THE PACHYTENE AND SOMATIC CHROMOSOME MORPHOLOGY OF *CYNODON DACTYLON* (L.) PERS.

by

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ABSTRACT

Diploid clones of C. dactylon were studied to determine their pachytene chromosome morphology. The chromosomes ranged in length from 20.5 µm to 44.6 µm. Arm ratios ranged from under 1.50 to 2.14. The chromosomes were identified by their length, arm ratios, relative length and position and number of prominent chromomeres. The shortest chromosome was designated chromosome 1 and the other chromosomes numbered on basis of chromosome length. There was little morphological variability between clones suggesting only one genome.

The tetraploid C. dactylon var. dactylon appears to have two sets of the pachytene chromosome complement found in the diploid. The variability that exists in the complement can be explained by the variability in the diploid. This suggests an autotetraploid origin or, if an allotetraploid, lack of genome differentiation at the pachytene level.

The somatic prophase pairs were matched with the corresponding pachytene bivalents. The relative lengths were found to vary due to differential contraction rates of heterochromatic and euchromatic areas. The fourth and sixth chromosomes in the pachytene complement were the fifth and fourth chromosomes respectively in the somatic cells. Chromosome 8 and 9 were reversed in the somatic complement.

INTRODUCTION

Cynodon dactylon (L.) Pers. is a perennial grass endemic to Africa, the Near East and Asia (Harlan and de Wet 1969). The tetraploid, C. dactylon var. dactylon, is the cosmopolitan weed. It was probably introduced into America by 1751 by Governor Ellis, who was credited with introducing it to Savannah (Kneebone 1966), and it is important throughout the world as a turf and forage. The diploid giant bermudagrass, C. dactylon var. aridus, has been introduced into Hawaii and Arizona where it is grown as a forage.

Genomic relationships in the genus Cynodon have been postulated on the basis of pairing at metaphase 1 of meiosis. An autopolyploid origin of C. dactylon var. dactylon from C. dactylon var. aridus with essentially no genomic differentiation has been suggested by many researchers (Harlan, de Wet, and Rawal 1970; Gupta and Srivastava 1970; Rawal and Chedda 1971). A segmental allopolyploid origin has also been suggested on the basis of quadrivalent numbers in the tetraploid and trivalents in the triploid hybrid of var. aridus x var. dactylon (Sengupta 1968; Tripathi, Sachdeva, and Malik 1977).

The only detailed study of pachytene chromosome morphology in the genus is one by Ourecky (1963), in which he describes the chromosome complement of one diploid clone of C. dactylon, probably var. afghanicus because it was from Afghanistan (P.I. 220385). Tripathi et al. (1977) noted differences in the knobs and chromomeres as reported by Ourecky although no specifics are given. There are apparently no published reports on somatic chromosome morphology although many researchers have reported on somatic chromosome numbers (Hurcombe 1946; Hurcombe 1947; Burton 1947; Brown 1950; Burton 1951; Tateoka 1954; Rochecouste 1962; Gould 1966; Powell, Forbes, and Burton 1968).

The purpose of the present investigation was to study 1) the pachytene and somatic chromosome morphology of various clones of C. dactylon var. aridus to identify each chromosome and to determine the variability in the complement and 2) the pachytene and somatic chromosome morphology of clones of C. dactylon var. dactylon to see if genomic differentiation was apparent.

LITERATURE REVIEW

The genus Cynodon has recently been revised using primarily morphological traits but grouping genetically allied units as a single species (Harlan et al. 1966; Clayton and Harlan 1969; Sengupta 1968; Harlan and de Wet 1969; de Wet and Harlan 1970; Harlan, de Wet, and Rawal 1970; Harlan, de Wet, et al. 1970; Rawal and Harlan 1971). This resulted in the two varieties in this study, tetraploid common bermudagrass and diploid giant bermudagrass, being classified as C. dactylon (L.) Pers. var. dactylon and C. dactylon (L.) Pers. var aridus Harlan et de Wet respectively. Also used in this study were two putative hybrids between C. dactylon (L.) Pers. var. afghanicus Harlan et de Wet and var. aridus. C. dactylon var. dactylon is further divided into three races: tropical, temperate and seleucidus. C. dactylon var. aridus is divided into two races, a large robust one and a small slow growing one found in Southern India and Ceylon (Harlan and de Wet 1969).

There have been conflicting reports on the basic chromosome number in the genus Cynodon. Hurcombe (1947) reported a basic number of x=10 from root tips, with C. bradleyi (2n=18) (now classified as C. incompletus) being of an aneuploid origin. Other reports of x=10

included C. dactylon with forty chromosomes (Tateoka 1954) and biotypes of C. dactylon with thirty and forty chromosomes (Rochecouste 1962). The basic number of x=9 was also reported by many researchers (Burton 1947; Brown 1950; Burton 1951; Forbes and Burton 1963; Ourecky 1963; Gould 1966). Forbes and Burton (1963) concluded that the previous reports of x=10 were due to the presence of "fragments" that were found to be satellites at the ends of long secondary constrictions. Later studies concluded that the basic number was x=9 and that reports of x=10 were due to the presence of B-chromosomes in some accessions of Cynodon (Hoff 1967; Sengupta 1968; Powell et al. 1968).

The cytogenetics of the genus has been studied at metaphase I of meiosis to elucidate specific relationships and to determine the relationship of pairing behavior to the fertility of specific cultivars. Hoff (1967) studied meiotic behavior in Arizona clones of bermudagrass (2n=36), giant bermudagrass (2n=18) and apparent natural triploid hybrids between them. Giant bermudagrass always had nine bivalents. Bermudagrass had averages that ranged from 1.13 to 2.07 quadrivalents in different clones, with 47% of all quadrivalents observed associated with the nucleolus. The triploids' averages ranged from 2.74 to 3.67 trivalents per cell with a maximum pairing of seven trivalents. Gupta and Srivastava (1971) reported a triploid from a natural stand with two to fourteen univalents and a mean of 6.10

univalents, two to ten bivalents with a mean of 7.15 bivalents, and zero to five trivalents with a mean of 2.20 trivalents. Hanna and Burton (1977) studied four tetraploid cultivars of bermudagrass in which pairing varied from five per cent of the cells having eighteen bivalents in one cultivar to sixty-three per cent of its cells with eighteen bivalents in another cultivar. These cultivars varied from .51 to .26 quadrivalents per cell.

Forbes and Burton (1963) reported a maximum of eight trivalents per cell in triploid hybrids between diploid and tetraploid C. dactylon and a maximum of nine trivalents in Tiffine, a triploid hybrid between C. transvaalensis (2n=18) and tetraploid C. dactylon (2n=36). A maximum of three quadrivalents was observed in tetraploid C. dactylon. The high rate of trivalent formation in the triploids suggested homology between the genomes of diploid and tetraploid C. dactylon and between those of tetraploid C. dactylon and diploid C. transvaalensis. The low quadrivalent formation in the tetraploid might indicate limited homology of two genomes or advanced diploidization of a doubled set. On the other hand the genomes could be completely homologous, but with low chiasmata frequency limiting formation of quadrivalents.

Sengupta (1968) found that C. dactylon var. dactylon showed fairly regular meiotic behavior with a quadrivalent, occasionally associated with the nucleolus,

and some univalents. Triploid hybrids between C. dactylon (2n=36) and C. transvaalensis (2n=18) showed primarily twelve bivalents with three univalents or thirteen bivalents with one univalent, occasionally eleven bivalents with five univalents, and nine bivalents with nine univalents. He postulated that there may be two partially homologous genomes, D'D' for C. transvaalensis and DDD'D' for C. dactylon, or there may be preferential pairing of the two sets.

Harlan, de Wet, et al. (1970) reported that hybrids between C. transvaalensis and C. dactylon var aridus (2n=18) showed essentially perfect pairing suggesting no genome differentiation. Hybrids between C. dactylon var. dactylon (2n=36) and C. transvaalensis (2n=18) showed seven to thirteen univalents (mean of 9.3) and seven to ten bivalents (mean of 8.8). Hybrids between var. aridus and var. dactylon showed six to nine univalents, seven to twelve bivalents, zero to two trivalents and zero to two quadrivalents. Hoff (1967) found in eight synthetically produced aridus (2n=18) x dactylon (2n=36) triploids the average number of trivalents per cell from each plant ranged from 3.04 to 3.67. The most common association was three trivalents, six bivalents, and six univalents. They concluded that there appears to be no significant genome differentiation in the species studied.

In triploids collected from natural stands of C. dactylon, Gupta and Srivastava (1970) found zero to six trivalents per cell with the maximum number in three different clones ranging from four to six. Univalents varied from four to thirteen with an average of 6.8 to 8.1 in the three clones. They concluded that the genomes in diploid and tetraploid C. dactylon were homologous. They speculated that the failure of univalents to associate in trivalents was due to the small size of the chromosomes which rarely formed more than two chiasmata.

Tripathi et al. (1977) observed eighteen bivalents and rarely one quadrivalent in natural tetraploids and three to eight quadrivalents in colchiploids from var. aridus. They believe that the few quadrivalents in natural tetraploids may be due to translocations or to partial homology of the genomes. The tetraploid may be a segmental allopolyploid from two diploids with considerable homology. They also found pairing in triploids of primarily nine bivalents and nine univalents which suggested to them allosyndetic pairing. The few trivalents found suggests either heterogenomic pairing, translocations or some homologous segments distributed among the two genomes of the tetraploid.

Rawal and Chedda (1971) produced hybrids between various diploid and tetraploids in Cynodon and concluded: 1) the genomes have considerable homology, 2) polyploids

are essentially autopolyploids and 3) chromosome pairing is essentially autosyndetic and therefore preferential. The small number of univalents in 2n hybrids and univalents and multivalents in polyploid hybrids suggest that genic and chromosomal changes have occured in the original genome during the course of evolution.

Using evidence from cytology, morphology and ecology Rawal and Chedda (1971) postulated that the genus evolved from an ancestor which was 2n, non-rhizomatous, and distributed from South and East Africa to the Middle East and India. A diploid form evolved rhizomes and invaded more arid regions of Africa, the Near East and India in which C. dactylon var. aridus represents the modern survivor (Rawal and Harlan 1971). This is the only diploid in the genome with rhizomes and the only likely source of C. dactylon var. dactylon (Harlan and de Wet 1969). They concluded that var. dactylon is an autopolyploid and that the morphologically smaller forms of var. aridus appear closer to var. dactylon than the "giant" race. Plants of the seleucidus race of var. dactylon, often selected for grazing and hay production, are probably an introgression product involving a tetraploid race of C. dactylon var. afghanicus and a temperate race of var. dactylon (Harlan, de Wet, et al. 1970).

Metaphase I of meiosis and quadrivalent formation are not always indicative of genome relationships and may

not be a reliable criterion for determining whether a species is an auto or an allopolyploid. Hossain (1976) found that in a random mating population of rye disomic association was the predominant type, where as in inbred materials the chromosome association pattern is predominantly tetrasomic. This suggested that autoploids which form multivalents with high frequency at the time of their origin during subsequent evolution may shift to the bivalent characteristic of allopolyploids, a phenomenon referred to as diploidization. Gottschalk (1972) found that in a Solanum stenotomum and "S. ajuscence" hybrid pachytene chromosomes varied in total length but euchromatic regions paired normally. The length differences were restricted to heterochromatic regions so loops formed. However, MI had normal bivalents, indicating that after spiralization the length differences had disappeared. He concluded that studying bivalent formation at MI only means that pairing and a certain amount of homology exists, but it does not mean that there is complete homology between the chromosomes. He suggests meiotic pachytene is better to elucidate genome relationships because spiralization has just started and all structural elements can be analyzed.

Ourecky (1963) studied pachytene in one diploid clone of C. dactylon from Afghanistan, which is probably var. afghanicus because Harlan, de Wet, and Rawal (1970)

determined that this variety comes from that area. Ourecky, using the entire chromosome complement of four different meiotic cells, characterized the nine bivalents on the basis of (1) linear length, (2) relative length, (3) arm ratio, (4) number of prominent chromomeres and (5) presence of terminal knobs and nucleolus. He found that the average linear length, relative length and presence and abscence of terminal knobs were the most useful criteria for determining specific chromosomes. Based on these characteristics he numbered the bivalents from 1, the smallest, to 9, the largest, although some bivalents in individual spreads showed variation. He states that the number of terminal knobs varies with the strain and ecotype.

Tripathi et al. (1977) observed chromosome polymorphism in Cynodon species with some differences in terminal knobs and relative lengths of knobs as compared to Ourecky. They concluded that several chromosomal biotypes exist within the two diploid and tetraploid races of C. dactylon, although they showed no chromosomes or measurements. They believe that the polymorphism can easily be explained by considering the wide geographic distribution of the species and the acclimitization to widely diversified areas.

Lima de Faria (1976a, 1976b, 1976c) has developed the concept of the chromosome field to examine chromosome morphology and the relationship of the various parts of the

chromosome to each other. In this concept all parts of the chromosome are interrelated and in equilibrium with every other part. He utilized the arm frame method, a triangle formed by placing the chromosome arms of a species with their centromeres on a vertical axis and their telomeres at 45 degrees, to bring out the relations between chromosome segments exhibiting a certain property in both meiotic and mitotic material. He found chromomere gradients in most species characterized by: 1) large chromomeres on both sides of the centromeres, 2) chromomeres gradually decreasing in size proceeding to the telomeres and, 3) maintenance of gradients regardless of chromosome length from tissue to tissue (Lima de Faria 1976c). Using his method, the secondary constriction can be found in a characteristic position on a short arm along a straight line, with the angle from the centromere axis constant in a given family (Lima de Faria 1976a). Knobs are a large chromomere or chromomere group found near the ends of chromosomes, in which their positions are influenced by the telomeres (Lima de Faria 1976b). He felt that all of these characteristics showing these patterns could be influenced by cultivation, particularly vegetative propagation, so that new rearrangements could be established and continue to survive.

In a study of pachytene chromosomes of Medicago species closely related to M. sativa L., Gillies (1972) found they had extremely similar chromosome arm ratios and

proportional lengths, suggesting one common cytogenetic unit. Absolute lengths differed, possibly due to differences in contraction, with achromatic regions shortening faster. Gillies and Bingham (1971) found that the chromosomes of diploid M. sativa were also considerably longer than the chromosomes of tetraploid alfalfa or 2x haploids. Haploids (2x) with pachytene chromosome lengths similar to the tetraploid suggest that greater chromosome contraction may be a property of the tetraploid state still present in the 2x haploid state. Ho and Kasha (1974) found that M. sativa pachytene chromosomes and their individual arms had different contraction rates that could change relative chromosome lengths and arm ratios. This was more noticeable in submedian chromosomes, which had decreasing arm ratios, and chromosomes with unequal distribution of heterochromatic and euchromatic regions.

Maguire (1962) found variability in the length and arm ratio of pachytene chromosomes of corn. This variability was of two kinds, one contributing approximately uniformly per unit length throughout the genome and the other a characteristic of each chromosome unrelated to length. She also studied the satellite region of chromosome 6 and found that it contained from one to five chromomeres, with expression varying from cell to cell within a sample (Maguire 1977). She suggested that missing chromomeres may reflect failure of condensation of differential aggregation

during condensation of subunits. Zecevic (1974) studied the knobs on pachytene chromosomes of maize and found that the number and position varied between populations.

A study of pachytene and somatic chromosome of tomato, Lycopersicum esculentum L., showed that both are differentiated into proximal chromatic and distal achromatic parts (Ramanna and Prakken 1967). All 12 bivalents and 12 somatic pairs could be identified and each somatic pair could be homologised with its corresponding bivalent. In comparing somatic and pachytene contraction, the chromatic parts in somatic tissues were contracted by a factor of 4 to 5 over pachytene contraction, whereas the achromatic parts were contracted by a factor of 30.

Hyde (1953) in studying the differentiated chromosomes of Plantago ovata (2n=8) in meiosis and mitosis found all chromosomes showed sharp and asymetric differentiation at pachytene which corresponded in detail to that in mitotic prophase. Both the meiotic and somatic chromosomes were made up of deeply staining proximal segments on either side of the centromere and much more weakly chromatic distal segments.

De and Krishnan (1966) studied the pachytene and somatic chromosomes of Phaseolus mungo (L.). They found that the chromosomes were of different relative lengths during the two phases. The two NOR chromosomes were the

second and third longest at somatic metaphase but only seventh and ninth longest during pachytene.

MATERIALS AND METHODS

Diploid and tetraploid clones of C. dactylon were obtained for study from the crossing block maintained by the University of Arizona at Casa Grande Highway Farm, Tucson, Arizona. Some clones failed to produce heads and others produced pachytene stages unsuitable for analysis. The clones used in this study and their origins are listed in Table 1.

Young spikes were collected, before the boot had split, between 10:00 a.m. and 2:00 p.m. for meiotic analysis and fixed in 3:1 95% ethanol:glacial acetic acid. These were stored in the refrigerator for at least one week and later transferred to 70% ethanol for longer storage. The anthers were removed from the spike and stained using standard acetrocarmine squash techniques. A trace of iron was added from an iron needle for better staining. Alternate heating over a hot water bath and squashing was used to flatten the chromosomes. Cells with excellent spreading and differentiation of all pachytene bivalents were selected for analysis. From these cells photomicrographs and, in some cases, camera lucida drawings were made. The chromosome measurements were made from the photomicrographs utilizing a fine wire that was bent to follow the curves of the chromosome pairs.

Table 1. C. dactylon clones used in this study with chromosome number, variety, and origin as shown.

Clone	2n	Variety	Origin
B442	18	afghanicus	Herat, Afghanistan (P.I. 223129)
$B4420P_1$	18	afghanicus x aridus	Open-pollinated offspring of B442
$B4420P_2$	18	afghanicus x aridus	Open-pollinated offspring of B442
B445	18	aridus	Buffelsvlei, S. Africa (P.I. 291616)
Yakima	18	aridus	Yakima, Washington A. Law, WSU, Pullman
I-77-1	18	aridus	Unknown
B434	27	aridus x dacytlon	Open-pollinated offspring of OSU 983
FB141	36	dactylon	Gainesville, Florida
R17P4	36	dactylon	Open-pollinated offspring of FB141
Fults	36	dactylon	Greeley, Colorado Jess Fults, CSU
Sunset 10	36	dactylon	Sunset Hills Golf Course, St. Louis, MO

Mitotic material was obtained from root tips that were produced by stolons in water. Root tips were removed and fixed in 3:1 95% ethanol:glacial acetic acid. After storage of at least one week, the root tips were removed and the acetocarmine squash technique outlined above was utilized for staining.

The position and number of prominent chromomeres and knobs and the presence of the NOR were used in identification of each of the chromosomes, although differences in staining did occur. Other major characteristics used to identify each chromosome were (1) linear length, (2) short arm length, (3) long arm length, (4) arm ratio and (5) relative length. The chromosomes were numbered from 1, the shortest, to 9, the longest, on the basis of average linear length.

RESULTS

Pachytene Morphology in Diploid C. dactylon

Variation in linear measurement of chromosomes occurred within and between clones due to the stage of the cell, chromosome contraction, and degree of spreading. Figure 1 shows a typical pachytene spread with the chromosome strands, chromomeres and knobs showing prominently. The schematic drawing shows the number of each chromosome and the position of the centromere. In this cell the NOR chromosome is stretched and the centromere is obvious.

The following tables show statistical characterizations of each chromosome from measurements made for each clone studied.

An analysis of variance showed that clones differed significantly in overall linear length ($F=22.72$, $P<.01$), short arm length ($F=14.14$, $P<.01$), and long arm length ($F=16.74$, $P<.01$). There was no chromosome by clone interaction for each of these characteristics and, as expected, differences between chromosomes were highly significant ($P<.01$). The two clones that showed the greatest differences in overall length were B445 and Yakima. Many of the cells studied from each clone came from one or a few heads. Many of the Yakima cells were in late

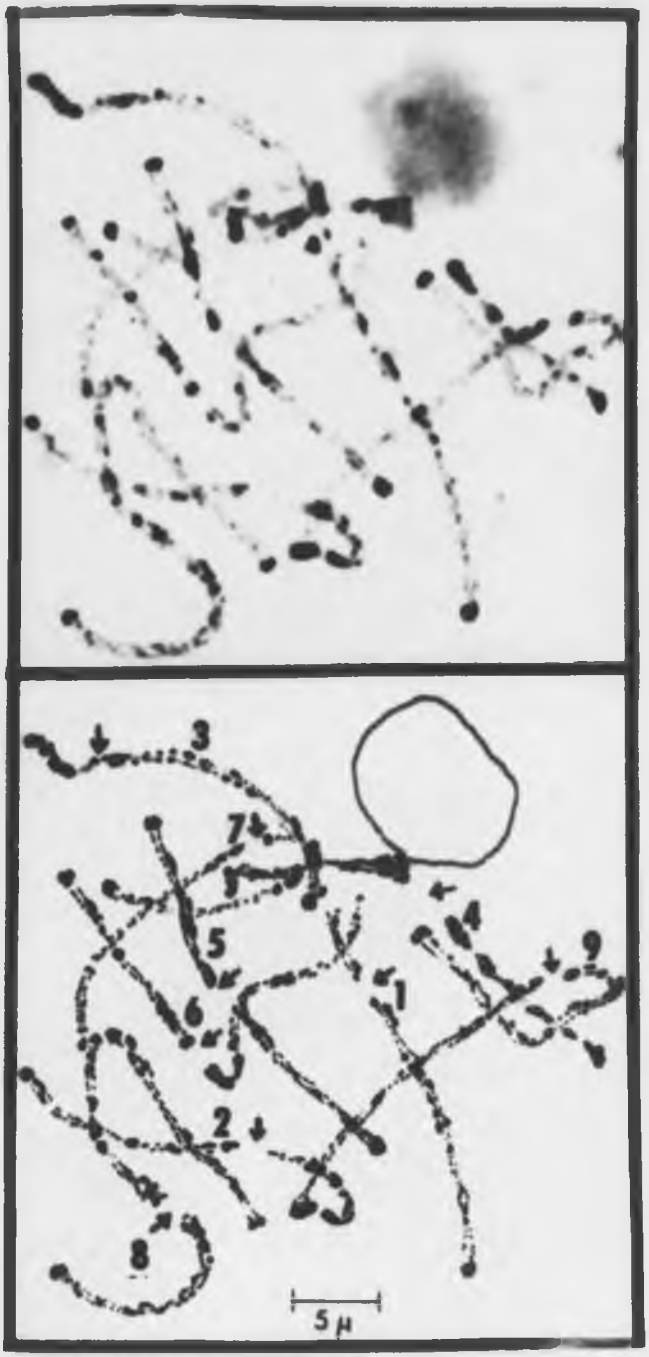

Figure 1. Pachytene chromosome complement of B445 with bivalents numbered and centromeres indicated.

pachytene as shown in Figure 2, with bivalents already beginning to separate. The B445 cells were all in early pachytene as in Figure 1. The range and standard deviation of each chromosome overlapped for the different clones, however, and since no significant morphological differences were noted between clones, mean values with their standard deviations are shown for each characteristic at the end of each table.

Figure 3 shows a composite drawing of the entire chromosome complement using the average measurements for each chromosome. The position and number of the prominent chromomeres and knobs are those usually found for each chromosome. Small variations in these characteristics were found between and within clones and these will be described and shown for those chromosomes involved.

Chromosome 1 (Table 2): The smallest chromosome of the complement was designated as chromosome 1 and given a relative length of 1. This is the chromosome upon which relative length measurements were based for each cell, although occasionally it was not the smallest in the complement. It was characterized by having a knob on the end of the long arm but no knob on the short arm, although it did end in a small chromomere. The long arm had three prominent chromomere pairs next to the centromere and two slightly less prominent pairs of chromomeres approximately

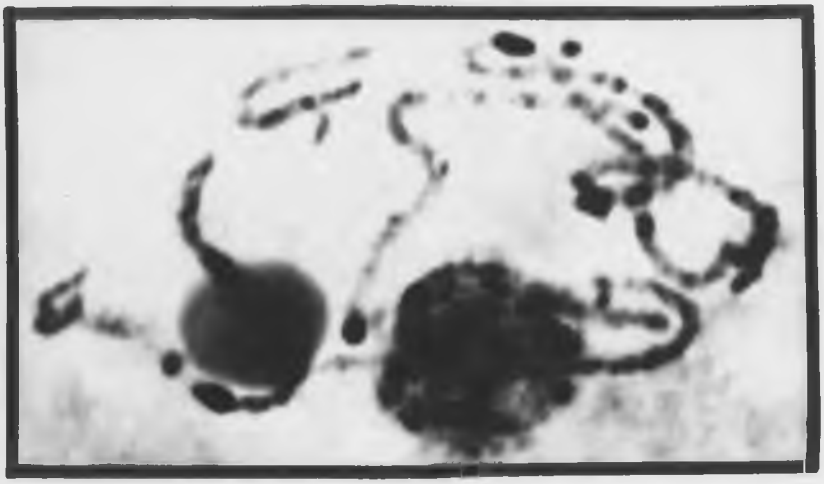

Figure 2. Yakima in late pachytene showing separation of the bivalents beginning.

Figure 3. Composite picture of the pachytene complement of diploid Cynodon dactylon.

Table 2. Mean values and ranges among measurements for pachytene lengths and arm ratios from five diploid clones: Chromosome 1.

Clone	No. Cells	Length (μm)			Arm ratio	Relative Length
		Whole Chromosome	Short arm	Long arm		
B445	(6)	23.8±1.5	9.0±0.9	14.8±1.0	1.66±0.19	1
		(21.5-25.5)	(7.2-9.6)	(13.3-16.3)	(1.48-1.99)	
I-77-1	(8)	20.3±3.4	6.9±1.5	13.4±2.2	1.99±0.31	1
		(16.6-25.8)	(5.0-9.9)	(10.0-15.9)	(1.52-2.32)	
Yakima	(7)	18.4±1.8	7.2±1.0	11.2±1.4	1.57±0.30	1
		(16.2-21.2)	(5.4-8.1)	(9.6-13.4)	(1.31-2.05)	
$B4420P_1$	(5)	19.0±3.0	7.9±1.5	11.1±1.9	1.44±0.28	1
		(16.0-22.6)	(5.6-9.6)	(9.6-13.9)	(1.22-1.86)	
$B4420P_2$	(8)	21.1±2.2	7.8±1.3	13.3±1.7	1.71±0.33	1
		(18.0-24.0)	(6.4-9.5)	(10.8-16.0)	(1.20-2.03)	
Average	(34)	20.5±2.9	7.7±1.4	12.8±2.1	1.70±0.33	1

one third of the distance to the end. The short arm had two chromomere pairs next to the centromere and two other pairs occurred slightly distal to the former. The chromosome's average length was 20.5 μm, with the short arm and long arm measuring 7.7 μm and 12.8 μm respectively, the arm ratio was 1.70, thus placing the centromere in a submedian position.

Chromosome 2 (Table 3): This chromosome was very similar to chromosome 1 and had to be compared closely to it for accurate identification. It was usually longer, the average length being 22.1 μm and the relative length 1.08, although in one cell of B445 it was shorter than chromosome 1. It usually had knobs on both arms (Figure 1) but occasionally the knob on the short arm did not stain as darkly and could be resolved into three chromomeres as in Figure 4. This chromosome had fewer prominent chromomeres next to the centromere than chromosome 1, two pairs on both arms. It also had one pair more a little further up the long arm. Both arms were also slightly longer than in chromosome 1, being 8.5 μm and 13.6 μm for a slightly less arm ratio of 1.64.

Chromosome 3 (Table 4): This was the chromosome most easily identified other than the NOR chromosome. Its short arm was primarily heterochromatin that could be resolved into four prominent pairs of chromomeres

Table 3. Mean values and ranges among measurements for pachytene lengths and arm ratios from five diploid clones: Chromosome 2.

Clone	No. Cells	Length (μm) Whole Chromosome	Short arm	Long arm	Arm ratio	Relative Length
B445	(6)	24.4±1.8	10.0±1.2	14.4±2.0	1.46±0.35	1.03±0.06
		(22.5-26.6)	(8.2-11.8)	(12.5-17.5)	(1.17-2.13)	(0.94-1.11)
I-77-1	(8)	22.1±3.3	8.8±2.1	13.3±2.1	1.58±0.40	1.10±0.11
		(16.9-27.9)	(6.4-12.5)	(8.8-15.4)	(1.09-2.16)	(1.00-1.32)
Yakima	(7)	20.2±1.5	7.7±1.4	12.5±0.6	1.66±0.32	1.10±0.09
		(18.5-22.6)	(5.7-10.0)	(11.7-13.4)	(1.36-2.25)	(1.01-1.28)
$B4420P_1$	(5)	21.4±2.4	8.4±0.8	13.0±1.9	1.55±0.21	1.14±0.99
		(19.0-24.6)	(7.2-9.6)	(10.9-15.8)	(1.33-1.88)	(1.03-1.27)
$B4420P_2$	(8)	22.5±2.1	7.9±1.2	14.6±1.6	1.88±0.32	1.07±0.06
		(19.1-24.9)	(6.4-9.7)	(12.7-15.6)	(1.54-2.46)	(1.02-1.19)
Average	(34)	22.1±2.6	8.5±1.6	13.6±1.8	1.64±0.35	1.08±0.09

Figure 4. Chromosome 2 showing no prominent chromomere at end from B4420P$_1$.

Figure 5. Contracted chromosome 4 from B4420P$_2$.

Figure 6. Chromosome 5 with a greater number of chromomeres from B445.

Figure 7. Chromosome 6 showing knobs on both arms from Yakima.

Figure 8. Chromomere gradients in pachytene chromosomes.

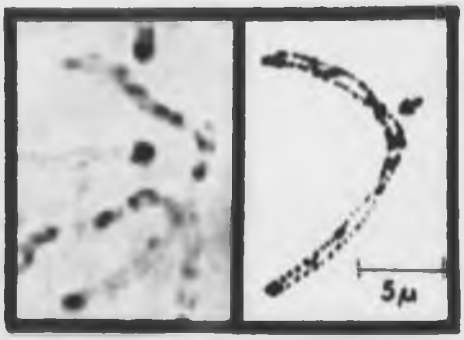

Figure 4. Chromosome 2

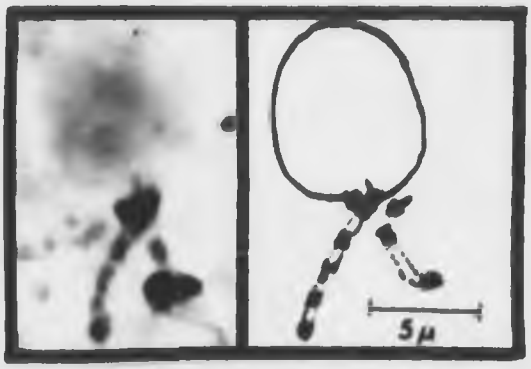

Figure 5. Chromosome 4

Figure 6. Chromosome 5

Figure 7. Chromosome 6

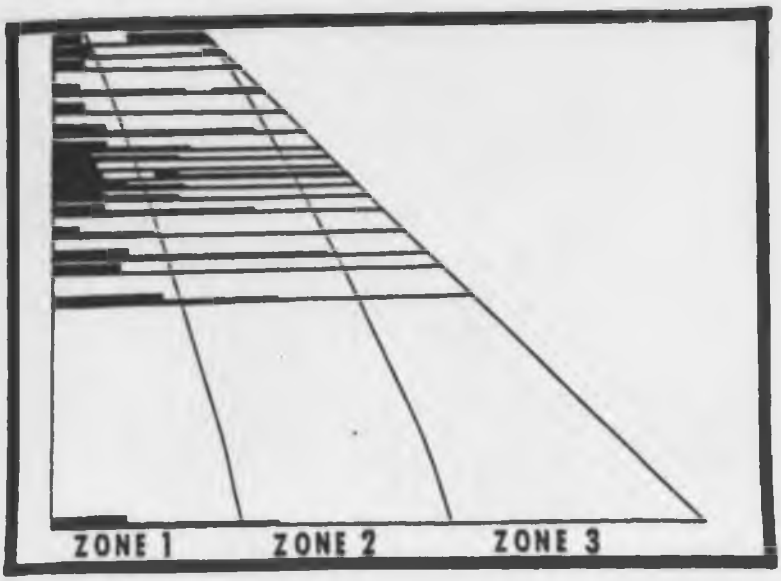

Figure 8. Chromomere gradients in pachytene chromosomes.

Table 4. Mean values and ranges among measurements for pachytene lengths and arm ratios from five diploid clones: Chromosome 3.

Clone	No. Cells	Length (μm)			Arm ratio	Relative Length
		Whole Chromosome	Short arm	Long arm		
B445	(6)	26.5±5.2	8.1±1.5	18.4±3.9	2.27±0.30	1.11±0.19
		(18.4-32.0)	(6.4-10.0)	(12.0-22.6)	(1.88-2.52)	(0.86-1.34)
I-77-1	(8)	24.2±4.5	7.9±1.8	16.3±3.2	2.10±0.44	1.20±0.16
		(17.5-29.5)	(5.6-10.1)	(10.8-20.2)	(1.46-2.73)	(1.00-1.48)
Yakima	(7)	21.3±1.7	6.6±0.8	14.7±1.7	2.24±0.41	1.15±0.09
		(19.2-23.8)	(6.4-7.9)	(12.5-16.8)	(1.68-2.73)	(1.05-1.33)
$B4420P_1$	(5)	22.7±2.9	6.9±0.6	15.8±2.4	2.28±0.28	1.20±0.15
		(19.2-25.2)	(6.4-7.9)	(12.8-18.0)	(2.00-2.65)	(1.10-1.44)
$B4420P_2$	(8)	24.9±3.2	8.6±1.1	16.3±2.5	1.92±0.30	1.18±0.09
		(20.9-30.2)	(7.0-10.4)	(13.9-21.8)	(1.65-2.59)	(1.04-1.31)
Average	(34)	23.9±3.9	7.7±1.4	16.2±2.9	2.14±0.37	1.17±0.13

or knobs followed by a short euchromatic segment and another chromomere pair next to the centromere. The average length was 23.9 µm and relative length was 1.17, although in two cells of B445 it was shorter than chromosome 1 and in one cell it was the same length. The arms were 7.7 µm and 16.2 µm, giving an average arm ratio of 2.14.

Chromosome 4 (Table 5): This was the NOR chromosome so it was easily distinguished. It was sometimes stretched out, making the chromosome much longer than chromosome 1 as shown in Figure 1 or sometimes contracted as shown in Figure 5. The average length was 25.1 µm and the average relative length was 1.23. The relative length varied from 1.75 when stretched out to 0.94 when contracted. Both arms had many chromomeres and both terminals had knobs. The long arm had two pairs of prominent chromomeres between the centromere and the NOR and two more pairs distal to the NOR. Three sets of less prominent chromomeres occurred between the latter two pairs of chromomeres and the knob. The short arm had two prominent chromomeres next to the centromere and a variable number beyond. The arms were 10.8 µm and 14.3 µm long, giving an arm ratio of 1.35.

Chromosome 5 (Table 6): Figure 6 shows the more typical appearance of chromosome 5 as used in drawing the composite. Occasionally fewer chromomeres were evident, especially near the knob on the short arm. This is illustrated by Figure 1. This chromosome had knobs on

Table 5. Mean values and ranges among measurements for pachytene lengths and arm ratios from five diploid clones: Chromosome 4, NOR chromosome.

Clone	No. Cells	Length (μm) Whole Chromosome	Short arm	Long arm	Arm ratio	Relative Length
B445	(6)	29.6±2.2	13.2±1.6	16.4±1.8	1.26±0.22	1.25±0.08
		(27.3-32.1)	(11.4-15.3)	(14.2-19.7)	(1.04-1.59)	(1.10-1.34)
I-77-1	(8)	24.5±3.8	10.0±2.3	14.5±1.7	1.48±0.20	1.22±0.24
		(19.4-29.8)	(7.0-14.0)	(12.4-17.6)	(1.13-1.77)	(1.02-1.75)
Yakima	(7)	22.7±2.7	9.7±1.2	13.0±1.7	1.35±0.15	1.25±0.20
		(18.9-26.3)	(7.4-11.1)	(11.0-15.2)	(1.17-1.55)	(0.94-1.47)
$B4420P_1$	(5)	22.3±1.9	9.5±1.2	12.8±1.3	1.36±0.19	1.19±0.20
		(20.0-25.2)	(8.2-10.8)	(11.6-14.8)	(1.07-1.59)	(0.94-1.44)
$B4420P_2$	(8)	26.5±4.9	11.8±2.3	14.7±2.8	1.26±0.14	1.23±0.31
		(19.2-32.0)	(8.3-14.5)	(10.9-18.2)	(1.04-1.48)	(1.04-1.58)
Average	(34)	25.1±4.1	10.8±2.2	14.3±2.3	1.35±0.19	1.23±0.22

Table 6. Mean values and ranges among measurements for pachytene lengths and arm ratios from five diploid clones: Chromosome 5.

Clone	No. Cells	Length (μm) Whole Chromosome	Short arm	Long arm	Arm ratio	Relative Length
B445	(6)	27.1±3.1	10.4±1.1	16.7±3.0	1.64±0.36	1.14±0.14
		(21.9-30.2)	(9.4-12.3)	(12.5-20.7)	(1.27-2.20)	(1.10-1.35)
I-77-1	(8)	25.6±4.0	9.5±2.0	16.1±2.5	1.72±0.27	1.28±0.23
		(19.0-30.1)	(7.2-12.9)	(11.8-20.2)	(1.28-2.15)	(1.12-1.81)
Yakima	(7)	22.4±4.0	7.6±1.1	14.8±2.4	1.97±0.33	1.22±0.15
		(18.7-27.5)	(5.6-8.8)	(11.1-18.7)	(1.37-2.34)	(1.03-1.45)
$B4420P_1$	(5)	23.6±1.9	9.5±1.1	14.1±1.0	1.49±0.15	1.26±0.17
		(21.4-25.7)	(8.3-11.1)	(12.6-15.3)	(1.32-1.73)	(1.04-1.46)
$B4420P_2$	(8)	26.6±3.4	9.8±2.3	16.8±2.7	1.80±0.44	1.27±0.17
		(20.8-31.0)	(6.4-14.6)	(14.4-21.8)	(1.03-2.37)	(1.07-1.53)
Average	(34)	25.1±3.5	9.3±1.9	15.8±2.5	1.74±0.35	1.24±0.17

both arms. The arms were 9.3 μm and 15.8 μm long, giving a submedian arm ratio of 1.74. The long arm had three chromomere pairs next to the centromere and two more groups further out. Another chromomere pair occurred near the knob. The short arm had three pairs of chromomeres near the centromere and two next to the knob. The average length was 25.1 μm, the same as chromosome 4.

Chromosome 6 (Table 7): This chromosome was definitely longer than the previous five of the complement, with an average length of 31.0 μm and a relative length of 1.53. The long arm was 18.4 μm and had three pairs of prominent chromomeres near the centromere. It had three pairs of less prominent chromomeres scattered up the arm which may (Figure 7) or may not (Figure 1) be knobbed. This variation was noted in different spreads in the same and different clones. The centromere was median with an arm ratio of 1.47.

Chromosome 7 (Table 8): This chromosome had the least number of prominent pairs of chromomeres; three occurred near the centromere on the long arm and three on the short arm with two of them near the centromere. There were two small knobs or large chromomeres at either end. The linear length was 33.5 μm, divided into 14 μm for the short arm and 19.5 μm for the long arm. The arm ratio was 1.43 and the relative length was 1.65.

Table 7. Mean values and ranges among measurements for pachytene lengths and arm ratios from five diploid clones: Chromosome 6.

Clone	No. Cells	Length (μm) Whole Chromosome	Short arm	Long arm	Arm ratio	Relative Length
B445	(6)	33.0±3.3	13.4±1.7	19.6±2.4	1.48±0.22	1.39±0.10
		(27.8-37.0)	(10.5-15.5)	(15.9-21.8)	(1.10-1.68)	(1.30-1.55)
I-77-1	(8)	29.3±4.6	11.7±1.8	17.6±3.4	1.52±0.25	1.47±0.32
		(22.2-36.1)	(8.8-14.0)	(12.9-22.7)	(1.16-1.85)	(1.12-2.05)
Yakima	(7)	29.2±4.2	12.2±1.7	17.0±2.7	1.40±0.15	1.60±0.23
		(24.3-37.5)	(9.3-15.1)	(15.0-22.4)	(1.20-1.61)	(1.23-1.93)
$B4420P_1$	(5)	30.7±5.5	13.2±3.1	17.5±2.7	1.36±0.25	1.62±0.24
		(21.6-36.2)	(8.8-16.2)	(12.8-20.0)	(1.13-1.75)	(1.35-1.87)
$B4420P_2$	(8)	33.1±3.4	13.1±1.4	20.8±3.0	1.53±0.27	1.58±0.19
		(25.5-37.7)	(11.4-15.6)	(14.1-24.7)	(1.23-1.90)	(1.38-1.94)
Average	(34)	31.0±4.3	12.6±1.9	18.4±3.0	1.47±0.23	1.53±0.23

Table 8. Mean values and ranges among measurements for pachytene lengths and arm ratios from five diploid clones: Chromosome 7.

Clone	No. Cells	Length (μm) Whole Chromosome	Short arm	Long arm	Arm ratio	Relative Length
B445	(6)	36.7±2.7	16.1±2.2	20.6±2.0	1.31±0.26	1.55±0.08
		(33.4-40.6)	(12.3-18.4)	(17.4-23.0)	(1.08-1.75)	(1.45-1.70)
I-77-1	(8)	31.0±4.1	13.6±2.0	17.4±2.4	1.29±0.13	1.55±0.23
		(23.4-38.2)	(9.4-16.2)	(14.0-22.0)	(1.10-1.49)	(1.20-1.94)
Yakima	(7)	31.8±3.5	12.3±1.3	19.5±3.1	1.60±0.27	1.74±0.21
		(25.9-37.4)	(9.8-13.8)	(15.9-25.1)	(1.15-2.04)	(1.31-1.98)
$B4420P_1$	(5)	32.2±3.4	13.1±2.2	19.2±2.8	1.51±0.37	1.71±0.16
		(26.6-35.2)	(10.5-16.3)	(14.6-21.9)	(1.16-2.09)	(1.53-1.89)
$B4420P_2$	(8)	35.8±2.5	14.7±1.3	21.1±1.6	1.44±0.13	1.71±0.19
		(32.6-38.7)	(12.6-15.9)	(18.2-22.9)	(1.18-1.58)	(1.45-1.95)
Average	(34)	33.5±3.9	14.0±2.1	19.5±2.7	1.43±0.25	1.65±0.20

Chromosome 8 (Table 9): This chromosome had the most median centromere with an arm ratio of 1.26 and the most chromomere pairs of the longer chromosomes. The long arm was 21.4 µm long, had a knob on the end, three spaced prominent chromomere pairs near the centromere, and three smaller pairs one-third further up the arm. The small arm was 17.1 µm long and terminated with a knob; four pairs of chromomeres occurred near the centromere. The average linear length was 38.5 µm and the relative length 1.89.

Chromosome 9 (Table 10): This was the longest of the chromosomes with an average linear length of 44.6 µm and a relative length of 2.20. Occasionally it was shorter than chromosome 8, but it could always be distinguished because its centromere was not as median as in chromosome 8. It had an arm ratio of 1.57. It also had smaller knobs on both arms and fewer chromomeres. The long arm, which was 17.6 µm long, had three pairs of chromomeres near the centromere and one group of two further up the arm. The short arm, which was 27 µm long, had three closely spaced pairs of chromomeres near the centromere.

Figure 8 shows the chromomere gradients found in the diploid C. dactylon. The bivalents are shown in the arm frame method (Lima de Faria 1976c) with the centromeres on the left edge. Zone 1 was the area where the largest chromomeres were found. Zone 2 had medium sized

Table 9. Mean values and ranges among measurements for pachytene lengths and arm ratios from five diploid clones: Chromosome 8.

Clone	No. Cells	Length (μm)			Arm ratio	Relative Length
		Whole Chromosome	Short arm	Long arm		
B445	(6)	43.6±6.2	18.3±3.8	25.3±2.7	1.41±0.22	1.84±0.24
		(32.4-49.8)	(11.9-22.2)	(20.5-27.6)	(1.24-1.72)	(1.45-2.08)
I-77-1	(8)	35.6±4.9	15.7±1.9	19.9±3.5	1.28±0.18	1.78±0.30
		(28.6-45.8)	(13.3-18.6)	(14.5-27.2)	(1.03-1.56)	(1.33-2.27)
Yakima	(7)	36.3±3.4	16.6±1.8	19.7±2.2	1.19±0.14	1.98±0.21
		(32.6-42.0)	(13.8-19.2)	(16.6-22.8)	(1.04-1.40)	(1.77-2.36)
$B4420P_1$	(5)	37.6±4.6	16.7±1.5	20.9±3.3	1.25±0.14	1.99±0.27
		(29.5-40.6)	(14.3-18.1)	(15.2-23.4)	(1.06-1.43)	(1.73-2.32)
$B4420P_2$	(8)	40.0±4.6	18.3±1.7	21.7±3.0	1.18±0.08	1.91±0.28
		(33.8-48.0)	(16.4-21.6)	(17.4-26.4)	(1.06-1.34)	(1.58-2.29)
Average	(34)	38.5±5.3	17.1±2.4	21.4±3.4	1.26±0.17	1.89±0.26

Table 10. Mean values and ranges among measurements for pachytene lengths and arm ratios from five diploid clones: Chromosome 9.

Clone	No. Cells	Length (μm) Whole Chromosome	Short arm	Long arm	Arm ratio	Relative Length
B445	(6)	51.9±5.7	19.2±2.3	32.7±6.6	1.74±0.48	2.20±0.31
		(44.1-58.4)	(16.9-22.2)	(22.3-40.7)	(1.02-2.30)	(1.73-2.62)
I-77-1	(8)	43.1±8.0	17.8±4.6	25.3±4.9	1.47±0.33	2.16±0.43
		(32.8-56.7)	(12.0-28.0)	(19.7-32.5)	(1.03-1.95)	(1.63-2.89)
Yakima	(7)	37.9±2.4	15.1±2.5	22.8±2.3	1.55±0.35	2.08±0.34
		(34.5-44.5)	(11.3-19.2)	(19.8-25.3)	(1.28-2.21)	(1.83-2.70)
$B4420P_1$	(5)	44.1±3.9	17.0±2.5	27.1±1.9	1.62±0.18	2.35±0.27
		(39.2-49.4)	(14.2-19.9)	(25.0-30.1)	(1.32-1.76)	(1.96-2.69)
$B4420P_2$	(8)	46.8±5.1	18.7±2.2	28.1±3.6	1.52±0.20	2.23±0.31
		(41.5-54.4)	(14.8-21.9)	(23.3-34.2)	(1.28-1.89)	(1.88-2.60)
Average	(34)	44.6±7.0	17.6±3.2	27.0±5.1	1.57±0.32	2.20±0.32

chromomeres in this area. Zone 3 was where the smallest chromomeres were found. The knobs, where they occur, were all on the ends of the arms.

Pachytene Morphology of C. dactylon var. dactylon

The tetraploid C. dactylon var. dactylon was more difficult to work with because of the increased number of bivalents present. It was very difficult to obtain spreads in which all of the bivalents could be positively separated and identified. Often all of the bivalents were not in the same plane and the knobs tended to associate as indicated by the arrow in Figure 10A. The observations below were based on many cells even though the references were only to those shown.

Figure 9 shows a well spread pachytene cell, which illustrates the problem of chromosome stretching when flattening of the cell was achieved. The chromosomes probably corresponding to the bivalents in the diploid complement were marked with the appropriate numbers. There were two of each except chromosome 8 which was probably in the darkened area of cytoplasm. The four chromosomes most easily identified in this cell were 2, 3, 5, and the NOR chromosome.

Chromosome 1 appeared the same as in the diploid in FB141 (Figure 9) and Fults (Figures 10D and E) and in one cell each of R17P4 (Figure 10A), and Sunset 10 (Figure 10C).

Figure 9. Tetraploid FB141 with chromosomes probably corresponding to diploid bivalents numbered.

Figure 10. Pachytene chromosomes of var. dactylon.

A. R17P4 showing association of knobs and bivalent pairs 5, 6, and 9. B. R17P4 showing contracted, heterochromatic chromosome 1. C. Sunset 10 with two bivalents associated with the nucleolus. D. Fults with pachytene bivalent pairs 3, 2, and 1. E. Fults with matching bivalent pairs 7 and 9. F. Triploid B434 showing mass of univalents usually formed. G. Two bivalents associated with the nucleolus from FB141. H. Quadrivalents associated with the nucleolus from FB141. I. Contracted and heterochromatic chromosome 1 from Sunset 10. J. Contracted chromosome 2 shorter than chromosome 1 from R17P4.

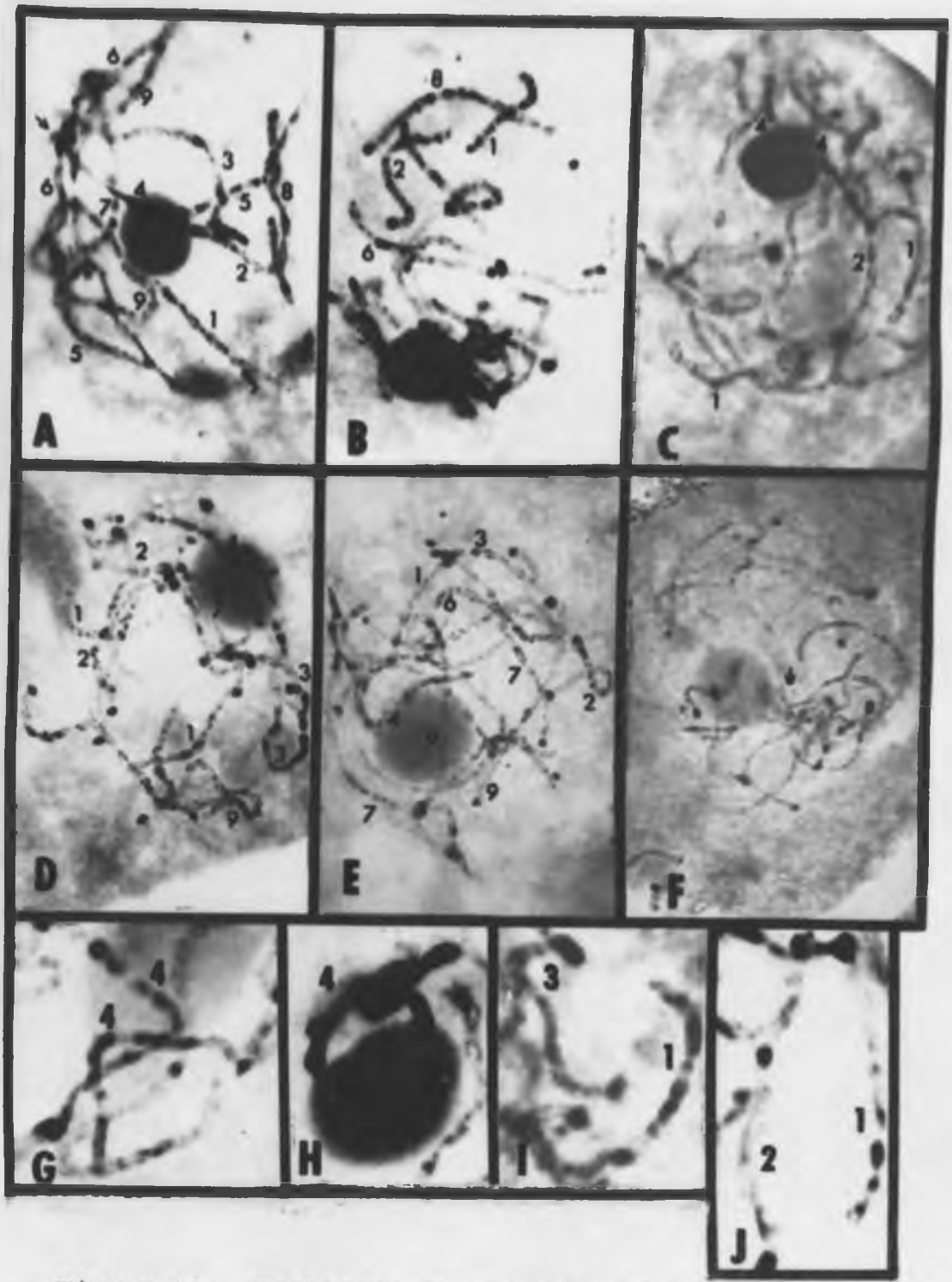

Figure 10. Pachytene chromosomes of var. dactylon.

In one cell each of R17P4 (Figure 10B) and Sunset 10 (Figure 10I) it appeared much more contracted and heterochromatic, so some differences in morphological appearance were evident.

Chromosome 2 usually appeared the same as in the diploid (Figures 9 and 10A to E). In one cell of R17P4 (Figure 10J) it was contracted and shorter than chromosome 1. This also occurred occasionally in the diploids.

Chromosome 3 was readily distinguishable because of its heterochromatic arm. In Figures 8 and 9D the two bivalents appeared to be identical. This chromosome did not appear to be different in the diploid or tetraploid.

Chromosome 4 could be distinguished because of its attachment to the nucleolus. It usually was attached as two bivalents as in Figures 9, 10C, and D. This was more clearly shown in Figure 10G of FB141, where both bivalents and their attachments were apparent. Figure 10H, also from FB141, showed a quadrivalent formed by these chromosomes, with only one point of attachment. This suggested complete homology.

Chromosome 5 in Figure 8 was as it normally appeared in the diploid (Figures 3 and 6). In Figure 10A, it had less chromomeres as it occasionally appeared in the diploid (Figure 1). Variability in the tetraploid was similar to that in the diploid.

Chromosome 6 showed the same variability as in the diploid. In Figures 9 and 10A, it had a knob on the long arm. In Figure 10E, the long arm had only a chromomere on the end.

Chromosome 7 appeared the same as in the diploid. It had only a few chromomeres on either side of the centromere and small knobs on the ends of both arms (Figure 9, 10A and E).

Chromosome 8 appeared to have more chromomeres than in the diploid. This is shown in Figures 9 and 10A.

Chromosome 9 in Figures 9 and 10A had many more chromomeres than in the diploid and may be more contracted or have a different origin. In Figures 10D and E, it appeared the same as in the diploid with a few chromomeres.

Figure 10F shows a natural triploid hybrid between the tetraploid and diploid. The arrow shows the mass the univalents usually formed at pachytene in this hybrid. Nine bivalents were usually found separated from this mass.

Somatic Prophase Chromosome Morphology

Figure 11 shows a well-defined spread of diploid C. dactylon B4420P$_2$. The chromosome complement was drawn below with pairs matched and numbered in increasing order of size on the left. The number on the right shows the number of the corresponding pachytene bivalent, based on the appearance of distinguishing heterochromatic areas.

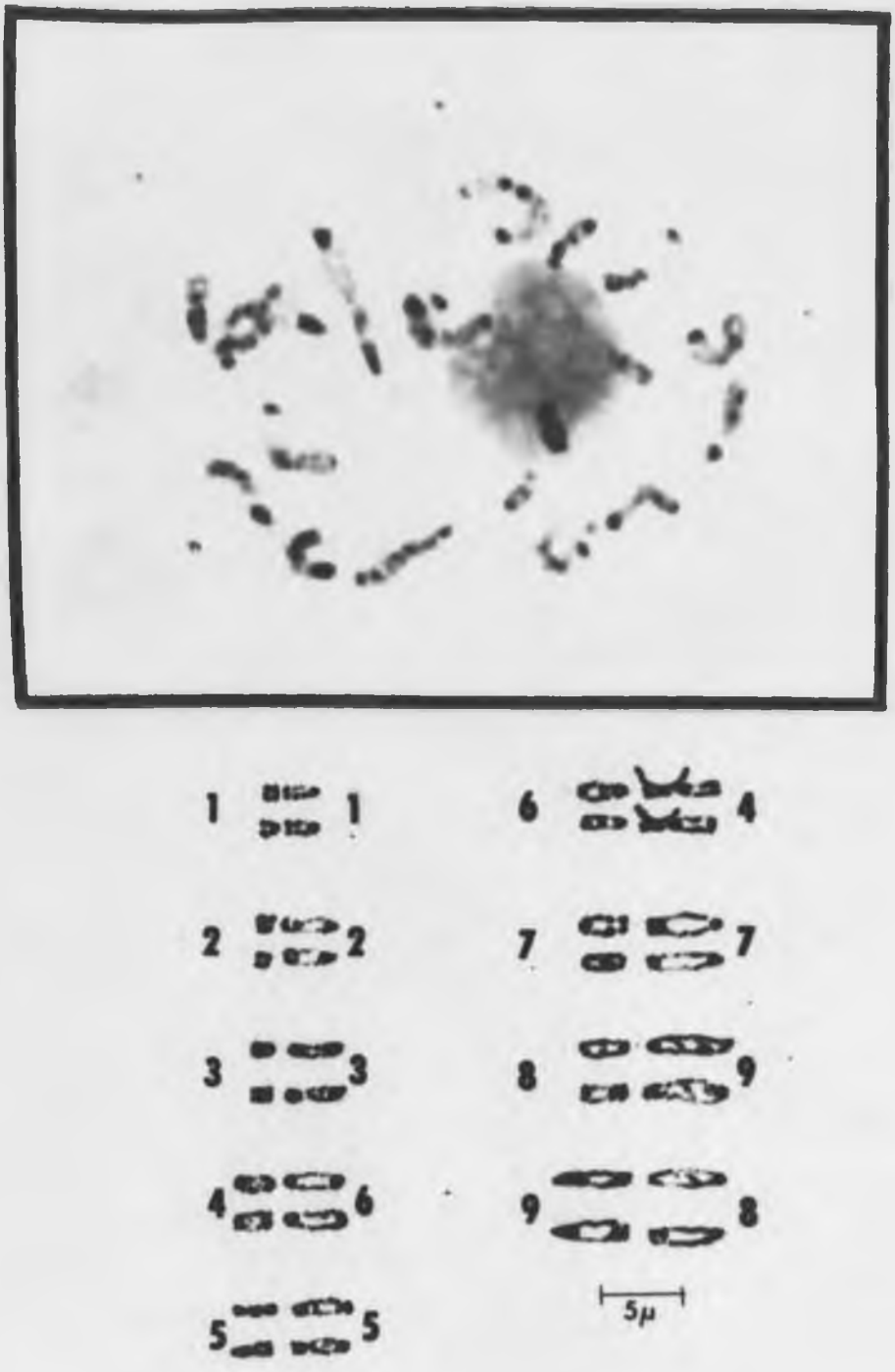

Figure 11. Mitotic chromosome complement of diploid B4420P$_2$ with matched pairs shown below. Mitotic number on left, number of corresponding pachytene bivalent on right.

Figure 12 shows spreads at a slightly more contracted stage of a diploid, triploid and tetraploid. It became difficult to distinguish pairs and correspondence of the intermediate size chromosomes in the tetraploid.

Table 11 shows differences in chromosome measurements in two cells of B4420P_2 due to differences in cell stage. The second cell of B4420P_2 was at the same stage as B442 in Figure 12. Different contraction rates were apparent for the different chromosomes.

Table 11. Two somatic prophase cells of B4420P$_2$ showing the effects of cell stage on chromosome measurements.-- The corresponding pachytene bivalent number is shown in parenthesis.

Chromosome	Cell	Length (µm)			Arm ratio	Relative Length
		Whole Chromosome	Short arm	Long arm		
1 (1)	1	4.25	1.60	2.65	1.66	1
	2	3.25	1.30	1.95	1.50	1
2 (2)	1	6.05	1.70	4.35	2.56	1.42
	2	4.60	1.50	3.10	2.07	1.42
3 (3)	1	6.25	1.80	4.45	2.47	1.47
	2	5.10	1.50	3.60	2.40	1.57
4 (6)	1	6.35	3.05	3.30	1.08	1.49
	2	4.90	1.70	3.20	1.88	1.51
5 (5)	1	6.50	3.15	3.35	1.06	1.53
	2	6.10	1.60	4.50	2.81	1.88
6 (4)	1	6.75	3.20	3.55	1.11	1.59
	2	6.40	2.40	4.00	1.67	1.51
7 (7)	1	7.90	3.10	4.80	1.55	1.86
	2	6.50	3.00	3.50	1.17	2.00
8 (9)	1	9.30	3.25	5.95	1.78	2.19
	2	7.10	3.20	3.90	1.22	2.18
9 (8)	1	9.70	4.30	5.40	1.26	2.28
	2	8.10	3.15	4.95	1.57	2.49

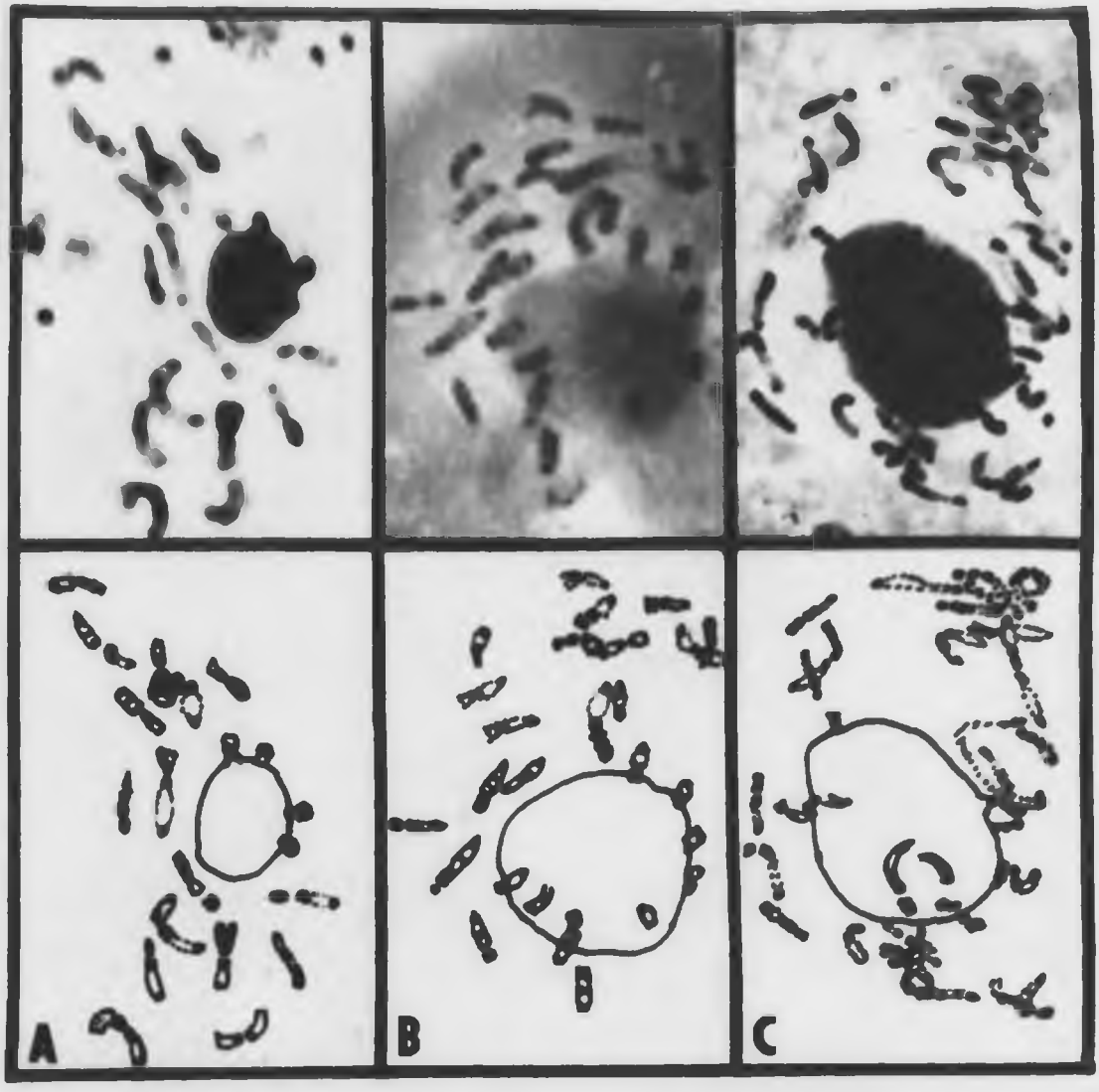

Figure 12. Mitotic complement of different ploidy levels of C. dactylon.-- A. Diploid B442. B. Triploid B434. C. Tetraploid FB141.

DISCUSSION

The diploid clone studied by Ourecky (1963) was probably C. dactylon var. afghanicus because it originated in Afghanistan. Harlan, de Wet, and Rawal (1970) determined that this variety exists in that area and C. dactylon var. aridus is not usually found there. Two of the clones, $B4420P_1$ and $B4420P_2$, used in this study are putative hybrids between var. afghanicus and var. aridus. In these clones complete pairing always occurred which suggests the two genomes are completely homologous. This agrees with pairing behavior observed by Harlan, de Wet, et al. (1970) in 64 hybrids between these two in which 8.98 bivalents (8-9) and 0.04 univalents (0-2) were formed. Rawal and Chedda (1971) also found that hybrids had regular pairing and eight or nine bivalents. Morphologically, $B4420P_1$ and $B4420P_2$ showed no major differences from the other three clones which were var. aridus; this suggests these results can be compared to Ourecky's results.

The differences in overall chromosome length of the various clones can probably be explained by differences in the stage of the cell from one spread to another. The cells of Yakima which were studied came from only two heads, with four of the cells in late pachytene as in Figure 2.

The cells of B445 all came from one head and were in early pachytene as in Figure 1. Ho and Kasha (1971) found significant regression of lengths on stage index when pachytene complements of Medicago sativa L. were designated as being in early, middle, and late pachytene. Gillies and Bingham (1971) found that chromosome lengths of the 2x haploid and tetraploid in M. sativa were shorter than in the diploid which suggested that genetic factors causing contraction may be on the chromosomes. If such factors exist, these could also explain differences in overall lengths. The similarity in morphology and overlap in ranges suggests that all of the clones possess the same genome.

In most cases Ourecky's observations agree with those in this study, his chromosome measurements were within the range of those in this study and the morphological descriptions were similar. Two major differences occur, the arm measurements and the arm ratios for chromosomes 3 and 4. In the case of the NOR chromosome, three of the four cells he reported on were close to the observed values. In one cell this chromosome was very contracted and this significantly affected his results because only four cells were studied. Chromosome 3 differences appear to be in different placement of the centromere and designation of which chromosome in the complement is represented. In cell A of his study I would change the designations of chromosome

2 and 3. Chromosome 9 in his study also appears to have more chromomeres than that from clones in this study. Tripathi et al. (1977) also found chromosome polymorphism in diploids compared to Ourecky with differences in terminal knobs and length of a knob observed.

The similarity of the genomes in the two studies suggests that they are homologous. The differences are due to differences in cell stage and possibly chromosomal biotypes. This supports Harlan and de Wet (1969) that only one genome exists for all crossable species in the genus. Rawal and Chedda (1971) concluded from cytomorphology studies that vars. afghanicus and aridus evolved from a common non-rhizomatous ancestor and that the barriers to gene flow are primarily ecological and geographical. Tripathi et al. (1977) believed that the existence of chromosomal biotypes could easily be explained by the wide geographic distribution and ecological adaptation.

Maguire (1977) found that in a specific region of chromosome 6 of maize the number of chromomeres varied from cell to cell within a sample and each type was seen in early, middle, and late pachytene. She believed missing chromomeres may reflect failure of condensation of differential aggregation during condensation of subunits. This may account for the variability observed in chromomere patterns and no actual morphological differences may exist.

Most researchers have observed variability in chromosome length, relative length and arm ratio for pachytene chromosomes. Chromosome length is the usual criterion for numbering chromosomes but Ho and Kasha (1974) found that there can be different rates of contraction for each chromosome. They found that longer chromosomes tended to vary more in length. In this study the only exception to this was the NOR chromosome which had a little more variability than chromosome 5. Ho and Kasha (1974) and Maguire (1962) found that longer arms and larger arm ratios also tend to vary more. This also was true in this study, the smallest variability of 1.4 μm being associated with the shortest arm of 7.7 μm and the largest variability of 5.1 μm being associated with the longest arm of 27.0 μm, however the percentage variability was only slightly larger in the later case.

Ho and Kasha (1974) and Gillies (1972) found that differences in contraction rate can change relative chromosome lengths and arm ratios. The differences in contraction rate may be due to differential contraction rates in euchromatic and heterochromatic regions. A chromosome with euchromatic regions in two arms may shorten faster than one with one arm largely heterochromatic. This could explain why, in certain cells that were especially contracted, chromosome 8, with more heterochromatin, was as

long or longer than chromosome 9. This could also explain why the variability in arm ratio is greater in chromosomes 3 and 5, which both have a short arm that is primarily heterochromatic.

The chromomere gradients approximately follow the patterns found by Lima de Faria (1976c). In different chromosome arms the gradient may be abrupt or gradual. Three zones can generally be drawn which correspond to his optimal zone (Zone 1), permitted zone (Zone 2), and forbidden zone (Zone 3).

The knobs, when present, are all found at the end of arms, which corresponds to Lima de Faria's optimal region. This is where 72.3 percent of all knobs are found (1976b). Cultivation and vegetative propagation do not seem to have influenced their position as has happened in maize. Lima de Faria (1976b) says knobs have a tendency not to appear in short arms of less than 15 µm, because they have not escaped the centromere influence. In this species knobs do appear in arms down to 7.7 µm long but the short arm of chromosome 1, where there is never a knob, the short arm of chromosome 2, and the long arm of chromosome 6, both often lacking knobs, are all under 15 µm long.

The position of the NOR does not follow the general pattern found by Lima de Faria (1976a). It is found on a long arm rather that a short arm and is on the borderline

between a forbidden and permitted region. He did find that cultivation and vegetative propagation allow new arrangement in the NOR to be preserved. In those cases the NOR becomes displaced toward the centromere, which is where the NOR is found in this species.

The tetraploid C. dactylon var. dactylon appears to have two sets of the same genome represented. There appears to be no differences in the bivalents and each is represented twice. They appear to be morphologically the same as bivalents represented in different cells of the diploid. Chromosome 9 in FB141, with more chromomeres than found in the diploids in this study, appears to be the same as chromosome 9 found by Ourecky (1963). The tetraploid from this analysis appears to be an autotetraploid. The formation of primarily nine bivalents and nine univalents in the triploid suggests preferential pairing or suggests genetic differences may be present. Murty (1973) states that genetic differences of specific magnitude may exist in chromosomes that appear very similar (or identical) at pachytene.

The pachytene bivalents and their corresponding somatic pairs can be matched on the basis of their chromomere patterns. The chromosomes are of different relative lengths during the two phases. The NOR chromosome is the fourth shortest during pachytene but the sixth shortest

during somatic prophase. The relative positions of chromosomes 5, 6, 8, and 9 are reversed during somatic prophase. This agrees with findings by De and Krishnan (1966) that in Phaseolus mungo L. the two NOR chromosomes are the second and third longest at somatic metaphase but only seventh and ninth longest during pachytene. Hyde (1953) found in Plantago ovata that the differentiation characteristic for each chromosome at pachytene corresponds in detail to that in mitotic prophase.

Ramanna and Prakken (1967) found, in comparing somatic and pachytene chromosomes, that heterochromatic parts of somatic chromosomes are contracted by a factor of four to five whereas the euchromatic parts are contracted by a factor of thirty. This could explain why the NOR chromosome is not as contracted because it is primarily heterochromatic. Pachytene chromosomes 5 and 8 also have more heterochromatin in them than 6 or 9 which may be why 5 and 8 are longer in somatic prophase than 6 and 9 respectively. The overall chromomere pattern remains the same, however, and can be recognized in pachytene and somatic prophase. This agrees with Lima de Faria's viewpoint (1976a) that the chromosome is a whole unit and when the chromosome is reduced or increased in length as the result of coiling and uncoiling it does not affect the pattern as a whole.

SUMMARY

The present study revealed that five diploid clones of C. dactylon, which included three var. aridus and two hybrids between var. aridus and var. afghanicus, had the same pachytene chromosome complement. These chromosomes were numbered 1 to 9 from shortest to longest and were the same as those described by Ourecky (1963).

The pachytene chromosomes had a chromosome gradient that could be divided into three zones, as expected from studies by Lima de Faria (1976c) in other species. The knobs were all in the optimal region as defined by Lima de Faria (1976b) but were found in arms under 15 um long. The NOR region was in a long arm instead of a short arm and was closer to the centromere than normally observed in other species (Lima de Faria 1976a).

The tetraploids (var. dactylon) appeared to have two sets of the same chromosomes observed in the diploids. This suggests an autotetraploid origin or that the genetic differences separating the two species that hybridized to form the autotetraploid are not evident in the pachytene chromosomes.

The somatic prophase pairs can be homologized with their corresponding pachytene bivalents by using their

chromomere patterns. Different rates of contraction are evident in the heterochromatic and euchromatic regions causing the relative lengths to be different in the two stages.

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