

# Disomic Inheritance, Suppressed Recombination, and Allelic Interactions Govern Apospory in Buffelgrass as Revealed by Genome Mapping

R. W. Jessup,\* B. L. Burson, G. B. Burrow, Y.-W. Wang, C. Chang, Z. Li, A. H. Paterson, and M. A. Hussey

## ABSTRACT

Molecular tools have not identified the gene(s) governing apomixis nor have they been used to successfully transfer the trait to important, sexually reproducing food crops. Several molecular studies addressing apomixis in grasses have used interspecific and intergeneric hybrids. The failure to recover specific  $F_1$  genotypes from these wide crosses can be caused by unfavorable interactions between the gametes, zygote, embryo, endosperm, and/or maternal tissue. These interactions can eliminate recombinant genotypes with valuable information towards linkage analyses of the trait. Buffelgrass [*Pennisetum ciliare* (L.) Link syn. *Cenchrus ciliaris* L.], a polymorphic species with interfertile apomictic and sexual genotypes, offers an opportunity to genetically map apomixis by means of intraspecific hybrids with euploid genomes. This study reports a linkage map of the apospory region in buffelgrass. Apospory, classified by progeny testing and cytologically observing megagametophytes, mapped to a single locus in the apomictic parent's genome. Two buffelgrass cDNAs (pPAP3A07 and pPAP8C08) and three previously reported apospory markers (UGT197, QH8, and OPC4) were tightly linked (1.4 centimorgans, cM) to the trait. As a tetraploid species ( $2n = 4x = 36$ ), four copies of each chromosome are expected in buffelgrass. A single homolog and two homeologs were identified for the chromosome carrying apospory, indicating the formation of two bivalents during meiosis and the disomic inheritance of apospory in buffelgrass. Allelic bridges between the parents revealed suppressed recombination in the apospory linkage group. Segregation distortion between a marker on the sexual parent's homolog to the apospory linkage group and a marker on a separate maternal linkage group suggested specific allelic combinations in female gametes affect offspring survival in buffelgrass.

**A**POMIXIS, asexual plant reproduction through seeds, results from the parthenogenetic development of an unreduced egg cell into a viable embryo (Bashaw and Hanna, 1990). Because apomixis permits the clonal propagation of hybrid genotypes, its transfer to cultivated crops is of major interest to plant breeding programs. Two types of apomixis, apospory and diplospory, occur in grasses. Unreduced embryo sacs arise from somatic nucellar cells in apospory and unreduced archeosporial cells in diplospory (Asker and Jerling, 1992).

The complete body of apomixis literature is abound with conflicting reports. This paper focuses on the molecular analyses of apomixis in grasses, while deferring the general subject of apomixis to a recent and extensive review (Savidan, 2000).

Wide hybridizations, which have been used extensively to obtain high polymorphism rates in linkage studies, have often resulted in segregation distortion of mo-

lecular markers within mapping populations (Wendel and Parks, 1984; Torres et al., 1985; McCouch et al., 1988; Paterson et al., 1988, 1991; Saito et al., 1991; Lyttle, 1991; Schon et al., 1991; Zivy et al., 1992; Causse et al., 1994; Chittenden et al., 1994). This loss of specific genotypes from the progeny may be due to interactions between genes of the two species within gametes, zygote, embryo, endosperm, or maternal tissue (Hadley and Openshaw, 1980). Differential viability of offspring because of allelic interactions has been reported in maize (*Zea mays* L.)-*Tripsacum* hybrids (Maguire, 1963), tomato, *Lycopersicon esculentum* Mill. (Rick, 1966), wheat, *Triticum aestivum* L. (Manabe et al., 1999), and rice, *Oryza sativa* L. (Sano, 1990; Cheng et al., 1996; Xu et al., 1997; Liu et al., 2001). Because detectable recombination in a mapping population is directly related to offspring survival, reports of segregation distortion in pearl millet, *Pennisetum glaucum* (L.) R. Br. (Liu et al., 1994) and maize (Helentjaris et al., 1986; Gardiner et al., 1993) may negatively affect genetic mapping studies of apomixis in these species.

Molecular markers have been associated with apomictic phenotypes in several grass species. One restriction fragment length polymorphism (RFLP) (UGT197) and one random amplified polymorphic DNA (RAPD) (OPC4) were syntenic with apospory in interspecific *Pennisetum* hybrids (Ozias-Akins et al., 1993). An alien addition line ( $2n + 1 = 29$ ) derived from a cross between tetraploid pearl millet ( $2n = 4x = 28$ ) and *P. squamulatum* Fresen. ( $2n = 6x = 54$ ) possessed one *P. squamulatum* chromosome, suggesting that a single chromosome could confer apospory. However, the lack of a homolog for this chromosome to pair with during meiosis precluded recombination and made this system unsuitable for genetic mapping.

Analyses of isozyme, protein, and RAPD markers did not identify associations with apospory in buffelgrass (Gustine et al., 1996). This can likely be attributed to the small population size and types of markers used. In contrast, a bulked-segregant analysis using RAPDs in two different half-sib buffelgrass populations revealed two markers (M02-680 and J16-800) tightly linked to apospory (Gustine et al., 1997). The two apomictic male parents used to develop the populations gave slightly different results. M02-680 and J16-800 flanked apospory in both populations, but at greater distances in the second population. Interestingly, UGT197 cosegregated with J16-800 in the first population and with apospory in the second population. Assuming the reproductive

R.W. Jessup, Y.-W. Wang, C. Chang, Z. Li, and M.A. Hussey, Dep. of Soil & Crop Sciences, Texas A&M Univ., College Station, TX 77843; B.L. Burson, USDA-ARS, College Station, TX 77843; G.B. Burrow and A.H. Paterson, Center for Applied Genetic Technologies, Dep. Crop and Soil Sciences, Botany, and Genetics, Univ. of Georgia, Athens, GA 30602. Received 7 July 2001. \*Corresponding author (tonoend@tamu.edu).

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**Abbreviations:** AFLP, amplified fragment length polymorphism; BAC, bacterial artificial chromosome; cM, centimorgan; PCR, polymerase chain reaction; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; SCAR, sequence characterized amplified region; SDRF, single dose restriction fragment.

behavior of each hybrid was classified correctly, such genotype-dependent differences in recombination are important in map-based analyses of apomixis.

Ozias-Akins et al. (1998) identified 12 sequence characterized amplified regions (SCARs) cosegregating with apospory in an interspecific population of *P. glaucum* × *P. squamulatum* hybrids. Roche et al. (1999) analyzed the 12 markers identified by Ozias-Akins et al. (1998) using the two buffelgrass populations developed by Gustine et al. (1997). Six of the 12 SCARs were present in the apomictic buffelgrass parents but absent in the sexual parent. Three of the six remaining SCARs showed polymorphisms upon RFLP analysis, indicating that these three SCARs were present in both parental phenotypes. This suggests that the sexual and apomictic parents contain alternative alleles for these markers. Unfortunately, amplification patterns between parents were not given for the three remaining SCARs. The presence of these markers in both parental types would have either strengthened the evidence for allelic counterparts between apomixis and sexuality or demonstrated a lack of linkage between the markers and the apospory gene(s). Their presence in both parental types, along with a lack of RFLP polymorphism, would suggest penetrance effects might occur between apomictic and sexual genotypes. The absence of these markers from both parental types would have indicated differences between the genomic regions controlling apospory in buffelgrass and *P. squamulatum*. Five of the six polymorphic SCARs, as well as the three having RFLP polymorphisms, cosegregated with apospory. However, one apomictic buffelgrass plant lacked SCAR X18R. This SCAR revealed different amplification products between buffelgrass and *P. squamulatum*, further suggesting genomic divergence between the two species. Roche et al. (1999) were unable to confirm the recombination between UGT197 and the apospory locus that was reported by Gustine et al. (1997). However, only 38 of the 53 hybrids from the relevant population were included. The recombinant plant could have been omitted from this already critically small population. The lack of recombination between UGT197 and the apospory locus was confirmed in 46 of the 62 hybrids from the second buffelgrass population reported by Gustine et al. (1997). The RAPDs showing recombination near apospory in this population were not analyzed, and the recombinant plant may have also been omitted from this partial population. Thus, the question regarding recombination near the apospory locus in buffelgrass remains unanswered.

Evidence supporting Gustine's et al. (1997) findings was reported in *Brachiaria*. A bulked-segregant analysis of RFLPs and RAPDs in *Brachiaria ruziziensis* R. Germ. R. C. Evrard ( $2n = 4x = 36$ ) × *Brachiaria brizantha* (Hochst. Ex A. Rich.) Stapf ( $2n = 4x = 36$ ) hybrids revealed recombination around the apospory locus (Pessino et al., 1997). OPC4 and several RFLPs were loosely linked to the trait, and comparative mapping suggested homology to maize chromosome 5. Two amplified fragment length polymorphisms (AFLPs), PAM525 and PAM4913, were subsequently found to be tightly linked to apospory in *Brachiaria* (Pessino et al., 1998). Both

the *Brachiaria* and buffelgrass reports of recombination around apospory involved euploid ( $2n = 4x = 36$ ) hybrids.

In the case of diplospory, bulked-segregant analysis of maize ( $2n = 2x = 20$ ) × *Tripsacum dactyloides* (L.) L. ( $2n = 4x = 72$ ) hybrids revealed that three RFLPs (UMC28, CSU68, and UMC62) were loosely linked to the trait (Leblanc et al., 1995). UMC71 and CDO202 also were loosely linked to the diplospory locus (Grimanelli et al., 1998a, 1999). These studies associated the long arm of maize chromosome 6 with diplospory. Kindiger et al. (1996) supported this finding by cytologically demonstrating that apomictic maize × *Tripsacum* backcross hybrids carried a translocation between chromosomes 6 of maize and 16 of *Tripsacum*.

Segregation analyses of molecular markers have indicated tetrasomic inheritance in apomictic *Paspalum simplex* Morong (Pupilli et al., 1997), maize-*Tripsacum* hybrids (Grimanelli et al., 1998a), and *Pennisetum* hybrids (Ozias-Akins et al., 1998). However, chromosome associations in the genomic region(s) harboring apomixis and linked markers have not been reported. Both in situ hybridization and genome mapping studies could provide definitive evidence regarding this subject.

Non-Mendelian transmission of apomixis-linked molecular markers has been reported in maize-*Tripsacum* hybrids (Grimanelli et al., 1998b) and *Pennisetum* hybrids (Roche et al., 2001). These findings support models that explain the close association between apomixis and polyploidy in which apomixis alleles are eliminated when transmitted through haploid gametes. However, the gene(s) involved in this system were not identified. Both studies involved wide hybridizations, which have been shown to result in chromosome rearrangements and selection against paternally inherited alleles (Song et al., 1995). Genomic incompatibilities between paternal nuclear and maternal cytoplasmic elements could cause the observed distortion autonomously. Segregation distortion through female gametophytes has been reported in interspecific *Triticum* hybrids, a strictly sexual species (Manabe et al., 1999).

Considering that recombination and flanking markers were found near the apospory locus in both buffelgrass and *Brachiaria*, it is possible that recombinants near the apomixis locus may only be recovered in intraspecific hybrids. Once recombination can be detected in the region of interest, larger population sizes can increase the resolution of linkage analyses. This study characterizes the genomic region controlling apospory in buffelgrass through genome mapping.

## MATERIALS AND METHODS

### Plant Material

Our mapping population consisted of 86 full-sib buffelgrass hybrids, derived from crossing a heterozygous, highly sexual plant (90C48507) with a heterozygous, highly apomictic plant (PI 409164) (Wang, 1996). Meiosis was normal in buffelgrass pollen mother cells, allowing apomictic plants to serve as paternal sources in hybrid crosses. The pedigree of the hybrids is shown in Fig. 1.

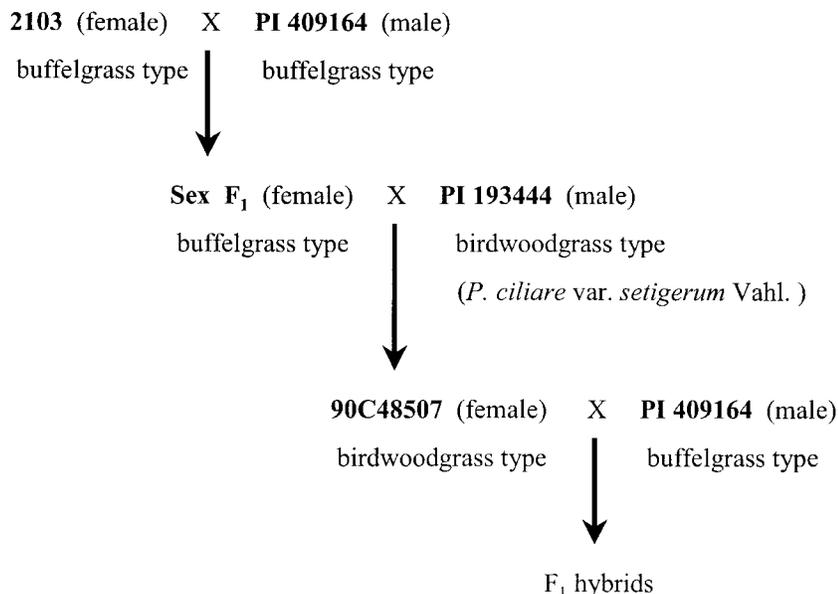


Fig. 1. Pedigree of the buffelgrass mapping population. The parental lines (90C48507 and PI 409164) differed in many traits, including inflorescence type (birdwoodgrass vs. buffelgrass), rhizomes (absence vs. presence), and method of reproduction (sexual vs. apomictic).

### Analysis of Molecular Markers

Genomic DNA extraction was adapted from the protocol of Causse et al. (1994). Ten micrograms of buffelgrass genomic DNA was digested with *EcoRI*, *HindIII*, or *XbaI*, according to the manufacturer's instructions. Southern blotting, radioactive labeling, and autoradiography were as described in Chittenden et al. (1994).

A cDNA library constructed from pistils of an apomictic buffelgrass plant selected from the full-sib population was developed with the Stratagene "ZAP-cDNA Synthesis Kit." Bacterial clones from the library were obtained by en masse phagemid excision, followed by two cycles of selection for recombinant clones on ampicillin plates containing X-gal (5-bromo-4-chloro-3-indoxyl-beta-D-galactopyranoside) and IPTG (isopropyl- $\beta$ -D-thiogalactoside) (Sambrook et al., 1989). Inserts were amplified by polymerase chain reaction (PCR) from bacterial lysate (McCabe, 1990), and an aliquot of the products was electrophoresed in 1% (w/v) agarose. Clones that gave multiple products were discarded. Sephadex G50 (Sigma) spun mini-columns were used to separate PCR products from excess reaction components (Sambrook et al., 1989). Dot blots with 20 ng of DNA from each probe were hybridized with leaf cDNA to identify and eliminate repetitive elements. A total of 443 suitable probes were designated "pPAP," for Plasmid-*Pennisetum*-Apomictic-Pistil.

In addition, heterologous DNA probes from several sources were surveyed: 21 barley cDNAs (BCD), 32 bermudagrass gDNAs (pCD), 37 johnsongrass cDNAs (pSHR), 250 maize cDNAs (CSU), 28 maize gDNAs (BNL and UMC), 52 pearl millet gDNAs (M, UGT, QH, and OPC), 105 rice cDNAs (C and RZ), 88 rice gDNAs (G, L, RG, V, and Y), 29 sorghum cDNAs (HHU), 148 sorghum gDNAs (pSB and SHO), 29 sugarcane cDNAs (CDSB, CDSC, and CDSR), and 46 oat cDNAs (CDO). Probes were generously provided by: A. Paterson (pCD, pSHR, pSB, and SHO), S. Tanksley, M. Sorrells, and S. McCouch (BCD, RZ, RG, and CDO), P. Moore (CDSB, CDSC, and CDSR), T. Sasaki and Y. Nagamura (G, L, V, and Y), P. Westhoff (HHU), M. Gale (M), and P. Ozias-Akins (UGT, QH, and OPC).

Probes revealing polymorphism between the parents on survey blots were hybridized to the mapping filters. Because both parental buffelgrass lines were highly heterozygous tetra-

ploids, each polymorphic band was treated as a locus with dominant gene action. Individual bands present in one parent and absent in the other parent were scored for presence or absence in the progeny. A  $\chi^2$  test was used to identify single dose restriction fragments (SDRFs) by their 1:1 segregation ratio at a significance level of 1% (Wu et al., 1992). A nonsignificant test indicated that a given band was an SDRF and could be considered in the linkage analysis.

Since an SDRF only reveals recombination in the gametes of one parent, each parent's respective SDRFs were analyzed independently by means of Mapmaker 3.0 (Lander et al., 1987). SDRFs were treated as backcross data. A LOD score of 4.0 and recombination fraction of 0.30 was set as the linkage threshold. Map units, in centimorgans, were derived by the Kosambi (1944) function. Maximum likelihood orders of markers were verified by the "ripple" function, with those at LOD  $\geq$  2.0 being placed on the framework map and all others added at the most likely interval between framework markers.

Linkage groups were checked for markers linked in repulsion to distinguish between random and preferential chromosome pairing. To detect repulsion-phase linkages, two-point linkage analyses were performed with SDRF scores in both inverted and noninverted allele states. Pairs of SDRFs for which the presence of an inverted score (absence when noninverted) was linked to presence of a noninverted score were counted as being linked in repulsion.

Allelic bridges were used to identify and orient analogous linkage groups in the paternal and maternal maps (Ritter et al., 1991). Such probes detect an SDRF unique to each parent and a fragment in common to both parents.

Segregation distortion was analyzed between each mapped SDRF and all other mapped SDRFs, excluding those on the same linkage group and its homolog. Deviations from the expected 1:1:1:1 ratio were considered significant at a LOD  $\geq$  4.0. Analyses of each class would identify which allelic combination occurred in excess.

### Phenotypic Classification for Method of Reproduction

The reproductive behavior of the F<sub>1</sub> hybrids was determined by two methods. The first method involved microscopically ob-

serving megagametophytes in cleared, mature pistils (Young et al., 1979). Twenty to 50 mature gametophytes from each hybrid were classified independently by Drs. E.C. Bashaw and B.L. Burson. Second, a field progeny test of the hybrids was conducted to confirm method of reproduction. A clonal ramet and 20 progeny of each hybrid were transplanted into a space-planted nursery on 1-m centers. Once the nursery was flowering, three observers independently classified the hybrids and progeny rows. Hybrids with morphologically uniform progeny were scored as apomictic. Hybrids with progeny exhibiting partial uniformity and a degree of variability were classified as facultative apomicts, and hybrids with completely variable progeny were scored as sexual. A consensus of cytological and progeny testing data was used for the final classification of each hybrid for method of reproduction.

### Linkage Analyses of Apospory

Contingency tests were performed to identify marker associations to the facultative mode of reproduction. Hybrids with any apomictic reproductive capacity (i.e., those scored as apomictic or facultative) were placed into a single class. All sexual hybrids were placed into a second class. These two reproductive classes were tested for a 1:1 segregation ratio, at a significance level of 1%, by a  $\chi^2$  test with SAS version 6.0 (SAS Institute, 1989). If a 1:1 ratio was confirmed, method of reproduction would be treated as an SDRF and analyzed for linkage relationships to markers using Mapmaker 3.0 (Lander et al., 1987).

## RESULTS

### Genetic Mapping of Apospory

The mapping population consisted of 38 sexual, 34 apomictic, and 14 facultative apomictic hybrids. Contingency tests failed to identify any markers associated with the facultative class. However, method of reproduction (obligate and facultative apomicts combined) did not deviate from the 1:1 segregation ratio expected for SDRFs ( $\chi^2 = 0.58$ ,  $\chi^2_{1,0.01} = 6.63$ ) and was included in the linkage analyses.

Linkage maps of each parent's genome were constructed (data not shown). Associations were found for apospory, which mapped to a single linkage group (7b) in the apomictic parent's map and was absent in the sexual parent's map (Fig. 2). Two buffelgrass probes (pPAP3A07 and pPAP8C08), as well as three previously identified markers (UGT197, QH8, and OPC4), were tightly linked to the trait. These markers maintained identical associations upon autoradiography of two additional Southern blots. Interestingly, two cDNAs (Pca2 and Pca3) previously associated with apospory in buffelgrass by differential display (Vielle-Calzada et al., 1996) remained unlinked in both parents' maps. However, a single cDNA (Pcs4) associated with sexuality in the earlier study mapped to 7b in the sexual parent's map. One plant scored as apomictic possessed none of the apomixis-linked markers. Field notes indicated this plant may have been a mixture, and it was subsequently removed from the analyses. Another plant scored as sexual contained all of the apomixis-linked markers. Additional cytological testing was conducted on this hybrid. Of 118 mature megagametophytes observed, none had aposporous development. Interestingly, an embryo sac failed to develop in 14 of the pistils. This

plant may have had penetrance–expressivity effects. The error detection function in MAPMAKER adjusted for this plant upon linkage analysis with apospory. The apospory linkage group was in repulsion to a single homolog (7a). Two duplicated loci (pPAP3A07 and pPAP8H05) identified the two homeologs (8a and 8b) that were expected in the tetraploid genome of buffelgrass, and they were also in repulsion with one another.

### Suppressed Recombination

An allelic bridge defined by heterologous markers HHU27 and M466 identified the homolog to the apomixis linkage group (7b) in the sexual parent's map (Fig. 2). As in the apomictic parent's map, a single homolog (7a) and two homeologs (8a and 8b) were found in the sexual parent's map. However, significantly greater recombination occurred between HHU27 and M466 in 7b of the sexual parent's map (15.8 cM) than in 7b of the apomictic parent's map (2.1 cM).

### Allelic Interactions

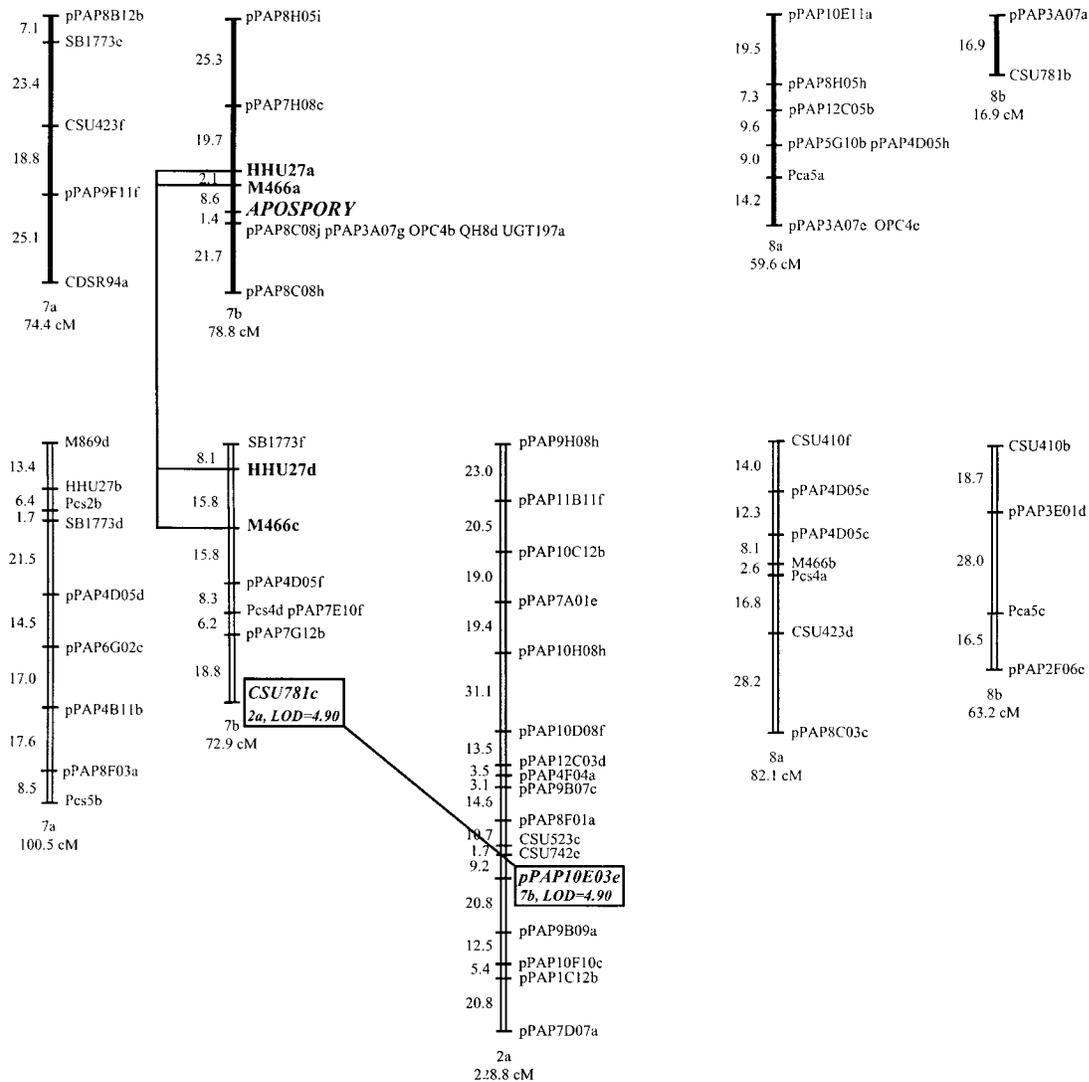
While absent from the apomictic parent's map, segregation distortion was detected in the sexual parent's map (Fig. 2). pPAP10E03e on linkage group 2a and CSU781c on linkage group 7b deviated significantly from the expected 1:1:1:1 (A/B: A/–: –/B: –/–) segregation ratio for markers on independent chromosomes (Table 1). The allelic heterozygote class pPAP10E03e/– was transmitted in excess to the progeny. Both homozygote classes were transmitted less than expected, and the –/CSU781c class was transmitted near predicted levels.

## DISCUSSION

This study provides supporting evidence for three established tenets concerning apomixis in grasses: (i) a single genomic region exerts major influence upon the trait; (ii) suppressed recombination occurs in this region; and (iii) this region is transmitted to progeny in a non-Mendelian fashion. The use of an established genome map also revealed new information regarding the genetics of this complex trait.

To our knowledge, this is the first molecular report of disomic inheritance for apospory. This finding has direct implications on buffelgrass breeding programs, because smaller effective population sizes can be used to produce desirable genotypes. It also suggests that the gene(s) controlling apomixis have diploid origin(s), with apospory mapping to a distinct subgenome in buffelgrass. Even though expression is quite low, reports of apomixis in diploid *Sorghum bicolor* (L.) Moench (Rao and Narayana, 1968; Elkonin et al., 1995) and *Brachiaria decumbens* (Stapf) R. D. Webster (Naumova et al., 1999) also suggest the presence of apomixis gene(s) in diploid genomes. These findings indicate potential to clone the apomixis gene(s) from bacterial artificial chromosome (BAC) libraries of the relatively small genomes in these species. By providing markers that are specific to the apomixis linkage group and flank the apospory locus, the buffelgrass genome map would streamline contig formation in such studies.

Detection of a defined linkage group with markers



**Fig. 2.** Paternal (solid line) and maternal (hollow line) RFLP maps of buffelgrass. Markers and map distances (Kosambi) are shown to the right and left of horizontal lines, respectively. Probes are denoted as follows: buffelgrass cDNAs (pPAP, Pca, and Pcs), sorghum cDNAs (HHU), sorghum gDNAs (pSB and SHO), barley cDNAs (BCD), bermudagrass gDNAs (pCD), johnsongrass cDNAs (pSHR), maize cDNAs (CSU), maize gDNAs (BNL and UMC), pearl millet gDNAs (M, UGT, QH, and OPC), rice cDNAs (C and RZ), rice gDNAs (G, L, RG, V, and Y), sugarcane cDNAs (CDSB, CDSC, and CDSR), and oat cDNAs (CDO). Markers followed by a, b, c, etc. correspond to probes detecting polymorphisms at multiple loci on the maps. Linkage group names with a or b indicate homologous (disomic) chromosome pairs. Allelic bridge loci between the maps are in bold with brackets, and markers with allelic interactions are italicized in boxes.

tightly linked to apospory in our population supports previous reports of recombination near the trait in buffelgrass (Gustine et al., 1997) and *Brachiaria* (Pessino et al., 1997). Because OPC4 is syntenic with apospory in *Pennisetum squamulatum* (Ozias-Akins et al., 1998), closely linked to apospory (1.4 cM) in buffelgrass, and loosely linked to apospory (19 cM) in *Brachiaria* (Pessino et al., 1998), gene conservation in this region is a possibility. However, comparative mapping of published linkage maps reveals a more complex evolution of

apomixis in grasses. Seven markers aligned the apospory linkage group in *Brachiaria* to chromosome 5 of maize (Pessino et al., 1998), and one marker (CSU134) aligned it to linkage group C of sorghum (Lin et al., 1995). HHU27 from our study mapped to sorghum linkage group D (Wyrich et al., 1998), which has homology to chromosomes 2 and 10 of maize (Paterson et al., 1995). Five markers aligned the diplospory linkage group in *Tripsacum* to the long arm of chromosome 6 in maize (Grimanelli et al., 1998a), which has homology to sorghum linkage groups G and I (Wyrich et al., 1998). With different maize and sorghum chromosomes implicated in each of these maps, it appears apomixis may have evolved independently at least three times in grasses. However, the limited number of available comparative markers makes this conclusion tentative. More detailed maps are needed for comparative analyses to reveal the

**Table 1.** Transmission distortion of pPAP10E03e/CSU781c allelic combinations in the sexual parent's map.†

	A/B	A/-	-/B	-/-
Expected (n = 86):	21.5	21.5	21.5	21.5
Observed:	8	40	25	13

†  $\chi^2 = 28.33, P \leq 0.0001$

evolutionary relationships of apospory and diplospory within grasses.

As was determined in this study, there is additional molecular evidence that segregation distortion can be limited to female gametophytes (Manabe et al., 1999). Relative to apomixis, a high transmission of a single *Tripsacum* chromosome through eggs of  $2n + 1 = 21$  maize-*Tripsacum* aneuploids also has been reported (Maguire, 1963). Our findings reveal that allelic interactions within female gametophytes affect offspring survival in sexual by apomictic buffelgrass crosses. Specifically, the pPAP10E03e/- heterozygote is favored over both pPAP10E03e/CSU781c and -/- homozygotes. The presence of pPAP10E03 and CSU781 in the apomictic parent's map suggests that parent-of-origin (imprinting) effects may be involved. The absence of dosage effects and imprinting requirements for *Tripsacum* endosperm formation (Grimanelli et al., 1997), as well as endosperm formation and seed set in *Paspalum simplex* (Quarin, 1999), could indicate the restricted evolution of apomixis in species without such requirements. However, the production of facultative apomicts via chromosome doubling of a sexual, diploid race of *Paspalum notatum* Flüge suggests that increased allele dosage may be sufficient to induce the expression of apomixis (Quarin et al., 2001). As an alternative to imprinting and allele dosage requirements, directional genome changes associated with polyploidization (Song et al., 1995) were evident between the parents. pPAP10E03 mapped to 2a in both parental maps; however, CSU781 mapped to 7b in the sexual parent and homeolog 8b in the apomictic parent. The duplication of pPAP8C08h 21.7 cM below pPAP8C08j further suggests that a genome rearrangement has occurred in 7b of the apomictic parent (Fig. 2). It is therefore possible that the chromosome region containing CSU781 must be removed (deleted or translocated) from 7b for apospory to be functional, and the alleles CSU781c and pPAP10E03e have partial lethality. Our findings agree with the hypothesis of an incompletely penetrant, *trans*-active system (Grimanelli et al., 1998b), and they indicate that the (hypothetical) lethal factor acts only through maternal gametes. The molecular markers associated with this mechanism can be used in future studies to select for the favored heterozygote and against the lethal genotypes, increasing the probability of obtaining apomictic diploids in breeding programs. With the segregation distortion from this allelic interaction being incompletely penetrant, possible effects from the entire genetic background and/or the environment cannot be dismissed.

The presence of exceptional genotypes in this and other studies (Ozias-Akins et al., 1993, 1998; Gustine et al., 1997; Roche et al., 1999) suggests that additional gene(s) may modify the expressivity-penetrance of apospory. Genetic factors that modify the time of initiation, spatial location, and developmental progression of apomixis have recently been discovered (Koltunow et al., 2000). With molecular reports of independent loci controlling parthenogenesis and diplospory (Noyes and Rieseberg, 2000), as well as six QTLs influencing nucellar embryony (Garcia et al., 1999), it is likely that modifiers affect the expression of apomixis in many plants.

Results from this study demonstrate that buffelgrass is a desirable organism to use for genetic linkage studies of apospory. Our buffelgrass genome map is a useful tool for map-based cloning and marker-assisted breeding programs involving this important reproductive trait. Identification of the gene(s) conferring apomixis, as well as its modifiers, will be necessary before functional apomixis can be successfully transferred into major crop species. With the rapid development of molecular technologies, identifying such genetic intricacies of apomixis is a realistic goal.

## ACKNOWLEDGMENTS

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