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Observation of Chromosomes in Some Orchid Species from Peru and Mexico

By

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田中隆荘*・前川文夫**： ペルーおよびメキシコ産のラン数種における染色体研究

(Communicated by Syo KUROKAWA)

Introduction

In the year 1960, Maekawa, one of the authors, made a botanical expedition to two areas of Peruvian Andes Mountains, Peru, and Sierra Madre in southern Chiapas, Mexico, as a member of The Second Scientific Expedition of the University of Tokyo to the Andes. The wild orchids collected from the two areas were planted in the Koishikawa Botanical Garden, the University of Tokyo. In 1964 and 1965, Tanaka made a cytological observation in some of these orchids. The results of the cytological observations have long been overdue to be published by the delay of the flowering of these orchids and the difficulty of taxonomic treatment without flower. These orchids transferred to the Tsukuba Botanical Garden, National Science Museum a few years ago, and some of them recently produced flowers. The present report was prepared by the suggestions of Messrs. T. Hashimoto and M. Nakata, staffs of the Tsukuba Botanical Garden and who did a taxonomic treatment on these orchids, and by the promotion of Dr. S. Kurokawa, the director of the Tsukuba Botanical Garden. The authors are grateful to them for their kind help.

Material and Method

The number of orchids observed cytologically were limited by an inevitable difficulty in having chance to collect cytological samples from distant place. But, 40 samples were obtained by the three times of sampling trips in June and July of 1964 and in July of 1965. Of the 40 samples 38 were successfully observed. As shown in Table 1, the present paper deals with 16 samples of them, dried specimens of which are preserved in TI. The remaining 22 will be reported successively following to taxonomic treatment. For the taxonomic treatment of subtribes and Genera, Dressler & Dodson (1960) have been followed.

The observation of chromosomes was made in the cells of root tips which were

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Table 1. Species and chromosome numbers of the orchids collected from Peruvian Andes and Chiapas, Mexico.

Species	Locality	TBG[1] accession number	Chromosome number (2n)	Dried specimen number in TI
Spiranthinae				
Cyclopogon conqestus (Vell.) Hoehne	Peru[2]	32696-32700	36	65-7-13-8
Pleurothallidinae				
Apatostelis rubens (Schltr.) Garay	Mexico[3]	32965	32	64-6-8-12
Masdevallia civilis Reichb. f. & Warsc.	Peru	32860, 32861	36	64-6-8-7
Pleurothallis matudiana C. Schweinf.	Peru	32894	72	64-6-8-10
P. obovata Lindl.	Peru	32950	44	64-6-8-8
P. restrepioides Lindl.	Peru	32960	78	64-6-8-5
Stelis argentata Lindl.	Peru	32974	30	65-7-13-7
	Peru	32976	30	65-7-13-9
Epidendrinae				
Epidendrum ciliare L.	Mexico	32659-32663	40	64-6-8-18
E. lanipes Lindl.	Peru	32617, 32618	40	64-6-8-4
Encyclia fragrans (Sw.) Lemée	Peru	32648	40	64-6-8-22
Neolehmannia difformis (Jacq.) Pabst	Peru	32634	40	64-7-31-6
Stanhopeinae				
Gongora quinquinervis Ruiz & Pav.	Peru	32979	40	64-7-31-1
Maxillariinae				
Maxillaria violaceo-punctata Reichb. f.	Peru	32755-32757	42	64-6-8-21
Xylobium variegatum (Ruiz & Pav.) Garay & Dunsterv.	Peru	32771-32773	40	64-6-8-17
	Peru	32774	40	65-7-13-4

1. Tsukuba Botanical Garden.
2. Peruvian Andes Mountains, Peru.
3. Sierra Madre, Chiapas, Mexico.

fresh and well growing. The results of chromosome countings are shown in Table 1.

Cytological preparation was carried out by the method described by Tanaka (1959). Root tip sections 0.5-1.0 mm in length were pretreated in 0.002M 8-hydroxyquinoline at 15°C for 4 hr. The pretreated root tip sections were fixed in 45% acetic acid at 10°C for 10 min. The fixed sections were macerated in the solution mixed two volumes of 1N hydrochloric acid and one volume of 45% acetic acid at 60°C for 30 sec. On a slide glass the macerated section was stained in 1% aceto-orcein for 10 min, then it was squashed softly under cover glass. A strong squashing was given after heating at about 70°C.

The morphology of chromosomes were studied in the three phases of nuclei, *i.e.*, resting, interphase and mitotic stages. The cells in young velamen were used to observe resting chromosomes, and the cells in periblem were used to study the chromosomes at interphase and mitotic stages. The results of the morphological studies were classified according to the proposals of Tanaka (1971a, 1971b, 1977, 1980, 1982). The classification of the mitotic metaphase chromosomes was made following to the system proposed by Levan *et al.* (1964). The terms, simple chromocenter type, complex chromocenter type and prochromosome type were used to classify the karyotype concerning resting stage. The terms, homogeneous, gradual, heterogeneous and bimodal were used to describe the characteristics of karyotype on chromosome length, and the terms, symmetry and asymmetry were used to describe the characteristics of karyotype concerning arm ratio. These terms were used as defined by Tanaka (1980, 1982).

Observation

Fourteen species belonging to 11 genera (Table 1) have been investigated cytologically. The results of observations on the morphology of chromosomes in each of the species are summarized as follows.

1. Spiranthinae

Cyclopogon conquestus (Vell.) Hochne, Fig. 1.

2n=36 chromosomes (the first record) were clearly discernible at mitotic prophase and metaphase. The chromosomes were medium-sized measuring from about 5 μm to 1 μm, varied bimodally and consisted of two long, 34 medium and small-sized chromosomes. The two longest chromosomes had median centromeres and condensed early at prophase in almost whole region, while the remaining 34 chromosomes had centromeres located at median, submedian or subterminal position and had early condensed small segments proximally or distally located which transformed gradually to late condensed segments.

Chromosomes at resting stage formed several chromocenters which varied in shape irregularly from round blocks to fibrous blocks.

According to proposal of Tanaka (1980, 1982) for the classification of the morphology of chromosomes, the morphology of the chromosomes of this species was regarded as sym-

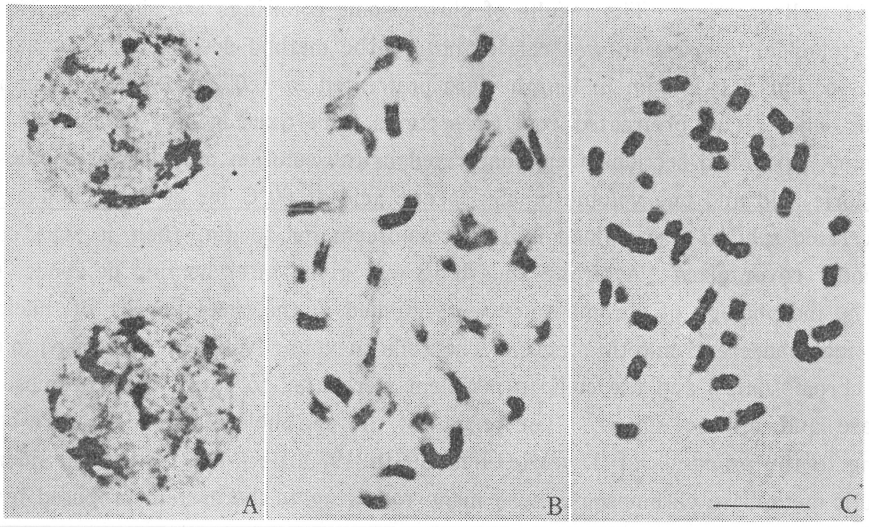

Fig. 1. Photomicrographs of the somatic chromosomes of *Cyclopogon congestus*. A, chromosomes in two nuclei at resting stage. B, chromosomes at mitotic late prophase, 2n=36. C, chromosomes at mitotic metaphase, 2n=36. Bar indicates 10 μm.

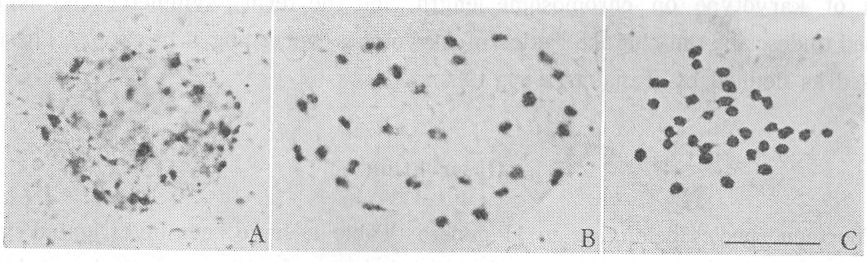

Fig. 2. Photomicrographs of the somatic chromosomes of *Apatostelis rubens*. A, chromosomes at resting stage. B, chromosomes at mitotic late prophase, 2n=32. C, chromosomes at mitotic metaphase, 2n=32. Bar indicates 10 μm.

metric type in arm ratio, bimodal type in the variation of the length of the complement, gradual type in condensation at mitotic prophase, and complex chromocenter type at resting stage.

2. Pleurothallidinae

Apatostelis rubens (Schltr.) Garay, Fig. 2.

2n=32 chromosomes (the first report) were counted at mitotic metaphase and prophase in the cells of root tips. The chromosomes were very short and rod-shaped, and showed homogeneous size variation measuring about 1.5-1.0 μm. Almost all of the chromosomes had centromeres at median or submedian positions, while some had at subterminal positions. All of the chromosomes had early condensed segments at proximal regions of both arms. The chromosomes at resting stage formed many chromocenters which varied in shape from rod to round.

Masdevallia civilis Reichb. f. & Warsc., Fig. 3.

2n=36 small chromosomes (a new count) were observed in root tip cells. All of the chromosomes were almost of the same size varying from about 2.0 μm to 1.0 μm in length and appeared to be median or submedian centromeric. The longest chromosome group in the complement was median centromeric and was composed of two members. The morphology of chromosomes at mitotic prophase was of the proximal type in early condensing segments and that of resting chromosomes belonged to loosely aggregated simple chromocenter type.

Pleurothallis matudiana C. Schweinf., Fig. 4.

Root tip cells revealed 2n=72 chromosomes (a new record). The chromosomes were round or rod-shape and very small and varied from about 2.0 μm to 0.7 μm. They appeared to be median or submedian centromeric. The longest chromosome of the complement was median. Four of the long chromosomes were classified together in the longest group. The second longest chromosome group had submedian centromeres and was composed of four members. The pattern of the condensation of chromosomes at resting stage and mitotic prophase was similar to that of the chromosomes of *Masdevallia civilis* described in the previous paragraph. *Pleurothallis matudiana* is regarded as a tetraploid related to *M. civilis*, since its 2n=72 chromosomes are considered to be the double of the 2n=36 of *M. civilis* in number and also resemble morphologically chromosomes of the latter species.

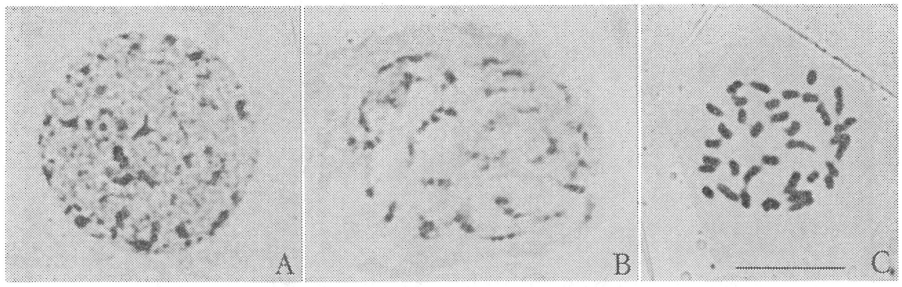

Fig. 3. Photomicrographs of the somatic chromosomes of *Masdevalia civilis*. A, chromosomes at resting stage. B, chromosomes at mitotic mid-prophase. C, chromosomes at mitotic metaphase, 2n=36. Bar indicates 10 μm.

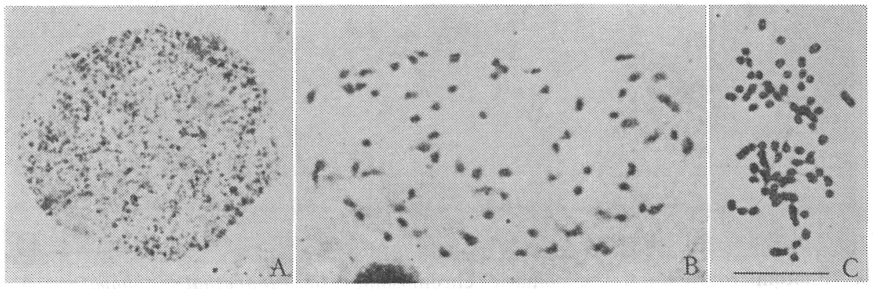

Fig. 4. Photomicrographs of the somatic chromosomes of *Pleurothallis matudiana*. A, chromosomes at resting stage. B, chromosomes at mitotic late prophase, 2n=72. C, chromosomes at mitotic metaphase, 2n=72. Bar indicates 10 μm.

P. obovata Lindl., Fig. 5.

Somatic chromosomes in root tip cells were counted to be 2n=44 (a new record). The chromosomes were very short and homogeneous, and measured about 1.5-1.0 μm in length. The centromeres were not clear in some chromosomes but a tentative observation showed median and submedian centromeres in many chromosomes. Chromosomes at resting stage showed many round medium-sized chromocenters which were also observed in interphase nuclei. The karyotype of this species was found to differ from that of *P. matudiana* in both mitotic and resting stages.

P. restrepioides Lindl., Fig. 6.

Somatic chromosome number 2n=78 (a new count) was determined from several nuclear plates at mitotic prophase and metaphase. The complement consisted of small chromosomes varied in graded series. The morphological features of the chromosomes at both mitotic and resting stages were observed to be similar to those of *P. obovata* described in the previous paragraph.

Stelis argentata Lindl., Fig. 7.

2n=30 chromosomes were counted in two plants of this species. This is a new number in the genus *Stelis* (Tanaka & Kamemoto, 1983). The size of the chromosomes

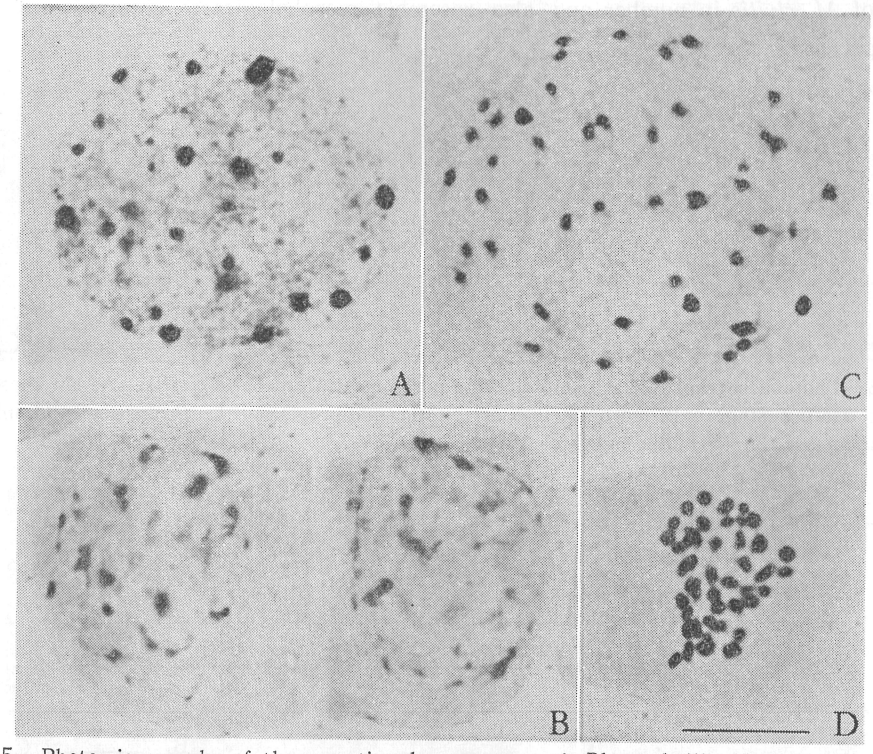

Fig. 5. Photomicrographs of the somatic chromosomes of *Pleurothallis obovata*. A, chromosomes at resting stage. B, chromosomes in two nuclei at interphase. C, chromosomes at mitotic late prophase, 2n=44. D, chromosomes at mitotic metaphase, 2n=44. Bar indicates 10 μm.

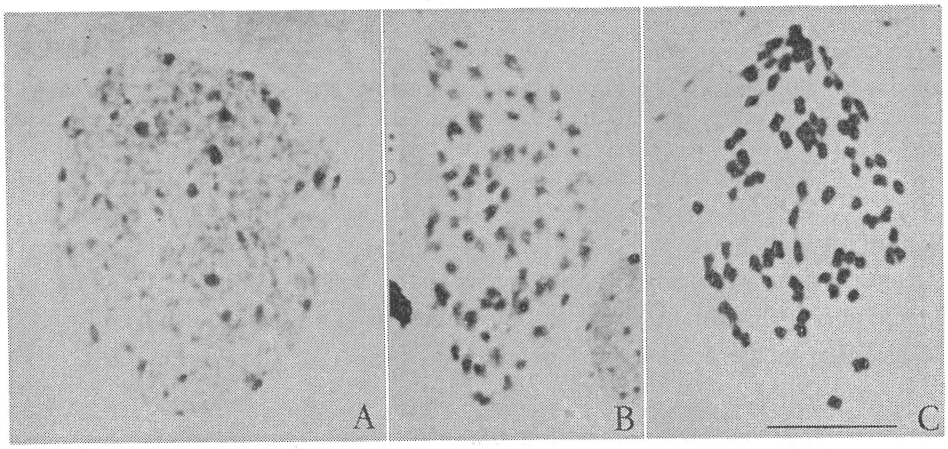

Fig. 6. Photomicrographs of the somotic chromosomes of *Pleurothallis restrepioides*. A, chromosomes at resting stage. B, chromosomes at mitotic late prophase, 2n=78. C, chromosomes at mitotic metaphase, 2n=78. Bar indicates 10 μm.

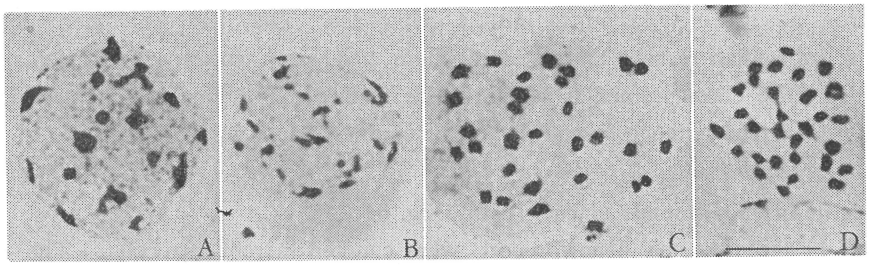

Fig. 7. Photomicrographs of the somatic chromosomes of *Stelis argentata*. A, chromosomes at resting stage. B, chromosomes at interphase. C, chromosomes at mitotic late prophase, 2n=30. D, chromosomes at mitotic metaphase, 2n=30. Bar indicates 10 μm.

within the complement was very small and varied from about 2.0 to 1.0 μm. The chromosomes were median or submedian centromeric and had early condensed large segments in proximal region. They formed several large chromocenters varied in size and shape from rod-shaped to the blocks with irregular surface. The karyotype at resting stage was regarded as compactly aggregated simple chromocenter type.

Six species belonging to four genera in subtribe Pleurothallidinae used in the present study were found to have common features in their karyotypes at resting and mitotic stages, while they showed difference in chromosome number.

3. Epidendrinae

Epidendrum ciliare L., Fig. 8.

2n=40 chromosomes were counted in root tip cells. This number confirms the previous reports by Geitler (1940), Kamemoto (1950) and others (Tanaka and Kamemoto 1983). The chromosomes were smaller and measured 2.0-1.5 μm showing gradual series and had centromeres located in median or submedian positions. They had early condensed small

segments in the proximal regions of both arms, while in some chromosomes there were no early condensed segments. The early condensed segments transformed gradually to late condensed segments. The chromosomes at resting stage formed few chromocenters which varied in size and shape irregulary. The karyotype in resting stage was regarded as of simple chromocenter type.

E. lanipes Lindl., Fig. 9.

The chromosome number 2n=40 (a new count) was determined. The morphology of chromosomes at both mitotic and resting stages was found to be similar to that of *E. ciliare* described in the previous paragraph.

Encyclia fragrans (Sw.) Lemée, Fig. 10.

2n=40 chromosomes (a new report) were counted in the cells of root tips, while the same chromosome number was reported in the other species of the same genus (Blumenschein, 1960). The somatic chromosomes were smaller in size varied gradually in length from about 2.0 to 1.0 μm at mitotic metaphase. They had centromeres located in median or submedian regions and some of them had early condensed segments located proximally. The chromosomes at resting stage formed a few chromocenters which were round or rod-shaped and were regarded as of simple chromocenter type.

Neolehmannia difformis (Jacq.) Pabst, Fig. 11.

2n=40 chromosomes (a new report) were counted in root tip cells. The chromo-

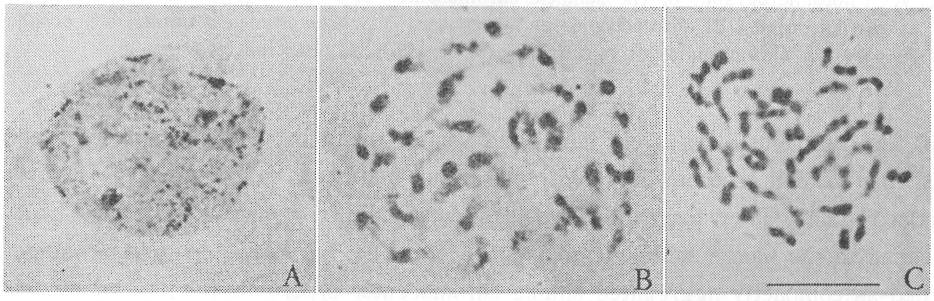

Fig. 8. Photomicrographs of the somatic chromosomes of *Epidendrum ciliare*. A, chromosomes at resting stage. B, chromosomes at mitotic mid-prophase, 2n=40. C, chromosomes at mitotic metaphase, 2n=40. Bar indicates 10 μm.

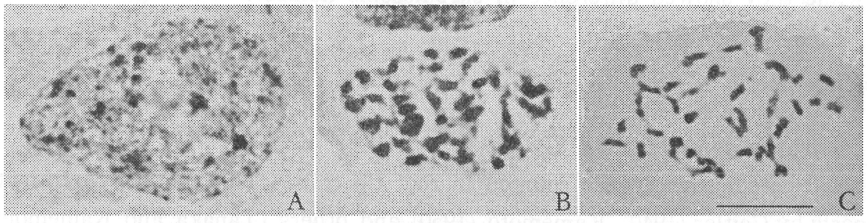

Fig. 9. Photomicrographs of the somatic chromosomes of *Epidendrum lanipes*. A, chromosomes at resting stage. B, chromosomes at mitotic mid-prophase. C, chromosomes at mitotic metaphase, 2n=40. Bar indicates 10 μm.

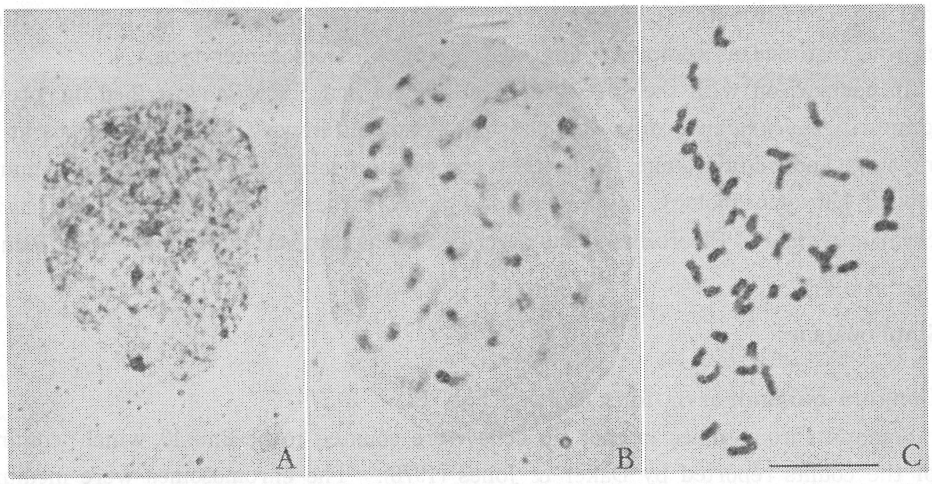

Fig. 10. Photomicrographs of the somatic chromosomes of *Encyclia fragrans*. A, chromosomes at resting stage. B, chromosomes at mitotic mid-prophase, 2n=40. C, chromosomes at mitotic metaphase, 2n=40. Bar indicates 10 μm.

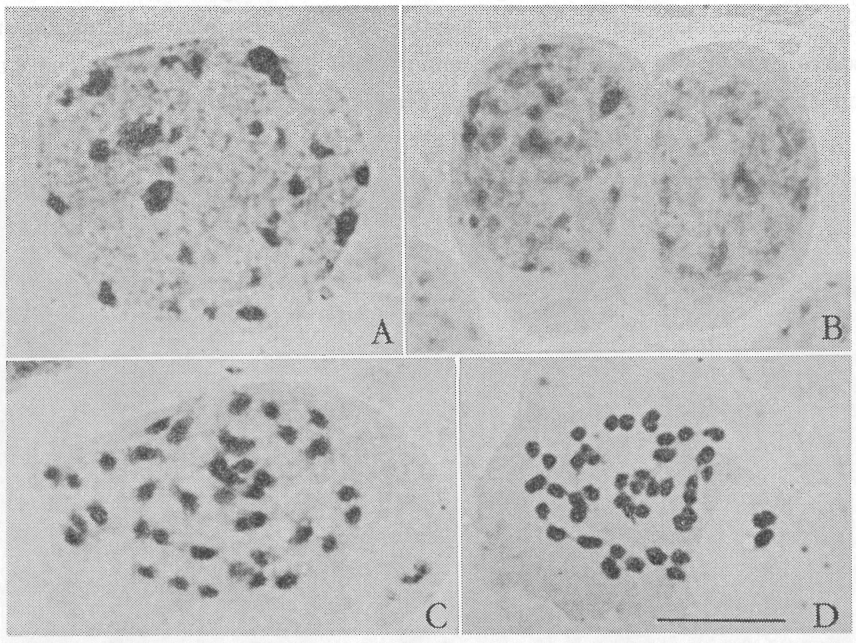

Fig. 11. Photomicrographs of the somatic chremosomes of *Neolehmannia difformis*. A, chromosomes at resting stage. B, chromosomes in two nuclei at interphase. C, chromosomes at mitotic late prophase, 2n=40. D, chromosomes at mitotic metaphase, 2n=40. Bar indicates 10 μm.

somes were small measured 1.5-0.5 μm, graded, round or rod-shaped at mitotic metaphase, and most of them were determined to have median or submedian centromeres. At mitotic prophase all of the chromosomes formed early condensed segments located in the proximal regions. At resting stage the chromosomes showed several compactly aggregated chromocenters which varied in size and shape irregularly, while the chromosomes at interphase

showed few chromocenters condensed compactly. The morphology of resting chromosomes was regarded as compactly aggregated simple chromocenter type.

In comparison with the species of *Epidendrum* and *Encyclia* described in previous paragraphs, *Neolehmannia differmis* showed great difference in the karyotypes at both resting stage and mitotic stage, while they showed the same 2n=40 chromosome number. In view of karyomorphology, *Neolehmannia differmis* rather resembled *Pleurothallis obovata* and *Stelis argentata* of Pleurothallidinae described in the previous paragraphs (Figs. 5 and 7).

4. Stanhopeinae

Gongora quinquinervis Ruiz & Pav., Fig. 12.

Somatic chromosomes in root tip cells were counted to be 2n=40 which confirmed one of the counts reported by Daker & Jones (1970). The chromosomes were medium in size and varied in length gradually from 3.0 to 1.0 μm. They had median or submedian centromeres, while a few of the short chromosomes had subterminal centromeres. Chromosomes at prophase had in proximal region early condensed segments, which transformed gradually to late condensed segments located in distal regions. At resting stage the chromosomes formed many small chromocenters with irregular surface. The karyotype of resting chromosomes was regarded as of complex chromocenter type.

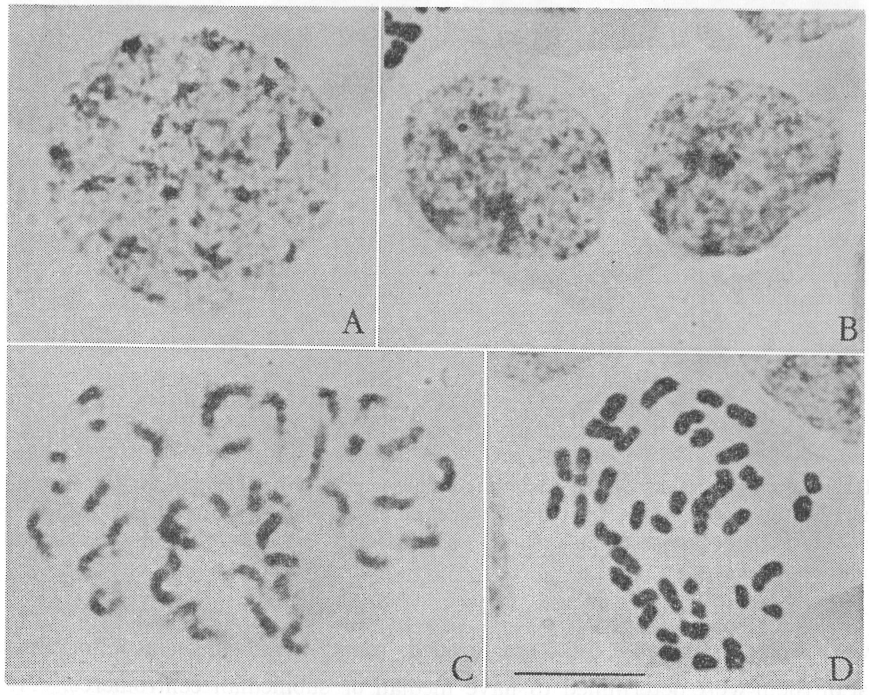

Fig. 12. Photomicrographs of the somatic chromosomes of *Gongora quinquinervis*. A, chromosomes at resting stage. B, chromosomes in two nuclei at interphase. C, chromosomes at mitotic mid-prophase, 2n=40. D, chromosomes at mitotic metaphase, 2n=40. Bar indicates 10 μm.

5. Maxillariinae

Maxillaria violaceo-punctata Reichb. f., Fig. 13.

Somatic chromosomes at mitotic metaphase were counted to be 2n=42 which was a new record in the species of *Maxillaria* (Tanaka & Kamemoto, 1983). Chromosomes at mitotic metaphase were observed to be medium in size and heterogeneous in length measured about 4.0-1.0 μm. The longest chromosome of the complement had median centromere. The shortest chromosome had subterminal centromere. Most of the chromosomes had the centromeres located in median or submedian positions.

Chromosomes at mitotic prophase formed early condensed segments located in the proximal regions and late condensed segments in the distal regions. Gradual transformation was observed between the two segments.

The chromosomes at resting stage formed several darkly stained chromatin blocks. The blocks varied in shape from round small spherule to irregular concave. Some of the chromatin formed chromonemic threads which aggregated to a fibrous block. The morphology of chromosomes at resting stage was regarded as of the complex chromocenter type.

Xylobium variegatum (Ruiz & Pav.) Garay & Dundterv., Fig. 14.

Two plants which were studied cytologically showed the same 2n=40 chromosome numbers (a new record).

Chromosomes at mitotic metaphase were relatively longer than those of *Maxillaria violaceo-punctata* described in the previous paragraph. The longest chromosome of the complement was about 5.0 μm and had centromere in median position. The shortest chromosome was about 1.5 μm and had centromere in median position. The length of the chromosomes varied gradually between the longest and the shortest ones showing successive variation in length, thus constituting a heterogeneous gradual karyotype. Of the 40

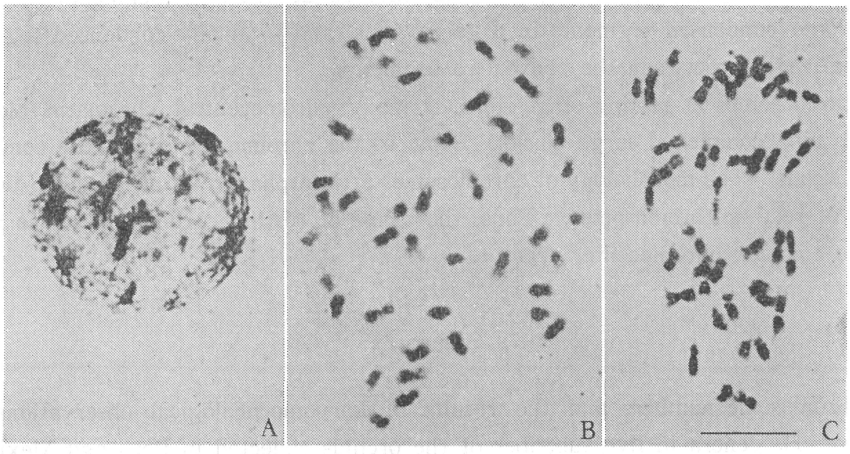

Fig. 13. Photomicrographs of the somatic chromosomes of *Maxillaria violaceo-punctata*. A, chromosomes at resting stage. B, chromosomes at mitotic late prophase, 2n=42. C, chromosomes at mitotic metaphase, 2n=42. Bar indicates 10 μm.

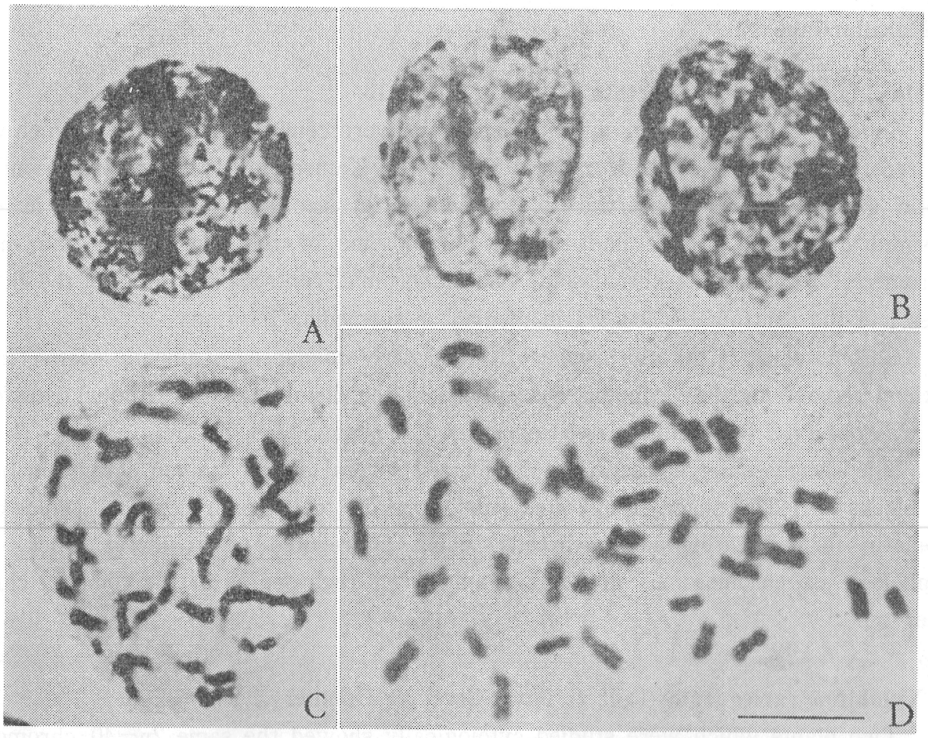

Fig. 14. Photomicrographs of the somatic chromosomes of *Xylobium variegatum*. A, chromosomes at resting stage. B, chromosomes in two nuclei at interphase. C, chromosomes at mitotic mid-prophase. D, chromosomes at mitotic metaphase, 2n=40. Bar indicates 10 μm.

chromosomes almost all had median or submedian centromeres, while some of relatively small chromosomes had subterminal centromeres. Thus, the shape of chromosomes of the complement was regarded as the symmetric karyotype in arm ratio.

Chromosomes at mitotic prophase had early condensed large segments in proximal regions, late condensed segments in distal regions, and gradually condensed segments in interstitial regions between the above two segments.

Chromosomes at resting stage formed many chromocentral chromatin blocks aggregated into complexed large blocks. Some of the chromatin blocks were composed of fibrous threads. The morphology of chromosomes at interphase was observed to be similar to that of resting chromosomes. Thus, the shape of resting chromosomes was regarded as of the complex chromocenter type.

Summary

Chromosome numbers and the results of karyomorphological observations on 14 species of 11 genera in five subtribes of the orchids collected in Peru and Mexico were reported. *Pleurothallis matudiana* with 2n=72 is regarded as a tetraploid related to *Masdevallia civilis* with 2n=36, rather than the species of the genera *Pleurothallis* and

Stelis studied. *Neolehmannia difformis* (2n=40) of Epidendrinae karyomorphologically rather resembled *Pleurothallis obovata* (2n=44) and *Stelis argentata* (2n=30) of Pleurothallidinae than to the two species of *Epidendrum* (2n=40) and *Encyclia fragrans* (2n=40) in Epidendrinae.

要　　約

筆者らは，東京大学ペルーアンデスおよびメキシコ，チアパス第2次学術調査において採集された野生ランのうち，11属14種（5亜連）の染色体数および核型を観察した（Table 1, Figs. 1～14)。

Pleurothallis matudiana (2n=72) は4倍体であり，本報で観察した *Pleurothallis* 属や *Stelis* 属の他種よりもむしろ *Masdevallia civilis* (2n=36) に類縁がある。

Epidendrinae の *Neolehmannia difformis* (2n=40) は核形態学的には Epidendrinae の *Epidendrum* (2n=40) の2種や *Encyclia fragrans* (2n=40) よりも Pleurothallidinae の *Pleurothallis obovata* (2n=44) や *Stelis argentata* (2n=30) に類似している。

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