KARYOMORPHOLOGICAL STUDIES IN *DRIMIOPSIS KIRKII* BAKER

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ABSTRACT

Karyomorphological studies in a local population of *D. kirkii* have revealed the somatic chromosome number of $2n = 68$. This is a new number and has made the cytological situation in the genus more complex in respect of the basic chromosome number. The chromosomes form a graded series with 4 pairs of satellited chromosomes. On the basis of the present observations a tetraploid level of a complex category has been suggested for this species. An analysis of meiotic stages and the chromosome number in the pollen grains is suggestive of the presence of 1–4 supernumerary chromosomes in the genome of the taxon.

THE genus *Drimiopsis* Lindl. *et* Paxt. is included in the tribe *Scilleae* of Liliaceae. It is relatively a small genus with about ten species all reported from tropical Africa, and is stated to be closely related to *Scilla* and *Ornithogalum* (Chittenden, 1956). Four species of the genus have been cytologically examined so far and these studies have revealed a rather intriguing situation with respect to the basic chromosome number for the genus and cytological pattern of speciation. Matsuura and Sato (1935) were the first to report the chromosome number of $2n = 80$ in *D. botryoides* while Sato (1942) working on *D. maculata* recorded the number $2n = 64$. DeWet (1957) made an extensive study of a number of genera belonging to the tribe Scilleae and has reported the chromosome number of two species of *Drimiopsis*, viz., *D. saundersiae* and *D. crenata* as $2n = 20$ and $n = 10$ for both. More recently Fernandes and Neves (1962) working on the cytology of *D. maculata* record the gametophytic and sporophytic number as $22_{II} + 16_{I}$ and 60 respectively. It was against this background that the present karyomorphological studies in *Drimiopsis kirkii* were undertaken to make a detailed analysis of its somatic and meiotic chromosomes in order to discover the cytological situation in the species. This study has revealed

further interesting cytological data in relation to other species already investigated by others and these observations are presented and discussed in the present contribution.

Material and Methods

Drimiopsis kirkii is a bulbous plant found growing as a cultivar in the gardens of Bangalore for some years. The bulbs are globose with long and pale green leaves narrowed to the base and with very characteristic dark green blotches on the upper surface. The flowers are borne on a long scape and are greenish-white. In spite of profuse flowering, normally no fruits are produced and occasionally each scape bears one to three fruits in which the carpels are either incompletely developed occasionally with a few crumpled seeds or hollow and as such the plants are sexually sterile. They, however, multiply vegetatively through daughter bulbs. The material was procured from Government Botanical Gardens, Bangalore (S. India) and grown in pots in the gardens attached to the Botany Department of the Bangalore University. Somatic chromosomes were studied from root tips. Healthy excised root tips were thoroughly washed and treated with either colchicine (0·0%) aqueous for about 90 minutes or with 8-hydroxyquinoline (0·002 M) at 10–12° C. for three hours or more. After pretreatment they were washed in tap-water and gently heated in a mixture of 2% acetic orcein and 1/N hydrochloric acid (9 : 1) for a few seconds over a spirit flame. They were kept in the mixture for a period of 30 minutes and then each tip was squashed separately in a drop of 2% acetic orcein. The squashes were sealed temporarily with a mixture of gum-mastic and paraffin and kept for 1–2 days for the stain to deepen. After preliminary observations the temporary squash preparations were made permanent following the N-butyl alcohol-acetic acid series and using euparal as the mountant. For the study of meiosis and meiotic chromosomes, temporary squashes of pollen mother cells from appropriate buds were prepared using the customary aceto-carmine squash technique.

They were made permanent following the N-butyl alcohol-acetic acid series method. For the study of pollen grain mitosis aceto-carmine was used as the stain fixative. Observations, drawings and photo-micrography were made with temporary as well as permanent preparations.

Observations

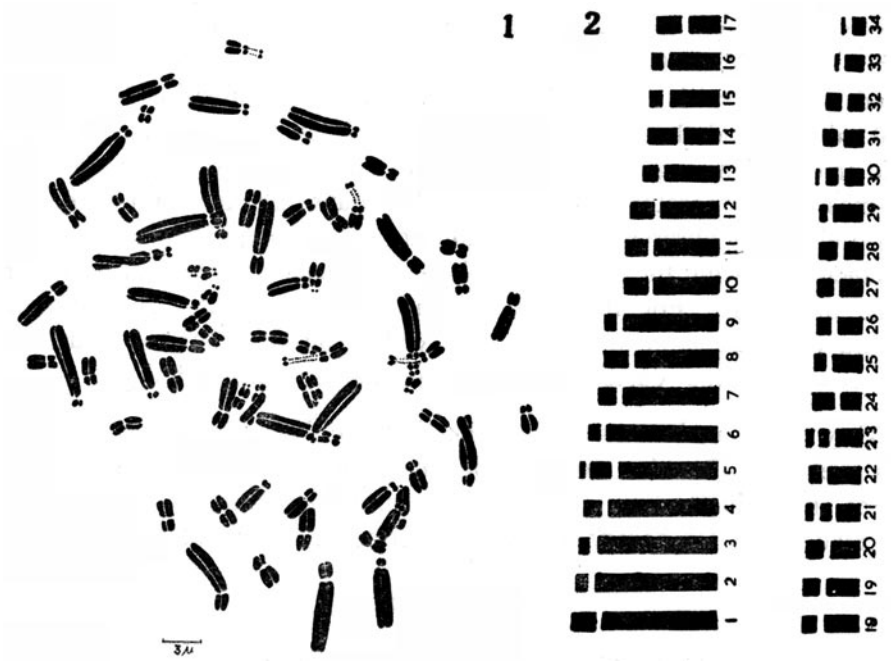
The karyotype as determined from the root tips consists of $2n = 68$ chromosomes. The chromosomes form a graded series. These can be

classified under the following four categories, on the basis of the position of the centric constrictions and the presence or absence of secondary constrictions:

1. Four pairs with terminal satellites (designated as 'S' in the karyotype formula).
2. Four pairs with median primary constrictions (designated as 'V' in the karyotype formula).
3. Ten pairs with sub-median primary constrictions (designated as 'J' in the karyotype formula).
4. Sixteen pairs with sub-terminal primary constrictions (designated as 'I' in the karyotype formula).

The karyotype formula is $2n = 68 = S_8 + V_8 + J_{20} + I_{32}$.

Text-Fig. 1 and Plate XI, Fig 1 show the 68 somatic metaphase chromosomes and Text-Fig. 2 is the idiogram of the somatic complement. The length of the chromosomes ranges from $0{\cdot}83\,\mu$ to $7{\cdot}25\,\mu$, and the absolute length of the chromosomes is $122{\cdot}77\,\mu$.



TEXT-FIGS. 1–2. Fig. 1. Somatic metaphase chromosomes. Fig. 2. Idiogram of the haploid somatic complement, × 1,455.

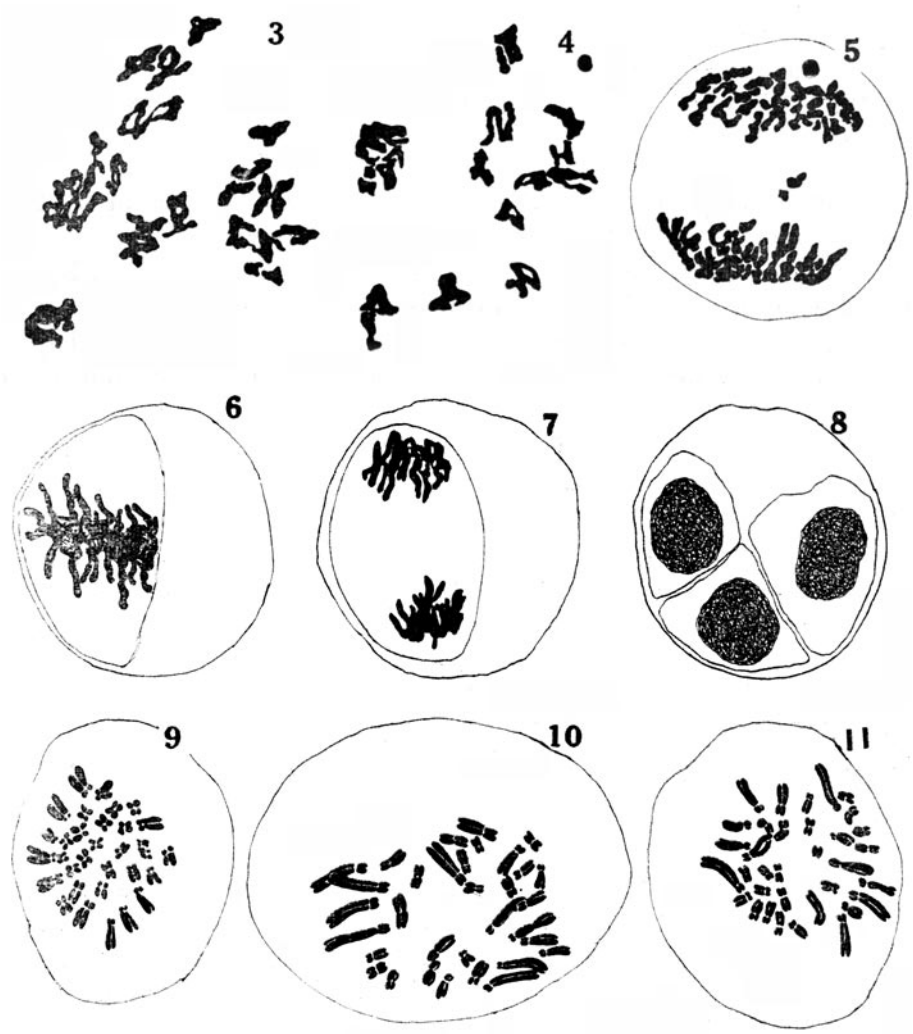
Meiosis was studied in considerable detail and has revealed a variety of irregularities. Diakinesis stages show the formation of bivalents, multivalents and univalents. Twenty pollen mother cells at diplotene and diakinesis were examined in respect of associations and these counts give a varying number of univalents, bivalents, trivalents and quadrivalents in different cells. Bivalents constitute the largest number, while the number of trivalents range from two to three and that of quadrivalents between two and four. Two to four univalents were encountered in some cells. Text-Figs. 3 and 4 and Plate XI, Figs. 2 and 3 show the different kinds of associations, the multivalents being in the form of chains or rings.

Orientation at Metaphase I is found to be generally normal (Plate XI, Fig. 4) Anaphase I shows apparently normal disjunction but, in certain cells one or more laggards are seen (Text-Fig. 5). In a few cells it was noticed that instead of regular anaphasic separation, the two groups of chromosomes form a restitution nucleus towards one half of the cell leaving the other half empty. The restitution nucleus thus formed would undergo the second division forming the two daughter nuclei leaving the other half of the cell empty (Text-Figs. 6 and 7 and Plate XI, Fig. 5). A similar situation appears to occur at the second division which results in the formation of two normal pollen grains on one side and only one large pollen grain on the other side giving rise to a triad (Text-Fig. 8). In spite of such irregularities the formation of pollen grains appears to be normal. In a great majority of pollen mother cells empty or shrunken pollen grains are rather rare. The first mitotic division could easily be studied in a large number of pollen grains and chromosomal counts at metaphase revealed three different chromosome numbers namely, 32, 33 and 34 in different cells (Text-Figs. 9, 10 and 11; Plate XI, Figs. 6 and 7).

Discussion

The available cytological information in the four species of *Drimiopsis* investigated by previous workers reveals a rather intriguing situation in regard to the basic chromosome number for the genus. Work of Matsuura and Sato (1935) on *D. botryoides* $2n = 80$ and of Sato (1942) on *D. maculata* $2n = 64$ are indicative of the basic chromosome numbers as $x = 8$ for both these species. It is on this basis that Darlington and Wylie (1956) have mentioned the basic number for the genus as $x = 8$. On the contrary de Wet (1957) working on *D. saundersiae* and *D. crenata* has recorded the chromosome number as $2n = 20$ and $n = 10$ for both the species and has also given an idiogram for the former species on the basis of his compre-

hensive study of the several genera of the tribe Scilleae. He has postulated the original basic number for the whole tribe Scilleae as $n = 5$. He has further opined that the other chromosome numbers met within the tribe may be regarded as polyploids or diploids on the basis of $n = 6, 7, 8, 9$ and that hybridization and allopolyploidy would have given rise to the basic



TEXT-FIGS. 3–11. Figs. 3–4. Diakienesis stages (part) showing different kinds of association: univalents, bivalents, trivalents and quadrivalents (Rings and chains may be noted. Fig. 5,) A PMC at Anaphase I showing laggards. Figs. 6–7. Two PMC's one at Metaphase II and the other at Anaphase II showing one half of the cell empty. Fig. 8. A PMC showing the formation of a triad leaving one quarter of the cell empty. Figs. 9–11. Three pollen grains at first mitotic metaphase showing 32, 33 and 34 chromosomes respectively, × 728.

numbers like $n = 11$ and $n = 13$. Fernandes and Neves (1962) working on *D. maculata* have recorded the somatic number as $2n = 60$ and the formation of 22 bivalents and 16 univalents at meiosis. This somatic number of $2n = 60$ could be reconciled with the basic number of $n = 5$ for Scilleae as postulated by de Wet. But the observation of 22 bivalents and 16 univalents introduces a confusion which is difficult to resolve and is not of much help in arriving at the basic number. It may be that two different genomes are involved in this species whose identity cannot be established.

The present study on *D. kirkii* with $2n = 68$ obviously introduces further difficulties in the already complex cytological picture of the genus. There are, however, certain indications in the karyotype which provide clues in resolving the cytological anomalies encountered in the genus. If chromosomes with secondary constrictions provide an index, the presence of four pairs of chromosomes all with terminal satellites is suggestive that this species may be at the tetraploid level of some category. This assumption is further borne out by the formation of a number of multivalents and univalents in addition to bivalents at diakinesis. Such a situation obviously introduces subsequent meiotic irregularities. The variation in the chromosome number in the pollen grains at the first mitotic division is significant. Although the somatic number is $2n = 68$, pollen grains clearly show either 32, 33 or 34 chromosomes. This variation in the number of chromosomes in the pollen grains is highly suggestive of the presence of 1–4 supernumerary chromosomes in the genome of the species.

On the basis of the present study it can be stated that *D. kirkii* represents a hybrid of complex ancestry in which karyotypically dissimilar forms are involved and the occurrence of probably 1–4 accessory chromosomes have made the species cytologically more complex.

ACKNOWLEDGEMENT

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EXPLANATION OF PLATE XI

FIGS. 1–7

FIG. 1. A root tip cell at Metaphase, × 1,265.

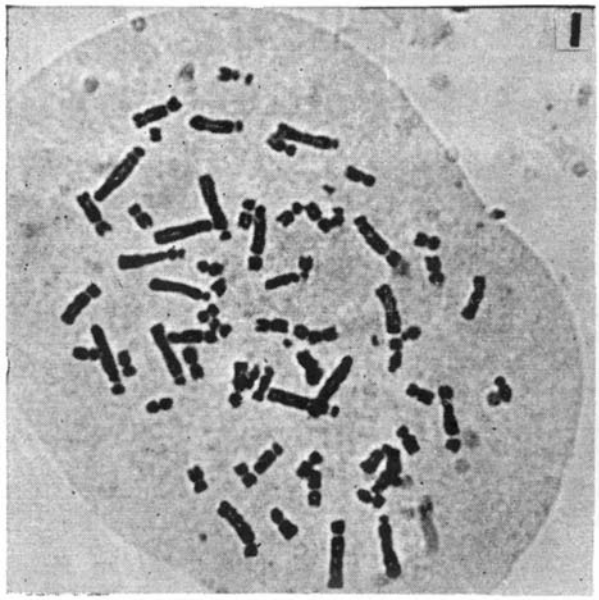
FIG. 2. A part of a PMC at diakinesis showing a multivalent along with bivalents (chain), × 1,164.

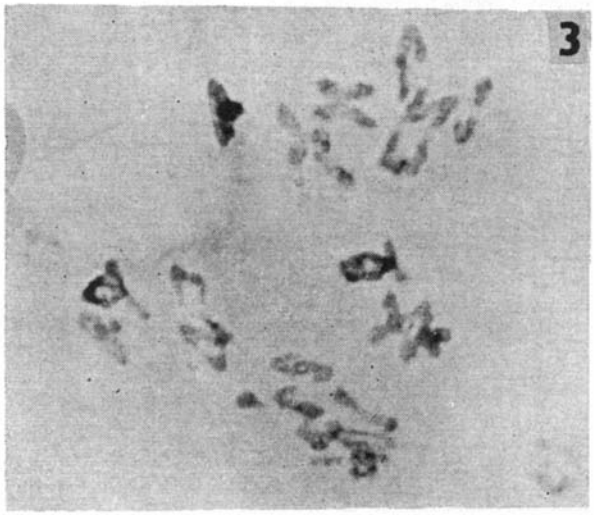
FIG. 3. A part of a PMC at diakinesis showing multivalents (rings along with other associations), × 1,164.

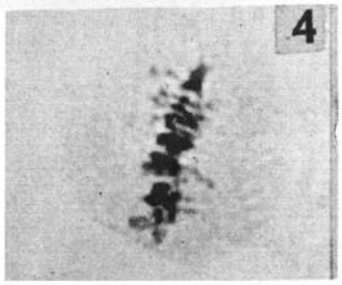
FIG. 4. A PMC at Metaphase I showing normal orientation, × 679.

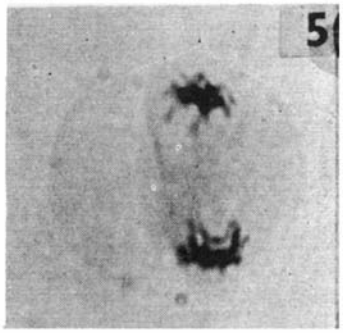
FIG. 5. A PMC at Anaphase II showing one half of the cell empty × 679.

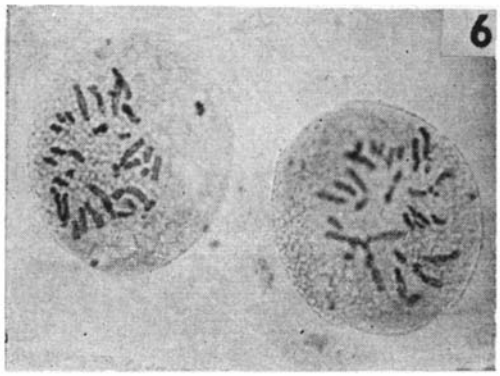
FIGS. 6–7. Pollen grains at first mitotic division showing different chromosome numbers. Fig. 6, 582; Fig. 7, × 1,132.

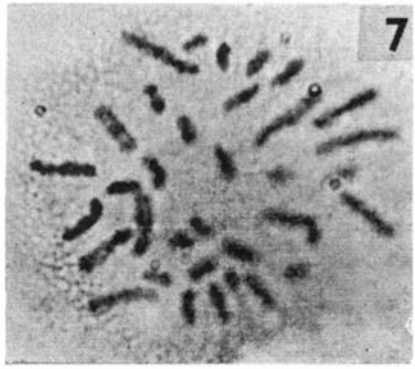












FIGS. 1–7