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Karyomorphological Studies in *Phragmipedium*, Orchidaceae

Kohji Karasawa

フラグミペディウム属の核形態学的研究

唐 澤 耕 司

Introduction

The genus *Phragmipedium* is considered one of the most primitive genus of the Orchidaceae as same as *Cypripedium*, *Paphiopedilum* and *Selenipedium*, which are classified into Cypripedioideae (Garay 1960).

We are studying karyomorphologically on Cypripedioideae for the elucidation of the origin of Orchidaceae and of the phylogenetic relationships of this sub-family. This paper is a report dealing with *Phragmipedium* following *Paphiopedilum* (Karasawa 1979).

The reports on the chromosome of *Phragmipedium* are in three species: *P. caudatum* $2n=32$ (Hoffman 1929, cited in Duncan 1959), *P. longifolium* var. *hartwegii* $2n=20$ (Brown 1959, cited in Duncan 1959) and *P. boisseranum* $2n=18$ (Karasawa & Tanaka 1976). However, there, is no report on the detail of the karyomorphological analysis.

In the present investigation the morphology of chromosomes was studied in 14 species and one variety in *Phragmipedium*.

Acknowledgements

I would like to acknowledge the continuing guidance and encouragement of Professor Dr. Ryuso Tanaka of Hiroshima University. I also wish to thank Dr. Yoshihiko Yonezawa for his helpful advice and Dr. Leslie A. Garay for identification of specimen. My special thanks are also due to Mr. Sigeaki Ichijo, Mr. Hiromichi Miyahara, Mr. Yoshio Udagawa, Mr. Kazuhito Uchida and Mr. Nobuhiro Hata for supplying the materials.

Materials and Methods

Materials, sources and the number of clones investigated are listed in Table 1. Taxonomical treatment was followed Garay (1979). Observation of somatic chromosomes in the root tip cells was made according to the previous report (Karasawa & Tanaka 1976).

Karyotype analysis was made on the chromosomes at interphase, prophase and metaphase. In interphase, the morphology of the condensed and diffused chromatin was

analysed, and the location of early condensing chromatin in prophase. In the metaphase chromosomes, the length of both long and short arms was measured, and relative length and arm ratio were estimated. The position of centromere was expressed by the terms of median, submedian, subterminal and terminal according to Levan *et al.* (1964).

Table 1. Sources, number of clones and chromosome number of the species of *Phragmipedium* studied

Species	Source	No. of clone	Chromosome number (2n)
Section MICROPETALUM			
<i>schlimii</i>	Colombia	4	30*
× <i>stenophyllum</i> ***		1	40*
Section PLATYPETALUM			
<i>lindleyanum</i>	Venezuela	2	22*
<i>sargentianum</i>	Brazil	4	22*
Section PHRAGMIPEDIUM			
<i>caudatum</i>	Peru, Ecuador	4	28**
<i>wallisii</i>	Ecuador	1	28*
<i>lindenii</i>	Venezuela, Colombia	4	28*
Section HIMANTOPETALUM			
<i>ecuadorensis</i>	Peru	2	22*
<i>pearcei</i>	Peru	3	20*, 21*, 22*
Section LORIFOLIA			
<i>boisseranum</i>	Peru	3	18
<i>vittatum</i>	Brazil	2	18*
<i>hartwegii</i>	Ecuador	2	20
<i>longifolium</i>	Colombia, Panama	2	23*(20+3f)
<i>longifolium</i> var. <i>gracile</i>	(Rands Orchids)***	1	21*(20+1f)
<i>roezlii</i>	Panama	2	22*(20+2f)

* first time record

** differ from previous record

*** cultured

Observations

In the preliminary study, the morphological changes of chromosomes in mitotic cell cycle were observed in root tip cells of *Phragmipedium hartwegii*, 2n=20 (Fig. 1). In the

interphase nuclei (Fig. 1A, B), many, darkly stained chromocenters were observed. These chromocenters occasionally showed aggregations forming several, large heteropycnotic blocks. These nuclei were categorized the complex chromocenter type of Tanaka's classification of resting nuclei (Tanaka, 1971). In early prophase, chromosomes changed to fibrous thread stained almost homogeneously (Fig. 1C), while in middle or late prophase, early condensing segments were observed at the proximal regions of both arms (Fig. 1D, E). These early condensing segments transformed abruptly into late condensing segments

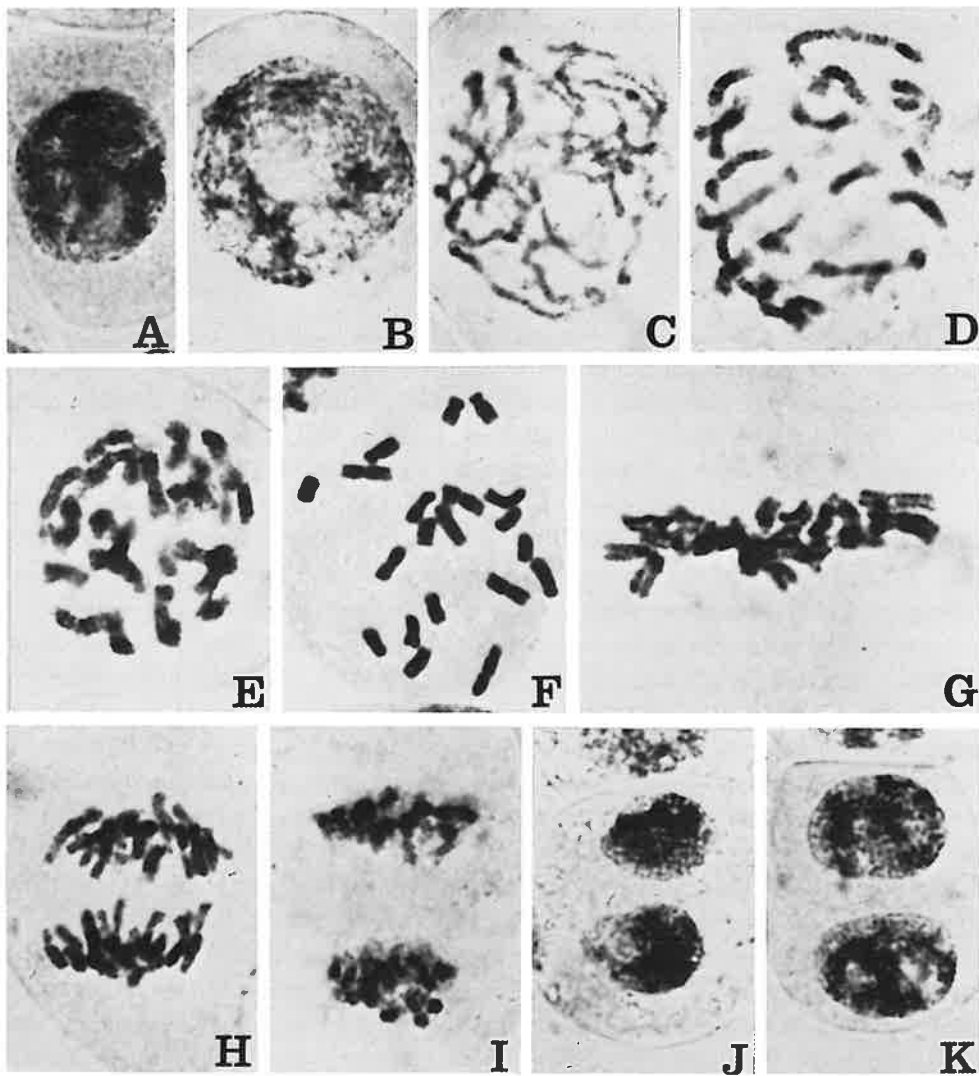


Fig. 1. Chromosomes in mitotic cell cycle in root-tip cells of *Phragmipedium hartwegii*, $2n=20$. A and B, interphase. C and D, prophase. E, prometaphase. F and G, metaphase. H, anaphase. I, telophase. J and K, early interphase of daughter cells. $\times 1600$.

located at the distal regions. These prophase nuclei were categorized the proximal type on the distribution of the early condensing chromatin (Tanaka 1977).

At metaphase pretreated with 8-hydroxyquinoline, $2n=20$ chromosomes were counted, and each chromosome was distinguished by their length and centromere position (Fig. 1F).

At anaphase to next interphase, no karyomorphological characteristic was observed (Fig. 1G-K).

Therefore, karyotype analysis was made in the chromosomes at interphase, prophase and metaphase as mentioned in Materials and Methods.

I. MICROPETALUM

1) *Phragmipedium schlimii* (Lindl. & Rchb. f.) Rolfe, $2n=30$

Varidated specimen No. BG-31, BG-32, M-1, I-1.

This species is native to Colombia and has the following distinct morphological characteristics: The dorsal sepal is oval. The petals are broad and not pointed. The upper sepal and petals are pinkish white in color. The natural spread of flower is about 4 cm in width. The pouch is oval and white with deep pinkish stripes. The middle part of rhombate staminode is brownish saffron-yellow in color (Fig. 2A).

The interphase nuclei were similar to those of *P. hartwegii* mentioned above showing the complex chromocenter type (Fig. 2B). Prophase chromosomes were the proximal type (Fig. 2C). At metaphase, $2n=30$ chromosomes were counted in four clones (Fig. 2D). The measurements of chromosome length and arm ratio are shown in Table 2.

Among the 30 chromosomes the two longest chromosomes (Nos. 1, 2) were distinguished. They were $5.8\mu\text{m}$ and $5.2\mu\text{m}$ in length, respectively. Arm ratio of these chromosomes were 3.5 and 3.0, and the position of centromeres were subterminal and submedian, respectively. Four chromosomes (Nos. 3-6) ranged from 4.0 to $3.6\mu\text{m}$ in length and the position of centromeres of these chromosomes were terminal. The 7th and 8th chromosomes were both $3.5\mu\text{m}$ in length. Arm ratio of these chromosomes were both 1.9, and the position of centromeres were submedian. Two chromosomes (Nos. 9, 10) were both $3.4\mu\text{m}$ in length, and the position of centromeres were terminal. Two chromosomes (Nos. 11, 12) were both $3.3\mu\text{m}$ in length. Arm ratio of these chromosomes were both 1.5, and the position of centromeres were median. Eight chromosomes (Nos. 13-20) ranged from 3.3 to $2.9\mu\text{m}$ in length, and the position of centromeres were terminal. Two chromosomes (Nos. 21, 22) had the satellites at the distal region of long arms. They were both $2.9\mu\text{m}$ ($2.0+0.9\mu\text{m}$), and the position of centromeres were terminal. The remaining eight chromosomes (Nos. 23-30) ranged from 2.9 to $2.1\mu\text{m}$ in length, and the position of centromeres were terminal.

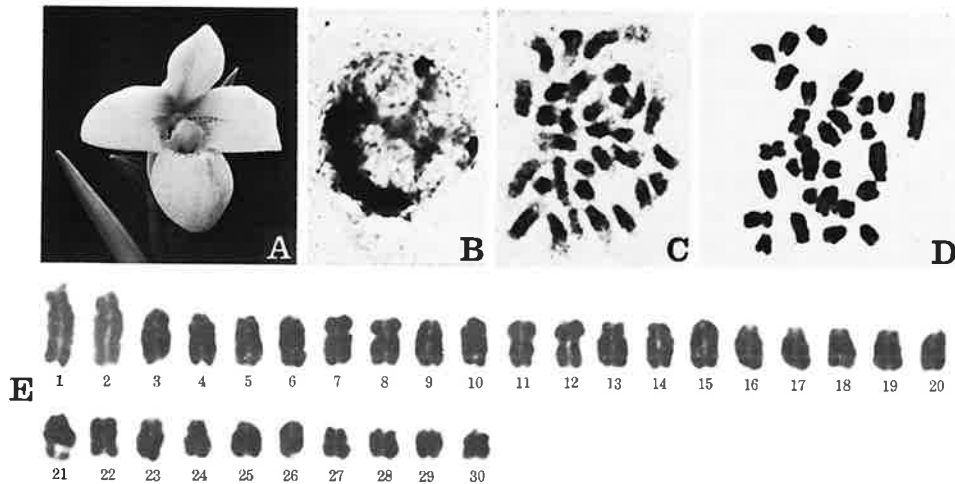


Fig. 2. *Phragmipedium schlimii*, $2n=30$. A, flower. B, interphase nucleus. C, prometaphase. D and E, metaphase. A, $\times 0.7$. B-D, $\times 1200$. E, $\times 1800$.

As mentioned above, the $2n=30$ chromosomes were divided into two quantitative groups showing bimodal karyotype: The one consisted of the two longest metacentric chromosomes and the other consisted of 28 small chromosomes which decreased gradually in size. Two telocentric chromosomes which had satellites were the marker chromosomes for the karyotype of this species.

2) *Phragmipedium x stenophyllum*, $2n=40$

Varidated specimen No.3014.

This species was nearly recorded as the new species in the magazine of the Orchid Digest (42: 232, 1978). In the horticultural world it was introduced as *P. schlimii*, but Garay (1979) considered it *P. x stenophyllum* based on the examination of the flower which showed to be identical with the original specimen of *P. x stenophyllum* in Reichenbach Harbarium (Fig. 3A).

In the present investigation the chromosome number of this species was examined to be $2n=40$. The chromosomes at prometaphase and metaphase are shown in Fig. 3B-D. The results of the measurement of chromosome length and arm ratio are shown in Table 3.

Among the 40 chromosomes the longest chromosome (No. 1) was $6.0\mu\text{m}$ in length. Arm ratio of this chromosome was 3.3, and the position of centromere was subterminal. The 2nd chromosome was $5.1\mu\text{m}$ in length and had the satellite on its long arm. The short arm was $2.1\mu\text{m}$ and the long arm was $3.0\mu\text{m}(2.3+0.7\mu\text{m})$ in length. Arm ratio of this chromosome was 1.4, and the position of centromere was median. The 3rd chromosome was $4.9\mu\text{m}$ in length. Arm ratio of the chromosome was 2.8, and the position of centromere

mere was submedian. The 4th chromosome was $4.3\mu\text{m}$ in length. Arm ratio of this chromosome was 1.3, and the position of centromere was median. The 5th chromosome was $4.1\mu\text{m}$ in length. Arm ratio of this chromosome was 1.3, and the position of centromere was median. The 6th chromosome was $4.1\mu\text{m}$ in length. Arm ratio of this chromosome was 2.4, and the position of centromere was submedian. The 7th chromosome was $4.0\mu\text{m}$ in length and had the small constriction near the middle region of the long arm. The position of centromere of this chromosome was terminal. The 8th chromosome was $4.0\mu\text{m}$ in length. Arm ratio of the chromosome was 2.1, and the position of centromere was submedian. Two chromosomes (Nos. 9, 10) were both $4.0\mu\text{m}$ in length. The position of centromeres of these chromosomes were terminal. The 11th chromosome was $4.0\mu\text{m}$ in length. Arm ratio of this chromosome was 1.2, and the position of centromere was median. Two chromosomes (Nos. 12, 13) were both $3.9\mu\text{m}$ in length. The position of centromeres of these chromosomes were terminal. The 14th chromosome was $3.6\mu\text{m}$ in length. Arm ratio of this chromosome was 1.4, and the position of centromere was median. The 15th chromosome was $3.6\mu\text{m}$ in length. Arm ratio of this chromosome was 2.0, and the position of centromere was submedian. The 16th chromosome was $3.6\mu\text{m}$ in length. The position of centromere of this chromosome was terminal. Two chromosomes (Nos. 17, 18) were both $3.5\mu\text{m}$ in length. Arm ratio of these chromosomes were both 1.9, and the position of centromeres were submedian. Two chromosomes (Nos. 19, 20) were both $3.4\mu\text{m}$ and $3.3\mu\text{m}$ in length, respectively. The position of centromeres of these chromosomes were terminal. Two chromosomes (Nos. 21, 22) were both $3.3\mu\text{m}$ in length. Arm ratio of these chromosomes were both 1.5, and the position of centromeres were median. The eight chromosomes (Nos. 23-30) ranged from 3.0 to $2.8\mu\text{m}$ in length, and the position of centromeres were terminal. Two chromosomes (Nos. 31, 32) were both $2.8\mu\text{m}$ in length,

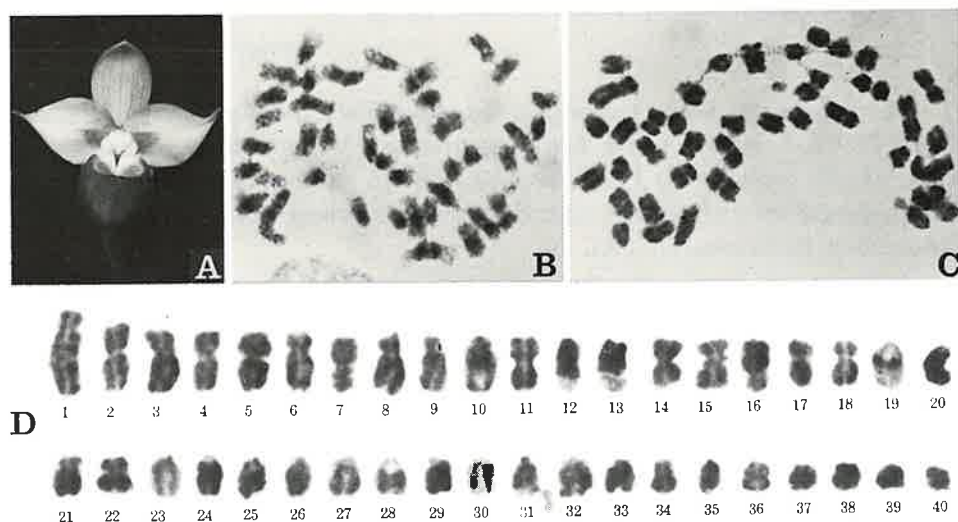


Fig. 3. *Phragmipedium* $\times$ *stenophyllum*, $2n=40$. A, flower. B, prophase. C and D, metaphase. A, $\times 0.4$. B and C, $\times 1200$. D, $\times 1800$.

and had satellites at the distal region of long arms. The position of centromeres of these chromosomes were terminal. The eight chromosomes (Nos. 33-40) ranged from 2.8 to $2.0\mu\text{m}$ in length and gradually decreased in size. The position of centromeres of these chromosomes were terminal.

According to the morphology of chromosome complement, the $2n=40$ chromosomes of this species consisted of 14 metacentric chromosomes and 26 telocentric chromosomes. The 16 chromosomes (Nos. 1-16) of them were heterogeneous pairs which did not make the matched chromosomes, while the rest 24 chromosomes (Nos. 17-40) of them were homogeneous pairs which made 12 matched chromosomes.

II. PLATYPETALUM

1) *Phragmipedium lindleyanum* (Schomb. ex Lindl.) Rolfe, $2n=22$

Varidated specimen No. BG-30, I-2

This large-sized species grow in Guyana and in Venezuela. External characteristics of this species are as follows: Leaves are lustrous and leathery, the tip of leaf is pointed and 50-60cm in length. The dorsal sepal is long oval. The petals are beltshape and their ends are round. The staminode is nearly lozenge (Fig. 4A).

The interphase nuclei were similar to those of above species showing the complex chromocenter type (Fig. 4B). Prophase chromosomes were the proximal type (Fig. 4C). At metaphase, $2n=22$ chromosomes were counted in two clones (Fig. 4D, E). The measurements of chromosome length and arm ratio are shown in Table 4.

Two chromosomes (Nos. 1, 2) which had the small constrictions near the middle of their short arms were both $5.7\mu\text{m}$ in length. Arm ratio of these chromosomes were both 1.3, and the position of centromeres were median. Two chromosomes (Nos. 3, 4) which had the satellites at the distal region of long arms were $5.3\mu\text{m}$ in length. The long arms of these chromosomes were $2.9\mu\text{m}$ ($1.8+1.1\mu\text{m}$) and the short arms were $2.4\mu\text{m}$ in length. Arm ratio of these chromosomes were 1.2, and the position of centromeres were median. Two chromosomes (Nos. 5, 6) were both $5.2\mu\text{m}$ in length. Arm ratio of these chromosomes were 3.0, and the position of centromeres were submedian. These chromosomes had the small constrictions near the middle region of their long arms. Two chromosomes (Nos. 7, 8) were both $5.2\mu\text{m}$ in length. Arm ratio of these chromosomes were both 1.2, and the position of centromeres were median. Two chromosomes (Nos. 9, 10) were both $4.2\mu\text{m}$ in length. Arm ratio of these chromosomes were both 2.2, and the position of centromeres were submedian. Two chromosomes (Nos. 11, 12) were $3.4\mu\text{m}$ and $3.1\mu\text{m}$ in length, and the position of centromeres were terminal. Two chromosomes (Nos. 13, 14) were both $3.1\mu\text{m}$ in length. Arm ratio of these chromosomes were both 1.6, and the position of centromeres were median. The remaining eight chromosomes (Nos. 15-22) ranged from 2.9 to $2.6\mu\text{m}$ in length. These chromosomes were decreased gradually in size and the position of centromeres were all terminal.

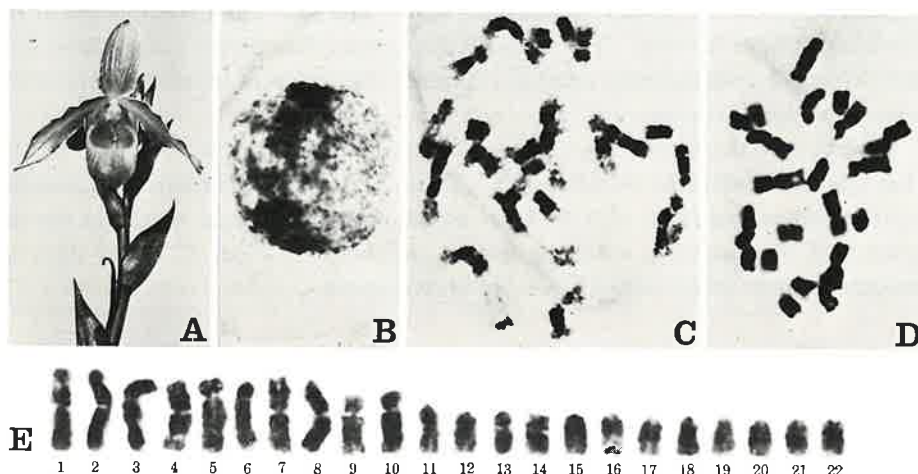


Fig. 4. *Phragmipedium lindleyanum*, $2n=22$. A, flower. B, interphase nucleus. C, prometaphase. D and E, metaphase. A, $\times 0.3$. B-D, 1200. E, $\times 1800$.

As mentioned above, the chromosome complement of this species consisted of 22 chromosomes which could be divided into two groups showing a bimodal karyotype: The one consisted of 10 large chromosomes and the other consisted of 12 small chromosomes which decreased gradually in size. Among the 12 small chromosomes, 10 chromosomes were terminal. Two large chromosomes (Nos. 3, 4) had the satellites at the distal regions of long arms and the 11th chromosome was longer than the 12th chromosome. The small constrictions were observed in the middle region of the short arms of the first and the second chromosomes and near the middle region of the long arms of the fifth and the sixth chromosomes.

2) *Phragmipedium sargentianum* (Rolfe) Rolfe, $2n=22$

Varidated specimen No. 3013, BG-35, M-2, M-3.

This species, native to Brazil, is similar to above *P. lindleyanum*. Color of the flower is more purplish red than the *P. lindleyanum*. The dorsal sepal has red stripes. The petals are 1.5 to 2.0 times as long as the dorsal sepal. The staminode is broad and nearly trapezoid (Fig. 5A).

The interphase nuclei were similar to those of above species showing the complex chromocenter type. Prometaphase chromosomes were the proximal type (Fig. 5B). At metaphase, $2n=22$ chromosomes were counted in four clones (Fig. 5C, 5D). The measurements of chromosome length and arm ratio are shown in Table 5.

Two chromosomes (Nos. 1, 2) which had small constrictions on the middle region of the short arms were both $5.9\mu\text{m}$ in length. Arm ratio of these chromosomes were both 1.3, and the position of centromeres were median. Two chromosomes (Nos. 3, 4) which had satellites in their long arms were both $5.4\mu\text{m}$ in length. The short arms were $2.2\mu\text{m}$ and the

long arms were $3.2\mu\text{m}$ ($2.2+1.0\mu\text{m}$) in length. Arm ratio of these chromosomes were both 1.5, and the position of centromeres were median. Two chromosomes (Nos. 5, 6) were both $5.4\mu\text{m}$ in length. Arm ratio of these chromosomes were both 2.9, and the position of centromeres were submedian. There were small constrictions near the middle region of their long arms of these two chromosomes. Two chromosomes (Nos. 7, 8) were both $4.8\mu\text{m}$ in length. Arm ratio of these chromosomes were both 1.1, and the position of centromeres were median. Two chromosomes (Nos. 9, 10) were both $4.6\mu\text{m}$ in length. Arm ratio of these chromosomes were both 2.1, and the position of centromeres were submedian. The 11th chromosome which was $3.5\mu\text{m}$ in length was longer than the 12th chromosome which was $3.2\mu\text{m}$ in length. The position of centromeres of these two chromosomes were both terminal. Two chromosomes (Nos. 13, 14) were both $2.9\mu\text{m}$ in length. Arm ratio of these chromosomes were both 1.4, and the position of centromeres were median. The remaining eight chromosomes (Nos. 15-22) which were decreased gradually in size ranged from 2.9 to $2.4\mu\text{m}$ in length, and the position of centromeres were terminal.

According to the morphology of chromosome complement, this species was extremely similar to above *P. lindleyanum*.

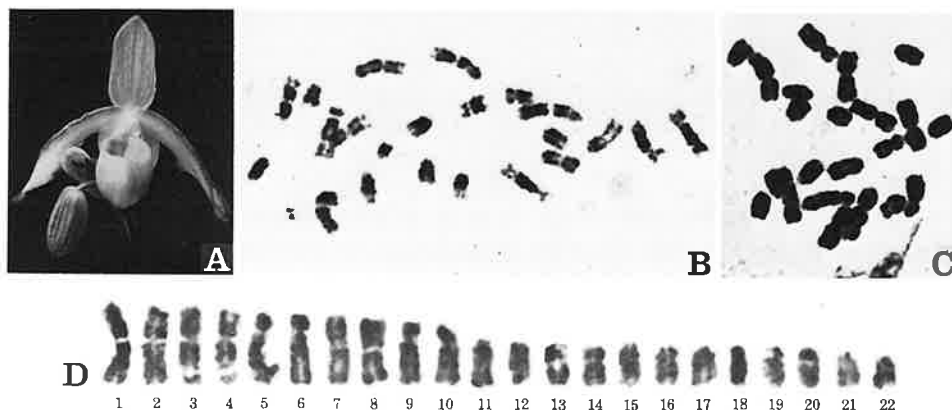


Fig. 5. *Phragmipedium sargentianum*, $2n=22$. A, flower. B, prometaphase. C and D, metaphase. A, $\times 0.3$. B and C, $\times 1200$. D, $\times 1800$.

III. PHRAGMIPEDIUM

1) *Phragmipedium caudatum* (Lindl.) Rolfe, $2n=28$

Varidated specimen No. 1156, 3006, 3020, BG-33.

This species grows in Central America, Ecuador, Peru and Colombia. The height of this species is different in accordance with the habitat. Several flowers which are on the erect peduncle bloom at the same time. The dorsal sepal is acute triangle and its edge is wave. Petals are narrow twisted ribbon and hang down about 50-80 cm in length (Fig. 6A).

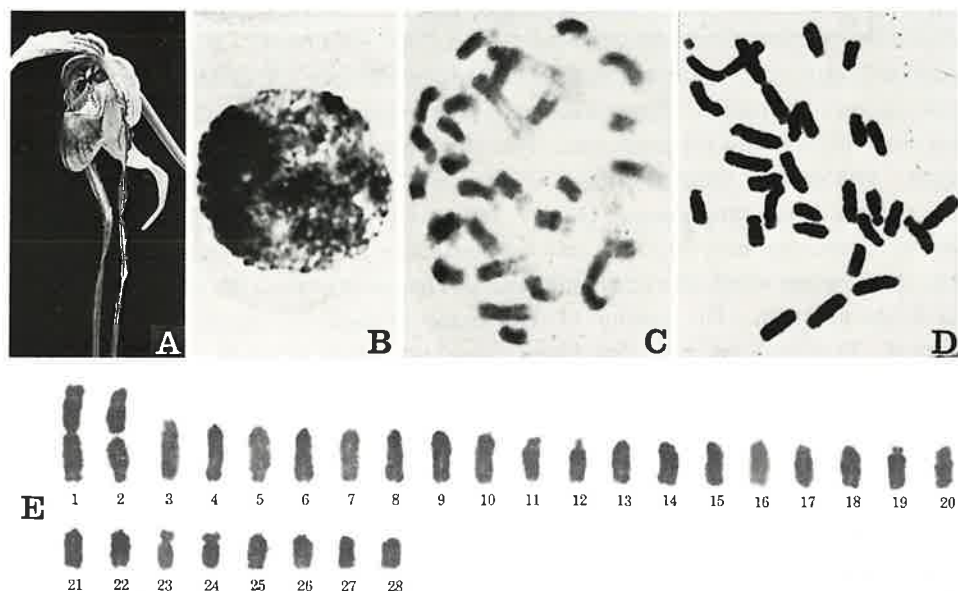


Fig. 6. *Phragmipedium caudatum*, $2n=28$. A, flower. B, interphase nucleus. C, prophase. D and E, metaphase. A, $\times 0.3$. B and C, $\times 1200$. D and E, $\times 1800$.

The interphase nuclei were similar to those of above species showing the complex chromocenter type (Fig. 6B). Prophase chromosomes were the proximal type (Fig. 6C). Chromosome number of $2n=32$ in this species was previously reported by Hoffman (1929, cited in Duncan 1959). In the present investigation the number of chromosomes were examined in four clones to be $2n=28$ (Fig. 6D, E). The measurements of chromosome length and arm ratio are shown in Table 6.

Among the 28 chromosomes the two longest chromosomes (Nos. 1, 2) were distinguished. The other 26 chromosomes (No. 3-28) ranged from 4.5 to $2.5\mu\text{m}$ in length, and decreased gradually in size. The 1st and 2nd chromosomes were both $7.0\mu\text{m}$ in length, and had satellites at the distal region of long arms. The short arms of them were $3.4\mu\text{m}$ and the long arms were $3.6\mu\text{m}(2.9+0.7\mu\text{m})$ in length. Arm ratio of these chromosomes were both 1.1, and the position of centromeres were median. There were small constrictions near the middle region of their arms of these two chromosomes. Eight chromosomes (Nos. 3-10) ranged from 4.5 to $3.7\mu\text{m}$ in length, and the position of centromeres were terminal. Two chromosomes (Nos. 11, 12) were $3.5\mu\text{m}$ in length. Arm ratio of these chromosomes were 4.0, and the position of centromeres were subterminal. Six chromosomes (Nos. 13-18) ranged from 3.3 to $3.1\mu\text{m}$ in length, and the position of centromeres were terminal. Two chromosomes (Nos. 19, 20) were both $3.1\mu\text{m}$ in length. Arm ratio of these chromosomes were 3.4, and the position of centromeres were subterminal. Two chromosomes (Nos. 21, 22) were both $2.9\mu\text{m}$ in length, and the position of centromeres were terminal. Two chromosomes (Nos. 23, 24) were both $2.9\mu\text{m}$ in length. Arm ratio of these chromosomes were

2.2, and the position of centromeres were submedian. The rest four chromosomes (Nos. 25-28) ranged from 2.7 to 2.5 μm in length, and the position of centromeres were terminal.

As mentioned above, the chromosome complement of this species consisted of 28 chromosomes which could be divided into two groups showing a bimodal karyotype: The one consisted of two large metacentric chromosomes and the other consisted of 26 small chromosomes which were decreased gradually in size. Among the 26 chromosomes six chromosomes (Nos. 11, 12, 19, 20, 23 and 24) were metacentric chromosomes and the rest 20 chromosomes were all telocentric chromosomes. Two large chromosomes (Nos. 1, 2) had the satellites at the distal region of long arms and small constrictions were in the middle region of their short arms.

2) *Phragmipedium wallisii* (Rchb. f.) Garay, $2n=28$

Varidated specimen No. M-4.

This species is native to Ecuador. The morphology of the flower is similar to that of *P. caudatum*, but lateral lobes of the staminode of this species is more acute (Fig. 7A).

The interphase nuclei were similar to those of above species showing the complex chromocenter type (Fig. 7B). Prophase chromosomes were the proximal type. At metaphase, $2n=28$ chromosomes were counted in one clone (Fig. 7C, D). The measurements of chromosome length and arm ratio are shown in Table 7.

Among the 28 chromosomes the two longest chromosomes (Nos. 1, 2) which were both 6.5 μm length were clearly distinguished. The other 26 chromosomes (Nos. 3-28) ranged from 4.1 to 2.3 μm in length, and decreased gradually in size. The longest two

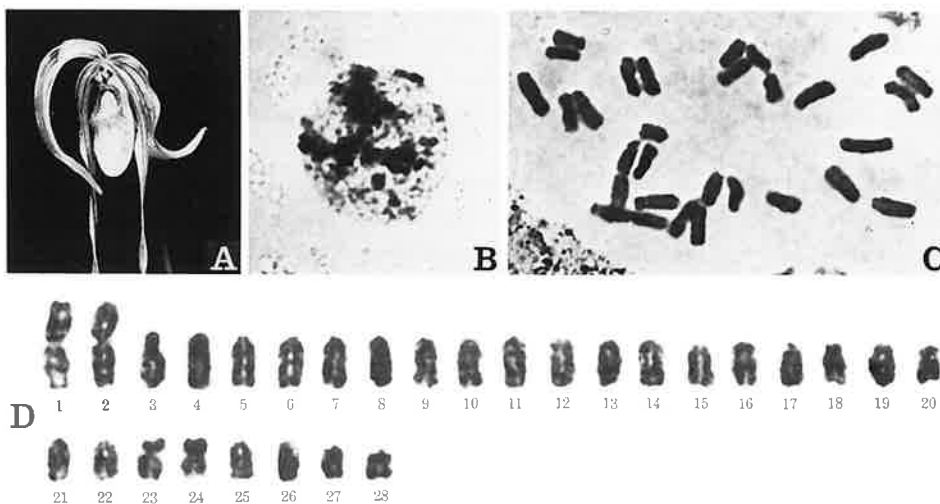


Fig. 7. *Phragmipedium wallisii*, $2n=28$. A, flower. B, interphase nucleus. C and D, metaphase. A, $\times 0.3$. B and C, $\times 1200$. D, $\times 1800$.

chromosomes (Nos. 1, 2) had satellites at the distal region of long arms. The short arms of them were $3.0\mu\text{m}$ and the long arms were $3.5\mu\text{m}(2.5+1.0\mu\text{m})$ in length. Arm ratio of these chromosomes were both 1.2, and the position of centromeres were median. There were small constrictions near the middle region of their short arms of these two chromosomes. Eight chromosomes (Nos. 3-10) ranged from 4.1 to $3.7\mu\text{m}$ in length, and the position of centromeres were terminal. Two chromosomes (Nos. 11, 12) were both $3.7\mu\text{m}$ in length. Arm ratio of these chromosomes were both 3.1, and the position of centromeres were subterminal. Six chromosomes (Nos. 13-18) ranged from 3.5 to $3.0\mu\text{m}$ in length, and the position of centromeres were terminal. Two chromosomes (Nos. 19, 20) were both $3.0\mu\text{m}$ in length. Arm ratio of these chromosomes were both 3.3, and the position of centromeres were subterminal. Two chromosomes (Nos. 21, 22) were $3.0\mu\text{m}$ in length, and the position of centromeres were terminal. Two chromosomes (Nos. 23, 24) were both $3.0\mu\text{m}$ in length. Arm ratio of these chromosomes were both 2.3, and the position of centromeres were submedian. The remaining four chromosomes (Nos. 25-28) ranged from 2.9 to $2.3\mu\text{m}$ in length, and the position of centromeres were terminal.

According to the morphology of chromosome complement, this species was similar to *P. caudatum* mentioned above.

3) *Phragmipedium lindenii* (Lindl.) Dressler & N. Wms., $2n=28$

Varidated specimen No. BG-34, BG-36, BG-37, I-3.

This species distributes in Venezuela, Colombia and Ecuador. The petals are all same form and color, and does not have a pouch (Fig. 8A).

The interphase nuclei were similar to those of above species showing the complex chromocenter type. Prometaphase chromosomes were the proximal type (Fig. 8B). At metaphase, $2n=28$ chromosomes were counted in four clones (Fig. 8C, D). The measurements of chromosome length and arm ratio are shown in Table 8.

Among the 28 chromosomes the two longest chromosomes (Nos. 1, 2) which were both $6.7\mu\text{m}$ in length were clearly distinguished. The other 26 chromosomes (Nos. 3-28) ranged from 4.7 to $2.4\mu\text{m}$ in length, and decreased gradually in size. The 1st and 2nd chromosomes had satellites at the distal region of long arms. The short arms of them were $3.0\mu\text{m}$ and the long arms were $3.7\mu\text{m}(2.9+0.8\mu\text{m})$ in length. Arm ratio of these chromosomes were both 1.2, and the position of centromeres were median. There were small constrictions near the middle region of their arms of these two chromosomes. Eight chromosomes (Nos. 3-10) ranged from 4.7 to $3.7\mu\text{m}$ in length, and the position of centromeres were terminal. Two chromosomes (Nos. 11, 12) were both $3.6\mu\text{m}$ in length. Arm ratio of these chromosomes were both 4.1, and the position of centromeres were subterminal. Six chromosomes (Nos. 13-18) ranged from 3.6 to $3.3\mu\text{m}$ in length, and the position of centromeres were terminal. Two chromosomes (Nos. 19, 20) were both $3.3\mu\text{m}$ in length. Arm ratio of these chromosomes were both 3.1, and the position of centromeres were subterminal. Two chromosomes (Nos. 21, 22) were both $3.3\mu\text{m}$ in length, and the position of cen-

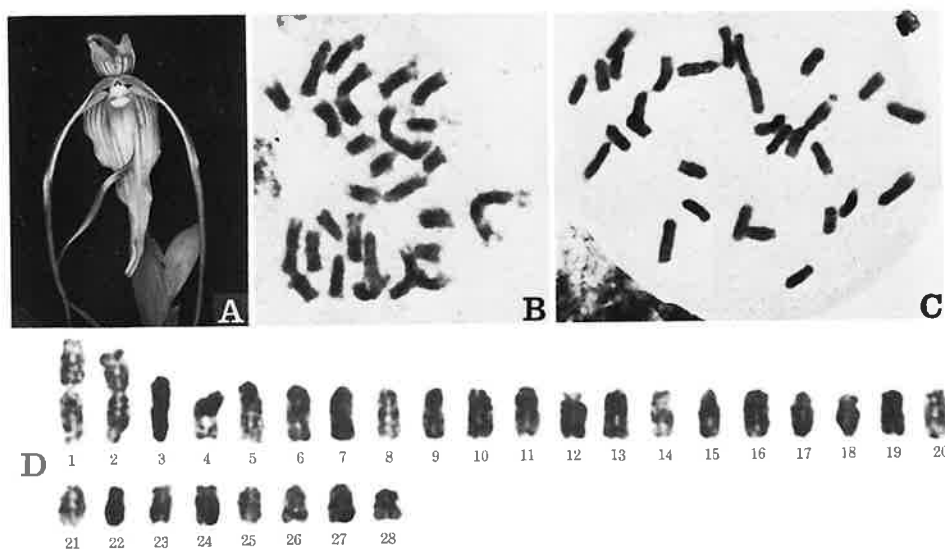


Fig. 8. *Phragmipedium lindenii*, $2n=28$. A, flower. B, prometaphase. C and D, metaphase. A, $\times 0.3$. B and C, $\times 1200$. D, $\times 1800$.

tromeres were terminal. Two chromosomes (Nos. 23, 24) were both $3.0\mu\text{m}$ in length. Arm ratio of these chromosomes were both 2.8, and the position of centromeres were submedian. The remaining four chromosomes (Nos. 25-28) ranged from 2.7 to $2.4\mu\text{m}$ in length, and the position of centromeres were terminal.

According to the morphology of chromosome complement, this species was similar to *P. caudatum* mentioned above.

IV. HIMANTOPETALUM

1) *Phragmipedium ecuadorensis* Garay, $2n=22$

Varidated specimen No. 3010, BG-38.

This species which distributes in Ecuador and in Peru is smaller than *P. longifolium* belonging to Sect. Lorifolia. The length of the leaves and peduncle are about half of *P. longifolium*. The color of flower is greenish pink. Petals are ribbon-type and twistingly hang down. The staminode has thick black hairs (Fig. 9A).

The interphase nuclei were similar to those of above species showing the complex chromocenter type (Fig. 9B). Prometaphase chromosomes were the proximal type (Fig. 9C). At metaphase, $2n=22$ chromosomes were counted in two clones (Fig. 9D, E). The measurements of chromosome length and arm ratio are shown in Table 9.

The 22 chromosomes of this species ranged from 5.6 to $2.4\mu\text{m}$ in length, and de-

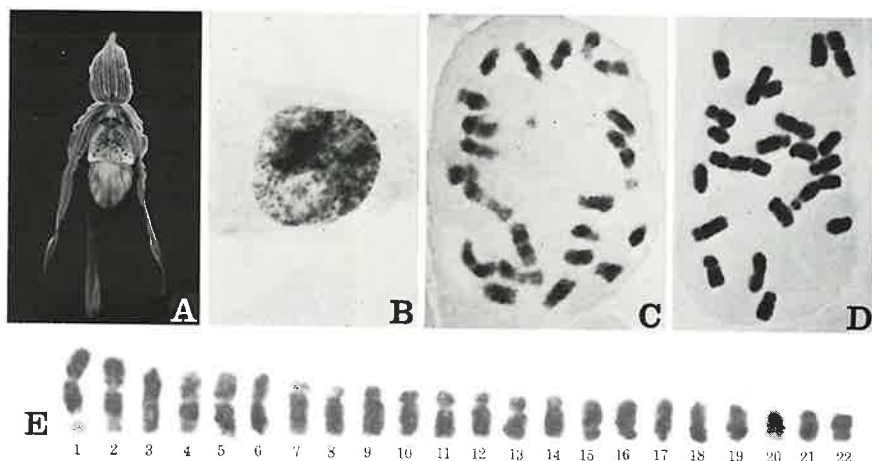


Fig. 9. *Phragmipedium ecuadorensis*, $2n=22$. A, flower. B, interphase nucleus. C, pro-metaphase. D and E, metaphase. A, $\times 0.5$. B-D, $\times 1200$. E, $\times 1800$.

creased gradually in size. The 1st and 2nd chromosomes had satellites at the distal region of long arms and were $5.6\mu\text{m}$ and $5.5\mu\text{m}$ in length, respectively. The short arms of them were each $2.3\mu\text{m}$ and $2.2\mu\text{m}$ and the long arms were both $3.3\mu\text{m}(2.5+0.8\mu\text{m})$ in length. Arm ratio of these chromosomes were 1.4 and 1.5 and the position of centromeres were median. Four chromosomes (Nos. 3-6) ranged from 5.1 to $4.9\mu\text{m}$ in length. Arm ratio of these chromosomes were 1.2 and 1.1, and the position of centromeres were median. Two chromosomes (Nos. 7, 8) were each 4.3 and $4.2\mu\text{m}$ in length. Arm ratio of these chromosomes were each 3.3 and 3.2, and the position of centromeres were subterminal. Four chromosomes (Nos. 9-12) ranged from 3.8 to $3.5\mu\text{m}$ in length. Arm ratio of these chromosomes varied from 2.8 to 2.9, and the position of centromeres were submedian. Two chromosomes (Nos. 13, 14) were both $3.4\mu\text{m}$ in length. Arm ratio of these chromosomes were 1.6, and the position of centromeres were median. Eight chromosomes (Nos. 15-22) which had the small constriction in the middle region of long arms varied from 3.3 to $2.4\mu\text{m}$ in length, and the position of centromeres were terminal.

As mentioned above, the chromosome complement of this species consisted of 22 chromosomes which decreased gradually in size. The two longest chromosomes (Nos. 1, 2) had the satellites at the distal region of long arms. Among the 22 chromosomes eight small chromosomes were the telocentric chromosomes.

2) *Phragmipedium pearcei* (Rchb. f.) Rauh & Senghas, Type 1, $2n=20$
 Varidated specimen No. 3016.

This species is native to Ecuador and Peru and has been classified into two types based on the differences of the shape and the color of flower by Garay (1979). The specimen in-

vestigated is suit to Type 1 and its peduncle reaches about 60 cm in height. Color of the dorsal sepal and petals are brownish green with brownish veins. The staminode of this species is a half-moon shape and its edge bears black hairs (Fig. 10A).

The interphase nuclei were similar to those of above species showing the complex chromocenter type (Fig. 10B). Prophase chromosomes were the proximal type (Fig. 10C). In the present investigation the chromosome number of this Type 1 was examined to be $2n=20$ (Fig. 10D, E). The measurements of chromosome length and arm ratio are shown in Table 10.

Among the 20 chromosomes the 1st chromosome (No. 1) was $7.2\mu\text{m}$ in length and was distinguished. The other 19 chromosomes (Nos. 2-20) ranged from 4.4 to $2.1\mu\text{m}$ in length, and decreased gradually in size.

Arm ratio of the 1st chromosome was 1.2, and the position of centromere was median. This chromosome had the small constrictions in the middle region of long and short arms. The 2nd chromosome had a satellite at the distal region of long arm. The short arm was $1.9\mu\text{m}$ in length and the long arm was $2.5\mu\text{m}$ ($1.7+0.8\mu\text{m}$) in length. Arm ratio of this chromosome was 1.3, and the position of centromere was median. The 3rd chromosome was $4.3\mu\text{m}$ in length and had the small constriction in the middle region of the long arm. Arm ratio of the chromosome was 1.0, and the position of centromere was median. The 4th chromosome was $4.3\mu\text{m}$ in length and had the small constriction in the middle region of the long arm. Arm ratio of this chromosome was 2.3, and the position of centromere was submedian. The 5th chromosome was $4.3\mu\text{m}$ in length and had the satellite at the distal region of the long arm. The short arm was $1.2\mu\text{m}$ in length and the long arm was $3.1\mu\text{m}$

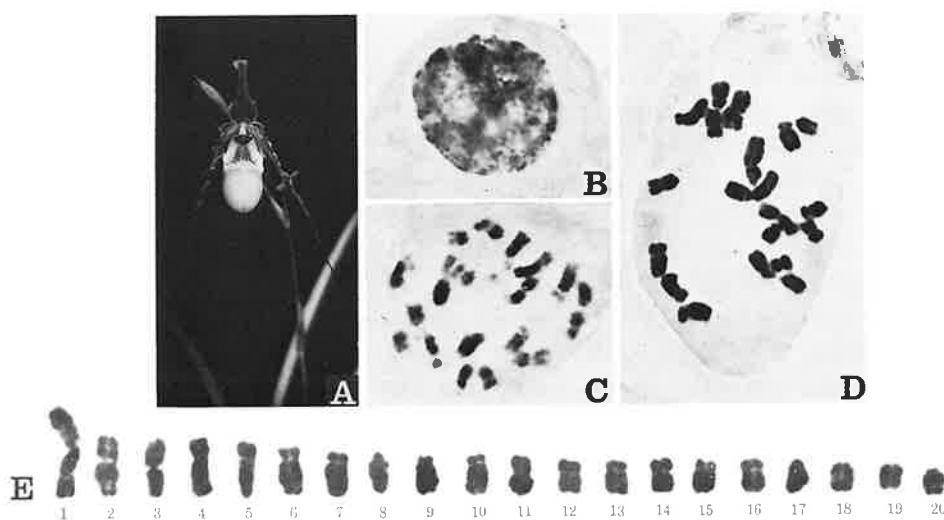


Fig. 10. *Phragmipedium pearcei* type 1, $2n=20$. A, flower. B, interphase nucleus. C, prometaphase. D and E, metaphase. A, $\times 0.4$. B and C, $\times 900$. D, $\times 1200$. E, $\times 1800$.

($2.3 \pm 0.8 \mu\text{m}$). Arm ratio of this chromosome was 2.6, and the position of centromere was submedian. Two chromosomes (Nos. 6, 7) were $3.7 \mu\text{m}$ and $3.3 \mu\text{m}$ in length and had small constrictions near the middle region of their long arms. Their arm ratio were 2.4 and 2.9, and the position of centromeres were submedian. The 8th chromosome was $3.1 \mu\text{m}$ in length. Arm ratio was 1.4, and the position of centromere was median. Two chromosomes (Nos. 9, 10) were both $3.0 \mu\text{m}$ in length. Arm ratio of these chromosomes were both 2.8, and the position of centromeres were submedian. The 11th chromosome was $2.9 \mu\text{m}$ in length. Arm ratio of this chromosome was 1.1, and the position of centromere was median. The 12th chromosome was $2.7 \mu\text{m}$ in length. Arm ratio of this chromosome was 1.5, and the position of centromere was median. The 13th chromosome was $2.7 \mu\text{m}$ in length. Arm ratio of this chromosome was 2.0, and the position of centromere was submedian. Three chromosomes (Nos. 14-16) ranged from 2.7 to $2.5 \mu\text{m}$ in length. Arm ratio of these chromosomes varied from 1.3 to 1.5, and the position of centromeres were median. Four chromosomes (Nos. 17-20) ranged from 2.4 to $2.1 \mu\text{m}$ in length, and the position of centromeres were terminal. Two chromosomes (Nos. 18, 20) among these four chromosomes had small constrictions in the middle region of the long arms.

As mentioned above the $2n=20$ chromosomes of this species consisted of one large chromosome and 19 small chromosomes which decreased gradually in size. The position of centromeres of the four shortest chromosomes were terminal. These 20 chromosomes did not form the pair of matched chromosomes morphologically, and were heterogeneous.

3) *Phragmipedium pearcei*, Type 2, $2n=21$

Varidated specimen No. 3033.

Comparing with Type 1, the leaves and the peduncle are shorter. The color of the flower is more purplish-red and the petals are shorter, too. Furthermore the petals of this species do not hang and do not firmly twist (Fig. 11A).

The interphase nuclei were similar to those of above species showing the complex chromocenter type (Fig. 11B). Prophase chromosomes were the proximal type. At metaphase, $2n=21$ chromosomes were counted (Fig. 11C, D). The measurements of chromosome length and arm ratio are shown in Table 11.

The length of the 21 chromosomes of Type 2 varied from the longest to the shortest chromosomes. Two chromosomes (Nos. 1, 2) had the satellites at the distal region of their long arms and were both $5.3 \mu\text{m}$ in length. The short arms were $2.3 \mu\text{m}$ and the long arms were $3.0 \mu\text{m}$ ($2.3 \pm 0.7 \mu\text{m}$) in length. Arm ratio of these chromosomes were both 1.3, and the position of centromeres were median. Two chromosomes (Nos. 3, 4) were $4.9 \mu\text{m}$ and $4.8 \mu\text{m}$ in length, respectively. Arm ratio of these chromosomes were 1.1 and 1.2, and the position of centromeres were median. These chromosomes had the small constrictions in the middle region of their long arms. Two chromosomes (Nos. 5, 6) were $4.4 \mu\text{m}$ and $4.2 \mu\text{m}$ in length, respectively. Arm ratio of these chromosomes were 1.3 and 1.2, and the position of centromeres were median. These chromosomes had the small constrictions in

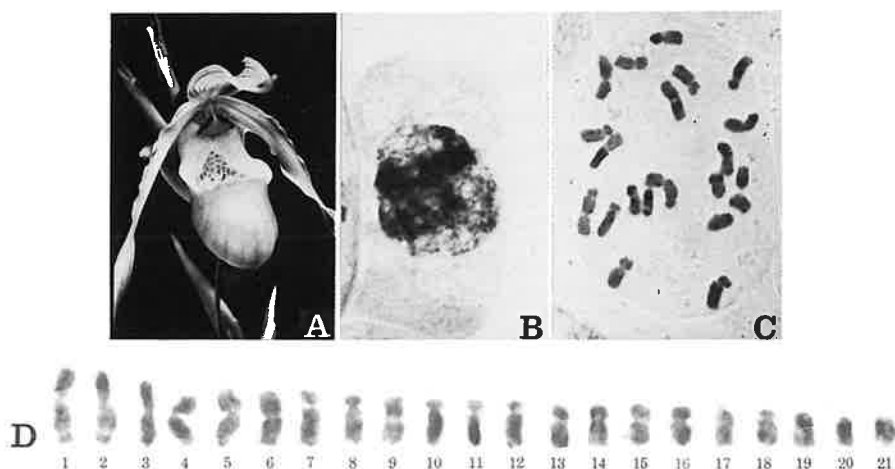


Fig. 11. *Phragmipedium pearcei* type 2, $2n=21$. A, flower. B, interphase nucleus. C and D, metaphase. A, $\times 0.6$. B and C, $\times 1200$. D, $\times 1800$.

the middle region of their long arms. Two chromosomes (Nos. 7, 8) were $3.8\mu\text{m}$ and $3.7\mu\text{m}$ in length, respectively. Arm ratio of these chromosomes were 2.5 and 2.7, and the position of centromeres were submedian. The 9th chromosome was $3.7\mu\text{m}$ in length. Arm ratio of the chromosome was 1.2, and the position of centromere was median. The 10th chromosome was $3.6\mu\text{m}$ in length. Arm ratio of this chromosome was 3.0, and the position of centromere was submedian. Two chromosomes (Nos. 11, 12) were both $3.6\mu\text{m}$ in length. Arm ratio of these chromosomes were both 2.6, and the position of centromeres were submedian. Two chromosomes (Nos. 13, 14) were both $3.3\mu\text{m}$ in length. Arm ratio of these chromosomes were both 1.8, and the position of centromeres were submedian. Two chromosomes (Nos. 15, 16) were both $3.2\mu\text{m}$ in length. Arm ratio of these chromosomes were both 1.5, and the position of centromeres were median. The 17th chromosome was $3.2\mu\text{m}$ in length, and the position of centromere was terminal. The 18th chromosome was $3.0\mu\text{m}$ in length. Arm ratio of this chromosome was 1.5, and the position of centromere was median. The rest three chromosomes (Nos. 19-21) ranged from 2.8 to $2.3\mu\text{m}$ in length, and the position of centromeres were terminal.

According to the morphology of chromosome complement, the $2n=21$ chromosomes did not formed the pair of matched chromosomes morphologically. Especially two pairs, Nos. 9-10 and Nos. 17-18 were heterogenous. The karyotype of Type 2 was different from that of above *P. pearcei* Type 1.

4) *Phragmipedium pearcei*, Type 3, $2n=22$

Varidated specimen No. 3020.

The external characteristics of this type are similar to those of above Type 2, and could not be distinguished each other. Petals of this type are longer than those of above Type 2 and twist (Fig. 12A).

The interphase nuclei were similar to those of above species showing the complex chromocenter type. Prophase chromosomes were the proximal type (Fig. 12B). At metaphase, $2n=22$ chromosomes were counted in one clone (Fig. 12C, D). The measurements of chromosome length and arm ratio are shown in Table 12.

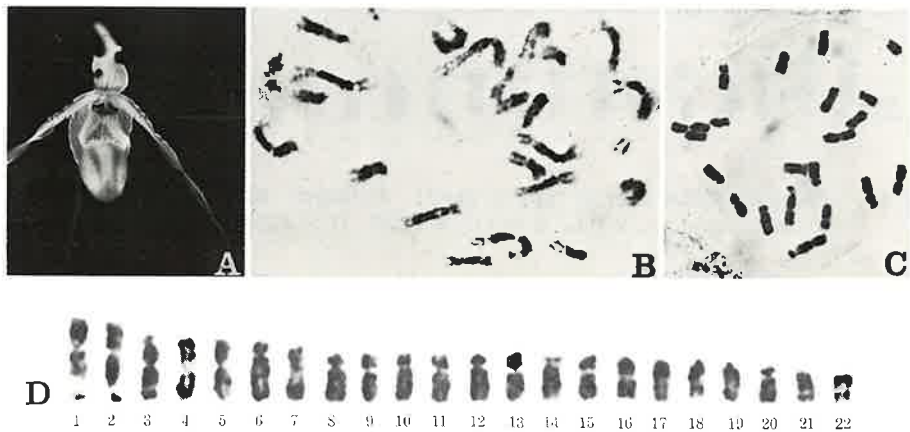


Fig. 12. *Phragmipedium pearcei* type 3, $2n=22$. A, flower. B, prometaphase. C and D, metaphase. A, $\times 0.4$. B and C, $\times 900$. D, $\times 1800$.

The length of the 22 chromosomes of Type 3 varied from the longest to the shortest chromosomes. Two chromosomes (Nos. 1, 2) had the satellites at the distal region of the long arms and were $5.3\mu\text{m}$ in length. The short arms were $2.2\mu\text{m}$ and the long arms were $3.1\mu\text{m}(2.4+0.7\mu\text{m})$ in length. Arm ratio of these chromosomes were both 1.4, and the position of centromeres were median. Two chromosomes (Nos. 3, 4) were $4.7\mu\text{m}$ and $4.3\mu\text{m}$ in length, respectively. Arm ratio of these chromosomes were 1.1 and 1.2, and the position of centromeres were median. These two chromosomes had the small constrictions in the middle region of the short arm of the 3rd chromosomes and near the middle region of the short arm of the 4th chromosome. The 5th chromosome was $4.3\mu\text{m}$ in length. Arm ratio of this chromosome was 1.2, and the position of centromere was median. The 6th chromosome was $4.3\mu\text{m}$ in length. Arm ratio of this chromosome was 3.3, and the position of centromere was subterminal. The 7th chromosome was $4.0\mu\text{m}$ in length. Arm ratio of this chromosome was 1.4, and the position of centromere was median. Two chromo-

somes (Nos. 8, 9) were $3.6\mu\text{m}$ and $3.5\mu\text{m}$ in length, respectively. Arm ratio of these chromosomes were 2.3 and 1.9, and the position of centromeres were submedian. The 10th chromosome was $3.4\mu\text{m}$ in length. Arm ratio of this chromosome was 2.4, and the position of centromere was submedian. The 11th chromosome was $3.4\mu\text{m}$ in length. Arm ratio of this chromosome was 2.1, and the position of centromere was submedian. The 12th chromosome was $3.4\mu\text{m}$ in length. Arm ratio of this chromosome was 2.4, and the position of centromere was submedian. The 13th chromosome was $3.4\mu\text{m}$ in length. Arm ratio of this chromosome was 1.6, and the position of centromere was median. The 14th chromosome was $3.2\mu\text{m}$ in length. Arm ratio of this chromosome was 3.6, and the position of centromere was subterminal. The 15th chromosome was $2.9\mu\text{m}$ in length. Arm ratio of this chromosome was 1.9, and the position of centromere was submedian. The 16th chromosome was $2.9\mu\text{m}$ in length. Arm ratio of this chromosome was 1.4, and the position of centromere was median. Three chromosomes (Nos. 17-19) ranged from 2.9 to $2.7\mu\text{m}$ in length, and the position of centromeres were terminal. These three chromosomes had the small constrictions on the middle region of their long arms. The 20th chromosome was $2.6\mu\text{m}$ in length. Arm ratio of this chromosome was 2.7, and the position of centromere was submedian. The remaining two chromosomes (Nos. 21, 22) were both $2.3\mu\text{m}$ in length, and the position of centromeres were terminal. These two chromosomes had the small constrictions near the middle region of the long arm of the 21th chromosome and in the middle region of the long arm of the 22th chromosome.

According to the morphology of chromosome complement, the $2n=22$ chromosomes did not form the pair of matched chromosomes morphologically. Especially two pairs, Nos. 5-6, and 7-8 were heterogenous. The karyotype of this Type was different from that of *P. pearcei* Type 1, while it was similar to that of *P. pearcei* Type 2.

V. LORIFOLIA

1) *Phragmipedium boisseranum* (Rchb. f.) Rolfe, $2n=18$

Varidated specimen No. 3034, I-5, I-6.

This species distributes in Peru. External characteristics of this species are as follows: Leaves are broad and are about 10cm in length. The peduncle is about 50-60cm in length and the width of flower is about 15cm. The color of flower is green. The dorsal sepal is lanceolate and the edge is wave. Petals are narrow and the edges are wave. The petals twist and obliquely spread towards the down ward. The staminode is rhombic-triangular (Fig. 13A).

The interphase nuclei were similar to those of above species showing the complex chromocenter type (Fig. 13B). Prophase chromosomes were the proximal type (Fig. 13C). The chromosome number of $2n=18$ in this species was previously reported by Karasawa & Tanaka (1976) except the details of the chromosome complement. The metaphase chromo-

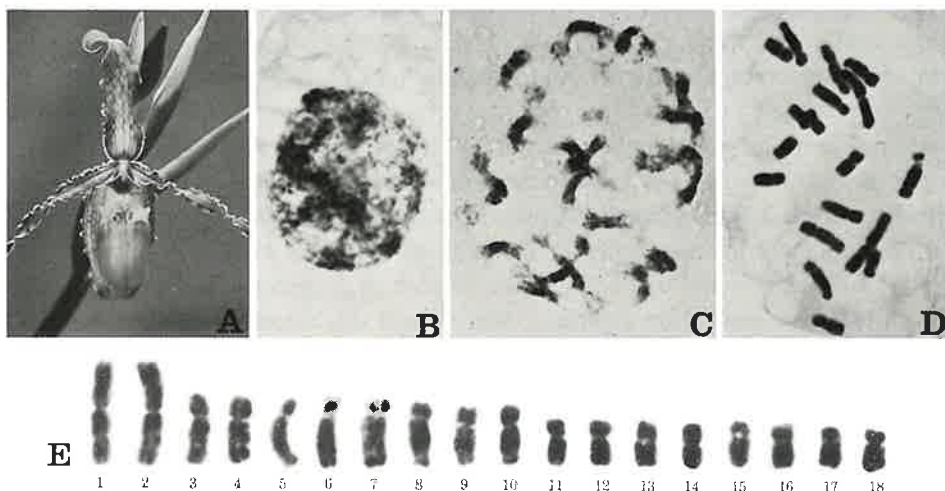


Fig. 13. *Phragmipedium boisseranum*, $2n=18$. A, flower. B, interphase nucleus. C, prometaphase. D and E, metaphase. A, $\times 0.3$. B D, $\times 1200$. E, $\times 1800$.

somes are shown in Fig. 13D and E. The measurements of chromosome length and arm ratio are shown in Table 13.

Among the 18 chromosomes the two longest chromosomes (Nos. 1, 2) which were both $7.3\mu\text{m}$ in length were clearly distinguished. Arm ratio of these chromosomes were both 1.1, and the position of centromeres were median. These two chromosomes had small constrictions on the middle region of the long and short arms. Two chromosomes (Nos. 3, 4) were both $5.0\mu\text{m}$ in length. Arm ratio of these chromosomes were both 1.9, and the position of centromeres were submedian. These two chromosomes had small constrictions near the middle region of their long arms. Two chromosomes (Nos. 5, 6) which had the small constrictions near the middle region of their long arms were both $4.7\mu\text{m}$ in length. Arm ratio of these chromosomes were both 2.6, and the position of centromeres were submedian. Two chromosomes (Nos. 7, 8) which had the satellites at the distal region of long arms were both $4.7\mu\text{m}$ in length. The short arms of these chromosomes were $1.0\mu\text{m}$ and the long arms were $3.7\mu\text{m}(2.9+0.8\mu\text{m})$ in length. Arm ratio of these chromosomes were both 3.6, and the position of centromeres were subterminal. Two chromosomes (Nos. 9, 10) were both $4.5\mu\text{m}$ in length. Arm ratio of these chromosomes were both 1.8, and the position of centromeres were submedian. These two chromosomes had small constrictions near the distal region of their long arms. Six chromosomes (Nos. 11-16) ranged from 3.5 to $3.3\mu\text{m}$ in length. Arm ratio of these chromosomes varied from 1.2 to 1.7, and the position of centromeres were median. Two chromosomes (Nos. 17, 18) were $3.1\mu\text{m}$ in length. Arm ratio of these chromosomes were both 1.8, and the position of centromeres were submedian.

Thus, the chromosome complement of this species consisted of 18 chromosomes which could be divided mainly into three groups showing a trimodal karyotype: The one consisted of two largest chromosomes and the second consisted of 8 medium-size chromosomes which were decreased gradually in size and the rest consisted of 8 small chromosomes which were decreased gradually in size, too. One pair of matched chromosomes (Nos. 7, 8) had the satellites on their long arms. Two large chromosomes (Nos. 1, 2) had the small constrictions on the long and short arms and another six chromosomes (Nos. 3-6, 9, 10) had them on their long arms.

2) *Phragmipedium vittatum* (Vell.) Rolfe, $2n=18$

Varidated specimen No. 3018, BG-39.

This species, distributing in Brazil, is similar to above *P. boisseranum*. Color of the edge of leaves is yellow. The edge of ventral sepals is not wave. The staminode is cordate-triangular (Fig. 14A).

The interphase nuclei were similar to those of above species showing the complex chromocenter type (Fig. 14B). Prometaphase chromosomes were the proximal type (Fig. 14C). At prometaphase and metaphase, $2n=18$ chromomes were counted in two clones (Fig. 14D, E). The measurements of chromosome length and arm ratio are shown in Table 14.

Among the 18 chromosomes the two longest chromosomes (Nos. 1, 2) were distinguished. They were both $7.5\mu\text{m}$ in length. Their arm ratio were both 1.2, and the position of centromeres were median. These two chromosomes had the small constrictions in

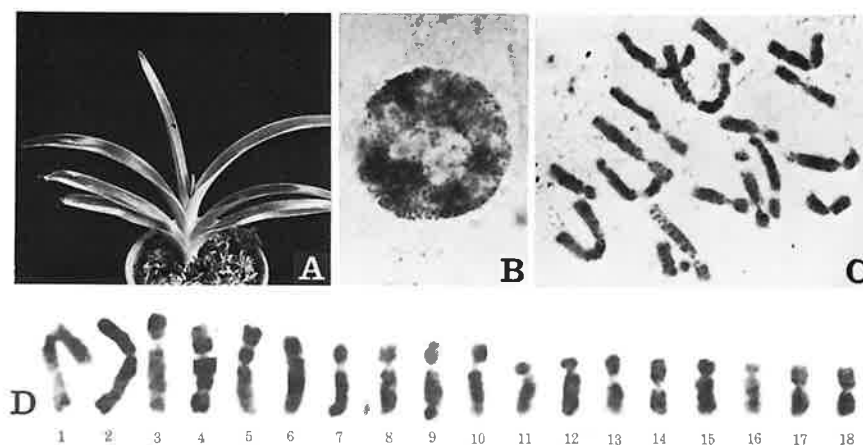


Fig. 14. *Phragmipedium vittatum*, $2n=18$. A, seedling. B, interphase nucleus. C, prometaphase. D, metaphase. A, $\times 0.1$. B and C, $\times 1200$. D, $\times 1800$.

middle region of each long and short arms. Two chromosomes* (Nos. 3, 4) were both $5.5\mu\text{m}$ in length. Arm ratio of these chromosomes were 2.1, and the position of centromeres were submedian. These two chromosomes had the small constrictions near the end of the long arms. Two chromosomes (Nos. 5, 6) were both $5.5\mu\text{m}$ in length. Arm ratio of these chromosomes were 2.3, and the position of centromeres were submedian. These two chromosomes had the small constrictions near the centromere of the long arms. Two chromosomes (Nos. 7, 8) which had the satellites at the distal region of the long arms were both $5.3\mu\text{m}$ in length. The short arms of these chromosomes were $1.3\mu\text{m}$ and long arms of them were $4.0\mu\text{m}(3.2+0.8\mu\text{m})$ in length. Arm ratio of these chromosomes were both 3.1, and the position of centromeres were subterminal. Two chromosomes (Nos. 9, 10) were both $4.9\mu\text{m}$ in length. Their arm ratio were both 2.5, and the position of centromeres were submedian. Two chromosomes (Nos. 11, 12) were both $4.2\mu\text{m}$ in length. Their arm ratio were both 3.2, and the position of centromeres were subterminal. Two chromosomes (Nos. 13, 14) were both $3.9\mu\text{m}$ in length. Their arm ratio were 1.3 and 1.2, respectively, and the position of centromeres were median. Two chromosomes (Nos. 15, 16) were both $3.9\mu\text{m}$ in length. Their arm ratio were both 2.5, and the position of centromeres were submedian. The remaining two chromosomes (Nos. 17, 18) were $3.8\mu\text{m}$ and $3.7\mu\text{m}$ in length, respectively. Arm ratio of these chromosomes were both 1.2, and the position of centromeres were median.

The similarity between the chromosome complement of this species and that of above *P. boisseranum* was observed. Thus, the chromosome complement of this species could be divided into three groups showing a trimodal karyotype: The first group consisted of two largest chromosomes and the second consisted of 8 medium-sized chromosomes and the rest consisted of 8 small chromosomes. The two longest chromosomes had the small constrictions on the middle region of the long and short arms. Six medium-sized chromosomes (Nos. 3-6, 9, 10) had the small constrictions and two chromosomes (Nos. 7, 8) had the satellites on their long arms.

Compared with the karyotype of *P. boisseranum*, two subtelocentric chromosomes of this species (Nos. 11, 12) were the characteristics.

3) *Phragmipedium hartwegii* (Rchb. f.) L. O. Wms., $2n=20$

Varidated specimen No. 3035, BG-40.

Phragmipedium hartwegii is native to Ecuador. The leaves are linear, archedly curved and 30cm in length. The erect peduncle bears several flowers which bloom by turns. The flower is horizontally about 7cm in diameter. The dorsal sepal is lanceolate and pale green, suffused with pale redish-purple and has redish-purple veins (Fig. 15A).

The interphase nuclei were similar to those of above species showing the complex chromocenter type. Prophase chromosomes were the proximal type. The chromosome number of $2n=20$ in this species was previously reported by Brown (1959, cited in Duncan

1959). In the present investigation the number of chromosomes was reexamined to be $2n=20$ (Fig. 15 B-F). The measurements of chromosome length and arm ratio are shown in Table 15.

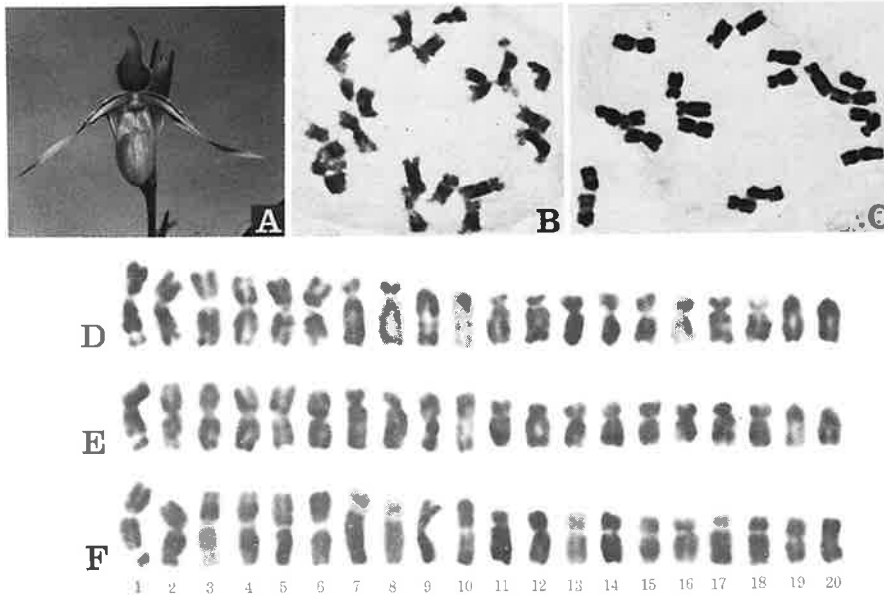


Fig. 15. *Phragmipedium hartwegii*, $2n=20$. A, flower. B, prometaphase. C-F, metaphase. A, $\times 0.5$. B, $\times 1200$. C, $\times 1000$. D-F, $\times 1800$.

The 20 chromosomes (Nos. 1-20) ranged from 5.4 to $3.4\mu\text{m}$ in length, and decreased gradually in size. The two longest chromosomes (Nos. 1, 2) had the satellites at the distal region of the long arms. The short arms of these chromosomes were $2.4\mu\text{m}$ and the long arms were $3.0\mu\text{m}(2.3+0.7\mu\text{m})$ in length. Arm ratio of these chromosomes were 1.2, and the position of centromeres were median. Two chromosomes (Nos. 3, 4) were both $4.9\mu\text{m}$ in length. Arm ratio of these chromosomes were both 1.2, and the position of centromeres were median. These two chromosomes had small constrictions near the middle region of the short and long arms. Two chromosomes (Nos. 5, 6) were both $4.9\mu\text{m}$ in length. Arm ratio of these chromosomes were both 1.1, and the position of centromeres were median. Two chromosomes (Nos. 7, 8) were $4.8\mu\text{m}$ and $4.7\mu\text{m}$ in length, respectively. Arm ratio of these chromosomes were 3.0 and 2.9, and the position of centromeres were submedian. These chromosomes had the small constrictions near the end of their long arms. The 9th and 10th chromosomes were both $4.4\mu\text{m}$ in length, and the position of centromeres were terminal. These chromosomes had the small constrictions near the middle region of the long arms. Two chromosomes (Nos. 11, 12) were both $3.8\mu\text{m}$ in length. Arm ratio of these chromosomes were 2.8, and the position of centromeres were submedian. Four chromosomes (Nos. 13-16) ranged from 3.7 to $3.5\mu\text{m}$ in length. Arm ratio of these chro-

mosomes varied from 1.6 to 1.7, and the position of centromeres were median. Two chromosomes (Nos. 17, 18) were both $3.5\mu\text{m}$ in length. Arm ratio of these chromosomes were 2.2, and the position of centromeres were submedian. The rest two chromosomes (Nos. 19, 20) were $3.5\mu\text{m}$ and $3.4\mu\text{m}$ in length, respectively, and the position of centromeres were terminal. These chromosomes had the small constrictions near the middle region of the long arms.

The chromosome complement of this species consisted of 10 pairs of morphologically matched chromosomes which decreased gradually in size. The longest pair of matched chromosomes had the satellites at the distal region of the long arms. Four chromosomes (Nos. 9, 10, 19 and 20) were telocentric chromosomes.

4) *Phragmipedium longifolium* (Rchb. f. & Warsc.) Rolfe, $2n=23$ ($=20+3f$)
Varidated specimen No. 1241, I-6.

The distribution of this species is recorded in Costa Rica, Panama, Colombia and Ecuador. External characteristics of this species are as follows: Leaves are linear lanceolate and are 40-60cm in length. The peduncle is 50-60cm in length. Natural spread of the flower is 7-10cm in width and its color is green or brownish-pinked green. The dorsal sepal is lanceolate and its edge curls toward outside. The petals are linear lanceolate and twistingly hang down slant. The upper edge of staminode has thick black hairs (Fig. 16A).

The interphase nuclei were similar to those of above species showing the complex chromocenter type (Fig. 16B). Prophase chromosomes were the proximal type (Fig. 16C). At metaphase, $2n=23$ chromosomes were counted in two clones (Fig. 16D, E). The measurements of chromosome length and arm ratio are shown in Table 16.

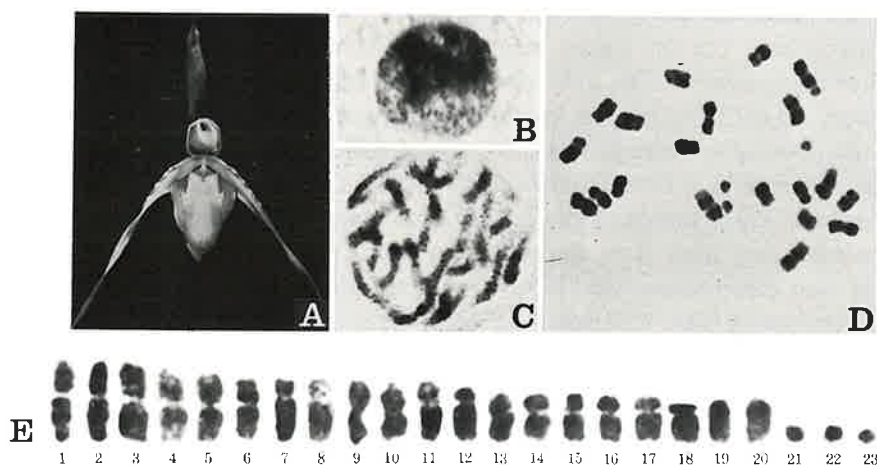


Fig. 16. *Phragmipedium longifolium*, $2n=23$. A, flower. B, interphase nucleus. C, prophase. D and E, metaphase. A, $\times 0.3$. B-D, $\times 900$. E, $\times 1800$.

Among the 23 chromosomes, 20 chromosomes ranged from 5.4 to 3.0 μm in length, and decreased gradually in size. The two longest chromosomes (Nos. 1, 2) had the satellites at the distal region of the long arms. The short arms of them were 2.3 μm and the long arms were 3.1 μm (2.1+1.0 μm) in length. Arm ratio of these chromosomes were 1.3, and the position of centromeres were median. Four chromosomes (Nos. 3-6) ranged from 5.0 to 4.8 μm in length, respectively. Arm ratio of these chromosomes were 1.2, and the position of centromeres were median. Two chromosomes (Nos. 3, 4) had the small constrictions near the middle region of the short and long arms. Two chromosomes (Nos. 7, 8) were both 4.4 μm in length. Arm ratio of these chromosomes were 3.0, and the position of centromeres were submedian. Two chromosomes (Nos. 9, 10) were both 4.1 μm in length, and the position of centromeres were terminal. These chromosomes had the small constrictions near the middle region of long arms. Two chromosomes (Nos. 11, 12) were 4.0 and 3.9 μm in length, respectively. Arm ratio of these chromosomes were 2.1 and 2.3, and the position of centromeres were submedian. Two chromosomes (Nos. 13, 14) were both 3.5 μm in length. Arm ratio of these chromosomes were 1.7, and the position of centromeres were median. Four chromosomes (Nos. 15-18) ranged from 3.2 to 3.0 μm in length. Arm ratio of them varied from 1.9 to 2.3, and the position of centromeres were submedian. Two chromosomes (Nos. 19, 20) were both 3.0 μm in length, and the position of centromeres were terminal. The remaining three chromosomes (Nos. 21-23) ranged from 1.5 to 1.4 μm in length, and the position of centromeres were terminal.

As mentioned above, the 23 chromosomes of this species consisted of 20 chromosomes which decreased in descending order of size and three minute chromosomes. Among the 23 chromosomes, 20 chromosomes (Nos. 1-20) of this species were similar to those of *P. hartwegii*. The difference between the chromosome complement of this species and that of *P. hartwegii* was the presence of three minute chromosomes in this species.

5) *Phragmipedium longifolium* (Rchb. f. & Warsc.) Rolfe var. *gracile* Rolfe,
2n=21 (=20+1f)

Varidated specimen No. 3015.

The specimen was obtained from Rands Orchids. The leaves of this species are more narrow and longer than those of above *P. longifolium*.

The interphase nuclei were similar to those of above species showing the complex chromocenter type. Prophase chromosomes were the proximal type. At prometaphase and metaphase, 2n=21 chromosomes were counted in one clone (Fig. 17A-C). The measurements of chromosome length and arm ratio are shown in Table 17.

Among the 21 chromosomes, 20 chromosomes (Nos. 1-20) ranged from 5.5 to 3.7 μm in length, and decreased gradually in size. The 1st and 2nd chromosomes were both 5.5 μm in length, and had the satellites at the distal region of the long arms. The short arms of their chromosomes were both 2.3 μm and the long arms were both 3.2 μm (2.3+0.9 μm) in

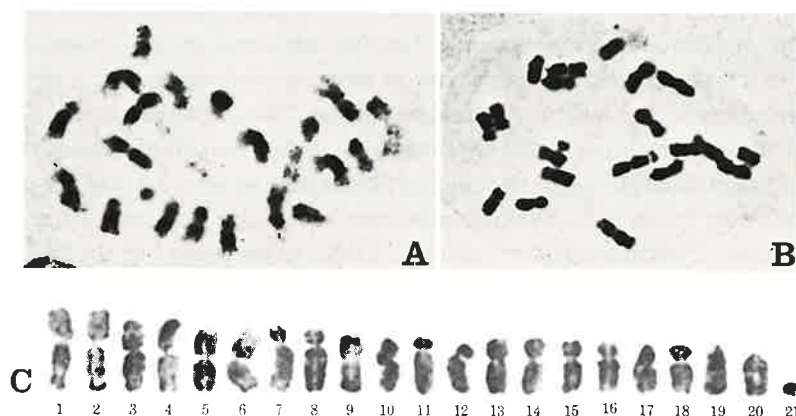


Fig. 17. *Phragmipedium longifolium* var. *gracile*, $2n=21$. A, prometaphase. B and C, metaphase. A and B, $\times 1200$. C, $\times 1800$.

length. Arm ratio of these chromosomes were 1.4, and the position of centromeres were median. Two chromosomes (Nos. 3, 4) were both $5.3\mu\text{m}$ in length. Arm ratio of these chromosomes were both 1.3, and the position of centromeres were median. These two chromosomes had the small constrictions near the middle region of each short and long arms. Two chromosomes (Nos. 5, 6) were both $4.7\mu\text{m}$ in length. Arm ratio of these chromosomes were both 1.1, and the position of centromeres were median. Two chromosomes (Nos. 7, 8) were both $4.5\mu\text{m}$ in length. Arm ratio of these chromosomes were both 2.8, and the position of centromeres were submedian. Two chromosomes (Nos. 9, 10) were both $4.3\mu\text{m}$ in length, and the position of centromeres were terminal. These two chromosomes had the small constrictions near the middle region of the long arms. Two chromosomes (Nos. 11, 12) were both $4.2\mu\text{m}$ in length. Arm ratio of these chromosomes were both 2.8, and the position of centromeres were submedian. Two chromosomes (Nos. 13, 14) were both $4.1\mu\text{m}$ in length. Arm ratio of these chromosomes were both 1.6, and the position of centromeres were median. Four chromosomes (Nos. 15-18) ranged from $3.8\mu\text{m}$ to $3.7\mu\text{m}$ in length. Arm ratio of these chromosomes ranged from 1.9 to 1.8, and the position of centromeres were submedian. Two chromosomes (Nos. 19, 20) were both $3.7\mu\text{m}$ in length, and the position of centromeres were terminal. The 21th chromosome was $1.0\mu\text{m}$ in length, and the position of centromere was terminal.

As mentioned above, among the 21 chromosomes 20 chromosomes (Nos. 1-20) of this species were similar to those of *P. hartwegii*. The difference between the chromosome complement of this species and that of *P. hartwegii* was the presence of minute chromosome in this species.

6) *Phragmipedium roezlii* (Rchb. f.) Garay, $2n=22$ ($=20+2f$)

Varidated specimen No. 3017, BG-41.

This species distributes in Costa Rica, Panama and Colombia. Compared with *P. longifolium*, the shape of this species is closely similar but a little small. Petals are short and spread horizontally. Color of dorsal sepal and petals are light yellowish green with red veins. The staminode is cordate and its upper edge has thick black hairs. (Fig. 18A).

The interphase nuclei were similar to those of above species showing the complex chromocenter type (Fig. 18B). Prophase chromosomes were the proximal type (Fig. 18C). At metaphase, $2n=22$ chromosomes were ocunted in two clones (Fig. 18D, E). The measurements of chromosome length and arm ratio are shown in Table 18.

Among the 22 chromosomes, 20 chromosomes (Nos. 1-20) ranged from 5.2 to $2.9\mu\text{m}$ in length, and decreased gradually in size. Two chromosomes (Nos. 1, 2) had the satellites at the distal region of long arms. These chromosomes were both $5.2\mu\text{m}$ in length. The short arms of their chromosomes were $2.2\mu\text{m}$ and the long arms were $3.0\mu\text{m}$ ($2.1+0.9\mu\text{m}$) in length. Arm ratio of their chromosomes were 1.4, and the position of centromeres were median. Two chromosomes (Nos. 3, 4) were both $5.1\mu\text{m}$ in length. Arm ratio of these chromosomes were both 1.1, and the position of centromeres were median. Two chromosomes (Nos. 5, 6) were $5.0\mu\text{m}$ and $4.5\mu\text{m}$ in length, respectively. Arm ratio of these chromosomes were 1.1 and 1.0, and the position of centromeres were median. Two chro-

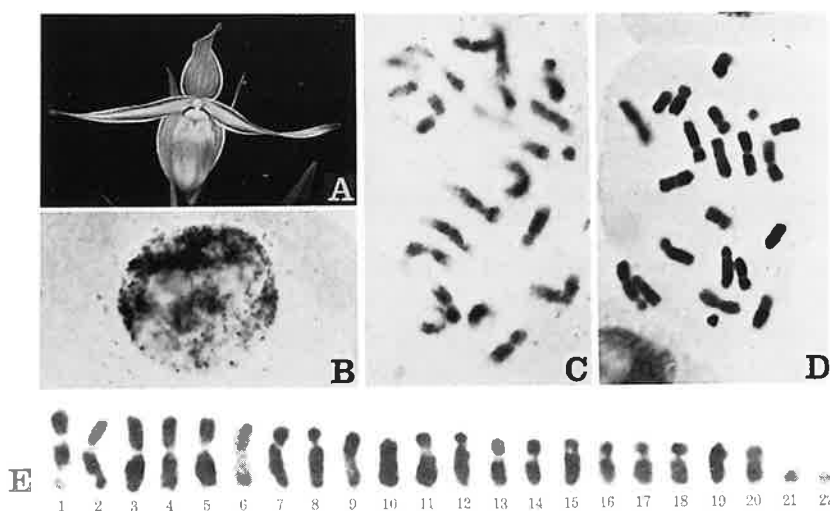


Fig. 18. *Phragmipedium roezlii*, $2n=22$. A, flower. B, interphase nucleus. C, prophase. D and E, metaphase. A, $\times 0.5$. B-D, $\times 1200$. E, $\times 1800$.

mosomes (Nos. 7, 8) were 4.5 μ m and 4.3 μ m in length, respectively. Arm ratio of these chromosomes were 2.5 and 2.6, respectively, and the position of centromeres were submedian. Two chromosomes (Nos. 9, 10) were 4.3 μ m and 4.1 μ m in length, respectively, and the position of centromeres were terminal. Two chromosomes (Nos. 11, 12) were both 3.9 μ m in length. Arm ratio of these chromosomes 2.5 and 2.9, respectively, and the position of centromeres were submedian. Two chromosomes (Nos. 13, 14) were both 3.4 μ m in length. Arm ratio of these chromosomes were both 1.4, and the position of centromeres were median. Four chromosomes (Nos. 15-18) ranged from 3.2 to 3.0 μ m in length. Arm ratio of these chromosomes were 2.2, and 2.0, respectively, and the position of centromeres were submedian. Two chromosomes (Nos. 19, 20) were 3.0 μ m and 2.9 μ m in length, respectively, and the position of centromeres were terminal. The rest two chromosomes (Nos. 21, 22) were 1.1 μ m and 1.0 μ m in length, respectively, and the position of centromeres were terminal.

As mentioned above, among the 22 chromosomes, 20 chromosomes (Nos. 1-20) of this species were similar to those of *P. hartwegii*, and the presence of two minute chromosomes was the characteristic of this species.

Discussion

I. Karyomorphological characteristics of *Phragmipedium*

All interphase nuclei of 15 taxa of *Phragmipedium* investigated were categorized into the complex chromocenter type (cf. Tanaka 1971). This is the same as the taxa of *Paphiopedilum* (Karasawa 1979), but the prophase chromosomes showing the proximal type (cf. Tanaka 1971) clearly differ from those of *Paphiopedilum* showing the interstitial type (Karasawa 1979).

Small constrictions were observed in certain chromosomes at prometaphase in all species of *Phragmipedium*. These small constrictions were more general and clear than those of *Paphiopedilum* (Karasawa 1979). In addition, two satellite chromosomes were observed in 14 taxa, and three satellite chromosomes were observed in one taxa investigated. These satellites were almost same size in 15 taxa, and existed in the long arms of metacentric chromosomes except for *P. schlimii* and *P. x stenophyllum* having in telocentric chromosomes.

The chromosome numbers of $2n=18$ and $2n=20$ in *Phragmipedium* were reexamined and those of $2n=21$, $2n=22$, $2n=28$, $2n=30$, $2n=20+1f$, $2n=20+2f$, $2n=20+3f$ and $2n=40$ were newly added. The $2n=32$ in *P. caudatum* by Hoffman (1929) was corrected as $2n=28$.

The chromosome complements of *P. boissierianum* and *P. vittatum* with $2n=18$ consisted of nine pairs of matched chromosomes with median, submedian and subterminal centromere, while those of the species with over $2n=20$ had several chromosomes with terminal centromere besides on above three types (Fig. 19).

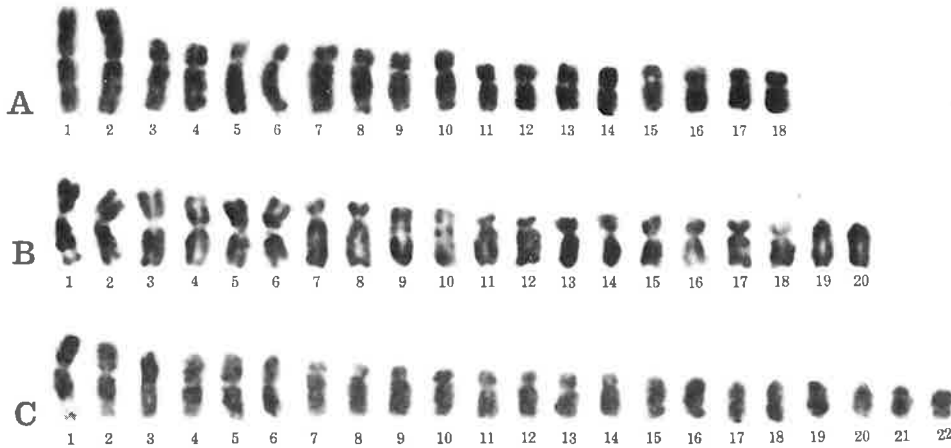


Fig. 19. Metaphase chromosomes of three species of *Phragmipedium*. A, *P. boisseranum* $2n=18$. B, *P. hartwegii* $2n=20$. C, *P. ecuadorensis* $2n=22$. $\times 1800$.

When we express the metacentric, submetacentric and subtelocentric chromosome as 'V' and the telocentric chromosome as 'I', the karyotype of each species can be shown as $2n=18=18V$ in *P. boisseranum* and *P. vittatum*, as $2n=20=16V+4I$ in *P. hartwegii*, as $2n=22=14V+8I$ in *P. ecuadorensis*, as $2n=28=8V+20I$ in *P. caudatum*, *P. wallisii* and *P. lindenii*, as $2n=30=6V+24I$ in *P. schlimii*. By this method of karyotype expression, it is found that the increase of 'I' chromosome originates from the decrease of 'V' chromosome. When we assume that two 'I' chromosomes derive from one 'V' chromosome by centric fission, $2n=20=16V+4I$ in *P. hartwegii* is converted into $2n=18V$. Similarly, $2n=22=14V+8I$ in *P. ecuadorensis* is converted into $2n=18V$, $2n=28=8V+20I$ in *P. caudatum*, *P. wallisii* and *P. lindenii* into $2n=18V$ and $2n=30=6V+24I$ in *P. schlimii* into $2n=18V$, while $2n=22=12V+10I$ in *P. lindleyanum* and *P. sargentianum* of section *Platypetalum* is converted into $2n=17V$. This may suggest the presence of the difference basic karyotype in *Phragmipedium*. However, the species with the karyotype of $2n=17=17V$ has not been found. Therefore, we assume that the karyotype of $2n=17V$ is derived not from the different basic karyotype but from the loss of telocentric chromosomes after centric fission, like *Paphiopedilum* (Karasawa 1979).

P. longifolium var. *gracile* ($2n=20+1f$), *P. roezlii* ($2n=20+2f$) and *P. longifolium* ($2n=20+3f$) had small fragment chromosomes of different number, while the morphology of rest 20 chromosomes were almost similar to those of *P. hartwegii* ($2n=20$). Consequently, the karyomorphological difference among them was due to the number of fragment chromosomes.

II. Cytotaxonomical comparisons in the species of *Phragmipedium*

The genus *Phragmipedium* had been classified into three subgenus and 11 species by Brieger (1971), while it has been classified into five sections, 12 species and one variety by Fowlie (1970). Recently Garay (1979) rearranged into five sections and 21 species. In the present study, karyomorphological relationships in this genus will be discussed according to Garay's classification.

(1) MICROPETALUM

P. schlimii is only one species in this section. The sepal and petals of this species are oval and their top are round. The chromosome number of $2n=30$ is the largest in this genus except for *P. x stenophyllum*, and the complement consists of two large metacentric chromosomes and 28 small chromosomes which are divided into four metacentric and 24 telocentric chromosomes. Thus, the karyotype of this species can be expressed as $2n=30=6V+24I$, and it could be converted into $2n=18V$. Satellites being at the distal region of the telocentric chromosomes are characteristics of this species.

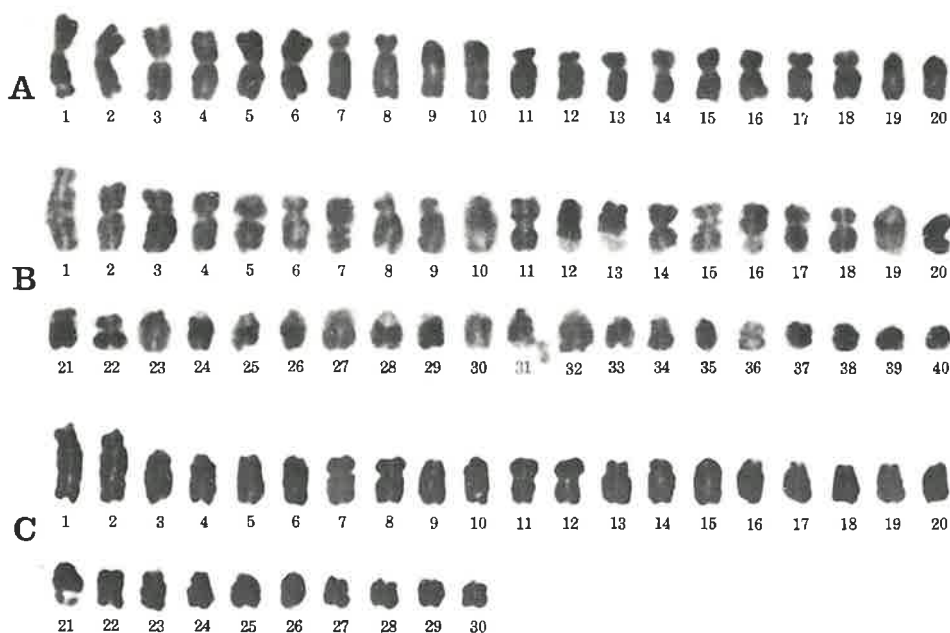


Fig. 20. Metaphase chromosomes of three species of *Phragmipedium*. A, *P. hartwegii* $2n=20$. B, *P. x stenophyllum* $2n=40$. C, *P. schlimii* $2n=30$. $\times 1800$.

The large-sized species which has been recognized as *P. x stenophyllum* by Garay (1979) is bigger than *P. schlimii*. The karyomorphological characteristics are as follows; Figure 20 shows the metaphase chromosomes of *P. hartwegii* ($2n=20$), *P. x stenophyllum* ($2n=40$) and *P. schlimii* ($2n=30$). Among the 40 chromosomes of *P. x stenophyllum*, 24 chromosomes (Nos. 17-40) were similar to the 24 chromosomes (Nos. 7-30) of *P. schlimii*. Six chromosomes (Nos. 1, 3, 9, 10, 12 and 13) in the other 16 chromosomes were similar to the six chromosomes (Nos. 1-6) of *P. schlimii* and the rest 10 chromosomes which consisted of one metacentric chromosome with satellite (No. 2), seven metacentric chromosomes (Nos. 4, 5, 6, 8, 11, 14 and 15) and two telocentric chromosomes (Nos. 7, 16) were similar to a half set of the complement of *P. hartwegii* ($2n=20$).

Namely the chromosome complement of *P. x stenophyllum* might be consist of one set of the complement of *P. schlimii* and a half set of the complement of *P. hartwegii* or another $2n=20$ species. Thus we assume that *P. x stenophyllum* is complex triploid as same as hybrid of *Paphiopedilum* (Atwood 1980).

(2) PLATIPETALUM

The species of section Platipetalum have an oval dorsal sepal, and petals are not sharp and longer than dorsal sepal. Two species of this section, *P. lindleyanum* and *P. sargentianum*, had the same chromosome number of $2n=22$, and their karyotype were similar to each other.

The complements of these species consisted of 11 pairs of matched chromosomes, and could be divided into two groups showing a bimodal karyotype. The karyotypes of these species can be expressed as $2n=22=12V+10I$ and can be converted into $2n=17V$. This is the characteristic of this section and differ from other sections.

(3) PHRAGMIPEDIUM

The ribbon-type petals are morphological characteristic of the species of this section. Fowlie (1970) recognized one species and one variety in this section; *P. caudatum* and *P. caudatum* var. *lindenii*, while Garay (1979) recognized four species by the difference of staminode; *P. caudatum*, *P. wallisii*, *P. warscewizii* and *P. lindenii*.

The chromosome numbers of three species, *P. caudatum*, *P. wallisii* and *P. lindenii*, were all $2n=28$, and the karyotype of these species were similar to each other. Therefore, we could not distinguish karyomorphologically among them.

The karyotype of these species could be expressed as $2n=28=8V+20 I$ and could be converted into $2n=18V$.

(4) HIMANTOPETALUM

The difference between Himantopetalum and following Lorifolia is the shape of lip. The opening of lip of this section had not a pair of hornlike protuberances on side.

The karyotype of *P. ecuadorensis* $2n=22$ are extremely similar to that of *P. hartwegii* $2n=20$ which is included section Lorifolia. The karyotype of *P. ecuadorensis* can be expressed as $2n=22=14V+8I$ and can be converted into $2n=18V$.

Two types of *P. pearcei* which were presented by Garay (1979) were different from each other in the color and the shape of flowers. Chromosome numbers of these types were $2n=20$, 21 and 22, respectively, and the chromosome complements of them consisted of heterogenous pairs suggesting hybrids.

Figure 21 shows the metaphase chromosomes of *P. boissierianum* ($2n=18$), *P. pearcei* Type 1 ($2n=20$) and *P. ecuadorensis* ($2n=22$). The largest chromosome (No. 1) and the satellited chromosome (No. 5) of *P. pearcei* Type 1 were similar to the largest chromosome (No. 1 or 2) and satellited chromosome (No. 7 or 8) of *P. boissierianum*, while the meta-centric chromosome with satellite (No. 2) and small telocentric chromosomes (Nos. 7, 8, 9 and 20) of *P. pearcei* Type 1 were similar to those of No. 1 or 2 and No. 15 or 16, 17 or 18, 19 or 20 and 21 or 22 of *P. ecuadorensis* ($2n=22$).

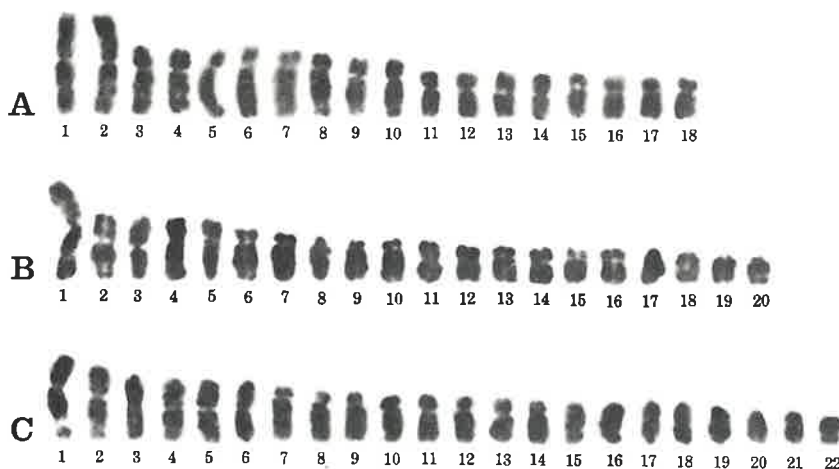


Fig. 21. Metaphase chromosomes of three species of *Phragmipedium*. A, *P. boissierianum* $2n=18$. B, *P. pearcei* Type 1 $2n=20$. C, *P. ecuadorensis* $2n=22$. $\times 1800$.

Namely, the chromosome complement of *P. pearcei* Type 1 might consisted of a half set of the complement of both *P. boissierianum* and *P. ecuadorensis*. Thus we assume that *P. pearcei* Type 1 is F1 hybrid between *P. boissierianum* and *P. ecuadorensis*.

The chromosome complements of *P. pearcei* Type 2 ($2n=21$) and Type 3 ($2n=22$) also showed heterogenous karyotype. These two types did not have any large metacentric chromosomes but had medium-sized metacentric chromosomes with satellite and four or five small telocentric chromosomes. According to their karyomorphological characteris-

tics, we assume that these two types are hybrids originated to the $2n=20$ species (e. g. *P. hartwegii*) and the $2n=22$ species (e. g. *P. ecuadorensis*).

Thus the species which is classified as *P. pearcei* may be hybrid group.

(5) LORIFOLIA

The morphological characteristic of this section is the opening of lip with a pair of horn-like protuberances on side.

P. boisseranum $2n=18$ and *P. vittatum* $2n=18$ which have the lowest chromosome number in this genus are included in this section. The complement of *P. boisseranum* consisted of nine pairs of matched chromosomes, and could be divided into three groups showing a trimodal karyotype: The first consisted of two large chromosomes and the second eight medium-sized chromosomes and the third eight small chromosomes. The position of centromeres were median, submedian and subterminal (Fig. 13E).

The karyotype of *P. vittatum* $2n=18$ showed a trimodal karyotype similar to *P. boisseranum*, except for centromeric position of each chromosome.

The complement of *P. hartwegii* $2n=20$ consisted of 16 metacentric and four telocentric chromosomes, and showed a gradient karyotype (Fig. 15D-F). The karyotype of this species can be expressed as $2n=20=16V+4I$ and can be converted into $2n=18V$, showing a trimodal karyotype.

Three specimen, *P. longifolium* $2n=20+3f$, *P. roezlii* $2n=20+2f$ and *P. longifolium* var. *gracile* $2n=20+1f$, are very similar to *P. hartwegii* $2n=20$ in external morphology, and their karyotype of 20 chromosomes (Nos. 1-20) except for fragment chromosomes were also similar.

As a conclusion of karyomorphological analysis in the *Phragmipedium*, each distinct karyotype is found to be useful for not only the validation species but also sectioning of genus. Especially section Himantopetalum and Lorifolia are closely related in the morphological characteristics. Thus, the phylogenetic differentiation of *Phragmipedium* can be considered as follow: The species with the chromosome number of $2n=18$ might be the most primitive. Karyomorphological differentiation in this genus might be led mainly by centric fission of chromosomes and partly by certain structural changes, e. g. chromosome loss.

Summary

1. Karyomorphological investigations were carried out in 15 taxa of *Phragmipedium*.
2. The chromosome numbers of 15 taxa investigated are as follows: *P. schlimii* $2n=30$, *P. x stenophyllum* $2n=40$, *P. lindleyanum* $2n=22$, *P. sargentianum* $2n=22$, *P. wallisii* $2n=28$, *P. lindenii* $2n=28$, *P. ecuadorensis* $2n=22$, *P. pearcei* $2n=20, 21, 22$, *P. vittatum* $2n=18$, *P. longifolium* $2n=23 (=20+3f)$, *P. longifolium* var. *gracile* $2n=21 (=20+1f)$ and

- P. roezlii* $2n=22$ ($=20+2f$); (new count), *P. caudatum* $2n=28$; (revised count), *P. boisseranum* $2n=18$ and *P. hartwegii* $2n=20$; (confirmed count).
3. *P. boisseranum* and *P. vittatum* with the lowest chromosome numbers of $2n=18$ showed trimodal karyotype: The first group consisted of two large metacentric chromosomes and the second eight medium-sized metacentric chromosomes and the third eight small metacentric chromosomes.
 4. The species with the chromosome number $2n=20, 22, 28$ and 30 including sect. Micropetalum, Phragmipedium, Himantopetalum and Lorifolia, showed the decrease of metacentric chromosomes and the increase of telocentric chromosomes. It might be caused directly by centric fission on a pair of metacentric chromosomes.
 5. Converting every two telocentric chromosomes into one metacentric chromosome, the chromosome numbers of the species of section Micropetalum, Phragmipedium, Himantopetalum and Lorifolia, can be connected to $2n=18$, while the chromosome number of $2n=22$ of the species of section Platypetalum can be connected to $2n=17$. It is assumed that the species with the chromosome number of $2n=22$ in section Platypetalum is derived from the loss of telocentric chromosomes after centric fission.
 6. The chromosome numbers of three types of *P. pearcei*, section Himantopetalum, of which color and shape of flowers were slightly different were examined as $2n=20, 21$ and 22 , respectively. These types were considered to be hybrids because the karyotypes of their chromosomes were heterogenous. By the comparison of karyotype among three types, it was found that the $2n=20$ type and the $2n=21, 22$ types were derived from different parental species.
 7. *P. x stenophyllum* ($2n=40$) was considered to be the complex triploid originated from the 30 chromosomes of *P. schilimii* ($2n=30$) and the 10 chromosomes of *P. hartwegii* ($2n=20$).
 8. Fragment chromosomes were observed in *P. longifolium*, *P. longifolium* var. *gracile* and *P. roezlii* including section Lorifolia. The 20 chromosomes except for fragment chromosomes of these three taxa were very similar to those of *P. hartwegii*.
 9. All species of *Phragmipedium* showed distinct karyotypes, and section Himantopetalum was resemble to section Lorifolia, karyomorphologically.
 10. The phylogenetic differentiation of *Phragmipedium* was considered as follows: The species with $2n=18$ might be the most primitive. Karyomorphological differentiation in this genus might be led mainly by centric fission of chromosomes and partly by certain structural changes, e. g. chromosome loss.

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Table 2. Measurements of somatic chromosomes of *Phragmipedium schlimii* at metaphase, $2n=30$

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	$1.3+4.5= 5.8$	6.0	3.5	s t
2	$1.3+3.9= 5.2$	5.4	3.0	sm
3	$d+4.0= 4.0$	4.1	$<\infty$	t
4	$d+3.7= 3.7$	3.8	$<\infty$	t
5	$d+3.6= 3.6$	3.7	$<\infty$	t
6	$d+3.6= 3.6$	3.7	$<\infty$	t
7	$1.2+2.3= 3.5$	3.6	1.9	sm
8	$1.2+2.3= 3.5$	3.6	1.9	sm
9	$d+3.4= 3.4$	3.5	$<\infty$	t
10	$d+3.4= 3.4$	3.5	$<\infty$	t
11	$1.3+2.0= 3.3$	3.4	1.5	m
12	$1.3+2.0= 3.3$	3.4	1.5	m
13	$d+3.3= 3.3$	3.4	$<\infty$	t
14	$d+3.3= 3.3$	3.4	$<\infty$	t
15	$d+3.3= 3.3$	3.4	$<\infty$	t
16	$d+3.3= 3.3$	3.4	$<\infty$	t
17	$d+3.3= 3.3$	3.4	$<\infty$	t
18	$d+3.0= 3.0$	3.1	$<\infty$	t
19	$d+2.9= 2.9$	3.0	$<\infty$	t
20	$d+2.9= 2.9$	3.0	$<\infty$	t
21	$d+2.0+0.9=2.9*$	3.0	$<\infty$	t
22	$d+2.0+0.9=2.9*$	3.0	$<\infty$	t
23	$d+2.9= 2.9$	3.0	$<\infty$	t
24	$d+2.9= 2.9$	3.0	$<\infty$	t
25	$d+2.7= 2.7$	2.8	$<\infty$	t
26	$d+2.5= 2.5$	2.6	$<\infty$	t
27	$d+2.3= 2.3$	2.4	$<\infty$	t
28	$d+2.1= 2.1$	2.2	$<\infty$	t
29	$d+2.1= 2.1$	2.2	$<\infty$	t
30	$d+2.1= 2.1$	2.2	$<\infty$	t

* Chromosome with secondary constriction

d : dot

Table 3. Measurements of somatic chromosomes of *Phragmipedium*
× stenophyllum at metaphase, $2n=40$

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	$1.4+4.6= 6.0$	4.4	3.3	s t
2	$2.1+2.3+0.7=5.1 *$	3.8	1.4	m
3	$1.3+3.6= 4.9$	3.7	2.9	sm
4	$1.9+2.4= 4.3$	3.2	1.3	m
5	$1.8+2.3= 4.1$	3.0	1.3	m
6	$1.2+2.9= 4.1$	3.0	2.4	sm
7	$d+4.0= 4.0$	3.0	$<\infty$	t
8	$1.3+2.7= 4.0$	3.0	2.1	sm
9	$d+4.0= 4.0$	3.0	$<\infty$	t
10	$d+4.0= 4.0$	3.0	$<\infty$	t
11	$1.8+2.2= 4.0$	3.0	1.2	m
12	$d+3.9= 3.9$	2.9	$<\infty$	t
13	$d+3.9= 3.9$	2.9	$<\infty$	t
14	$1.5+2.1= 3.6$	2.7	1.4	m
15	$1.2+2.4= 3.6$	2.7	2.0	sm
16	$d+3.6= 3.6$	2.7	$<\infty$	t
17	$1.2+2.3= 3.5$	2.6	1.9	sm
18	$1.2+2.3= 3.5$	2.6	1.9	sm
19	$d+3.4= 3.4$	2.5	$<\infty$	t
20	$d+3.3= 3.3$	2.4	$<\infty$	t
21	$1.3+2.0= 3.3$	2.4	1.5	m
22	$1.3+2.0= 3.3$	2.4	1.5	m
23	$d+3.0= 3.0$	2.2	$<\infty$	t
24	$d+3.0= 3.0$	2.2	$<\infty$	t
25	$d+2.9= 2.9$	2.1	$<\infty$	t
26	$d+2.9= 2.9$	2.1	$<\infty$	t
27	$d+2.9= 2.9$	2.1	$<\infty$	t
28	$d+2.9= 2.9$	2.1	$<\infty$	t
29	$d+2.8= 2.8$	2.1	$<\infty$	t
30	$d+2.8= 2.8$	2.1	$<\infty$	t
31	$d+2.0+0.8=2.8 *$	2.1	$<\infty$	t
32	$d+2.0+0.8=2.8 *$	2.1	$<\infty$	t
33	$d+2.8= 2.8$	2.1	$<\infty$	t
34	$d+2.8= 2.8$	2.1	$<\infty$	t
35	$d+2.5= 2.5$	1.8	$<\infty$	t
36	$d+2.5= 2.5$	1.8	$<\infty$	t
37	$d+2.2= 2.2$	1.6	$<\infty$	t
38	$d+2.2= 2.2$	1.6	$<\infty$	t
39	$d+2.0= 2.0$	1.5	$<\infty$	t
40	$d+2.0= 2.0$	1.5	$<\infty$	t

* Chromosome with secondary constriction
d : dot

Table 4. Measurements of somatic chromosomes of *Phragmipedium lindleyanum* at metaphase, $2n=22$

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	$2.5+3.2= 5.7$	6.6	1.3	m
2	$2.5+3.2= 5.7$	6.6	1.3	m
3	$2.4+1.8+1.1=5.3 *$	6.2	1.2	m
4	$2.4+1.8+1.1=5.3 *$	6.2	1.2	m
5	$1.3+3.9= 5.2$	6.1	3.0	sm
6	$1.3+3.9= 5.2$	6.1	3.0	sm
7	$2.4+2.8= 5.2$	6.1	1.2	m
8	$2.4+2.8= 5.2$	6.1	1.2	m
9	$1.3+2.9= 4.2$	4.9	2.2	sm
10	$1.3+2.9= 4.2$	4.9	2.2	sm
11	$d+3.4= 3.4$	4.0	$<\infty$	t
12	$d+3.1= 3.1$	3.6	$<\infty$	t
13	$1.2+1.9= 3.1$	3.6	1.6	m
14	$1.2+1.9= 3.1$	3.6	1.6	m
15	$d+2.9= 2.9$	3.4	$<\infty$	t
16	$d+2.9= 2.9$	3.4	$<\infty$	t
17	$d+2.8= 2.8$	3.3	$<\infty$	t
18	$d+2.8= 2.8$	3.3	$<\infty$	t
19	$d+2.7= 2.7$	3.1	$<\infty$	t
20	$d+2.7= 2.7$	3.1	$<\infty$	t
21	$d+2.6= 2.6$	3.0	$<\infty$	t
22	$d+2.6= 2.6$	3.0	$<\infty$	t

* Chromosome with secondary constriction

d : dot

Table 5. Measurements of somatic chromosomes of *Phragmipedium sargentianum* at metaphase, $2n=22$

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	$2.6+3.3= 5.9$	6.8	1.3	m
2	$2.6+3.3= 5.9$	6.8	1.3	m
3	$2.2+2.2+1.0=5.4 *$	6.2	1.5	m
4	$2.2+2.2+1.0=5.4 *$	6.2	1.5	m
5	$1.4+4.0= 5.4$	6.2	2.9	sm
6	$1.4+4.0= 5.4$	6.2	2.9	sm
7	$2.3+2.5= 4.8$	5.5	1.1	m
8	$2.3+2.5= 4.8$	5.5	1.1	m

Table 5. (continued)

9	1.5+3.1= 4.6	5.3	2.1	sm
10	1.5+3.1= 4.6	5.3	2.1	sm
11	d+3.5= 3.5	4.0	<∞	t
12	d+3.2= 3.2	3.7	<∞	t
13	1.2+1.7= 2.9	3.3	1.4	m
14	1.2+1.7= 2.9	3.3	1.4	m
15	d+2.9= 2.9	3.3	<∞	t
16	d+2.9= 2.9	3.3	<∞	t
17	d+2.8= 2.8	3.2	<∞	t
18	d+2.8= 2.8	3.2	<∞	t
19	d+2.8= 2.8	3.2	<∞	t
20	d+2.8= 2.8	3.2	<∞	t
21	d+2.5= 2.5	2.9	<∞	t
22	d+2.4= 2.4	2.8	<∞	t

* Chromosome with secondary constriction
d: dot

Table 6. Measurements of somatic chromosomes of *Phragmipedium caudatum* at metaphase, 2n=28

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	3.4+2.9+0.7=7.0 *	6.9	1.1	m
2	3.4+2.9+0.7=7.0 *	6.9	1.1	m
3	d+4.5= 4.5	4.5	<∞	t
4	d+4.3= 4.3	4.3	<∞	t
5	d+4.2= 4.2	4.2	<∞	t
6	d+4.0= 4.0	4.0	<∞	t
7	d+3.9= 3.9	3.9	<∞	t
8	d+3.9= 3.9	3.9	<∞	t
9	d+3.8= 3.8	3.8	<∞	t
10	d+3.7= 3.7	3.7	<∞	t
11	0.7+2.8= 3.5	3.5	4.0	st
12	0.7+2.8= 3.5	3.5	4.0	st
13	d+3.3= 3.3	3.3	<∞	t
14	d+3.3= 3.3	3.3	<∞	t
15	d+3.3= 3.3	3.3	<∞	t
16	d+3.3= 3.3	3.3	<∞	t
17	d+3.1= 3.1	3.1	<∞	t
18	d+3.1= 3.1	3.1	<∞	t

Table 6. (continued)

19	0.7+2.4=	3.1	3.1	3.4	s t
20	0.7+2.4=	3.1	3.1	3.4	s t
21	d+2.9=	2.9	2.9	<∞	t
22	d+2.9=	2.9	2.9	<∞	t
23	0.9+2.0=	2.9	2.9	2.2	sm
24	0.9+2.0=	2.9	2.9	2.2	sm
25	d+2.7=	2.7	2.7	<∞	t
26	d+2.7=	2.7	2.7	<∞	t
27	d+2.6=	2.6	2.6	<∞	t
28	d+2.5=	2.5	2.5	<∞	t

* Chromosome with secondary constriction

d : dot

Table 7. Measurements of somatic chromosomes of *Phragmipedium wallisii* at metaphase, 2n=28

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	3.0+2.5+1.0=6.5 *	6.5	1.2	m
2	3.0+2.5+1.0=6.5 *	6.5	1.2	m
3	d+4.1=	4.1	<∞	t
4	d+4.1=	4.1	<∞	t
5	d+3.8=	3.8	<∞	t
6	d+3.8=	3.8	<∞	t
7	d+3.8=	3.8	<∞	t
8	d+3.8=	3.8	<∞	t
9	d+3.7=	3.7	<∞	t
10	d+3.7=	3.7	<∞	t
11	0.9+2.8=	3.7	3.1	s t
12	0.9+2.8=	3.7	3.1	s t
13	d+3.5=	3.5	<∞	t
14	d+3.5=	3.5	<∞	t
15	d+3.3=	3.3	<∞	t
16	d+3.3=	3.3	<∞	t
17	d+3.0=	3.0	<∞	t
18	d+3.0=	3.0	<∞	t
19	0.7+2.3=	3.0	3.3	s t
20	0.7+2.3=	3.0	3.3	s t
21	d+3.0=	3.0	<∞	t
22	d+3.0=	3.0	<∞	t

Table 7. (continued)

23	0.9+2.1=	3.0	3.0	2.3	sm
24	0.9+2.1=	3.0	3.0	2.3	sm
25	d+2.9=	2.9	2.9	<∞	t
26	d+2.9=	2.9	2.9	<∞	t
27	d+2.4=	2.4	2.4	<∞	t
28	d+2.3=	2.3	2.3	<∞	t

* Chromosome with secondary constriction

d : dot

Table 8. Measurements of somatic chromosomes of *Phragmipedium lindenii* at metaphase, 2n=28

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	3.0+2.9+0.8=6.7 *	6.4	1.2	m
2	3.0+2.9+0.8=6.7 *	6.4	1.2	m
3	d+4.7=	4.5	<∞	t
4	d+4.5=	4.3	<∞	t
5	d+4.4=	4.2	<∞	t
6	d+4.3=	4.1	<∞	t
7	d+4.0=	3.8	<∞	t
8	d+3.9=	3.7	<∞	t
9	d+3.7=	3.5	<∞	t
10	d+3.7=	3.5	<∞	t
11	0.7+2.9=	3.6	4.1	st
12	0.7+2.9=	3.6	4.1	st
13	d+3.6=	3.4	<∞	t
14	d+3.6=	3.4	<∞	t
15	d+3.6=	3.4	<∞	t
16	d+3.6=	3.4	<∞	t
17	d+3.3=	3.2	<∞	t
18	d+3.3=	3.2	<∞	t
19	0.8+2.5=	3.3	3.1	st
20	0.8+2.5=	3.3	3.1	st
21	d+3.3=	3.2	<∞	t
22	d+3.3=	3.2	<∞	t
23	0.8+2.2=	3.0	2.8	sm
24	0.8+2.2=	3.0	2.8	sm
25	d+2.7=	2.6	<∞	t
26	d+2.7=	2.6	<∞	t

Table 8. (continued)

27	d+2.7= 2.7	2.6	<∞	t
28	d+2.4= 2.4	2.3	<∞	t

* Chromosome with secondary constriction

d : dot

Table 9. Measurements of somatic chromosomes of *Phragmipedium ecuadorensis* at metaphase, 2n=22

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	2.3+2.5+0.8=5.6 *	6.6	1.4	m
2	2.2+2.5+0.8=5.5 *	6.5	1.5	m
3	2.3+2.8= 5.1	6.0	1.2	m
4	2.3+2.8= 5.1	6.0	1.2	m
5	2.3+2.6= 4.9	5.8	1.1	m
6	2.3+2.6= 4.9	5.8	1.1	m
7	1.0+3.3= 4.3	5.1	3.3	st
8	1.0+3.2= 4.2	5.0	3.2	st
9	1.0+2.8= 3.8	4.5	2.8	sm
10	1.0+2.8= 3.8	4.5	2.8	sm
11	0.9+2.6= 3.5	4.2	2.9	sm
12	0.9+2.6= 3.5	4.2	2.9	sm
13	1.3+2.1= 3.4	4.0	1.6	m
14	1.3+2.1= 3.4	4.0	1.6	m
15	d+3.3= 3.3	3.9	<∞	t
16	d+3.3= 3.3	3.9	<∞	t
17	d+3.2= 3.2	3.8	<∞	t
18	d+3.2= 3.2	3.8	<∞	t
19	d+2.7= 2.7	3.2	<∞	t
20	d+2.7= 2.7	3.2	<∞	t
21	d+2.5= 2.5	3.0	<∞	t
22	d+2.4= 2.4	2.8	<∞	t

* Chromosome with secondary constriction

d : dot

Table 10. Measurements of somatic chromosomes of *Phragmipedium pearcei* Type 1. at metaphase, $2n=20$

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	$3.3+3.9= 7.2$	10.9	1.2	m
2	$1.9+1.7+0.8=4.4 *$	6.7	1.3	m
3	$2.1+2.2= 4.3$	6.5	1.0	m
4	$1.3+3.0= 4.3$	6.5	2.3	sm
5	$1.2+2.3+0.8=4.3 *$	6.5	2.6	sm
6	$1.1+2.6= 3.7$	5.6	2.4	sm
7	$0.9+2.6= 3.5$	5.3	2.9	sm
8	$1.3+1.8= 3.1$	4.7	1.4	m
9	$0.8+2.2= 3.0$	4.6	2.8	sm
10	$0.8+2.2= 3.0$	4.6	2.8	sm
11	$1.4+1.5= 2.9$	4.4	1.1	m
12	$1.1+1.6= 2.7$	4.1	1.5	m
13	$0.9+1.8= 2.7$	4.1	2.0	sm
14	$1.2+1.5= 2.7$	4.1	1.3	m
15	$1.1+1.6= 2.7$	4.1	1.5	m
16	$1.0+1.5= 2.5$	3.8	1.5	m
17	$d+2.4= 2.4$	3.6	$<\infty$	t
18	$d+2.3= 2.3$	3.5	$<\infty$	t
19	$d+2.1= 2.1$	3.2	$<\infty$	t
20	$d+2.1= 2.1$	3.2	$<\infty$	t

* Chromosome with secondary constriction

d : dot

Table 11. Measurements of somatic chromosomes of *Phragmipedium pearcei* Type 2. at metaphase, $2n=21$

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	$2.3+2.3+0.7=5.3 *$	6.8	1.3	m
2	$2.3+2.3+0.7=5.3 *$	6.8	1.3	m
3	$2.3+2.6= 4.9$	6.3	1.1	m
4	$2.2+2.6= 4.8$	6.2	1.2	m
5	$1.9+2.5= 4.4$	5.7	1.3	m
6	$1.9+2.3= 4.2$	5.4	1.2	m
7	$1.1+2.7= 3.8$	4.9	2.5	sm
8	$1.0+2.7= 3.7$	4.8	2.7	sm
9	$1.7+2.0= 3.7$	4.8	1.2	m
10	$0.9+2.7= 3.6$	4.6	3.0	sm
11	$1.0+2.6= 3.6$	4.6	2.6	sm

Table 11. (continued)

12	1.0+2.6=	3.6	4.6	2.6	sm
13	1.2+2.1=	3.3	4.3	1.8	sm
14	1.2+2.1=	3.3	4.3	1.8	sm
15	1.3+1.9=	3.2	4.1	1.5	m
16	1.3+1.9=	3.2	4.1	1.5	m
17	d+3.2=	3.2	4.1	< ∞	t
18	1.2+1.8=	3.0	3.9	1.5	m
19	d+2.8=	2.8	3.6	< ∞	t
20	d+2.3=	2.3	3.0	< ∞	t
21	d+2.3=	2.3	3.0	< ∞	t

* Chromosome with secondary constriction

d : dot

Table 12. Measurements of somatic chromosomes of *Paphiopedilum pearcei* Type 3. at metaphase, 2n=22

Chromosome	Length (μ m)	Relative length	Arm ratio	Form
1	2.2+2.4+0.7=5.3 *	6.8	1.4	m
2	2.2+2.4+0.7=5.3 *	6.8	1.4	m
3	2.2+2.5=	6.1	1.1	m
4	2.0+2.3=	5.6	1.2	m
5	2.0+2.3=	5.6	1.2	m
6	1.0+3.3=	5.6	3.3	s t
7	1.7+2.3=	5.2	1.4	m
8	1.1+2.5=	4.7	2.3	sm
9	1.2+2.3=	4.5	1.9	sm
10	1.0+2.4=	4.4	2.4	sm
11	1.1+2.3=	4.4	2.1	sm
12	1.0+2.4=	4.4	2.4	sm
13	1.3+2.1=	4.4	1.6	m
14	0.7+2.5=	4.1	3.6	s t
15	1.0+1.9=	3.7	1.9	sm
16	1.2+1.7=	3.7	1.4	m
17	d+2.9=	3.7	< ∞	t
18	d+2.7=	3.5	< ∞	t
19	d+2.7=	3.5	< ∞	t
20	0.7+1.9=	3.4	2.7	sm
21	d+2.3=	3.0	< ∞	t
22	d+2.3=	3.0	< ∞	t

* Chromosome with secondary constriction

d : dot

Table 13. Measurements of somatic chromosomes of *Phragmipedium boisseranum* at metaphase, $2n=18$

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	$3.4+3.9= 7.3$	9.2	1.1	m
2	$3.4+3.9= 7.3$	9.2	1.1	m
3	$1.7+3.3= 5.0$	6.3	1.9	sm
4	$1.7+3.3= 5.0$	6.3	1.9	sm
5	$1.3+3.4= 4.7$	5.9	2.6	sm
6	$1.3+3.4= 4.7$	5.9	2.6	sm
7	$1.0+2.9+0.8=4.7 *$	5.9	3.7	s t
8	$1.0+2.9+0.8=4.7 *$	5.9	3.7	s t
9	$1.6+2.9= 4.5$	5.7	1.8	sm
10	$1.6+2.9= 4.5$	5.7	1.8	sm
11	$1.3+2.2= 3.5$	4.4	1.7	m
12	$1.3+2.2= 3.5$	4.4	1.7	m
13	$1.6+1.9= 3.5$	4.4	1.2	m
14	$1.6+1.9= 3.5$	4.4	1.2	m
15	$1.3+2.0= 3.3$	4.2	1.5	m
16	$1.3+2.0= 3.3$	4.2	1.5	m
17	$1.1+2.0= 3.1$	3.9	1.8	sm
18	$1.1+2.0= 3.1$	3.9	1.8	sm

* Chromosome with secondary constriction

Table 14. Measurements of somatic chromosomes of *Phragmipedium vittatum* at metaphase, $2n=18$

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	$3.4+4.1= 7.5$	8.4	1.2	m
2	$3.4+4.1= 7.5$	8.4	1.2	m
3	$1.8+3.7= 5.5$	6.2	2.1	sm
4	$1.8+3.7= 5.5$	6.2	2.1	sm
5	$1.7+3.8= 5.5$	6.2	2.3	sm
6	$1.7+3.8= 5.5$	6.2	2.3	sm
7	$1.3+3.2+0.8=5.3 *$	6.0	3.1	s t
8	$1.3+3.2+0.8=5.3 *$	6.0	3.1	s t
9	$1.4+3.5= 4.9$	5.5	2.5	sm
10	$1.4+3.5= 4.9$	5.5	2.5	sm
11	$1.0+3.2= 4.2$	4.7	3.2	s t
12	$1.0+3.2= 4.2$	4.7	3.2	s t
13	$1.7+2.2= 3.9$	4.4	1.3	m

Table 14. (continued)

14	1.8+2.1= 3.9	4.4	1.2	m
15	1.1+2.8= 3.9	4.4	2.5	sm
16	1.1+2.8= 3.9	4.4	2.5	sm
17	1.7+2.1= 3.8	4.3	1.2	m
18	1.7+2.0= 3.7	4.2	1.2	m

* Chromosome with secondary constriction

Table 15. Measurements of somatic chromosomes of *Phragmipedium hartwegii* at metaphase, 2n=20

Chromosome	Length (μ m)	Relative length	Arm ratio	Form
1	2.4+2.3+0.7=5.4 *	6.4	1.3	m
2	2.4+2.3+0.7=5.4 *	6.4	1.3	m
3	2.2+2.7= 4.9	5.8	1.2	m
4	2.2+2.7= 4.9	5.8	1.2	m
5	2.3+2.6= 4.9	5.8	1.1	m
6	2.3+2.6= 4.9	5.8	1.1	m
7	1.2+3.6= 4.8	5.7	3.0	sm
8	1.2+3.5= 4.7	5.6	2.9	sm
9	d+4.4= 4.4	5.2	< ∞	t
10	d+4.4= 4.4	5.2	< ∞	t
11	1.0+2.8= 3.8	4.5	2.8	sm
12	1.0+2.8= 3.8	4.5	2.8	sm
13	1.4+2.3= 3.7	4.4	1.6	m
14	1.4+2.3= 3.7	4.4	1.6	m
15	1.3+2.2= 3.5	4.1	1.7	m
16	1.3+2.2= 3.5	4.1	1.7	m
17	1.1+2.4= 3.5	4.1	2.2	sm
18	1.1+2.4= 3.5	4.1	2.2	sm
19	d+3.5= 3.5	4.1	< ∞	t
20	d+3.4= 3.4	4.0	< ∞	t

* Chromosome with secondary constriction

d : dot

Table 16. Measurements of somatic chromosomes of *Phragmipedium longifolium* at metaphase, $2n=23$

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	$2.3+2.1+1.0=5.4 *$	6.4	1.3	m
2	$2.3+2.1+1.0=5.4 *$	6.4	1.3	m
3	$2.3+2.7= 5.0$	5.9	1.2	m
4	$2.3+2.7= 5.0$	5.9	1.2	m
5	$2.2+2.6= 4.8$	5.7	1.2	m
6	$2.2+2.6= 4.8$	5.7	1.2	m
7	$1.1+3.3= 4.4$	5.2	3.0	sm
8	$1.1+3.3= 4.4$	5.2	3.0	sm
9	$d+4.1= 4.1$	4.8	$<\infty$	t
10	$d+4.1= 4.1$	4.8	$<\infty$	t
11	$1.3+2.7= 4.0$	4.7	2.1	sm
12	$1.2+2.7= 3.9$	4.6	2.3	sm
13	$1.3+2.2= 3.5$	4.1	1.7	m
14	$1.3+2.2= 3.5$	4.1	1.7	m
15	$1.1+2.1= 3.2$	3.8	1.9	sm
16	$1.1+2.1= 3.2$	3.8	1.9	sm
17	$1.0+2.1= 3.1$	3.7	2.1	sm
18	$0.9+2.1= 3.0$	3.5	2.3	sm
19	$d+3.0= 3.0$	3.5	$<\infty$	t
20	$d+3.0= 3.0$	3.5	$<\infty$	t
21	$d+1.3= 1.3$	1.5	$<\infty$	t
22	$d+1.3= 1.3$	1.5	$<\infty$	t
23	$d+1.2= 1.2$	1.4	$<\infty$	t

* Chromosome with secondary constriction

d : dot

Table 17. Measurements of somatic chromosomes of *Phragmipedium longifolium* var. *gracile* at metaphase, $2n=21$

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	$2.3+2.3+0.9=5.5 *$	6.2	1.4	m
2	$2.3+2.3+0.9=5.5 *$	6.2	1.4	m
3	$2.3+3.0= 5.3$	6.0	1.3	m
4	$2.3+3.0= 5.3$	6.0	1.3	m
5	$2.2+2.5= 4.7$	5.3	1.1	m
6	$2.2+2.5= 4.7$	5.3	1.1	m
7	$1.2+3.3= 4.5$	5.1	2.8	sm

Table 17. (continued)

8	1.2+3.3=	4.5	5.1	2.8	sm
9	d+4.3=	4.3	4.9	<∞	t
10	d+4.3=	4.3	4.9	<∞	t
11	1.1+3.1=	4.2	4.7	2.8	sm
12	1.1+3.1=	4.2	4.7	2.8	sm
13	1.6+2.5=	4.1	4.6	1.6	m
14	1.6+2.5=	4.1	4.6	1.6	m
15	1.3+2.5=	3.8	4.3	1.9	sm
16	1.3+2.5=	3.8	4.3	1.9	sm
17	1.3+2.4=	3.7	4.2	1.8	sm
18	1.3+2.4=	3.7	4.2	1.8	sm
19	d+3.7=	3.7	4.2	<∞	t
20	d+3.7=	3.7	4.2	<∞	t
21	d+1.0=	1.0	1.1	<∞	t

* Chromosome with secondary constriction

d : dot

Table 18. Measurements of somatic chromosomes of *Phragmipedium roezlii* at metaphase, 2n=22

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	2.2+2.1+0.9=5.2 *	6.3	1.4	m
2	2.2+2.1+0.9=5.2 *	6.3	1.4	m
3	2.4+2.7= 5.1	6.2	1.1	m
4	2.4+2.7= 5.1	6.2	1.1	m
5	2.4+2.6= 5.0	6.1	1.1	m
6	2.2+2.3= 4.5	5.5	1.0	m
7	1.3+3.2= 4.5	5.5	2.5	sm
8	1.2+3.1= 4.3	5.2	2.6	sm
9	d+4.3= 4.3	5.2	<∞	t
10	d+4.1= 4.1	5.0	<∞	t
11	1.1+2.8= 3.9	4.7	2.5	sm
12	1.0+2.9= 3.9	4.7	2.9	sm
13	1.4+2.0= 3.4	4.1	1.4	m
14	1.4+2.0= 3.4	4.1	1.4	m
15	1.0+2.2= 3.2	3.9	2.2	sm
16	1.0+2.2= 3.2	3.9	2.2	sm
17	1.0+2.0= 3.0	3.6	2.0	sm
18	1.0+2.0= 3.0	3.6	2.0	sm

Table 18. (continued)

19	d+3.0= 3.0	3.6	$<\infty$	t
20	d+2.9= 2.9	3.5	$<\infty$	t
21	d+1.1= 1.1	1.3	$<\infty$	t
22	d+1.0= 1.0	1.2	$<\infty$	t

* Chromosome with secondary constriction

d : dot

生長点培養法によって生じた カトレヤ系6倍体の観察

青山 幹男・唐澤 耕司

Observations of the hexaploid in *Sophrolaeliocattleya*
occurred by the meristematic cloning

Mikio Aoyama and Kohji Karasawa

ラン科植物の生長点培養法は、ウイルス・フリー株の作出を目的として Morel (1960) により始められた。この方法は、今日ランの増殖法として広く用いられている。

生長点培養による増殖法は同一形質をもつ株を多量に得るための方法であるが、大量増殖の過程において、形質の異なる個体が稀に見出されることが報告されている。(田中・長久1979, 加古1979)。

今回、生長点培養法によって生じた変異株について、組織学および核形態学的観察を行なったので、ここに報告する。

材 料

Sophrolaeliocattleya Jewell Box 'Scheherazade'

国際園芸株式会社において生長点培養法によって得られた多数の苗の中から見出された変異株(明らかに他の株と外部形態が異なる個体)と、普通株(標準的な外部形態をもつ個体)とを用いた。

材料を提供された同社社長合田弘之氏に御礼申し上げます。

実 験 方 法

葉の組織の観察は、十分生長した葉の先端より1/3の部分を用い、ハンドセクション法により切片を作成した。

染色体の観察は、生育中の根の先端を1mm切りとり、18℃の8-オキシキノリン液に5時間浸漬した

後、3:1アルコール酢酸で固定処理し、アセトオルセイン染色による押しつぶし法でプレパラートを作成した。

観 察 結 果

1 外部形態

普通株と変異株の各部の形態および測定値は図1, 表に示した。

変異株の花は普通株に比べて、弁質が厚く、花弁の縁に不規則で小さな切れ込みが見られた。花色は普通株よりやや淡く、部分的に濃淡が見られた(図1A, B)。

葉は、普通株ではすべて一葉をつけるが、変異株では7茎中3茎において2葉をつけていた。

葉肉中の柔細胞および維管束は変異株において約2倍の大きさになり、その結果、葉の厚さも約2倍になっていた(図1C, D)。

葉の裏面表皮における孔辺細胞は変異株の方がやや大きく、気孔数は変異株において半数近くに減少していた(図1E, F)。

表 各部の測定値

材 料	普通株	変異株
花の自然開張幅	8.5 cm	8.0 cm
葉 の 厚 さ	1.4 mm	2.9 mm
気 孔 数	132 個/mm ²	78 個/mm ²
孔辺細胞の大きさ	31 μm	34 μm

変異株の根は伸長生長にむらを生じ、表面に凹凸がみられた。

2 核形態

普通株と変異株の静止期核および中期染色体を

図2に示した。

(1) 普通株

静止期核は直径が $20\sim 25\mu\text{m}$ あり、大きさが不揃いで小さな凝縮塊を多数もつ単純染色中央粒

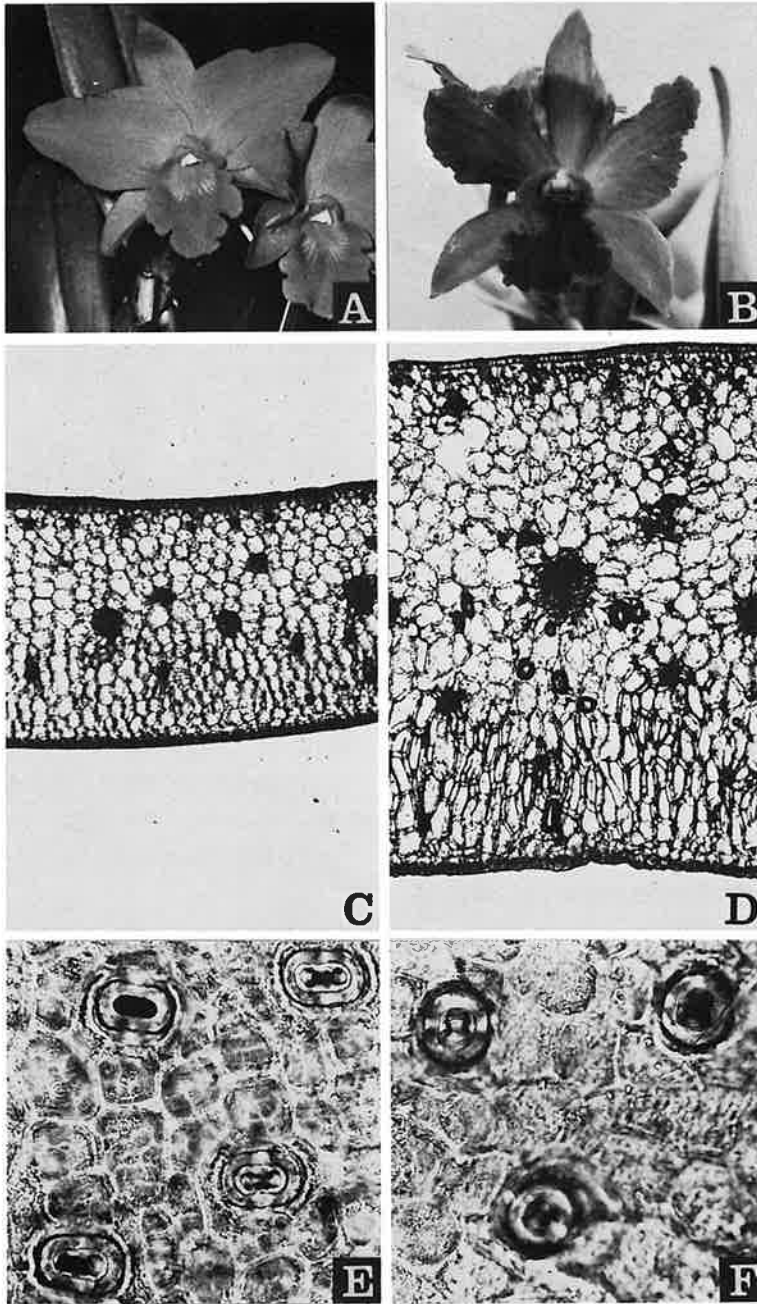


図 1. *Schl. Jewell Box 'Scheherazade'* の普通株 (A, C, E) と変異体 (B, D, F).
A - B, 花 ($\times 0.48$). C - D, 葉の横断面 ($\times 24$). E - F, 気孔 ($\times 240$).

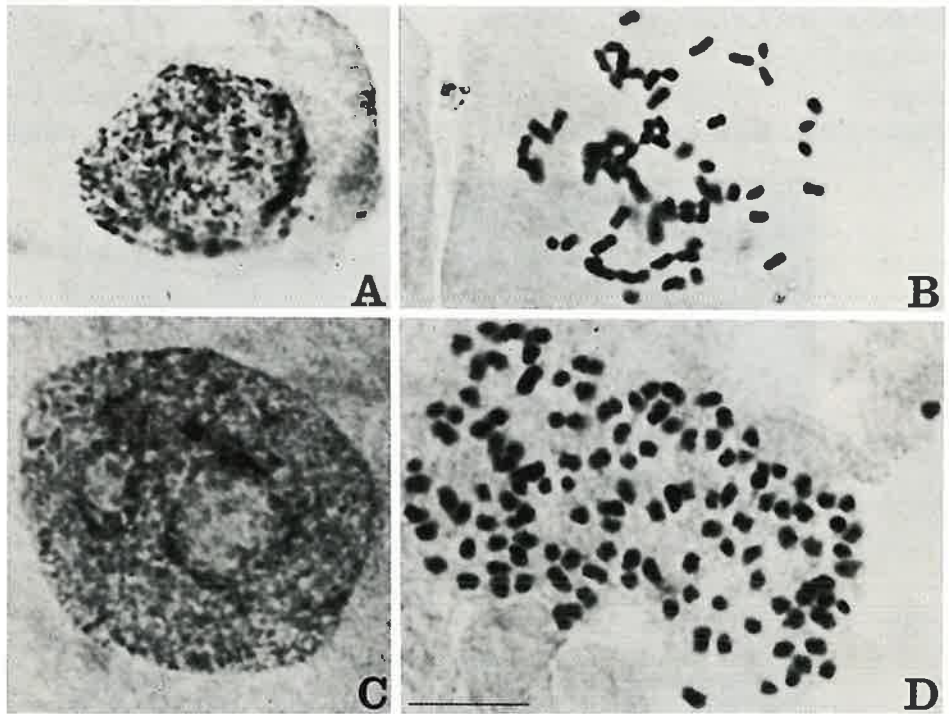


図 2. *Slc. Jewell Box 'Scheherazade'* ($2n=60$, ca. 120) $\times 1600$.
A, B, 普通株 ($2n=60$). C, D, 変異株 ($2n=ca. 120$).
A, C, 静止期核. B, D, 中期染色体.

型 (Tanaka 1971) を示し、核内には仁が 1 個観察された (図 2 A)。

観察した個体の染色体数は中期染色体において $2n=60$ を算定した。

(2) 変異株

静止期核は直径が $27\sim 32\ \mu\text{m}$ あり、前記の普通株と同様に単純染色中央粒型を示し、核内には大小 2 個の仁が観察された (図 2 C)。

観察した個体の染色体数は中期染色体において $2n=約 120$ を算定した (図 2 D)。

考 察

本交雑種の変異株は、3 倍体の培養組織の一部が倍数化を起こして 6 倍体の組織を生じた結果出現したものと思われる。

カトレヤ系交雑種では、2 倍体 $\times$ 5 倍体および 3 倍体 $\times$ 4 倍体の実生苗の中から 6 倍体の個体が報告されている (Vajrabhaya & Randolph 1961, Tanaka 1966) が、生長点培養からの 6 倍体の出現の報

告は見あたらない。シンビジウム属およびデンドロビウム属においては同様な現象がすでに報告されている (Vajrabhaya 1977, 田中・長久 1979)。

今回観察した変異株は、他の植物の高次倍数体で見られる多肉化の現象が強度に現われており、実用的には園芸的価値がむしろ低下していた。

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セッコク交雑種に関する細胞遺伝学的研究

唐澤耕司・橋本清美

Cytogenetic studies in the hybrids of *Dendrobium moniliforme*

Kohji Karasawa and Kiyoshi Hashimoto

栽培ランの品種の分化が、細胞遺伝学的な変化、特に倍数性と異数性によって進行していることは、すでに多くの報告がある(Ito & Mutsuura 1957)。わが国に最も普通の栽培ランのうち、*Dendrobium* の占める役割は大きく、なかでも *nobile* 系の *Dendrobium* の早咲き(12月咲き)品種の市場性は高いが、*Dendrobium* も倍数性と異数性によって品種分化が進行している(Kamemoto 1950, Kamemoto & Tanaka 1961)。

この *Dendrobium* では、早咲きで大輪、花もちよく、花つき、株立がよく、特に鉢物用としては、強健で、茎が低く倒れない品種の作出が望まれている。また耐寒性品種の作出も重要な課題である。

早咲き性については、日本産のセッコク(*D. moniliforme*)を片親に使用した子孫、*D. Cassiope* (*D. moniliforme* × *D. nobile*)や *D. Nagasaki* (*D. moniliforme* × *D. Sagimusume*)などで、その雑種第1代が早咲きになることが知られている。しかし、これら雑種第1代は、強健で早咲き、耐寒性は強いが、花が小さく、花肉が薄く、花もちがよくない。これら雑種第1代に、さらに *nobile* 系大輪種を交配して、より大輪で花もちのよい、早咲き品種の作出を目標に、多くの交配が試みられている。また倍数体が花を大きく、花肉を厚くすることは、他のラン、*Cattleya* や *Cymbidium* でもよく知られ、交配親としても積極的に用いられている。

本研究は、早咲き性の強い *Dendrobium* の選抜された優良個体を選んで、その染色体を調べ、優れた倍数体の早咲き交配親を見出すことを目的とした

ものである。

材料および方法

今回観察に用いた *Dendrobium* の原種と交配種は表1に示した通りである。各材料株を提供された藤尾謙吾氏、森本春一氏、神代昌典氏、谷口通生氏および米沢耕一氏に深く御礼申し上げる。

染色体の観察には根端細胞を用い、Kamemoto & Tanaka(1960)の方法によった。すなわちよく伸長しつつある根の先端を2mmの長さに切り取り、0.002 mol の8オキシキノリン溶液に、約18℃で4時間浸漬した後、クロロフォルム・エタノール・45%酢酸溶液(1:1:2)を用い、5℃で15分間固定した。ついで60℃の1規定塩酸で解離処理を行った後、45%酢酸にもどし、1%酢酸オルセインで30分間染色し、スライドガラス上にて押しつぶし標本とした。

外部形態の観察は、よく開花した株の代表的な花を選んで測定した。

観 察 結 果

Dendrobium の原種および交配種における根端細胞の染色体を表1および図1に、花の形態を表2および図2に示す。

染色体数は *D. moniliforme* および *D. nobile* の両原種は、いずれも $2n = 38$ であった。雑種第1代(mF_1)では、*Cassiope* 'Beppu' と *Nagasaki*

表 1. *Dendrobium* の染色体数

世 代 Generation	種および品種 Species and Hybrids	体細胞染色 体数(2n) Chromosome number (2n)	所 有 者 Owner
Original Species	<i>D. moniliforme</i>	38	Hiroshima Botanical Garden
	<i>D. nobile</i>	38	Hiroshima Botanical Garden
mF ₁	<i>D. Cassiope</i> 'Beppu' (<i>moniliforme</i> × <i>nobile</i>)	38	Morimoto
	<i>D. Nagasaki</i> (<i>moniliforme</i> × <i>Sagimusume</i>)	38	Kumashiro
	<i>D. Unnamed</i> (<i>moniliforme</i> × <i>Yukidaruma</i> 'King')	57	Taniguchi
	<i>D. Unnamed</i> (<i>moniliforme</i> × <i>Snowflake</i>)	64	Taniguchi
mF ₂	<i>D. Miss Nagasaki</i> (<i>Cassiope</i> × <i>Kongoh</i>)	76	Yonezawa
	<i>D. Snowflake</i> 'Otome' (<i>Cassiope</i> × <i>nobile</i>)	76	Taniguchi
	<i>D. Snowflake</i> 'Red Star' (<i>Cassiope</i> × <i>nobile</i>)	78	Taniguchi
mF ₃	<i>D. Hojun</i> (<i>Snowflake</i> × <i>Merry Christmas</i>)	38	Taniguchi
	<i>D. Tomoflake</i> 'Seto' (<i>Snowflake</i> × <i>Misstomopink</i>)	76	Taniguchi
	<i>D. Tomoflake</i> 'Momoyama' (<i>Snowflake</i> × <i>Misstomopink</i>)	74	Hiroshima Botanical Garden
mF _n	<i>D. Yukidaruma</i> 'King' (<i>Shiranami</i> × <i>Pinocchio</i>)	76	Hiroshima Botanical Garden
	<i>D. Misstomopink</i> (<i>Kobayashi</i> × <i>Taketane</i>)	76	Hiroshima Botanical Garden

は $2n = 38$ であったが, *Unnamed* (*moniliforme* × *Yukidaruma* 'King') は $2n = 57$ で三倍体, *Unnamed* (*moniliforme* × *Snowflake*) は $2n = 64$ で異数性三倍体であった。

雑種第2代 (mF₂) では, *Snowflake* 'Otome' と *Miss Nagasaki* がいずれも $2n = 76$ の四倍体であ

ったが, *Snowflake* 'Red Star' は $2n = 78$ で異数性四倍体であった。

さらにセッコクの雑種第3代 (mF₃) では, *Hojun* が $2n = 38$ の二倍体, *Tomoflake* 'Seto' が $2n = 76$ の四倍体, *Tomoflake* 'Momoyama' は $2n = 74$ の異数性四倍体であった。

交配をくり返したmF_n世代では、Yukidaruma 'King' と Misstomopink がいずれも2n = 76の四倍体であった。

以上の観察結果において、雑種第1代において三倍体が現われ、雑種第2代では、すでに四倍体が現われたことが確認された。また交配がくり返されることによって、異数体の出現や低倍数体への変化が起っていた。

外部形態の観察においては、花および上萼片・花弁・唇弁の長さと幅を測定した。雑種第1代より第2代が、さらに雑種第2代より第3代の方が、一般に上萼片・花弁・唇弁ともに大きくなっている。mF_n世代においては、必ずしも花の大きさについて最大とはなっていないが、花弁は厚く、花もち、花着きなどについて優れた形質をもっている。

この度材料として用いた植物は、さまざまな観点から選抜された株であり、それぞれ優れた形質をもっ

ている。一般に交配がくり返されるほど、花も大きく、花肉が厚く、花もちのよい品種が得られている。今後の交配親としての観点からみれば、mF₁世代のCassiope 'Beppu' mF₂世代のSnowflake 'Otome' mF₃世代のTomoflake 'Seto'のように、整倍数体のものの方が、異数性倍数体にくらべ平均的に子孫に優れたものが多く得られるであろうと考えられる。

要 約

- 1 *Dendrobium* 2種10品種2栽培品種について、染色体数および外部形態を記載した。
- 2 選抜された優良品種は、二倍体、三倍体、四倍体およびそれらの異数体であることがわかった。
- 3 交配親としては、これらのうち整倍数体のものの方が優秀であると考えられる。

表 2. *Dendrobium*の花の測定値比較

世 代 Generation	種および品種 Species and Hybrids	花の各部の測定値(長さ×幅)(mm) Length and Width of flower (mm)			
		自然開長 Natural spread	上 萼 片 Upper Sepal	花 弁 Petal	唇 弁 Lip
Original Species	<i>D. moniliforme</i>	31 × 22	23 × 5	23 × 7	24 × 13
mF ₁	<i>D. Cassiope</i> 'Beppu' (<i>moniliforme</i> × <i>nobile</i>)	52 × 56	32 × 8	32 × 13	28 × 20
	<i>D. Unnamed</i> (<i>moniliforme</i> × Yukidaruma 'King')	58 × 50	31 × 11	31 × 19	30 × 20
mF ₂	<i>D. Snowflake</i> 'Otome' (<i>Cassiope</i> × <i>nobile</i>)	57 × 65	32 × 14	34 × 24	38 × 20
	<i>D. Snowflake</i> 'Red Star' (<i>Cassiope</i> × <i>nobile</i>)	61 × 65	33 × 15	33 × 30	35 × 29
	<i>D. Hojun</i> (<i>Snowflake</i> × Merry Christmas)	50 × 47	28 × 10	28 × 17	27 × 19
mF ₃	<i>D. Tomoflake</i> Seto (<i>Snowflake</i> × Misstomopink)	70 × 65	39 × 20	37 × 19	35 × 29
	<i>D. Tomoflake</i> 'Momoyama' (<i>Snowflake</i> × Misstomopink)	65 × 75	40 × 17	40 × 29	40 × 34
mF _n	<i>D. Yukidaruma</i> 'King' (Shiranami × Pinocchio)	64 × 53	32 × 18	33 × 25	32 × 28

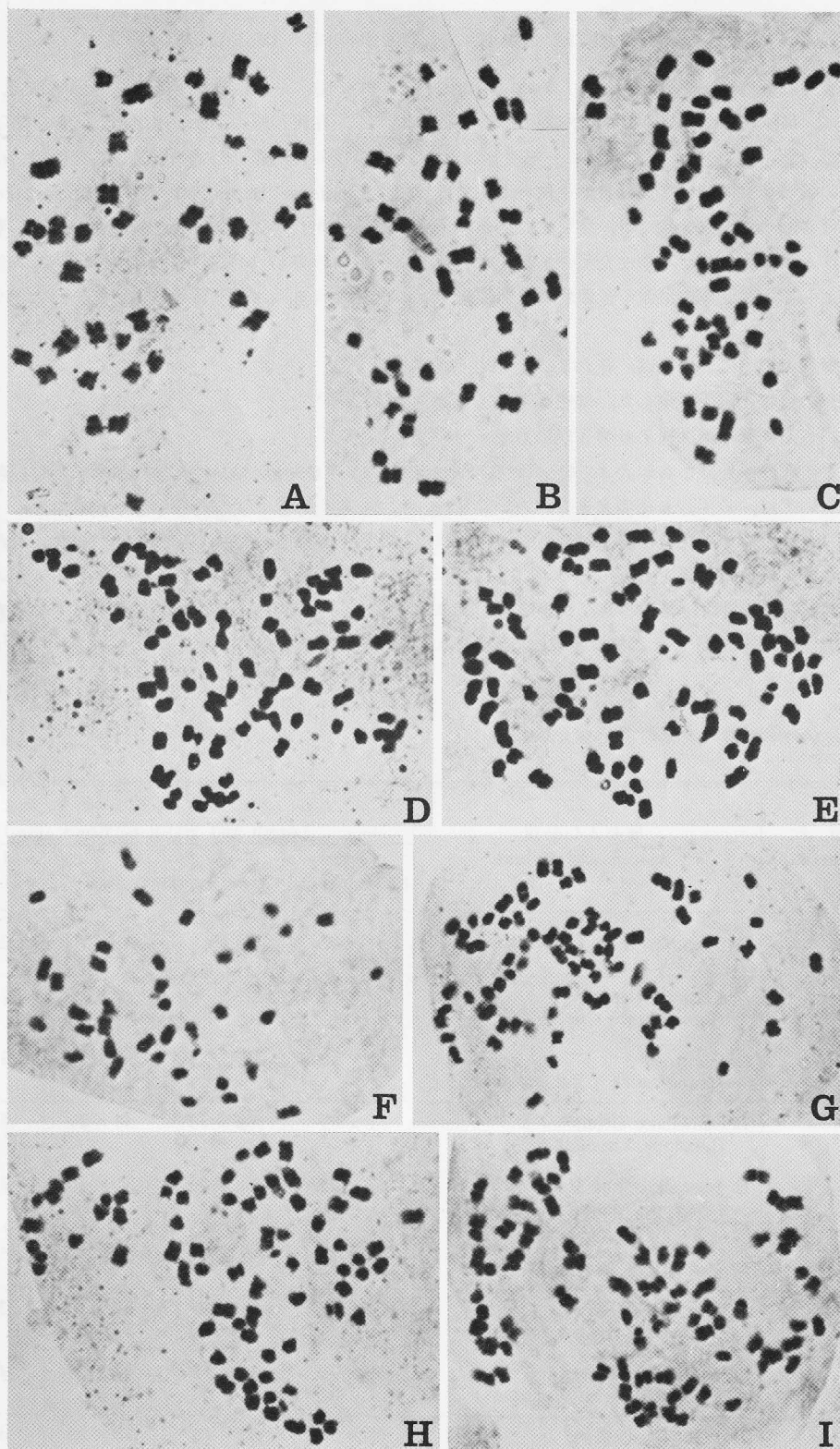


図 1. *Dendrobium* 1種5品種2栽培品種における体細胞分裂中期染色体, ($\times 2500$) A.*D. moniliforme* ($2n=38$). B.*D. Cassiope* 'Beppu' ($2n=38$). C.*D.* Unnamed ($2n=57$). D.*D.* Snowflake 'Otome' ($2n=76$). E.*D.* Snowflake 'Red Star' ($2n=78$). F.*D.* Hojun ($2n=38$). G.*D.* Tomoflake 'Seto' ($2n=76$). H.*D.* Tomoflake 'Momoyama' ($2n=74$). I.*D.* Yukidaruma 'King' ($2n=76$).

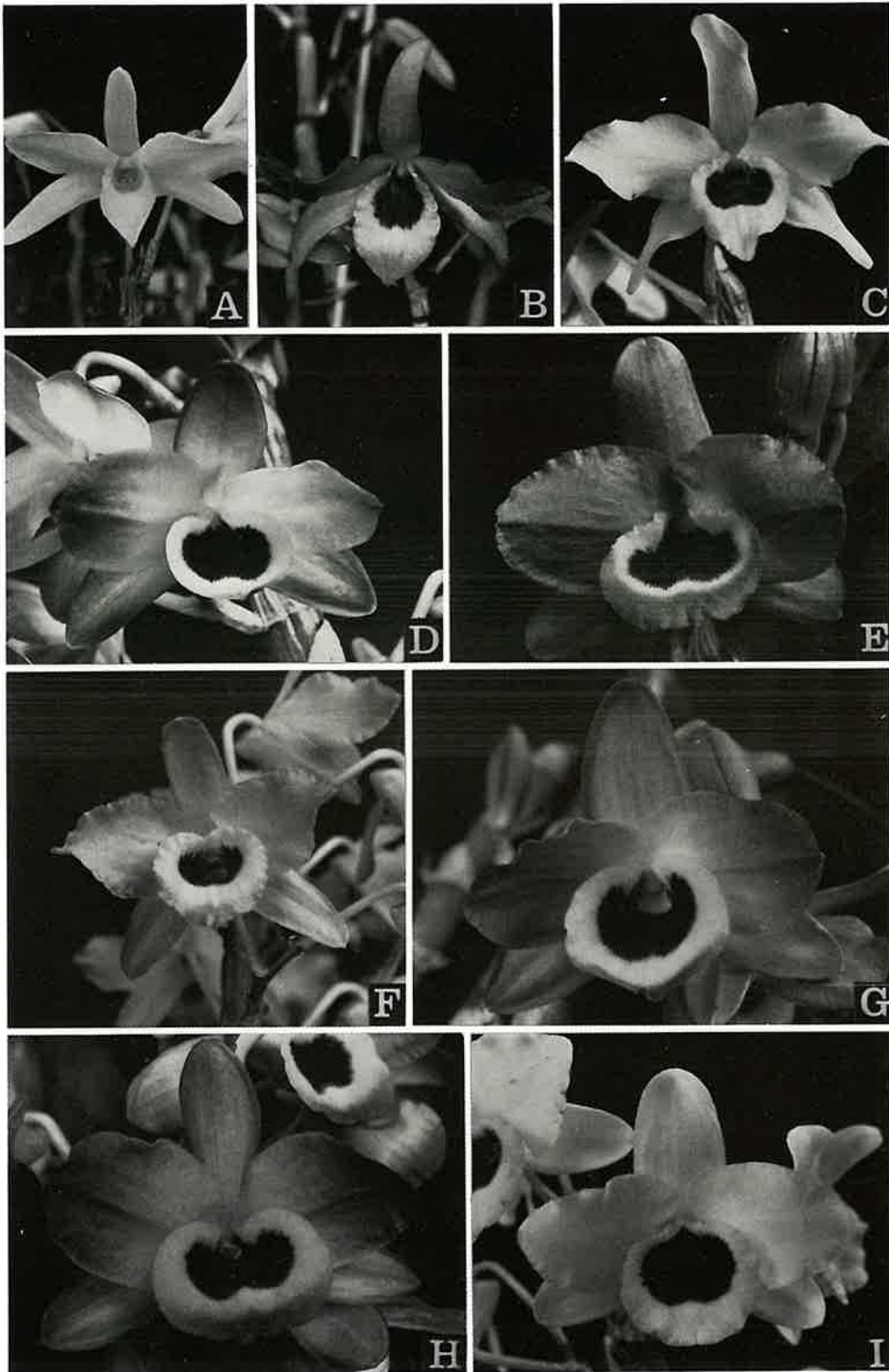
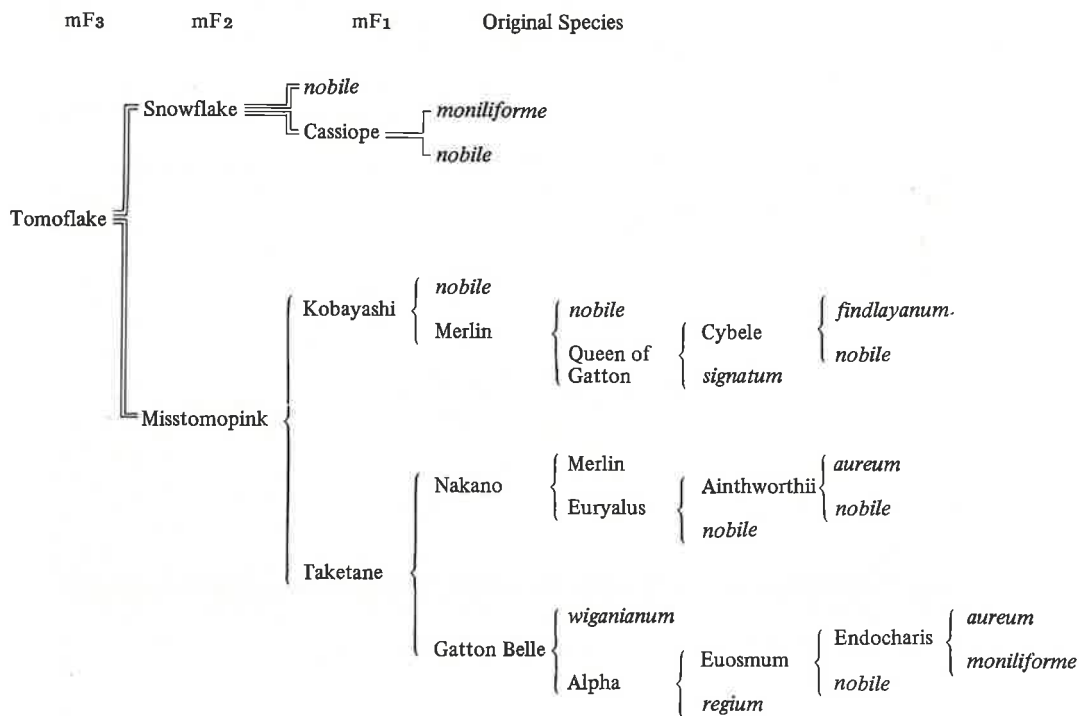


図 2. *Dendrobium* 1 種 5 品種 2 栽培品種における花. ($\times 1$) A.*D. moniliforme* ($2n=38$). B.*D. Cassiope* 'Beppu' ($2n=38$). C.*D. Unnamed* ($2n=57$). D.*D. Hojun* ($2n=38$). G.*D. Tomoflake* 'Seto.' ($2n=76$). H.*D. Tomoflake* 'Momoyama' ($2n=74$). I.*D. Yukidaruma* 'King' ($2n=76$).

表 3. セッコク系デンドロビウムの系統



Summary

1. Chromosome numbers and morphological features were described in the species and the hybrids of *Dendrobium*. The results were shown in Table 1.
2. Chromosome numbers of 12 selected hybrids of *D. moniliforme* were recorded for the first time.
3. It was confirmed that euploids is superior to aneuploids as crossing parents.

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Bifrenaria × *Cymbidium* の属間雑種に関する 細胞学的・形態学的研究

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Cytological and morphological studies on an intergeneric hybrid of
Bifrenaria × *Cymbidium*

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ラン科植物では、異種間ではもとより異属間でも人為雑種が容易に生じ、本科の種や属の間には著しい遠縁交雑能力があることがわかる。現在まで、属間雑種を生じている属はおおよそ100属にのぼり、その大部分は同一亜連内に含まれる属の間においてであるが、田中(1971b)はこれらの属間雑種及び異亜連間の属間雑種(*Arundina sinensis* × *Bletilla striata*, *Bletilla striata* × *Eleorchis japonica*)の核形態学的研究にもとづいて、遠縁交雑を起こしている属の間では、外部形態におけるよりも核形態学的な類似性がみられること、および遠縁交雑の可能性は、染色体および染色質の形態的類似性で判定できることを指摘した。さらに、多くの属の静止期核の染色質を比較し、*Cymbidium* の染色質の形態に強く類似している属として、*Maxillaria*, *Grammatophyllum*, *Ansellia*, *Bifrenaria*, *Zygopetalum*, また、およそ類似している属として、*Calanthe*, *Phaius*, *Crematstra*, *Oreorchis*, *Bletia*, *Oncidium*, *Promenaea*などをあげている。この指摘をきっかけとして、育種家の中に *Cymbidium* を用いた属間交雑を実行する人達が現われているが、本論文はその中の一つとして、高木誠作氏によって交雑実験された *Bifrenaria* × *Cymbidium* の人為雑種について、この雑種が細胞学的にも、また、形態学的にも確かに雑種第一代株であることを確認できたことを報告するものである。*Cymbidium* の今後の育種には属間雑種の利用による新遺伝子を導入することが是非とも必要と考えら

れるが、本属間雑種もその一連の研究の一つである。

材料および方法

材料植物は、交雑に用いられた両親株の *Bifrenaria harrisoniae* (Hk.) Rchb. f., *Cymbidium* Rosalita 'Dos Pueblos' と、その雑種第一代株である。これらの材料は高木農園の高木誠作氏および小島勝也氏から提供を受けた。両氏に対しここに御礼申し上げる。

形態的観察は、植物体の外観的特徴、および葉の組織について行った。いずれも十分に生長した株を用い、葉の組織は、葉身の先端より1/4の部分のものをを用いた。組織の標本は徒手切片により作成した。

染色体の観察は、生育中の根の先端を約2mm切りとり、18℃0.002モル8-オキシキノリン液に5時間浸漬した後、5℃45%酢酸で5分間固定処理し、塩酸による解離の後、アセトオルセイン染色によるおしつぶし法でプレパラートを作成した。

観 察 結 果

1. 外部形態および組織の形態

観察した株の形態的観察結果を図1および表1に示した。

A. *Bifrenaria harrisoniae* (Hk.) Rchb. f., 表1, 図1A, D, G.

偽球茎は長卵形で4稜があり、先端に長だ円形で

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両端がとがった葉を1枚つけていた(図1A)。

葉の葉脈の上部・下部に多数の厚膜細胞が観察され、特に上部で著しく発達していた。表皮の内側の柔組織内には厚膜細胞束が観察されなかった(図1D)。

裏面表皮における気孔の数は83個/mm²であって、1つの気孔を構成する孔辺細胞の形は円形で、その直径は34μmであった。孔辺細胞に接する表皮細胞は小型で細長い細胞に変化していた(図1G)。

B. *Cymbidium* Rosalita 'Dos Pueblos', 表1, 図1B, E, H.

偽球茎は卵形で大きく、線状被針形の葉を約15枚つけていた(図1B)。

葉の葉脈の下部に多数の厚膜細胞が発達しており、上部にはこの細胞が微少であった。葉の上下両面の表皮のすぐ内側の柔組織内には直径40~70μmの厚膜細胞束が多数観察された(図1E)。

裏面表皮における気孔の数は171個/mm²であっ

表 1

材 料		<i>Bifrenaria harrisoniae</i>	<i>Cymbidium</i> Rosalita 'Dos Pueblos'	属 間 雑 種 <i>Bifrenaria</i> × <i>Cymbidium</i>
花	輪 数	1~2	約 15	未開花
	花茎の 出る位置	偽球茎の下部	偽球茎の下部	未開花
偽球茎	形	長卵形 4稜をもつ	卵 形	長卵形
	長 さ	8 cm	9 cm	4 cm
葉	形	長だ円形 両端はとがる	線状被針形	線状被針形
	長さ×幅	28 × 7.5 cm	73 × 1.9 cm	51 × 2.4 cm
	葉 数	1 枚	約 12 枚	約 5 枚
	厚 さ	0.42 mm	0.45 mm	0.38 mm
	厚膜細胞	葉脈の上部に多数 下部に少数 表皮内側に厚膜細胞束が ない	葉脈の上部に多数 下部に少数 表皮内側に厚膜細胞束が 点在	葉脈の上下に多数 表皮内側に厚膜細胞束が 少数ある
	裏面表皮 の気孔数	83 個/mm ²	171 個/mm ²	129 個/mm ²
	孔辺細胞 の大きさ	34 μm	31 μm	32 μm
	気孔の形	円形	だ円形	だ円形

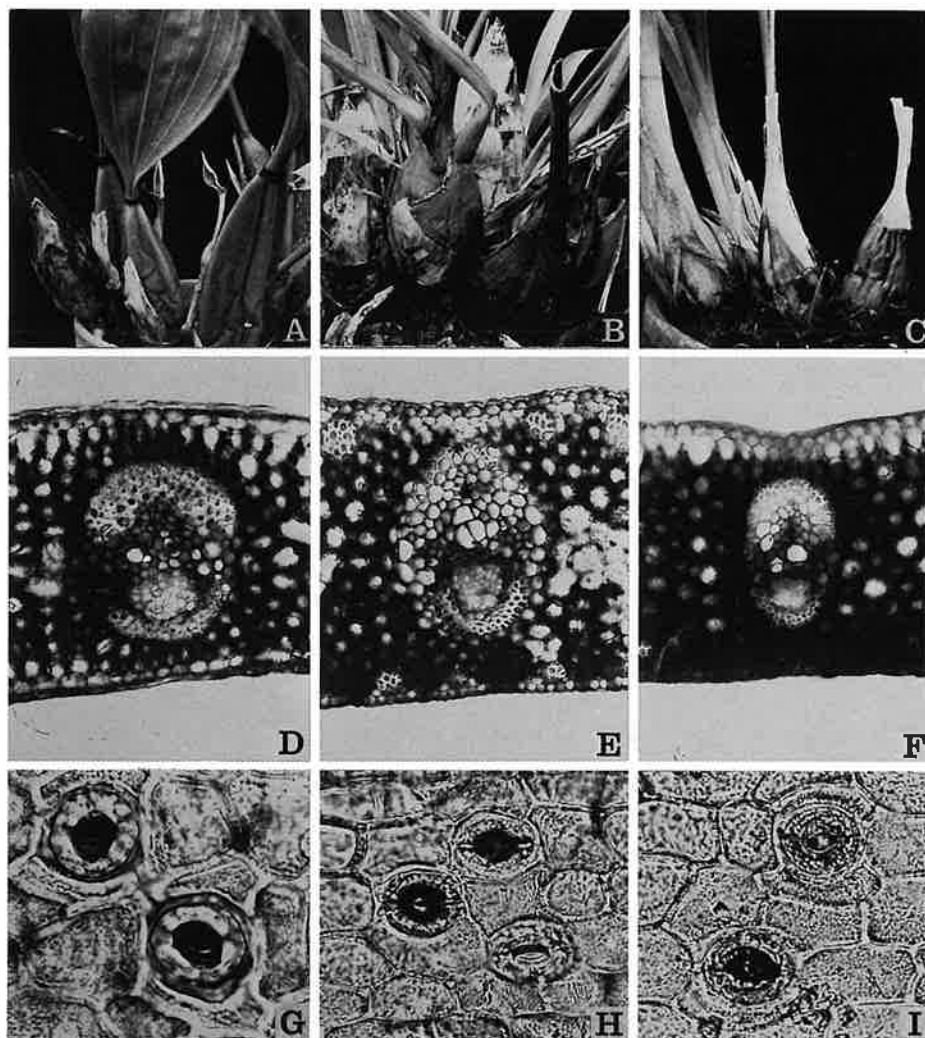


Fig. 1. *Bifrenaria harrisoniae* (A, D, G), *Cymbidium Rosalita* 'Dos Pueblos' (B, E, H) and intergeneric hybrid (C, F, I).

A (× 0.36). B (× 0.3). C (× 0.48). D-F (× 90) cross section of leaf.
G-I (× 360) stomata in lower epidermis of leaf.

て、1つの気孔を構成する孔辺細胞の形はだ円形で、その長径は $31\mu\text{m}$ であった。孔辺細胞に接する表皮細胞の形態は他の部分の表皮細胞とほぼ同じであった(図1H)。

C. 属間雑種 (*Bifrenaria harrisoniae* × *Cymbidium Rosalita* 'Dos Pueblos') 表1, 図1C, F, I.

偽球茎は長卵形で小さく、線状被針形の葉を約5枚つけていた。偽球茎より葉が出る部位は上

部と下部に多く、中部では少なかった(図1C)。

葉の葉脈の上部・下部に多数の厚膜細胞が観察された。葉の上下両面の表皮のすぐ内側の柔組織内には弱い厚膜細胞束が多数観察された(図1F)。

裏面表皮における気孔の数は $129\text{個}/\text{mm}^2$ であって、1つの気孔を構成する孔辺細胞の形はだ円形で、その長径は $32\mu\text{m}$ であった。孔辺細胞に接する表皮細胞はやや小型で細長い細胞に変化していた(図1I)。

2. 核形態

観察した株の静止期核、前期染色体、中期染色体を図2に示した。中期染色体を長さの順に並べて図3に示し、その測定値を表2～4に示した。

A. *Bifrenaria harrisoniae* (Hk.) Rchb.f., $2n =$

38, 図2 A, D, G, 図3 A, 表2.

静止期核は直径が $18\mu\text{m}$ で、染色質は多数の凝縮部を形成していた。凝縮部は形や大きさが不揃いで、染色中央粒として観察され、Tanaka (1971 a) の分類による複雑染色中央粒型に相当した(図

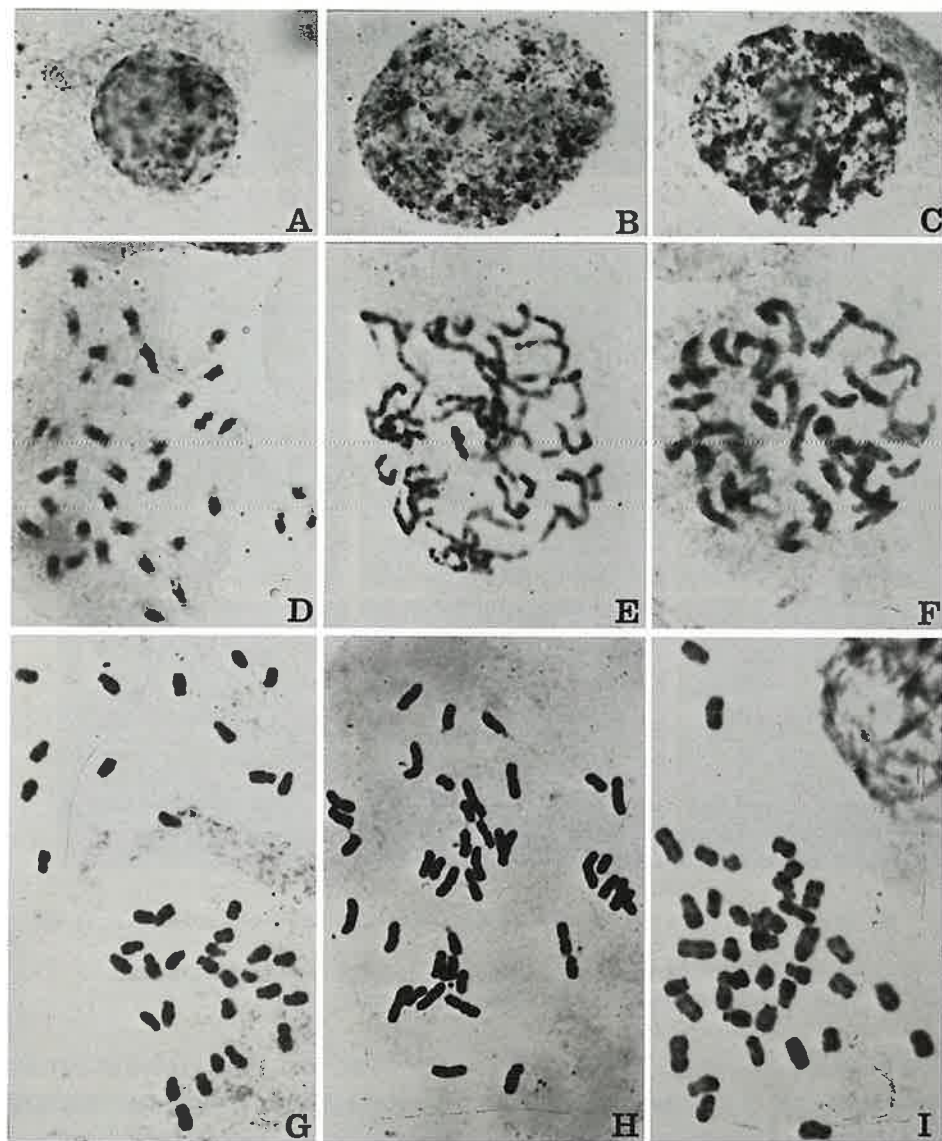


Fig. 2. *Bifrenaria harrisoniae* ($2n=38$, A, D, G), *Cymbidium* Rosalita 'Dos Puebles' ($2n=41$, B, E, H) and intergeneric hybrid ($2n=39$, C, F, I).
A-C, resting nuclei. D-F, prophase chromosomes.
G-I, metaphase chromosomes. $\times 1200$.

2 A)。

前期染色体において、早期凝縮部は、基部寄り、介在位で観察され、末端寄りでは観察されなかった(図2 D)。

本交雑に用いられた親株は、染色体数が $2n = 38$ であって、田中(1962)の報告と一致した(図2 G)。分裂期中期染色体について長さを測定し、その結果を表2に示した。

38個の染色体は、第1番目から第36番目までの漸变的に短くなる36個の大型染色体と、第37、38番目の2個の小型染色体とで構成される2様相核型を示していた(図3 A)。

36個の大型染色体は長さが $3.1 \mu\text{m}$ から $2.0 \mu\text{m}$ までの範囲にあった。これらのうち第1、2、5、6、23、24、29-32、35、36番目の12個の染色体は、腕比が1.0から1.7までの範囲にあり、動原体は中部に位置していた。第3、4、7-20、25-28、33、34番目の22個の染色体は、腕比が1.8から2.5までの範囲にあり、動原体は次中部に位置していた。第21、22番目の2個の染色体は腕比がいずれも3.2で、動原体は次端部に位置していた。

最小の染色体の1対は、共に長さが $1.6 \mu\text{m}$ 、腕比が2.2であって、動原体は次中部に位置していた。この小型染色体はいずれも短腕の末端部に極く小さい付随体をもっていた。

以上の観察結果から、本種は2個ずつの同一の形態をもつ染色体19対で構成される2倍体であることがわかる。また、小型染色体の2個は本種の核型を特徴づけるものである。

B. *Cymbidium Rosalita* 'Dos Pueblos', $2n = 41$, 図2 B, E, H, 図3 B, 表3。

静止期核は直径が約 $25 \mu\text{m}$ で、染色質は多数の凝縮部を形成していた。凝縮部は形や大きさが不揃いで、染色中央粒として観察され、複雑染色中央粒型に相当した(図2 B)。

前期染色体において、早期凝縮部は、末端寄り、基部寄り、介在位の各部で観察された(図2 E)。

本交雑に用いられた親株は、染色体数が $2n = 41$ であった(図2 H)。分裂期中期染色体について長さを測定し、その結果を表3に示した。

41個の染色体は長さが $4.5 \mu\text{m}$ から $1.9 \mu\text{m}$ までの範囲にあり、漸变的に短くなっていった(図3 B)。

腕比は、41個の染色体のうち、第6、16、23、26、28、34番目の6個の染色体が1.8から3.0までの範囲にあり、動原体は次中部に位置していた。残りの35個の染色体が1.0から1.7までの範囲にあり、動原体は中部に位置していた。

第28、34番目の2個の染色体は短腕の末端部に小さな付随体をもっていた。

以上の観察において、 $2n = 41$ 個の染色体は、相同染色体として形態的に対応する染色体が、少ないことがわかる。このことから、本雑種は核型的に不揃いな高異数体であるといえる。

C. 属間雑種 (*Bifrenaria harrisoniae* × *Cymbidium Rosalita* 'Dos Pueblos'), $2n = 39$, 図2 C, F, I, 図3 C, 表4。

静止期核は直径が約 $23 \mu\text{m}$ で、染色質は多数の凝縮部を形成していた。凝縮部は形や大きさが不揃いで、前記の2種と同様に複雑染色中央粒型であった(図2 C)。

前期染色体において、早期凝縮部は *Cymbidium* と同様に末端寄り、基部寄り、介在位の各部で観察された(図2 F)。

観察した個体は、染色体数が $2n = 39$ であった(図2 I)。分裂期中期染色体について厚さを測定し、その結果を表4に示した。

39個の染色体は、第1番目から第38番目までの漸变的に短くなる38個の大型染色体と、第39番目の1個の小型染色体とで構成されていた(図3 C)。

38個の大型染色体は長さが $3.9 \mu\text{m}$ から $2.0 \mu\text{m}$ までの範囲にあった。これらのうち第1-10、12、22-24、26、29、30、33-38番目の24個の染色体は、腕比が1.0から1.7までの範囲にあり、動原体は中部に位置していた。第11、13-15、17-21、25、27、28、31、32番目の14個の染色体は、腕比が1.8から2.5までの範囲にあり、動原体は次中部に位置していた。

最小の染色体は、長さが $1.5 \mu\text{m}$ 、腕比が2.0であって、動原体は次中部に位置していた。この小



Fig. 3. Metaphase chromosomes of *Bifrenaria harrisoniae* ($2n=38$, A), *Cymbidium Rosalita* 'Dos Pueblos' ($2n=41$, B) and intergeneric hybrid ($2n=39$, C). $\times 2400$.

型染色体は短腕の末端部に極く小さい付随体をもっていた。

以上の観察結果から、本属間雑種は、極めて異質な染色体で構成されていることがわかる。小型染色体は、*Bifrenaria* がもつ2個の小型染色体と同様の形態であった。

考 察

本属間雑種は、偽球茎の大きさ、葉の厚さにおいて両親より少ない数値を示すが、気孔数、孔辺細胞の大きさ、葉の長さはすべて両親の中間的値におよそ相当する。葉の厚膜細胞は、*Bifrenaria* では葉脈の上部に強く発達しており、*Cymbidium* では葉脈の下部と表皮内側の柔組織内に発達しているが、属間雑種では葉脈の上下と表皮内側の柔組織内のいずれにおいても観察される。しかし、属間雑種のこれらの細胞の発達の程度は両親より弱い。

以上のごとく、本属間雑種の外部形態はおよそ両親の中間形である。

Cymbidium Rosalita は漸变的に短くなる41個の染色体をもち、動原体が次中部に位置する6個の染色体をもつ。これに対して、*Bifrenaria* は染色体長においては、*Cymbidium* と同様に漸变的に短くなる染色体を36個もつが、最小の染色体の2個(小型染色体)がその大きさ、次中部動原体、付随体をもつなどの点で特にマークされる。また、38個の染色体のうち、動原体が次中部もしくは次端部に位置している染色体は26個あり、*Cymbidium* に比べて平均的に高い腕比を示す。属間雑種は、 $2n=39$ 個の染色体数をもち、これは *Bifrenaria* の半数染色体数 $n=19$ と、*Cymbidium* の半数染色体数の20と21のうちの正常染色体組 $n=20$ とが結合したものである。さらにその核型は、*Bifrenaria* に見られる小型染色体と同じ形態の小型染色体を1個含み、また、動原体が次中部に位置する染色体は15個あり、その

数は両親の中間的である。

以上の外部形態と核型の観察結果から、本属間雑種は *Bifrenaria* の染色体の半数 $n = 19$ 個と, *Cymbidium* の染色体の半数 $n = 20$ 個とで構成される雑種第一代であることがわかる。

Schlechter (1926) および Dressler & Dodson (1960) によると *Bifrenaria* はマキシラリア亜連 (Maxillariinae) に, *Cymbidium* はシンビジウム亜連 (Cymbidiinae) に属している。分類学的にこのような遠縁な位置にある両属が交雑能力を持っていることは極めて注目すべきことである。

両属は、染色体数においては $2n = 40$ と $2n = 38$ との相違があり、また、分裂期中期染色体の形態においては、大きさの不均等性、動原体部位、付随体染色体および最小染色体等のマーカー染色体において顕著な相違を示すものであるが、一方、静止期核の染色体の形態においては、両親とその属間雑種とのいずれも同じ複雑染色中央粒型であった。

この事実が示すように、本属間雑種の形成は、田中 (1971b) が指摘した染色質の形態の類似性による遠縁交雑の可能性の一つの実証となるものである。

摘 要

1. *Bifrenaria harrisoniae* ($2n = 38$), *Cymbidium* Rosalita 'Dos Pueblos' ($2n = 41$), およびその属間雑種第一代 ($2n = 39$) の形態学的、解剖学的、および核形態学的比較研究を行った。
2. 外部形態および組織の形態において本属間雑種は両親種の中間形を示した。
3. 本属間雑種の体細胞染色体 $2n = 39$ は, *Bifrenaria* の半数染色体 $n = 19$ と, *Cymbidium* の半数染色体 $n = 20$ との和で構成されており、正常な属間雑種第一代である。
4. 本属間雑種の作出により、田中 (1971b) が指摘した静止期染色体の形態の類似性による *Bifrenaria* と *Cymbidium* との遠縁交雑の可能性が実証された。

Summary

1. Morphological, anatomical and cytological studies were carried out in *Bifrenaria harrisoniae* ($2n=38$), *Cymbidium* Rosalita 'Dos Pueblos' ($2n=41$) and its intergeneric hybrid (*Bifrenaria* × *Cymbidium*, $2n=39$).
2. The external and anatomical morphology of the intergeneric hybrid was found to be intermediate between parental species.
3. The $2n=39$ chromosomes of the intergeneric hybrid were consisted of the $n=19$ chromosomes of *Bifrenaria* and the $n=20$ chromosomes of *Cymbidium*. It could be concluded that the intergeneric hybrid investigated was a F_1 hybrid.
4. The occurrence of this intergeneric hybrid substantiated the hypothesis proposed karyomorphologically by Tanaka (1971 b) that the two genera, *Cymbidium* and *Bifrenaria*, could be crossed.

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Table 2. Measurements of somatic chromosomes of *Bifrenaria harrisoniae* at metaphase, $2n=38$

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	$1.3 + 1.8 = 3.1$	3.2	1.4	m
2	$1.1 + 1.9 = 3.0$	3.1	1.7	m
3	$1.0 + 1.9 = 2.9$	3.0	1.9	sm
4	$1.0 + 1.8 = 2.8$	2.9	1.8	sm
5	$1.1 + 1.8 = 2.9$	3.0	1.6	m
6	$1.1 + 1.6 = 2.7$	2.8	1.5	m
7	$0.9 + 1.9 = 2.8$	2.9	2.1	sm
8	$0.9 + 1.9 = 2.8$	2.9	2.1	sm
9	$0.9 + 1.9 = 2.8$	2.9	2.1	sm
10	$0.9 + 1.9 = 2.8$	2.9	2.1	sm
11	$0.9 + 1.9 = 2.8$	2.9	2.1	sm
12	$0.8 + 2.0 = 2.8$	2.9	2.5	sm
13	$0.8 + 2.0 = 2.8$	2.9	2.5	sm
14	$0.8 + 2.0 = 2.8$	2.9	2.5	sm
15	$0.9 + 1.8 = 2.7$	2.8	2.0	sm
16	$0.9 + 1.8 = 2.7$	2.8	2.0	sm
17	$0.8 + 1.9 = 2.7$	2.8	2.4	sm
18	$0.8 + 1.8 = 2.6$	2.7	2.3	sm
19	$0.8 + 1.8 = 2.6$	2.7	2.3	sm
20	$0.8 + 1.8 = 2.6$	2.7	2.3	sm
21	$0.6 + 1.9 = 2.5$	2.6	3.2	st
22	$0.6 + 1.9 = 2.5$	2.6	3.2	st
23	$1.1 + 1.4 = 2.5$	2.6	1.3	m
24	$1.1 + 1.3 = 2.4$	2.5	1.2	m
25	$0.8 + 1.6 = 2.4$	2.5	2.0	sm
26	$0.8 + 1.6 = 2.4$	2.5	2.0	sm
27	$0.8 + 1.6 = 2.4$	2.5	2.0	sm
28	$0.8 + 1.5 = 2.3$	2.4	1.9	sm
29	$0.9 + 1.4 = 2.3$	2.4	1.6	m
30	$0.9 + 1.4 = 2.3$	2.4	1.6	m
31	$1.0 + 1.3 = 2.3$	2.4	1.3	m
32	$0.9 + 1.3 = 2.2$	2.3	1.4	m
33	$0.8 + 1.5 = 2.3$	2.4	1.9	sm
34	$0.6 + 1.4 = 2.0$	2.1	2.3	sm
35	$1.0 + 1.1 = 2.1$	2.2	1.1	m
36	$1.0 + 1.0 = 2.0$	2.1	1.0	m
37	$0.5 + 1.1 = 1.6^*$	1.7	2.2	sm
38	$0.5 + 1.1 = 1.6^*$	1.7	2.2	sm

* Chromosome with secondary constriction

Table 3. Measurements of somatic chromosomes of *Cymbidium* Rosalita 'Dos Pueblos' at metaphase, $2n=41$

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	$2.1 + 2.4 = 4.5$	3.4	1.1	m
2	$2.0 + 2.4 = 4.4$	3.3	1.2	m
3	$2.0 + 2.4 = 4.4$	3.3	1.2	m
4	$2.0 + 2.1 = 4.1$	3.1	1.1	m
5	$1.9 + 2.1 = 4.0$	3.0	1.1	m
6	$1.1 + 2.9 = 4.0$	3.0	2.6	sm
7	$1.9 + 2.0 = 3.9$	3.0	1.1	m
8	$1.8 + 2.1 = 3.9$	3.0	1.2	m
9	$1.8 + 1.9 = 3.7$	2.8	1.1	m
10	$1.8 + 1.9 = 3.7$	2.8	1.1	m
11	$1.8 + 1.9 = 3.7$	2.8	1.1	m
12	$1.8 + 1.8 = 3.6$	2.7	1.0	m
13	$1.8 + 1.8 = 3.6$	2.7	1.0	m
14	$1.8 + 1.8 = 3.6$	2.7	1.0	m
15	$1.6 + 2.0 = 3.6$	2.7	1.3	m
16	$1.3 + 2.3 = 3.6$	2.7	1.8	sm
17	$1.6 + 1.8 = 3.4$	2.6	1.1	m
18	$1.5 + 1.9 = 3.4$	2.6	1.3	m
19	$1.4 + 2.0 = 3.4$	2.6	1.4	m
20	$1.5 + 1.8 = 3.3$	2.5	1.2	m
21	$1.5 + 1.8 = 3.3$	2.5	1.2	m
22	$1.4 + 1.8 = 3.2$	2.4	1.3	m
23	$0.8 + 2.4 = 3.2$	2.4	3.0	sm
24	$1.4 + 1.6 = 3.0$	2.3	1.1	m
25	$1.4 + 1.6 = 3.0$	2.3	1.1	m
26	$0.9 + 2.1 = 3.0$	2.3	2.3	sm
27	$1.4 + 1.5 = 2.9$	2.2	1.1	m
28	$0.1 + 0.8 + 2.0 = 2.9^*$	2.2	2.2	sm
29	$1.3 + 1.5 = 2.8$	2.1	1.2	m
30	$1.3 + 1.4 = 2.7$	2.0	1.1	m
31	$1.1 + 1.6 = 2.7$	2.0	1.5	m
32	$1.3 + 1.3 = 2.6$	2.0	1.0	m
33	$1.3 + 1.3 = 2.6$	2.0	1.0	m
34	$0.1 + 0.8 + 1.6 = 2.5^*$	1.9	1.8	sm
35	$1.1 + 1.3 = 2.4$	1.8	1.2	m
36	$1.1 + 1.3 = 2.4$	1.8	1.2	m
37	$1.1 + 1.3 = 2.4$	1.8	1.2	m
38	$0.9 + 1.5 = 2.4$	1.8	1.7	m
39	$1.0 + 1.3 = 2.3$	1.7	1.3	m
40	$0.9 + 1.3 = 2.2$	1.7	1.4	m
41	$0.8 + 1.1 = 1.9$	1.4	1.4	m

* Chromosome with secondary constriction

Table 4. Measurements of somatic chromosomes of intergeneric hybrid (*Bifrenaria* × *Cymbidium*) at metaphase, 2n=39

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	1.9 + 2.0 = 3.9	3.7	1.1	m
2	1.6 + 2.0 = 3.6	3.4	1.3	m
3	1.5 + 2.0 = 3.5	3.4	1.3	m
4	1.4 + 1.8 = 3.2	3.1	1.3	m
5	1.4 + 1.8 = 3.2	3.1	1.3	m
6	1.5 + 1.5 = 3.0	2.9	1.0	m
7	1.5 + 1.5 = 3.0	2.9	1.0	m
8	1.4 + 1.6 = 3.0	2.9	1.1	m
9	1.4 + 1.5 = 2.9	2.8	1.1	m
10	1.1 + 1.8 = 2.9	2.8	1.6	m
11	0.9 + 2.0 = 2.9	2.8	2.2	m
12	1.3 + 1.5 = 2.8	2.7	1.2	m
13	1.0 + 1.8 = 2.8	2.7	1.8	sm
14	1.0 + 1.8 = 2.8	2.7	1.8	sm
15	0.8 + 2.0 = 2.8	2.7	2.5	sm
16	1.1 + 1.6 = 2.7	2.6	1.5	m
17	0.9 + 1.8 = 2.7	2.6	2.0	sm
18	0.9 + 1.8 = 2.7	2.6	2.0	sm
19	0.9 + 1.8 = 2.7	2.6	2.0	sm
20	0.8 + 1.9 = 2.7	2.6	2.4	sm
21	0.8 + 1.9 = 2.7	2.6	2.4	sm
22	1.1 + 1.5 = 2.6	2.5	1.4	m
23	1.1 + 1.5 = 2.6	2.5	1.4	m
24	1.0 + 1.6 = 2.6	2.5	1.6	m
25	0.8 + 1.8 = 2.6	2.5	2.3	sm
26	1.1 + 1.4 = 2.5	2.4	1.3	m
27	0.9 + 1.6 = 2.5	2.4	1.8	sm
28	0.9 + 1.6 = 2.5	2.4	1.8	sm
29	1.1 + 1.3 = 2.4	2.3	1.2	m
30	0.9 + 1.5 = 2.4	2.3	1.7	m
31	0.8 + 1.6 = 2.4	2.3	2.0	sm
32	0.8 + 1.6 = 2.4	2.3	2.0	sm
33	1.0 + 1.3 = 2.3	2.2	1.3	m
34	1.1 + 1.1 = 2.2	2.1	1.0	m
35	0.9 + 1.3 = 2.2	2.1	1.4	m
36	1.0 + 1.1 = 2.1	2.0	1.1	m
37	1.0 + 1.1 = 2.1	2.0	1.1	m
38	1.0 + 1.0 = 2.0	1.9	1.0	m
39	0.5 + 1.0 = 1.5*	1.4	2.0	sm

* Chromosome with secondary constriction

Paphiopedilum 属 3 種の核形態学的研究

唐澤 耕 司 ・ 青 山 幹 男

Karyomorphological studies on three species of *Paphiopedilum*

Kohji Karasawa and Mikio Aoyama

Paphiopedilum 属の核形態学的研究は Karasawa (1979) により、53種 5 亜種 1 変種 1 品種について報告されている。

今回新たに未報告の 3 種について核形態学的観察を行ったので報告する。

研究方法は Karasawa (1979) に準じた。

観 察 結 果

1 *Paphiopedilum elliotianum* (O'Brien)

Fowl., $2n = 26$

本種はフィリップピンで採集されたものである。

観察した個体の染色体数は $2n = 26$ であった。

前中期染色体は図 1 A, 図 2 A-B に示した。

前中期染色体 (図 2 B) の長さ動原体の位置は表 1 に示した。

本種の 26 個の前中期染色体のうち、第 1 番目から第 4 番目までの 4 個の染色体は長さが $18.1\mu\text{m}$ から $16.1\mu\text{m}$ までの範囲にあって、他の染色体に比べて明らかに長く、腕比が 1.2 から 1.3 までの範囲にあり、動原体は中部に位置していた。

残りの 22 個の染色体は、長さが $15.3\mu\text{m}$ から $5.6\mu\text{m}$ までの範囲にあり漸次短くなっていた。第 5 番目から第 10 番目までと、第 13 番目から第 26 番目までの 20 個の染色体は、腕比が 1.0 から 1.7 までの範囲にあり、動原体は中部に位置していた。第 11 番目と第 12 番目の染色体は、腕比がそれぞれ 1.8 と 1.9 あり、動原体は次中部に位置していた。

以上のごとく、本種の 26 個の染色体は、4 個の

大型染色体と、漸变的に短くなっている 22 個の小型染色体とから構成される二様相核型を示していた。

本種では早い時期の前中期染色体 (図 2 A) において、第 19, 20 番目の染色体の長腕の中部、および第 21, 22 番目の染色体の長腕の次端部に小狭窄が観察された。

本種の核型は、*P. rothschildianum* ($2n = 26$) の染色体の動原体がいずれも中部に位置しているのに比べ、第 11, 12 番目の 2 個の染色体の動原体が次中部に位置している点で明らかに区別できる。

2 *Paphiopedilum victoria-mariae* (Rolfe)

Hook. f., $2n = 30$

本種はスマトラで採集された。

観察した個体の染色体数は $2n = 30$ であった。

前中期染色体は図 2 C に、中期染色体は図 1 B, 図 2 D に示した。中期染色体の長さ動原体の位置は表 2 に示した。

本種の 30 個の染色体のうち、第 1 番目から第 20 番目までの 20 個の染色体は長さが $10.3\mu\text{m}$ から $4.8\mu\text{m}$ までの範囲にあり漸次短くなっていた。これらの染色体の腕比は 1.0 から 1.6 までの範囲にあり、動原体はいずれも中部に位置していた。

第 21, 22 番目の 2 個の染色体は長さがそれぞれ $5.5\mu\text{m}$ と $5.3\mu\text{m}$ で第 23 番目以下の染色体に比べて明らかに長く、動原体は頂端部に位置していた。

第 23 番目から第 30 番目までの 8 個の染色体は長さが $3.8\mu\text{m}$ から $3.5\mu\text{m}$ までの範囲にあって漸次

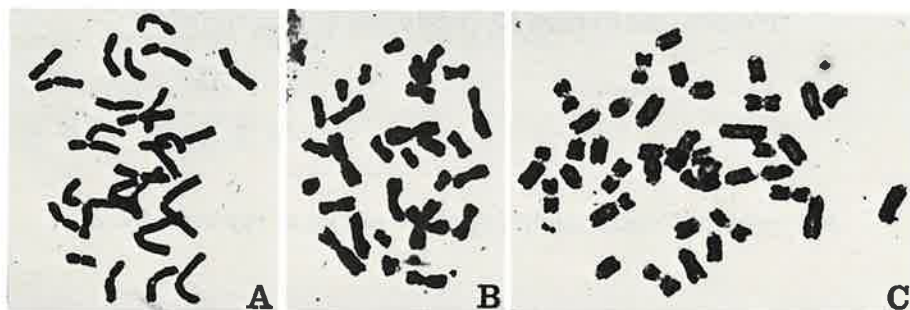


Fig. 1. Chromosomes of *P. elliotianum* (A, prometaphase, $2n=26$), *P. victoria - mariae* (B, metaphase, $2n=30$) and *P. linii* (C, metaphase, $2n=40$) $\times 720$.

短くなっており、動原体は頂端部に位置していた。

前中期において、第1, 2, 9-30番目の24個の染色体の動原体寄りに小狭窄が観察された(図2C)。

以上のごとく、本種は *Cochlopetalum* 節では最も少ない染色体数を持ち、 $2n = 30$ 個の染色体は20個の中部動原体的染色体と10個の頂端部動原体的染色体とで構成されていた。

この頂端部動原体的染色体2個を中部動原体的染色体1個に換算すると、その合計は25個の中部動原体的染色体となる。これは Karasawa (1979) の指摘したように、動原体切断により生じた頂端部動原体的染色体の1対が減少したものと考えられる。

3 *Paphiopedilum linii* Schoser., $2n = 40$

本種はボルネオで採集されたものである。

観察した個体の染色体数は $2n = 40$ であった。

中期染色体は図1C, 図2Eに示した。中期染色体の長さや動原体の位置は表3に示した。

本種の40個の染色体のうち、第1番目と第2番目の2個の染色体は長さがそれぞれ $8.8\mu\text{m}$ と $8.5\mu\text{m}$ あり、腕比がそれぞれ2.1と2.4で、動原体は次中部に位置していた。

第3番目から第12番目までの10個の染色体は長さが $7.8\mu\text{m}$ から $5.3\mu\text{m}$ までの範囲にあり漸次短かくなっていた。これらの染色体の腕比は1.0から1.3までの範囲にあり、動原体はいずれも中部に位置していた。

第13番目から第16番目までの4個の染色体は長

さが $9.0\mu\text{m}$ から $8.3\mu\text{m}$ までの範囲にあり、第17番目以下の染色体に比べ明らかに長く、動原体は頂端部に位置していた。

第17番目から第40番目までの24個の染色体は長さが $7.3\mu\text{m}$ から $3.5\mu\text{m}$ までの範囲にあって漸次短かくなっており、動原体はいずれも頂端部に位置していた。

以上のごとく、本種の $2n = 40$ 個の染色体は12個の中部動原体的染色体と28個の頂端部動原体的染色体とで構成されていた。

本種の核型は、*P. bullenianum* ($2n = 40$) の核型とよく一致し、核形態学的に両種は区別できない。

要 約

1. *Paphiopedilum* 属3種の核形態学的研究を行い、新たに *P. elliotianum* $2n = 26$, *P. victoria-mariae* $2n = 30$, *P. linii* $2n = 40$ の染色体数を算定した。
2. *P. elliotianum* の核型は、第11, 12番目の2個の染色体において動原体が次中部に位置する点で、*P. rothschildianum* の核型と異なる。
3. *P. victoria - mariae* は *Cochlopetalum* 節では最も少ない染色体数を持ち、その構成は $2n = 30 = 20V + 10I = 25V$ で、Karasawa (1979) が指摘した本節特有の切断様式に一致する。
4. *P. linii* と *P. bullenianum* の核型はよく一致し、核形態学的に両種は区別できない。



Fig. 2. Karyotype of *P. elliotianum* (A, B, prometaphase. $2n=26$), *P. victoria-mariae* (C, prometaphase. D, metaphase. $2n=30$) and *P. linii* (E, metaphase. $2n=40$) $\times 1200$.

Summary

1. The chromosome numbers of 3 species (*P. elliotianum* $2n=26$, *P. victoria-mariae* $2n=30$, *P. linii* $2n=40$) were recorded for the first time.
2. The karyotype of *P. elliotianum* was clearly different from *P. rothschildianum*. Two submetacentric chromosomes (Nos. 11, 12) of *P. elliotianum* were the marked chromosomes of the chromosome complement of this species.
3. The chromosome numbers of *P. victoria-mariae* $2n=30$ was the least in the species of Section Cochlopetalum, and could be corresponded 25 metacentric chromosomes by con-

verting every two telocentric chromosomes into one metacentric chromosomes. It was confirmed that the species of Section Cochlopetalum was derived from the $2n=26$ species by the loss of a pair of telocentric chromosomes (Karasawa 1979).

4. The karyotype of *P. linii* was extremely similar to that of *P. bullenianum*, and it supported that both taxa were conspecific.

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- Karasawa, K. 1979. Daryomorphological studies in *Paphiopedilum*, Orchidaceae. Bull. of the Hiroshima Botanical Garden 2 : 1-149.

Table 1. Measurements of somatic chromosomes of *Paphiopedilum elliotianum* at prometaphase, $2n=26$

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	$8.3 + 9.8 = 18.1$	6.4	1.2	m
2	$7.8 + 9.8 = 17.6$	6.2	1.3	m
3	$7.5 + 8.8 = 16.3$	5.7	1.2	m
4	$7.3 + 8.8 = 16.1$	5.6	1.2	m
5	$6.8 + 8.5 = 15.3$	5.4	1.3	m
6	$5.8 + 7.8 = 13.6$	4.8	1.3	m
7	$5.8 + 7.3 = 13.1$	4.6	1.3	m
8	$5.5 + 7.3 = 12.8$	4.5	1.3	m
9	$4.8 + 6.0 = 10.8$	3.8	1.3	m
10	$4.0 + 6.3 = 10.3$	3.6	1.6	m
11	$3.8 + 7.0 = 10.8$	3.8	1.8	sm
12	$3.5 + 6.5 = 10.0$	3.5	1.9	sm
13	$4.3 + 5.5 = 9.8$	3.4	1.3	m
14	$3.8 + 5.8 = 9.6$	3.4	1.5	m
15	$4.8 + 4.8 = 9.6$	3.4	1.0	m
16	$4.8 + 4.8 = 9.6$	3.4	1.0	m
17	$3.5 + 6.0 = 9.5$	3.3	1.7	m
18	$3.5 + 6.0 = 9.5$	3.3	1.7	m
19	$4.0 + 5.0 = 9.0$	3.2	1.3	m
20	$3.3 + 5.5 = 8.8$	3.1	1.7	m

Table 1. (continued)

21	$3.5 + 5.3 = 8.8$	3.1	1.5	m
22	$3.3 + 4.5 = 7.8$	2.7	1.4	m
23	$4.0 + 4.0 = 8.0$	2.8	1.0	m
24	$3.3 + 4.5 = 7.8$	2.7	1.4	m
25	$2.8 + 4.0 = 6.8$	2.4	1.4	m
26	$2.8 + 2.8 = 5.6$	2.0	1.0	m

Table 2. Measurements of somatic chromosomes of *Paphiopedilum victoria-mariae* at metaphase, $2n=30$

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	$4.3 + 6.0 = 10.3$	5.5	1.4	m
2	$4.8 + 5.3 = 10.1$	5.4	1.1	m
3	$4.5 + 5.0 = 9.5$	5.1	1.1	m
4	$4.5 + 5.0 = 9.5$	5.1	1.1	m
5	$4.3 + 5.0 = 9.3$	5.0	1.2	m
6	$4.0 + 4.5 = 8.5$	4.6	1.1	m
7	$3.5 + 4.5 = 8.0$	4.3	1.3	m
8	$3.3 + 4.0 = 7.3$	3.9	1.2	m
9	$3.0 + 4.8 = 7.8$	4.2	1.6	m
10	$2.8 + 4.5 = 7.3$	3.9	1.6	m
11	$3.3 + 4.0 = 7.3$	3.9	1.2	m
12	$3.0 + 3.8 = 6.8$	3.7	1.3	m
13	$3.0 + 3.8 = 6.8$	3.7	1.3	m
14	$3.0 + 3.5 = 6.5$	3.5	1.2	m
15	$2.8 + 3.0 = 5.8$	3.1	1.1	m
16	$2.5 + 2.8 = 5.3$	2.9	1.1	m
17	$2.5 + 2.8 = 5.3$	2.9	1.1	m
18	$2.5 + 2.5 = 5.0$	2.7	1.0	m
19	$2.5 + 2.5 = 5.0$	2.7	1.0	m
20	$2.3 + 2.5 = 4.8$	2.6	1.0	m
21	$d + 5.5 = 5.5$	3.0	1.1	t
22	$d + 5.3 = 5.3$	2.9	$<\infty$	t
23	$d + 3.8 = 3.8$	2.0	$<\infty$	t
24	$d + 3.8 = 3.8$	2.0	$<\infty$	t
25	$d + 3.5 = 3.5$	1.9	$<\infty$	t
26	$d + 3.5 = 3.5$	1.9	$<\infty$	t
27	$d + 3.5 = 3.5$	1.9	$<\infty$	t
28	$d + 3.5 = 3.5$	1.9	$<\infty$	t
29	$d + 3.5 = 3.5$	1.9	$<\infty$	t
30	$d + 3.5 = 3.5$	1.9	$<\infty$	t

Table 3. Measurements of somatic chromosomes of *Paphiopedilum linii* at metaphase, $2n=40$

Chromosome	Length (μm)	Relative length	Arm ratio	Form
1	$2.8 + 6.0 = 8.8$	3.6	2.1	sm
2	$2.5 + 6.0 = 8.5$	3.4	2.4	sm
3	$3.8 + 4.0 = 7.8$	3.2	1.1	m
4	$3.8 + 3.8 = 7.6$	3.1	1.0	m
5	$3.3 + 3.8 = 7.1$	2.9	1.2	m
6	$3.3 + 3.5 = 6.8$	2.8	1.1	m
7	$3.3 + 3.5 = 6.8$	2.8	1.1	m
8	$3.3 + 3.3 = 6.6$	2.7	1.0	m
9	$2.8 + 3.0 = 5.8$	2.3	1.1	m
10	$2.8 + 3.0 = 5.8$	2.3	1.1	m
11	$2.5 + 3.3 = 5.8$	2.3	1.3	m
12	$2.5 + 2.8 = 5.3$	2.1	1.1	m
13	$d + 9.0 = 9.0$	3.6	$<\infty$	t
14	$d + 9.0 = 9.0$	3.6	$<\infty$	t
15	$d + 8.5 = 8.5$	3.4	$<\infty$	t
16	$d + 8.3 = 8.3$	3.4	$<\infty$	t
17	$d + 7.3 = 7.3$	3.0	$<\infty$	t
18	$d + 7.3 = 7.3$	3.0	$<\infty$	t
19	$d + 7.0 = 7.0$	2.8	$<\infty$	t
20	$d + 6.5 = 6.5$	2.6	$<\infty$	t
21	$d + 6.3 = 6.3$	2.6	$<\infty$	t
22	$d + 5.8 = 5.8$	2.3	$<\infty$	t
23	$d + 5.5 = 5.5$	2.2	$<\infty$	t
24	$d + 5.5 = 5.5$	2.2	$<\infty$	t
25	$d + 5.5 = 5.5$	2.2	$<\infty$	t
26	$d + 5.5 = 5.5$	2.2	$<\infty$	t
27	$d + 5.3 = 5.3$	2.1	$<\infty$	t
28	$d + 5.3 = 5.3$	2.1	$<\infty$	t
29	$d + 5.3 = 5.3$	2.1	$<\infty$	t
30	$d + 5.3 = 5.3$	2.1	$<\infty$	t
31	$d + 5.3 = 5.3$	2.1	$<\infty$	t
32	$d + 5.3 = 5.3$	2.1	$<\infty$	t
33	$d + 5.0 = 5.0$	2.0	$<\infty$	t
34	$d + 5.0 = 5.0$	2.0	$<\infty$	t
35	$d + 4.8 = 4.8$	1.9	$<\infty$	t
36	$d + 4.8 = 4.8$	1.9	$<\infty$	t
37	$d + 4.3 = 4.3$	1.7	$<\infty$	t
38	$d + 4.3 = 4.3$	1.7	$<\infty$	t
39	$d + 3.8 = 3.8$	1.5	$<\infty$	t
40	$d + 3.5 = 3.5$	1.4	$<\infty$	t

d : dot

スズフリエビネの受粉機構の観察

唐澤耕司・石田源次郎

Histological observation on the pollination of
Calanthe lyroglossa Reichb.f.

Kohji Karasawa and Genjiro Ishida

スズフリエビネは、わが国南西諸島から台湾、フィリピン、ビルマにかけて分布するやや大型の地生ランで、3月中旬ごろ約40~50cmの花茎を伸ばし、30~50個の黄色花をつける。花被は、半開もしくは全く展開しないが、天然で多数の果実をつけることが知られている(図1)。しかし、この機構は未だ明らかでない。

今回、スズフリエビネの受粉の機構を明らかにするため観察を行ったので、その結果を報告する。

材料および方法

本研究に用いたスズフリエビネ3株は、鹿児島県奄美大島で採集し、鉢に植え、広島市植物公園栽培温室で栽培し開花させたものである。柱頭及び花粉

塊の形態観察は、実体顕微鏡下で行い、組織はアルコール・氷酢酸・ホルマリン混液で固定したのち、常法のパラフィン法により切片を作成し、トルイジンブルーまたはデラフィールド・ヘマトキシリンで染色し検鏡に供した。

結 果

エビネ属では一般に葯床は洞穴状に凹み、その下面がくちばし体となり、花粉塊と柱頭とを隔てているが、スズフリエビネでは、くちばし体が欠除しており、花粉塊と柱頭が隣接していた(図2)。また、花粉塊は8小塊から成りたっていたが、粘着盤を持っていなかった(図3)。

柱頭部分を小花の発達段階を追って観察すると、

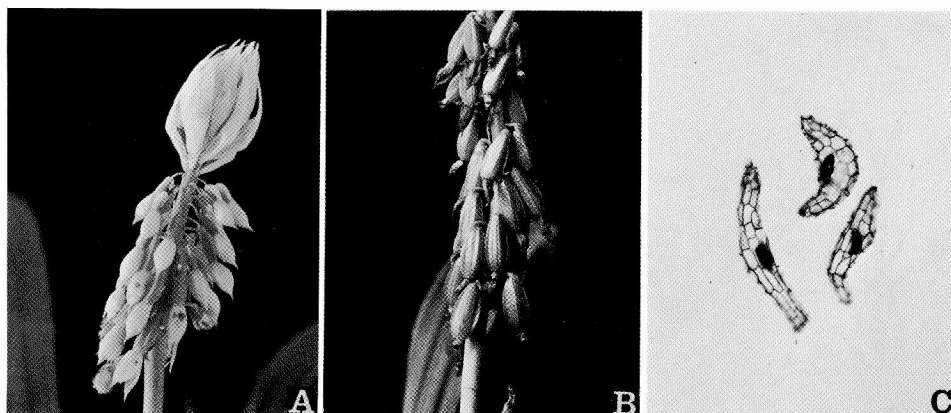


図1. スズフリエビネ *C. lyroglossa* A. 花(×0.6) B. 果実(×0.6) C. 種子(×90)

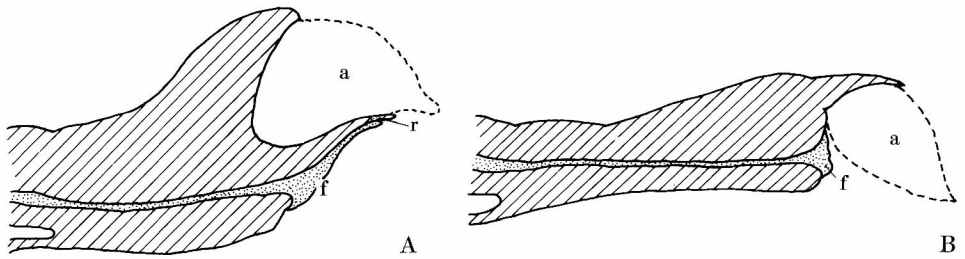


図2. ずい柱縦断面(模式) A. エビネ *C. discolor*, B. スズフリエビネ *C. lyroglossa*, a: 葯帽, f: 柱頭粘液, r: くちばし体。

子房長が4.8mm以下のものでは、花粉塊と柱頭粘液の間に隔たりがあったが、子房長が5.0mmのものでは、粘液が増大し花粉塊に達するのが見られた(図4)。この粘液が増大し花粉塊に達するころ、それまで花粉塊を完全に包み込んでいた葯帽の下面に亀裂が生じ、なかの花粉塊が現われた。子房長7.0mmの段階では、花粉塊のほぼ半分が粘液に埋った状態となっており、コットンブルー染色液で染色すると、粘液表面に無数の花粉管が伸長しているのが観察された。この段階のずい柱の切片を作成し、検鏡したところ柱頭粘液に接した花粉は、発芽し多数の花粉管を子房に向けて伸長させていた(図5)。

本種では、小花の発達にしたがい子房の花茎に対する角度が、 $0 \sim 180^\circ$ 、すなわち上向きから下向きへと変化するが、角度がほぼ直角となる時期の子房長は、5.2~5.8mmであり、この時期は、柱頭粘液が花粉塊に達するころとほぼ一致していた。

開花時における子房長は、8.2から8.6mmの範囲内にあった。

子房長が4.5mm以下の小花から花粉塊を除去したものは、すべて子房が発達せずに落下した。

花粉母細胞における四分体形成は正常に行われており、また、成熟果実から得た種子は、胚を持ち完全な形態を示していた。

摘 要

以上のとおりスズフリエビネでは、くちばし体が欠除しているため、増大した柱頭粘液が直接花粉塊に達することがみられた。柱頭粘液が花粉塊に達する時期は、外観的には子房が花茎に対しほぼ直角となる段階でとらえることができる。受粉が花被の展開前に始まり、開花時にはすでに完了し、花粉管がずい柱内を伸長していたこと及び花粉塊が粘着盤を持っていないことから、本種では昆虫による受粉は行われず、柱頭粘液の増大による自家受粉のみを行っていると考えられる。

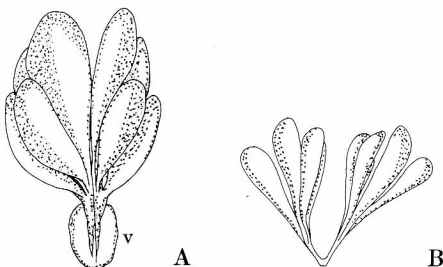


図3. 花粉塊($\times 10$) A. エビネ *C. discolor*, B. スズフリエビネ *C. lyroglossa*, v: 粘着盤。

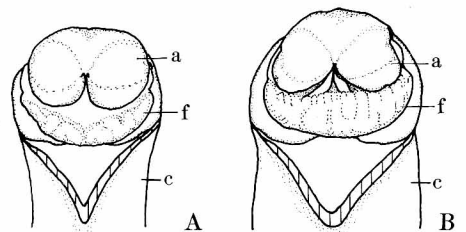


図4. スズフリエビネ *C. lyroglossa* の柱頭($\times 8$) A. 子房長3.0mmの時期, B. 子房長7.0mmの時期, a: 葯帽, f: 柱頭粘液, c: ずい柱

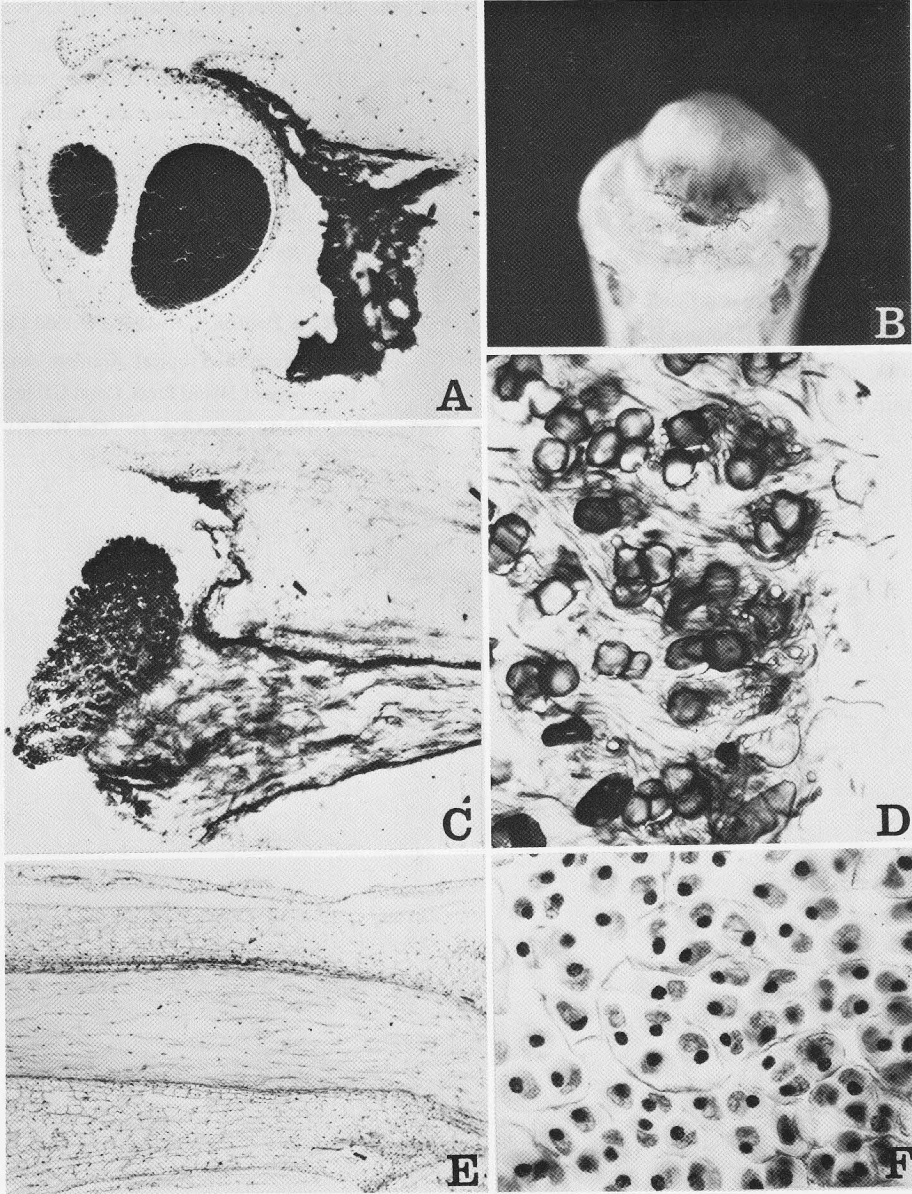


図5. スズフリエビネ *C. lyroglossa* A. 柱頭縦断面, 子房長 4.0 mm ($\times 180$) B. 柱頭, 子房長 7.0 mm ($\times 12$) C. 柱頭縦断面, 子房長 7.0 mm ($\times 180$) D. C. の拡大花粉塊と粘液接触部分 ($\times 480$) E. ずい柱縦断面, 子房長 7.0 mm ($\times 120$) F. 四分子 ($\times 160$)

Summary

Pollination mechanism of *Calanthe lyroglossa* was observed.

The stigma of this species lacked the rostellum and was close to anther which contained eight pollinia lacking the viscidium with the development of the floret, stigmatic fluid became so abundant that it reached the pollinia. That is, the pollination of this species is self-pollination by the abundance of stigmatic fluid, like *Vanilla*. The pollination completed before the opening of the periantha, and the pollen tube grew down in the column to the ovule at the time of blooming.

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