

PLANT GENETIC RESOURCES

RAPD Marker Diversity among Creeping Bentgrass Clones

Michael D. Casler,* Yolibeth Rangel, John C. Stier, and Geunhwa Jung

ABSTRACT

Creeping bentgrass (*Agrostis stolonifera* L.) is currently one of the most desirable grasses for golf courses in temperate regions. Old golf courses often have populations of creeping bentgrass clones that have persisted for many years, possibly surviving since the original construction. Over time, environmental stresses and diseases may cause shifts in the genetic composition of bentgrass populations as natural selection occurs. The objective of this study was to conduct a survey of RAPD (random amplified polymorphic DNA) marker diversity within a population of 110 creeping bentgrass clones. The clones were collected from putting greens or fairways of 17 golf courses that were at least 75 yr old. Thirteen RAPD primers resulted in 99 polymorphic bands. Two multidimensional scales varied among golf courses for fairway-collected clones. Much of this variation was attributed to presettlement historic vegetation (northern coniferous, hardwood-prairie, or transition). The most unique population of creeping bentgrass clones was collected on the visually unique fairways at the Marshfield Country Club, containing the greatest diversity of perennial grass species. Clones collected from putting greens were not differentiated by golf courses, suggesting that the putting green environment was sufficiently homogeneous across golf courses that its impact on natural selection was largely uniform.

CREeping BENTGRASS is the most widely used temperate grass species on golf course putting greens. It is often used on fairways at relatively low mowing heights, but the adaptation of many other grasses to various fairway management regimes results in a wider array of species and a reduced frequency of creeping bentgrass on fairways compared to greens. Creeping bentgrass has been commonly used on golf courses in North America since the end of the 19th century. Creeping bentgrass is highly self-incompatible and seeded populations are highly heterozygous and heterogeneous (Warnke et al., 1997).

Old golf courses (≥ 75 yr) contain mixed populations of creeping bentgrass that are likely the product of several forces acting to change the composition of a turf. Original populations of bentgrasses used to plant many golf courses in temperate North America were derived from highly heterogeneous populations of South German bentgrass mixtures. Decades of natural selection have eliminated many unadapted plants in favor of plants with pest resistances and stress tolerances needed to survive management and edaphic factors that define

their local environment (Casler et al., 1996). New cultivars can be added to an existing golf course by overseeding, sodding, sprigging, or contamination on equipment or shoes. Because new cultivars may not be well adapted to all management and edaphic factors that define the environment of a particular golf course, natural selection will continue to be the driving force controlling the genetic composition of a mixed bentgrass turf.

Many old golf courses in the northcentral USA possess populations of creeping bentgrass that are visually heterogeneous and patchy on both greens and fairways. Visually homogeneous patches may exceed a diameter of 3 m on fairways and 1.5 m on greens. Perennial grass clones are considered capable of surviving for hundreds of years (Harberd, 1961, 1967), suggesting the existence of some extremely old bentgrass clones on these golf courses. Natural variation present on old golf courses has been extremely useful as a foundation of several creeping bentgrass breeding programs (Engelke et al., 1995; Hurley et al., 1994). Our objective was to conduct a survey of RAPD marker diversity of creeping bentgrass clones collected from fairways or greens of 17 old golf courses in Wisconsin.

MATERIALS AND METHODS

Over 700 clones of creeping bentgrass were collected from golf courses throughout Wisconsin in summer 1996, spring 1997, and summer 1997. Putting-green clones were collected during the summer on the basis of three principal criteria: clone size, absence of *Poa annua* L. contamination, and fine texture. Fairway clones were collected in early spring, just after snowmelt on the basis of reaction to snow mold fungi [*Typhula* spp. and *Microdochium nivale* (Fries) Samuels & Hallett]. Fairway collections were made only on courses with reliable snow cover, without a history of fungicide applications for snow mold prevention, and at a time when snow mold symptoms were severely evident.

All clones were potted in the greenhouse as single tillers or small groups of connected tillers. Clones were maintained in the greenhouse by clipping, frequent thinning, and occasional repotting to maintain vigor and purity of each clone. Approximately half of the 700 clones were screened for reaction to one isolate of *Typhula ishikariensis* Imai under controlled environmental conditions (Wang et al., 2000).

One hundred ten clones, representing 17 golf courses, were chosen for this study (Fig. 1, Table 1). A minimum of three clones were selected from each golf course on the basis of high or low mean snow mold reaction as determined by Wang et al. (2000). All available clones from Hillcrest Golf and Country Club and Marshfield Country Club, the two courses that contributed both fairway and green collections, were included in this study. All golf courses represented in this study are at least 75 yr old. Most of the northern Wisconsin courses are dominated by *Poa annua* and creeping bentgrass has not been intentionally introduced within the past 30 to 40 yr (verified by records and/or memory of superintendents or owners).

Michael D. Casler, Dep. of Agronomy, University of Wisconsin-Madison, Madison, WI 53706-1597; Yolibeth Rangel and Geunhwa Jung, Dep. of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706; John C. Stier, Dep. of Horticulture, University of Wisconsin-Madison, Madison, WI 53706. This research was supported by the Wisconsin Sod Producers, Inc. and the College of Agric. and Life Sci. and Hatch formula funds. Received 26 Nov. 2001. *Corresponding author (mdcasler@facstaff.wisc.edu).

The three golf courses in southern Wisconsin have active overseeding programs to maintain high populations of creeping bentgrass on putting greens.

Genomic DNA was extracted from fresh leaves (0.1–0.2 g) of individual greenhouse-grown plants that were macerated in potassium ethyl xanthogenate (PEX) DNA extraction buffer with a FastPrep FP120 machine from BIO 101 Inc. (Carlsbad, CA) using ceramic beads. The remainder of the DNA extraction procedure followed Johns et al. (1997) with minor modification. The samples were grounded in 450 μ L of DNA extraction buffer in 2.0-mL microcentrifuge tubes. All remaining DNA extraction procedures were performed in 1.5-mL microcentrifuge tubes.

Reactions for RAPD analysis were performed in 10- μ L volumes in 96-well plates in an MJ PTC-100 incubator (MJ Research, Watertown, MA) following the methods of Johns et al. (1997). An arbitrary set of plants representing 10 cultivars each of creeping bentgrass and colonial bentgrass (*Agrostis capillaris* L.) were previously evaluated for polymorphism by means of 30 RAPD primers (Operon Technologies, Alameda, CA). Thirteen primers (C11, D2, D20, E14, F13, G11, G13, G19, O6, O15, P8, Y5, and Y9) were selected for this study because of the consistent clarity and reproducibility of polymorphic bands and polymorphisms on *Agrostis* species. All RAPD reaction products were electrophoresed on agarose gels as described by Johns et al. (1997). Gels were run for 2 h at 300 V, stained with ethidium bromide, illuminated by UV light, photographed, and manually scored for presence/absence of clear bands.

Comigrating polymorphic fragments, possessing unambiguous differences among the clonal DNA samples and ranging from 0.3 to 2.0 kb, were visually scored for presence (1) or absence (0) of the band. Each scorable band was named according to the primer and approximate band size in base pairs (e.g., C11.200). Genetic distance among the 110 clones in all pairwise combination was estimated as the complement to the simple matching coefficient and Jaccard's similarity coefficient (Gower, 1972). The 110 \times 110 genetic distance matrix was fitted in two-dimensional coordinates by a multidimensional scaling (MDS) procedure (SAS PROC MDS; SAS Institute, 1990). The MDS procedures have been used to represent genetic relationships among genotypes using molecular-marker-derived genetic distances (Beebe et al., 1995; Skroch et al., 1998; Rodriguez et al., 1999). The MDS variables (MDS1 and MDS2) were also analyzed by mixed models analysis (Littel et al., 1996) to identify sources of variation among the 110 clones. Golf courses, sod types (green vs. fairway), snow mold reaction classes, and their interactions were assumed to be fixed, while clones within groups were assumed to be random.

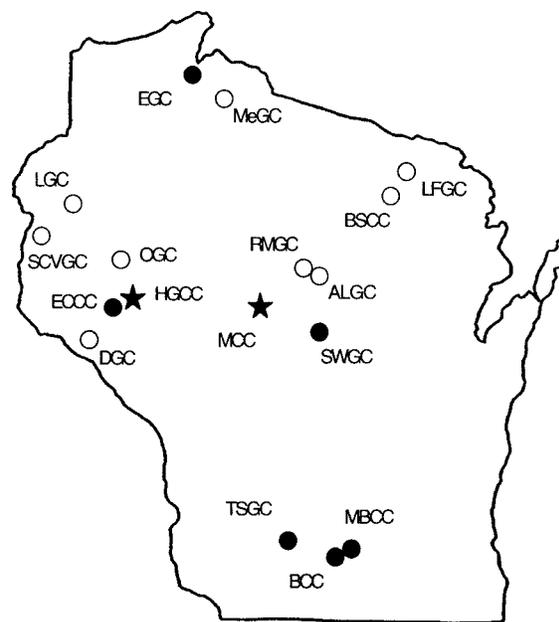


Fig. 1. Map of Wisconsin, showing locations of the 17 golf courses that served as sources of creeping bentgrass germplasm for the study of RAPD marker diversity. Open circles represent fairway collections, filled circles represent putting green collections, and stars represent both fairway and putting green collections. See Table 1 for golf course and city names.

RESULTS AND DISCUSSION

The 13 RAPD primers produced a total of 99 scorable bands, with size of amplified segments ranging from approximately 300 to 2000 bp. Frequencies of individual bands ranged from 3 to 97% with a mean of 53% and a median of 55%. Overall frequencies for the 99 bands were uniformly distributed across the range of values (amodal).

The clones from each golf course were selected for divergent snow mold reaction based on an artificial inoculation and incubation under simulated winter conditions (Wang et al., 2000). Mixed models analysis showed that the two multidimensional scales did not differ between "resistant" and "susceptible" classes of clones. Thus, there was no evidence that genetic variability, estimated from RAPD-derived genetic distances, was

Table 1. Names, locations, and abbreviations used for 17 Wisconsin golf courses that served as the sources of creeping bentgrass germplasm for the study of RAPD marker diversity (Fig. 1).

Name	Nearest city/town	Abbreviation	Type of collection
American Legion Golf Course	Wausau	ALGC	Fairway
Big Stone Country Club	Three Lakes	BSCC	Fairway
Blackhawk Country Club	Madison	BCC	Green
Durand Golf Course	Durand	DGC	Fairway
Eau Claire Country Club	Eau Claire	ECCC	Green
Elks Golf Course	Ashland	EGC	Green
Hillcrest Golf & Country Club	Altoona	HGCC	Fairway & Green
Lake Forest Golf Course	Eagle River	LFGC	Fairway
Luck Golf Course	Luck	LGC	Fairway
Maple Bluff Country Club	Madison	MBCC	Green
Marshfield Country Club	Marshfield	MCC	Fairway & Green
Mellen Golf Course	Mellen	MeGC	Fairway
Ojibwa Golf Course	Chippewa Falls	OGC	Fairway
Rib Mountain Golf Course	Wausau	RMGC	Fairway
SentryWorld Golf Course	Stevens Point	SWGCC	Green
St. Croix Valley Golf Course	Dresser	SCVGC	Fairway
The Springs Golf Course	Spring Green	TSGC	Green

associated with snow mold reaction phenotypes of these clones. Either the original screening for snow mold was unreliable, snow mold resistance in creeping bentgrass is highly polygenic, or the RAPD primers did not adequately saturate the creeping bentgrass genome. Because the clonal classifications for snow mold reaction were largely repeatable (Wang et al., 2000), one or both of the latter two explanations is more likely.

Mixed models analysis showed that turf types were significantly different for means of MDS1 ($P < 0.05$). Creeping bentgrass clones collected from putting greens tended to cluster at above-average values of MDS1, while clones collected from fairways were uniformly distributed throughout the range of values for MDS1, resulting in a relatively small number of clones with unique fairway-type RAPD profiles (Fig. 2). This is consistent with results from *P. annua*, which had RAPD marker profiles much more variable among fairway-collected clones than among green-collected clones (Sweeney and Danneberger, 1995). In the latter study, frequencies of three RAPD markers differed among fairway-collected clones ($P < 0.01$), but did not differ among green-collected clones ($P > 0.15$). Sweeney and Danneberger (1995) favored the explanation that fairway clones had diverged because of differential selection pressures. Because *P. annua* plants are generally annual and capable of producing seed several times throughout the growing season (Johnson et al., 1993), natural selection should create genetic changes in populations at a much faster rate than for creeping bentgrass. Because of lack of sexual recombination on golf courses, creeping bentgrass must rely strictly on mortality/survival differentials or immigration (introduction of new plants by seed or contamination) as a basis for genotypic change.

Creeping bentgrass clones collected from different golf courses were significantly different for means of both multidimensional scales ($P < 0.01$). For bentgrass clones collected from fairways, means among golf courses ranged from -0.31 ± 0.03 to 0.17 ± 0.03 for MDS1 and

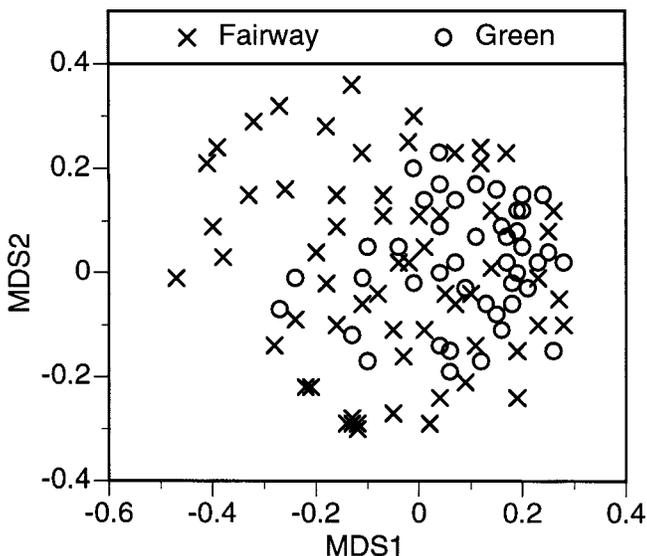


Fig. 2. Scatterplot of the first two multidimensional scales for 110 creeping bentgrass clones evaluated for 99 random amplified polymorphic DNA (RAPD) markers.

from -0.19 ± 0.01 to 0.24 ± 0.03 for MDS2. Together, the two multidimensional scales discriminated among fairway-collected bentgrass clones on the basis of geographic regions of Wisconsin (Fig. 1 and Fig. 3).

The 66 fairway-collected clones were visually organized into three nondiscrete clusters on the basis of the majority of clones within each of three regions: NE, NW, and NC (Fig. 3). The NE cluster contained 25 clones, 17 (68%) collected from golf courses in northeastern Wisconsin. This cluster contained 89% of the 19 clones collected on these five golf courses (ALGC, BSCC, LFGC, MeGC, and RMGC in Fig. 1). The NW cluster contained 30 clones, 23 (77%) collected from golf courses in northwestern Wisconsin. This cluster contained 92% of the 25 clones collected on these five golf courses (DGC, HGCC, LGC, OGC, and SCVGC in Fig. 1). The NC cluster contained 28 clones, 20 (71%) collected from the Marshfield Country Club (MCC in Fig. 1) in northcentral Wisconsin. This cluster contained 91% of the 22 clones collected at MCC, many of which are hidden in the two tight groups at the bottom of Fig. 3. These two groupings suggest that the MCC fairway collections contained some redundancy, possibly because of vegetative spread of a relatively small number of highly adapted clones. Overall, the origin of 60 out of 66 fairway clones (91%) was correctly identified by these three nondiscrete clusters. A chi-square test of independence between geographic source (NE, NC, and

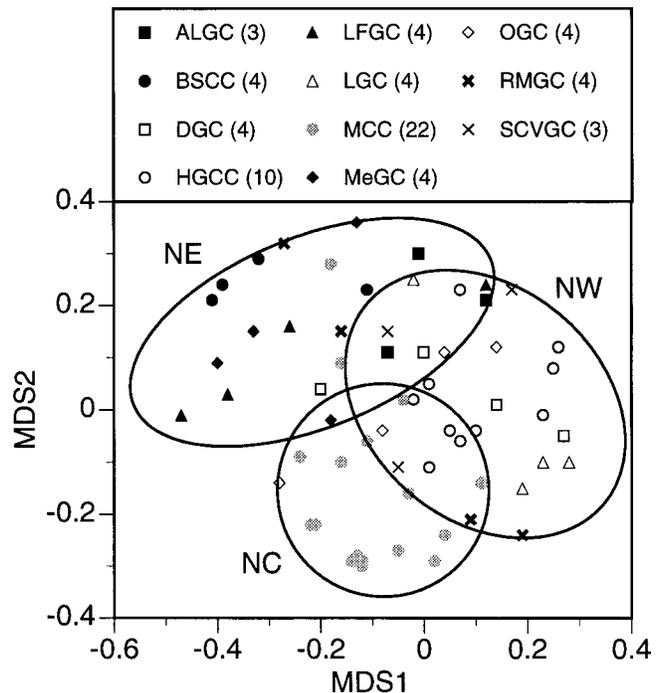


Fig. 3. Scatterplot of the first two multidimensional scales for 66 creeping bentgrass clones collected from fairways of 11 golf courses and evaluated for random amplified polymorphic DNA (RAPD) markers. Golf course abbreviations are identified in Table 1. Number of clones per golf course are indicated in parentheses. Nondiscrete clusters identify the majority of clones collected from northeastern (NE) Wisconsin golf courses (filled or bold symbols: ALGC, BSCC, LFGC, MeGC, and RMGC in Fig. 1), northwestern (NW) Wisconsin golf courses (open or non-bold symbols: DGC, HGCC, LGC, OGC, and SCVGC in Fig. 1), and one northcentral (NC) Wisconsin golf course (gray symbols: MCC in Fig. 1).

NW) and cluster composition had $P < 0.01$. Three clones were the most notable outliers: two clones from RMGC with extremely low values of MDS2 and high values of MDS1, and one clone from MCC with an unusually high value of MDS2.

The simplest explanation for genotypic differentiation of creeping bentgrass clones among these three regions of northern Wisconsin would be different sources of seed or vegetative propagules used to plant fairways of these old golf courses. It is highly possible that golf course architects and superintendents within a region shared information about seed sources and planted seed from identical or similar sources. Seed sources in the latter 19th and early 20th centuries were limited (Duich, 1985). Sources of "South German Bent," which contained a small amount of creeping bentgrass, were commonly used in Wisconsin before 1915. Later in the 20th century, natural and naturalized sources of creeping bentgrass from the Pacific Northwest and coastal regions of eastern North America were utilized (S.E. Warnke, 2002, personal communication), possibly resulting in differential seed sources among golf courses. Unfortunately, lack of records and imprecise information about germplasm sources used to establish these old golf courses prevent this hypothesis from being investigated. The genotypic differentiation within golf courses (Fig. 3) suggests that creeping bentgrass on any individual golf course did not arise from propagation of a single clonal cultivar.

Geographic or geologic information may explain part of the genetic differentiation among fairway clones of northern Wisconsin. Soil types of the three geographic regions (NE, NC, and NW) do not show consistent patterns. Golf courses in the NE region are located on soils that range from sandy loam to silt loam to clay loam, with a wide range of sand and silt contents, but all low to moderate clay content (10 to 30%). Golf courses in the NW region are located on soils with a narrower range from loamy sand to silt loam, generally with clay content less than 15%. The Marshfield Country Club (MCC) is located on a Withee silt loam (fine, loamy, mixed, superactive, frigid Aquic Glossudalfs) that tends toward the higher clay content for this series (30%). The Withee soil is characterized by a seasonally high water table and has consistently slow permeability (Bartelme, 1977).

Conversely, presettlement vegetation of Wisconsin corresponds closely to these three geographic regions. The five golf courses in northeastern Wisconsin are all sited on historic northern coniferous forest, dominated by *Pinus*, *Tsuga*, *Betula*, *Fagus*, and *Populus*. These soils tend to be highly leached and acidic. The five golf courses in northwestern Wisconsin are all sited on historic hardwood forest (dominated by *Quercus* or *Acer*), tallgrass prairie, or oak (*Quercus*) savanna. These soils tend to be near neutral in pH and have higher organic matter content than soils from northeastern Wisconsin. Marshfield lies in the transition zone between these two historic vegetation types, with a mixture of coniferous and hardwood forest as presettlement vegetation.

The pattern of RAPD variation among fairway-collected clones may have resulted from differential selection pressures related to soil characteristics that derived from differences in presettlement vegetation. Soil pH

and/or organic matter content (including moisture retention capacity) may have been the specific forces driving selection coefficients of creeping bentgrass populations. Creeping bentgrass populations that were planted on these golf courses likely contained large amounts of genetic variation, being largely derived from natural grasslands in Europe (Duich, 1985). Genetically heterogeneous populations of bentgrass species respond to soil stress factors by natural selection, resulting in changes in genotype and phenotype frequency. Natural selection pressures within bentgrass populations can be modified by heavy metals, salinity, or fertilizer (Casler et al., 1996; Helgadóttir and Snaydon, 1986). Genetic variation for tolerance to numerous heavy metals occurs in several *Agrostis* species, including creeping bentgrass (Archambault and Winterhalder, 1995; Bradshaw, 1952; Hogan et al., 1977; Walley et al., 1974; Wu et al., 1975). Soils with high concentrations of heavy metals are invariably extremely low in pH, suggesting that heavy-metal tolerant bentgrass plants are also highly acid tolerant and, by extension, there is genetic variation for acid tolerance within *Agrostis* species. Low calcium content, a characteristic of acidic forest soils, may also be a stress factor for creeping bentgrass (Kuo, 1993), driving selection coefficients on these soils.

Creeping bentgrass clones collected from putting greens did not generally vary among golf courses (Fig. 4). For bentgrass clones collected from greens, means among golf courses ranged from -0.03 ± 0.03 to 0.17 ± 0.03 for MDS1 and from -0.08 ± 0.03 to 0.12 ± 0.03 for MDS2. Most of the variation among creeping bentgrass clones collected from putting greens was observed within golf courses. It is possible that many of these clones derived from interseedings with 'Penncross'. The moderate to high degree of variability within Penncross

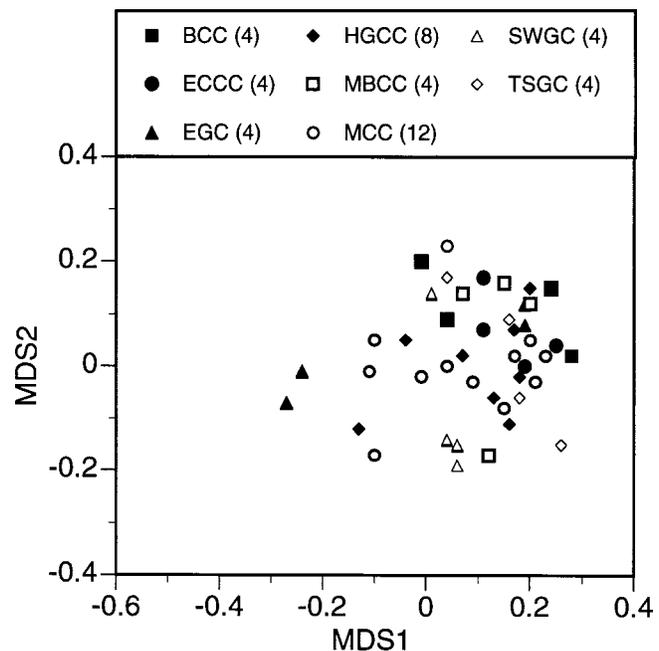


Fig. 4. Scatterplot of the first two multidimensional scales for 44 creeping bentgrass clones collected from putting greens of eight golf courses and evaluated for random amplified polymorphic DNA (RAPD) markers. Golf course abbreviations are identified in Table 1. Number of clones per golf course are indicated in parentheses.

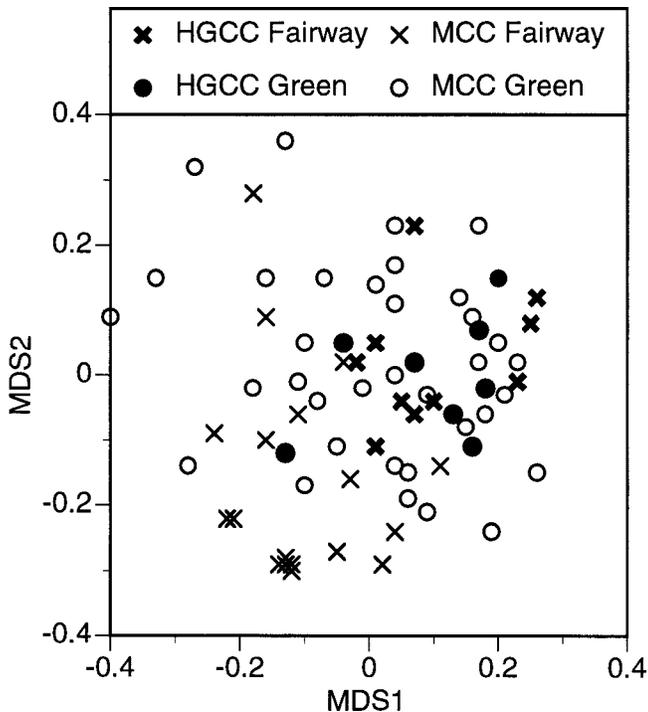


Fig. 5. Scatterplot of the first two multidimensional scales for 52 creeping bentgrass clones collected from Hillcrest Golf and Country Club (HGCC) or Marshfield Country Club (MCC) and evaluated for random amplified polymorphic DNA (RAPD) markers. The two tight clusters in the lower left corner contain several hidden data points with nearly identical values for both dimensions.

(Warnke et al., 1997) could cause considerable variation among bentgrass clones collected from putting greens within a golf course. Uniformity among golf courses in putting green management may reduce the opportunity for course-specific natural selection, resulting in mortality-survival of similar genotypes across golf courses in northern Wisconsin. The universal practice of topdressing putting greens may also contribute toward selection of more uniform genotypes within narrow geographic regions such as the state of Wisconsin. For these northern Wisconsin golf courses, putting greens were much more phenotypically (visually) uniform than fairways across golf courses. Mowing height and contamination by *P. annua* were similar on putting greens across golf courses, while fairways of different golf courses differed widely in both mowing height, contamination by *P. annua*, and presence of other perennial grasses (e.g., *Poa* spp, *Festuca* spp, and *Lolium perenne* L.).

Table 2. Means and *t* tests of multidimensional scales (MDS1 and MDS2) for fairway and green creeping bentgrass collections from Hillcrest Golf and Country Club (HGCC) and Marshfield Country Club (MCC).

Golf course	Turf type	n	MDS1	MDS2
Hillcrest	Fairway	10	0.107	0.023
Hillcrest	Green	8	0.082	0.001
Marshfield	Fairway	22	-0.098	-0.186
Marshfield	Green	12	0.064	0.004
<i>t</i> -test comparisons				<i>P</i> -values
Hillcrest	Fairway vs. Green		0.69	0.84
Marshfield	Fairway vs. Green		<0.01	<0.01
HGCC vs. MCC	Fairway		<0.01	<0.01
HGCC vs. MCC	Green		0.77	0.98

Creeping bentgrass clones were collected from both fairway and putting green environments on two golf courses, Hillcrest Golf and Country Club and Marshfield Country Club (Fig. 1). Clones collected from fairways and putting greens at HGCC were similar to each other on the basis of both multidimensional scales (Fig. 5). Both the range of variation (Fig. 5) and the means (Table 2) were similar for these two groups of clones. Conversely, at MCC, clones collected from fairways and putting greens were significantly different from each other (Table 2) and there were numerous clones collected on MCC putting greens that showed marker profiles unique from clones collected on MCC fairways. Clones collected from putting greens on HGCC and MCC did not differ from each other, on average, but MCC-green clones were much more variable than HGCC-green clones. Conversely, fairway-collected clones from HGCC and MCC showed almost complete separation (Fig. 5), including statistical significance of the mean difference between golf courses for both multidimensional scales (Table 2).

A chi-square test for independence of each individual RAPD marker was performed for the four clonal groups reported in Table 2. Eighteen of 99 markers showed lack of independence at *P* < 0.05, indicating differences in RAPD marker frequencies among these four clonal groups (Table 3). For 16 of these 18 markers, MCC-fairway clones had the most unusual frequency, with half of the 16 deviations showing a higher frequency of the marker. Several of the markers showed surprisingly large differences in frequency among groups. Marker F13.1250 was observed only within the MCC-fairway group, at a frequency of 0.64. Markers D2.550 and O6.1150 had a frequency of 0.64 in the MCC-fairway group, but only one band among the 30 clones in the other three groups (overall frequency = 0.03). Markers D20.1650 and P8.600 had a frequency of 0.73 in the MCC-fairway group, but only two bands or one band, respectively, among the 30 clones in the other three groups (overall frequencies = 0.07 and 0.03, respectively). Markers D2.1200 and G11.850 had no bands present among the 22 MCC-fairway clones, while marker Y9.650 had one band and marker E14.550 had two bands in the MCC-fairway group.

Table 3. Frequencies of 18 RAPD markers within four groups of creeping bentgrass clones collected from fairways or putting greens of Hillcrest Golf and Country Club (HGCC) or Marshfield Country Club (MCC).

Primer	Size (bp)	HGCC Fairway	HGCC Green	MCC Fairway	MCC Green	Chi-square P-value
D2	350	0.80	0.13	0.14	0.42	0.012
D2	500	0.90	0.88	0.14	0.92	0.008
D2	550	0.00	0.00	0.64	0.08	<0.001
D2	1100	0.00	0.13	0.50	0.08	0.012
D20	720	0.20	0.38	0.68	0.08	0.045
D20	1650	0.10	0.00	0.73	0.08	<0.001
E14	550	0.90	0.50	0.09	0.50	0.014
F13	520	0.00	0.13	0.00	0.42	0.022
F13	1225	0.00	0.00	0.27	0.00	0.013
F13	1250	0.00	0.00	0.64	0.00	<0.001
F13	1500	1.00	0.75	0.23	0.58	0.038
G11	650	0.30	0.50	0.14	0.83	0.016
G11	850	0.20	0.88	0.00	0.58	0.002
O6	1150	0.00	0.00	0.64	0.08	<0.001
O15	875	0.10	0.13	0.68	0.33	0.002
P8	600	0.00	0.00	0.73	0.08	<0.001
Y5	775	0.90	1.00	0.27	0.92	0.040
Y9	650	0.60	0.88	0.05	1.00	0.001

The fairways at Marshfield Country Club are an eclectic mixture of *P. annua*, fine fescues (*Festuca* spp. subgenus and section *Festuca*), Kentucky bluegrass (*P. pratensis* L.), and occasional creeping bentgrass plants. Fairways have been mown at approximately a 2-cm height for over 25 yr. Fairways are noticeably patchy, with large clones, many over 2-m diam, of several species. At the time of collection, fairways of the original nine holes at MCC were largely a result of 75 yr of natural selection. Golf course records indicate that fairways of the original nine holes have not been seeded since their establishment. Thus, it seems likely that the unique RAPD marker profiles of most MCC-fairway clones are a result of 75 yr of natural selection. Although there are many confounding factors among the golf courses in this experiment, it seems likely that the long-term low mowing height at MCC and the poorly drained soil have been important factors guiding selection pressures within these fairways. The predominance of large and dense fine fescue patches is one other highly unique aspect to fairways at MCC, possibly creating a unique aspect to the natural selection pressure on creeping bentgrass plants. Grasses, including *Festuca* spp., may interfere with growth of other species, and possibly each other, by leaching of phenolic acids from decaying organic matter (Norrington-Davies and Buckeridge, 1994). It is also possible that the original seed source used to establish MCC was unique among the 17 golf courses in this study, but that is unlikely due to limited bentgrass seed sources available in 1922 (Duich, 1985) and to the observation that MCC-green clones were not particularly unique, relative to green-collected clones from other golf courses (Fig. 4).

In conclusion, RAPD markers were used successfully to demonstrate the existence of genetic polymorphisms within a population of 110 creeping bentgrass plants. For fairway-collected plants, much of this variation was associated with different golf courses which belonged to three regions of northern Wisconsin. Variation in RAPD markers among these regions were associated with presettlement vegetation zones, which may have influenced natural selection pressures on creeping bentgrass populations through variation in soil properties. Plants collected on putting greens showed little differentiation among golf courses, suggesting that homogeneity of the putting-green environment among golf courses reduced the potential for differential selection pressures. There was some differentiation between fairway and green clones on one golf course, possibly resulting from extreme selection pressures on the fairway population of creeping bentgrass. The variation in RAPD marker phenotypes and their apparent associations with geographic, edaphic, and cultural history suggest that the RAPD technique is an excellent technique for assessing variability among creeping bentgrass genotypes.

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