

The chromosomes of *Chortolirion* and *Poellnitzia* (Asphodelaceae: Alooideae)

G.F. SMITH*

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ABSTRACT

The somatic chromosomes of *Chortolirion angolense* (Baker) A. Berger and *Poellnitzia rubriflora* (L. Bol.) Uitewaal have been studied. Both taxa are monotypic genera in the subfamily Alooideae of the Asphodelaceae. Prior to this study *Chortolirion* had not been cytologically examined, while karyograms and idiograms have never been presented for *Poellnitzia*. *Chortolirion* and *Poellnitzia* are diploid with $2n = 14$ chromosomes and with a bimodal karyotype typical of the entire subfamily, comprising four pairs of long chromosomes and three pairs of short chromosomes. For *Poellnitzia* minute structural differences in karyotype morphology were noted between observed and published data, especially with regard to the presence of satellites.

UITTREKSEL

Die somatiese chromosome van *Chortolirion angolense* (Baker) A. Berger en *Poellnitzia rubriflora* (L. Bol.) Uitewaal is bestudeer. Beide takson is monotipiese genusse in die subfamilie Alooideae van die Asphodelaceae. *Chortolirion* is nog nooit voorheen sitologies ondersoek nie, terwyl kariogramme en idiogramme van *Poellnitzia* nog nie voorheen aangebied is nie. *Chortolirion* en *Poellnitzia* is beide diploïed met $2n = 14$ chromosome en elk beskik oor 'n bimodale kariotipe wat uit vier pare lang en drie pare kort chromosome bestaan. Hierdie kariotipe is soortgelyk aan dié van die oorgrote meerderheid takson in die Alooideae. Veral ten opsigte van die teenwoordigheid van satelliete bestaan daar by *Poellnitzia* fyn, strukturele verskille in kariotipe-morfologie tussen waargenome en gepubliseerde data.

INTRODUCTION

The subfamily Alooideae of the Asphodelaceae (*sensu* Dahlgren *et al.* 1985) includes about seven genera and more than 450 species. Most Alooideae taxa are found in subSaharan Africa, with a marked concentration of species and genera in southern Africa. All species of Alooideae are petaloid, succulent-leaved, rosulate or distichous perennials. They differ in size from miniatures barely 10 mm high (*Haworthia parksiana* Von Poellnitz) to trees of massive bulk up to 20 m tall (*Aloe bainesii* Thiselton-Dyer). In spite of this notable morphological variation the Alooideae is reasonably homogeneous in an evolutionary sense, unifying characters being the widespread occurrence of secondary thickening growth, leaf succulence, usually tubular petaline flowers and fusion of the perianth segments. Furthermore, in the entire subfamily the basic diploid karyotype ($2n = 14$; four pairs of long chromosomes and three pairs much shorter) is only very rarely altered (Brandham 1969).

The majority of species of Alooideae has been investigated cytologically. These studies were initiated early in the 20th century and have resulted in an extensive bibliography on the cytology of this group (for reviews see Muller 1941; Riley 1959a,b,c; Brandham 1971, 1983; Riley & Majumdar 1979). The intention of the present paper is to contribute to the cytotaxonomic knowledge of the Alooideae by the chromosome complement analysis of *Chortolirion* Berger and *Poellnitzia* Uitewaal, two monotypic genera. Chromosome studies have not been reported for *Chortolirion* to date and for *Poellnitzia* only four previous counts have been published, one of which is not readily accessible (Majumdar 1968 quoted by Riley

& Majumdar 1979). The previous cytological observations of *Poellnitzia* were all made on specimens cultivated in botanic gardens (Resende 1937; Viveiros 1949; Majumdar & Riley 1972). These early cytological studies therefore had no connection with natural populations. Problems which arise from such a practice have been discussed by Riley & Majumdar (1979).

C. angolense (Baker) A. Berger is a perennial, deciduous herb with a subterranean bulb and is widely distributed in the summer rainfall region of southern Africa. With short, annual, succulent shoots arising from the bulb in early spring, *C. angolense* presents a combination of geophytic and succulent habits (Figure 1). *P. rubriflora* (L. Bol.) Uitewaal is a low-growing, caulescent, succulent herb. Stems are up to 250 mm long and densely leaved. The ovate leaves are pungent-acuminate and up to 40 mm long (Figure 2). *P. rubriflora* has a restricted distribution in the south-western Cape Province of South Africa. This region receives its precipitation mainly during the winter months.

Chortolirion and *Poellnitzia* have been the subject of taxonomic confusion (Bayer 1974; cf. Smith 1988). A revision of the smaller genera of Alooideae currently under way has shown that both taxa warrant recognition as monotypic genera.

MATERIAL AND METHODS

The origin of the material of *C. angolense* studied is:

TRANSVAAL. — 2528 (Pretoria): in habitat in the Botanic Garden of the National Botanical Institute, Pretoria. (—CB). Smith 8 (PRU).

Material of *P. rubriflora* was collected at:

CAPE — 3320 (Montagu): southern side of farm 'Langverwagt 169', 5 km W of Bonnevale. (—CC). Stagner s.n. sub Smith 9 (PRU).

* Department of Plant Sciences, Potchefstroom University for Christian Higher Education, Potchefstroom 2520.
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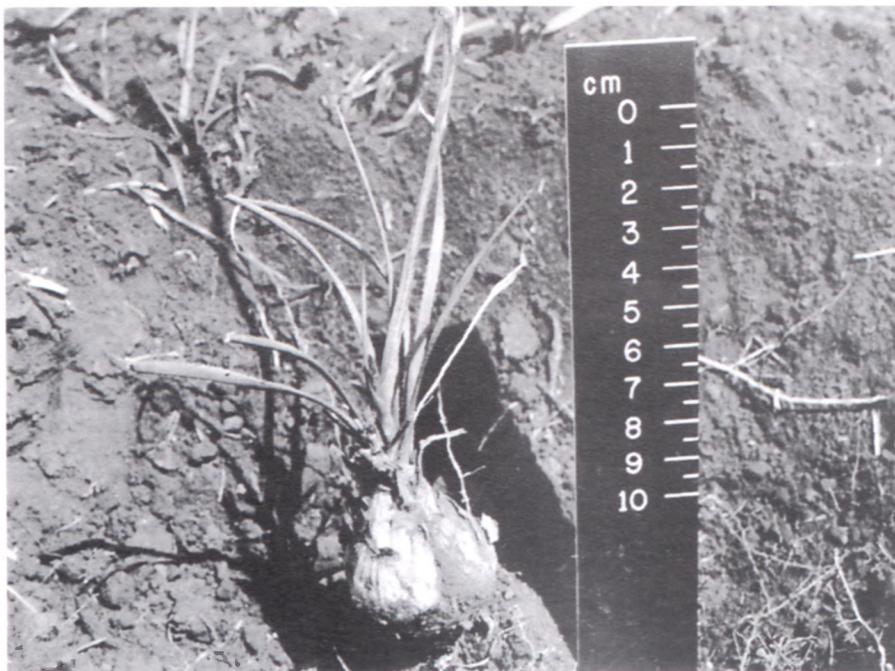


FIGURE 1.—Growth form of *Chortolirion angolense* in habitat in grassland at Potchefstroom, south-western Transvaal, South Africa. The soil has been removed to show the bulb and narrowly linear leaves.

Plants for cytological study were grown in the greenhouse of the Department of Botany, University of Pretoria. Somatic chromosomes were studied in root tips collected during late winter/early spring from vigorously growing potted plants. Actively elongating root tips (2–5 mm) were collected at 14:00 and pretreated with freshly prepared colchicine (0,05% in distilled water) for 2h and fixed in Pienaar's (1955) fixative (methanol: chloroform: propionic acid in the ratio 6:3:2) for 16h. Root tips were hydrolysed for 12 minutes in a 1M hydrochloric acid solution kept at 60°C and stained in Feulgen for 2,5 h. Squash preparations were made using a standard technique (Van der Schijff & Robberste 1976). Photographs were taken with a Nikon FX 35A microscope fitted with a Nikon Optiphot camera. Measurements were made directly from mitotic preparations using an eyepiece micrometer. For chromosome nomenclature, the terminology introduced by Levan

et al. (1964) has been followed. The karyograms (Figures 3B; 4B) and idiograms (Figures 3C; 4C) are arranged according to chromosome length.

RESULTS

In *Chortolirion* and *Poellnitzia* the somatic chromosome number $2n = 14$ was constant in all metaphases with karyotypes as in Figure 3B (*Chortolirion*) and Figure 4B (*Poellnitzia*). For both genera the haploid set is asymmetrical, producing a distinctly bimodal karyotype which consists of four long and three short chromosomes. No major chromosomal differences between *Chortolirion* and *Poellnitzia* could be detected. Furthermore, no marked size variation was encountered within the sets of long and short chromosomes in the respective genera.



FIGURE 2.—*Poellnitzia rubriflora* (arrowed) in habitat at Langverwacht near Bonnievale, south-western Cape Province, South Africa. The species usually grows in association with sclerophyllous xerophytes.

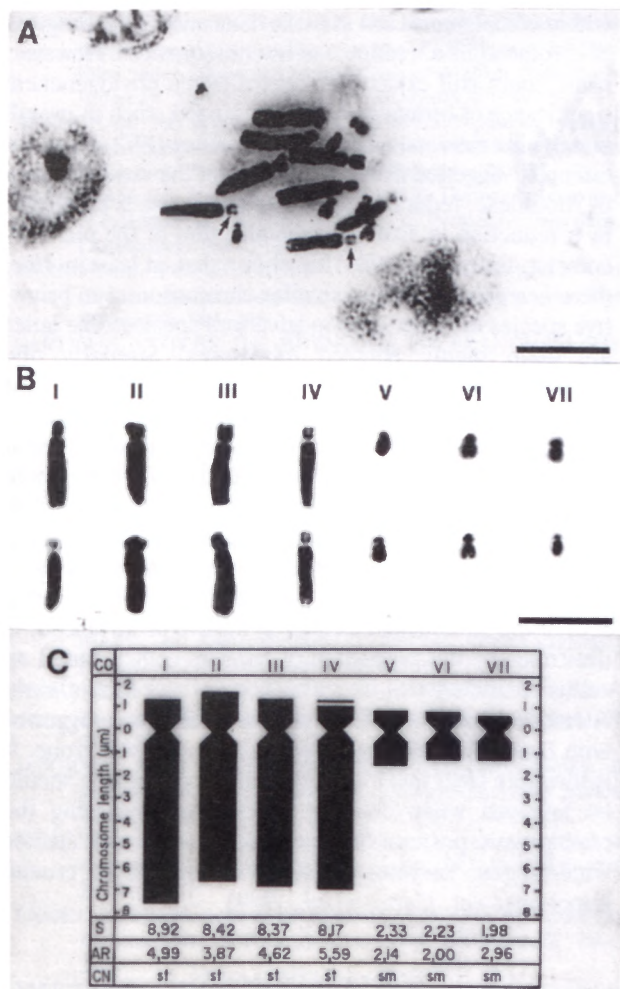


FIGURE 3.—*Chortolirion angolense*. A, mitotic metaphase, arrows indicate satellites; B, karyogram; C, idiogram. CO, chromosome ordering (overall length); S, chromosome size; AR, arm ratio (= long arm/short arm); CN, chromosome nomenclature after Levan *et al.* (1964). Chromosome II is clearly the L1 (long chromosome with longest short arm) and chromosome I is the L2, which is often longer than the L1.

Karyotype of *C. angolense*

Each of the long chromosomes (I–IV) is of the st type, while all the short chromosomes (V–VII) are of the sm type (Figure 3C). Satellites were detected at the ends of the short arms of the shortest long chromosome (IV) (Figure 3A, B). Secondary chromosome constrictions are absent from the long arms of the short chromosomes (V–VII). Such constrictions occur widespread in taxa of the Alooideae (Brandham 1971).

One chromosome I has a short arm much smaller than that of the other (Figure 3A, B). Since this phenomenon was not encountered consistently, it is probably not associated with an interchange or a deletion, but rather due to the orientation of the chromosome on the slide.

Karyotype of *P. rubriflora*

The longest long chromosome (I) is of the sm type while the three other long chromosomes (II–IV) are of the st type. The longest short chromosome (V) is of the m type and the two remaining short chromosomes (VI–VII) are of the sm type (Figure 4C). Satellites were not detected

on any of the chromosomes (Figure 4A, B). Secondary constrictions of unknown nature were observed on the short arms of the long chromosomes (except I) and the long arms of all the short chromosomes.

DISCUSSION

The chromosomes of all plants studied were found to match the markedly bimodal karyotype which has been observed in every species of Alooideae to date. The basic number is $\times = 7$ and comprises four long and three short chromosomes. To date a large number of intra- and intergeneric hybrids produced in the Alooideae have been described and figured (Riley 1948; Jacobsen & Rowley 1955, 1973; Rowley 1968, 1976; Graf 1980). Although some of the hybrids are sterile, others are fully fertile (Brandham 1973). This testifies to the close cytogenetical relationship which exists amongst taxa of the Alooideae (Rollins 1953). *Chortolirion* and *Poellnitzia* are no exceptions to this rule, *Chortolirion* having been crossed successfully with *Aloe* and *Poellnitzia* with *Gasteria* (Rowley 1980, 1982; Brandham 1990).

Both *Chortolirion* and *Poellnitzia* are diploid with $2n = 14$ chromosomes, in common with the great majority of species in the subfamily Alooideae as a whole. Although *Aloe*, *Gasteria* and *Haworthia* are known to include a

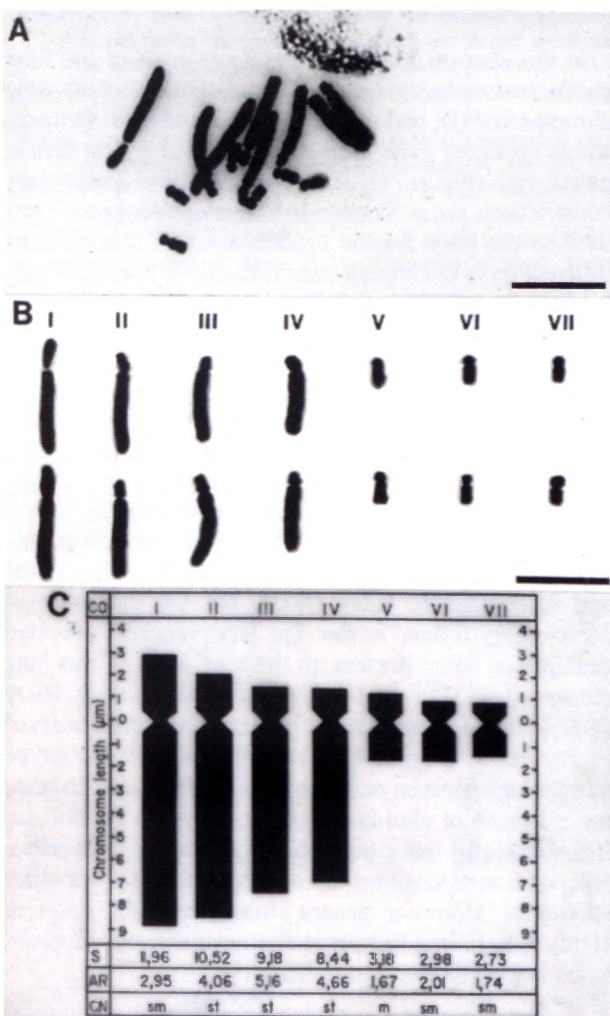


FIGURE 4.—*Poellnitzia rubriflora*. A, mitotic metaphase; B, karyogram; C, idiogram. CO, chromosome ordering (overall length); S, chromosome size; AR, arm ratio (= long arm/short arm); CN, chromosome nomenclature after Levan *et al.* (1964).

variety of polyploids (Riley & Majumdar 1965; Brandham & Johnson 1977a, 1982; Cutler *et al.* 1980; Brandham 1982; Motohashi *et al.* 1985), none of the individuals included in the present study contained different levels of ploidy. In the Alooideae polyploidy can give rise to local bursts of speciation (Brandham & Cutler 1981; Cutler *et al.* 1980).

Some authors have previously established a nomenclature for the chromosomes of taxa of the Alooideae (L1 through L4 for the long chromosomes and S1 through S3 for the short chromosomes) (Snoad 1951; Riley & Majumdar 1966; cf. Brandham 1971 for an explanation; Brandham & Johnson 1977b; Brandham & Cutler 1978, 1981). However, the identification of individual chromosomes (especially the short chromosomes) and their subsequent classification is very difficult and no attempt was made to do so here. Brandham (1983) eventually concluded that only L1 (long chromosome with longest short arm) could be identified with certainty in somatic and meiotic cells. However, recognition even of the L1 chromosome, which usually is the only submetacentric long chromosome, can occasionally yield difficulties as is shown in reports of Mogford & Rautenbach (1981), Vosa & Bayer (1981) and Vosa & Mogford (1981). In *Chortolirion* chromosome II is clearly the L1 and chromosome I is the L2, which is often longer than the L1 (Figure 3C). In *Poellnitzia* chromosome I is the L1 (Figure 4C).

In the case of *Poellnitzia* the short arms of the long chromosomes, with the exception of the longest long chromosome (I), and the long arms of the short chromosomes appeared to be thinner in their mid-region than at either end (Figure 4). These thin regions [secondary constrictions *sensu* Sharma & Mallick (1966)] have been used as the basis for the establishment of a number of different types of chromosomes (Sharma & Mallick 1966). As Brandham (1971) justifiably remarks, the constrictions could possibly be due to variations in the degree of coiling of the chromatids and are structurally insignificant. Thinner regions on the chromosomes were virtually absent from *Chortolirion*, with the exception of satellites present on the short arms of the two shortest long chromosomes (IV) (Figure 3).

The chromosome count of *Poellnitzia* agrees with that reported by Resende (1937), Viveiros (1949), Riley (1961) and Majumdar & Riley (1972), but the chromosome morphology differs in that the latter workers reported satellites as being present on the long arms of two long chromosomes [L1 and L4 *sensu* Majumdar & Riley (1972)]. In the present study no satellites were observed in *Poellnitzia* (Figure 4). The differences in karyotype morphology between published and observed data indicate the existence of chromosomal variability in *Poellnitzia*. It is noteworthy that considerable variation in the number and position of satellites has also been reported for other species of Alooideae genera (Brandham 1971; Spies & Hardy 1983). This karyotype character therefore appears to be taxonomically insignificant.

Knowledge of chromosome morphology in closely related taxa is of primary importance in biosystematic and taxonomic studies. In the Alooideae studies of karyotypes are particularly informative, where the asymmetry in size

within complements can assist in determining the progress of chromosome alteration and its consequences. However, some doubt still exists with regard to the phylogenetical significance of certain karyological aspects, such as overall size of chromosomes. Majumdar & Riley (1972) have, for example, suggested that in the species of the various genera of Alooideae, reduction in chromosome size is paralleled by a reduction in size and specialization of the plant. In contrast, Brandham (1983) has shown that, at least in *Aloe*, there is a gradation from smaller chromosomes in primitive species to larger ones in advanced species. The latter are often highly adapted miniatures. Similarly, the chromosomes of *Gasteria* are larger than those of *Aloe* (Brandham 1990). *Gasteria* is generally regarded as phylogenetically more advanced than *Aloe* (Van Jaarsveld 1989). The karyotypes of species of *Gasteria* are also more acutely bimodal than those of species of the more primitive *Aloe*. Based on chromosome size and increased bimodality *Poellnitzia* would therefore appear to be more advanced than *Chortolirion*. However, the complexity of interpreting taxa of the Alooideae as either primitive or advanced, is illustrated by the suggestion of Hayashi (1987), based on callus characteristics, that *Poellnitzia* is a relict of early Alooideae. For *Chortolirion*, a close affinity was suggested with *Haworthia* subgenus *Haworthia*, a derived group. It is therefore clear that a multidisciplinary approach should be followed when drawing conclusions regarding the phylogenetic position of Alooideae taxa. In such studies wide-ranging karyological analyses will be of crucial importance (cf. Carter *et al.* 1984).

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