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Chromosome Evolution and Affinity of Certain Genera of Orchidaceae

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In India the Orchids are widely represented in the Himalayas, particularly in subtropical and temperate zones of the Eastern Himalayas as well as in Khasia hills. Cytological data on Indian genera of this family is rather meagre (Sharma and Chatterjee 1966, Chatterjee 1966, Panigrahi 1966). No systematic attempt has so far been made in working out the evolutionary tendencies and affinities of the different taxa. The promise of chromosome research in Orchidaceous genera has already been indicated by the records of intraspecific occasional chromosome races (Sharma and Chatterjee 1966). Several genera such as, *Coelogyne*, *Vanda* etc. though epiphytes, occur under varying environmental conditions. The homogeneity of the chromosome numbers 2n=38, 40 and 42, with occasional chromosome races in such a large family as Orchidaceae raises the very problem of the extent to which gene mutation and structural alterations have influenced the process of speciation. In view of the scanty work on Indian species, as well as the cytological interest already indicated in the family, the present investigation was undertaken, involving a detailed karyotype analysis and chromosome behaviour of several genera viz., *Coelogyne*, *Pholidota*, *Dendrobium*, *Aerides*, *Sarcochilus*, *Hexisea*, *Cleisostoma*, *Rhynchostylis*, *Stauropsis* and *Vanda* belonging to three subtribes Coelogyninae, Dendrobiinae and Sarcanthineae.

Materials and methods

35 species including cytotypes of Orchidaceae belonging to 10 genera under the tribe Kerosphaereae (Schweinfurth 1959) have been studied. They were collected from different altitudes in the Terai to subtropical regions of Eastern Himalayas and Khasia hills at different seasons (Table 1).

Studies on somatic chromosomes were made from squash preparations of root tips. In majority of species, a mixture of saturated solution of para-dichlorobenzene and 0.001 M hydroxyquinoline was found to be effective as a pretreatment chemical at 8–12°C (Sharma and Mookerjea 1955), whereas in case of *Dendrobium* and *Coelogyne*, treatment with saturated aqueous solution of aesculine for 3 hrs. at 10–12°C gave best results. The usual propiono-orcein staining schedule was followed. For meiotic study propiono-carmine stain was used.

Table 1. Comparison of somatic and meiotic chromosomes within the genus of the family Orchidaceae**

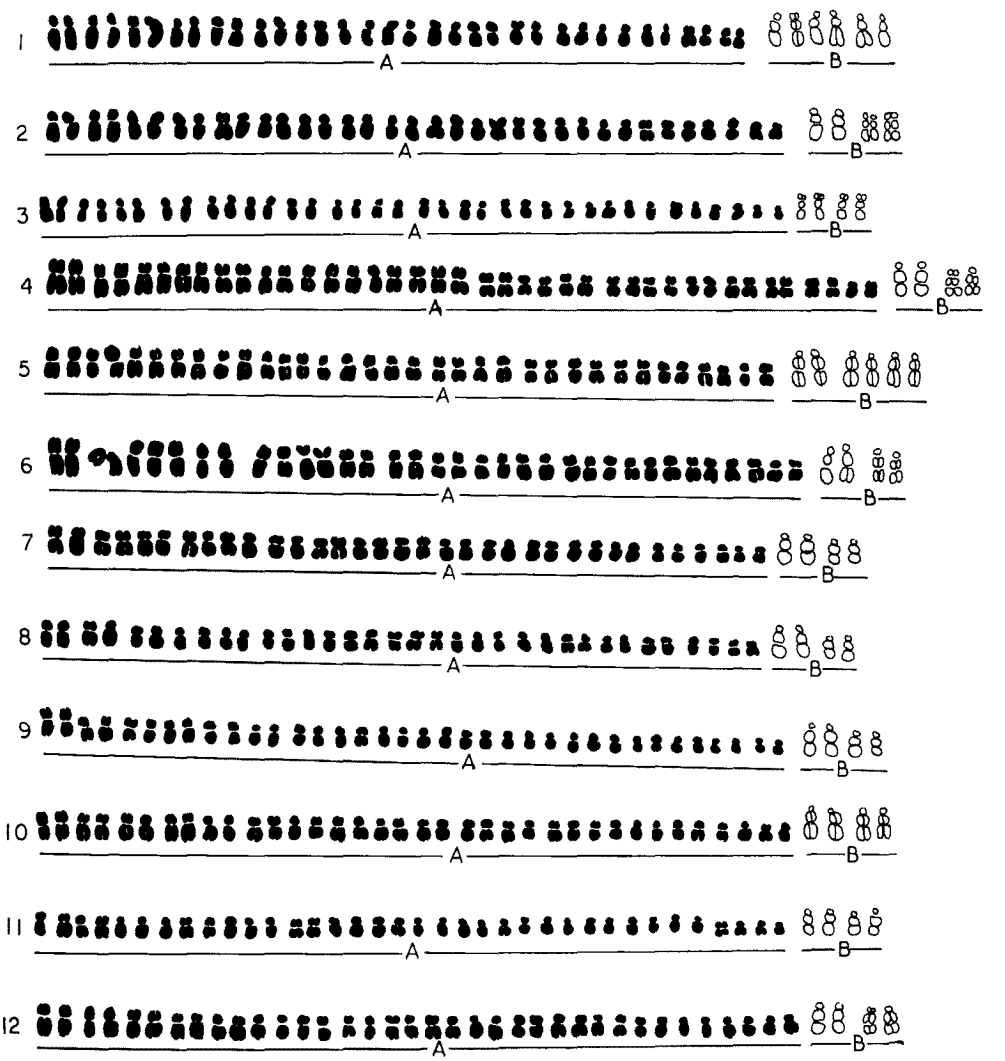
Name of the species	Locality and altitude in meters	Chromosome number		Karyotype formula	Range of chromosome length in μ	Total chromosome length (2n) in μ	Reference Fig. no.
		n	2n				
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Coelogyne flaccida Lindl.	Darjeeling (E.H.), 1844		*40	A34, B6	1.53–3.07	84.38	1
C. fuscescence Lindl.	Kalimpong (E.H.), 914		40	A36, B4	1.53–2.83	68.96	2
C. ochracea Lindl.	Shillong, 2133		40	A36, B4	1.15–2.30	60.24	3
Pholidota bicolor Lindl.	Tonglu (E. H.), 3048		*44	A40, B4	1.15–3.26	91.10	4
Dendrobium chrysanthum Wall.	Tagda (E. H.), 1844	20	*40	A34, B6	1.92–3.26	85.22	5
D. chrysanthum Wall.	Shillong, 1539		38	A34, B4	1.87–3.46	88.16	6
D. densiflorum Wall.	Tagda (E. H.), 1844		*38	A34, B4	1.34–2.69	58.56	7
D. fimbriatum Hook.	Darjeeling (E. H.), 2133		40	A36, B4	1.25–2.98	72.50	8
D. gibsonii Lindl.	Tagda (E. H.), 1844		*40	A36, B4	1.25–2.98	65.02	9
D. hookerianum Lindl.	Shillong, 1844	20	40	A36, B4	1.53–3.07	72.50	10
D. moschatum Wall.	Shillong, 1499		*40	A36, B4	1.15–2.40	59.72	11
D. nobile Lindl.	Tagda (E. H.), 1539		40	A36, B4	1.73–2.88	79.12	12
D. ochreatum Lindl.	Shillong, 1222		40	A38, B2	1.05–2.06	56.68	13
D. pierardi Roxb.	Shillong, 1234	19	38	A34, B4	1.73–3.94	81.74	14
D. stauposum Lindl.	Khasi hills, 1539		40	A36, B4	1.34–2.69	66.92	15
D. transparence Wall.	Shillong, 1495	19	38	A34, B4	1.29–2.88	68.96	16
D. wardianum Warner.	Shillong, 1222	20	*40	A36, B4	1.39–3.46	73.32	17
Dendrobium sp.	Shillong, 1222		40	A34, B6	1.68–3.50	69.40	18
Aerides affine Wall.	Kalimpong (E. H.), 1539		*38	A34, B4	2.50–6.81	137.56	19
A. crassifolium Par. & Reichb. f.	Shillong, 1222		38	A34, B4	1.25–3.26	63.54	20
A. fieldingii Lodd	Shillong, 1222		38	A34, C4	2.01–4.70	92.80	21
A. odoratum Lour	Shillong, 1222		38	A34, B4	1.53–3.46	79.04	22
A. vandarum Reichb. f.	Shillong, 1222		38	A36, B2	1.77–3.12	65.04	23
Cleisostoma mannii Reichb. f.	Khasia hills, 914		*38	A34, B4	1.96–3.94	107.36	24
Hexisea reflexa Reichb. f.	Kalimpong (E. H.), 1844	19	*38	A34, B4	1.39–3.21	65.20	25
Rhynchostylis retusa Blume	Sukhna (E. H.), 914		38	A30, B8	1.82–4.27	104.06	26
R. violacea Reichb. f.	Serampore (W. B.), 6		*38	A30, B8	1.63–3.84	86.18	27
Sarcochilus mannii Hook. f.	Khasia hills, 914		*38	A34, B4	1.58–3.17	74.98	28
Stauropsis undulata Benth.	Darjeeling (E. H.), 2133		*38	A34, B4	1.34–3.36	66.26	29
Vanda alpina Lindl.	Khasia hills, 1539		38	A34, B4	1.44–2.83	58.10	30
V. cerulescence Griff	Kalimpong (E. H.), 1539		*40	A36, B4	1.73–3.07	73.94	31
V. perviflora Lindl.	Kalimpong, 1539		38	A34, B4	1.25–2.69	61.66	32
V. pumila Hook. f.	Kalimpong, 609		*40	A36, B4	1.63–3.07	67.92	33
V. roxburghii Br.	Barrackpore (W.B.), 6	19	38	A34, B4	1.44–2.83	58.10	34
V. teres Lindl (Type I)	Darjeeling (E. H.), 1844		*38	A34, B4	1.34–3.07	70.42	35
V. teres Lindl (Type II)	Tagda (E. H.), 1844		*38	A34, B4	1.82–3.89	94.00	36

* Indicates new report.

** Arranged in order as followed in key to the Orchids by C. Schweinfurth in *The Orchids.* A Scientific Survey. Edited by C. Withner, 1959, Ronald Press.

Observation

The somatic chromosome number ranges from 2n=38 to 44. According to the length of the chromosome and to the position of primary and secondary constriction, the chromosomes are classified as follows:



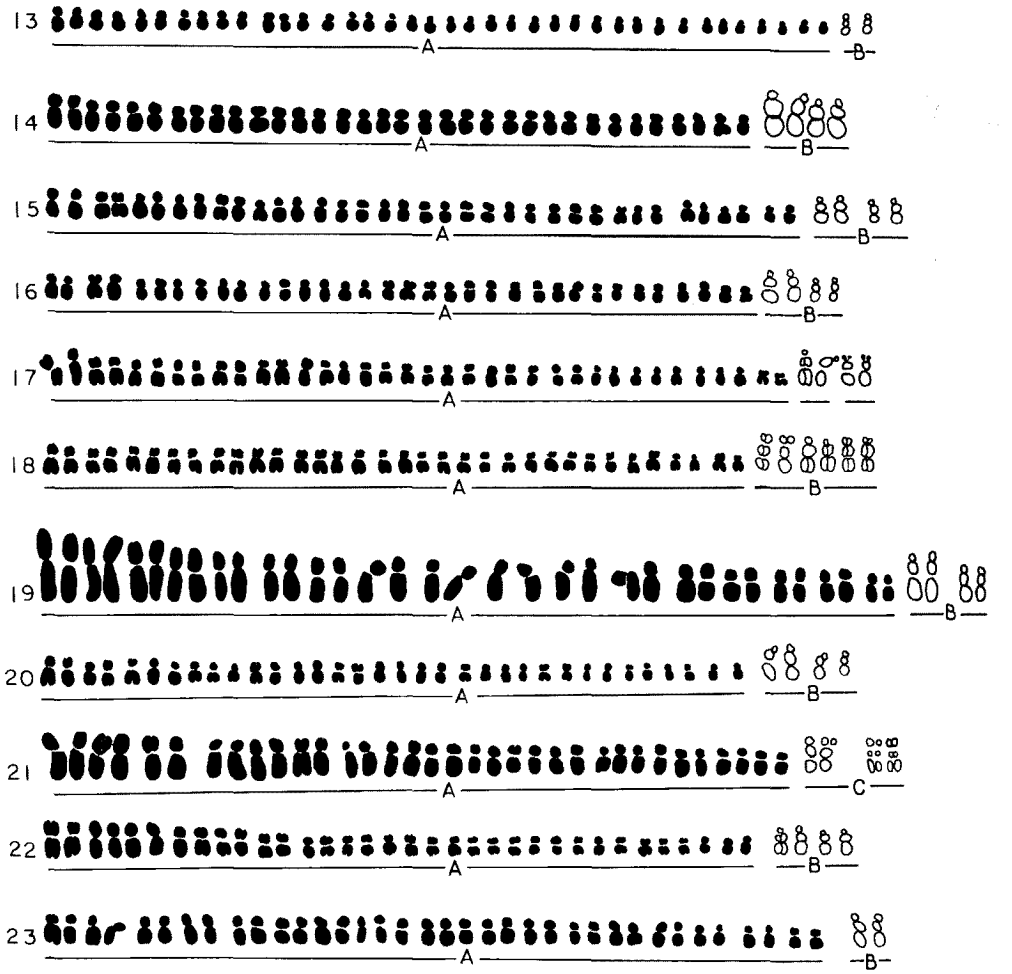
Figs. 1–2. 1, *Coelogyne flaccida* Lindl., 2n=40. 2, *C. fuscesence* Lindl. 2n=40. 3, *C. ochracea* Lindl. 2n=40. 4, *Pholidota bicolor* Lindl. 2n=44. 5, *Dendrobium chrysanthum* Wall. (Tagda) 2n=40. 6, *D. chrysanthum* Wall. (Shillong) 2n=38, 7. *D. densiflora* Wall. 2n=38. 8, *D. fimbriatum* Hook. 2n=40. 9 *D. gibsonii* Lindl. 2n=40. 10 *D. hookerianum* Lindl. 2n=40. 11, *D. moschatum* Wall. 2n=40. 12, *D. nobile* Lindl. 2n=40.

Type A—Comparatively long to short (6.81–1.05 μ) chromosomes with mostly median or nearly median to nearly submedian primary constriction.

Type B—Comparatively medium to short chromosomes (4.5–2.11 μ) with primary and secondary constrictions; one nearly median to nearly

submedian and the other nearly submedian to subterminal in position.

Type C—Comparatively medium chromosome (3.64 μ) with two constrictions, primary and secondary; nearly median and submedian at the opposite ends of chromosome dividing it into one middle short and two outer larger segments (e. g., *Aerides fieldingii*).



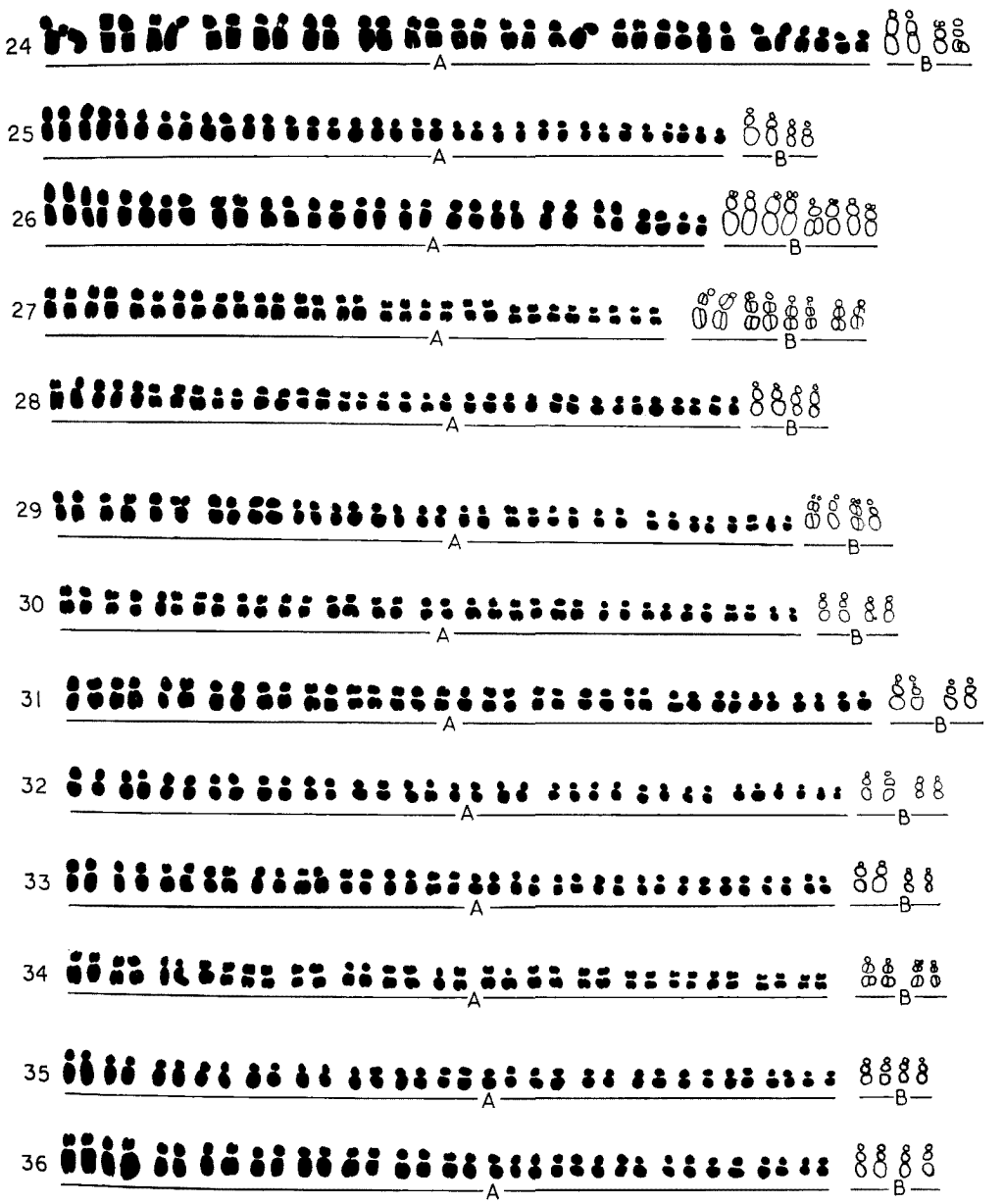
Figs. 13–23. 13, *D. ochreatum* Lindl. 2n=40. 14, *D. pierardi* Roxb. 2n=38. 15, *D. stauposum* Lindl. 2n=40. 16, *D. transparence* Wall. 2n=38. 17, *D. wardianum* Warner 2n=40. 18, *Dendrobium* sp. 2n=40. 19, *Aerides affine* Wall. 2n=38. 20, *A. crassiflium* Par. & Reichb. f. 2n=38. 21, *A. fieldingii* Lodd. 2n=38. 22, *A. odoratum* Lour. 2n=38. 23, *A. vandarum* Reichb. f. 2n=38.

A comparative description of the karyotypes of different species are given in Table 1.

While preparing the idiograms (Figs. 1–36) at least five well-spread plates were compared and whenever the size and arm ratios of chromosomes varied, a mean length was taken to calculate its F% and TF value of each species as well as for the genus. The centromeric index or F% or total TF values were calculated as follows:

F% or TF value=The ratio in percentage of the total of short arm length to the total length of chromosome, i. e.

$$\frac{\text{Short arm length of the chromosome}}{\text{Total length of the chromosome}} \times 100$$



Figs. 24–36. 24, *Cleisostoma mannii* Reichb. f. 2n=38. 25, *Hexisea reflexa* Reichb. f. 2n=38. 26, *Rhynchostylis retusa* Blume. 2n=38. 27, *R. violacea* Reichb. f. 2n=38. 28, *Sarcochilus mannii* Hook. f. 2n=38. 29, *Stauropsis undulata* Benth. 2n=38. 30, *Vanda alpina* Lindl. 2n =38. 31, *V. cerulescence* Griff. 2n=40. 32, *V. parviflora* Lindl. 2n=38. 33, *V. pumila* Hook. f. 2n=40. 34, *V. roxburghii* Br. 2n=38. 35, *V. teres* Lindl. (Type I) 2n=38. 36, *V. teres* Lindl. (Type II) 2n=38.

Figs. 1–36. Showing idiograms. ×1500 approx.

Discussion

Subtribe Coelogyninae

The genus *Coelogyne* as studied by previous authors shows a constant chromosome number, 2n=40, in all the species, excepting in *C. corymbosa*, where 2n=38 (Titz 1966) chromosomes have been reported. In the present investigation too 40 chromosomes have been recorded in 3 species of *Coelogyne* indicating further the homogeneity of the genus. The total chromosome length, the F% or TF value (Table 1, Figs. 1–3) shows their close similarity. All species of this subtribe have almost similar values, for example, *Coelogyne flaccida* Lindl., *C. fuscescence* Lindl. have values (approx.) 43.2% and 45.7% respectively. The numbers of secondary constrictions vary from species to species indicating that cryptic structural alterations have played an important role in speciation. The occurrence of polyspory as in *C. cristata* in Orchidaceae is however not uncommon (Chatterjee 1966).

The genus *Pholidota* shows, 2n=44 (Fig. 4) chromosomes but the chromosomes follow more or less the same pattern as of *Coelogyne*. The F% or TF value also indicates its close similarity with *Coelogyne*. In species of *Pholidota* the TF value is approximately 40.9%, more or less similar to that of *Coelogyne*. All these features may suggest not only the affinity of *Pholidota* with *Coelogyne*, but homogeneity of this section, as could be deduced from this investigation.

Subtribe Dendrobeae

The genus *Dendrobium* has in majority of the species a basic set of 19 chromosomes (Jones 1963, Pancho 1965 and Sharma and Chatterjee 1966). Though n=19 has also been reported, a few species show polyploidy and chromosome number as high as 114 too has been found in *D. kingianum* (Jones 1963). Triploid variety of *D. kingianum* var. *sileockii* with 57 chromosomes has also (Jones 1963) been obtained. Intraspecific polyploidy and aneuploidy are on record as in *D. transparens* (Jones 1963 and Sharma and Chatterjee 1966) and *D. tosaense* (Mutsuura and Nakahira, 1959; Tanaka, 1965, 1971). Species with fragments or accessory chromosomes have been observed by several authors (Kasaki 1958, Chardard 1963, Jones, 1963 and Pancho 1965). Of all the species of *Dendrobium* studied, ten show n=20 chromosomes, whereas the rest are n=19, excepting the *D. longicornu* (n=22). This record is in contrast to n=20 chromosomes reported by previous authors. The present Himalayan form (n=22), where polyspory has been observed, is a distinct cytological race. In *D. chrysanthum*, the population collected from Tagda (Eastern Himalayas) shows 2n=40 as against 2n=38 from the population of Shillong (Khasia hills). These two cytological races differ markedly in their phenotypic characters. The Shillong population develops spongy bulbs at the tip of the mature roots, which are not present in the other. Similarly the population from Tagda has bushy habit with profuse leaves, whereas that of Shillong is characterised by a few divergent leaves. Differences in chromosome number associated with phenotypic difference of two populations growing in distinct areas are quite likely correlated with their ecological adaptations. In *D. gibsonii*, previous records show 2n=38 as against 2n=40 chromosomes of the present record. This is another example of intraspecific aneuploid race.

The chromosomes of *Dendrobium* could be categorised under 2 types 'A' and 'B' (Table 1, Figs. 5–18). The general similarity of the F% and TF value shows the homogeneity of the genus. The minute details of karyotype however vary from species to species. The difference in minute details of the karyotype is an indication of the karyotypic structural alterations affecting the evolution of species. On the basis of the present and previous records, n=19 and 20 are the predominant numbers of which n=19 is comparatively deep seated. But that the two numbers are quite related is indicated in the occurrence of intraspecific variation.

Subtribe Sarcanthineae

All the species of *Aerides* show 2n=38 chromosomes, a character which is constant for the genus *Aerides* as a whole. Of the fourteen species and varieties, only in one of them viz., *A. hitchongii* 2n=40 chromosomes (Chardard 1963) have been found. In *A. lawrence*, in addition to 2n=38 (Shindo and Kamemoto 1963a, Kamemoto *et al.* 1964a) a chromosome race with 2n=40 is also on record (Eftimiu-Hein 1941). Excepting these two, multiples of 19 chromosomes are uniformly found throughout the genus.

The karyotypes of the species of *Aerides* show gross similarity with each other and *A. affine* has slightly longer chromosomes than the rest. In *A. fieldingii* a specialized type of chromosome with intercalary primary and secondary constrictions (Type 'C') is present (Fig. 21). It is evident that the genus is quite homogeneous and principally minute structural alterations involving changes in karyotype have been effective factors in evolution of species.

The chromosomes of *Sarcochilus* in general are medium to short and do not show much difference in gross morphology from species of *Aerides* excepting *A. affine* and *A. fieldingii*. The affinity of *Sarcochilus* and *Aerides* is indicated in F% and TF value as well. The inclusion of *Sarcochilus* (Fig. 28) as an allied genus of *Aerides* is justified on cytological grounds.

On the morphological grounds, *Cleisostoma* is very much alike to *Saccolabium* and *Stauropsis* to *Vanda*, with which most of their species are merged.

In *V. cerulescence*, previous reports show 2n=38 chromosomes (Jones 1967, Sharma and Chatterjee 1966). All species of *Vanda* have remarkable homogeneity in the gross morphology of the chromosome and size difference between members is rather small (Figs. 30–36). It is remarkable that even the two varieties of *V. teres* viz., Type I and Type II, differ in relation to minute changes in karyotype. Some of the 'A' type chromosomes are rather longer in Type II than those of the Type I. From a cytological standpoint, the homogeneity of the *Vanda* cannot be questioned even though minute alterations have been effective factors in evolution.

The genus *Vanda*, shows chromosome races in several species (Chardard 1963, Sharma and Chatterjee 1966). Even then, n=19 is certainly a deep seated number in the genus which is present in species inhabiting different climatic regions including alpine and temperate zones. High degree of polyploidy too has been observed in *V. spathulata, V. tricuspidata* (Storey 1952, 1953) etc. Polyploidy, occasional aneuploidy as well as structural alterations have therefore played important roles in successful speciation of the genus *Vanda*.

Two species of *Rhynchostylis* show difference from each other in karyotype. There is slight difference in chromosome size, though otherwise general karyotype pattern is identical. Number of secondary constrictions too, is the same not withstanding slight difference in morphology. In general, they fall in line with karyotype characteristics of the subtribe Sarcanthineae as a whole.

Along with the subribe Sarcanthineae, one genus which deserves special mention is *Hexisea*. The species—*H. reflexa* has been reported from Trinidad (Schultes 1960). Individuals of this species were collected from Kalimpong. So far there is no record of this species in India. The leaves are very alike to those of *Vanda* including the nature of insertion, though the hanging nature differentiates them clearly from *Vanda*. The flowers are extremely small and even smaller than those of *Cleisostoma mannii*. The chromosomes in general show similarity with those of the section Sarcanthineae. In view of this similarity, its affinity with members of Sarcanthineae is obvious.

The two more species of the section Sarcanthineae studied are *Stauropsis undulatus* and *Cleisostoma mannii*. In both of them, $2n=38$ chromosomes in graded karyotype have been found. The general morphology too, finds parallel to other genera of section Sarcanthineae (Figs. 24 and 29).

All the species of Sarcanthineae provide ample cytological evidences of the homogeneity of the group as a whole. In the evolution of the different taxonomic units, cryptic structural alterations have no doubt been effective. But it is not possible to identify the genera on the basis of certain karyotype characteristics. This is because alterations have been equally effective at inter and intraspecific levels.

Considerable amount of non-homology has been noted between chromosomes in several species (Table 1). In such cases the non-homology could be detected in the presence of unpaired chromosomes in the karyotype. The difference may involve both or either of the long and short arms. Such wide non-homology suggesting structural alteration has evidently survived through vegetative propagation.

Abstract

The present paper deals with a detailed chromosome study of 35 species including cytotypes of the tribe Kerosphaereae of Orchidaceae. There are 17 new reports from Eastern Himalayas and the Khasia hills. On the basis of the chromosome behaviour and karyotype analysis it has been concluded that in the subtribe Coelogyneae, the genus *Pholidota* is allied to *Coelogyne* and those two represent homogeneous assemblages. In the subtribe Dendrobeae, the genus *Dendrobium* is quite homogeneous in which polyploidy and aneuploidy along with chromosome changes have played an important role in evolution. Intraspecific chromosome races are common and a clear case has been demonstrated in *D. chrysanthum* where such cytotypes are correlated with distinct environmental conditions. Dressler (1961) described the northern epiphytes of Mexico and also recorded the severe frost-damage in them excepting *Epidendrum conopseum* R. Br. (Dressler 1964). In the subtribe Sarcanthineae, the affinity of *Aerides* with *Sarcachilus* has been revealed and presence of supernumerary constriction indicates the extent to which structural changes of chro-

mosomes are operating in evolution. The cytological affinity of *Stauropsis* with *Vanda* has been shown. The position of the species *Hexisea reflexa*, newly recorded here from India, has been assigned under Sarcanthineae. In *Vanda*, polyploidy and aneuploidy occurring at intra and interspecific level as well as cryptic structural alterations have been considered to be the factors which have contributed to the wide geographical distribution and adaptation of the genus. In general, genera cannot be categorised on the basis of their karyotypes. In broad features of the karyotype, the family as a whole represents a normal grouping, as karyotype alterations, though playing a role in evolution, are equally effective at an intra and interspecific level. An illustrated work was published on Orchid pollination and evolution (Dodson 1961).

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