DOI: 10.1534/genetics.110.113910

Complete Switchgrass Genetic Maps Reveal Subgenome Collinearity, Preferential Pairing and Multilocus Interactions

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Manuscript received January 14, 2010
Accepted for publication April 7, 2010

ABSTRACT

Polyploidy is an important aspect of the evolution of flowering plants. The potential of gene copies to diverge and evolve new functions is influenced by meiotic behavior of chromosomes leading to segregation as a single locus or duplicated loci. Switchgrass (*Panicum virgatum*) linkage maps were constructed using a full-sib population of 238 plants and SSR and STS markers to access the degree of preferential pairing and the structure of the tetraploid genome and as a step toward identification of loci underlying biomass feedstock quality and yield. The male and female framework map lengths were 1645 and 1376 cM with 97% of the genome estimated to be within 10 cM of a mapped marker in both maps. Each map coalesced into 18 linkage groups arranged into nine homeologous pairs. Comparative analysis of each homology group to the diploid sorghum genome identified clear syntenic relationships and collinear tracts. The number of markers with PCR amplicons that mapped across subgenomes was significantly fewer than expected, suggesting substantial subgenome divergence, while both the ratio of coupling to repulsion phase linkages and pattern of marker segregation indicated complete or near complete disomic inheritance. The proportion of transmission ratio distorted markers was relatively low, but the male map was more extensively affected by distorted transmission ratios and multilocus interactions, associated with spurious linkages.

POLYPLOIDY is common among plants (Masterson 1994; Levin 2002) and is an important aspect of plant evolution. Widespread paleopolyploidy in flowering plant lineages suggests that ancient polyploidization events have contributed to the radiation of angiosperms (Soltis *et al.* 2009; Van de Peer *et al.* 2009a). Whole genome duplications are thought to be the sources of evolutionary novelty (Osborn *et al.* 2003; Freeling and Thomas 2006; Chen 2007; Hegarty and Hiscock 2008; Flagel and Wendel 2009; Leitch and Leitch 2008). Other attributes of polyploids considered to promote evolutionary success include increased vigor, masking of recessive alleles, and reproductive barriers arising from loss of one of the duplicate genes (Soltis and Soltis 2000; Comai 2005; Otto 2007; Van de Peer *et al.* 2009b). Among crop species, polyploidy likely contributed to trait improvement under artificial selection (Paterson 2005; Udall and Wendell 2006; Dubcovsky and Dvorak 2007; Hovav *et al.* 2008).

Disomic inheritance in polyploids, in contrast to polysomic inheritance, presents opportunities for duplicated genes to diverge and evolve new functions. The relative age of whole genome duplications and the extent of homology between subgenomes greatly influence chromosomal pairing at meiosis (Soltis and Soltis 1995; Wolfe 2001; Ramsey and Schemske 2002). Polysomic inheritance resulting from random chromosome pairing is associated with doubling of a single set of chromosomes. Disomic inheritance resulting from preferential pairing is often associated with polyploidy arising from combinations of divergent genomes. The evolutionary process of diploidization leads to a shift from random to preferential pairing that is not well understood but is genetically defined in systems such as *Ph1* of wheat (*Triticum aestivum*) and *PrBn* of *Brassica napus* (Riley and Chapman 1958; Vega and Feldman 1998; Jenczewski *et al.* 2003). The degree of preferential pairing also affects allelic diversity and the ability to detect linkage. Accurate information about chromosome pairing and whole or partial genome duplications is thus important for both evolutionary studies and in linkage analysis.

Such information is extremely limited in the C4 panicoid species *Panicum virgatum* (switchgrass), which is now viewed as a promising energy crop in the United

Supporting information is available online at http://www.genetics.org/cgi/content/full/genetics.110.113910/DC1.

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States and Europe (Lewandowski *et al.* 2003; McLaughlin and Kszos 2005) and is planted extensively for forage and soil conservation (Vogel and Jung 2001). Little is known about either its genome structure or inheritance. Much current bioenergy feedstock development is focused on tetraploid cytotypes ($2n = 4x = 36$) due to their higher yield potentials, and an initial segregation study indicated a high degree of preferential pairing in a single F1 mapping population (Missaoui *et al.* 2005). A once-dominant component of the tallgrass prairie in North America, switchgrass is largely self-incompatible (Martinez-Reyna and Vogel 2002) with predominantly tetraploid or octoploid cytotypes (Hultquist *et al.* 1997; Lu *et al.* 1998). Limited gene flow appears possible between different cytotypes suggested by DNA content variation within collection sites and seed lots (Nielsen 1944; Hultquist *et al.* 1997; Narasimhamoorthy *et al.* 2008). True diploids appear to be rare (Nielsen 1944; Young *et al.* 2010). Multivalents in meiosis have not been observed in tetraploids or F1 hybrids between upland and lowland tetraploids, although rare univalents occurred (Barnett and Carver 1967; Martinez-Reyna *et al.* 2001). However, polysomic inheritance may occur with random bivalent pairing (Howard and Swaminathan 1953).

Sustainable production of switchgrass for bioenergy to meet the goal of reducing greenhouse gas emissions will require advances in feedstock production that include improvements in yield (Carroll and Somerville 2009). Switchgrass has extensive genetic diversity and potential for genetic improvements, but each cycle of phenotypic selection can take several years (McLaughlin and Kszos 2005; Parrish and Fike 2005; Bouton 2007). Detailed understanding of genome structure to enable efficient marker-assisted selection (MAS) can speed this process considerably. Complete linkage maps are therefore required to both understand chromosome pairing and allow MAS.

We report the construction of the first complete linkage maps of two switchgrass genotypes. The linkage maps provide genetic evidence for disomic inheritance in lowland, tetraploid switchgrass. Gene-derived markers enabled a comparative analysis to sorghum, revealing syntenic relationships between the diploid sorghum genome and the tetraploid switchgrass subgenomes. Transmission ratio distortion and multilocus interactions were analyzed in detail to document their potential influence on map accuracy and map-based studies in switchgrass.

MATERIALS AND METHODS

Mapping population: A full-sib mapping population derived from a cross between selected genotypes of switchgrass Kanlow as the female parent and Alamo as the male parent was produced and initial genotyping identified self-pollinated and diploid individuals comprising 5% of the population. These were eliminated and 238 F1 plants were used for mapping. The male parent was an individual with good response to tissue culture and was from seed obtained from colleagues in Lincoln, Nebraska. The female parent was a randomly selected individual from a commercial seed source (Osenbaugh Grass Seed, Lucas, IA). The cultivars Kanlow and Alamo are of the lowland ecotype and are expected to be tetraploid ($2n = 4x = 36$).

DNA extraction and molecular marker analysis: Total genomic DNA was extracted from young leaves dried in silica using the CTAB method of Chen and Ronald (1999). Previously identified simple sequence repeat (SSR) PCR primers in existing switchgrass expressed sequence tags (ESTs) (Tobias *et al.* 2006, 2008) were used and an additional 205 EST–SSR primers were developed from the same EST data. EST sequence-tagged-site (EST–STS) primer pairs were developed by designing primers spanning predicted conserved introns (supporting information, Table S1). Genomic SSR primers were developed by sequencing GA/CT enriched genomic libraries constructed from a pooled population of cultivar Alamo as well as sequences submitted to GenBank by Lee Gunter (Oak Ridge National Laboratory, TN) (Table S1).

Products were amplified in 5- to 10-μl PCR reactions, PEG precipitated, and sized on an ABI3730xl using PET-labeled size standards. Amplicons were scored using Genemapper v. 3.7 (Applied Biosystems, Foster City, CA). The EST–SSR and EST–STS markers were tested for amplification and polymorphism in the parents and six random F1 individuals. Markers that amplified one or more amplicons that were polymorphic in the parents and/or the F1 were used for genotyping the whole population. Markers with greater than 15% missing data were omitted from analysis.

Segregation and linkage analysis: Determining allelic segregation at a locus is difficult in polyploids, since not all genotypes can be determined on the basis of marker phenotype alone (Sorrells 1992; Wu *et al.* 1992). Thus, the linkage analysis was conducted using polymorphic single-dose amplicons (SDAs) following Wu *et al.* (1992). SDAs were identified on the basis of presence in only one parent and goodness-of-fit to a 1:1 presence to absence ratio in the F1 using the chi-square test ($\alpha = 0.01$). The next higher segregation ratio for polymorphism between parents is 3:1, which is the ratio expected if double-dose amplicons were present in each of the subgenomes under disomic inheritance. In addition, transmission ratio distorted (TRD) SDAs were defined as polymorphisms that were present in either one of the parents and did not fit the 1:1 ratio, but were below a 1.73:1 ratio, which represents equal chi-square values for 1:1 and 3:1 ratios (Mather 1957). Remaining polymorphisms were tested for 3:1 and 5:1 ratios. The next higher ratio beyond 3:1 is 5:1, which is expected for double-dose amplicons under tetrasomic inheritance. Amplicons that were shared between parents and single-dose in both (SDxSD) were identified using the fit to the 3:1 ratio by chi-square test ($\alpha = 0.01$). See File S1 for the raw genotypes.

The data were analyzed with JoinMap 4 (Van Ooijen 2006) using the outbreeder full-sib family (CP) as the population type. Maternal and paternal maps were analyzed separately following the two way pseudo-testcross strategy (Grattapaglia and Sederoff 1994). The SDAs were grouped into linkage groups at the minimum independence test LOD score of 8.0. TRD–SDAs were excluded during construction of the framework maps. Loci within linkage groups were ordered using the maximum-likelihood mapping algorithm (Jansen *et al.* 2001). Separate SDAs within a linkage group with equivalent or nearly equivalent map positions with regard to the variation in the data (Jansen *et al.* 2001) were binned together and map order was then determined using only one SDA from each bin. The grouped SDAs that were not used in the linkage analysis were

TABLE 1

Amplification, polymorphism, and segregation ratios of molecular markers in the switchgrass mapping population

	Markers	Total amplicons	Polymorphic amplicons	Female parent				Male parent				Both parents
				1:1	1:1 (TRD[a])	3:1	5:1	1:1	1:1 (TRD)	3:1	5:1	3:1 (SDxSD[b])
EST–SSR	420	1364	945	354	13	2	0	342	62	11	0	91
Genomic SSR	181	620	509	209	6	2	0	191	24	8	3	29
EST–STS	36	109	55	20	0	0	0	22	4	0	0	6
Total	637	2093	1509	583	19	4	0	555	90	19	3	126

[a] TRD, transmission ratio distorted. Markers were considered TRD if segregation ratio was less than 1.73:1 and deviated significantly from 1:1 by the chi-square test ($\alpha = 0.01$)

[b] Markers monomorphic between parents and segregating in 3:1.

treated as accessory loci to those that were mapped. SDAs were placed on the map using the regression mapping algorithm as described in Stam (1993), with the relative order obtained above fixed at the minimum LOD score of 8.0 and maximum recombination fraction of 0.35. The Kosambi mapping function was used to obtain map distance. When two groups of SDAs within the LG were not linked at the threshold, they were accepted as linked if at least one SDA was linked to two or more SDAs in the other group. To allow linkage of the two groups, *i.e.,* the presence of at least two independent linkages at the threshold, lower LOD thresholds were used. Subsequently, SDxSD amplicons and TRD–SDAs were added to the framework map as additional accessory loci at the strongest cross link (SCL) parameter threshold of 8.0.

Each linkage group (LG) is expected to belong to one of nine homology groups (HGs) based on the basic chromosome number in switchgrass. LGs were grouped into HGs when at least two pairs of loci derived from the same SSR or STS marker were shared (Ming *et al.* 1998). Within each HG, LGs from two parental maps were identified as homologous on the basis of one or more shared SDxSD amplicons.

Genome length (G) was estimated on the basis of the framework map by the method of Hulbert *et al.* (1988) as modified by method 3 of Chakravarti *et al.* (1991). A pairwise linkage threshold of LOD 8.0 was used for the estimation of G, because the LGs were grouped at LOD 8.0. Genome coverage was calculated for each framework map using

$$c = 1 - e^{-2dn/G},$$

where c is the proportion of the genome within d cM of a framework marker, G is the estimated genome length, and n is the number of framework markers in the map (Lange and Boehnke 1982).

Disomic and polysomic polyploids differ in the population size that is required to detect repulsion phase linkages because of recombination resulting from independent assortment in repulsion linkages under polysomic inheritance (Wu *et al.* 1992; Qu and Hancock 2001). For the population size of 238, the expected ratio of coupling to repulsion phase linkages is 1:1 and 1.55:1 for disomic and tetrasomic inheritance, respectively (Wu *et al.* 1992). The observed ratio of the loci linked in coupling phase *vs.* repulsion phase at LOD greater than 3.0 was tested for fit to the expected ratios by the chi-square test. The homogeneity chi-square test was used to assess whether the ratios across LGs were homogeneous within each parents (d.f. = 17) prior to pooling the data.

The proportion of SSR or STS markers with amplicons that mapped across subgenomes was assessed on the maps. The expected proportion of markers with SDAs and/or SDxSD amplicons in both subgenomes was estimated under no subgenome differentiation, using parental genotypes simulated with SPIP v1.0 (Anderson and Dunham 2005) on the basis of allele frequencies obtained at 21 of the mapped EST–SSR markers in 38 and 40 individuals from Kanlow and Alamo (Table S2). The 21 markers produced amplicons consistent with a single disomic locus, enabling estimation of allele frequencies within cultivars. Of the 21 markers used, 9 and 12 had dinucleotide and trinucleotide SSR motifs, respectively. To simulate the effect of no divergence between subgenomes, genotypes in both subgenomes were randomly sampled from the same allele pool. The proportion of markers with SDAs and/or SDxSD amplicons from both subgenomes was obtained out of the 21 markers. The mean and standard deviation for the proportion of markers with amplicons mapping in both subgenomes were estimated using 1000 simulated parental pairs.

Transmission distorted markers in multilocus interactions: To assess the extent of multilocus interactions between unlinked loci associated with single-locus transmission ratio distortions, all mapped TRD–SDAs were tested for independence against all SDAs in other LGs in both male and female maps using the two-locus genotypes in the two-by-two contingency chi-square test. Since comparisons were made among unlinked SDAs, the significance was evaluated with a correction for 770 comparisons of 22 LGs with at least one TRD–SDA with 35 other LGs ($P < 6.49 \times 10^{-5}$).

Comparative mapping: Assembled EST sequences corresponding to EST–SSR and EST–STS markers used for mapping were aligned to predicted protein coding sequences of rice (*Oryza sativa* ssp. *japonica*) present in release 6.1 of the MSU rice genome annotation project and sorghum (*Sorghum bicolor*) predicted coding sequences released in genome annotation Sbi1.4 (http://www.phytozome.net). BLAST nucleotide alignments with an *E*-value threshold of 1×10^{-10} or lower were considered. Orthologous pairs of sequences in switchgrass and sorghum were defined as the best matches that both matched the same rice gene. The corresponding sorghum and rice genome coordinates were then used for comparison to the map positions of all loci detected by the corresponding EST–SSR or EST–STS marker excluding alternate alleles of allelic pairs linked in repulsion on the same LG. Naming of LGs was based on published comparative maps between *Setaria italica* (foxtail millet) and rice that allowed alignment using rice as a common reference (Devos *et al.* 1998). For determining syntenic relationships and collinearity with sorghum, putative sorghum–switchgrass orthologs were counted for each switchgrass LG and grouped by HG. For assessing gene collinearity, only framework SDAs were evaluated, while all TRD, SDxSD, and accessory loci were omitted.

RESULTS

Marker segregation: A total of 420 EST–SSR, 181 genomic SSR, and 36 EST–STS markers generated 2093 amplicons, of which 1509 were polymorphic between the parents (Table 1). Of the polymorphic amplicons, 583 segregated as SDAs in the female parent and 555 in the male parent ($\alpha = 0.01$). In addition, 19 (3%) and 90 (14%) amplicons were identified as TRD–SDAs for the female and male parent, respectively. The remaining 236 polymorphic amplicons were tested for segregation ratios consistent with double dose across subgenomes (A–A–*x*– – – –) under disomic (3:1) or tetrasomic (5:1) inheritance. Only three (0.2%) amplicons fit the 5:1 ratio, whereas 23 (1.5%) amplicons segregated 3:1, and three (0.2%) fit both ratios ($\alpha = 0.01$). All three amplicons that fit the 5:1 ratio and two of the three that fit both ratios segregated in the male parent. On the basis of the 3:1 ratio ($\alpha = 0.01$) and presence in both parents, 126 amplicons were classified as SDxSD (A– – –*x*A– – – –).

Switchgrass linkage maps are complete and highly collinear: In both female and male maps, 18 linkage groups formed at the minimum LOD score of 8.0. Seven and three SDAs in the female and male parent, respectively, remained ungrouped. Of the equivalent or nearly equivalent SDAs, 39 and 28 in the female and male maps, respectively, were apparently allelic and an additional 225 nonallelic SDAs were combined into 89 bins in the female and 162 into 80 bins in the male maps (Figure 1). A total of 299 SDAs in the female map and 352 in the male map were ordered and placed on each LG (Table 2) at the threshold of LOD 8.0 and recombination fraction of 0.35, with the exception of three LGs: *IIa-f, VIIIb-f,* and *IIIa-m*. These groups consisted of two clusters of SDAs at the threshold but were joined at lower LOD scores of 6.0, 6.0, and 4.0, respectively. Naming of linkage groups was based on inferred syntenic relationships between switchgrass and foxtail millet (see below), with the addition of an arbitrary subgenome designation and male (*m*) or female (*f*) designation. Excellent correspondence between male and female maps was observed ($r = 0.896$, $P < 0.0001$).

Including accessory SDAs there were 563 SDAs in the female framework map spanning 1376 cM and 542 SDAs in the male framework map spanning 1645 cM (Tables 2 and 3). The average length of LGs and interlocus distance per LG were 76.5 and 5.5 cM in the female map and 91.4 and 5.0 cM in the male map. The total number of SDAs that were not grouped into a LG or placed on the maps was 9 (1.4%) and 4 (0.7%) in the female and male framework maps, respectively.

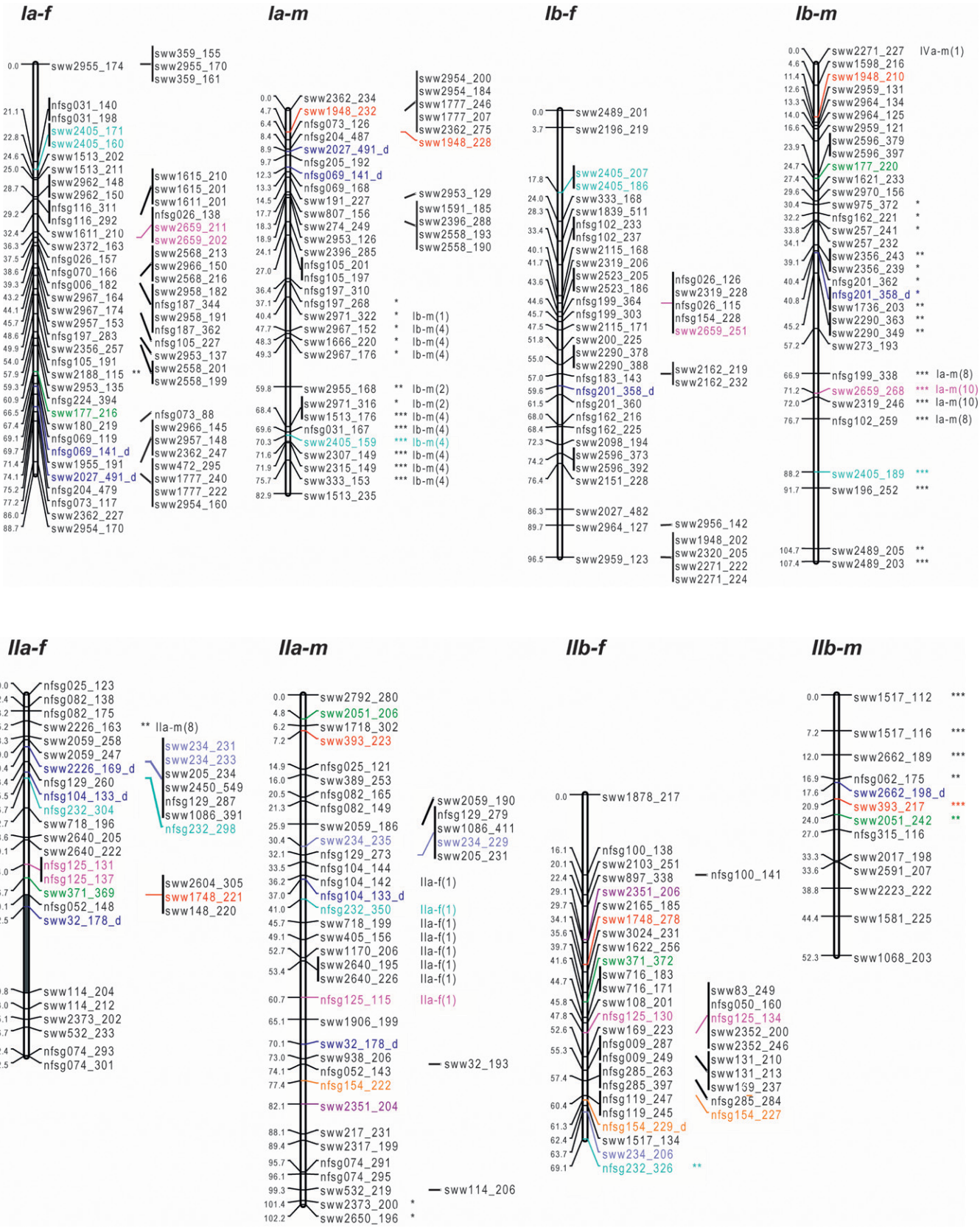
Estimated total genome size for switchgrass using the method of Hulbert *et al.* (1988) were 1515 and 1935 cM for the female and male genomes, respectively, based on the framework maps. For both framework maps, 97% of the genome was estimated to be within 10 cM of a mapped marker.

Fourteen of the 19 maternal TRD–SDAs were mapped to the framework map, and 75 of the 90 paternal TRD–SDAs were mapped to the framework map (Table 2). Of the 126 SDxSD amplicons, 38 and 40 were placed on the female and male maps, respectively. One additional SDA that did not map in the framework map was placed on each of the maps when the TRD and SDxSD amplicons were added. The addition of these 53 loci on the female map and 116 loci to the male map increased the overall map length by 24 and 103 cM, respectively.

The LGs in both female and male maps grouped into nine HGs on the basis of marker sharing, as expected from the tetraploid genome structure of $2n = 4x = 36$. Within seven of the nine HGs, two pairs of homologs were identified on the basis of shared SDxSD amplicons. In the remaining two HGs, II and VII, only one of the homologous pairs shared SDxSD amplicons.

The subgenomes within all HGs did not significantly differ in the number of mapped EST–SSR amplicons with the exception of HG VIII (Table S3). This allowed the estimated allelic frequencies at 21 of the mapped markers from a sample of the parental cultivars to be used for assessing subgenome differentiation. The expected proportion of markers present in both subgenomes was 0.629 ± 0.100 (SD) assuming no subgenome divergence. The total number of amplicons detected in the parental cultivars varied across markers

Figure 1.—Female and male parental linkage maps of tetraploid switchgrass. The gray segments in LGs, *IIa-f, VIIIb-f,* and *IIIa-m*, indicate linkage identified at lowered LOD scores of 6.0, 6.0, and 4.0, respectively. The accessory loci are listed next to mapped loci, but apparently allelic accessory and framework SDAs with identical map positions are shown together. Accessory loci are those assessed as equivalent or nearly equivalent to the mapped loci based on the plausible map position in the maximum-likelihood algorithm in JoinMap4 (Van Ooijen 2006). The linkage groups were grouped into homology groups based on shared markers. The homologs were identified on the basis of the single-dose by single-dose amplicons shared by the parents. The Roman numeral designation of each homology group (I–IX) follows the foxtail millet chromosomes. The name of each linkage group includes the homology group, and the letter a or b arbitrarily designates the subgenomes. The letters f and m at the end of the linkage group name denote female and male parental map, respectively. TRD–SDAs are noted with asterisks: (*) $P < 0.01$, (**) $P < 0.001$, and (***) $P < 0.0001$. Significant two-locus interactions detected for TRD–SDAs are denoted next to the LG on which interacting loci are located and the number of loci is indicated beside it in brackets. SDxSD amplicons are in dark blue color with the letter d appended to the name. All loci mapped across subgenomes with the same SSR or STS marker on the maps are highlighted with matching colors.



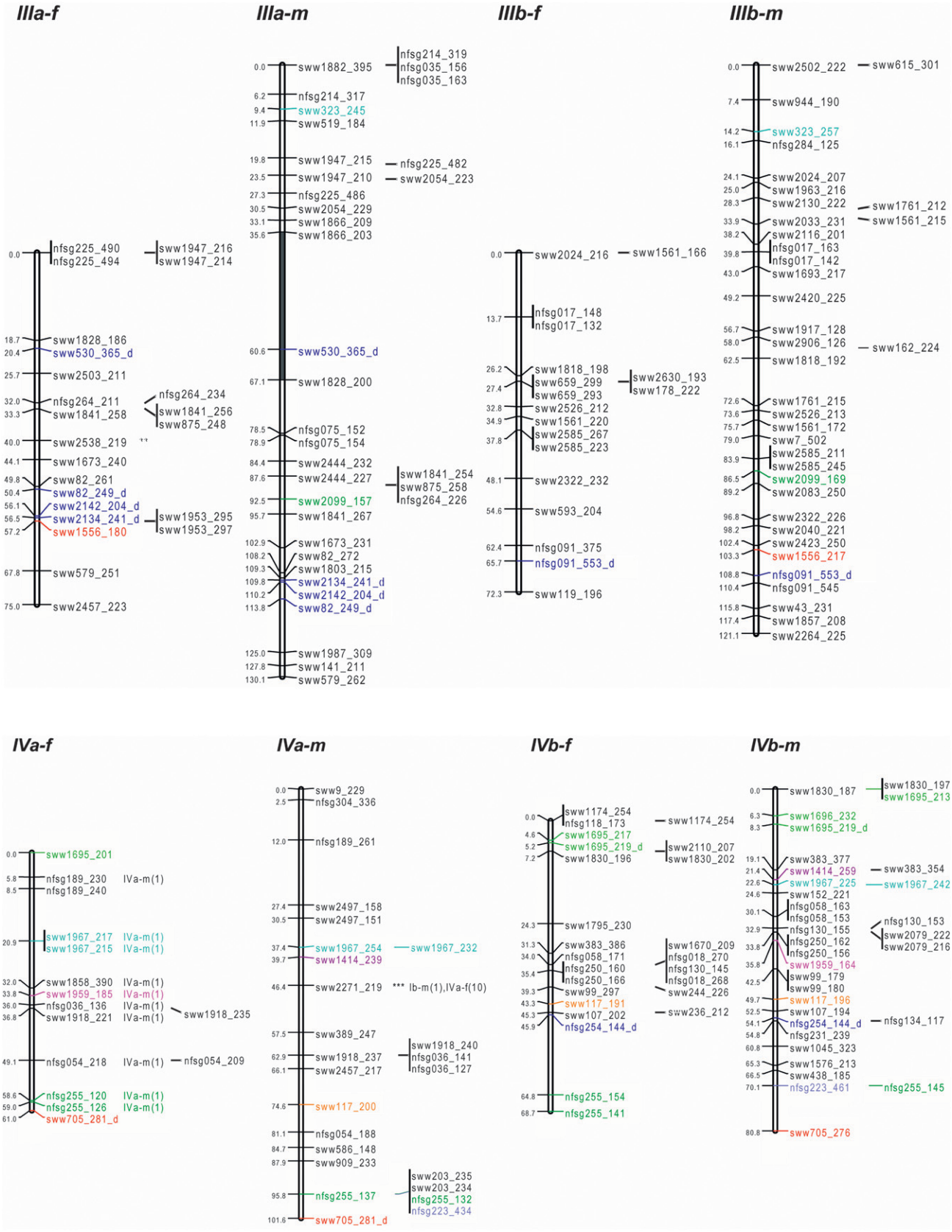


FIGURE 1.—(*Continued*)

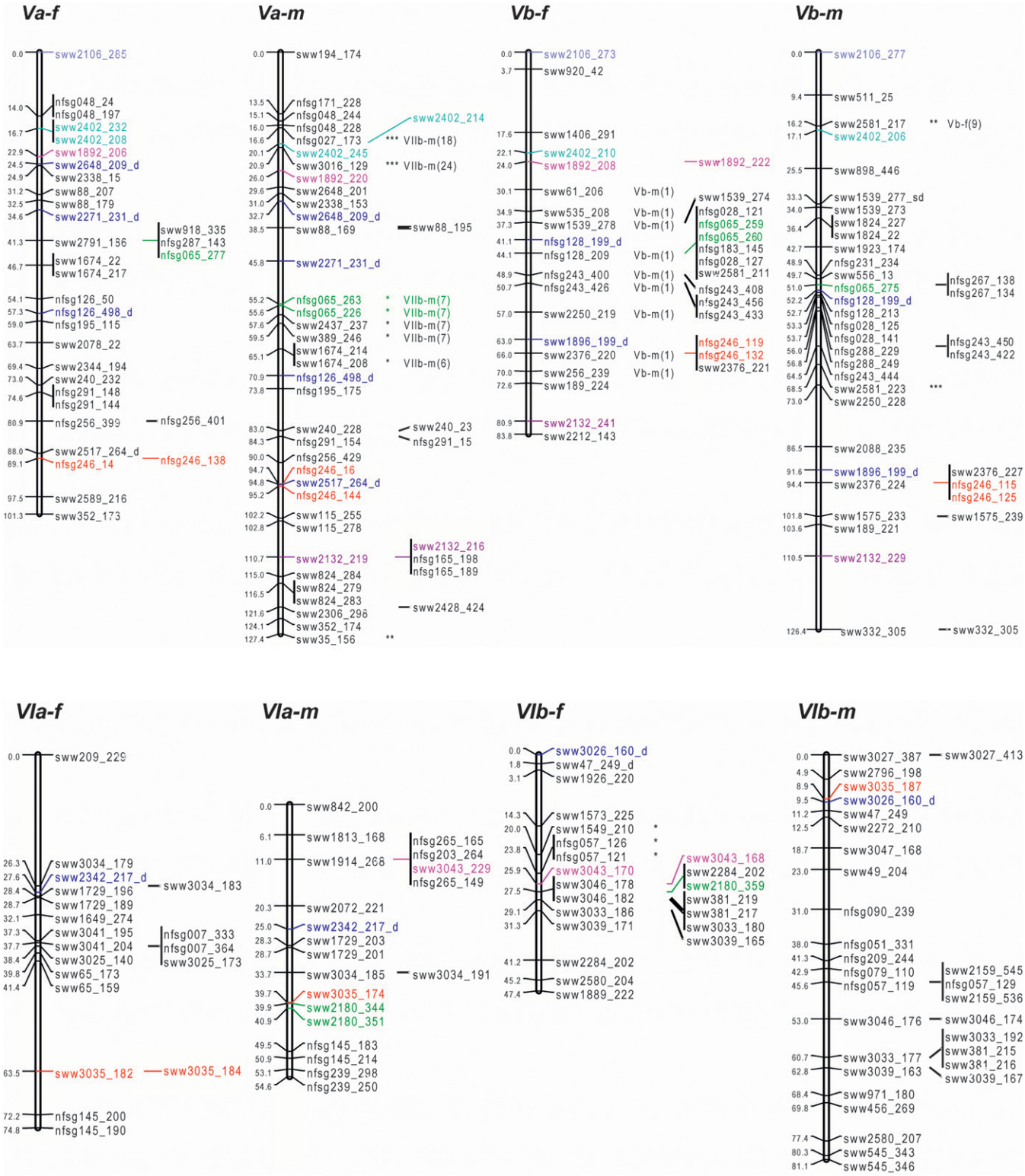


FIGURE 1.—(*Continued*)

from 4 to 31 (mean 13, SD 9.35). The frequency of the presence in both subgenomes varied between 0.035 and 0.979 (mean 0.558, SD 0.320) across markers. After weighting the SDAs and SDxSD amplicons by the proportions mapped in this study, and adjusting the proportions of dinucleotide *vs.* other SSR motif types, the expected proportion of markers present in both subgenomes was lower at 0.569 (SD 0.106). In the linkage maps constructed 68 of 613 (0.111) mapped across subgenomes. When only EST–SSR markers were considered, as in the simulation, 47 of 393 (0.120) mapped across subgenomes indicating significant divergence.

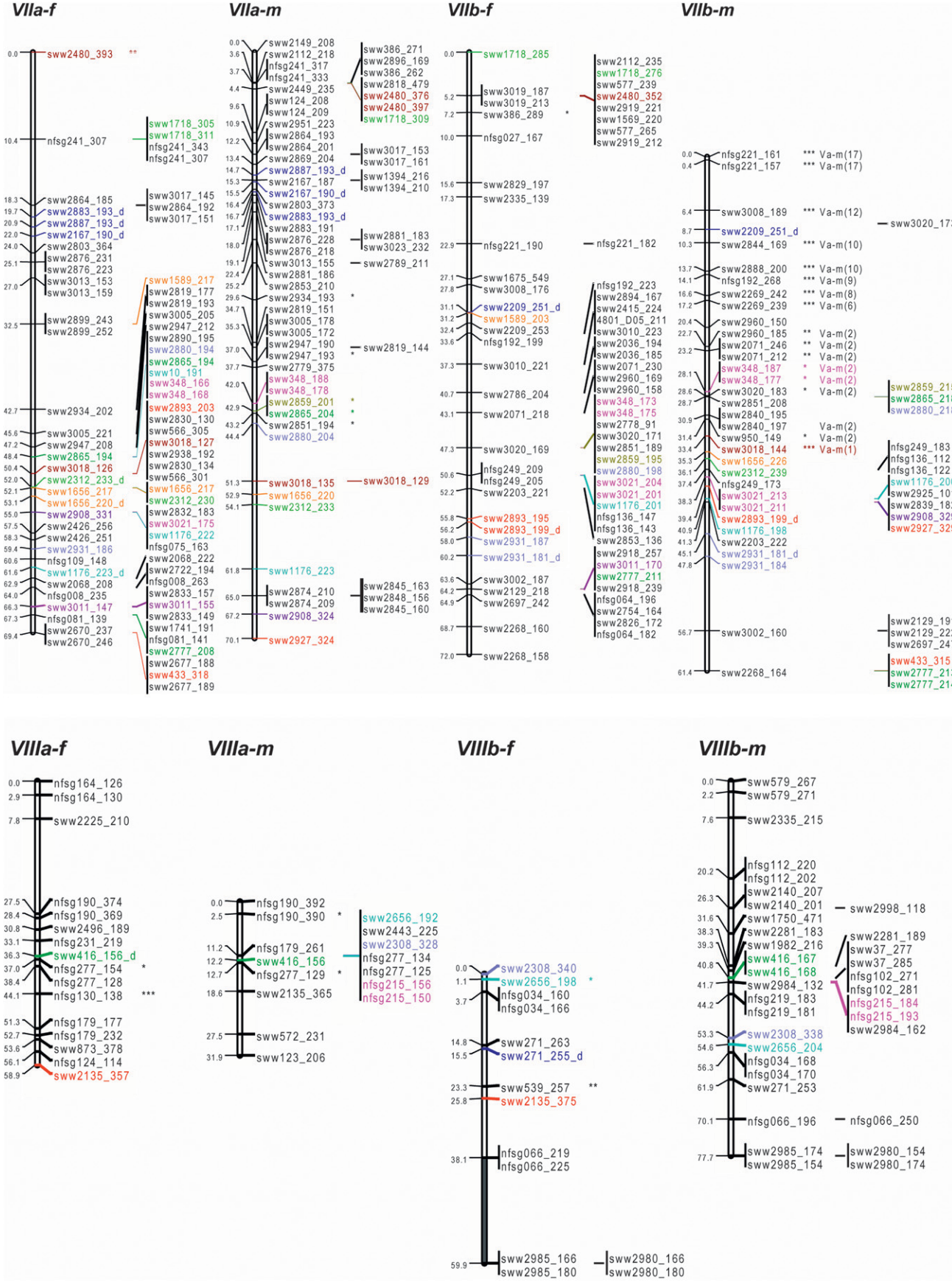


FIGURE 1.—(*Continued*)

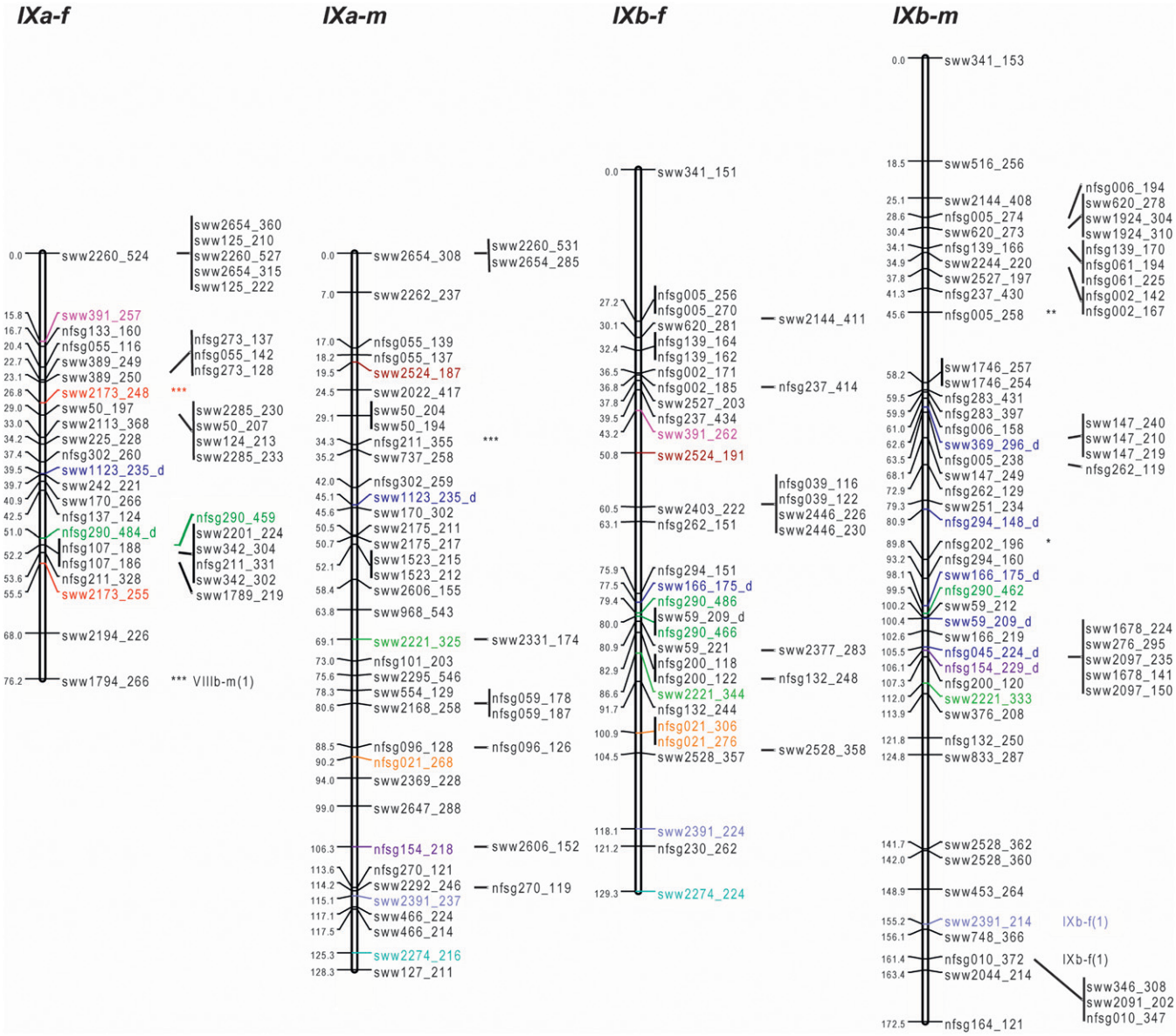


FIGURE 1.—(*Continued*)

Inheritance in switchgrass is disomic: The ratio of coupling to repulsion phase linkages detected at the minimum LOD score of 3.0 in each map was tested for homogeneity across LGs, and pooled data were tested for fit to the expected 1:1 and 1.55:1 ratios for disomic and tetrasomic inheritance, respectively. The LGs within each parent showed homogeneity with respect to the test for both ratios. The observed ratio of coupling to repulsion phase linkages conformed to the 1:1 ratio in both female and male maps but significantly deviated from the 1.55:1 ratio (Table S4).

Putative allelic pairs amplified with the same SSR or STS marker were considered individually for evidence of rare nonhomologous pairing. Although most of the LGs displayed indications of complete preferential pairing, all LGs had at least one putative allelic pair that was not completely complementary, *i.e.,* presence or absence of both alleles in the same individual (Table S5). Twenty-nine out of 36 LGs displayed at least one fully complementary allelic pair and 5 of the remaining LGs had at least one putative allelic pair with only one noncomplimentary genotype. In all LGs with two or more pairs of allelic markers, the number of non-complimentary genotypes varied with the average per LG ranging from 0.3 in *VIIIb-f* to 7.8 in *IVb-f*.

The extent and distribution of TRD loci on the maps: *Single-locus distortion:* Among the segregating SDAs in the female and male parents, 3 and 14% were classified as TRD, respectively (Table 2). At least one TRD–SDA mapped on 10 and 12 LGs on the female and male maps, respectively (Figure 1). In the male map, 65 out of 75 TRD–SDAs mapped in 14 clusters of two or more markers on seven LGs within four HGs (I, II, V, and VII). The number of markers per cluster ranged be-

TABLE 2

Female and male framework maps and the two parental maps after adding transmission distorted and shared single-dose amplicons between parents to the framework map

	Framework		With distorted and shared single-dose amplicons included	
	Female map	Male map	Female map	Male map
Number of mapped polymorphisms				
Total	563	542	616	658
Single dose (accessory)	299 (264)	352 (190)	300 (264)	353 (162)
Distorted	N/A	N/A	14	75
Single dose × single dose	N/A	N/A	38	40
Per linkage group, average (range)	17 (6–28)	20 (5–34)	20 (9–31)	26 (8–42)
Unmapped single dose	9	4	8	3
Total number of linkage groups	18	18	18	18
Total map length (cm)	1376	1645	1400	1748
Average linkage group length (range) (cm)	76 (45–129)	91 (27–176)	78 (47–129)	97 (32–173)

tween two and eight. In the female map, there was only a single cluster of three markers on LG *VIb-f*, and the remaining 11 did not cluster with another TRD–SDA.

Two-locus interactions: Overall, significant two-locus interactions between unlinked loci were found at only 1 of 14 TRD–SDAs in the female map (7%), whereas 39 of 75 (52%) TRD–SDAs were involved in interactions with unlinked marker(s) in the male map. No within-parent interactions were detected in the female map. In contrast, within the male map, two-locus interactions involved 38 of the 39 interacting TRD–SDAs and totaled 160 pairs. These involved 42 interactions between *Ia-m* and *Ib-m*, and 117 between *Va-m* and *VIIb-m*, while a single pair of interacting loci was found on LGs *IVa-m* and *Ib-m*.

Between-parent interactions were detected at one TRD–SDA on the female map with two on the male map. A total of 27 marker pairs were significant for two-locus interactions, including eight between *IIa-f* and *IIa-m*, 10 between *IVa-f* and *IVa-m*, and nine between *Vb-f* and *Vb-m*. All between-parent interactions detected were on homologous chromosomes in HGs II, IV, and V.

Comparative mapping: The map positions of EST–SSR or STS markers designed against assembled switch-

TABLE 3

Locus frequency and length comparison between female and male linkage groups

Female						Male					
LG	Total Length (cM)	Average interlocus	Mapped	Accessory	Total	LG	Total Length (cM)	Average intermarker	Mapped	Accessory	Total
Ia-f	88	3.1	28	32	60	*Ia-m*	76	3.3	15	12	27
Ib-f	97	4.4	24	17	41	*Ib-m*	60	5.1	13	1	14
IIa-f	84	4.9	18	11	29	*IIa-m*	100	3.8	29	8	37
IIb-f	65	6.3	19	15	34	*IIb-m*	27	5.7	6	0	6
IIIa-f	90	5.2	12	7	19	*IIIa-m*	133	8.1	23	8	31
IIIb-f	73	8.2	11	6	17	*IIIb-m*	121	5.7	30	6	36
IVa-f	59	2.8	11	4	15	*IVa-m*	96	6.0	15	8	23
IVb-f	69	7.3	12	10	22	*IVb-m*	81	5.3	19	12	31
Va-f	102	4.2	19	9	28	*Va-m*	125	3.6	23	9	32
Vb-f	84	5.6	17	14	31	*Vb-m*	126	4.5	23	9	32
VIa-f	76	3.6	13	5	18	*VIa-m*	90	4.5	14	5	19
VIb-f	45	5.6	9	7	16	*VIb-m*	81	5.4	19	9	28
VIIa-f	59	6.3	22	48	70	*VIIa-m*	73	3.3	23	26	49
VIIb-f	72	4.7	24	42	66	*VIIb-m*	41	6.9	12	20	32
VIIIa-f	57	5.3	13	0	13	*VIIIa-m*	32	5.0	5	7	12
VIIIb-f	57	11.4	6	5	11	*VIIIb-m*	77	5.5	17	18	35
IXa-f	71	3.3	17	19	36	*IXa-m*	129	4.2	32	10	42
IXb-f	129	5.9	24	13	37	*IXb-m*	176	4.2	34	22	56
Total	1376		299	264	563		1645		352	290	542
Average	76.5	5.5	16.6	14.7	31.3		91.4	5.0	19.6	10.6	30.1

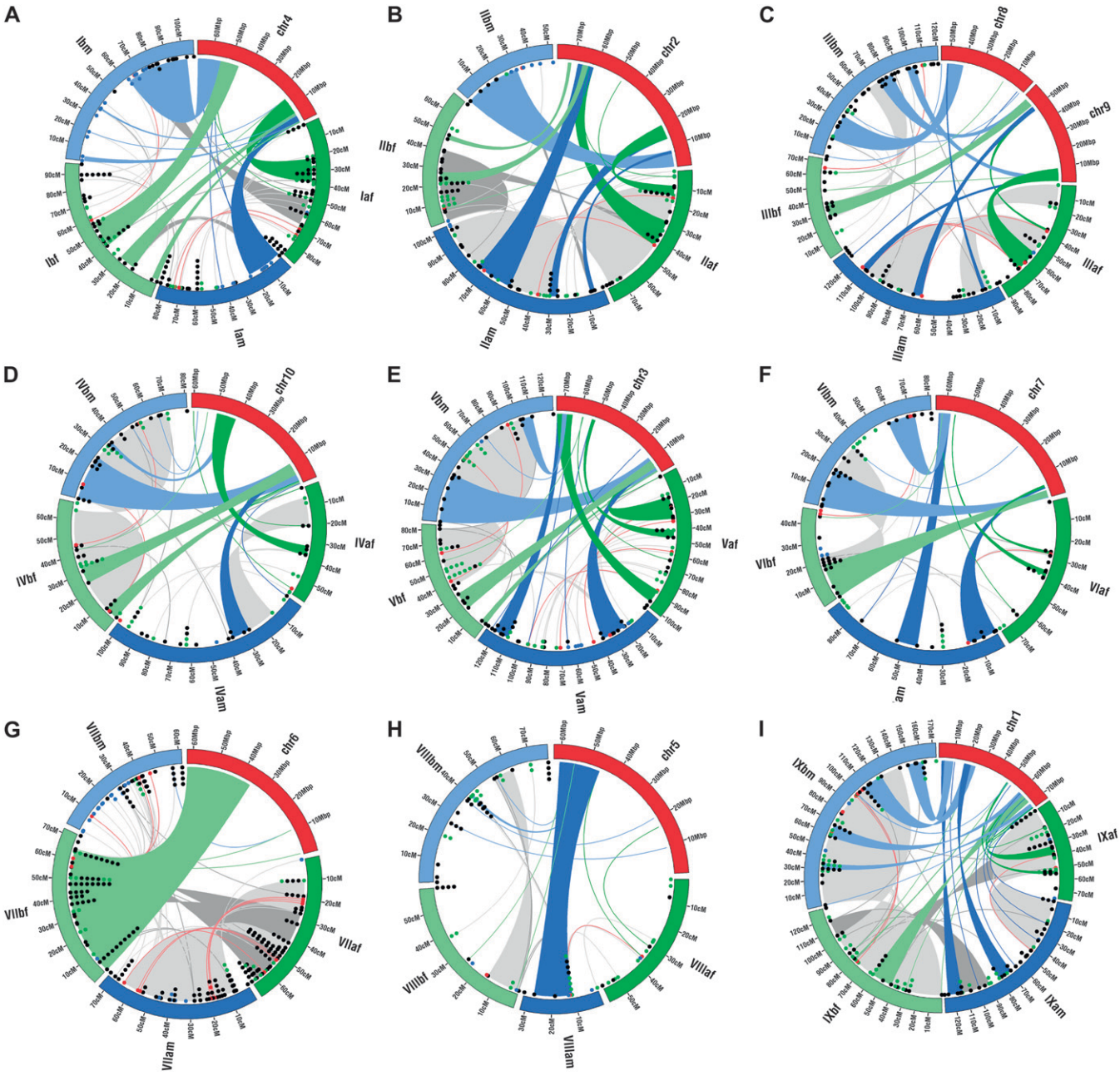


FIGURE 2.—Comparison of linkage groups to sorghum genome. Each homology group was drawn around a radius along with the syntenic chromosome(s) of sorghum. Sorghum chromosomes are indicated in red while switchgrass linkage groups are depicted in green or light green for the female linkage group's A and B subgenomes, respectively, or blue and light blue for the male A and B subgenomes, respectively. Individual loci are plotted around the radius with likely allelic pairs plotted as one unit and accessory loci stacked in their respective map locations. Black dots indicate nondistorted EST–SSR markers, green dots indicate nondistorted genomic SSR, light red dots indicate SDxSD markers, and blue dots indicate TRD–SDA markers. Links between maps were bundled together if the distance between individual links (either markers detected with the same SSR or STS marker or based on sequence identity) were less than 10 Mbp (sorghum) or 12.3cM (switchgrass) on both chromosomes/linkage groups. Light gray link color indicates correspondence between male and female linkage groups; dark gray indicates correspondence between different subgenomes. Links derived from SDxSD markers are indicated in light red. Linkages between sorghum chromosomes and individual switchgrass linkage groups are colored on the basis of the corresponding switchgrass linkage group. Homology group I (A), II (B), III (C), IV (D), V (E), VI (F), VII (G), VIII (H), IX (I).

grass EST sequences were compared to the genome coordinates of sorghum genes determined to be orthologs. This allowed a global assessment of synteny between switchgrass and sorghum and determination of gene collinearity to the extent that our map resolution would allow. Table 4 shows the number of orthologous pairs for each switchgrass homology group across all sorghum chromosomes. There exists a one-to-one relationship between the 10 sorghum chromosomes and 9 switchgrass homology groups with sorghum chromo-

TABLE 4

Likely orthologous gene pairs in Sorghum and switchgrass

Switchgrass homology group	Sorghum chromosome									
	1	2	3	4	5	6	7	8	9	10
I	3[a]	0	0	40 (27)[b]	0	2	0	1	0	1
II	2	34 (20)	0	1	0	4	0	0	5	0
III	2	1	2	2	0	0	1	12 (5)	15 (4)	0
IV	0	0	0	0	0	0	0	0	0	22 (17)
V	1	2	26 (31)	5	1	2	3	2	1	1
VI	0	0	0	0	0	2	19 (11)	0	0	0
VII	3	2	2	1	2	64 (45)	2	0	1	0
VIII	0	0	1	0	11 (7)	0	0	0	1	0
IX	41 (27)	6	4	4	1	0	1	0	1	6
Grand Total	52	45	35	53	15	74	26	15	24	30

[a] Number of loci (excluding alternate alleles of allelic pairs) present in both subgenomes A and B that had likely orthologs in sorghum.

[b] Number of loci participating in collinear stretches of four or more indicated in parentheses.

somes 8 and 9 both matching switchgrass homology group III (Figure 2 and Table 4). Two large stretches of complete map collinearity to sorghum chromosomes 6 and 1 were found. Collinearity with chromosome 6 extended for 13 gene/marker pairs and represented 58 cM of linkage group *VIIb-f*, which encompassed 22 Mbp of the long arm of sorghum chromosome 6, or 35% of the overall chromosome length. Collinearity with sorghum chromosome 1 extended for 11 gene/marker pairs over 124 cM of linkage group *IXa-m*, which encompassed 71 Mbp, or 91% of the chromosome.

Comparative maps have been published for foxtail millet interspecific crosses (Devos *et al.* 1998) and rice using RFLP loci. For the purposes of applying a consistent nomenclature between switchgrass linkage groups and foxtail millet we compared these published data and our mapped STS and EST–SSR markers through comparison of the matrices of shared map features using rice as a common reference genome. The data indicated a close one-to-one relationship between linkage groups I–IX of foxtail millet and individual switchgrass homology groups.

DISCUSSION

Linkage maps: Switchgrass linkage maps were constructed using a full-sib population derived from a cross between two heterozygous genotypes sampled from lowland tetraploid cultivars Kanlow and Alamo as female and male, respectively. Each of the parental maps contained a complete representation of 18 LGs in which the expected nine pairs of homeologues were identified. These linkage maps are the first to have been constructed for switchgrass representing all LGs. A thorough coverage of the genome is indicated by the estimate of 97% of the genome residing within 10 cM of the mapped markers in both framework maps. Accordingly, the number of unmapped SDAs was small for both female (1.5%) and male (0.7%) parents. Construction of two separate parental maps, each with two subgenomes, allowed detailed comparisons between maps and subgenomes within the study to identify false linkages and to confirm linkages identified at lower LOD scores. Good coverage of the genome enabled a robust analysis of the meiotic behavior, genome structure, and syntenic relationships to other grasses in this study.

Comparative analysis: The syntenic relationships evident through our usage of switchgrass expressed sequences for marker design and the high levels of gene sequence conservation within the Poaceae provide the first high level genome structural information available for switchgrass. Marker collinearity with sorghum, the most closely related reference genome available, was particularly strong within homology groups VII and IX. Comparative maps have been published for foxtail millet (Devos *et al.* 1998), pearl millet (*Pennisetum glaucum*) (Devos *et al.* 2000), and rice using RFLP loci. When evaluating these published data with our map comparisons to the rice genome it was clear that segments of collinearity to rice could be used as a basis for maintaining consistent nomenclature. A close relationship between each of the switchgrass homology groups and linkage groups I–IX of foxtail millet existed; thus we adopted the same group designations.

Preferential pairing at meiosis and disomic inheritance: The ratio of coupling to repulsion phase linkages, marker segregation ratios, and recombination fractions between putative allelic markers are consistent with complete or near complete preferential pairing. None of the LGs deviated significantly from the ratio of 1:1 coupling to repulsion phase linkages, but all deviated significantly from 1.55:1 (Table S4), indicating that linkages in both phases were equally efficiently detected as expected under complete preferential pairing in disomic inheritance (Wu *et al.* 1992; Qu and Hancock

2001). Concordant with the preferential pairing indicated by the ratio of the two linkage phases detected, only three polymorphic amplicons fit the tetrasomic double-dose segregation ratio of 5:1. The three amplicons were segregating in the male parent, which was more severely affected by TRD than the female parent. Thus, they may be SDAs (1:1) or double-dose amplicons (3:1) with distorted segregation rather than tetrasomic double-dose amplicons (5:1). Meiotic behavior inferred in this study agrees with the published cytological observations of meiotic chromosomes (Barnett and Carver 1967; Martinez-Reyna *et al.* 2001) and the linkage mapping analysis (Missaoui *et al.* 2005).

Although 29 of the 36 LGs displayed at least one completely complementary putative allelic pair suggesting complete preferential pairing of homologous chromosomes (Qu and Hancock 2001), a substantial number of putatively allelic pairs were noncomplementary to various degrees. These inconsistent genotypes could be caused by low frequency pairing of homeologous chromosomes. However, for the LGs with the most frequent occurrences of the inconsistent genotypes, their homeologues did not display correspondingly high frequencies in most cases, suggesting that homeologous pairing is not the cause. Furthermore, there are a variety of other possible causes for the genotypes deviating from disomic inheritance. The observed nonparental genotypes may actually be recombination between closely linked duplicated loci that appear to be allelic markers. Mutations and genotyping artifacts including allele dropouts, PCR-mediated recombination (Cronn *et al.* 2002), and experimental error could result in nonparental genotypes. Chromosomal rearrangements may also underlie the genotypes deviating from complete preferential pairing (Cloutier *et al.* 1997).

Substantial subgenome differentiation is indicated by the small proportion of markers detecting alternate alleles across subgenomes, which was significantly lower than the simulated proportion that accounted for estimated allele frequencies in the parental cultivars, probability of SDA and SDxSD amplicons being included in the linkage map, and the proportion of EST–SSR motif types. The observed cross subgenomic EST–SSR markers would represent 20% transferability between the subgenomes on the basis of the simulated proportion as 100%. Between species within a genus, EST–SSR marker transferability reported varies from 26 to 100% (Ma *et al.* 2009; Moccia *et al.* 2009; Sharma *et al.* 2009; Tang *et al.* 2009). Transferability reported between species from different genera varies between 11 and 86% (Saha *et al.* 2004; Varshney *et al.* 2005; Heesacker *et al.* 2008; Chapman *et al.* 2009; Choudhary *et al.* 2009; Raji *et al.* 2009; Sim *et al.* 2009). The subgenome transferability of EST–SSR markers in switchgrass estimated in this study is comparable to transferability between distantly related species within a genus or species from closely related genera in other plants. If the alleles within a subgenome are more closely related to each other than between subgenomes (Huang *et al.* 2003), the low cross subgenome transferability would be largely due to divergence between homeologues attributable to mutations, sequence losses, and chromosomal rearrangements leading to diploidization and/or divergence prior to polyploidization. Complete preferential pairing at meiosis, or a pattern of inheritance that is at least effectively disomic, would be expected in switchgrass to maintain the highly distinct subgenomes.

Transmission ratio distortion: TRD and interactions between unlinked markers resulting in transmission distortions can introduce inaccuracies in linkage maps due to spurious linkages, biased estimates of recombination fractions, and incorrect marker order (Lorieux *et al.* 1995; Cloutier *et al.* 1997). Inaccurate linkage maps negatively affect the identification of quantitative trait loci (Vogl and Xu 2000). In this study, although the proportion of TRD–SDAs for both parents was within the lower range found in intraspecific linkage analyses (Jenczewski *et al.* 1997; Anhalt *et al.* 2008), the TRD–SDAs appeared to be a significant source of map inaccuracies in the male map. The male map was more severely affected by TRD, and the TRD–SDAs in the male map were clearly associated with spurious linkages that prompted their exclusion from the framework map. Heterozygosity for translocations may underlie such spurious linkages if they are linked to transmission distorters (Santos *et al.* 2006). In addition, if more than one transmission distorter locus underlie TRD or TRD is due to epistatic interactions, estimates of recombination fractions are biased (Lorieux *et al.* 1995; Lu *et al.* 2002). Significant two-marker interactions in the male often involved nondistorted markers, suggesting that biased estimation of recombination fractions may affect the framework map. The longer male map may be interpreted as greater rate of recombination in the male parent, but could also be an artifact of the biased estimates of recombination fractions caused by interacting TRD markers.

TRD is commonly observed in linkage mapping studies and is caused by a variety of processes underlying meiotic drive or pre- or postzygotic viability differences (Lyttle 1991; Zamir and Tadmor 1986). Because of the structure of the full-sib mapping population, interactions detected between parents indicate postzygotic mechanisms causing TRD. All the detected interactions were between homologs in similar regions of the LGs, suggesting that these interacting markers may be linked to alleles at a single locus. Since the parents of the mapping population are from two cultivars of geographically distant origins, in Oklahoma (Kanlow) and Texas (Alamo), the interactions are less likely due to deleterious recessive alleles (Diwan *et al.* 2000) but may represent a developing reproductive barrier between diverging populations (Muller 1939; Dobzhansky 1951). Alternatively, they may be linked to

transmission distorter loci common to populations within the species.

Identification of map regions with consistent TRD within the species would benefit the design of marker-assisted breeding strategies (Lu *et al.* 2002; Anhalt *et al.* 2008). Map regions containing self-incompatibility loci are often TRD and exhibit consistent interactions with other LGs across mapping populations in *Lolium perenne* (Thorogood *et al.* 2002, 2005; Anhalt *et al.* 2008).

Many examples of TRD in grasses are cross specific, but we investigated possible prezygotic effects on the basis of published mapping studies of *S* and *Z* self-incompatibility loci in rye (*Secale cereale*). Hackauf and Wehling (2005) performed comparative mapping between rye and rice to identify genes colocalized to the *Z* locus. They found that rice BACs carried orthologs of the three rye STS markers cosegregating with *Z* in their mapping populations. When aligned with switchgrass the closest distorted marker was sww3008_189 present on linkage group *VIIb-m*, suggesting that the TRD at the marker may be related to the *Z* locus. Analysis of wheat–rye–rice–switchgrass synteny placed the *S* locus tentatively on HGIII; however, there were no TRD markers in the region expected.

The complete linkage maps have indicated that the subgenomes of the tetraploid, lowland switchgrass ecotypes should be considered independent due to a high degree of preferential pairing. Furthermore, conserved gene order between switchgrass and other grass taxa indicated by our comparison with sorghum is likely to facilitate map-based cloning and translational approaches using information from other grasses and cereals. However, the presence of segregation distortion, multilocus interactions, and an unknown degree of selfing can complicate interpretation of the genetic maps. The marker resources presented should provide impetus for development of further populations and QTL studies.

We thank Scott Sattler and Kenneth Vogel for critical reading of the manuscript. This research was supported by the Office of Science (B.E.R.), U. S. Department of Energy, Interagency Agreement No. DE-AI02-07ER64453 and by the U. S. Department of Agriculture–Agricultural Research Service (USDA–ARS), CRIS 9235-21000-013-00D, National Program 213/307, Bioenergy. The USDA–ARS is an equal opportunity/affirmative action employer, and all agency services are available without discrimination. Mention of commercial products and organizations in this manuscript is solely to provide specific information. It does not constitute endorsement by USDA–ARS over other products and organizations not mentioned.

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Communicating editor: J. A. Birchler