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KARYOTYPIC STUDY IN THE GENUS *CROTALARIA*. II.

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INTRODUCTION

In evaluating phylogenetic relationships and cytotaxonomical characteristics amongst species, karyotype studies are of much importance. These are intensively studied now-a-days with great improvements in cytological techniques.

In 1960 GANGULY and DATTA (*in press*) studied the karyotypes of *Crotalaria juncea* Linn., *C. anagyroides* Retz. and *C. sericia* H. B. K. They noted close similarities in morphological characteristics among chromosome complements in these three species and observed five types of chromosomes in *Crotalaria juncea* and six types in two other species. They further noted that three pairs of chromosomes have only primary constrictions and the rest five pairs have secondary constrictions. The chromosome length varied from long size to short with $2n = 16$ in all of them.

SRIVASTAVA (1958) observed seven types of chromosomes in somatic metaphases of *C. Brownei* Ber. ex D. C. and *C. saltiana* Andr. In the former 1 pair of long chromosomes with submedian constrictions and 3 pairs of medium chromosomes with submedian constrictions are observed. In the latter, 1 pair of long chromosome with submedian constrictions, 2 pairs of median chromosomes with median constrictions and 5 pairs of medium chromosomes with submedian constrictions occur. The pair of large chromosome A is satellited in both the cases but the second pair of SAT-chromosomes is different in the two species being E in *C. Brownei* and the much longer pair C in *C. saltiana*. In *C. Brownei* the duplicated pair in the complement is G, whereas E is duplicated in the other. A critical examination of the idiograms reveals that both of them do not belong to the same species. Such variations arise due to hybridization between species with distinct karyotypes or as a result of non-homologous translocations resulting in the transference of satellites to different chromosomes. Both phenomena are known to be operative in plants and are of considerable significance in course of evolution and origin of new species. Besides, chances of spontaneous breakage and reunions resulting in the alteration of gross chromosome morphology, though rare, cannot be ruled out altogether.

Chromosome morphology often reflects genetic relationship accurately. In any hybridization programme, these studies will be of much importance. With that end in view the present piece of work was undertaken in 1961.

MATERIALS AND METHODS

Difficulties were experienced in germinating the seeds, particularly the seeds of *Crotalaria ferruginea* Grah. Pretreatments with varying concentrations of HCl or in hot water etc. were of no avail. Finally as a last resort a fine slit was made on the hard testa with a sharp knife, facilitating easy penetration of water, when no further difficulties were experienced in germinating the seeds. This method enhanced the germination time and good healthy seedlings were obtained within 36-48 hours.

Before fixation root tips were pretreated in saturated aqueous asculine solution for 1-2 hours at 6-8° C. Hydroxyquinoline 0.001 M to 0.002 M was also tried. But the former chemical gave better result.

After pretreatment the root tips were fixed in acetic-alcohol (1:2) for one hour.

Finally they are warmed in a mixture of 2% aceto-orcein + N. HCl (9:1) a few seconds and then kept for 30 minutes and squashed in 1% aceto-orcein.

OBSERVATIONS

The somatic chromosome numbers in two species *Crotalaria saltiana* (= *C. striata*) and *C. verrucosa* are found to be $2n=16$ as recorded previously (cf.

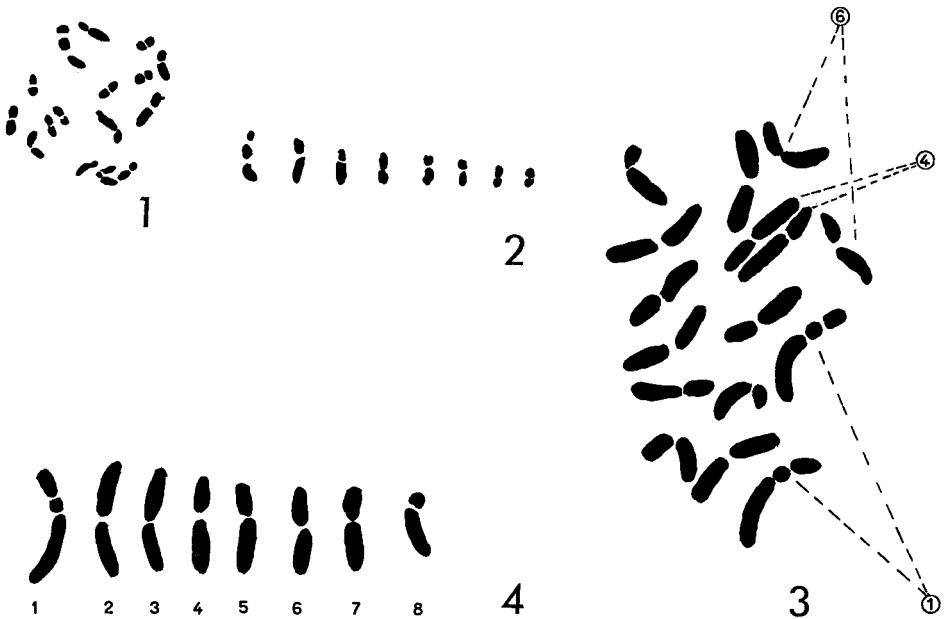


Fig. 1. — Metaphase in root tip cell of *Crotalaria saltiana* showing 16 chromosomes. $\times 800$.

Fig. 2. — Idiogram of the haploid set of chromosomes of *C. saltiana*.

Fig. 3. — Metaphase in a root tip cell of *C. verrucosa* showing 16 chromosomes. $\times 2000$.

Fig. 4. — Idiogram of the haploid set of chromosomes of *C. verrucosa*.

DARLINGTON and WYLIE 1955, CHANDRASEKHARAN and PARTHASARATHY 1950, RAO 1943 and SRIVASTAVA 1958).

The somatic chromosome number $2n=16$ of *C. ferruginea* Grah. is a new record.

Karyotypic studies were carried out in other two species of *Crotalaria*. Detailed observations regarding the structure of the chromosomes, position of the centromere etc. are recorded in Table I.

DISCUSSIONS

Study of the ratio of the median and the submedian positions of the centromere in the chromosomes are important.

According to LEWITSKY (1931) the karyotypes are broadly of two types: (1) Symmetrical — where the chromosomes are essentially similar to one another and possess median or nearly median centromere; such karyotype is primitive in nature; (2) Asymmetrical or specialized — where chromosomes do not have uniformity in size and the centromere is shifted to a relatively more submedian, subterminal or terminal position; this according to LEWITSKY is brought about as a result of reduction of some or all of the chromosomes in length leading to unequal length of the arms and considered by him to be more advanced. According to this hypothesis, therefore, the larger number of medianly constricted chromosomes suggests the relative primitivity of the species. Gross variations in such specialised karyotypes were reported by LEWITSKY 1931a, b; BABCOCK 1947, GATES 1951, CLAUSEN 1951, MITRA 1956 etc. These conspicuous differences are found to occur in the karyotypes of different species with the same or different base numbers. In certain cases, the differences in chromosomes are due to position of centromere, chromosome length and secondary constrictions. On the other hand, MALIK (1961) pointed out that while *Arisaema wallichianum* and *A. intermedium* show important morphological differences, both possess a similar karyotype. Since the summary by STEBBINS (1951) it has been increasingly appreciated that karyotypic similarity may not necessarily mean genic likeness.

It will automatically follow therefore that karyotypic dissimilarity automatically means genic unlikeness.

In the present investigation the occurrence of median (m) and submedian (sm) positions of the centromere are almost equal in both the species studied, suggesting that the two species are intermediate in position between advanced and primitive stages.

Reduction in size of one of the arms resulting in the shifting of the centromeric position is the loss in the absolute size of the chromosomes on the complement. This fact was independently brought to light by DELAUNAY (1926)

TABLE I
Measurement of somatic chromosomes.

Species	Types	Chro- moso- mes	Length in μ	Av. length	R. length	F.%	T. F.%	Centro- mere	No. of Sec. constrict- ed chro- mosomes	
<i>C. saltiana</i> Andr. (= <i>C. striata</i> L.)	A	1	4.40		100	18		nm st		
	B	2	4.00		90	33		sm		
	C	3	3.39	3.00	77	29		sm	1	
		4	3.22		73	28		sm		
	ABC ₂ D ₂ E ₂	D	5	2.59		58	50		m	
			6	2.40		54	33	$\frac{7.98 \times}{100}$	sm	
		E	7	2.00		45	50	$\frac{24}{= 33.35}$	m	
			8	2.00		45	50		m	
	5	2n=16	24.00					4m, 4sm, 1st		
<i>C. verrucosa</i> Linn.	A	1	6.09		100	27		sm st		
	B	2	5.73		94	50		m		
	ABC ₂ D ₂ E ₂	C	3	5.38		88	50		m	
			4	5.38	4.97	88	37	$\frac{15.84 \times}{100}$	sm	1
		5	5.02		82	35	$\frac{39.83}{= 39.7}$	sm		
	D	6	4.67		76	50		m		
		7	4.31		70	38		sm		
	E	8	3.25		53	25		st		
	5		39.83					3m, 4sm, 2st		

Relative length (R. length) used to represent the ratio in percentage of the length of the individual chromosome to that of the largest.

F% represents the percentage of the short arm length to the entire chromosome length.

TF% represents the ratio in percentage of the total sum of short arm length to the total sum of chromosome length. *nm* = nearly medium; *m* = medium; *sm* = submedium; *st* = subterminal.

from which he concluded that there is phylogenetic reduction in the length of the chromosomes in course of evolution. The short chromosomes are predominantly advanced characters.

In the present investigation it is noted that the relative length of the chromosomes varied only about 50% in both the species or in other words the reduction in the chromosome length has been only about 50% suggesting the same fact that the two species stand intermediate between primitive and advanced stages in evolution.

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SUMMARY

1. Five types of chromosome pairs were noted in *Crotalaria saltiana* and *C. verrucosa* the number of pair or pairs of chromosomes included in each type varied in the two species.
2. Length of the chromosome in *C. verrucosa* was relatively longer than *C. saltiana*.
3. Both the species have only one pair of secondary constricted chromosomes.
4. In *C. saltiana* out of the 9 constrictions 4 are median, 4 are submedian and one subterminal in position whereas in *C. verrucosa* 3 median, 4 submedian and 2 subterminal were in position.
5. All these relevant data suggest that the two species stand intermediate between primitive and advance stages in evolution.
6. Diploid chromosome number in *C. ferruginea* ($2n = 16$) is a new record