

# Meiotic chromosome behaviour in *Cenchrus ciliaris* (Poaceae: Panicoideae)

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**Keywords:** *Cenchrus ciliaris*, chromosomes, meiosis, Panicoideae, Poaceae, polyploidy

## ABSTRACT

A basic chromosome number of  $x = 9$  has been confirmed for *Cenchrus ciliaris* L. Polyploidy is common and levels vary from tetraploid to hexaploid. Aneuploidy is reported for a single specimen, where two chromosomes of a single genome were lost. Various meiotic irregularities were observed. The highest incidence of meiotic abnormalities was observed in the pentaploid specimens. This was attributed to their uneven polyploid level. All specimens varied from segmental allopolyploid to allopolyploid.

## INTRODUCTION

The classification of the genus *Cenchrus* L. is complex (De Lisle 1963) and it is sometimes difficult to describe species on gross morphological characters alone (Chapman 1990). Meiotic chromosome behaviour can make a vast contribution to the classification process.

The aim of the present study was to use meiotic chromosome behaviour in *C. ciliaris* in order to deduce the polyploid origin of the specimens studied, as well as the chromosomal variation within and among populations of *C. ciliaris* in South Africa.

## MATERIALS AND METHODS

Specimens were collected and fixed in the veld. Voucher herbarium specimens are housed in the Geo Potts Herbarium, Department of Botany and Genetics, University of the Orange Free State, Bloemfontein (BLFU) (Table 1).

Slides were prepared for meiotic analysis (Visser & Spies 1994). A minimum of 20 cells of each of the following stadia were studied: diakinesis, metaphases I and II, anaphases I and II and telophases I and II. The following were recorded: gametic chromosome numbers, the number of univalents, laggards and micronuclei during metaphase I, anaphase I and II, and telophase I and II, respectively. The number of chiasmata per cell was inferred from the chromosome configurations observed during diakinesis or metaphase I.

The genomic constitution of some of the tetraploid plants was calculated, based on the models proposed by Kimber & Alonso (1981). Expected chromosome configurations for each of the proposed models (4:0, 3:1, 2:1:1 and 2:2) were calculated and compared with those observed. The average sum of squares (SS) between the expected and the observed values were calculated. The

relative affinity between the pairs of genomes was expressed as an  $x$ -value. An  $x$ -value of 0.5 indicated a close affinity between corresponding (homologous) genomes, whereas non-homologous genomes were represented by an  $x$ -value of 1. The model with the lowest SS-value provided the best 'fit' for that particular specimen. The specific model was then considered to represent the genomic constitution of the specimen.

## RESULTS

From all the meiotic stages studied, only metaphase I, anaphase I and telophase I, proved to contribute to meiotic analyses. Four ploidy levels, namely aneuploidy ( $n = 2x - 1 = 17$ ), tetraploidy ( $n = 2x = 18$ ), pentaploidy ( $n = 5/2x = 45/2$ ) and hexaploidy ( $n = 3x = 27$ ) were observed, based on a basic chromosome number of  $x = 9$  (Table 2).

A wide range of meiotic abnormalities were observed for *Cenchrus ciliaris*. These abnormalities included the presence of univalents during metaphase I, chromosome and chromatid laggards during anaphases I and II respectively, uneven segregation of chromosomes during anaphase I, anaphase I bridges and micronuclei during telophases I and II. Two additional meiotic abnormalities were observed, namely precocious disjunction of chromosomes into chromatids during anaphase I and the absence of cytokinesis at the end of telophase I.

The presence of univalents, chromosome and/or chromatid laggards and micronuclei, were compared among the tetraploid specimens. A histogram was plotted, based on the percentage of cells containing the various numbers of univalents, chromosome and/or chromatid laggards and micronuclei (Figure 1). The topography of the curves corresponded. This fact indicated that, for *C. ciliaris*, the number of univalents present during metaphase I, is the most likely reason why there is an increase in the chromosome and/or chromatid laggards observed during anaphase I. This in turn, could have contributed to the formation of micronuclei during telophase I. The absence of various peaks in the hyperboles indicates that no distinct chromosomal groups exist in the tetraploid specimens. Therefore, the average percentages for each

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TABLE 1.—List of *Cenchrus ciliaris* specimens studied, voucher specimen numbers and localities according to the degree reference system (Edwards & Leistner 1971)

n	Locality	Voucher
17	NORTH-WEST.—2627 (Potchefstroom): in Potchefstroom, on route to Orkney. (–CA)	Spies 5883
18	GAUTENG.—2528 (Pretoria): near Pretoria. (–CC)	Spies 5645
	NORTH-WEST.—2627 (Potchefstroom): in Potchefstroom, on route to Orkney. (–CA)	Spies 5653, 5654
	GAUTENG.—2628 (Johannesburg): Grassmere Garage, Johannesburg. (–AB)	Spies 5646
	NORTH-WEST.—2724 (Taung): 101 km from Kuruman to Vryburg. (–AB)	Spies 5527
	NORTH-WEST.—2725 (Bloemhof): in Amalia, on route to Schweizer-Reneke. (–AA)	Spies 5538
	NORTH-WEST.—2725 (Bloemhof): 2 km from Britten to Christiana. (–CB)	Spies 5542, 5543
	FREE STATE.—2726 (Odendaalsrus): 8 km from Wesselsbron to Bultfontein. (–CD)	Spies 5659
	FREE STATE.—2726 (Odendaalsrus): 46 km from Bothaville to Wesselsbron. (–DA)	Spies 5657
	FREE STATE.—2727 (Kroonstad): 61 km from Kroonstad to Parys. (–AC)	Spies 5650
	FREE STATE.—2727 (Kroonstad): 7 km from Kroonstad to Kroonvaal. (–CA)	Spies 5649
	KWAZULU-NATAL.—2732 (Ubombo): Mhlosinga, on route to Sordwana. (–CC)	Venter 9286
	NORTHERN CAPE.—2822 (Glen Lyon): 7 km from Smidtsdorp to Postmasburg. (–DA)	Spies 5521
	FREE STATE.—2826 (Brandfort): 57 km from Wesselsbron to Bultfontein. (–AA)	Spies 5662
	FREE STATE.—2826 (Brandfort): 30 km from Wesselsbron to Bultfontein. (–BB)	Spies 5660
	FREE STATE.—2826 (Brandfort): 25 km from Bloemfontein to Brandfort. (–CD)	Spies 5576, 5577
	FREE STATE.—2826 (Brandfort): 27 km from Bloemfontein to Brandfort. (–CD)	Spies 5574, 5575
	FREE STATE.—2826 (Brandfort): 38 km from Bloemfontein to Brandfort. (–CD)	Spies 5849, 5850
	FREE STATE.—2826 (Brandfort): 32 km from Bloemfontein to Abrahamskraal. (–CD)	Spies 5638
	FREE STATE.—2925 (Jagersfontein): 44 km from Petrusburg to Kimberley. (–AB)	Spies 5508
	FREE STATE.—2926 (Bloemfontein): near Bloemfontein. (–AA)	Spies 5643, 5664
	FREE STATE.—2926 (Bloemfontein): 16 km from Bloemfontein to Winburg. (–AA)	Spies 5847
	EASTERN CAPE.—3125 (Steynsburg): 30 km from Steynsburg to Hofmeyr. (–BC)	Spies 5669
	EASTERN CAPE.—3125 (Steynsburg): near Hofmeyer. (–DC)	Spies 5587
	EASTERN CAPE.—3125 (Steynsburg): 12 km from Hofmeyer to Cradock. (–DC)	Spies 5670
	WESTERN CAPE.—3222 (Beaufort West): 5 km from Beaufort West. (–BC)	Spies 5487, 5489
	EASTERN CAPE.—3224 (Graaff-Reinet): 58 km from Jansenville to Graaff-Reinet. (–BC)	Spies 5240
	EASTERN CAPE.—3224 (Graaff-Reinet): 131 km from Uitenhage to Graaff-Reinet. (–DC)	Spies 5236
	EASTERN CAPE.—3224 (Graaff-Reinet): 145 km from Uitenhage to Graaff-Reinet. (–DC)	Spies 5237
	EASTERN CAPE.—3224 (Graaff-Reinet): 122 km from Patensie to Willowmore. (–DD)	Spies 5215
	EASTERN CAPE.—3225 (Somerset East): 57 km from Cradock to Cookhouse. (–DB)	Spies 5591
	EASTERN CAPE.—3225 (Somerset East): Kokskraal, Cookhouse. (–DB)	Spies 5594, 5676
	WESTERN CAPE.—3320 (Ladismith): 4 km from Calitzdorp to Oudtshoorn via Kuilsrivier. (–DC)	Spies 5226
	EASTERN CAPE.—3324 (Steytlerville): 102 km from Uitenhage to Graaff-Reinet. (–BD)	Spies 5232
	EASTERN CAPE.—3325 (Port Elizabeth): 40 km from Uitenhage to Graaff-Reinet. (–CD)	Spies 5230
18+0-2B	NORTH-WEST.—2624 (Vryburg): near Vryburg, on route to Kuruman. (–DC)	Spies 5531
	NORTH-WEST.—2627 (Potchefstroom): 10 km from Parys to Potchefstroom. (–CD)	Spies 5652
	FREE STATE.—2925 (Jagersfontein): 60 km from Petrusburg to Kimberley. (–AA)	Spies 5512
	FREE STATE.—2926 (Bloemfontein): 25 km from Bloemfontein to Winburg. (–AA)	Spies 5848
	EASTERN CAPE.—3125 (Steynsburg): 10 km from Steynsburg to Hofmeyr. (–BC)	Spies 5584, 5585
	EASTERN CAPE.—3125 (Steynsburg): 24 km from Steynsburg to Hofmeyr. (–BC)	Spies 5586
	WESTERN CAPE.—3222 (Beaufort West): 5 km from Beaufort West. (–BC)	Spies 5488
	EASTERN CAPE.—3225 (Somerset East): Kokskraal, Cookhouse. (–DB)	Spies 5675
	EASTERN CAPE.—3324 (Steytlerville): 68 km from Uitenhage to Graaff-Reinet. (–DA)	Spies 5231
	EASTERN CAPE.—3325 (Port Elizabeth): 30 km from Uitenhage to Graaff-Reinet. (–CD)	Spies 5229
45/2	NORTH-WEST.—2522 (Sanie): in the riverbed at Watersend. (–DB)	Spies 5497
	FREE STATE.—2925 (Jagersfontein): Spitskop farmyard. Fauresmith. (–DA)	Du Preez 2758
	NORTHERN CAPE.—3024 (Colesberg): 27 km from Verwoerddam to Venterstad. (–DA)	Spies 5581, 5583
	EASTERN CAPE.—3224 (Graaff-Reinet): 39 km from Jansenville to Graaff-Reinet. (–DA)	Spies 5239
	EASTERN CAPE.—3224 (Graaff-Reinet): 15 km from Jansenville to Graaff-Reinet. (–DC)	Spies 5238
	EASTERN CAPE.—3224 (Graaff-Reinet): 76 km from Patensie to Willowmore. (–DD)	Spies 5210
27	NORTHERN CAPE.—2824 (Kimberley): 1 km from Kimberley to Griekwastad. (–DA)	Spies 5513, 5514
	FREE STATE.—2925 (Jagersfontein): 44 km from Petrusburg to Kimberley. (–AB)	Spies 5510
27+0-1B	NORTHERN CAPE.—2824 (Kimberley): 1 km from Kimberley to Griekwastad. (–DA)	Spies 5517

TABLE 2.—Meiotic chromosome behaviour of *Cenchrus ciliaris* specimens showing voucher specimen no.; gametic chromosome no. (n); average frequency of univalents (I); frequency of chromosome laggards; percentage of cells studied containing anaphase I bridges; frequency of micronuclei during telophase I. All ranges are included in brackets

Voucher no.	n	I	# laggards	% bridges	# micronuclei
<i>Spies 5883</i>	17	1.5 (0-4)	2.9 (0-8)	20	0.9 (0-2)
<i>Spies 5215</i>	18	-	3.2 (0-6)	0	3.8 (0-8)
<i>Spies 5226</i>	18	0.3	-	0	1.0 (0-2)
<i>Spies 5229</i>	18	2.0 (1-3)	4.0 (0-6)	0	3.2 (0-8)
<i>Spies 5230</i>	18	5.1 (0-18)	2.9 (0-12)	6.3	2.7 (0-5)
<i>Spies 5231</i>	18	1.2 (0-2)	4.6 (0-9)	9.1	1.3 (0-4)
<i>Spies 5232</i>	18	3.2 (0-10)	2.0 (0-6)	9.1	3.0 (0-5)
<i>Spies 5236</i>	18	0.6 (0-4)	2.2 (0-6)	9	1.1 (0-5)
<i>Spies 5237</i>	18	1.5 (0-3)	3.9 (0-8)	6.3	1.9 (0-6)
<i>Spies 5240</i>	18	6.5 (0-8)	8.8 (0-16)	0	1.1 (0-5)
<i>Spies 5487</i>	18	0.5 (0-2)	1.1 (0-5)	30	0
<i>Spies 5488</i>	18	0.6 (0-2)	0.8 (0-2)	33.3	0.5 (0-2)
<i>Spies 5489</i>	18	0	4.0 (0-6)	-	0.4 (0-1)
<i>Spies 5508</i>	18	2.3 (0-5)	1.4 (0-5)	0	1.8 (0-5)
<i>Spies 5509</i>	18	-	-	-	-
<i>Spies 5512</i>	18	2.0 (0-6)	4.1 (0-9)	7.1	2.4 (0-5)
<i>Spies 5521</i>	18	2.7 (0-6)	1.3 (0-5)	15.4	2.9 (0-9)
<i>Spies 5522</i>	18	-	-	-	-
<i>Spies 5525</i>	18	2.0 (0-4)	-	-	0.2 (0-1)
<i>Spies 5527</i>	18	-	0	0	0.9 (0-2)
<i>Spies 5529</i>	18	-	4.2 (1-8)	0	0
<i>Spies 5531</i>	18	2.8 (0-6)	1.3 (0-5)	0	0.5 (0-4)
<i>Spies 5538</i>	18	-	0.2 (0-2)	0	0.2 (0-1)
<i>Spies 5539</i>	18	-	-	-	-
<i>Spies 5542</i>	18	1.4 (0-5)	2.2 (0-5)	10	3.3 (1-7)
<i>Spies 5543</i>	18	2.7 (1-4)	4.0	-	0.7
<i>Spies 5553</i>	18	-	-	-	-
<i>Spies 5574</i>	18	0.4 (0-1)	0.3 (0-1)	0	0.7 (0-3)
<i>Spies 5575</i>	18	1.6 (0-4)	0.5 (0-10)	8.3	0.7 (0-3)
<i>Spies 5576</i>	18	-	0.7 (0-3)	0	0.8 (0-4)
<i>Spies 5577</i>	18	-	1.0 (0-3)	0	1.0 (0-4)
<i>Spies 5584</i>	18	2.3 (0-8)	1.5 (0-6)	14.3	0.1 (0-1)
<i>Spies 5585</i>	18	0.6 (0-2)	0	0	1.0 (0-3)
<i>Spies 5586</i>	18	0.7 (1)	0.8 (0-2)	0	1.1 (0-3)
<i>Spies 5587</i>	18	-	0	0	0.2 (0-1)
<i>Spies 5591</i>	18	-	1.9 (0-5)	27.2	1.2 (0-3)
<i>Spies 5594</i>	18	-	1.7 (0-5)	30	2.1 (1-5)
<i>Spies 5638</i>	18	1.7 (0-4)	2.8 (0-5)	0	1.0 (0-6)
<i>Spies 5642</i>	18	-	-	-	-
<i>Spies 5643</i>	18	-	4.0 (0-6)	0	3.0 (0-3)
<i>Spies 5645</i>	18	2.3 (0-8)	1.5 (0-3)	-	1.0 (0-3)
<i>Spies 5646</i>	18	-	0	12.5	1.6 (0-1)
<i>Spies 5649</i>	18	2.5 (0-8)	0.4 (0-3)	0	1.1 (0-1)
<i>Spies 5650</i>	18	2.7 (1-4)	0.9 (0-10)	10	0.4 (0-2)
<i>Spies 5652</i>	18	1.9 (0-4)	1.2 (0-5)	71.4	3.3 (2-6)
<i>Spies 5653</i>	18	2.3 (0-5)	0	0	0.5 (0-2)
<i>Spies 5654</i>	18	0.7 (0-3)	0	0	1.5 (0-4)
<i>Spies 5655</i>	18	-	0.3 (0-1)	20	0.9 (0-4)
<i>Spies 5657</i>	18	-	0	0	0.4 (0-1)
<i>Spies 5659</i>	18	1.4 (0-4)	0.2 (0-1)	10	0.1 (0-1)
<i>Spies 5660</i>	18	1.8 (0-4)	0	10	0.5 (0-2)
<i>Spies 5662</i>	18	-	0.9 (0-6)	0	1.0 (0-3)
<i>Spies 5664</i>	18	2.4 (0-5)	1.5 (0-6)	0	0.4 (0-2)
<i>Spies 5668</i>	18	-	-	-	-
<i>Spies 5669</i>	18	3.3 (1-4)	0.3 (0-1)	0	0.4 (0-2)
<i>Spies 5670</i>	18	0.2 (0-1)	1.5 (0-5)	0	0.5 (0-3)
<i>Spies 5671</i>	18	-	-	-	-
<i>Spies 5675</i>	18	-	-	-	1.5 (0-5)
<i>Spies 5676</i>	18	-	3.4 (0-5)	0	1.6 (0-6)
<i>Spies 5847</i>	18	1.4 (0-4)	0	-	0.4 (0-2)
<i>Spies 5848</i>	18	2.8 (0-4)	0	16.7	0.6 (0-2)
<i>Spies 5849</i>	18	-	-	-	0.8 (0-3)
<i>Spies 5850</i>	18	1.4 (0-3)	1.4 (0-2)	11.1	0.7 (0-4)
<i>Venter 9286</i>	18	1.8 (0-4)	3.1 (0-10)	30	1.8 (0-4)
Average		1.9	1.7	8.3	1.1
<i>Du Preez 2758</i>	45/2	4.0 (3-9)	8.2 (7-12)	0	3.5 (2-5)
<i>Spies 5210</i>	45/2	4.6 (3-8)	11.6 (7-14)	0	4.8 (0-11)
<i>Spies 5238</i>	45/2	4.8 (1-10)	7.1 (1-18)	0	2.3 (0-7)
<i>Spies 5239</i>	45/2	3.5 (2-8)	11.8 (1-20)	0	4.0 (1-5)
<i>Spies 5497</i>	45/2	3.4 (2-4)	3.0 (0-6)	0	3.6 (0-5)
<i>Spies 5581</i>	45/2	-	10.7 (1-32)	0	4.6 (2-9)
<i>Spies 5583</i>	45/2	4.0 (2-9)	10.0 (1-20)	0	6.2 (1-10)
Average		4.0	9.2	0	4.1

TABLE 2. (cont.)—Meiotic chromosome behaviour of *Cenchrus ciliaris* specimens showing voucher specimen no.; gametic chromosome no. (n); average frequency of univalents (I); frequency of chromosome laggards; percentage of cells studied containing anaphase I bridges; frequency of micronuclei during telophase I. All ranges are included in brackets

Voucher no.	n	I	# laggards	% bridges	# micronuclei
<i>Spies 5510</i>	27	4.1 (2-7)	2.5 (0-8)	0	2.8 (0-8)
<i>Spies 5513</i>	27	-	2.0 (0-4)	0	4.8 (0-8)
<i>Spies 5514</i>	27	2.5 (0-8)	6.1 (1-9)	24.3	4.5 (0-8)
<i>Spies 5515</i>	27	1.8 (0-5)	2.4 (0-5)	10	3.1 (0-6)
<i>Spies 5517</i>	27	1.3 (0-3)	5.2 (0-20)	0	2.2 (0-6)
Average		2.8	3.3	6.9	3.8

-, cells in the particular meiotic stage have not been observed, or a complete meiotic analysis (involving at least 10 cells of the particular stage) could not be concluded.

of the three chromosome abnormalities will be representative of all the tetraploid specimens. This process was not repeated for the pentaploid and hexaploid specimens, due to the limited number of specimens available.

The average numbers of univalents observed in the specimens investigated were 1.5, 1.9, 4.0 and 2.8 respectively for the  $n = 17$ ,  $n = 18$ ,  $n = 45/2$ , and  $n = 27$  specimens. The variation in the number of univalents is indicated in Table 2. The highest number of univalents (0-18) was observed for *Spies 5230* ( $n = 18$ ) (Figure 2B, C).

The univalents present were usually situated near the equator (Figure 2A-D) and moved onto the plate where they orientated themselves both syntelically and amphitelically. This led to the centromeres of the two chromatids either undergoing reductional distribution or equational distribution. The chromatids segregated to opposite poles.

Since the B-chromosomes were mostly indistinguishable from the normal euchromosome complement, specimens containing B-chromosomes were excluded during the calculation of the univalent, laggard and micronuclei averages.

Chromosome and chromatid laggards were frequently observed during anaphase I (Figure 3A-E). The average

numbers of chromosome laggards observed, were 2.9, 1.7, 9.2 and 3.3 for the  $n = 17$ ,  $n = 18$ ,  $n = 45/2$ , and  $n = 27$  specimens, respectively (Table 2). The variation in numbers is indicated in Table 2. The highest number of laggards (1-32) was observed for *Spies 5581* ( $n = 45/2$ ).

The number of micronuclei observed during telophase I varied (Figure 4A-D). The average numbers of micronuclei observed were 0.9 ( $n = 17$ ), 1.1 ( $n = 18$ ), 4.1 ( $n = 45/2$ ) and 3.8 ( $n = 27$ ) (Table 2). The variation in numbers is indicated in Table 2. The highest number of micronuclei (11) was observed for *Spies 5210* ( $n = 45/2$ ) (Figure 4B). The size of these micronuclei varied (Figure 4B, D).

Genome interpretation was performed on three tetraploid specimens (*Spies 5215*, *5240* and *5649*). These analyses revealed that the 2:2 model (Kimber & Alonso 1981) fitted the specimens to the greatest degree, with  $x$ -values of 1 or approximately 1 (Table 3).

Anaphase bridges were observed in 26 specimens of *C. ciliaris* (Table 2) (Figure 5A-C). The average number of cells per specimen which contained bridges, varied from nought to a maximum of 71.4% (*Spies 5652*) (Table 2). The acentric fragment could most often be observed (Figure 5A, B), and varied in size among the various specimens.

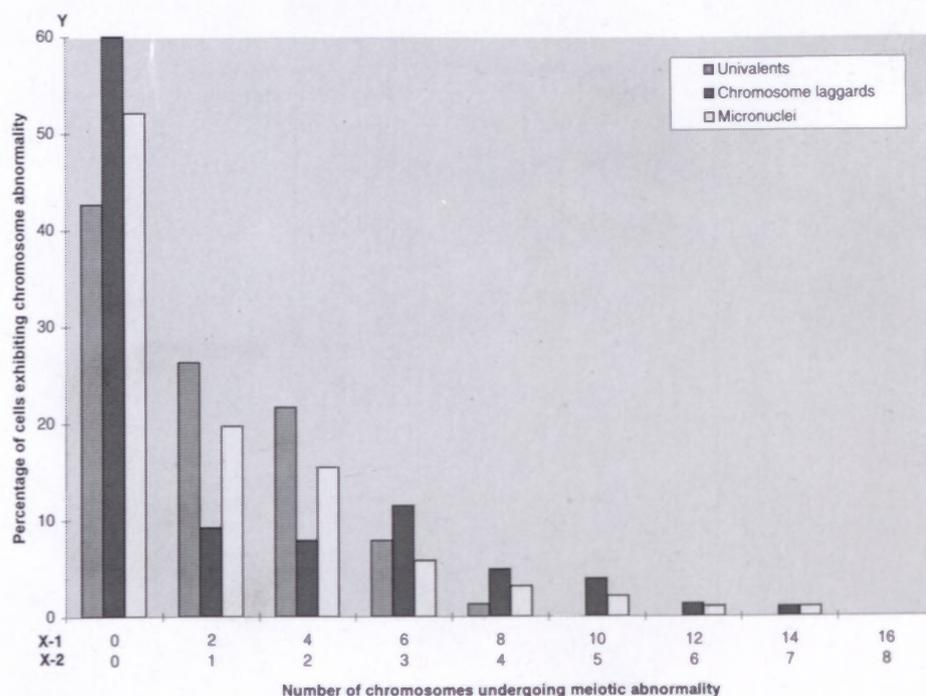


FIGURE 1.—Histogram, indicating percentage of tetraploid cells exhibiting univalents during metaphase I, chromosome laggards during anaphase I, and micronuclei during telophase I. X-1, number of chromosomes involved in formation of univalents and chromosome laggards; X-2, number of micronuclei observed per cell.

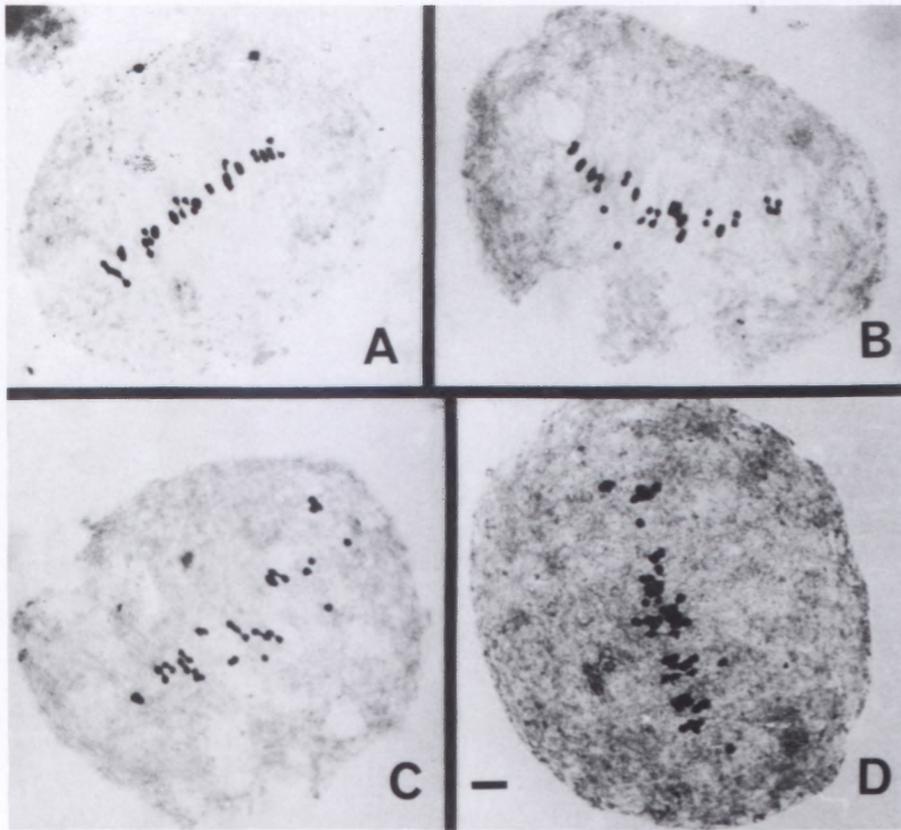


FIGURE 2.—Variation in number of univalents observed during metaphase I in *Cenchrus ciliaris*. A, *Spies 5240*,  $n = 2x = 18$ , with two univalents; B, *Spies 5230*,  $n = 2x = 18$ , with two univalents; C, *Spies 5230*,  $n = 2x = 18$ , with eight univalents; D, *Spies 5514*,  $n = 3x = 27$ , with numerous univalents. Scale bar: 10  $\mu\text{m}$ .

#### DISCUSSION

Basic chromosome numbers constitute the core of any meiotic study, as they are essential for confirming the presence of polyploidy. *Cenchrus ciliaris* has a basic chromosome number of  $x = 9$  and polyploidy is present. Three polyploid levels have been observed, with the most abundant being the tetraploids (82.9%). The penta-

and hexaploids were observed at much lower frequencies (9.2% and 6.6% respectively).

Polyploidy is prominent in the plant kingdom (Stebbins 1982). Polyploid levels for *C. ciliaris*, taking the published aneuploid chromosome numbers into account, include diploidy, triploidy, tetraploidy, pentaploidy, hexaploidy and nanoploidy. References for these

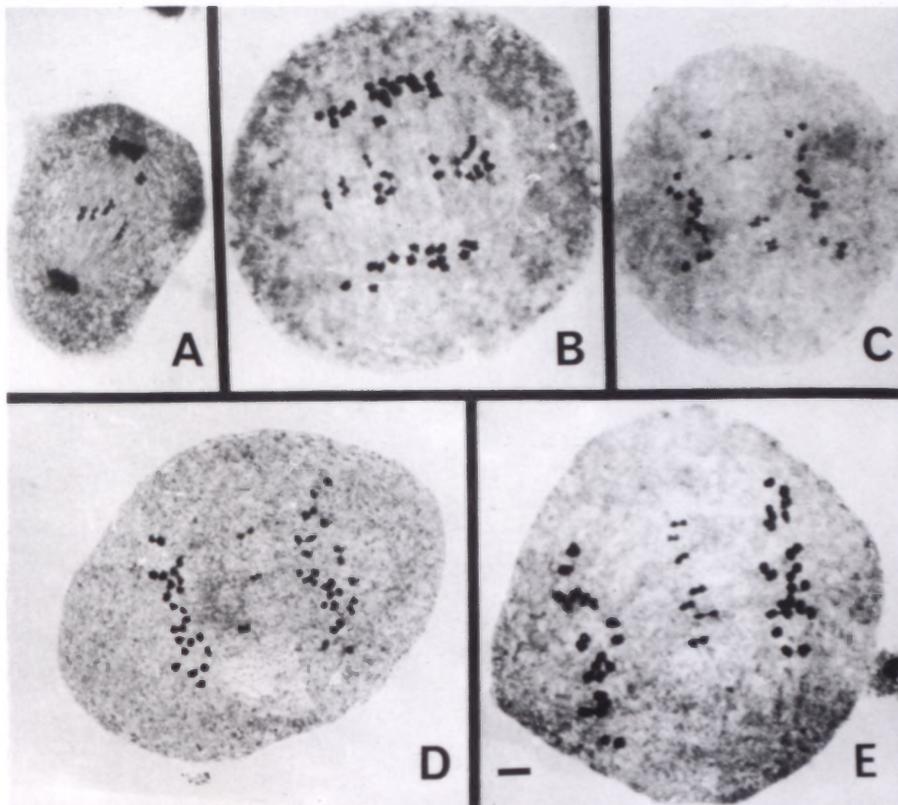


FIGURE 3.—Variation in number of chromosome and chromatid laggards observed during anaphase I in *Cenchrus ciliaris*. A, *Spies 5883*,  $n = 17$ ; B, *Spies 5508*,  $n = 2x = 18$ ; C, *Spies 5583*,  $n = 5/2x = 45/2$ ; D, *Spies 5514*,  $n = 3x = 27$ ; E, *Spies 5514*,  $n = 3x = 27$ . Scale bar: 10  $\mu\text{m}$ .

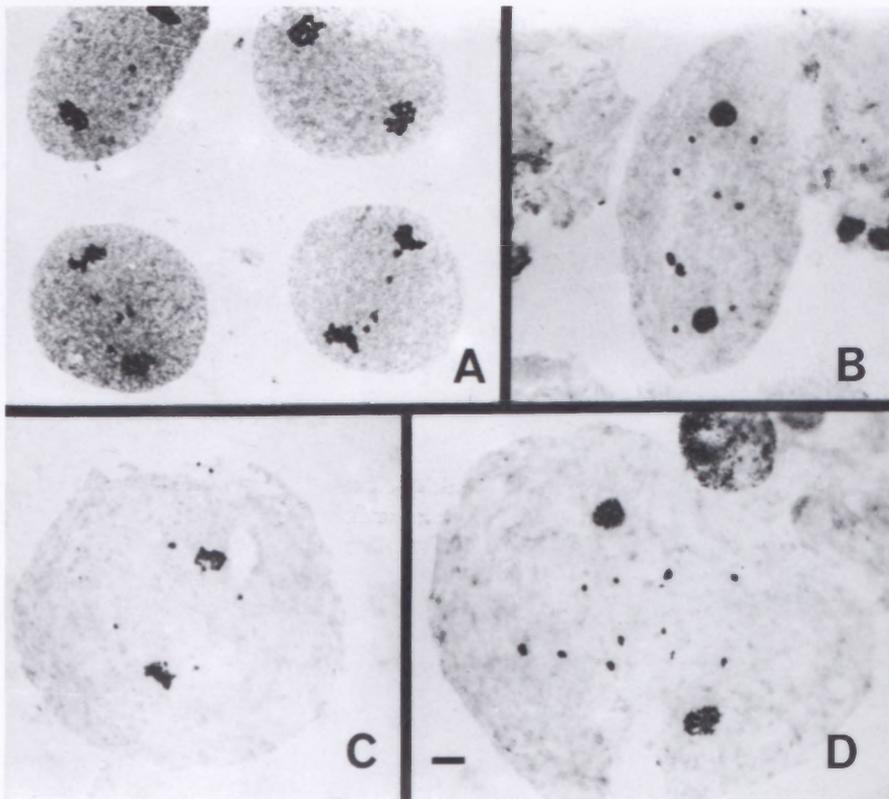


FIGURE 4.—Variation in number and size of micronuclei observed during telophase I and II in *Cenchrus ciliaris*. A, Spies 5542,  $n = 2x = 18$ , telophase II, with 0–4 per cell; B, Spies 5210,  $n = 5/2x = 45/2$ , telophase I, with nine micronuclei; C, Spies 5583,  $n = 5/2x = 45/2$ , telophase I, with minimum of four micronuclei; D, Spies 5514,  $n = 3x = 27$ , telophase I, with 10 micronuclei. Scale bar: 10  $\mu\text{m}$ .

chromosome numbers are presented in Visser *et al.* (in prep.). The wide range of polyploid levels and very low frequency of diploids indicate that this species forms a mature polyploid complex.

The meiotic chromosome behaviour of the polyploid specimens varied from being normal to highly irregular, depending on the number of genomes present and their homology. Polyploids with an even number of genomes are influenced less dramatically during meiosis than those with an uneven number of genomes. Uneven polyploid levels have more meiotic abnormalities, due to the presence of an uneven number of sets of chromosomes which complicates chromosome pairing. Genome homology also plays an important role in the normality of meiosis. It can affect chromosome pairing to such an extent that from univalents to multivalents are formed.

In order to attest the proposed basic chromosome number of  $x = 9$  for *C. ciliaris*, a comparison was made between the observed and the expected meiotic chromosome behaviour of each of the polyploid levels studied. The presence of univalents during metaphase I was reg-

ularly observed. A variation in the number of univalents present occurred within and among the polyploid levels (Figure 2A–D). The highest average numbers of univalents were observed in the pentaploid specimens (4.0), whereas for the hexaploid specimens, they were 2.8 (Table 2). Approximately similar average numbers of univalents were observed for the aneuploid and tetraploid specimens (1.5 versus 1.9).

The increased averages of univalents present during metaphase I for the pentaploids and the hexaploids were expected, as a higher incidence of meiotic irregularities is closely associated with uneven and higher polyploid levels (Stebbins 1950). The variation in the number of univalents observed within each of these polyploid levels, indicates chromosomal differences among the specimens.

The number of univalents present during metaphase I corresponds with the numbers of chromosome laggards and micronuclei observed (Figure 1). The data suggest that the univalents observed during metaphase I were mostly lagging during anaphase I (Figure 3A–E), and led

TABLE 3.—Genomic constitution of tetraploid *Cenchrus ciliaris* specimens, according to models of Kimber & Alonso (1981): voucher nos, chiasma frequencies, relative  $x$ -values and the appropriate average sum of squares for each of the various models in brackets (M)

Voucher	Chiasma frequency	M			
		4:0	3:1	2:2	2:1:1
Spies 5215	1.3	- (16.778)	0.889 (21.192)	1 (8.429)	0.828 (16.18)
Spies 5240	1.2	- (14.026)	8.874 (17.866)	1 (3.564)	0.872 (12.801)
Spies 5649	1.4	- (9.876)	0 (14.324)	0.84 (4.465)	0.632 (8.03)

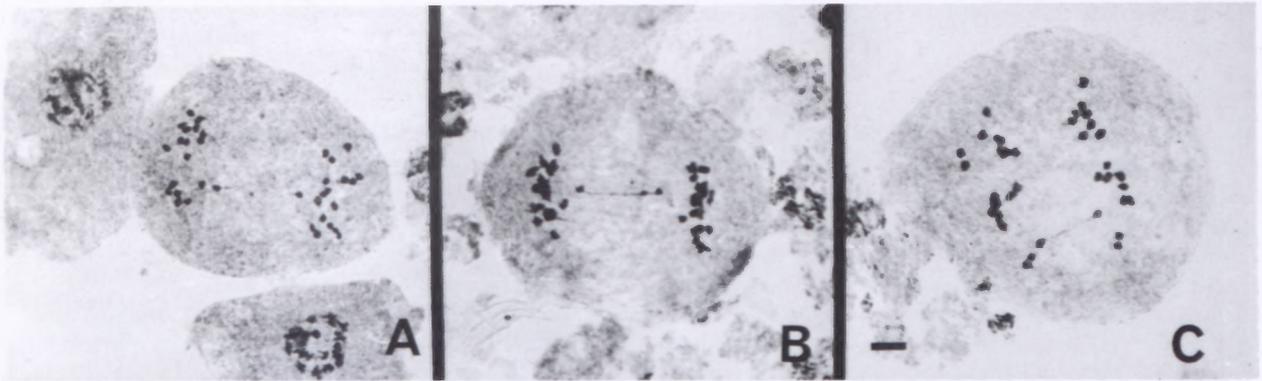


FIGURE 5.—Some anaphase I bridges observed in *Cenchrus ciliaris*. A, *Spies 5652*,  $n = 2x = 18$ ; B, *Spies 5594*,  $n = 2x = 18$ ; C, *Spies 5574*,  $n = 2x = 18$ . Scale bar: 10  $\mu\text{m}$ .

to the formation of micronuclei during telophase I. The average number of laggards was the highest for the pentaploid specimens (9.2 laggards per cell), followed by the hexaploid and aneuploid specimens (3.3 and 2.9 respectively) (Table 2). The number of chromosome and chromatid laggards varied among the specimens of the various polyploid levels, emphasizing genetic differences among specimens within each of the polyploid levels.

The average number of micronuclei also varied among the specimens of each polyploid level (Table 2). The micronuclei were not incorporated into the daughter nuclei at the time of cell division (Figure 4A–D). The highest average number was observed in the pentaploid specimens (4.1), followed by the hexaploid (3.8), tetraploid (1.1) and aneuploid (0.9) specimens respectively. The highest number and greatest variation of micronuclei were observed in the pentaploid specimens (Figure 4B, C) (Table 2).

The highest average numbers of univalents, chromosome laggards and micronuclei have been observed in the pentaploid specimens (Table 2). This polyploid level was followed by the hexaploids, whereas for the tetraploids and the single aneuploid specimen, the level was approximately similar. The high incidence of meiotic abnormalities in the pentaploid specimens was expected, due to their uneven polyploid levels. An average number of 9.2 chromosomes lagged during anaphase I and was representative of an entire genome. A basic chromosome number of  $x = 9$  for *C. ciliaris*, due to the presence of a fifth genome lagging during anaphase I, is hereby confirmed.

Genome variation in the tetraploid specimens was confirmed during this study. Meiosis was normal in some specimens (*Spies 5522*), whereas for others, it was highly irregular (*Spies 5230*) (Table 2). The average numbers of univalents present and chromosome laggards observed, were almost similar (1.9 and 1.7, respectively). These averages suggest two univalents observed during metaphase I, lagging during anaphase I, and finally forming a single micronucleus (1.1 per cell) (Table 2).

Genome interpretation of three tetraploid specimens (*Spies 5215*, *5240* & *5649*) revealed that the 2:2 model (Kimber & Alonso 1981) agreed best with these specimens, with  $x$ -values of 1 (*Spies 5215* & *5240*) and 0.84 (*Spies 5649*) (Table 3). An  $x$ -value of 1 inferred two dis-

tinctly different sets of genomes, therefore, *Spies 5215* and *5240* were classified as allotetraploids (for example AABB). Although the 2:2 model fitted *Spies 5649* the best, a lower  $x$ -value of 0.84 was calculated (Table 3). This specimen was classified as a segmental allopolyploid, based on the lower  $x$ -value and the occasional presence of quadrivalents (for example AAA'A').

The specimens' genomic constitutions were attested by their meiotic chromosome behaviour. The specimens differed in respect of their meiotic behaviour. Quadrivalents were observed in *Spies 5215* and *5649*, whereas for *Spies 5240*, univalents and bivalents were observed. The number of quadrivalents observed in the two specimens varied. One to three quadrivalents were observed for *Spies 5649*, whereas for *Spies 5215*, an infrequent quadrivalent was observed.

*Spies 5240* was confirmed as an allopolyploid (for example AABB), based on the presence of bivalents only, observed during metaphase I. The presence of an occasional quadrivalent in cells of *Spies 5215* and one to three quadrivalents per cell in *Spies 5649*, justify segmental allopolyploidy (for example AAA'A') for both these specimens. However, the presence of genes, controlling homoeologous chromosome pairing in *Cenchrus*, should be studied before these genomic constitutions can be accepted.

The two allopolyploid specimens were collected in the Eastern Cape, whereas *Spies 5649* was collected in the Free State (Table 1). Taking their different geographical localities into consideration, it is suggested that the Eastern Cape specimens represent a genetically different group (or hybrid swarm) from those in the Free State. Therefore, chromosomal variation in these three tetraploid specimens confirm the presence of genetic variability in *C. ciliaris* in South Africa.

The highest incidences of meiotic abnormalities were recorded in the pentaploid specimens (Table 2). These abnormalities were mostly the result of an uneven polyploid level. The average number of univalents observed, was approximately half of the average number of chromosome laggards observed (4.0 versus 9.2) (Table 2). There was an increase in the average number of laggards observed (Table 2). This could have been due to amphitelic orientation and equational distribution of the univalents observed during metaphase I, as various chromatid lag-

gards were observed during anaphase I (Figure 3C). These anaphase I laggards were mostly included in more than one micronucleus per cell (Figure 4B, C), as an average frequency of 4.1 micronuclei per cell has been observed.

The average number of laggards observed for this polyploid level, was representative of a complete genome lagging during anaphase I. It is suggested that the genomic constitution of the pentaploid specimens includes one unrelated genome. This suggestion is based on the average number of univalents present, the absence of trivalents and the high average number of laggards observed during anaphase I. A genomic constitution of, for example AAA'A'A", is proposed for this polyploid level. Segmental allopolyploidy is justified by the high and the low occurrence of bivalents and quadrivalents respectively, observed during prophase and metaphase I. Chromosomal variation is evident in this polyploid level, for meiotic chromosome behaviour varied among the specimens studied (Table 2).

The average number of univalents present, the chromosome and chromatid laggards and the micronuclei observed for the hexaploid specimens (2.8, 3.3 and 3.8 respectively), were relatively low when compared to that of the pentaploid specimens (Table 2). A genomic constitution of, for example AAA'A'A"A", is proposed for the hexaploids. Segmental allopolyploidy, based on the abundant bivalent and occasional quadrivalent chromosome configurations found (Figure 2D), is proposed. The presence of quadrivalents indicates a degree of homology between the A and A' genomes.

The genomic constitutions of the various polyploid levels ( $n = 17$ —segmental allopolyploidy;  $n = 18$ —segmental polyploidy to allopolyploidy;  $n = 45/2$ —allopolyploidy and  $n = 27$ —segmental allopolyploidy) indicate the presence of hybridisation in this species.

Hybridisation among plant individuals is usually characterised by various changes in chromosome structure (Darlington 1937; Dobzhansky 1941). For *C. ciliaris*, these changes include the presence of paracentric inversions. These inversions were mostly observed in the tetraploid specimens (Figure 5A–C). The higher occurrence of these inversions could be due to the high number of tetraploid specimens which were cytogenetically studied. The highest average number of cells containing anaphase I bridges was observed in *Spies 5652* (71.4%) (Figure 5A) (Table 2). Different paracentric inversions were found among the specimens, as the chromosome fragments differed in size.

Occasional or recurrent hybridisation and the complete local breakdown of reproductive isolation between sympatric species result in the production of hybrid swarms. These swarms include the whole range of genetic variability of the parental species. This scenario could be representative of *C. ciliaris*, for a wide range of chromosomal, morphological and genetic variation is evident in this species.

## CONCLUSIONS

With the aid of meiotic analyses, a basic chromosome number of  $x = 9$  has been confirmed for *Cenchrus ciliaris*. Polyploidy is common and varies from tetraploid to hexaploid. Aneuploidy was observed in a single specimen. It is suggested that this specimen is the result of loss aneuploidy from two chromosomes of a single genome.

Various meiotic irregularities were observed for this species. The highest incidences of meiotic abnormalities were observed for the pentaploid specimens. This was attributed to their uneven polyploid and chromosome number.

The chromosome abnormalities observed during meiosis were an indication of genomic relationships. These relationships varied among the specimens and the polyploid levels. Segmental allopolyploidy was suggested for the aneuploid specimen, whereas for the tetraploid specimens, it varied from segmental allopolyploidy to allopolyploidy. A genomic constitution of allopolyploidy and segmental allopolyploidy is suggested for the pentaploid and hexaploid levels respectively. The nature of the genomic relationships indicated the presence of hybridisation. Hybridisation in *C. ciliaris* was confirmed by the chromosomal variation observed among specimens in each of the polyploid levels.

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