B-chromosomes in some Lachenalia species and hybrids

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Varying chromosome numbers within a species and a series of diploid, polyploid and basic numbers have been reported for the genus Lachenalia. In a cytogenetical study conducted on this genus, B-chromosomes were observed in somatic and meiotic material of some species and F₁ hybrids. The size of the B-chromosomes in somatic material was found to be similar, or slightly smaller than the smallest chromosome of the normal complement. In pollen mother cells (PMCs), having univalent B-chromosomes, the B-chromosomes divided precociously during the first meiotic division. In a few PMCs of the hybrids where more than one B-chromosome was observed, the B-chromosomes associated to produce bivalents indicating that they are homologous or homeoeologous. The B-chromosome bivalents tended to disjoin normally during the first meiotic division and the resulting chromosomes divided normally during the second meiotic division. The discovery of B-chromosomes in the genus helps to account for some of the variation in chromosome number that has been reported for the genus, and specifically for the species L. orchioides (L.) Ait.

Variation in chromosomes amongst a group of 'n spesies, asook 'n reeks diploïëde, poliploïëde en basiese aantalle is vir die genus Lachenalia aangeteken. In 'n sitogenetiese ondersoek wat op die genus gedoen is, is B-chromosome in somatiese sowel as mikrospoormoederselle (MMSe) van sommige spesies en F₁-basters gevind. Die grootte van die B-chromosome, in somatiese weefsel was min of meer dieselfde, of 'n bietjie kleiner as die kleinste chromosoom van die normale komplement. In MMSe waar monovalente B-chromosome gevind is, deel die B-chromosome vroeg gedurende meiose. In 'n paar MMSe van die hibriede waar meer as een B-chromosoom waargeneem is, paar die B-chromosome om bivalente te vorm wat impliseer dat die B-chromosome homoloog is of homoeoloog is. Die B-chromosome bivalente reël normaal tydens die eerste meiotiese deling en die resulterende chromosomes deel normaal tydens die tweede meiotiese deling. Die ontdekking van B-chromosome in die genus Lachenalia bied 'n verklaring vir sommige van die variasie in chromosoomaantalle wat vir die genus opgeteken is, veral in die spesie L. orchioides (L.) Ait.

Keywords: B-chromosome, cytogenetics, Lachenalia, meiosis, mitosis

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Introduction

Lachenalia is a member of the Liliaceae [Hyacinthaceae (sensu stricta)] family. These small bulbous plants are endemic to southern Africa with a concentration of species in the south-western Cape Province. The genus incorporates approximately 90 species. It is currently under revision and according to Duncan (1988), the number of species is more likely to near 110. The plants are characterized by tubular or bell-shaped flowers arranged in a spike on a fleshy stem that grows 200 to 250 mm high. The colours of the flowers range from shades of red, green, blue, purple, yellow and white. Breeding work on this little-known, but one of South Africa’s most beautiful wild flowers, commenced in 1965 at the Vegetable and Ornamental Plant Research Institute at Rooedeplaat near Pretoria.

Several workers conducted chromosomal studies on the genus (Moffett 1936; de Wet 1957; Riley 1962; Ornduff & Watters 1978; Nordenstam 1982; Coertze pers. comm.) and their results revealed chromosome numbers of 2n = 14, 16, 22, 24, 26, 28, 32, 42, 56 and n = 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 18, 22 and 28. Variation in chromosome number, in addition to ploidy level differences, have been reported in L. orchioides (L.) Ait. and L. unifolia (Jacq.) W.F. Baker. The numbers reported in L. orchioides are 2n = 16 (de Wet 1957; Coertze pers. comm.) and n = 7, 14 (Ornduff & Watters 1978). For L. orchioides var. glauca (Jacq.) W.F. Baker, the reported chromosome numbers are 2n = 18 (Riley 1962), 2n = 24 (Coertze pers. comm.) and 2n = 28 (de Wet 1957; Moffett 1936). In the other species with variation in chromosome number namely L. unifolia the reported chromosome numbers are 2n = 16 (Coertze pers. comm.), 2n = 21, 22, 24, 26 (de Wet 1957) and 2n = 22 (Moffett 1936).

This paper deals with the presence and behaviour of the B-chromosomes discovered in some of the Lachenalia species and hybrids.

Materials and Methods

The nine F₁ hybrids of the six species most frequently used in the breeding programme and their surviving parents which were investigated, are given in Table 1. The numbers allocated to the hybrids, and those in brackets after the specific names are reference numbers used by the Vegetable and Ornamental Plant Research Institute.

For somatic chromosome studies young root tips were pretreated with 0.05% colchicine for 3 h and fixed in 1 : 3 acetic–alcohol for 24 h. Subsequently, the root tips were hydrolyzed in 1N HCl for 5–7 min at 60°C and squashed in propionic acid. For meiotic studies, flower spikes were dissected from the bulbs as soon as the first flowers became visible between the leaves. In this process the plants were
Table 1  Chromosome numbers for the species and F1 hybrids studied in the genus Lachenalia. The numbers of the hybrids and those in brackets after the specific names are reference numbers used by the Vegetable and Ornamental Plant Research Institute

| Hybrid number | Species or F1 hybrid | Somatic material | Meiotic material |
|---------------|----------------------|------------------|-----------------|
|               |                      | Local           | Plant number    | 2n   | Plant number | 2n   |
| 7504          | L. aloides (45)      | Unknown         | 1 and 2         | 28   | –            | not determined |
| 7512          | L. viridiflora (194) | –                | 0* and 2        | 14   | 1            | 14 + 0 - 2B   |
| 7513          | L. mutabilis (15)    | –                | 3, 4 and 10     | 14   | 1,9          | 28            |
| 7516          | L. reflexa (181)     | –                | 2, 8 and 9      | 28   | –            | –             |
| 7530          | L. splendidida (30)  | –                | 1               | 21 + 0 - 1B | 1          | 21            |
| 7531          | L. splendidida (30)  | –                | 1               | 15 + 0 - 1B | –          | –             |
| 7537          | L. reflexa (181)     | –                | 1               | 14 | 1, 2 and 5   | 14            |
| 7553          | L. bulbifera (Cyrillo) Engl. (175) | – | 1 and 3 | 21 | – | not determined |
| 7554          | L. orchioides var. glaucina (143) | – | 2, 3 and 5 | 14 | 3 | 14 + 0 - 3B |
| 7556          | L. mutabilis (161)   | –                | 5               | 14 | 3 | 14 + 0 - 2B |

*Plant number 'O' indicates the first plants that have been studied.

destroyed. This made it difficult to use the same plants for mitotic as well as for meiotic studies (Table 1). However, in the case of the hybrids, meiotic studies were conducted in PMCs from the same clone that were used for chromosome counts in the root tips, except for the hybrid 7529 namely L. splendidida Diels (30) × L. aloides (L.f.) Engl. cv. Pearsonii (22) where two different clones were used (Table 1). For the species it was not possible to do the meiotic studies on the same clone used for chromosome counts as more than one plant had been collected on a locality spot and were allocated the same number. After dissection, the inflorescences were fixed in a 6:3:2 mixture (v/v) of methanol, chloroform and propionic acid (Pienaar 1955) and the anthers were squashed in propionic carmine.

As mentioned by Moffet (1936) the chromosomes of Lachenalia fixed and stained poorly at the prophase I stages of meiosis. This investigation was therefore limited to metaphase and anaphase of the first and second meiotic division. At least 100 pollen mother cells (PMCs) of each species and hybrid were studied.

Results and Discussion

Extra chromosomes were observed in some somatic and meiotic material of different Lachenalia species and hybrids (Table 1). According to Jones & Rees (1982) B-chromosomes differ morphologically from the chromosomes of the normal complement in that they are smaller and are heterochromatic. However, these characteristics are not invariable (Jones & Rees 1982). In this investigation of Lachenalia species and hybrids the size of the extra chromosomes was more or less similar to the smallest chromosome of the normal complement. Further, the latter chromosomes have no definite staining pattern. In some cells they stained slightly lighter or darker and in others similar to the chromosomes of the normal complement. The variation in staining did not correlate with specific material or developmental stages.

According to Jones & Rees (1982) the mitotic transmission of B-chromosomes to daughter cells during vegetative growth, is in most species disjunctional such that all cells carry the same number of B-chromosomes. There are exceptions where nondisjunction of the B-chromosomes causes variation in the number of B-chromosomes among cells within the same individual. The extra chromosomes observed in some somatic cells of Lachenalia presumably revealed the latter behaviour. In the species and hybrids investigated most of the cells have the normal chromosome number, but in addition to the normal complement, one or two extra chromosomes were observed in some cells. These unstable distribution patterns of B-chromosomes have been reported in different species by several workers (Price 1963; Shopova 1966; Joshi & Raghuvanshi 1969; Lewis et al. 1971; Sen 1974). This manner of transmission and distribution of the extra chromosome in Lachenalia, accounts for the
variation in chromosome number between vegetatively similar plants (Table 1) and is reflected in meiotic material as well. Jones & Rees (1982) stated that while the behaviour and transmission of B-chromosomes during mitosis is, in most cases, orthodox and regular, the distribution of B-chromosomes to gametes during reproduction is unorthodox in the majority of species. They found that the main cause is the nondisjunction of B-chromosomes either during meiosis or, in higher plants particularly, during mitotic divisions in gametophytes subsequent to meiosis.

Jones & Rees (1982) are of the opinion that B-chromosomes descended from members of the normal complement, which initially existed as trisomics or trisomic fragments and became modified in structure. This affects their pairing behaviour at meiosis and prevents homologous associations with their ancestral normal chromosomes. These trisomics and trisomic fragment can arise in various ways, e.g. non-disjunction or unequal interchanges. The centric fragment may be lost or evolve into a B-chromosome.

Since the Lachenalia plants with the extra chromosomes did not exhibit phenotypic abnormalities, the variation in basic number in this genus is probably not due to trisomy or partial trisomy, and must be ascribed to B-chromosomes. These extra chromosomes are not particularly small, as mentioned earlier. Mogford (1978) found that the chromosomes of L. aloides have large amounts of centric heterochromatin. Thus, relatively large inert centric fragments could have evolved in some of the species.

B-chromosomes in somatic material of Lachenalia

The somatic chromosome numbers are listed in Table 1. These are in accordance with the literature for all species except for L. reflexa Thunb. (181). The chromosome numbers of the F1 hybrids indicated that the chromosome numbers of their parents must have been in accordance with the literature except for the hybrids 7516, namely L. reflexa (222) × L. aloides (45); 7529, namely L. splendida (30) × L. aloides cv. Pearsonii (22); 7504, namely L. orchioides (61) × L. aloides var. (41) and 7554, namely L. orchioides var. glaucina (143) × L. aloides var. quadricolor (155) and 7556, namely L. mutabilis Sweet (161) × L. aloides var. quadricolor (155) (Table 1). The frequency and behaviour of B-chromosomes observed in the latter species and hybrids are given in Table 2. The total number of pollen mother cells (PMCs) having B-chromosomes ranged from 2.8% in the hybrid 7504, namely L. orchioides (61) × L. aloides var. (41) to 6.1% in the species L. aloides var. quadricolor (122). The meiosis of the hybrids 7556, namely L. mutabilis (161) × L. aloides var. quadricolor (155) and 7529, namely L. splendida (30) × L. aloides cv. Pearsonii (22) are abnormal. Due to the staining and the size of the observed extra chromosomes it was difficult to determine whether they resulted from the abnormality, or were in fact true B-chromosomes. The statistics of these stages were therefore omitted from the calculation of the total number of PMCs having B-chromosomes for the latter two hybrids; Their B-chromosome values in Table 2 are therefore only approximate, namely 4.1% and 3.3% respectively.

PMCs having one B-chromosome were most frequently observed. A few PMCs with more than one B-chromosomes were, however, also observed.

The behaviour of B-chromosomes in PMCs having one B-chromosome

B-chromosomes were observed as univalents in up to 7.8% of the PMCs at metaphase I (Table 2 and Figure 1a). The B-chromosomes of hybrids 7504 and 7554 underwent precocious chromatid separation during metaphase I in 1.0% and 0.6% of the PMCs respectively (Table 2 and Figure 1b). Further, up to 11.1% of the PMCs at anaphase I had a B-chromosome with only one chromatid at each pole as the result of precocious chromatid separation. In up to 2.2% of the PMCs at anaphase I, the B-chromosome laggards were included with the normal chromosomes at a pole. No B-chromosomes laggards were found on the equatorial plate of anaphase I (Table 2). Therefore, the B-chromosome univalents either underwent precocious chromatid separation or were included intact at one pole, but did not lag during the first meiotic division. In up to 3.4% of the PMCs at metaphase II, B-chromosomes with only one chromatid (resulting from precocious chromatid separation during the first division) were observed (Table 2 and Figure 1c). These B-chromosomes were incapable of division at anaphase II and were either included with the normal chromosomes at a pole or remained stranded on the equatorial plate. According to Jones & Rees (1982), B-chromosomes univalents are successfully transmitted to the gametes if they are incorporated undivided within a telophase I nucleus. If not,
Table 2  The frequency and behaviour of the B-chromosomes observed in pollen mother cells (PMCs) of some Lachenalia species and F₁ hybrids. Number of cells (N) and percentage (P)

| Species/F₁ hybrids | 122* | 7504* | 7554* | 7556* | 7529* |
|--------------------|------|-------|-------|-------|-------|
| N                  |      |       |       |       |       |
| P                  |      |       |       |       |       |

The B-chromosomes observed as a:

### Metaphase I

| Behaviour | 122* | 7504* | 7554* | 7556* | 7529* |
|-----------|------|-------|-------|-------|-------|
| univalent | 5    | 7.8   | 1     | 0.5   | 8     | 2.3   | 2     | 2.1   | 4     | 4.0   |
| univalent undergoing precocious chromatid separation | -    | -     | 2     | 1.0   | 2     | 0.6   | -     | -     | -     | -     |
| bivalent, disjoins precociously | -    | -     | 1     | 0.5   | -     | -     | -     | -     | -     | -     |
| bivalent | -    | -     | -     | -     | 1     | 0.5   | 1     | 0.3   | 2     | 2.1   |
| bivalent + univalent | -    | -     | -     | -     | 1     | 0.3   | -     | -     | -     | -     |
| Total number of cells with B-chromosomes | 5    | 7.8   | 5     | 2.6   | 12    | 3.4   | 4     | 4.2   | 4     | 4.0   |
| Total number of cells studied | 64   | -     | 193   | -     | 352   | -     | 95    | -     | 100   | -     |

### Anaphase I

- One B-chromatid at each pole after precocious chromatid separation
- One B-chromosome at each pole after normal bivalent disjunction

| Behaviour | 122* | 7504* | 7554* | 7556* | 7529* |
|-----------|------|-------|-------|-------|-------|
| Total number of cells with B-chromosomes | 6    | 13.3  | -     | -     | 2     | 5.7   | 2     | 4     | -     | -     |
| Total number of cells studied | 45   | -     | 17    | -     | 35    | -     | 50    | -     | 20    | -     |

### Metaphase II

B-chromosome (with only one chromatid after chromatid separation during the first meiotic division)

| Total number of cells with B-chromosomes | 2    | 1.8   | -     | -     | 2     | 3.4   | -     | -     | -     | -     |
| Total number of cells studied | 109  | -     | 41    | -     | 58    | -     | -     | -     | -     | -     |

### Anaphase II

- B-chromosome, one at each pole after normal chromatid separation
- B-chromosome + univalent

| Total number of cells with B-chromosomes | 2    | 7.1   | 3     | 8.6   | 2     | 20    | -     | -     | -     | -     |
| Total number of cells studied | 28   | -     | 35    | -     | 10    | -     | -     | -     | -     | -     |
| Total number of cells in which B-chromosomes were observed | 15   | 6.1   | 8     | 2.8   | 18    | 3.9   | 6     | ±4.1  | 4     | ±3.3  |
| Total number of cells studied | 246  | -     | 286   | -     | 455   | -     | 145   | -     | 120   | -     |

122* = L. aloides var. quadricolor (122)
7554* = L. orchioides var. glaucina (143) × L. aloides var. quadricolor (155)
7556* = L. mutabilis (161) × L. aloides var. quadricolor (155)
7504* = L. orchioides (61) × L. aloides var. (41)

they are eliminated. In the case of Lachenalia it appeared that the B-chromosome univalent more often divided during first meiotic division (Table 2). Therefore it could be expected that the latter behaviour of the B-chromosomes in Lachenalia will reduce the transmission of B-chromosomes to the next generation especially in plants with only one B-chromosome. However, Jones & Rees (1982) stated that B-chromosomes univalents are not always inevitably eliminated if they do divide at the first division. An exception is described in Festuca pratensis Huds. by Bosemark (1954). He found that in 15 to 30% of the PMCs the B-chromosomes divide at the first anaphase I. The B-chromosomes with only one chromatid lag at anaphase II, but only a few fail to be incorporated into telophase II nuclei with the result that the number eliminated was small. It was not possible to make any similar conclusions for the genus Lachenalia from the present results. The exact transmission mechanism of univalent B-chromosomes will have to be determined.

The behaviour of B-chromosomes in PMCs having more than one B-chromosome

In a few PMCs of the hybrids, more than one B-chromosome was observed and the B-chromosomes appeared as bivalents at metaphase I (Table 2 and Figure 1d). In 0.3% of the PMCs at metaphase I of the hybrid 7554, namely L. orchioides var. glaucina (143) × L. aloides var. quadricolor(155), three B-chromosomes were observed (Table 2). The latter associated to produce a bivalent and a univalent. These B-chromosomes are therefore homologous or homoeologous. The disjunction of the B-chromosome bivalents were presumably normal during the first and second meiotic division. In the hybrid 7504 0.5% of the PMCs at metaphase I had precocious
Figure 1  The B-chromosomes, marked with an arrow observed (1a) as a metaphase I univalent in the hybrid 7556, namely *L. mutabilis* (161) × *L. aloides* var. *quadricolor* (155); (1b) as a univalent dividing precociously at metaphase I in the hybrid 7504, namely *L. orchioides* (61) × *L. aloides* var. (41); (1c) as a B-chromosome with only one chromatid (resulting from precocious chromatid separation during the first meiotic division) at metaphase II in the species *L. aloides* var. *quadricolor* (122); (1d) as a bivalent at metaphase I in the hybrid 7556 and (1e) as a bivalent, disjoining precociously at metaphase I in the hybrid 7504.
disjoining B-chromosome bivalents (Table 2 and Figure 1e). In up to 2.9% of the PMCs at anaphase I, one B-chromosome was observed at each pole as a result of normal bivalent disjunction (Table 2). However, at metaphase II, only B-chromosomes with one chromatid, (resulting from precocious chromatid separation during the first division) were found. This observation is probably due to the relatively low number of cells studied at metaphase II. In up to 20% of the PMCs at anaphase II, the B-chromosomes divided normally and a B-chromosome was observed at each pole (Table 2). Therefore, it appears that in PMCs having more than one B-chromosome their behaviour during first and second meiotic division are normal and the B-chromosomes are distributed, like the chromosomes of the normal complement, one to each nucleus of the tetrad of microspores. In this way the B-chromosomes could be transmitted from one generation to the next.

Jones & Rees (1982) are of the opinion that a higher average number of B-chromosomes in the offspring than in the parental generation indicates a B-chromosome accumulation mechanism. The accumulation mechanisms of B-chromosomes could be operative before, during or after meiosis. In this study no accumulation of B-chromosomes occurred prior to or during meiosis. PMCs having more than one B-chromosome were observed in hybrids only (Table 2). Therefore an accumulation mechanism, if present, should be postmeiotic.

Conclusions

One to three B-chromosomes were found in L. aloides var. quadricolor (122), L. reflexa (181) and the hybrids 7504, namely L. orchiodides (61) x L. aloides var. (41); 7516, namely L. reflexa (222) x L. aloides (45); 7529, namely L. splendida (30) x L. aloides cv. Pearsonii (22); 7554, namely L. orchiodides var. glauca (143) x L. aloides var. quadricolor (155) and 7556, namely L. mutabilis (161) x L. aloides var. quadricolor (155). Since the number of PMCs with B-chromosomes is low, no pre-meiotic B-chromosome accumulation mechanism is operative. No accumulation mechanism was detected during meiosis. The presence of an accumulation mechanism in Lachenalia will have to be proved by cytological investigations post-meiotic mitosis in gametophytes, as well as chromosome counts in the progeny of plants possessing different numbers of B-chromosomes.

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