Karyotypes of Paphiopedilum Species of Thailand[1]

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Species of the genus *Paphiopedilum*, commonly known as Cypripediums or Lady Slippers, are distributed south of the Himalayas along the Indo-Malayan region to the Philippines. About 9 species are known to occur in Thailand. Also a few putative natural hybrids have been recorded, but their taxonomic relationships are not clearly defined (Seidenfaden and Smitinand, 1959).

The chromosomes of the Paphiopedildums are relatively favorable for cytological examinations because of their large size and strong affinity for stains. Duncan and MacLeod (1948a, 1948b, 1950) examined several species including some native species of Thailand and constructed idiograms illustrating the similarities and differences of chromosome complements. Since a comparison of karyotypes often provides an insight into species relationships and evolution the present investigation was initiated to determine whether karyotype analysis employing a slightly modified technique from that of Duncan and MacLeod might shed some light on the phylogenetic relationships of the *Paphiopedilum* species of Thailand.

MATERIALS AND METHODS

The karyotypes of 8 species, *P. bellatulum*, *P. concolor*, *P. niveum*, *P. godefroyae*, *P. exul*, *P. villosum*, *P. parishii* and *P. callosum* were analyzed following the method described earlier by Shindo and Kamemoto (1963). Active root tips were severed and pretreated for 5 to 6 hours in .002 M 8 oxyquinoline, fixed in 1 : 1 : 2 mixture of chloroform, 95% ethyl alcohol, and glacial acetic acid for about 18 hours, hydrolyzed in 1 : 1 mixture of 95% ethyl alcohol and concentrated hydrochloric acid for 5 minutes, transferred to 45% acetic acid for at least 10 minutes, and squashed and stained in 1% acetoorcein.

Photomicrographs were taken of the best plates, and the negatives were projected to give a magnification of 4800X. Individual chromosomes were carefully traced, measured in millimeters, and cut out and arranged in descending order of length.

The length of the chromosomes was expressed as the mean, range, and ratio of the longest and shortest chromosomes of the complement. The morphology was expressed as the Mean F % derived as the mean percentage of the short arm length over the total length of each chromosome.

OBSERVATIONS

External morphology

A brief description of the *Paphiopedilum* species studied is given below:

P. bellatulum Pfitz. (fig. 1)—Massive flower 5.8 by 5 cm, white with large maroon spots; petals 3.5 by 4 cm; dorsal sepal 4.5 by 3 cm; pouch 1.5 cm across, 2.5 cm long; flower stem short and weak carrying one or two flowers; leaves 14 by 6 cm, dark green mottled with pale green, purple on lower surface.

P. concolor Pfitz. (fig. 2)—Flower 5.3 by 5.3 cm, pale yellow with small purplish spots; petals 3.8 by 2.2 cm; dorsal sepal 3 by 3 cm, broad; pouch 1.5 cm across, 2.5 cm long with small spots on the incurved lateral lobes; staminode dark yellow spotted with purple; flower stems short, 6 cm, bearing 2 to 3 flowers; leaves tasselated, lower surface light purple.

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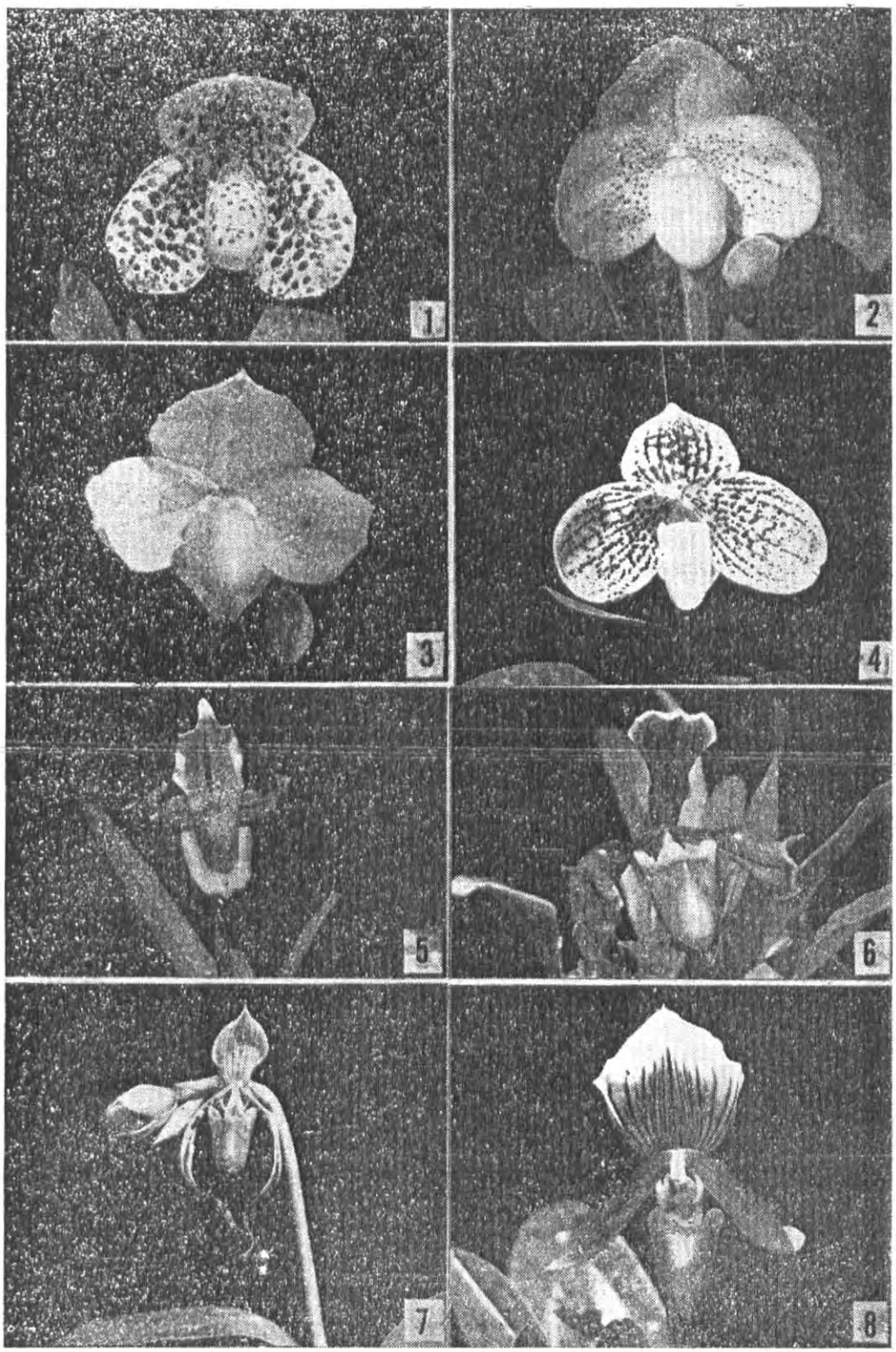

Fig. 1 - 8. *Paphiopedilum* species. 1, *P. bellatulum*. 2, *P. concolor*. 3, *P. niveum*. 4. *P. godefroyae*. 5. *P. exul*. 6. *P. villosum*. 7. *P. parishii*. 8. *P. callosum*.

P. niveum Pfitz. (fig. 3)—Flower 6.5 by 5 cm, white stippled with purple near the base; petals 3.5 by 2.3 cm; dorsal sepal 3 by 3.5 cm; pouch 1.6 cm across, 3 cm long, white with minute purplish spots; staminode lemon yellow; flower stem relatively long, 17 cm, often carrying 2 flowers; leaves 10 by 3 cm, mottled, with purple on lower surface.

P. godfroyae Pfitz. (fig. 4)—Flower 7.5 by 6 cm, pale yellow with spots of variable size some of which are fused; petals 4.5 by 3 cm; dorsal sepal 3 by 3 cm; pouch 1.5 cm across, 2.5 cm long, minute spots on incurved lobes; staminode spotted with purple; flower stem short, 2.5 cm, bearing 1 or 2 flowers; leaves 9 by 2.5 cm, mottled, purple on lower surface.

P. exul Pfitz. (fig 5)—Flower 5 by 6.5 cm; petals 4.5 by 1.2 cm, narrow, curved forward, sides undulating, brownish yellow with brown mid-rib, hairy towards base; dorsal sepal 4.3 by 2.7 cm, hooded, white at the periphery, green toward base and center, spotted with purplish black; pouch 1.6 cm across, brownish purple; flower stem 20 cm, bearing a single flower; leaves 26 by 2 cm, light green, long and narrow.

P. villosum Pfitz. (fig. 6)—Flower 7.7 by 8.5 cm; petals 5.1 by 2.5 cm, midrib dark purple, the upper half purple while the lower half tasselated and light lavendar, curving forward; dorsal sepal 4.8 by 2.7 cm, upper area greenish with white edge, lower area purple with dark veins; pouch 2.7 cm across, 3.4 cm long, light lavendar; staminode white; flower stem 15 cm bearing a single flower; leaves 38 by 3.5 cm, solid green with purple spots at base; habit epiphytic.

P. parishii Pfitz. (fig. 7)—Flower 7.5 by 11 cm; petals 8.3 by 1 cm, twisted, greenish yellow with dark purple spots near the base, solid purple toward tip, warts with hair on lower edge, at least twice as long as the dorsal sepal; dorsal sepal 3.7 by 2 cm, slightly twisted charyreuse with green stripes; pouch 2 by 2.4 cm, green with purplish tinge; staminode white with green center, notched; leaves 38 by 4 cm, green; habit epiphytic.

P. callosum Pfitz. (fig. 8)—Flower 8 by 8.3 cm; petals 6 by 1.5 cm, green toward base, purple toward tip with dark purple warts, hairy along the edges; dorsal sepal 4.5 by 5.5 cm, sides reflexed, white with purple veins toward, tip, greenish veins toward base; pouch 2.5 cm across, 5 cm long, purplish green; staminode green with lavender tinge; flower stem 23 cm bearing a solitary flower; leaves 23 by 4.5 cm, mottled.

Cytology

The karyotypes of *P. bellatulum*, *P. concolor*, *P. niveum*, and *P. godefroyea* of the Brachypetalum group and *P. exul* and *P. villosum* of the Otopedilum group were remarkably similar (fig. 9-12, 15-20; table 1). The chromosome number was uniformly 2n=26. With the possible exception of one pair, all chromosomes had a mediun or sub-median centromere with a mean F% of about 45. The karyotypes were characterized by two pairs of extremely long chromosomes and the rest in continuous sizes. The longest chromosomes were approximately 3 times the length of the shortest. Satellites were not always evident, but when visible, they were attached to a medium or small-size chromosome.

The chromosome length varied from cell to cell, probably influenced by the degree of penetration of the pre-treatment and the slight variation in the stage of metaphase. Also, the ratio between the longest and shortest chromosome was not constant, and there was as much variation among cells within a species as between species.

Some fine differences among the karyotypes, however, were detectable. Both *P. niveum* and *P. godefroyae* had a pair of relatively small chromosomes with conspicuously low F% of 26 to 32, while the lowest F% of individual chromosomes in *P. bellatulum* and *P. concolor* was about 38. The karyotypes of *P. exul* and *P. villosum* differed from the Brachypetalums in that the satellities were distinctly larger. *P. exul* showed a much more uniform F% than *P. villosum*.

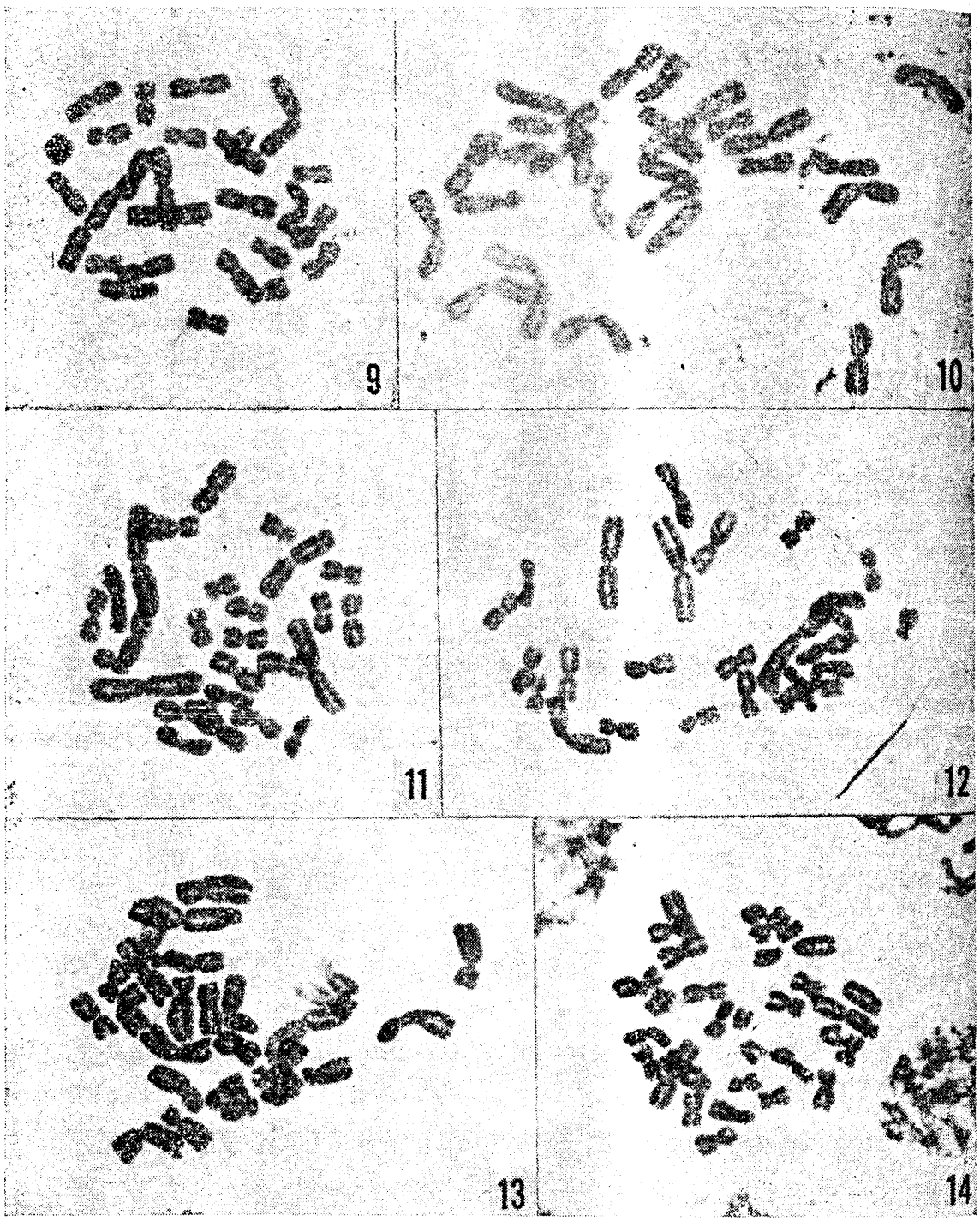

Fig. 9-14. Photomicrographs of somatic metaphase chromosomes of *Paphiopedilum* species, X1500. 9, *P. concolor*, 2n=26. 10, *P. godefroyae*, 2n=26. 11, *P. exul*, 2n=26. 12, *P. villosum*, 2n=26. 13, *P. parishii*, 2n=26. 14, *P. callosum*, 2n=32.

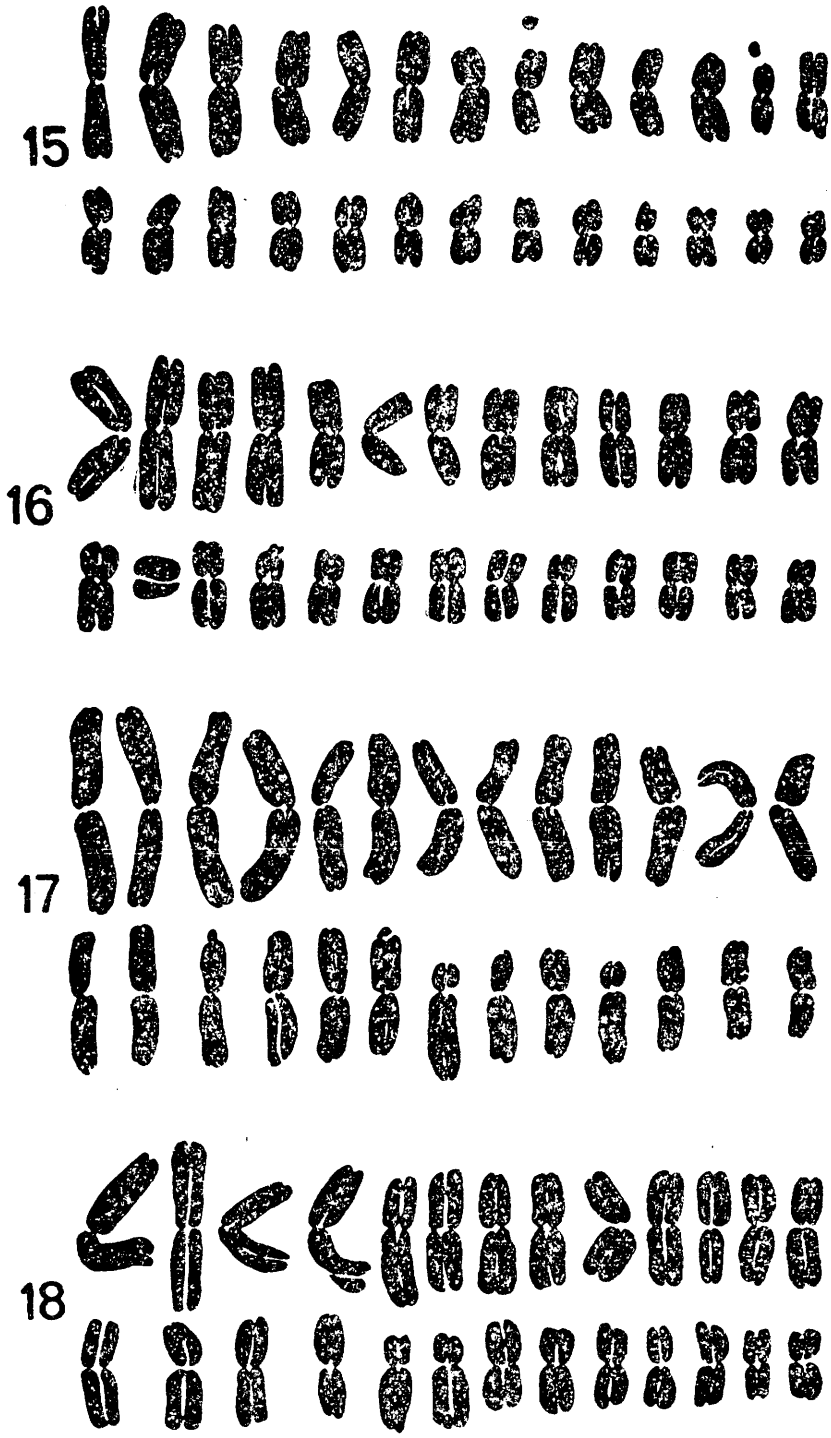

Fig. 15 - 18. Serial arrangement of somatic chromosomes of *Paphiopedium* species. X2,000. 15, *P. bellatulum* 16, *P. concolor.* 17, *P. niveum.* 18, *P. godefroyae.*

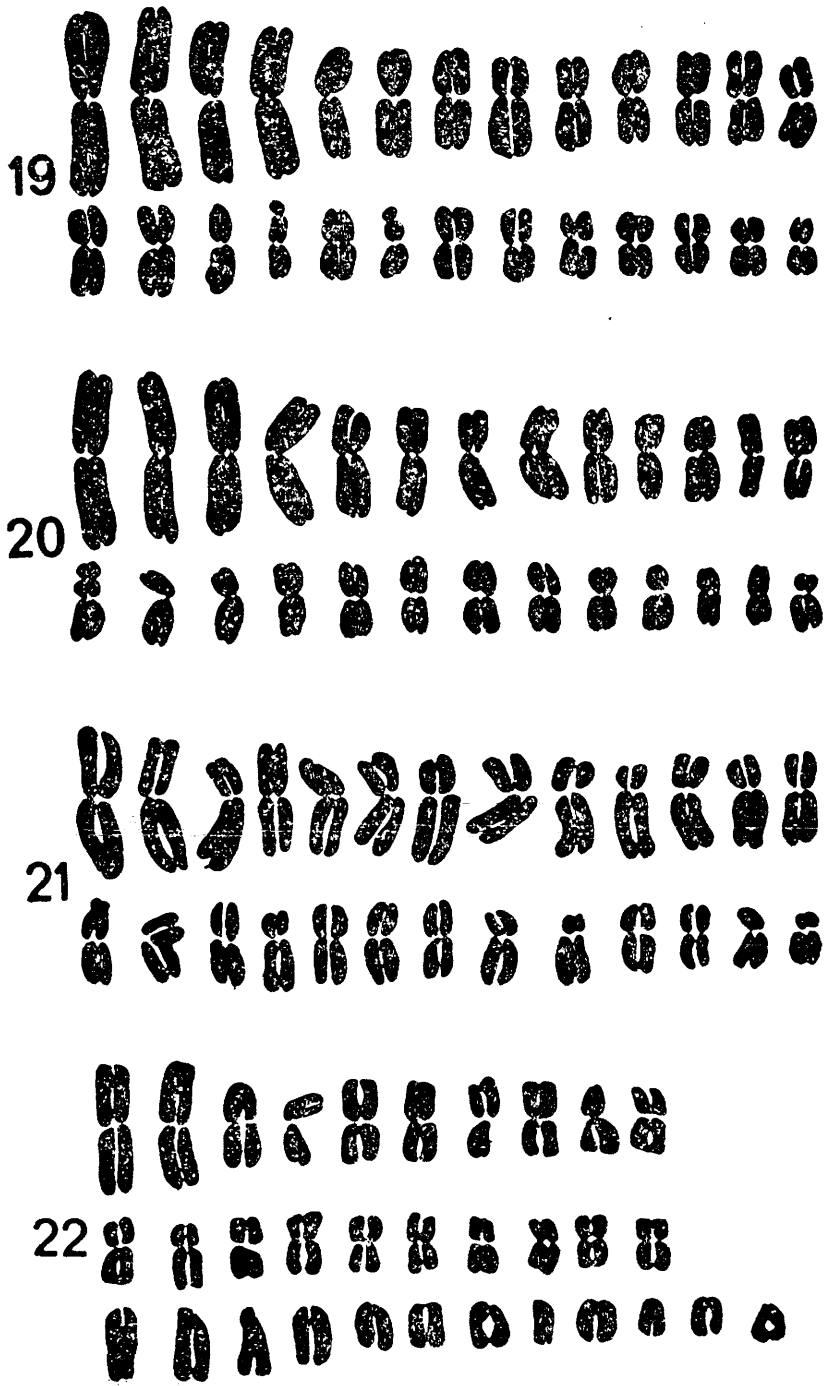

Fig. 19-22. Serial arrangement of somatic chromosomes of *Paphiopedilum* species. X2,000. 19, *P. exul.* 20, *P. villosum.* 21, *P. parishii.* 22, *P. callosum.*

Table 1. Karyotype analysis of eight Paphiopedilum species of Thailand

Species	Chromosome Number	Cell Sample	Chromosome Length*			F %		
			Mean	Range	Longest / Shortest	Mean	Range	No. of Chr. with F% < 40
P. bellatutum	26	A	27.3	16 – 47	2.9	46.4	38.3 – 50.0	1
P. concolor	26	A	35.7	26 – 65	3.1	44.9	36.7 – 50.0	3
	26	B	29.7	20 – 49	2.4	45.6	39.5 – 50.0	1
P. niveum	26	A	44.9	30 – 66	2.3	44.7	26.3 – 49.6	2
P. godefroyae	26	A	34.8	21 – 55	2.6	45.3	31.6 – 50.0	2
P. exul	26	A	30.7	20 – 57	2.8	46.4	40.0 – 49.1	0
	26	B	29.3	19 – 57	3.0	47.5	43.3 – 50.0	0
	26	C	31.5	19 – 56	2.9	46.9	41.7 – 50.0	0
	26	D	30.3	19 – 58	3.2	46.3	37.5 – 50.0	1
	26	E	33.6	20 – 62	3.1	45.1	38.0 – 50.0	1
P. villosum	26	A	28.2	19 – 54	3.1	45.1	34.3 – 50.0	2
	26	B	38.6	23 – 73	3.2	44.4	33.3 – 49.3	5
P. parishii	26	A	29.4	16 – 46	2.9	40.3	27.2 – 50.0	11
		B	28.7	17 – 50	2.9	38.1	17.7 – 48.2	14
P. callosum	32	A	20.3	12 – 41	3.4	28.9 (46.2+)	0 – 50.0	13
	32	B	28.2	16 – 50	3.1	28.3 (45.3+)	0 – 50.0	13
	32	C	31.0	18 – 64	3.6	30.5 (44.4+)	0 – 50.0	14

* Expressed as millimeters at a magnification of 4800 X.

\+ Mean after excluding the 12 telocentric chromosomes.

Although the chromosome number of *P. parishii* was identical and the chromosome size was comparable to those of the above species, its chromosome morphology showed considerable variation. The mean F% was much lower, and 11 to 14 chromosomes had an F% under 40 with a few under 30.

The chromosome number of *P. callosum* was 2n=32 instead of 2n=26. Twenty chromosomes of the complement had median or sub-median centromeres, while the remaining 12 chromosomes had terminal or sub-terminal centromeres. The mean F% was about 29, but if the 12 telocentric chromosomes were excluded in the calculation, the mean was about 45 which did not deviate from those of the Brachypetalums. It is interesting to note that an increase in number from 26 to 32 was accompanied by a decrease of two-armed chromosomes and an increase of 12 one-armed chromosomes.

Whereas the 26 chromosome groups had 2 pairs of large chromosomes, *P. callosum* exhibited only a single pair of large two-armed chromosomes. However, the four exceptionally long one-armed chromosomes were comparable in size to the single arms of the longest pair of the two-armed chromosomes.

DISCUSSION

The results of this investigation corroborates the earlier conclusion of Duncan and MacLeod (1948a, 1948b) that in general aspects the karyotypes of the Brachypetalums and the insigne complex which includes *P. exul* and *P. villosum* are difficult to distinguish. The chromosome numbers are identical and the individual chromosomes have median or sub-median centromeres. The mean F% of all species ranged from 44.8 to 46.4 indicating a uniformly high karyotype symmetry.

Fine differences in individual chromosomes of the karyotypes might well be expected. Duncan and MacLeod (1948a, 1948b) recorded differences involving relative lengths of the longest and shortest chromosomes, the number of very short chromosomes, the chromosomes with secondary constrictions, and relative length of arms in respect to the set. Some of these differences, however, were not apparent in the present investigation. The ratio of the longest to the shortest chromosome was found to differ from cell to cell, and there was as much variation within as between species. Differences were found in the size of the satellites and the relative arm lengths of certain chromosomes within the set. It appears that *P. niveum* and *P. godefroyae* can be distinguished from *P. bellatulum* and *P. concolor* by a pair of chromosomes having a relatively low F% of about 30. *P. exul* and *P. villosum* might be distinguished from the Brachypetalums by a pair of chromosomes with large satellite.

Only a slight difference in the karyotypes of *P. exul* and *P. villosum* was observed, despite the fact that the latter is epiphytic rather than terrestrial. There was a slight trend toward asymmetry in *P. villosum* the mean F% and the F% of a single pair of chromosomes were lower than for **P. exul.**

The karyotype of the epiphytic *P. parishii* differed considerably from that of the Brachypetalums and *P. exul* and *P. villosum* of the Otopedilum group. The mean F% was about 39, and there were around 12 chromosomes with an F% under 40. It can be generallized that plants with asymmetrical karyotypes are more specialized morphologically than those with symmetrical karyotypes (Stebbins, 1950). The divergent and asymmetrical karyotype of *P. parishii* suggests that this species is a more advanced form than the species of the Barchypetalum or the Insigne group. P. *parishii* has been included in Pfitzer's Otopedilum by Seidenfaden and Smitinand (1959), but as Holttum (1953) has pointed out, Pfitzer's classification of *Paphiopedilum* into three sub-genera is not a natural one, particularly in light of the recent genetical work. Karyotype similarities and differences should greatly aid a reclassification of the heterogeneous and unnatural Otopedilum subgenus.

P. callosum, another member of the Otopedilum can be easily distinguished cytologically. As mentioned earlier, the chromosome number and morphology were distinctive. Because the increase in number from 26 to 32 was accompanied by the decrease in number of two-armed chromosomes from 26 to 20, and the addition of 12 one-armed chromosomes, it might be inferred that the break of chromosomes at the region of the centromere in 6 preexisting chromosomes gave rise to the 12 one-armed chromosomes. The four largest telocentric chromosomes are about the same size as the arms of the two largest chromosomes and furthermore, the karyotype comprises only two large chromosomes instead of the usual four seen in other species, suggesting that one of the two largest pairs of chromosomes was involved in the evolution of the 4 largest one-armed chromosomes

If the telocentric chromosomes are excluded in calculating the F% of *P. callosum*, the mean F% is remarkably similar to that of other species excepting *P. parishii*. Thus it appears that the karyotype of *P. callosum*. is essentially similar to that of the Brachypetalums, except that 6 pairs of chromosomes with median to sub-median centromeres are substituted by 12 telocentric chromosomes.

The other native species that is closely related to *P. callosum* is *P. barbatum* which occurs in Peninsular Thailand and Malaya. *P. barbatum* should be easy to distinguish cytologically, for according to Duncan and MacLeod (1950), it posesses 38 chromosomes, 24 of which are one-armed. Again, like *P. callosum*, the increase in number from 26 to 38 corresponds with a decrease in two-armed chromosomes from 26 to 14.

It might be concluded that the evolution of the Brachypetalums and *P. exul* and *P. villosum* occurred without pronounced changes in the chromosomes complements, while in *P. parishii*, evolution accompanied a change in symmetry of karyotype morphology, and in *P. callosum*, an increase in chromosome number from 26 to 32 which corresponds with a decrease of two-armed chromosomes from 26 to 20 and an increase of 12 one-armed chromosomes.

SUMMARY

The karyotypes of eight indigenous *Paphiopedilum* species of Thailand were investigated *P. bellatulum*, *P. concolor*, *P. niveum*, *P. godefroyae*, *P. exul*, and *P. villosum* exhibited remarkably similar karyotypes. Chromosome number was uniformly 2n = 26. With the exception of a single pair, all chromosomes had median or sub-median centromeres and the mean F% was about 45. Fine differences were observed for the size of satellites and the relative arm length of individual chromosomes of the set.

The karyotype morphology of *P. parishii* differed from that of the above species. The mean F% was much lower, and about 12 chromosomes had an F% value under 40.

The chromosome number of *P. callosum* was 2n=32 instead of 2n=26. Twenty chromosomes had median and sub-median centromeres while the remaining 12 chromosomes had terminal or sub-terminal centromeres. The mean F% was about 29 but if the 12 telocentric chromosomes were excluded, the mean F% was comparable to that for species other than *P. parishii*.

Thus, speciation in *P. bellatulum*, *P. concolor*, *P. niveum*, *P. godefroyae*, *P. exul* and *P. villosum* proceeded without pronounced changes in the karyotype, while in *P. parishii* speciation accompanied a divergence in karyotype symmetry, and in *P. callosum*, an increase in chromosome number associated with a corresponding decrease in two-armed chromosomes and an increase in one-armed chromosomes.

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