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Karyomorphological Studies on Species of *Pleurothallis*, Orchidaceae

By

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中田政司*・橋本　保*： プレウロタリス属（ラン科）の核形態

Introduction

The genus *Pleurothallis* R. Br., one of the largest genera in Orchidaceae, comprises about 1,000 species or more with many diverse forms. Species of the genus are distributed in tropical and subtropical Americas. The genus has been considered to be a complex group, and subdivisional classifications have been proposed by Lindley (1842, 1859), Pfitzer (1889), Misas & Arango (1974), Garay (1974) and Pabst & Dung (1977). However, these classifications seem to be still artificial and have not had acceptance with most orchidologists. Although such studies may give some suggestions to the classification of *Pleurothallis*, karyomorphological studies have scarcely been made in the genus. According to Tanaka & Kamemoto (1974), n=21 in *P. vittata* (Chardard, 1963) is the only report of chromosome number of the genus.

In the Tsukuba Botanical Garden, National Science Museum, which is abbreviated in this paper as TBG, 28 species and some unnamed species of *Pleurothallis* are cultivated. Most of them were collected by the overseas expeditions such as The First Botanical Expedition to the Andes, University of Tokyo, 1965-1966, The Scientific Expedition to South America, University of Tokyo, 1971, and La Expedición Botánica a los Andes, Universidad de Chiba, 1974.

In the present paper, results of karyomorphological observations will be reported in 23 species of *Pleurothallis* preserved in TBG, in order to clarify karyomorphological characteristics of this genus.

Materials and Methods

Materials and their TBG accession numbers are listed in Table 1. Except for one unnamed species (TBG 32886) bearing only one leaf, all voucher herbarium specimens are deposited in TNS. All slides have been made permanent and are preserved in TNS.

For the observations of somatic chromosomes, growing root tips were used. Young flower buds were also used in some species. Materials were cut into small pieces of 0.5-1.0 mm and placed in 0.002M 8-hydroxyquinoline for 5 hr at about 20°C. They were fixed

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Table 1. Material, TBG accession number and chromosome number of the *Pleurothallis* studied.

Species	Source	TBG accession number	2n
P. alopex Luer.	Bolivia	32923	36
		32924	36
		32925	36
		32926	36
P. aurantio-lateritia Speg.	Bolivia	32944	40
P. bivalvis Lindl.	Bolivia	32929	77
P. brevipes Focke	Surinam	14053	43
P. carinata C. Schweinf.	Peru	32881	40
		32882	40
P. chanchamayoensis C. Schweinf.	Peru	32899	84
P. gelida Lindl.	Peru	32919A	32
		32905	64
		32907	64
		32910	64
		32919	64
P. grobyi Batem. ex Lindl.	Bolivia	32943	20
	Surinam	14005	20
P. luteola Lindl.	Brazil	32118	40
P. matudiana C. Schweinf.	Mexico	32894	68
		32895	68
P. obovata Lindl.	Bolivia	32946	42
		32950	43
		32947	45
		32948	45
		32951	45
P. pachyglossa Lindl.	Mexico	32865	38
		32867	38
		32874	38
P. restrepioides Lindl.	Peru	32953	76
		32954	76
		32956	76
P. revoluta (Ruiz & Pav.) Garay	Bolivia	32875	40
		32876	80
		32878	80
P. ruscifolia (Jacq.) R. Br.	Peru	32945	38
P. saccatilabia C. Schweinf.	Mexico	32891	38
P. segoviensis Reichb. f.	Mexico	32893	42
P. teres Lindl.	Brazil	33137	40
P. tridentata Klotzsch	Bolivia	32903	36
P. velaticaulis Reichb. f.	Peru	32937	34
		32938	34
P. xanthochlora Reichb. f.	Bolivia	32896	40
		32897	40
		32898	40
P. aff. *coffeicola* Schltr.	Bolivia	32886	40
P. sp.	Peru	32889A	38

in modified Carnoy's solution (99% ethanol: chloroform: 99.5% acetic acid=2: 1: 1) for 24 hr at 5°C and then transferred to 45% acetic acid for about 10 min at 5°C. The fixed materials were macerated in a mixture of 1N Hydrochloric acid and 45% acetic acid (2: 1) for 20 sec at 60°C, and finally stained in 1% aceto-orcein and squashed.

Observations

Many mitotic cell divisions were observed in root tips. In a small parts of the meristematic region, several enlarged and polygonal shaped cells with rich intracellular contents were observed. They were regarded as the differentiated rootcap cells at resting stage. For the observations of the resting nucleus, these cells were used. Observations were also made in mitotic prophase and metaphase.

Results of chromosome counts are shown in Table 1.

Morphology of the chromosomes in 23 taxa was as follows.

1) **Pleurothallis alopex** Luer, 2n=36, Table 1 and Fig. 1.

Four plants collected in Bolivia (Hashimoto, 1978) were investigated.

Chromosomes at resting stage formed many chromomeric granules and several small chromocentral blocks scattered within the nuclear space. These chromocentral blocks varied in number from 11 to 18 per nucleus and were almost round in shape, measuring 0.2-0.6 μm in diameter.

At mitotic prophase, chromosomes had early condensed segments situated in the proximal regions and had late condensed segments located in the distal ends. The size of the early condensed segments varied by chromosomes.

At metaphase 2n=36 chromosomes were counted in all of the four plants. These chromosomes varied in length from 0.3 to 0.7 μm. Among the 36 chromosomes, four longer chromosomes were distinguished. These four chromosomes were median centromeric. All of the remaining 32 chromosomes, except for four chromosomes of subterminal, had a centromere situated in median or submedian positions.

2) **Pleurothallis aurantio-lateritia** Speg., 2n=40, Table 1 and Fig. 2.

Chromosomes at resting stage formed many chromomeric granules and several large chromatin blocks scattered in the nuclear space. These condensed chromocentral blocks varied in number from 30 to 36 per nucleus and were almost round in shape, measuring

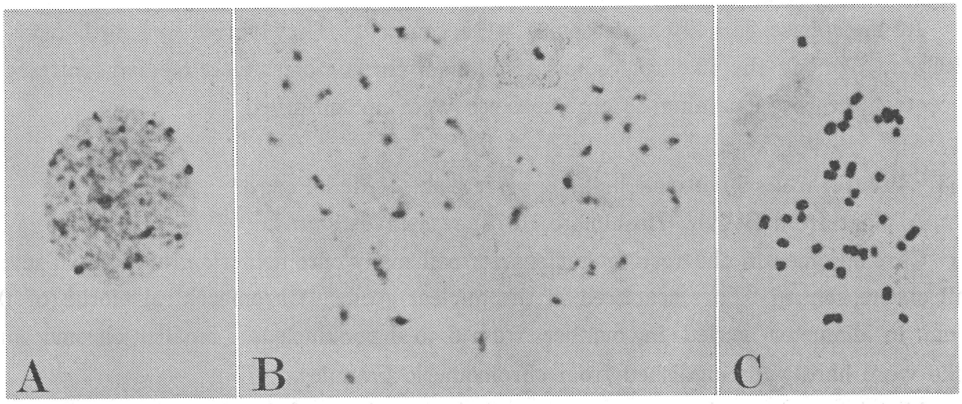

Fig. 1. Chromosomes of *Pleurothallis alopex*, TBG 32923, 2n=38. A, resting stage. B, prophase. C, metaphase. A-C, ×1,750.

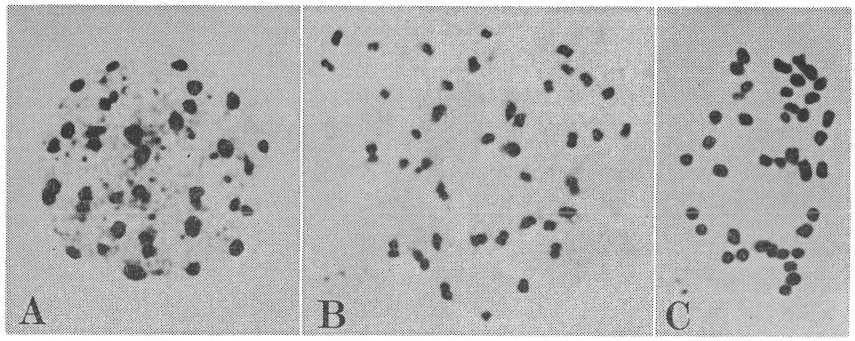

Fig. 2. Chromosomes of *Pleurothallis aurantio-lateritia*, TBG 32944, 2n=40. A, resting stage. B, prophase. C, metaphase. A-C, ×1,750.

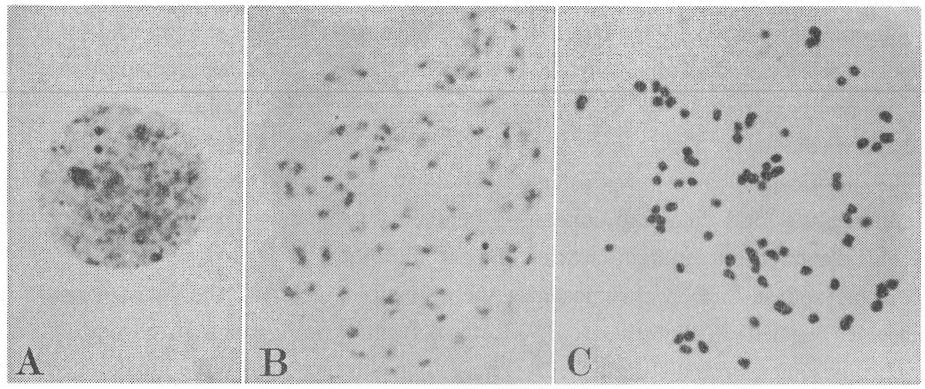

Fig. 3. Chromosomes of *Pleurothallis bivalvis*, TBG 32929, 2n=77. A, resting stage. B, prophase. C, metaphase. A-C, ×1,750.

0.8 to 1.6 μ m in diameter. The morphology of the resting nuclei quite differed in size of chromocentral blocks from that of *P. alopex*.

At mitotic prophase all chromosomes had early condensed segments situated in the proximal regions and had late condensed segments located in the distal ends with distinct border between them.

At metephase 2n=40 chromosomes were counted. They varied in length from 0.6 to 1.4 μm. Among the 40 chromosomes two longer chromosomes were median centromeric, but centromeres of remaining 38 chromosomes were not observed.

3) **Pleurothallis bivalvis** Lindl., 2n=77, Table 1 and Fig. 3.

A plant from Bolivia (Hashimoto, 1978) was investigated.

Chromosomes in the resting nucleus formed many chromomeric granules and several small chromocentral blocks scattered in the nuclear space. Chromocentral blocks of 0.2-0.6 μm in diameter varied in number from 5 to 9 per nucleus. Smaller chromocentral blocks were hardly distinguished from chromomeric granules.

Mitotic prophase chromosomes were similar to those of *P. alopex* in morphology.

The chromosome number of 2n=77 was counted. These chromosomes varied in

length from 0.5 to 1.4 μm, showing gradual size variation. The four longer chromosome had a centromere situated in median positions. Centromeres of the remaining chromosomes were not observed.

4) **Pleurothallis brevipes** Focke, 2n=43, Table 1 and Fig. 4.

Chromosomoes at resting stage were similar to those of *P. alopex*. The number of these blocks, measuring 0.3-1.0 μm in diameter, varied from 23 to 29 per mucleus.

At mitotic prophase several chromosomes had early condensed segments in the proximal regions. The size of these early condensed regions varied widely by each chromosome. The smallest segment occurred as a dot and the largest one was about 1.0 μm in length.

At metaphase 2n=43 chromosomes were counted. They varied in length from 0.6 to 1.4 μm, showing gradually changed homogeneous karyotype. Most of these chromosomes had a centromere situated in median or submedian positions.

5) **Pleurothallis carinata** C. Schweinf., 2n=40, Table 1 and Fig. 5.

Chromosomes at resting stage were similar to those of *P. aurantio-lateritia*. The chromocentral blocks varied in number from 22 to 29 per nucleus. They were round or elliptical, measuring 0.3-1.6 μm long in the major axis.

Chromosomes at mitotic prophase had early condensed segment at the proximal

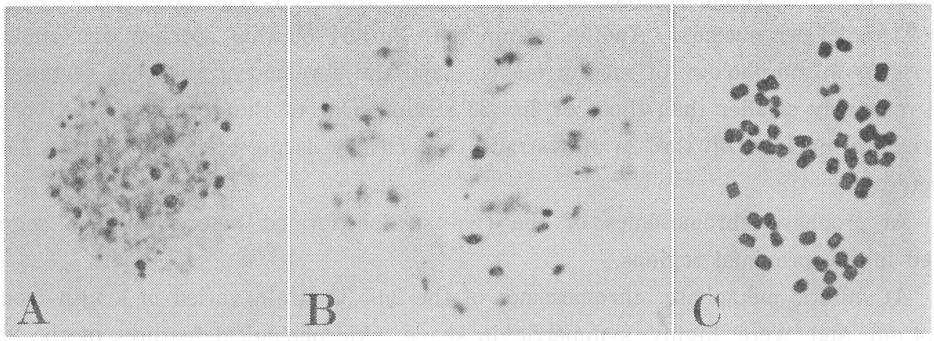

Fig. 4. Chromosomes of *Pleurothallis brevipes*, TBG 14053, 2n=43. A, resting stage. B, prophase. C, metaphase. A-C, ×2,000.

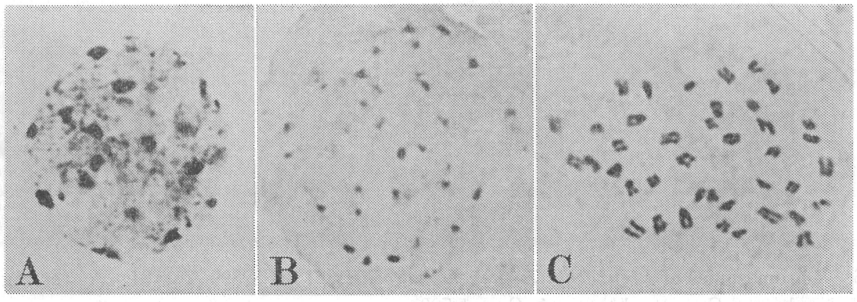

Fig. 5. chromosomes of *Pleurothallis carinata*, TBG 32881, 2n=40. A, resting stage. B, prophase. C, metaphase. A-C, ×1,750.

regions. More or less 10 chromosomes of a complement had large and strongly condensed segments.

Two plants collected in Peru showed 2n=40 chromosomes, which varied in length from 0.8 to 1.6 μm. Some chromosomes were submedian or subterminal centromeric, while the centromeres of the most chromosomes were not be found.

6) **Pleurothallis chanchamayoensis** C. Schweinf., 2n=84, Table 1 and Fig. 6.

Chromosomes at resting stage were similar to those of *P. bivalvis*. Small chromocentral blocks of 0.2-0.8 μm in diameter varied in number from 17 to 23 per nucleus.

The morphology of chromosomes at mitotic prophase was similar to that of *P. alopex*. The compactly condensed segments were found in 8-12 chromosomes.

The 2n=84 chromosomes were counted at mitotic metaphase. They varied in length from 0.3 to 0.8 μm showing bimodal karyotype. The longer four chromosomes were median centromeric. Centromeric constrictions were not observed in the remaining chromosomes.

7) **Pleurothallis gelida** Lindl., 2n=32, 64, Table 1 and Fig. 7.

Five plants from Peru were investigated. One of them showed 2n=32 chromosomes, while the other four showed 2n=64 chromosomes.

Resting nucleus of 2n=32 strain had large chromocentral blocks of 0.3-0.9 μm in diameter and was similar to that of *P. aurantio-lateritia*. The number of the blocks varied from 23 to 39 per nucleus. Another strain with 2n=64 of this species was similar to *P. brevipes* in morphology of resting nucleus, and the chromocentral blocks of this strain were relatively smaller than those of 2n=32 strain. One of the chromocentral blocks in the resting nucleus of the 2n=64 strain was clearly large mesuring about 1.7 μm in diameter.

At prophase chromosomes of the two strains formed heterochromatic segments located in the proximal regions.

At mitotic metaphase, chromosomes of the 2n=32 strain varied in length from 0.9 to 1.4 μm and were highly symmetric in shape. Morphological features of the 2n=64 chromosomes were very similar to those of 2n=32 strain.

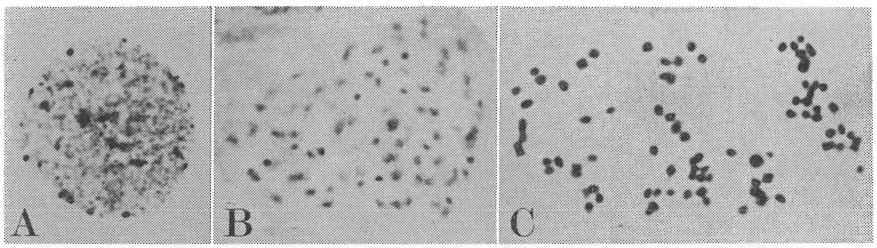

Fig. 6. Chromosomes of *Pleurothallis chanchamayoensis*, TBG 32899, 2n=84. A, resting stage. B, prophase. C, metaphase. A-C, ×1,500.

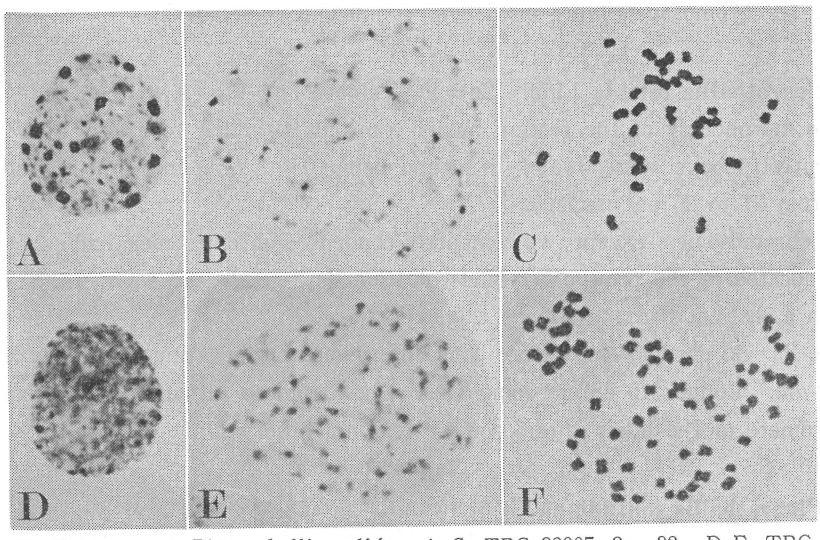

Fig. 7. Chromosomes of *Pleurothallis gelida*, A-C, TBG 32905, 2n=32. D-F, TBG 32919A, 2n=64. A and D, resting stage. B and E, prophase. C and F, metaphase. A-F, ×1,500.

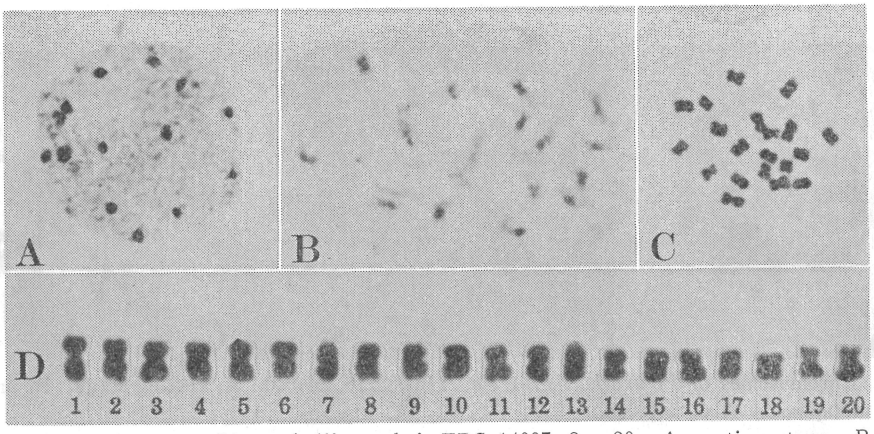

Fig. 8. Chromosomes of *Pleurothallis grobyi*, TBG 14005, 2n=20. A, resting stage. B, prophase. C, metaphase. D, individual chromosomes at metaphase. A-C, ×1,750. D, ×3,500.

8) **Pleurothallis grobyi** Batem. ex Lindl., 2n=20, Table 1 and Fig. 8.

One plant from Bolivia (Hashimoto, 1978) and another plant from Surinam were investigated.

In the resting nuclei 16 conspicuous chromocentral blocks were observed with many chromomeric granules scattered homogeneously within the nuclear space. These blocks varied in size from 0.3 to 1.2 μm in diameter.

Chromosomes at mitotic prophase had early condensed segments at the proximal regions. About five to seven chromosomes were stained much lighter than others.

The two strains mentioned above had the same chromosome number, 2n=20. These chromosomes varied in length from 0.8 to 1.5 μm with the successive gradual variation. The shorter two chromosomes had a centromere situated in subterminal position and the

remaining 18 chromosomes were median or submedian centromeric.

9) **Pleurothallis luteola** Lindl., **2n=40**, Table 1 and Fig. 9.

The morphology of the resting nuclei was quite similar to those of *P. brevipes*. Chromocentral blocks were round in shape being 0.4-1.0 μm in diameter and varied in number from 21 to 25 per nucleus.

All chromosomes, except two compactly condensed chromosomes, had early condensed segments located in the proximal regions and had late condensed segments at the distal parts with a distinct border between them.

The 2n=40 chromosomes were counted. These chromosomes varied in length from 0.6 to 1.2 μm. Longer 10 chromosomes had a centromere situated in median position. The centromere of the other chromosomes were not observed.

10) **Pleurothallis matudiana** C. Schweinf., 2n=68, Table 1 and Fig. 10.

Chromosomes at resting stage were similar to those of *P. alopex*. The number of the chromocentral blocks varied from 25 to 32 per nucleus.

The morphology of the chromosomes at prophase was similar to that of *P. bivalvis*.

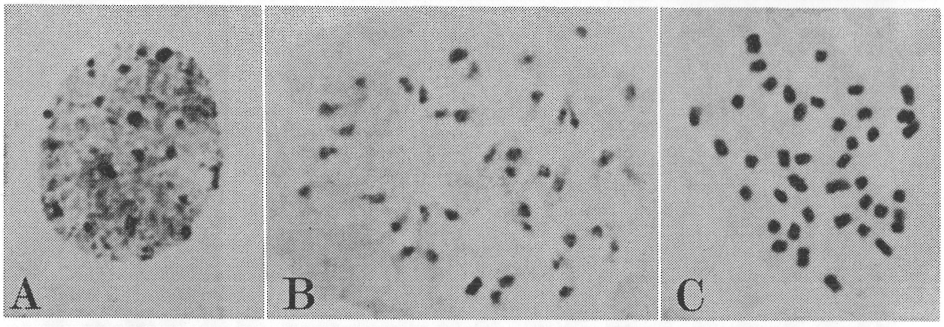

Fig. 9. Chromosomes of *Pleurothallis luteola*, TBG 33116, 2n=40. A, resting stage. B, prophase. C, metaphase. A-C, ×2,000.

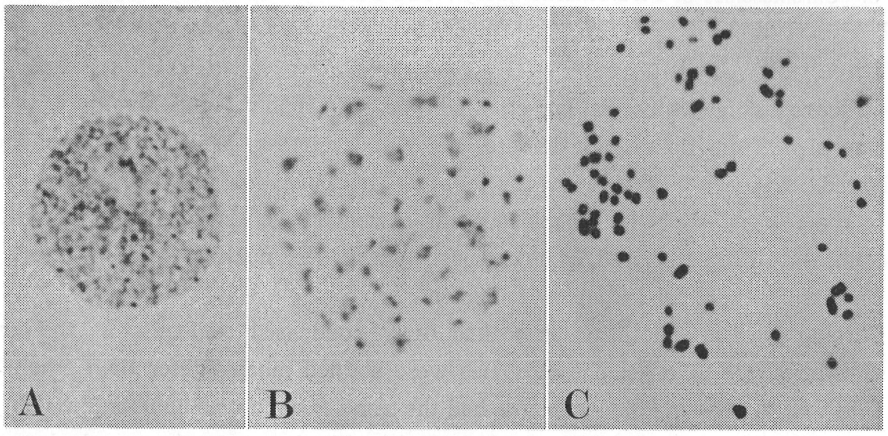

Fig. 10. Chromosomes of *Pleurothallis matudiana*, TBG 32895, 2n=68. A, resting stage. B, prophase. C, metaphase. A-C, ×1,500.

At metaphase the 2n=68 chromosomes were observed. These chromosomes varied in length from 0.3 to 0.9 μm. Among the 68 chromosomes, eight longer chromosomes of about 0.9 μm in length were distinguished. These chromosomes were median centromeric. The centromeres were not observed in the remaining shorter chromosomes. The chromosomes showed heterogeneous variation in length and were categorized as the bimodal karyotype.

11) Pleurothallis obovata Lindl., 2n=42, 43, 45, Table 1 and Fig. 11.

Five plants collected in Bolivia were investigated. One plant showed 2n=42 chromosomes and another one 2n=43, whereas the remaining three individuals were counted to be 2n=45.

Chromosomes at resting nuclei of all three strains formed many chromomeric granules scattered homogeneously within the nuclear space and large chromocentral blocks varying in size from 0.4 to 1.6 μm in diameter. The morphology of the resting nuclei of the three strains was similar to those of *P. aurantio-lateritia*. Chromatin blocks were variable

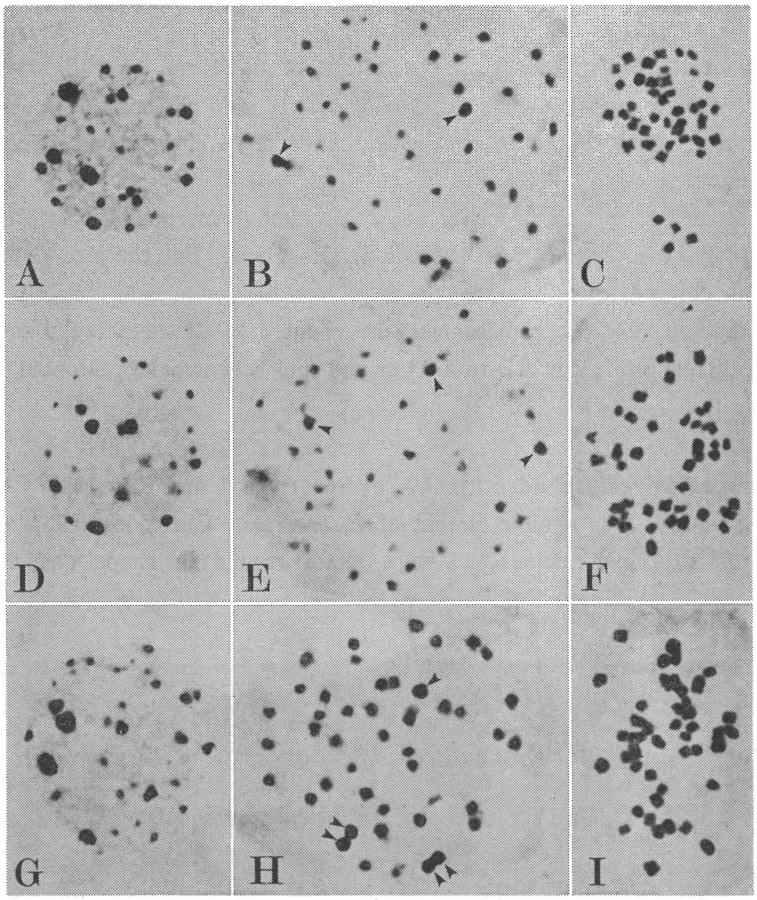

Fig. 11. Chromosomes of *Pleurothallis obovata*, A-C, TBG 32946, 2n=42. D-F, TBG 32950, 2n=43. G-I, TBG 32948, 2n=45. A, D and G, resting stage. B, E and H, prophase. C, F and I, metaphase. A-I, ×1,750. arrows show condensed bodies (see text).

in number per nucleus and the range of number was overlapped in these three strains. However, these strains differed from each ofher in the means and the standard deviations (n=10) of the number of chromocentral blocks 23.9±2.3 in 2n=42 strain, 26.5±2.5 in 2n=43 strain and 28.3±3.2 in 2n=45 strain, respectively.

As in the previous 10 species, the chromosomes at mitotic prophase formed early condensed segments located in the proximal regions. With these chromosomes, remarkable heterochromatic bodies were observed in each strain. Two condensed bodies were found in 2n=42 strain, and three in the 2n=43 strain. The most numerous blocks, five in number, were observed in 2n=45 strain.

At metaphase, more or less 18 shorter chromosomes measuring approximately 0.6 μm in length were observed in each chromosome complement of the three strains. The number of the longer chromosomes measuring 1.0-1.2 μm in length varied among these three strains; two in 2n=42 strain, three in 2n=43 strain and five in 2n=45 strain, respectively. Most chromosomes of these strains were median or submedian centromeric. Two satellites were found on medium-sized median chromosomes in 2n=43 strain.

12) **Pleurothallis pachyglossa** Lindl., 2n=38, Table 1 and Fig. 12.

Resting nucleus was similar to that of *P. aurantio-lateritia*. Chromocentral blocks varied in number from 15 to 24 per nucleus and were almost round in shape and 0.4-1.4 μm in diameter.

Chromosomes at mitotic prophase showed a similar morphology as in *P. obovata*, having early condensed segments in the proximal regions. But the size of the segments varied by each chromosome.

At metaphase 2n=38 chromosomes were counted in all the three plants examined. They varied in length from 0.5 to 1.4 μm and had a centromere situated in median or submedian position.

13) **Pleurothallis restrepioides** Lindl., 2n=76, Table 1 and Fig. 13.

Resting nucleus was similar to that of *P. brevipes*. Chromocentral blocks varied in number from 33 to 47 per nucleus and were almost round in shape and 0.3-1.0 μm in diameter.

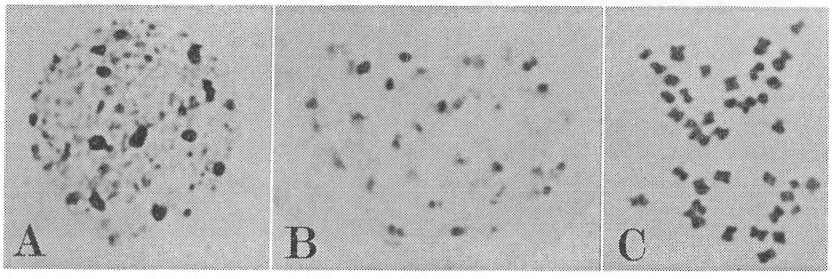

Fig. 12. Chromosomes of *Pleurothallis pachyglossa*, TBG 32874, 2n=38. A, resting stage. B, prophase. C, metaphase. A-C, ×2,000.

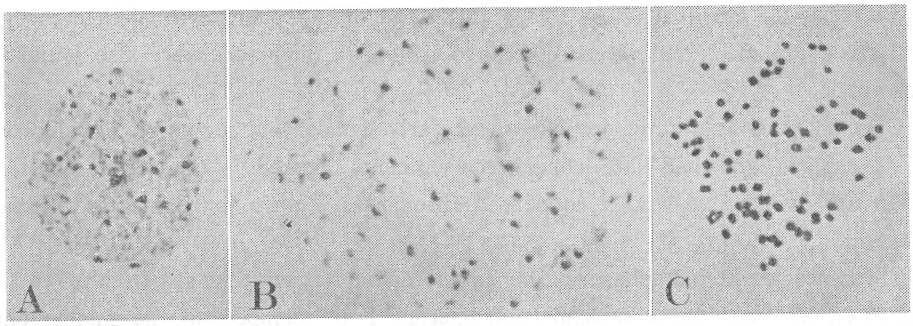

Fig. 13. Chromosomes of *Pleurothallis restrepioides*, TBG 32959, 2n=76. A, resting stage. B, prophase. C, metaphase. A-C, ×1,500.

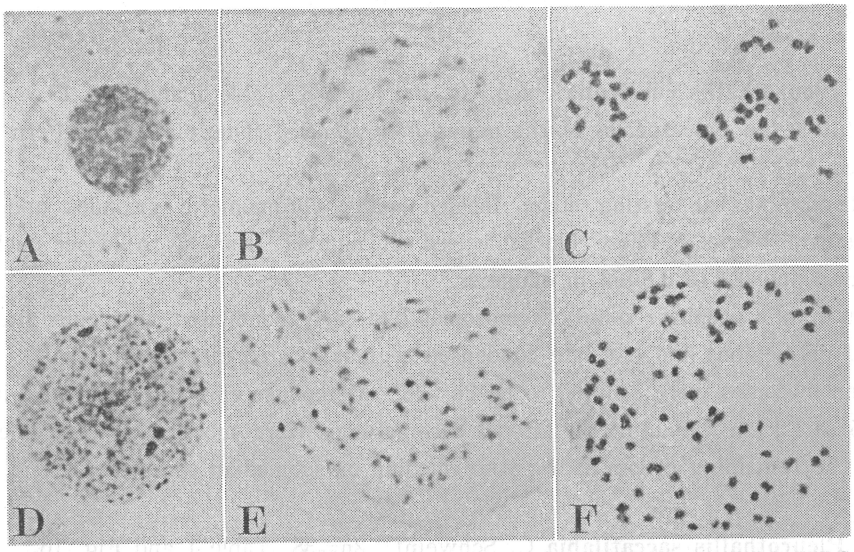

Fig. 14. Chromosomes of *Pleurothallis revoluta*, A-C, TBG 32875, 2n=40. D-F, TBG 32878, 2n=80. A and D, resting stage. B and E, prophase. C, metaphase. F, prometaphase. A-F, ×1,500.

Mitotic prophase chromosomes closely resembled those of *P. pachyglossa*.

Four plants investigated showed the same chromosome number, 2n=76. Chromosomes are divided into three groups by their length. Longer two chromosomes were about 1.2 μm and had a centromere at median position. Medium-sized 20 chromosomes were approximately 0.8 μm. The remaining shorter chromosomes varied in length from 0.4 to 0.6 μm with gradual size variation. The karyotype of this species, thus was found to be trimodal. Chromosomes of the latter two groups were median or submedian.

14) **Pleurothallis revoluta** (Ruiz & Pav.) Garay, 2n=40, 80, Table 1 and Fig. 14.

Three plants collected in Bolivia were investigated. The one had 2n=40 chromosomes, while the other two showed 2n=80 chromosomes.

In both strains chromosomes at resting stage formed many chromomeric granules dispersed homogeneously within the nuclear space and several condensed chromocentral

blocks of about 0.3 μm in length. Large chromatin blocks, which varied in number from two to five per nucleus and measured 1.0 to 1.5 μm in diameter, were also found in 2n =80 strain.

Chromosomes at mitotic prophase had early condensed segments situated in the proximal regions and had late condensed segments located in the distal ends. In the 2n=80 strain two to five wholly condensed chromosomes were found in the chromosome complement.

Chromosomes at metaphase in the 2n=40 strain varied in length from 0.5 to 1.3 μm. Longer 10 chromosomes were mediam centromeric. Four members of the smaller chromosome group were subterminal centromeric. In the 2n=80 strain, no countable metaphase chromosomes could be observed. Chromosome number of 2n=80 were counted at prometaphase stage.

15) Pleurothallis ruscifolia (Jacq.) R. Br., 2n=38, Table 1 and Fig. 15.

This is the type species of the genus *Pleurothallis*. One plant from Peru was investigated.

Chromsomes at resting stage formed many chromomeric granules and several chromocentral blocks. These blocks varied in number from 20 to 26 per nucleus and were round and 0.4 to 0.8 μm in diameter.

At mitotic prophase some chromosomes had early condensed segments situated in the proximal regions and had late condensed regions located distal ends.

Chromosomes were counted to be 2n=38. Metaphase chromosomes varied in length from 0.5 to 1.1 μm. Four of the 2n=38 chromosomes were subterminal centromeric, but most of the remaining chromosomes were median or submedian centromeric.

16) Pleurothallis saccatilabia C. Schweinf., 2n=38, Table 1 and Fig. 16.

In the resting nucleus, rod or round shaped chromocentral blocks were observed with many chromomeric granules scattered within the nuclear space. The chromocentral blocks varied in number from 10 to 20 per nucleus and varied in length from about 0.5 to 1.2 μm.

Mitotic prophase chromosomes had early condensed segments located proximally. Two of the complement had conspicuously large segments.

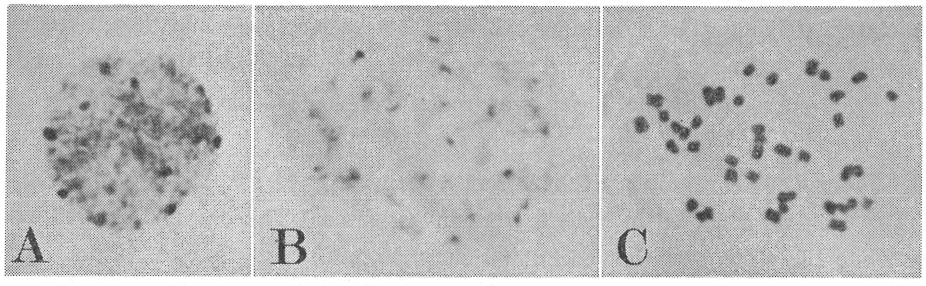

Fig. 15. Chromosomes of *Pleurothallis ruscifolia*, TBG 32945, 2n=38. A, resting stage. B, prophase. C, metaphase. A-C, ×2,250.

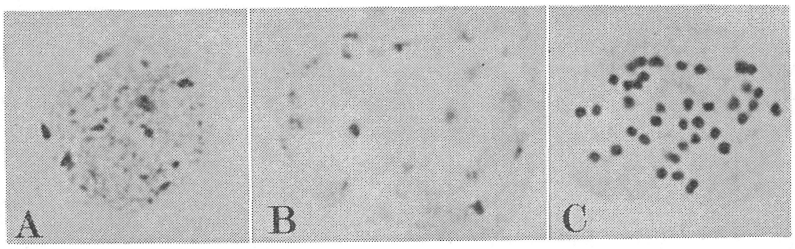

Fig. 16. Chromosomes of *Pleurothallis saccatilabia*, TBG 32891, 2n=38. A, resting stage. B, prophase. C, metaphase. A-C, ×1,750.

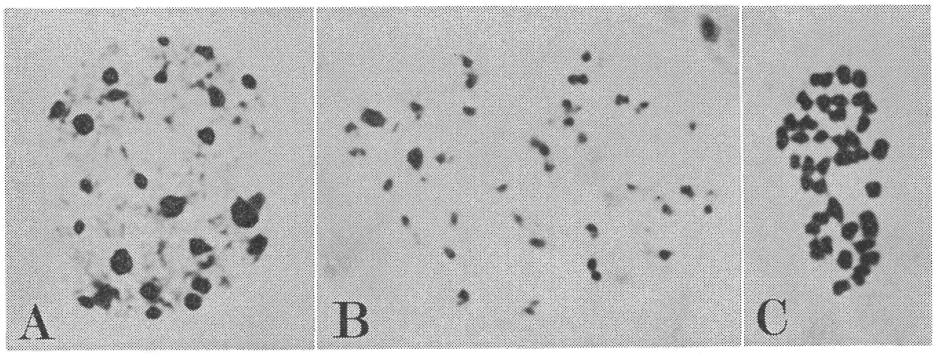

Fig. 17. Chromosomes of *Pleurothallis segoviensis*, TBG 32893, 2n=42. A, resting stage. B, prophase. C, metaphase. A-C, ×2,250.

At metaphase 2n=38 chromosomes were observed. They were almost homogeneous in length, measuring 0.6-0.8 μm. No clear centromeric constrictions were observed in them.

17) **Pleurothallis segoviensis** Reichb. f., 2n=42, Table 1 and Fig. 17.

The karyotype of resting nucleus of this species was similar to that of *P. aurantiolateritia*. Large condensed bodies measuring 1.6 to 1.8 μm and smaller ones of 0.6-1.2 μm were observed. Total number of these bodies varied from 24 to 27 per nucleus.

Chromosomes at mitotic prophase were found to form early condensed segments located in the proximal regions. Among these chromosomes the two chromosomes had large heterochromatic segments of about 1.2 μm in length.

The chromosome number of this species was found to be 2n=42. They varied in length from 0.6 to 1.2 μm. Ten chromosomes of a complement were median or submedian centromeric. Centromeric constrictions were not observed in the remaining 32 chromosomes.

18) **Pleurothallis teres** Lindl., 2n=40, Table 1 and Fig. 18.

A large chromatin block stained darkly was observed in the resting nucleus. This chromatin block was measured about 1.6 μm or more in diameter. Many chromomeric granules and several chromocentral bodies were also observed in the resting nucleus.

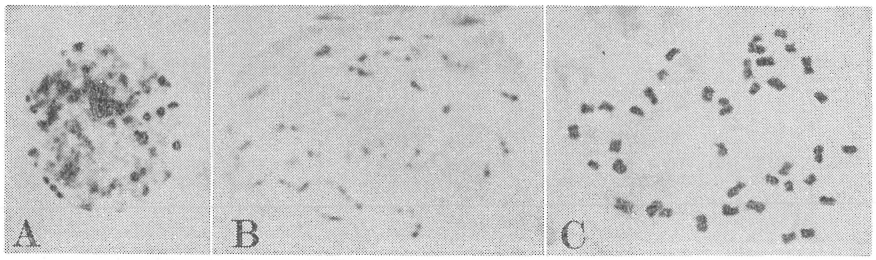

Fig. 18. Chromosomes of *Pleurothallis teres*, TBG 33137, 2n=40. A, resting stage. B, prophase. C, metaphase. A-C, ×1,750,

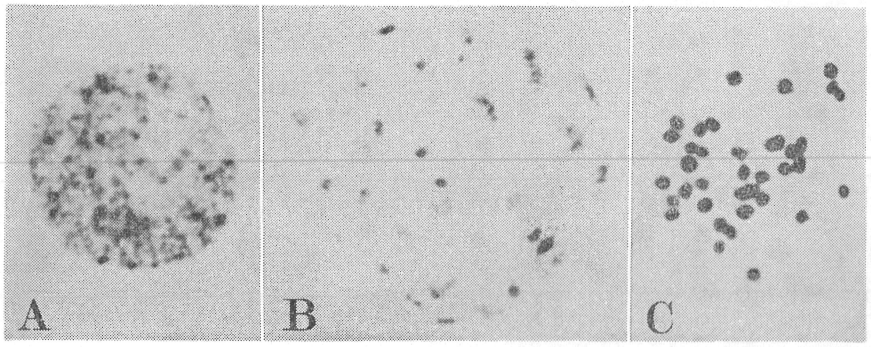

Fig. 19. Chromosomes of *Pleurothallis tridentata*, TBG 32903, 2n=36. A, resting stage. B, prophase. C, metaphase. A-C, ×2,000.

These chromocentral blocks varied in number from 28 to 35 per nucleus and almost round in shape of 0.4-0.8 μm in diameter.

At mitotic prophase, chromosomes formed early condensed segments situated in the proximal regions, which were replaced gradually late condensed segments towards the distal regions.

The 2n=40 chromosomes were counted. They varied in length from about 0.8 to 1.2 μm. Most chromosomes of a complement were metacentric or submetacentric.

19) **Pleurothallis tridentata** Klotzsch., 2n=36, Table 1 and Fig. 19.

A plant from Bolivia (Hashimoto, 1976) was studied.

The morphology of the resting chromosomes was quite similar to that of *P. bivalvis*. Small chromocentral blocks of 0.2-0.5 μm varied in number from 12 to 18 per nucleus.

At mitotic prophase, several chromosomes formed early condensed segments situated in the proximal regions, but they differed widely in size of the segments.

At metaphase 2n=36 chromosomes were counted. These chromosomes varied gradually in length from 0.5 to 1.1 μm, showing a homogeneous karyotype. The centromeric constrictions were not observed in all 36 chromosomes.

20) **Pleurothallis velaticaulis** Reichb. f., 2n=34, Table 1 and Fig. 20.

The morphology of the resting nucleus was similar to that of *P. brevipes*. The

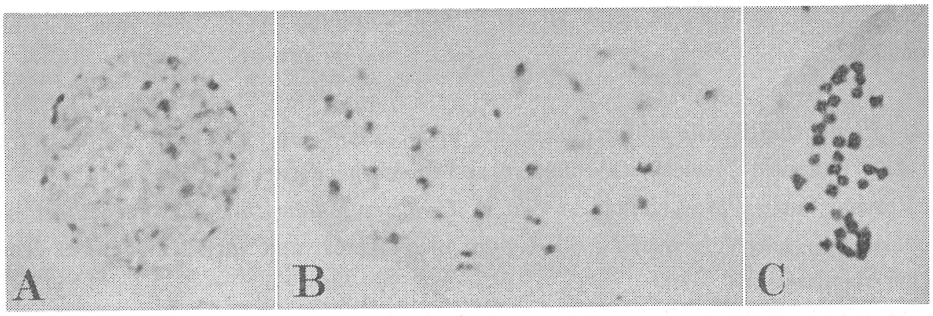

Fig. 20. Chromosomes of *pleurothallis velaticaulis*, TBG 32937, 2n=34. A, resting stage. B, prophase. C, metaphase. A-C, ×2,000.

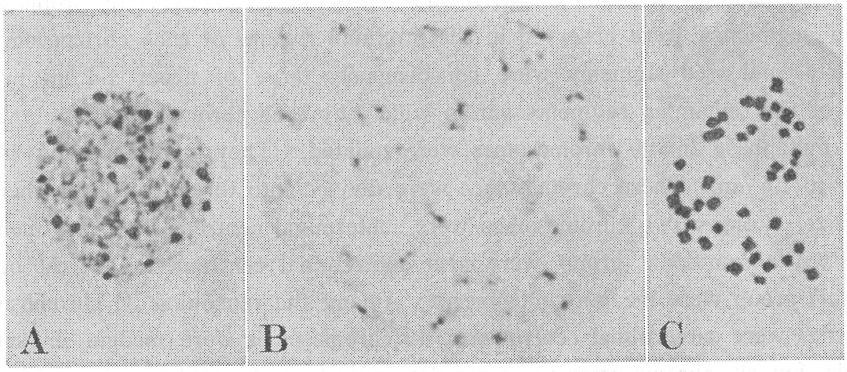

Fig. 21. Chromosomes of *Pleurothallis xanthochlora*, TBG 32896, 2n=40. A, resting stage. B, prophase. C, metaphase. A-C, ×1,750.

chromocentral blocks were almost round in shape and 0.3-0.6 μm in diameter and varied in number from 16 to 25 per nucleus.

Chromosomes at mitotic prophase had early condensed segments situated in the proximal regions which abruptly replaced by late condensed segments towards the distal ends. About five chromosomes of a complement were stained much lighter than the other chromosomes.

At metaphase 2n=34 chromosomes were counted. They ranged from 0.4 to 1.0 μm in length, showing gradual size variation. Most of the 34 chromosomes had a centromere situated in median or submedian positions.

21) **Pleurothallis xanthochlora** Reichb. f., 2n=40, Table 1 and Fig. 21.

Chromosomes at resting stage were similar to those of *P. brevipes*. Medium-sized chromocentral blocks varied in number from 24 to 30 per nucleus and were almost round in shape, measuring 0.4 to 1.0 μm in diameter.

Morphological feature of the chromosomes in mitotic prophase was similar to that of *P. brevipes*.

At metaphase 2n=40 chromosomes were counted. They varied in length from 0.4 to 1.1 μm. The shortest two chromosomes were fragment-like and were easily distinguished from the other 38 chromosrmes. Among the 38 chromosomes, eight shorter ones

were subterminal centromeric and longer 10 chromosomes were median or submediam centromeric.

22) **Pleurothallis aff. coffeicola** Schltr. (TBG 32886), 2n=40, Table 1 and Fig. 22.

This unnamed species was collected at the southern part of Bolivia.

Resting nucleus was similar to that of *P. aurantio-lateritia*. Chromocentral blocks were relatively large, measuring 0.3-1.4 μm in diameter, and varied in number from 23 to 28 per nucleus.

At mitotic early prophase, four condensed chromosomes were observed. Among these chromosomes two smaller ones were half the size of two longer ones. The remaining 36 chromosomes were stained lightly and still elongated. At late prophase, early condensed segmentss were observed in the proximal regions of each chromosome. Two of the early condensed segments were conspicuously large of about 1.4 μm in length. The size of the remaining segments varied widely by each chromosome.

At metaphase 2n=40 chromosomes were counted. They varied widely in length from 0.5 to 1.6 μm. These chromosomes were divided into three groups by their differences in size. Longer two chromosomes were subterminal centromeric. Two members of medium-sized chromosome group were also subterminal centromeric, but the other two ones in this group were median centromeric. Among the remaining 34 chromosomes, 12 chromosomes were subterminal centromeric, 16 chromosomes were median or submedian centromeic, but six chromosomes were not determined on the centromeric positions.

23) **Pleurothallis sp.** (TBG 32889A), 2n=38, Table 1 and Fig. 23.

One unnamed plant, which somewhat resembled *P. trialata*, from Peru was investigated.

In the resting nucleus, several chromocentral blocks, which varied in number from 11 to 15 per nucleus and were round in shape of 0.4-0.6 μm in diameter, were observed with many chromomeric granules.

At mitotic prophase, chromosomes had early condensed segments situated in the pro-

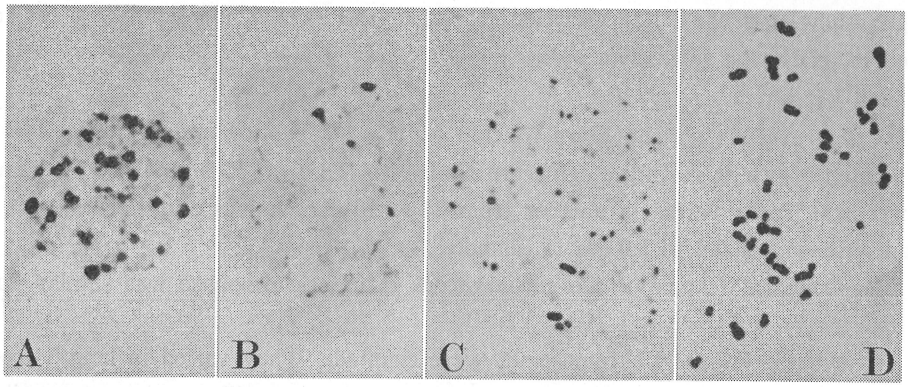

Fig. 22. Chromosomes of *Pleurothallis* aff. *coffeicola*, TBG 32886, 2n=40. A, resting stage. B, early prophase. C, mid-prophase. D, metaphase. A-D, ×1,500.

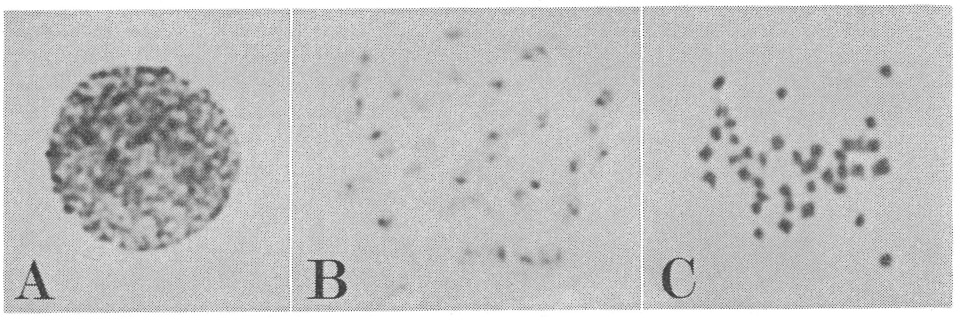

Fig. 23. Chromosomes of *Pleurothallis* sp., TBG 32889A, 2n=38. A, resting stage. B, prophase. C, metaphase. A-C, ×2,500.

ximal regions. The size of the early condensed blocks varied widely by each chromosomes.

The 2n=38 chromosomes were counted. They varied in length from about 0.4 to 0.8 μm. Longer two chromosomes were subterminal centromeric. Centromeres of the remaining 36 chromosomes were not observed.

Discussion

1. Karyomorphological characteristics of chromosomes

1) Chromosome number

A wide range of chromosome numbers of *Pleurothallis* (2n=20, 32, 34, 36, 38, 40, 42, 43, 45, 64, 68, 76, 77, 80 and 84) has been found in the present study (Table 1, Figs. 1-23). All these chromosome numbers of the species examined are reported here for the first time. The multiplication of chromosome number is found in the following series; 2n=20, 40 and 80, 2n=32 and 64, 2n=34 and 68, 2n=36 and 72, and 2n=42 and 84, respectively. But the basic chromosome number for the present genus has not yet been determined.

2) Resting chromosomes

The size and the number of chromocentral blocks in resting nuclei varied widely between the species. Being based on the results of the present observations, the following three types (Fig. 24) can be recognized.

Type A: Chromocentral blocks of the nucleus of this type are the smallest among the three types, measuring 0.2-0.6 μm in diameter, with some exceptions. They are heterogeneous in size and the smallest ones can not be distinguished from chromomeric granules. The number of blocks per nucleus is relatively low. This type was observed in *P. bivalvis*, *P. chanchamayoensis*, *P. matudiana*, *P. tridentata*, *P. velaticaulis*, *P.* sp. (TBG 32889A), and both 2n=40 and 2n=80 strains of *P. revoluta*.

Type B: Nucleus of this type has medium-sized chromocentral blocks. Chromocentral blocks are 0.3-1.0 μm in diameter and are clearly distinguished from chromomeric granules. This type was observed in *P. alopex*, *P. brevipes*, *P. grobyi*, *P. luteola*, *P. restrepioides*, *P. ruscifolia*, *P. saccatilabia*, *P. teres*, *P. xanthochlora* and 2n=64 strain of *P. gelida*.

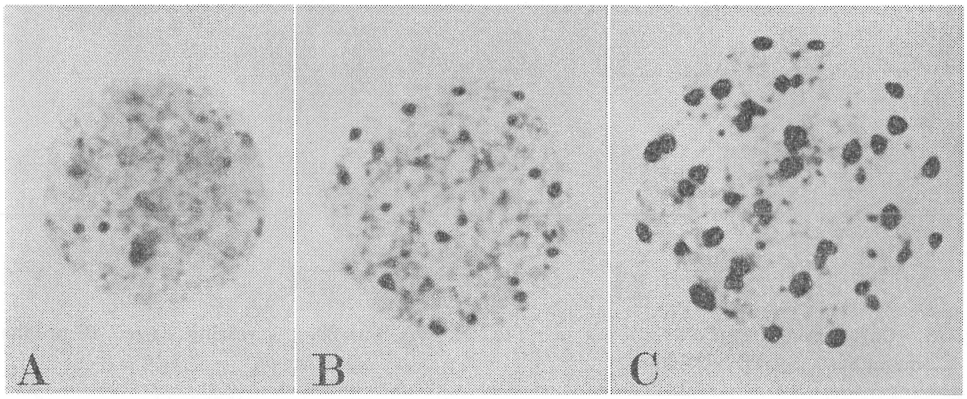

Fig. 24. Three types of the resting nuclei of the *Pleurothallis* studied. A, type A in *P. bivalvis*, characterized by some small chromocentral blocks. B, type B in *P. xanthochlora*, characterized by several medium-sized chromocentral blocks. C, type C in *P. aurantio-lateritia*, characterized by several large chromocentral blocks. (see text). A-C, ×2,400.

Type C: Distinct large chromocenters are observed in nuclei of this type. They are round or elliptical in shape and are 0.4-1.6 μm long in the long axis. This type was found in *P. aurantio-lateritia, P. carinata, P. pachyglossa, P. segoviensis, P.* aff. *coffeicola,* all the three strains of *P. obovata* and 2n=32 strain of *P. gelida.*

The nucleus of the type A is quite comparable to that of the simple chromocenter type proposed by Tanaka (1971), while the nuclei of the type B and the type C are rather similar to those of the round prochromosome type reported by him, because the ratios of the chromocentral blocks to chromosome numbers of these species are 50-70% and are relatively high. Especially in the three taxa, *P. teres* (type B), *P. aurantio-lateritia* (type C), 2n=32 strain of *P. gelida* (type C), the ratios of numbers of chromocentral blocks to chromosome numbers are 70-90%. These three species are considered to belong to the category of the round prochromosome type proposed by Tanaka (1971).

The type A and type B nuclei are found in both low-chromosome-numbered taxa as well as high-numbered taxa (2n=20-80), while the type C is found in low-numbered taxa (2n=32-45). This may indicate that in *Pleurothallis,* the resting chromosome morphology is related to the chromosome number in some extent.

3) Mitotic prophase chromosomes

Prophase chromosomes of *Pleurothallis* have the early condensed segments located in the proximal regions at least. The size and number of the segments varies widely in chromosomes as well as in species. Compactly condensed chromosomes and wholly less stained chromosomes are often observed within one cell.

4) Mitotic metaphase chromosomes

The metaphase chromosomes of *Pleurothallis* are round or rod in shape and are very small, measuring 0.4-1.6 μm. Most members of a chromosome complements of the *Pleurothallis* species have centromeres situated in median or submedian positions, and show symmetric karyotype in chromosome shape. In *P.* aff. *coffeicola,* in contrast, half or more chromosomes of the complement have subterminal centromeres and are asymmetric.

Most species of the genus show homogeneous karyotype in chromosome length, while *P. chanchamayoensis* and *P. matudiana* are bimodal, and *P. restrepioides* and *P.* aff. *coffeicola* are trimodal.

All species studied except *P. obovata,* have no satellite on metaphase chromosomes.

5) Intraspecific polyploidy

In Orchidaceae, intraspecific polyploidy has been reported in *Orchis, Epidendrum, Vanda,* etc. accoding to Tanaka & Kamemoto (1974). In addition to these genera, intraspecific polyploidy is found in two taxa of the present genus; *P. gelida,* 2n=32, 64 and *P. revoluta,* 2n=40, 80 (Fig. 7, 14). In these two species, no notable morphological difference has been generally found between two ploidy types. As mentioned before, however, the resting nuclei of the 2n=32 strain of *P. gelida* differ from that of the 2n=64 strain; 2n=32 strain is of the type C and 2n=64 strain of the type B. In contrast, both 2n=40 and 2n=80 strains of *P. revoluta* are of the same nuclear type, the type A. These facts may suggest that the polyploidization of the two species has followed the different pathways.

6) Intraspecific aneuploidy

According to Tanaka & Kamemoto (1974), intraspecific aneuploidy has been reported by various authors in *Epidendrum, Dendrobium, Gongora, Listera, Oncidium, Pogonia,* and so on of the Orchidaceae. In the present genus, the intraspecific aneuploidy was found in *P. obovata,* 2n=42, 43, and 45 (Fig. 11). In these three strains, the mean number of condensed bodies in the resting nuclei seems to be increased by the addition of chromosome number. In mitotic prophase, condensed chromatin blocks were observed in all the three strains, but the number of blocks was two in the 2n=42 strain, three in 2n=43, and five in 2n=45.

Accoding to Tanaka & Matsuda (1972), in *Tainia laxiflora* which was an orchid with 2n=36+B (0-9B) chromosomes, the accessory chromosomes formed strong condensation at interphase and were composed of heterochromatin. They reported that the number of condensed bodies at prophase cells corresponded to the number of accessory chromosomes. In accordance with the facts mentioned above, *P. obovata* may be considered to have some additional chromosomes.

2. Karyomorphology and taxonomy

The results of karyomorphological studies on 23 species of *Pleurothallis* are reported in this paper. Some aspects related to the taxonomy of the genus will be reported below, even though the results of the present study are not enough to apply to the taxonomy and many other species have not been studied karyomorphologically yet.

The range of karyomorphological variation of *Pleurothallis* is apparently wider than those of the other well-studied genera in Orchidaceae, such as *Paphiopedilum* (Karasawa, 1979) and *Calanthe* (Tanaka, Karasawa & Ishida, 1981). In spite of both genera, *Paphiopedilum* and *Calanthe,* are definitely differentiated in the gross morphology and those species may be rather easily given their right subgeneric or sectional position in each genera, their karyomorphological features are rather uniform espcially in the inter-

phase or the resting nuclei.

These facts indicates that the genus *Pleurothallis* of a traditional concept would be heterogeneous.

Among the subdivisional taxa of the genus proposed by previous authors, Lindley's (1859) Sect. *Macrophyllae-Fasciculatae* may be obviously recognized by having very short columns, short fascicled inflorescences, basically cordate leaves and elongate secondary stems. Thus, *Pleurothallis alopex, P. bivalvis* (presumably should be adopted as the type species), *P. chanchamayoensis, P. matudiana* and *P. tridentata* which are studied in our report, are included in this group. And, the resting nuclear type of these species is of type A and is rather uniform. The resting nuclear type of *P. alopex* might be considered to be of type B, but it is not clearly separated from type A, and is more or less similar to type A. The correlation of gross morphology with karyomophology in this taxonomic group indicates that Sect. *Macrophyllae-Fasciculatae* may be considered to be a natural group.

Acknowledgements

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Summary

1. Karyomorphological observations were made on 23 species of *Pleurothallis* cultivated in TBG.

2. A wide range of chromosome numbers, 2n=20, 32, 34, 36, 38, 40, 42, 43, 45, 64, 68, 76, 77, 80, and 84, was found. These chromosome numbers were newly reported here.

3. Chromosomes at resting stage showed a wide range of variation in the morphology. They could be divided into three groups by the size of chromocentral blocks. On the other hand, the simple chromocenter type (Tanaka, 1971), the round prochromosome type (Tanaka, 1971) and the type between these two are recognized as types of resting nuclei.

4. Intraspecific polyploidy was found in two species; 2n=32 and 64 in *P. gelida* and 2n=40 and 80 in *P. revoluta.*

5. In *P. obovata,* intraspecific aneuploidy, 2n=42, 43, and 45, was found. In this species, condensed bodies seemed to form some accessory chromosomes at prophase.

6. An example of the correlation of gross morphology with karyomorphology was given to Lindley's Sect. *Macrophyllae-Fasciculatae.*

要　　約

筑波実験植物園には，東京大学第1次東亜関連植物調査・環太平洋班（1965～1966），同・第2次（1968～1971）などによって採集された多数の南米産ラン科植物が系統保存されている。*Pleurothallis* 属は熱帯・亜熱帯アメリカに産し，約1,000種を含む多形的な植物群で，当園には28種と未同定の数種が栽培されている。本属の核形態学的性質を明らかにする目的で，このうち23種（未同定の2種を含む）の静止期，分裂期前期，分裂期中期の染色体の観察を行ない，次のような結果を得た。

1. 23種において，2n=20, 32, 34, 36, 38, 40, 42, 43, 45, 64, 68, 76, 77, 80, 84 の染色体数が明らかになった（Table 1, Figs. 1～23）。

2. 観察した全ての種の静止期核には，多数の染色小粒と，それぞれの染色体数より少ない数の，大小の染色中央粒が観察された。染色中央粒の大きさは種によって異なり，次の3型を区別することができた（Fig. 24）。Type A：染色中央粒は直径 0.2～0.6 μm と小型で，小さいものは染色小粒と区別がつきにくい。*P. bivalvis, P. chanchamayoensis, P. matudiana, P. revoluta, P. tridentata, P. velaticaulis, P.* sp.（TBG 32889A）に見られた。Type B：明瞭に区別できる直径 0, 3～1.0 μm の中型の染色中央粒をもつ。*P. alopex, P. brevipes, P. grobyi, P. luteola, P. restrepioides, P. ruscifolia, P. saccatilabia, P. teres, P. xanthochlora* と *P. gelida* の 2n=64 の個体に見られた。Type C：直径 0, 4～1.6 μm の，大型で顕著な染色中央粒を持つ。*P. aurantio-lateritia, P. carinata, P. obovata, P. pachyglossa, P. segoviensis, P.* aff. *coffeicola* と *P. gelida* の 2n=32 の個体に見られた。Type A は Tanaka（1971）の単純染色中央粒型と見なされる。Type B と Type C は，観察される染色中央粒の数がそれぞれの染色体数の 50～70% と比較的高く，球形前染色体型に近い。*P. aurantio-lateritia, P. teres* および *P. gelida* の 2n=32 の個体では，染色中央粒が各々の染色体数の 70～90% 観察され，Tanaka（1971）の球形前染色体型と見なされる。

3. 分裂期前期では，動原体の基部よりに早期凝縮部が観察されたが，凝縮部の大きさ，凝縮の強さ，端部の晩期凝縮部への移行のしかたには染色体間で，また種間で変異が見られた。

4. 分裂期中期染色体は長さ 0.4～1.6 μm と小型で，多くの種は染色体間の長さの差も小さかった。*P.* aff. *coffeicola* では，約半数の染色体が次端部ないし次中部原体型であったが，その他の種の，動原体が観察された染色体の多くは，中部ないし次中部動原体型であった。

5. 種内倍数性が *P. gelida*（2n=32, 64）（Fig. 7）と *P. revoluta*（2n=40, 80）（Fig. 14）に認められた。このうち *P. gelida* の両系統間では静止期核形態にも違いが見られた。

6. *P. obovata* で，2n=42, 43, 45 の種内異数性が観察された。これらの系統間では静止期核の染色中央粒の数に差があり，分裂期前期ではそれぞれに，2, 3, 5個の凝縮塊が観察された（Fig. 11）。このことから，異数性の原因としてB染色体の存在が推察される。

Pleurothallis は外部形態の多様な属として知られているが，以上の結果から，核形態，特に染色体数と静止期核の形態においても多様性を示すことが明らかとなった。一方，属内で外部形態的にまとまった群と考えられている Lindley（1859）の Sect. *Macrophyllae-Fasciculatae* に入る *P. alopex, P. bivalvis, P. chanchamayoensis, P. matudiana, P. tridentata* の5種は，静止期核形態でも互いによく似ており，Sect. *Macrophyllae-Fasciculatae* が自然分類群であるとする考え方が支持される。

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