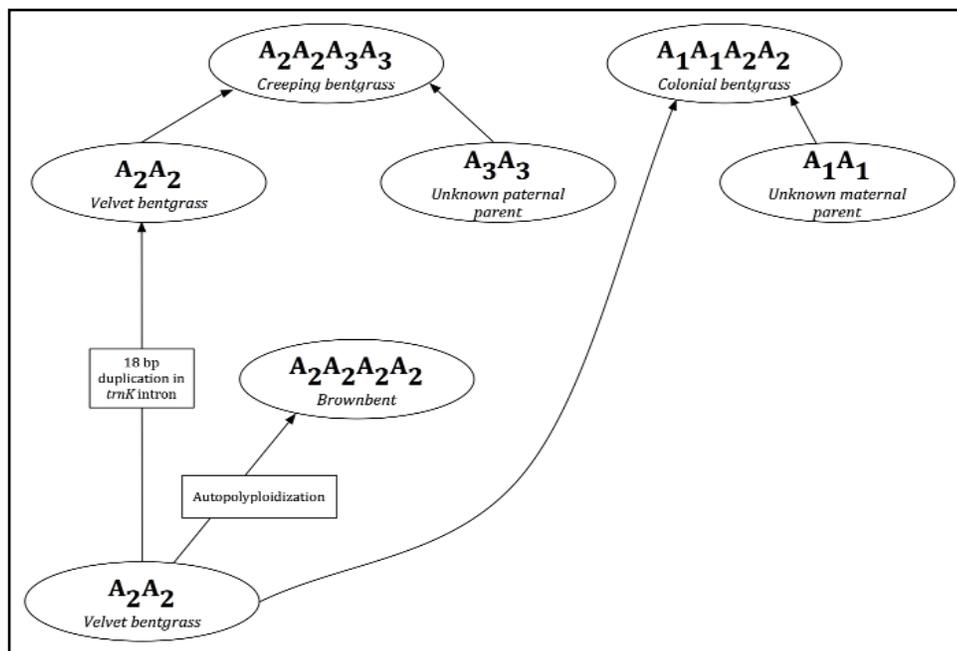


Turfgrass and Environmental Research Online

...Using Science to Benefit Golf



Scientists at Rutgers University continue to investigate the genetic relationships among the important bentgrass species: velvet bentgrass, creeping bentgrass, and colonial bentgrass. Using DNA sequence analyses, they developed a new model of the relationships of these species to each other—that velvet bentgrass is the likely ancestral maternal parent of creeping bentgrass and the ancestral paternal parent of colonial bentgrass.

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 400 projects at a cost of \$30 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***.

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A New Model of the Genomic Relationships Among Creeping, Colonial, and Velvet Bentgrasses

Faith C. Belanger, David Rotter, and Karen V. Ambrose

SUMMARY

Better understanding of the genetic relationships among the important bentgrass species (velvet bentgrass, creeping bentgrass, and colonial bentgrass) may be useful in future breeding efforts in these species.

- We used DNA sequence analysis to investigate the relationships among these species.
- We sequenced regions of the nuclear and chloroplast DNA of velvet, creeping, colonial, and brown bentgrass and developed a new model of the relationships of these species to each other.
- Based on this analysis, we propose that velvet bentgrass is the likely ancestral maternal parent of creeping bentgrass and the ancestral paternal parent of colonial bentgrass.

Of the 220 bentgrass species (16), creeping bentgrass, colonial bentgrass, and velvet bentgrass are the three commercially most important species (2, 14, 15). All three are used on golf courses in the United States. Creeping bentgrass is used the most often, but colonial bentgrass and velvet bentgrass are also used, particularly in the Northeastern regions of the US. Turfgrass breeders are working on developing improved cultivars of all three species.

Knowledge of the evolutionary relationships among species is of inherent interest and practical importance in breeding efforts for crop improvement. For example, understanding the relationships among cereal grains and related wild species has been critical in introducing important traits into cultivated cereals (6). A considerable amount of research has been devoted to understanding the evolutionary relationships among all species (10; <http://tolweb.org/tree/phylogeny.html>).

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There have also been several large studies focused on the evolutionary relationships among grass species (1, 3, 11). Current research investigating the evolutionary relationships among species includes extensive DNA sequence comparisons. The more similar the DNA sequences are between two species, the closer the evolutionary relationship between the two. There are several computer programs available that can compare large DNA sequence data sets and generate trees of the relationships among the species tested.

Some bentgrass species have been included in investigations into the relationships of grasses in general. These studies placed the bentgrasses in the relatively recently evolved (46 million years ago) tribe Aveneae (4). Other familiar cool-

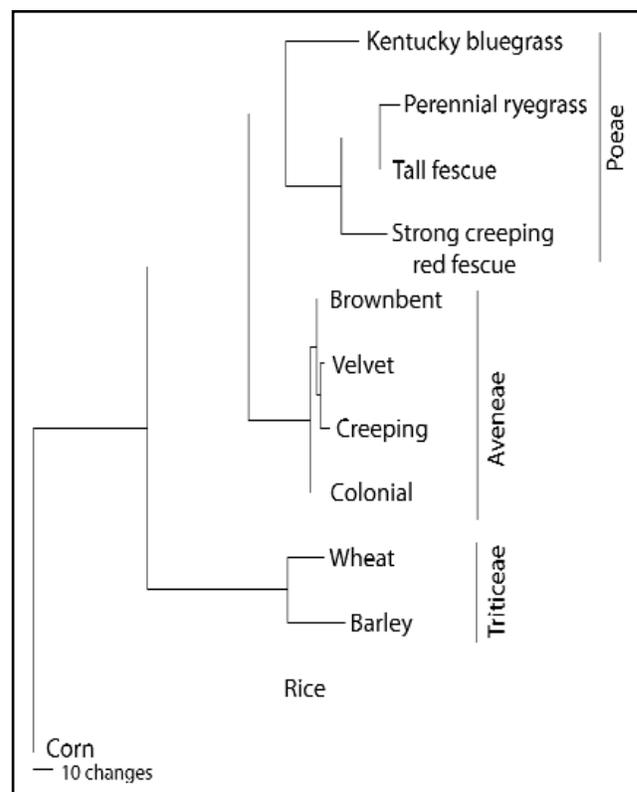


Figure 1. Phylogenetic tree illustrating the relationships among some important turfgrass species.

Species	Common Name	Chromosome Number	Genome Composition
<i>A. canina</i>	Velvet bentgrass	14	A1 A1
<i>A. vinealis</i>	Brown bentgrass	28	A1 A1 A1 A1
<i>A. capillaris</i>	Colonial bentgrass	28	A1 A1 A2 A2
<i>A. stolonifera</i>	Creeping bentgrass	28	A2 A2 A3 A3

Table 1. Genome organization, as proposed by Jones (7, 8, 9), of some *Agrostis* spp.

season turfgrasses, Kentucky bluegrass, tall fescue, perennial ryegrass, and strong creeping red fescue, were placed in the sister tribe Poeae (11). A simplified tree showing the relationships of some of the important turfgrass species to each other is shown in Figure 1.

Previously, however, there was not a DNA sequence-based consideration of the relationships among the many bentgrass species. The only previous studies of the relationships among bentgrasses were published in 1956 (7, 8, 9). In these studies, microscopic examination of chromosome pairing at meiosis of some bentgrass species and some interspecific bentgrass hybrids was used to develop a model for the relationships among four bentgrass species. Jones proposed that velvet bentgrass was a diploid with a total of 14 chromosomes. Brown bentgrass, a common grassland species was considered to be an autotetraploid of velvet bentgrass, having originated from a chromosome doubling of the 14 velvet chromosomes to 28 chromosomes. Creeping bentgrass and colonial bentgrass were determined to be allotetraploid species, each with a total of 28 chromosomes.

In contrast to the autotetraploid brown-bent, creeping and colonial bentgrass were each considered to have originated from hybridization between different diploid species. Velvet bentgrass was considered to be one of the diploid par-

ents of colonial bentgrass and was designated as having the A1 genome. The other unknown diploid parent of colonial bentgrass was designated as having the A2 genome. Creeping bentgrass was considered to share the A2 genome with colonial bentgrass, and its other genome was designated the A3 genome. The two diploid parents of creeping bentgrass were not known. Table 1 summarizes the relationships among some bentgrass species as proposed by Jones.

We used DNA sequence analysis to reexamine the evolutionary relationships among the bentgrass species (13). We isolated DNA from velvet bentgrass, brown bentgrass, creeping bentgrass, and colonial bentgrass and sequenced regions from the nuclear DNA and from chloroplast DNA. Some sequence data from other bentgrass species was available from other researchers that could also be included in our analysis. In contrast to the model proposed by Jones, based on the DNA sequence analysis, we concluded that velvet bentgrass was the likely maternal diploid parent of creeping bentgrass.

In plants, the chloroplast is generally inherited from the maternal parent. Our analysis of chloroplast DNA sequences revealed that the velvet bentgrass sequences were closely related to those of creeping bentgrass. In the region of the chloroplast DNA that we sequenced, there is an 18-base pair duplication in velvet bentgrass and

Velvet bentgrass	TTTCTTTTTTTGAATGGAATAAAAATTTTGAATGGAATAAAAATTACGAT
Creeping bentgrass	TTTCTTTTTTTGAATGGAATAAAAATTTTGAATGGAATAAAAATTACGAT
Colonial bentgrass	TTTCTTTTTTTGAATGGAATAAAAA-----TTACGAT
Brown bentgrass	TTTCTTTTTTTGAATGGAATAAAAA-----TTACGAT

Figure 2. Sequence alignment of the region of the 18-base pair duplication in the chloroplast trnK intron of velvet, creeping, colonial, and brown bentgrasses.

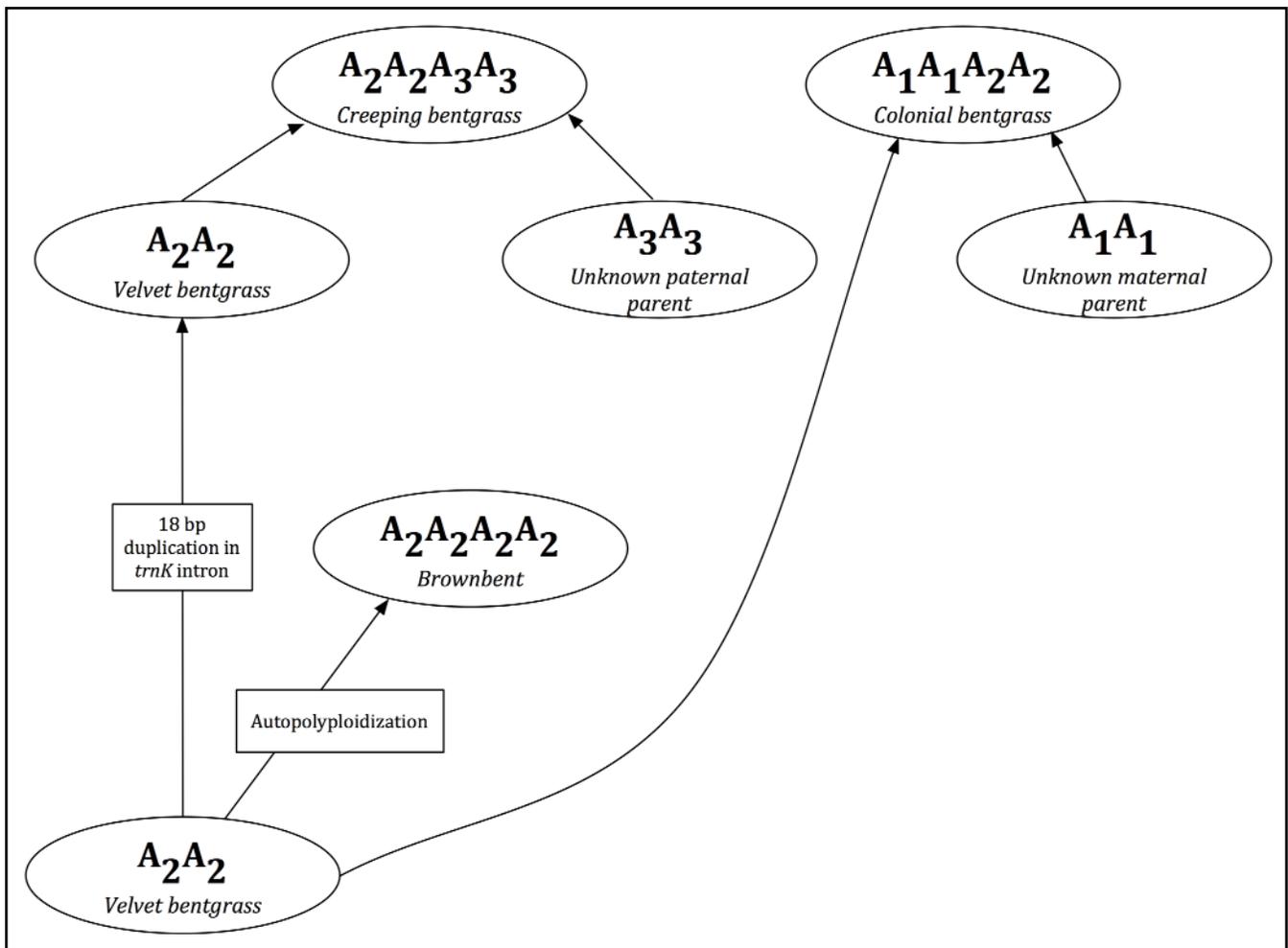


Figure 3. A new model of genome relationships among velvet, creeping, colonial and brown bentgrasses. This model was based on the DNA sequence analyses reported by Rotter et al. (13). This figure was used with permission from Springer.

creeping bentgrass that is not found in the brown bentgrass or colonial bentgrass sequences (Figure 2). Although brown bentgrass did not have the duplication, an analysis of the larger region of chloroplast DNA revealed brown bentgrass is closely related to velvet bentgrass and creeping bentgrass.

Analysis of the nuclear DNA regions also indicated that velvet bentgrass was closely related to the A2 genome of creeping bentgrass. Based on the interpretation from the DNA sequence data, we propose a new model for the genome relationships among velvet, creeping, and colonial bentgrasses (Figure 3). In this model, velvet bentgrass is proposed as the ancestral diploid maternal parent of creeping bentgrass. Previously, analysis of gene sequences from colonial and creeping bentgrass confirmed the close relationship of the A2

genomes in these species (12). Since colonial bentgrass shares the A2 genome with creeping bentgrass, we propose that diploid velvet bentgrass was the paternal parent of colonial bentgrass.

There are 220 bentgrass species (16), but the ploidy status of most of them is unknown. Of the bentgrasses that are readily available for analysis, only velvet bentgrass is a known diploid. In the future, identification of additional bentgrass diploid species combined with DNA sequence analysis could be used to possibly identify the A1 and A3 ancestral diploid parental species. This could be very useful for turfgrass breeders since they could potentially have new germplasm sources to incorporate into their programs. It would be very interesting to generate interspecific hybrids between the diploid species identified as

ancestral parents of creeping and colonial bentgrass and compare the progeny to today's colonial bentgrass and creeping bentgrass species.

Recently there has been a report using the chromosome labeling techniques of genomic *in situ* hybridization and fluorescence *in situ* hybridization to examine the chromosomes of creeping and colonial bentgrasses (5). The data the authors presented is consistent with the DNA sequence data from our study in that the DNA from velvet bentgrass hybridized with that of both creeping and colonial bentgrass nuclear DNA (5). In the future, these methods, in addition to DNA sequence analysis, could also be used to expand our knowledge of the relationships of the many bentgrass species.

Acknowledgments

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