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

Josh A. Honig1, Vincenzo Averello, Stacy A. Bonos, and William A. Meyer

Department of Plant Biology and Pathology, School of Environmental and Biological Sciences, Rutgers University, 59 Dudley Road, Foran Hall, New Brunswick, NJ, 08901-8520

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Abstract. Kentucky bluegrass (Poa pratensis L.) is an important facultative apomorphic 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, Sydsport, 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 apomorphic 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; Grazzi 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 apomorphic 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 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., 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., 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).
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.

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, 2008). 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).

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 (simple sequence repeat data) and selection

| Classification type | Cultivar or selection |
|---------------------|-----------------------|
| BVMG                | Abbey, Baron, Baritza, Envicta, Goldrush, Marquis, Raven, DLF769036* |
| Compact             | Alpine, Ascot, Barocone, Baronie, Blacksburg II, Blackstone, BlueSapphire, Hallmark, Hampton, Rita, Wildwood, NA-K992, PST-B4-246, PST-B5-125, PST-H15-35, PST-YorkHarbor4, SRX2114|
| Compact-America     | Apollo, Arrow, Barnique, Baroness, Bedazedele, 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, S2284, Unique, Valor, VoyagerII, A000-1400, A000-430, A000-999, A03-132, A03-141, A03-335, A03-66, A03-77, A05-314, A05-322, A05-332, A05-335, A05-336, A96-363, A96-739, A97-1294, A97-1303, A97-1328, A97-1409, A98-365, A98-407, A99-2427, A99-2678, A99-3116, A99-3122, A99-3245, A99-523, BAR P0566, BAR P057, B99-2103, DLF769037, H00-99 × H98-767, H98-203, PST-222, PST-604, PST-H6-150, SIA938636 |
| Cynthia             | Bodacious, Boomerang, Cheetah, Pf7H907, Pf7H929, SRX27921 |
| Eurasian1           | A04-1735PargasFin, A04-1740SialiaLith, A04-1745SialiaLith, A04-1748KurseniLith, A04-1745KurseniLith, A04-1569MoletLith, A98-3366RekrovoPol, A98-3384AnimalPKSwe, H02-608UlreichannSwe, H04-535KorpoFin, H07-909NorriksPew |
| Eurasian2           | Barzan, Bluemight, Chelsea, A04-1324KusvatiFin, A04-1324PotemoFin, A04-1384ArloFin, A05-847ColdePortFra, A98-3369TeologPol, GO9LM9, H04-376KaisterFin, U2989Uxe |
| Eurasian3           | Eagleton, Jewell, Kenblue, Limerick, Wellington, A04-1271Porkalafin, A04-1272Porkalafin, A04-1415KrudonisLith, A04-1423BuktaLith, A04-3380PoriaRoman, A04-387PasulrisipolRom, A04-394PetrojaniRom, A96-742, A98-183, A98-3297GrodziecicPol, A98-332ZycharyPol, A98-332ZychalnPol, DLF769032, DLF769034, H01-847SomcutaMareRom, H01-869HongyanCh, H05-315H99-1723Uze |
| Jefferson/Wash.     | Jefferson, Washington |
| Julia               | Barris, Bartone, Julia, Pick 453, Rampart, Ulysses, A04-1283HankoFin, A05-894VillaboneSp, CVB20631, H01-912FrankfurtGer, H02-558 |
| Limousine           | Freedom II, Julius, Limousine, A00-4083Porfin, Pf6H366, SRX2651 |
| Mid-Atlantic         | Appalachian, Cabernet, Starburst, A00-1254, A03-37, A03-6, H03-180xA99-2874, PST-161 |
| Midnight             | Alexa, Arcadia, Award, Awesome, Barrister, Beyond, Bluestone, Blue Velvet, Chicago II, Courtyard, Everest, EverGlade, Excursion, Freedom III, FrontPage, Ginny, Impact, Liberator, Midnight, Midnight II, NuDestiny, NuGlade, Odyssey, Perfection, Quantum Leap, Ramsey II, Skyge, Total Eclipse, Tsunami |
| P-105               | Avid, P-105, A95-410 |
| Shamrock            | Aries, Brooklawn, Lakeshore, Mongoose, Moonshine, Shamrock, A01-701, A01-703, A93-201, A97-1799, A98-1028, Bd0384 |
| Sydsport            | Allure, Chateau, Coventry, Fairfax, Lily, Serena, A04-1315FinstromFin, B5144, B543, B545, H04-389SodderRuderFin, SRXQ0245 |
| Other               | Misty, NorthStar, 99AN53, A04-1268Porkalafin, A04-1374LepainenFin, A04-1427JurbarkaLith, A04-1494MH49, A97-857, A98-332FicalPorkalafin, A98-3367RekrovoPol, H140, H01-804ComaricRom, H02-603UlreichannSwe, H04-390ArloFin |

*DDenotes experimental selection.
*Original ‘Blackstone’.
*Denotes collection (Ch = China; Fin = Finland; Fra = France; Ger = Germany; Lith = Lithuania; Pol = Poland; Rom = Romania; Sp = Spain; Swe = Sweden; Uz = Uzbekistan).
*Original ‘Serene’.

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 Hapludalf). Plants were maintained in the field, in nursery
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 \(q_j\). 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 Prt(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 distances \((\Phi_{CT}\)). 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 dendrogram 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 values exist 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 Cheetal as well as the experimental selections S RX27921, 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 Al- lure, 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
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).
The majority of cultivars and accessions were uniquely identified with the current panel of 25 Kentucky bluegrass SSR markers. Exceptions included three clusters of cultivars within the Midnight classification type (Fig. 1A, Panel a). The first of these clusters consisted of the cultivars NUGlade (Brede, 2001a), Courtyard (Brede, 2011), Awesome (Brede, 2011), and Award (Brede, 2001b); the second consisted of the cultivars Tsunami (Brede, 2004a), Alexa (Brede, 2006a), Everest (Brede, 2006b), Freedom III...
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 in the UPGMA dendrogram (Fig. 1, Panel a) for the Midnight, BVMG, Cynthia, Sydapot, 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 (Supplementary Table 4). The STRUCTURE admixture analysis (bootstrap value 1.00; Fig. 1B) identified ‘Skye’ as a group of (K) = 12 supported by the UPGMA clustering analysis (Fig. 1, Panel a) and the Jefferson/Washington classification type. The STRUCTURE analysis at (K) = 14 did not uniquely identify the Jefferson/Washington classification type, as identified in the UPGMA clustering analysis (Supplementary Table 4). The STRUCTURE admixture analysis (bootstrap value 1.00; Fig. 1B) identified ‘Skye’ as a group of (K) = 12 supported by the UPGMA clustering analysis (Fig. 1, Panel a) and the Jefferson/Washington classification type. The STRUCTURE analysis at (K) = 14 did not uniquely identify the Jefferson/Washington classification type, as identified in the UPGMA clustering analysis (Supplementary Table 4). The STRUCTURE admixture analysis (bootstrap value 1.00; Fig. 1B) identified ‘Skye’ as a group of (K) = 12 supported by the UPGMA clustering analysis (Fig. 1, Panel a) and the Jefferson/Washington classification type.

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. Exceptions, model-based clustering analysis (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.
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 ecotype 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 in old pastures. Additional entries in the third Eurasian cluster that fit this description were H-14-305, 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 ΦST 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 the 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 ΦST 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 $\Phi_{ST}$ 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., 1981; 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 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.\(^z\)

| 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 [*Ustilago striiformis* (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 [*Drechslera poae* (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 [*Sclerotinia homoeocarpa* 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 (*Erysiphe graminis* 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 (*Sphenophorous parvulus* 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 |

\(^z\)A brief description of each classification type and some example cultivars are also shown.
### Supplementary Table 2. Kentucky bluegrass 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_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)
| SSR_allele size (bp) | PIC value |
|----------------------|-----------|
| GA1095_282          | 0.460     |
| GA1095_285          | 0.212     |
| GA1095_287          | 0.201     |
| GA1095_289          | 0.480     |
| GA1095_290          | 0.256     |
| GA1095_292          | 0.207     |
| GA1095_298          | 0.412     |
| GA1095_300          | 0.432     |
| GA1095_306          | 0.493     |
| GA1095_329          | 0.165     |
| GA1101_285          | 0.207     |
| GA1101_286          | 0.485     |
| GA1101_287          | 0.227     |
| GA1101_289          | 0.498     |
| GA1101_290          | 0.492     |
| GA1101_291          | 0.173     |
| GA1101_292          | 0.275     |
| GA1101_293          | 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          | 0.227     |
| 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 3. Pedigree, common Turf performance, and Morphological traits (PTM) classification of Kentucky bluegrass cultivars, experimental selections, and collections in the current study.a

| Classification type | Cultivar or selection |
|---------------------|-----------------------|
| BVMG                | Abbey, Baron, Envicta, Goldrush, Marquis, Raven |
| CELA                | Jefferson             |
| Common              | Kenblue, GO9LM9†      |
| Compact             | Alpine, Ascot, Blackstone,† Bluetastic, Chicago II, Diva, Goldstar, Hallmark, Hampton, Moonlight, Skye, P-105, Wildwood, PST-B4-246, PST-B5-125† |
| Compact-America     | Apollo, Arrow, Barnique, Barones, Bedazzled, Bordeaux, Boutique, Brilliant, Casablanca, Delight, Dynamo, Glenmont, Kingfisher, Langara, Mallard, Moonshadow, Royale, Showcase, Sonoma, SR 2284, Unique, Valor, BAR Pp 0566,† BAR Pp 0573,† PST-222,† PST-604,† PST-H6-150† |
| Compact-Midnight    | Alexa, Arcadia, Award, Awesome, Barrister, Beyond, Bluetone, Blue Velvet, Courtyard, Everest, EverGlade, Excursion, Freedom II, Freedom III, FrontPage, Gmne, Impact, Liberator, Midnight, Midnight II, NuDestiny, NuGlade, Odyssey, Perfection, Quantum Leap, Rambo, Rugby II, Total Eclipse, Tsunami |
| High Density        | Bariris, HV140,† Julius, Limousine, NorthStar |
| Julia               | Julia, Pick 453,† Rampart, H92-558† |
| Mid-Atlantic        | Appalachian, Cabernet, Eagleton, Starburst, PST-161† |
| Shamrock            | Brooklawn, Durham, Katie, Lakeshore, Mongoose, Moonshine, Shamrock, A98-1028* |
| Other (and unclassified) | Allure, Aries, Avid, Baritone, Baronette, Baronie, Barititia, Barzan, Blacksburg II, Blueemax, Bluennight, BlueRidge, BlueSapphire, Boddous, Boomerang, Chateau, Cheetah, Chelsea, Coventry, Fairfax, Jewel, Limerick, Mercury, Misty, MonteCarlo, Rta, Royce, Serena,† Shiraz, Ulysses, Voyager II, Washington, Wellington, 99AN53,† A00-1254,† A00-1400,† A00-4083PoriFin,† A00-430,† A00-999,† A01-701,† A03-332,† A03-141,† A03-335,† A03-337,† A03-338,† A04-1268PorkalaFin,† A04-1271PorkalaFin,† A04-1272PorkalaFin,† A04-1283HankoFin,† A04-1315FinstromFin,† A04-1342KustaviFin,† A04-1347LepainenFin,† A04-1354PetsamoFin,† A04-1375PargasFin,† A04-1384AristoFin,† A04-1415KrudonisLith,† A04-1423BuktaLith,† A04-1427JurbarkasLith,† A04-1470SiauliaiLith,† A04-1474SiauliaiLith,† A04-1484KursenaiLith,† A04-1547SpitrenaiLith,† A04-1569MoletLith,† A04-383PojorataRom,† A04-387PulsuploRom,† A04-394PetrojanRom,† A05-313,† A05-314,† A05-322,† A05-332,† A05-335,† A05-336,† A05-847ColdeFhra,† A05-894VillaBoneSp,† A93-201,† A94MH94,† A95-410,† A96-363,† A96-739,† A96-742,† A97-1924,† A97-1303,† A97-1738,† A97-1909,† A97-1799,† A98-857,† A98-183,† A98-3297GrodziecicPol,† A98-3320PilicaWarkaPol,† A98-3322ZywardPol,† A98-3323ZycholinPol,† A98-3366RekowoPol,† A98-3367RekowoPol,† A98-3369TeogolPol,† A98-3384AnimalPkswe,† A98-365,† A98-407,† A99-2427,† A99-2678,† A99-3116,† A99-3122,† A99-3245,† A99-523,† B5144,† B543,† B545,† Bd0384,† Bd09-2103,† CB20631,† DLF769032,† DLF769034,† DLF769036,† DLF769037,† H01-804ComarnicRom,† H01-847SomcutaMareRom,† H01-894HongyanCh,† H01-912FrankfurttGer,† H02-601UlricehamnSwe,† H02-605UlricehamnSwe,† H02-99xH98-767,† H03-180xA99-2874,† H04-376KaisterFin,† H04-389SoderRuderFin,† H04-390AristoFin,† H04-5315KorpoFin,† H07-697NorrkopingSwe,† H92-203,† H94-305,† H99-1722Luz,† NA-K992,‡ PpH6366,‡ PpH7907,‡ PST-YorkHarbor4,‡ SIA96386,‡ SRX2114,‡ SRX26351,‡ SRX27921,‡ SRXQG245,‡ U2998Uz,‡ PST-H5-356† |

*Kentucky 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).
†Denotes experimental selection.
‡Original 'Blackstone'.
§Original 'Serene'.
‖Denotes 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.  

| UPGMA order | Entry and Classification Type | Breeding History or Classification Type summary |
|-------------|-------------------------------|-----------------------------------------------|
| 1           | *Midnight*                    | 'Midnight' is the 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). |
| 2           | NuGlade                        | 'Limousine' x *Midnight* (Brende, 2003a). |
| 3           | Courtyard                      | 'Limousine' x *Midnight* (Brende, 2011). |
| 5           | Awesome                        | 'Limousine' x *Midnight* (Brende, 2011). |
| 6           | Award                          | 'Limousine' x *Midnight* (Brende, 2008b). |
| 7           | Tsunami                        | 'Freedom' x 'Limousine' (Brende, 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' (Brende, 2006a). |
| 9           | Everett                        | Open pollinated progeny of *Midnight* (Brende, 2006b). |
| 10          | Freespirit                      | 92-0076 x'Midnight' (Brende, 2006c). |
| 11          | Beyond                         | 'Limousine' x 'Midnight' (Brende, 2004b). |
| 12          | Excursion                      | 'Limousine' x *Midnight* (Brende, 2004c). |
| 13          | Barrister                      | 'Limousine' x *Midnight* (Brende, 2006d). |
| 14          | Everglade                      | 92-0076 x'Midnight' (Brende, 2006e). |
| 15          | Ginney                         | 'Limousine' x *Midnight* (Brende, 2011). |
| 16          | NOrthwest                     | 90-0416 x'Midnight' (Brende, 2004d). |
| 17          | Bluegrass                      | 'Limousine' x 'Midnight' (Brende, 2004c). 'Midnight' is a derivative of Julia (W. A. Meyer and S. A. Bonos, personal communication). |
| 18          | BlueVelvet                     | 'Limousine' x 'Midnight' (Brende, 2011). |
| 19          | Rambo                          | 'Rambo' originated from a highly apomictic, single-plant selection from an open-pollinated cross in the Jacklin Seed, Post Falls breeding nursery. The parentage of Rambo traces to breeding line 90-0499, which originated from a naturally occurring hybrid from *Midnight* Kentucky bluegrass (Brende, 2003a). |
| 20          | Impact                         | 'Limousine' x 'Midnight' (Brende, 2003b). |
| 21          | Midnight                       | 'Midnight' is a derivative of Julia (W. A. Meyer and S. A. Bonos, personal communication). |
| 22          | Total Eclipse                  | 'Limousine' x 'Midnight' (Brende, 2001c). |
| 23          | Rugby                          | 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 'Blackkburg' and 'Midnight'.' (Brende, 2001d) |
| 24          | FrontPage                      | 92-0076 x'Midnight' (A. D. Brede, personal communication). |
| 25          | Perfection                    | 'Limousine' x *Midnight* (Brende, 2011). |
| 26          | Odyssey                        | 'Limousine' x *Midnight* (Brende, 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*.' (Brende, 2001e) |
| 28          | Quantum Leap                   | 'Limousine' x 'Midnight' (Brende, 2003c). |
| 29          | Arriva                         | 'Midnight' x 'Limousine' (Brende, 2004d). |
| 30          | Giegold                        | 'Midnight' x 'Limousine' (Brende, 2004e). |
| 31          | Strye                          | 96-4644-6 1078 x'Midnight' (W. A. Meyer and S. A. Bonos, personal communication). |
| 32          | Baron                          | 'Baron' is one of the type cultivars for the BVMA (Baron, Vital, 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 (Hutley and Ghijzen, 1980). |
| 33          | Goldrush                       | BVMG type cultivar (Park et al., 2005; Shortell et al., 2009). |
| 34          | DLF765306                      | Collection from Holland (Niel Ch. Nielsen, personal communication); Direct ectopic selection - unknown breeding origin. |
| 35          | Bartita                        | BVMG type based on UPGMA and Structure analysis. |
| 36          | Emvicta                        | Ecotypic selection from Sweden (Alderson and Sharp, 1994). BVMG type based on UPGMA and Structure analysis. |
| 37          | Raven                          | BVMG type cultivar (Park et al., 2005; Shortell et al., 2009). |
| 38          | Abbey                          | BVMG type cultivar (Park et al., 2005; Shortell et al., 2009). |
| 39          | Marquis                        | BVMG type cultivar (Bara et al., 1993; Park et al., 2005; Shortell et al., 2009). |
| 40          | HD-C960                        | Collection from Ulriehormann, Sweden (W. A. Meyer and S. A. Bonos, personal communication). |
| 41          | A01-1427Lil                      | Collection from Jaburkas, Lithuania (W. A. Meyer and S. A. Bonos, personal communication). |
| 42          | Mutz                           | Unknown breeding origin; European cultivar (W. A. Meyer, personal communication) |
| 43          | 90AM53                         | Unknown breeding origin. |
| 44          | Bedauxa                        | Origin traces back to 'Cymbria' (Kenneth Hignight, personal communication). |
| 45          | Bloomerang                     | Origin traces back to 'Cymbria' (Kenneth Hignight, personal communication). |
| 46          | SCP7921                        | Origin traces back to 'Cymbria' (Kenneth Hignight, personal communication). |
| 47          | PS7920                         | Origin traces back to 'Cymbria' (Kenneth Hignight, personal communication). |
| 48          | P97929                         | Origin traces back to 'Cymbria' (Kenneth Hignight, personal communication). |
| 49          | Cheetah                        | 'Cymbria' x 'Limousine' (L. A. Brilman, personal communication). |
| 50          | NR300                          | Ecotypic selection from Canada (Kenneth Hignight, personal communication). Direct ectopic selection - unknown breeding origin. |
| 51          | A01-126Fln                     | Vermont x*Midnight* based on UPGMA and Structure analysis. |
| 52          | A98-3323Pol                    | Direct ectopic selection based on description. |
| 53          | A98-5837Pol                    | Systory type based on UPGMA and Structure analysis. |
| 54          | Lily                           | 'Gnome's unreleased variety in O. M. Scott breeding program (Alderson and Sharp, 1994) - Unknown (incomplete) breeding origin based on description. Systory type based on UPGMA and Structure analysis. Ecotypic selection from Holly nursery in New Brunswick, NJ in 1985 (Crystal Rose-Fricker, personal communication). Direct ectopic selection - unknown breeding origin. Systory type based on UPGMA and Structure analysis. Origin likely traces back to 'Systory' (L. A. Brilman, personal communication). |
| 55          | BS141                          | Origin likely traces back to 'Systory' (L. A. Brilman, personal communication). 'Systory' hybrid x 9189 (Crystal Rose-Fricker, personal communication). |
| 56          | Fairfax                        | First generation hybrid cross of two selections (Alderson and Sharp, 1994) - Unknown breeding origin based on description. Systory type based on UPGMA and Structure analysis. 'Systory' open pollinated (Crystal Rose-Fricker, personal communication). |
| 57          | Allure                         | Collection from Finnstrom, Finland (W. A. Meyer and S. A. Bonos, personal communication). Likely a Systory type based on UPGMA and Structure analysis. |
| 58          | Chateau                        | Collection from SuddlerRader, Finland (W. A. Meyer and S. A. Bonos, personal communication). Likely a Systory type based on UPGMA and Structure analysis. 'Systory' hybrid x 9189 (Crystal Rose-Fricker, personal communication). |
| 59          | Serene                         | Origin traces back to 'Systory' (L. A. Brilman, personal communication). |
| 60          | A06-1315Fin                    | (Continued on next page)
Supplementary Table 4. (Continued)

| Variety | Synonym | Description |
|---------|---------|-------------|
| 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 apomorphic plant selected from the open-pollinated progeny of 'A80-336'. A80-336 is an exceptionally vigorous, moderately apomorphic F1 hybrid selected from the progeny of the cross 'Warran'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). 'Shamrock' x 'Midnight' (W. A. Meyer and S. A. Bonos, personal communication) |
| 'Sydsport' | 'Sydsport' | 'Sydsport' (W. A. Meyer and S. A. Bonos, personal communication) |
| 'Baron' | 'Baron' | 'Baron' (W. A. Meyer and S. A. Bonos, personal communication) |
| 'Julia' | 'Julia' | 'Julia' (W. A. Meyer and S. A. Bonos, personal communication) |

| Variety | Synonym | Description |
|---------|---------|-------------|
| 'Lakeshore' | 'Lakeshore' | 'Lakeshore' (W. A. Meyer and S. A. Bonos, personal communication) |
| 'Alpine' | 'Alpine' | 'Alpine' (W. A. Meyer and S. A. Bonos, personal communication) |
| 'Midnight' | 'Midnight' | 'Midnight' (W. A. Meyer and S. A. Bonos, personal communication) |

(Continued on next page)
| Code | Description |
|------|-------------|
| A04-3354Fn | Collection from Petros, Finland (W. A. Meyer and S. A. Bonos, personal communication) |
| G09-M5 | Previously classified as a Common or Midwest Ecotype - unknown breeding origin (W. A. Meyer, personal communication) |
| A05-842Fn | Collection from Calde Port, France (W. A. Meyer and S. A. Bonos, personal communication) |
| Barzan | Dutch ecotype (Alderson and Sharp, 1994) |
| A06-3100Fn | Collection from Tuedo, Poland (W. A. Meyer and S. A. Bonos, personal communication) |
| A04-3320Fn | Collection from Kostinci, Finland (W. A. Meyer and S. A. Bonos, personal communication) |
| U2998U | Collection from Utsukashi (W. A. Meyer and S. A. Bonos, personal communication) |
| A04-1384Fn | Collection from Aristo, Finland (W. A. Meyer and S. A. Bonos, personal communication) |
| Limerick | Unknown breeding origin; European cultivar (W. A. Meyer, personal communication) |
| A04-3381Fn | Collection from Pasuajärv, Romania (W. A. Meyer and S. A. Bonos, personal communication) |
| H01-8478om | Collection from Somcuta Mare, Romania (W. A. Meyer and S. A. Bonos, personal communication) |
| A04-1771Fn | Collection from P urgency, Romania (W. A. Meyer and S. A. Bonos, personal communication) |
| A04-3940om | Unknown breeding origin; from DLF International Seeds, Holland (W. A. Meyer, personal communication) |
| DL795634 | Collection from Ogrodzieniec, Poland (W. A. Meyer and S. A. Bonos, personal communication) |
| A08-3295Pl | Collection from Zephyria, Poland (W. A. Meyer and S. A. Bonos, personal communication) |
| H94-3205 | Valsburg Park open pollinated (W. A. Meyer and S. A. Bonos, personal communication); "Oberb" type - a collection from remnant pastures / old fields - would have been previously classified as a Common or Midwest Ecotype (W. A. Meyer, personal communication); ecotype selection - unknown breeding origin |
| Jewell | Unknown breeding origin; European cultivar (W. A. Meyer, personal communication) |
| A06-742 | 'Unique' open pollinated - pollen source unknown (W. A. Meyer and S. A. Bonos, personal communication) |
| A04-1432Lith | Collection from Balta, Lithuania (W. A. Meyer and S. A. Bonos, personal communication) |
| Kehlaue | 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 (Barr et al., 1993; Park et al., 2005; Shoffet et al., 2000); ecotype selection - unknown breeding origin |
| H05-1772 | Collection of unknown origin - likely central Asia (W. A. Meyer and S. A. Bonos, personal communication) |
| A04-3381om | Collection from Pojama, Romania (W. A. Meyer and S. A. Bonos, personal communication) |
| A08-183 | 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) |
| A08-3322Pl | Collection from Zyrnow, Poland (W. A. Meyer and S. A. Bonos, personal communication) |
| A04-1771Fn | Collection from Parki, Finland (W. A. Meyer and S. A. Bonos, personal communication) |
| Eagleton | 'Eagleton' originated as a single, highly apomorphic plant selected from the grounds of Woodlawn, home of the Eagleton Institute, on the Douglas College Campus of Rogers University at New Brunswick, NJ, during the spring of 1975 (Hurley et al., 1997) - a collection from a remnant pasture / old field; ecotype selection - unknown breeding origin |
| Wellington | Accession from the Nordic Gene Bank (Niels Chr. Nielsen, personal communication) |
| A04-1431Lith | Ecotypic selection from Oregon (Tom Brentano, personal communication) - a collection from a remnant pasture / old field; ecotype selection - unknown breeding origin |
| H01-8464 | Collection from Krudnok, Lithuania (W. A. Meyer and S. A. Bonos, personal communication) |
| A04-3381om | Collection from Hongyan, China (W. A. Meyer and S. A. Bonos, personal communication) |
| Bedazzled | 'Unique' open pollinated by typical plants of SR 2109, + selections identified as A38-1091, A82-1095, and H86-788, which were collections typical of "Cheli" (Bonos et al., 2008) |
| PST22 | '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) |
| AST-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) |
| Katie | 'Unique' x 'Lakeshore' (W. A. Meyer and S. A. Bonos, personal communication) |
| Mallard | 'Unique' open pollinated by typical plants of 'Moonlight', + SR 2109, + selections identified as A466 and A81-2183 (S. A. Bonos, 2011) |
| 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) |
| Voyager | Abarient from 'Voyager' (Crystal Rose-Fricker, personal communication); Compact America type based on UPGMA and Structure analysis |
| Apollo | Unique x A33-285 or A84-803 (Crystal Rose-Fricker, personal communication) |
| Dynamo | 'Unique' x 'Blackburn' (Crystal Rose-Fricker, personal communication) originated as a single highly apomorphic plant selected from 'Unique' (Rose-Fricker, C. A. and M. L. Frmer, 2004) |
| Showcase | Ditwood fine collection from Ditwood Country Club) x 'Unique' (W. A. Meyer and S. A. Bonos, personal communication) |
| H92-203 | 'Unique' x 'Rid', + C-727 (Crystal Rose-Fricker, personal communication) |
| Brilliant | 'Unique' x 'Lakeshore' (W. A. Meyer and S. A. Bonos, personal communication) |
| Valor | 'Unique' x BM3 (BM 3 - collection from Florence, Italy), + 'Limosiaus', + 'Denim' (Jerry Hall, personal communication); Jamaican type based on UPGMA and Structure analysis |
| Casablanca | Ecotypic selection Denmark (Niels Chr. Nielsen, personal communication); Compact America type based on UPGMA and Structure analysis |
| DL796537 | Unknown breeding origin. Compact America type based on UPGMA and Structure analysis; derivative of 'Unique' x 'Midnight' (W. A. Meyer and S. A. Bonos, personal communication) |
| Boronez | Unique x 'Moonlight', + SR 2109, + A466 + A81-2183 ('Warren's A25 x 'Blacksburgh') (W. A. Meyer and S. A. Bonos, personal communication) |
| A09-3116 | Unique x 'Moonlight', + SR 2109, + A466 + A81-2183 (Warren's A25 x 'Blacksburgh') (W. A. Meyer and S. A. Bonos, personal communication) |
| Barrique | Typical Compact America type cultivar (Park et al., 2005; Shoffet et al., 2009); Single plant collection from Exeter, Rhode Island (Rose-Fricker et al., 1999) |
| A06-2678 | Unique x 'Midnight', + Muddy Park (Mid-Atlantic ecotype), + 'Laguna' (W. A. Meyer and S. A. Bonos, personal communication) |
| A05-1294 | Unique x 'Shamrock' (W. A. Meyer and S. A. Bonos, personal communication) |
| Royce | Unique x 'Monopoly', + 'NorthStar', + one plant similar to 'Voyager', + one plant derived from 'Ulge' Kentucky bluegrass (W. A. Meyer and S. A. Bonos, personal communication) |
| A03-141 | 'Unique' x 'Moonlight', + SR 2109, + A466, + A81-2183 ('Warren's A25 x 'Blacksburgh') (W. A. Meyer and S. A. Bonos, personal communication) |
| A05-1294 | 'Unique' x 'Shamrock' (W. A. Meyer and S. A. Bonos, personal communication) |
| A03-335 | 'Unique' x 'Lakeshore' (W. A. Meyer and S. A. Bonos, personal communication) |
| A05-335 | 'Unique' x 'Lakeshore' (W. A. Meyer and S. A. Bonos, personal communication) |
| A05-336 | 'Unique' x 'Lakeshore' (W. A. Meyer and S. A. Bonos, personal communication) |
| A00-99 | 'Unique' x 'Lakeshore' + plant similar to 'Lakeshore' (W. A. Meyer and S. A. Bonos, personal communication) |
| A09-523 | 'Unique' x 'RSP' (Mid-Atlantic type) + 'Midnight' (W. A. Meyer and S. A. Bonos, personal communication) |

(Continued on next page)
| Varietal Name | Characteristics |
|--------------|----------------|
| A00-430      | Acriolo (type cultivar for Compact-America classification type) x AE3-204 (experimental Mid-Atlantic type) |
| Goldstar     | Unique x several Julia hybrids, + 'Monopoly', + 'Cohort', + two collections from New Jersey (W.A. Meyer and S.A. Bonos, personal communication) |
| A06-739      | 'Tara', + plants collected from the Mid-Atlantic region, Delaware, Maryland, New Jersey and Pennsylvania, + four plants of P. ampla and P. ampla s. P. pratinax (W.A. Meyer and S.A. Bonos, personal communication) |
| PSTHB1150    | Unique open-pollinated (Crystal Rose-Fricker, personal communication) |
| A98-365      | 'Tara', + plants collected from the Mid-Atlantic region, Delaware, Maryland, New Jersey and Pennsylvania, + four plants of P. ampla and P. ampla s. P. pratinax (W.A. Meyer and S.A. Bonos, personal communication) |
| Kingfisher   | Unique x 'Tara', + plants collected from the Mid-Atlantic region, Delaware, Maryland, New Jersey and Pennsylvania, + four plants of P. ampla and P. ampla s. P. pratinax (W.A. Meyer and S.A. Bonos, personal communication) |
| A09-3245     | 'Tara', + plants collected from the Mid-Atlantic region, Delaware, Maryland, New Jersey and Pennsylvania, + four plants of P. ampla and P. ampla s. P. pratinax (Ford et al., 2004a) |
| Bordeaux     | Unique x 'Monopoly' - other Mid-Atlantic ecotypes collected from Delaware, Maryland, New Jersey and Pennsylvania (W.A. Meyer and S.A. Bonos, personal communication) |
| Langara      | Unique x 'Midnight' (W.A. Meyer and S.A. Bonos, personal communication) |
| A05-314      | 'Lakshmi' (W.A. Meyer and S.A. Bonos, personal communication) |
| A96-363      | Unique open-pollinated with 152 Kentucky bluegrass ecotypes (W.A. Meyer and S.A. Bonos, personal communication) |
| ACO-1400     | Unique x 'Midnight' (W.A. Meyer and S.A. Bonos, personal communication) |
| A09-3177     | Unique x 'Monopoly' + other Mid-Atlantic ecotypes collected from Delaware, Maryland, New Jersey and Pennsylvania (W.A. Meyer and S.A. Bonos, personal communication) |
| Montecarlo   | Unique x 'Monopoly' + other Mid-Atlantic ecotypes collected from Delaware, Maryland, New Jersey and Pennsylvania (W.A. Meyer and S.A. Bonos, personal communication) |
| SAR39266     | Unique x 'Lakshmi' (W.A. Meyer and S.A. Bonos, personal communication) |
| Bluehx      | Unique x 'Lakshmi' (W.A. Meyer and S.A. Bonos, personal communication) |
| Keylight     | Unique x 'Lakshmi' (W.A. Meyer and S.A. Bonos, personal communication) |
| Biostar      | Unique x 'Lakshmi' (W.A. Meyer and S.A. Bonos, personal communication) |
| Arrow        | Unique x 'Lakshmi' (W.A. Meyer and S.A. Bonos, personal communication) |
| BARKP0566    | Unique x 'Midnight' (W.A. Meyer and S.A. Bonos, personal communication) |
| BARKP0573    | Unique x 'Monopoly' + other Mid-Atlantic ecotypes collected from Delaware, Maryland, New Jersey and Pennsylvania (W.A. Meyer and S.A. Bonos, personal communication) |
| Royal        | Unique x 'Lakshmi' (W.A. Meyer and S.A. Bonos, personal communication) |
| Diva         | Unique x 'Lakshmi' (W.A. Meyer and S.A. Bonos, personal communication) |
| A09-3177     | Unique x 'Lakshmi' (W.A. Meyer and S.A. Bonos, personal communication) |
| Sonoma       | Unique x 'Lakshmi' (W.A. Meyer and S.A. Bonos, personal communication) |
| A03-132      | Unique x 'Lakshmi' (W.A. Meyer and S.A. Bonos, personal communication) |
| Durban       | Unique x 'Lakshmi' (W.A. Meyer and S.A. Bonos, personal communication) |
| Shiraz       | Unique x 'Lakshmi' (W.A. Meyer and S.A. Bonos, personal communication) |
| A03-66       | Unique x 'Monopoly' + other Mid-Atlantic ecotypes collected from Delaware, Maryland, New Jersey and Pennsylvania (W.A. Meyer and S.A. Bonos, personal communication) |
| Bd922103     | Unique x 'Lakshmi' (W.A. Meyer and S.A. Bonos, personal communication) |
| A98-2417     | Unique x 'Lakshmi' (W.A. Meyer and S.A. Bonos, personal communication) |
| A98-407      | Unique x 'Lakshmi' (W.A. Meyer and S.A. Bonos, personal communication) |
| STS004       | Unique x 'Lakshmi' (W.A. Meyer and S.A. Bonos, personal communication) |
| STS2284      | Unique x 'Lakshmi' (W.A. Meyer and S.A. Bonos, personal communication) |
| Delight      | Unique x 'Lakshmi' (W.A. Meyer and S.A. Bonos, personal communication) |
| Glencourt    | Unique x 'Lakshmi' (W.A. Meyer and S.A. Bonos, personal communication) |
| Blackburg    | Unique x 'Monopoly' + other Mid-Atlantic ecotypes collected from Delaware, Maryland, New Jersey and Pennsylvania (W.A. Meyer and S.A. Bonos, personal communication) |
| Baromette    | Unique x 'Monopoly' + other Mid-Atlantic ecotypes collected from Delaware, Maryland, New Jersey and Pennsylvania (W.A. Meyer and S.A. Bonos, personal communication) |
| Lakshmi      | Unique x 'Monopoly' + other Mid-Atlantic ecotypes collected from Delaware, Maryland, New Jersey and Pennsylvania (W.A. Meyer and S.A. Bonos, personal communication) |
| PSH535       | Unique x 'Monopoly' + other Mid-Atlantic ecotypes collected from Delaware, Maryland, New Jersey and Pennsylvania (W.A. Meyer and S.A. Bonos, personal communication) |
| Barone       | Unique x 'Monopoly' + other Mid-Atlantic ecotypes collected from Delaware, Maryland, New Jersey and Pennsylvania (W.A. Meyer and S.A. Bonos, personal communication) |
| MoosShadow   | Unique x 'Monopoly' + other Mid-Atlantic ecotypes collected from Delaware, Maryland, New Jersey and Pennsylvania (W.A. Meyer and S.A. Bonos, personal communication) |
| Wildwood     | Unique x 'Monopoly' + other Mid-Atlantic ecotypes collected from Delaware, Maryland, New Jersey and Pennsylvania (W.A. Meyer and S.A. Bonos, personal communication) |
| Hallmark     | Unique x 'Monopoly' + other Mid-Atlantic ecotypes collected from Delaware, Maryland, New Jersey and Pennsylvania (W.A. Meyer and S.A. Bonos, personal communication) |
| PSB1246      | Unique x 'Monopoly' + other Mid-Atlantic ecotypes collected from Delaware, Maryland, New Jersey and Pennsylvania (W.A. Meyer and S.A. Bonos, personal communication) |
| PSTY675R     | Unique x 'Monopoly' + other Mid-Atlantic ecotypes collected from Delaware, Maryland, New Jersey and Pennsylvania (W.A. Meyer and S.A. Bonos, personal communication) |
| SKX2114      | Unique x 'Monopoly' + other Mid-Atlantic ecotypes collected from Delaware, Maryland, New Jersey and Pennsylvania (W.A. Meyer and S.A. Bonos, personal communication) |

(Continued on next page)
Supplementary Table 4. (Continued)

238 PST1561 Cabernet
239 Starburst Appalachian
240 AO-1254
241 AO-97
242 AO-37
243 H031806292874
244 Unique 'Lakeshore'
245 Unique 'Lakeshore'
246 H03-1328
247 H03-1328
248 RSP open-pollinated ('Crystal Rose-Fricker, personal communication) - RSP in a known Mid-Atlantic ecotype (Bonos et al., 2004b)
239 RSP 'P-105' - 'Wili', 'Unique' x plants collected from the Mid-Atlantic region, Delaware, Maryland
240 New Jersey and Pennsylvania. x four plants of Poa annua and P. annua x P. pratensis (Bonos et al., 2004b)
241 RSP open-pollinated with 132 Kentucky bluegrass ecotypes (W. A. Meyer and S. A. Bonos, personal communication)
242 'Wabash' open pollinated with 132 Kentucky bluegrass ecotypes (W. A. Meyer and S. A. Bonos, personal communication)
243 'Wabash' considered a Mid-Atlantic cultivar (Bonos et al., 2000)
244 RSP 'x Midnight' (W. A. Meyer and S. A. Bonos, personal communication)
245 RSP 'x Moonshadow' (W. A. Meyer and S. A. Bonos, personal communication)
246 H03-180 = A99-2874, A99-2874 = 'RSP's 'Midnight' x 'Unique' (W. A. Meyer and S. A. Bonos, personal communication)
247 'Unique x Slamrock' = plant similar to 'Lakeshore' (W. A. Meyer and S. A. Bonos, personal communication)
248 RSP 'x Moonshadow' (W. A. Meyer and S. A. Bonos, personal communication)

"Entry 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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