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B CHROMOSOMES IN SPONTANEOUS AND INDUCED INTERCELLULAR CHROMOSOME MIGRATION OF *PAPAVER SOMNIFERUM* L.

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ABSTRACT

Cytomictic subpopulations raised from mutagen-treated and untreated populations of *Papaver somniferum* (2n=22) show intraindividual variability for A chromosome numbers at both somatic and meiotic levels. Three B chromosomes, of which the first two are submetacentric (B_1 and B_2) and the third one telocentric (B_3, have been identified in these subpopulations. The B chromosomes, besides indicating their inhibitory effects on A chromosome size, appeared to be involved with their pioneering functions in intercellular chromosome migration. The heterochromatic elements identified in B chromosomes may be responsible for their kinetic activity and sticky associations with other chromosomes. Specific breakage at the proximity of the heterochromatic block of B_1 was considered to be due to intracellular mobile function of the heterochromatic block. B chromosome-pioneered cytomixis is the possible underlying cause of derangements in chromosome number (4–39 in meiocytes, binucleate PMC, and large variations in pollen size in cytomictic subpopulations. The data on numerical variations in chromosomes coupled with low gametic sterility (12.63–38.45%) of cytomictic plants point to the fact that B-pioneered cytomixis may be playing an important role in the evolution of *P. somniferum*.

Key words: Cytomixis, B chromosome, *Papaver somniferum*, opium poppy.

The potential of mutagenic treatment for creation of genetic variation in the morphinane alkaloids of *Papaver* species has not been exploited. Morphine is the drug extracted from *Papaver somniferum*, however, because of its additive effects, codeine is the analgesic commonly used in medicine, hence 90% of the legal morphine is converted into codeine by the drug industry. A codeine chemotype or a thebaine chemovariety, in which the demethylation to morphine is metabolically blocked/suppressed, would be most valuable both for the drug industry and prevention of illegal uses of morphine. Such chemotypes have not yet been evolved. The main objective for applying the mutagenic treatment in this study was to evolve desirable "chemovar" and "chemotype" in opium poppy. However, the initial cytological investigation towards better understanding of the chromosome comportment in *Papaver somniferum* revealed the presence and involvement of B chromosomes in both spontaneous and induced intercellular chromosome migration.

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The wide occurrence of B chromosomes in diploid outbreeding plant species has been established [1]. The body of literature on B chromosomes reveals no essential function for them during normal plant growth and development. They have, however, been considered to influence various phenotypic and macromolecular expression, namely, cell size [2], DNA quantity and mitotic cycle [3], protein and RNA contents [4], chiasma frequency and distribution [5], and allopolyploidization of autopolyploids [6]. Despite these findings, as regards the role(s) of B chromosomes, their possible involvement in intercellular chromosome movement (cytomixis) has not yet been reported, notwithstanding the recognized kinetic functions of the supernumerary segment in the form of heterochromatic knob at intracellular segment in the form of heterochromatic knob at intracellular level [7–10]. We earlier demonstrated the genesis of syncytes with the ploidy levels 2n to 10n through intercellular chromatin/chromosome migrations during premeiotic phase of meiocytes in *Papaver somniferum* L. (2n=22) [11]; the results indicated the involvement of heterochromatic chromosomes in pioneering the cytomictic events. The data presented here provide further evidence for the functional involvement of three heterochromatic B chromosomes in pioneering the intracellular migratory process of A chromosomes in meiotic meiocytes.

MATERIALS AND METHODS

Three types of populations of opium poppy, *Papaver somniferum* L. (2n=22), were made: (i) 5 kR-M_1, which comprised 45 plants raised from seeds exposed to 5 kR gamma rays with dose rate 0.38 Mrad/h, (ii) combined dose-M_1, which comprised 45 plants raised from seeds exposed to 5 kR gamma rays+6 h treatment with 0.6% EMS, and (iii) untreated control of 48 plants. The seeds were sown in contiguous 9 m^2 plots in an experimental field with normal cultural practices. Each of the three populations was further divided for convenience into two subpopulations of cytomictic and noncytomictic plants. Cytomictic plants, in contrast to the noncytomictic ones, had earlier exhibited cytomixis (intercellular chromosome migration) [11]. Three cytomictic subpopulations are likewise described in Table 1. For cytological analysis,

Table 1. Characterization of three experimental subpopulations [11] for the B chromosome studies in *Papaver somniferum* L. (size of original population comprising cytomictic and noncytomictic plants in parentheses)

Subpopulation	No. of plants		Range of somatic chromosome number	Range of PMC chromosome number	Somatic cells with diploid chromosome number (2n=22), %	PMC with diploid chromosome number (2n=22), %
	total	cytomictic				
Cytomictic control	48	16	17–26	12–32	95.33	92.42
Cytomictic 5 kR-M_1	40	18	19–29	4–24	88.61	87.15
Cytomictic combined dose-M_1	45	20	16–25	2–26	83.41	81.24

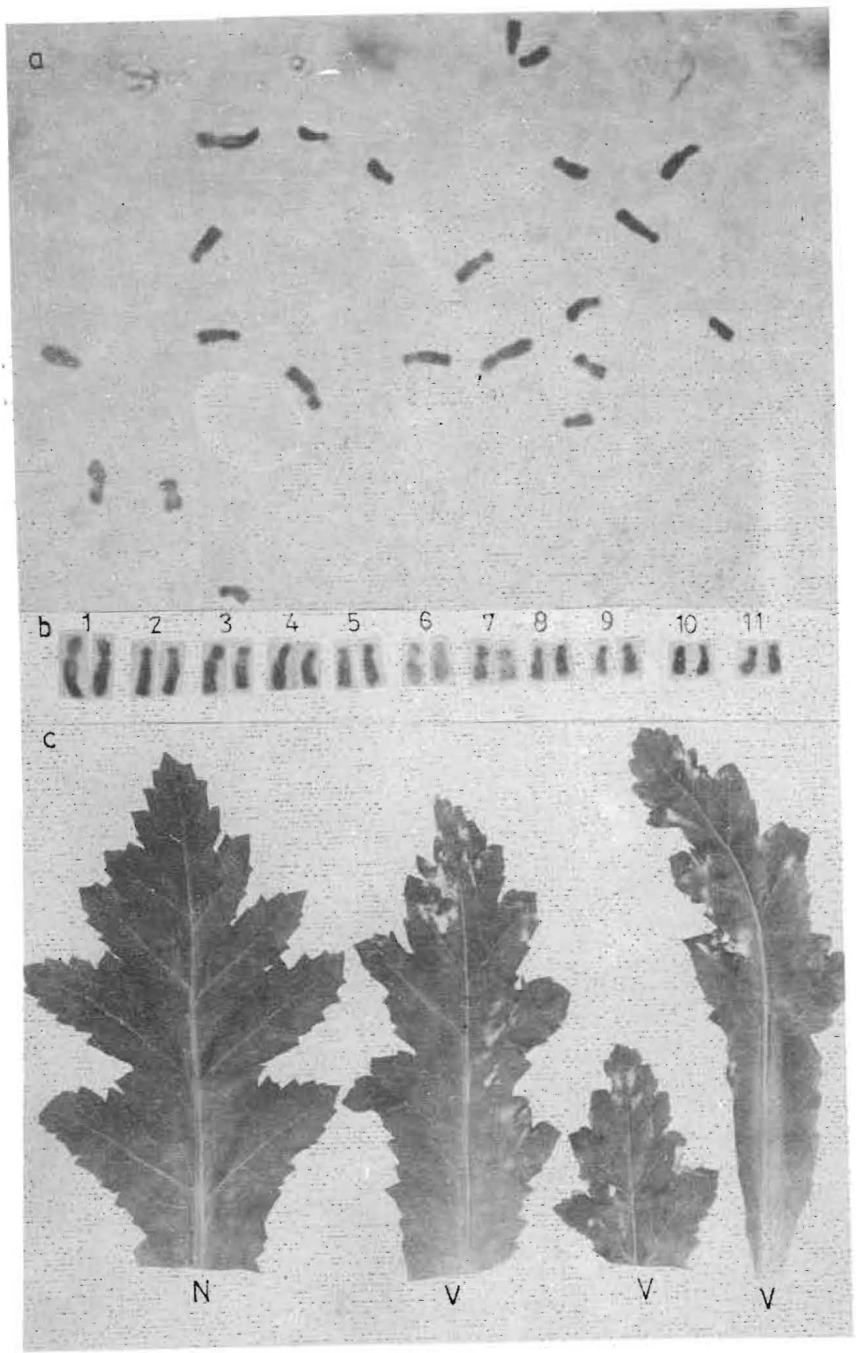

Fig. 1. Somatic chromosomes and induced leaf variation in *P. somniferum:* a&b) 11 pairs of somatic chromosomes, of the normal (noncytomictic) plant, and c) normal (N) and cytomictic variegated (V) leaves.

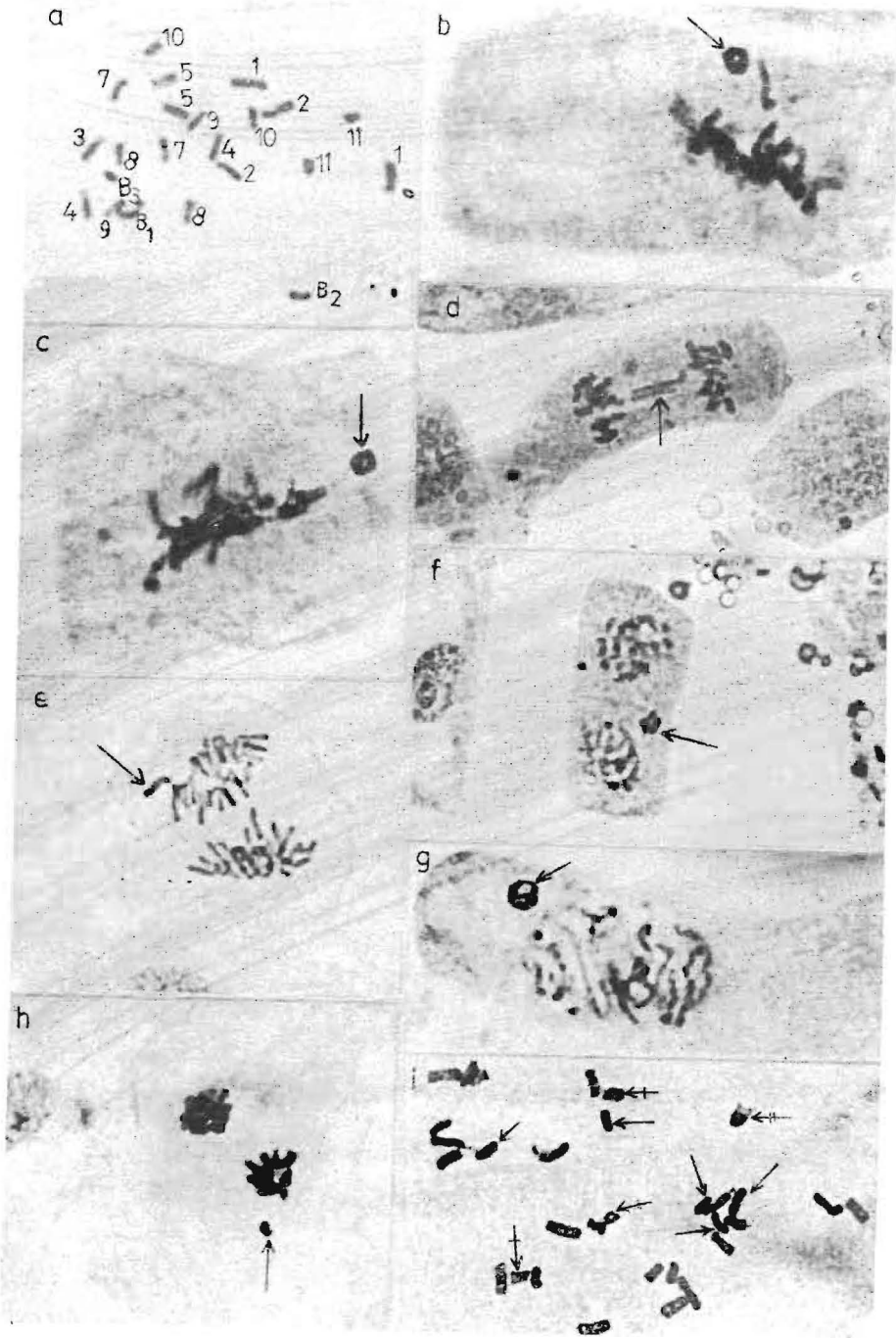

Fig. 2. Mitotic behaviour of B chromosomes in cytomictic subpopulations: a) cell with 19 A + 3 B chromosomes in dwarf variant, chromosome size reduced and unequal members of A chromosomes 2, 5 and 7; b-f) B chromosomes (marked by arrow); g) micronucleus formed by the B chromosomes (arrow); and h) deleted heterochromatic block with a minute segment of B_1 (arrow); i) a cell with 22 A+5, B+4 deleted fragments, B chromosomes indicated by arrows, acentric fragments by arrows with single bars, and centric fragments by arrows with double bars.

flower buds, petal base and root tips were fixed in Carnoy's fluid (6 ethyl alcohol: 3 chloroform: 1 glacial acetic acid) and squashed in 2% propionic carmine. Feulgen technique was also employed to confirm the heterochromatic nature of the chromosomes. Cytological observations were recorded on temporary slides. The slides were made permanent by the method described elsewhere [12]. Pollen sterility was studied by staining fresh pollen grains in 2% iodine solution (2 g I+4g KI+100 ml distilled water). Nonstainable pollen grains were considered sterile. Microphotographs were taken from either temporary or permanent slides. The nature and identity of B chromosomes were ascertained following the specific B characteristics defined by Jones and Rees [cf.12].

RESULTS AND DISCUSSION

Cytomictic plants, in contrast to normal plants, were characterized particularly by the presence of B chromosomes (Fig. la-b and Fig. 2a, e). The presence of B chromosomes did not affect normal morphological features of the cytomictic plants, barring rare variants like leaf variegated type (Fig. 1c), twin shooted type, and dwarf type. The mean numbers of B chromosomes in the three subpopulations—cytomicitc 5 kR-M_1, combined dose-M_1, and control—were 1.83, 1.86 and 1.75, respectively (Table 2). Numerical variations in A chromosomes, as observed

Table 2. Frequency of B chromosome pioneered cytomixis, multivalent association of B and A chromosomes and pollen sterility in three cytomictic subpopulations drawn from treated and untreated populations of *P. somniferum* L.

Subpopulation	Average No. of PMC	Average number of B chromosomes	PMC with cytomixis, %	Mutivalent associations of B_3 and A chromosomes, %	Pollen sterility, %
Cytomictic	250	1.75	18.76	7.61	12.63
Cytomictic 5 kR-M_1	290	1.83	26.16	12.18	16.82
Cytomictic combined dose-M_1	228	1.86	35.13	18.24	38.45

in the cytomictic plants (Table 3), were sometimes found to impede the identity of their homologues. Even so, comparative count of chromosomes from a large number of cells established the cytomictic karyotype (2n=22+3B), (Fig. 2a, e): the chromosome size, in general, was considerably reduced in the cytomictic individuals (Fig. la, b; Fig 2a). The chromosomes within the complement varied widely in length ranging from 2.24–7.82 μm, as opposed to the normal range of 3.92–8.30 μm in the noncytomictic control. Another marked karyotypic alteration in the cytomictic plants was observed in terms of length in the unequal members of A chromosome pairs: the chromosomes 2, 5 and 7 provided examples for such deviations.

The three B chromosomes were indentified as B_1, B_2 and B_3 (Fig. 2a). The first two chromosomes were of standard submedian type, whereas the third one was a telocentric, chromosome B_1, besides being heterochromatic for its entire short

Table 3. Numerical variations in A and B chromosomes in PMC and somatic cells in three representative plants of three cytomictic subpopulations of *P. somniferum* L. (2*n*=22)

Chromosomes	No. of PMC	No. of somatic cells
Cytomicitic control:		
14	2	—
16	1	—
18	2	4
$19 + B_1 + B_2 + B_3$	8	6
$22 + B_1$	113	95
$22 + B_1 + B_2$	137	82
$22 + B_1 + B_2 + B_3$	143	91
$24 + B_1 + B_2$	4	2
$35 + B_1 + B_2 + B_3 + B_3$	2	—
Cytomictic 5kR-M_1:		
$6 + B_1$	3	—
$14 + B_1 + B_2$	4	—
$19 + B_1$	5	2
$22 + B_1 + B_2$	138	83
$22 + B_1 + B_2 + B_3$	146	96
$23 + B_1 + B_2 + B_3$	1	1
Cytomictic combined dose-M_1:		
4	2	—
6	4	—
10	1	—
12	3	—
16	1	—
$18 + B_1$	1	1
$19 + B_1 + B_2$	—	2
$20 + B_1 + B_2$	3	2
$22 + B_1 + B_2$	136	79
$22 + B_1 + B_2 + B_3$	169	87
$26 + B_2 + B_3$	12	1

arm, contained a distinct heterochromatic block at the terminal point of the long arm (Fig. 2e, Fig. 3a). In contrast, both B_2 and B_3 were entirely heterochromatic. Apart from having these marked morphological features, the B chromosomes were endowed with specific mitotic and meiotic comportments which unequivocally distinguished them from the normal autosomes. Chromosomes B_1 and B_2 were often found fused together in the form of a ring at metaphase (Fig. 2b-c). The association of B_1 and B_2 was further reflected from the data on their nonrandom distribution (Table 3). The B chromosomes, besides showing attenuated laggards at anaphase (Fig. 2d), indicated nondisjunction of chromatids (Fig. 2e–f). As is apparent from Fig. 2f–g, they sometimes remained extranuclear, condensing precociously or assembling together to form micronuclei. Thus, the possibility of extinction of B chromosomes from the cells is suggested [12]. However, B_1 as opposed to B_2 and B_3 has been

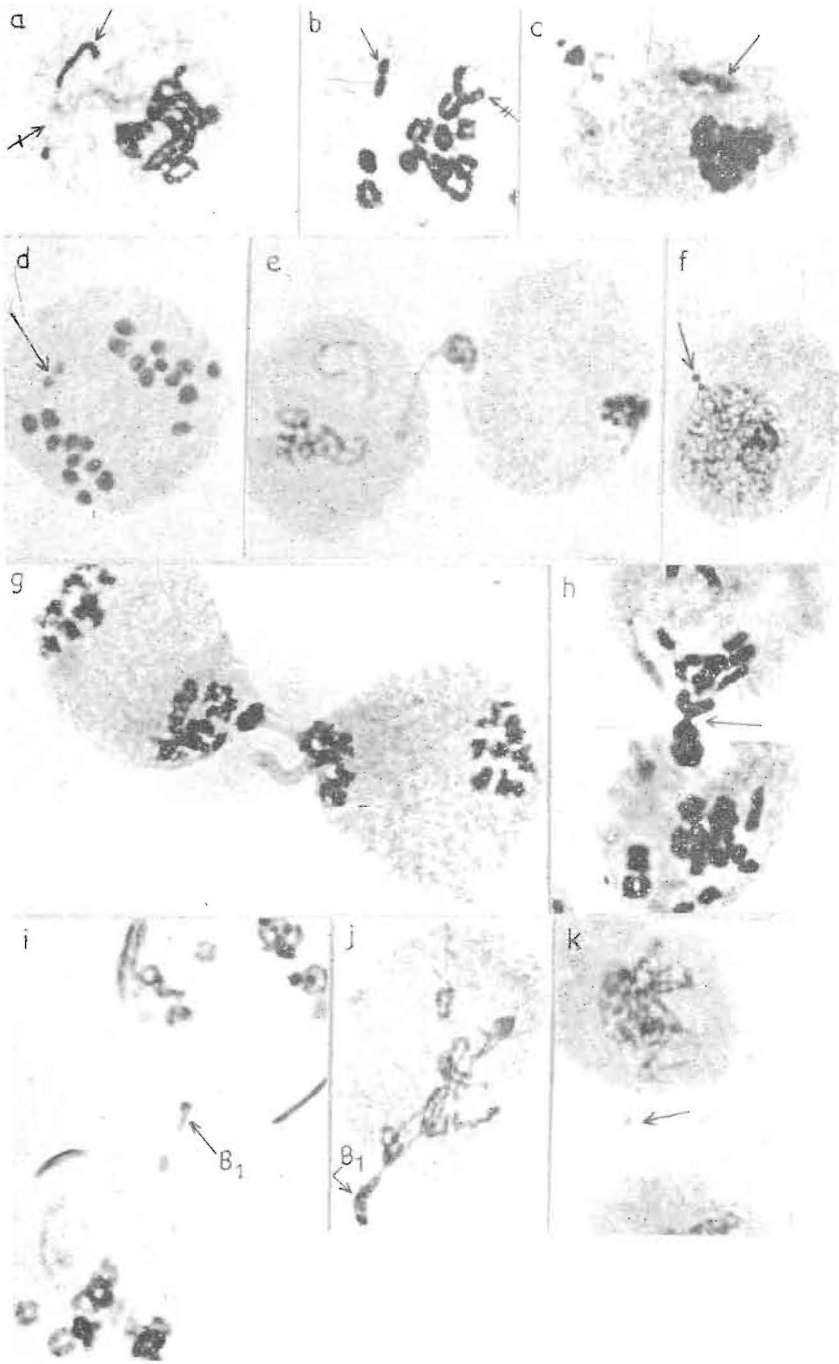

Fig. 3. Meiotic behaviour of B chromosomes in cytomictic subpopulations: a-c) the bivalent B_1 B_2 (arrows) remaining distinct from the A chromosomes at pachytene, diakinesis and metaphase I, and also showing breakage near its heterochromatic block (arrow with bar), note the multivalent association formed by B_3 (arrow with double bar) and A chromosomes; d) B chromosomal bivalent as laggard; e-j) chromosomes in intercellular migration; f) precociously condensed B chromosomal group at early pachytene, h) pioneering function of a group comprising basically B chromosomes (arrow), i) migration of single B_1 chromosome and, j) dragging of whole pachytene chromosome mass of a meiocyte by B_1; and k) mobility function of disarticulated heterochromatic element (arrow).

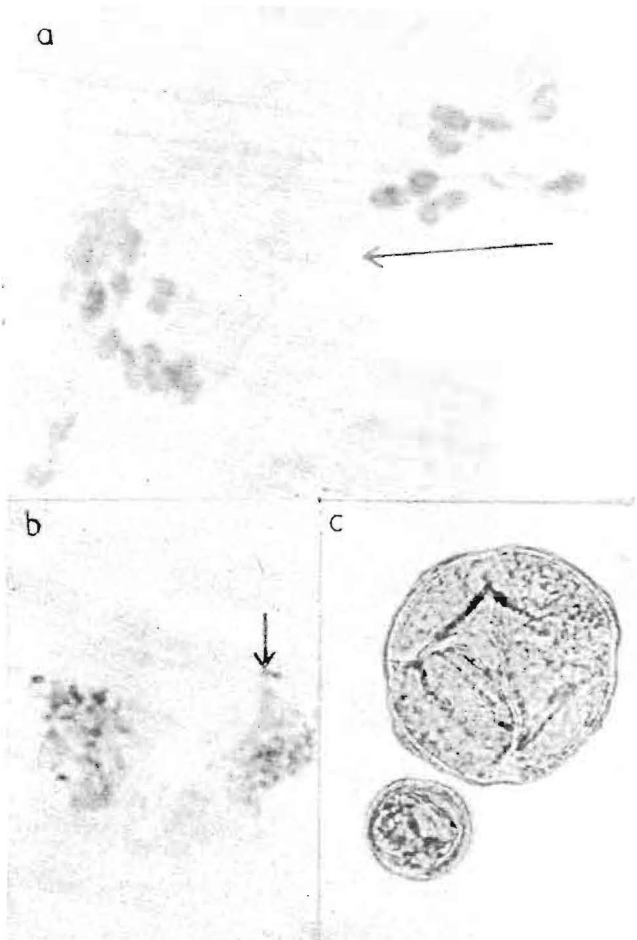

Fig. 4. Consequences of B chromosome pioneered cytomixis in cytomictic subpopulations: a) massive chromosome migration, arrow indicating the direction of migration, b) binucleate PMC, the migrated nucleus being indicated by the marker chromosome B_2, and c) large variation in size of fertile pollen grains.

marked more often in the nucleus (Fig. 2e). The cytomictic plants commonly exhibited chromosome fragments (Fig. 2h–i) and the fragments, with rare exceptions, were acentric. A deleted heterochromatic block along with a minute segment of the accessary chromosome B_1 was noticed very frequently (Fig. 2h). Hence, it was indicated that B_1 might have a specific breakage site in the proximity of the heterochromatic block. It is worth mentioning that such behaviour in B_1 was marked only in variegated cytomictic plants drawn from combined dose-M_1.

Figures 3a–k display the features and behaviour of three B chromosomes in meiocytes. Despite large derangements of A chromosome number in PMC, B chromosomes could be distinguished clearly by their unique morphology and functions. Intensive pachytene analyses on two variegated plants and the control (novariegated cytomictic) of combined dose-M_1 were conducted. Chromosome B_1 in most cytological

preparations from the variegated plants showed breakage proximally to the heterochromatic block (Fig. 3a), whereas B_1 from the nonvariegated plant did not exhibit such breakage. As revealed by Fig. 3a-c, B_1 and B_2 had been marked to pair with each other and remain distinctly sequestered from the A chromosomes and B_3 at pachytene, diakinesis and metaphase I. On the contrary, B_3 was found to pair with A chromosomes, resulting into a multivalent configuration at diakinesis (Fig. 3b). The corresponding data of multivalent association in diakinesis in the three subpopulations—cytomictic control, 5kR-M_1 and combined dose-M_1—were 7.61%, 12.8% and 18.24%, respectively (Table 2). As regards their anaphase behaviour, the B chromosomes behaved as laggard bivalents (Fig. 3d).

The cytomictic behaviour of B chromosomes was interesting. Here for the sake of brevity in discussing the results. First some general points on the nature of cytomixis in the material are worth considering. Cytomixis was noted in meiocytes but not in the somatic cells of roots. The percentage of cytomixis in the three subpopulations, i.e. cytomicitc control, 5kR-M_1 and combined dose-M_1 was 18.76, 26.16 and 35.13, respectively (Table 2). Though cytomixis was observed at all meiotic stages, it was more frequent at prophase; chromosome(s) of one cell stretched across and invaded the cytoplasm of the neighbouring cell (Fig. 3e). The cytoplasmic channel between contiguous cells, earlier presumed to be functioning as passage for chromatin/chromosome migration [13–15], was rarely observed in this study. The chromosomes moved more or less in an invasive manner from one cell to another. A close look of Fig. 3e–j indicates that B chromosomes, while remaining distinct from A chromosomes at all meiotic stages, perhaps lead the intercellular chromosome migration in groups, the B chromosomes pioneered migratory function in singles (Fig. 3i–j). During their exodus from the cell, the B chromosomes had a tendency to involve other chromosomes in the process by dragging them in bulk through formation of sticky segmental associations (Fig. 3j). In view of this, it appeared convincing that the massive chromosome migration in cells (Fig. 4a) and the occurrence of binucleate PMC (Fig. 4b) were the consequences of B chromosome maneuvered cytomixis. Table 3 reflects the numerical deviations in chromosomes in the PMC and somatic cells of cytomictic plants. Although PMC were mostly normal, sometimes their chromosome number varied from 4 to 39. Such a large derangements in chromosome number indicated that cytomixis in different PMC may have occurred with variable degrees. Chromosome number variations in meiocytes of cytomictic individuals have been recorded earlier in *Gossypium* [13] and *Panax* [15]. The numerical variations in somatic chromosomes, especially A chromosomes, even in the absence of cytomictic disturbances was interesting. Somewhat similar intraindividual variability in chromosome number has been reported in cytomictic plants [16]. As regards the B chromosome distribution in *Papaver somniferum*, the three B chromosomes occur in most cells in either of the two combinations, namely, $B_1/B_2/B_3$ and B_1/B_2. Thus, it was clear that there was no random distribution of B chromosomes in different cells.

The observations on pollen size and fertility were recorded in order to have an idea about the repercussion of cytomixis at gametic level. All the three cytomictic subpopulations had exhibited large variation in pollen size (Fig. 4c). Unexpectedly,

pollen sterility was generally low in all the three classes of cytomictic plants: 12.63% in cytomictic control, 16.82% in cytomictic 5 kR-M_1, and 38.45% in cytomictic combined dose-M_1. Hence cytomixis may not have serious repercussions on gametic viability despite the lack of parity in chromosome number in the gametes.

Thus, we have demonstrated the nature and function of three B chromosomes in relation to cytomixis in *Papaver somniferum*, an intriguing plant with numerical variations for both A and B chromosomes. The features and behaviour of B chromosomes observed, namely, their heterochromatic nature, stickiness, nondisjunction, precocity of condensation, lagging and clubbing with each other to form micronuclei are in concordance with the B characterizations as described by Jones and Rees [12]. However, the existence of a specific breakage site near the heterochromatic block, as has been noticed in B_1 chromosome, has not been reported so far. It appears plausible that the mobile function of the heterochromatic block B_1 is presumably-responsible for its breakage phenomenon. Such likelihood, it may be pointed out here, stems from our specific observations on the heterochromatic elements which have exhibited intercellular mobility following their disarticulation from their carrier chromosomes (Fig. 3k). These results indicates some association between leaf variegation and the breakage of B_1 in the vicinity of heterochromatic block, but the mode of breakage, mobility and variegations requires further investigation.

While this study has revealed that the B chromosome maneuvered cytomixis is one of the underlying causes of numerical variation in PMC chromosomes, it does not form the cause of numerical variation of A chromosomes in somatic cells. The observations on the heterochromatic nature of the three B chromosomes vis-a-vis their intercellular migration suggest that their heterochromatic elements play a significant role in ensuring kinetic activity and sticky associations with other chromosomes [15]. As regards the cytoplasmic mechanism of chromosome migration between two contiguous cells, based on the formation of sticky anaphase bridges at chromosome as well as chromatid levels, we have proposed that the sticky chromosomes of opium poppy may facilitate weak/incomplete cell wall formation by staying between the two poles of dividing cells so as to leave a provision for the formation of cytoplasmic anastomoses for chromosome migration [11]. Large variation in chromosome number coupled with low gametic sterility of the cytomictic plants suggest that B chromosome-pioneered cytomixis may have a significant role in causing variation in opium poppy. Therefore, it turns out that the three B chromosomes studied here could be dispensable chromosomes in so far as the evolution of *P. somniferum* is concerned. From the reflected homology between B_1 and B_2 it appears likely that they have similar coancestry in origin. The property of B_3 to form association with autosomes suggests that B_3 has been derived from an A chromosome. [11].

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