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Karyomorphological Studies on Five Rare Species of *Paphiopedilum*, Orchidaceae

by

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唐澤耕司*・青山幹男**・神村　武***：ラン科 *Paphiopedilum* 属の稀少な5種の核形態学的研究

Paphiopedilum is widely distributed in the subtropical to tropical regions of South-east Asia and consists of about 80 species. Chromosome numbers and karyotypes in 70 species, five subspecies, two varieties and one forma in the genus have been reported by Karasawa (1979, 1982, 1986) and Karasawa and Aoyama (1980, 1988).

Some rare endemic species in the genus were naturally rediscovered or subsequently and additionally described after the genus was established with the major species. In the present paper, we report here karyomorphological characters on five among these species for the first time.

Materials and Methods

The materials, sources, numbers of clones used and the chromosome numbers obtained are listed in Table 1.

For the observations of somatic chromosomes, growing root tips were used. Materials were cut into small pieces of 1.0 mm and immersed in 2mM 8-hydroxyquinoline for 4 hours at 18℃. They were fixed in 45% acetic acid at 5 ℃ for 5 minutes, hydrolyzed in 1N HCl at 60℃ for 20 seconds, and finally stained in 1% aceto-orcein and squashed. The terminology for description of karyotypes followed Levan *et al.* (1964) and Tanaka (1971).

Results and Discussion

The resting chromosomes in the five species of *Paphiopedilum* studied here commonly formed numerous chromomeric granules and several dark-stained chromocentral blocks which varied in shape and size. Thus, the morphology of the resting chromosomes for the species was commonly categorized to be the complex chromocenter type according to Tanaka (1971), same as that of the species of the

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genus previously reported (cf. Karasawa 1979). Chromosomes at mitotic prophase were condensed homogeneously.

Chromosomes at mitotic metaphase performed karyotype in length and form specific to respective species as follows:

1. *Paphiopedilum sanderianum* (Rchb. f.) Stein, 2n=26, Fig. 1 and Table 2.

This species grown in the northern part of Borneo was described by Reichenbach (1886) as *Cypripedium sanderianum* Rchb. f. and was replaced taxonomically to the genus *Paphiopedilum* by Stein (1892). The natural habitats and living specimen had been lost for a long time, and the existence of the species had been mistreated. Fortunately, a new habitat of the species was recently rediscovered (Fowlie 1983). This epiphytic species belonged to the subgenus Polyantha is distinctively with hanged and twisted petals up to 60 cm in length.

In three clones of the species, the chromosome number was counted to be 2n=26 for the first time. The chromosome complement consisted of four large chromosomes varied from 17.2-13.3 μm and 22 small chromosomes varied gradually from 10.7-5.5 μm , and thus, showed a bimodal karyotype. The four large chromosomes were median-centromeric with arm ratios ranged from 1.0-1.1. Among the 22 small chromosomes, 20 were median-centromeric with arm ratios ranged from 1.0-1.7 and the other two (Nos. 21-22) were submedian-centromeric with arm ratios of 2.5 and 1.9. Fourteen chromosomes (Nos. 9, 11-12, 16-26) had small secondary constriction on the interstitial region of the long arm.

Thus, the karyotype of the species was similar to that of the allied *P. stonei* (2n=26) previously reported by Karasawa (1979) with respect to low arm ratios and existence of small secondary constrictions, excepting one pair of small chromosomes was submedian-centromeric.

2. *Paphiopedilum kolopakingii* Fowlie, 2n=26, Fig. 2 and Table 3.

This species was discovered in the central part of Borneo in 1982 and described by Fowlie (1984). The species is belonged to the subgenus Polyantha. It is a large epiphyte and its flower is similar to that of *P. stonei*.

The chromosome number of 2n=26 for the species was counted in the four clones studied here. The chromosome complement consisted of four large chromosomes varied from 14.0-11.5 μm in length and 22 small chromosomes varied gradually from 8.8-4.0 μm , and showed a bimodal karyotype. Arm ratios of the 26 chromosomes ranged from 1.0-1.6, and thus, positions of their centromeres were all median. Thirteen chromosomes (Nos. 2-3, 7-8, 12, 15, 17-21, 23, 26) had small secondary constriction on the proximal region of either long or short arm.

Thus, the karyotype of the species was similar to that of *P. stonei* (Karasawa 1979) with respect to low arm ratios and existence of small secondary constrictions excepting two large chromosomes had small secondary constriction.

3. *Paphiopedilum tigrinum* Koopowitz and Hasegawa, 2n=26, Fig. 3 and Table 4.

This species found in the southern part of Yunnan, China, was described as a new species placed in the subgenus Paphiopedilum by Koopowitz and Hasegawa (1990). The species is terrestrial and similar to *P. henryanum* in green leaves but different in broad stripes on dorsal sepal and petals.

The 2n=26 chromosomes were counted in three clones of the species. Among them, four large chromosomes varied from 13.1-11.5 μm and 22 small chromosomes varied gradually from 9.5-4.3

Table 1. Sources, numbers of clones and chromosome numbers of the species of *Paphiopedilum* studied

Species	Locality	Source	Clone numbers	Chromosome number (2n)
P. sanderianum (Rchb. f.) Stein	Borneo Is.	Kohji Karasawa	1	26
		Chuyo Fukui	2	
P. kolopakingii Fowlie	Borneo Is.	Kohji Karasawa	2	26
		Chuyo Fukui	2	
P. tigrinum Koopowitz	Yunnan	Kohji Karasawa	2	26
		Chuyo Fukui	1	
P. sangii Braem	Sulawesi Is.	Shigenobu Nishida	2	28
P. shoseri Braem and Mohr	Bachan Is.	Kohji Karasawa	1	35

Table 2. Measurements of somatic chromosomes of *Paphiopedilum sanderianum*, 2n=26 at metaphase

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	8.5+8.7=17.2	7.5	1.0	m
2	7.5+7.8=15.3	6.7	1.0	m
3	7.0+7.5=14.5	6.3	1.0	m
4	6.2+7.2=13.3	5.8	1.1	m
5	4.8+5.8=10.7	4.7	1.2	m
6	4.8+5.7=10.5	4.6	1.1	m
7	4.7+5.5=10.2	4.4	1.1	m
8	4.3+5.2= 9.5	4.1	1.1	m
9	4.5+4.7= 9.2	4.0	1.0	m
10	4.0+4.3= 8.3	3.6	1.0	m
11	3.8+4.3= 8.2	3.6	1.1	m
12	4.0+4.2= 8.2	3.6	1.0	m
13	3.7+4.3= 8.0	3.5	1.1	m
14	3.5+4.3= 7.8	3.4	1.2	m
15	3.8+4.0= 7.8	3.4	1.0	m
16	3.7+4.0= 7.7	3.3	1.0	m
17	3.2+3.8= 7.0	3.1	1.3	m
18	3.0+3.7= 6.7	2.9	1.1	m
19	2.8+3.7= 6.5	2.8	1.2	m
20	3.0+3.5= 6.5	2.8	1.1	m
21	1.8+4.7= 6.5	2.8	2.5	sm
22	2.2+4.2= 6.3	2.8	1.9	sm
23	2.2+3.8= 6.0	2.6	1.7	m
24	2.3+3.7= 6.0	2.6	1.5	m
25	2.3+3.7= 6.0	2.6	1.5	m
26	2.2+3.3= 5.5	2.4	1.5	m

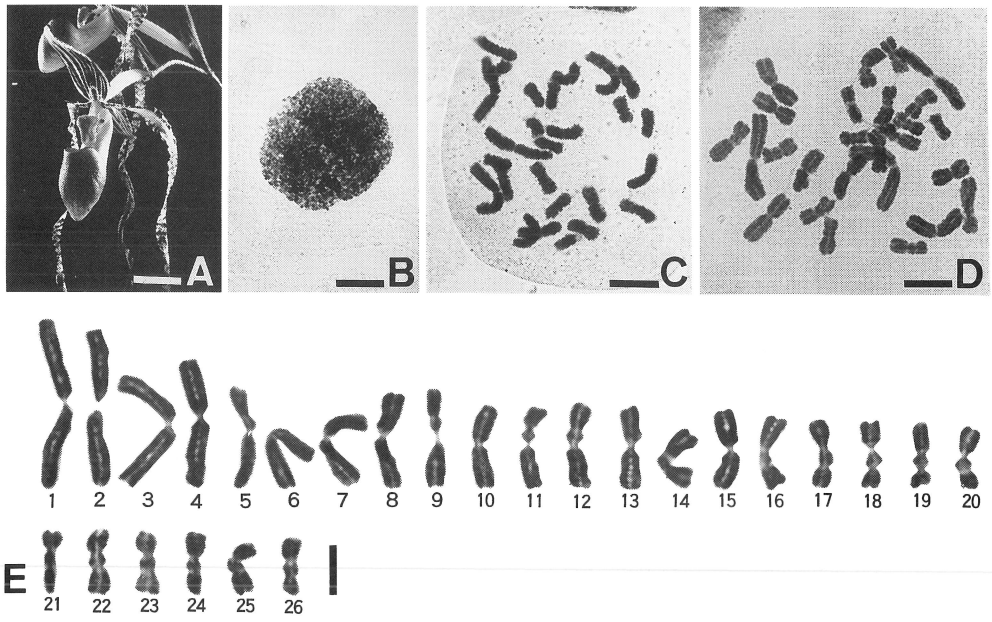

Fig. 1. Karyomorphology of *Paphiopedilum sanderianum*, 2n=26. **A,** a flower. **B,** chromosomes at resting stage. **C,** chromosomes at mitotic late prophase. **D** and **E,** chromosomes at mitotic metaphase. Bars indicate 20 mm in **A** , 10 μm in **B-D** and 5 μm in **E.**

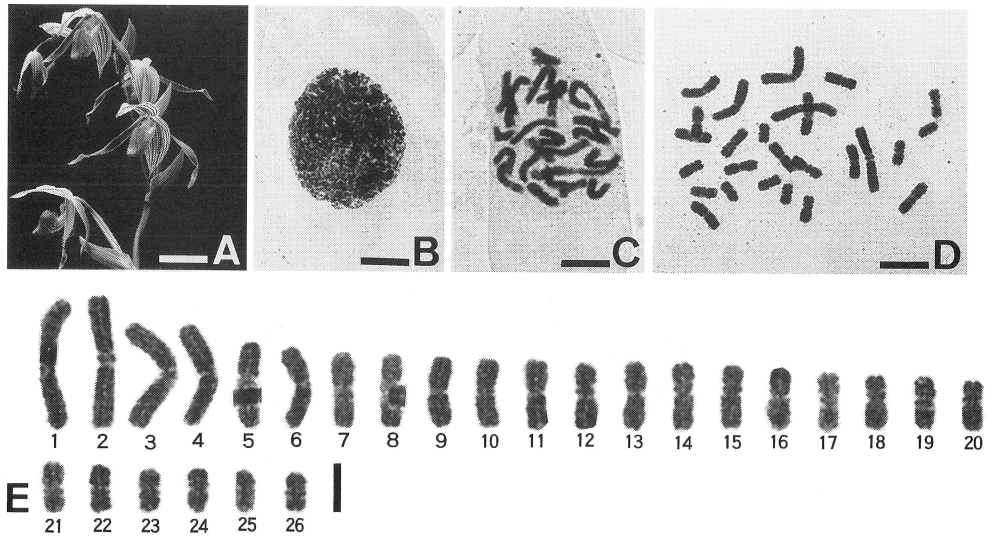

Fig. 2. Karyomorphology of *Paphiopedilum kolopakingii*, 2n=26. **A,** flowers. **B,** chromosomes at resting stage. **C,** chromosomes at mitotic prophase. **D** and **E,** chromosomes at mitotic metaphase. Bars indicate 40 mm in **A,** 10 μm in **B-D** and 5 μm in **E.**

Table 3. Measurements of somatic chromosomes of *Paphiopedilum kolopakingii*, 2n=26 at metaphase

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	7.0+7.0=14.0	7.5	1.0	m
2	6.0+7.7=13.7	7.3	1.2	m
3	6.0+6.8=12.8	6.8	1.1	m
4	5.7+5.8=11.3	6.1	1.0	m
5	3.7+5.2= 8.8	4.7	1.4	m
6	3.7+4.8= 8.5	4.5	1.3	m
7	3.5+4.0= 7.5	4.0	1.1	m
8	2.8+4.7= 7.5	4.0	1.6	m
9	3.3+3.8= 7.2	3.8	1.1	m
10	3.2+3.8= 7.0	3.7	1.2	m
11	3.3+3.7= 7.0	3.7	1.1	m
12	3.3+3.5= 6.8	3.6	1.0	m
13	3.2+3.7= 6.8	3.6	1.1	m
14	3.2+3.7= 6.8	3.6	1.1	m
15	3.0+3.5= 6.5	3.5	1.1	m
16	3.2+3.3= 6.5	3.5	1.0	m
17	2.3+3.7= 6.0	3.2	1.5	m
18	2.7+3.0= 5.7	3.0	1.1	m
19	2.3+3.0= 5.3	2.8	1.2	m
20	2.5+2.7= 5.2	2.8	1.0	m
21	2.3+2.5= 4.8	2.6	1.0	m
22	2.3+2.3= 4.7	2.5	1.0	m
23	2.0+2.3= 4.3	2.3	1.1	m
24	2.0+2.3= 4.3	2.3	1.1	m
25	1.8+2.3= 4.2	2.2	1.2	m
26	1.8+2.2= 4.0	2.1	1.1	m

μm . Arm ratios of the 26 chromosomes ranged between 1.0 and 1.5, and thus, the positions of their centromeres were all median. Two chromosomes (Nos. 15-16) had secondary constrictions on the interstitial region of the short arm, and the satellites were both 1.3 μm in length.

Thus, the chromosome complement showed a symmetric and bimodal karyotype, and it was quite similar to that of *P. henryanum* (2n=26) described by Karasawa and Aoyama (1988).

4. *Paphiopedilum sangii* Braem, 2n=28, Fig. 4 and Table 5.

This species was discovered in Sulawesi Is., Indonesia and described by Braem (1987). The species belonged to the subgenus Sigmatopetalum is a mottled-leaved terrestrial plant. The well hanged petals are distinguished from the closely related *P. hookerae* distributed in Borneo.

The chromosome number of 2n=28 was observed at mitotic metaphase in two clones of the species. The chromosome complement was constituted from two large chromosomes 16.7 and 15.0 μm long and 26 small chromosomes varied gradually from 11.7-7.0 μm , and showed an indistinct bimodal

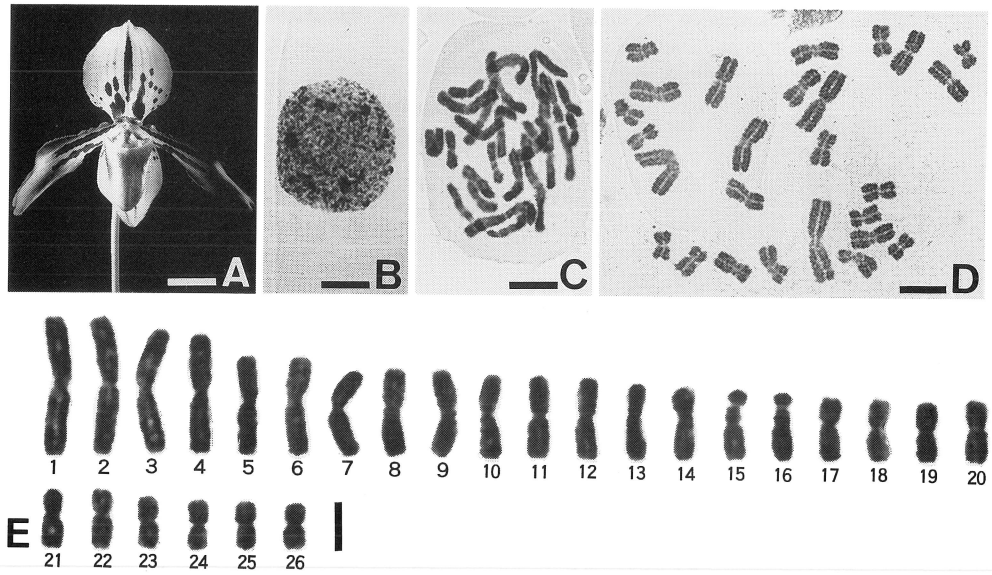

Fig. 3. Karyomorphology of *Paphiopedilum tigrinum*, 2n=26. **A,** a flower. **B,** chromosomes at resting stage. **C,** chromosomes at mitotic prophase. **D** and **E,** chromosomes at mitotic metaphase. Bars indicate 20 mm in **A**, 10 μm in **B-D** and 5 μm in **E.**

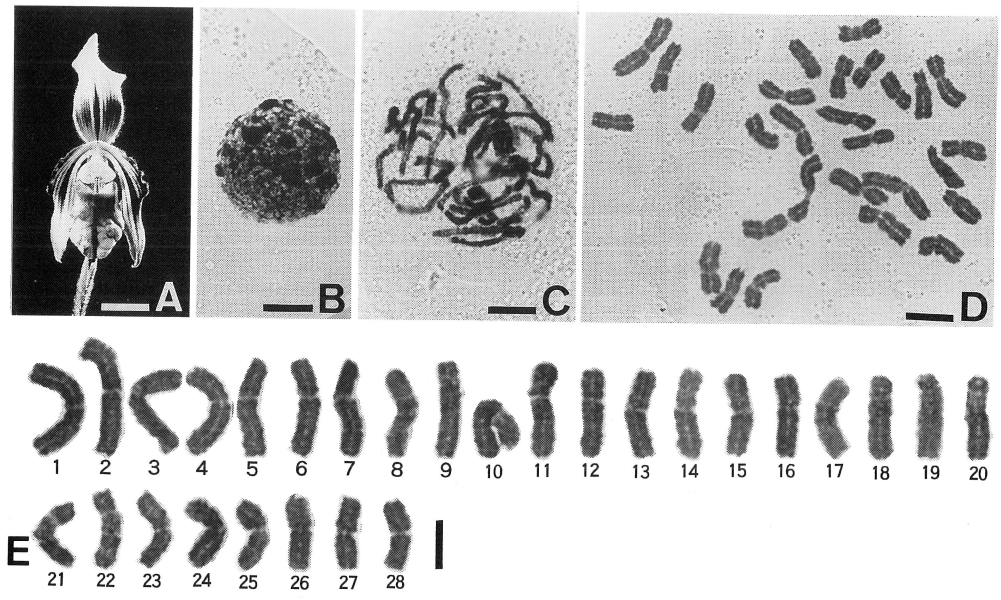

Fig. 4. Karyomorphology of *Paphiopedilum sangii*, 2n=28. **A,** a flower. **B,** chromosomes at resting stage. **C,** chromosomes at mitotic prophase. **D** and **E,** chromosomes at mitotic metaphase. Bars indicate 20 mm in **A**, 10 μm in **B-D** and 5 μm in **E.**

Table 4. Measurements of somatic chromosomes of *Paphiopedilum tigrinum*, 2n=26 at metaphase

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	6.5+6.6=13.1	6.7	1.0	m
2	6.4+6.8=13.1	6.7	1.0	m
3	5.8+6.3=12.0	6.2	1.0	m
4	5.5+6.0=11.5	5.9	1.0	m
5	4.3+5.3= 9.5	4.9	1.2	m
6	4.0+5.4= 9.4	4.8	1.3	m
7	4.1+4.8= 8.9	4.6	1.1	m
8	3.4+4.8= 8.1	4.2	1.4	m
9	3.8+4.4= 8.1	4.2	1.1	m
10	3.5+4.3= 7.8	4.0	1.2	m
11	3.9+3.9= 7.8	4.0	1.0	m
12	3.6+4.0= 7.6	3.9	1.1	m
13	3.0+4.0= 7.0	3.6	1.3	m
14	2.9+4.1= 7.0	3.6	1.4	m
15	1.3+1.5+3.6= 6.4*	3.3	1.3	m
16	1.3+1.5+3.5= 6.3*	3.2	1.2	m
17	2.4+3.6= 6.0	3.1	1.5	m
18	2.8+3.0= 5.8	3.0	1.0	m
19	2.8+2.9= 5.6	2.9	1.0	m
20	2.5+3.1= 5.6	2.9	1.2	m
21	2.4+3.1= 5.5	2.8	1.3	m
22	2.3+2.8= 5.0	2.6	1.2	m
23	2.1+2.5= 4.6	2.4	1.1	m
24	2.1+2.4= 4.5	2.3	1.1	m
25	2.0+2.4= 4.4	2.2	1.1	m
26	2.0+2.3= 4.3	2.2	1.1	m

*Chromosomes with secondary constriction

karyotype. Among the 28 chromosomes, 18 were median with arm ratios ranged from 1.0–1.6, six (Nos. 10–12, 17, 21–22) were submedian with arm ratios ranged from 1.8–2.6 and the other four (Nos. 9, 18–20) were subterminal with arm ratios ranged from 3.1–5.2. Two chromosomes (Nos. 21–22) had small secondary constriction on the interstitial region of the long arm.

Thus, the karyotype of this species lacked the terminal-centromeric chromosome was similar to the 2n=28 chromosome complement of *P. hookerae* (Karasawa 1979). The karyotype could be distinguished by higher arm ratios and no satellite chromosome.

5. *Paphiopedilum shoseri* Braem and Mohr, 2n=35, Fig. 5 and Table 6.

This species found in Bachan Is., Indonesia was described by Braem and Mohr (1988). The species belonged to the subgenus Sigmatopetalum is mottled-leaved terrestrial plant similar to *P. javanicum* distributed in Java Is. and Flores Is., Indonesia.

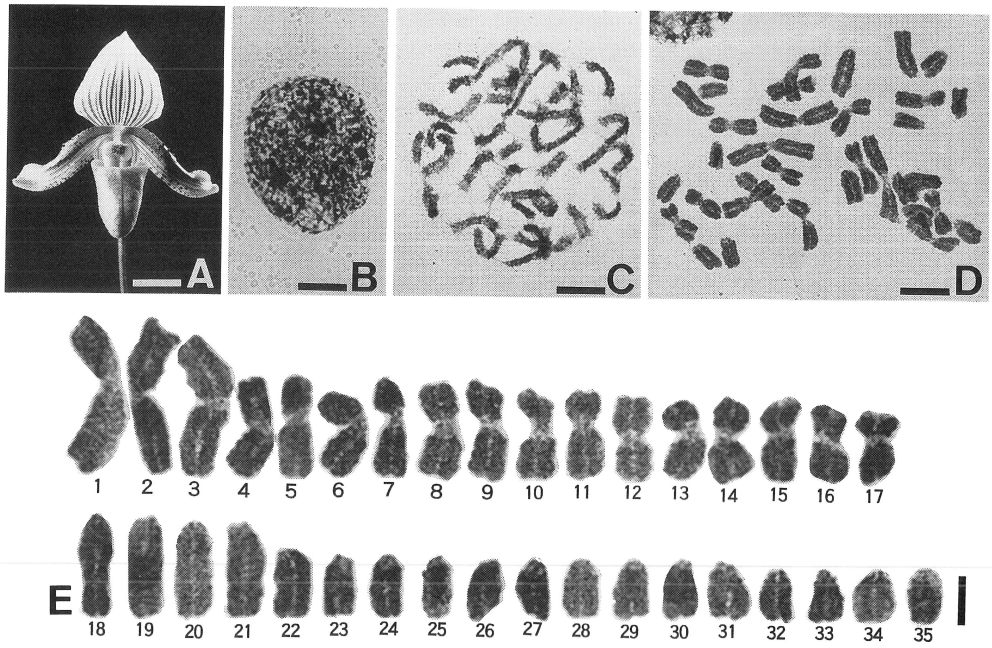

Fig. 5. Karyomorphology of *Paphiopedilum shoseri*, 2n=35. **A,** a flower. **B,** chromosomes at resting stage. **C,** chromosomes at mitotic prophase. **D** and **E,** chromosomes at mitotic metaphase. Bars indicate 20 mm in **A**, 10 μm in **B-D** and 5 μm in **E.**

The chromosome number of 2n=35 was counted in one clone of the species. Among the 35 chromosomes, three large chromosomes (Nos. 1-3) varied from 16.7-15.0 μm in length and were median-centromeric with arm ratios ranged from 1.1-1.3. Fourteen chromosomes (Nos. 4-17) varied gradually from 10.3-7.3 μm and the positions of their centromeres were median with arm ratios ranged between 1.1 and 1.5. The remaining 18 chromosomes (Nos. 18-35) varied from 10.3-5.3 μm and thus, the positions of their centromeres were terminal. These 18 chromosomes consisted of four long and 14 small chromosomes.

Thus, the karyotype of the species was consisted of heterogeneous pairs and odd chromosomes. The karyotype of 2n=35=17V+18I in the species might be originated from 2n=26=26V by the Robertsonian fission that agreed with the basic chromosome number of the genus (Karasawa 1979). The species might be karyomorphologically a natural hybrid. For instance, *P. javanicum* (2n=38) and *P. tonsum* (2n=32) grow near the habitat of *P. shoseri* and thus, are the candidates of parentage to make hybridization with 2n=35 chromosomes. However, *Paphiopedilum shoseri* has three large chromosomes and thus, there may be another undiscovered parental species near there.

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Table 5. Measurements of somatic chromosomes of *Paphiopedilum sangii*, 2n=28 at metaphase

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	6.0+6.7=12.7	5.0	1.1	m
2	5.7+6.7=12.3	4.8	1.1	m
3	4.7+7.0=11.7	4.6	1.5	m
4	4.3+6.3=10.7	4.2	1.4	m
5	4.0+6.3=10.3	4.0	1.5	m
6	4.0+6.0=10.0	3.9	1.5	m
7	4.0+6.0=10.0	3.9	1.5	m
8	4.7+4.7= 9.3	3.6	1.0	m
9	2.3+7.3= 9.7	3.8	3.1	st
10	2.7+7.0= 9.7	3.8	2.6	sm
11	3.3+6.0= 9.3	3.6	1.8	sm
12	3.2+5.8= 9.0	3.5	1.8	sm
13	4.0+5.2= 9.2	3.6	1.2	m
14	4.0+5.0= 9.0	3.5	1.2	m
15	4.0+4.7= 8.7	3.4	1.1	m
16	3.3+5.3= 8.7	3.4	1.6	m
17	2.7+6.0= 8.7	3.4	2.2	sm
18	2.0+6.3= 8.3	3.3	3.1	st
19	1.3+7.0= 8.3	3.3	5.2	st
20	1.7+6.7= 8.3	3.3	4.0	st
21	2.3+6.0= 8.3	3.3	2.5	sm
22	2.3+5.7= 8.0	3.1	2.4	sm
23	4.0+4.3= 8.3	3.3	1.0	m
24	3.7+4.3= 8.0	3.1	1.1	m
25	3.3+4.3= 7.7	3.0	1.3	m
26	3.3+4.0= 7.3	2.9	1.2	m
27	3.7+3.7= 7.3	2.9	1.0	m
28	3.3+3.7= 7.0	2.7	1.1	m

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Summary

The karyotypes of a species of *Paphiopedilum* naturally-rediscovered and four species subsequently, additionally-described after the genus was established with the major species were dealt with: The morphology of the resting chromosomes of five species were commonly categorized to be the complex chromocenter type; *P. sanderianum* (2n=26) had a pair of small, submedian-centromeric chromosomes, differed slightly from the karyotype of *P. stonei; P. kolopakingii* (2n=26) had two large

Table 6. Measurements of somatic chromosomes of *Paphiopedilum shoseri*, 2n=35 at metaphase

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	7.3+ 9.3=16.7	5.6	1.2	m
2	7.7+ 8.7=16.3	5.5	1.1	m
3	7.0+ 8.0=15.0	5.0	1.1	m
4	5.0+ 5.3=10.3	3.5	1.0	m
5	4.3+ 6.0=10.3	3.5	1.3	m
6	4.3+ 6.0=10.3	3.5	1.3	m
7	4.0+ 6.0=10.0	3.4	1.5	m
8	4.0+ 5.7= 9.7	3.2	1.4	m
9	4.3+ 5.3= 9.7	3.2	1.1	m
10	4.0+ 5.0= 9.0	3.0	1.2	m
11	3.7+ 5.3= 9.0	3.0	1.4	m
12	4.0+ 4.3= 8.3	2.8	1.0	m
13	3.3+ 5.0= 8.3	2.8	1.5	m
14	3.7+ 4.7= 8.3	2.8	1.2	m
15	3.3+ 5.0= 8.3	2.8	1.5	m
16	3.3+ 4.3= 7.7	2.6	1.3	m
17	3.0+ 4.3= 7.3	2.5	1.4	m
18	d+10.3=10.3	3.5	$<\infty$	t
19	d+10.3=10.3	3.5	$<\infty$	t
20	d+10.0=10.0	3.4	$<\infty$	t
21	d+ 9.3= 9.3	3.1	$<\infty$	t
22	d+ 7.0= 7.0	2.4	$<\infty$	t
23	d+ 6.3= 6.3	2.1	$<\infty$	t
24	d+ 6.3= 6.3	2.1	$<\infty$	t
25	d+ 6.3= 6.3	2.1	$<\infty$	t
26	d+ 6.0= 6.0	2.0	$<\infty$	t
27	d+ 6.0= 6.0	2.0	$<\infty$	t
28	d+ 6.0= 6.0	2.0	$<\infty$	t
29	d+ 6.0= 6.0	2.0	$<\infty$	t
30	d+ 6.0= 6.0	2.0	$<\infty$	t
31	d+ 5.7= 5.7	1.9	$<\infty$	t
32	d+ 5.3= 5.3	1.8	$<\infty$	t
33	d+ 5.3= 5.3	1.8	$<\infty$	t
34	d+ 5.3= 5.3	1.8	$<\infty$	t
35	d+ 5.3= 5.3	1.8	$<\infty$	t

d=dot

chromosomes which contain small secondary constriction, differed slightly from the karyotype of *P. stonei: P. tigrinum* (2n=26) had the karyotype extremely similar to *P. henryanum; P. sangii* (2n=28) had the chromosomes with high arm ratios and no satellite, differed slightly from the karyotype of *P. hookerae*; and *P. shoseri* (2n=35) had heterogeneous pairs of chromosomes. *Paphiopedilum shoseri* might be either a natural hybrid between *P. javanicum* (2n=38) and *P. tonsum* (2n=32) or an offspring of undiscovered parental species grown near there.

摘　　要

ラン科 *Paphiopedilum* 属の稀少な5種の核形態学的研究を行い，新たに *P. sanderianum* 2n=26, *P. kolopakingii* 2n=26, *P. tigrinum* 2n=26, *P. sangii* 2n=28, *P. shoseri* 2n=35の染色体数を算定した。

静止期染色体の形態はすべて複雑染色中央粒型であった。 *P. sanderianum* は小型の次中部型染色体を1対もち，近縁な *P. stonei* の核型とは異なった。*P. kolopakingii* は小狭窄を有する大型染色体を1対もち，近縁な *P. stonei* の核型とは異なった。*P. tigrinum* の核型は近縁な *P. henryanum* の核型に極めて類似した。*P. sangii* は腕比が高く付随体染色体を有しないことで近縁な *P. hookerae* の核型と異なった。*P. shoseri* は異質な染色体構成を示し，*P. javanicum* (2n=38) と *P. tonsum* (2n=32) との雑種であるか，または近くに分布する未記載種との雑種であると推察される。

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