MEIOTIC ANALYSIS IN *RAUVOLFIA SERPENTINA* (L.) BENTH. EX KURZ

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A detailed analysis of the chromosome complement of *Rauvolfia serpentina* at pachytene has revealed that there is a common basic structural pattern for all the chromosomes except the smallest one. The chiasma frequency and the coefficient of terminalization have been observed to be less per bivalent in this perennial plant. Observations on the bivalents in the pollen mother cells of five plants coming from different localities have indicated that their chiasma frequencies are negatively correlated. These plants differ in their internuclear variance at each stage but not in the inherent variance. The test of homogeneity of variances has indicated that plants coming from different localities resemble very closely in their inherent variance but not in the internuclear variance. Meiotic abnormalities include precocious movement of bivalents and occasional occurrence of univalents.

INTRODUCTION

Rauvolfia serpentina (L.) Benth. ex Kurz belonging to the family Apocynaceae has now earned wide reputation as a source of a group of curative alkaloids used successfully as a remedy in hypertension and insanity. It has also recently attracted the attention of botanists. But, however, a perusal of the literature on the cytology of this species indicates that the work is almost negligible. Although several papers (Bowden 1945; Witkus 1951; Raghavan 1957; Roy Tapadar and Sen 1960; Roy Tapadar 1964) have appeared on the karyological data of different species of the family Apocynaceae, there are only two papers dealing with *R. serpentina*, one by Bhattacharjee and Bhaduri (1959) who have studied the karyotype of *R. serpentina* and the other by Roy Tapadar (1964) who has also given the karyological data. However, the data given by these authors were not sufficient and hence the present authors had undertaken the study of its karyotype (Dnyansagar and Torne 1967) and meiosis in detail.

MATERIAL AND METHODS

Seedlings raised from the seeds of *R. serpentina* were planted in pots as well as in plots in the garden of the Institute of Science, Bombay. Floral buds were fixed for 48 hours in 4 : 3 : 1 chloroform-alcohol-propionic acid mixture. A small amount of $Fe(OH)_2$ was dissolved in propionic acid before a fresh

mixture was prepared. Finally the material was stored in 70 per cent alcohol in a refrigerator. Aceto-carmine was used for staining temporary smear preparations and was found satisfactory. The slides were made permanent by separating the cover-slip and the slide in a mixture of acetic acid and normal butyl alcohol in equal proportions followed by two quick changes in normal butyl alcohol and mounting in euparal.

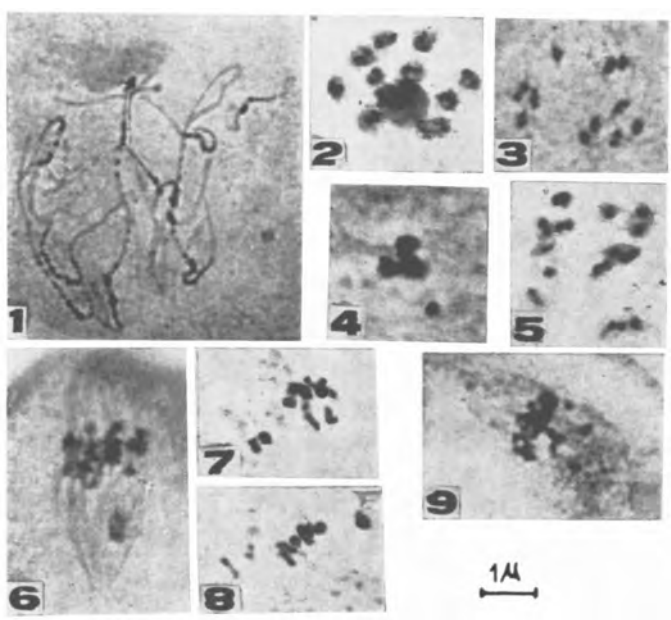
OBSERVATIONS

Prophase of the first division

Owing to the small size of chromosomes and the delicate nature of the threads, it is difficult to study them in detail. This stage begins with an increase in size of the nucleus. A clear leptotene stage is seen. Chromosomes are slightly thick, long and beaded.

Pachytene

Chromosomes appear distinctly thicker and shorter but are still longer to make out all the bivalents. In the clearest cases 11 bivalents could be counted. One bivalent is distinctly attached to the nucleolus (Fig. 1).



FIGS. 1 to 9. 1, pachytene chromosomes showing one chromosome attached to the nucleolus; 2, PMC showing late diakinesis; 3, PMC showing metaphase (polar view); 4, PMC showing lack of orientation of a bivalent; 5, PMC showing 8 bivalents and 6 univalents; 6, PMC showing precocious movement of two chromosomes; 7 and 8, PMC's showing groups of bivalents arranged at the equator; 9, PMC showing precocious movement of three chromosomes.

Based upon the average values of length, obtained by complete analysis of chromosomes in 10 entire nuclei, the 11 pachytene chromosomes of *R. serpentina* have been designated in the order of their length as chromosome I to chromosome XI, chromosome I being the longest and chromosome XI being the shortest in the complement. Table I indicates the measurements based on the analysis of all the pachytene chromosomes in each of the 10 nuclei.

TABLE I

Summary of means from 10 camera lucida drawings of each of the 11 chromosomes of R. serpentina
(All lengths are given in microns)

Chromosome	Total length	Long arm length	Short arm length	Arm ratio
I	46·40	28·74	11·82 +5·84 (sat)	1·62
II	35·00	21·50	13·50	1·58
III	33·30	20·15	13·15	1·53
IV	30·70	21·40	9·30	2·31
V	28·00	15·70	12·30	1·27
VI	26·30	16·70	9·60	1·09
VII	25·40	14·10	11·30	1·24
VIII	21·00	11·20	9·80	1·14
IX	18·90	10·21	8·69	1·17
X	17·90	11·67	6·23	1·87
XI	11·60	05·89	5·71	1·03

Diplotene

No information was available regarding any possible difference in external morphological variations amongst the plants of *R. serpentina* from different places and their chiasma frequency.

Table II gives the chiasma frequencies at diakinesis and metaphase I. In order to find whether the chiasma frequency amongst the chromosomes of the same nucleus indicates any correlation with the chiasma frequency of chromosomes of different nuclei, a correlation coefficient has been calculated (Table III). This method involves the determination of internuclear variance which measures the variations in the total number of chiasmata amongst the pollen mother cells, and the inherent variance which measures the variation in the distribution of chiasmata between the bivalents of the individual cells. If the internuclear variance exceeds the inherent variance, a positive correlation is suggested. A negative correlation is achieved, on the other hand, if the inherent variance is greater than the internuclear variance.

The minimum number of observations, which were to be made in order to give statistically significant differences, was first determined. Hundred cells

were taken from each of the five plants for each stage. The observations on mean chiasma frequencies of each of the five plants are summarized in Table III.

TABLE II

Chiasma frequencies at diakinesis to metaphase I stages

Stage	Number of cells analysed	Bivalents with				Total number of chiasmata	Average number of Xta per nucleus	Average number of Xta per bivalent	S.D.	Coefficient of terminalization
		4 Xta	3 Xta	2 Xta	1 chiasma					
Early diakinesis	14	–	58	92	4	362	25·8	2·3	0·14	0·22
Late diakinesis	20	–	–	96	124	316	15·8	1·4	0·12	0·45
Metaphase	16	–	–	47	129	223	13	1·1	0·10	0·53

S.D.—standard deviation; Xta—chiasmata.

TABLE III

Plant No.	Number of cells	Mean Xta per nucleus	Inherent variance (mean)	Internuclear variance (mean)	*r*
	(*a*) *Correlation coefficient between inherent and internuclear chiasmata at late diplotene*				
1	100	31·5	0·4167	0·1238	−0·9762*
2	100	30·8	0·4192	0·1235	−0·9694*
3	100	30·9	0·4427	0·1361	−0·9735*
4	100	31·7	0·4338	0·2103	−0·9751*
5	100	31·8	0·4501	0·2157	−0·9708*
	(*b*) *Correlation coefficient between inherent and internuclear chiasmata at early diakinesis*				
1	100	26·2	0·3651	0·1129	−0·9356*
2	100	25·5	0·3549	0·1188	−0·9452*
3	100	26·2	0·3689	0·0998	−0·9539*
4	100	25·4	0·3591	0·1229	−0·9486*
5	100	25·7	0·3603	0·1243	−0·9531*
	(*c*) *Correlation coefficient between inherent and internuclear chiasmata at metaphase*				
1	100	13	0·3267	0·1237	−0·7684*
2	100	14	0·3208	0·1231	−0·7701*
3	100	13	0·3187	0·1351	−0·7691*
4	100	12	0·3189	0·1307	−0·7723*
5	100	13	0·3202	0·1286	−0·7689*

* Significant at 1 per cent level.

Also given in this table are the corresponding internuclear and inherent variances.

A variation in the shape of the nucleolus from pachytene (bilobed, Fig. 1) to diplotene-diakinesis (globular to slightly oval, Fig. 2) has been observed.

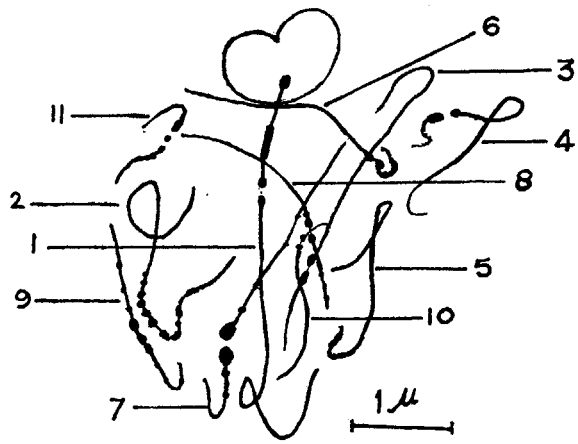


FIG. 10. Camera lucida drawing of Fig. 1.

Diakinesis

The chromosomes at this stage are short and thick and reveal lightly stained distal ends of the chromosome arms. One bivalent in most of the cases remains attached to the nucleolus. The chiasma frequency and the terminalization coefficient have been given in Table II.

The early diakinesis is marked by the repulsion of the bivalents. The number of chiasmata per bivalent varies from 3 to 1. It is 2·3 per bivalent and the coefficient of terminalization is 0·22. The percentage of the chiasma classes at late diplotene, early diakinesis, late diakinesis and metaphase I is plotted as a graph (Fig. 11).

In the late diakinesis (Fig. 2) and early metaphase, the number of chiasmata varies from 2 to 1. In the metaphase only one or two chiasmata may be distinguished, of which one terminal chiasma is of more frequent occurrence. Some of the pairs of chromosomes are characterized by weak conjugation so that most probably chiasmata are not formed and, as a result, a varying number of univalents occur. The maximum number of univalents has been found to be 14 in one plate. Usually, however, a single pair of univalent occurs.

Prometaphase

The nuclear membrane now disappears and the spindle appears in the region of the nucleus. The cells show a transition from a type of spindle formation and chromosome movement, that seems to be completely regular, to

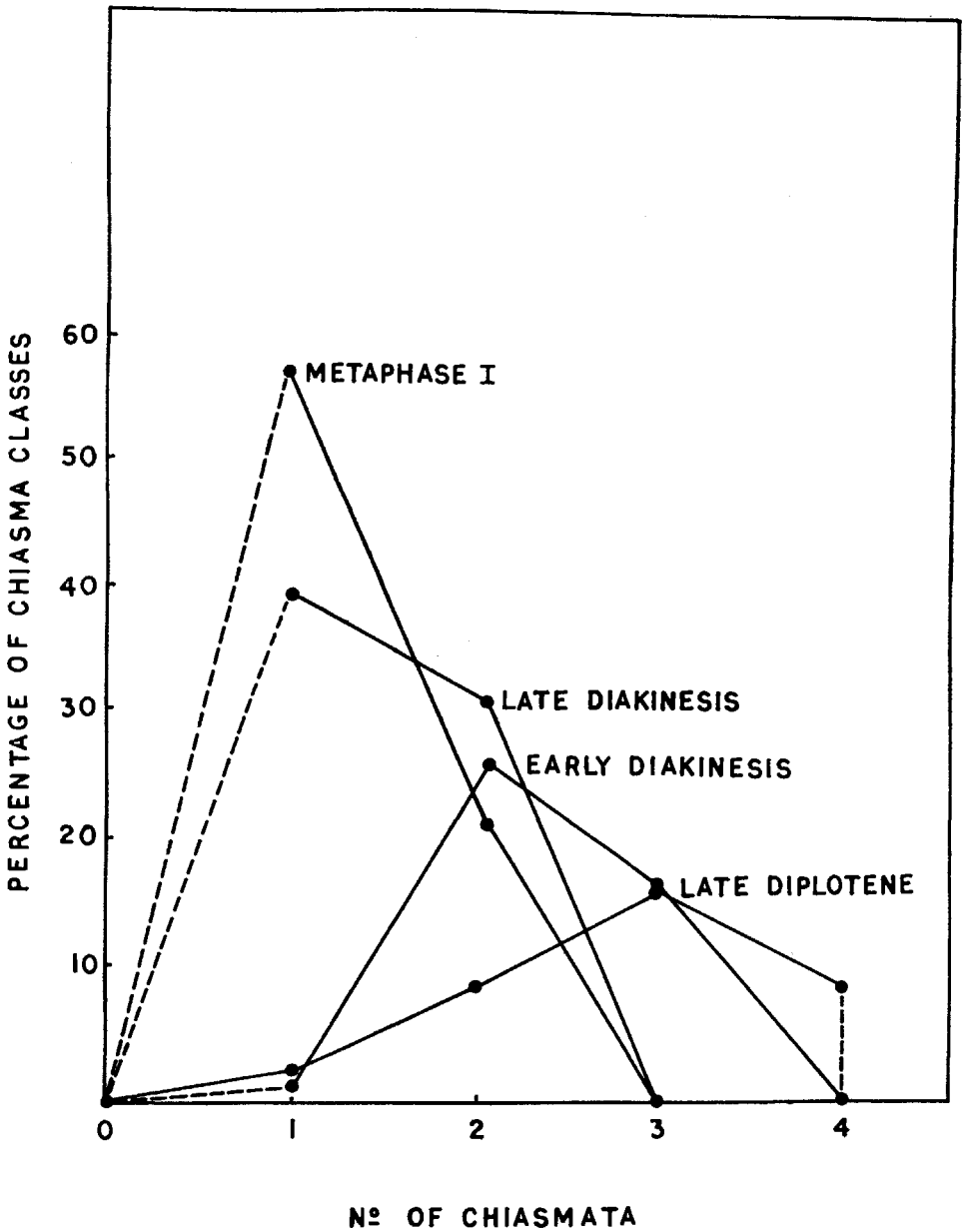


FIG. 11. Percentage frequency polygons of numbers of chiasmata in bivalents of *R. serpentina* at late diplotene, early diakinesis, late diakinesis and metaphase of the first division in PMC's.

an irregular type. A compact spindle is formed at metaphase, with two approximately defined poles, and the bivalent chromosomes have their pairs of centromeres co-oriented on the arcs of this spindle.

Irregularity shows itself in its mildest form either by lack of compactness at one or both poles or by lack of orientation of one or more bivalents (Fig. 4) and univalents. Where compactness is found to be lacking, it is noticed that the groups of bivalents are arranged at the equator of the spindle (Figs. 7, 8).

The polar view of the metaphase indicates 11 bivalents (Fig. 3). Fig. 5 shows 8 bivalents and 6 univalents, while a side view of the metaphase shows a varying number of rings and rods. The longest or the shortest chromosome might be forming rod or ring bivalents. There is a frequent occurrence of rod bivalents than of ring bivalents. Their percentage has been calculated from 16 early anaphase plates and is given below (Table IV).

TABLE IV

Percentage frequency of occurrence of rod bivalents or ring bivalents

Stage	Ring bivalents	Percentage	Rod bivalents	Percentage	Total bivalents
Early anaphase	47	27·7	129	73·3	176

The persistence of the chiasmata sometimes results in the lagging of bivalents.

The precocious movement of some chromosomes towards the pole was observed in some cases when others were at the equatorial plate (Figs. 6, 9).

Anaphase I

Anaphase I is for the most part regular. The smallest chromosome, which contracts at the metaphase to a short rod, separates as soon as the rest of the complements are arranged at the equator.

Telophase I

At telophase I sometimes precocious movements of univalents are observed.

DISCUSSION

There is no doubt that the chromosome I, as designated in the chromosome complement of *R. serpentina* in this work, is the satellited or nucleolar organizer. Although Roy Tapadar and Sen (1960) have not mentioned the number of SAT-chromosomes in pollen mother cells of *R. serpentina*, even then the camera lucida figure in their paper clearly shows a single SAT-chromosome.

Data given in Table III indicate that, from the last diplotene to metaphase, inherent variance exceeds internuclear variance. This is true for all the five plants coming from different localities and the difference has been found to be statistically significant in all the five plants at three stages. The analysis at three

stages thus provides an evidence for a negative correlation between chiasmata in the large and small groups of bivalents. Also it has been shown that the five plants differ in their internuclear variance at each stage but not in the inherent variance. At the same time, it has been indicated that the mean chiasma frequency per nucleus is directly proportional to the increase in the inherent variance but not so with the internuclear variance.

A negative correlation must indicate some influence of a bivalent over the chiasma formation in others of the same nucleus irrespective of an outside influence. This phenomenon has been given the name as competition by Mather (1936).

The almost linear increase in inherent variance with an increasing number of chiasmata indicates that, at lower levels of chiasma frequency, the inter-bivalent distribution of chiasmata is to a large extent independently determined. In other words, each of the bivalents is adapted to form a minimum number of chiasmata beyond which the competition sets in for the extra number of chiasmata formed in the nucleus. Lamm (1936) found that inbred rye with a low chiasma frequency failed to show the interchromosomal competition, whereas ordinary rye with a higher chiasma frequency exhibited it often. Also, the recent finding of Wilson (1959), that experimentally induced changes in the chiasma frequency in *Endymion* affect the long chromosomes more than the short ones, is understandable on the basis of the above conclusion.

The test of homogeneity of variance (Table III) in *R. serpentina* indicates that the five plants coming from different localities resemble very closely in their inherent variance but not in the internuclear variance.

In *R. serpentina*, which is a perennial plant, the chiasma frequency per bivalent, as observed from Table II, is very low. Recent investigations in *Lolium* (Rees and Ahmed 1963) show that annual and other short-lived populations have higher chiasma frequencies than in the case of perennial populations. The variation in the chiasma frequency is inferred to be adaptive. Jones and Rees (1966) have suggested, however, that the variation in the chiasma frequency is directly dependent on the longevity of the population.

The nucleolus has been found to vary in its shape from the pachytene (bilobed) to diplotene (globular-oval). The variation in the shape of the nucleolus from pachytene to diplotene is difficult to interpret. The polarization of the nucleolar shapes to the sites of attachment of bivalents suggests the operation of some mechanical forces. The most probable explanation is the hindrance to terminalization in the regions proximal to satellites which might be operative in the mechanical forces imposed upon the shapes of nucleoli (Misra and Shastry 1966).

The above-mentioned anomalies, together with a low chiasma frequency and a low terminalization coefficient, are considered as an evidence that

R. serpentina differentiation might have been accompanied by great structural changes of the chromosomes. The same types of observations have been recorded by Sanudo (1960) in *Solanum pinnatisectum* and *S. sambucinum.*

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