CYTOGENETICS OF SOME HEXAPLOID X TETRAPLOID HYBRIDS IN *CYNODON*

By

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CYTOGENETICS OF SOME HEXAPLOID X TETRAPLOID HYBRIDS IN CYNODON

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CHAPTER I

INTRODUCTION

An understanding of the reproductive mechanisms is essential to a plant improvement program and consequently, plant breeders have turned to cytologists for information on chromosome activity. Thus, the study of chromosomes and their relation to fertility and the transmission and recombination of genetic information has become an integral and fundamental part of plant breeding and genetics. Before the initiation of any breeding program, the breeder must become familiar with the plant material in question. Of utmost importance is knowledge of its taxonomy and the degree of morphological and cytological variation encompassed within the limits of the breeding population (7). Cytological information that is fundamental to genetic and breeding studies is chromosome number and morphology, ploidy level, pairing behavior, genomic relationships and occurrence of chromosome abnormalities.

The genus Cynodon Rich. is a relatively small taxon, containing nine species and ten varietal subdivisions (17). Its distribution is primarily limited to the tropics and subtropics. Bermudagrass, Cynodon dactylon (L.) Pers., however; has an expanded area of distribution extending almost continuously between 45°N and 45°S latitude (8). The vast genetic diversity present in this species prompted Harlan and de Wet to call it "one of the most dynamic, aggressive and cosmopolitan species in the world' (15, p. 778). The extensive genetic diversity

within C. dactylon and its ability to hybirdize with other species of the genus has been important in facilitating its agronomic improvement.

The genus Cynodon has been the object of several cytological studies. Ploidy levels that have been reported to occur within the genus thus far are diploid, triploid, tetraploid, and hexaploid (5, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22, 23, 25, 26). In the Cynodon improvement program at Oklahoma State University, hexaploid by tetraploid crosses have been made and viable F_1 hybrids obtained. The objectives of this study were to determine the unreduced chromosome number, study meiotic stability and observe chromosome behavior and pairing in six hexaploid x tetraploid hybrids in Cynodon Rich.

CHAPTER II

REVIEW OF LITERATURE

General

Myers (24), in his study on the cytology and genetics of forage grasses, states two primary reasons for the initiation of cytological and genetic investigations of the grasses: a) to serve as an adjunct to morphological data in studies of the taxonomy and phylogeny of the Gramineae and b) to provide fundamental information for the improvement of species by breeding. The formation of interspecific hybirds to improve vigor, yield, resistance, and other desirable characters was begun when breeders learned that several speces of wild grasses are closely related to crop species (33). Early literature regarding grass cytogenetics pertained primarily to biosystematic investigations aimed at revision and elucidation of grass taxonomy and phylogeny (1, 35). Other studies (19, 21) followed, and the relative constancy of chromosome number and morphology within a species became well documented.

The potential value of bermudagrass as a forage crop was realized well over 200 years ago (6). Now, after extensive germ plasm collection and breeding, it has become one of the most widely used forage grasses in the southeastern United States (11). Initial breeding programs with bermudagrass chiefly involved introduction, selection, and hybridization through chance cross-pollination. A more exact method of hybridization

in species with small florets was made possible by Richardson's development of a hand emasculation technique (29). Since that time, the technique has been used extensively in making controlled crosses between grasses at Oklahoma State University in Stillwater. It was also the method used in the biosystematic studies of the genus Cynodon by Harlan and de Wet (8, 15, 16). Information from these and other studies was obtained on the crossability within and between species (5) and genomic relationships between the different ploidy levels (10, 11, 15, 16). Breeders and cytologists are interested in chromosomal behavior of hybrids derived from crosses involving different ploidy levels. Such crosses often increase plant vigor and environmental adaptability. Hybrids at the triploid and pentaploid level, due to their odd number of chromosome sets, generally have irregular meiosis and are sterile. Such hybrids are important, however, in the production of aneuploids (such as trisomics) and creating genetic bridges between species.

Cytology of Cynodon

The presence of extensive morphological variability and the prevalence of inter- and intraspecific hybridization between many of its members, have made classification of the genus Cynodon difficult. Derivation of an explicit and complete taxonomic scheme for this genus has been the objective of several biosystematic studies (15, 16, 20, 22). Interpretations and revisions were made on the basis of data collected in the following areas: a) gross plant morphology, b) ecological behavior and distribution, c) inter- and intraspecific hybridization and d) plant cytogenetics.

In an early study, Hurcombe (20) reported 2N chromosome numbers for the species Cynodon bradleyi Stent; C. transvaalensis, C. magennisii, C. dactylon (L.) Pers. and C. dactylon (L.) Pers. var densus, to be 18, 20, 30 and 40 respectively. She interpreted C. bradleyi Stent (2N = 18) to be an aneuploid produced by the union of two 2N-1 gametes. Thus, the basic chromosome number in the genus Cynodon was thought to be ten. Later investigations by several workers showed the correct basic chromosome number to be nine and substantiated the existence of 2x, 3x, 4x and 6x ploidy levels (5, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 26). Forbes and Burton (11) found what appeared to be chromosome fragments in some Cynodon ascessions which they interpreted to be satellites belonging to the nucleolar organizing chromosomes. They concluded that those investigators who reported a basic number of ten (20, 23) were misinterpreting these satellites to be whole chromosomes because the secondary constrictions were long, poorly visible and and easily broken by cytological manipulation.

Studies of interspecific hybrids within the genus have been done. Burton (5) studied F_1 plants from crosses between C. dactylon x C. plectostachyus and C. transvaalensis x C. dactylon and found them to be sterile triploids. Extensive biosystematic studies of the genus were conducted by Harlan et al. (16, 18) in 1969 and 1970. They reported three species, C. barberi, C. arcuatus and C. plectostachyus, to be completely isolated from each other and the rest of the genus. C. aethiopecus was also well isolated genetically, with only occasional success in crossing with other species. Hybridization between the rest of the species in the genus could be done with relative ease. These biosystematic investigations led to the current revision of the genus

(17) which includes nine species and ten varieties (Table I).

Tetraploidy and other ploidy levels that are whole number multiples of 2x, usually are sexually stable and undergo normal meiosis, 4x by far the most common. Forbes and Burton (11) reported average chromosome pairing at diakinesis in Coastal bermudagrass (2N = 36) to be .15 I's, 16.00 II's and .96 IV's, with no more than 2 I's or 2 IV's per cell. Another 4x plant introduction (P.I. 226011), also scored at diakinesis, averaged 1.36 I's, 15.69 II's, .18 III's and .68 IV's, with no more than 4 I's, 2 III's or 3 IV's per cell. They reported 49.4% of the cells at anaphase I had laggards and 50.7% of the tetrads contained micronuclei in Costal. P.I. 226011 had 39% laggards at anaphase I with only an occasional micronuclei in the tetrads.

Hexaploid autopolyploids have been found within the genus Cynodon, but are quite uncommon. Moffett and Hurcombe (23) reported a 2N = 54 race in C. plectostachyus and Felder (10) reported obtaining hexaploid offspring from a cross between C. dactylon (2N = 36) and C. plectostachyus (2N = 18). He attributed this to fertilization of an unreduced egg. Powell et al. (26) also reported finding a vigorous hexaploid among F_1 seedlings resulting from a cross between C. dactydon (2N = 36) and C. transvaalensis (2N = 18). They theorized that the hexaploid was formed, instead of an expected triploid, due to endomitosis in early zygote development. Clear cut analysis of chromosome pairing was difficult but associations greater than tetravalents were rare. The most common associations were bivalents and tetravalents in the form of rings or chains. Chromosomes associated as trivalents were less frequently observed. As many as eight univalents per cell

TABLE I

A REVISED CLASSIFICATION OF THE GENUS CYNODON*

Taxon	Chromosome number
C. aethiopicus Clayton et Harlan	18, 36
C. arcuatus J.S. Presl. ex. C.B. Presl.	36
C. barberi Rang et Tad.	18
C. Dactylon (L.) Pers. var dactylon	36
var. afganicus Harlan et de Wet	18, 36
var. aridus Harlan et de Wet	18
var. coursii (A. Camus) Harlan et de Wet	36
var. elegans Rendle	36
var. polevansii (Stent) Harlan et de Wet	36
C. incompletus Nees var. incompletus	18
var. hirsutus (Stent) de Wet et Harlan	18, rarely 36
C. nlemfuensis Vanderyst var. nlemfuensis	18, rarely 36
var. robustus Clayton et Harlan	18, 36
C. plectostachyus (K. Schum.) Pilger	18
C. transvaalensis Burtt-Davy	18
C. magennisii Hurcombe	27

*From Harlan et al. Okla. Agr. Exp. Sta. Bull. B-673, 1970.

were reported and most microsporocytes contained lagging chromosomes at late anaphase I. However, the occurrence of micronuclei, was low indicating that the laggards were included in the telophase nucleus.

Triploid plants have been reported in Cynodon dactylon (L.) Pers. (13, 14). Due to the odd number of chromosome sets, these polyploids have irregular meiosis, are unstable and do not persist in nature in the sexual form. They do, however, exist by asexual reproduction. The occurrence of triploids in nature is usually a result of diploid x tetraploid hybridization, but they may arise when unreduced eggs are formed in a diploid then fertilized by a haploid spore. In triploids, the chromosomes may associate during meiosis either as trivalents or as bivalents with univalents. Segregation from such configurations generally results in gametes with a chromosome number somewhere between the diploid and haploid number. Not only is segregation apt to be irregular, but very often univalents and bivalents lag on the metaphase plate and consequently are not included in the daughter nuclei (3). All of the triploid hybrids obtained by Forbes and Burton (11) were sterile. One triploid hybrid had a maximum of eight III's per cell at diakinesis, which suggests homology between the chromosomes of the diploid and tetraploid parent. In several of their triploid hybrids, chromosome stickiness at metaphase I was common. Stickiness may also account for the apparent multivalents. Investigations by Guptka and Srivastava (13, 14) revealed that bivalent and trivalent associations were common at metaphase I in naturally occurring triploid races of C. dactylon. The number of trivalents in these clones ranged from zero to six per cell. The number of univalents per cell ranged from 4 to 13, with the average number of univalents per cell being 6.8, 7.1 and 8.1 for three clones

studied. Micronuclei were common in the microspore tetrads. Most univalents divided at anaphase I, then lagged at anaphase II creating micronuclei. Felder (10) noted, in his study of triploid hybrids, that chromosome associations were essentially the same in all crossing combinations. He observed a maximum of 12 bivalents occurring in one cell, but most commonly found nine bivalents and nine univalents. An occasional trivalent or tetravalent was observed but often disassociated by early metaphase.

The occurrence of accessory, supernumerary, or B-chromosomes are now known to exist in over 150 species of flowering plants (34). They are primarily differentiated from the basic or A-chromosomes with respect to inconstancy in number, smaller size, a greater degree of heterochromatinization and they regularly undergo nondisjunction. In some plants accessory chromosomes may not be found in root or leaf tissue, even though they are present in the stem or flower tissue (3). Brown (3) states that accessories are probably chromosome remnants that have persisted. He attributes their occurrence to three natural phenomenon:

1. centric fusion
2. misdivision and fragmentation, producing tiny telocentrics of isochromosomes
3. subsequent modification of normal species into which accessories are introduced by hybridization

Most results indicate that in small numbers (1 to 3), accessories have no detectable effects or produce only slightly unfavorable effects. One possible effect is preferential fertilization, where the pollen nuclei containing the accessory chromosomes unites more often with the egg than it does the normal pollen (3).

Accessory chromosomes in Cynodon have been reported by several workers. Hurcombe (20) in her determination of ten as the basic number, was according to Forbes and Burton (11), probably counting a fragmented chromosome satellite. Gould (12) later reported finding races of C. dactylon with one and two accessories. Differences vary between reports as to whether the extra chromosomes are actually accessories or fragments. Powell et al. (26) state that even though differences in stainability may not be observable, accessories should be distinguishable due to their differences in meiotic behavior. In his study of one clone of C. dactylon, five accessories were detected. Their occurrence was uniform throughout the plant, being in both somatic tissue and in the meiocytes. At meiosis the accessories formed multiple associations among themselves, but were not seen to associate with the A-chromosomes. These accessories were characteristically heterochromatic.

Genomes are said to be exactly homologous if they contain the same genetic loci in identical sequence in their linkage structures, and homeologous, if only a part of the segments is identical in localization, due to structural rearrangements (30). Strict distinction between homology and non-homology is difficult, however, because genomes of a polyploid are not necessarily identical with respect to genic content and structural arrangement, unless they arose from chromosome doubling. Consequently, a species may be termed as autopolyploid (which signifies homology) and differ in genic content but be very similar in chromosome morphology (32). Currently, the prevailing criterion for homology is chromosome morphology and pairing behavior. Genomes having structurally similar chromosomes which commonly associate as multivalents are termed homologous.

Reports of chromosomal homology between genomes in Cynodon have been reported, but thus far the total evidence is inconclusive. Forbes and Burton (11) observed a high frequency of trivalents per cell in intraspecific triploid hybrids and a maximum of three tetravalents per cell in tetraploid hybrids of C. dactylon, indicating at least partial homology. In two studies (16, 18) regarding intra- and interspecific hybridization within Cynodon hybrid chromosome behavior indicated that all the crossable species in the genus share the same genome. Contuining these hybridization studies, Harlan et al. (18) found no evidence of genomic differentiation among diploids that could be hybridized. Felder (10) reported common chromosome configurations in triploid hybrids to be nine I's and nine II's. Harlan et al. (18) and Malik and Tripathi (22) also produced triploid hybrids and found the mode of pairing to be the same. In a study of three naturally occurring triploid clones of C. dactylon, Guptka and Srivastava (13, 14) reported apparent homology between genomes. They found from eight to ten univalents or up to six trivalents occurring per cell. Their assumption of homology is explained on the basis that the small size of the chromosomes made it difficult for chiasma to form, thus decreasing the number of multivalent formations. The average number of chiasmata per chromosome in their triploid clones ranged from .53 to .63. Forbes and Burton (11) reported chiasmata frequency in the tetraploid C. dactylon of 12 to 18 per cell, which coincides with the aforementioned averages per chromosome. Felder (10) suggests the presence of a common genome among diploids (DD). He theorizes that the genome constitution of polyploids is derived from the basic DD genome and refers to it as D^1D^1. Supporting this theory Harlan and de Wet (15) state that morphological and

cytological evidence seem to suggest that the tetraploid C. dactylon var. dactylon evolved from the diploid C. dactylon var. aridus. Irregular pairing in meiosis of the tetraploids indicates some variability between genomes. The differences between these genomes possibly arising through inversions or interchanges of short chromosome segments (32). Studies of chromosome morphology at pachytene, like that of Ourecky (25), would be instrumental in further studies on the degree of chromosome homology. Chromosome morphological features in his study of a diploid C. dactylon included, total chromosome length, chromosome arm lengths, arm ratios, number of prominent chromomeres per arm, presence or absence of terminal knobs and centromere position. Comparative chromosome analysis in polyploids, utilizing this type of information, would reveal structural changes which would effect chromosome pairing and homology.

Gamete viability is a primary concern of plant breeders who deal with polyploids. Stebbins (34) reports that low fertility and seed set are common among polyploids. Reasons he sites for this are: a) irregular meiotic pairing, b) abnormal chromosome segregation and c) various kinds of physiological unbalance. A quick, easy method that is commonly used to check gamete viability is the percent of well filled pollen. The tetraploid bermudagrass "Coastal" showed 40% pollen viability while another tetraploid (P.I. 220611) had 92% (11). Guptka and Srivastava (14, 14) reported pollen viability in triploid clones of C. dactylon ranging from 41% to 69%, with no seed set. Powell et al. (26) reported apparent pollen viability in one hexaploid of Cynodon to range from 30% to 90%, with most samples being 80% to 90% viable. Data of this type

is in no way conclusive but does indicate to a certain extent, the relative fertility of the plant.

A review of the literature shows no evidence of a pentaploid bermudagrass being produced artificially or occurring naturally. Meiosis would be expected to be about as irregular as occurs in triploids, with varying numbers of I's, II's, III's, IV's and V's. Dewey (9) found irregular chromosome behavior in pentaploid Agropyron hybrids. These hybrids contained various combinations of univalents, bivalents, and multivalents, which ranged from trivalents to decavalents. The prevalence of such multivalent associations provides good evidence of chromosome homology within genomes existing in the tetraploid and hexaploid species.

CHAPTER III

MATERIALS AND METHODS

Plant materials used in this study were obtained from a bermudagrass holding nursery, at Oklahoma State University, which contained cloned progeny from several crosses made by W. L. Richardson in 1971. Six F_1 plants which were derived from a hexaploid x tetraploid cross were selected and examined. The seed parent (6x) was derived from interspecific hybridization of Cynodon barberi (Okla. accession #10574) and a hybrid C. Dactylon with the numerical designation 66-x-198. C. barberi (2N = 18) is a weak, nonvigorous plant but a reasonably good seed producer. This species was found to be almost completely isolated genetically from the rest of the genus by Harlan et al. (18). The female parent (numerical designation 7-x-4) in this study is apparently an autohexaploid, originating in the same manner as described by previous authors (10, 26). The plant resembles C. barberi morphologically, being nonvigorous but having good fertility. Experimental strain 66-x-198, used as the male parent was derived from a tetraploid x tetraploid cross between C. dactylon (Okla. accession #10254a) x C. dactylon var. afganicus (Okla. accession 7R). Both C. dactylon and C. dactylon var. afganicus were vigorous tetraploids with a relatively high level of fertility. The following table gives a brief description of the F_1's used in this study.

TABLE II

HYBRID ORIGIN, NOMENCLATURE AND DESCRIPTION

Cross			Numerical Designation for F_1 Hybrid	General Growth Habit
Hexaploid		Tetraploid		
(7-x-4)	x	Coastal	36-7	Nonvigorous, weak
(7-x-4)	x	Coastal	36-6	Moderately vigorous
(7-x-4)	x	(SS-21)	36-5	Vigorous
(7-x-4)	x	(SS-16)	36-2	Vigorous
(7-x-4)	x	(Guymon x 8153)	35-8	Moderately vigorous
(7-x-4)	x	8153	35-4	Moderately vigorous

The plant material used for cytological study was collected in the field on sunny mornings and fixed for a minimum of 24 hours in Carnoy's solution. The pollen mother cells were prepared and stained using the standard acetocarmine smear technique described by Smith (31). If, after initial observation, a slide was deemed good it was made permanent by applying Venetian turpentine beneath the coverslip. It was found that better results were obtained when the plant material and stain were kept refrigerated until used.

Meiotic analysis and photographs were made using the oil imersion objective. Meiotic analysis included observations of chromosome pairing behavior at diakinesis and metaphase I, disjunction at anaphase I, lagging chromosomes at anaphase I, occurrence of accessory chromosomes and presence or absence of micronuclei in the tetrads. The data

presented in this study was not based on a minimum or maximum number of cells scored due to the extreme difficulty in some strains of finding cytologically analyzable cells. In some strains the PMC's were small, and the chromosomes spread poorly and were very sticky.

Positive determination of chromosome number for each strain was made by the use of somatic counts from leaf tip meristems. The procedure used was a modification of the technique for leaf tip preparation described by Bennett (2). Tissue dissection was done in the same manner but the pretreatment was for 90 minutes at room temperature in a saturated aqueous solution of 8-hydroxyquinoline acid, instead of αbromonaphthalene. The material was then washed in tap water for 30 seconds and fixed in Carnoy's solution for a minimum of 24 hours. After fixing, the material was again washed, then placed in a pectic enzyme solution[1] for ten hours at 29.5C. Excellent tissue maceration was obtained with this procedure. The tissue was allowed to stain for five to eight minutes before applying the coverslip. If the slide was good, it was made permanent with Venetian turpentine. Again, best results were obtained when pretreatment and fixing was done under refrigeration and when the stain and enzyme were kept cold.

Infloresences used for pollen were collected at the first sign of anthers extruding. The heads were cut and placed in a vial of water. After one day, several anthers had become exposed and the pollen was dusted on a slide. Pollen was stained with KI_2 solution and considered viable if completely stained.

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[1]Pectinol 59-L (Rhom and Haas).

Photographs were made through the oil imersion lens. The film used was Kodak High Contrast Copy.

CHAPTER IV

RESULTS

Analysis of dividing meristematic leaf tip cells from each of the six F_1 bermudagrass plants revealed unreduced (2N) chromosome numbers of 45 (plants 36-6, 36-5, and 35-4), 46 (plants 36-7 and 36-2) and 48 (plant 35-8). Relative differences in size of the somatic chromosomes were variable among plants with total length averaging 1.7 microns. Morphological characteristics such as arm length, arm ratios or satellites were indistinguishable. Photomicrographs of the somatic chromosomes along with interpretive drawings are presented in Figures 1 and 2.

The six plants differed greatly with respect to the ease with which suitable meiotic cells were found. Plants 36-7 and 36-5 provided the best opportunities for chromosomal analysis during meiosis. Each plant contained ample numbers of large pollen mother cells (PMC's) in which the chromosomes spread fairly well. The degree of difficulty in obtaining good chromosome spreading in the PMC's increased with meiotic studies of the remaining four plants. Due to the scarcity of cells with well spread chromosomes, observations were made with no minumum number of cells in mind, but with the idea of recording as many suitable cells as possible. A brief summary of the pairing associations in the hexaploid x tetraploid hybrids is presented in Table III. A more detailed description of the plants, their meiotic behavior and pollen viability follows.

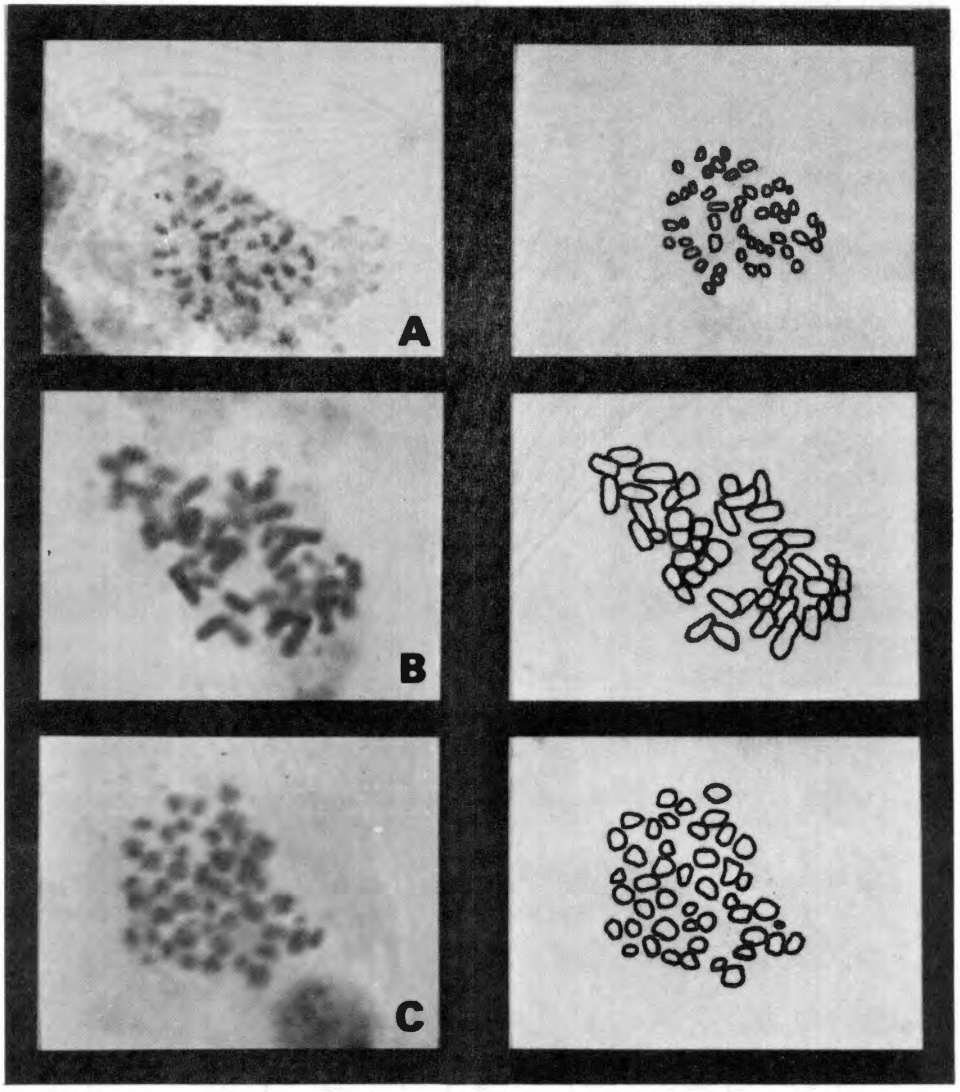

Figure 1. Somatic Chromosomes with Descriptive Drawings (1000x); A) Hybrid 36-7 (2N = 46); B) Hybrid 36-6 (2N = 45); C) Hybrid 36-5 (2N = 45)

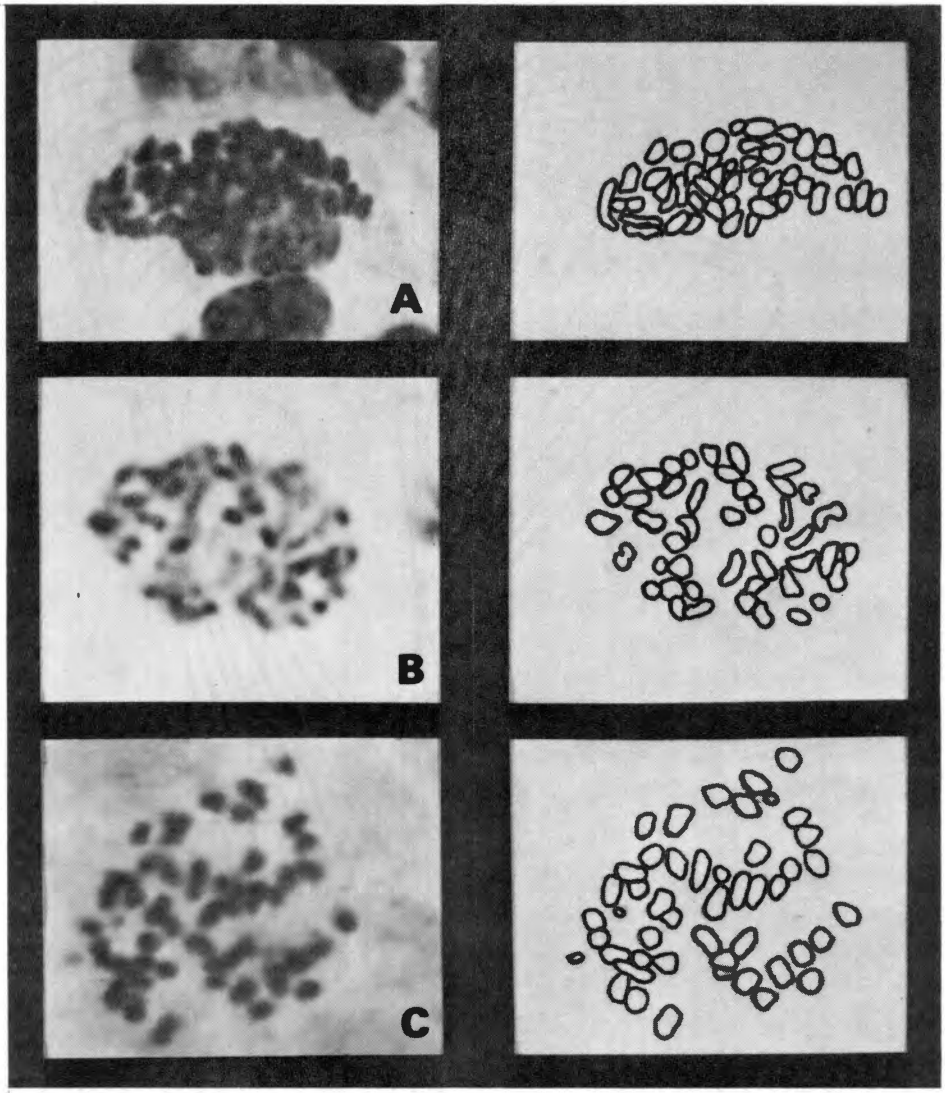

Figure 2. Somatic Chromosomes with Descriptive Drawings (1000x); A) Hybrid 36-2 (2N = 46); B) Hybrid 35-8 (2N = 48); C) Hybrid 35-4 (2N = 45)

TABLE III

CHROMOSOME ASSOCIATIONS IN PENTAPLOID CYNODON HYBRIDS

Plant	Somatic Chromosome Number	Cells	Diakinesis* I	II	III	IV	VI	Cells	Metaphase I* I	II	III	IV	Pollen Viability %
36-6	45	1	9.00 9	18.00 18				4	9.00 9	17.00 16-18		0.50 0-1	19.5
36-5	45	46	8.74 7-9	17.59 11-18	0.07 0-2	0.76 0-3		6	9.00 9	18.00 18			26.8
35-4	45	16	7.88 5-9	10.12 7-16	0.38 0-1	2.88 0-5	0.18 0-1	0					32.0
36-7	46	37	7.86 5-9	13.14 8-18	0.22 0-2	2.54 0-5		4	8.50 7-9	13.00 11-16	0.50 0-2	2.25 0-3	34.0
36-2	46	4	9.75 6-13	13.50 9-18	0.75 0-3	1.50 0-3		4	8.25 6-9	17.25 15-18		0.50 0-2	19.0
35-8	48	9	8.22 5-9	14.11 11-16	0.11 0-1	2.00 0-3	0.11 0-1	2	8.00 8	16.50 16-17	0.50 0-1	1.00 0-2	29.0

*Average above and range below for each entry.

The F_1 hybrids 36-7 and 36-6 were both progeny of the hexaploid x tetraploid cross involving Coastal as the pollen parent. Plant 36-7 was very weak and nonvigorous, capable of growth only in pots. The somatic chromosome number was 46. Chromosome pairing in meiosis was irregular, with cells containing various combinations of univalents, bivalents and multivalents. Cells at diakinesis had averages of 7.86 I's, 13.14 II's, .22 III's and 2.54 IV's. In the 37 cells examined there were 5-9 I's, 8-18 II's, 0-2 III's and 0-5 IV's per cell. Tetravalents occurred as chains and rings. Examples of chromosomal associations are shown (Figure 3, A and B). Only four cells were found with good metaphase I configurations. The pairing averages remained very much the same with never more than 9 I's, 16 II's, 2 III's, and 3 IV's per cell. At anaphase I, 75% of the cells contained lagging chromosomes (Figure 3, A and B). The laggards were generally disoriented, occurring outside the spindle. It was assumed that these chromosomes were eventually included in the diad and tetrad nuclei because the occurrence of micronuclei was practically nonexistant. Thirty-four percent of the pollen appeared viable. The second hybrid from this cross, 36-6, had a somatic chromosome number of 45 and was moderately vigorous. Study of meiosis in this plant was very difficult due to very poorly spread chromosomes. Only one cell was scored at diakinesis and four cells at metaphase I. Meiosis was termed slightly irregular with 9 I's and 18 II's at diakinesis and 9 I's, 17 II's and .5 IV's at metaphase I (Figure 4, A and B). The single multivalent found may be attributable to chromosome stickiness. At anaphase I, 72% of the cells contained laggards (Figure 4, C and D) with micronuclei very seldom occurring. Approximately 19.5% of the pollen was viable.

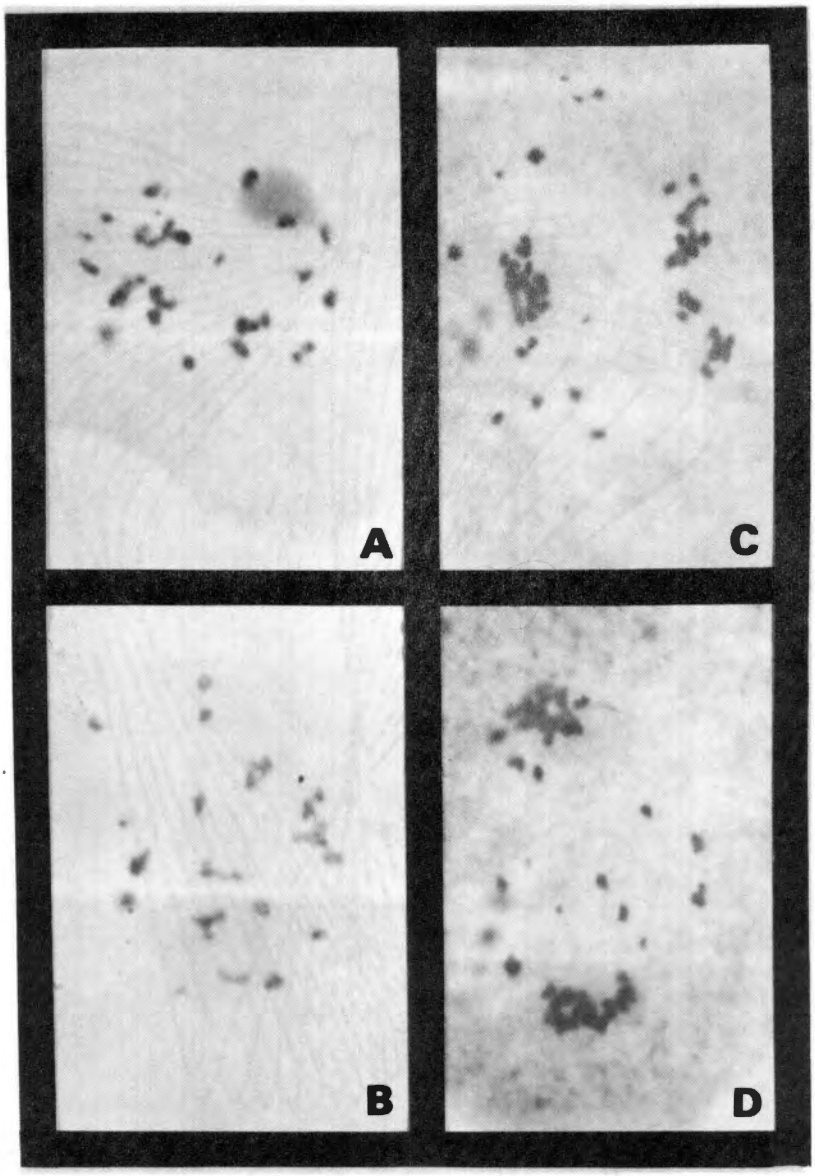

Figure 3. Stages in Meiosis of Hybrid 36-7 (1000x); A & B) Diakinesis; C) Early Anaphase I, Showing Disoriented Lagging Chromosomes; D) Lagging Chromosomes of Late Anaphase I

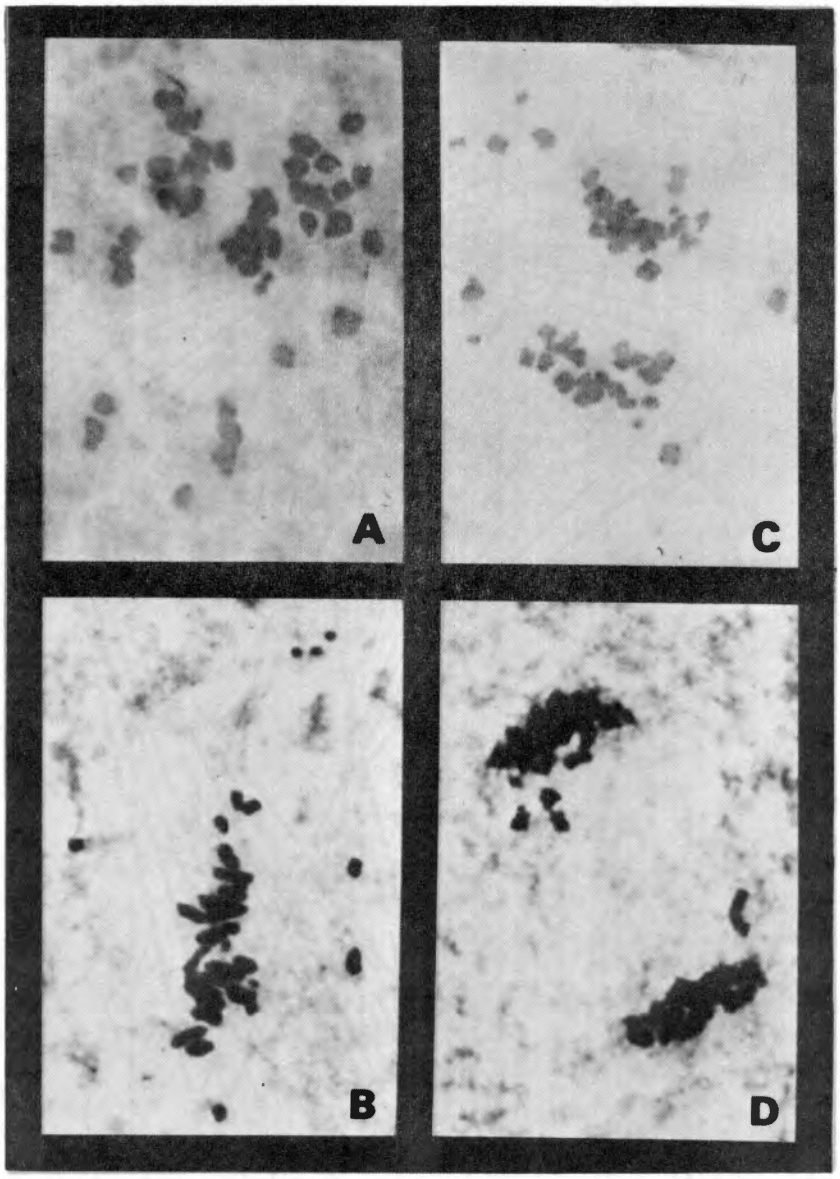

Figure 4. Stages in Meiosis of Hybrid 36-6 (1000x); A) Diakinesis; B) Metaphase I; C) Early Anaphase I; D) Early Telophase

Hybrid 36-5, the F_1 from the cross with SS-21 as the tetraploid pollen plant, was very vigorous and had a somatic chromosome count of 45. Cells at diakinesis were easily obtainable and had averages of 8.74 I's, 17.59 II's, .07 III's, and .76 IV's (Figure 5, A and B). There were 7-9 univalents, 11-18 bivalents, 0-2 trivalents and 0-3 tetravalents per cell. Six cells were studied at metaphase I all having 9 I's and 18 II's. Cells with laggards at anaphase I occurred approximately 80% of the time (Figure 5, C and D). These laggards were often disoriented with only slight attraction for the poles, but due to the lack of micronuclei, apparently were eventually included in the newly formed nuclei. About 26.8% of the pollen appeared viable.

The other hybrid with a vigorous growth habit, 36-2, was the result of a hexaploid x tetraploid cross with SS-16 as the male parent. The unreduced somatic chromosome numer was 46, the extra chromosome being considered an accessory. Chromosome pairing was irregular, with averages of 9.75 I's, 13.5 II's, .75 III's and 1.5 IV's, occurring at diakinesis (Figure 6, C and D). At metaphase I the averages were 8.25 I's, 17.25 II's, 0 III's and .5 IV's, with never more than 9 I's, 18 II's or 2 IV's occurring in a single cell. Chromosome stickiness was also a problem in this plant, possibly accounting for the different averages of bivalents and tetravalents in diakinesis and metaphase I. Approximately 88% of the cells at anaphase I contained laggards (Figure 6, A and B) but no micronuclei were observed. Well filled pollen grains occurred approximately 19% of the time.

Hybrid 35-8, the F_1 from the cross using (Guymon x 8153) as the male parent, was moderately vigorous and had a somatic chromosome number of 48. Cells studied at diakinesis (Figure 7, A) had irregular pairing

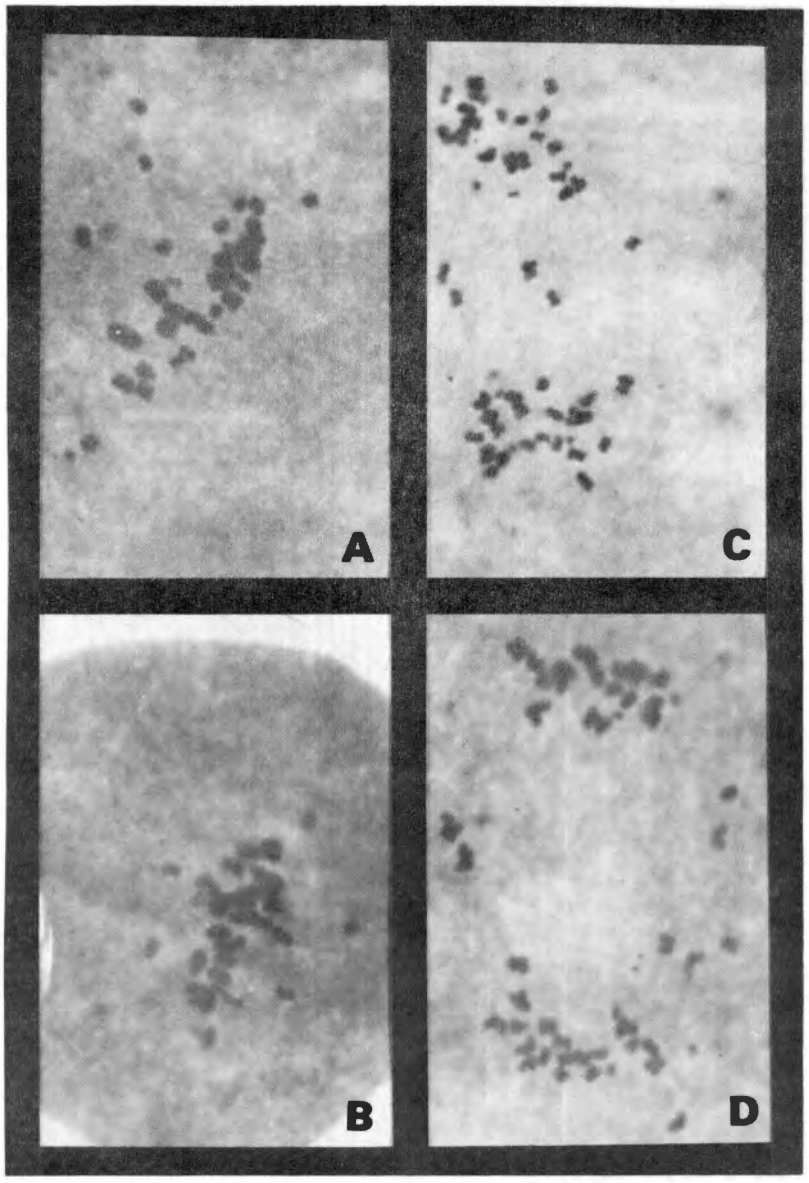

Figure 5. Stages in Meiosis of Hybrid 36-5 (1000x); A & B) Late Diakinesis, Chromosomes Approaching Metaphase I Orientation; C & D) Lagging Chromosomes at Anaphase I

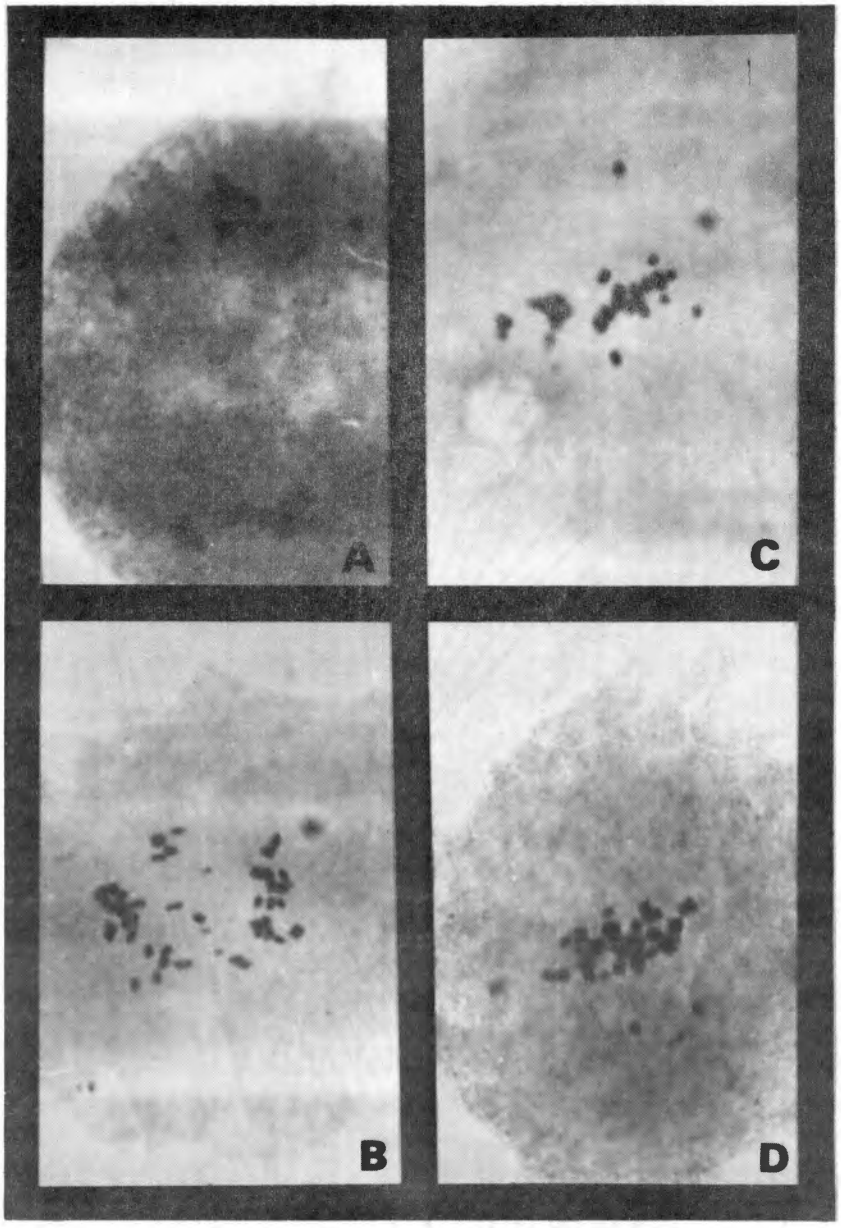

Figure 6. Stages in Meiosis of Hybrid 36-2 (1000x); A) Disoriented Chromosomes at Early Telophase; B) Lagging Chromosomes at Anaphase I; C & D) Chromosomes Approaching Metaphase Orientation

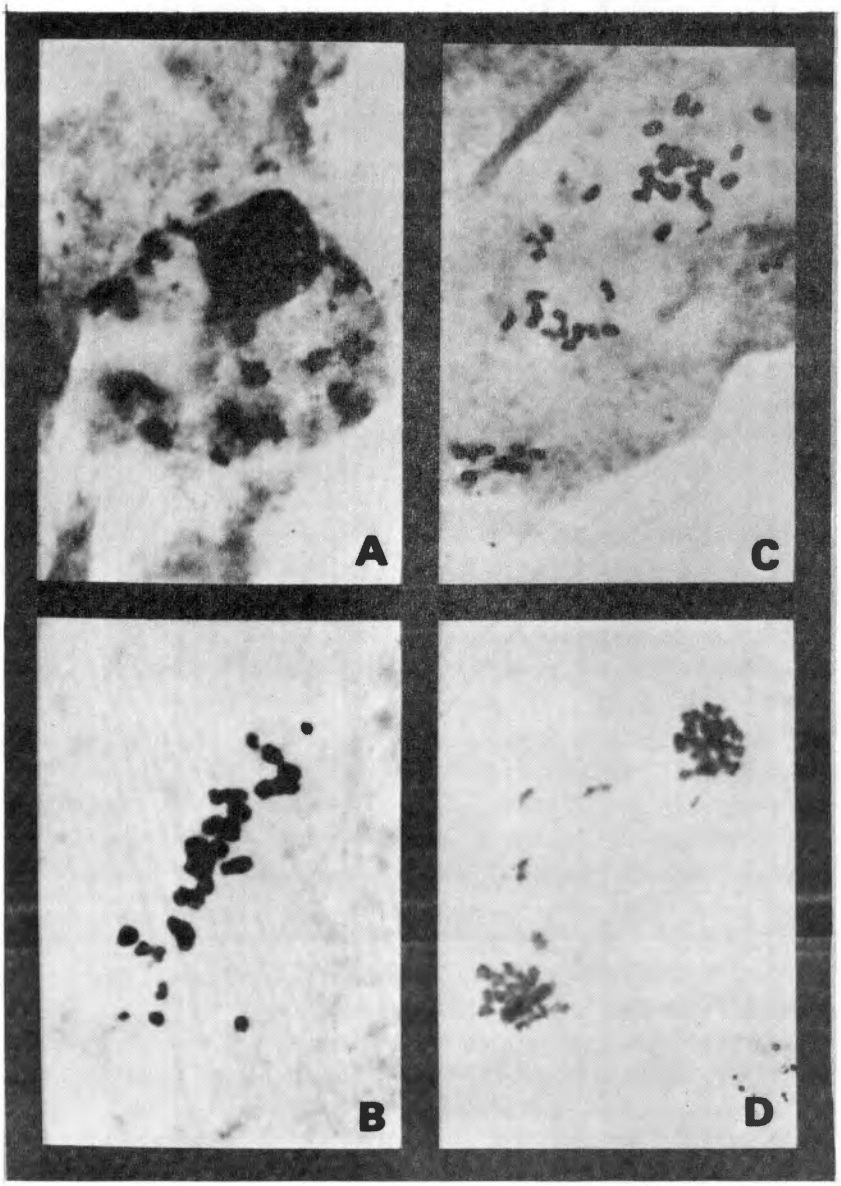

Figure 7. Stages in Meiosis of Hybrid 35-8 (1000x); A) Diakinesis; B) Metaphase I; C) Anaphase I; D) Lagging Chromosomes at Early Telophase

with averages of 8.22 I's, 14.22 II's, .11 III's and 2.0 IV's. A chain of six chromosomes was found in one cell. The occurrence of univalents, bivalents, trivalents and tetravalents ranged from 5-9, 11-16, 0-1, and 0-3, respectively. Only two cells were scored at metaphase I (Figure 7, B) with averages of 8 I's, 16.5 II's, .5 III's and 1 IV. At anaphase I, 79% of the cells had lagging chromosomes and no micronuclei were observed in the tetrads (Figure 7, C and D). Pollen viability was about 29%.

The sixth hybrid studied, 35-4, was a moderately vigorous plant resulting from a cross between (7-x-4) x 8153. Its somatic chromosome number was 45. Pairing at diakinesis (Figure 8, C and D) was irregular with averages of 7.88 I's, 10.12 II's, .38 III's and 2.88 IV's. There were never more than 9 I's, 16 II's, 1 III or 5 IV's per cell. No cells were scored at metaphase I due to extreme stickiness (Figure 8, B). At anaphase I, 77% of the cells had laggards but micronuclei occurred only occasionally in the tetrads (Figure 8, A). Approximately 32% of the pollen appeared viable.

Disjunction at anaphase I usually resulted in diad nuclei with 22 and 23 chromosomes, but unbalanced nuclei ranging from 17 to 28 chromosomes were also found. A summary of the observations on chromosome disjunction at anaphase I is presented (Table IV).

The presence of possible accessory chromosomes was noted in three of the six hybrids. The chromosomes were much smaller than the A-chromosomes and always heterochromatic. These chromosomes were found in both somatic and reproductive tissue. There seemed to be no apparent secondary associations between the accessory chromosomes and chromosomes of the normal complement.

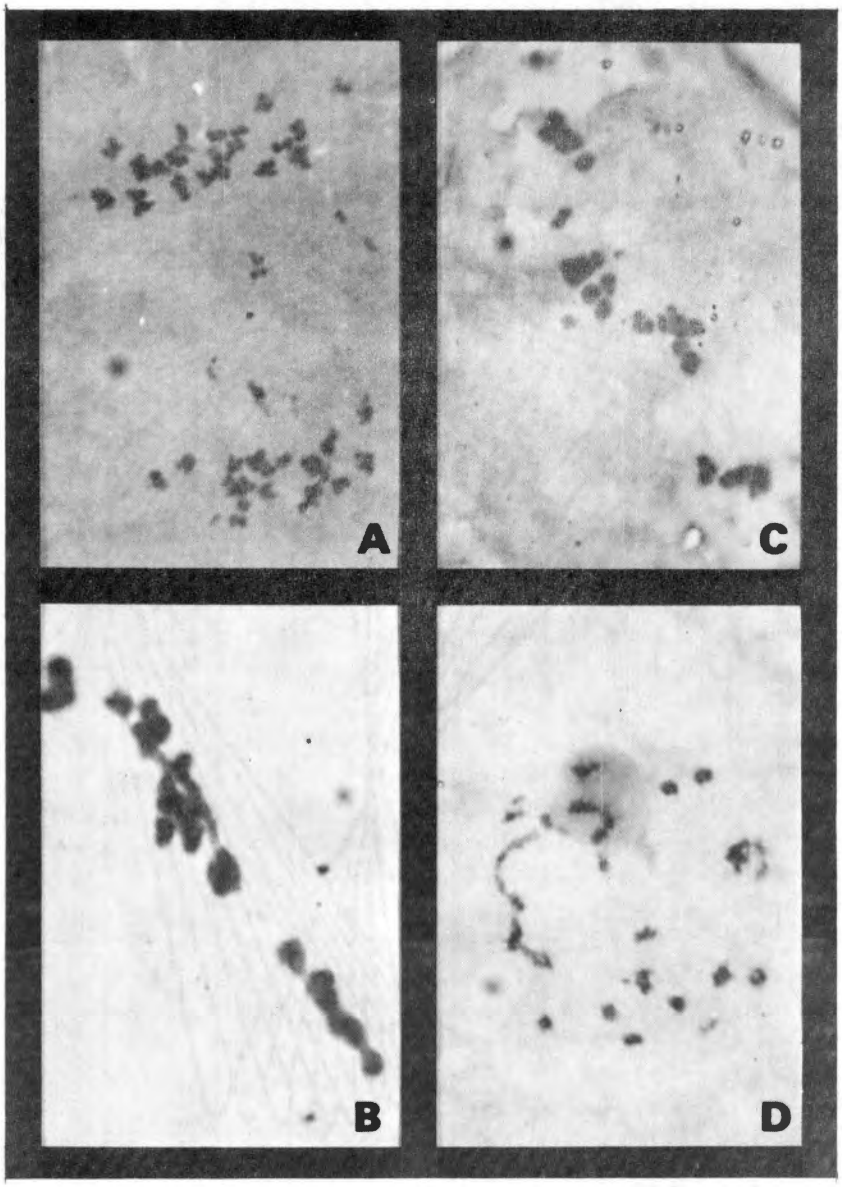

Figure 8. Stages in Meiosis of Hybrid 35-4 (1000x); A) Anaphase I; B) Metaphase I; C) Chromosomes Approaching Metaphase I Orientation; D) Diplotene

TABLE IV

CHROMOSOME DISJUNCTION AT ANAPHASE I IN CYNODON HEXAPLOID X TETRAPLOID HYBRIDS

Plant	Disjunction Ratios					
	17:28	18:27	19:26	20:25	21:24	22:23
	No. Cells	No. Cells	No. Cells	No. Cells	No. Cells	No. Cells
36-7	...	...	...	1	1	3
36-6	...	...	...	...	2	1
36-5	...	...	...	3	4	15
36-2	...	...	...	2	2	4
35-8	...	...	1	...	2	5
35-4	1	1	1	...	5	5

CHAPTER V

DISCUSSION

The present study revealed three of the hexaploid x tetraploid hybrids to be euploids and have the expected unreduced chromosome number of 45. Chromosome numbers of 46, 46, and 48 were found in the remaining three hybrids. These odd numbers could be explained by the presence of accessory chromosomes, which have been reported to occur in Cynodon by other workers (12, 27). These chromosomes are thought to be accessories instead of fragments because of: a) relative uniformity in appearance (darkly stained and small) and number, and b) common occurrence in both somatic and reproductive tissue. The meiotic behavior of the accessories was not closely studied, but disjunction was observed in a few cells at anaphase I. Brown (3) states that nondisjunction is a common characteristic of accessory chromosomes, but adds, that in some species the ability to disjoin is controlled by such things as the presence of a knob on the B-chromosome (rye) or the degree of heterochromatinization in the accessory (maize). The loss of accessory chromosomes from one generation to the next is not usual but was reported to occur in Cynodon dactylon (L.) Pers., P.I. 290899 (27). This loss was attributed to occasional lagging of the accessory chromosomes at anaphase II. Transmissability of the accessory chromosomes reported here was not studied and it is not presently known if accessories are present in the parent material.

As outlined by Stebbins (32), polyploids are generally of three types: allopolyploids, those with almost exclusive bivalent pairing; segmental allopolyploids, where bivalent pairing is accompanied by multivalent associations and autopolyploids, which are characterized by a high frequency of multivalent associations. The existence of multivalence along with bivalents and univalents indicates the hexaploid x tetraploid hybrids in this study could probably best be defined as segmental allopolyploids. Stebbins (32) further states that segmental allopolyploids may commonly resemble morphologically one or other of their diploid ancestral species. The resemblance of hybrid 36-7 to its diploid ancestor Cynodon barberi substantiates his statement.

If Cynodon barberi (found to be almost completely isolated [18]) introduced a new genome into the hexaploid upon its crossing with a tetraploid you would expect 9 I's and 18 II's. If its genome varied only slightly, occasional trivalents and pentavalents would be expected, and if the same, III's and V's should occur in high numbers. The occurrence of multivalents in these plants indicates the genomes are at least partially homologous. Even though there was a high frequency of univalents occurring, their failure to pair may have been related to small chromosome size and the inability of chiasmata to form (13, 14) or to possible genic influence (28). Cytological studies have shown that triploid hybrids contain high frequencies of trivalent formation (11) or associations in the form of 9 I's and 9 II's (18, 22). As Harlan et al. (18) state, there seems to be no significant difference between genomes of species that can be hybridized.

The low production of viable gametes by these hybrids indicates that they are meiotically unstable. The cause of gamete infertility

stems from the disorientation of chromosomes at anaphase I. These chromosomes were randomly included in the diad nuclei, resulting in unbalanced chromosome compliments in the tetrads. Micronuclei were seldom present. It is highly probable that the viable gametes produced were those with or very near the N chromosome number (17, 18, 19 or 26, 27, 28). The studies of disjunction at anaphase I (Table IV) point out that these numbers are infrequent. The presence of accessory chromosomes seem to have no notable effects on chromosome pairing or gamete viability. Brown (3) states that effects from accessories is little or none when they occur in small numbers (from 1-3).

CHAPTER VI

SUMMARY AND CONCLUSIONS

Cytological investigations of six Cynodon hexaploid x tetraploid F_1 hybrids revealed the somatic chromosome number of three plants to be 45. Two plants had chromosome numbers of 46, and one plant had 48. Observations of the somatic cells and meiocytes of the aneuploids indicates that the extra chromosomes are most likely accessories. To substantiate this hypothesis, studies of the cytology of the parent material and of the disjunction behavior in meiosis should be pursued. No effects, detrimental or otherwise, were noted in relation to the presence or absence of accessory chromosomes.

In all hybrids, the occurrence of univalents, bivalents, and multivalents was common. Tetravalent associations were the most common multivalent configurations, generally appearing as rings or chains. Chains of six chromosomes were noted in two hybrids but were very uncommon. Strict bivalent, univalent configurations in a single cell were infrequent, indicating that the genomes were at least partially homologous.

The hybrids in this study were determined to be relatively sterile due to irregular pairing behavior, abnormal disjunction, and low pollen viability. The common occurrence of disoriented chromosomes at anaphase I, generally resulted in diad nuclei with reduced chromosome numbers occurring somewhere between those expected for a tetraploid or a

hexaploid. Viable pollen was produced, but in low numbers. It is suspected that these hybrids, if cloned out, would eventually revert to the tetraploid form due to gametophytic screening of unbalanced gametes. They could possibly be of value as possible genetic bridges between the hexaploid and tetraploid Cynodons. Two of the reported hexaploids were derived from species considered to be genetically isolated (C. barberi and C. plectostachyus [10, 23]). A possible means of tapping the germ plasm of these species could be by the hexaploid x tetraploid hybrids. To further determine the stability of the hybrids, continued study of pollen viability and seed production are under way.

SELECTED BIBLIOGRAPHY

1. Avdulov, N. P. 1931 Karyo-systematisch untersuchung der familie Gramineen. Bull. Appl. Bot. Genet. and Pl. Breed. Suppl. 43: 428 pp.

2. Bennett, Erna 1964 A rapid modification of de Lautor's technique for grass leaf chromosomes. Euphytica 13: 44-48.

3. Brown, W. V. 1972 Textbook of Cytogenetics. The C. V. Mosby Co.

4. Burton, G. W. 1947 Breeding bermudagrass for the southeastern United States. J. Amer. Soc. Agron. 39: 551-569.

5. Burton, G. W. 1951 Intra- and interspecific hybrids in bermudagrass. J. Hered. 42: 152-156.

6. Burton, G. W. 1973 "Bermudagrass" In Forages ed. by M. E. Heath, D. S. Metcalfe, and R. E. Barns. Iowa State University Press, Third Edition, p. 321.

7. Carnahan, H. L. and H. D. Hill 1961 Cytology and genetics of forage grasses. Bot. Rev. 27: 1-162.

8. de Wet, J. M. J. and J. R. Harlan 1971 South African species of Cynodon. J. of S. Afr. Bot. 37: 53-56.

9. Dewey, R. D. 1969 Hybrids between tetraploid and hexaploid crested wheatgrasses. Crop Sci. 9: 787-791.

10. Felder, M. R. 1967 Chromosome association in triploid Cynodon hybrids. (unpublished M.S. thesis, Okla. State Univ.)

11. Forbes, Ian and G. W. Burton 1963 Chromosome numbers and meiosis in some Cynodon species and hybrids. Crop Sci. 3: 45-49.

12. Gould, F. W. 1966 Chromosome numbers of some Mexican grasses. Can. J. of Bot. 44: 1683-1696.

13. Guptka, P. K. and A. K. Srivastava 1970 Natural triploids in Cynodon dactylon (L.) Pers. Caryologia 23: 29-35.

14. Guptka, P. K. and A. K. Srivastava 1971 Formation of restitution nucleus and chromatin migration in a triploid clone of Cynodon dactylon (L.) Pers. J. of Indian Bot. Soc. 50(2): 132-137.

15. Harlan, J. R. and J. M. J. de Wet 1969 Sources of variation in Cynodon dactylon (L.) Pers. Crop Sci. 9: 774-778.

16. Harlan, J. R., J. M. J. de Wet and W. L. Richardson 1969 Hybridization studies with species of Cynodon from East Africa and Malagasy. Amer. J. Bot. 56(8): 944-950.

17. Harlan, J. R., J. M. J. de Wet, W. W. Huffine and J. R. Deakin 1970 A Guide to the Species of Cynodon (Gramineae). Okla. State Ag. Exp. Stat. Bull. B-673.

18. Harlan, J. R., J. M. J. De Wet, K. M. Rawal, M. R. Felder and W. L. Richardson 1970 Cytogenetic studies in Cynodon L. C. Rich. (Gramineae) Crop. Sci. 10: 288-291.

19. Hunter, A. W. S. 1934 A karyosystematic investigation in the Gramineae. Can. J. Res. 11(2): 213-241.

20. Hurcombe, Ruth 1947 A cytological and morphological study of cultivated Cynodon species. J. of S. Afr. Bot. 13: 107-116.

21. Krishnaswamy, N. 1940 Untersuchungen zur cytologie and systematik der Gramineen. Beih. Bot. Centralbl. Abt. A, 60: 1-56.

22. Malik, C. P. and R. C. Tripathi 1968 Cytogenetical evolution within the Cynodon dactylon complex. Biol. Zbl. 87: 625-627.

23. Moffett, A. A. and R. Hurcombe 1949 Chromosome numbers of South African grasses. Heredity 3: 369-373.

24. Myers, W. M. 1947 Cytology and genetics of forage grasses. Bot. Rev. 13: 319-421.

25. Ourecky, D. K. 1963 Pachytene chromosome morphology in Cynodon dactylon (L.) Pers. Nucleus 6(1): 63-82.

26. Powell, J. B., G. W. Burton and C. M. Taliaferro 1968 A hexaploid clone from a tetraploid x diploid cross in Cynodon. Crop Sci. 8: 184-185.

27. Powell, J. B., I. Forbes and G. W. Burton 1968 A Cynodon dactylon (L.) Pers. clone with five accessory chromosomes. Crop Sci. 8: 267-268.

28. Rees, H. 1961 Genotypic control of chromosome form and behavior. Bot. Rev. 27: 288-318.

29. Richardson, W. L. 1958 A technique of emasculating small grass florets. Indian J. of Genet. and Pl. Breed. 18(1): 69-75.

30. Rieger, R., A. Michaelis, M. M. Green 1968 A Glossary of Genetics and Cytogenetics. Springer-Verlag New York, Inc.

31. Smith, L. 1947 The acetocarmine smear technique. Stain Tech. 22: 17-31.

32. Stebbins, G. L. 1947 Types of polyploids: their classification and significance. Adv. in Genet. 1: 403-429.

33. Stebbins, G. L. 1956 Cytogenetics and evolution in the grasses. Amer. J. Bot. 43: 890-895.

34. Stebbins, G. L. 1971 Chromosomal Evolution in Higher Plants. Addison-Wesley Publishing Co.

35. Tateoka, T. 1953 Karyotaxonomic studies in Poaceae I. Ann. Rept. Natl. Inst. Genet., Japan No. 4, pp. 45-47.

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