and the chromosome number of $2n=20$ was reported here for the first time in the genus.

The karyomorphological characteristics of this species, i.e. karyotype of resting chromosome, length of metaphase chromosomes and position of the secondary constriction, were clearly different from other three genera in subfamily Cypridioideae.

Table 2. Comparison of cytological characters in Cyripedioideae.

Genus (taxon)	Karyotype of resiting chromosome	Chromosome number (2n) (Basic number)	Average of total chromosome length (μm)	Position of secondary constriction
<i>Selenipedium</i> (1)	Simple chromocenter	20 (20V)	40	Interstitial region of short arm
<i>Phragmipedium</i> (16)	Complex chromocenter	18, 20, 22, 26, 30 (18V)	86	Distal region of long arm
<i>Paphiopedilum</i> (73)	Complex chromocenter	26, 28, 30, 32, 36, 38, 40, 42 (26V)	202	Distal region of short arm
<i>Cyripedium</i> (15)	Simple chromocenter	20, 22, 30 (20V)	283	Proximal region of short arm

摘要

Selenipedium aequinoctiale の核形態学的観察を行い、染色体数 $2n=20$ を本属では初めて報告した。本種の核形態は、静止期染色体の核型、中期染色体の大きさおよび二次狭窄の位置においてアツモリソウ亜科の他の3属とは異なっていた。

References

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