A Preliminary Study of Interspecific Hybrids in *Lachenalia* (Hyacinthaceae)

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Abstract

***Lachenalia* is a bulbous geophyte mainly found in the western parts of southern Africa and especially in the 'fynbos' and Namaqualand regions of the Western Cape Province. These plants are very well adapted to their environment. The genus *Lachenalia* encompasses a great deal of variation. The ARC started with a breeding programme in 1966, in order to produce better flowers and bigger leaves and to provide for the ever-increasing demand for pot plant and flower production. This study was done to determine the effect of interspecific hybridization on the genetic constitution of hybrids between *Lachenalia unicolor* and four other species. The cytogenetic study shows that all the species used and their hybrids have somatic chromosome numbers of 2n = 2x = 16. The meiotic study indicates that the genomic constitutions of these species correspond to such an extent that chromosome pairing still occurs. The chiasma frequencies of the hybrids are usually slightly lower than in the parental species, thus indicating that these species are closely related.**

INTRODUCTION

The genus *Lachenalia* Jacq.f. ex Murray is a genus with considerable beauty and much variation. The genus is closely related to the genus *Polexyna* Kunth and is endemic to the Southwestern area of southern Africa (Duncan 1988). Approximately 110 species have been described and Crosby (1986) divided 42 species in five different species groups. The *Lachenalia* species grow mainly in the winter and flower during the period from April to November.

The species included in this study, i.e. *Lachenalia framesii* W.F.Barker, *L. namaquensis* Schltr. ex W.F.Barker and *L. unicolor* Jacq., are classified in the *L. unicolor* group. The two other species used in this study, i.e. *L. carnosa* Bak. and *L. splendida* Diels, are ungrouped. It is believed that these species are very similar to the species in the *L. unicolor* group and may possibly be placed in this group. These species were crossed at the Agricultural Research Council (ARC) at the Roodeplaat Vegetable and Ornamental Plant Institute near Pretoria and 45 of these interspecific hybrids were used in this study.

The variation present in this genus is used in the breeding of new hybrids by the ARC-Roodeplaat. This breeding programme was initiated in 1966 for the vast international pot plant market, which demanded better and more glamorous pot plants (Niederwieser et al. 1997). Since then 25 cultivars have been registered (Kleynhans 1997). A number of interspecific hybrids were made, but there are isolation barriers amongst many species (Lubbinge 1980). Various factors like flower size, polyploidy, reciprocal combinations and genetical differences, have to be considered in breeding *Lachenalia* (Lubbinge 1980). Crosby (1978) stated that the seedlings of interspecific hybrids show very little variation in comparison with the parental species. It also takes about three years to produce a flowering plant from a seedling.

Many different basic chromosome numbers have been described for *Lachenalia*

species (Moffett 1936; De Wet 1957; Riley 1962; Mogford 1978; Ornduff and Watters 1978; Nordenstam 1982; Crosby 1986; Hancke and Liebenberg 1990; Hancke 1991; Johnson and Brandham 1997; Kleynhans 1997; Hancke and Liebenberg 1998; Kleynhans and Spies 1999; Spies et al. 2000). The *L. unicolor* group has a basic chromosome number of x = 8 and most specimens are diploid, with 2n = 2x = 16 (Crosby 1986; Spies et al. 2000). Occasionally a tetraploid was observed and Crosby (1986) even found some plants with a somatic number of 2n = 18. This could possibly be attributed to the presence of B-chromosomes. Hancke and Liebenberg (1990), as well as Johnson and Brandham (1997) observed B-chromosomes in various *Lachenalia* species.

The aim of this study is to infer the relationships between the species by comparing the meiotic chromosome behaviour in the parental species and interspecific hybrids.

MATERIALS AND METHODS

Twenty-nine specimens, representing five species, were collected in the field. The material was grown at the ARC-Roodeplaat and the Department of Botany and Genetics, University of the Orange Free State, Bloemfontein. Voucher herbarium specimens are housed in the Geo Potts Herbarium (BLFU) at the university (Table 1). Interspecific crosses were made at Roodeplaat and nine of the hybrids are included in this study.

Bulbs from the specimens were placed on Deco gel™ and actively growing roots tips were cut and placed in water (4°C) for 24 hours. The mitotic studies were done on the root tips, which were fixed in Carnoy's (1886) fixative for 24-36 hours, hydrolyzed in 1N HCl at 60°C for 7 minutes and stained with 0.5% leucobasic fuchsin for two hours (Darlington and LaCour 1976). Mitotic squashes were done in 1% aceto-orcein (Darlington and LaCour 1976).

Meiotic studies were done collecting young inflorescences and fixing them in Carnoy's (1886) fixative for 24-72 hours, before storing them in 70% ethanol. The anthers were squashed in aceto-carmine and ferri-acetate (Darlington and LaCour 1976). The slides from both the mitotic and meiotic squashes were permanently mounted in Euparal (Darlington and LaCour 1976).

RESULTS AND DISCUSSION

During this study 29 specimens, representing five species, as well as nine interspecific hybrids, were studied cytogenetically (Table 1). All specimens were diploid with 2n = 2x = 16 (Table 1). The chromosome configurations of the pollen mother cells were usually five or six rod bivalents, with one terminal chiasma, and two or three ring bivalents, with two chiasmata. Occasionally univalents were observed. The occasional trivalent were observed in some of the hybrids and corresponded with the presence of univalents. Chromosome associations and the chiasma frequencies of the five parental species and five F_1-hybrids were studied.

The hybrids showed some more variation in terms of pairing configurations, than the parental species. This is normal behaviour for hybrids. An interesting factor however is the fact that the chiasma frequencies are, between means of fluctuation, very similar to that of the parental species. This is proof that there are very little genetic differences between the species and lead to the formation of hybrids that have almost the same genetical constitution than that of the parental species.

This study shows that, because of chromosome numbers and hybridization studies, the two ungrouped species, *L. carnosa* and *L. splendida* may well be grouped with the other species within the *L. unicolor* group. However, molecular work should be done to determine the real phylogenetic relationships between these species.

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Tables

Table 1. Somatic chromosome numbers of specimens of the *Lachenalia* species used as parents in the different crosses, with their voucher numbers and localities. Specimens are listed according to their locality from north to south and from west to east.

Species/Voucher	2n	Locality
L. carnosa		
Spies 6999	16	NORTHERN CAPE.—2917 (Springbok): Nababiep (-DB)
Spies 6998	16	NORTHERN CAPE.—2917 (Springbok): Springbok (-DB)
Spies 6992, 6993	16	NORTHERN CAPE.—3017 (Hondeklipbaai): Kamieskroon (-BB)
Spies 6996	16	NORTHERN CAPE.—3018 (Kamiesberg): between Garies and Leliefontein (-AD)
Spies 7003	16	NORTHERN CAPE.—3018 (Kamiesberg): between Platbakkies en Garies (-AD)
Spies 6991, 7000, 7007	16	Unknown – grown from seeds
L. framesii		
Spies 7008, 7009, 7010	16	Unknown – grown from seeds
L. namaquensis		
Spies 6969	16	NORTHERN CAPE.—2917 (Springbok): Steinkopf (-BC)
Spies 6970	16	NORTHERN CAPE.—2917 (Springbok): Springbok (-DB)
Spies 6971, 6972	16	Unknown – grown from seeds
L. splendida		
Spies 6977	16	WESTERN CAPE.—3118 (Vanrhynsdorp): along road to Vredendal (-DA)
Spies 6976	16	WESTERN CAPE.—3119 (Calvinia): Soutpans koppies (-CD)
Spies 6975	16	Unknown
L. unicolor		
Spies 6838	16	WESTERN CAPE.—3118 (Vanrhynsdorp): Clanwilliam (-BB).
Spies 6979	16	WESTERN CAPE.—3118 (Vanrhynsdorp): Karingmelkfontein (-DA)
Spies 6981	16	WESTERN CAPE.—3118 (Vanrhynsdorp): Bottervlei (-DA).
Spies 6982	16	WESTERN CAPE.—3118 (Vanrhynsdorp): Gifberg (-DA)
Spies 6980	16	WESTERN CAPE.—3218 (Clanwilliam): between Pakhuis Pass and Biedouvallei (-DD).
Spies 6833	16	WESTERN CAPE.—3318 (Potsberg): Porterville (-BB)
Spies 6985, 6986	16	WESTERN CAPE.—3319 (Worcester): Brandvleidam (CB)
Spies 6983, 6984	16	WESTERN CAPE.— 3418 (Simonstown): Langkloof (-AB)

Table 2. Somatic chromosome numbers of *Lachenalia* hybrids with their voucher numbers and parents.

Voucher	2n	Ovule parent	Pollen parent
Spies 7013	16	*L. carnosa* (*Spies 6993*)	*L. splendida* (*Spies 6977*)
Spies 7014	16	*L. carnosa* (*Spies 6993*)	*L. splendida* (*Spies 6977*)
Spies 7015	16	*L. carnosa* (*Spies 6993*)	*L. splendida* (*Spies 6977*)
Spies 7025	16	*L. splendida* (*Spies 6977*)	*L. carnosa* (*Spies 6993*)
Spies 7035	16	*L. splendida* (*Spies 6977*)	*L. carnosa* (Unknown)
Spies 7052	16	*L. unicolor* (*Spies 6838*)	*L. carnosa* (*Spies 6993*)
Spies 7053	16	*L. unicolor* (*Spies 6838*)	*L. carnosa* (*Spies 6993*)
Spies 7054	16	*L. unicolor* (*Spies 6838*)	*L. carnosa* (*Spies 6993*)
Spies 8120	16	*L. carnosa* (Unknown)	*L. framesii* (Unknown)

Table 3. Chiasma frequency of some of the hybrids and their parents.

Voucher	Species	Chiasma frequency
Spies 6825	*L. unicolor*	1.25
Spies 6843	*L. unicolor*	1.15
Spies 6972	*L. namaquaensis*	1.24
Spies 6996	*L. carnosa*	1.29
Spies 7003	*L. carnosa*	1.13
Spies 7015	*L. carnosa x L. splendida*	1.19
Spies 7025	*L. splendida x L. carnosa*	1.18
Spies 7052	*L. unicolor x L. carnosa*	1.2
Spies 7054	*L. unicolor x L. carnosa*	1.12
Spies 8120	*L. carnosa x L. framesii*	1.28

Figures

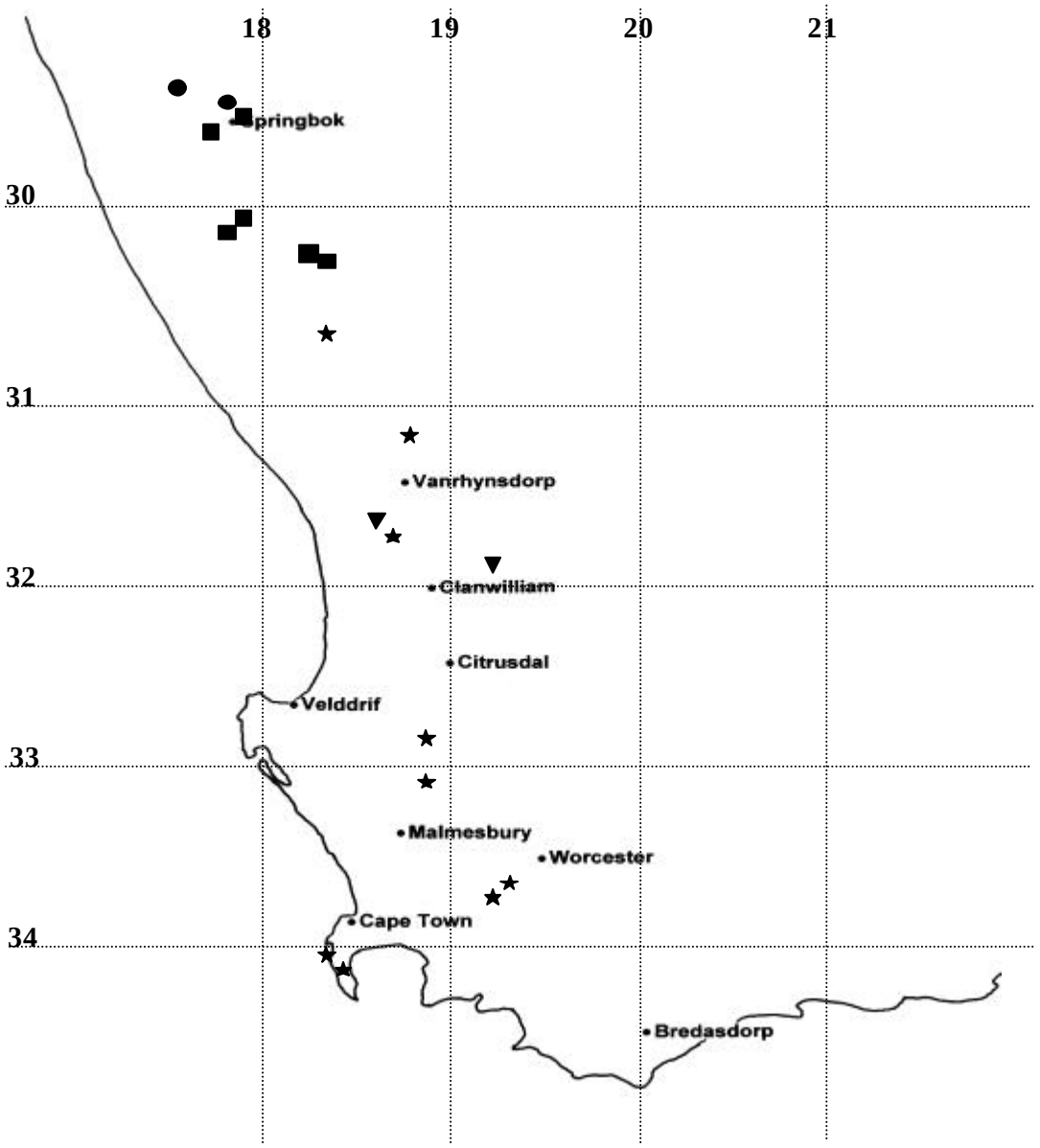

Fig. 1. Map of the South-Western Cape, showing the localities where the specimens were collected. Squares indicate the localities of *L. carnosa*, circles *L. namaquensis*, triangles *L. splendida* and stars *L. unicolor*.

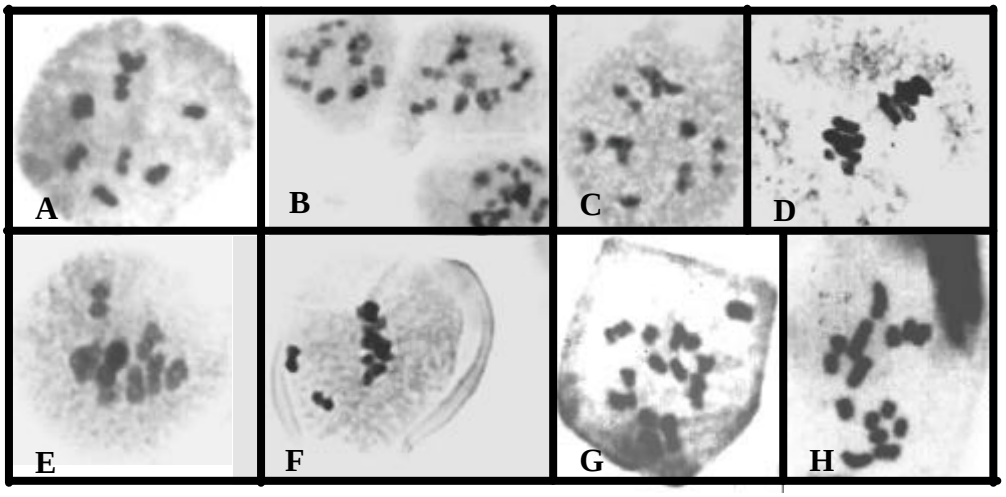

Fig 2. Somatic and meiotic chromosomes in some of the hybrids and the *Lachenalia carnosa* and *L. unicolor* parental specimens. A, *Spies 7052*, *L. unicolor* x *L. carnosa* (diakinesis); B, *Spies 6996*, *L. carnosa* (diakinesis); C, *Spies 7015*, *L. carnosa* x *L. splendida* (diakinesis); D, *Spies 7052*, *L. unicolor* x *L. carnosa* (metaphase I); E, *Spies 7052, L. unicolor* x *L. carnosa* (diakinesis); F, *Spies 6825, L. unicolor* (metaphase I); G-H, = *Spies 6838, L. unicolor* (mitotic material).