Natural Hybrids and Gene Flow between Upland and Lowland Switchgrass

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ABSTRACT

Switchgrass (*Panicum virgatum* L.) is a perennial grass native to the North American tallgrass prairie and savanna habitats and is broadly adapted to the central and eastern United States. Upland and lowland ecotypes represent the two major taxa within switchgrass, which have distinct but overlapping distributions. The purpose of this study was to survey a broad array of putative upland and lowland accessions for the possible presence of natural hybrids or hybrid derivatives and evidence of historic gene flow between the two ecotypes. All plants were classified as upland, lowland, or intermediate based on visual assessment of phenotype, using large nurseries of known upland or lowland plants as controls. A total of 480 plants were surveyed for 19 simple sequence repeat (SSR) markers and sequenced using five chloroplast DNA (cpDNA) segments. Genetic structure analysis revealed 21 individuals with strong evidence for intertaxa hybrid origin and another 25 individuals with moderate evidence for intertaxa hybrid origin. All but two of these individuals originated from remnant populations of the central or eastern Gulf Coast or along the Atlantic Seaboard, a region that is populated with significant quantities of both upland and lowland ecotypes. We propose the central and eastern Gulf Coast glacial refuge as the primary center of origin and diversity for switchgrass, with the western Gulf Coast as the secondary center of origin and diversity. Much of this diversity appears to have been preserved along one of the major northward postglacial migration routes, the Atlantic Seaboard.

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Abbreviations: cpDNA, chloroplast DNA; PCR, polymerase chain reaction; p_H, probability of any type of hybrid ancestry; PIC, polymorphism information content; ROX, carboxy-X-rhodamine; SSR, simple sequence repeat.

What happens to all of our familiar habitats and ecosystems during the Ice Ages? "…unless one can believe that the typical temperate species were pushed south of the Rio Grande and deep into peninsular Florida, the alternative is an extraordinary intermingling of boreal, temperate, and subtropical elements along the Gulf coast." (Deevey, 1949) Edward Smith Deevey Jr. is largely responsible for converting the field of paleolimnol-

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ogy into a quantitative science that unlocked the vast treasure of chronological data buried in lakebed sediments.

"The role of climate in vegetational selection is nowhere more evident than in grassland studies.…The result of selection within the grassland climax has been the creation of geographic continua made up of site climax communities which are self maintaining and have reached a partial stability with high productivity under the existing habitat pressures." (McMillan, 1959). Calvin McMillan performed pioneering research on the ecology and biogeography of dominant species of the tallgrass prairie. He was the first to propose multiple glacial refugia for the dominant tallgrass prairie species.

Switchgrass (*Panicum virgatum* L.) is one of the dominant grasses of the tallgrass prairie and associated ecosystems. These associated ecosystems include oak savanna, pine barrens, forest margins, and some wetlands that form transition habitats between the ancient tallgrass prairie and the eastern forest. Switchgrass can be found in thousands of prairie or savanna remnants scattered through much of the original species range. Germplasm collected from many of these remnants has been the genetic foundation of early cultivars, which were source-identified random seed increases, and modern cultivars resulting from selection and breeding both within and among these original accessions.

Two taxa form the dominant phenotypic groups in switchgrass. Lowland ecotypes are found on flood plains and other areas subject to inundation, while upland ecotypes are found in upland areas that are not subject to flooding but may experience frequent droughts. Lowland switchgrass is taller, has longer leaf blades, fewer tillers per plant, larger stem diameter, and later heading and flowering than upland switchgrass (Cortese et al., 2010). Lowland ecotypes also typically possess a bluish waxy bloom on leaf blades and sheaths. Lowland ecotypes are the dominant form in the southern portion of the range (Kansas, Oklahoma, and Texas to the eastern Gulf Coast and the Atlantic Seaboard), while upland ecotypes are the dominant form in the northern portion of the range (Nebraska and the Dakotas to New England), with a transition zone in which both ecotypes are well adapted. In general, upland ecotypes are well adapted to USDA hardiness zones 3 to 7 and lowland ecotypes are well adapted to USDA hardiness zones 5 to 9 (Casler et al., 2011). Minor exceptions to this may derive from microclimate effects due to altitude, soil type, moisture availability, or specific pest problems.

Discrimination between upland and lowland switchgrass ecotypes, including classification of unknown germplasm, is relatively straightforward with DNA markers, having successfully been applied to numerous germplasm collections and using several marker systems (Cortese et al., 2010; Gunter et al., 1996; Hultquist et al., 1996; Martinez-Reyna et al., 2001; Missaoui et al., 2006; Narasimhamoorthy et al., 2008; Zalapa et al., 2011). Nevertheless, some ambiguities have arisen in these studies, such as two unclassified accessions (Missaoui et al., 2006; Narasimhamoorthy et al., 2008), disagreement in classification between nuclear and chloroplast markers for some accessions (Gunter et al., 1996), and mixed ploidy levels within some lowland accessions (Narasimhamoorthy et al., 2008). Lowland ecotypes are exclusively tetraploid ($2n = 4x = 36$), while many upland ecotypes are tetraploid ($2n = 4x = 36$) or octaploid ($2n = 8x = 72$) with hexaploids ($2n = 6x = 54$) reported very rarely (Narasimhamoorthy et al., 2008; Nielsen, 1944). Aneuploidy is frequent in switchgrass, especially at higher ploidy levels (Costich et al., 2010).

The presence of occasional hexaploids, mixed ploidies within some lowland accessions, and inconsistency between nuclear and cytoplasmic DNA markers all suggest the possibility of gene flow between upland and lowland ecotypes of switchgrass. Further circumstantial evidence for possible gene flow derives from the rapid increase in germplasm exploration, collection, evaluation, and enhancement efforts since 1992 (Sanderson et al., 2007). Increases in both the number and size of switchgrass breeding programs have led to massive efforts to sample germplasm from a wider array of environments and habitats (e.g., Casler, 2005; Hopkins et al., 1996). Unusual plants that defy simple classification as either upland or lowland types are becoming increasingly frequent in many of these breeding nurseries (e.g., Casler, 2005; Hultquist et al., 1997).

Knowledge of gene flow between the two switchgrass ecotypes is critical for two reasons. Because of the differential adaptation regions of upland and lowland ecotypes, this discrimination and classification of plants according to ecotype is critical for development and identification of regionally adapted gene pools and cultivars (Casler et al., 2007a, b; Zalapa et al., 2011). Second, upland and lowland ecotypes are sufficiently differentiated to have an a priori biologically significant heterotic pattern (Martinez-Reyna and Vogel, 2008; Vogel and Mitchell, 2008), warranting development of independent, but complementary, gene pools for the purposes of interpopulation improvement for development of commercial hybrids. Therefore, the objectives of this study were to determine if gene flow has occurred and to identify the origins of gene exchange between upland and lowland switchgrass ecotypes.

MATERIALS AND METHODS

Plant Materials

The germplasm for this study consisted of 67 switchgrass accessions made up of improved cultivars, source-identified cultivars, and wild populations (Table 1). All accessions are members of two switchgrass association panels, one originating in Madison, WI, from northern-adapted germplasm, and one originating in Athens, GA, from southern-adapted germplasm. All plants have been observed as spaced plants in field environments in either Wisconsin or Georgia, in direct comparison to a wide range of other accessions (upland ecotypes in Wisconsin and lowland

Table 1. Names, abbreviations, number of individuals, and passport information for 67 switchgrass cultivars or accessions used in DNA marker evaluations.

Cultivar or accession	Abbreviation	*n*	Status†	Origin	Latitude, north	Longitude, west	Phenotype	Seed source‡
Shawnee	SE	4	Bred	Illinois	37.47	88.17	Upland	USDA-ARS
Summer	SU	20	Bred	Nebraska	40.68	95.86	Upland	SDCIA
Sunburst	SB	13	Bred	South Dakota	42.87	97.40	Upland	SDCIA
Trailblazer	TB	8	Bred	Nebraska	39.92	98.07	Upland	USDA-ARS
WS4U	4U	6	Bred	Wisconsin	43.30	89.35	Upland	SW776
Blackwell	BL	28	SIC	Oklahoma	35.96	97.07	Upland	PI 421520
Carthage	CT	8	SIC	North Carolina	35.35	79.45	Upland	PI 421138
Cave-in-Rock	CR	24	SIC	Illinois	37.47	88.17	Upland	PI 469228
Dacotah	DC	20	SIC	North Dakota	46.38	100.94	Upland	NRCS-PMC
Forestburg	FB	17	SIC	South Dakota	44.02	98.10	Upland	NRCS-PMC
KY1625	K16	4	SIC	West Virginia	37.87	81.23	Upland	PI 431575
Pathfinder	PF	13	SIC	Kansas	39.82	98.48	Upland	USDA-ARS
Shelter	SH	9	SIC	West Virginia	39.40	81.20	Upland	NRCS-PMC
Albany, NY	ALB	4	Wild	New York	42.72	73.83	Upland	ECS-12
Allegheny River, PA	ALG	4	Wild	Pennsylvania	40.95	79.62	Upland	ECS-10
Apache Road	APR	3	Wild	Wisconsin	44.20	89.67	Upland	SW110
Camp Dawson	CDW	4	Wild	Kentucky	38.02	82.67	Upland	SW809
Chippewa	CHP	4	Wild	Minnesota	45.52	95.31	Upland	SW48
Chiwaukee 1	CH1	4	Wild	Wisconsin	42.55	87.80	Upland	SW124
Columbiana	COL	4	Wild	Ohio	40.62	80.70	Upland	SW64
Genesee	GEN	4	Wild	New York	42.99	78.15	Upland	SW63
Ipswich Prairie 2	IP2	4	Wild	Wisconsin	42.57	90.40	Upland	SW115
Jackson	JCK	4	Wild	Michigan	42.25	84.31	Upland	SW43
Leaches Crossing	LEC	3	Wild	Wisconsin	43.20	90.33	Upland	SW122
Montgomery	MON	5	Wild	Indiana	40.03	86.82	Upland	SW38
Morrison	MOR	4	Wild	Minnesota	46.63	93.65	Upland	SW50
NCC Campus PV#1	NCC	4	Wild	New York	40.74	73.65	Upland	SW797
NRCS 9064224	U38	4	Wild	Indiana	40.61	86.25	Upland	NRCS-PMC
NRCS 9084291	U37	4	Wild	Michigan	42.98	86.06	Upland	NRCS-PMC
Rocky Run 1	RR1	5	Wild	Wisconsin	43.47	89.43	Upland	SW112
Rt 72/563 NJ	R72	10	Wild	New Jersey	39.82	74.53	Upland	ECS-1
Shenandoah NP	SNP	5	Wild	Virginia	38.59	78.38	Upland	SW782
Staten Island	STI	8	Wild	New York	40.59	74.15	Upland	SW781
Sterling Barrens 3	SB3	4	Wild	Wisconsin	45.08	92.83	Upland	SW99
Tipton	TIP	4	Wild	Indiana	40.28	86.04	Upland	SW31
Toledo, OH	TOL	4	Wild	Ohio	41.58	83.67	Upland	ECS-2
TRC Park PV#4	TR4	7	Wild	New York	40.83	72.98	Upland	SW800
Wadena	WAD	4	Wild	Minnesota	46.44	95.13	Upland	SW60
Waterford	WAT	4	Wild	Wisconsin	42.78	88.30	Upland	SW123
Alamo	AL	21	SIC	Texas	28.33	98.12	Lowland	PI 420006
Kanlow	KA	23	SIC	Oklahoma	35.33	96.24	Lowland	PI 421521
AW-314/MS-155	AMS	7	Wild	Arkansas	35.43	91.84	Lowland	PI 421999
BN-12323-69	L11	7	Wild	Kansas	38.81	98.27	Lowland	PI 414070
Pangburn	PNG	7	Wild	Arkansas	35.43	91.84	Lowland	PI 414065
Hoffman	HOF	7	Wild	North Carolina	35.03	79.55	Lowland	PI 315723
Hillsborough River S.P.	HRP	7	Wild	Florida	28.15	82.24	Lowland	UGA-HRP
Miami	MI	2	Wild	Florida	25.54	80.63	Lowland	PI 421901
Oscar Scherer S.P.	OSP	7	Wild	Florida	27.19	82.46	Lowland	UGA-OSP
Pasco County	PCF	7	Wild	Florida	28.33	82.42	Lowland	UGA-PCF
PI 422016	L19	7	Wild	Florida	27.00	81.00	Lowland	PI 422016
PMT-785	PMT	7	Wild	Florida	27.00	81.00	Lowland	PI 422003
SG5	SG5	9	Wild	Oklahoma	34.50	95.50	Lowland	NRCS-PMC
Sprewell Bluff	SPB	7	Wild	Georgia	32.90	84.44	Lowland	UGA-SPB
Stuart	ST	2	Wild	Florida	27.20	80.23	Lowland	PI 422001

Table 1. Continued.

Cultivar or accession	Abbreviation	*n*	Status†	Origin	Latitude, north	Longitude, west	Phenotype	Seed source‡
Sumter National Forest	SNF	7	Wild	South Carolina	34.52	81.57	Lowland	UGA-SNF
T 2086	WIL	7	Wild	North Caroline	34.24	77.94	Lowland	PI 476290
Timber	TI	13	Wild	North Caroline	35.54	79.28	Lowland	NRCS-PMC
Wabasso	WB	6	Wild	Florida	27.75	80.44	Lowland	PI 422000
MS SG Cycle 5	MS5	3	Bred	Mississippi	33.53	88.75	Mixed§	MSU
WSK4	K4	2	Bred	Wisconsin	35.33	96.24	Mixed	SW1302
Bald Bluff	BBF	4	Wild	Wisconsin	42.85	88.63	Mixed	SW128
Buena Vista	BUV	4	Wild	Wisconsin	44.27	89.67	Mixed	SW109
Escambia	ESC	3	Wild	Alabama	31.13	87.30	Mixed	ECS-3
Howard	HOW	1	Wild	Indiana	40.45	86.13	Mixed	SW33
Hwy 59	H59	5	Wild	Wisconsin	42.90	87.55	Mixed	SW127
MSPMT-PAVI2	PAV	3	Wild	Mississippi	33.53	88.75	Mixed	MSU
SWG39	S39	3	Wild	Georgia	31.00	84.50	Mixed	NRCS-PMC

†Status: Wild, seed harvested on a prairie-remnant population that is likely to represent local germplasm; SIC, source-identified cultivar derived from a random seed increase, without conscious selection and breeding, within a prairie-remnant population; Bred, a product of one or more cycles of selection and breeding.

‡Seed sources: USDA-ARS, switchgrass breeding program at Lincoln, NE; SDCIA, South Dakota Crop Improvement Association (Brookings, SD); NRCS-PMC, NRCS Plant Materials Centers (Bismarck, ND; Rose Lake, MI; Big Flats, NY; Cape May, NJ; Americus, GA; Coffeeville, MS); PI-xxxxxx (NRCS-GRIN; Germplasm Resources Information Network, USDA-ARS, Beltsville, MD); MSU, Mississippi State University (Mississippi State, MS); ECS-xx, Ernst Conservation Seeds, Meadville, PA; SWxxx, seeds collected directly from prairie remnant site and all processing conducted by hand in Madison, WI; and UGA-xxx, seeds collected directly from prairie remnant site and all processing conducted by hand in Athens, GA.

§Mixed, all plants of an intermediate phenotype or accession of mixed phenotype, based on visual observations of 30 plants from accession in a nursery of 90 accessions made during 2005–2009 at Arlington, WI.

ecotypes in Georgia). A total of 480 plants were used to represent these accessions. The goal was to represent each accession with a minimum of four plants, but severe germination problems, seedling mortality, and field survival issues reduced the number of individuals in some accessions (Table 1).

Very few of the accessions are capable of surviving at both Arlington, WI, and Athens, GA, due to the differential stress at each location. Thus, any phenotypic assessment of individuals could only be conducted in reference to other individuals at each location. Accessions were selected from each panel to represent as much of the geographic adaptation of upland and lowland ecotypes as possible. A few upland accessions were chosen in Wisconsin specifically for their unusual phenotype, either an intermediate phenotype between upland and lowland or a mixture of phenotypes (Table 1; see the last nine entries). These accessions generally possessed one or more of the following "lowland" traits, relative to the majority of upland plants in the nursery: extremely late flowering, obvious bluish waxy bloom to leaf blades and sheaths, reduced tiller density and larger tiller diameter.

DNA Isolation and Polymerase Chain Reaction

Total genomic DNA was isolated from approximately 0.5 cm^2 of leaf tissue using a DNeasy kit (QIAGEN, Valencia, CA). Nineteen simple sequence repeat (SSR) primer pairs from Zalapa et al. (2011; Table 2) were used in this study. Laboratory procedures were described in detail by Zalapa et al. (2011). Briefly, polymerase chain reactions (PCRs) were performed in 8 μL total volume using 3.5 μL 1x JumpStart REDTaq ReadyMix (Sigma, St. Louis, MO), 2 μL 5 ng μL^{-1} genomic DNA, 1.25 μL of H_2O, 0.5 μL 5 μM M13-FAM/HEX primer, 0.5 μL 5 μM reverse, 0.5 μM forward primer, 0.125 μL 5 M betaine (Sigma), and 0.125 μL 50 mg ml^{-1} bovine serum albumin (BSA) (CHIMERx, Milwaukee, WI). Thermocycling conditions consisted of an initial melting step (94°C for 3 min), followed by 30 cycles of 94°C for 15 s, 55°C for 90 s, and 72°C for 2 min, and a final elongation step (72°C for 20 min), followed by an indefinite soak at 4°C. Polymerase chain reaction products combined with 15 μL Hi-Di formamide (Applied Biosystems, Foster City, CA) and 0.5 μL of carboxy-X-rhodamine (ROX) standard (GeneFlo-625 ROX; CHIMERx). Simple sequence repeat allele genotyping was performed using an ABI 3730 fluorescent sequencer (POP-6 and a 50-cm array; Applied Biosystems). Amplicons were scored using GeneMarker Software version 1.5 (SoftGenetics, 2006). Polymerase chain reaction amplifications were repeated on approximately 10% of the samples, and we obtained 99% repeatability.

Flow Cytometry

Flow cytometry was performed on leaves from individual plants prepared using the CyStain PI Absolute P kit (Partec, Swedesboro, NJ) with the modifications described by Zalapa et al. (2011). The mean DNA content per plant cell for each sample was analyzed using ModFit software (Verity Software House, 2008). Ploidy levels were estimated following Zalapa et al. (2011) for all plants originally identified as the upland ecotype and those identified as having an intermediate or mixed phenotype (Table 1). A small number of individual plants, including all plants classified as "unusual," were analyzed independently in a second laboratory using the methods of Costich et al. (2010). The latter group included euploid controls of known chromosome number: $2n = 2x = 18$, $2n = 4x = 36$, $2n = 6x = 54$, and $2n = 8x = 72$.

Chloroplast DNA Sequence Analysis

Chloroplast DNA was amplified from five intergenic regions: trnL(UAA) and trnT(UGU)-trnL(UAA) 5′ (Taberlet et al.,

1991); trnH(GUG)-psbA (Hamilton, 1999); and psbJ-petA and atpI-atpH ndhA (Shaw et al., 2007). Polymerase chain reaction amplifications were performed in 6-μL volumes containing 1x JumpStart REDTaq ReadyMix (Sigma), 0.2 μM each primer, 1 M betaine (Sigma), and 10 ng template DNA. Thermocycling conditions were as follows: 80°C for 5 min; 35 cycles of 95°C for 1 min, 50°C for 1 min with a ramp of 0.3°C s^{-1}, and 65°C for 5 min. Polymerase chain reaction products were purified by adding 2 μL of 0.1 U μL^{-1} Exonuclease I (USB Corp., Cleveland, OH) and 0.1 U μL^{-1} Shrimp Alkaline Phosphatase (USB Corp.) and incubating 30 min at 37°C followed by 20 min at 80°C and 30 sec at 4°C. Purified PCR products were sequenced in both directions using separate sequencing reactions. Sequencing reactions were performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to the method of Platt et al. (2007) and resolved on an ABI 3730 Genetic Analyzer (Applied Biosystems). Sequences were aligned and contigs were compared using Codon Code Aligner version 3.5 (CodonCode Corp., 2009) using the MUSCLE algorithm (Edgar, 2004). Haplotypes among sequences were identified using GeneAlEx 6.0 (Peakall and Smouse, 2006) and submitted to GenBank (accessions HQ110705–HQ110715 and JF901564–JF901577). Plants were classified as either upland or lowland cytotype based on cluster analysis of five cpDNA regions totaling 3708 bp (Zalapa et al., 2011).

Data Analysis

All amplicon products from each SSR primer pair were treated as single-locus alleles for the purpose of estimating the following genetic parameters: observed number of amplicons, number amplicons unique to upland or lowland ecotypes, total number of unique amplicons, and polymorphism information content (PIC) (Table 2). Polymorphism information content was calculated as follows: $PIC = 1 - \Sigma f_i^2$, where f_i is the frequency of the *i*th allele.

Individuals with hybrid ancestry were identified using three methods: the Bayesian clustering algorithm implemented in STRUCTURE v. 2.2 (Pritchard et al., 2000), the Bayesian clustering algorithm implemented in NewHybrids v. 1.1 β (Anderson and Thompson, 2002), and principal component analysis as implemented in GenAlEx 6.4 (Peakall and Smouse, 2006). The ecotype of each individual was classified using the Bayesian clustering algorithm implemented in the program STRUCTURE (v. 2.2) (Pritchard et al., 2000). The algorithm was run for one million Markov chain Monte Carlo iterations following a 100,000-iteration burn-in for three independent runs. Model parameters assumed admixture, $K = 2$ taxonomic groups, and no other prior population information. Zalapa et al. (2011) determined that an assumption of $K = 2$ subpopulations successfully discriminated upland and lowland ecotypes. Using individuals of known ecotype as anchors, the samples of unknown origin were classified as upland, lowland, or putative hybrids. Individuals with less than 95% assignment to either the upland or lowland groups were identified as putative hybrids. In addition, individuals with hybrid ancestry were identified using the Bayesian algorithm implemented in the software NewHybrids v. 1.1 β (Anderson and Thompson, 2002). Six genotypic classes were used when running NewHybrids: the two parental ecotypes, F_1 and F_2 generations, and first-generation backcrosses to each parental ecotype. The algorithm was run for 600,000 iterations following a 100,000-iteration burn-in. Two categories of putative hybrid individuals were identified from this output. The first category included individuals with a probability of any type of hybrid ancestry (p_H) greater than 0.5, suggesting strong evidence of hybrid ancestry. The second category included individuals with marginal or moderate support for hybrid ancestry where $0.05 < p_H < 0.50$. The NewHybrids algorithm is designed to estimate probabilities of membership as F_1 hybrids (first-generation upland × lowland crosses), F_2 hybrids (selfed progeny of an upland × lowland cross), or backcrosses of an F_1 hybrid to a representative of either ecotype (Anderson and Thompson, 2002).

To further provide evidence of the hybrid ancestry of these individuals, a principal coordinates analysis was performed using GenAlEx 6.4 (Peakall and Smouse, 2006). Individuals were grouped as being of upland, lowland, or strong hybrid ancestry based on the NewHybrids (Anderson and Thompson, 2002) classification. The geographic location of each cultivar or accession was mapped using ArcGIS 9.3 (Esri, 2009). Each cultivar is represented by a pie chart, proportional in size to the number of individuals of each accession; the different colors of each pie represent the proportion of individuals within each accession classified as having upland, lowland, or strong hybrid ancestry ($p_H > 0.50$).

Table 2. Number of amplicons and polymorphism information content (PIC) for 19 simple sequence repeat (SSR) markers used to differentiate switchgrass cultivars and accessions.

Locus	Number of amplicons	Unique to lowland	Unique to upland	Total unique amplicons	PIC
				%	
sww150	13	2	5	54	0.826
sww175	8	3	0	38	0.661
sww185	11	2	0	18	0.779
sww210	11	7	1	73	0.596
sww274	4	1	0	25	0.519
sww651	19	6	7	68	0.747
sww687	10	2	3	50	0.619
sww2309	19	7	7	74	0.821
sww2312	17	4	2	35	0.794
sww2320	8	1	3	50	0.698
sww2341	11	1	3	36	0.565
sww2376	15	1	4	33	0.777
sww2377	11	2	4	55	0.534
sww2385	14	2	6	57	0.850
sww2387	11	5	3	73	0.666
sww2393	21	5	3	38	0.826
sww2394	13	5	4	69	0.771
sww2403	17	9	2	65	0.836
sww2415	19	10	2	63	0.829
Mean	13.3	3.9	3.1	53	0.717

RESULTS

The 19 primer pairs used in this study produced amplification products corresponding to the expected lengths (Tobias et al., 2008). Overall, we detected 252 amplicons, with a range of 2 to 21 amplicons per locus and an average of 12.7 amplicons per locus across all individuals (Table

2). The polymorphism information content ranged from 0.519 to 0.850 for the 19 loci. Of the 252 amplicons, 139 were unique within either the upland or lowland ecotypes (52.8%), corresponding to 0.26 unique amplicons per individual in the upland ecotype and 0.35 unique amplicons per individual in the lowland ecotype.

Principal coordinates analysis revealed two clear taxonomic groups, associated with the upland and lowland ecotypes, but also included several ambiguous individuals (Fig. 1). An initial STRUCTURE (Pritchard et al., 2000) cluster analysis with $K = 2$ groups resulted in clear upland and lowland groups but a small number of individuals with unclear taxonomic membership (Fig. 2A). The hybrid cluster analysis performed in NewHybrids (Anderson and Thompson, 2002) with $K = 2$ parental groups and four hybrid or backcross groups revealed a clear separation between upland and lowland groups and 21 individuals with strong support ($p_H > 0.50$) for an upland × lowland hybrid origin (Fig. 2B). In the principal coordinates analysis (Fig. 1), these 21 individuals were represented as intermediate types, located between upland and lowland clusters.

The NewHybrids (Anderson and Thompson, 2002) cluster diagram revealed several structural groupings for the 21 plants of putative hybrid origin (Fig. 2B). Most of these individuals had strong membership support for the F_1 and upland-backcross categories, but a few plants had strong membership support for the lowland and lowland-backcross categories. Of the 21 plants with $p_H > 0.50$, 13 had cpDNA of upland origin and eight had cpDNA of lowland origin (Table 3). Within the upland cytotype, phenotype ranged from upland to lowland, with the majority of plants (10 of 13) having an intermediate phenotype that could not be definitively classified as either upland or lowland. Within the lowland cytotype, seven of eight plants had the lowland phenotype and one was classified as intermediate. Most of these "intermediate" plants had flowering time, height, tiller number, and tiller size intermediate between obvious upland and lowland phenotypes. Waxy bloom varied from absent to strong and was not consistent.

Two plants with the upland cytotype had strong support for backcrosses of an upland × lowland hybrid to an individual of lowland ecotype (Table 3). One of these plants was a hexaploid (#1.5 from Hoffman) and one was an octoploid ('Carthage'). The remaining upland plants with strong support for hybrid origin were derived from four accessions: Escambia, MSPMT-PAVI2, Sprewell Bluff, and PMT-785. These plants all had $p_H \geq 0.999$, indicating the strongest support for hybrid origin, but with a range of hybrid structures from strongly upland to roughly equal contributions of upland and lowland. Each of these plants was octoploid.

Within the lowland cytotype, values of p_H ranged from 0.624 to 0.952 and the upland-backcross category had the strongest support for all eight individuals (Table 3). Two accessions, PMT-785 and MSPMT-PAVI2, were split across cytotypes and structures. For MSPMY-PAVI2, this was due to its origin as a composite of 92 accessions from Mississippi, Arkansas, and Alabama (Brian Baldwin, personal communication, 2010). Overall, the 21 individuals in Table 3 represent half of the 42 individuals from these seven accessions and all three of the available individuals from Escambia and MSPMT-PAVI2. Flow cytometry revealed that the seven individuals with strong support for hybrid ancestry, combined with both lowland cytotype and phenotype, were all octoploids.

The NewHybrids (Anderson and Thompson, 2002) clustering results revealed a second group of individuals for which there was marginal to moderate support for hybrid origin: $0.05 < p_H < 0.50$ (Fig. 2B). Eight of these plants had the upland cytotype: three of eight had an upland phenotype while five of eight had a contradictory lowland phenotype (Table 4). The SSR NewHybrids output for these eight upland plants was largely split according to their phenotype: three of the four upland phenotypes had support for a moderate level of lowland introgression and all four lowland phenotypes had support for a moderate level of upland introgression. The remaining 17 individuals in this group had the lowland cytotype, five of which had a contradictory

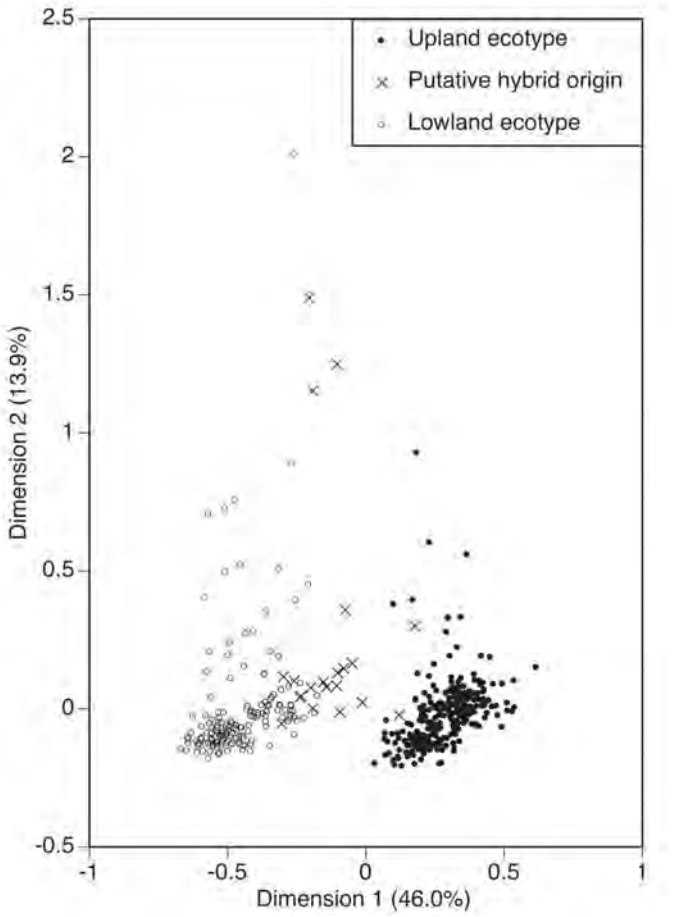

Figure 1. Principal coordinates analysis of 480 switchgrass plants in two dimensions, based on 19 simple sequence repeat (SSR) markers, showing differentiation of upland and lowland groups and 21 plants of hybrid origin.

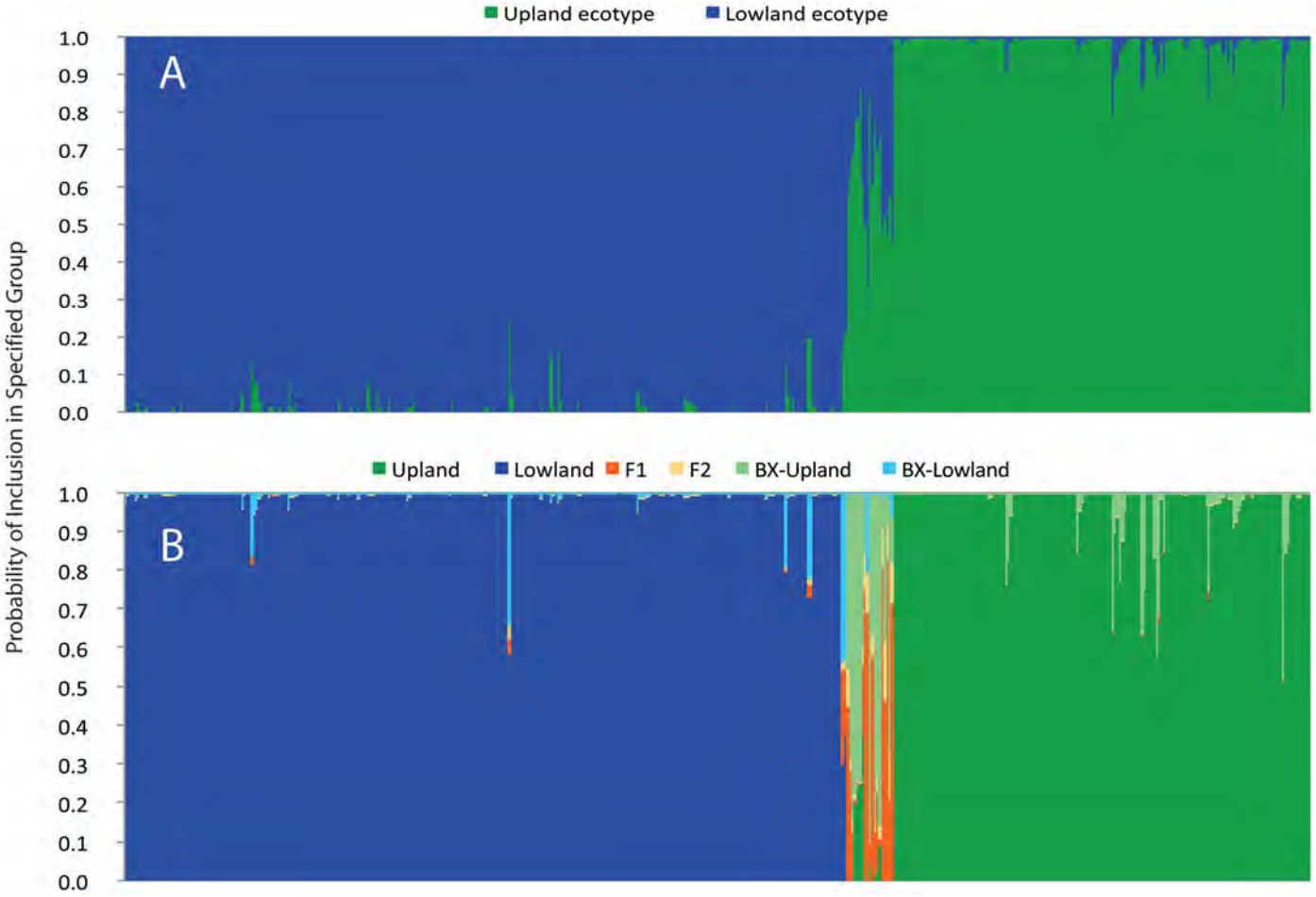

Figure 2. Bayesian cluster analysis of 480 switchgrass plants belonging to 67 accessions. Each plant is represented by a thin vertical line divided into *K*-colored segments representing the individual's estimated membership probability for each of *K* clusters or populations labeled at the top of each panel. Panel A: STRUCTURE (Pritchard et al., 2000) output assuming $K = 2$ (upland and lowland). Panel B: NewHybrids (Anderson and Thompson, 2002) output assuming $K = 2$ parent populations (upland and lowland) and four types of hybrid derivatives (F_1, F_2, upland backcross [BX-Upland], or lowland backcross [BX-Lowland]).

upland phenotype. Hybrid structures of these 17 individuals were fairly uniform, with strong membership in the lowland ecotype and moderate support for membership in the upland-backcross group, suggesting a small amount of upland introgression. Of the 12 individuals in Table 4 with both lowland cytotype and phenotype, six were tetraploids and six were octoploids, to our knowledge the first octoploids reported for the lowland ecotype of switchgrass.

The 21 individuals with strong membership support in the hybrid categories originated from a fairly narrow region that extends from Mississippi in the west to Florida in the southeast and North Carolina in the north (Fig. 3). This region largely corresponds to Bailey's Southern Mixed Forest and Outer Coastal Plain Provinces (Bailey, 1998). Adding the additional 25 individuals with marginal to moderate membership support in the hybrid categories results in an additional 18 plants within this narrow region and seven plants outside the region (four from New York, specifically Staten Island and Long Island, and one each from New Jersey, Nebraska, and Wisconsin). The Wisconsin plant is of unknown origin, because it originated from a broad germplasm pool that traces to many accessions of diverse origin (Casler et al., 2006). The Nebraska plant traces to germplasm that originated in eastern Nebraska. The New Jersey and New York plants originated in a region of switchgrass diversity that appears to be a physical extension of the Florida–Georgia–North Carolina axis of diversity, expanding this region of diversity into Bailey's Eastern Broadleaf Forest Province (Fig. 3).

Within the entire group of 46 individuals with partial support for hybrid ancestry, the overall picture of phenotype, genotype, and cytotype classifications reflected clear evidence of gene flow in both directions between the upland and lowland taxa (Fig. 4). Within this group, plants classified as upland phenotype were equally split between upland and lowland cytotypes. Plants with the upland phenotype and cytotype were classified as mixed ancestry, indicating varying levels (strengths) of introgression of lowland genes into upland cytoplasm. Plants with the upland phenotype and lowland cytotype represented relatively small amounts

Table 3. Identity, origin, and NewHybrids (Anderson and Thompson, 2002) estimated membership probabilities for 21 switchgrass plants identified by STRUCTURE (Pritchard et al., 2000) analysis as having strong support as upland × lowland hybrid derivatives (probability of any type of hybrid ancestry [p_H] > 0.50 probability of being classified as an F_1, F_2, or backcross—upland backcross (BX Up) or lowland backcross (BX Low)—between the upland and lowland ecotype groups).

					Pure ecotype, hybrid, and backcross categories							
Identifier	Cultivar or accession	Origin	cpDNA†	Phenotype‡	Lowland	Upland	F_1	F_2	BX Up	BX Low	F + BX§	Ploidy¶
1.5	Hoffman	North Carolina	Upland	Upland	0.000	0.299	0.246	0.019	0.000	0.435	0.701	6*x*
Jcar.7	Carthage	North Carolina	Upland	Upland	0.000	0.375	0.175	0.024	0.000	0.425	0.625	8*x*
17.9	PMT-785	Florida	Upland	Lowland	0.000	0.000	0.580	0.055	0.363	0.002	1.000	8*x*
7607	Escambia	Alabama	Upland	Intermediate	0.005	0.000	0.120	0.037	0.837	0.000	0.995	8*x*
7615	Escambia	Alabama	Upland	Intermediate	0.001	0.000	0.282	0.031	0.686	0.000	0.999	8*x*
7619	Escambia	Alabama	Upland	Intermediate	0.000	0.000	0.449	0.095	0.451	0.005	1.000	8*x*
4017	MSPMT-PAVI2	Mississippi	Upland	Intermediate	0.000	0.001	0.691	0.098	0.008	0.202	0.999	8*x*
4012	MSPMT-PAVI2	Mississippi	Upland	Intermediate	0.000	0.000	0.750	0.100	0.145	0.006	1.000	8*x*
35.1	Sprewell Bluff	Georgia	Upland	Intermediate	0.000	0.000	0.462	0.154	0.378	0.006	1.000	8*x*
35.4	Sprewell Bluff	Georgia	Upland	Intermediate	0.000	0.000	0.810	0.102	0.070	0.018	1.000	8*x*
35.5	Sprewell Bluff	Georgia	Upland	Intermediate	0.000	0.000	0.716	0.102	0.121	0.061	1.000	8*x*
35.6	Sprewell Bluff	Georgia	Upland	Intermediate	0.000	0.000	0.208	0.122	0.668	0.002	1.000	8*x*
35.7	Sprewell Bluff	Georgia	Upland	Intermediate	0.000	0.000	0.828	0.086	0.068	0.018	1.000	8*x*
32.7	OSSP-FL	Florida	Lowland	Lowland++	0.372	0.004	0.178	0.051	0.381	0.014	0.624	8*x*
32.9	OSSP-FL	Florida	Lowland	Lowland	0.199	0.000	0.009	0.013	0.779	0.000	0.801	8*x*
32.10	OSSP-FL	Florida	Lowland	Lowland	0.251	0.000	0.003	0.001	0.746	0.000	0.749	8*x*
32.13	OSSP-FL	Florida	Lowland	Lowland	0.244	0.000	0.012	0.012	0.732	0.000	0.756	8*x*
17.7	PMT-785	Florida	Lowland	Lowland	0.029	0.000	0.200	0.047	0.722	0.001	0.971	8*x*
17.10	PMT-785	Florida	Lowland	Lowland	0.092	0.000	0.019	0.028	0.861	0.000	0.908	8*x*
17.15	PMT-785	Florida	Lowland	Lowland	0.013	0.000	0.115	0.010	0.862	0.000	0.987	8*x*
4004	MSPMT-PAVI2	Mississippi	Lowland	Intermediate	0.048	0.000	0.052	0.005	0.895	0.000	0.952	4*x*

†cpDNA, chloroplast DNA.

‡Phenotypic assessment made by visual observation post heading, in direct comparison to other plants in each respective association panel, based on leaf, sheath, and stem color; plant height; tiller number and diameter, and heading date.

§Sum of probabilities for F_1 + F_2 (F) plus both backcrosses (BX).

¶Determined by flow cytometry using known 2*x*, 4*x*, 6*x*, and 8*x* individuals as controls.

of introgression of upland alleles into the lowland cytoplasm. Of the plants classified as the lowland phenotype, 19 of 25 (76%) had the lowland cytotype and 15 of 25 (60%) a distinct lowland trend for their hybrid origin. There was some strong mixed parentage in both groups and a considerable range of introgression in both groups. Somewhat surprisingly, the group with the greatest variation in possible hybrid strength and origin was the lowland phenotype and lowland cytotype group, suggesting that "looks can be deceiving." Plants with the intermediate phenotype mainly had the upland cytotype (10 of 11), strong support for hybrid origin (11 of 11), and either an upland or mixed trend, largely indicating introgression of lowland genes into plants with upland cytoplasm and phenotype. Of the 46 plants of putative hybrid origin, 16 were tetraploid, one hexaploid, and 29 octoploids.

DISCUSSION

Our results indicate that significant gene flow has occurred, and is continuing to occur, between upland and lowland ecotypes of switchgrass. Twenty-one of 480 plants (4.4%) showed strong evidence of a hybrid ancestry, while an additional 25 plants (5.2%) showed marginal or moderate support for a hybrid ancestry. These plants represented a diverse and inconsistent mixture of cytotypes and phenotypes, suggesting a long history of hybridization and gene flow. The upland and lowland cytotypes, characterized by specific cpDNA sequences (Missaoui et al., 2006; Zalapa et al., 2011), were equally represented in this group, indicating that gene flow has occurred in both directions, from upland to lowland and from lowland to upland.

The presence of past introgression cannot be predicted from a simple phenotypic assessment, as only 11 of 46 (24%) of the hybrid-derived individuals were scored as having an intermediate phenotype. Similarly, the direction of past gene flow cannot be predicted from a phenotypic assessment, because upland and lowland cytotypes were represented in nearly equal frequencies. Within upland and intermediate phenotypes there was a distinct trend toward lowland-into-upland introgression and within lowland phenotypes there was a distinct trend toward upland-into-lowland introgression, but neither direction was the exclusive domain of any of the three phenotypic classes. Both tetraploids and octoploids were present within upland, lowland, and intermediate phenotypic categories, as well as within both upland and lowland cytotypes, represented within the 46 individuals of hybrid ancestry. In addition, one hexaploid individual of hybrid origin was discovered within an otherwise tetraploid lowland accession. These results suggest that gene flow has occurred across ploidy boundaries, from

Table 4. Identity, origin, and NewHybrids (Anderson and Thompson, 2002) estimated membership probabilities for 25 switchgrass plants identified by STRUCTURE (Pritchard et al., 2000) analysis as having marginal to moderate support as upland × lowland hybrid derivatives (0.05 < probability of any type of hybrid ancestry [p_H] < 0.50 probability of being classified as an F_1, F_2, or backcross— upland backcross (BX Up) or lowland backcross (BX Low)—between the upland and lowland ecotype groups).

Identifier	Cultivar or accession	Origin	cpDNA†	Phenotype‡	Pure Ecotype, Hybrid, and Backcross Categories							
					Lowland	Upland	F_1	F_2	BX Up	BX Low	F + BX§	Ploidy¶
Jcar.2	Carthage	North Carolina	Upland	Upland	0.000	0.814	0.018	0.005	0.000	0.162	0.186	8*x*
Jtr.2	Trailblazer	Nebraska	Upland	Upland	0.000	0.795	0.008	0.002	0.000	0.195	0.205	8*x*
U518	WS4U	Wisconsin	Upland	Upland	0.000	0.727	0.038	0.016	0.000	0.219	0.273	4*x*
Jm.1	Miami	Florida	Upland	Lowland	0.636	0.000	0.000	0.001	0.363	0.000	0.364	4*x*
Jm.2	Miami	Florida	Upland	Lowland	0.755	0.000	0.000	0.000	0.245	0.000	0.245	4*x*
33.6	Pasco Co-FL	Florida	Upland	Lowland	0.000	0.584	0.044	0.031	0.000	0.341	0.416	4*x*
33.9	Pasco Co-FL	Florida	Upland	Lowland	0.879	0.000	0.000	0.000	0.120	0.000	0.121	8*x*
35.2	Sprewell Bluff	Georgia	Upland	Lowland	0.725	0.000	0.023	0.015	0.236	0.001	0.275	8*x*
4703	NCC Campus PV#1	New York	Lowland	Upland	0.832	0.000	0.003	0.003	0.162	0.000	0.168	4*x*
7414	Rt 72/563 NJ	New Jersey	Lowland	Upland	0.848	0.000	0.002	0.002	0.148	0.000	0.152	4*x*
5001	TRC Park PV#4	New York	Lowland	Upland	0.513	0.000	0.006	0.008	0.472	0.000	0.487	4*x*
5008	TRC Park PV#4	New York	Lowland	Upland	0.848	0.000	0.000	0.001	0.151	0.000	0.152	4*x*
5011	TRC Park PV#4	New York	Lowland	Upland	0.874	0.000	0.002	0.001	0.123	0.000	0.126	4*x*
1.1	Hoffman	North Carolina	Lowland	Lowland	0.827	0.000	0.001	0.001	0.171	0.000	0.173	4*x*
1.3	Hoffman	North Carolina	Lowland	Lowland	0.761	0.000	0.002	0.002	0.234	0.000	0.239	4*x*
1.6	Hoffman	North Carolina	Lowland	Lowland	0.942	0.000	0.000	0.000	0.057	0.000	0.058	4*x*
32.8	OSSP-FL	Florida	Lowland	Lowland	0.939	0.000	0.000	0.000	0.061	0.000	0.061	8*x*
32.11	OSSP-FL	Florida	Lowland	Lowland	0.642	0.000	0.001	0.002	0.355	0.000	0.358	8*x*
32.14	OSSP-FL	Florida	Lowland	Lowland	0.902	0.000	0.000	0.000	0.098	0.000	0.098	8*x*
33.4	Pasco Co-FL	Florida	Lowland	Lowland	0.773	0.000	0.001	0.001	0.225	0.000	0.227	8*x*
19.2	PI422016	Florida	Lowland	Lowland	0.843	0.000	0.003	0.002	0.151	0.000	0.157	4*x*
17.13	PMT-785	Florida	Lowland	Lowland	0.666	0.000	0.023	0.006	0.305	0.000	0.334	8*x*
17.14	PMT-785	Florida	Lowland	Lowland	0.584	0.000	0.001	0.000	0.415	0.000	0.416	8*x*
21.8	T 2086	North Carolina	Lowland	Lowland	0.913	0.000	0.000	0.000	0.086	0.000	0.087	4*x*
21.9	T 2086	North Carolina	Lowland	Lowland	0.925	0.000	0.000	0.000	0.075	0.000	0.075	4*x*

†cpDNA, chloroplast DNA.

‡Phenotypic assessment made by visual observation post heading, in direct comparison to other plants in each respective association panel, based on leaf, sheath, and stem color; plant height; tiller number and diameter, and heading date.

§Sum of probabilities for F_1 + F_2 (F) plus both backcrosses (BX).

¶Determined by flow cytometry using known 2*x*, 4*x*, 6*x*, and 8*x* individuals as controls.

8*x* to 4*x* via polyembryony and from 4*x* to 8*x* via 2*n* gametes (Harlan and de Wet, 1975; Young et al., 2010). Recent evidence suggests that octoploids suffer very high rates of aneuploidy, biased significantly toward chromosome loss (Costich et al., 2010). Hexaploids, arising from one or more of these mechanisms, may act as a gene-flow bridge between the two most common ploidy levels, tetraploid and octoploid.

The presence of both upland and lowland phenotypes and cytotypes at the 8*x* level suggests that 2*n* gametes have acted as a polyploidization mechanism within both ecotypes. This is the first report of possible octoploid individuals within the lowland switchgrass ecotype, previously thought to be exclusively tetraploid (Hopkins et al., 1996; Hultquist et al., 1996; Vogel, 2004). The extreme rarity of octoploids within the lowland ecotype suggests a possible relationship between ecotype, geographic region (climate), and ploidy. It is not within the scope of this paper, nor do we have access to explanatory data, to speculate on the mechanisms or reasons for the rarity of octoploids within the lowland ecotype.

The presence of multidirectional introgression, the varying degrees of introgression, and the various genetic plans that can result in a similar phenotype all suggest that these plants derive from historically varying hybridizations. While we cannot date these putative hybridization events, the nature of the genetic polymorphisms identified by the NewHybrids (Anderson and Thompson, 2002) software suggests relatively recent events. How is it possible that nearly 10% of this collection could have arisen from past hybridization events between two ecotypes with a typical difference in flowering time of 3 to 4 wk and a large disparity in their most frequent geographic range? To answer this question, we must go back in time at least one million years.

The diploid progenitors of switchgrass are thought to have diverged from their closest relatives approximately two million years ago, while polyploidization occurred sometime within the last one million years (Huang et al., 2003). During the past one million years, there have been

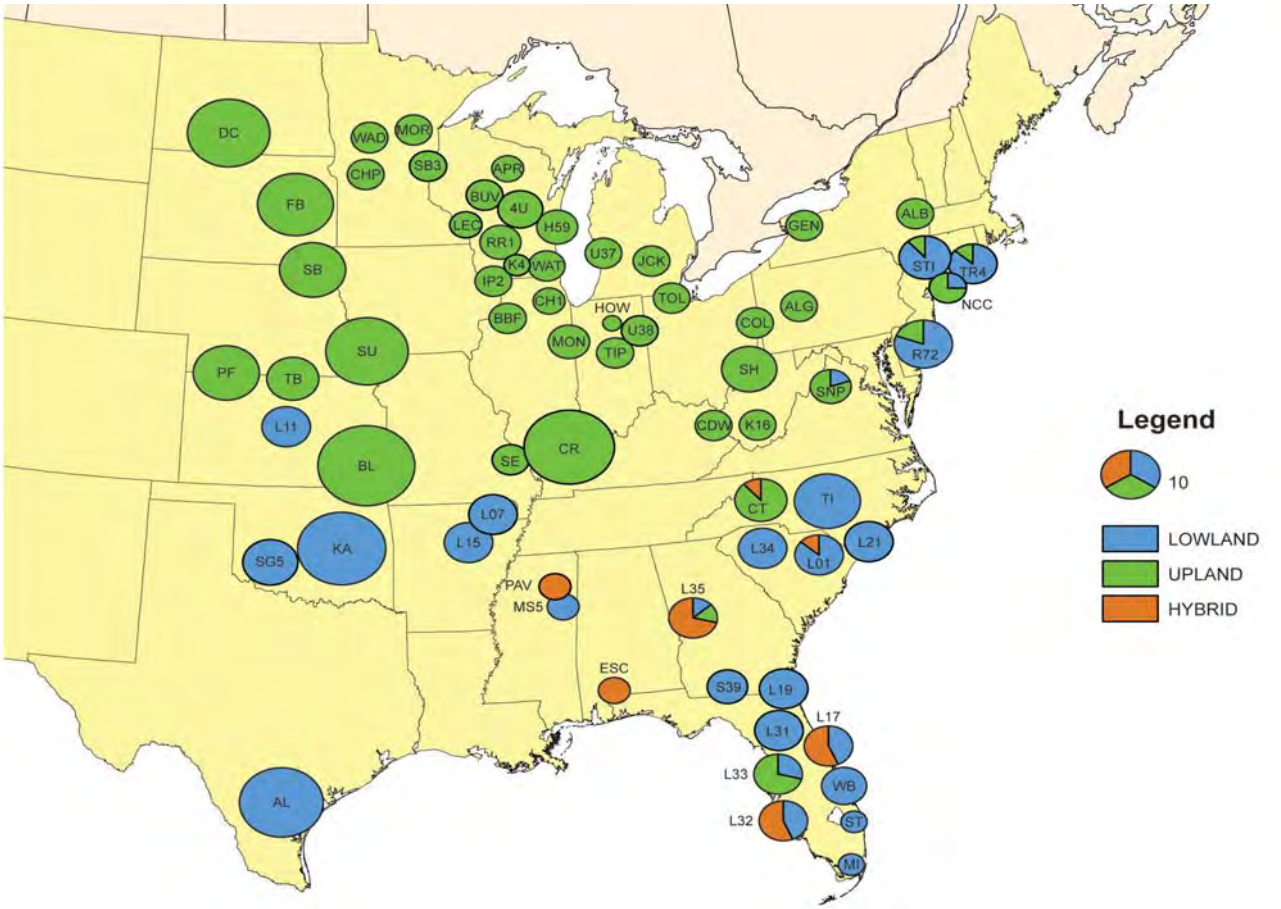

Figure 3. Partial map of the United States, showing the approximate location of each switchgrass cultivar or accession and the membership of plants evaluated within each accession in one of three groups: lowland, upland, or hybrid. The size of each circle represents the sample size for each accession, with *n* = 10 shown in the legend. Each cultivar or accession is identified by a two- or three-character code from Table 1.

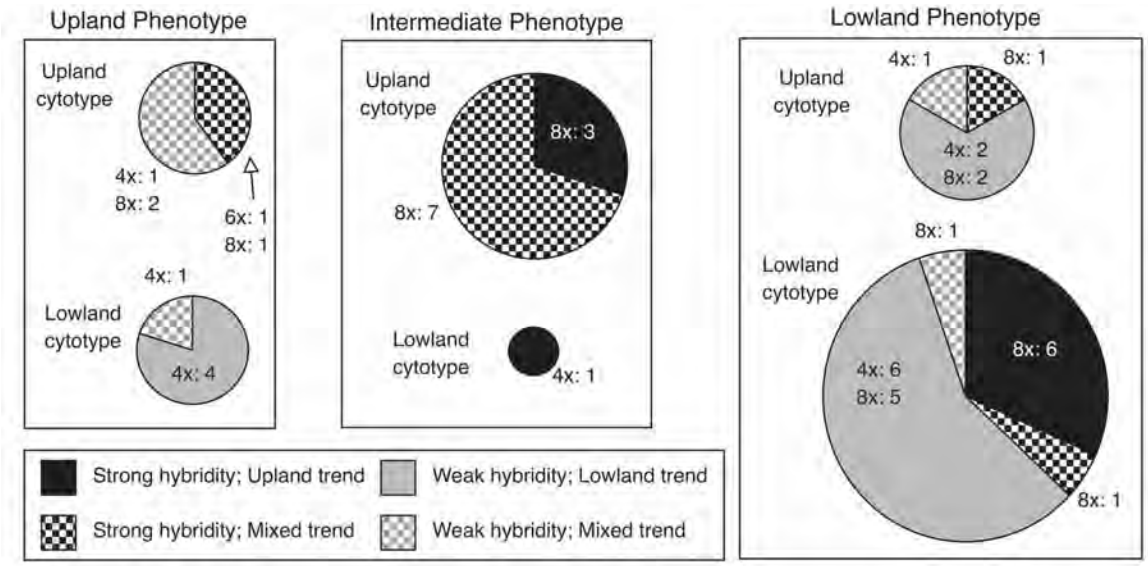

Figure 4. Pie diagrams showing the relationships among phenotype, cytotype (chloroplast DNA [cpDNA] sequence), and nuclear simple sequence repeat (SSR) marker profiles for 46 switchgrass plants that were identified as having marginal to high probabilities of any type of hybrid ancestry ($p_H > 0.05$) and presented in Tables 3 and 4. Each pie chart refers to one group defined by cytotype and phenotype and the size of each pie represents group size, with *n* = 1 for the smallest and *n* = 19 for the largest group. The strength of hybrid support is indicated by black (strong; $p_H > 0.50$) or gray (moderate; $0.05 < p_H < 0.50$) and the predominant ecotypic trend of the hybrid-origin genotypes by the pattern (solid for dominant upland or lowland SSR pattern or trend; checkered for mixed SSR pattern or trend). Number of tetraploids (4*x*) and octoploids (8*x*) are identified for each slice of pie.

12 major continental glaciation events in North America, with distinct interglacial (ice-free) periods (Bintanja and van de Wal, 2008). Repeated glaciation of a large portion of North America has caused massive cyclic migrations of all the major North American ecosystems, including the major grasslands, essentially a repeated cycle of retreat to southern North American and Central American refugia during glaciation events, followed by recolonization of central and eastern North America during postglacial warming cycles (Deevey, 1949). Both the extinction and recolonization phases required thousands of years due to the slow rate of temperature change (Bintanja and van de Wal, 2008) and the relatively sessile nature of perennial plants (Collingham et al., 1996).

The last of these events, the Pleistocene Glaciation, ended approximately 14,000 to 12,000 yr BP, essentially marking the Wisconsin–Holocene boundary (Berger et al., 1987). Deglaciation was an extremely slow process, driven by thousands of years of incremental and cyclic changes in temperature, precipitation, and atmospheric CO_2 concentrations (Bintanja and van de Wal, 2008). This process was punctuated by frequent long-term cold reversals (Berger et al., 1987; Cwynar and Levesque, 1995; Jakobsen, 2009; Levesque et al., 1993) that impacted the process and rate of plant migration and recolonization into newly forming habitats (Kneller and Peteet, 1999). These climatic changes resulted in a gradual reestablishment of tallgrass prairie and savanna habitats in the northern United States between 9000 and 5000 yr BP (Clark et al., 2001; Jacobson and Grimm, 1986; Kelley et al., 2006). However, continued cyclic climate change is reflected in pollen records from lakebed sediments that show frequent and continual cyclical changes in the grassland–forest boundary during the early and mid-Holocene postglacial warming between 9000 and 3000 yr BP (Clark et al., 2001; Kelley et al., 2006; Kneller and Peteet, 1999). As a net result, tallgrass prairie and savanna, the dominant habitats of switchgrass in North America, did not reestablish in a single recolonization event but in multiple events over thousands of years in which dominant forms of vegetation shifted between grassland, savanna, mixed forest, and coniferous or boreal forests.

Switchgrass is thought to have survived the Pleistocene Glaciation in three refugia located in the southern United States, perhaps including parts of northern Mexico. Based on morpho-geographic analyses of many switchgrass accessions covering much of its distributional area, McMillan (1959, 1964) recognized clear relationships of several distinct morphological types with both latitude and longitude. Recognizing that the Gulf Coast region served as a refuge for plants and animals from many widely divergent ecosystems across North America (Deevey, 1949), McMillan (1959, 1964) proposed a western semimontane refuge as the dominant germplasm source for the arid and semiarid Great Plains region, the eastern Gulf Coast as the dominant germplasm source for the Atlantic Seaboard, and a central region of high diversity as a source of germplasm for both the central and eastern United States. Long-term pollen records from soil and lakebed cores in central Florida, the Florida panhandle, and the Coastal Plain of Georgia and the Carolinas indicate that grasses and other C4 species were most frequent in the southeastern landscape during the previous glacial maximum, ranging from 60,000 to 30,000 yr BP (Grimm et al., 1993; Huang et al., 2006; LaMoreaux et al., 2009; Leigh, 2008; Watts, 1971; Watts et al., 1992). As the climate warmed during deglaciation, southeastern landscapes were gradually converted from grassland to savanna or from savanna to forest as the grasslands began their slow migration northward. According to the pollen record in sediments, what was left behind may have been a very close representation to what survives today—hundreds to perhaps thousands of small, fragmented populations of switchgrass, representing a huge array of genetic and phenotypic diversity.

Modern switchgrass survives in thousands of refugia scattered across the landscape, occupying a wide range of habitats (Casler, 2005; Hopkins et al., 1995). These habitats range from tallgrass prairie to wetlands and riparian zones and include a wide range of intermediate or transition habitats, such as oak savanna and pine barrens. Agriculture and development have severely fragmented these refugia, reducing their size to relatively small patches of land ranging from a few plants up to about 5 to 10 ha. A few extensive prairies still survive, for example, the Flint Hills of Kansas, the Osage Prairie of Oklahoma, and the Sand Hills of Nebraska (Vogel, 2004), but they are exceptions. Partly due to these large and famous grasslands, partly due to breeding history, and partly due to the simple association of switchgrass with the tallgrass prairie ecosystem, we tend to think of the Great Plains as the center of origin and likely center of diversity for switchgrass. Our data show that the Great Plains is a secondary center of diversity for switchgrass, not the primary center.

Using nuclear SSR markers and cpDNA sequences, our previous results established the existence of two lowland clades (eastern Gulf Coast and southern Great Plains) and three upland clades (central Great Plains, northern Great Plains, and eastern Savanna) (Zalapa et al., 2011). Remarkably, these results are completely consistent with McMillan's hypothesis of three refugia, in which the western montane refuge was the source of upland ecotypes for the arid and semiarid Great Plains, the eastern Gulf Coast refuge was the source of lowland plants colonizing the Atlantic Seaboard, and the western Gulf Coast refuge contained both upland and lowland ecotypes that populated the more humid regions of the Great Plains (Zalapa et al., 2011). Our current results lend further support for this hypothesis, demonstrating that the eastern Gulf Coast is the primary center of diversity for switchgrass, acting as

the only currently identifiable source of switchgrass plants that appear to be of hybrid origin, in addition to identifiable upland and lowland plants (Fig. 3). The presence of hybrid-derived individuals with weak or moderate support in several accessions of the New York and New Jersey region (Table 4) suggests that hybrid plants from the eastern refuge acted as founder plants for switchgrass accessions that established along the Atlantic Seaboard migration route. These individuals likely served to maintain genetic diversity at relatively high levels all along the Atlantic Seaboard, a characteristic that appears to have been preserved in modern switchgrass remnant populations (Fig. 3).

Due to limited sampling at this time, we cannot make any specific statements about the western Gulf Coast refuge (McMillan's "central, highly diverse" refuge), except to indicate that it contained both upland and lowland types in relatively high frequencies. There is no compelling evidence at this time for hybridization and gene flow between upland and lowland ecotypes in this region, either within the refuge region itself or along the northward migration routes into the central and northern United States. Only two plants in our study support the hypothesis of hybrid origin within the western Gulf Coast refuge, one plant from 'WS4U' and one plant from 'Trailblazer', both of which were moderately supported as being of hybrid origin (Table 4). Nevertheless, "absence of evidence is not evidence of absence" (Sagan, 1995, p. 213), so the genetic makeup and diversity of this refuge remains in doubt until more accessions are collected and evaluated.

During deglaciation, switchgrass migrated north very slowly, colonizing new regions to form species-rich grasslands with a highly diverse species assemblage that had been constrained to these refugia (Deevey, 1949). Many of these new grasslands were likely transitory, due to the presence of cyclic cold reversions (Berger et al., 1987; Cwynar and Levesque, 1995; Jakobsen, 2009; Levesque et al., 1993). Birds and mammals were the most likely primary agents of this northward migration. Numerous bird species are generally considered the prime candidates for reforestation of glaciated areas during the early to mid Holocene (Clark et al., 1998; Webb, 1986; Wilkinson, 1997). Species such as the passenger pigeon are very likely to have participated in switchgrass seed dispersal, due to their abundance, capacity for delayed digestion of seeds, and nomadic habits (Webb, 1986). Viable switchgrass seeds were likely transported by bison and other ungulates, by the processes of endozoochory (ingestion and passage through the gut) or ectozoochory (adherence to hair or skin) (Ernst et al., 1992; Ocumpaugh et al., 1996; Pakeman, 2001). While any individual seed has an extremely low probability of being transported to another site and becoming a founder plant, repetition of this process in large flocks or herds over thousands of years results in a high probability of colonization and estimated migration rates that match the paleoecological record (Clark et al., 1998; Pakeman, 2001; Wilkinson, 1997).

Seed dispersal by birds or mammals provides an obvious mechanism for multiple introductions of switchgrass into any particular site. Multiple introductions from the highly diverse eastern Gulf Coast refuge could very simply explain the relatively high frequency of switchgrass accessions containing plants of both upland and lowland ecotypes along the northern Atlantic Seaboard (Fig. 3). This region has a very mild climate, favoring long-term survivorship of both upland and lowland ecotypes, acting largely as a physical extension of the center of diversity originating on the southern Coastal Plain. The Atlantic Seaboard northward migration route possesses a relatively minor change in hardiness zone, ranging from USDA hardiness zone 9 in the Florida central panhandle to Zone 6 in Connecticut and Rhode Island, an average temperature reduction of 15°C (Cathey, 1990). Conversely, the Great Plains northward migration routes, both arid and humid regions, span a change of hardiness zones from Zone 8 in central Texas to Zone 3 in North Dakota, Minnesota, Wisconsin, and Michigan, an average temperature reduction of 25°C (Cathey, 1990).

We hypothesize that most gene flow between upland and lowland ecotypes occurred during glaciation events rather than during deglaciation or stable interglacial periods. Glaciation forced a highly diverse assemblage of switchgrass genotypes and phenotypes into a relatively small area, increasing the likelihood of occasional matings between plants of different ecotypes. Northward migration would have led to radiation and dispersion, rapidly decreasing the likelihood of such matings, especially as distance from the refuge increases. Northward migrations that mimic mammalian migration routes or avian flyways would preserve traits of the founder plants within each migration route, preserving some level of phenotypic differentiation across a longitudinal gradient, as observed by McMillan (1959, 1964). Longitudinal migration of switchgrass pollen and seeds undoubtedly occurred during the northward postglacial migrations, but the preservation of distinct phenotypes (McMillan, 1959; 1964) and genotypic clades (Zalapa et al., 2011) suggests that east-west migrations were sufficient to mitigate founder effects that occurred at numerous points along these northward migration routes.

During the long northward migrations, switchgrass populations differentiated along latitudinal gradients, responding to shorter frost-free periods and longer daylengths of the northern latitudes (McMillan, 1959). These adaptive selection responses have created an adaptive gradient, driven by temperature and photoperiod. Lowland populations are limited by their relatively low cold tolerance and extremely late flowering time, which may actually be related through a physiological cause-and-effect. Mortality of southern-adapted populations generally occurs in winter

(Casler et al., 2002), but it is not known if this is due to an inherent lack of cold or freezing tolerance or because delayed flowering results in impaired or incomplete hardening. The general southern range of lowland accessions, which largely corresponds to USDA hardiness zones 5 to 9, is illustrated in Fig. 3. Similarly, upland ecotypes are generally adapted to USDA hardiness zones 3 to 7, with a few notable exceptions in Georgia and Florida that have not previously been described (Fig. 3). A transition zone, approximately corresponding to USDA hardiness zones 5 to 7, supports adaptation of southern upland strains and northern lowland strains (Casler et al., 2004, 2007b). This adaptive gradient applies to genetic differentiation within both upland and lowland ecotypes as well essentially creating a gradient of early-flowering and cold-hardy genotypes in the extreme north to late-flowering and subtropical genotypes in the extreme south.

Finally, human influences on these evolving prairie and savanna communities began shortly after the arrival of humans from Siberia (Hoffecker et al., 1993). For most of this interglacial period, fire was the dominant, perhaps only, form of human influence on prairie and savanna communities, although McMillan (1959) discounted the effect of fire as "not basically directed toward the vital populational pressures which have molded grassland vegetation." Of more importance has been the influence of modern humans to fragment the various habitats of switchgrass during the past 200 yr. It is highly remarkable that this massive fragmentation process, for example, reducing the tallgrass prairie to less than 1% of its early-19th century size, has done little to erode the genetic base of switchgrass. Molecular marker studies have uniformly demonstrated that genetic variation is highly conserved within nearly all of these remnant populations (e.g., Zalapa et al., 2011), suggesting that archaic populations of switchgrass functioned across a broad landscape and that many remnant prairie or savanna populations are functional duplications (Casler et al., 2007a). A strong self-incompatibility system, encouraging a high rate of outcrossing (Martinez-Reyna and Vogel, 2002; Talbert et al., 1983), combined with postcolonization migration facilitated by wind-aided pollen transport and animal-aided seed dispersal have served to promote continued gene flow across a broad landscape.

The most important influence of humans on the switchgrass landscape has been the movement of germplasm from one region to another. In many cases, without proper land records it cannot be verified that some accessions represent true local genotypes that evolved in that region. We recognize that some accessions may not represent truly local genotypes or may represent admixtures created by human efforts to establish nonlocal switchgrass strains and that contamination of seed lots is always a possible explanation for accessions that appear to have multiple origins. Despite any doubts raised by these phenomena, seeds from the majority of accessions evaluated in this experiment were either completely under our control or were increased by professionals who understand the need for care and control of contamination. We feel that the explanations offered herein represent the most plausible explanation for our observations, partly due to the low probability that such a large number of accessions would be contaminated in such a clear geographic pattern and partly because our results provide logical and unequivocal support of McMillan's 1959 hypothesis.

CONCLUSIONS

Numerous modern switchgrass accessions contain admixtures of genotypes that represent upland ecotypes, lowland ecotypes, and a range of upland × lowland hybrid descendents. Nearly all of these admixtures were found in remnant populations of the central and eastern Gulf Coast region of the United States and along the Atlantic Seaboard, suggesting that the central and eastern Gulf Coast region served as the primary center of origin and diversity for modern switchgrass. A secondary center of origin and diversity was located along the western Gulf Coast. These two refugia, plus a third western montane refuge, formed the basis for all northward migrations of switchgrass into postglacial habitats.

Functional gene pools for use in germplasm improvement, habitat restoration, or soil conservation should be designed to account for genotypic and phenotypic variability on both latitudinal and longitudinal transects. Switchgrass germplasm can generally be moved one hardiness zone north or south of its origin without severe adaptation consequences, creating roughly four effective gene pools on a north–south transect of the United States. On the east–west transect, there are minimally two divisions: germplasm of Great Plains origin and germplasm of eastern origin. A third division may exist between the humid central United States and the Atlantic Seaboard, but there is insufficient data to resolve that question at this time. Combining the latitudinal and longitudinal divisions results in a general concept of eight regional gene pools (four north–south Great Plains pools and four north–south eastern pools) that could serve as logical and functional sources of local germplasm for a wide variety of purposes. Within some of these eight regions, care must be taken to maintain upland and lowland plants in separate gene pools to support the development of heterotic gene pools and the possible commercialization of upland × lowland hybrids.

Finally, we make a plea to our colleagues who are involved in germplasm exploration, collection, and maintenance. With recent increases in funding and interest for breeding switchgrass, many new collections have been created by intensive exploration into many parts of the eastern United States. Due to incomplete coverage and significant gaps that still exist in our geographic SSR and cpDNA sequence database for switchgrass (Fig. 3), there

is still a need to access small numbers of plants of specific source-identified accessions. We recognize that bulking or compositing accessions within regions greatly simplifies maintenance and storage requirements for seed lots, but it vastly obscures valuable data on the origin, migration routes, and life history of switchgrass within these regions. We ask all germplasm collectors who wish to create bulk or composite populations to split their seed collections and maintain at least a small quantity of source-identified seed in long-term storage, preferably by donating a small sample to USDA-Germplasm Resources Information Network.

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References

Anderson, E.C., and E.A. Thompson. 2002. A model-based method for identifying species hybrids using multilocus genetic data. Genetics 160:1217–1229.

Bailey, R.G. 1998. Ecoregions: The ecosystem geography of the oceans and continents. Springer-Verlag, New York, NY.

Berger, W.H., J.S. Killingley, and E. Vincent. 1987. Time scale of the Wisconsin/Holocene transition: Oxygen isotope record in the Western Equatorial Pacific. Quat. Res. 28:295–306. doi:10.1016/0033-5894(87)90068-8

Bintanja, R., and R.S.W. van de Wal. 2008. North American ice-sheet dynamics and the onset of 100,000-year glacial cycles. Nature 454:869–872. doi:10.1038/nature07158

Casler, M.D. 2005. Ecotypic variation among switchgrass populations from the northern USA. Crop Sci. 45:388–398. doi:10.2135/cropsci2005.0388

Casler, M.D., D.R. Buxton, and K.P. Vogel. 2002. Genetic modification of lignin concentration affects fitness of perennial herbaceous plants. Theor. Appl. Genet. 104:127–131. doi:10.1007/s001220200015

Casler, M.D., R.B. Mitchell, and K.P. Vogel. 2011. (in press) Switchgrass. *In* C. Kole et al. (ed.) Bioenergy crops. Vol. 1. Commercialized Bioenergy Crops. Taylor & Francis Group, LLC, London, UK.

Casler, M.D., C.A. Stendal, L. Kapich, and K.P. Vogel. 2007a. Genetic diversity, plant adaptation regions, and gene pools for switchgrass. Crop Sci. 47:2261–2273. doi:10.2135/cropsci2006.12.0797

Casler, M.D., K.P. Vogel, and A.C. Beal. 2006. Registration of WS4U and WS8U switchgrass germplasms. Crop Sci. 46:998–999. doi:10.2135/cropsci2005.04-0020

Casler, M.D., K.P. Vogel, C.M. Taliaferro, N.J. Ehlke, J.D. Berdahl, E.C. Brummer, R.L. Kallenbach, C.P. West, and R.B. Mitchell. 2007b. Latitudinal and longitudinal adaptation of switchgrass populations. Crop Sci. 47:2249–2260. doi:10.2135/cropsci2006.12.0780

Casler, M.D., K.P. Vogel, C.M. Taliferro, and R.L. Wynia. 2004. Latitudinal adaptation of switchgrass populations. Crop Sci. 44:293–303.

Cathey, H.M. 1990. USDA Plant hardiness zone map. USDA Misc. Publ. No. 1475. Available at http://www.usna.usda.gov/Hardzone/ushzmap.html (verified 20 June 2011). U.S. Natl. Arboretum, USDA-ARS, Washington, DC.

Clark, J.S., C. Fastie, G. Hurtt, S.T. Jackson, C. Johnson, G.A. King, M. Lewis, J. Lynch, S. Pacala, C. Prentice, E.W. Schupp, T. Webb, III, and P. Wycoff. 1998. Reid's paradox of rapid plant migration. Bioscience 48:13–24. doi:10.2307/1313224

Clark, J.S., E.C. Grimm, J. Lynch, and P.G. Mueller. 2001. Effects of Holocene climate change on the C_4 grassland/woodland boundary in the northern Plains, USA. Ecology 82:620–636.

CodonCode Corp. 2009. CodonCode Aligner. Release 3.5. CodonCode Corp., Dedham, MA.

Collingham, Y.C., M.O. Hill, and B. Huntley. 1996. The migration of sessile organisms: A simulation model with measurable parameters. J. Veg. Sci. 7:831–846. doi:10.2307/3236461

Cortese, L.M., J. Honig, C. Miller, and S.A. Bonos. 2010. Genetic diversity of twelve switchgrass populations using molecular and morphological markers. Bioenerg. Res. 3:262–271. doi:10.1007/s12155-010-9078-2

Costich, D.E., B. Friebe, M.J. Sheehan, M.D. Casler, and E.S. Buckler. 2010. Genome-size variation in switchgrass (*Panicum virgatum*): Flow cytometry and cytology reveal rampant aneuploidy. Plant Gen. 3:130–141. doi:10.3835/plantgenome2010.04.0010

Cwynar, L.C., and A.J. Levesque. 1995. Chironomid evidence for late-glacial climatic reversals in Maine. Quat. Res. 43:405–413. doi:10.1006/qres.1995.1046

Deevey, E.S., Jr. 1949. Biogeography of the Pleistocene: Part I: Europe and North America. Geol. Soc. Am. Bull. 60:1315–1416. doi:10.1130/0016-7606(1949)60[1315:BOTP]2.0.CO;2

Edgar, R.C. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792–1797. doi:10.1093/nar/gkh340

Ernst, W.H.O., E.M. Veenendaal, and M.M. Kebakile. 1992. Possibilities for dispersal in annual and perennial grasses in a savanna in Botswana. Vegetatio 102:1–11. doi:10.1007/BF00031700

Esri. 2009. ArcGIS. Release 9.3. Esri, Redlands, CA.

Grimm, E.C., G.L. Jacobson, Jr., W.A. Watts, B.C.S. Hansen, and K.A. Maasch. 1993. A 50,000-year record of climate oscillations from Florida and its temporal correlation with the Heinrich Events. Science 261:198–200. doi:10.1126/science.261.5118.198

Gunter, L.E., G.A. Tuscan, and S.D. Wullshcleger. 1996. Diversity of switchgrass based on RAPD markers. Crop Sci. 36:1017–1022.

doi:10.2135/cropsci1996.0011183X003600040034x
Hamilton, M.B. 1999. Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. Mol. Ecol. 8:513–525. doi:10.1046/j.1365-294X.1999.00510.x
Harlan, J.R., and J.M.J. de Wet. 1975. On Ö. Winge and a prayer: The origins of polyploidy. Bot. Rev. 41:361–369. doi:10.1007/BF02860830
Hoffecker, J.F., W.R. Powers, and T. Goebel. 1993. The colonization of Beringia and the peopling of the New World. Science 259:46–53. doi:10.1126/science.259.5091.46
Hopkins, A.A., C.M. Taliaferro, C.D. Murphy, and D. Christian. 1996. Chromosome numbers and nuclear DNA content of several switchgrass populations. Crop Sci. 36:1192–1195. doi:10.2135/cropsci1996.0011183X003600050021x
Hopkins, A.A., K.P. Vogel, K.J. Moore, K.D. Johnson, and I.T. Carlson. 1995. Genotypic variability and genotype × environment interactions among switchgrass accessions from the midwestern USA. Crop Sci. 35:565–571. doi:10.2135/cropsci1995.0011183X003500020047x
Huang, S., X. Siu, R. Haselkorn, and P. Gornicki. 2003. Evolution of switchgrass (*Panicum virgatum* L.) based on sequences of the nuclear gene encoding plastic acetyl-CoA carboxylase. Plant Sci. 164:43–49. doi:10.1016/S0168-9452(02)00327-8
Huang, Y., B. Shuman, Y. Wang, T. Webb, III, E.C. Grimm, and G.L. Jacobson, Jr. 2006. Climatic and environmental controls on the variation of C_3 and C_4 plant abundances in central Florida for the past 62,000 years. Palaeogeogr. Palaeoclimatol. Palaeoecol. 237:428–435. doi:10.1016/j.palaeo.2005.12.014
Hultquist, S.J., K.P. Vogel, D.J. Lee, K. Arumuganathan, and S. Kaeppler. 1996. Chloroplast DNA and nuclear DNA content variations among cultivars of switchgrass, *Panicum virgatum* L. Crop Sci. 36:1049–1052. doi:10.2135/cropsci1996.0011183X003600040039x
Hultquist, S.J., K.P. Vogel, D.E. Lee, K. Arumuganathan, and S. Kaeppler. 1997. DNA content and chloroplast DNA polymorphisms among accessions of switchgrass from remnant Midwestern prairies. Crop Sci. 37:595–598. doi:10.2135/cropsci1997.0011183X003700020047x
Jacobson, G.T., and E.C. Grimm. 1986. A numerical analysis of Holocene forest and prairie vegetation in central Minnesota. Ecology 67:958–966. doi:10.2307/1939818
Jakobsen, B.H. 2009. Holocene climate change and environmental reconstruction in East Greenland. IOP Conf. Series: Earth Environ. Sci. 6 (2009) 072028 doi:10.1088/1755-1307/6/7/072028.
Kelley, D.W., S.A. Brachfeld, E.A. Nater, and H.E. Wright, Jr. 2006. Sources of sediment in Lake Pepin on the Upper Mississippi River in response to Holocene climate changes. J. Paleoclim. 35:193–206. doi:10.1007/s10933-005-8686-x
Kneller, M., and D. Peteet. 1999. Late-glacial to early Holocene climate changes from a central Appalachian pollen and macrofossil record. Quat. Res. 51:133–147. doi:10.1006/qres.1998.2026
LaMoreaux, H.K., G.A. Brook, and J.A. Knox. 2009. Late Pleistocene and Holocene environments of the southwestern United States from the stratigraphy and pollen content of a peat deposit on the Georgia Coastal Plain. Palaeogeogr. Palaeoclimatol. Palaeoecol. 280:300–312. doi:10.1016/j.palaeo.2009.06.017
Leigh, D. 2008. Late Quaternary climates and river channels of the Atlantic Coastal Plain, southeastern USA. Geomorphology 101:90–108. doi:10.1016/j.geomorph.2008.05.024
Levesque, A.J., F.E. Mayle, I.R. Walker, and L.C. Cwynar. 1993. A previously unrecognized late-glacial cold event in eastern north. Am. Nat. 361:623–626.
Martinez-Reyna, J.M., and K.P. Vogel. 2002. Incompatibility systems in switchgrass. Crop Sci. 42:1800–1805. doi:10.2135/cropsci2002.1800
Martinez-Reyna, J.M., and K.P. Vogel. 2008. Heterosis in switchgrass: Spaced plants. Crop Sci. 48:1312–1320. doi:10.2135/cropsci2007.12.0695
Martinez-Reyna, J.M., K.P. Vogel, C. Caha, and Donald J. Lee. 2001. Meiotic stability, chloroplast DNA polymorphisms, and morphological traits of upland × lowland switchgrass reciprocal hybrids. Crop Sci. 41:1579–1583. doi:10.2135/cropsci2001.4151579x
McMillan, C. 1959. The role of ecotypic variation in the distribution of the central grassland of North America. Ecol. Monogr. 29:285–308. doi:10.2307/1942132
McMillan, C. 1964. Ecotypic differentiation within four North American prairie grasses. I. Morphological variation within transplanted community fractions. Am. J. Bot. 51:1119–1128. doi:10.2307/2439961
Missaoui, A.M., A.H. Paterson, and J.H. Bouton. 2006. Molecular markers for the classification of switchgrass (*Panicum virgatum* L.) germplasm and to assess genetic diversity in three synthetic switchgrass populations. Genet. Resour. Crop Evol. 53:1291–1302. doi:10.1007/s10722-005-3878-9
Narasimhamoorthy, B., M.C. Saha, T. Swaller, and J.H. Bouton. 2008. Genetic diversity in switchgrass collections assessed by EST-SSR markers. BioEnergy Res. 1:136–146. doi:10.1007/s12155-008-9011-0
Nielsen, E.L. 1944. Analysis of variation in *Panicum virgatum*. J. Agric. Res. 69:327–353.
Ocumpaugh, W.R., S. Archer, and J.W. Stuth. 1996. Switchgrass recruitment from broadcast seed vs. seed fed to cattle. J. Range Manage. 49:368–371. doi:10.2307/4002599
Pakeman, R.J. 2001. Plant migration rates and seed dispersal mechanisms. J. Biogeogr. 28:795–800. doi:10.1046/j.1365-2699.2001.00581.x
Peakall, R., and P.E. Smouse. 2006. Genalex 6: Genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6:288–295. doi:10.1111/j.1471-8286.2005.01155.x
Platt, A.R., R.W. Woodhall, and A.L. George. 2007. Improved DNA sequencing quality and efficiency using an optimized fast cycle sequencing protocol. Biotechniques 43:58–62. doi:10.2144/000112499
Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.
Sagan, C. 1995. The demon-haunted world: Science as a candle in the dark. Random House, New York, NY.
Sanderson, M.A., P.R. Adler, A.A. Boateng, M.D. Casler, and G. Sarath. 2007. Switchgrass as a biofuels feedstock in the USA. Can. J. Plant Sci. 86:1315–1325. doi:10.4141/P06-136
Shaw, J., E.B. Lickey, E.E. Schilling, and R. Small. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. Am. J. Bot. 94:275–288. doi:10.3732/ajb.94.3.275
SoftGenetics. 2006. GeneMarker. Release 1.5. SoftGenetics, State College, PA.
Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol. Biol. 17:1105–1109. doi:10.1007/BF00037152

Talbert, L.E., D.H. Timothy, J.C. Burns, J.O. Rawlings, and R.H. Moll. 1983. Estimates of genetic parameters in switchgrass. Crop Sci. 23:725–728. doi:10.2135/cropsci1983.0011183X002300040029x

Tobias, C.M., G. Sarath, P. Twigg, E. Lindquist, J. Pangilinan, B. Penning, K. Barry, N. Carpita, and G.R. Lazo. 2008. Comparative genomics in switchgrass using 61,585 high-quality EST. Plant Gen. 1:111–124. doi:10.3835/plantgenome2008.08.0003

Verity Software House. 2008. ModFit LT. Release 3.2. Verity Software House, Topsham, ME.

Vogel, K.P. 2004. Switchgrass. p. 561–588. *In* L.E. Moser et al. (ed.) Warm-season (C4) grasses. ASA, CSSA, SSSA, Madison, WI.

Vogel, K.P., and R.B. Mitchell. 2008. Heterosis in switchgrass: Biomass yield in swards. Crop Sci. 48:2159–2164. doi:10.2135/cropsci2008.02.0117

Watts, W.A. 1971. Postglacial and interglacial vegetation history of southern Georgia and central Florida. Ecology 52:676–690. doi:10.2307/1934159

Watts, W.A., B.C.S. Hansen, and E.C. Grimm. 1992. Camel Lake: A 40,000-yr record of vegetational and forest history from Northwest Florida. Ecology 73:1056–1066. doi:10.2307/1940180

Webb, S. 1986. Potential role of passenger pigeons and other vertebrates in the rapid Holocene migrations of nut trees. Quat. Res. 26:367–375. doi:10.1016/0033-5894(86)90096-7

Wilkinson, D.M. 1997. Plant colonization: Are wind dispersed seeds really dispersed by birds at larger spatial and temporal scales? J. Biogeogr. 24:61–65. doi:10.1111/j.1365-2699.1997.tb00050.x

Young, H.A., B.J. Hernlem, A.L. Anderton, C.L. Lanzatella, and C.M. Tobias. 2010. Dihaploid stocks of switchgrass isolated by a screening approach. Bioenergy Res. 3:305–313. doi:10.1007/s12155-010-9081-7

Zalapa, J.E., D.L. Price, S.M. Kaeppler, C.M. Tobias, M. Okada, and M.D. Casler. 2011. Hierarchical classification of switchgrass using SSR and chloroplast sequences: Ecotypes, ploidies, gene pools, and cultivars. Theor. Appl. Genet. doi:10.1007/s00122-010-1488.1