A CYTOTAXONOMIC STUDY OF SOME REPRESENTATIVES OF THE TRIBE CYNODONTEAE (CHLORIDOIDEAE)

Poaceae is divided into five major subfamilies. One of these subfamilies, Chloridoideae Rouy, is represented by approximately 50 genera and 232 species in southern Africa (Gibbs Russell *et al.* 1990). One of the tribes of the Chloridoideae, Cynodonteae Dumort., commonly occurs in unstable communities (Clayton & Renvoize 1986). Cynodonteae has four subtribes, of which two were studied, namely Chloridinae Presl and Zoysiinae Benth. The following genera of the subtribe Chloridinae are included in this study: *Chloris* Sw., *Cynodon* Rich., *Eustachys* Desv., *Harpochloa* Kunth, *Microchloa* R. Br., and *Rendlia* Chiov. The only genus in the subtribe Zoysiinae studied, is *Tragus* Haller.

The aim of this study is to establish the chromosome numbers of the different genera and species.

MATERIAL AND METHODS

The material was collected and fixed in the field. Voucher herbarium specimens are housed in the Geo Potts Herbarium, Department of Botany and Genetics, University of Orange Free State, Bloemfontein (BLFU) or the National Herbarium, Pretoria (PRE).

SPECIMENS EXAMINED

Chloris gayana Kunth: **n = 10**.

TRANSVAAL.—2428 (Nylstroom): Soutpan Experimental Station, (–CD), *Spies 3727*. 2528 (Pretoria): Sphinx Station, (–CA), *Spies 2021*.

C. virgata Swartz: **n = 10**.

ORANGE FREE STATE.—2827 (Senekal): 6 km from Clocolan to Peka bridge, (–DC), *Spies 4799*. 2926 (Bloemfontein): on the U.O.F.S. campus, (–AA), *Spies 5151, 5161, 5164, 5165, 5174*; 26 km from Dewetsdorp to Hobhouse, (–DB), *Spies 4783*.

CAPE.—3026 (Aliwal North): Aliwal North, (–DA), *Spies 5245, 5249*.

Cynodon dactylon (L.) Pers.: **n = 18**.

CAPE.—3026 (Aliwal North): Aliwal North, (–DA), *Spies 5248*. *3318* (Cape Town): on the top of Botmaskloof Pass, (–BD), *Spies 4424*. 3420 (Bredasdorp): De Hoop Nature Reserve, (–DC), *Spies 4626*.

Eustachys paspaloides (Vahl) Lanza & Mattei: **n = 20**.

TRANSVAAL.—2530 (Lydenburg): 10 km from Boshoek to Buffelsvlei, (–AC), *Spies 1521*.

Harpochloa falx (L. f.) Kuntze: **n = 20, 25, 30**.

TRANSVAAL.—2430 (Pilgrim's Rest): 4 km from Pilgrim's Rest to Graskop, (–BA), *Spies 5134* (n = 20); 25 km from Sabie to Lydenburg, (–BA), *Spies 5140* (n = 20). 2530 (Lydenburg): Nederhorst turnoff on Lydenburg-Roossenekal road, (–AA), *Spies 5128* (n = 30); 11 km from Dullstroom to Lydenburg via Frischgewaagd, (–AC), *Spies 5118* (n = 25); 16 km from Dullstroom to Lydenburg via Frischgewaagd, (–AC), *Spies 5125* (n = 30); 5 km from Belfast to Dullstroom, (–CA), *Spies 5113* (n = 20).

ORANGE FREE STATE.—2729 (Volksrust): 53 km from Harrismith to Newcastle via Normandien Pass, (–DC), *Spies 5063* (n = 20); 92 km from Harrismith to Normandien Pass, (–DC), *Spies 5065* (n = 20); 97 km from Harrismith to Normandien Pass, (–DC), *Spies 5078* (n = 20).

CAPE.—3027 (Lady Grey): 45 km from Barkly East to Rhodes, (–DD), *Spies 3986* (n = 20); 52 km from Rhodes via Lundeansnek, (–DD), *Spies 4729* (n = 25). 3028 (Matatiele): 65 km from Rhodes via Naudesnek, (–CC), *Spies 4695* (n = 20); 69 km from Rhodes, (–CC), *Spies 4701* (n = 20). 3128 (Umtata): 38 km from Maclear to Elliot, (–AC), *Spies 4712* (n = 30).

Microchloa caffra Nees: **n = ± 50**.

TRANSVAAL.—2430 (Pilgrim's Rest): 4 km from Pilgrim's Rest to Graskop, (–BA), *Spies 5132*; 25 km from Sabie to Lydenburg, (–BA), *Spies 5141*. 2530 (Lydenburg): 49 km from Lydenburg to Machadodorp, (–CB), *Spies 5146*.

CAPE.—3128 (Umtata): 38 km from Maclear to Elliot, (–AC), *Spies 4714*.

Rendlia altera (Rendle) Chiov.: **n = 20**.

TRANSVAAL.—2430 (Pilgrim's Rest): 4 km from Pilgrim's Rest to Graskop, (–BA), *Spies 5133*; 25 km from Sabie to Lydenburg, (–BA), *Spies 5142*. 2530 (Lydenburg): Nederhorst turnoff on the Lydenburg-Roossenekal road, (–AA), *Spies 5129*; 11 km from Dullstroom to Lydenburg via Frischgewaagd, (–AC), *Spies 5120*; 49 km from Lydenburg to Machadodorp, (–AD), *Spies 5147*.

ORANGE FREE STATE.—2729 (Volksrust): 93 km from Harrismith to Normandien Pass, (–DC), *Spies 5072*; 97 km from Harrismith to Normandien Pass, (–DC), *Spies 5077*.

CAPE.—3028 (Matatiele): 69 km from Rhodes, (–CC), *Spies 4700*. 3128 (Umtata): 38 km from Maclear to Elliot, (–AC), *Spies 4713*.

Tragus berteronianus Schult.: **n = 10**.

SWAZILAND.—2631 (Mbabane): Pikiti in the Lebombo Mountains, (–BB), *Spies 2605*.

CAPE.— 3026 (Aliwal North): Aliwal North, (–DA), *Spies 5246*.

Tragus racemosus (L.) All.: **n = 10**.

CAPE.—3026 (Aliwal North): Aliwal North, (–DA), *Spies 5244*.

Young inflorescences were fixed in Carnoy's fixative for 24–48 hours and the fixative was subsequently replaced by 70% ethanol. Anthers were squashed in 2% aceto-carmine (Darlington & La Cour 1976) and small aliquots of iron acetate. Slides were made permanent by freezing them with liquid CO_2 (Bowen 1956), followed by dehydration in ethanol and mounting in Euparal. Meiotic chromosome behaviour for each specimen, was examined during diakinesis, metaphase I, anaphase I and telophase I. At least 20 cells, representative of each of these meiotic stages, were examined per specimen.

RESULTS AND DISCUSSION

The genus *Chloris* (Cynodonteae Dumort.; Chloridinae Presl) usually has a chromosome base number of ten, occasionally nine (Gibbs Russell *et al.* 1990). Both *Chloris gayana* and *C. virgata* have somatic chromosome numbers of 20 (Figure 1A–G). We accept that the basic chromosome number is ten, because that is the lowest haploid chromsosome number observed in this study, and described for this genus.

The chromosome numbers published, range from 2n = 20 to 40 for *C. gayana* and from 2n = 14 to 36 for *C. virgata*, with 2n = 20 being the most frequent (Darlington & Wylie 1955; Ornduff 1967–1969; Fedorov 1969; Moore 1970, 1971, 1972, 1974, 1977; Goldblatt 1981, 1983, 1985, 1988; Goldblatt & Johnston 1990, 1991). The genus *Chloris* is either diploid, as observed in this study, or polyploid, with the polyploid levels ranging from triploid to tetraploid (Spies & Jonker 1987). There were almost no meiotic abnormalities in any of the specimens.

The *Cynodon dactylon* (Cynodonteae; Chloridinae) specimens studied have haploid chromosome numbers of 9 (Figure 1H), 18 and 20. The basic chromosome number is nine, because most published chromosome numbers are multiples of nine. These chromosome numbers ranged from 2n = 18 to 54 (Darlington & Wylie 1955; Malik 1967; Ornduff 1967–1969; Fedorov 1969; Moore 1970–1977; Goldblatt 1981–1988; Goldblatt & Johnston 1990, 1991). Malik (1967) described three cytological races in this species: diploid (2n = 18 + 0 – 3B), tetraploid (2n = 36 + 0 – 2B) and hexaploid (2n = 54).

A few cells of *Spies 4626* were diploid and in all these cells some extent of desynapsis occurred (Figure 1H). Just why desynapsis should occur in the diploid cells, is not known. Since some of the cells in this specimen contained 40 chromosomes, the specimen is regarded as a tetraploid specimen, with four additional chromosomes. The additional chromosomes were similar in size to the euchromosomes. During metaphase I these univalents lay on the metaphase plate and showed no lagging. Since the number of additional chromosomes varied from 0–4 per cell, we have regarded them as B-chromosomes.

The polyploid specimens in this study form only bivalents and no multivalents and can, therefore, be regarded as allopolyploids. The fact that only rod bivalents were observed, however, indicates that only one chiasma forms per chromosome. Multivalent formation is thus impossible, since a multivalent requires more than one chiasma per chromosome. There were no meiotic abnormalities in

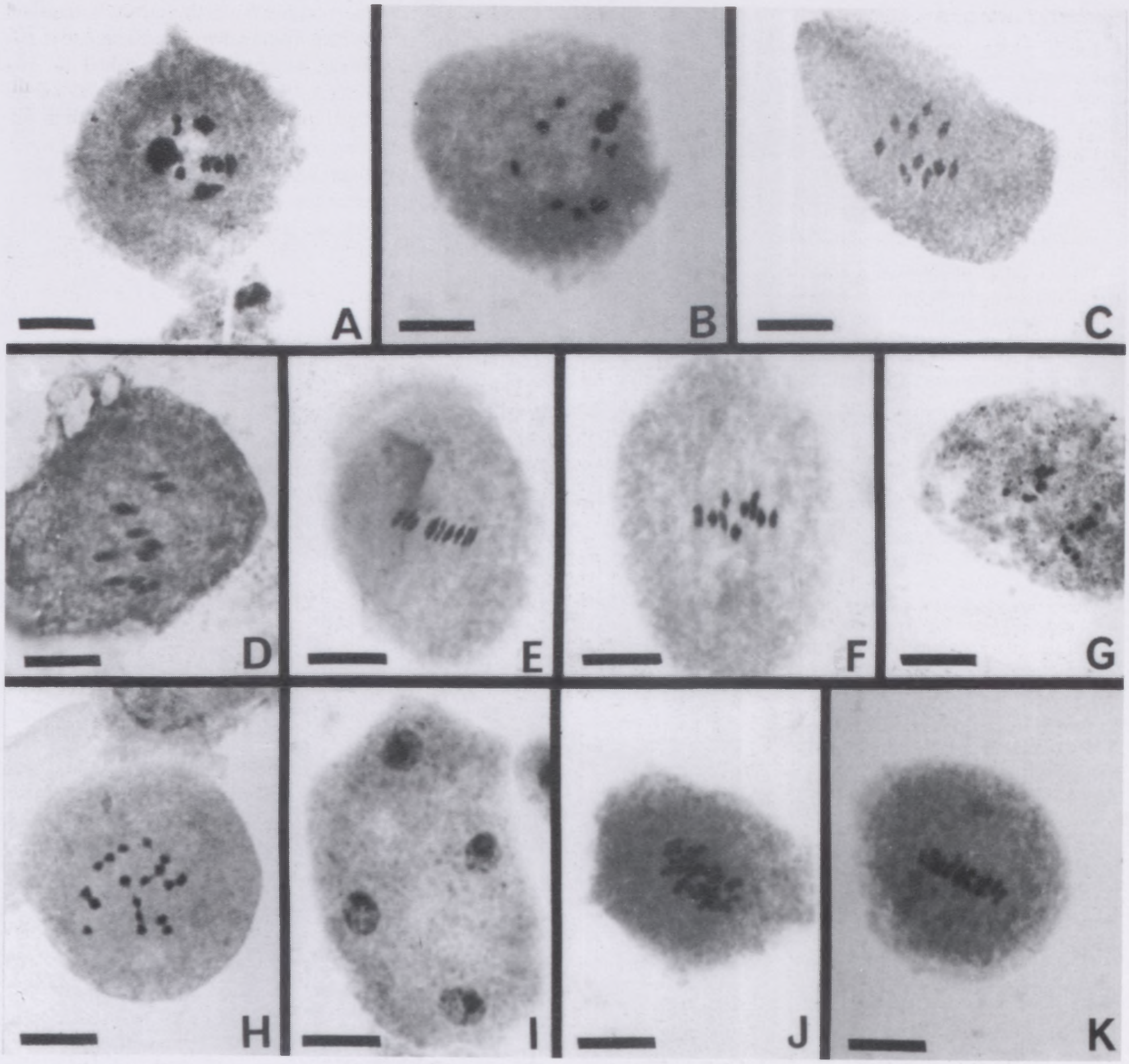

FIGURE 1.—Photomicrographs of meiotic chromosomes in Cynodonteae. A, *Chloris gayana*, *Spies 3727*, n = 10, diakinesis with 10_{II}; B, C, *C. virgata*, *Spies 4783*, n = 10, diakinesis with 10_{II}; D, E, *C. virgata*, *Spies 4799*, n = 10, metaphase I with 10_{II}; F, *C. virgata*, *Spies 4783*, n = 10, metaphase I with 10_{II}; G, *C. gayana*, *Spies 3727*, n = 10, anaphase I with 10-10 segregation; H, *C. dactylon*, *Spies 4626*, n = 9, metaphase I with $5_{II}8_{I}$; I, *C. dactylon*, *Spies 4424*, tetrad after meiosis II; J, K, *Eustachys paspaloides*, *Spies 1521*, n = 20, metaphase I with 20_{II}. Scale bar: 20 µm.

the specimens studied, except for desynapsis in the few diploid cells observed in *Spies 4626*.

The genus *Eustachys* (Cynodonteae; Chloridinae) was represented by *Eustachys paspaloides* with a haploid chromosome number of 20 (Figure 1J, K). Therefore the basic chromosome number of ten described by Gibbs Russell *et al.* (1990), is substantiated. De Wet (1960), however, reported a specimen with 2n = 36. This could possibly be attributed to loss aneuploidy, as these grasses are usually tetraploid. *Spies 1521* is an allopolyploid, since no multivalents were observed. Only rod bivalents were observed and the evidence for alloploidy is, consequently, not conclusive. There were no meiotic abnormalities.

Harpochloa falx (Cynodonteae; Chloridinae) has haploid chromosome numbers of 20, 25 and 30 (Figure 2). The basic chromosome number is ten and ploidy levels range from tetraploid to hexaploid. Only rod bivalents were observed, which is an indication that only one chiasma forms per bivalent and, therefore, no multivalents can be formed. Therefore, it is impossible to determine the type of polyploidy present since the number of chiasmata play a restricting role. Abnormalities observed included univalents (Figure 2M), laggards (Figure 2N–P) and an anaphase I bridge (Figure 2Q). These abnormalities occurred at very low frequencies.

Rendlia altera (Cynodonteae; Chloridinae) has haploid chromosome numbers of 18 and 20 (Figure 3A–D). The basic chromosome number can be either nine or ten. No other chromosome numbers were determined or published. Loss aneuploidy from a basic chromosome number of ten can result in a somatic chromosome number of 36, or gain aneuploidy from a basic chromosome number of nine can result in a somatic chromosome number of 40. There were no meiotic abnormalities. Further studies are needed to determine the basic chromosome number and to establish the range of polyploid levels.

Tragus (Cynodonteae; Zoysiinae) has a basic chromosome number of nine or ten (Gibbs Russell *et al.* 1990). *Tragus berteronianus* has a haploid chromosome number of 10 (Figure 3E, F).

In conclusion, this study indicates that Cynodonteae have polyploid or agamic complexes, with the somatic chromosome numbers ranging from diploid to hexaploid. Two different basic chromosome numbers (i.e. 9 and 10) are present. Further studies are needed to determine the phylogenetic relationship between the two base numbers and to establish the polyploid levels in each taxon.

ACKNOWLEDGEMENTS

We thank the National Botanical Institute for material of *Chloris virgata* and *Eustachys paspaloides* provided during this study. Financial support by the Foundation for

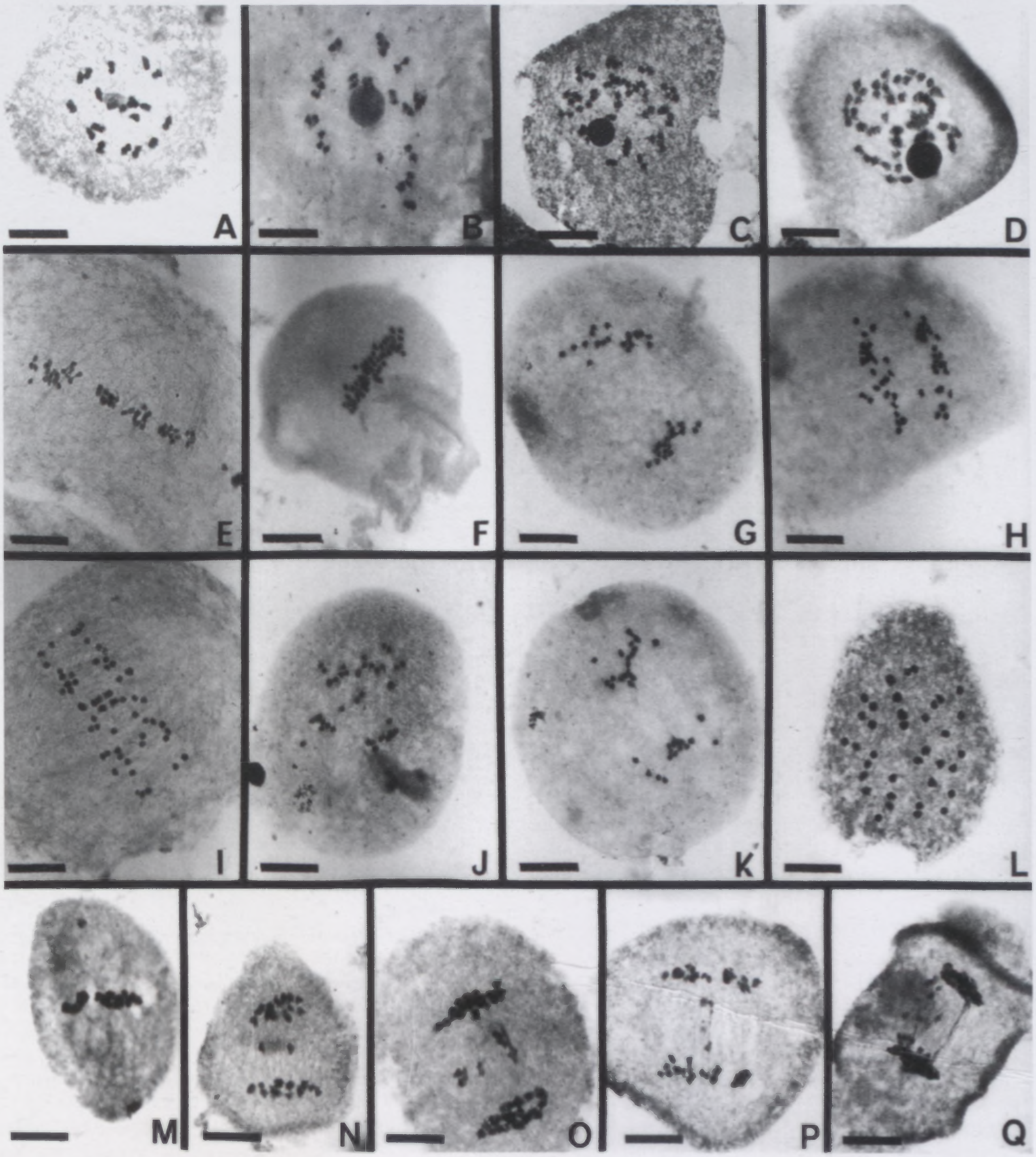

FIGURE 2.—Photomicrographs of meiotic chromosomes in *Harpochloa falx*. A, *Spies 4695*, n = 20, diakinesis with 20_{II}; B, *Spies 5065*, n = 20, diakinesis with $19_{II}2_{I}$; C, *Spies 4712*, n = 30, diakinesis with 30_{II}; D, *Spies 5128*, n = 30, diakinesis with 30_{II}; E, *Spies 5125*, n = 30, metaphase I with 30_{II}; F, *Spies 5128*, n = 30, metaphase I with 30_{II}; G, *Spies 5078*, n = 20, anaphase I with a 20-20 segregation; H, *Spies 5128*, n = 30, anaphase I with a 30-30 segregation; I, *Spies 5125*, n = 30, anaphase I with a 30-30 segregation; J, *Spies 5078*, n = 20, anaphase I with 40 chromosomes; K, *Spies 5078*, n = 20, anaphase I with a 20-20 segregation; L, *Spies 4695*, n = 20, metaphase I with desynapsis; M, *Spies 4729*, n = 25, metaphase I with a B-chromosome in each pole; N, *Spies 4695*, n = 20, anaphase I with two laggards; O, *Spies 4712*, n = 30, anaphase I with laggards; P, *Spies 4695*, n = 20, anaphase I bridge; Q, *Spies 4712*, n = 30, anaphase I bridge. Scale bar: 20 µm.

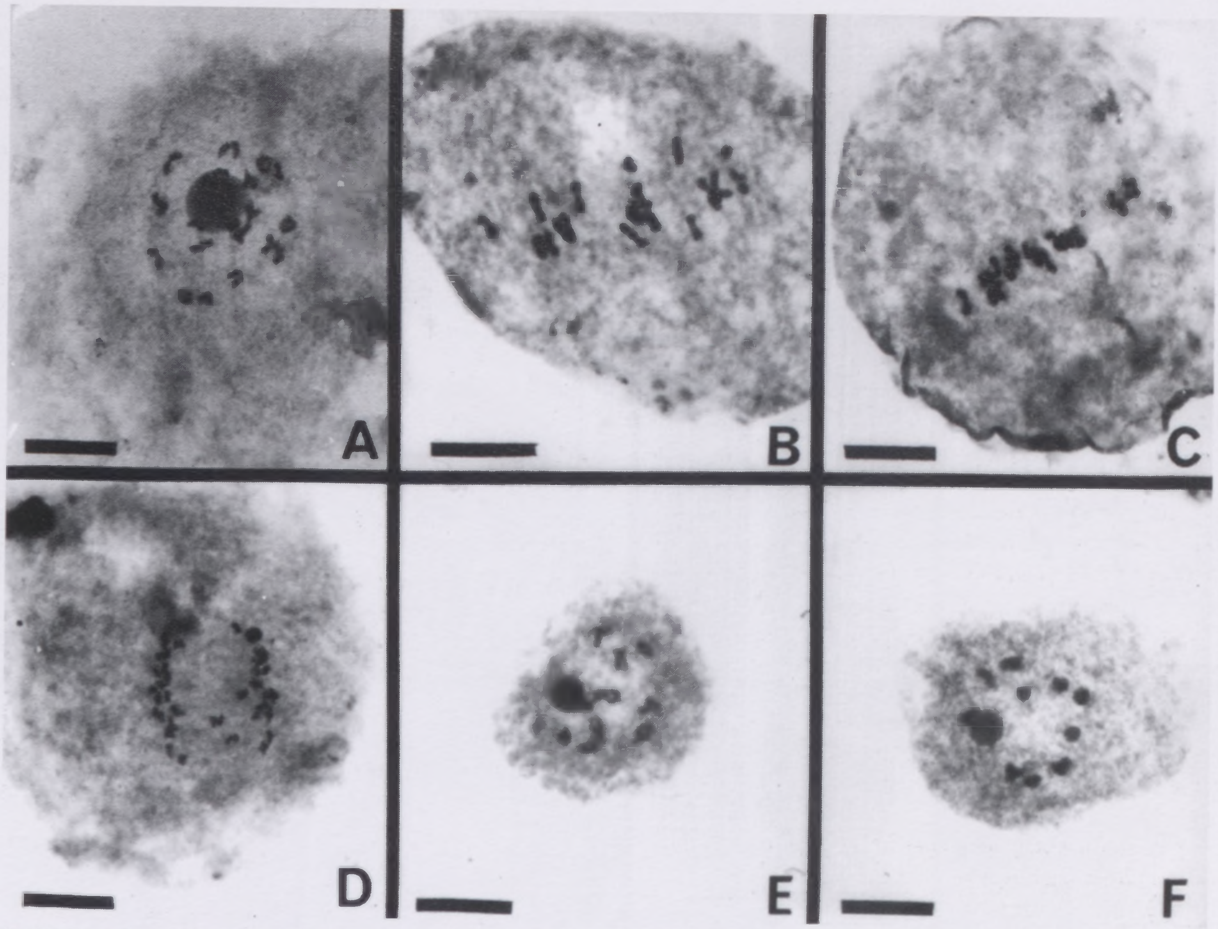

FIGURE 3.—Photomicrographs of meiotic chromosomes in *Rendlia altera* and *Tragus berteronianus*. A–D, *R. altera*: A, *Spies 5077*, n = 20, diakinesis with 20_{II}; B, *Spies 5077*, n = 20, metaphase I; C, *Spies 5072*, n = 20, metaphase I with 20_{II}; D, *Spies 5077*, n = 20, anaphase I with a 20-20 segregation. E, F, *T. berteronianus, Spies 2605*, n = 10, diakinesis with 10_{II}. Scale bar = 20 µm.

Research and Development and the University of the Orange Free State is gratefully acknowledged.

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MS. received: 1992-12-14.