Genome and Karyotype Relationships in the Genus *Dendrobium* (Orchidaceae) II. Karyotype relationships[1]

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The genus *Dendrobium* Swartz of the Orchidaceae comprises between 800 and 1600 species distributed from Japan to Tasmania and India to Polynesia, and has been subdivided into 41 sections on the basis of floral and vegetative characteristics (Schlechter 1912). Although the first chromosome counts of *Dendrobium* species were recorded by Hoffman in 1929, very little was known about the cytology of this genus until the middle of this century. Chromosome numbers of 133 species have been recorded to date, 110 of which are 2n=38, 20 are 2n=40, 2 are 2n=76, and one, *D. kingianum*, is variable from 2n=38 to 2n=114 (Hoffman 1929, 1930, Miduno 1940, Eftimiu-Heim 1941, Ito and Mutsuura 1957, Kosaki 1958, Mutsuura and Nakahira 1958, 1959, Blumenschein 1960, Vajrabhaya and Randolph 1960, Kosaki and Kamemoto 1961, Dorn and Kamemoto 1962, Jones 1963, Chardard 1963, Shindo and Kamemoto 1963, Pancho 1965a, 1965b, Kamemoto and Sagarik 1967).

Relatively little work has been done on the size and morphology of the chromosomes of *Dendrobium* species. Ito and Mutsuura (1957) noticed a difference in size of chromosomes in different species. Kosaki (1958) observed that most of the species he examined had minute chromosomes, but that *D. macrophyllum*, *D. spectabile* and *D. anosmum* (*superbum*) had chromosomes 3 to 4 times as large as the others. Shindo and Kamemoto (1963) observed conspicuous interspecific differences in the chromosome size of three species. The chromosomes of *D. formosum* were twice as large as those of *D. sanderae*, while those of *D. draconis* (Figs. 4, 10) were of intermediate size.

In an earlier paper (Wilfret and Kamemoto 1969), the crossability of species in the genus was presented. In the present investigation the number, size, and morphology of chromosomes were closely examined in order to establish the usefulness of karyotype analyses in the classification of the genus.

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Materials and methods

Thirty four species representing 11 sections of the genus *Dendrobium* were investigated. These species, assembled from various sources, are part of the living collection of orchids at the University of Hawaii.

Root-tip smears of actively dividing roots were made using the aceto-orcein squash technique. The excised root-tips were pretreated in 0.002 M hydroxyquinoline at 16°C for 4 hours, fixed in a modified Carnoy's solution (1:1:2) for 15 minutes at 16°C, hydrolyzed in 1 N HCl at 60°C for 2 minutes, transferred to 45% acetic acid for 3 minutes, and squashed and stained in 1% aceto-orcein.

Photomicrographs of selected metaphase figures were taken and enlarged to a magnification of 5500×. Each chromosome was carefully traced and measured as to the length of both the long and short arms, using 0.5 mm as the unit. The chromosomes were then arranged in descending oder of length. The mean chromosome length was determined for each karyotype. The morphology of the individual chromosomes of the karyotype was expressed as the mean F% with standard deviation. The F% is the percentage of the short arm over the total length of the chromosome. The chromosomes were segregarted into 3 groups according to the F%: $0<F\%\leq30$ (subterminal), $30<F\%\leq45$) (submedian), and $45<F\%\leq50$ (median). The mean S%, which is the percentage of the length of the smallest chromosome over the length of the largest chromosome within the karyotype, was also calculated.

Observation and discussion

Among the 34 *Dendrobium* species examined representing 11 of Schlechter's 41 sections, 32 were 2n=38 and only 2, *D. leonis* and *D. dixanthum*, were 2n=40 (Table 1, Figs. 1-6). The chromosome numbers of the following 7 species are recorded for the first time: *D. leonis*, 2n=40, *D. trigonopus*, 2n=38; *D. canaliculatum*, 2n=38; *D, bullenianum*, 2n=38; *D. mirbelianum*, 2n=38; and *D. sutepense*, 2n=38; *D. atroviolaceum*, 2n=38.

The data from this study together with those tabulated by Tanaka and Kamemoto (1963, 1964) show that 115 species of *Dendrobium* have a somatic chromosome number of 38 and only 21 have a somatic number of 40. Both numbers are represented in the sections *Aporum*, *Callista*, *Eugenanthe*, *Nigrohirsutae*, *Pedilonum*, and *Rhopalanthe*, while only a somatic number of 38 has been recorded for the sections *Ceratobium*, *Eleutheroglossum*, *Latourea*, and *Stachyobium*. In the section *Phalaenanthe* somatic numbers of 38, 57, and 76 have been reported (Tanaka and Kamemoto 1963, 1964). The latter two numbers might have been horticultural variants. In *D. kingianum* of the section *Dendrocoryne*, diploid (38), tetraploid (76), and hexaploid (114) numbers exist (Vajrabhaya and Randolph 1961, Jones 1963, Maxwell 1967). Thus, despite the occurrence of some variation in chromosome numbers among

Table 1. Karyotype analysis of *Dendrobium* species

Section and species	Author	2n	Mean chromosome* length (microns)	Mean S%*
Aporum				
distichum	H. Reichenbach	38	1.27±0.02	46.1±4.6
leonis	H. Reichenbach	40	1.62±0.13	58.3±5.3
Callista				
aggregatum	W. Roxburgh	38	1.56±0.04	50.9±3.7
chrysotoxum	J. Lindley	38		
trigonopus	H. Reichenbach	38	1.69±0.04	43.2±1.8
Ceratobium				
d'albertsii	H. Reichenbach	38		
gouldii	H. Reichenbach	38	1.48±0.04	35.3±2.2
grantii	C. T. White	38	1.54±0.15	45.7±4.1
mirbelianum	Gaudichaud	38		
stratiotes var. *giganteum*	H. Reichenbach	38		
strebloceras	H. Reichenbach	38		
undulatum	R. Brown	38	1.54±0.07	29.5±1.4
Eleutheroglossum				
canaliculatum	R. Brown	38	1.93±0.05	50.5±3.5
Eugenanthe				
anosmum	J. Lindley	38	2.51±0.11	47.1±0.4
arachnites	H. Reichenbach	38	1.84±0.07	52.0±2.1
dixanthum	H. Reichenbach	40		
heterocarpum	N. Wallich	38	1.62±0.08	54.2±4.2
linguella	H. Reichenbach	38	1.38±0.05	43.8±2.3
macrostachyum	J. Lindley	38		
monile	F. Kränzlin	38	1.43±0.05	50.1±2.2
moschatum	O. Swartz	38	1.69±0.05	56.3±4.1
senile	Parish	38		
tortile	J. Lindley	38		
Latourea				
atroviolaceum	Rolfe	38	1.88±0.16	47.5±3.0
spectabile	F. Miguel	38		
Nigrohirsutae				
draconis	H. Reichenbach	38	2.18±0.03	47.5±3.0
formosum var. *giganteum*	W. Roxburgh	38	2.28±0.08	50.1±2.5
sutepense	R. Rolfe	38	2.00±0.05	42.9±3.2
Pedilonum				
bullenianum	H. Reichenbach	38		
victoria-reginae	Loher	38	1.53±0.05	40.5±2.5
Phalaenanthe				
biggibum	J. Lindley	38	1.62±0.05	47.1±3.3
phalaenopsis	Fitzgerald	38	1.47±0.05	41.9±2.4
Rhopalanthe				
crumenatum	O. Swartz	38	1.48±0.05	37.6±2.2
Stachyobium				
delacourii	A. Guillaumin	38	1.64±0.10	48.1±3.4

* with standard deviation

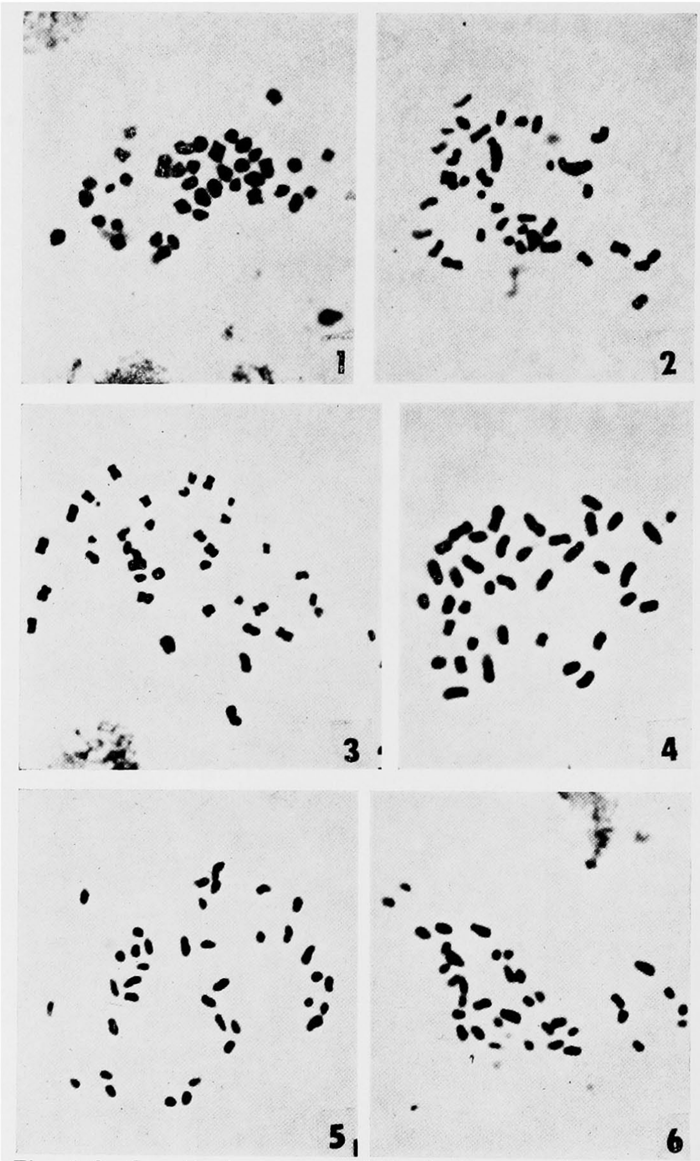
Table 1. (Cntiuned) Karyotype analysis of *Dendrobium* species.

Section and species	Mean number of chromosomes with*			Mean F%
	0<F%≤30	30<F%≤45	45<F%≤50	
Aporum				
distichum	0.0	21.3±1.5	16.7±1.5	43.8±0.6
leonis	0.0	17.3±2.1	22.7±2.1	44.8±0.7
Callista				
aggregatum	0.0	16.7±1.1	21.3±1.1	44.8±0.6
trigonopus	0.0	23.0±1.0	15.0±1.0	41.9±0.5
Ceratobium				
gouldii	4.0±0.0	19.0±2.6	15.0±2.6	41.3±0.6
grantii	0.0	24.7±2.3	13.3±2.3	43.0±0.8
undulatum	2.0±0.0	20.7±3.5	15.3±3.5	41.5±1.2
Eleutheroglossum				
canaliculatum	2.0±0.0	28.3±1.5	7.7±1.5	41.0±0.9
Eugenanthe				
anosmum	0.0	29.0±2.7	9.0±2.7	41.9±0.8
arachnites	0.0	23.7±0.6	14.3±0.6	41.7±0.4
heterocarpum	0.0	16.7±3.5	21.3±3.5	45.0±1.3
linguella	0.0	15.7±1.1	22.3±1.1	45.1±0.4
monile	0.0	21.7±2.1	16.3±2.1	44.3±0.6
moschatum	0.0	22.7±1.5	15.3±1.5	43.6±1.6
Latourea				
atroviolaceum	0.0	24.3±2.0	13.7±2.0	43.3±0.4
Nigrohirsutae				
draconis	0.0	24.3±2.1	13.7±2.1	42.3±0.9
formosum var. *giganteum*	0.0	26.0±3.0	12.0±3.0	42.4±1.0
sutepense	0.0	28.5±1.5	9.5±1.5	41.0±0.9
Pedilonum				
victoriae-reginae	0.0	19.3±1.1	18.7±1.1	44.0±0.5
Phalaenanthe				
biggibum	0.0	21.3±2.5	16.7±2.5	42.9±0.6
phalaenopsis	6.0±0.0	21.7±1.5	10.3±1.5	39.7±0.2
Rhopalanthe				
crumenatum	0.0	26.5±2.1	11.5±2.1	41.7±0.6
Stachyobium				
delacourii	0.0	20.0±2.6	18.0±2.6	44.8±0.5

* with standard deviation

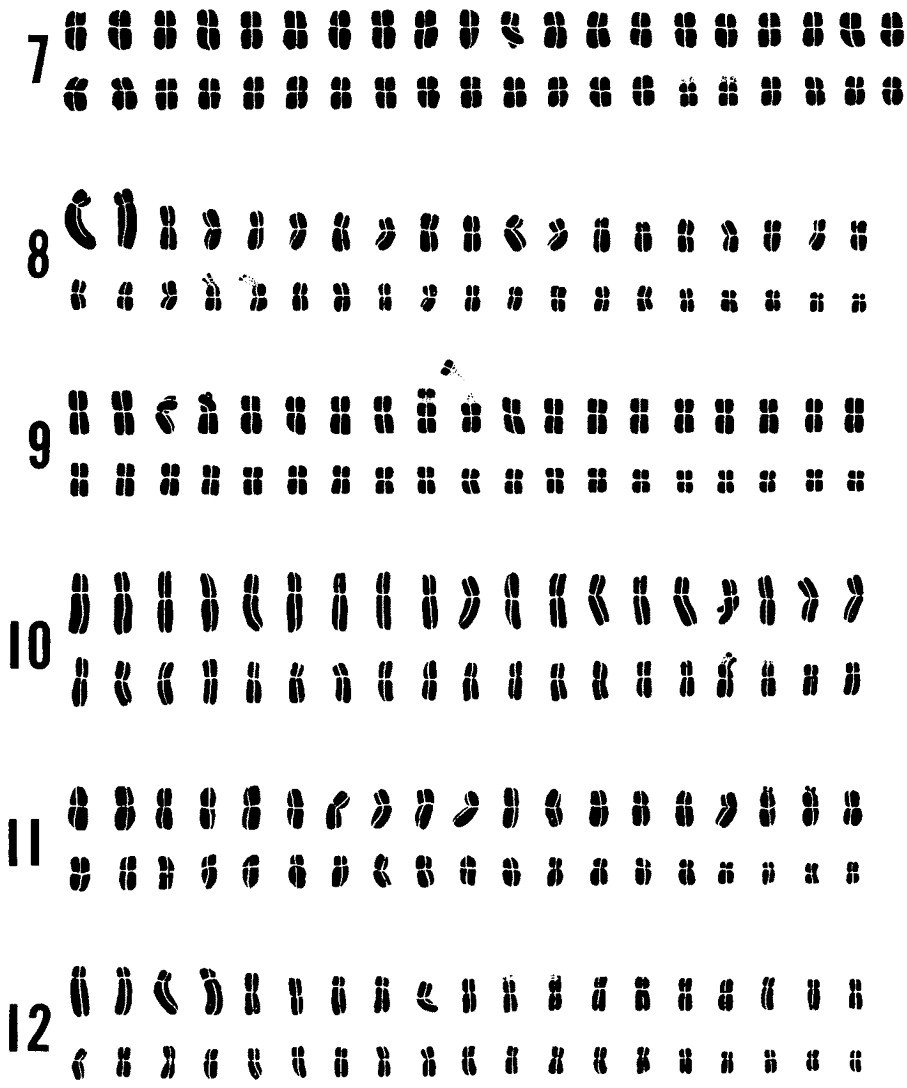
species, numbers in themselves do not appear to have any significance in Schlechter's sectional classification of the genus.

Somatic chromosome numbers of both 38 and 40 have been recorded for some *Dendrobium* species (Jones 1963). Earlier studies suggested a relative abundance of species with 2n=40, but the more recent studies appear to disagree with some of the earlier determinations. For example, *D. chrysotoxum*, *D. parishii*, and *D. nobile* were recorded as 2n=40 in the earlier accounts, but more recent reports, including the present investigation, have shown that 2n=38 is the representative number for these species. On the other hand, some counts might have represented atypical individuals of the species, such as an aneuploid *D. dixanthum* with 41 chromosomes (Jones 1963), an aneuploid *D. moschatum* with 39 chromosomes (Kamemoto and Sagarik 1967) and plants with fragment or accessory chromosomes such as 3 additional small chromosomes in *D. moschatum* (Kosaki and Kamemoto 1961).



Figs. 1-6. Somatic chromosomes of *Dendrobium* species (1,600×). 1, *D. leonis* 2, *D. undulatum*. 3, *D. monile* 4, *D. draconis* 5, *D. biggibum*. 6, *D. phalaenopsis*.

The chromosome morphology of 23 *Dendrobium* species is shown in Table 1. At least five somatic cells were analyzed for each karyotype. Mean chromosome length with standard deviation varied among species from 1.27 ±0.018 microns to 2.51±0.109 microns. The smallest chromosome set was that of *D. distichum* in the section *Aporum* and the largest was that of *D. anosmum* in the section *Eugenanthe.*



Figs. 7-12. Karyotypes of *Dendrobium* species (2,850×). 7, *D. leonis*. 8, *D. undulatum*. 9, *D. monile*. 10, *D. draconis*. 11, *D. biggibum*. 12, *D. phalaenopsis*

Although several species exhibited significant differences in mean chromosome size, the examined sections could not be distinguished by this parameter. There was almost as much variation within some of the sections as between all of the sections. The maximum mean difference, including standard deviation, between all of the species examined was 1.36 microns. In the Eugenanthe section alone, the difference between the means of the smallest and largest karyotypes was 1.29 microns. *D. anosmum* had one of the largest chromosome complements in the genus while *D. linguella* had one of the smallest. With such a wide variation in mean chromosome size within sections, comparisons among sections in the genus are meaningless for use in correlating chromosome size with Schlechter's classification system. However, species in the section *Nigrohirsutae* generally exhibited large chromosomes and could be separated from those of most of the other sections on the basis of mean chromosome size.

The karyotypes of species exhibited a range of S% with standard deviation from 29.5±1.4 to 58.3±5.3 (Table 1). The two species at either end of the range were *D. undulatum* (Figs. 2, 8) and *D. leonis* (Figs. 1, 7), respectively. *D. leonis* had very uniform, medium-sized chromosomes with a difference of only 0.94 microns between the largest and smallest chromosomes. In comparison, *D. undulatum* exhibited a difference of 2.04 microns, over twice that found in *D. leonis*. The high S% of *D. undulatum* was produced by the presence of two large subterminal chromosomes accompanied by exceedingly small median to submedian chromosomes. The karyotype of this species was distinguishable from all other species examined by the existence of this one pair of very large, heterochromatic, subterminal chromosomes. As with the mean chromosome size, it was not possible to distinguish individual sections on the basis of S%. Some species could be distinguished by S% in conjunction with other morphological characteristics of the karyotypes.

The mean F% (Table 1), which is another measure of the symmetry of the karyotype, varied from that of *D. phalaenopsis* (Figs. 6, 12) with the lowest F% of 39.7±0.2 to that of *D. linguella* with the highest F% of 45.1±0.4. It was not possible to separate the sections on the basis of mean F%, although a few species exhibited significant differences. *D. phalaenopsis* showed a significant difference in mean F% compared to the rest of the species examined, including *D. biggibum* (Figs. 5, 11), which is in the same *Phalaenanthe* section.

Although the sections cannot be distinguished on the basis of chromosome number, mean chromosome size, S%, or F%, certain characteristics of individual karyotypes make it possible to distinguish certain species. As previously mentioned, the chromosome complement of *D. undulatum* can be distinguished by the large pair of heterochromatic subterminal chromosomes accompanied by small, median to submedian chromosomes. *D. phalaenopsis* can be distinguished by the low F%, and *D. grantii* can be distinguished easily from *D. gouldii* by the absence of 4 subterminal chromosomes found in the latter species.

The position of the satellite chromosomes was inconsistent among all of the species studied. The satellites ranged from being on the second chromosome pair to the eighteenth pair. *D. aggregatum* consistently showed 2 pairs of satellite chromosomes. The size of the satellites was highly variable among the species. *D. anosmum* and *D. atroviolaceum* had very large and distinctive satellites, while those in the *Ceratobium* section were very small and sometimes indistinguishable. The satellites were either heterochromatic, as in *D. monile* (Figs. 3, 9), or euchromatic, as in *D. phalaenopsis.* In general, the species and sections cannot be separated on the basis of the position, size or stainability of the satellites.

Karyotype analysis makes it possible to distinguish between two closely related species in the *Phalaenanthe* section. *D. biggibum*, a small flowered species native to northern Queensland, has pseudobulbs to 1-1/2 feet tall and produces inflorescences of 4 to 12 flowers extending about 12 inches. *D. phalaenopsis* native of the Tanimbar Islands, is similar in habit but much more robust in all parts with the pseudobulbs 4 feet tall or more (Wilfret and Kamemoto 1969). For many years taxonomists have considered these to be separate species (Kranzlin 1910, Schlechter 1927). The major floral differences are as follows (Blake 1964):

D. biggibum	*D. phalaenopsis*
petals broadly rounded	petals distinctly acuminate
veins on disc thickened and contiguous	veins on disc slender and distant by more than their width
calli usually numerous and dense	calli commonly small and not dense

However, Hawkes (1965) recently considered these two variants to be conspecific, with the former the typical variety and the latter *D. biggibum* var. *phalaenopsis* (Fitzg.) F. M. Bail. The two species have the same somatic number, 38. There is no significant difference in either mean chromosome length or in the S% (Table 1). The greatest differences in the karyotypes of the two species are found in a comparison of the mean F% and the morphology of the individual chromosomes in each karyotype. There is a significant

Table 2. Comparison of number of median, submedian and subterminal chromosomes in *D. biggibum* and *D. phalaenopsis*

Type of chromosome	*D. biggibum**	*D. phalaenopsis**
Subterminal	0.0	6.0±0.0
Submedian	21.3±2.5	21.7±1.5
Median	16.7±2.5	10.3±1.5

* with standard deviation

difference in mean F%, with that of *D. biggibum* being 42.9±0.6 and that of *D. phalaenopsis* 39.7+0.2 (Table 1). The two species can be distinguished from one another by F% alone, but a comparison of the two species also shows differences in the number of median and subterminal chromosomes (Table 2). The two karyotrpes can be distinguished by the presence of the 3 pairs of large subterminal chromosomes found in the *D. phalaenopsis* complement. Since this species has 3 more pairs of subterminal chromosomes and 3 less pairs of median chromosomes than *D. biggibum*, the differences might have resulted through simple translocations or inversions. However, the structural changes in the chromosomes appear to have had little effect creating differences in the external morphology of the two species. Analysis of F_1 hybrids between these species might grealty elucidate the taxonomic status of the two species.

Summary

The chromosome numbers of 34 species representing 11 of Schlechter's sections were determined, of which 32 were $2n=38$ and 2 were $2n=40$. The chromosome numbers of 7 of these species had not been reported previously. Detailed examinations of chromosome morphology were made of species representing 11 sections. The mean chromosome size, S%, and F% were as variable among species within a section as between the sections. Although karyotype analysis of species did not prove to be useful in delineating sections in the genus *Dendrobium*, it was utilized in an attempt to clarify the taxonomic relationships between *D. biggibum* and *D. phalaenopsis*.

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