Bothalia 19,1: 125–132 (1989)

Chromosome studies on African plants. 9. Chromosome numbers in *Ehrharta* (Poaceae: Ehrharteae)

J. J. SPIES*, E. J. L. SAAYMAN*, S. P. VOGES** and G. DAVIDSE***

Keywords: aneuploidy, chromosome numbers, *Ehrharta*, meiosis, polyploidy, southern Africa, winter rainfall area

ABSTRACT

Cytogenetic studies of 53 specimens of 14 species of the genus *Ehrharta* Thunb. confirmed a basic chromosome number of 12 for the genus. Chromosome numbers for 13 species are described for the first time. The highest ploidy level yet observed in the genus (2n = 10x = 120) is reported for *E. villosa* var. *villosa*. B chromosomes were observed in several specimens of four different species.

UITTREKSEL

Sitogenetiese studies van 53 eksemplare, verteenwoordigend van 14 spesies van die genus *Ehrharta* Thunb., bevestig 'n basiese chromosoomgetal van 12 vir die genus. Chromosoomgetalle van 13 spesies word vir die eerste keer beskryf. Die hoogste poliploïede vlak wat nog in die genus beskryf is (2n = 10x = 120), is by *E. villosa* var. *villosa* gevind. B-chromosome is by verskeie eksemplare van vier verskillende spesies waargeneem.

INTRODUCTION

The presentation of chromosome numbers in this report continues with the format established in the first publication in this series (Spies & Du Plessis 1986). However, contrary to previous publications in this series which all dealt with several different genera of the family Poaceae, this paper covers the single genus, *Ehrharta* Thunb. The results presented here are limited to the species from the winter rainfall area of southern Africa.

The winter rainfall region of southern Africa, with its Mediterranean climate, is located in the extreme south-west of the continent. Though small in area, less than 4% of the total land surface of southern Africa, it is floristically extremely important, being recognized as one of the six Floral Kingdoms of the world (Goldblatt 1978). The flora of the Cape Floristic region, or Fynbos Biome, is unique in many respects, being particularly rich in species (Goldblatt 1978), with high levels of endemism. Thus, in the 87 244 km^2 of the Cape Region (Fynbos and Succulent Karoo Biomes combined), there are over 8 000 species of which 73% are endemic (Gibbs Russell 1987). High species diversity characterizes this fynbos vegetation (Gibbs Russell 1987) and this is also the case with the grasses of this biome. Although relatively inconspicuous in this vegetation, the fynbos grasses are particularly interesting as many of them are restricted to this region and are poorly known taxonomically.

Ehrharta is one of the grass genera with a high proportion of its species endemic to the Fynbos Biome. All but three of the southern African species are endemic to the Fynbos Biome (Gibbs Russell pers. comm.). The genus belongs to the tribe Ehrharteae Nevski and, as currently classified, is represented by 30 species (with 16 infraspecific taxa) in southern Africa (Gibbs Russell & Ellis 1987). Gibbs Russell & Ellis (1987) subdivided the South African representatives of the genus into seven species groups, i.e. the Setacea, Capensis, Erecta, Calycina, Villosa, Ramosa and Dura groups. The higher classification of the tribe Ehrharteae, which has been reviewed by Gibbs Russell & Ellis (1987), is still unresolved. Most recently Clayton & Renvoize (1986) included the tribe in the subfamily Bambusoideae. However, both Soderstrom & Ellis (1987) and Kellogg & Campbell (1987) excluded it from the Bambusoideae, to which they considered the Ehrharteae to be a sister group.

The aim of this study was to determine the chromosome numbers and the meiotic chromosome behaviour of as many different species as possible. Future studies will determine whether this information can contribute to the delimitation of species in the genus, as well as to clarifying the position of the Ehrharteae in the Poaceae.

MATERIALS AND METHODS

The material used during this study was collected and fixed in the field. A list of the material used and the localities are listed under results. Voucher herbarium specimens are housed in PRE.

Inflorescences were fixed in Carnoy's fixative (1886). After 24–48 hours of fixation, the fixative was replaced by 70% ethanol. Anthers were squashed in aceto-carmine (Darlington & La Cour 1976). Slides were permanently mounted by freezing them with liquid CO_2, followed by dehydration in ethanol and mounting in Euparal.

* Botanical Research Institute, Department of Agriculture and Water Supply, Private Bag X101, Pretoria 0001.

** Current address: Biotechnological Research Centre, Private Bag X293, Pretoria 0001.

*** Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166-0299, USA.

MS. received: 1988.09.12.

RESULTS

Family Poaceae, Tribe Ehrharteae, Genus *Ehrharta*.

Capensis group

Capensis subgroup

Ehrharta barbinodis Nees ex Trin.: **n = 12.**

CAPE.—2917 (Springbok): 1 km from Springbok to Hondeklip Bay (−DD), *Spies 3000*.

E. capensis Thunb.: **n = 12.**

CAPE.—3218 (Clanwilliam): Versveld Pass (−DC), *Spies 3168*.

Longifolia subgroup

No results.

Erecta group

E. erecta Lam. var. *erecta*: **n = 12.**

CAPE.—3325 (Port Elizabeth): King Neptune Beach (−DC), *Spies 3478*.

E. erecta Lam. var. *natalensis* Stapf: **n = 12.**

CAPE.—3323 (Willowmore): Blouwkransrivier Pass (−DC), *Davidse 33656*.

E. triandra Nees ex Trin.: **n = 12 & 24.**

CAPE.—2917 (Springbok): 21 km from Springbok to Kleinsee (−DA), *Davidse 33240* (n = 24). 3018 (Kamiesberg): 6 km east of Kamieskroon (−BD), *Davidse 33320* (n = 12).

E. longiflora J. E. Sm.: **n = 12 & 24.**

CAPE.—2918 (Gamoep): 21 km from Springbok to Kleinsee (−CA), *Spies 3343* (n = 12). 3018 (Kamiesberg): 52 km from Springbok to Loeriesfontein (−AA), *Spies 3375* (n = 12); Buffelsrivier Valley between Pedroskloof and Bobbejaanshoek (−AA), *Davidse 33308* (n = 12); 6 km east of Kamieskroon (−BD), *Spies 2992* (n = 12). 3418 (Simonstown): Redhill, 4 km from Simonstown (−AB), *Spies 3233* (n = 24).

Calycina group

E. brevifolia Schrad. var. *brevifolia*: **n = 12.**

CAPE.—3017 (Hondeklipbaai): coastal dunes near Groenrivier (−DC), *Davidse 33365*.

E. brevifolia Schrad. var. *cuspidata* Nees: **n = 12 + 0−1B.**

CAPE.—2917 (Springbok): 36 km from Port Nolloth to Kleinsee (−AC), *Davidse 33273*; *Spies 3361*, *3362*. 3017 (Hondeklipbaai): 15 km east of Hondeklip Bay (−AB), *Spies 3028*; 42 km west of Garies towards Groenriviersmond (−DC), *Spies 3383*.

E. calycina J. E. Sm.: **n = 12, 12 + 0−2B, 24 & 24 + 2B.**

CAPE.—2917 (Springbok): 17 km from Okiep to Goodhouse (−BD), *Spies 2995* (n = 12); 1 km from Springbok to Hondeklip Bay (−DD), *Spies 3003* (n = 12 + 0−2B), *3005* (n = 24). 3017 (Hondeklipbaai): 6 km east of Kamieskroon in Kamiesberg Pass (−BB), *Davidse 33321* (n = 24), *33326* (n = 12 + 0−2B); *Spies 3380* (n = 12); 42 km from Garies to Groenrivier (−DC), *Spies 3393* (n = 12); 3 km south of Groenrivier on road to Kotzerus (−DD), *Davidse 33371* (n = 12 + 2B). 3018 (Kamiesberg): 16 km east of Kamieskroon (−AC), *Spies 3058* (n = 24), *3062* (n = 12); Rooiberg in Kamiesberg Range (−AC), *Davidse 33337* (n = 12), *33349* (n = 24 + 2B). 3118 (Vanrhynsdorp): Gifberg Pass (−DC), *Spies 3094* (12 + 2B), *3095* (n = 12); 24 km from Clanwilliam to Elizabethfontein (−DD), *Davidse 33417* (n = 12). 3119 (Calvinia): Vanrhyns Pass (−AC), *Spies 3110* (n = 12); top of Botterkloof Pass (−CD), *Davidse 33401* (n = 12). 3318 (Cape Town): near Stellenbosch turnoff on road between Paarl and Franschhoek (−DD), *Spies 3198* (n = 12 + 2B). 3321 (Ladismith): Seweweekspoort Pass (−AD), *Spies 3272* (n = 12 + 0−2B). 3323 (Willowmore): 5 km from Avontuur to Uniondale (−CA), *Davidse 33687* (n = 24). 3419 (Caledon): 5 km from Hermanus to Onrustrivier (−AC), *Davidse 33828* (n = 12 + 0−2B). 3421 (Riversdale): 4 km from Kweekkraal to Droërivier (−AA), *Spies 3549* (n = 12 + 0−2B); 2 km from Vermaaklikheid to Puntjie (−AC), *Spies 3562* (n = 12). 3424 (Humansdorp): Skuitbaai (−AA), *Davidse 33638* (n = 12).

E. delicatula (Nees) Stapf: **n = 12.**

SWA/NAMIBIA.—2616 (Aus): near Aus (−CB), *Spies 2927*.

CAPE.—2917 (Springbok): 57 km from Kleinsee to Springbok (−DA), *Davidse 33285*.

E. melicoides Thunb.: **n = 12.**

CAPE.—3018 (Kamiesberg): Rooiberg in the Kamiesberg Range (−AC), *Davidse 33346*. 3119 (Calvinia): 25 km from Calvinia to Clanwilliam (−CA), *Spies 3122*. 3420 (Bredasdorp): 6 km from Malgas to Bredasdorp (−AD), *Spies 3251*.

E. pusilla Nees ex Trin.: **n = 12.**

CAPE.—2918 (Gamoep): 53 km from Springbok to Gamoep (−CC), *Davidse 33302*. 3018 (Kamiesberg): Buffelsrivier Valley between Pedroskloof and Bobbejaanshoek (−AA), *Davidse 33310*; 52 km from Springbok to Loeriesfontein (−AA), *Spies 3374*.

Villosa group

E. villosa Schult. f. var. *villosa*: **n = 60.**

CAPE.—3424 (Humansdorp): 27 km from Humansdorp to Cape St. Francis (−BB), *Spies 3505*.

Ramosa group

E. ramosa (Thunb.) Thunb. subsp. *ramosa*: **n = 12.**

CAPE.—3324 (Steytlerville): 71 km from Rocklands to Elandsrivier (−DB), *Spies 3490*.

E. rehmannii Stapf subsp. *rehmannii*: **n = 12 + 0−5B.**

CAPE.—3322 (Oudtshoorn): Montagu Pass (−CD), *Davidse 33708*.

E. rehmannii Stapf subsp. *subspicata* Stapf: **n = 36.**

CAPE.—3323 (Willowmore): 9 km from Coldstream to Humansdorp (−DD), *Spies 3515*.

Dura group

E. dura Nees ex Trin.: **n = 12 + 0−4B.**

CAPE.—3322 (Oudtshoorn): Robinson Pass (−CC), *Davidse 33534*.

DISCUSSION

According to Watson *et al.* (1986) the basic chromosome number of the genus *Ehrharta* is 12 and ploidy levels vary from diploid to tetraploid. This study confirms the basic chromosome number but increases the

range of polyploidy from diploid to decaploid. In order to place the cytogenetic results in a taxonomic context, the results are discussed according to the species groups of Gibbs Russell & Ellis (1987).

Both species studied in the Capensis subgroup of the Capensis group, i.e. *E. barbinodis* and *E. capensis*, were diploid with no meiotic abnormalities (Figure 1A). This is the first reported chromosome number for both species.

The described diploid and tetraploid levels in the Erecta group (Parthasarathy 1939; De Wet & Anderson 1956; Tateoka 1965; Raven *et al.* 1965; Fernandes & Queiros 1969; Davidse *et al.* 1986; Spies & Du Plessis 1986; Hoshino & Davidse 1988) are supported by this study. Chromosome numbers are reported for the first time for *E. erecta* var. *natalensis* (n = 12) and *E. triandra* (n = 12 & 24) (Figure 1B), as well as a new ploidy level for *E. longiflora* (n = 12). Results reported up to now indicate that 72 % of the sampled plants are diploid. With the exception of *E. erecta* var. *natalensis*, of which only one specimen has been studied, the four other taxa of the group have both diploid and tetraploid specimens. *E. triandra* and *E. longiflora* are closely related and have many morphological similarities, with *E. longiflora* being larger than *E. triandra*. However, since both species have been shown to have diploid and tetraploid populations, this size difference cannot be attributed to different ploidy levels.

The Calycina group was the most thoroughly studied with counts from 45 plants, representing six of the seven taxa. Both diploid (84%) and tetraploid (16%) specimens were observed (Figure 2D–L) which corroborates published results (Parthasarathy 1939; Love 1948; Tateoka 1957; De Wet 1960; Tothill & Love 1964; Spies & Du Plessis 1986; Hoshinso & Davidse 1988; Spies & Voges 1988). The polymorphic *E. calycina* diploid forms include prostrate coastal plants with short, broad leaf blades as well as the very common, widely distributed plants with erect growth habit and flat leaf blades. The tetraploid form of *E. calycina* seems to be restricted to densely tufted plants with erect, narrow, often rolled leaf blades. The chromosome numbers reported here for *E. brevifolia* var. *brevifolia* and *E. brevifolia* var. *cuspidata* (Figure 2A–C), as well as *E. delicatula* and *E. melicoides*, are the first reported numbers for the taxa.

A very high frequency of specimens from the Calycina group were aneuploid (24%) with one or two additional chromosomes, although most had two. With the exception of one *E. brevifolia* var. *brevifolia* specimen, these additional chromosomes were restricted to *E. calycina*. Love (1948), Tothill & Love (1964) and Spies & Voges (1988) have previously reported one to six additional chromosomes beyond the basic complement(s) in *E. calycina*. Tothill & Love (1964) referred to these additional chromosomes as supernumerary chromosomes and Spies & Voges (1988) called them B chromosomes. Both groups of authors observed no size or behavioural differences between the additional and standard chromosomes. As in these three previous studies, we observed meiotic irregularities, including late disjunction of bivalents during the first division (Figure 2L) and non-alignment of chromosomes on the metaphase plate (Figure 2J). As was also observed in these previous studies, the extra chromosomes may sometimes pair, either as multivalents or as bivalents (Figure 2F). Additional abnormalities observed during meiosis include the presence of univalents in a diploid specimen *Davidse 33371* (n = 12) and up to six anaphase I laggards in *Davidse 33321* (n = 24) (Figure 2L).

A very narrow spindle on one side of the cell was observed in an *E. delicatula* specimen *(Davidse 33285)* (Figure 3A & B). Specimens of both *E. brevifolia* and *E. delicatula* had two chromosome pairs associated with the nucleolus (Figure 2A & B).

Only one specimen of the Villosa group was successfully analysed. This specimen, *Spies 3505 (E. villosa* var. *villosa)*, provides the first chromosome number report for a member of this group and it proved to have the highest ploidy yet described in the Ehrharteae, i.e. n = 60 (Figure 4A & B). Apart from a few multivalents and univalents observed in this specimen, meiosis in this decaploid was normal and no laggards were observed, which is surprising in view of the high ploidy level.

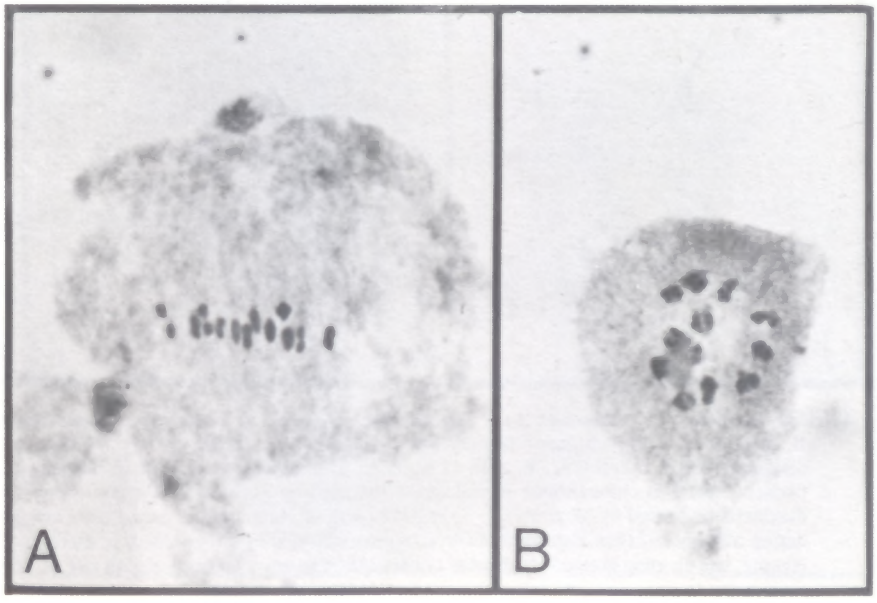


FIGURE 1.—Meiotic chromosomes in: A, the Capensis subgroup, *E. capensis, Spies 3168*, metaphase I with 12 bivalents; B, the Erecta group, *E. triandra, Davidse 33320*, diakinesis with 12 bivalents of which four are associated with the nucleolus, × 1300.

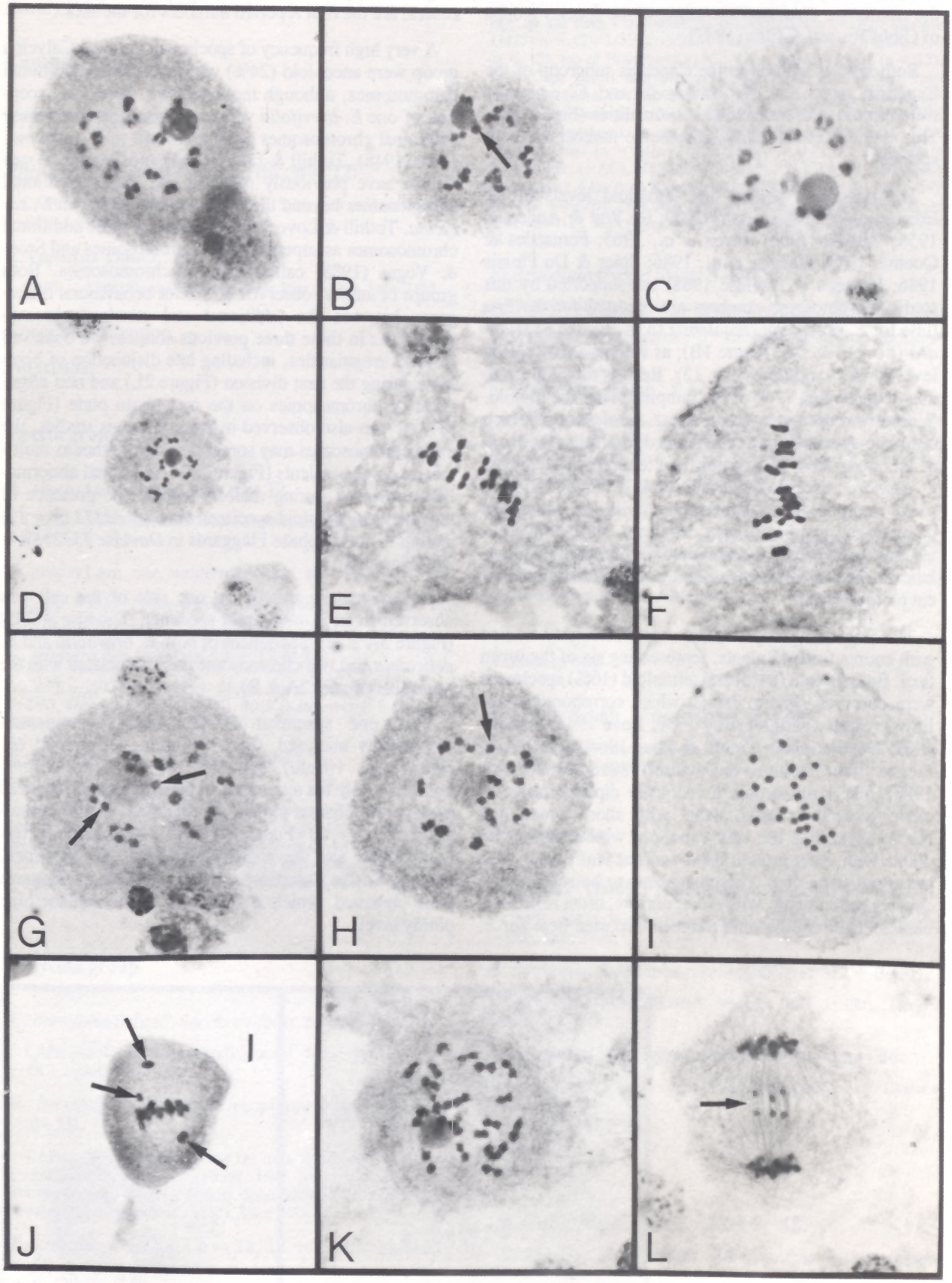


FIGURE 2.—Meiotic chromosomes in the Calycina group. A–C: *E. brevifolia* var. *cuspidata*, with 12 bivalents at diakinesis, A, *Spies 3028*; B, *Spies 3362*, with B chromosome (indicated by an arrow); C, *Davidse 33273*. D–L: *E. calycina*, D, *Spies 2995*, with 12 bivalents at diakinesis; E&F, *Spies 3272*, E, with 12 bivalents at metaphase I; F, with 13 bivalents at metaphase I; G, *Davidse 33828* with 12 bivalents and 2 B chromosomes at diakinesis (indicated by arrows); H, *Davidse 33371*, with 12 bivalents and a B chromosome at diakinesis (indicated by an arrow); I, *Spies 3198*, with 26 chromosomes during early anaphse I; J, *Spies 3003*, with 2 B chromosomes during metaphase I (non-alignment of chromosomes indicated by arrows); K & L, *Davidse 33321*, with 24 bivalents at diakinesis and 3 lagging, late dividing bivalents at anaphse I (indicated by an arrow). D & J, × 730; rest, × 1200.

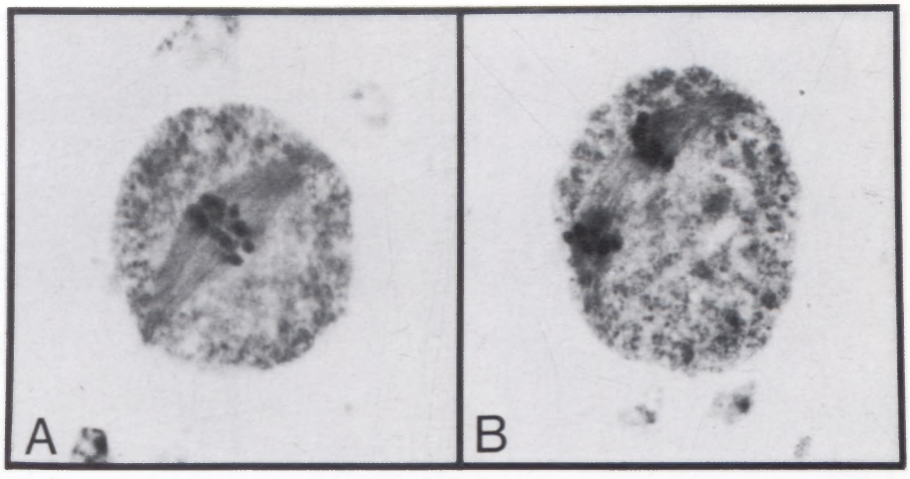


FIGURE 3.—Meiotic chromosomes in the Calycina group: A & B, *E. delicatula*, *Davidse 33285*, with 12 bivalents and a very distinct and narrow spindle, which may be off-centre, B, × 1100.

Three specimens of the Ramosa group were counted and the counts represent the first chromosome number reports for these species. The *E. ramosa* subsp. *ramosa* specimen was a normal diploid plant (Figure 5A & B). The *E. rehmannii* subsp. *rehmannii* specimen was a diploid with 0−5 B chromosomes (Figure 5C − E). During metaphase I the B chromosomes were indistinguishable from the euchromosomes but at the end of anaphase I they seemed to despiralize (Figure 5D). It seems as if the division of these normal bivalents into chromosomes and the division of the univalents into chromatids is not synchronized. The *E. rehmannii* subsp. *subspicata* specimen had the second highest ploidy level found in the Ehrharteae, with n = 36. A low frequency of multivalents (up to hexavalents) were observed in this subspecies (Figure 5F & G). In addition,

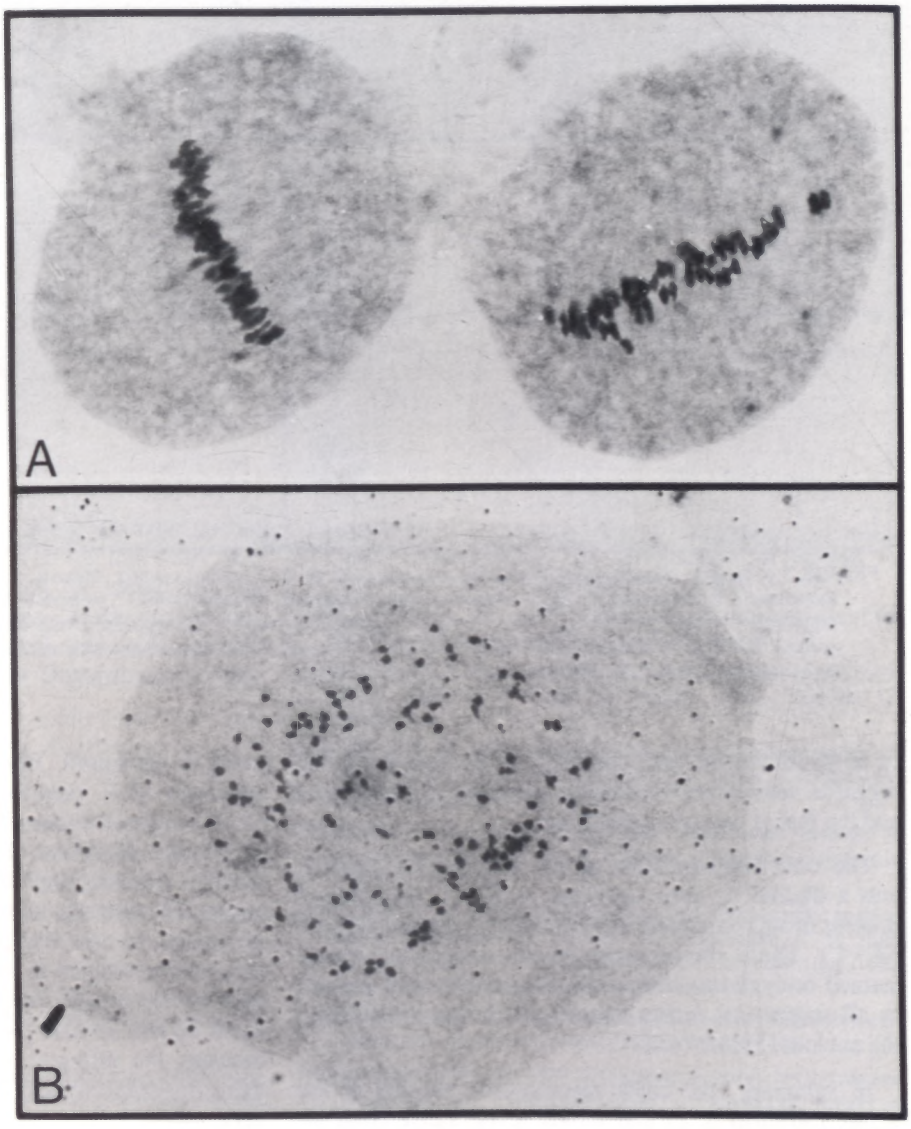


FIGURE 4.—Meiotic chromosomes in the Villosa group. A & B, *E. villosa* var. *villosa*, *Spies 3505*, A, 60 bivalents in metaphase; B, 120 chromosomes during anaphase I. Additional chromosome-like images in the photograph were caused by crystalization of stain which can be differentiated from the chromosomes under the microscope, × 930.

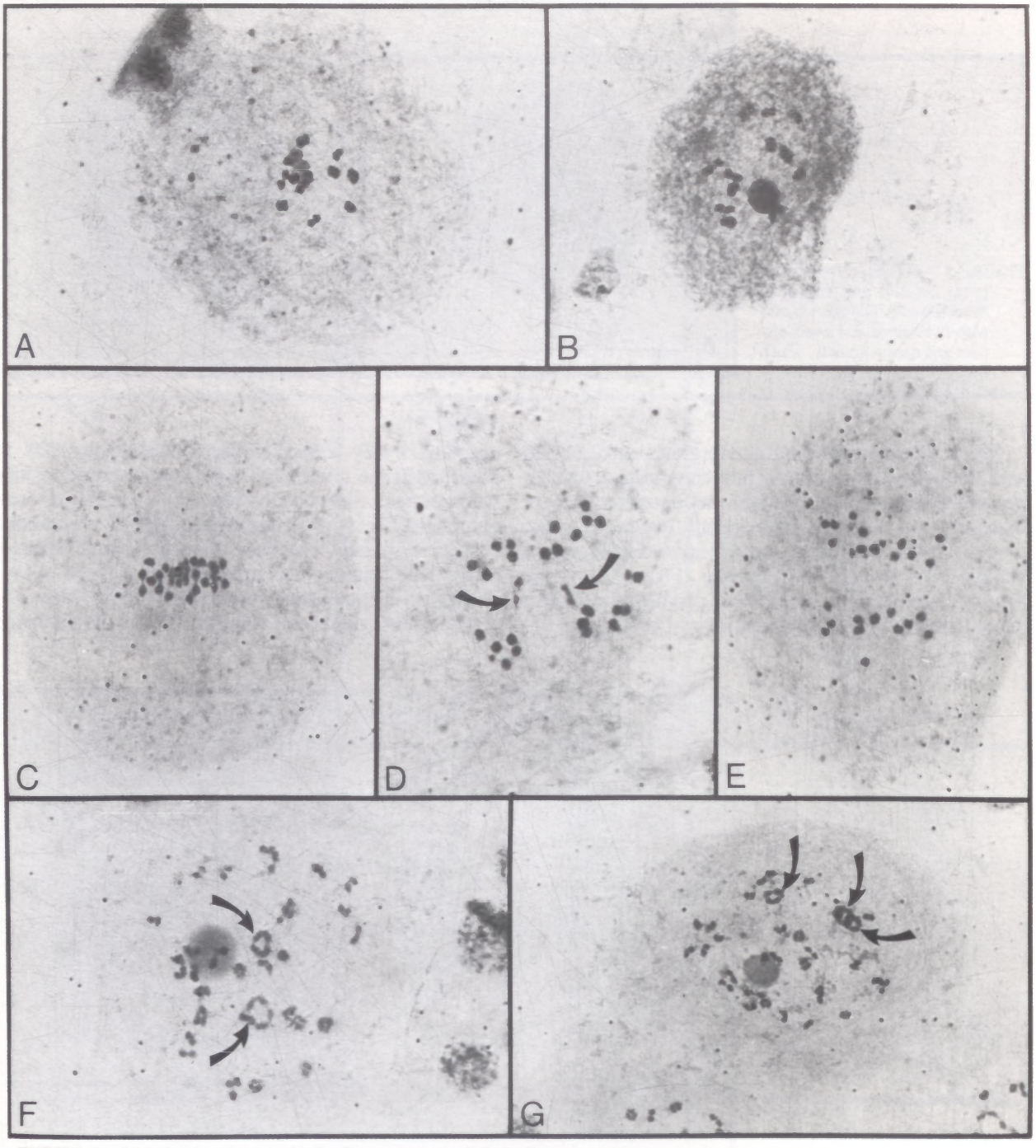


FIGURE 5.—Meiotic chromosomes in the Ramosa group. A & B: *E. ramosa* subsp. *ramosa*, *Spies 3490*, with 12 bivalents, A, early metaphase I; B, diakinesis. C–E: *E. rehmannii* subsp. *rehmannii*, *Davidse 33708*: C, metaphase I with 12 bivalents; D, anaphase I with 2 univalents dividing precociously into chromatids (indicated by arrows); E, anaphase I with unequal (12–14) distribution of chromosomes). F & G, *E. rehmannii* subsp. *subspicata*, *Spies 3515*, with 72 chromosomes which include multivalents (indicated by arrows). G, × 745; A, B, C, E & F, × 1000; D, × 1200.

up to six univalents were observed during diakineses. No laggards were observed in this specimen at anaphase I and the rest of meiosis was normal.

The only representative of the Dura group studied, was a diploid *E. dura* specimen with a B chromosome present in approximately 90% of the cells studied (Figure 6A–C). The B chromosome appears as a round, lightly stained body during diakinesis (Figure 6B). It is present in all stages and during telophase I it is excluded from the nucleus (Figure 6C).

In summary, the basic chromosome number of the genus *Ehrharta* is 12 and ploidy levels range from diploid to decaploid. Based on one earlier count for one specimen of *E. calycina* with 2n=30, Spies & Voges (1988) suggested that a basic chromosome number of six is possible. However, the current, much more extensive results, indicate that the 2n=30 plant is probably an aneuploid with six additional chromosomes. It is also interesting to note that ploidy levels exceeding tetraploidy, were restricted to the eastern part of the distribution area of the genus. Further cytogenetic studies on *Ehrharta* should include the production of artificial hybrids between the different species and meiotic analyses of these hybrids to clarify the genomic relationships in the genus.

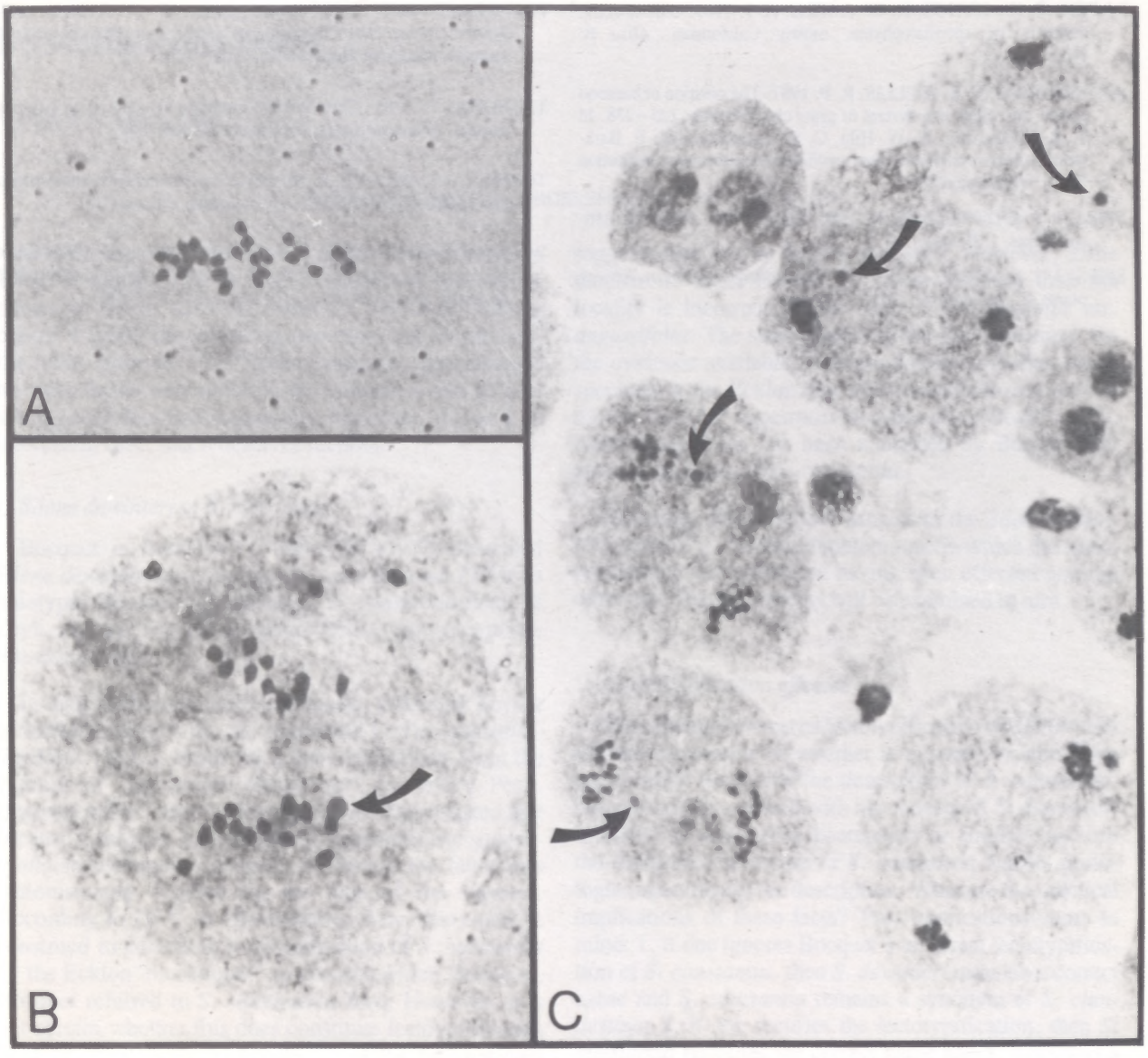


FIGURE 6.—Meiotic chromosomes in the Dura group: *E. dura*, *Davidse 33534*, with 12 bivalents and up to four B chromosomes (indicated by arrows). A, × 1400; B, × 1700; C, × 1000.

ACKNOWLEDGEMENTS

Dr R. P. Ellis is thanked for his comments on the manuscript and useful suggestions during this study. We also thank Dr G. E. Gibbs Russell for identifying the plant specimens, as well as for many useful suggestions during the study. Dr Davidse's work was made possible by a research fellowship from the Department of Agriculture and Water Supply.

REFERENCES

CARNOY, J. B. 1886. La cytodierèse de l'oeuf. *Cellule* 3: 1–92.

CLAYTON, W. D. & RENVOIZE, S.A. 1986. Genera graminum—grasses of the world. *Kew Bulletin Additional Series* 13: 1–389.

DARLINGTON, C. D. & LA COUR, L. F. 1976. *The handling of chromosomes*. Allen & Unwin, London.

DAVIDSE, G. HOSHINO, T. & SIMON, B. K. 1986. Chromosome counts of Zimbabwean grasses (Poaceae) and an analysis of polyploidy in the grass flora of Zimbabwe. *South African Journal of Botany* 52: 521–528.

DE WET, J. M. J. 1960. Chromosome numbers and some morphological attributes of various South African grasses. *American Journal of Botany* 47: 44–50.

DE WET, J. M. J. & ANDERSON, L. J. 1956. Chromosome numbers in Transvaal grasses. *Cytologia* 21: 1–10.

FERNANDES, A. & QUEIROS, M. 1969. Contribution à la connaissance cytotaxonomique des Spermatophyta du Portugal. I. Gramineae. *Boletim da Sociedade Broteriana* 43: 3–140.

GIBBS RUSSELL, G. E. 1987. Preliminary floristic analysis of the major biomes in southern Africa. *Bothalia* 17: 213–227.

GIBBS RUSSELL, G. E. & ELLIS, R. P. 1987. Species groups in the genus *Ehrharta* (Poaceae) in southern Africa. *Bothalia* 17: 51–65.

GOLDBLATT, P. 1978. Analysis of the flora of southern Africa: its characteristics, relationships and origins. *Annals of the Missouri Botanical Garden* 65: 369–436.

HOSHINO, T. & DAVIDSE, G. 1988. Chromosome numbers of grasses from southern Africa. I. *Annals of the Missouri Botanical Garden* 75. In press.

KELLOGG, E. A. & CAMPBELL, C. S. 1987. Phylogenetic analysis of the Gramineae: 310–322. In T. R. Soderstrom, K. W. Hilu, C. S. Campbell & M. E. Barkworth, *Grass systematics and evolution*. Smithsonian Institution Press, Washington D.C.

LOVE, R. M. 1948. Preliminary cytological studies of *Ehrharta calycina* Smith. *American Journal of Botany* 35: 358–360.

PARTHASARATHY, N. 1939. Cytogenetical studies in Oryzeae and Phalarideae. II. Cytological studies in Phalarideae. *Annals of Botany* 3: 43–58.

RAVEN, P. H., KYHOS, D. W. & HILL, A. J. 1965. Chromosome numbers of spermatophytes, mostly Californian. *Aliso* 6: 105–113.

SODERSTROM, T. R. & ELLIS, R. P. 1987. The position of bamboo genera and allies in a system of grass classification: 225–238. In T. R. Soderstrom, K. W. Hilu, C. S. Campbell & M. E. Barkworth, *Grass systematics and evolution*. Smithsonian Institution Press, Washington D.C.

SPIES, J. J. & DU PLESSIS, H. 1986. Chromosome studies on African plants. 1. *Bothalia* 16: 87–88.

SPIES, J. J. & VOGES, S. P. 1988. Chromosome studies on African plants. 7. *Bothalia* 18: 114–119.

TATEOKA, T. 1957. Notes on some grasses. VI. (9. *Coelachne* and *Sphaerocaryum*. 10. Chromosomes and leaf structure of several species). *Botanical Magazine* 827: 119–125.

TATEOKA, T. 1965. Chromosome numbers of some East African grasses. *American Journal of Botany* 52: 864–869.

TOTHILL, J. C. & LOVE, R. M. 1964. Supernumerary chromosomes and variation on *Ehrharta calycina* Smith. *Phyton* 21:21–28.

WATSON, L., DALLWITZ, M. J. & JOHNSTON, L. R. 1986. Grass genera of the world: 728 detailed descriptions from an automated database. *Australian Journal of Botany* 34: 223–230.