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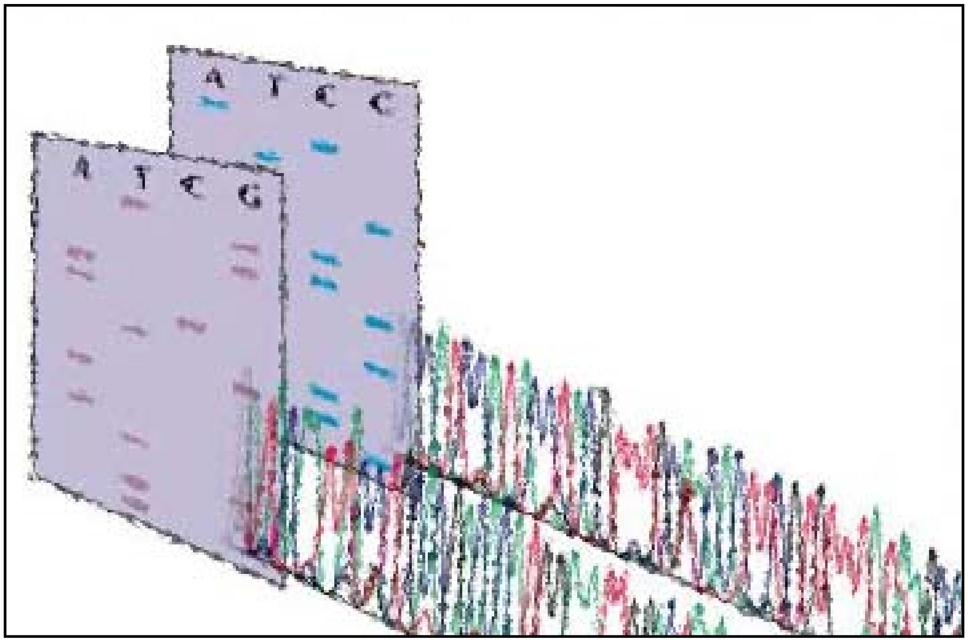

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PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 225 projects at a cost of $25 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

Editor

Jeff Nus, Ph.D.
904 Highland Drive
Lawrence, KS 66044
jnus@usga.org
(785) 832-2300
(785) 832-9265 (fax)

Research Director

Michael P. Kenna, Ph.D.
P.O. Box 2227
Stillwater, OK 74076
mkenna@usga.org
(405) 743-3900
(405) 743-3910 (fax)

Creating a Genetic Roadmap for Bermudagrass

Andrew H. Paterson, Wayne Hanna, Casey Bethel, and Erica Sciara

SUMMARY

Research at the University of Georgia has begun to reveal the repertoire and organization of genes along the chromosomes of bermudagrass, and their relationships to those of well-studied models such as rice. In particular:

- The bermudagrass genome map is based on analysis of a cross between the two species that have contributed most substantially to today's elite turf cultivars.
- To date, researchers have screened a total of 665 "DNA probes" to identify a subset of 230 that revealed from 1-7 differences (an average of 1.95) between the parents of the mapping cross.
- Using these probes, they have determined the arrangements of 449 different genetic loci including 306 from the *C. dactylon* parent and 143 from the *C. transvaalensis* parent.
- With the completion of the consensus map, they will next explore its alignment to the well-mapped genomes of sorghum and maize, and largely-sequenced genome of rice.
- Although the population is small, they are conducting an exploratory search for DNA markers that are diagnostic of genes related to turf quality.
- The comparative approach that they describe is primarily of value to work out the similarities among diverse grasses -- they and others are beginning to explore the differences from other grasses for which bermudagrass is prized through *de novo* sequencing of large numbers of genes from bermudagrass itself.
- Complete sequencing of the bermudagrass genome would reveal to researchers its entire genetic potential.

Study of the hereditary information of all organisms has been revolutionized by spinoffs of the Human Genome Project. Many of the tools and approaches used in mapping and sequencing genomes were pioneered in other organisms. At the chemical level, however, the hereditary information (DNA, deoxyribonucleic acid) of all organisms is very similar, and the substantial investments made in the human genome drove the development of efficient technologies and instrumentation that are applicable to all organisms.

The detailed analysis of a few carefully selected plant genomes has provided a springboard for quickly advancing knowledge of many other plants including major crops with complex genomes. Most major crops now enjoy detailed "genetic maps" that provide DNA markers that represent unique mileposts along the roadmap of the genome. Curiously, although genetic maps of different plant only vary over a range of perhaps 10-fold in length, the amount of DNA in different plant genomes varies over more than a 1000-fold range. The genomes of *Arabidopsis thaliana* and *Oryza sativa* (rice), two plants with relatively small amounts of DNA and few chromosomes, have been almost fully sequenced, providing a template for the comparative analysis of many other more complex plant genomes (4).

The discovery that most plants share similar sets of genes, and further that the genes are arranged along the chromosomes in similar orders, has suggested a general approach by which detailed knowledge of the hereditary information in selected botanical models can be "leveraged" to quickly advance knowledge of the genomes of

ANDREW H. PATERSON, Ph.D., Distiguished Professor; WAYNE HANNA, Ph.D., Professor; CASEY BETHEL, graduate student; and ERICA SCIARA, former technician; Department of Crop and Soil Sciences at the University of Georgia, Athens, GA.

Figure 1. Plots representing individual progeny of the population used for genetic mapping, growing in Tifton, GA.

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many other plants. Specifically, by mapping the same genes in different plants, one can align the chromosomes of the respective plants to varying degrees. Plants within a single species, different bermudagrass cultivars for example, have virtually identical gene orders along the chromosomes. More distant relatives such as maize (corn) and rice, still retain sufficient similarities that their comparison is useful (3). Even comparisons between monocots (such as bermudagrass) and dicots, such as *Arabidopsis*, show valuable parallels that have endured the roughly 200-million-year tenure of flowering plants on our planet (4).

To accelerate progress in learning about the arrangement of the bermudagrass genes, we used many genes that had been previously mapped in several other grasses, primarily sorghum, but also including maize and rice (1). In addition, we mapped a substantial population of bermudagrass genes that had not previously been characterized - we also determined the DNA sequences ("spelling") of most of these bermudagrass genes so we could search the rice genome for genes with similar spelling that are likely to be the rice versions of our bermudagrass genes.

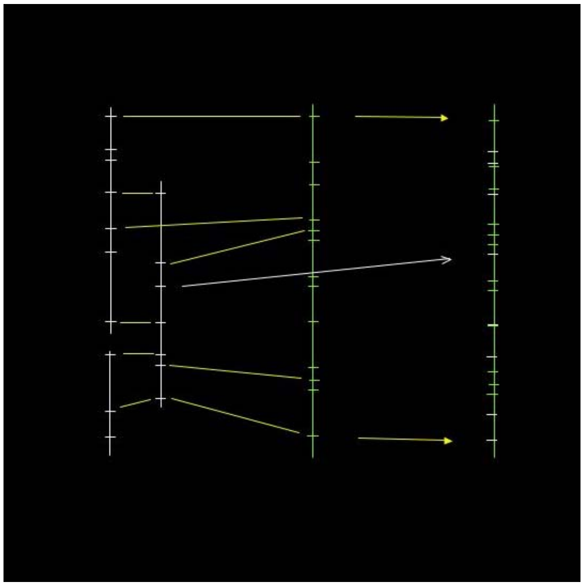

Figure 2. Consensus map of the bermudagrass chromosomes. *Cynodon dactylon* (white chromosomes at left), with 36 chromosomes, is expected to contribute two somewhat different versions of each chromosome to each of the 113 progeny in our study, thus yielding two maps of each chromosome. *C. transvaalensis*, (green chromosome in center), with 18 chromosomes, is expected to contribute only one to each of our progeny, yielding a single genetic map. In some cases, we are able to map the same genes on both sets of chromosomes, providing a scaffold by which to align the respective chromosomes to one another (horizontal lines). This permits us to interpolate the locations of the remaining genes that can only be mapped in a subset of the chromosomes, yielding a "consensus map" (at right).

The bermudagrass genome map is based on analysis of a cross between the two species that have contributed most substantially to today's elite turf cultivars. Specifically, we have analyzed 113 progeny of a cross between *C. dactylon* accession 'T89', and *C. transvaalensis* accession 'T574' (Figure 1). Other crosses between these two species have led to many of today's elite bermudagrass cultivars such as 'Tifdwarf', 'Tifway', 'TifSport', and 'TifEagle'. One reason for the success of these cultivars is that the parental species differ in chromosome number. Consequently, the progeny are sterile. This results in male and female sterile interspecific hybrids that have to be propagated vegetatively. Clonal propagation of cultivars helps to assure the fidelity of reproduction preserving the desirable genotype from one generation to the next.

Genetic mapping involves first identifying differences (polymorphisms) between the parental genotypes in the sizes of specific DNA fragments in their genomes, and then using the size differences to determine which parent contributed each fragment to each progeny individual. Our work used the RFLP (restriction fragment length polymorphism) method to identify and reveal differences between progeny although several other methods are available as well.

To date, we have screened a total of 665 DNA probes to identify a subset of 230 that revealed from 1-7 differences (an average of 1.95) between the parents of our mapping cross. Using these probes, we have determined the arrangements of 449 different genetic loci including 306 from the *C. dactylon* parent and 143 from the *C. transvaalensis* parent. We are now aligning the maps of the two parents to one another to produce an interleaved consensus map that will be our best available representation of the bermudagrass chromosomes (Figure 2).

With the completion of the consensus map we will explore its alignment to the well-mapped genomes of sorghum and maize and largely-sequenced genome of rice. Based on the findings of similar efforts we have conducted in other grasses such as sorghum (3), we expect this to have a high degree of predictive value. In other words, we should be able to predict the locations in bermudagrass of many genes that have previously been mapped, sequenced and assigned to functions in the rice genome, thus permitting study and improvement of bermudagrass to benefit cheaply and quickly from a host of intensive research in these botanical models.

Although our population is small, we are conducting an exploratory search for DNA markers that are diagnostic of genes related to turf quality. Dr. Wayne Hanna has used a selection index to assess turf quality of each of the 113 progeny, which varies widely (Figure 3). Next, we will use established statistical techniques to seek correlations between turf quality and specific DNA markers. While the small size of our population and the genetic complexity of turf quality (representing a composite of many different traits) may be some hindrance, this will provide a pilot study-of our ability to identify specific DNA markers that are diagnostic of traits directly related to bermudagrass improvement.

Our map, while a valuable step, provides only a first glimpse into the genetic potential of bermudagrass. Further, the comparative approach that we describe is primarily of value to work out the similarities among diverse grasses such as bermudagrass, sorghum, and rice. Capturing the inherent genetic potential of bermudagrass in the form of improved cultivars will require that we also have knowledge of the genes that account for differences among such grasses. We and others are beginning to explore these differences through *de novo* sequencing of large numbers of genes from bermudagrass itself.

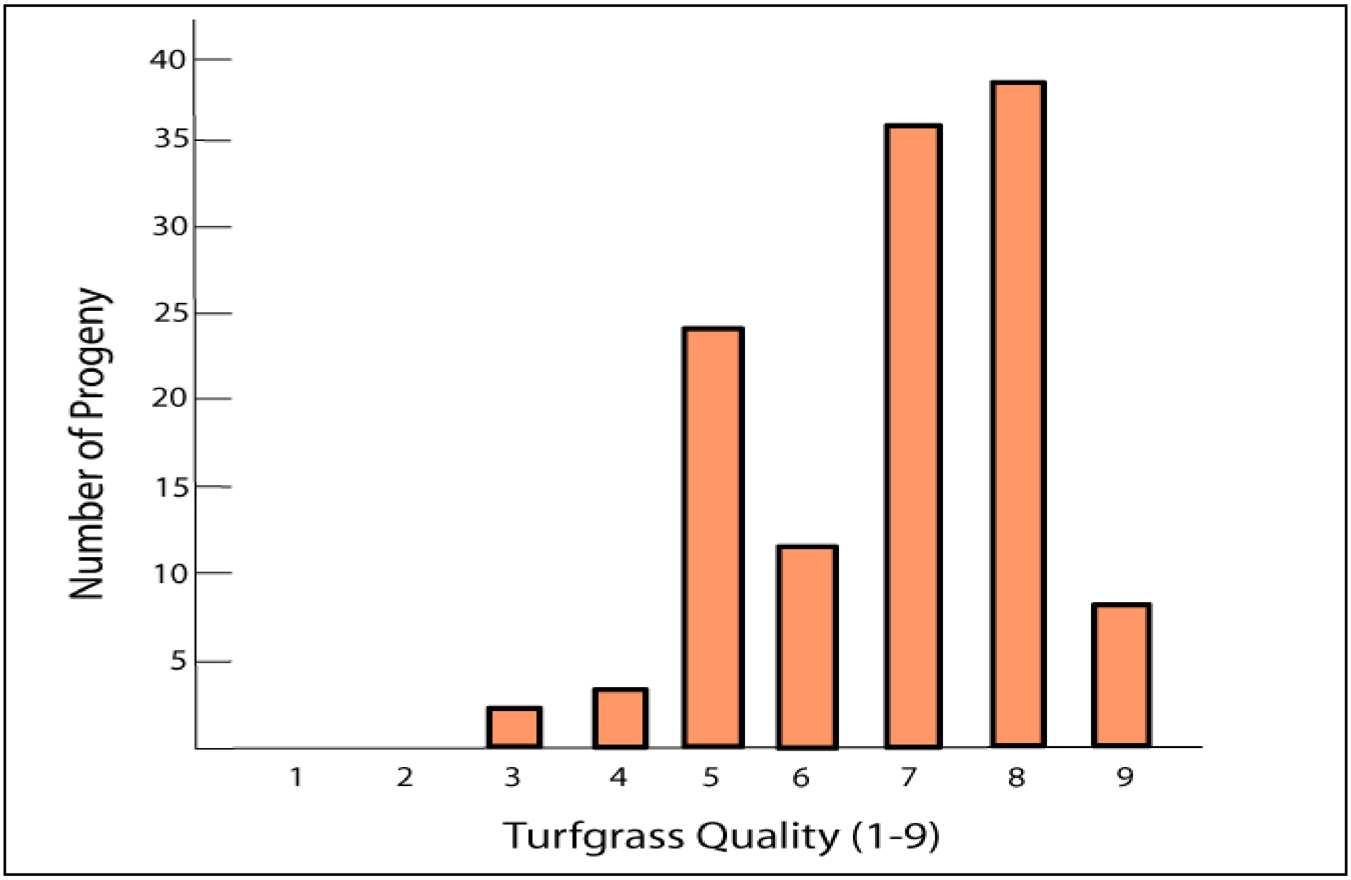

Figure 3. Distribution of turf quality among the progeny of a cross between *Cynodon dactylon* and *C. transvaalensis.* Turf quality was measured on a composite scale reflecting mainly texture, density, seedheads, and leaf color, and rated from 1 (poorest) through 9 (best).

Moreover, genetic mapping has practical limits to resolution that will eventually necessitate a transition to physical mapping. Physical mapping uses large-insert DNA cloning vectors (vehicles) such as “bacterial artificial chromosomes” (BACs) which can carry DNA segments that span perhaps 0.1% of a chromosome (2). Bacterial artificial chromosomes offer important efficiencies in that they permit gene mapping without the difficult and tedious step of finding differences (polymorphisms) between the parental genotypes in the sizes of specific DNA fragments in their genomes. While the present study permitted us to map a large number of genes that broadly sample the bermudagrass chromosomes, the lack of differences prevented us from mapping nearly two-thirds of the 665 genes that we attempted, and cost us time and resources to find the subset that could be mapped.

Finally, BAC-based physical maps are a valuable foundation for complete sequencing of a genome. While the cost of such an effort was measured in billions of dollars only a decade ago, today the cost is down to the tens of millions and falling. New methods permit us to capture much of the novel sequence information in a genome for even less (5, 6). Complete sequencing of the bermudagrass genome will reveal to researchers its entire genetic potential, and one can envision the time when the benefits will certainly justify the cost.

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