Chromosome associations of three interspecific, dibasic *Lachenalia* hybrids

FL Hancke*†, WS Jansen van Rensburg† and H Liebenberg†

*† ARC-Roodeplaat, Private Bag X293, Pretoria 0001, South Africa
† Department of Genetics, University of Pretoria, Pretoria 0002, South Africa
* Corresponding author, e-mail: hancke@cybertrade.co.za

Received 11 January 2000, accepted in revised from 26 May 2000

The ARC-Roodeplaat started to develop some endemic species as commercial pot plants and cut flowers several years ago. One of the genera selected for pot plant breeding was *Lachenalia*. For proper planning of the breeding programme the relationship between and within species is of utmost importance. Determining these relationships is an ongoing process at the ARC-Roodeplaat. The present study was done on three interspecific dibasic hybrids, that resulted from two crosses made between the species *L. splendida* (x = 8) and *L. unicolor* (x = 8) as female parents and *L. aloides* (x = 7) as male parent. Chromosome associations were used to draw conclusions on the relationship between species with different basic chromosome numbers. On morphological grounds the species *L. aloides* does not seem to be closely related to *L. splendida* and *L. unicolor*. Chromosome pairing in the interspecific hybrids, however, revealed that these species are more closely related than expected. The study further revealed homology between two chromosomes of the x = 7 karyotype and three chromosomes of the x = 8 karyotype. It indicates that the x = 7 plants differ from the x = 8 plants by at least two exchanges of chromosome material and involves also the loss of one centromere from the x = 8 karyotype. It further indicates that the change in the basic chromosome number of *Lachenalia* involves a reduction in chromosome number. The process of change is, however, not straight forward, since the x = 8 karyotype has no acrocentric chromosomes, it was not just the result of simple centric fusion.

Introduction

In the world of flowers the search for something new, something different and interesting is never ending. South Africa has a wonderfully rich flora which is well known and used by many botanists, horticulturists and flower breeders from countries all over the world. It is especially the bulbous plants which have attracted attention. The South African bulbous plants include approximately 4 000 species and genera such as *Freesia*, *Babiana*, *Ixia*, *Tritonia*, *Gladiolus*, *Sparaxis*, *Ornithogalum* and *Zantedeschia*.

The ARC-Roodeplaat realised the potential of these plants and started to develop some endemic species as commercial pot plants and cut flowers several years ago. One of the genera selected for pot plant breeding was *Lachenalia* (Jacq.f. ex Murray). This genus exhibits a huge amount of phenotypic diversity. Hundreds of crosses have been made and several reports published on the breeding work done by the ARC-Roodeplaat (Lubbinge 1980, Malan et al. 1983, Lubbinge et al. 1983a, b, c, d; Ferreira and Hancke 1985, Hancke and Coertze 1998, Coertze et al., 1992). However, it was only during 1998 that the first commercial pot plants were sold in the Netherlands, offering these growers something completely new, interesting and different. To hold the interest of the market in *Lachenalia* it is essential that new cultivars must be made available continuously. In order to do proper planning of the breeding programme the relationship between and within species is of utmost importance. Determining these relationships is an ongoing process at the ARC-Roodeplaat (Hancke and Liebenberg 1998, Kleynhans and Spies 1999, Kleynhans and Spies 2000).

Various methods have been developed to assess genome affinity between different taxa. Some of these methods are karyotype analysis, chromosome pairing in interspecific hybrids, chromosome banding techniques, seed protein profiles, iso-enzyme studies, DNA hybridisation and restriction endonuclease studies (Hiremath *et al.* 1990). However, the more classical method of assessing genome affinities through chromosome pairing in interspecific hybrids is still considered to be the best in assessing genome homologies between different taxa (Hiremath *et al.* 1990).

Several authors have done cytogenetic studies on the genus *Lachenalia* and reported different basic chromosome numbers (Moffett 1936, De Wet 1957, Riley 1962, Nordenstam 1982, Crosby 1986, Mogford 1978, Ornduff and Watters 1978, Hancke and Liebenberg 1990, Johnson and Brandham 1997). The present study was done on three interspecific hybrids that resulted from crosses made
between species with basic chromosome numbers of \( x = 7 \) and \( x = 8 \). Chromosome pairing was used to draw conclusions on the relationship between species with different basic chromosome numbers.

**Material and Methods**

Hybrids used in this study form part of the breeding program and resulted from two crosses. The first being between the species *L. splendida* (30) (\( x = 8 \)), as the female parent, and *L. aloides* cv. Pearsonii (22) (\( x = 7 \)), as the male parent. The hybrid resulting from this cross has the accession number 75/29. Numbers in brackets behind species names indicate accession numbers used by the ARC-Rooideplaat. At the time of the study the parent *L. splendida* (30) no longer existed. The parent *L. aloides* cv. Pearsonii (22) is an intraspecific *L. aloides* hybrid.

The second cross is between the species *L. unicolor* (109) (\( x = 8 \)), as the female parent, and 66/3/3 (\( x = 7 \)) as the male parent. The parent 66/3/3 is an intraspecific hybrid between *L. aloides* cv. Pearsonii (22) and *L. aloides* var. aurea. The hybrids 76/16/20 and 76/16/74 resulted from this cross from two different seeds.

Pollen fertility estimation and the preparation of pollen mother cells (PMC’s) for observation was done as previously reported (Hancke and Liebenberg 1998). Ideograms of the hybrid 75/29, the species *L. splendida* and *L. aloides* are extracts from the Masters thesis of Hancke (1991). Meiosis of all studied parents was normal. In the case of the *L. aloides* parents seven bivalents, two large and five small, were present in all PMC’s. The five small bivalents were commonly rod shaped with one terminal chiasma. The one large bivalent has in most of the PMC’s, two chiasmata on the same side of the centromere, one interstitial and the other terminal. In rare cases it has one interstitial chiasma and in very rare cases one terminal chiasma. The other large bivalent has one chiasma, either terminal or subterminal. In all PMC’s of *L. unicolor* (109) eight bivalents were observed, seven with one chiasma only and one having either one or two chiasmata per bivalent.

The hybrid 75/29 revealed a fairly normal meiosis, in spite of the fact that it was completely pollen sterile. The number of chromosomes that associated as bivalents were 6.53 per PMC and a very high GAI of 0.93 is calculated (Table 1). At metaphase I the chromosomes associate either as \( 7n + l \) (Figure 2a and d) or \( 6n + 1 \) (Figure 2b, c, f and g) and rarely as \( 6n + 3 \) (Figure 2e). The frequencies of the different pairing associations are summarised in Table 2.

The *L. unicolor* x *L. aloides* (76/16/20 and 76/16/74) hybrids were also completely pollen sterile and exhibited a much more variable meiosis (Table 2). However, most chromosomes still associated as bivalents. In the hybrids 76/16/20 and 76/16/74, the number of chromosomes per PMC associated as bivalents was 6.16 and 6.06 respectively (Table 1). Consequently the GAI’s for these two hybrids...
had previously and is manifested in the heteromorphic bivalents and the high morphological splendida (1897, Crosby 1986, Duncan 1988). Chromosome pairing in a/aides explains slightly. It the meiosis of the hybrids different pairing configurations were observed (Table 2 and Figure 3b, e and f). From Table 2 it seems that the pairing configurations of the hybrid 76/16/20 and 76/16/74 differ slightly. It was observed that cells that are grouped together tend to have the same meiotic configurations. This might explain why the frequencies of the different pairing configurations differ for the two hybrids, 76/16/20 and 76/16/74.

**Discussion**

On morphological grounds, the species L. aloides does not seem to be closely related to L. splendida and L. unicolor. L. aloides has been classified under different taxa than L. splendida and L. unicolor by several taxonomists (Baker 1897, Crosby 1986, Duncan 1988). Chromosome pairing in the interspecific hybrids, however, revealed that these species are more closely related than expected, especially if the high GAI's and frequency of chromosomes associated as bivalents (Table 1) are taken into account.

A great deal of chromosome divergence is revealed as well, which is apparent in a difference in number and length and is manifested in the heteromorphic bivalents and multivalents. In the hybrid 75/29, 43% of PMC's at metaphase I had 7+1, of which the two largest bivalents are conspicuously heteromorphic (Figure 2a). The acrocentric chromosome of the x = 7 karyotype is involved in one of these large heteromorphic bivalents. The two satellite chromosomes most probably form the other large heteromorphic bivalent. Since the satellite chromosome of the x = 8 karyotype is the longest (Figure 1b) and would form a heteromorphic bivalent with any of the chromosomes of the x = 7 karyotype (Figure 1c). However, in 10% of the PMC's with 7+1 only the bivalent in which the acrocentric chromosome is involved, is conspicuously heteromorphic and all other bivalents are relatively homomorphic (Figure 2c). This indicated that the satellite chromosome of the x = 7 karyotype is not only homologous to the satellite chromosome of the x = 8 karyotype, but also to a second chromosome of the x = 8 karyotype which is more or less equal in length to the satellite chromosome of the x = 7 karyotype. It is thus possible to obtain a trivalent between these three chromosomes. This trivalent was observed in 34% PMC's at metaphase I (Figure 2b and c). In 7% of PMC's a second trivalent was observed in which the acrocentric chromosome was involved. These cells also included five homomorphic bivalents and one large heteromorphic bivalent (Figure 2f and g). This indicated that both the acrocentric and satellite chromosomes of the x = 7 karyotype are homeologous to the same chromosome of the x = 8 karyotype. Figure 4 illustrates the proposed homology and homeology between the chromosomes of the karyotypes of the species L. splendida and L. aloides. With this hypothesis it should be theoretically possible to have a quadrivalent and a pentavalent. These configurations were not observed in

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**Table 1:** Meiotic pairing, including mean and range (in brackets), of metaphase I and the genome affinity index (GAI) of three dibasic Lachenalia hybrids

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Ovule parent x Pollen parent</th>
<th>No. of PMC's studied</th>
<th>Mean and range (in brackets) per PMC</th>
<th>GAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>75/29: L. splendida (30)(x = 8) x 76/16/20: L. unicolor (109)(x = 8)</td>
<td>100</td>
<td>0.7 (0-3)</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td><strong>L. aloides cv. Pearsonii (22)(x = 7)</strong></td>
<td><strong>L. aloides cv. Pearsonii (22)(x = 7)</strong></td>
<td>105</td>
<td>1.1 (0-5)</td>
<td>0.90</td>
</tr>
<tr>
<td><strong>66/3/3 (x = 7)</strong></td>
<td><strong>66/3/3 (x = 7)</strong></td>
<td><strong>66/3/3 (x = 7)</strong></td>
<td><strong>66/3/3 (x = 7)</strong></td>
<td><strong>66/3/3 (x = 7)</strong></td>
</tr>
</tbody>
</table>

were very high as well (Table 1). In 75% and 85% of PMC's, the meiosis of the hybrids 76/16/20 and 76/16/74 was similar to that of the hybrid 75/29. In the rest of the PMC's different pairing configurations were observed (Table 2 and Figure 3b, e and f). From Table 2 it seems that the pairing configurations of the hybrid 76/16/20 and 76/16/74 differ slightly. It was observed that cells that are grouped together tend to have the same meiotic configurations. This might explain why the frequencies of the different pairing configurations differ for the two hybrids, 76/16/20 and 76/16/74.

**Table 2:** The frequency of metaphase I pairing configurations in three dibasic Lachenalia hybrids

<table>
<thead>
<tr>
<th>Pairing configuration</th>
<th>75/29: L. splendida (30)(x = 8) x 76/16/20: L. unicolor (109)(x = 8)</th>
<th>76/16/74: L. unicolor (109)(x = 8) x 66/3/3 (x = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pairing configuration</td>
<td>No. of PMC's</td>
<td>% of PMC's</td>
</tr>
<tr>
<td>71 + II</td>
<td>53</td>
<td>53</td>
</tr>
<tr>
<td>61I + III</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>61I + III</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>51I + IV</td>
<td>5</td>
<td>4.7</td>
</tr>
<tr>
<td>51I + 1IV + 1I</td>
<td>6</td>
<td>5.7</td>
</tr>
<tr>
<td>51I + 1IV + 2I</td>
<td>6</td>
<td>5.7</td>
</tr>
<tr>
<td>51I + 1I</td>
<td>3</td>
<td>2.8</td>
</tr>
<tr>
<td>41I + 1IV + 2I</td>
<td>2</td>
<td>1.9</td>
</tr>
<tr>
<td>41I + 1IV + 3I</td>
<td>3</td>
<td>2.8</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
the hybrid 75/29. However, they were observed in the hybrids 76/16/20 and 76/16/74. The absence of quadrivalents and peniavalents in the meiosis of 75/29 might be the result of cryptic karyotype evolution in *L. splendida*. This should make *L. splendida* further divergent from *L. aloides* than *L. unicolor*. However, a study of the meiosis of interspecific hybrids between *L. unicolor* and *L. splendida* will shed more light on this theory.

In 75% and 85% PMC's studied for the hybrids 75/16/20 and 75/16/74 respectively, pairing configurations were similar to that of the hybrid 75/29 (Figure 3a–c). The rest of the PMC's studied for these hybrids have configurations that support the opinion that two chromosomes of the *x = 7* karyotype, of which one is the acrocentric chromosome, share homeology to three chromosomes of the *x = 8* karyotype (Table 1 and Figure 3d and e). These chromosomes are most possibly the chromosomes involved in the change of the basic chromosome number. Jones and Rees (1982) were of the opinion that B-chromosomes are the result of a reduction of the basic chromosome number. If the presence of B-chromosomes in *Lachenalia* (Hancke and Liebenberg 1990), the presence of heterochromatin around the centromeres (Mogford 1978) and the fact that two chromosomes of the *x = 7* karyotype are homeologous to three
chromosomes of the x = 8 karyotype, are taken into account, it is most likely that the change in the basic chromosome number of *Lachenalia* is a reduction in chromosome number. However, this process is not straightforward as was concluded from the meiosis of the intraspecific dibasic hybrids. More than one translocation was, at least, involved and these translocations were not the result of simple centric fusion, since the x = 8 karyotype has no acrocentric chromosomes.

**Conclusion**

Chromosome pairing of the dibasic interspecific hybrids *L. unicolor* x *L. aloides* and *L. splendida* x *L. aloides* revealed that the species are more closely related than expected. Hybrids between the different karyotypes are completely pollen sterile and for the purpose of a breeding program careful planning should be done if fertility is needed. With such a high homology between the different karyotypes it is also questionable whether chromosome doubling will restore fertility. However, crosses between species with different chromosome numbers are a tool that can be used to obtain sterility if needed. Pot plants and cut flowers with sterile flowers have a better keeping ability for instance.

The study further revealed homology between two chromosomes of the x = 7 karyotype and three chromosomes of the x = 8 karyotype. It indicates that the x = 7 plants differ from the x = 8 plants by at least two exchanges of chromo-

**Figure 3:** Metaphase I pairing associations for the hybrids 76/16. (a) 7→1 in the hybrid 76/16/74; (b) 5→1→6→1 in the hybrid 76/16/20; (c) 6→1→6/16/74 and the acrocentric chromosome is involved in the trivalent; (d) 6→1→6 in the hybrid 76/16/74 and the acrocentric chromosome is not involved in the trivalent; (e and f) 5→1→6 in the hybrid 76/16/74.
some material and involves also the loss of one centromere from the \( x = 8 \) karyotype. Thus, the change in the basic chromosome number of *Lachenalia* involves a reduction in number.

### References


