CYTOLOGICAL STUDIES OF INDIAN ORCHIDS

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Thirty-five species belonging to nineteen genera covering all the four tribes of Orchidaceae, collected from different areas of India at different altitudes, have been studied cytologically.

Detailed karyotype study of all the species shows chromosome numbers ranging from $2n=20$ to 62, the most common ones being multiples of $X=19$, 20, 21, 22. They show more or less homogeneity in size with mainly medium and short chromosomes. In *Cypripedium elegans* of the primitive tribe Cypripediloideae belonging to the subfamily Diandrae, very long chromosomes with symmetrical karyotype have been noted.

Interrelationships between various genera, their taxonomic status and the relative roles of aneuploidy and polyploidy in their evolution have been discussed. The significance of structural alterations in the evolution of different species has also been indicated. In case of different species of *Habenaria* though numerical alterations in the chromosomes are noted, yet the presence of different combinations of the chromosome types indicates the possible role of structural alterations in addition to the numerical changes in their origin. The distinct role of structural alterations in speciation can be clearly seen in the seven species of *Calanthe* studied. From the cytological data observed, the positions of the different genera as observed in Schlechter's system of classification can be justified.

The general chromosome morphology in the members of the tribe Polychondreae and the marked size difference of chromosomes in *Epipactis* may show the relatively advanced nature of this tribe and the probable hybrid origin of the genus.

INTRODUCTION

The family Orchidaceae is a very successful one with a wide distribution throughout the world, mainly in the temperate and tropical regions. Almost 20,000 species under 735 genera have been recorded in addition to a vast number of horticultural forms raised through selection and hybridisation by orchidologists over a long period (Willis 1966). In India itself approximately 1600 species have been reported occupying habitats from the tropical plains to the temperate Himalayas even up to 13,000 feet (Maheshwari *et al.* 1965). Though mainly epiphytic, yet a large number of terrestrial forms also occur.

Cytological data on the Indian members are available only for a relatively few genera (Sampath Kumaran and Rangaswamy 1931; Swamy 1941; Arora 1960; Sharma and Chatterji 1961, 1956; Mehra and Yash Pal 1961; Mehra and Bawa 1962; Chennaveeraiah and Jorapur 1966). Most of them are records of chromosome numbers. Sharma and Chatterji (1966), from investigations on 35 species of orchids under 17 genera to study the interrelationships and the lines of evolution, showed the presence of a wide spectrum of basic numbers within each tribe and often within the same genus.

The classification of the family has been a highly controversial one. Bentham and Hooker (1883) divided the entire Orchidaceae into five distinct tribes. Pfitzer (1889) and Schlechter (1926), however, have included the genera under two principal subfamilies Diandrae and Monandrae. But their classifications differ mainly on the positions of different taxa under different subtribes. The system of classification by Dressler and Dodson (1960) has considerably changed the positions of different taxa from those advocated in the previous classifications. The debated taxonomic positions of the different genera, the paucity of cytological knowledge of the Indian members of these genera and the possibility of finding a relationship between the chromosome constitution and the ecological preference prompted the initiation of this project.

Materials and Methods

The present work was undertaken on wild orchids collected from different areas of Northern India and the Himalayan ranges. Thirty-five species of the family Orchidaceae belonging to nineteen genera were studied during the present investigation. Four species namely, *Cypripedium elegans*, *Epipactis latifolia*, *Habenaria latilabris* and *Orchis latifolia* were collected from higher altitudes of the Western Himalayas; *Pogonia plicata* from the suburbs of Calcutta, and the remaining from different localities of the Eastern Himalayas. They were identified through the courtesy of authorities at the herbarium of the National Botanic Gardens at Sibpore. The plants were grown in the glass-houses of the Department of Botany at the University of Calcutta.

For the study of the somatic chromosomes, healthy young root tips were taken. A saturated aqueous solution of *p*-dichlorobenzene (Sharma and Mookerjea 1955) was found to be the most effective pretreating agent in all the materials investigated. The pretreatment was carried out at 10–12°C for three hours. The root tips were then kept in acetic acid–ethyl alcohol mixture (1:2) for one hour after rinsing in distilled water, heated in a mixture of 2 per cent acetic-orcein–(N) HCl (9:1) over a flame for a few seconds and squashes were made in 1 per cent acetic-orcein solution, applying uniform pressure over the cover glass. For meiotic studies flower buds were fixed in acetic acid-ethyl alcohol mixture (1:2) for 24 hours in the field and then transferred to 70 per cent alcohol. Temporary squash preparations were made following Belling's 1 per cent aceto-carmine squash technique.

Figures were drawn at a table magnification of approximately ×2000 using a Zeiss Winkel microscope with an eyepiece of 12.5×, objective of 100× and an aplanatic condenser of 1.3 N.A. In the figures, the chromosomes with secondary constrictions are drawn by outlines only.

Observations

The different members of Orchidaceae under investigation show chromosome numbers ranging from 2n=20 in *Cypripedium elegans* to 2n=62 in *Pogonia plicata*. In species showing more than one number, like *Habenaria decipiens* (2n=42, 84), that occurring in the higher frequency is taken to be the normal one. A wide range has been found in the size of the chromosomes. Very long chromosomes have been found in *Cypripedium elegans* of the first tribe Cypripediloideae and also in one member of the tribe Kerosphaereae. Otherwise, the chromosomes are, in general, medium to short in size.

Due to wide variation in the karyotypes, the common types have been described first within a genus where more than one species have been studied and their minor differences have been dealt with separately for each species.

For the sake of convenience, the observations were tabulated on the basis of the chromosome morphology of the different genera studied.

TABLE I

Name of the species	Chromosome number and types	Range of variation in length of chromosomes
Subfamily : DIANDRAE		
Tribe : Cypripediloideae		
Cypripedium elegans Reichb. f.	$2n=20=A_8+B_2+C_{10}$	37.5 μ–19.2 μ
Subfamily : MONANDRAE		
Division I : BASITONAE		
Tribe : Ophrydoideae		
Habenaria arietina Hook. f.	$n=24$; $2n=48=A_2+C_{38}+D_8$	2.0 μ–1.4 μ
	$2n=42=A_4+C_{32}+D_6$	5.0 μ–2.5 μ
H. decipiens Wight	$2n=84=A_6+C_{72}+D_6$	
H. latilabris Hook. f.	$n=19$	
H. mullaeformis Hook. f.	$n=24$; $2n=48=A_4+B_2+C_{40}+D_2$	4.0 μ–2.0 μ
Orchis latifolia Linn.	$n=21$	
Division II ACROTONAE		
Tribe : Kerosphaereae		
Microstylis khasiana Hook. f.	$2n=42=A_4+B_4+C_{34}$	3.0 μ–1.5 μ
Coelogyne elata Lindl.	$2n=44=A_2+A'_2+B_8+C_{26}+D_6$	4.5 μ–2.0 μ
Pleione praecox Don.	$2n=40=A_6+B_{34}$	4.0 μ–1.5 μ
Thunia alba Reichb. f.	$2n=44=A_4+B_{40}$	4.0 μ–1.7 μ
T. marshalliana Reichb. f.	$2n=38=A_2+B_{36}$	2.2 μ–1.2 μ
Dendrobium pierardii Roxb.	$2n=40=A_4+B_{36}$	2.5 μ–1.5 μ
D. transparens Wall. ex Lindl.	$n=20$	
Arachnanthe Clarkei Rolye	$2n=38=A_8+B_2+C_{28}$	15.5 μ–8.5 μ
Doritis taenialis Benth.	$2n=40=A_2+B_2+C_{36}$	3.0 μ–1.8 μ
Phalaenopsis rosea Lindl.	$n=19$	
P. mannii Reichb. f.	$2n=38=A_4+B_2+C_4+D_{22}+E_6$	4.0 μ–2.0 μ
Calanthe alismaefolia Lindl.	$n=22$; $2n=44=A_2+B_4+C_{38}$	6.6 μ–2.7 μ
C. biloba Lindl.	$2n=38=A_8+B'_4+D_{22}+E_4$	3.5 μ–2.0 μ
C. brevicorum Lindl.	$n=24$	
C. chloroleuca Lindl.	$2n=28=A_2+C'_2+D_{24}$	3.4 μ–1.8 μ
C. herbacea Lindl.	$n=21$; $2n=42=B''_2+C_2+D_{36}+E_2$	8.6 μ–3.1 μ
C. masuca Lindl.	$2n=52=A_2+B_4+B'_2+D_{40}+E_4$	6.5 μ–2.0 μ
C. puberula Lindl.	$2n=42=A_4+B_2+D_{36}$	5.5 μ–2.5 μ
C. tricarinata Lindl.	$2n=42=C''_2+D_{40}$	5.5 μ–2.8 μ
Phaius mishmensis Reichb. f.	$n=31$	
P. wallichii Lindl.	$2n=48=A_2+A'_4+B_2+C_{36}+D_4$	6.5 μ–1.5 μ

P. wallichii Lindl. var. *assamica*	n=21	
Spathoglottis plicata Blume	$2n=42=A_2+B_2+C_2+D_{28}+E_8$	5.7 μ–3.0 μ
Cymbidium longifoliumloman D. Don	$2n=42=A_2+A'_2+B_{28}+C_{10}$	4.0 μ–1.5 μ
C. lowianum Reichb. f.	n=22	
C. pendulum Sw.	$2n=40=A'_4+B_{26}+C_{10}$	3.5 μ–2.0 μ
Tribe : Polychondreae		
Anthogonium gracile Lindl.	n=27	
Epipactis latifolia All.	$2n=36=A_2+B_6+C_4+D_{16}+E_8$	9.0 μ–1.7 μ
Pogonia plicata Lindl.	2n=62	6.5 μ–1.5 μ
Spiranthes sinensis Amer.	$2n=30=A_2+B_4+C_{12}+D_2+E_{10}$	3.0 μ–1.8 μ

Discussion

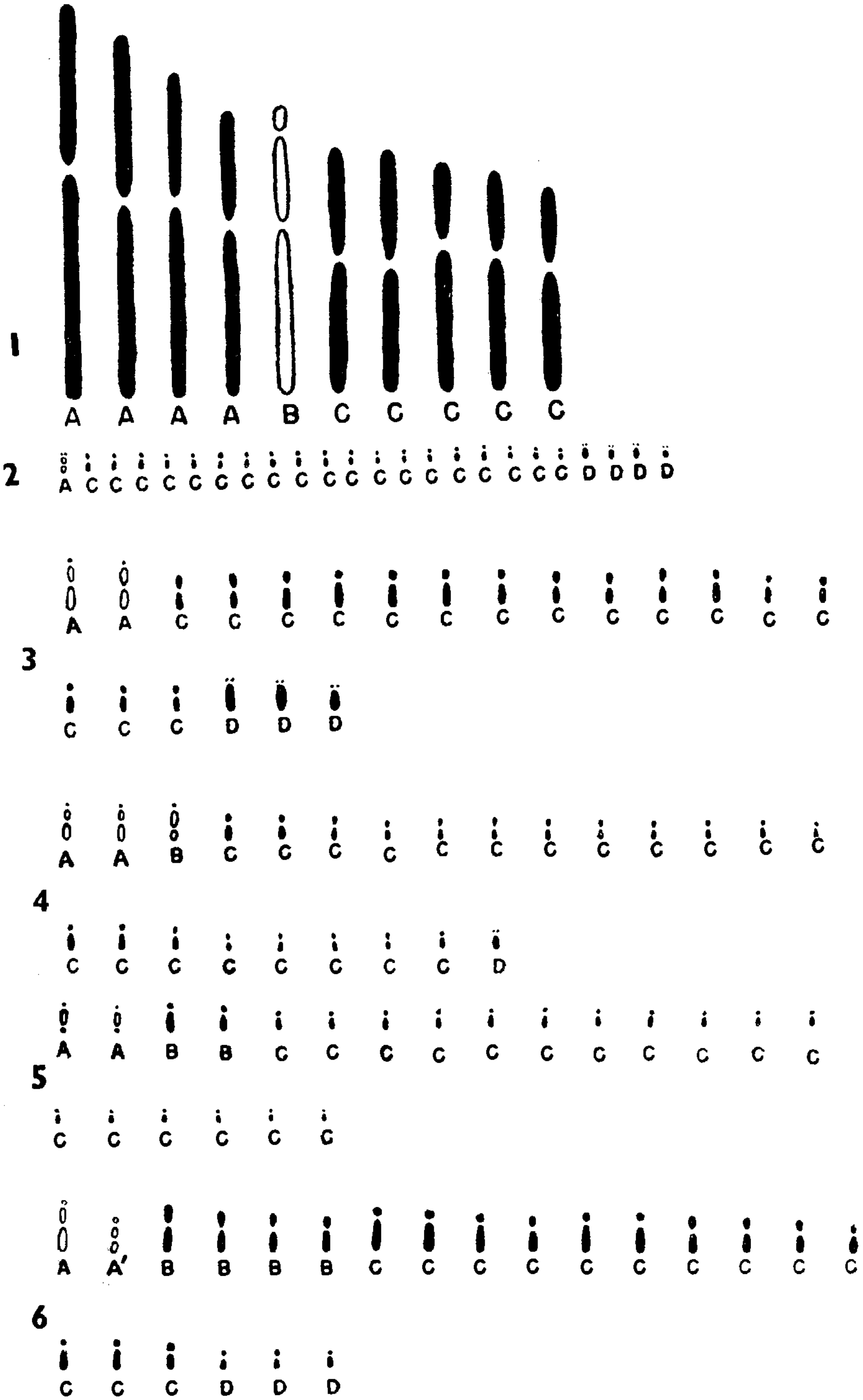
Within the tribe Cypripediloideae, one species of *Cypripedium* namely, *C. elegans* was investigated with 2n=20 chromosomes. N=19 and 2n=22 and 26 have been reported in *C. barbatum, C. spectabile* and *C. speciosum* respectively by Chardard (1963), Hoffmann (1930) and Mulay and Panikkar (1953). Both 2n=20 and 22 chromosomes have been observed in *C. calceolus* (Francini 1931; Humphrey 1932; Love 1954 and Skalinska *et al.* 1957). These numbers indicate that aneuploidy has been an associated feature in evolution within this genus, though the genus, as a whole, forms a very homogeneous complex.

Four species of *Habenaria* studied under the tribe Ophrydoideae namely, *H. arietina, H. decipiens, H. latilabris* and *H. mullaeformis* show 2n=48, 42, n=19 and 2n=48 chromosomes respectively. Of these, previous records on *H. arietina* show 42 and 46 chromosomes respectively (Mehra and Bawa 1962; Chatterji unpublished). In *H. decipiens* somatic cells are seen with a polyploid number of 2n=84 chromosomes as well. Other numbers reported within this genus are 2n=28, 32, 40, 62 and 64 chromosomes. The most common number is 2n=42 showing that the others are probably derived ones and as far as the chromosome numbers are concerned the genus forms a more or less homogeneous group. The only species of *Orchis* studied, *O. latifolia,* shows n=21 chromosomes. Previous reports on this genus indicate two predominant haploid numbers 20 and 21. Possibly, one has been derived from the other. The similarity of the haploid numbers observed within *Orchis* and *Habenaria* indicates their affinity and justifies their inclusion within the same tribe.

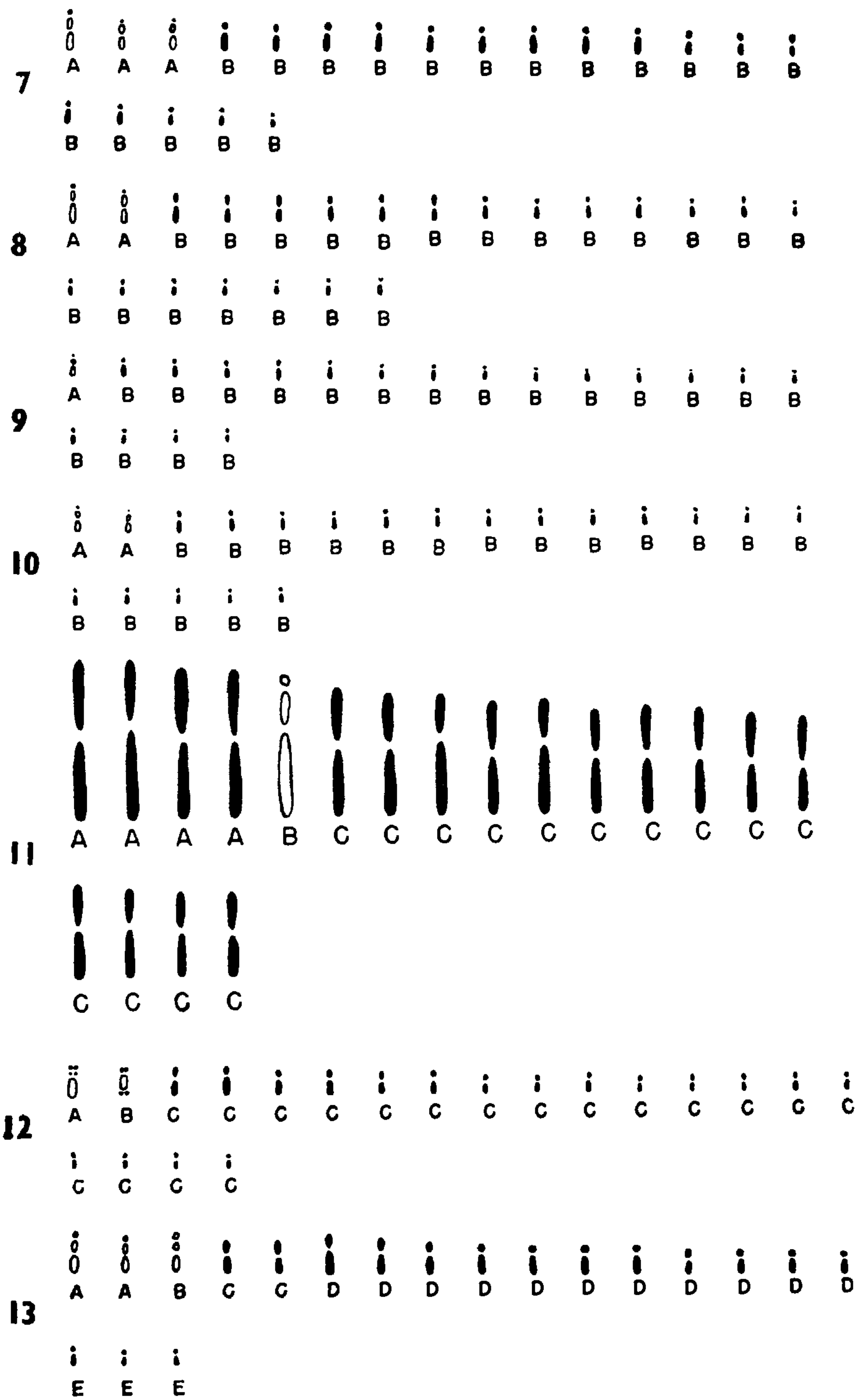
Within the tribe Kerosphaereae, twenty six species under twelve genera have been investigated, from the temperate Himalayas ranging between 6000 to 12,000 feet. One species of *Microstylis* studied, *M. khasiana,* has 2n=42 chromosomes which is new for this genus where 38 to 42 and 44 chromosomes have been observed previously (Chardard 1963; Tanaka 1965; Sharma and Chatterji 1966). *Coelogyne elata* shows 2n=44 as against a widespread occurrence of 2n=40 chromosomes in other species studied. *Pleione praecox* has a chromosome complement of 2n=40 tallying with earlier reports made on other members of this genus. Within *Thunia,* 2n=38 and 44 chromosomes have been observed here in *T. alba* and *T. marshalliana* respectively in collections made in the same locality. This genus has not been investigated previously. Both n=19 and 20 chromosomes are equally prominent within *Dendrobium.* n=20 in *D. transparens* reported here agrees with earlier observations

(Sharma and Chatterji 1966). In *D. pierardii*, however, 2n=40 has been observed here as against reports of 2n=38 made by previous workers (Vajrabhaya and Randolph 1960; Sharma and Chatterji 1966). *Arachnanthe clarkei* shows 2n=38 chromosomes and it is the only report as yet available on this genus. *Doritis taenialis* from the Eastern Himalayas has 2n=40 chromosomes. Sharma and Chatterji (1966) showed both 2n=40 and 60 chromosomes in the same species suggesting the presence of a polyploid series. Other species within this genus have shown 2n=38 and 76 indicating the role of aneuploidy in evolution of the different species (Woodard 1951, Chardard 1963; Kamemoto *et al.* 1964). The records of 2n=38 chromosomes in *Phalaenopsis mannii* and *P. rosea* fit in nicely with previous literature indicating a predominance of this number within the large number of species studied under this very homogeneous taxon. Eight species belonging to the genus *Calanthe* studied, show a series of chromosome numbers ranging from 2n=28, 38, 42, 44 to 52. Other workers have observed a dominance of the number 2n=40. These different numbers indicate that in this genus as well both aneuploidy and polyploidy have been effective in evolution and that some of the numbers are derived ones. The members of *Phaius* studied namely, *P. mishmensis* and the two varieties of *P. wallichii* show n=31, 2n=48 and n=21 respectively. In the other species of the same genus, 2n=38, 42, 44 and 50 chromosomes have been observed (Matsuura and Nakahira 1964, Tanaka 1965; Larsen 1966; Arora 1968). Within this genus, therefore, numerical alterations followed by aneuploidy have possibly played the major role in evolution. *Spathoglottis plicata* collected from the Eastern Himalayas is seen to have 2n=42 chromosomes as against a previous record of 2n=40 made by Tanaka (1965). The genus *Cymbidium* forms a very homogeneous group with 2n=40 chromosomes in a large majority of species including *C. pendulum* studied here. A few discrepant reports like 2n=32 in *C. aloifolium* (Sampathkumaran and Rangaswamy 1931) and 2n=42 in *C. longifolium* and n=22 in *C. lowianum* made by the present author are also available. The high frequency of 2n=40 chromosomes denotes, however, that structural alterations of chromosomes rather than a numerical alteration have been responsible for the evolution in different species within this genus. Within the tribe Kerosphaereae, as a whole, the predominant chromosome numbers are n=19, 20 and 21. Cytological data show that both structural and numerical alterations followed by aneuploidy as well as polyploidy have been active in evolution within the genera belonging to the tribe.

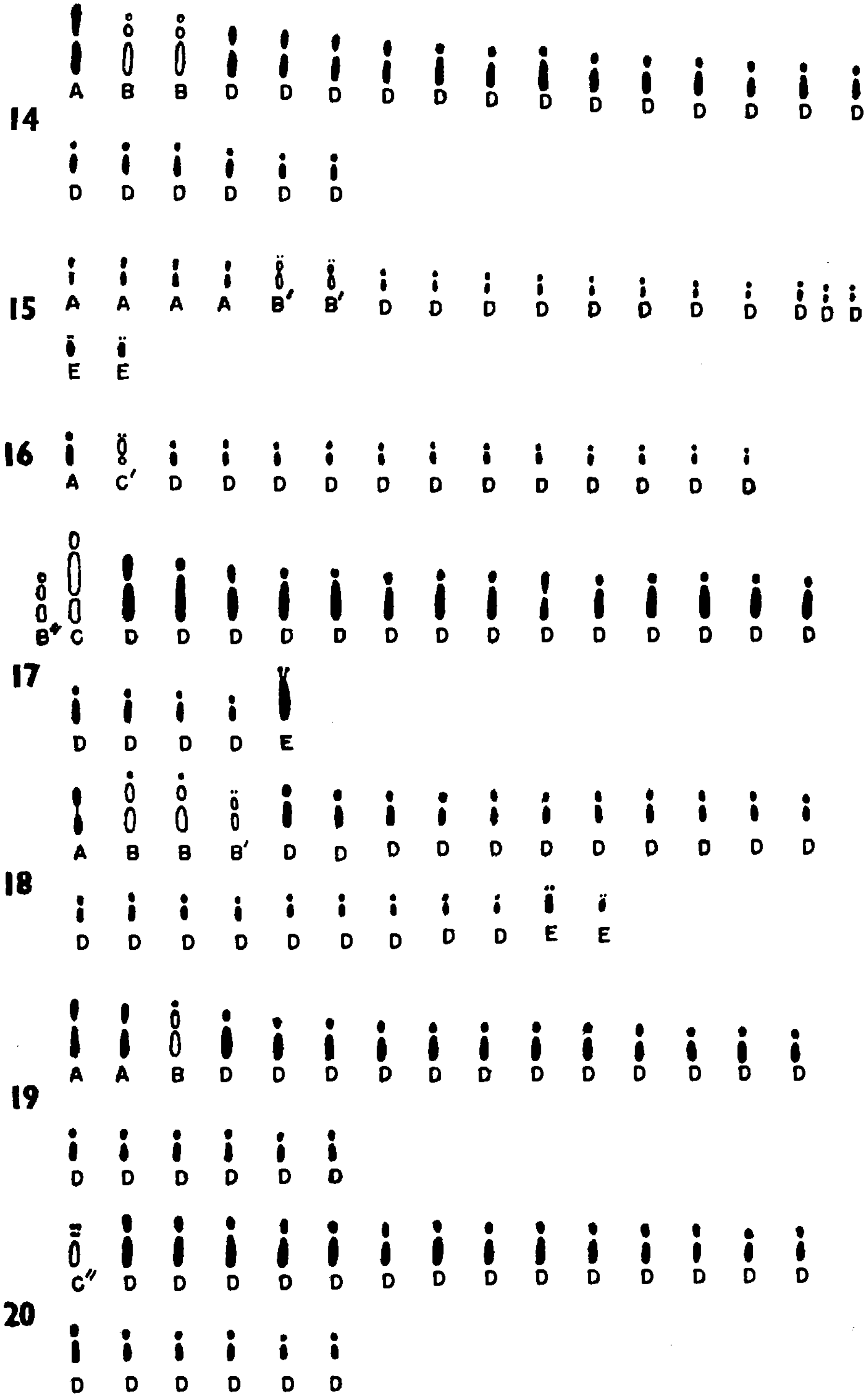
In the tribe Polychondreae, the observation of n=27 chromosomes made here disagrees with a previous report of n=21 (Chatterji 1968) made from collections obtained from the same altitude in the Eastern Himalayas. Evidently, therefore, distinct cytotypes occur in this species. The present record of 2n=36 chromosomes in *Epipactis latifolia* does not tally with 2n=38 and 40 chromosomes observed by Hagerup (1945,1947) in the same species. Other reports on this genus show a preponderance of 2n=40 chromosomes though stray records of 2n=24, 32, 36 and 38 are also available (Gadella and Kliphuis 1963, Tanaka 1965; and Arora 1968). *Pogonia plicata* from the tropics is found to have 2n=62 chromosomes while other species have numbers ranging from 2n=18, 20 to 21 chromosomes. *Spiranthes sinensis* from the Eastern Himalayas has 2n=30 chromosomes which agrees well with reports from other species of the same genus. Other numbers found with this genus



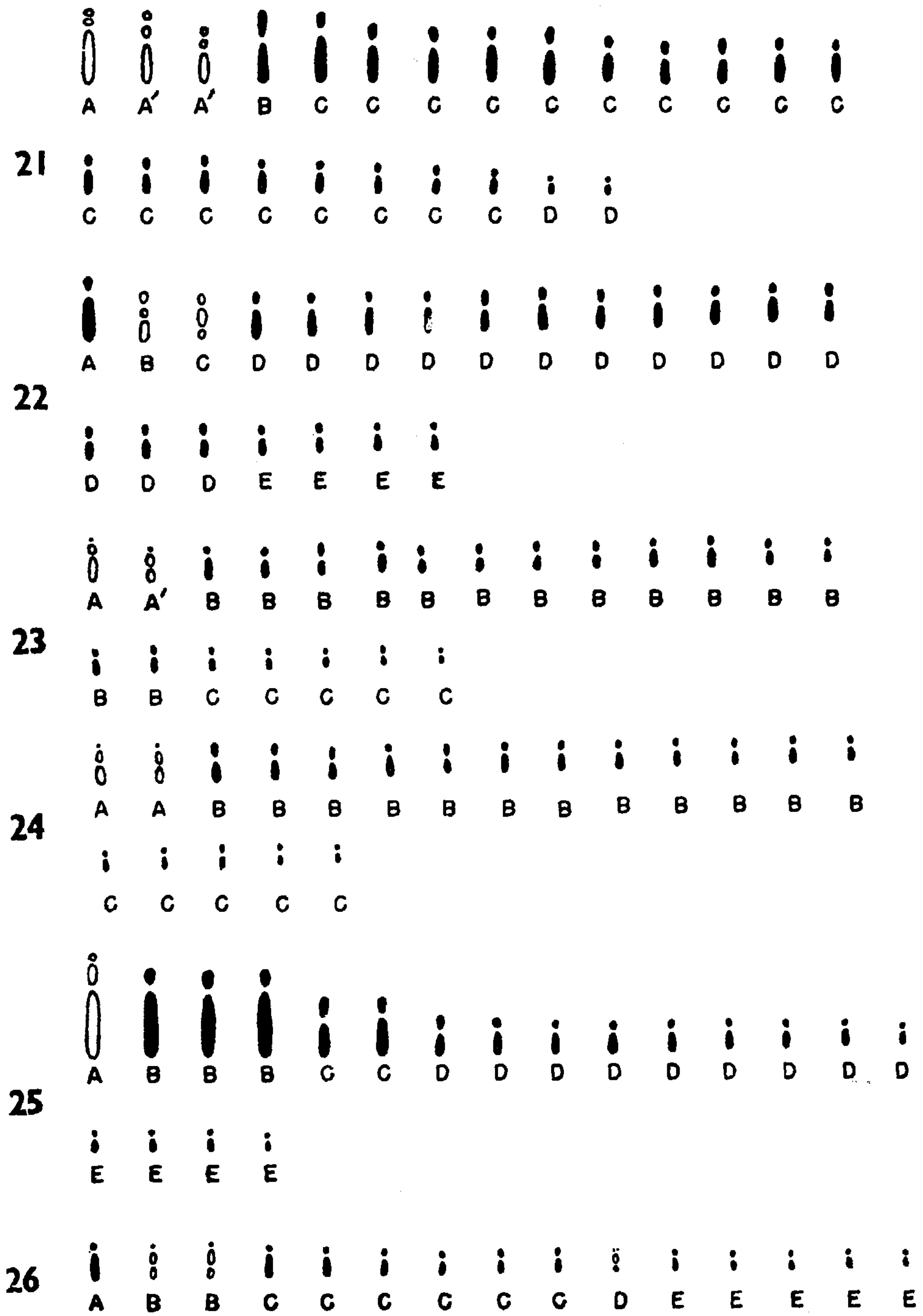
FIGS. 1–6. Idiograms of *Cypripedium elegans*, *Habenaria arietina*, *H. decipiens*, *H. mullaeformis*, *Microstylis khasiana*, and *Coelogyne elata* respectively.



FIGS. 7–13. Idiograms of *Pleione praecox*, *Thunia alba*, *T. marshalliana*, *Dendrobium pierardii*, *Arachnanthe clarkei*, *Doritis taenialis* and *Phalaenopsis mannii* respectively.



FIGS. 14–20. Idiograms of different species of *Calanthe*.



FIGS. 21–26. Idiograms of *Phaius wallichii*, *Spathoglottis plicata*, *Cymbidium longifolium* *C. pendulum*, *Epipactis latifolia* and *Spiranthes sinensis* respectively.

are 2n=24, 26, 35 and 50. The occurrence of multiples of 30 chromosomes shows that the polyploidy has been also a factor in addition to aneuploidy in evolution of the members belonging to this genus.

The importance of structural alterations in chromosomes in the evolution of species can be seen clearly in those cases where more than one species have been investigated cytologically within a single genus. In the four species of *Habenaria* studied, as mentioned before, numerical alterations have been of greater importance in their evolution. The karyotypes however differ as well in having different combinations of the chromosome types showing that structural alterations have also played a part in their origin. *H. arietina*, particularly with 2n=42, has much smaller chromosomes than the other three species showing an overall reduction in the chromatin matter.

Seven species of *Calanthe* studied, show chromosome numbers ranging from 2n=28, 38, 42, 44 and 52 chromosomes. A gross similarity is observed within the chromosome types so that the chromosomes present can be classified into five general groups. The karyotypes, however, differ from each other in different combinations of the different types of chromosomes. In the species, *C. puberula*, *C. herbacea* and *C. tricarinata* all with 2n=42, the karyotypes are completely different showing the distinct role of structural alterations in their evolution.

The two species of *Cymbidium*, *C. longifolium* and *C. pendulum*, have different chromosome numbers 2n=42 and 40 respectively. The chromosome types, however, are similar in the two species. They differ in different combinations of these types. Thus both minor structural alterations and numerical ones have been active within this genus.

Systematic status of different genera as justified through karyotypic analysis

Bentham and Hooker (1883) distributed the orchids under five tribes Epidendreae (9 subtribes), Vandeae (8 subtribes), Neottieae (6 subtribes), Ophrydoideae (4 subtribes) and Cypripedieae on the basis of floral characters. Of them the last one Cypripedieae is regarded to be the most primitive. The classification followed by Pfitzer emphasizes the importance of vegetative characters including the stem, leaf and inflorescence. He has divided the entire family into two groups Diandrae and Monandrae on the basis of number of stamens present. The former includes one tribe cypripediloideae. Monandrae has been further divided into two divisions Basitonae and Acrotonae. The group Basitonae has one large diverse tribe while Acrotonae has twenty eight tribes under two subgroups Acranthae and Pleuranthae.

Schlechter's system of classification is a synthesis in the sense that floral structure as emphasized by Bentham and Hooker and vegetative characters as emphasized by Pfitzer have both been taken into consideration. It follows Pfitzer's classification up to the level of the division into Acrotonae and Basitonae.

The position of the genus *Cypripedium* is the same under both Schlechter's and Pfitzer's systems of classification as a member of the tribe Cypripediloideae under the subfamily Diandrae, while under Bentham and Hooker's system it has been included under the fifth and last tribe Cypripedieae. The species studied here has 2n=20 chromosomes which are very long and form a graded series. The karyotype is comparatively primitive with a predominance of metacentric chromosomes. These characters fully justify the inclusion of this genus in a separate group and show its primitiveness in the evolutionary scale.

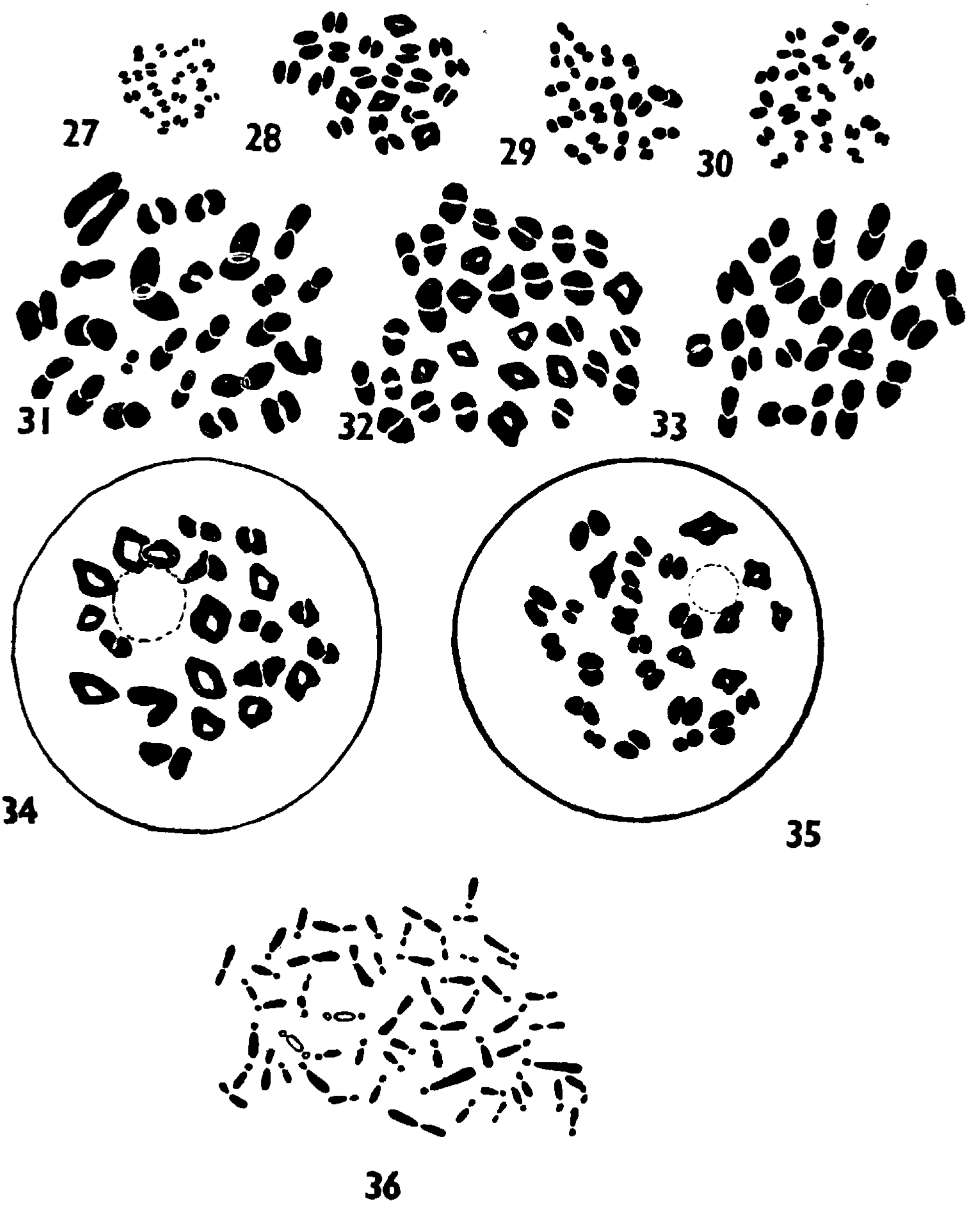
Under the tribe Ophrydoideae, the genus *Habenaria* has rather small chromosomes, almost half of them being acrocentric, showing the comparatively advanced nature of this genus. Other reports on species of *Orchis* also show the small size and relative asymmetry of their karyotypes. The haploid number ranges from 21 to 24 in both these genera justifying their inclusion within the same tribe.

Under the tribe Kerosphaereae, twelve genera have been included. The chromosome numbers observed range from 2n=28 to 52. The only representative of the subtribe Liparideae studied, *Microstylis khasiana* has 2n=42 chromosomes, which are comparatively very small. All the chromosomes are acrocentric showing the advanced status of this genus. Within the subtribe Coelogyneae, the two members studied viz., *Coelogyne elata* and *Pleione praecox* have 2n=44 and 40 chromosomes respectively, ranging from medium size to short, being slightly larger in the former species than in the latter. The types are also similar. The overall similarity of the karyotypes possibly justifies the inclusion of these genera within the same subtribe. Under the subtribe Thunieae, two species of the genus *Thunia* studied show chromosome numbers as 2n=38 and 44, ranging from medium size to very short with a higher number of acrocentrics. The small size and the comparatively asymmetrical karyotypes place this genus amongst the more advanced ones of this group. Within Dendrobieae, the chromosomes of *Dendrobium pierardii* (2n=40) are also very small with a majority of them having submedian to subterminal primary constrictions.

Taken as a whole, the inclusion of these subtribes within the series Acranthae under the tribe Kerosphaereae finds support from the cytological data since almost all of them show a gross similarity in the comparative asymmetry of the karyotype, the general types of chromosomes present and their relatively small size.

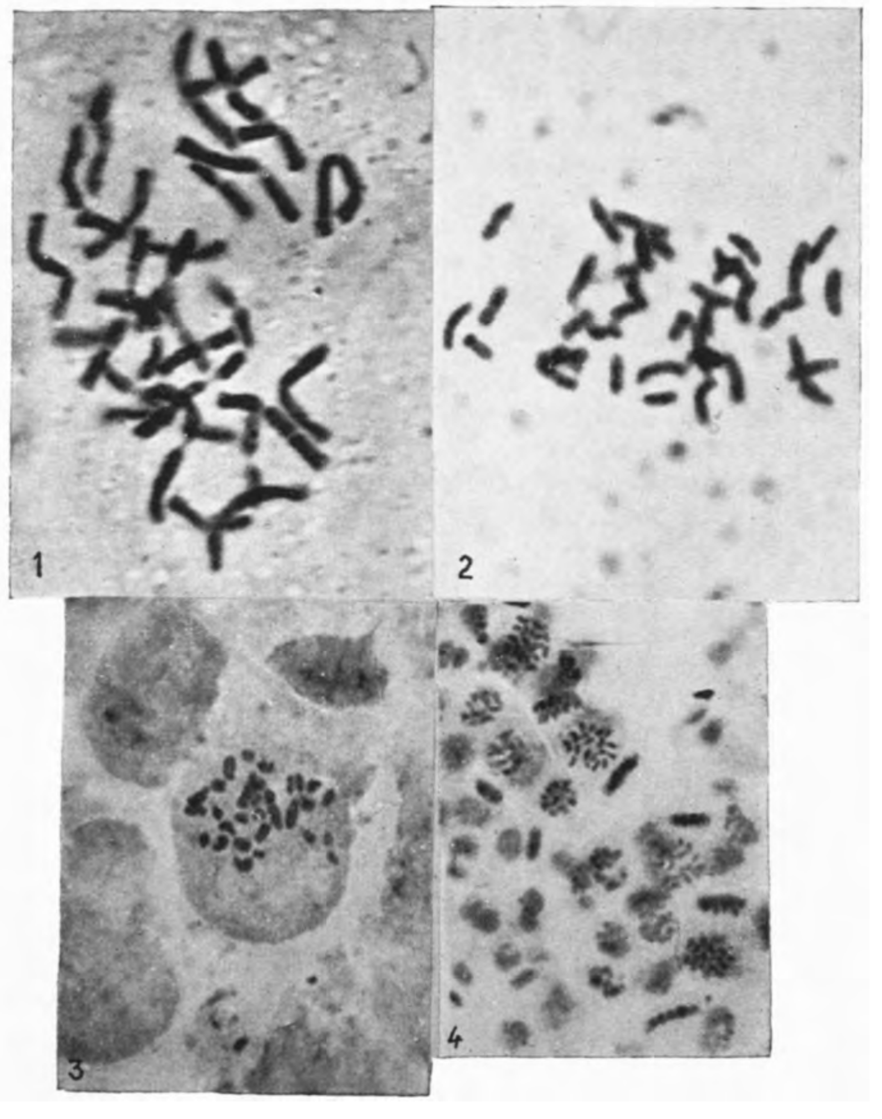
Only one representative of the subtribe Sarcantheae under the subseries Monopodiales has been studied here namely *Phalaenopsis mannii* with 2n=38 chromosomes. A large amount of data is available on the members of this substribe from previous works (Sagawa 1962; Shindo and Kamemoto 1963). The species of *Phalaenopsis* studied previously, show mostly a haploid set of 19 with small chromosomes as compared to the other genera of the same subtribe. The karyotype is relatively symmetrical with an almost equal numbers of acrocentric and metacentric chromosomes. Six chromosomes bear secondary constrictions. The karyotype agrees with previous reports by Shindo and Kamemoto (1963) made on the same species in which they observed the chromosomes to be larger and the karyotype to be more asymmetrical as compared to the Philippine species of *Phalaenopsis* studied. Further data, however, is required before the position of *Phalaenopsis*, the climax of the Monopodiales in Schlechter's system of classification, can be justified.

Under the subseries Sympodiales, three subtribes have been investigated. Within Phajeae, in the twelve species under three genera studied, the chromosome size ranges from almost long to short with marked size difference. The different species of *Calanthe* show an overall similarity in the types of chromosomes present, justifying their inclusion within the same genus. On an average the greater number of chromosomes within a karyotype have submedian to subterminal primary constrictions. A wide range of structural alterations is observed showing that these, in addition to numerical ones, have been effective in speciation, In *C. biloba* and *C. masuca* particularly, even some of the short chromosomes show subterminal constrictions so that the end segment is very small. *C. tricarinata* and *C. herbacea* both with 2n=42 chromosomes indicate remarkable structural alterations in that



FIGS. 27–36. Meiotic studies of *Dendrobium transparens* (n=20), *Habenaria latilabris* (n=21), *Phalaenopsis rosea* (n=19), *Cymbidium lowianum* (n=22), *Calanthe brevicorum* (n=24), *Phaius mishmensis* (n=31), *P. wallichii* var. *assamica* (n=21), *Orchis latifolia* (n=21), *Anthogonium gracile* (n=27) and somatic cells showing 2 n=62 chromosomes in *Pogonia plicata* respectively.

the former has a unusual pair of "C" chromosomes and the latter an unusually large "C" type chromosome. *Phaius wallichii* shows a rather remarkable size difference and a pair of chromosomes (A type) with submedian and subterminal constrictions located very close to each other at one end. *Spathoglottis plicata* with 2n=42 has a more symmetrical karyotype than the other genera with the size grading from comparatively long to comparatively short. Thus the karyotypes of *Phaius* and



Figs. 1–4. 1, somatic metaphase showing 2n=38 chromosomes in *Arachnanthe clarkei*; 2, somatic metaphase showing 2n=42 chromosomes in *Calanthe herbacea*; 3, somatic metaphase showing 2n=30 chromosomes in *Spiranthes sinensis*; 4, P.M.C. of *Phalaenopsis rosea* showing n=19 chromosomes in metaphase I. × 1900

Spathoglottis, though exhibiting a gross similarity in their morphology with *Calanthe* possibly support their inclusion within the same subtribe, yet they differ sufficiently from the latter and from each other to justify their existence as separate genera. The only other genus studied here belonging to the subseries Sympodiales is *Cymbidium* under the subtribe Cymbidieae. As noted from both previous and present observations (Wimber 1957; Withner 1959) it is a homogeneous one with medium-sized to small chromosomes and more or less similar chromosome types forming a graded series. The two species *C. longifolium* (2n=42) and *C. pendulum* (2n=40) have very similar karyotypes structurally. As far as can be seen from the cytological data, the members of Cymbidieae can be included within the same subseries Sympodiales as those of the subtribe Phajeae. Only one species of the genus *Arachnanthe* namely, *A. clarkei* (2n=38) has been investigated here. The karyotypes show a remarkable difference from the other members of the tribe Kerosphaereae. The chromosomes range from very long to medium sized, only one pair showing secondary constrictions. Primary constrictions are mostly median and size difference is graded. This primitive relatively similar karyotype marks out this species as a comparatively primitive one within this tribe. Further studies on other species of this genus are necessary for determining its systematic position in relation to the other genera of the same tribe.

In the other tribe Polychondreae, karyotype study has been carried out on two taxa namely, *Spiranthes sinensis* (2n=30) and *Epipactis latifolia* (2n=36). The chromosomes in both these genera show a rather marked size difference though those of *Epipactis* are much larger than those of *Spiranthes.* Chromosomes are mainly acrocentric showing the relatively advanced nature of this tribe. The size difference and general chromosome morphology justify the inclusion of these genera in a separate tribe. Further work is necessary to elucidate their interrelationships. The abrupt size difference observed in the species of *Epipactis* and *Spiranthes* studied indicates a possibility that these karyotypes might have arisen by hybridisation between two forms, one with long and the other with short chromosomes. However, in the absence of detailed data the suggestion can remain only a tentative one.

Thus in the present investigation on the different members of orchids studied, both numerical and structural alterations in the chromosomes have played important roles in the evolution of different species. The present cytological data also gives support to the classification most widely accepted namely, that of Schlechter (1926).

References

Arora, C. M. (1960). New chromosome report. *Bull. bot. Surv. India,* **2**, 305.

———(1968). I.O.P.B. chromosome number reports, XVI *Taxon,* **17**, 199–200

Bentham, G., and Hooker, J. D. (1883). Genera Plantarum, L. Reeve & Co., London.

Chardard, R. (1963). Contribution àl'étude cytotaxonomique des Orchideés. (A contribution to the cytotaxonomy of the Orchidaceae). *Rev. Cytol. Biol. Vég.,* **26**, 1–58.

Chatterji, A. K. (1968). Chromosome numbers and karyotypes of some orchids. *Bull. Am. Orchid Soc.,* **37**, 202–205.

Chennaveeraiah, M. S., and Jorapur, S. M. (1966). Chromosome number and morphology in five species of *Nervilia* Gaud. *Nucleus, Calcutta,* **9**, 39–44.

Dressler, R. L., and Dodson, C. H. (1960). Classification and phylogeny in the Orchidaceae. *Ann. Mo. bot. Gdn.,* **47**, 25–68.

Francini, E. N. (1931). Ricerche embryologische a cariologische sul genere *Cypripedium. Nuovo G. bot. ital. (N.S.),* **38**, 154–212.

Gadella, T. W. J., and Kliphuis, E. (1963). Chromosome numbers of flowering plants in the Netherlands. *Acta Bot. Neerl.*, **12**, 195–230.

Hogerup, O. (1945). Facultative parthenogenesis and haploidy in *Epipactis latifolia*. *K. Dansk. Vidensk. Selsk. Biol. Medd.*, **19**, 1–3.

————(1947). The Spontaneous formation of polyploid and aneuploid embryos in some orchids. *K. Dansk. Vidensk. Selsk. Biol. Medd.*, **20**, 1–22.

Hoffmann, K. (1930). Beitrage Zur cytologie der Orchideen. *Planta*, **10**, 523-595.

Humphrey, L. M. (1932). Somatic chromosomes in certain Minnesota Orchids. *Am. Nat.*, **66**, 471-474.

Kamemoto, H., Sagarik, R., and Kasemsap, S. (1964). Chromosome numbers of Sarcanthine Orchid species of Thailand. *Nat. Hist. Bull. Siam Soc.*, **20**, 235–241.

Larsen, K. (1966). Studies in the flora of Thailand 40. Cytology of Vascular plants II. *Dansk. Bot. Ark.*, **23**, 376-379.

Love, A. (1954). Cytotaxonomical evaluation of corresponding taxa. *Vegetatio, ActaGeobotanica*, **5-6**, 212–224

Maheshwari, P., Sengupta, J. C., and Venkatesh, C. S. (1965). Flora—The Gazetter of India. I. Publications Division, Govt. of India.

Matsuura, O., and Nakahira, R. (1964). Chromosome numbers of the family orchidaceae in Japan (4). *Sci. Rep. Kyoto Perfectual Univ.*, **15**, 11–14.

Mehra, P. N., and Bawa, K. S. (1962). Chromosome studies in Orchidaceae. *Proc. 49th Indian Sci. Cong.*, Part III, 326–327.

Mehra, P. N., and Yash Pal, (1961). Cytological observations on some Indian members of Orchidaceae. *Proc. 48th Indian Sci. Cong.*, Part III, 294.

Mulay, B. N., and Panikkar, T. K. B. (1953). The chromosome number and morphology in *Cypripedium speciosum* L. *Proc. Rajasthan Acad. Sci.*, **4**, 29–31.

Pfitzer, E. (1889). Orchidaceae in Engl.—Prantl *Die Naturlichen Pflanzenfamilien*. **2**, 52-218..

Sagawa, Y. (1962). Cytological studies of the genus *Phalaenopsis*. *Bull., Am. Orchid. Soc.* **31**, 459–465.

Sampathkumaran, M., and Rangaswamy (1931). Referred in Withner, C. L.—The Orchids—a scientific survey. Ronald Press Co., N. Y., 1959.

Schlechter, R. (1926). *Notizbl. bot. Gart. Mus. Berl.* **9**, 563–591.

Sharma, A. K., and Mookerjea, A. (1955). Paradichlorobenzene and other chemicals in chromosome work. *Stain Tech.*, **30**, 1–7.

Sharma, A. K., and Chatterji, A. K. (1961). The chromosome numbers of a few more Orchid genera. *Curr. Sci.*, **30**, 75.

————(1966). Cytological studies in Orchids with respect to their evolution and affinities *Nucleus*, **9**, 177-203.

Shindo, K., and Kamemoto, H. (1963). Karyotype analysis of some species of *Phalaenopsis*. *Cytologia*, **28**, 390–398.

Skalinska, M., Banach-Pogan, E., and Wcislo, *H. et al.* (1957). Further studies in chromosome numbers of Polish Angiosperms. *Acta. Polsk. Towarz. Bot.*, **30**, 463–489.

Swamy, B. G. L. (1941). The development of the male gamete in *Cymbidium bicolor* Lindl. *Proc. Indian Acad. Sci.*, **B14**, 454-460.

Tanaka, R. (1965). Chromosome numbers of some species of Orchidaceae from Japan and its neighbouring areas. *J. Jap. Bot.*, **40**, 65–77.

Vajrabhaya, T., and Randolph, L. F. (1960). Chromosome studies in *Dendrobium Bull. Am. Orchid Soc.*, **29**, 507–517.

Willis, J. C. (1966). A Dictionary of the Flowering Plants and Ferns. Cambridge University Press, Cambridge.

Wimber, D. E. (1957). Cytogenetic studies in the genus *Cymbidium*. I. Chromosome numbers within the genus and related genera. *Bull. Am. Orchid Soc.*, **26**, 356–358.

Withner, C. L. (1959) The Orchids—a scientific survey. Ronald Press Co., N. Y.

Woodard, J. W. (1951). Some chromosome numbers in *Phalaenopsis*. *Bull. Am. Orchid Soc.*, **20**, 356–358.