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Cytogenetic studies in some representatives of the subfamily Pooideae (Poaceae) in South Africa. 2. The tribe Aveneae, subtribes Phalaridinae and Alopecurinae

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ABSTRACT

This is a report on chromosome numbers for the subtribes Phalaridinae and Alopecurinae (tribe Aveneae) which are, to a large extent, naturalized in South Africa. Chromosome numbers of 34 specimens, representing nine species and four genera, are presented. These numbers include the first report on *Agrostis avenacea* Gmel. (n = 4x = 28). New ploidy levels are reported for *Phalaris aquatica* L. (n = x = 7), *Agrostis barbuligera* Stapf var. *barbuligera* (n = 2x = 14 and n = 4x = 28) and *A. lachnantha* Nees var. *lachnantha* (n = 3x = 21).

INTRODUCTION

The first paper in this series on chromosome numbers of representatives of the tribe Aveneae in South Africa, indicated the importance of determining the ploidy levels and basic chromosome numbers of naturalized and endemic flora in South Africa (Spies *et al.* 1996). This second paper in the series is restricted to the subtribes Phalaridinae and Alopecurinae.

The subtribe Phalaridinae Rchb. consists of three genera (Clayton & Renvoize 1986), of which two are represented in South Africa, i.e. *Anthoxanthum* L. and *Phalaris* L. (Gibbs Russell *et al.* 1990). The subtribe Alopecurinae Dumort. consists of 27 genera (Clayton & Renvoize 1986), with only six being represented in South Africa (Gibbs Russell *et al.* 1990), i.e. *Agrostis* L., *Ammophila* Host, *Calamagrostis* Adans., *Gastridium* P.Beauv., *Lagurus* L. and *Polypogon* Desf. (Gibbs Russell *et al.* 1990).

The aim of this study was to determine the chromosome numbers, polyploid levels and meiotic chromosome behaviour of the South African representatives of the tribe Aveninae. These results will eventually be compared with results of indigenous and endemic taxa to compare the frequency of polyploidy between indigenous and introduced grasses.

MATERIALS AND METHODS

The material was either collected and fixed in the field, or living material was collected in the field and planted in the nurseries of either the Department of Botany and Genetics, University of the Orange Free State (Bloemfontein), or the National Botanical Institute (Pretoria), where the cytogenetic material was collected and fixed. The material used and the collecting localities are listed in Table 1. Voucher specimens are housed in the Geo Potts Herbarium, Department of Botany and Genetics, University of the Orange Free State, Bloemfontein (BLFU) or the National Herbarium, Pretoria (PRE).

Anthers were squashed in aceto-carmine and meiotically analysed (Spies *et al.* 1996). Chromosome numbers are presented as haploid chromosome numbers to conform to previous papers on chromosome numbers in this journal (Spies & Du Plessis 1986). These numbers were compared with results published in the chromosome atlasses of Fedorov (1969), Ornduff (1967–1969), Moore (1970–1972, 1974, 1977), Goldblatt (1981, 1983, 1985, 1988) and Goldblatt & Johnson (1990, 1991, 1994). Genome homology was determined in some tetraploid specimens according to the models described by Kimber & Alonso (1981).

RESULTS AND DISCUSSION

The subtribe Phalaridinae Rchb. consists of three genera, of which two are represented in South Africa (Clayton & Renvoize 1986). The genus *Anthoxanthum* consists of 18 species, four of which are endemic to South Africa [*A. brevifolium* Stapf, *A. dregeanum* (Nees) Stapf, *A. ecklonii* (Nees ex Trin.) Stapf & *A. tongo* (Trin.) Stapf] and a fifth species, *A. odoratum* L., which is naturalized (Gibbs Russell *et al.* 1990). The genus *Phalaris* L. comprises 15 species, of which six are naturalized in this country [*P. angusta* Nees ex Trin., *P. aquatica* L., *P. arundinacea* L., *P. canariensis* L., *P. minor* Retz. and *P. paradoxa* L.] (Gibbs Russell *et al.* 1990). No *Phalaris* species is indigenous to South Africa (Gibbs Russell *et al.* 1990).

Apparently only one specimen of the genus *Anthoxanthum* has sofar been cytogenetically studied and reported in South Africa, namely of *A. tongo* (Trin.) Stapf for which a haploid chromosome number of 20 + 0–5B has been established by one of our laboratories (Spies & Voges 1988). Reports of haploid chromosome numbers of n = 5 and 10 from other parts of the world are frequent but numbers such as n = 20, 35 or 45 are rarely reported

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TABLE 1.—Haploid chromosome numbers of representatives of the subtribes Phalaridinae, Alopecurinae (Poaceae, Pooideae, Aveneae) in southern Africa, with their voucher specimen numbers and specific localities [arranged according to the system of Edwards & Leistner (1971)]

Taxon	Voucher	n =	Locality
Subtribe Phalaridinae Rchb.			
Phalaris aquatica L.	*Spies 3676*	7&14	WESTERN CAPE.—3318 (Cape Town): 5 km from Riebeek-Kasteel to Malmesbury in Bothmaskloof Pass, (–BD)
P. minor Retz.	*Davidse 33237*	14	NORTHERN CAPE.—2917 (Springbok): 6 km from Springbok to Kleinsee, (–DB)
	Davidse 33272	7	NORTHERN CAPE.—2917 (Springbok): 6 km from Springbok to Kleinsee, (–DB)
	Spies 4284	14	NORTHERN CAPE.—2917 (Springbok): 17 km from Springbok to Hondeklipbaai, (–DB)
	Spies 4880	14	NORTHERN CAPE.—2917 (Springbok): 20 km from Springbok to Kleinsee, (–DB)
	Spies 3108	14	WESTERN CAPE.—3118 (Vanrhynsdorp): Gifberg, (–DC)
	Spies 4566	14	WESTERN CAPE.—3318 (Cape Town): 7 km from Yzerfontein to Darling, (–AC)
	Spies 4572	14	WESTERN CAPE.—3318 (Cape Town): 5 km from Langebaan to Langebaanweg, (–DC)
	Spies 5306	14	NORTHERN CAPE.—3119 (Calvinia): 10 km from Nieuwoudtville to Clanwilliam, (–AC)
	Spies 4978	14	NORTHERN CAPE.—3119 (Calvinia): 88 km from Nieuwoudtville to Clanwilliam in Botterkloof, (–CC)
	Spies 4593	14	WESTERN CAPE.—3320 (Montagu): 22 km from Villiersdorp to Worcester via Koppies, (–AD)
	Spies 4509	14	WESTERN CAPE.—3420 (Bredasdorp): 1 km north of De Hoop Nature Reserve, (–CA)
P. paradoxa L.	*Spies 5395*	7	WESTERN CAPE.—3319 (Worcester): 11 km from Ceres in Mitchell's Pass, (–AD)
Subtribe Alopecurinae Dumort.			
Agrostis avenacea Gmel.	*Saayman 78*	28	MPUMALANGA (EASTERN TRANSVAAL).—2430 (Pilgrim's Rest): 10 km from Graskop to Bosbokrand, (–DD)
	Spies 3922	28	WESTERN CAPE.—3319 (Worcester): McGregor FM Tower, (–DB)
	Spies 3522	28	WESTERN CAPE.—3323 (Willowmore): Spitskop, in Prince Alfred Pass on road between Knysna and Uniondale, (–CC)
A. barbuligera Stapf var. *barbuligera*	*Saayman 88*	28	MPUMALANGA (EASTERN TRANSVAAL).—2430 (Pilgrim's Rest): 2 km from Graskop to Pilgrim's Rest, (–DD)
	Saayman 57	14	MPUMALANGA (EASTERN TRANSVAAL).—2530 (Lydenburg): 23 km from Lydenburg to Sabie, (–BA)
	Spies 2467	14	EASTERN CAPE.—3126 (Queenstown): Penhoek Pass, (–BC)
A. lachnantha Ness var. *lachnantha*	*Saayman 89*	28	MPUMALANGA (EASTERN TRANSVAAL).—2430 (Pilgrim's Rest): 2 km from Graskop to Pilgrim's Rest, (–DD)
	Saayman 76	28	MPUMALANGA (EASTERN TRANSVAAL).—2430 (Pilgrim's Rest): 14 km from Graskop to Sabie, (–DD)
	Spies 2662	21	(ORANGE) FREE STATE.—2826 (Brandfort): Glen Agricultural College, (–CD)
	Spies 2504	21	EASTERN CAPE.—3027 (Lady Grey): near Barkly East, (–DC)
	Spies 4726	21	EASTERN CAPE.—3027 (Lady Grey): 49 km from Rhodes to Lundean's Nek, (–DD)
	Spies 2467	21	EASTERN CAPE.—3126 (Queenstown): Penhoek Pass, (–BC)
Lagurus ovatus L.	*Davidse 33570*	7	EASTERN CAPE.—3325 (Port Elizabeth): King Neptune Beach, (–DC)
	Spies 5227	7	EASTERN CAPE.—3325 (Port Elizabeth): Greenacres, (–DC)
	Spies 3894	7	WESTERN CAPE.—3418 (Simonstown): Silvermine Nature Reserve, (–AD)
Polypogon monspeliensis (L.) Desf.	*Spies 2895*	7	NAMIBIA.—2617 (Bethanie): Fish River Bridge on road to Lüderitz, (–DD)
	Spies 2945	14	NORTHERN CAPE.—2816 (Oranjemund): on riverbank at crossing of Orange River (Dreigratdrif), (–BB)
	Spies 3083a	14	WESTERN CAPE.—3118 (Vanrhynsdorp): Gifberg Pass, (–DC)
	Spies 5199	14	EASTERN CAPE.—3324 (Steytlerville): 34 km from Patensie to Willowmore, (–CB)
P. viridis (Gouan) Breistr.	*Spies 2912*	14	NAMIBIA.—2615 (Lüderitz): in Lüderitz, (–CB)
	Spies 5201	14	EASTERN CAPE.—3324 (Steytlerville): 34 km from Patensie to Willowmore, (–CB)

(Goldblatt 1981, 1983, 1985, 1988; Goldblatt & Johnson 1990, 1991, 1994). In addition to these numbers based on x = 5, three reports of n = 28 for *A. borii*, therefore x = 7, have been published (Goldblatt 1981, 1985, 1988). With only three reports suggesting a basic chromosome number of seven rather than the five indicated in 55 other reports, we suggest that the basic chromosome number of the genus *Anthoxanthum* is five, as our research indicates. However, a re-investigation of this genus will help to clarify the position of *A. borii* in the genus.

Three species of the genus *Phalaris* were included in this study (Table 1). The only specimen of *P. aquatica* investigated, had both diploid (n = x = 7) and tetraploid (n = 2x = 14) chromosome numbers (Figure 1A, B). We attribute this apparent discrepancy to cell fusion (Spies & Van Wyk 1995). This process involves the formation of polynucleate microspores after cell fusion and the consequent formation of polyhaploid pollen. This phenomenon is frequently observed in grasses and was discussed by Spies & Van Wyk (1995). *Phalaris aquatica* was represented by a diploid specimen (n = x = 7) in this study. This is a lower chromosome number than the tetraploid one previously described (Moore 1971; Goldblatt 1983, 1988). Meiosis was normal at both ploidy levels with only bivalents observed.

One specimen of *P. minor* was diploid (Figure 1C, D) and the rest (10 specimens) tetraploid (Figure 1F–H). With the exception of an anaphase I bridge in one cell of one specimen, meiosis was normal and only bivalents were observed in the *P. minor* specimens. *Phalaris minor* specimens proved to be either diploid (n = x = 7) or tetraploid (n = 2x = 14) in this study, confirming the tetraploid level previously described (Moore 1971; Goldblatt 1983, 1985; Goldblatt & Johnson 1990). In a previous study by one of our laboratories a hexaploid specimen (n = 3x = 21) was observed (Spies & Voges 1988). In addition to these ploidy levels octoploid specimens (n = 4x = 28) have also been described in the literature (Moore 1977). The presence of diploid and possibly allotetraploid specimens in the same species, should render a morphological study of this species worthwhile.

The only *P. paradoxa* specimen studied proved to be a diploid (n = x = 7) with normal meiosis, thus supporting previous reports on this species (Moore 1971, 1977; Goldblatt 1981, 1983). A few species of *Phalaris* with a basic chromosome number of six have been reported (Fedorov 1969; Goldblatt 1981, 1983, 1985, 1988; Goldblatt & Johnson 1994). However, in South Africa all species studied had a basic chromosome number of seven.

The subtribe Alopecurinae Dumort. consists of 27 genera (Clayton & Renvoize 1986), with only six being represented in South Africa (Gibbs Russell *et al.* 1990). *Agrostis* L. consists of 220 species worldwide (Clayton & Renvoize 1986), eight of which are indigenous to South Africa [*A. barbuligera* Stapf, *A. bergiana* Trin., *A. continuata* Stapf, *A. eriantha* Hack., *A. lachnantha* Nees, *A. polypogonoides* Stapf, *A. schlechteri* Rendle and *A. subulifolia* Stapf] and three species are naturalized [*A. avenacea* Gmel., *A. gigantea* Roth and *A. montevidensis* Spreng. ex Nees] (Gibbs Russell *et al.* 1990). Twelve specimens, representing three species of the genus *Agrostis*, were included in this study (Table 1). The three *A. avenacea* specimens were octoploid (n = 4x = 28) (Figure 2A, B). This seems to be the first chromosome number report for this species. Two *A. barbuligera* var. *barbuligera* specimens were tetraploid (Figure 2D) and one octoploid. Both ploidy levels differ from the previously described hexaploid (n = 3x = 21) level from one of our laboratories (Spies & Du Plessis 1986). Four hexaploid specimens were observed in *A. lachnantha* var. *lachnantha*, as well as two octoploid specimens (Figure 2E, F). The octoploid specimens conform with the number pre-

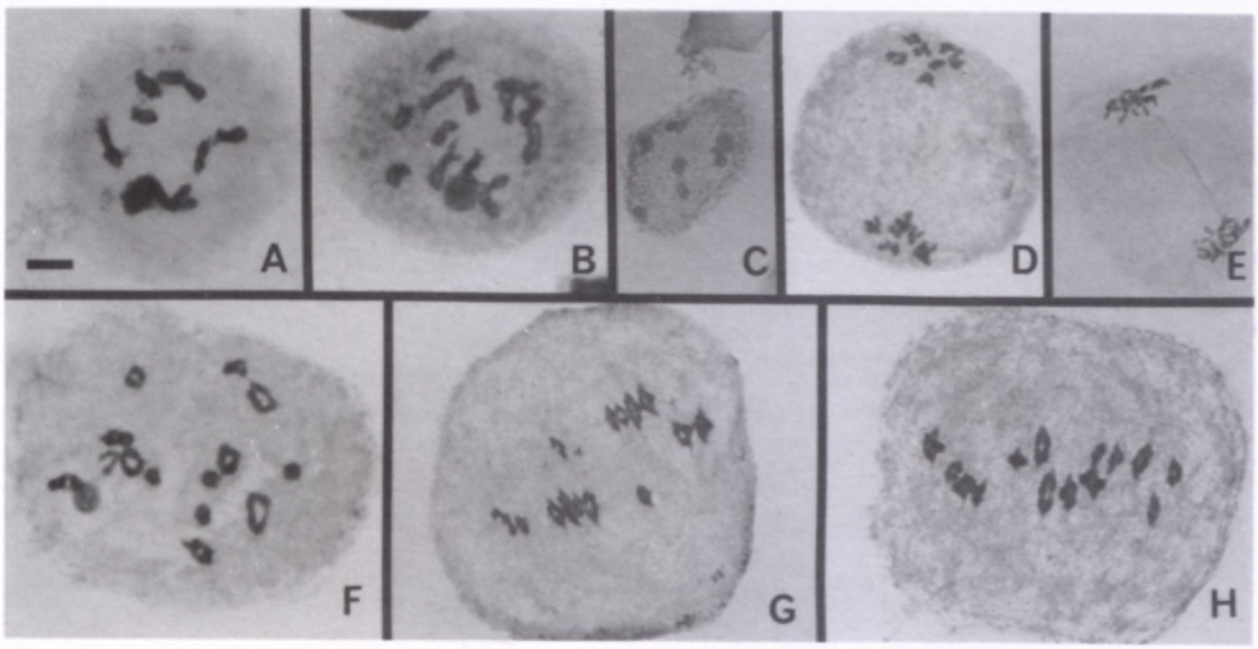

FIGURE 1.—Photomicrographs of meiotic chromosomes in the genus *Phalaris*. A, B, *P. aquatica*, *Spies 3676*, diplotene/diakinesis with 7_{II} and 14_{II} respectively; C, *P. minor*, *Davidse 33272*, diakinesis with 7_{II}; D, *P. paradoxa*, *Spies 5395*, anaphase I with 7 chromosomes in each pole; E, *P. minor*, *Spies 4593*, late anaphase I with a chromatid bridge; F, *P. minor*, *Spies 4284*, diakinesis with 14_{II}; G, *P. minor*, *Spies 4978*, metaphase I with 14_{II}; H, *P. minor*, *Davidse 33237*, metaphase I with 14_{II}. Scale bar: 10 μm.

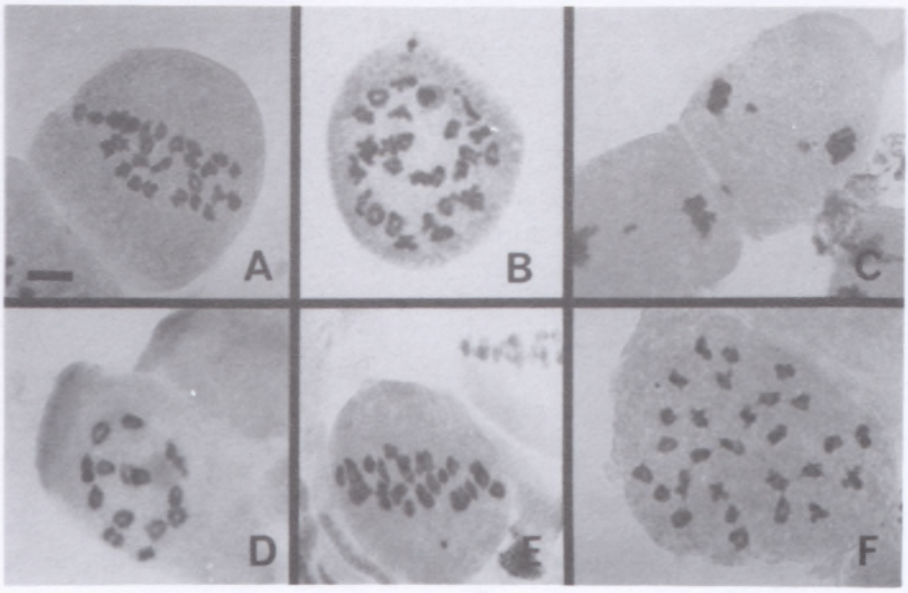

FIGURE 2.—Photomicrographs of meiotic chromosomes in the genus *Agrostis*. A, *A. avenacea*, *Saayman 78*, early metaphase I with 28_{II}; B, *A. avenacea*, *Spies 3522*, diakinesis with 28_{II}; C, D, *A. barbuligera* subsp. *barbuligera*, *Saayman 57*; C, telophase I cells with micronuclei; D, diakinesis with 14_{II}; E, F, *A. lachnantha* subsp. *lachnantha*; E, *Saayman 89*, metaphase I with 28_{II}; F, *Saayman 89*, diakinesis with 28_{II}. Scale bar: 10 μm.

viously described by us (Spies & Du Plessis 1986). Meiosis was normal in almost all specimens, excepting one telophase II cell of *A. barbuligera*, where micronuclei were observed (Figure 2C).

Ammophila Host is a north temperate genus with two species, with *A. arenaria* (L.) Link naturalized in South Africa (Gibbs Russell *et al.* 1990). Although this species was not represented in this study, all reports indicate that representatives from the rest of the world are tetraploid 2n = 4x = 28 (Ornduff 1967; Moore 1970, 1972, 1977; Goldblatt 1983; Goldblatt & Johnson 1994).

Calamagrostis Adans. is a very large genus with ± 270 species (Clayton & Renvoize 1986) with only one species represented in South Africa, *C. epigeios* (L.) Roth (Gibbs Russell *et al.* 1990). Chromosome number reports indicate the presence of tetraploid to octoploid specimens for this species (Moore 1977; Goldblatt 1981, 1983, 1985, 1988; Goldblatt & Johnson 1990, 1994).

Gastridium phleoides (Nees & Meyen) C.E.Hubb. is the only species of *Gastridium* P.Beauv. introduced to South Africa (Gibbs Russell *et al.* 1990). The only chromosome number report found for this species, indicates 2n = 4x = 28 (Goldblatt 1981).

The monotypic genus *Lagurus* L. was also introduced to South Africa (Gibbs Russell *et al.* 1990). All *L. ovatus* specimens studied were diploid (Figure 3) with normal meiosis in most cells, excepting a bivalent lying away from the metaphase plate in one cell (Figure 3D) and a laggard in a late anaphase II cell (Figure 3E). The diploid chromosome number observed during this study supports previous reports (Moore 1970, 1972, 1977; Goldblatt 1981, 1983; Goldblatt & Johnson 1994).

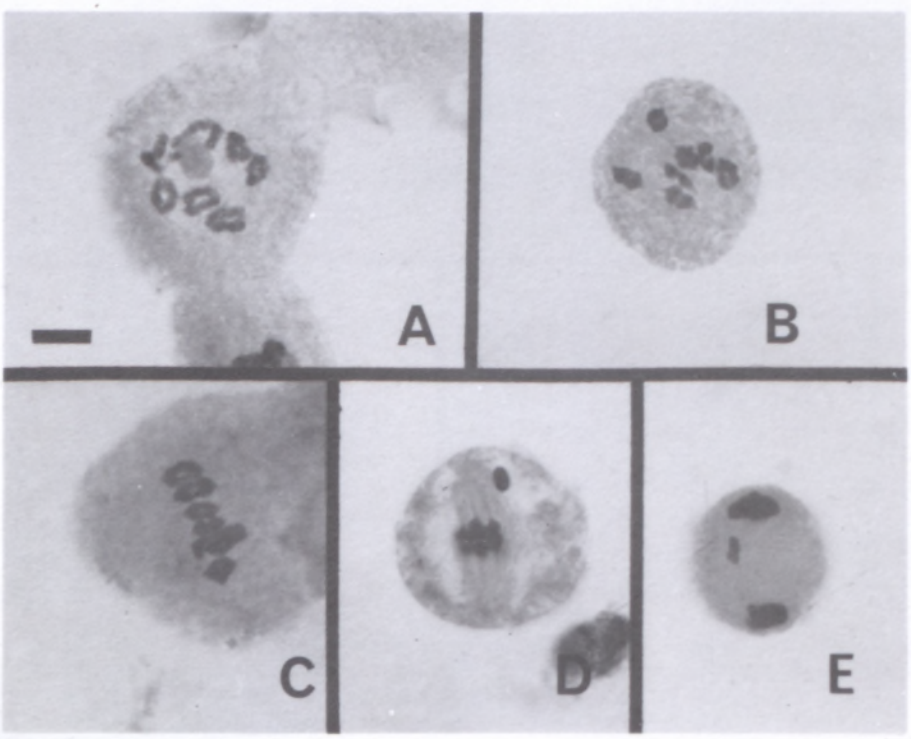

FIGURE 3.—Photomicrographs of meiotic chromosomes in *Lagurus ovatus*. A, *Spies 3894*, diakinesis with 7_{II}; B, *Davidse 33570*, diakinesis with 7_{II}; C, *Spies 5227*, metaphase I with 7_{II}; D, *Davidse 33570*, metaphase I with one bivalent away from the metaphase plate; E, *Spies 3894*, telophase I with a laggard. Scale bar: 10 μm.

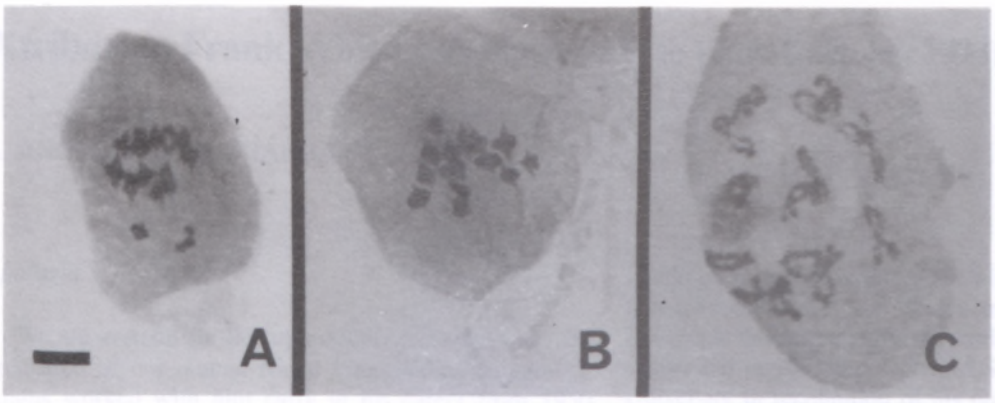

FIGURE 4.—Photomicrographs of meiotic chromosomes in the genus *Polypogon*. A, *P. monspeliensis*, *Spies 5199*, early metaphase I with 14_{II}; B, *P. monspeliensis*, *Spies 3083*, early metaphase I with 14_{II}; C, *P. viridis*, *Spies 5201*, diplotene with 14_{II}. Scale bar: 10 μm.

The last genus of the Alopecurinae represented in this country is *Polypogon* Desf. This genus comprises 18 species worldwide and is represented by two naturalized [*P. monspeliensis* (L.) Desf. and *P. viridis* (Gouan) Breistr.] and two indigenous [*P. griquensis* (Stapf) Gibbs Russ. and *P. strictus* Nees] species in South Africa (Gibbs Russell *et al.* 1990). Two species of *Polypogon* were studied (Table 1). Three specimens of *P. monspeliensis* were tetraploid (Figure 4A, B), whereas a single specimen was found to be diploid. This confirms previous reports on this species in which diploid to hexaploid specimens have been described (Moore 1970–1972, 1974, 1977; Goldblatt 1981, 1983, 1985; Goldblatt & Johnson 1990, 1994). Both *P. viridis* specimens studied were tetraploid (Figure 4C), thus confirming previous reports (Goldblatt & Johnson 1990, 1994). Meiosis was normal and only bivalents were formed in all specimens.

The two genera representing the subtribe Phalaridineae appear to have two different basic chromosome numbers, i.e. *Anthoxanthum* with five and *Phalaris* with both six and seven. A thorough phylogenetic study based on molecular data of this subtribe could be very useful in determining the evolutionary significance of the change in chromosome number. A basic chromosome number of seven is present in all the genera of the subtribe Alopecurinae present in South Africa.

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