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Nature and Behaviour of B Chromosomes in *Allium stracheyii* Baker and *Urginea indica* Kunth.

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Several species of *Allium* are known to possess accessory chromosomes. They occur in most sections of the genus and may have different shape and size (Bothmer 1970). Sharma and Aiyanger (1961) reported that in *A. stracheyii*, extra chromosomes with telocentric centromere are present. They also noted, that, these extra chromosomes disappear consequent to polyploidisation. The detailed cytological behaviour of these extra chromosomes in different organs has not yet been studied.

In the present study, this species was collected too from Darjeeling for cytological study along with other species of the genus *Allium*. Besides the normal number 2n=14, the extra chromosomes were also found and in the present paper their nature, behaviour and morphology, in tissues having different functions, have been studied in detail. During another collection tour, another species viz. *Urginea indica* with B chromosomes belonging to the same family Liliaceae was collected from Peninsular India (Sen 1972). The behaviour of B chromosomes of this species too has been carried out and a comparative observation has been presented in this paper.

Materials and methods

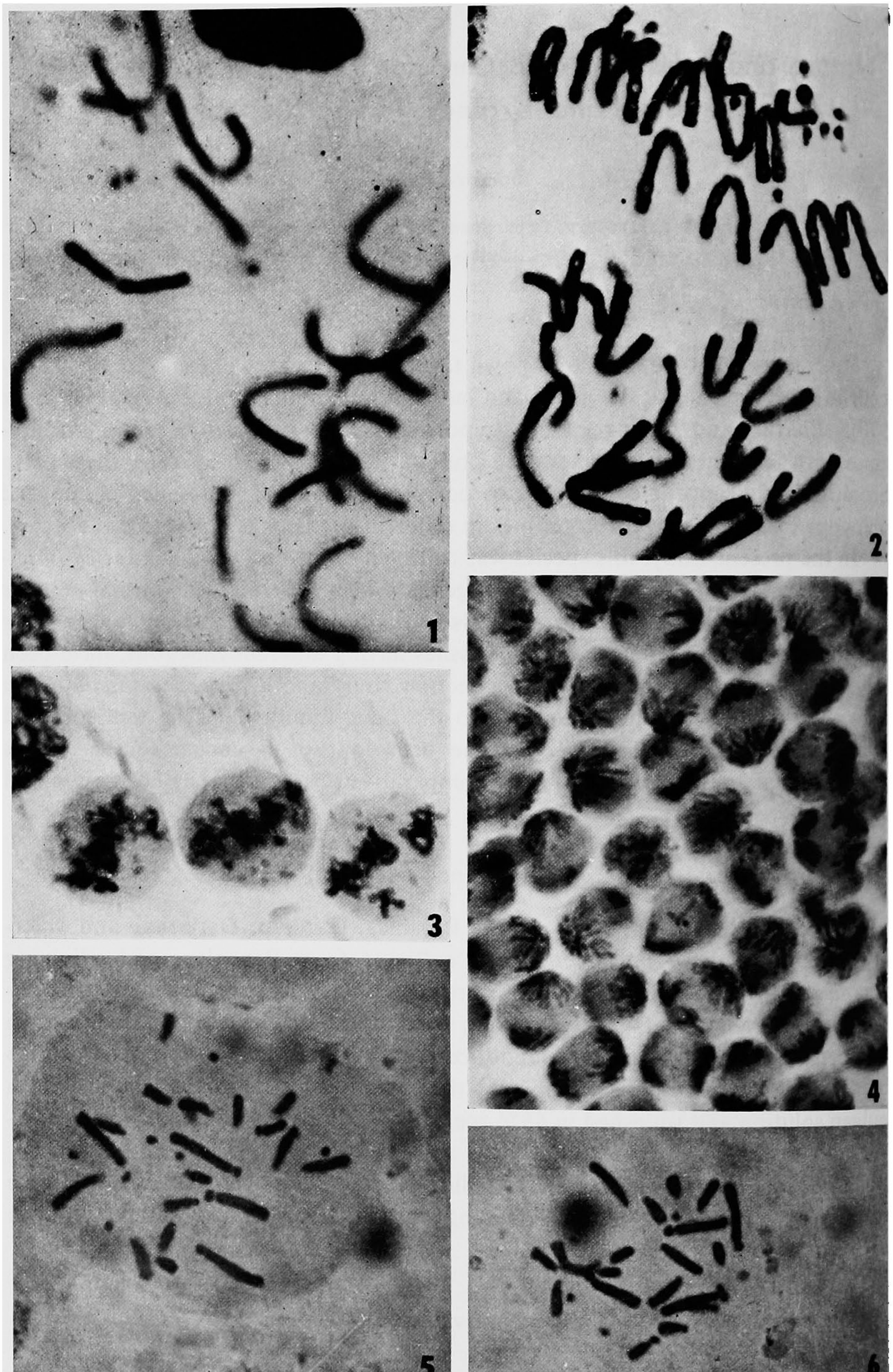
A. stracheyii was obtained from the Botanical Gardens, Darjeeling and different populations of *Urginea indica* were collected from Bangalore and adjoining areas in South India. Root tips were pretreated in 0.25% colchicine and paradichlorobenzene solution (1 part: 3 parts), fixed in acetic alcohol (1:2), stained in 2% acetic acid. Some roots were directly fixed without any pretreatment to observe the behaviour at anaphase. At the same time root-tips were stained with Feulgen solution and squashed in 45% acetic acid to note the DNA positive behaviour.

For study of meiosis, as well as pollen grain mitosis acetocarmine technique was applied. In *Urginea indica*, flowering was not noted in this population even in the wild state.

Observation

Allium stracheyii

For the study of B chromosomes in *A. stracheyii*, observations were carried out on roots, pollen mother cells as well as pollen grains. In all cases, cells were



Figs. 1–6. 1–4, B chromosomes of *Allium stracheyii*. 1 and 2, somatic metaphase and anaphase respectively. 3 and 4, meiotic stages. 5 and 6, B chromosomes of *Urginea indica* of Type I and II respectively.

found to contain variable number of Bs and as such a frequency count of B chromosomes were carried out in all these organs (Figs. 1–4). This attempt was aimed at finding out the extent to which the B chromosomes of somatic cells persist upto the germinal line. B chromosomes are mostly acrocentric in this species, the size being 1.5 μ to 1.25 μ approx.

Somatic tissue

In the root-tip cells, the separation in anaphase has been found to be mostly irregular going to either of the poles in a split or unsplit conditions. Consequently, there is unequal number of Bs in daughter nuclei. In a number of cases, the chromosome number in two poles has been found to be 8 and 7 indicating that 7 Bs have undergone splitting, whereas the eighth one has gone to one of the poles, without separation. No case of lagging at the equator has been observed in the somatic cells. This may imply B chromosomes have the functioning centromere. In a large number of cells, most of the B chromosomes have been found to be associated together giving the appearance of stickiness, indicating their possible heterochromatic nature. The B chromosomes were not only found to be brightly stained with Feulgen solution at metaphase/anaphase stages, but the same appearance was observed in early prophase as well, confirming their heterochromatic constitution. Even in interphase, brightly stained bodies were observed which presumably were the B chromosomes. All these facts show, that the B chromosomes in *A. stracheyii* represent a condensed heterochromatic state throughout the divisional cycle.

The Table 1 would show that of the cells containing variable number of B chromosomes, a very large percentage of cells (32% approx.) contain 6 or 7 Bs.

Pollen mother cells

During meiosis, no bivalent formation of B chromosomes has been observed. This might be due to the absence of qualitative genes and/or in addition, the lack of homology between the different B chromosomes. Their heterochromatic nature is indicated by bright staining throughout the divisional cycle even in meiosis.

In contrast to mitosis, behaviour on the spindle of the B chromosomes in meiosis has been found to be rather different. Here in the first meiotic division, some of the B chromosomes were found to lag in a few mother cells. In fact, in about 52% of the cells (vide Table 1), one B chromosome was found lagging, whereas two chromosomes were lagging in 36% of cells. The lagging behaviour of a few B chromosome may suggest the presence of defective, though functioning centromere. The regular behaviour of these chromosomes in the mitotic spindle as against their slightly irregular behaviour in the meiotic cells may be explained on the basis of the fact, that in meiosis, spindle formation and separation of bivalent chromosomes are quite likely guided by more subtle timing sequence as compared to the mitotic ones. Some of the B chromosomes may fail to maintain this timing cycle and so lag at the equator. It has been pointed out in a previous paper (Sharma and Aiyanger 1961) that these B chromosomes are absent in a tetraploid *A. stracheyii*. In several other varieties of *A. stracheyii*, having different chromosome number (Sen 1972) no B chromosomes were noted.

Pollen grains

The behaviour in the pollen grains too was studied. It has been noted that the pollen grains possess B chromosomes in varying number (vide Table 1) and grains with two B chromosomes occur in highest frequency. In this connection, it is significant that, in the first division of meiosis, five B chromosomes were found

Table 1. Percentage of B chromosomes of *Allium stracheyii* in different tissues

Number of Bs	Root (%)	PMC 1st div. (%)	PMC 2nd div. (%)	PMC Laggers (%)	Pollen grains
1	—	—	22	52	8
2	—	—	32	36	32
3	2.5	16	22	8	16
4	10	18	13	4	20
5	20	26	7	—	8
6	32	18	4	—	8
7	32	10	2	—	—
8	5	8	—	—	—
9	—	2	—	—	—

in maximum percentage of cells whereas majority of the cells in the second division of meiosis had either two or three B chromosomes. One B chromosome was also noted in a very high frequency of cells.

The observation of a high frequency of pollen grains containing two B chromosomes tallies well with the meiotic data. But the frequency of cells containing one B chromosome is very low as compared to those noted in the meiotic cells.

In addition to normal behaviour, irregular separation of B chromosomes in the first division of pollen grain too has been recorded. It is, therefore, expected that such cells may give rise to sperm with higher or lower number of B chromosomes.

Urginea indica

In this species, eight different populations were studied from southern part of India (Sen 1972). Out of these, five populations show the diploid chromosome number 2n=20, two are characterised by triploid constitution having thirty chromosomes and one tetraploid with forty chromosomes has been noted in one of the populations. Detailed chromosome analysis has been carried out in all the populations and has been presented in the above paper.

In Type I—(2n=20+6B) where B chromosomes have been noted (Figs. 5 and 6), the number has been found to be six in 90% of the cells, excepting one or two less in the rest ten percent. They are metacentric and size variations is not very marked (1 μ–1.5 μ). As no flowering has been noticed in this form even in wild state, the behaviour of B chromosomes could not be studied.

In Type II, which is also a diploid population (2n=20+7B), seven B chromosomes have been noted (Fig. 5). They are also metacentric though size difference is absent (1 μ approx.). Frequency of cells with 7 B chromosomes is approximately 80%, the rest showing lower number of Bs. No flowering could be noted even under wild condition.

No polyploid population with B chromosomes has been observed. There are other diploid populations without any B chromosomes.

Discussion

The study of the nature, behaviour and function of B chromosomes, specially in *Secale cereale* and other members of Gramineae have been extensively carried out by Müntzing (1958, 1963, 1964, 1966, 1967). It has been shown that in several cases they are heterochromatic in nature, but their selective advantage at least in certain strains cannot be disregarded.

Battaglia (1964) published a detailed list of species showing B chromosomes in plants. Extensive literature on this aspect has been accumulated and it has been recorded that Bs are most frequent in diploids rather than in polyploids (Darlington 1956).

In species of *Allium*, accessory chromosomes have been reviewed by Shopova (1966; vide Fedarov 1969). Generally, two types of B chromosomes have so far been observed viz. 1) small bodies with centromere at different positions and 2) larger bodies equal to or nearly half the length of the normal chromosomes, mostly having centromere near the tip (Bothmer 1970). B chromosomes of *A. stracheyii* fall in the first category.

It has been observed, that both in *A. stracheyii* the number of B chromosomes vary from cell to cell, and in *Urginea indica*, such variation though present, is comparatively less. In *A. stracheyii* population from Darjeeling, no plant could be observed without any B chromosome. In that case, it may be suggested that in these cases, B chromosomes confer certain amount of adaptive value to this temperature population.

Even though several other varieties of *A. stracheyii* were studied, showing high chromosome number, B chromosomes could not be noted in any of them. This may be taken to indicate that tetraploids having a high tolerance range do not require the presence of Bs for adaptation. It was pointed out by Sharma and Aiyanger (1961) that induction of tetraploidy in *A. stracheyii* results in complete elimination of B chromosomes.

With regard to this behaviour, parallelism to some extent has been noted in *Urginea indica* as well. In this species, tetraploids are devoid of any B chromosomes. The absence of Bs in tetraploids may be due to the same reason as suggested for *A. stracheyii*.

In *Urginea indica* however, diploid populations without B chromosomes too have been noted. The genic constitution of such a population may have the adaptive value, not requiring to be supplemented by the B chromosomes. But in population with Bs, the existence of accessories in every individual, point to the adaptive value of such accessories, for the population concerned.

In *A. stracheyii*, a comparative analysis of B chromosomes in different organs shows that the frequency of B chromosomes in cells undergoing mitosis and meiosis can be correlated. This fact may be taken to indicate that there is no selective barrier for the cells with B chromosomes to participate in the formation of pollen mother cells. But this frequency shows deviations in the pollen grains. In second meiotic division, a high frequency of cells was found to contain B chromosomes, though on the contrary in the pollen grains, the frequency has been shown to be very low. Similarly, pollen grains with high number of B chromosomes are rather rare. These data may be interpreted in terms of the fact that even though the Bs of *A. stracheyii* have a certain adaptive value, their selective advantage is controlled by a threshold range. With too high or too low a number of B chromosomes, there is a restriction of their dividing capacity in the pollen grain.

The mode of origin of the B chromosomes of *A. stracheyii* and *Urgines indica* is difficult to visualise. In *A. porrum* L., Vosa (1966) suggested the origin of subtelocentric B chromosomes from submetacentric Bs through deletion of part of a short arm. Different other mechanisms too have been suggested by various authors (vide Müntzing 1967). In case of *A. stracheyii*, it is significant that though diploids and polyploids have been observed, low aneuploids have not been recorded. The same is true for *Urginea indica*. It is likely that B chromosomes might have originated through deletion of euchromatic parts of duplicated individual chromosomes, which might have originated possibly through non-disjunction or triploidy. Through such an elimination of euchromatic parts of aneuploid or triploid cells, which otherwise might have disturbed the genic balance, the plants have been able to stand in competition. The retention of heterochromatic segments of such duplicated chromosomes on the other hand has enabled these plants to have a further advantage in selection. This mechanism may be considered as responsible for the origin of B chromosomes in *A. stracheyii* and *Urginea indica*.

Abstract

The nature and behaviour of B chromosomes in *Allium stracheyii* and *Urginea indica* have been studied in detail. In *A. stracheyii*, a correlated analysis of their behaviour in root cells, pollen mother cells and pollen grains has been carried out. The presence of defective but functioning centromere has been suggested. The heterochromatic constitution of the Bs has been demonstrated. It has been shown that there is no selection barrier for the Bs to enter into the germinal line, though in the pollen grains, over and below a threshold, the B chromosomes are not favoured.

The selective value of B chromosomes is exhibited by their presence in every individual of the population. As in *A. stracheyii*, in the *U. indica* as well, tetraploids are devoid of B chromosomes indicating that the adaptability conferred by Bs is not needed in tetraploids with increased tolerance range. The origin of B chromosomes has been suggested through elimination of euchromatic segments from triploids or aneuploids.

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