Chromosome Studies in Different Species and Varieties of *Sida* with Special Reference to Accessory Chromosomes

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Received November 12, 1969

Introduction

The genus *Sida* of the family Malvaceae is profusely represented in India. It has a wide range of tolerance and its distribution extends from the subtemperate regions of the Himalayas to the plains. Though only six species are recorded (Prain 1903) from the plains of Bengal and nine (Hooker 1872) from all over India, a wide range of form variations is frequent in this genus. Of the different species, in *S. rhombifolia* and *S. acuta*, the maximum phenotypic variations occur. A study of such variations is of special interest, not only from the academic viewpoint, but also due to the high medicinal importance of species of *Sida*.

Almost all the species, occurring in India and specially in Bengal, (Kirtikar and Basu 1918, Chopra 1933 and Chopra *et al.* 1956), contain medicinal principles which are obtained differentially from different plant parts, namely roots, stems, leaves, flowers, fruits and seeds. These principles have been used widely for the cure of nervous diseases, facial paralysis, rheumatism, gonorrhoea, pulmonary tuberculosis, ophthalmia and heart diseases.

Cytological data, so far worked out in this genus, have presented facts of fundamental importance. Different chromosome numbers have been reported from different species (Roy and Sinha 1961, Adhikary 1965, etc.) and the exact basic number is unknown. Further interest is provided by a report of accessory chromosomes in *Sida rhombifolia*.

In view of the medicinal importance of the genus, the extensive phenotypic variations providing facts of taxonomic importance, the scanty cytological data indicating variations in chromosome numbers, the present investigation on a detailed cytotaxonomic studies of *Sida* was undertaken. In order to attain this objective, improved methods had to be adopted for the study of detailed chromosome morphology. Investigations have been centered on population studies of each taxonomic unit from different areas in Bengal.

Materials and methods

Nine species and varieties of *Sida*, occurring in wild state, were collected from different localities of West Bengal and regions adjoining it, mostly from the suburbs of Calcutta.

S. rhombifolia has a wide occurrence with different varieties adapted to different conditions of the soil and other environmental conditions. Under the present study five distinct forms have been observed.

1. *S. rhombifolia* var. A (*typica*): Leaves comparatively long rhomboid, narrow, thick, acute and branches green or greyish green.

2. *S. rhombifolia* var. B (*rhomboidea*): Leaves comparatively large, broad, rhomboid, acute or sub-acute, branches and leaves green.

3. *S. rhombifolia* var. C (*obovata*): Leaf blades obovate.

4. *S. rhombifolia* var. D: Leaves comparatively short, rhomboid narrow, thin, acute or sub-acute, branches green.

5. *S. rhombifolia* var. E: Leaves comparatively small, short rhomboid, broad, thick, acute or sub-acute; leaves and branches green or light green; distinctly reddish, when young. Forty-eight populations, from different localities, of these varieties were studied.

Somatic and meiotic preparations, both temporary and permanent, were made. Pollen sterility was also noted. Much difficulty was encountered in studying both somatic and meiotic chromosomes as they are small and the cells are full of inclusions. Various pretreating agents, such as, oxyquinoline, p-dichlorobenzene, aesculin, isopsoralene, etc. (Sharma and Ghosh 1951, Sharma and Mookerjea 1955, Sharma and Sarkar 1955, Chaudhuri, Chakraborty and Sharma 1962) were tried. The most effective ones were p-dichlorobenzene and aesculin on different species at 10–20°C for periods ranging from 1 to 3 hours.

After pretreatment, the root tips were fixed in acetic acid-ethyl alcohol mixture (1:2) for one hour. They were then hydrolysed and stained in a mixture of 2% aceto-orcein and (N) HCl (9:1) by heating over a flame for a few seconds. Due to the heavy cytoplasmic contents the root tips were kept in the acid-dye mixture for 24 hours and subsequently squashed in 1% aceto-orcein solution, exerting uniform pressure over the coverglass. Squash preparations were inverted in normal butyl alcohol until coverslips were detached and permanent preparations were made by mounting the slides and coverslips separately in euparal.

For meiotic studies flower buds of suitable size were fixed in acetic acid-ethyl alcohol mixture (1:2) for 24 hours and then kept in 70% alcohol for future observation. Temporary smear preparations were made following Belling's 1% aceto-carmine technique. Pollen preparations by aceto-carmine method for sterility count and Feulgen test for differences in intensity of staining in the B chromosomes, were made.

Figures were drawn at a table magnification of approximately ×3000 using a Zeiss compensating eyepiece ×20 and 1.3 apochromatic condenser. In the figures the chromosomes with secondary constrictions were drawn in outline.

Observations

The somatic chromosome numbers of the different species and varieties under present investigation range from 2n=14 to 2n=28. The presence of B chromosomes characterises some of the populations of *S. rhombifolia* var. A. Polysomy and variations in the pollen sterility are also observed in some of them.

A detailed analysis of the karyotype of the different species and varieties reveals similarity in their gross morphology. On the basis of the relative length of the chromosomes, three groups—comparatively long, medium and short—can be identified. A uniform gradation in size is observed amongst the members of a karyotype. The longer chromosomes usually bear secondary constrictions and the medium and short ones bear median to sub-median primary constrictions. On the basis of their relative size and the positions of primary and secondary constrictions, a general description of the types of chromosomes occurring under this genus is given below. An account on their detailed karyotypes will be given separately for each.

Type A—Comparatively long chromosome with two constrictions, primary and secondary, one nearly median in position and the other submedian at the distal end of the longer arm.

Type B—Medium sized chromosome with two constrictions, primary and secondary, one median to nearly submedian in position and the other nearly submedian to nearly subterminal at the distal end of the slightly longer arm.

Type C—Medium sized chromosome with two constrictions, one nearly submedian and the other nearly submedian to nearly subterminal on the longer arm. The chromosome arm between the constrictions is much longer than the end segments.

Type D—Medium sized chromosome with two constrictions, primary and secondary, both located at submedian to nearly submedian positions at the two opposite ends of the chromosome, dividing it into three equal parts.

Type E—Medium sized to short chromosome with median to nearly submedian primary constriction.

Type F—Short to very short chromosome with median to nearly median primary constriction.

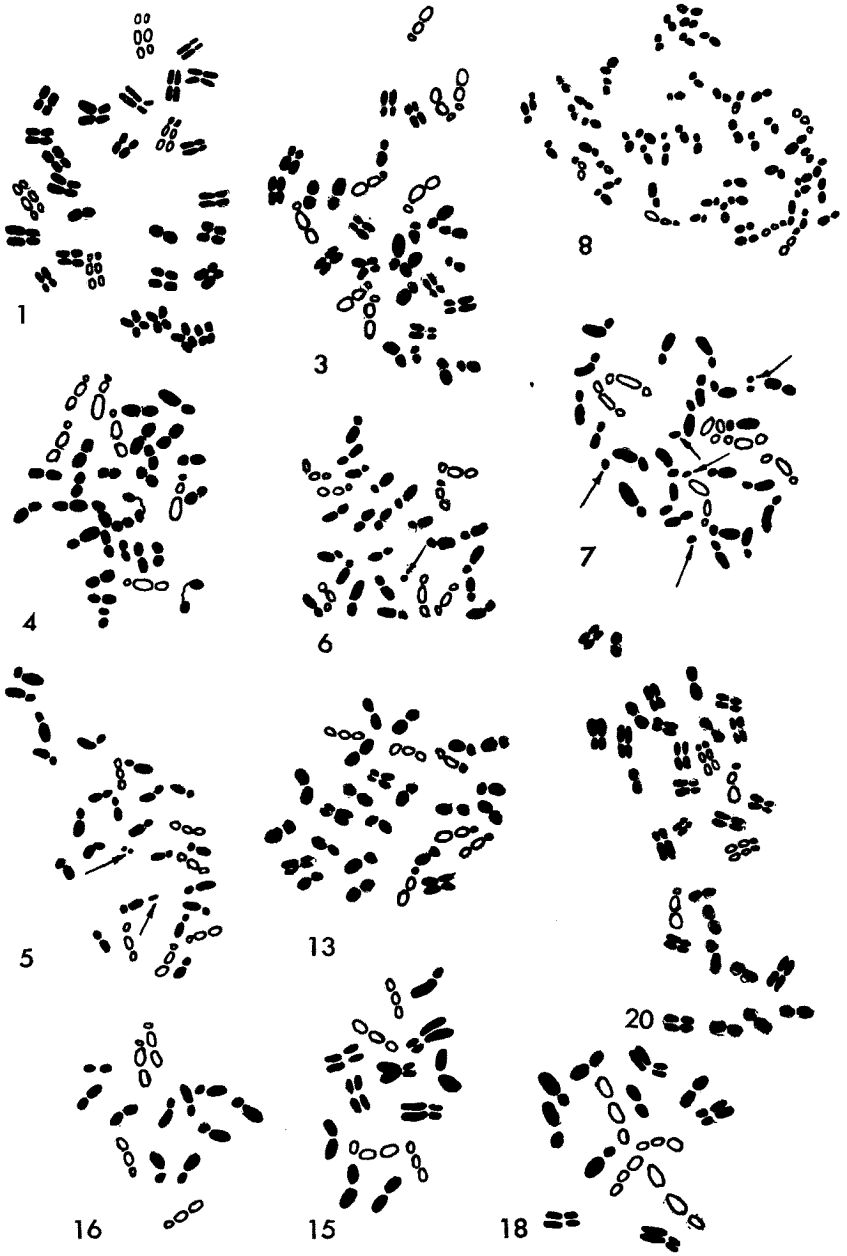
1. *S. acuta* Burm.

$2n=28=C_2+D_2+E_4+F_{20}=2.9\mu$ to 1.3μ (Figs. 1 and 1a).

Both the constrictions of the C pair are nearly submedian in position. E and F types of chromosomes are larger than the general E and F types.

2. *S. cordifolia* L.

$2n=32=B_8+C_2+E_{18}+F_6=2.5\mu$ to 1.3μ (Figs. 3 and 3a).

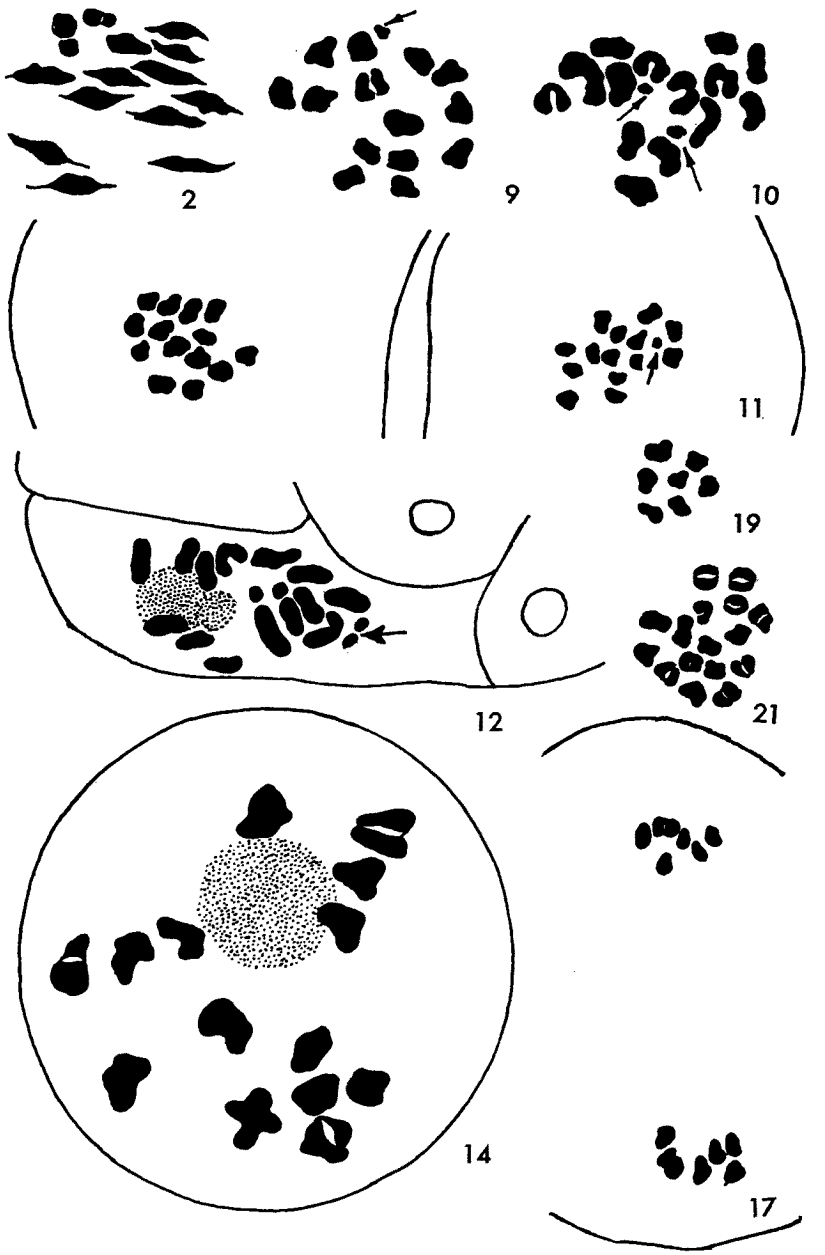


Figs. 1, 3, 4, 5, 6, 7, 8, 13, 15, 16, 18 and 20. Somatic metaphase plates of *Sida acuta*, *S. cordifolia*, *S. glutinosa*, *S. rhombifolia*, varieties A (Figs. 5 to 8), B, C, D and E and *S. veronicaefolia* respectively. Arrows indicate B chromosomes.

3. *S. glutinosa* Cav.

$2n=32=B_2+C_4+E_{16}+F_{10}=3\mu$ to 1.1μ (Figs. 4 and 4a).

The B type chromosomes are larger in size than the normal B type. One pair of C type is smaller than the other.

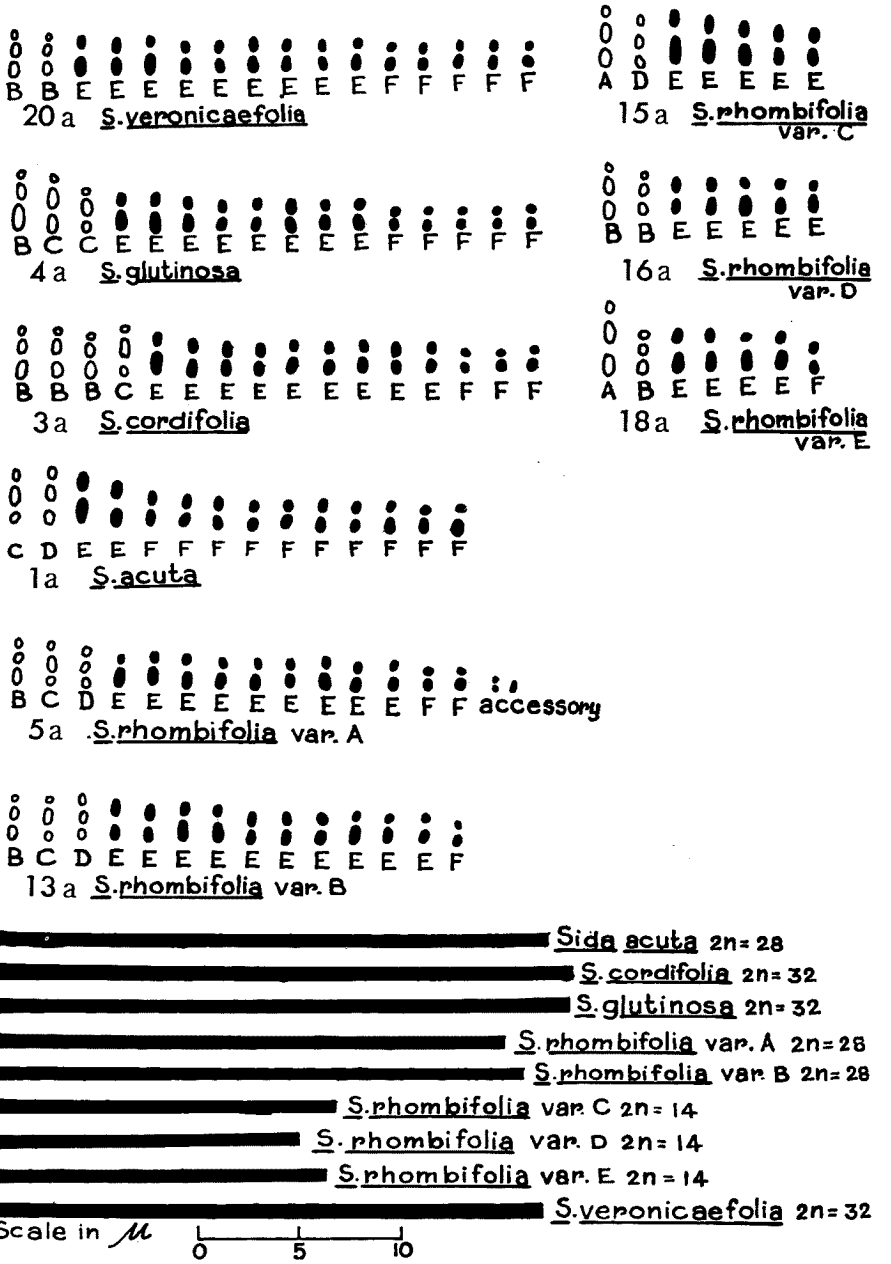


Figs. 2, 9, 10, 11, 12, 14, 17, 19 and 21. Meiotic stages in *Sida acuta*, *S. rhombifolia* varieties A (Figs. 9 to 12), B, D and E and *S. veronicaefolia* respectively. Arrows indicate B chromosomes.

4. *S. rhombifolia* var. A

$2n=28+0-8B=B_2+C_2+D_2+E_{18}+F_4=2.3\mu$ to 1μ.

The normal somatic complement shows twenty-eight chromosomes and accessory chromosomes upto 8 in number (Figs. 5 and 5a). In addition to



Figs. 1a, 3a, 4a, 5a, 13a, 15a, 16a, 18a and 20a. Idiograms of different species and varieties of *Sida* and histogram showing the total amount of chromatin matter in haploid complements.

the normal complement, somatic nuclei with 2n=56 have been observed. Different nuclei also contain different numbers of accessory chromosomes which may be either with or without constrictions and may range from 0.58μ to 0.83μ in length (Figs. 6–8).

5. *S. rhombifolia* var. B

   $2n=28=B_2+C_2+D_2+E_{20}+F_2=2.3\mu$ to 1μ (Figs. 13 and 13a).

6. *S. rhombifolia* var. C

   $2n=14=A_2+D_2+E_{10}=3\mu$ to 1.9μ (Figs. 15 and 15a).

7. *S. rhombifolia* var. D

   $2n=14=B_4+E_{10}=2.6\mu$ to 1.6μ (Figs. 16 and 16a).

8. *S. rhombifolia* var. E

   $2n=14=A_2+B_2+E_3+F_2=3.5\mu$ to 1.6μ (Figs. 18 and 18a).

   A pair is distinctive in being comparatively much longer than the rest.

9. *S. veronicaefolia* Lamk.

   $2n=32=B_4+E_{18}+F_{10}=2.3\mu$ to 1.2μ (Figs. 20 and 20a).

Table 1. Showing variation in the number of B chromosomes in the somatic cells of the variety A (*typica*) collected from different places and different populations of the same place

Population	Somatic cells with Bs in %	% of cells with							
		1B	2Bs	3Bs	4Bs	5Bs	6Bs	7Bs	8Bs
*Bot 1	30.40	8.19	9.00	—	7.10	5.05	—	1.06	—
Bot 2	25.52	5.05	9.10	11.05	—	—	0.32	—	—
Bot 3 to 6	nil	—	—	—	—	—	—	—	—
Bot 7	18.25	4.09	6.08	7.05	—	—	1.03	—	—
Bot 8	31.28	7.05	9.09	8.10	3.00	—	2.03	—	2.01
Bot 9	48.30	15.08	13.10	9.02	5.00	3.03	—	2.03	1.04
**Eden 1 to 5	nil	—	—	—	—	—	—	—	—
***Zoo 1 to 4	nil	—	—	—	—	—	—	—	—

* Bot=populations collected from Indian Botanic Gardens (adjacent localities)
** Eden=populations collected from Eden Gardens (adjacent localities)
*** Zoo=populations collected from Zoological Gardens (adjacent localities)

Meiotic study reveals normal n=16 (*S. veronicaefolia*), n=14 (*S. rhombifolia* var. B and *S. acuta*), n=14+0−4B (*S. rhombifolia* var. A), n=7 (*S. rhombifolia* var. D and var. E) in metaphases I and II without appreciable abnormalities (Figs, 21, 14, 2, 9, 17 and 19). Pollen mother cells of *S. rhombifolia* var. A show variations in the number of B chromosome (Figs. 9–12). B chromosomes are lesser in number than those present in the somatic cells. Irregularities, like lagging and irregular distribution of the B chromosomes, have been observed in the pollen mother cells. Feulgen test for the B chromosomes is found to be negative. Pollen sterility difference is observed in the different populations of *S. rhombifolia* var. A.

Table 2. Showing B chromosomes in the meiotic cells of different populations of the variety A (*typica*)

Population	% of PMC with Bs	% of PMC with			
		1B	2Bs	3Bs	4Bs
Bot 1	18.92	13.57	5.35	—	—
Bot 2	13.26	9.15	3.10	—	1.01
Bot 5	10.56	7.25	2.19	1.12	—
Bot 8	12.83	8.19	4.05	—	0.59
Bot 9	22.48	17.18	5.30	—	—

Discussion

Analysis of chromosome numbers

A glance at the previous records of chromosome numbers in different species of *Sida* shows that in addition to 7 and 8 chromosomes in the haploid set (Skovsted 1935, Nascimento 1941, Covas and Schnack 1946, Krapovikas 1957, Basak 1959, Raghavan and Arora 1958, Diers 1961), occasional cases have been recorded with $2n=34$ chromosomes in a variety of *S. veronicaefolia* (Adhikary 1965), $2n=22$ chromosomes in *S. hederacea* (Heiser and Whitaker 1948) and $2n=18$ in a variety of *S. acuta* (Roy and Sinha 1961). In the present investigation, out of the nine species and varieties studied, six have shown a basic set of 7 chromosomes, whereas multiples of 8 have been found in the other three. As most of the species of this genus are characterised by $n=7$ chromosomes which appears to be deep seated in this taxon, this number is apparently basic for this genus. A further confirmation of the above statement may be obtained from the fact that such varying numbers like $n=9$, may be found in occasional individuals of the same species showing otherwise $n=7$ chromosomes in different populations, as in *S. acuta*. This may indicate that $n=8$ and 9 chromosomes are derived from $n=7$. The occasional occurrence of $n=17$ and $n=11$ chromosomes in some species may be regarded as accidental aneuploidy. However as accessory chromosomes have been found in certain species, extra caution is necessary to count chromosome numbers of the genus which otherwise may lead to an erroneous conclusion.

A feature worth noting in this genus is the occurrence of intraspecific polyploids reported earlier as well as during the present investigation in *S. rhombifolia* (Skovsted 1941, Adhikary 1965 and Harvey 1966). Evidently polyploidy has played an important role, either directly or indirectly, in evolution.

Interpretation of the meiotic behaviour

Meiotic studies carried out have shown regular behaviour with the formation of bivalents. Even in intraspecific tetraploids bivalent formation has been recorded instead of multivalents.

The short size of the chromosomes as well as other genic factors, specially the genes controlling the formation of chiasma (Riley and Law 1965), may possibly stand against the formation of multivalents even in an autotetraploid. Different views (vide Stebbins 1947), as is well known, are on record in this regard. On the other hand, even in an intraspecific tetraploid, cryptic structural alterations in certain members of the duplicate chromosomes may prevent a typical multivalent formation. The evidence of such alterations is borne out also by certain minute morphological differences in the phenotypes in addition to giganticism in certain characters, between the tetraploids and diploids within the species. It may therefore be presumed that in addition to tetraploidy cryptic structural changes too have been an important factor in evolution of intraspecific varieties.

Data on the karyotype, histogram and their implications

A detailed karyotype analysis has been performed on all species of *Sida* investigated here. Heavy cytoplasmic contents and small size of the chromosomes presented much difficulty in their analysis. After several trials improved methods had to be adopted which yielded successful results. Inspite of variation in chromosome numbers, the karyotypes of all the species indicate a common general pattern in their morphology, proving that they form a homogeneous assemblage originating from a single basic set as suggested here. The chromosomes, in general, are graded, being mostly medium to short in size and the constrictions are either median or submedian in position. However, each species or variety shows certain minute chromosomal differences from allied taxa, suggesting its distinct status. This is a further indication of the fact that structural rearrangements have also been important factors in evolution. As each variety has a distinct phenotype of its own, such minor differences in their karyotypes may suggest that such structural alterations have been associated with changes in phenotypic characters as well.

The homogeneity of the genus *Sida* is further borne out in the study of the histogram of the total chromatin length of the different species and varieties. The total chromatin length, excepting slight variations, is very similar. The increase or decrease in chromosome number is also reflected in the increase or decrease in their length in the histogram.

The individual chromosome size does not show any reduction even in intraspecific polyploids. Diminution of individual chromosomes, along with an increase in chromosome number, has been found to be an associated feature in the evolution of several taxa (Stebbins 1951, Sharma and Sharma 1959) for which different theories have been suggested. However, opposite cases are on record where no such diminution is involved. The genus *Sida* too is an example where polyploidy in speciation has not been associated with diminution in chromosome size.

Accessory chromosomes in S. rhombifolia

Skovsted in 1941 reported the occurrence of accessory chromosomes in diploid individuals of *S. rhombifolia*, whereas in tetraploids they were found to be absent. In the present investigation, where different varieties of *S. rhombifolia* have been collected from various localities, interesting data could be gathered on the accessory chromosomes. Moreover a detailed analysis of the behaviour of the accessories and their morphology and chemical nature has been worked out.

The varieties of *S. rhombifolia* collected from different areas, have distinct phenotypic differences. Of these three are diploids and two tetraploids. Out of them again, accessory chromosomes have been found in one of the varieties.

It is often stated that diploids show more frequent occurrence of accessories as compared to tetraploids (Müntzing 1967, Sharma and Aiyangar 1961 and Darlington 1956). In *S. rhombifolia* Skovsted (1941) too did not find any accessory chromosomes in the tetraploids. On the other hand, in the present observations accessory chromosomes have been found only in the tetraploids. Such constant presence in a population with distinct phenotypic characters indicates some selective advantage being conferred by the accessories in those individuals and as such they exist in that population. On the basis of previous and present records apparently at least in *S. rhombifolia* the presence of accessories is not dependent on its ploidy level.

The accessory chromosomes, noted in *S. rhombifolia*, were found to possess either a median centromere or an indistinguishable centromere. That the apparently acentric ones are in fact centric, is proved by their regular maintenance in the cells. Extremely small size of these accessories did not possibly permit resolution of the centromere even if present. Their irregular separation and variability in number confirm their accessory nature.

With regard to their chemical nature, they are Feulgen positive in metaphase. It does not preclude the possibility of their being heterochromatic in nature, as often heterochromatic segments remain condensed throughout the divisional cycle (Vanderlyn 1949). However, as the staining of the segments could not be followed in the metabolic stage, their heterochromatic behaviour could not be fully ascertained.

That the accessory chromosomes, even within an individual, undergo certain screening is evidenced by the higher frequency of such accessories in somatic cells than in germinal line. Evidently an increased number of accessory chromosomes, resulting into a change of equilibrium thus affecting the germ cells, is avoided by selection of cells entering into the germinal line.

With regard to the origin of accessories different theories have so far been proposed (Müntzing 1949 and 1959, Battaglia 1963, 1964 a, b). In *S. rhombifolia* though it is not possible to ascertain precisely the mode of origin, the behaviour of chromosomes may give a clue in this direction. In the

variety *typica* occasional cells have been recorded with octaploid constitution. It may be suggested that an increase in chromosome constitution may often prove to be not beneficial to the individual. In such cases a gradual loss of function of some of the extra chromosomes and often heterochromatization may lead to the origin of accessories. Even though originating in a high polyploid individual, the accessories may be retained in lower polyploid and diploid progeny of such individuals. The suggestion however requires confirmation through planned experimental approach involving induction of high polyploidy in *S. rhombifolia* and observations of the fate of the additional genomes.

The existence of accessories as well as the presence of diploids and tetraploids in *S. rhombifolia* are of special interest. The medicinal importance of *S. rhombifolia* is well established. It needs to be seen whether these different varieties have differential content of the medicinal principle. Such an investigation should also be planned along with the study of response of the different varieties to varied environmental conditions. This may lead to a particular type, genotypically and phenotypically distinct, growing under the specific environmental conditions and leading to the optimum production of the medicinal principle.

Summary

Detailed cytological studies have been carried out on nine species and varieties under the genus *Sida*. Some varieties of *S. rhombifolia* showed the presence of accessory chromosomes and so populations of these varieties from different ecological habitats were collected. Paradichlorobenzene and aesculin were found to be the most effective pretreating chemicals in studying their somatic chromosomes. The chromosome numbers reveal that polyploidy, both inter- and intraspecific, has played a role in evolution in the genus, in addition to aneuploidy. Of the different basic numbers (n=7, 8, 9) 7 is found to be deep seated.

In addition to polyploidy, structural rearrangements of chromosomes have been observed in all cases showing their prominent role in speciation within this genus. Moreover, regular bivalent formation even in intraspecific tetraploids suggests structural changes during evolution. However homogeneity of the genus as a whole is indicated in the general pattern of the karyotypes of different species and varieties and also in the similarity in their total chromatin length. Within this genus at least polyploidy in speciation has not been associated with diminution in chromosome size.

The constant presence of accessory chromosomes in a phenotypically distinct population of *S. rhombifolia*, indicates some selective advantage. Regarding the origin of accessory chromosomes, it has been suggested that some of the extra chromosomes of high polyploid cells which are often found in the individuals bearing accessories, may lose their function and undergo

heterochromatinization and as such are transformed into accessory ones.

Acknowledgments

The authors would like to express their deep gratitude to Prof. A. K. Sharma, Head of the Department of Botany, University of Calcutta for suggestions, encouragement and facilities provided during the course of the investigation.

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