A FRAMEWORK LINKAGE MAP OF

BERMUDAGRASS (*Cynodon dactylon* x *transvaalensis*)

BASED ON SINGLE-DOSE RESTRICTION FRAGMENTS

by

CASEY M. BETHEL

(Under the direction of Andrew H. Paterson)

ABSTRACT

Bermudagrass (*Cynodon* sp.) is a perennial, warm season grass that is widely used in the turfgrass industry because of its rapid growth rate, its ability to tolerate very close mowing and its potential to generate a variety of textures needed for multiple uses. Genome mapping of this organism has proceeded at a slow pace however, due to its polyploid nature, which imposes limitations on conventional strategies. This study describes the first linkage maps of two bermudagrass species, *Cynodon dactylon* and *Cynodon transvaalensis*, based on single-dose restriction fragments (SDRFs). The maps cover 1978 cM and 973.4 cM respectively, with average marker spacing of 15.5 cM and 17.2 cM. We also demonstrate the utility of these maps for use in determining attributes of the bermudagrass genome, including recombinational length and mode of inheritance. Finally, comparisons of these maps to the genomes of *Sorghum* and rice reveal regions of conserved gene order.

Index words: Cynodon, bermudagrass, autopolyploid, single-dose restriction fragments, genome, linkage

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# DEDICATION

This work is very humbly dedicated to:

*Mummy* – For the incredible personal sacrifices that you made over the years to give me the opportunities that you did. And for always believing in me. I owe this all to you.

*Mama* – For ALWAYS being there for me.

*Braddie, Shannen, Christian, De'Anthea, Antja, Annja, Aliage, De'Shaun, Chelsea, Carika, De'Andre, Shavaughn, Shonnette, Carrison Jr, Raven, Kelyen, Larry Jr., Kenny, Jonothan, Keisha, Gerran, Kishera, Sherrard, Emiko, Scott, Giovanni, Eric, Dave, Gerrard Jr., Lauren, Navas, Jodie and Jade* – In your academic pursuits, let my modest accomplishment here serve as proof that higher education is possible for each of you. In fact, each of you has the potential to go much further than I did. I believe in you all.

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# CHAPTER 1

## INTRODUCTION AND LITERATURE REVIEW

### *Bermudagrass*

Bermudagrass was first introduced to the United States from Africa in 1751 at Jekyll Island, Georgia (Beard 1973), but has since spread throughout the southern region of the USA. By the early 1900's it had become one of the most troublesome weed pests of cotton, alfalfa and other southern crops (Kneebone 1966). It was eventually used for forage because of its high growth rate and ability to maintain growth through the warm summer months when most other forage grasses temporarily decline. Its earliest forage use is not recorded, but its agronomical importance was highlighted by the release of 'Coastal', the first bermudagass forage cultivar, in 1943 (Burton 1943). In more recent times, however, the principle use of bermudagrass has been in the turfgrass industry. Although it has been planted in lawns and parade grounds since soon after its first introduction (Staten 1952), its first uses for golf and other sports turfs are not documented. The first turf bermudagrass breeding program was started in 1946 by Glenn Burton at the Coastal Plains Experiment Station in Tifton, Georgia (Burton 1991). As the golf and turfgrass industries expanded, so did bermudagrass use and importance. In 1990, the National Golf Foundation estimated the direct economic impact of golf course operations at over 5.5 billion dollars (NGF 1992). Since 1983, the United States Golf Association, USGA, reports that it has funded 215 bermudagrass improvement projects that total 21 million dollars (USGA 2002). Similarly, this project was designed to address three research interests related to bermudagrass. The central focus of this study was to construct the first genetic map of the bermudagrass genome using the single dose restriction fragment (SDRF) method (Wu et al 1992). In addition,

a detailed analysis of this map was conducted to answer questions about bermudagrass genome organization, including the nature of polyploidy. Finally, by comparing this map to the maps of other important grass species we could explore evolutionary relationships within the *Poaceae* family.

Bermudagrass is the common name used to refer to several species of the genus *Cynodon* (L.) Rich. The *Cynodon* Rich. genus is a member of the grass family (*Poaceae*), the subfamily *Chloridoideae*, the tribe *Cynodonteae* and the sub-tribe *Chloridinae* (Clayton and Renvoize 1986). Historically, there has been uncertainty regarding the number of species in the genus. A taxonomic revision of the *Cynodon* genus published in 1970 (Harlan et al. *a*) listed nine species and ten varieties. A more recent classification (Royal Botanic Gardens 1999) reduced the number of *Cynodon* species to eight by omitting *C. X magennisii* Hurcombe. The two species of most scientific, industrial and economic importance are *C. dactylon* and *C. transvaalensis*.

Bermudagrass is a resilient, perennial grass that is native to the warmer temperate and tropical regions of the world. The natural geographical distributions of individual members of the genus range from regions of south and east Africa and southeast Asia for more confined species, to cosmopolitan, being found in any area with climatic conditions conducive to its survival (Harlan et al. *a* 1970). It is best suited for fertile, loamy soils with pH between 6 and 7, and with adequate drainage, but is able to grow well in a wide range of edaphic conditions (Casler and Duncan 2003). It can also be bred to increase its salt tolerance (Dudeck et al 1983; Francois 1988). It uses the C4 photosynthetic pathway and is exceptionally tolerant of heat in comparison to other grasses. It is generally intolerant of shade (McBee and Holt 1966) and chilling temperatures (Dudeck and Peacock 1985; White and Schmidt 1989). In warmer climatic regions it is capable of maintaining active growth throughout the year, although growth is slowed

during the cooler months. In colder regions, however, where temperatures frequently reach 0$^{o}$C, foliage dies and regenerative tissues take on a dormant state until warmer temperatures return and new growth is initiated from crown buds, rhizomes and stolons (Beard 1973). It is very drought tolerant. The main attributes connected to its ability to tolerate drought conditions are root depth, root density and root biomass (Carrow 1996). The bermudagrass plant can have rhizomes below the soil surface, or 'prostrate' stolons above the surface, that may establish roots and shoots at any node, which contributes to its rapid growth potential and invasiveness. Under ideal conditions, seeded bermudagrass can spread to provide full coverage of 1000 ft$^2$ within four to six weeks after planting (CTAHR 1998). This low growing habit of bermudagrass also allows some cultivars to tolerate very close mowing. Substantial variation exists between species for a variety of physical characteristics, such as stem diameter, internode length, leaf length and width. As a result, hybrids can be produced and selected to generate a wide range of textures suitable for multiple uses.

Bermudagrass reproduces sexually, but can also be easily asexually propagated. Although flowers are perfect, bermudagrass is mostly cross-pollinated because of strong genetic self-incompatability. Extreme outcrossing and the inability to inbreed maintain heterozygosity in the genomes of vegetatively propagated lines. When desired, controlled crosses between plants can be done by hand emasculation and pollination (Richardson 1958) or other techniques (Burton 1965). Clonal propagation of non-seeded varieties can be done easily by planting rhizomes, stolons, crown buds, or any combination thereof.

Taliaferro et al (1997) estimated the average 2C nuclear DNA content of diploid, triploid and tetraploid bermudagrass species using flow cytometry to be 1.10, 1.60 and 2.25 picograms (pg) respectively. These estimates were supported by the findings of Arumuganathan et al

(1999). From the estimates of Taliaferro et al we can calculate the average 1X nuclear DNA content for bermudagrass species to be 0.547 pg. Dolezel et al (2003) offers a formula to convert DNA content in pg into genome size in base pairs (bp):

$$\text{DNA content (pg)} \quad X \quad 0.978 \quad X \quad 10^9 \quad = \quad \text{genome size (bp)}$$

This formula assumes that the ratio of AT to GC base pairs is 1:1, which is incorrect for plants since plants typically have genomes with higher GC contents. It also ignores the presence of modified nucleotides, which plants may potentially be rich in because of DNA methylation. However, Dolezel et al estimate that the error associated with applying this formula should be smaller than 1 % in any case. Therefore, using this formula, the average haploid (1X) genome size of bermudagrass plants is ~ 540 mega base pairs. Thus, in comparison, the bermudagrass genome is larger that that of *Arabidopsis* (~130 Mb) (Meyerowitz and Somerville 1994), and rice (~430 Mb) (Arumuganathan and Earle 1991), but orders of magnitude smaller than the genomes of humans (~3,000 Mb) (Venter et al. 2001) and wheat (~5,600 Mb) (Furata et al. 1986). Early studies of the bermudagrass genome suggested that it has a base chromosome number of x = 9 (Advulow 1931; Burton 1947; Darlington and Wylie 1956). This was confirmed by pachytene chromosome karyotypes (Ourecky 1963; Brillman 1982). Bermudagrass is most commonly tetraploid (Bogdon 1977), however, *Cynodon* plants can range from diploid to hexaploid, and have 2n chromosome numbers from 18 to 54 (Forbes and Burton 1963; Malik and Tripathi 1968; Harlan et al. *b* 1970; de Silva and Snaydon 1995). De Silva and Snaydon (1995) found some bermudagrass plants that appeared to be aneuploid (i.e. with chromosome numbers one or two less than the euploid number), but these may represent incorrect counts caused by overlapping chromosomes. Variation in chromosome number exists both interspecifically and intraspecifically (Table 1.1). De Silva and Snaydon demonstrated a cause-effect relationship

between intraspecific variation in chromosome number and ecological conditions by comparing different populations of *C. dactylon* collected from varying habitat types and climactic regions. From their findings, they concluded that bermudagrass ploidy level is related to soil pH ($p < 0.001$). Only diploid populations occurred in very acidic soils (pH < 5.0), while only tetraploid populations occurred in non-acidic soils (pH > 6.5). Diploid and tetraploid populations of *C. dactylon* were sympatric where soil pH was between 5.0 and 6.5 (de Silva and Snaydon 1995). Triploid bermudagrass plants are the result of intra- and interspecific hybridizations that can either occur naturally or be done artificially (Burton 1951; Harlan and de Wet 1969; Harlan et al. 1970 *b*). During meiosis, chromosomes form mainly bivalents (II) in diploids and tetraploids, but multivalents (III and IV) and univalents are also observed in tetraploids (Forbes and Burton 1963; Harlan et al. 1970 *b*). In the case of triploids, chromosome associations are far more complicated. Forbes and Burton (1963) studied chromosome behavior in one intraspecific and five interspecific triploid hybrids, including crosses of *C. dactylon* (4x) and *C. transvaalensis* (2x), and observed that meiosis was irregular in all six cases. Irregular meioses consisted of multivalent chromosomal associations, univalent chromosomes, sticky chromosomes or a combination of these factors. Harlan et al found that the chromosomes in 20 unique interspecific triploids tend to form nine bivalents (II) and nine univalents (I). They postulated that chromosomal structural differences between parents caused meiotic irregularity in triploid hybrids (Harlan et al 1970 *b*). In contrast, it is possible that the patterns of chromosome association observed at all ploidy levels can be explained by the 'natural' organization of the bermudagrass genome.

*Genetic Maps*

A linkage map of the entire genome of an organism provides a valuable foundation for understanding and manipulation of the organism's genome. Linkage maps, often referred to as genetic maps, have been constructed for many scientifically important and economically valuable organisms such as mice, fruit flies, chickens, pigs and humans among animals, and *Arabidopsis*, rice, sorghum, maize, wheat and cotton among plants. This study describes the first linkage map of the bermudagrass (*Cynodon* sp.) genome, an important tool for scientific research and bermuda turfgrass biotechnology.

"Genetic maps are the theoretical placement, based on experimental data, of markers along a conceptual linkage group" (Knox and Ellis 2002), where markers are morphological or molecular traits associated with genes, and a linkage group represents a chromosome, or a chromosomal segment. The first genetic map was done in 1913 to describe the linear order of six morphological markers on a segment of a *Drosophila* chromosome (Sturtevant 1913). Genetic maps have several theoretical and practical uses. One principal use of genetic maps is to track the transmission of genes by recording the passage of markers through subsequent generations, which provides a better understanding of heredity. Genetic maps can be used to shed light on the relationship between genes and traits. They also facilitate the study of morphological, physiological or developmental processes that are influenced by genetic variation (Arumuganathan & Earle 1991). While some genetic maps focus on a chromosomal segment or a single chromosome, most genetic maps are built with the purpose of assembling linkages for all of the organism's chromosomes. Whole genome maps can aid in determining various attributes including the nature and level of ploidy, genome size and the recombination rate of different regions of the genome. Also, as in the case of *Arabidopsis* and rice, detailed genetic maps

represent a useful step along the conventional path toward complete sequencing of the genome. Additionally, practical applications involving genetic maps offer significant advantages to some industries; in agriculture, for example, the maps of some important plants have several valuable uses, such as map based cloning and marker assisted selection

Map based cloning, or positional cloning, is the process of identifying the genetic basis of a particular phenotype by searching for molecular markers that co-segregate with the trait, and whose locations in the genome are known. It allows for isolation of favorable genes and their eventual transfer to organisms that lack the desired trait. Further, map based cloning on the basis of molecular markers permits the identification of important genes even when the corresponding phenotype is obscured by epistasis or masked by genotype-environmental interaction. Although later steps in the process of map based cloning may involve physical mapping and complementation, the critical first step entails the use of a genetic map to locate the gene of interest on a specific chromosomal segment. This strategy has been used to isolate a number of useful genes from important plants. For example, map based cloning was used to isolate the ABI1 gene in *Arabidopsis*, which is involved in abscisic acid sensitivity (Meyer et al 1994). In this case, 3 restriction fragment length polymorphism (RFLP) markers on the *Arabidopsis* genetic map were used to pinpoint the gene. This paper introduces the framework for a more detailed genetic map of the bermudagrass genome that may eventually be used in this strategy to isolate its valuable genes, like those for cold, heat and drought tolerance. The potential for map based cloning to drastically influence plant biotechnology through the isolation of more helpful genes further reinforces the usefulness and value of genetic maps.

For generations, crops have been improved via classical breeding, which involves selection of phenotypically elite individuals for use in subsequent mating cycles, encouraging the

passage of favorable traits and the creation of novel genotype combinations. While this approach has been very successful overall, some limitations to the strategy exist that make it inefficient and time consuming. Choosing superior individuals based on phenotype can be misleading because of epistasis, genotype-environmental interaction and measurement error. Also, since morphological traits are the main criteria, plants must be grown until such time as the trait selected for becomes apparent. DNA marker assisted selection has the potential to address these limitations of conventional breeding, enhancing the efficiency of, and accelerating crop improvement. In one example, molecular markers were used to successfully select for a bacterial blight resistance gene (*xa5*) in rice (Davierwala et al 2001). Like rice, detailed genetic maps including markers for a vast number of important characteristics have been constructed for most major crops, making them easily amenable to marker assisted selection. With increased saturation, the genetic map presented here can open the door to marker assisted selection of bermudagrass cultivars.

Comparative mapping has become an especially significant use of genetic maps. Over the past few decades, studies have revealed that the order of genes, in addition to their sequence, is conserved over long periods of time. The results of such inquiries also demonstrate that the genomes of related taxa may be differentiated on the basis of relatively few, rare chromosomal rearrangements. Comparative mapping refers to the analysis of these regions of conserved gene order based on the arrangement of a set of common (orthologous) DNA markers on the genetic maps of two or more different taxa. Colinearity in these regions can then be used to infer the evolutionary relatedness of the taxonomic groups included. An early comparative mapping study in plants looked for colinearity within the Solonaceae family by comparing the linkage maps of the tomato and potato genomes (Bonierbale et al 1988). However, the grass family

(Poaceae) has been the subject of far more comparative mapping studies than any other plant family to date, and has led to the identification of a set of well conserved anchor-probes for use in grass mapping (Van Deynze et al 1998) and encouraged the selection of rice as a model genome for the grasses (Feuillet and Keller 2002; Devos and Gale 2000). A linkage map of the bermudagrass genome is a beneficial new tool for inclusion in grass genome comparisons. *Cynodon* is a member of the small subfamily Chloridoideae, within the much larger Poaceae family. As a result of bermudagrass's relative position in the phylogeny of the grasses (Kellogg 1998; Fig. 1.1), it is assumed that the map of its genome can be compared to the maps of sorghum and maize to resolve more internal branches within the Panicoideae, Arundinoideae, Centothecoideae and Chloridoideae (PACC) clade. Also, it is anticipated that this map can be used as an intermediate in comparative studies to bridge the gap between rice and its more distant relatives.

*Genetic Mapping of Polyploids*

In comparison to diploid species, genetic mapping of polyploids is appreciably more difficult. Three major obstacles hinder the progress of linkage mapping in polyploid (Wu et al 1992). First, having multiple genomes drastically increases the number of possible genotypes at a single ancestral locus. Second, for mapping approaches that rely on agarose gel electrophoresis (e.g. RFLP), the co-migration of fragments of similar size makes it very difficult, and sometimes impossible to distinguish the several genotypes at each ancestral locus. Third, allopolyploids and autopolyploids have distinctly different patterns of inheritance, which substantially influences which mapping approach is ideal. The genome constitution (nature of ploidy) of many polyploids is unclear. These three complexities impose limitations on conventional mapping strategies and must be overcome in order for genetic mapping of polyploids to proceed.

In allopolyploids, multiple divergent genomes occupy the same nucleus, with strict disomic pairing of chromosomes at meiosis (e.g. Paterson et al 2000). As a result of the fidelity of meiotic pairing of homologous chromosomes in allopolyploids, their genetics is very similar to diploids, except for the presence of multiple genomes. On this basis, linkage maps for the genomes of wheat (Kam-Morgan et al 1989) and cotton (Rong et al 2004) have been constructed.

Single dose restriction fragment (SDRF) mapping is a strategy that addresses the difficulties of mapping polyploids. An SDRF is a restriction fragment that is only present as a single copy in one parent. One selects SDRF's on the basis of the one to one (1:1) ratio for presence or absence that is expected in the progeny. This way, the genetics of polyploids is simplified to resemble that of diploids so that mapping can proceed efficiently. Since the map reflects segregation and recombination in each parent separately, it is never necessary to identify the corresponding allele/fragment in the other parent. Together, these features of SDRF mapping eliminate the complexities owing to the number of possible genotypes, and the co-migration of fragments. In principle, the SDRF method is amenable to mapping of auto- and allopolyploids, however in allopolyploids it would sacrifice valuable information that can easily be obtained by identifying allelic restriction fragments. SDRF mapping allows the detection of both coupling and repulsion phase linkages, which can lead to the elucidation of the nature of ploidy (genome constitution) of many species. Finally, a single DNA probe has the potential to yield multiple SDRF's, which serve as a mechanism to group homologous chromosomes.

The single dose restriction fragment approach has been successfully applied to genetic mapping of sugarcane (da Silva et al 1993) and strawberry, *Fragaria* sp. (Lerceteau-Kohler et al 2003), and here we present a linkage map of the bermudagrass genome (*Cynodon* sp.) constructed using this technique.

This thesis is arranged as follows. The first chapter gives important background information on the goal of the project, the species, and the strategy. The second chapter is a manuscript to be submitted to the journal "Theoretical and Applied Genetics", which describes the map itself. The final chapter is an overall discussion of the project and its findings.

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Table 1.1. A revised classification of the *Cynodon* genus. (After Harlan et al. 1970). [a] Species of importance to this study.

| Epithet | Chromosome number |
|---|---|
| *Cynodon aethiopicus* Clayton et Harlan | 18, 36 |
| *Cynodon arcuatus* J. S. Presl ex C. B. Presl | 36 |
| *Cynodon barberi* Rang. et Tad. | 18 |
| *Cynodon dactylon* (L.) Pers. | |
| var. *dactylon*[a] | 36 |
| var. *afghanicus* 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 |
| *Cynodon incompletus* Nees | |
| var. *incompletus* | 18 |
| var. *hirsutus* (Stent) de Wet et Harlan | 18, 36 |
| *Cynodon nlemfuensis* Vanderyst | |
| var. *nlemfuensis* | 18, 36 |
| var. *robustus* Clayton et Harlan | 18, 36 |
| *Cynodon plectostachus* (K. Shum.) Pilger | 18 |
| *Cynodon transvaalensis* Burtt-Davy[a] | 18 |
| *Cynodon x magennisii* Hurcombe | 27 |

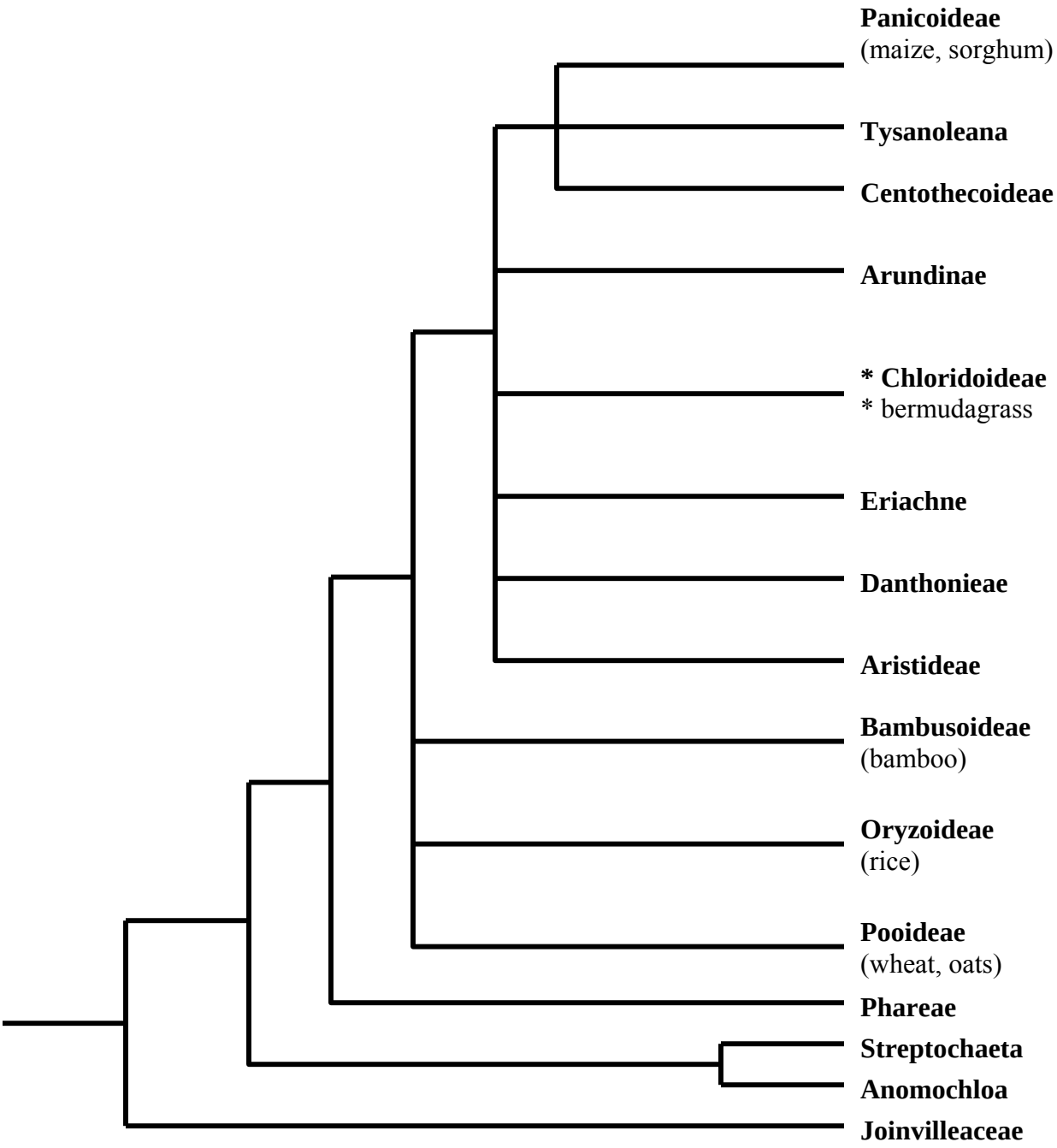


Figure 1.1 A phylogenetic map of the grass (*Poaceae*) family (derived from Kellogg 1998). Based on a summary of the comparative data, both morphological and molecular, available for the grass family. Bermuda grass and its subfamily (*Chloridoideae*) are highlighted by asterisks.

# CHAPTER 2

## A FRAMEWORK LINKAGE MAP OF BERMUDAGRASS (*CYNODON DACTYLON* X *TRANSVAALENSIS*) BASED ON SINGLE-DOSE RESTRICTION FRAGMENTS

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**Abstract**

This study describes the first linkage maps of two bermudagrass species, *Cynodon dactylon* (T89) and *Cynodon transvaalensis* (T574), based on single-dose restriction fragments (SDRFs). The mapping population consisted of 113 F1 progeny of a cross between the two parents. Loci were generated using 179 bermudagrass genomic clones and 50 heterologous cDNAs from *Pennisetum* and rice. The map of T89 is based on 155 SDRFs and 17 double-dose restriction fragments on 35 linkage groups, with an average marker spacing of 15.5 cM. The map of T574 is based on 77 SDRF loci on 18 linkage groups with an average marker spacing of 17.2 cM. Sixteen T89 linkage groups were arranged into 4 complete and 8 into 4 incomplete homologous sets, while 15 T574 linkage groups were arranged into 7 complete homologous sets, all on the basis of multi-locus probes and repulsion linkages. Eleven T89, and 3 T574 linkage groups remain un-assigned. In each parent, consensus maps were built based on alignments of homologous linkage groups. Four ancestral chromosomes were inferred after aligning T89 and T574 parental consensus maps using multi-locus probes. The inferred ancestral marker orders were used in comparisons to a detailed *Sorghum* linkage map using 40 common probes, and to the rice genome sequence using 98 significant BLAST hits, to find regions of colinearity. Using these maps we have estimated the recombinational length of the T89 and T574 genomes at 5419.5 and 2637 cM respectively, which are 38% and 39% covered by our maps.

**Introduction**

Bermudagrass (*Cynodon* sp.) is a resilient, perennial grass native to the warmer temperate and tropical regions of the world that is found throughout the southern United States. Its many uses are directly related to the favorable traits it possesses. For instance, it is used for

forage because of its ability to maintain active growth through the warm summer months when most other forage grasses temporarily decline. Also, it is widely used in landscaping because of its ability to grow well in a wide range of soil conditions (Caslet and Duncan 2003) and its high growth rate and extreme invasiveness. Seeded bermudagrass can spread to provide full coverage of 1000 $ft^2$ within four to six weeks after planting (CTAHR 1998). More recently however, bermudagrass's principle use has been in the golf and turfgrass industries, owing to its ability to generate a variety of textures, rapid recovery, and its low growing nature, which allows it to tolerate very close mowing.

*Cynodon* species are members of the *Cynodonteae* tribe and the *Chloridoideae* sub-family, within the grass (*Poaceae*) family (Clayton and Renvoize 1986). The grasses are among the better studied plant families at the level of comparative mapping (Feuillet and Keller 2002). A genetic map of the bermudagrass genome is a beneficial new tool for inclusion in grass genome comparisons. Based on its location in the phylogeny of the grasses (Kellogg 1998), it is anticipated that bermudagrass can be used as an intermediate in comparative studies to bridge the gap between important agronomic crops like sorghum, maize and rice, and their more distant relatives. Comparisons to sorghum and maize individually should also resolve more internal branches of the PACC clade.

Bermudagrass species have an average 1X nuclear DNA content of 0.547 pg (derived from Taliaferro et al 1997) equating to a 1X genome size of ~ 540 Mbp. They have a base chromosome number of 9 (Advulow 1931; Burton 1947; Darlington and Wylie 1956), and are mostly tetraploid (Bogdon 1977), but can range from diploid to hexaploid (Forbes and Burton 1963; Malik and Tripathi 1968). Triploid plants are the natural or synthetic products of intra-

and interspecific hybridizations of diploid and tetraploid species (Burton 1951; Harlan and de Wet 1969), and are widely used as turf genotypes.

In comparison to diploids, genetic mapping of polyploids has advanced at a much slower pace due to major obstacles, including more possible genotypes as a result of multiple genomes, the co-migration of similar size fragments during electrophoresis, and the complexity of unclear genome constitution (auto vs. allo) for many species, hinder the progress of mapping in polyploids (Wu et al 1992). Single-dose restriction fragment (SDRF) mapping addresses these difficulties. The strategy evaluates the presence or absence of a restriction fragment that is only present in one parent, and in the progeny at a 1:1 ratio. In this way, the genetics is similar to that of diploids, and mapping can proceed efficiently. Also, SDRF mapping permits detection of both coupling and repulsion phase linkages, which can lead to the clarification of the genome constitution of many species. Finally, the multiple segregating loci that may be produced from a single probe serve as a means to group homologous chromosomes. This approach has been successfully applied to genetic mapping of sugarcane (da Silva et al 1993) and strawberry (*Fragaria* sp.; Lercteau-Kholer et al 2003), and here we present a linkage map of the bermudagrass genome (*Cynodon* sp.) constructed using this technique.

## Materials and Methods

### *Mapping population*

The mapping population consisted of 113 progeny of a synthetic cross of T89 (*C. dactylon*, 4x) and T574 (*C. transvaalensis*, 2x). Similar crosses between other members of these two species have led to the production of numerous leading bermudagrass cultivars, including Tifway and Tifgreen. Triploid (2n = 3x = 27) F1 progeny were used for mapping, based on

heterozygosity within each parent. The cross was done by Wayne Hanna, and the progeny are maintained in the field at the USDA Coastal Plains Experiment Station, in Tifton, GA.

*RFLP probes*

Four sets of probes, either bermudagrass-derived or heterologous, were used in this mapping experiment. Probes labeled T574 were hypo-methylated (*Pst*-I digested) genomic clones made from *Cynodon transvaalensis* parental DNA following the protocol detailed by Chittenden (1994), except that fragments were isolated from a 0.8 % agarose gel using the Geneclean Kit (Bio-101). PCD probes were hypo-methylated (*Pst*-I digested) genomic clones from *Cynodon dactylon* cultivar 'Arizona Common'. pPAP probes were cDNA clones isolated from apomictic pistils of *Pennisetum ciliare* for use in a previous map (Jessup et al 2003), and RZ probes were cDNA clones from *Oryza sativa* cultivar IR36 (Causse et al 1994).

*Laboratory procedures*

The molecular methods used are as previously described by Chittenden et al (1994), with minor adjustments. Young leaf tissue was harvested from both parents and each of 113 progeny, lyophilized, ground in a tissue mill, and stored at – 80 $^{o}$C until further use. DNA was extracted from each, following the protocol for CTAB extraction outlined by Chittenden et al (1994). Approximately 5 μg of DNA from each sample was digested with either *Eco*R1 or *Hind*III, and run overnight at 22 V in a 1% agarose gel in 1X TAE buffer. Gels were then blotted onto Hybond N+ (Amersham), using 0.4N NaOH, overnight, after which blots were rinsed in 2X SSC and stored at 4 $^{o}$C until use. About 20 – 50 ng of each probe was labeled with [$^{32}$P]dCTP and hybridized to a blot. Following this the blots were washed of excess probe, exposed to X-ray film, and the films were developed.

*Identification of SDRFs and linkage analysis*

The presence or absence of each polymorphic fragment was visually determined and recorded for each individual in the mapping population. SDRF mapping necessitates building separate maps for each of the parental genomes. Therefore, the parental origin of each polymorphic fragment was ascertained based on comparison to parental DNA and the scoring convention adjusted so that loci from the two parents could be separated. Next, a 1:1 ratio of presence or absence in the progeny was verified for each polymorphic fragment by a chi-squared test with 99 % confidence. Coupling linkage was analyzed separately for loci from each parent using MAPMAKER/EXP v. 3.0b, with error detection. Map distances, in centiMorgans (cM) were calculated using the Kosambi function. Initial linkage groups were constructed using the 'group' command with a LOD score of 4.0 and a recombination fraction of 0.4 as the linkage thresholds. Previously unlinked markers were individually added to linkage groups at the most likely interval using the 'try' command with a threshold of LOD $\geq$ 2.0. Local maximum likelihood orders of markers were confirmed using the 'ripple' command.

To find repulsion linkages, a new data set was generated for each parent by inverting the map scores for each marker (Al-Janabi et al 1994; Grivet et al 1996; Ming et al 1998). Then, in each case, the two data sets were combined and analyzed in MAPMAKER/EXP v. 3.0b.

*Double Dose Restriction Fragment (DDRF) Mapping in T89*

Forty-four RFLP loci were identified as DDRF's by a chi-squared test to confirm a 5:1 segregation ratio in the progeny, as described by Ripol et al (1999). These loci were tested for asymmetric coupling linkage against the T89 SDRFs using the method described by Ripol et al. These loci were also tested for repulsion linkage to T89 SDRF loci, also as described by Ripol et al (1999).

*Estimated recombinational length and percent genome coverage*

The approximate length of each parental genome was estimated using the method-of-moment formula (Hulbert et al 1988), as modified in method 3 of Chakravarti et al (1991).

$E(G) = [n(n-1)2d]/2k$ ; where

E (G) represents the estimated genome length in centiMorgans, n equals the number of markers on the map, d equals the largest distance between two loci at a given LOD score, and k equals the actual number of pairs of loci linked at the specified LOD score or greater.

The percent of genome coverage, $E(C_n)$ was estimated using the method of Bishop et al (1983).

$E(C_n) = 1 - P_{1,n}$ ; where

$P_{1,n} = (2r / n + 1) * [(1 - d / 2g)^{n+1} - (1 - d / g)^{n+1}] + (1 - r d / g)(1 - d / g)^n$

In this case, r is the number of linkage groups, d is the maximum distance used to detect linkage at the same preset LOD score, and g is the estimated genome length in cM.

*Homology between linkage groups*

Wherever possible, linkage groups were assigned to homologous sets on the basis of 32 shared multi-locus probes and on shared repulsion linkages. Homologous groups were then merged to infer a consensus for each homologous group.

*Inferred Ancestral Chromosomes*

Inferred ancestral chromosomes were constructed by comparing the consensus groups and un-assigned linkage groups of the two parents, aligning them on the basis of multi-locus probes, and interleaving markers specific to any one group based on relative recombinational distances.

*Comparative Mapping*

Bermudagrass-rice

The sequences of marker generating probes from both maps were BLASTed against the TIGR rice pseudo-molecule v. 2.0 (http://www.tigr.org) at a significance threshold $\leq 1e^{-10}$. Colinearity was inferred by comparing the chromosomal locations of the best rice hits to the orders of the corresponding markers on the inferred *Cynodon* ancestral chromosomes.

Bermudagrass-*Sorghum*

Eighty-three of the probes used in this study were also applied to the *Sorghum* map of Bowers et al (2003), and consequently 67 new markers were added to the already dense genetic map. Twenty-eight of these markers also mapped in bermudagrass, and were added to the 12 common markers that already existed on the *Sorghum* map. On this basis, the *Sorghum* genetic map was compared to the inferred *Cynodon* ancestral chromosomes to find regions of conserved marker order.

*Mode of inheritance*

The ratio of SDRF coupling to repulsion linkages was calculated for each testable linkage group for both genomes. Linkage groups for which no repulsion linkages were found were considered non-testable and not included. A chi-squared test with 95% confidence was used to determine if the calculated ratios fit the 1:1 or 0.25:1 models expected for disomic and polysomic inheritance respectively.

**Results**

*Marker screening and mapping*

A total of 666 probes were screened, of which 330 detected at least one polymorphic fragment in the parents, and 229 produced at least one SDRF that was mapped to a bermudagrass

linkage group. This data is summarized in Table 2.1. A total of 440 segregating loci were scored, but the chi-squared test confirmed only 299 as SDRF's.

*Map construction*

T89 - *Cynodon dactylon* (2n = 4x = 36)

Construction of the map for T89 was based on the analysis of 202 SDRF loci. The map is composed of 35 linkage groups, with 16 organized into 4 complete homologous groups where all 4 chromosomes are represented, 8 into 4 incomplete homologous groups with only two chromosomes, and 11 un-assigned. The map covers a total of 1978 cM, with 155 markers separated by an average distance of 15.5 cM (Fig. 2.1). Forty-seven markers remain unlinked. On average, linkage groups are defined by about 4 markers and cover ~ 52.5 cM (Table 2.2).

By applying the method of Ripol et al (1999), we analyzed 44 candidate double-dose fragments, and were able to find linkages involving 17 (39%) of them. In principle, these detect 34 segregating loci (by definition), however we could only link 31 of them to SDRF markers. Twenty-five of the 31 were already on our linkage groups, and the remaining 6 were previously unlinked. Since this approach does not allow for the calculation of map distances, or the identification of specific map locations for the DDRF's, these loci were super-imposed onto the SDRF maps, in alignment with the SDRF to which they showed the tightest linkage (Fig. 2.1). In addition, these DDRF loci were involved in numerous repulsion linkages that are also shown in Fig. 2.1.

T574 – *Cynodon transvaalensis* (2n = 2x = 18)

The T574 genetic map was constructed on the basis of 97 SDRF loci. It is composed of 18 linkage groups, with 15 of them organized into 8 homologous groups and only 1 group un-assigned. The map covers a total of 973.4 cM with 77 markers separated by an average of 17.2

cM (Fig. 2.2). Twenty markers remain un-linked. On average, linkage groups are defined by about 4 markers and cover ~ 54 cM (Table 2.2).

*Estimated recombinational length and percent genome coverage*

For each parent, the LOD scores used for recombinational length and genome coverage estimates were consistent with the LOD score threshold used for map construction. The total recombinational length of the *Cynodon dactylon* genome was estimated to be 5419.5 cM, which is ~39 % covered by the map of T89. Meanwhile, the *Cynodon transvaalensis* genome was estimated to be 2637 cM, which is ~38 % covered by the map of T574. The estimated recombinational length of the T89 genome is about twice that of T574, which is consistent with their ploidy levels. Directly relating the estimated genome sizes to actual centiMorgrans in the maps gives coverage estimates of 34% and 37% for T89 and T574 respectively.

*Inferred Ancestral Chromosomes*

After constructing consensus groups of homology sets in each parent, in three cases T89 consensus groups were aligned with T574 consensus groups, and in one case a T89 consensus group was aligned with the un-assigned T574 linkage group 9, on the basis of shared multi-locus probes. Markers on each pair of groups were interleaved to construct 4 inferred *Cynodon* ancestral chromosomes (Fig. 2.3).

*Comparative mapping*

Bermudagrass - rice

The sequences of 71 of the probes that generated markers on the T89 map, and 36 from the T574 map produced 98 significant hits when BLASTed against the rice genome sequence data. With these hits, inferred *Cynodon* ancestral chromosomes and homology consensus groups were compared to the rice genome. In 3 separate comparisons inferred *Cynodon* ancestral

chromosomes shared at least 3 significant BLAST hits with regions of rice chromosomes 1, 2 and 9 (Fig. 2.3). In another comparison, an inferred ancestral chromosome from an alignment of T89 consensus group 4 and T574 consensus 1, shared 3 significant hits each to regions of rice chromosomes 1 and 7 (Fig. 2.3). Further, using the numbered base pair location of the 50 Kb block of rice sequence that gave each hit, it was possible to determine that in 4 out of 5 comparisons at least 3 of the significant hits between bermudagrass and rice showed conserved order in the two genomes (Fig. 2.3).

Bermudagrass – *Sorghum*

The bermudagrass-*Sorghum* comparison relied on 40 common probes. An inferred *Cynodon* ancestral chromosome from the alignment of T89 consensus group 3 and T574 consensus group 3 showed significant colinearity with *Sorghum* linkage group A (Fig. 2.3; Bowers 2003). Also, in separate comparisons the 3 other inferred ancestral chromosomes shared 2 common probes each with *Sorghum* linkage groups A, B and D (Table 2.3). The *Sorghum* comparisons relied on substantially less data points than the bermudagrass-rice, and only produced one alignment of a bermudagrass and a *Sorghum* linkage group with at least 3 conserved loci, yet this finding is noteworthy because it demonstrates the potential for comparisons using a more saturated bermudagrass map to detect more regions of colinearity between the two genomes.

*Mode of inheritance*

Twenty-four T89 and 17 T574 linkage groups were testable for mode of inheritance using the ratio of coupling to repulsion phase linkages. The ratio was exactly 1:1, or not significantly different by Chi-squared analysis, for all linkage groups tested, suggesting that both T89 and T574 follow disomic inheritance that is characteristic of diploids and allopolyploids.

**Discussion**

We report an early investigation of chromosomal transmission in bermudagrass, and present the first genetic maps of the *Cynodon dactylon* and *Cynodon transvaalensis* genomes. With approximately 39% and 38% estimated genome coverage, and 15.5 cM and 17.2 cM average marker spacing, these maps are still incipient, but represent the foundation for construction of more saturated maps. Moreover, in the case of T574, the genetic map is organized into 8 of the 9 expected homologous groups, suggesting that we have partial coverage of most *Cynodon transvaalensis* chromosomes. At the same time, although three linkage groups make up T574 homologous group 3, it is possible that linkage groups 7a-1 and 7a-2 represent two regions of the same chromosome that are linked by a segment for which we lack DNA markers. This is based on the observation that 7a-1 and 7a-2 align to separate ends of linkage group 7b (Fig. 2.2). Therefore, improving map density in this region may cause the two linkage groups to join, and T574 homologous group 3 may be reduced to two linkage groups, as expected. In the case of T89, the genetic map includes 35 of the 36 expected linkage groups. However, since some markers remained unlinked to the map, we cannot yet assume that we have partial coverage of all except one of the *Cynodon dactylon* chromosomes. Also, in constructing the map of T89, we detected repulsion linkages that involved single unlinked markers. Presumably, as marker density increases, these markers will adjoin to our linkage groups and allow us to complete the homology sets.

Regardless of map density or genome coverage, we have demonstrated the usefulness of bermudagrass genetic maps for comparative analysis with other grasses. Notably, comparisons conducted here using our framework map produce significant hits to regions of all 12 rice chromosomes, and common probes with regions of all 10 *Sorghum* linkage groups (Table 2.3).

The results from our comparative analyses were able to validate predictions of homology between linkage groups. Fifteen cases exist where separate linkage groups in the same homology set share significant hits to the same rice chromosome, and 6 cases where they share common probes with the same *Sorghum* linkage group (Table 2.3). For instance, T574 3a and T574 3b each share 3 significant hits to rice chromosome 1 and *Sorghum* linkage group A. Further, the results of the independent comparisons of bermudagrass to rice and *Sorghum*, when taken together, are consistent with Paterson et al (2004) where *Sorghum* and rice were compared directly. Our findings here underscore the importance and potential of bermudagrass linkage maps in contributing to evolutionary studies of the grass family.

Finally, for genomes of uncertain constitution, such as bermudagrass, the ratio of coupling and repulsion linkages found in SDRF mapping can be used to determine auto versus allopolyploidy. A 1:1 ratio of coupling to repulsion linkages for the markers on each linkage group would predict disomic inheritance characteristic of diploids and allotetraploids, while a ratio of 0.25: 1 would indicate polysomic inheritance of an autotetraploid (Wu et al 1992). In T574, the ratio for all 17 testable linkage groups is not significantly different from 1:1, which is expected for a diploid species, and validates the ability of this test to correctly predict genome constitution. However, in T89, the ratios of coupling to repulsion linkages for all testable linkage groups are also 1:1, or not significantly different from it. This would suggest that all of the T89 chromosomes that are represented in the current map follow the disomic behavior that is expected for an allotetraploid, which is contrary to the expected results. First, such results conflict with the findings of Forbes and Burton (1963), upon whose observation of multivalent chromosomes during meiosis, we classify bermudagrass as mainly an autotetraploid. Further, this level of disomic inheritance directly conflicts with the number of double-dose restriction

fragments present initially in our mapping population because two copies of the same fragment on the genomes of an allopolyploid would yield no polymorphism and no scoreable segregation in the progeny. Though these results remain anomalous, we offer two possible explanations. First, 8 of the 24 testable T89 linkage groups were members of partial homologous sets that included only 2 of the 4 expected members were assigned, which resembles a diploid state. Since repulsion phase linkages exist between chromosomes of the same homologous set, this caused a decrease in the number of repulsion linkages that we could detect in these 8 cases, and could be responsible for keeping ratio of coupling to repulsion linkages at 1:1. Therefore, as more homologous groups are completed, we will be able to more adequately compare the ratio of coupling to repulsion linkages and therefore distinguish the true nature of bermudagrass polyploidy. This does not affect the members of the 4 complete homologous sets, however. On the other hand, the size of the mapping population affects the ability of the SDRF strategy to detect repulsion linkages in all cases (Wu et al 1992). Applying this strategy in an auto-tetraploid using a mapping population of 113, only allowed us to detect repulsion linkages where the recombination fraction was 0.15 or less (Wu et al 1992), while the threshold recombination fraction for coupling linkage detection in this study was 0.45. This further explains why the ratio of coupling to repulsion linkage was 1:1, instead of 0.25:1. With a much larger mapping population we would be able to detect a higher number of repulsion linkages in comparison to coupling, and approach the ratio that expected of auto-tetraploids.

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Table 2.1. Summary of probes used in this study. 'Successful ' refers to probes that generated at least one SDRF locus mapped on a linkage group in either parent.

| | | | | | Mapped SDRF Loci | |
|---|---|---|---|---|---|---|
| Probes | Source | Screened | Polymorphic | Successful | T89 | T574 |
| T574 | *C. transvaalensis* | 298 | 155 | 143 | 92 | 53 |
| pCD | *C. dactylon* (A.C.) | 121 | 51 | 36 | 26 | 10 |
| pPAP | *Pennisetum ciliare* | 122 | 53 | 15 | 9 | 5 |
| RZ | *Oryza sativa* | 125 | 71 | 35 | 28 | 9 |
| Total | | 666 | 330 | 229 | 155 | 77 |

Table 2.2 Summary of statistics for the T89 and T574 genetic maps. Map lengths are in cM.

| | Length | | # of loci | | cM / pair | |
|---|---|---|---|---|---|---|
| | T89 | T574 | T89 | T574 | T89 | T574 |
| Total | 1978 | 973.4 | 155 | 77 | | |
| Average | 52.5 | 54 | 4.4 | 4.3 | 15.5 | 17.2 |
| Largest group | 191.2 | 149 | 12 | 7 | 17.4 | 24.8 |
| Unlinked | | | 47 | 20 | | |

Table 2.3. Bermudagrass-rice and bermudagrass-*Sorghum* genome comparisons.

| Marker | Bermudagrass Lg. | Rice Chr. | *Sorghum* Lg. |
|---|---|---|---|
| RZ476b | T89 1a | 6 | I |
| RZ242 | T89 1a | 6 | |
| PAP08B06 | T89 1b | 6 | I |
| PCD022 | T89 1b | 6 | |
| RZ242 | T89 1b | 6 | |
| RZ588 | T89 1b | 6 | |
| T5746C08 | T89 1b | 6 | |
| T5748F09 | T89 1b | | B |
| PCD008 | T89 1c | 3 | |
| RZ142 | T89 1c | 3 | |
| RZ575 | T89 1c | 3 | |
| T5745C11 | T89 1c | 6 | |
| RZ142 | T89 1d | 3 | |
| T5741C07 | T89 1d | 3 | |
| RZ497 | T89 1d | 5 | C |
| PAP04A06 | T89 1d | | G |
| RZ596a | T89 2a | 9 | B |
| T5741A04 | T89 2a | | B |
| PCD083 | T89 2b | 2 | I |
| PAP07B10 | T89 2b | 9 | |
| RZ912 | T89 2c | 3 | C |
| RZ912 | T89 2c | 3 | |
| T5745B09 | T89 2d | 10 | |
| RZ460 | T89 3a | 1 | |
| RZ460 | T89 3b | 1 | |
| RZ717 | T89 3b | 4 | D |
| RZ717 | T89 3b | 4 | F |
| RZ717 | T89 3b | 4 | |
| PAP07C04 | T89 3c | 1 | A |
| RZ538 | T89 3c | 1 | A |
| RZ444 | T89 3c | 1 | |
| RZ460 | T89 3c | 1 | |
| T5741A07 | T89 3c | 2 | |
| T5743C03 | T89 3c | | F |
| T5746B12 | T89 4a | 1 | |
| RZ740 | T89 4a | 4 | D |
| RZ740 | T89 4a | 4 | |
| RZ460 | T89 4b | 1 | |
| T5746B12 | T89 4b | 1 | |
| T5748G05 | T89 4b | 4 | |
| T5741E07 | T89 4c | 5 | |
| PCD012 | T89 4c | 7 | |
| PCD131 | T89 4c | 7 | |

Table 2.3 (continued). Bermudagrass-rice and bermudagrass-*Sorghum* genome comparisons.

| Marker | Bermudagrass Lg. | Rice Chr. | *Sorghum* Lg. |
|---|---|---|---|
| PCD132 | T89 4c | 7 | |
| T5742D09 | T89 4c | 7 | |
| T5743B03 | T89 4c | 11 | B |
| T5742F09 | T89 4c | | D |
| T5742F09 | T89 4c | | B |
| T5748G02 | T89 4d | 7 | |
| T5741A10 | T89 5a | 2 | |
| T5742C08 | T89 5a | 2 | |
| PAP03E08 | T89 5a | 3 | |
| T5741A08 | T89 5b | 2 | |
| T5742C08 | T89 5b | 2 | |
| PAP07H09 | T89 6a | 8 | C |
| T5746B02 | T89 6a | 8 | |
| T5746E01 | T89 6a | 8 | |
| PAP07H09 | T89 6a | | J |
| T5745F06 | T89 6b | | J |
| RZ545 | T89 7a | 3 | |
| RZ399 | T89 7b | 3 | C |
| RZ900 | T89 7b | 11 | H |
| RZ900 | T89 7b | 11 | |
| PAP06A07 | T89 8a | 2 | |
| PCD095 | T89 8a | 2 | |
| T5741A08 | T89 8a | 2 | |
| T5745F10 | T89 8a | 3 | |
| PCD057 | T89 8a | 10 | |
| RZ446a | T89 8a | | A |
| RZ446b | T89 8b | 2 | A |
| T5741A08 | T89 8b | 2 | |
| T5745F10 | T89 8b | 3 | |
| RZ261c | T89 9 | 12 | E |
| RZ557c | T89 10 | 11 | H |
| T5742F04 | T89 10 | 12 | E |
| RZ557b | T89 11 | 11 | H |
| PAP02D11 | T89 12 | 1 | A |
| RZ543 | T89 12 | 1 | |
| T5746E10 | T89 12 | 1 | |
| T5741H06 | T89 14 | 8 | |
| T5746E01 | T89 14 | 8 | |
| PCD032 | T89 14 | 9 | |
| PAP10A04 | T89 15 | 2 | F |
| PCD001 | T89 15 | 2 | |
| RZ288 | T89 16 | 1 | |
| RZ543 | T89 16 | 1 | |

Table 2.3 (continued). Bermudagrass-rice and bermudagrass-*Sorghum* genome comparisons.

| Marker | Bermudagrass Lg. | Rice Chr. | *Sorghum* Lg. |
|---|---|---|---|
| T5742A10 | T89 16 | 2 | |
| RZ390 | T89 17 | 5 | |
| PCD132 | T89 18 | 7 | |
| PCD142 | T89 18 | 7 | |
| T5743B03 | T89 19 | | B |
| T5741C03 | T574 1a | 7 | |
| PCD030 | T574 1b | 1 | |
| PCD131 | T574 1b | 7 | |
| PCD132 | T574 1b | 7 | |
| T5742F09 | T574 1b | | B |
| T5742F09 | T574 1b | | D |
| T5743G06 | T574 2a | 4 | |
| T5742G09 | T574 2b | 4 | |
| PAP07C04 | T574 3a | 1 | A |
| T5743D06 | T574 3a | 1 | A |
| T5743C03 | T574 3a | | F |
| T5743D06 | T574 3b | 1 | A |
| T5741H06 | T574 4a | 8 | |
| PCD032 | T574 4a | 9 | |
| PAP05H08 | T574 5a | 6 | |
| PAP10A04 | T574 5b | 2 | F |
| T5741D11 | T574 5b | 2 | |
| RZ574 | T574 6a | 3 | |
| T5741D12 | T574 6a | 3 | |
| T5745B01 | T574 6a | 3 | |
| RZ455 | T574 7a-1 | 5 | |
| RZ995 | T574 7a-2 | 1 | A |
| RZ995 | T574 7a-2 | 1 | C |
| RZ995 | T574 7a-2 | 1 | |
| RZ324 | T574 7a-2 | 2 | |
| RZ401 | T574 7a-2 | 2 | |
| T5741A08 | T574 7a-2 | 2 | |
| T5745F10 | T574 7a-2 | 3 | |
| T5748F06 | T574 7a-2 | 7 | |
| RZ401 | T574 7b | 2 | |
| T5741A08 | T574 7b | 2 | |
| T5745F10 | T574 7b | 3 | |
| PAP07G03 | T574 7b | 5 | |
| T5743B10 | T574 7b | 5 | |
| T5743B03 | T574 8 | 11 | B |
| RZ103 | T574 9 | 2 | |
| RZ140 | T574 9 | 6 | |
| RZ596c | T574 9 | 9 | B |

Figure 2.1. Linkage maps of *Cynodon dactylon* (4x=36) linkage groups. Map distances in centiMorgans (cM). Markers in red are DDRF loci super-imposed on the SDRF frame-work map. Where possible, homologous groups are aligned on the basis of multi-locus probes (solid lines), and repulsion repulsion linkages (dashed lines) to infer a consensus. In both cases, red lines involve DDRF loci.

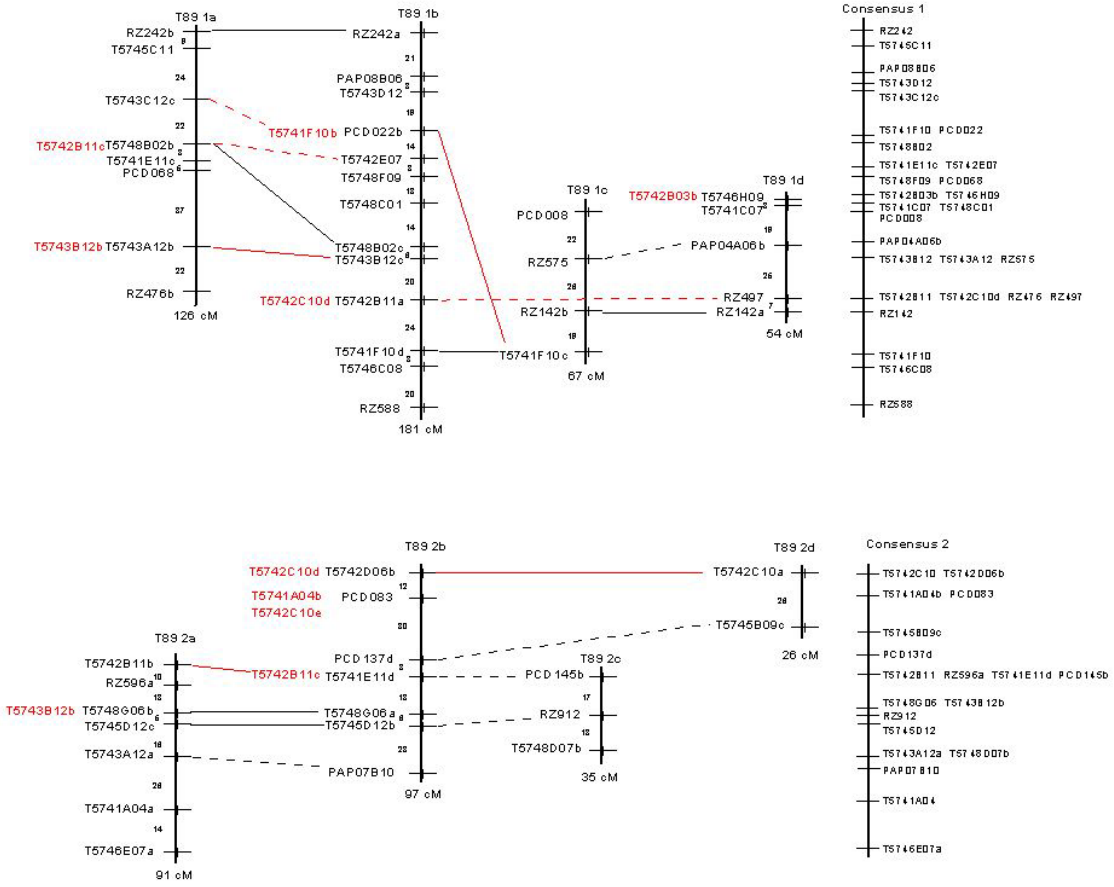


Figure 2.1 (continued). Linkage maps of *Cynodon dactylon* (4x = 36) linkage groups. (Shown is linkage between DDRFs PCD065b and T5748G02a and unlinked SDRF T5741E07b).

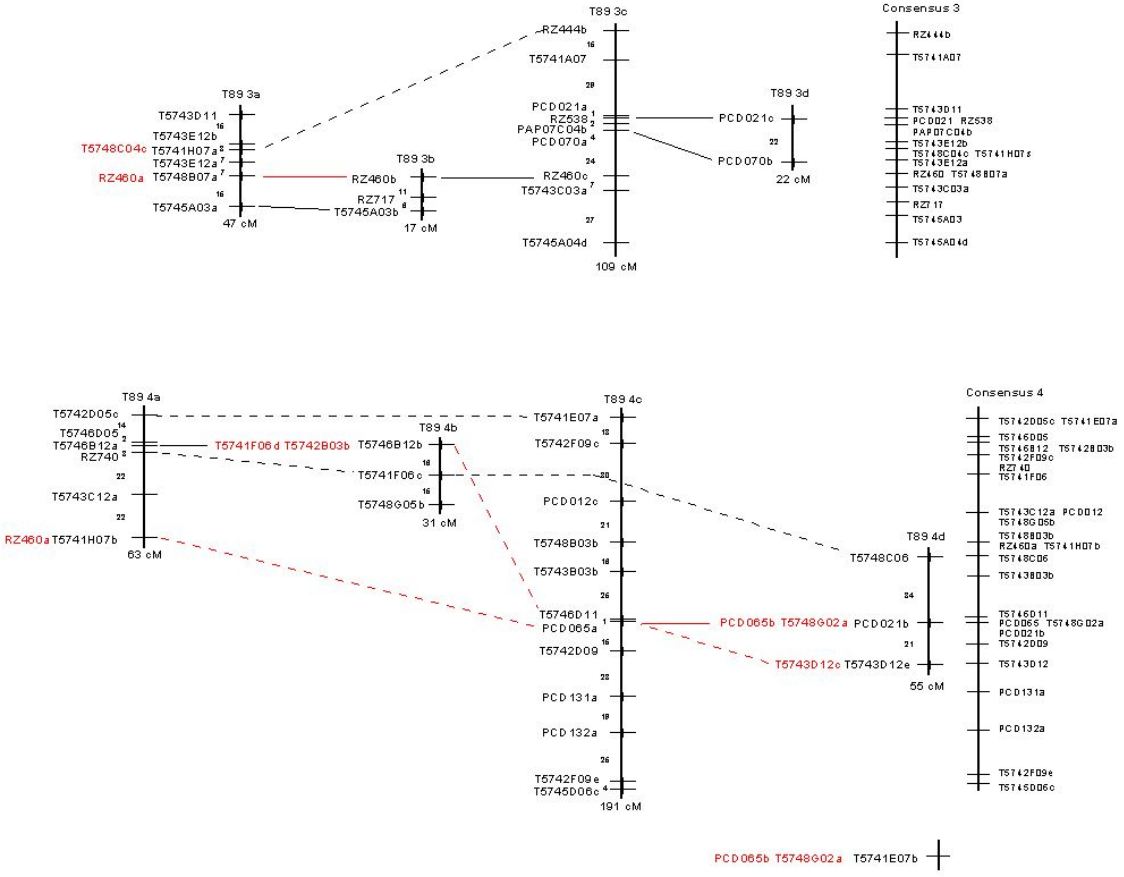


Figure 2.1 (continued). Linkage maps of *Cynodon dactylon* (4x = 36) linkage groups.

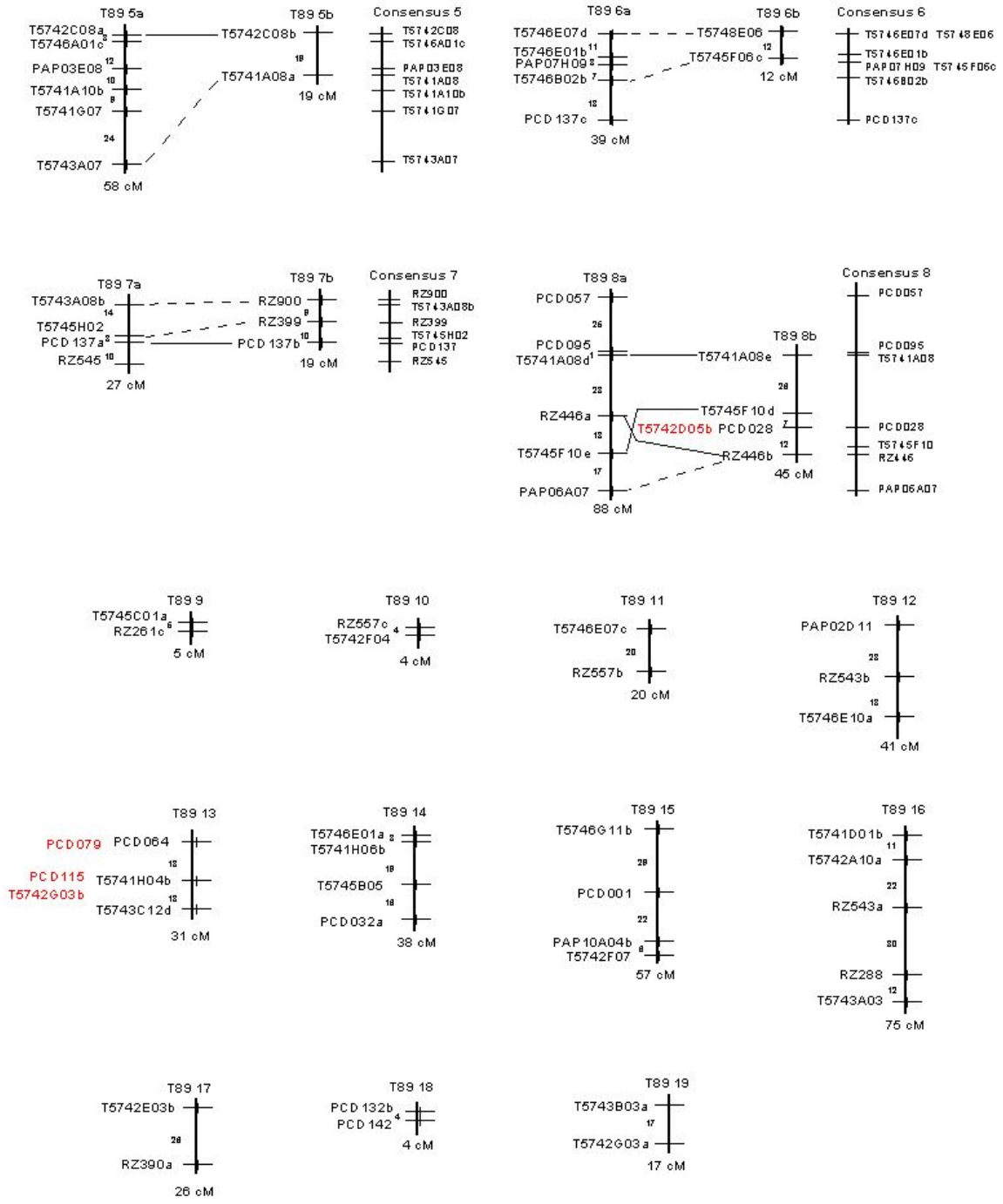


Figure 2.2. Linkage maps of *Cynodon transvaalensis* (2x=18) linkage groups. Map distances in centiMorgans (cM). Where possible, homologous groups are aligned on the basis of multi-locus probes (solid lines), and repulsion repulsion linkages (dashed lines) to infer a consensus.

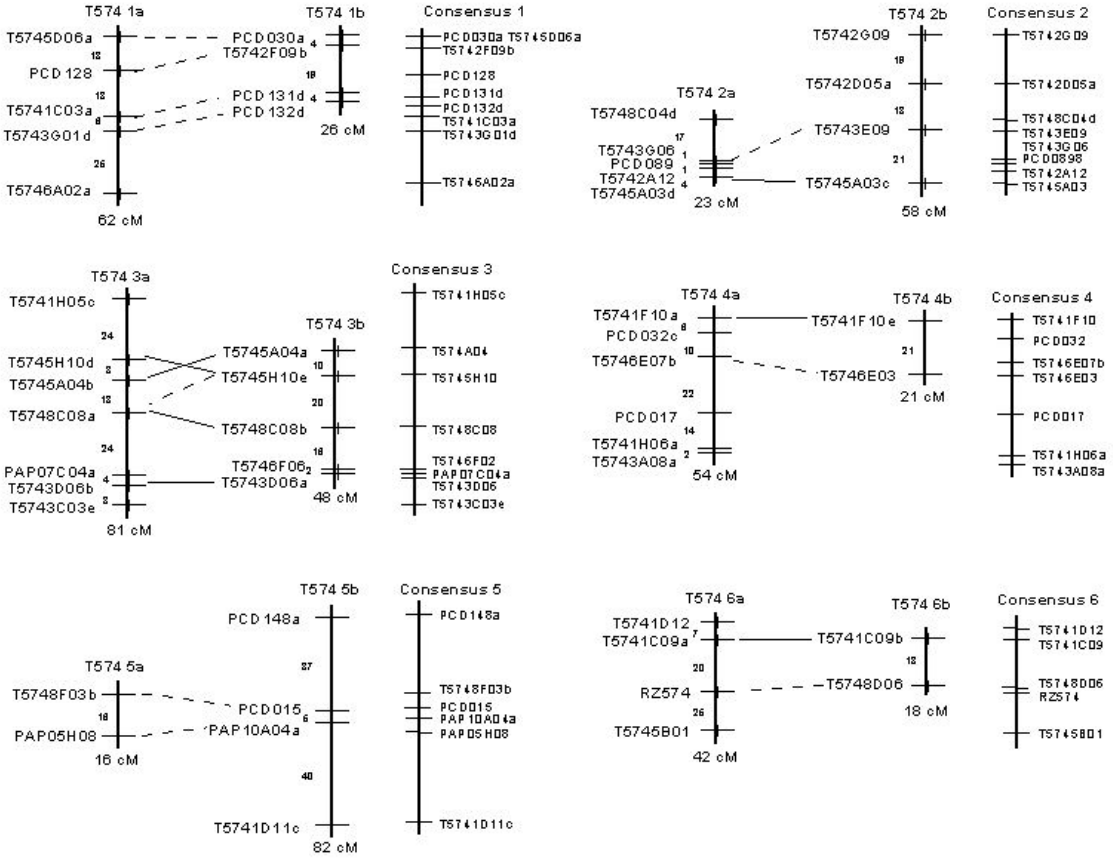


Figure 2.2 (continued). Linkage maps of *Cynodon transvaalensis* (2x=18) linkage groups.

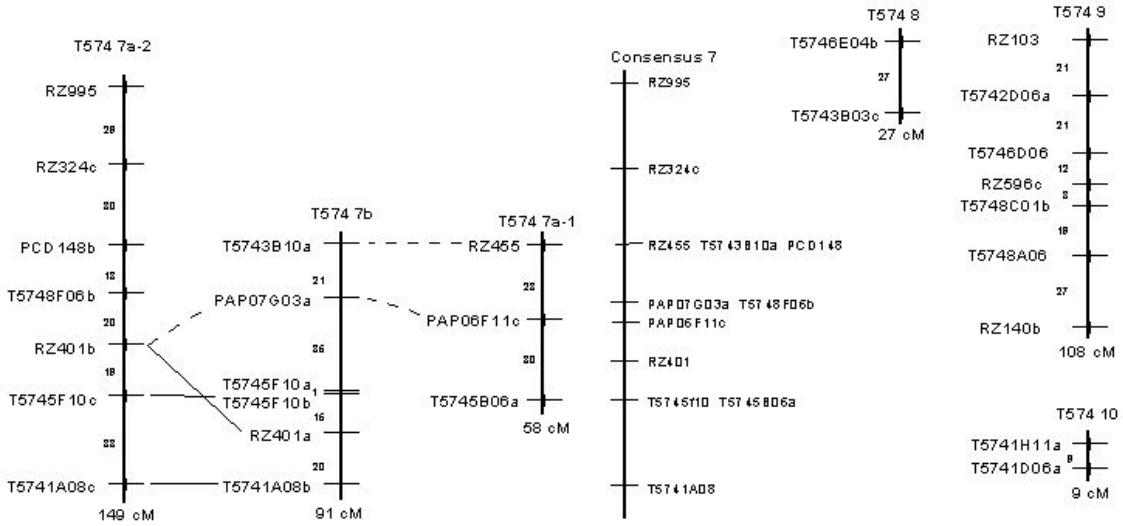


Figure 2.3. Construction of inferred bermudagrass ancestral chromosomes between the two parents T89 and T574, with comparisons to rice and *Sorghum*. Circles show the relative location of centromeres on rice chromosomes.

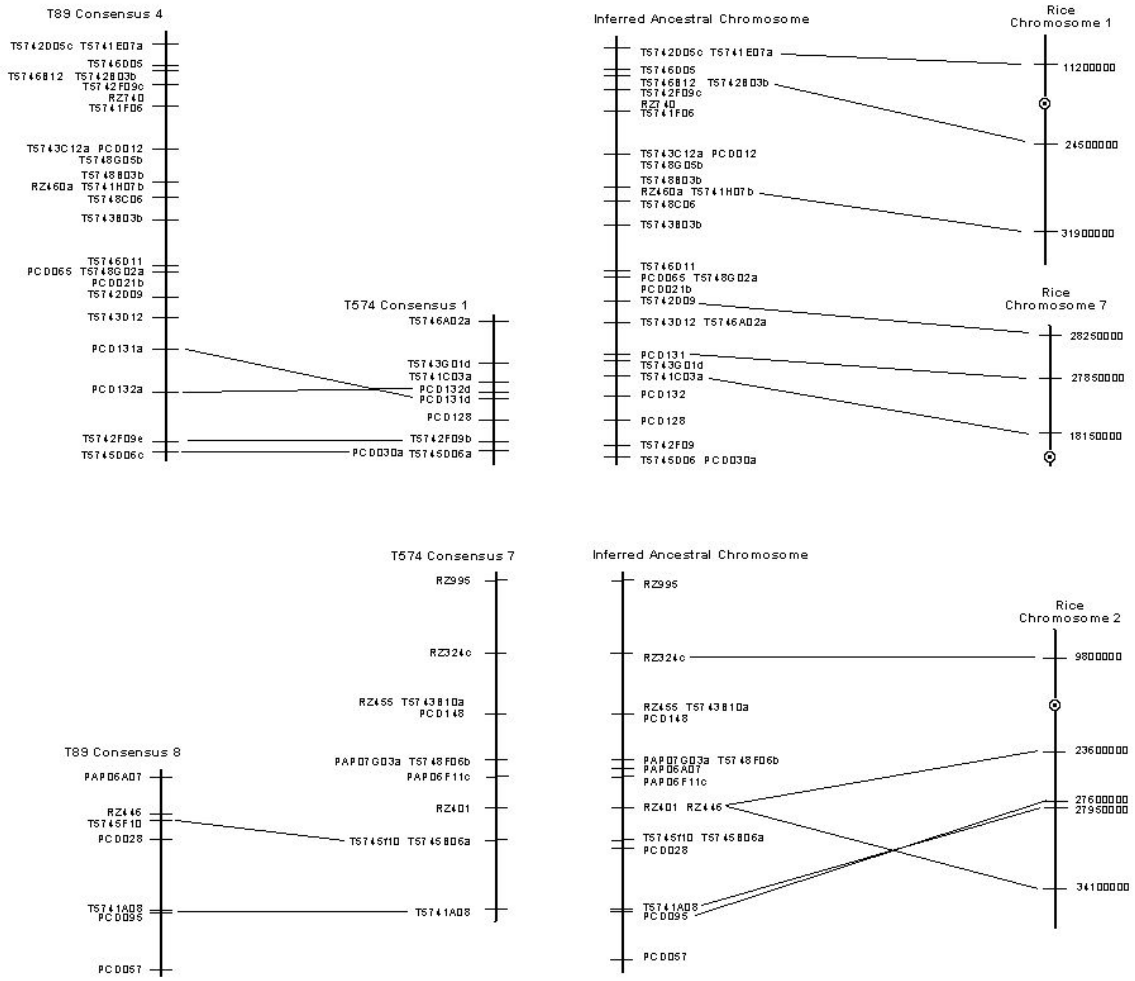
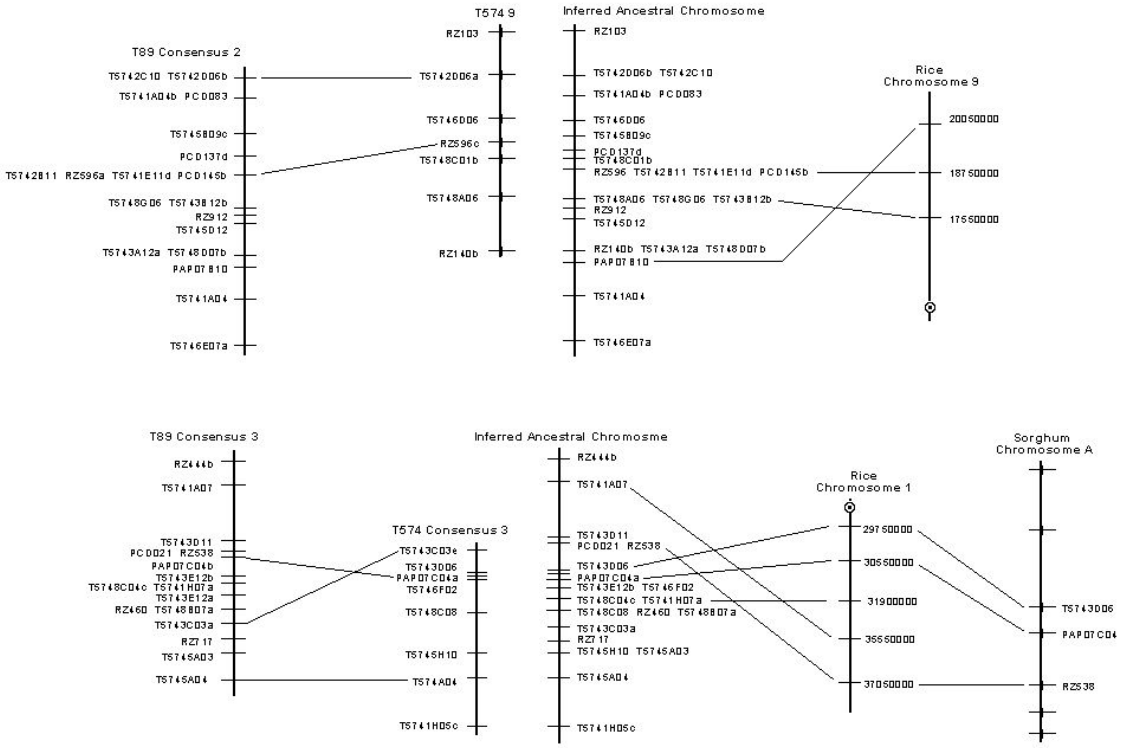


Figure 2.3 (continued). Construction of inferred bermudagrass ancestral chromosomes between the two parents T89 and T574, with comparisons to rice and *Sorghum*.



# CHAPTER 3

# CONCLUSIONS

## *SDRF Mapping*

Single-dose restriction fragment mapping offers an approach to linkage analysis that overcomes many of the difficulties related to mapping of polyploids. Use of this strategy allows one to circumvent complications that arise from multiple fragments at the same locus and the co-migration of same-sized fragments by requiring that only the segregation patterns of fragments that exist in single copy be considered for analysis. This however necessitates the removal of all non-single dose loci, and decreases the informativeness of the map data and the overall efficiency of the project. In our case, removal of non-SDRF loci eliminated 103 segregating loci from the T89 analysis, and 39 from T574, which combined represent 32 % of the initial map data. Even after combining with the strategy of Ripol et al (1999) to map double-dose restriction fragments, still only 343 of the 440 loci generated were considered for map construction and analysis. The promise of distinguishing auto versus allopolyploidy using the ratio of coupling and repulsion linkages found in SDRF mapping is another key advantage of this strategy. However, confident predictions may depend, at least in part, on relatively high marker density, which is in turn directly related to marker generation. Correspondingly, determination of the dosage level of each segregating fragment, which is the core strength of this approach, is also its main limitation. Accordingly, the SDRF strategy is one of the best tools available for framework map construction in difficult to map polyploids, as in our case, but is not well suited for high-density map saturation.

*Map utility and marker density*

Marker density is also a very important factor in map utility. We have presented the first framework genetic maps of the *Cynodon dactylon* and *Cynodon transvaalensis* genomes, with approximately 39% and 38% genome coverage, and average marker spacing of around 15.5 cM and 17 cM, respectively. The current maps are useful for preliminary investigations of conserved gene order with other grasses. However, more in depth comparative evolutionary studies involving bermudagrass would rely on a significant increase in the number of data points for comparison. Also, a considerable increase in the marker densities of these two maps would make them more suitable for use in applications such as map-based cloning and marker-assisted selection, which ideally require more complete, saturated maps with no more than 10 cM average marker spacing (Meyer et al 1994; Davierwala 2001).

*Future direction of the project*

Lange and Boehnke (1982) offer a formula to calculate the minimum number of randomly distributed markers (n) required to cover a proportion (p) of a genome of (k) at a maximum distance (2d) between markers.

$$n = [\log (1 - p)] / \log [1 - (2d / k)$$

Using this formula, it would take a total of 394 and 813 probes to achieve 95% coverage and 10cM average marker spacing for the *Cynodon dactylon* (4x) and *Cynodon transvaalensis* (2x) genomes respectively. That corresponds to 611 and 297 additional markers for the two genomes. Since only 68% of RFLP markers in this study are SDRF's, this would mean that 908 and 437 additional markers have to be generated for the two maps. It is with this regard that we must turn our attention to alternative methods.

Heteroduplex DNA analysis is an alternative approach which is designed for large-scale, expeditious marker generation for mapping (Nataraj et al 1999). The strategy depends on the ability to separate DNA fragments based on the conformation change that occurs between two complementary strands that have a single base-pair mis-match. In this way, it is and indirect means to examine sequence dissimilarity in a short region. More importantly however, since sequence dissimilarity replaces fragment size, it is possible to view all alleles present, which drastically increases the informativeness of the map data generated, and the efficiency of the project. Also, since polymorphism can be detected and scored directly on a gel, it significantly overcomes some of the time constraints of other mapping techniques. Short PCR-fragments from both parents are mixed, denatured and re-annealed, creating heteroduplex fragments. When electrophoresed in a poly-acrylamide like gel, heteroduplex fragments will migrate at different speeds based on the type of conformational change that has occurred, which is related to the degree of mis-match. After all possible genotype combinations are distinguished, the same is repeated using the individuals of the mapping population, and segregation patterns are recorded (Fukuoka et al 1994). Similar strategies have already been used to successfully contribute to mapping in rice (Fukuoka et al 1994), *Beta vulgaris* (Schneider et al 1999) and bulb onion (*Allum cepa* L.) (McCallum et al 2001), and exploratory attempts have been made to test the applicability of this strategy to mapping our bermudagrass population.

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