Classification of Kentucky Bluegrass (*Poa pratensis* L.) Cultivars and Accessions Based on Microsatellite (Simple Sequence Repeat) Markers

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Abstract. Kentucky bluegrass (*Poa pratensis* L.) is an important facultative apomictic temperate perennial grass species used for both forage and cultivated turf. Through apomixis, this species is able to propagate diverse and odd ploidy levels, resulting in many genetically distinct phenotypes. A wide range of diverse cultivars and accessions of kentucky bluegrass have been previously characterized based on pedigree, common turf performance, and morphological characteristics to create a kentucky bluegrass cultivar classification system. The objectives of the current study were to assess the amount of genetic divergence among kentucky bluegrass cultivars, experimental selections, and plant collections and revise/update the original pedigree, turf performance, and morphological characteristics kentucky bluegrass classification system using recently described kentucky bluegrass microsatellite [simple sequence repeat (SSR)] markers. In this study, 247 kentucky bluegrass cultivars, experimental selections, and collections were genotyped using 25 SSR markers. SSR markers showed a strong correlation between genetic relatedness as assessed by molecular markers and the original kentucky bluegrass classification system and also provided justification for a revision/update of the classification system. Traditional classification types that were supported by the current SSR analysis include BVMG, Compact, Compact-America, Julia, Mid-Atlantic, Midnight, and Shamrock types. Newly proposed classification types included Cynthia, Jefferson/Washington, Limousine, P-105, Sydspout, and three Eurasian types. The majority of cultivars, experimental selections, and collections were uniquely identified with the current set of SSR markers. Genetic relationships of individuals as assessed by SSR markers closely matched known pedigrees. The current set of SSR markers can be used to rapidly genotype and assign new cultivars/accessions to kentucky bluegrass classification types and assess genetic relatedness among individuals and should be considered for use in a kentucky bluegrass plant variety protection program.

Kentucky bluegrass (*Poa pratensis* L.) is a facultative apomictic cool-season perennial grass species widely used for forage and turf in the United States and Canada (Huff, 2003, 2010). Although sexuality in kentucky bluegrass can be variable, the species reproduces mainly through apomixis resulting in a high percentage of offspring that are genetically identical to the mother plant (Huff and Bara, 1993; Mazzucato et al., 1996). The chromosome number of kentucky bluegrass is also variable with reports of both polyploidy and aneuploidy ranging from $2n = 28$ to 154 (Akerberg, 1939; Grazi et al., 1961; Huff,

2003; Love and Love, 1975; Meyer and Funk, 1989; Muntzing, 1933; Nielson, 1946; Tinney, 1940). This complex polyploidy and aneuploidy can complicate kentucky bluegrass breeding efforts; however, apomixis allows this species to propagate diverse and odd ploidy levels, which results in many genetically distinct individuals (Huff, 2010). This high level of diversity has allowed for the development of numerous unique kentucky bluegrass cultivars directly selected from promising apomictic plants from natural populations (Bashaw and Funk, 1987; Bonos et al., 2000; Pepin and Funk, 1971). Additional cultivars have been developed from the improvement of intraspecific hybridization techniques (Pepin and Funk, 1971).

A classification system, based on varying combinations of pedigree information, common turf performance characteristics, and morphological traits, was developed to characterize the large number of diverse kentucky bluegrass cultivars and accessions (Bara et al.,

1993; Bonos et al., 2000; Murphy et al., 1997; Shortell et al., 2009). This classification system was developed to group cultivars and accessions into various classification types to provide an overview of the similarities and differences between cultivars and cultivar types and as a guide to help turf managers develop blends of cultivars that are genetically diverse yet still uniform in morphological and performance characteristics (Shortell et al., 2009). A detailed discussion of the original classification scheme, the description of the classification types as well as subsequent updates are available in previous publications (Bara et al., 1993; Bonos et al., 2000; Murphy et al., 1995, 1997; Shortell et al., 2004, 2009). A brief summary and description of the most recent version of the Pedigree, Turf performance, and Morphological (PTM) kentucky bluegrass classification system (adapted from Shortell et al., 2009) is shown in Supplementary Table 1.

The PTM kentucky bluegrass classification system has been useful to turf researchers and turf managers; however, the use of morphological traits and cultivar performance characteristics to distinguish varieties has several limitations. Morphological characterization requires large reference collections for comparative analyses, oftentimes with a limited number of morphological descriptors available to distinguish cultivars. Additionally, assessment of morphological and cultivar performance characteristics requires field-based measurements of large numbers of samples and replicates with the potential for the expression of these traits to be influenced by environmental conditions (Giancola et al., 2002; Ibanez et al., 2009; Kwon et al., 2005; Lombard et al., 2000; Roldan-Ruiz et al., 2001). Finally, common morphological or performance characteristics may not necessarily equate to genetic relatedness. As a result of these drawbacks, numerous researchers have proposed using molecular markers for variety discrimination, genetic diversity studies, and Plant Variety Protection (Cooke and Reeves, 2003; Gunjaca et al., 2008; Ibanez et al., 2009; Tommasini et al., 2003). Molecular markers offer a number of advantages over morphological characters and the assessment of common performance characteristics including a high degree of polymorphism, ease of scoring, a large potential number of characters for discrimination, and the fact that molecular markers are unaffected by environmental conditions (Lombard et al., 2001; Smykal et al., 2008; Tommasini et al., 2003).

Kentucky bluegrass cultivars and accessions have previously been characterized using random amplified polymorphic DNA (RAPD) markers (Curley and Jung, 2004; Huff, 2001; Johnson et al., 2002); however, these studies either did not attempt to correlate (Huff, 2001; Johnson et al., 2002) or failed to find a strong correlation (Curley and Jung, 2004) between genetic diversity assessed by RAPD markers and the PTM kentucky bluegrass classification system. In the current study we used microsatellite, or SSR, markers to study the genetic relationships of 247 kentucky

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bluegrass cultivars and accessions. The specific objectives of this study were to assess the amount of genetic divergence between kentucky bluegrass cultivars, experimental selections, and plant collections, and revise/update the original PTM kentucky bluegrass classification system using recently described kentucky bluegrass SSR markers (Honig et al., 2010).

Materials and Methods

Simple sequence repeat markers and genotyping

The development of 88 kentucky bluegrass SSR markers was previously described by Honig et al. (2010). Primer sequences, characteristics of the SSR repeat motifs, number of alleles per SSR marker, and polymorphism information content (PIC) of the SSR marker alleles for all 88 SSR markers are available in Honig et al. (2010). In the current study, 25 SSR markers from Honig et al. (2010) (Supplementary Table 2), with the highest average PIC values across all alleles in a given SSR marker, were used to genotype a new set of 247 kentucky bluegrass cultivars, accessions, and collections and one wild ecotype of *Poa annua* L. Although the calculation of PIC is not strictly accurate in

the case of polyploidy, it is still a useful method for discriminating appropriate primers. To minimize polymerase chain reaction (PCR) artifacts, only alleles with a PIC value greater than 0.15, for any SSR marker, were used in the current analysis.

Plant genomic DNA was isolated from the 247 entries of the current study using Sigma GenElute Plant Genomic DNA Miniprep Kit (St. Louis, MO) according to the manufacturer's instructions. PCR genotyping reactions followed the protocol outlined in Honig et al. (2010). PCR products were run on an ABI 3130xl capillary electrophoresis genetic analyzer (Applied Biosystems, Foster City, CA), sized using the LIZ 500(-250) size standard, and analyzed using Genemapper 3.7.

SSR markers can generate codominant data; however, problems may arise during allele scoring of polyploid individuals because of difficulties in assigning alleles and inferring allelic dosage over two or more homoeoloci (George et al., 2006; Liao et al., 2008; Markwith et al., 2006; Saltonstall, 2003); thus, DNA banding patterns at any given locus are more accurately scored as "allele phenotypes" (Becher et al., 2000). Individual alleles of the 25 SSR markers used in the current study of polyploid kentucky bluegrass were treated as "allele phenotypes"

and scored as dominant markers to create a binary data matrix (band absence = 0; band presence = 1).

Plant material

Two hundred forty-seven kentucky bluegrass cultivars, experimental selections, and collections were evaluated in this study and are listed in Table 1 according to the newly revised/updated version of the kentucky bluegrass classification system based on SSR markers. A duplicate list is provided in Supplementary Table 3, organized according to previous versions of the PTM kentucky bluegrass classification system, with particular emphasis given to the most recent report by Shortell et al. (2009). Forty-eight single seedlings or single tillers of each entry were transplanted into 48-cell flats (90 cm × 45 cm) and allowed to establish in the greenhouse. Plants were screened in the greenhouse for apomixis, and off-types (aberrant plants) were discarded. All remaining plants from each entry were then established in various spaced-plant nurseries at the Rutgers University Plant Biology and Pathology Research and Extension Farm at Adelphia, NJ, in Apr. 2003 through Apr. 2008 on a well-drained Freehold sandy loam (fine-loamy, mixed, mesic, Typic Hapludult). Plants were maintained in the field, in nursery

Table 1. Kentucky bluegrass classification system based on a combination of pedigree, unweighted pair group method using arithmetic average cluster analysis, and model-based clustering analysis of microsatellite (simple sequence repeat) data.

Classification type	Cultivar or selection
BVMG	Abbey, Baron, Bartitia, Envicta, Goldrush, Marquis, Raven, DLF769036 ^z
Compact	Alpine, Ascot, Baronette, Baronia, Blackburg II, Blackstone, ^y BlueSapphire, Hallmark, Hampton, Rita, Wildwood, NA-K992, ^z PST-B4-246, ^z PST-B5-125, ^z PST-H5-35, ^z PST-YorkHarbor4, ^x SRX2114 ^z
Compact-America	Apollo, Arrow, Barnique, Baroness, Bedazzeled, Bluemax, BlueRidge, Bluetastic, Bordeaux, Boutique, Brilliant, Casablanca, Delight, Diva, Durham, Dynamo, Glenmont, Goldstar, Katie, Kingfisher, Langara, Mallard, Mercury, MonteCarlo, Moonlight, Moonshadow, Royale, Royce, Shiraz, Showcase, Sonoma, SR 2284, Unique, Valor, VoyagerII, A00-1400, ^z A00-430, ^z A00-99, ^z A03-132, ^z A03-141, ^z A03-335, ^z A03-66, ^z A03-77, ^z A05-314, ^z A05-322, ^z A05-332, ^z A05-335, ^z A05-336, ^z A06-363, ^z A06-739, ^z A97-1294, ^z A97-1303, ^z A97-1328, ^z A97-1409, ^z A98-365, ^z A98-407, ^z A99-2427, ^z A99-2678, ^z A99-3116, ^z A99-3122, ^z A99-3127, ^z A99-3245, ^z A99-523, ^z BAR Pp 0566, ^z BAR Pp 0573, ^z Bd99-2103, ^z DLF769037, ^z H02-99 × H98-767, ^z H92-203, ^z PST-222, ^z PST-604, ^z PST-H6-150, ^z SIA96386 ^z
Cynthia	Bodacious, Boomerang, Cheetah, PpH7907, ^z PpH7929, ^z SRX27921 ^z
Eurasian1	A04-1375PargasFin, ^x A04-1470SiauliaiLith, ^x A04-1474SiauliaiLith, ^x A04-1484KursenaiLith, ^x A04-1547SpitrenaiLith, ^x A04-1569MoletLith, ^x A98-3366RekorvoPol, ^x A98-3384AnimalPKSwe, ^x H02-608UlricehamnSwe, ^x H04-535KorpoFin, ^x H07-697NorrkopingSwe ^x
Eurasian2	Barzan, Bluenight, Chelsea, A04-1342KustaviFin, ^x A04-1354PetsmoFin, ^x A04-1384AristoFin, ^x A05-847ColdePortFra, ^x A98-3369TeologPol, ^x GO9LM9, ^z H04-376KaisterFin, ^x U2998Uz ^z
Eurasian3	Eagleton, Jewel, Kenblue, Limerick, Wellington, A04-1271PorkalaFin, ^x A04-1272PorkalaFin, ^x A04-1415KrudonisLith, ^x A04-1423BuktaLith, ^x A04-383PojarataRom, ^x A04-387PasulprislopRom, ^x A04-394PetroojaniRom, ^x A96-742, ^z A98-183, ^z A98-3297OgrodzieniecPol, ^x A98-3322ZyrardowPol, ^x A98-3323ZychlinPol, ^x DLF769032, ^z DLF769034, ^z H01-847SomcutaMareRom, ^x H01-894HongyanCh, ^x H94-305, ^z H99-1722Uz ^z
Jefferson/Wash.	Jefferson, Washington
Julia	Bariris, Baritone, Julia, Pick 453, ^z Rampart, Ulysses, A04-1283HankoFin, ^x A05-894VillaboneSp, ^x CVB20631, ^z H01-912FrankfurtGer, ^x H92-558 ^z
Limousine	Freedom II, Julius, Limousine, A00-4083PoriFin, ^x PpH6366, ^z SRX26351 ^z
Mid.-Atlantic	Appalachian, Cabernet, Starburst, A00-1254, A03-37, ^z A03-6, ^z H03-180xA99-2874, ^z PST-161 ^z
Midnight	Alexa, Arcadia, Award, Awesome, Barrister, Beyond, Bluestone, Blue Velvet, Chicago II, Courtyard, Everest, EverGlade, Excursion, Freedom III, FrontPage, Ginney, Impact, Liberator, Midnight, Midnight II, NuDestiny, NuGlade, Odyssey, Perfection, Quantum Leap, Rambo, Rugby II, Skye, Total Eclipse, Tsunami
P-105	Avid, P-105, A95-410 ^z
Shamrock	Aries, Brooklawn, Lakeshore, Mongoose, Moonshine, Shamrock, A01-701, ^z A05-313, ^z A93-201, ^z A97-1799, ^z A98-1028, ^z Bd0384 ^z
Sydspout	Allure, Chateau, Coventry, Fairfax, Lily, Serene, ^w A04-1315FinstromFin, ^x B5144, ^z B543, ^z B545, ^z H04-389SodderRuderFin, ^x SRXQG245 ^z
Other	Misty, NorthStar, 99AN53, ^z A04-1268PorkalaFin, ^x A04-1347LepainenFin, ^x A04-1427JurbarkasLith, ^x A94MH94, ^z A97-857, ^z A98-3320PilicaWarkaPol, ^x A98-3367RekorvoPol, ^x HV140, ^z H01-804ComarnicRom, ^x H02-603UlricehamnSwe, ^x H04-390AristoFin ^x

^zDenotes experimental selection.

^yOriginal 'Blackstone'.

^xDenotes collection (Ch = China; Fin = Finland; Fra = France; Ger = Germany; Lith = Lithuania; Pol = Poland; Rom = Romania; Sp = Spain; Swe = Sweden; Uz = Uzbekistan).

^wOriginal 'Serene'.

rows, for a minimum of 2 years for further apomixis screening, and all off-types were discarded. This screening method was used to ensure that only the repeating apomictic mother clone, as intended by the breeder, was chosen to represent the cultivar, selection, or collection. A single transplant of each entry, representing the repeating apomictic mother clone, was established in the greenhouse and maintained for DNA extraction in the laboratory. Additional replication (beyond the initial field apomixis screening) was achieved by including multiple entries from each of the traditional kentucky bluegrass classification types. This methodology was chosen as a means to primarily assess population (kentucky bluegrass classification type) -level differences among the kentucky bluegrass cultivars, selections, and collections. An additional single entry of a wild ecotype of annual bluegrass, collected from the Rutgers University Plant Biology and Pathology Research and Extension Farm at Adelphia, NJ, was included in this study as an outgroup for the phylogenetic analysis.

Data analysis

Genetic similarity and unweighted pair group method using arithmetic average clustering. SSR marker allele phenotypes (Supplementary Table 2) were scored for presence/absence and assembled into a binary data matrix. Genetic similarity and clustering methods were performed using the Numerical Taxonomy System, NTSYSpc Version 2.21m (Rohlf, 2011). Pairwise comparisons of the proportion of shared alleles between individual genotypes (plants) were determined by the Jaccard (1908) coefficient, and ordered into a similarity matrix using the SIMQUAL module. Cluster analysis was generated from the similarity matrix by the unweighted pair group method using arithmetic averages (UPGMA) algorithm option in the SAHN module to create a dendrogram. The COPH (cophenetic values as a matrix) and MXCOMP (Mantel test statistic) modules were used to test the goodness of fit between the UPGMA dendrogram and the original similarity matrix (cophenetic correlation) using 999 permutations for the matrix correlations. The data set was resampled 1000 times using the RESAMPLE module with the bootstrap option. Bootstrap values for the dendrogram were calculated using the CONSENS module using the majority rule method with a minimum support value set to 0.500.

Model-based clustering. Model-based clustering analysis using a Bayesian algorithm was applied to infer the genetic structure and to define the number of clusters (kentucky bluegrass classification types/populations) in the data set using the computer program STRUCTURE 2.3.3 (Falush et al., 2003; Pritchard et al., 2000). In this analysis, genotyped individuals are allocated to a predetermined number of clusters/populations (K), where (K) is chosen in advance and can be varied across different runs. Plants can have membership in several clusters with the membership coefficient of individuals equaling 1.0 across clusters. This method uses a Markov

chain Monte Carlo algorithm to estimate the allele frequencies in each of the (K) clusters/populations and, for each individual, the proportion of its genome derived from each cluster/population (q_i). We assumed that all loci were independent and in linkage equilibrium. An admixture ancestry model was used and allele frequencies were correlated with a burnin length of 20,000 iterations followed by 50,000 run iterations at each (K). For other settings, program defaults were used, and no prior population information was assumed to define the clusters (K). (K) values were set from two to 25 with 20 replicate runs at each value of (K). The most parsimonious number of clusters/populations was identified using the maximal value of the average estimated log probability $\Pr(X|K)$ output from 20 independent runs at each (K) value (Pritchard et al., 2000). The wild ecotype of *P. annua* was excluded from this analysis.

Kentucky bluegrass classification type revision. Based on a combination of known pedigrees, the results of the UPGMA cluster analysis, and the results of the model-based clustering analysis, we assigned all 247 individuals of the current study into 16 revised kentucky bluegrass classification types and one outgroup (*P. annua*). The 247 kentucky bluegrass cultivars, experimental selections, and collections are listed in Table 1 according to this newly revised/updated classification scheme. This newly revised classification scheme is, therefore, a representation of genetic relatedness based on the current SSR marker data and known pedigrees and differs from the previous PTM kentucky bluegrass classification system (Supplementary Table 3) that was based on pedigree, turf performance data, and morphological characters.

Analysis of molecular variation. The revised/updated classification types, exactly as described in Table 1, were treated as populations in an analysis of molecular variation (AMOVA), performed in GenAlEx 6 (Peakall and Smouse, 2006), to examine the distribution of variation among and within populations (classification types) and to assess the interpopulation pairwise genetic distance (Φ_{ST}). Statistical significance was tested by random permutation with the number of permutations set to 999. Kentucky bluegrass entries classified as "other" type (Table 1) and the wild ecotype of *P. annua* were excluded from this analysis.

Results

Simple sequence repeat markers. The 25 SSR markers used in the current study produced 401 allele phenotypes in the 247 entries (Supplementary Table 2). The number of allele phenotypes for individual SSR markers ranged from seven to 25 with an average of 16.04 allele phenotypes per SSR marker. Additional details about individual SSR markers can be found in Honig et al. (2010).

Unweighted pair group method using arithmetic average clustering. The results of the UPGMA clustering analysis are presented in Fig. 1, Panel a. The Mantel test for the goodness of fit between the UPGMA dendro-

gram and the original similarity matrix (cophenetic correlation) was $r = 0.91$, where an r value above 0.90 indicates a very good fit (Rohlf, 2011, user documentation). Bootstrap values for the delineation of the major kentucky bluegrass classification types are shown, whereas additional bootstrap values are excluded for clarity of the figure (Fig. 1, Panel a). Cultivars and accessions in the UPGMA diagram are color-coded according to a combination of known pedigrees and the newly revised SSR classification type assignment.

The UPGMA clustering analysis grouped the kentucky bluegrass entries into several distinct classification types. Bootstrap support exists for the delineation of the traditional kentucky bluegrass types Midnight (0.971), BVMG (1.000), Shamrock (1.000), Julia (0.901), Compact-America (0.799), and Mid-Atlantic types (0.805) (Fig. 1, Panel a). The cultivars Bodacious, Boomerang, and Cheetah as well as the experimental selections SRX27921, PpH7907, and PpH7929 were part of a supported group in (Fig. 1A, Panel a). The pedigrees of these entries can all trace, in part, back to the cultivar Cynthia (Supplementary Table 4). Bootstrap support (0.978; Fig. 1A, Panel a) and unique morphological characteristics (e.g., lower seed yield compared with BVMG type) provide justification for a new Cynthia classification type. The cultivars Allure, Fairfax, and Serene as well as the experimental selections B545 and B543 were part of a bootstrap-supported (1.000) group at the bottom of Fig. 1A, Panel a, and the top of Fig. 1B, Panel a. The pedigrees of these entries can all trace, in part, back to the cultivar Sydsport (Supplementary Table 4) providing justification for a new Sydsport classification type. The majority of European as well as a few Asian plant collections formed three supported clusters in the UPGMA analysis (Fig. 1B–1C, Panel a), providing justification for newly defined classification types referred to as the Eurasian types. Additional new types with bootstrap support included Limousine (0.997) and P-105 (0.992) (Fig. 1B, Panel a and supporting breeding history information in Supplementary Table 4). Cultivars that had previously been referred to as Compact-type cultivars were split into two supported clusters: one cluster containing the cultivars Alpine, Blue Sapphire, Blackstone (Rose-Fricker et al., 2002), and Hampton (Fig. 1B, Panel a) and the other comprised of a number of cultivars and experimental selections closely related to the Compact-America-type cultivars (Fig. 1D, Panel a). A final potential supported new classification type was the Jefferson/Washington type (1.000) (Fig. 1B, Panel a); however, this current classification type would only be comprised of two cultivars in the current data set. Fourteen entries that have low bootstrap support, and consequently did not strongly cluster with defined classification groups, were classified as "other" type (Table 1).

The three clusters of Eurasian collections and cultivars were basal, being most closely related to the outgroup, *P. annua* (Fig. 1, Panel a). The remainder of the groups and cultivars, which were predominantly developed

Fig. 1. Unweighted pair group method using arithmetic average (UPGMA) and model-based clustering analysis of 247 Kentucky bluegrass cultivars, experimental selections, collections, and one outgroup entry using 25 microsatellite [simple sequence repeat (SSR)] markers. (**Panel a**) Diagram is a UPGMA dendrogram based on the average Jaccard similarity of 401 SSR alleles between individuals, color-coded according to known pedigrees. Black color indicates unknown pedigree. Bootstrap values (based on 1000 replicate runs) for major groupings are shown in parenthesis. (**Panel b**) Estimated population (Kentucky bluegrass classification type) structure output from STRUCTURE 2.3.3 for Kentucky bluegrass cultivars, experimental selections, and collections using 25 microsatellite (SSR) markers. Each individual is represented by a horizontal colored line, which can be partitioned into (*K*) segments that represent the individual's estimated membership fractions in (*K*) clusters. Color coding is based on the output from STRUCTURE 2.3.3. Groupings of cultivars from this model-based clustering analysis most closely resembled pedigree relationships and the groupings delineated in the UPGMA dendrogram at (*K*) = 14.

Fig. 1. (Continued).

from U.S. breeding programs, appeared to be derived from the more basal Eurasian cultivars. These derived classification types appeared to form two distinct larger groupings: one group

comprised of the Midnight, BVMG, Cynthia, Sydsport, Shamrock, Limousine, and P-105 classification types (as well as the potential Jefferson/Washington classification type)

(Fig. 1A–B, Panel a) and the other large group comprised of the Mid-Atlantic, Compact, and Compact-America classification types (Fig. 1C–D, Panel a).

Awesome (Brede, 2011), and Award (Brede, 2001b); the second consisted of the cultivars Tsunami (Brede, 2004a), Alexa (Brede, 2006a), Everest (Brede, 2006b), Freedom III

(Brede, 2006c), and Beyond (Brede, 2004b); and the third consisted of the cultivars Excursion (Brede, 2004c) and Barrister (Brede, 2006d) (Fig. 1A, Panel a).

Model-based clustering. The results of the model-based clustering (STRUCTURE) analysis are presented in Fig. 1, Panel b. Color coding is separated based on the groupings produced from the STRUCTURE output. The maximal value for the first plateau of the average estimated log probability $\Pr(X|K)$ [used to identify the most parsimonious number of clusters/populations (K)] from 20 independent runs at each (K) occurred at (K) = 14 (Supplementary Fig. 1). This (K) value very closely matched known pedigree relationships (Supplementary Table 4) and the major kentucky bluegrass classification groupings delineated in the UPGMA dendrogram (Fig. 1, Panel a). Other values of (K), particularly those at (K) = 11, 12, 13, 15, 16, and 17, were also considered; however, the biological interpretation (congruence with pedigree information and the UPGMA analysis) of the current data were most appropriate at (K) = 14 (Pritchard et al., 2000). At (K) = 14, clusters identified by STRUCTURE were very closely matched to pedigree (Supplementary Table 4) and clusters in the UPGMA dendrogram (Fig. 1, Panel a) for the Midnight, BVMG, Cynthia, Sydsport, Shamrock, Limousine, P-105, Julia, Compact-America, and Mid-Atlantic classification types. The STRUCTURE analysis split the Eurasian classification type into three distinct clusters (which were also supported by the UPGMA clustering analysis) (Fig. 1B–C). A number of cultivars and accessions that had been traditionally classified as Compact cultivars were found to have highly diverse membership in multiple (K) clusters. This included the cultivars Alpine, Blue Sapphire, Blackstone, and Hampton (Fig. 1B, Panel b) as well as the cultivars Blacksburg II, Rita, and Ascot and the experimental selections PST-H5-35 and NA-K992 (Fig. 1D, Panel b). The STRUCTURE analysis at (K) = 14 did not uniquely identify the Jefferson/Washington classification type, as identified in the UPGMA clustering analysis (bootstrap value 1.000; Fig. 1B). Entries defined as “other” type (Table 1) exhibited a combination of both high population level admixture in the STRUCTURE analysis and low bootstrap support in the UPGMA analysis (Fig. 1).

In some instances, the STRUCTURE analysis (Fig. 1, Panel b) provides additional detail not captured in the UPGMA analysis (Fig. 1, Panel a), specifically in relation to interbreeding or admixture between classification types. This was evident in some of the recognizable “hybrid” cultivars or selections exhibiting population-level admixture, where the admixture pattern matched known pedigree records (Supplementary Table 4). For example, ‘Chicago II’ (Brede, 2004d) (Fig. 1A, Panel b) was a cross between ‘Midnight’ (Meyer et al., 1984) $\times$ ‘Limousine’ (Supplementary Table 4); the STRUCTURE analysis showed admixture between these two classification types for

this cultivar (Fig. 1A, Panel b). Similar results, where STRUCTURE admixture accurately reflected known pedigrees (Supplementary Table 4), were observed for Bd0384, A05-313, SRX26351, ‘Freedom II’ (Brede, 2004e), Alpine (Fig. 1B, Panel b); A98-183 (Fig. 1C, Panel b); A00-1400, ‘Royale’, ‘Diva’, ‘Sonoma’ (Ford et al., 2004), A03-132, ‘Durham’, ‘Shiraz’, A03-66, Bd992103, A99-2427, PST-604, A00-1254, and A03-37 (Fig. 1D, Panel b).

In some instances, lower admixture vs. higher admixture in individual entries in the STRUCTURE analysis (Fig. 1, Panel b) helped to visually clarify relationships between major classification types and sister entries or sister clades in the UPGMA analysis (Fig. 1, Panel a). For example, the Midnight-type cultivars exhibited zero to relatively low levels of admixture with other populations from Midnight through Chicago II (Fig. 1A, Panel b). This grouping of entries had bootstrap support of 1.000 in the UPGMA analysis (Fig. 1A, Panel a). The cultivar Skye, defined here as a Midnight-type cultivar, could alternatively be considered sister to the other Midnight-type entries because of lower UPGMA similarity and lower bootstrap support when this cultivar was included in the Midnight classification type (Fig. 1A, Panel a). Skye exhibited a relatively high level of admixture in the STRUCTURE analysis (Fig. 1A, Panel b), which was consistent with the relationship of Skye to the remainder of the Midnight-type entries in the UPGMA analysis (Fig. 1A, Panel a). Other examples of STRUCTURE admixture accurately reflecting sister grouping relationships in the UPGMA analysis included the core Shamrock type entries vs. A01-701, Bd0384, A05-313, and A97-857 (Fig. 1B) and a core set of Compact-type entries that includes ‘Wildwood’, ‘Hallmark’, PST-B4246, PST-YorkHarbor4, SRX2114, and PST-B5125 vs. Blacksburg II, ‘Baronette’, ‘Rita’, PST-H535, NA-K992, and Ascot (Fig. 1D).

Kentucky bluegrass classification type revision. The combination of pedigree information, UPGMA cluster analysis, and model-based clustering analysis all supported the proposed revised kentucky bluegrass classification system outlined in Table 1. With just a few exceptions, kentucky bluegrass pedigree (Supplementary Table 4), UPGMA clustering analysis (Fig. 1, Panel a), and model-based clustering analysis at (K) = 14 (Fig. 1, Panel b) produced similar grouping results for the Midnight, BVMG, Cynthia, Sydsport, Shamrock, Limousine, P-105, Julia, Compact-America, Compact, and Mid-Atlantic classification types. Three clusters composed of predominantly Eurasian cultivars and collections were supported in the model-based clustering and UPGMA clustering. The potential Jefferson/Washington classification type had strong bootstrap support in the UPGMA analysis (1.000) (Fig. 1B) but had no support in the model-based clustering analysis. Taken together with pedigree, the combination of the results of these analyses indicated that the

revised 14 supported classification types (not including the potential Jefferson/Washington type and the catchall Other type) described in Table 1 are a reasonable representation of the genetic relationships among kentucky bluegrass classification types, cultivars, experimental selections, and collections.

Analysis of molecular variance. The AMOVA was conducted treating the newly revised classification types (as listed in Table 1) as populations. The “other” type entries (Table 1) were not included in the AMOVA, because this classification group is not a genetically related population. The AMOVA of the remaining 15 revised classification types (Table 1) was, in part, meant to serve as a validation step for the newly revised classification scheme. AMOVA results showed that the majority of the SSR marker variation (52%) observed among the kentucky bluegrass individuals from the 15 populations was accounted for by within population variance, although a significant portion (48%) was attributable to differences between populations. Within-population variance was spread relatively uniformly among the various classification types (data not shown); however, the Midnight type had the lowest within population variance (data not shown).

Pairwise Φ_{ST} values derived from the AMOVA highlighted a large number of differences between classification types when individual pairs of classification types were compared (Table 2). Only one pairwise Φ_{ST} comparison, between the Jefferson/Washington type and the P-105 type, was not significant ($P > 0.05$), whereas all other pairwise Φ_{ST} comparisons were significant at $P < 0.05$ (Table 2). This indicates that the vast majority of classification types were differentiated from other classification types, the Φ_{ST} distance values are not random, and the AMOVA supported the revised classification scheme presented in Table 1.

Discussion

Validation and revision of kentucky bluegrass classification types. The UPGMA depiction (Fig. 1, Panel a) of the genetic relatedness of kentucky bluegrass cultivars, experimental selections, and collections, based on SSR marker data, represents a cogent argument for the grouping of kentucky bluegrass entries into several distinct populations/classification types. With only a few exceptions, model-based clustering (Fig. 1, Panel b) corroborated the classification type grouping scenario depicted in the UPGMA analysis. Both of these analyses were in agreement with known pedigree information (Supplementary Table 4) and, therefore, supported a revision of the previous PTM kentucky bluegrass classification system, resulting in a newly proposed classification scheme outlined in Table 1. The results of the AMOVA (Table 2) validated the revised classification system, indicating that the supported proposed types were composed of distinct populations or genetically related classification groups.

Table 2. Pairwise Φ_{ST} values calculated by analysis of molecular variation (AMOVA) illustrating differences between populations (kentucky bluegrass classification types) of kentucky bluegrass (Φ_{ST} values are given below the diagonal and the associated P values are given above the diagonal).^z

	BVMG	Compact	Comp.-America	Cynthia	Eurasian1	Eurasian2	Eurasian3	Jeff/Wash	Julia	Limousine	Mid-At.	Midnight	P-105	Shamrock	Sydsport
BVMG	—	0.001	0.001	0.001	0.001	0.001	0.001	0.024	0.001	0.001	0.001	0.001	0.002	0.001	0.001
Compact	0.426	—	0.001	0.001	0.001	0.001	0.001	0.028	0.001	0.001	0.001	0.001	0.002	0.001	0.001
Comp.-Am.	0.571	0.318	—	0.001	0.001	0.001	0.001	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Cynthia	0.529	0.327	0.519	—	0.001	0.001	0.001	0.041	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Eurasian1	0.515	0.313	0.504	0.404	—	0.001	0.001	0.022	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Eurasian2	0.371	0.167	0.390	0.217	0.222	—	0.001	0.012	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Eurasian3	0.352	0.177	0.377	0.248	0.246	0.067	—	0.005	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Jeff/Wash	0.639	0.180	0.428	0.432	0.394	0.151	0.153	—	0.010	0.001	0.001	0.001	0.116	0.012	0.013
Julia	0.588	0.332	0.528	0.434	0.404	0.312	0.289	0.449	—	0.001	0.001	0.001	0.001	0.001	0.001
Limousine	0.644	0.338	0.499	0.529	0.458	0.285	0.292	0.542	0.571	—	0.002	0.001	0.005	0.001	0.001
Mid-At.	0.528	0.293	0.426	0.405	0.405	0.233	0.223	0.374	0.401	0.474	—	0.001	0.006	0.001	0.001
Midnight	0.741	0.607	0.665	0.728	0.678	0.600	0.548	0.802	0.742	0.781	0.705	—	0.001	0.001	0.001
P-105	0.600	0.270	0.465	0.470	0.424	0.229	0.257	0.396	0.508	0.544	0.390	0.789	—	0.001	0.001
Shamrock	0.534	0.332	0.463	0.463	0.462	0.290	0.305	0.420	0.526	0.477	0.441	0.706	0.428	—	0.001
Sydsport	0.613	0.428	0.520	0.586	0.539	0.374	0.367	0.659	0.619	0.655	0.568	0.781	0.643	0.455	—

^zThe AMOVA was conducted treating the kentucky bluegrass classification types, exactly as listed in Table 1, as populations.

A significant improvement over the PTM kentucky bluegrass classification system was the dramatic reduction in the number of entries in the current study from the “other” type (Supplementary Table 3 vs. Table 1, respectively). In the current study, the combination of low bootstrap support in the UPGMA analysis and high population admixture in the STRUCTURE analysis was used to define entries belonging to the “other” type (Table 1). Part of the original definition of the “other” type was that this group possessed traits that are intermediate between two or more of the defined classification types (Murphy et al., 1995); thus, high population-level admixture, in combination with poor UPGMA clustering resolution, can be used as a means to define “other” type cultivars/accessions. This significantly reduced the number of entries that were considered “other” type, because the vast majority of entries clustered in defined classification groups. It is interesting to note that the STRUCTURE analysis showed some weak associations between the “other” type entries and defined classification groups. For example, common or shared STRUCTURE admixture components likely explained why H02-603, A04-1427, ‘Misty’, and 99AN53 grouped with the BVMG-type entries (Fig. 1A); HV140, A04-1268, A98-3320, and A98-3367 grouped with the Cynthia-type entries (Fig. 1A); ‘NorthStar’ grouped with the Limousine-type entries (Fig. 1B); and A94MH94 grouped with the Julia-type entries (Fig. 1B). However, all of the “other” type entries that have these associations with defined classification types are best described as trends as a result of low bootstrap support in the UPGMA analysis (Fig. 1, Panel a).

The UPGMA cluster analysis (Fig. 1, Panel a) and model-based clustering (Fig. 1, Panel b) provide support for the traditional classification types of Midnight, BVMG, Shamrock, Julia, Compact-America, and Mid-Atlantic. New classification types with UPGMA and model-based clustering support include Cynthia, Sydsport, Limousine, and P-105 (Fig. 1). These analyses were in agreement with pedigree information (Supplementary Table 4).

The new Eurasian classification types were split into three supported clusters in the STRUCTURE analysis (Fig. 1B–C, Panel b), which were also supported in the UPGMA analysis (Fig. 1B–C, Panel a). The split Eurasian groups indicate that there was significant genetic diversity in the Eurasian collections and that continuing to collect Eurasian plant material could lead to novel germplasm sources and new kentucky bluegrass classification types. Although the genetic evidence clearly separated these new Eurasian types, it is difficult to assign specific attributes to these groups as a result of heretofore limited information about the performance and morphological characteristics of these collections. That being stated, one interesting observation was the presence of a higher percentage of entries that were older direct ecotypic selections from the United

States or could be traced back to older direct ecotypic selections from the United States (Supplementary Table 4) in the third Eurasian cluster (Fig. 1C) vs. the first and second Eurasian clusters (Fig. 1B–C). For example, the cultivar Kenblue (Fig. 1C) was previously classified as a Common (or Mid-Western) ecotype. The original description of the Common type (Bara et al., 1993) stated that cultivars in this group were commonly selected from naturalized ecotypes surviving within old pastures. Additional entries in the third Eurasian cluster that fit this description were H94-305, A98-183, ‘Eagleton’ (Hurley et al., 1997), and ‘Wellington’ (Brentano, 2004) (Fig. 1C; Supplementary Table 4). A plausible explanation for these entries grouping together, within the third Eurasian type, is that these cultivars, although clearly ecotypic selections made in the United States, were all descendants from remnant populations of pasture grasses originally seeded by early European settlers. The third Eurasian cluster could, therefore, represent the genetic cluster most closely related to some of the earliest Eurasian germplasm introduced into the United States. SSR marker genotyping of additional Common-type cultivars and other direct ecotypic selections from the United States will be needed to support this hypothesis.

The Compact-type was split among multiple clades in the UPGMA analysis (Figs. 1B and 1D, Panel a) and multiple (*K*) groupings in the model based clustering analysis (Figs. 1B and 1D, Panel b). This was interesting in that the current SSR marker data indicated that the Compact type, as previously defined in the PTM classification scheme, may not represent a distinct genetic group. The original description of Compact-type cultivars included cultivars that exhibited a “low compact growth habit, forming a highly attractive turf” (Bara et al., 1993). Although pedigree information was considered when assigning cultivars to this grouping, a number of genetically divergent cultivars may have been included in the Compact group based simply on a common low, compact morphological growth habit. The current data set indicated that the cultivars Alpine, Blue Sapphire, Blackstone, and Hampton (Fig. 1B) have a different genetic background than the remainder of the Compact-type entries (Fig. 1D). These four cultivars exhibited relatively high population-level admixture with the third Eurasian classification type (Fig. 1B, Panel b). These results may follow pedigree information for two of the cultivars, in which Alpine was a cross between Warren’s A-25 × a collection from Iceland, and Blue Sapphire was a cross between a derivative of Wildwood × ‘Baron’ (Hurley and Ghilsen, 1980) (Baron is a European cultivar) (Supplementary Table 4). In the future, admixture with the Eurasian 3 type could be a basis for creating a new classification type for these cultivars and any new hybrids created from this group.

The cultivars Jefferson (Bonos et al., 2003) and Washington form a distinct group in the UPGMA analysis (bootstrap support of 1.000 for this two cultivar clade, but much

weaker bootstrap support when grouped with the P-105 type cultivars) (Fig. 1B, Panel a); however, the STRUCTURE analysis did not uniquely identify this group, and the AMOVA pairwise Φ_{ST} comparisons indicate that although Jefferson/Washington were significantly different from most other groups, they were not significantly different from the P-105 classification type (Table 2). These discrepancies indicate that Jefferson/Washington should be referred to as a potential or incipient classification type. This terminology may be justified based on the fact that the morphological and performance characteristics of Jefferson and Washington are very different from P-105 type cultivars and also generally very different from all other Kentucky bluegrass classification types (authors’ personal observations). An additional line of evidence that provides some support for a potential Jefferson/Washington classification type included a higher level of population-level admixture in Jefferson and Washington vs. the P-105 type entries (Fig. 1B, Panel b). Finally, recent breeding efforts, using Jefferson and Washington as parents, have produced new experimental selections (not included in the current study) that might later be grouped in this classification type (authors’ personal observations). SSR marker genotyping of these additional selections will be required to determine if additional support can be found for this potential classification type.

Genetic relationships among classification types. Overall, the genetic relationships among Kentucky bluegrass classification types (populations) were more difficult to define than the delineations of the individual classification types/populations. This was evident in the comparatively lower bootstrap values of groupings larger than the main Kentucky bluegrass classification types in the UPGMA diagram (Fig. 1, Panel a). Additional SSR marker genotyping could improve resolution; however, lower bootstrap support for relationships among classification types could also be the result of reticulate relationships resulting from repeated hybridization between classification types in Kentucky bluegrass breeding programs. That being stated, there were still a number of genetic relationships among Kentucky bluegrass classification types that were supported by a combination of UPGMA clustering analysis (Fig. 1, Panel a), model-based clustering analysis (STRUCTURE) (Fig. 1, Panel b), AMOVA results (Table 2), and pedigree relationships (Supplementary Table 4).

The main grouping of the three Eurasian types (Fig. 1B–C, Panel a), being most closely related to the outgroup, *P. annua*, was basal to the remainder of the Kentucky bluegrass cultivars, experimental selections, and collections in the current study. The UPGMA placement of the Eurasian collections as basal to the remainder of the entries was supported by the AMOVA results, where the pairwise Φ_{ST} genetic distance values were always lowest between the Eurasian 2/Eurasian 3 groups and all other classification types (Table 2). These results suggest that

Eurasia was the likely center of origin for the germplasm in the current study. This grouping scenario fits with observations that Kentucky bluegrass is native to the old world, being distributed naturally throughout the temperate and cooler regions of Europe and Asia (Bashaw and Funk, 1987) and with the proposed center of origin of the genus *Poa*, which based on morphological, cytological, and species diversity is considered to be Eurasia (Huff, 2003).

The Julia classification type was closely aligned with the main grouping of Eurasian entries (Fig. 1B, Panel a). There was bootstrap support (0.848) for the grouping of Julia-type cultivars and accessions with the first Eurasian grouping (Fig. 1B, Panel a). This grouping scenario follows pedigree information, because ‘Julia’ originated as a collection from northern Germany (Alderson and Sharp, 1994).

A large grouping with bootstrap support includes the Midnight, BVMG, Cynthia, Sydsport, and Shamrock classification types (Fig. 1A–B, Panel a). This large lineage was composed of a mixture of cultivars developed in U.S. breeding programs, cultivars of European origin, and a limited number of individual Eurasian plant collections (now classified as “other” type) weakly aligned with the BVMG and Cynthia classification types. The individual Eurasian collections aligned with the BVMG and Cynthia classification types followed pedigree information, because Cynthia originated in England (Alderson and Sharp, 1994) and Baron (BVMG type) originated in Holland (Hurley and Ghilsen, 1980). The cultivar Sydsport, which originated in Sweden (Alderson and Sharp, 1994), was one of the earlier improved Kentucky bluegrass cultivars used in U.S. breeding programs, likely accounting for the placement of the new Sydsport classification type in this section of the UPGMA diagram, which includes both U.S. and European germplasm (Fig. 1A–B, Panel a). The cultivar Shamrock (Baily et al., 1995) (type name for the Shamrock type) was a single plant progeny from A80-336, pollinated in a polycross that included the cultivar Sydsport, which likely explains the proximity of the Shamrock and Sydsport classification types in the UPGMA clustering analysis (Fig. 1B, Panel a).

The cultivar Midnight (type name for the Midnight type) originated from the progeny of F64-603, a selection made from an old lawn in Washington, DC, crossed with the cultivar Glade (Alderson and Sharp, 1994; Jacklin et al., 1977). There were no clear pedigree relationships that explained the placement of the Midnight classification type in this large lineage, because both F64-603 and Glade were direct ecotypic selections (Alderson and Sharp, 1994; Meyer et al., 1984); however, it is very interesting that the Midnight type was placed within this lineage, as opposed to the lineage that includes the Compact and Compact-America types (Fig. 1A vs. Fig. 1D). Earlier PTM Kentucky bluegrass classification systems implied that the Midnight type was related to the Compact and Compact-America classification

types based on the inclusion of the name “Compact” associated with the Midnight type name (see Supplementary Table 1). The current SSR marker data dispute this based on the location of the Midnight type in the UPGMA analysis (Fig. 1A, Panel a) and comparatively large pairwise Φ_{ST} genetic distance values calculated by AMOVA (Table 2) between the Midnight type and all other classification types in the current study. Taken together, these findings suggest that the “Compact-Midnight” type was one of the most distinct classification groups relative to all other revised classification groups, and that from this point forward, this group should be named the Midnight type, because the “Compact” designation for this specific type seems to refer only to growth habit and not genetic relationships among groups.

Another large lineage depicted in the UPGMA diagram was comprised of the Mid-Atlantic, Compact, and Compact-America classification types (Fig. 1C–D, Panel a). There was bootstrap support for the association between the Compact and Compact-America classification types but lower bootstrap support when including the Mid-Atlantic type with the latter classification types (Fig. 1D, Panel a). Although bootstrap support in the UPGMA analysis was lower for all three classification types together, it is interesting to note that this large lineage was almost exclusively comprised of cultivars and accessions from U.S. breeding programs with the possible exceptions of ‘Baronie’ and Baronette (unknown pedigrees). This situation was different from the remaining large lineages, which were a mixture of U.S. and Eurasian, or only Eurasian, germplasm.

An association between the Compact and Compact-America types has existed in the kentucky bluegrass classification system since 1995 (Murphy et al., 1995). An early description of the Compact-America type was written as “within the compact type, a number of cultivars exhibit growth and performance characteristics similar to the cultivar ‘America’” (Funk et al., 1982) (Murphy et al., 1995). At the time, this description was intended to imply that the Compact-America type was a newly defined subtype within the Compact type. As numerous new cultivars were developed within the Compact-America subtype, this new group came to be considered a separate classification grouping. The UPGMA cluster analysis indicated a genetic relationship between the Compact and Compact-America classification types (0.909 bootstrap support for the combined groups) (Fig. 1D, Panel a). This was different from the previously described situation between the Compact and Midnight classification types in that the assumed relationship between the Compact and Compact-America classification types in previous PTM kentucky bluegrass classification systems appears to have a genetic basis. The delineation between the Compact and Compact-America classification types was also interesting. There was a core set of Compact-type entries in Wildwood through PST-B5125 (1.000

bootstrap support) (Fig. 1D, Panel a). Blacksburg II through ‘Moonshadow’ (Bonos et al., 2005) exhibited various levels of admixture between the two classification types (Fig. 1D, Panel b), whereas the remainder of the Compact-America types, from ‘Bedazzled’ (Bonos et al., 2008) through ‘Glenmont’, exhibited very low to zero levels of admixture between the two classification types (Fig. 1C–D, Panel b).

The remaining large lineage in the UPGMA diagram included the Limousine, Jefferson/Washington, and P-105 classification types as well as the Compact-type cultivars Alpine, Blue Sapphire, Blackstone, and Hampton (Fig. 1B, Panel a). Relative to some of the other larger lineages, this grouping was not supported in the UPGMA analysis (approaching minimum bootstrap support of 0.500) (Fig. 1B, Panel a). Although better described as a trend than a strong relationship, it is possible that these entries were grouping in this location as a result of an association with the Eurasian and Julia classification types. The cultivar Limousine was originally a European cultivar (collection from Germany) (Alderson and Sharp, 1994), whereas the entries NorthStar through ‘P-105’ (Hurley et al., 2000) exhibit various levels of admixture with the third Eurasian classification type (Fig. 1B, Panel b).

Kentucky bluegrass classification types not included in the current classification revision. There were four previous classification types that were not represented in the current classification revision based on SSR markers (Supplementary Table 3 vs. Table 1, respectively): CELA (‘Challenger’, ‘Eclipse’, ‘Liberty’, ‘Adelphi’) type, Common type, High Density (Aggressive) type, and texas bluegrass × kentucky bluegrass hybrids. As discussed previously, a number of the Common-type cultivars/accessions in the current study clustered with the third Eurasian type. SSR marker genotyping of additional Common-type cultivars will be needed to determine if all of these cultivars should remain in the third Eurasian type. High Density (Aggressive) type cultivars were underrepresented in the current study. Bonos et al. (2000) reported that this type may only be related by the common growth characteristic of high shoot density, indicating that this type may not be a genetically related group. This observation was supported by the fact that Limousine and ‘Julius’ (both previously High Density) are now classified as Limousine-type cultivars (Table 1; Fig. 1), whereas NorthStar (previously High Density) is now classified as an “other” type cultivar (Table 1; Fig. 1). SSR marker genotyping of additional cultivars that were previously considered to be High Density (Aggressive) type cultivars will be needed to determine whether this group should be retained or removed in the revised classification system. The CELA type, represented by the type cultivars Challenger (Meyer et al., 1987), Eclipse (Funk et al., 1981), Liberty (Brilman et al., 1989), and Adelphi (Funk et al., 1973), was also underrepresented in the current study. The situation with this group was

different from the High Density (Aggressive) type in that the CELA type may have been a genetically related classification type. The cultivars Adelphi and Eclipse shared a common pedigree (Alderson and Sharp, 1994) as did Challenger and Liberty (Alderson and Sharp, 1994). It will be problematic to determine the genetic relationships of the CELA classification type using SSR markers because many of the cultivars in this group currently have very limited or no commercial production and/or have infrequently been used in the development of new cultivars, meaning that this group has effectively been discontinued. The final classification type, texas bluegrass × kentucky bluegrass hybrids, was not included in the current study because hybrid cultivars in this classification type may be populations rather than apomicts.

Conclusions

The SSR markers in the current study are the first DNA markers that showed a correlation between genetic relatedness as assessed by molecular markers and the previously described PTM kentucky bluegrass cultivar classification system based on pedigree information, cultivar performance characteristics, and morphological traits. Additionally, SSR marker analysis in the current study provided justification for a revision/update of the kentucky bluegrass cultivar classification system. This revision included a significant reduction in the number of entries in the current study from the “other” type. Classification types that were supported by the current SSR marker analysis included Midnight, BVMG, Cynthia, Sydsport, Shamrock, Limousine, P-105, Julia, Compact-America, Compact, Mid-Atlantic, and three Eurasian types. The Jefferson/Washington classification type was best described as a potential or incipient classification type. The vast majority of all cultivars, experimental selections, and collections were uniquely identified with the current set of SSR markers. Genetic relationships of individuals as assessed by the current set of SSR markers very closely matched known pedigrees (Supplementary Table 4). The current set of SSR markers can be used to rapidly genotype and assign new cultivars/accessions to kentucky bluegrass classification types and assess genetic relatedness among individuals.

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Supplementary Fig. 1. Graphical representation of average Ln Pr(X|K) output from 20 independent STRUCTURE runs at each K value for K = 2 through 25. The earliest plateau for Ln Pr(X|K) occurs at K = 14 (highlighted red). Pritchard et al. (2000) note that it can be common for Ln Pr (X|K) values to continue to increase slightly after an initial plateau. In these cases, the authors state that the real K is the smallest plateau value of K that captures the major structure in the data (Pritchard et al., 2000), providing justification for choosing K = 14 for the current data set. The structure at K = 11, 12, 13, 15, 16, and 17 were also considered; however, the biological interpretation (congruence with pedigree and the unweighted pair group method using arithmetic average analysis) of the current data were most appropriate at K = 14 (Pritchard et al., 2000).

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Supplementary Table 1. The previous Pedigree, common Turf performance, and Morphological traits (PTM) kentucky bluegrass classification system.²

Classification type	Attributes of cultivars in classification type	Example cultivars
BVMG	Medium- to low-growing turf with medium density and medium-wide leaves; moderate to good turf quality; high seed yield potential; high susceptibility to certain races of stripe smut [<i>Ustilago striiformis</i> (Westend.) Niessl]	Baron, Victa, Merit, Gnome
CELA	Cultivars with similar growth and performance characteristics to the cultivars Challenger, Eclipse, Liberty, and Adelphi; early spring greenup; moderate to good stripe smut resistance; good resistance to leaf spot [<i>Drechslera poae</i> (Baudys) Shoemaker]; variable winter color and dormancy	Challenger, Eclipse, Liberty, Adelphi
Common (Midwest Ecotype)	Erect growth habit; narrow leaf blades; early flowering; high seed yields; high susceptibility to leaf spot; used for pastures, conservation purposes, and lower maintenance utility turf	Arboretum, Kenblue, SD Certified, Ginger
Compact	Low compact growth habit forming highly attractive turf; good to excellent resistance to leaf spot; slight purple winter color; later spring greenup	Wildwood, Hallmark, Goldstar, Blacksburg, Blackstone
Compact-America	Cultivars with similar growth and performance characteristics to the cultivar America; low compact growth forming highly attractive turf; moderate winter dormancy with some purpling; moderate recovery from summer stress; good resistance to dollar spot (<i>Sclerotinia homoeocarpa</i> F. T. Bennett), leaf spot, and stripe smut diseases	America, Bedazzled, Brilliant, Delight, Mallard, Arrow, Boutique, Showcase, Kingfisher, Barnique, Sonoma, Bordeaux, Unique
Compact-Midnight	Cultivars with similar growth and performance characteristics to the cultivar Midnight; low compact growth forming highly attractive turf; long winter dormancy/late spring greenup; dark green color; susceptibility to powdery mildew (<i>Erysiphe graminis</i> D. C.) when grown in the shade	Midnight, Midnight II, Arcadia, Quantum Leap, Impact, Beyond, Total Eclipse, Odyssey, NuGlade, Perfection, Tsunami, Awesome
High Density (Aggressive)	Aggressive lateral growth resulting in a high density turf; cultivars may dominate a turf stand when used in blends with other bluegrass types or other turf species; high thatch production under certain environmental and management conditions	Princeton 104, A-34, Limousine Touchdown
Julia	Cultivars with similar growth and performance characteristics to the cultivar Julia (a European variety); medium to dark green color; medium leaf texture; good winter color and spring greenup; good wear tolerance; poor resistance to dollar spot	Julia, Ulysses, Pick 453, H92-558, Rampart
Mid-Atlantic	Vigorous turf of medium to high density with a deep and extensive rhizome system; tolerance or good recovery from billbug damage; excellent summer stress tolerance; moderate to good leaf spot resistance	Cabernet, Starburst, Appalachian, PST-161, Riverside Park, Valsburg Park
Shamrock	Cultivars with similar growth and performance characteristics to the cultivar Shamrock; moderate resistance to leaf spot and billbugs (<i>Sphenophorus parvulus</i> Gyllenhal); high seed yield production	Shamrock, Lakeshore, Moonshine, Mongoose, Champlain
Texas × kentucky bluegrass hybrids	Intraspecific hybrids between texas bluegrass and kentucky bluegrass	Thermal Blue, Longhorn
Other	A large group of cultivars falling under the category of “other turf types”; wide range of characteristics; this group possesses traits that are intermediate between two or more of the previously discussed groups; cultivars that require further study to accurately classify or assign to different groups	Lily, Limerick, Bodacious, Allure, PST-York Harbor 4, Jewel, Barzan, Baronie, Rita

²A brief description of each classification type and some example cultivars are also shown.

Supplementary Table 2. Kentucky bluegrass microsatellite [(simple sequence repeat (SSR)) marker alleles and associated Polymorphism Information Content (PIC) values, used in the current study.

SSR_allele size (bp)	PIC value
GA1_218	0.449
GA1_222	0.289
GA1_228	0.166
GA1_230	0.427
GA1_232	0.388
GA1_234	0.323
GA1_239	0.209
GA1_250	0.475
GA1_252	0.483
GA1_262	0.214
GA1_267	0.415
GA1_279	0.154
GA1_298	0.444
GA1_305	0.203
GA9_203	0.332
GA9_211	0.495
GA9_213	0.268
GA9_215	0.157
GA9_219	0.345
GA9_223	0.206
GA9_225	0.485
GA9_227	0.349
GA9_229	0.246
GA9_231	0.169
GA9_233	0.230
GA9_235	0.169
GA9_251	0.432
GA9_253	0.429
GA107_211	0.424
GA107_213	0.357
GA107_214	0.499
GA107_215	0.253
GA107_216	0.449
GA107_220	0.214
GA107_222	0.466
GA107_224	0.496
GA107_231	0.433
GA107_241	0.424
GA107_244	0.209
GA107_250	0.226
GA107_252	0.475
GA107_254	0.279
GA107_256	0.220
GA125_198	0.409
GA125_203	0.418
GA125_207	0.438
GA125_209	0.485
GA125_211	0.497
GA125_216	0.309
GA125_230	0.405
GA125_232	0.179
GA125_241	0.259
GA125_243	0.226
GA125_249	0.166
GA125_251	0.237
GA125_253	0.349
GA125_255	0.494
GA125_257	0.479
GA125_259	0.274
GA125_261	0.332
GA125_262	0.357
GA125_264	0.179
GA125_266	0.203
GA125_268	0.285
GA125_270	0.304
GA125_272	0.357
GA125_274	0.365
GA125_276	0.179
GA403_231	0.367
GA403_233	0.171

(Continued on next column)

Supplementary Table 2. (Continued)

SSR_allele size (bp)	PIC value
GA403_265	0.236
GA403_267	0.434
GA403_269	0.393
GA403_271	0.491
GA403_273	0.478
GA403_277	0.494
GA446_156	0.216
GA446_187	0.227
GA446_189	0.334
GA446_206	0.155
GA446_223	0.500
GA446_231	0.168
GA446_242	0.342
GA446_243	0.286
GA446_265	0.198
GA446_277	0.404
GA446_288	0.276
GA446_290	0.478
GA446_292	0.296
GA446_294	0.450
GA446_306	0.498
GA446_336	0.155
GA480_176	0.334
GA480_181	0.494
GA480_188	0.320
GA480_191	0.411
GA480_193	0.459
GA480_221	0.351
GA480_224	0.355
GA480_225	0.210
GA480_229	0.483
GA480_232	0.174
GA480_236	0.338
GA480_238	0.470
GA480_240	0.445
GA480_242	0.499
GA748_271	0.217
GA748_275	0.287
GA748_281	0.352
GA748_285	0.398
GA748_287	0.199
GA748_289	0.245
GA748_290	0.499
GA748_292	0.181
GA748_294	0.408
GA748_296	0.500
GA748_298	0.438
GA748_300	0.411
GA748_303	0.307
GA748_304	0.234
GA748_307	0.456
GA748_309	0.364
GA748_311	0.438
GA748_313	0.245
GA748_315	0.316
GA748_317	0.472
GA748_319	0.487
GA748_321	0.484
GA748_323	0.446
GA748_325	0.343
GA748_327	0.199
GA749_276	0.419
GA749_281	0.158
GA749_283	0.458
GA749_285	0.152
GA749_288	0.399
GA749_290	0.281
GA749_292	0.335
GA749_294	0.490
GA749_296	0.494
GA749_298	0.373
GA749_300	0.238
GA749_302	0.202

(Continued on next column)

Supplementary Table 2. (Continued)

SSR_allele size (bp)	PIC value
GA749_329	0.330
GA749_331	0.158
GA914_277	0.455
GA914_280	0.480
GA914_283	0.192
GA914_286	0.276
GA914_289	0.276
GA914_292	0.155
GA914_293	0.311
GA914_297	0.390
GA914_298	0.363
GA914_300	0.500
GA914_302	0.255
GA914_305	0.440
GA914_308	0.161
GA914_311	0.325
GA914_313	0.315
GA914_327	0.423
GA914_328	0.445
GA947_260	0.375
GA947_266	0.236
GA947_269	0.494
GA947_273	0.346
GA947_276	0.430
GA947_277	0.290
GA947_280	0.479
GA947_281	0.460
GA947_284	0.454
GA947_287	0.355
GA947_290	0.280
GA1023_291	0.173
GA1023_293	0.419
GA1023_295	0.481
GA1023_297	0.481
GA1023_299	0.192
GA1023_300	0.265
GA1023_307	0.209
GA1023_308	0.482
GA1023_310	0.314
GA1023_314	0.243
GA1023_318	0.192
GA1023_321	0.260
GA1071_241	0.231
GA1071_245	0.231
GA1071_288	0.372
GA1071_289	0.500
GA1071_291	0.487
GA1071_293	0.214
GA1071_295	0.481
GA1071_297	0.380
GA1071_300	0.473
GA1071_301	0.214
GA1071_302	0.493
GA1071_304	0.294
GA1071_305	0.435
GA1071_306	0.477
GA1071_308	0.184
GA1071_310	0.263
GA1071_311	0.236
GA1071_312	0.236
GA1071_314	0.190
GA1071_315	0.298
GA1071_317	0.184
GA1071_318	0.284
GA1071_319	0.473
GA1071_322	0.488
GA1071_324	0.160
GA1095_258	0.152
GA1095_260	0.183
GA1095_262	0.207
GA1095_264	0.462
GA1095_272	0.177
GA1095_275	0.152

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Supplementary Table 2. (Continued)

SSR_allele size (bp)	PIC value
GA1095_282	0.460
GA1095_285	0.212
GA1095_287	0.201
GA1095_289	0.480
GA1095_298	0.256
GA1095_300	0.412
GA1095_306	0.493
GA1095_329	0.165
GA1095_335	0.207
GA1101_285	0.485
GA1101_286	0.494
GA1101_287	0.227
GA1101_289	0.498
GA1101_291	0.492
GA1101_292	0.173
GA1101_293	0.275
GA1101_295	0.366
GA1101_305	0.154
GA1101_307	0.179
GA1101_323	0.215
GA1101_326	0.203
GA1119_276	0.246
GA1119_280	0.312
GA1119_282	0.278
GA1119_284	0.298
GA1119_288	0.471
GA1119_290	0.236
GA1119_291	0.165
GA1119_292	0.419
GA1119_295	0.461
GA1119_297	0.379
GA1119_299	0.497
GA1119_301	0.474
GA1119_303	0.485
GA1119_306	0.407
GA1119_308	0.496
GA1119_310	0.500
GA1119_312	0.246
GA1119_314	0.316
GA1119_316	0.207
GA1119_319	0.224
GA1119_321	0.472
GA1119_323	0.252
GA1148_235	0.208
GA1148_236	0.322
GA1148_239	0.459
GA1148_241	0.202
GA1148_243	0.331
GA1148_245	0.352
GA1148_249	0.466
GA1148_251	0.432
GA1148_253	0.473
GA1148_257	0.468
GA1148_258	0.391
GA1148_262	0.360
GA1153_285	0.499
GA1153_287	0.312
GA1153_290	0.425
GA1153_296	0.224
GA1153_304	0.213
GA1153_308	0.454
GA1153_311	0.219
GA1153_313	0.343
GA1153_317	0.219
GA1153_319	0.459
GA1153_327	0.184
GA1153_331	0.419
GA9307_270	0.166
GA9307_274	0.405
GA9307_276	0.228
GA9307_278	0.185
GA9307_282	0.245
GA9307_288	0.418

(Continued on next column)

Supplementary Table 2. (Continued)

SSR_allele size (bp)	PIC value
GA9307_292	0.375
GA9307_296	0.415
GA9307_298	0.172
GA9307_300	0.191
GA9307_304	0.204
GA9307_308	0.191
GA9307_310	0.283
GA9307_311	0.444
GA9307_326	0.421
GA9307_330	0.185
GA9307_332	0.198
GA9307_351	0.332
GA9307_353	0.313
GA9324_251	0.479
GA9324_256	0.231
GA9324_261	0.220
GA9324_263	0.279
GA9324_267	0.464
GA9324_269	0.166
GA9324_271	0.377
GA9324_273	0.449
GA9324_275	0.237
GA9324_277	0.279
GA9324_279	0.474
GA9324_289	0.253
GA9324_301	0.299
GT110_205	0.482
GT110_207	0.448
GT110_211	0.453
GT110_219	0.329
GT110_222	0.393
GT110_226	0.477
GT110_227	0.296
GT110_228	0.492
GT110_230	0.455
GT110_232	0.499
GT110_234	0.244
GT110_236	0.494
GT110_238	0.342
GT110_242	0.494
GT123_153	0.460
GT123_155	0.497
GT123_157	0.472
GT123_159	0.462
GT123_161	0.251
GT123_165	0.484
GT123_171	0.246
GT135_148	0.292
GT135_150	0.189
GT135_157	0.430
GT135_159	0.430
GT135_161	0.409
GT135_163	0.354
GT135_165	0.287
GT135_167	0.177
GT135_169	0.500
GT135_173	0.177
GT135_177	0.240
GT135_178	0.392
GT135_179	0.177
GT135_182	0.246
GT135_183	0.346
GT135_186	0.415
GT135_188	0.165
GT135_190	0.472
GT135_192	0.195
GT135_194	0.235
GT135_196	0.177
GT135_198	0.382
GT135_200	0.262
GT135_210	0.165
GT9314_244	0.187
GT9314_245	0.187

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Supplementary Table 2. (Continued)

SSR_allele size (bp)	PIC value
GT9314_260	0.384
GT9314_265	0.481
GT9314_267	0.372
GT9314_269	0.205
GT9314_270	0.162
GT9314_277	0.339
GT9314_279	0.446
GT9314_281	0.297
GT9314_282	0.335
GT9314_286	0.436
GT9314_287	0.418
GT9314_288	0.287
GT9314_290	0.272
GT9314_292	0.316
GT9314_297	0.330
GT9314_304	0.245
GT9434_212	0.446
GT9434_248	0.250
GT9434_250	0.348
GT9434_252	0.449
GT9434_254	0.339
GT9434_256	0.498
GT9434_258	0.266
GT9434_260	0.250
GT9434_262	0.391
GT9434_264	0.193
GT9434_267	0.228
GT9434_268	0.436
GT9434_270	0.245
GT9434_275	0.282
GT9434_277	0.211
GT9434_279	0.155
GT9434_281	0.250
GT9434_283	0.302
GT9434_287	0.261
GT9434_296	0.245
GT9434_300	0.250
GT9434_311	0.199

Supplementary Table 3. Pedigree, common Turf performance, and Morphological traits (PTM) classification of kentucky bluegrass cultivars, experimental selections, and collections in the current study.^z

Classification type	Cultivar or selection
BVMG	Abbey, Baron, Envicta, Goldrush, Marquis, Raven
CELA	Jefferson
Common	Kenblue, GO9LM9 ^y
Compact	Alpine, Ascot, Blackstone, ^x Bluetastic, Chicago II, Diva, Goldstar, Hallmark, Hampton, Moonlight, Skye, P-105, Wildwood, PST-B4-246, ^y PST-B5-125 ^y
Compact-America	Apollo, Arrow, Barnique, Baroness, Bedazzled, Bordeaux, Boutique, Brilliant, Casablanca, Delight, Dynamo, Glenmont, Kingfisher, Langara, Mallard, Moonshadow, Royale, Showcase, Sonoma, SR 2284, Unique, Valor, BAR Pp 0566, ^y BAR Pp 0573, ^y PST-222, ^y PST-604, ^y PST-H6-150 ^y
Compact-Midnight	Alexa, Arcadia, Award, Awesome, Barrister, Beyond, Bluestone, Blue Velvet, Courtyard, Everest, EverGlade, Excursion, Freedom II, Freedom III, FrontPage, Ginney, Impact, Liberator, Midnight, Midnight II, NuDestiny, NuGlade, Odyssey, Perfection, Quantum Leap, Rambo, Rugby II, Total Eclipse, Tsunami
High Density	Bariris, HV140, ^y Julius, Limousine, NorthStar
Julia	Julia, Pick 453, ^y Rampart, H92-558 ^y
Mid-Atlantic	Appalachian, Cabernet, Eagleton, Starburst, PST-161 ^y
Shamrock	Brooklawn, Durham, Katie, Lakeshore, Mongoose, Moonshine, Shamrock, A98-1028 ^y
Other (and unclassified)	Allure, Aries, Avid, Baritone, Baronette, Baronie, Bartitia, Barzan, Blacksbury II, Bluemax, Bluenight, BlueRidge, BlueSapphire, Bodacious, Boomerang, Chateau, Cheetah, Chelsea, Coventry, Fairfax, Jewel, Lily, Limerick, Mercury, Misty, MonteCarlo, Rita, Royce, Serene, ^w Shiraz, Ulysses, Voyager II, Washington, Wellington, 99AN53, ^y A00-1254, ^y A00-1400, ^y A00-4083PoriFin, ^y A00-430, ^y A00-99, ^y A01-701, ^y A03-132, ^y A03-141, ^y A03-335, ^y A03-37, ^y A03-6, ^y A03-66, ^y A03-77, ^y A04-1268PorkalaFin, ^y A04-1271PorkalaFin, ^y A04-1272PorkalaFin, ^y A04-1283HankoFin, ^y A04-1315FinstromFin, ^y A04-1342KustaviFin, ^y A04-1347LepainenFin, ^y A04-1354PetsmoFin, ^y A04-1375PargasFin, ^y A04-1384AristoFin, ^y A04-1415KrudonisLith, ^y A04-1423BuktaLith, ^y A04-1427JurbarkasLith, ^y A04-1470SiauliaiLith, ^y A04-1474SiauliaiLith, ^y A04-1484KursenaiLith, ^y A04-1547SpitrenaiLith, ^y A04-1569MoletLith, ^y A04-383PojoaraRom, ^y A04-387PasulprislopRom, ^y A04-394PetroojaniRom, ^y A05-313, ^y A05-314, ^y A05-322, ^y A05-332, ^y A05-335, ^y A05-336, ^y A05-847ColdePortFra, ^y A05-894VillaboneSp, ^y A93-201, ^y A94MH94, ^y A95-410, ^y A96-363, ^y A96-739, ^y A96-742, ^y A97-1294, ^y A97-1303, ^y A97-1328, ^y A97-1409, ^y A97-1799, ^y A97-857, ^y A98-183, ^y A98-3297OgrodzieniecPol, ^y A98-3320PilicaWarkaPol, ^y A98-3322ZyrardowPol, ^y A98-3323ZychlinPol, ^y A98-3366RekorvoPol, ^y A98-3367RekorvoPol, ^y A98-3369TeologPol, ^y A98-3384AnimalPKSwe, ^y A98-365, ^y A98-407, ^y A99-2427, ^y A99-2678, ^y A99-3116, ^y A99-3122, ^y A99-3127, ^y A99-3245, ^y A99-523, ^y B5144, ^y B543, ^y B545, ^y Bd0384, ^y Bd99-2103, ^y CVB20631, ^y DLF769032, ^y DLF769034, ^y DLF769036, ^y DLF769037, ^y H01-804ComarnicRom, ^y H01-847SomcutaMareRom, ^y H01-894HongyanCh, ^y H01-912FrankfurtGer, ^y H02-603UlrichhamnSwe, ^y H02-608UlrichhamnSwe, ^y H02-99xH98-767, ^y H03-180xA99-2874, ^y H04-376KaisterFin, ^y H04-389SodderRuderFin, ^y H04-390AristoFin, ^y H04-535KorpoFin, ^y H07-697NorrkopingSwe, ^y H92-203, ^y H94-305, ^y H99-1722Uz, ^y NA-K992, ^y PpH6366, ^y PpH7907, ^y PpH7929, ^y PST-YorkHarbor4, ^y SIA96386, ^y SRX2114, ^y SRX26351, ^y SRX27921, ^y SRXQG245, ^y U2998Uz, ^y PST-H5-35 ^y

^zKentucky bluegrass entries are grouped according to historical classification typing (pedigree, common turf performance characteristics, and morphological traits) with particular emphasis given to the most recent report by Shortell et al., (2009).

^yDenotes experimental selection.

^xOriginal 'Blackstone'.

^wOriginal 'Serene'.

^zDenotes collection (Ch = China; Fin = Finland; Fra = France; Ger = Germany; Lith = Lithuania; Pol = Poland; Rom = Romania; Sp = Spain; Swe = Sweden; Uz = Uzbekistan).

Supplementary Table 4. Breeding history and classification type summaries for 247 Kentucky bluegrass cultivars, accessions, and collections in the current study.²

UPGMA order	Entry and Classification Type	Breeding History or Classification Type summary
1	<i>Poa annua</i> (outgroup)	
2	Midnight	'Midnight' is the type cultivar for the Midnight type classification group (Bara et al., 1993; Park et al., 2005; Shortell et al., 2009). Midnight originated as a single, highly apomictic, aberrant plant selected from the open-pollinated progeny of F64-603, a selection made from an old lawn located near the Natural History Museum in Washington, D. C. in 1963. (Meyer et al., 1984).
3	NuGlade	'Limousine' x 'Midnight'. (Brede, 2001a)
4	Courtyard	'Limousine' x 'Midnight'. (Brede, 2011)
5	Awesome	'Limousine' x 'Midnight'. (Brede, 2011)
6	Award	'Limousine' x 'Midnight'. (Brede, 2001b)
7	Tsunami	'Freedom' x 'Limousine'. (Brede, 2004a). Performance and morphological characteristics of 'Tsunami' are similar to Midnight type cultivars (W. A. Meyer and S. A. Bonos, personal communication).
8	Alexa	92-0076 x 'Midnight' (Brede, 2006a)
9	Everest	Open pollinated progeny of 'Midnight'. (Brede, 2006b)
10	FreedomIII	92-0076 x 'Midnight' (Brede, 2006c)
11	Beyond	'Midnight' x 'Limousine' (Brede, 2004b)
12	Excursion	'Limousine' x 'Midnight'. (Brede, 2004c)
13	Barrister	'Limousine' x 'Midnight'. (Brede, 2006d)
14	EverGlade	92-0076 x 'Midnight' (Brede, 2006e)
15	Gunney	'Limousine' x 'Midnight'. (Brede, 2011)
16	NuDestiny	90-0416 x 'Midnight' (Brede, 2004d)
17	Bluestone	'Midnight' x derivative of Julia (W. A. Meyer and S. A. Bonos, personal communication)
18	BlueVelvet	'Limousine' x 'Midnight'. (Brede, 2011)
19	Rambo	'Rambo' originated from a highly apomictic, single-plant selection from an open-pollinated cross in the Jacklin Seed, Post Falls breeding nurseries. The parentage of Rambo traces to breeding line 90-0499, which originated from a naturally occurring hybrid from 'Midnight' Kentucky bluegrass (Brede, 2003a)
20	Impact	'Limousine' x 'Midnight'. (Brede, 2003b)
21	MidnightII	'Midnight' x derivative of Julia (W. A. Meyer and S. A. Bonos, personal communication)
22	TotalEclipse	'Limousine' x 'Midnight'. (Brede, 2001c)
23	RugbyII	Single-plant selection from an apomictic Kentucky bluegrass found growing in a closely mowed cemetery in southern Washington State; registration article describes as "similar in appearance to 'Blacksburg' and 'Midnight'" (Brede, 2001d)
24	FrontPage	92-0076 x 'Midnight' (A. D. Brede, personal communication)
25	Perfection	'Limousine' x 'Midnight'. (Brede, 2011)
26	Odyssey	'Limousine' x 'Midnight'. (Brede, 2002)
27	Liberator	Pollen from 'Glade' Kentucky bluegrass was used to pollinate plants of breeding line '50-14'. Breeding line 50-14 originated from a collection by the late Arden Jacklin made in the northeastern USA during the 1970s. Registration article states that Liberator "is most similar in form and appearance to the cultivar, 'Midnight'" (Brede, 2001e)
28	QuantumLeap	'Limousine' x 'Midnight'. (Brede, 2003c)
29	Arcadia	'Midnight' x 'Limousine' (Brede, 2003d)
30	ChicagoII	'Midnight' x 'Limousine' (Brede, 2004e)
31	Skye	96-4664-6 1070 x 'Midnight' (W. A. Meyer and S. A. Bonos, personal communication)
32	Baron	'Baron' is one of the type cultivars for the BVMG ('Baron', 'Victa', 'Merit', 'Gnome') classification type (Bara et al., 1993; Park et al., 2005; Shortell et al., 2009). Baron is the result of a clonal selection taken from a meadow in eastern Holland (Hurley and Ghijsen, 1980).
33	Goldrush	BVMGtype cultivar (Park et al., 2005; Shortell et al., 2009)
34	DLF769036	Collection from Holland (Niels Chr. Nielsen, personal communication); Direct ecotypic selection - unknown breeding origin. BVMG type based on UPGMA and Structure analysis
35	Bartitia	Ecotypic selection from Sweden (Alderson and Sharp, 1994). BVMG type based on UPGMA and Structure analysis.
36	Envicta	BVMGtype cultivar (Park et al., 2005; Shortell et al., 2009)
37	Raven	BVMGtype cultivar (Park et al., 2005; Shortell et al., 2009)
38	Abbey	BVMGtype cultivar (Bara et al., 1993; Park et al., 2005; Shortell et al., 2009)
39	Marquis	BVMGtype cultivar (Bara et al., 1993; Park et al., 2005; Shortell et al., 2009)
40	H02-603Swe	Collection from Ulricehamn, Sweden (W. A. Meyer and S. A. Bonos, personal communication)
41	A04-1427Lith	Collection from Jurbarkas, Lithuania (W. A. Meyer and S. A. Bonos, personal communication)
42	Misty	Unknown breeding origin; European cultivar (W. A. Meyer, personal communication)
43	99AN53	Unknown breeding origin.
44	Bodacious	Origin traces back to 'Cynthia' (Kenneth Hignight, personal communication)
45	Boomerang	Origin traces back to 'Cynthia' (Kenneth Hignight, personal communication)
46	SRX27921	Origin traces back to 'Cynthia' (Kenneth Hignight, personal communication)
47	PpH7907	Origin traces back to 'Cynthia' (Kenneth Hignight, personal communication)
48	PpH7929	Origin traces back to 'Cynthia' (Kenneth Hignight, personal communication)
49	Cheetah	'Cynthia' x 'Limousine' (L. A. Brilman, personal communication)
50	HV140	Ecotypic selection from Canada (Kenneth Hignight, personal communication). Direct ecotypic selection - unknown breeding origin. Eurasian type based on UPGMA and Structure analysis.
51	A04-1268Fin	Collection from Porkkala, Finland (W. A. Meyer and S. A. Bonos, personal communication)
52	A98-3320Pol	Collection from PilicaWarka, Poland (W. A. Meyer and S. A. Bonos, personal communication)
53	A98-3367Pol	Collection from Rekoro, Poland (W. A. Meyer and S. A. Bonos, personal communication)
54	Lily	Unknown breeding origin; European cultivar (W. A. Meyer, personal communication). Sydsport type based on UPGMA and Structure analysis.
55	Coventry	'Gnome' x unreleased variety in O. M. Scott breeding program (Alderson and Sharp, 1994) - Unknown (incomplete) breeding origin based on description. Sydsport type based on UPGMA and Structure analysis.
56	B5144	Ecotypic selection from Holly nursery in New Brunswick, NJ in 1985 (Crystal Rose-Fricker, personal communication). Direct ecotypic selection - unknown breeding origin. Sydsport type based on UPGMA and Structure analysis.
57	Fairfax	Origin likely traces back to 'Sydsport' (L. A. Brilman, personal communication).
58	Allure	Origin likely traces back to 'Sydsport' (L. A. Brilman, personal communication).
59	B545	'Sydsport' hybrid x 9189 (Crystal Rose-Fricker, personal communication).
60	Chateau	First generation hybrid cross of two selections (Alderson and Sharp, 1994) - Unknown breeding origin based on description. Sydsport type based on UPGMA and Structure analysis.
61	Serene	'Sydsport' open pollinated (Crystal Rose-Fricker, personal communication).
62	A04-1315Fin	Collection from Finstrom, Finland (W. A. Meyer and S. A. Bonos, personal communication). Likely a Sydsport type based on UPGMA and Structure analysis.
63	H04-389Fin	Collection from SodderRuder, Finland (W. A. Meyer and S. A. Bonos, personal communication). Likely a Sydsport type based on UPGMA and Structure analysis.
64	B543	'Sydsport' hybrid x 9189 (Crystal Rose-Fricker, personal communication).
65	SRXQG245	Origin traces back to 'Sydsport' (L. A. Brilman, personal communication).

(Continued on next page)

Supplementary Table 4. (Continued)

66	Shamrock	'Shamrock' is the type cultivar for the Shamrock classification type (Park et al., 2005; Shortell et al., 2009). Shamrock originated as a single, highly apomictic plant selected from the open-pollinated progeny of A80-336. A80-336 is an exceptionally vigorous, moderately apomictic F1 hybrid selected from the progeny of the cross 'Warren's A-25' x 'Touchdown'. A80-336 was pollinated by selected plants from 'Sydsport', 'Baron', and 'Julia' during the late winter of 1985 in a greenhouse located on the Cook College campus of Rutgers University (Bailey et al., 1995).
67	Moonsh	'Shamrock' x 'Midnight' (W. A. Meyer and S. A. Bonos, personal communication)
68	Brooklawn	'Shamrock' x 'America' (W. A. Meyer and S. A. Bonos, personal communication)
69	Lakeshore	'Shamrock' type cultivar (Park et al., 2005; Shortell et al., 2009); ' Lakeshore ' Kentucky bluegrass originated as a single, highly apomictic (>95%) plant selected from the open-pollinated progeny of A80-336. A80-336 is an exceptionally vigorous, moderately apomictic F1 hybrid selected from the progeny of the cross Warren's A-25 x 'Touchdown'. A80-336 was pollinated by typical plants of 'Sydsport', 'Baron' and 'Julia' during late winter of 1985 in a greenhouse located on the Cook College campus of Rutgers University, New Brunswick, NJ (Bonos et al., 2004a).
70	Mongoose	'Shamrock' type cultivar (Park et al., 2005; Shortell et al., 2009); ' Lakeshore ' x 'Unique' (W. A. Meyer and S. A. Bonos, personal communication)
71	A98-1028	'Lakeshore' x 'Alpine' (W. A. Meyer and S. A. Bonos, personal communication)
72	Aries	'Lakeshore' x 'Midnight' (W. A. Meyer and S. A. Bonos, personal communication)
73	A93-201	Derivative of ' Lakeshore ' (W. A. Meyer and S. A. Bonos, personal communication)
74	A97-1799	'Shamrock' x 'Midnight' (W. A. Meyer and S. A. Bonos, personal communication)
75	A01-701	A95-284 open pollinated (W. A. Meyer and S. A. Bonos, personal communication); Shamrock type based on UPGMA and Structure analysis; pollen source may have been from a Shamrock type (W. A. Meyer and S. A. Bonos, personal communication)
76	Bd0384	'Lakeshore' x 'Unique' (W. A. Meyer and S. A. Bonos, personal communication)
77	A05-313	'Lakeshore' x 'Unique' (W. A. Meyer and S. A. Bonos, personal communication)
78	A97-857	H87-337 open pollinated (W. A. Meyer and S. A. Bonos, personal communication); Possible Shamrock type based on UPGMA and Structure analysis; pollen source may have been from a Shamrock type (W. A. Meyer and S. A. Bonos, personal communication)
79	PpH6366	'Limousine' x 'Merit' (L. A. Brilman, personal communication).
80	Limousine	'Limousine' is the type cultivar for the ' Limousine ' classification type. Original collection from Volgelsborg region of Germany (Alderson and Sharp, 1994).
81	Julius	'Limousine' type (W. A. Meyer and S. A. Bonos, personal communication)
82	SRX26351	'Limousine' x 'Emprimo' (L. A. Brilman, personal communication); where 'Emprimo' is a European cultivar
83	A00-4083Fin	Collection from Pori, Finland (W. A. Meyer and S. A. Bonos, personal communication). Likely a Limousine type based on UPGMA and Structure analysis.
84	FreedomII	'Limousine' x 'Midnight' (Brede, 2004f)
85	NorthStar	'Merit' x 'Eagleton' (Crystal Rose-Fricker, personal communication)
86	Alpine	'Warren's A-25' x Iceland 412; Iceland 412 collected from old turf in Iceland (W. A. Meyer and S. A. Bonos, personal communication)
87	BlueSapphire	93KB3 x 'Baron'; 93KB3 = derivative of 'Wildwood' (Tom Brentano, personal communication)
88	Blackstone	'Warren's A-25' x 'Blacksburg' (Rose-Fricker et al., 2002)
89	Hampton	experimental # H98-30; H98-30 traces to H92-582 open pollinated; H92-582 was a cross of 'Warren's A-25' x derivative of 'Blacksburg' (W. A. Meyer and S. A. Bonos, personal communication)
90	Jefferson	'Jefferson' originated from a single, highly apomictic plant, A84-811-4, selected from the open-pollinated progeny of Warren's A20-6A Kentucky bluegrass (Bonos et al., 2003);
91	Washington	Warren's A20 was an ecotypic selection - unknown breeding origin (Funk, 1969) Single highly apomictic plant selected from AL-17 open pollinated (W. A. Meyer and S. A. Bonos, personal communication); AL-17 was an ecotypic selection - unknown breeding origin
92	P-105	'P-105' is the type cultivar for the P-105 classification type. Warren's A-25 open pollinated (Hurley et al., 2000)
93	Avid	'P-105' x 'Rita', + numerous plants collected from the Mid-Atlantic region of the US, Delaware, Maryland, New Jersey and Pennsylvania, + four plants of <i>Poa ampla</i> and <i>P. ampla</i> x <i>P. pratensis</i> (W. A. Meyer and S. A. Bonos, personal communication)
94	A95-410	'P-105' open pollinated with 152 Kentucky bluegrass ecotypes (W. A. Meyer and S. A. Bonos, personal communication)
95	H04-390Fin	Collection from Aristo, Finland (W. A. Meyer and S. A. Bonos, personal communication)
96	Ulysses	'Julia' x 'Baron' (L. A. Brilman, personal communication).
97	Julia	'Julia' is the type cultivar for the Julia classification type (Park et al., 2005; Shortell et al., 2009). Collection from northern Germany (Alderson and Sharp, 1994)
98	Baritone	Unknown breeding origin; Julia type based on UPGMA and Structure analysis.
99	H01-912Ger	Collection from Frankfurt, Germany (W. A. Meyer and S. A. Bonos, personal communication); Julia type based on UPGMA and Structure analysis.
100	A04-1283Fin	Collection from Hanko, Finland (W. A. Meyer and S. A. Bonos, personal communication); Julia type based on UPGMA and Structure analysis.
101	Bariris	Unknown breeding origin; Julia type based on UPGMA and Structure analysis.
102	A05-894Sp	Collection from Villabone, Spain (W. A. Meyer and S. A. Bonos, personal communication); Julia type based on UPGMA and Structure analysis.
103	Pick453	'Pick453' = A93-453. A93-453 is a derivative of A88-1145. A88-1145 is an open pollinated hybrid of 'Julia' (W. A. Meyer and S. A. Bonos, personal communication)
104	Rampart	'Rampart' = A93-417. A93-417 is a derivative of A88-1145. A88-1145 is an open pollinated hybrid of 'Julia' (W. A. Meyer and S. A. Bonos, personal communication)
105	H92-558	H92-558 is a derivative of A88-1145. A88-1145 is an open pollinated hybrid of 'Julia' (W. A. Meyer and S. A. Bonos, personal communication)
106	CV820631	Unknown breeding origin; possible Julia type based on UPGMA and Structure analysis.
107	A94MH94	Baron B1 hybrid (W. A. Meyer and S. A. Bonos, personal communication)
108	A98-3384Swe	Collection from an animal park in Sweden (W. A. Meyer and S. A. Bonos, personal communication)
109	H07-697Swe	Collection from Norrköping, Sweden (W. A. Meyer and S. A. Bonos, personal communication)
110	A04-1375Fin	Collection from Pargas, Finland (W. A. Meyer and S. A. Bonos, personal communication)
111	H02-608Swe	Collection from Ulricehamn, Sweden (W. A. Meyer and S. A. Bonos, personal communication)
112	H04-535Fin	Collection from Korpo, Finland (W. A. Meyer and S. A. Bonos, personal communication)
113	A04-1474Lith	Collection from Siauliai, Lithuania (W. A. Meyer and S. A. Bonos, personal communication)
114	A04-1470Lith	Collection from Siauliai, Lithuania (W. A. Meyer and S. A. Bonos, personal communication)
115	A04-1484Lith	Collection from Kursenai, Lithuania (W. A. Meyer and S. A. Bonos, personal communication)
116	A04-1569Lith	Collection from Molet, Lithuania (W. A. Meyer and S. A. Bonos, personal communication)
117	A04-1547Lith	Collection from Spitrenai, Lithuania (W. A. Meyer and S. A. Bonos, personal communication)
118	A98-3366Pol	Collection from Rekorvo, Poland (W. A. Meyer and S. A. Bonos, personal communication)
119	A04-1347Fin	Collection from Lepainen, Finland (W. A. Meyer and S. A. Bonos, personal communication)
120	Chelsea	Unknown breeding origin; European cultivar (W. A. Meyer, personal communication)
121	H04-376Fin	Collection from Kaister, Finland (W. A. Meyer and S. A. Bonos, personal communication)
122	BlueKnight	Unknown breeding origin; European cultivar (W. A. Meyer, personal communication)

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Supplementary Table 4. (Continued)

123	A04-1354Fin	Collection from Petsmo, Finland (W. A. Meyer and S. A. Bonos, personal communication)
124	GO9LM9	Previously classified as a Common or Midwest Ecotype - unknown breeding origin (W. A. Meyer, personal communication)
125	A05-847Fra	Collection from Colde Port, France (W. A. Meyer and S. A. Bonos, personal communication)
126	Barzan	Dutch ecotype (Alderson and Sharp, 1994)
127	A98-3369Pol	Collection from Teolog, Poland (W. A. Meyer and S. A. Bonos, personal communication)
128	A04-1342Fin	Collection from Kustavi, Finland (W. A. Meyer and S. A. Bonos, personal communication)
129	U2998Uz	Collection from Uzbekistan (W. A. Meyer and S. A. Bonos, personal communication)
130	A04-1384Fin	Collection from Aristo, Finland (W. A. Meyer and S. A. Bonos, personal communication)
131	Limerick	Unknown breeding origin; European cultivar (W. A. Meyer, personal communication)
132	A04-387Rom	Collection from Pasulprislup, Romania (W. A. Meyer and S. A. Bonos, personal communication)
133	H01-847Rom	Collection from Somcuta Mare, Romania (W. A. Meyer and S. A. Bonos, personal communication)
134	A04-1271Fin	Collection from Porkala, Finland (W. A. Meyer and S. A. Bonos, personal communication)
135	A04-394Rom	Collection from Petroojani, Romania (W. A. Meyer and S. A. Bonos, personal communication)
136	DLF769034	Unknown breeding origin; from DLF International Seeds, Holland (W. A. Meyer, personal communication)
137	A98-3297Pol	Collection from Ogródzieniec, Poland (W. A. Meyer and S. A. Bonos, personal communication)
138	A98-3323Pol	Collection from Zychlin, Poland (W. A. Meyer and S. A. Bonos, personal communication)
139	H94-305	Valsburg Park open pollinated (W. A. Meyer and S. A. Bonos, personal communication); "Oberly" type - a collection from remnant pastures / old fields - would have been previously classified as a Common or Midwest Ecotype (W. A. Meyer, personal communication); ecotypic selection - unknown breeding origin
140	Jewel	Unknown breeding origin; European cultivar (W. A. Meyer, personal communication)
141	A96-742	'Unique' open pollinated - pollen source unknown (W. A. Meyer and S. A. Bonos, personal communication)
142	A04-1423Lith	Collection from Bukta, Lithuania (W. A. Meyer and S. A. Bonos, personal communication)
143	Kenblue	Collection from seed farms in central Kentucky (Alderson and Sharp, 1994); collection from remnant pastures / old fields - one of the original Common or Midwest Ecotypes (Bara et al., 1993; Park et al., 2005; Shortell et al., 2009); ecotypic selection - unknown breeding origin
144	H99-1772	Collection of unknown origin - likely central Asia (W. A. Meyer and S. A. Bonos, personal communication)
145	A04-383Rom	Collection from Pojorata, Romania (W. A. Meyer and S. A. Bonos, personal communication)
146	A98-183	Muddy Park x 'Unique' + 418 (W. A. Meyer and S. A. Bonos, personal communication); Muddy Park was a single plant collection from Maddox Park in Atlanta, GA in 1975 - a collection from remnant pastures / old fields - (W. A. Meyer, personal communication)
147	A98-3322Pol	Collection from Zyrardow, Poland (W. A. Meyer and S. A. Bonos, personal communication)
148	A04-1272Fin	Collection from Porkala, Finland (W. A. Meyer and S. A. Bonos, personal communication)
149	Eagleton	'Eagleton' originated as a single, highly apomictic plant selected from the grounds of Woodlawn, home of the Eagleton Institute, on the Douglas College Campus of Rutgers University at New Brunswick, NJ, during the spring of 1975 (Hurley et al., 1997) - a collection from a remnant pasture / old field; ecotypic selection - unknown breeding origin
150	DLF769032	Accession from the Nordic Gene Bank (Niels Chr. Nielsen, personal communication)
151	Wellington	Ecotypic selection from Oregon (Tom Brentano, personal communication) - a collection from a remnant pasture / old field; ecotypic selection - unknown breeding origin
152	A04-1415Lith	Collection from Krudonis, Lithuania (W. A. Meyer and S. A. Bonos, personal communication)
153	H01-894Ch	Collection from Hongyan, China (W. A. Meyer and S. A. Bonos, personal communication)
154	Bedazzled	'Unique' open pollinated by typical plants of SR 2109, + selections identified as A82-1091, A82-1095, and H86-788, which were collections typical of 'Cheri' (Bonos et al., 2008)
155	PST222	'Unique' x several Julia hybrids, + an improved common type collected on the grounds of Johnson & Johnson in NJ, + 'Cobalt', + 4 ecotypic selections from the Mid-Atlantic region (W. A. Meyer and S. A. Bonos, personal communication)
156	A97-1409	'Unique' x several Julia hybrids, + an improved common type collected on the grounds of Johnson & Johnson in NJ, + 'Cobalt', + 4 ecotypic selections from the Mid-Atlantic region (W. A. Meyer and S. A. Bonos, personal communication)
157	Katie	'Unique' x 'Lakeshore' (W. A. Meyer and S. A. Bonos, personal communication)
158	Mallard	'Unique' open pollinated by typical plants of 'Moonlight', + SR 2109, + selections identified as A466 and A81-2183 (S. A. Bonos, 2011)
159	BlueRidge	'Unique' open pollinated by typical plants of 'Moonlight', + SR 2109, + selections identified as A466 and A81-2183 (W. A. Meyer and S. A. Bonos, personal communication)
160	VoyagerII	Aberrant from 'Voyager' (Crystal Rose-Fricker, personal communication) - Compact America type based on UPGMA and Structure analysis
161	Apollo	'Unique' x A83-285 or A84-803 (Crystal Rose-Fricker, personal communication)
162	Dynamo	'Unique' x 'Blacksburg' (Crystal Rose-Fricker, personal communication)
163	Showcase	originated as a single highly apomictic plant selected from 'Unique' (Rose-Fricker, C. A. and M. L. Fraser, 2004)
164	H92-203	Dellwood fine (collection from Dellwood Country Club) x 'Unique' (W. A. Meyer and S. A. Bonos, personal communication)
165	Brilliant	'Unique' x 'Rita', + C-727 (Crystal Rose-Fricker, personal communication)
166	Valor	'Unique' x Lakeshore (W. A. Meyer and S. A. Bonos, personal communication)
167	Casablanca	'Unique' x BM3 (BM3 = collection from Florence, Italy), + 'Limousine', + 'Denim' (Jerry Hall, personal communication)
168	DLF769037	Ecotypic selection Denmark (Niels Chr. Nielsen, personal communication); Compact America type based on UPGMA and Structure analysis
169	Baroness	Unknown breeding origin. Compact America type based on UPGMA and Structure analysis
170	A99-3116	derivative of 'America' (type cultivar for Compact-America classification type) (W. A. Meyer and S. A. Bonos, personal communication)
171	Barnique	'Unique' x 'Moonlight', + SR 2109, + A466 + A81-2183 ('Warren's A25' x 'Blacksburg') (W. A. Meyer and S. A. Bonos, personal communication)
172	Unique	Typical Compact America type cultivar (Park et al., 2005; Shortell et al., 2009); Single plant collection from Exeter, Rhode Island (Rose-Fricker et al., 1999)
173	A99-2678	'Unique' x 'Midnight', + Muddy Park (Mid-Atlantic ecotype), + 'Langara' (W. A. Meyer and S. A. Bonos, personal communication)
174	A97-1294	'Unique' x 'Shamrock' (W. A. Meyer and S. A. Bonos, personal communication)
175	Royce	'Unique' x 'Monopoly', + 'NorthStar', + one plant similar to 'Voyager', + one plant derived from 'Glade' Kentucky bluegrass (W. A. Meyer and S. A. Bonos, personal communication)
176	A03-141	'Unique' x 'Moonlight', + SR 2109, + A466, + A81-2183 ('Warren's A25' x 'Blacksburg') (W. A. Meyer and S. A. Bonos, personal communication)
177	A97-1303	'Unique' x 'Shamrock' (W. A. Meyer and S. A. Bonos, personal communication)
178	A05-322	Derivative of 'Unique' x 'Midnight' (W. A. Meyer and S. A. Bonos, personal communication)
179	H0299H98767	'America' hybrid (W. A. Meyer and S. A. Bonos, personal communication)
180	A05-332	'Unique' x 638 (W. A. Meyer and S. A. Bonos, personal communication)
181	A03-335	'Unique' x 'Lakeshore' (W. A. Meyer and S. A. Bonos, personal communication)
182	A05-335	'Unique' x 'Lakeshore' (W. A. Meyer and S. A. Bonos, personal communication)
183	A05-336	'Unique' x 'Lakeshore' (W. A. Meyer and S. A. Bonos, personal communication)
184	A00-99	'Unique' x 'Shamrock' + Plant similar to 'Lakeshore' (W. A. Meyer and S. A. Bonos, personal communication)
185	A99-523	'Unique' x 'RSP' (Mid-Atlantic type) + 'Midnight' (W. A. Meyer and S. A. Bonos, personal communication)

(Continued on next page)

Supplementary Table 4. (Continued)

186	A00-430	'America' (type cultivar for Compact-America classification type) x A82-204 (experimental Mid-Atlantic type) (W. A. Meyer and S. A. Bonos, personal communication)
187	Goldstar	'Unique' x several Julia hybrids, + 'Monopoly', + 'Cobalt', + two collections from New Jersey (W. A. Meyer and S. A. Bonos, personal communication)
188	A96-739	'Unique' x 'P-105', + 'Rita', + plants collected from the Mid-Atlantic region, Delaware, Maryland, New Jersey and Pennsylvania, + four plants of <i>Poa ampla</i> and <i>P. ampla</i> x <i>P. pratensis</i> (W. A. Meyer and S. A. Bonos, personal communication)
189	PSTH6150	'Unique' open-pollinated (Crystal Rose-Fricker, personal communication)
190	A98-365	'Unique' open pollinated by three different plants: one collected from a naturalized turfgrass area at Hort Farm II in North Brunswick, NJ in 1980; the second collection designated as W-1; and the third plant was an open-pollinated hybrid from a cross between 'Warren's A-25' x 'Touchdown'. (W. A. Meyer and S. A. Bonos, personal communication)
191	Boutique	'Unique' x 'P-105', + 'Rita', + plants collected from the Mid-Atlantic region, Delaware, Maryland, New Jersey and Pennsylvania, + four plants of <i>Poa ampla</i> and <i>P. ampla</i> x <i>P. pratensis</i> (W. A. Meyer and S. A. Bonos, personal communication)
192	Kingfisher	'Unique' x 'P-105', + 'Rita', + plants collected from the Mid-Atlantic region, Delaware, Maryland, New Jersey and Pennsylvania, + four plants of <i>Poa ampla</i> and <i>P. ampla</i> x <i>P. pratensis</i> (W. A. Meyer and S. A. Bonos, personal communication)
193	A99-3245	derivative of 'Kingfisher' (W. A. Meyer and S. A. Bonos, personal communication)
194	Bordeaux	'Unique' x 'P-105', + 'Rita', + plants collected from the Mid-Atlantic region, Delaware, Maryland, New Jersey and Pennsylvania, + four plants of <i>Poa ampla</i> and <i>P. ampla</i> x <i>P. pratensis</i> (Ford et al., 2004a)
195	Langara	'Langara' Kentucky bluegrass originated as a single, highly apomictic plant selected from the open-pollinated progeny of C-74. C-74 is a vigorous, apomictic plant that originated from a plant collected from an old turf area in Exeter, RI, in 1987. C-74 is similar in appearance and performance to 'Unique' Kentucky bluegrass (Bonos et al., 2005a)
196	A05-314	'Unique' x 'Lakeshore' (W. A. Meyer and S. A. Bonos, personal communication)
197	A96-363	'Unique' open pollinated with 152 Kentucky bluegrass ecotypes (W. A. Meyer and S. A. Bonos, personal communication)
198	A00-1400	'Unique' x 'Midnight' (W. A. Meyer and S. A. Bonos, personal communication)
199	A99-3127	derivative of 'America' (type cultivar for Compact-America classification type) (W. A. Meyer and S. A. Bonos, personal communication)
200	Mercury	'Unique' x 'P-105', + 'Rita', + plants collected from the Mid-Atlantic region, Delaware, Maryland, New Jersey and Pennsylvania, + four plants of <i>Poa ampla</i> and <i>P. ampla</i> x <i>P. pratensis</i> (W. A. Meyer and S. A. Bonos, personal communication)
201	MonteCarlo	'Unique' open pollinated with 152 Kentucky bluegrass ecotypes (W. A. Meyer and S. A. Bonos, personal communication)
202	SIA96386	'Unique' open pollinated with 152 Kentucky bluegrass ecotypes (W. A. Meyer and S. A. Bonos, personal communication)
203	Bluemax	'Dragon' open-pollinated (Crystal Rose-Fricker, personal communication); where 'Dragon' is open pollinated progeny of A85-4993; where A85-4993 was F1 hybrid of 'Warren's A-20' x 'Blacksburg' (W. A. Meyer and S. A. Bonos, personal communication)
204	Moonlight	'America' x experimental strain Tenn-27 (Crystal Rose-Fricker, personal communication)
205	Bluetastic	'Moonlight' x 'Blacksburg', + 'Blue Star' (Crystal Rose-Fricker, personal communication)
206	Arrow	'America' x 'Monopoly' + other Mid-Atlantic ecotypes collected from Delaware, Maryland, New Jersey and Pennsylvania (W. A. Meyer and S. A. Bonos, personal communication)
207	BARPp0566	'America' x 'Monopoly' + other Mid-Atlantic ecotypes collected from Delaware, Maryland, New Jersey and Pennsylvania (W. A. Meyer and S. A. Bonos, personal communication)
208	BARPp0573	'America' x 'Monopoly' + other Mid-Atlantic ecotypes collected from Delaware, Maryland, New Jersey and Pennsylvania (W. A. Meyer and S. A. Bonos, personal communication)
209	Royale	'Unique' x 'Lakeshore' (W. A. Meyer and S. A. Bonos, personal communication)
210	Diva	'Unique' x 'Shamrock' (W. A. Meyer and S. A. Bonos, personal communication)
211	A99-3122	derivative of 'America' (type cultivar for Compact-America classification type) (W. A. Meyer and S. A. Bonos, personal communication)
212	Sonoma	C-74 x 'Lakeshore' - C-74 is a medium-dark, low-growing, vigorous apomictic plant collected by C.R. Skogley from an old lawn at Exeter, RI, in 1987. C-74 is similar in appearance and performance to 'Unique' (Ford et al., 2004b)
213	A03-132	'America' x 'Shamrock' (W. A. Meyer and S. A. Bonos, personal communication)
214	Durham	'Unique' x 'Shamrock' (W. A. Meyer and S. A. Bonos, personal communication)
215	Shiraz	'Unique' x 'Shamrock', + 'Lakeshore' (W. A. Meyer and S. A. Bonos, personal communication)
216	A03-66	'Unique' x 'Lakeshore' (W. A. Meyer and S. A. Bonos, personal communication)
217	Bd992103	'Unique' x 'Midnight' (W. A. Meyer and S. A. Bonos, personal communication)
218	A99-2427	'Unique' x 'Midnight' (W. A. Meyer and S. A. Bonos, personal communication)
219	A98-407	H86-247 x A82-1095 - where H86-247 is 'A-20' x 'Blacksburg', and A82-1095 is a collection similar to 'Cheri' (W. A. Meyer and S. A. Bonos, personal communication)
220	PST604	'America' x BM3 (BM3 = collection from Florence, Italy) (Crystal Rose-Fricker and W. A. Meyer, personal communication)
221	SR2284	'Unique' x 'P-105', + 'Rita', + plants collected from the Mid-Atlantic region, Delaware, Maryland, New Jersey and Pennsylvania, + four plants of <i>Poa ampla</i> and <i>P. ampla</i> x <i>P. pratensis</i> (W. A. Meyer and S. A. Bonos, personal communication)
222	Delight	'Unique' x A82-1091, + A82-1095, + H86-788 (which are collections similar to the cultivar 'Cheri'), + A82-232 NB-70 (W. A. Meyer and S. A. Bonos, personal communication)
223	Glenmont	'Unique' open pollinated with 152 Kentucky bluegrass ecotypes (W. A. Meyer and S. A. Bonos, personal communication)
224	BlacksburgII	repeating aberrant from 'Blacksburg' (Crystal Rose-Fricker, personal communication) - where 'Blacksburg' is a known Compact type (Bonos et al., 2000)
225	Baronette	unknown breeding origin - Compact or Compact America type based on UPGMA and Structure analysis
226	Rita	single apomictic clone collected from the University of Denver, 1986 (Crystal Rose-Fricker, personal communication) - Compact or Compact America based on UPGMA and Structure analysis
227	PSTH535	collection from claremont cemetery x 'Unique' + 'Blacksburg' (Crystal Rose-Fricker, personal communication); cross between Compact and Compact America
228	Baronie	unknown breeding origin - possible Compact or Compact America type based on UPGMA and Structure analysis
229	MoonShadow	100% apomictic plant selected from the progeny of a highly sexual maternal plant, identified as an aberrant derivative of H86-974, pollinated by C-74. H86-974 was a moderately apomictic F1 hybrid selected from the progeny of the cross 'Warren's A-25' x 'Blacksburg' Kentucky bluegrass. C-74 is a vigorous, apomictic plant that originated from a plant collected from an old turf area in Exeter, RI, in 1987. It is similar in appearance and performance to 'Unique' (Bonos et al., 2005b)
230	Wildwood	Compact type (Park et al., 2005; Shortell et al., 2009)
231	Hallmark	Compact type (Shortell et al., 2009)
232	PSTB4246	0514 x 'Glade', + A83-865 (Crystal Rose-Fricker, personal communication) - Compact type based on UPGMA and Structure analysis
233	PSTYorkHarbor4	Collection from York Harbor, Maine (Crystal Rose-Fricker, personal communication) - Compact type based on UPGMA and Structure analysis
234	SRX2114	apomictic progeny of single off-type plant from P-46. P-46 originated from cross of <i>Poa autumnalis</i> x <i>Poa pratensis</i> from CA with a resulting progeny crossed with Dellwood fine (Leah Brilman, personal communication); very unique breeding origin tracing back to ecotypic selections with unknown breeding origins - Compact type based on UPGMA and Structure analysis
235	PSTB5125	collection from the University of Denver, 1986 (Crystal Rose-Fricker, personal communication) - Compact type based on UPGMA and Structure analysis
236	NAK992	Aberrant selection out of 'Wildwood' (Tom Brentano, personal communication)
237	Ascot	Compact type (Shortell et al., 2009)

(Continued on next page)

Supplementary Table 4. (Continued)

238	PST161	RSP open-pollinated (Crystal Rose-Fricker, personal communication) - RSP is a known Mid-Atlantic ecotype (Bonos et al., 2004b)
239	Cabernet	RSP x 'P-105', + 'Rita', + 'Unique', + plants collected from the Mid-Atlantic region, Delaware, Maryland, New Jersey and Pennsylvania, + four plants of <i>Poa ampla</i> and <i>P. ampla</i> x <i>P. pratensis</i> (Bonos et al., 2004b)
240	Starburst	RSP open-pollinated with 152 Kentucky bluegrass ecotypes (W. A. Meyer and S. A. Bonos, personal communication)
241	Appalachian	Wabash ¹ open pollinated with 152 Kentucky bluegrass ecotypes (W. A. Meyer and S. A. Bonos, personal communication)
242	A00-1254	¹ Wabash ¹ considered a Mid-Atlantic cultivar (Bonos et al., 2000)
243	A03-6	RSP x 'Midnight' (W. A. Meyer and S. A. Bonos, personal communication)
244	H03180A992874	RSP x 'Moonshadow' (W. A. Meyer and S. A. Bonos, personal communication)
245	A03-77	H03-180 = A99-2874; A99-2874 = RSP x 'Midnight' + 'Unique' (W. A. Meyer and S. A. Bonos, personal communication)
246	A03-37	'Unique' x 'Shamrock' + plant similar to 'Lakeshore' (W. A. Meyer and S. A. Bonos, personal communication)
247	A97-1328	RSP x 'Moonshadow' (W. A. Meyer and S. A. Bonos, personal communication)
248	H01-804Rom	'Unique' x 'Lakeshore' (W. A. Meyer and S. A. Bonos, personal communication)
		collection from Comarnic, Romania (W. A. Meyer and S. A. Bonos, personal communication)

¹Entry order follows the order presented in the unweighted pair group method using arithmetic average (UPGMA) diagram (Fig. 1A). Female parent written before the "x" and pollinator(s) written after the "x."

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