

Karyomorphological observations on *Cypripedium kentuckiense* Reed, Orchidaceae*

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ラン科, シプリペジウム ケンタッキエンセの核形態学的観察*

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Cypripedium is a terrestrial orchid genus widely distributed in temperate regions of the Northern Hemisphere with high concentration in eastern Asia and North America, containing about 38 species (Chen & Xi 1987). The chromosome numbers of the genus have been reported for 19 species, most of them being uniformly $n = 10$ or $2n = 20$ (cf. Tanaka & Kamemoto 1984, Karasawa & Aoyama 1986).

Based on comparative studies on the karyotypes of Japanese and Formosan species, Karasawa & Aoyama (1986) reported that the karyomorphological characteristics are quite consistent with the subgeneric classification of the genus proposed by Brieger (1973).

Cypripedium kentuckiense was recently described from North America (Reed 1981, 1982). In the present paper, we report karyomorphological observations on this species for the first time.

Material and Method

A plant (Fig. 1A) obtained from state of Louisiana, U.S.A. was used in the present study. Method for observation of chromosomes and terminology for description of karyotypes followed Karasawa & Aoyama (1986).

Observations

The chromosome number of $2n = 20$ was counted in ten mitotic metaphase cells. Measurements of mitotic metaphase chromosomes are shown in Table 1.

Resting chromosomes (Fig. 1B) contained chromonemata and numerous darkly stained

* Contribution from the Hiroshima Botanical Garden No. 39.

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Bulletin of the Hiroshima Botanical Garden, No. 10: 73-76, 1988.

chromomeres which were scattered in the nuclear space, and no conspicuous heteropycnotic bodies were observed. The morphology of the resting chromosomes is best described as the densely diffuse type of Tanaka (1971), same as the other species of the genus reported by Karasawa & Aoyama (1986).

At mitotic prophase (Fig. 1C), numerous early condensed segments were equally formed in many parts of chromosomes, which condensed gradually and uniformly.

At mitotic metaphase (Fig. 1E), $2n=20$ chromosomes ranged 10.1–23.0 μm in length, and changed their length gradually. Of them, five chromosomes (nos. 13, 14, 16, 17, 20) were submetacentric with arm ratio 1.8–2.9, and the other 15 metacentric with arm ratio 1.0–1.5. Two chromosomes (nos. 5, 6) had secondary constrictions at the proximal region of the short arm, whose satellites were 5.3 and 5.0 μm long, respectively.

Thus, the karyotype of *Cypripedium kentuckiense* is characterized as being monomodal and gradual in length, and symmetrical in arm ratio.

Discussion

Although the chromosome numbers of $n=10, 11$; $2n=20, 21, 22, 30$ ($3x$) are counted in 22 species out of the genus *Cypripedium* (Tanaka & Kamemoto 1984, Karasawa & Aoyama 1986). Most of the counts are $n=10$ or $2n=20$, while a plant of *C. formosanum* with $2n=30$ chromosomes was regarded as a triploid of $x=10$, and a plant of *C. macranthum* var.

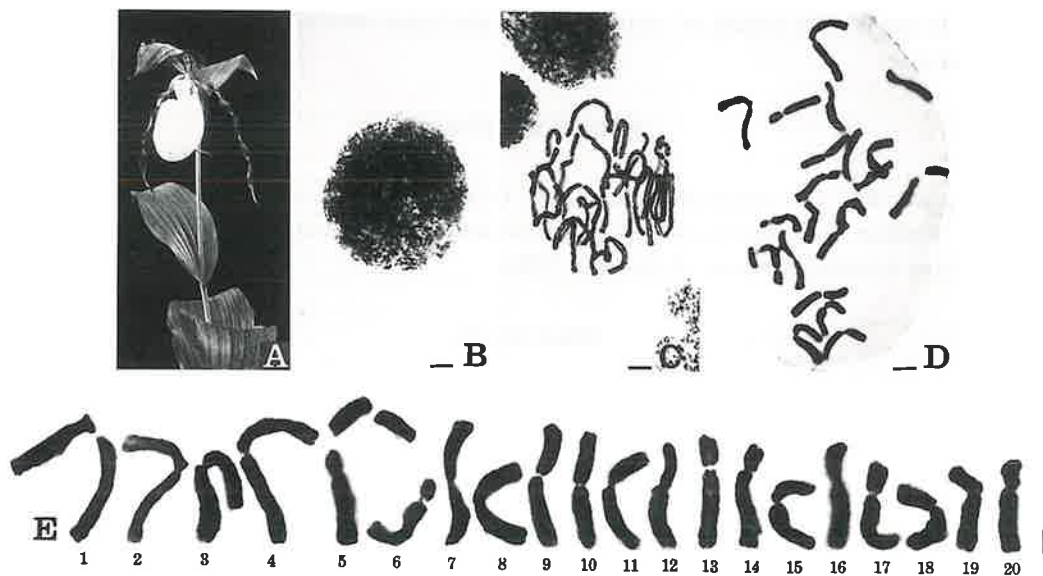


Fig. 1. *Cypripedium kentuckiense* $2n=20$. A, a plant. B, chromosomes at resting stage. C, chromosomes at mitotic prophase. D and E, chromosomes at mitotic metaphase. Bar indicates 5 μm for B-D and 2.5 μm for E.

speciosum with $2n=21$ chromosomes an aneuploid with structural change of chromosomes (Karasawa & Aoyama 1986). The chromosome number of $2n=20$ of the present species is the same as for most published reports. Compared with the results reported by Karasawa & Aoyama (1986), the present species is similar in the morphology of chromosomes at resting stage and mitotic prophase, the size of satellites, the centromeric position of medium-sized and small chromosomes within the complements to the subgenus *Cypripedium*, although the mean arm ratio of the species was lower than the subgenus *Cypripedium*.

Summary

1. Karyomorphological observations on *Cypripedium kentuckiense* are reported. It was found that resting chromosomes were the densely diffuse type, metaphase chromosomes were monomodal and gradual in length, and symmetrical in arm ratio.
2. The chromosome number of $2n=20$ in the present species is reported for the first time. The similarity in the morphology of metaphase chromosomes showed a close relationship with other species of the subgenus *Cypripedium*.

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Table 1. Measurements of somatic chromosomes of *Cypripedium kentuckiense* $2n=20$ at metaphase

Chromosome	Length(μm)	Relative length	Arm ratio	Form
1	10.5+12.5=23.0	8.3	1.1	m
2	10.3+10.5=20.8	7.5	1.0	m
3	8.3+ 9.5=17.8	6.4	1.1	m
4	8.5+ 8.8=17.3	6.2	1.0	m
5	5.3+2.7+ 8.0=16.0*	5.7	1.0	m
6	5.0+2.3+ 8.3=15.6*	5.6	1.1	m
7	7.0+ 7.3=14.3	5.1	1.0	m
8	6.5+ 7.0=13.5	4.8	1.0	m
9	5.5+ 8.0=13.5	4.8	1.4	m
10	5.8+ 7.0=12.8	4.6	1.2	m
11	5.0+ 7.5=12.5	4.5	1.5	m
12	5.0+ 7.3=12.3	4.4	1.4	m
13	3.3+ 8.8=12.1	4.3	2.6	sm
14	3.0+ 8.8=11.8	4.2	2.9	sm
15	5.3+ 6.5=11.8	4.2	1.2	m
16	4.0+ 7.3=11.3	4.1	1.8	sm
17	3.3+ 7.8=11.1	4.0	2.3	sm
18	4.3+ 6.3=10.6	3.8	1.4	m
19	4.0+ 6.3=10.3	3.7	1.5	m
20	3.3+ 6.8=10.1	3.6	2.0	sm

* Chromosome with secondary constriction