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# Determining genetic origins of aberrant progeny from facultative apomictic Kentucky bluegrass using a combination of flow cytometry and silver-stained RAPD markers

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**Abstract.** Seeded plants that reproduce through facultative apomixis produce two types of progeny: (1) apomictic progeny genetically identical to the maternal genotype, and (2) aberrant progeny genetically different from the maternal genotype. Aberrant progeny have at least nine different genetic origins depending on gametic ploidy level and whether fertilization was self, cross, or absent. Multiple genetic origins of aberrant progeny complicate the results of basic and applied genetic studies. Determining the genetic origin of progeny plants using traditional techniques, such as cytology, embryology, and segregational studies, is technically difficult in Kentucky bluegrass. We have found that two relatively new techniques, flow cytometry and silver-stained RAPD (ssRAPD) markers, are powerful tools for rapidly determining the genetic origins of aberrant Kentucky bluegrass progeny. Our application of these techniques demonstrate that (1) flow cytometry accurately distinguishes progeny ploidy levels, and (2) ssRAPD markers distinguish progeny resulting from cross-fertilization. Therefore, a combination of flow cytometry and ssRAPD data would be useful for most genetic studies of aberrant individuals. Moreover, ssRAPDs were found to be of value for measuring the loss of genetic markers from polyhaploids and quantifying the inheritance of parental genomes in polydiploid $B_{II}$ (n + n) and polytriploid $B_{III}$ (2n + n) hybrids. Quantifying shared ssRAPD markers may also be useful for determining genetic relatedness between varieties and germplasm sources.

**Key words:** Apomixis – Polyhaploid – Hybridization – *Poa pratensis*

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## Introduction

Kentucky bluegrass (*Poa pratensis* L. Gramineae) is an important agronomic crop which serves simultaneously as nutrious forage, as unexcelled turf, and as a soil stabilizer against erosion throughout temperate climates world-wide. It has a facultative apomictic breeding system, and reproduces mainly by a form of gametophytic apomixis known as pseudogamous apospory (Muntzing 1933; Tinney 1940; Akerberg 1943). Apomixis is a complex form of asexual reproduction through seed that raises interesting questions in basic evolutionary studies and has important agronomic applications as a means of maintaining heterosis, stability, and uniformity in crop cultivars (reviews: Petrov 1986; Asker and Jerling 1992; Elgin and Miksche 1992). The reproductive biology of apomixis presents extreme difficulties for detailed genetic studies. Segregation analysis of obligate apomicts is impossible unless sexual germplasm is available; whereas progeny from facultative apomicts regularly segregate into apomictic and non-apomictic (i.e. genetically aberrant) classes. However, the aberrant class of progeny may actually be composed of individuals derived from a number of different genetic origins. Aberrant progeny may develop either through a completely meiotic sexual process or from a partial breakdown of the apomixis pathway. A major obstacle in the genetic analysis of facultative apomicts is determining the genetic origin of non-apomictic, aberrant progeny.

Two main steps in the apomixis pathway are (1) the production of unreduced eggs, and (2) the avoidance of pollen fertilization coupled with either parthenogenic or pseudogamous development. Different genetic origins of aberrant progeny result from the variable frequencies of reduced versus unreduced gametes (eggs and/or pollen), whether or not union of gametes occurs and, if union

occurs, whether or not fertilization is within or between genotypes. Multiple genetic origins may even occur within the same ovary. The common occurrence of polyembryonic seed in aposporous apomicts often results in the production of non-identical twin, triplet, and quadruplet seedlings (Nielsen 1946).

After careful consideration, we have estimated that there are at least nine different genetic origins for aberrant progeny through the facultative apomictic pathway (Table 1). The use of traditional genetic nomenclature, such as "n" and haploid, diploid, triploid, etc., are not easily applied to apomictic species. In Kentucky bluegrass, there exists a complex series of polyploidy and aneuploidy suggesting an allopolyploid origin. Within the species, 2n chromosome numbers range anywhere between 24 and 124 with skewed distributional modes in the 49–56, 63–70, and 84–91 chromosome number classes (Nielsen 1945, 1946; Love and Love 1975). A convenient nomenclature used to represent aberrant progeny is "$B_{II}$" for n+n polydiploid hybrids and "$B_{III}$" for 2n+n polytriploid hybrids as suggested by Rutishauser (1948) and adopted by Bashaw and Hignight (1990). Here, "B" stands for bastard, defined as "a hybrid of unknown origin", and the Roman numerals represent parental genomic contributions. We have adopted and expanded this nomenclature system by including a distinction for self- vs cross-fertilization. Moreover, precision in genetic origin determination requires that "$B_{III}$-cross" hybrids be further subdivided into "maternal-$B_{III}$" (2n+n) and "paternal-$B_{111}$" (n+2n) hybrid classes depending upon which parent produces the unreduced gamete; whereas, "$B_{III}$-self" seems sufficient to describe either class of polytriploid selfed-progeny.

The determination of genetic origin requires an ability to ascertain ploidy level and whether or not fertilization between genotypes has occurred. In the past, apomixis research in Kentucky bluegrass has primarily utilized traditional embryological, cytogenetic, and segregational methods of analysis (van Dijk 1991). Although these techniques have been rigorously applied, and in some cases improved (Young et al. 1979), there are several inherent reasons why they are technically difficult to perform in Kentucky bluegrass (Huff 1992). Recent developments in molecular biology have provided two new techniques applicable to aberrant progeny analysis. Flow cytometry is a method of determining nuclear 2C DNA content that is both rapid and accurate (Galbraith et al. 1983; Arumuganathan and Earle 1991; Costich et al. 1991). Values of nuclear DNA content determined by flow cytometry are highly positively-correlated with values determined by the microdensitometer technique using Feulgen stain (Michaelson et al. 1991) and have been proven useful for estimating ploidy levels of the prairie grass big bluestem (*Andropogon gerardii* Vitman, (Keeler et al. 1987). Various classes of marker systems have been used for determining effective hybridization in apomixis research and breeding programs, including morphological traits (Hiesey and Nobs 1982), isoenzymes (Wu et al. 1984), and restriction fragment length polymorphisms (Ozias-Akins et al. 1992). A new class of DNA markers, mediated through the polymerase chain reaction (PCR), has become available and is most commonly referred to as random amplified polymorphic DNA or RAPD (Welsh and McClelland 1990; Williams et al. 1990; Caetano-Anolles et al. 1991). Advantages of the RAPD technique are that it is technically simple to perform, does not require prior molecular genetic information, does not use radioactive isotopes or recombinant DNA, supplies a virtually unlimited number of markers, and is extremely versatile for other genetic applications such as mapping (Williams et al. 1990), marker-assisted selection (Michelmore et al. 1991), and phylogenetic analysis of outbreeding germplasm (Huff et al. 1993).

The specific objective of the present study was to determine the genetic origins of aberrant progeny from facultative apomictic Kentucky bluegrass using a combination of flow cytometry and silver-stained RAPD (ss-RAPD) marker data.

**Table 1.** Theoretical ploidy levels (in parentheses) and suggested nomenclature of Kentucky bluegrass progeny derived through different genetic origins from facultative apospory. Genetic origins vary depending upon reduced (n) versus unreduced (2n) gametic ploidy level and pseudogamous development versus self or cross pollen fertilization. Nomenclature: B = bastard (i.e., of questionable origin), P = paternal, M = maternal, while Roman numerals represent the parental genomic contribution

| Egg | Pseudo-gamous develop-ment | Pollen fertilization | | | |
|---|---|---|---|---|---|
| | | Reduced (n) | | Unreduced (2n) | |
| | | Self | Cross | Self | Cross |
| Reduced (n) | (n) Poly-haploid | (n+n) $B_{II}$ | (n+n) $B_{II}$ | (n+2n) $B_{III}$ | (n+2n) P-$B_{III}$ |
| Unreduced (2n) | (2n) Apo-sporous apomixis | (2n+n) $B_{III}$ | (2n+n) M-$B_{III}$ | (2n+2n) $B_{IV}$ | (2n+2n) $B_{IV}$ |

## Materials and methods

### *Plant sources*

Genetic origin determination of self-fertilized progeny used the Kentucky bluegrass cultivar (cv) 'Baron' (certified seed lot # Z1-9-552). Progenies derived from polyembryonic seed were observed, carefully separated, and identified by a lettered epithet following the seed identification number. Two sets of twins, 3a and 3b and 27a and 27b, were chosen for detailed analysis. Genetic origin determination of cross-fertilized progeny used single progenies from artificial crosses between cv 'Mystic' (maternal) cv 'America' (paternal) and between an experimental line

NJE P-59 (maternal) × cv Baron (paternal). Progeny plants from hybridization were visually selected on the basis of morphology as being aberrant to the maternal genotype. After establishment in the greenhouse, all plant progenies along with parental material were transferred to the experimental field at the Adelphia Research Farm of Rutgers University.

*Flow-cytometry*

Young, fully expanded leaves were collected and washed with distilled water. Samples of 100 mg of leaf blade were placed in plastic Petri dishes containing 1 ml of chopping buffer (per liter: 50 ppm propidium iodide, 4.26 g $MgCl_2$, 8.84 g sodium citrate, 4.23 g MOPS, pH 7.3, 1.0 ml Triton X-100). Leaves were chopped with a scalpel into pieces approximately 0.5 × 0.5 mm in size. Chopping buffer containing leaf nuclei was filtered through 30-micron nylon mesh into 1.5-ml microcentrifuge tubes. Tubes were centrifuged at 13 k g for 30 s. Pelleted nuclei were resuspended in 150 µl of fresh chopping buffer with the addition of 20 µg ml/l of RNAse and 0.6 µl ml/l of chicken red blood cell stock ($10^7$ cells/ml). Tubes were incubated for 15 min at 37 °C. Each procedural step was performed on ice and in a cold room. Samples of suspended leaf nuclei were analyzed using an EPICS Profile flow cytometer at 488 nm. For each sample, the plant nuclear 2C DNA content, measured in picograms (PS), was determined by taking the ratio of the $G_0/G_1$ peak mean and the chicken red blood cell peak mean and multiplying by the known nuclear DNA content of chicken red blood cells, i.e., 2.33 pg (Galbraith et al. 1983; Costich et al. 1991).

*Template DNA isolation*

Template DNA was prepared by grinding approximately 300 mg of fresh leaf tissue in liquid nitrogen, placing an 0.6 µl volume of ground tissue into a 1.7-µl microfuge tube, adding 1/3 volume of buffer (100 mM Tris, 50 mM EDTA, pH 8.0, 100 mM NaCl, 1% SDS, 10 mM mercaptoethanol, 0.1% PVP) and 1/3 volume of 5 M potassium acetate. After mixing with a sterile pipette tip, and incubation in a 65 °C water bath for 15 min, tubes were spun at 15 k rpms for 20 min. The supernatant was transferred into a clean tube using a micropipette. After adding 1/5 volume of 10 M ammonium acetate and 1 volume of −20 °C isopropanol, tubes were mixed and stored at −20 °C for 20 min. The DNA was pelleted and washed in 70% ethanol, re-spun, allowed to dry, and then redissolved in 100 µl of double-distilled $H_2O$. DNA concentration was determined using a TKO-100 fluorometer and diluted to 10 ng $\mu l^{-1}$.

*PCR protocol*

A modification of the original RAPD technique (Williams et al. 1990) was used with DNA markers that we will refer to as silver-stained RAPD, or ssRAPD. The optimized polymerase chain reaction (PCR) conditions for producing ssRAPD markers of Kentucky bluegrass template DNA were 7 min at 94 °C; 45 cycles of 1 min at 94 °C followed by 1 min at 36 °C followed by 2 min at 72 °C; finishing with 5 min at 72 °C. Ramp times for heating and cooling were 0.3 degrees per s, and one degree per s, respectively. PCR reactions were carried out in a 12 µl volume containing 1 x buffer (Promega) adjusted to 2.5 mM of $MgCl_2$, 0.2 mM of each dNTP, 1 µg of primer, 0.024 U $\mu l^{-1}$ of *Taq* polymerase, and 1 ng $\mu l^{-1}$ of template DNA. Amplification fragments were separated in 7.5% acrylamide/bis gel (Bio-Rad) in 0.375 M Tris, pH 8.8, buffer, using a Mini-Protean II (Bio-Rad) and visualized using silver stain (Bio-Rad kit) following the prescribed protocol (Bio-Rad), except that fixation was performed in 10% acetic acid for 30 min. ssRAPD fingerprints were produced from each of 20 10-mer primers (set-A, Operon) for at least two replicate PCR amplification runs for each individual progeny plant. ssRAPD fingerprints of all parental cultivars were replicated using different, apomictically-derived, individuals. Only replicated ssRAPD markers between fragment sizes of 0.1 to 2.6 kb were scored.

*Cytology*

Cytological investigations were performed on at least two individuals from each modal peak of the DNA content distribution. Fresh, vegetative meristem cells were squashed in acetocarmine stain and chromosomes were viewed using phase microscopy.

## Results

*Self-fertilized progeny*

A trimodal distribution of nuclear 2C DNA contents was observed for the Kentucky bluegrass cv Baron (Fig. 1). The overall mean DNA content of 11.03 pg coincided with the central mode. The additional modes were 5.2 pg and 16.0 pg. Somatic chromosome counts confirmed that individuals possessing approximately 11 pg had 82 to 90 chromosomes. The reported 2n chromosome number of Baron is about 84 (Wu and Japametes 1986). Individuals possessing approximately 5 pg of nuclear DNA had 38 to 44 chromosomes and were determined to be polyhaploid progeny resulting from the pseudogamous development of a reduced egg. Individuals possessing approximately 16 pg had at least 125 chromosomes and were determined to be $B_{III}$-self progenies resulting from the self-fertilization of an unreduced egg. Multivalent formation at metaphase, as well as lagging chromosomes at anaphase, were commonly observed confirming earlier reports of these events in Kentucky bluegrass (Nielsen 1946; Wu and Japametes 1986); however, no data were collected as to the individual frequencies of these events. Both sets of polyembryonic twins were found to be "haplo-diplo"

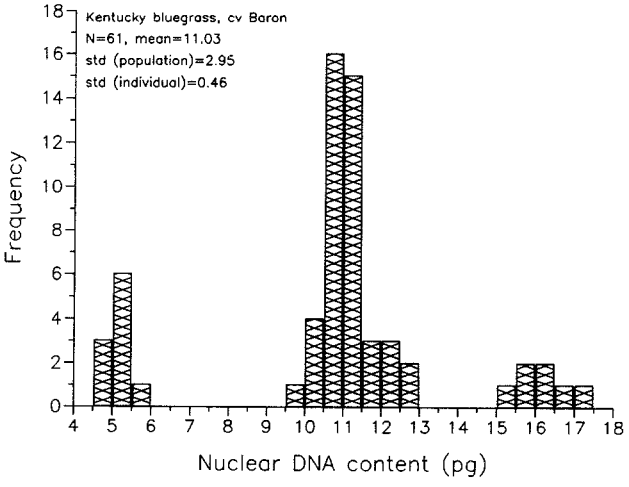


**Fig. 1.** Distribution of nuclear 2C DNA content of individual genotypes within the Kentucky bluegrass cultivar Baron

twins. The DNA content of each twin set was 5.0 pg for 3a and 10.8 pg for 3 b, 5.2 pg for 27 a and 11.4 pg for 27 b. These results indicate that flow cytometry was a useful technique for distinguishing the genetic origin of polyhaploids, haplo-diplo twins, and $B_{III}$-self progenies from a larger population of individuals within a single variety of Kentucky bluegrass.

ssRAPD fingerprints for two typical, apomictic Baron individuals and one $B_{III}$-self individual did not reveal any observable deviations from one another and thus demonstrate the reproducibility of ssRAPD fingerprints (Fig. 2 and Table 2). These three individuals were, therefore, considered to represent the typcial Baron ssRAPD fingerprint. The two polyhaploids, 3 a and 27 a, exhibited the most notable departure from the typical Baron banding pattern, but nevertheless shared over 92% of the 450 total ssRAPD band size positions observed for typical Baron. The two polydiploids, 3 b and 27 b, each showed slight deviations from typical Baron, sharing over 97% of ssRAPD marker positions with it. Several unique bands were observed to be shared only by twins within a set. This observation suggests that each set of twins was derived from a maternal parent whose past genetic lineage had involved either a low level of outcrossing, a recombinational event through selfing, or carried a chromosome addition atypical of Baron. Pairwise comparisons within twin sets indicated that, as a result of meiosis, polyhaploid 3 a had lost 4.1% of those ssRAPD markers observed in polydiploid 3 b, and polyhaploid 27 a had lost 6.7% of those ssRAPD markers observed in polydiploid 27 b. While ssRAPD marker loss in polyhaploids from the polydiploid state is genetically interesting (see Discussion) no additional genetic origin information was gained using ssRAPD markers. These results indicate that ssRAPD markers were less informative than flow cytometry within a single variety of Kentucky bluegrass.

**Table 2.** Percentage of 450 ssRAPD markers, produced from 20 10-mer primers, that are share between pairwise comparisons of selected individuals of Kentucky bluegrass cv 'Baron'. Selected individuals include a typical apomictic individual, a $B_{III}$-self progeny, and two pairs of haplo-diplo twins (3a and b and 27a and b) obtained from polyembryonic seed

| Comparison | Baron | $B_{III}$ self | Hap3a | Dip3b | Hap27a | Dip27b |
|---|---|---|---|---|---|---|
| Baron | – | | | | | |
| $B_{III}$-self | 100.0 | – | | | | |
| Haploid 3a | 92.7 | 92.7 | – | | | |
| Diploid 3b | 97.1 | 97.1 | 96.7 | – | | |
| Haploid 27a | 92.7 | 92.7 | 98.9 | 94.7 | – | |
| Diploid 27b | 99.3 | 99.3 | 92.9 | 96.7 | 93.2 | – |

*Cross-fertilized progeny*

Nuclear 2C DNA content values were found to have low standard deviations among replicate samples of hybrid and parental genotypes (Table 3). The DNA content of

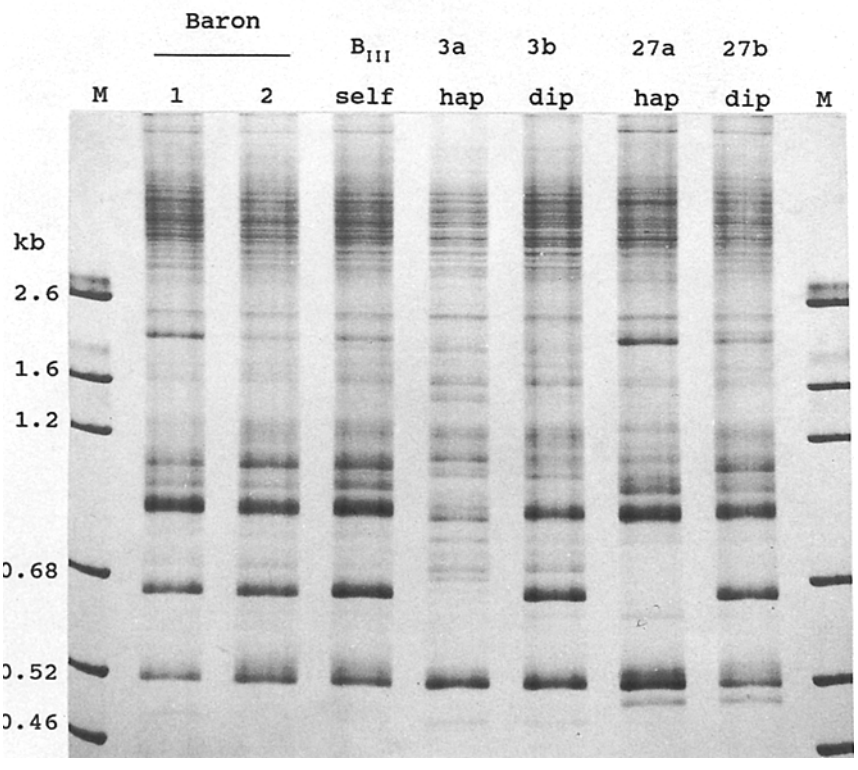


**Fig. 2.** ssRAPD fingerprints of selected individuals of Kentucky bluegrass cv 'Baron' using primer OPA-16. Selected individuals include two typical apomictic individuals, a $B_{III}$-self progeny, and two sets of haplo-diplo twins 3a and b and 27a and b. ssRAPD fingerprints for the two apomictic individuals of Baron also represent replicated ssRAPD fingerprints with Baron. DNA size standards (*M*) measured in kilobases (kb)

Hybrid-1 (10.33 pg) was intermediate between parental values and was closest to a theoretical value of a $B_{II}$-cross hybrid (9.53 pg) and next closest to a theoretical maternal-$B_{III}$ hybrid (13.54 pg). Based on flow-cytometry alone, Hybrid-1 was most likely a $B_{II}$-cross hybrid. The DNA content of Hybrid-2 (9.86 pg) exceeded either parental value and was similar to theoretical expectations of a $B_{III}$-self progeny (9.03 pg), a maternal $B_{III}$-cross hybrid (9.46 pg), and a paternal $B_{III}$-cross hybrid (9.88 pg). These results suggest that Hybrid-2 was a $B_{III}$ individual derived from union between an unreduced and a reduced gamete. However, because the parental varieties, America and Mystic, have similar values of nuclear DNA content, flow cytometry data alone was insufficient to distinguish between the three genetically distinct origins of $B_{III}$ individuals.

**Table 3.** Mean and standard deviation of nuclear 2C DNA content of parental genotypes and $F_1$ hybrids from two crosses of Kentucky bluegrass

| Crosses | | Nuclear 2C DNA content | |
|---|---|---|---|
| | | Mean (pg) | Std (pg) |
| Cross-1 | | | |
| Hybrid-1 | | 10.33 | 0.26 |
| Male parent | cv Baron | 11.03 | 0.16 |
| Female parent | line NJE P-59 | 8.03 | 0.14 |
| Cross-2 | | | |
| Hybrid-2 | | 9.86 | 0.20 |
| Male parent | cv America | 6.87 | 0.12 |
| Female parent | cv Mystic | 6.02 | 0.61 |

Comparison of Hybrid-2 with replicate parental genotypes demonstrates the repeatability and usefulness of the ssRAPD fingerprinting technique (Fig. 3). For each hybrid progeny, the observed paternal to maternal inheritance ratio of ssRAPD markers was tested against an expected ratio of either 1:1 ($B_{II}$ expectation), 1:2 (maternal-$B_{III}$ expectation), or 2:1 (paternal-$B_{III}$ expectation), using chi-square (Table 4). For Hybrid-1, the observed paternal to maternal ratio of 228 polymorphic markers was highly significantly different ($P<0.001$) from 1:2 and 2:1 expectations but was not significantly different ($P>0.05$) from a 1:1 expected ratio. Thus, one-half of the parental polymorphisms were derived from the paternal parent and one-half were derived from the maternal parent. This result confirms the above flow cytometry data that Hybrid-1 was a $B_{II}$-cross hybrid derived from the union of a reduced egg from NJE P-59 and a reduced pollen nucleus from Baron. Hybrid-2 exhibited inheritance of ssRAPD markers unique to the male America parent and, thus, did not result from a $B_{III}$-selfing event. For Hybrid-2, the observed paternal to maternal ratio of

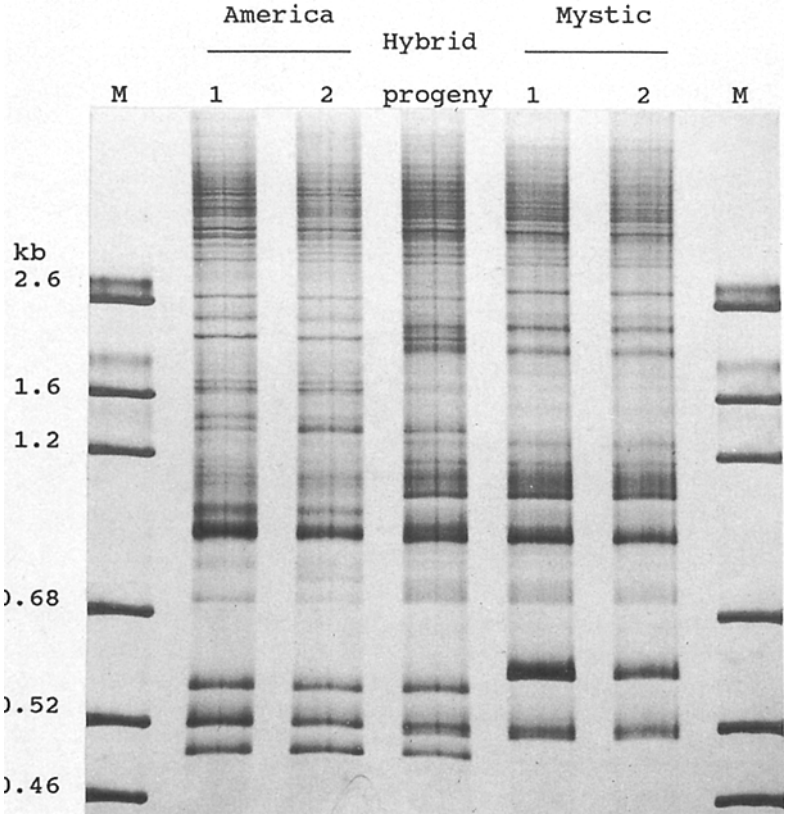


**Fig. 3.** ssRAPD fingerprints of a hybrid $F_1$ progeny (Hybrid-2) and parental varieties of Kentucky bluegrass using primer OPA-16. Hybrid-2 was produced from an artificial cross between the varieties 'America' (male parent) and 'Mystic' (female parent). ssRAPD fingerprints for Ameria and Mystic were replicated using two apomictically-derived individual plant within each variety. DNA size standards (*M*) measured in kilobases (kb)

**Table 4.** Shared ssRAPD polymorphisms between hybrid $F_1$ progeny and parental varieties of Kentucky bluegrass. Observed paternal to maternal ratios of polymorphic ssRAPD markers for each hybrid were tested against expected ratios of 1:1, paternal (n) to maternal (n); 1:2, paternal (n) to maternal (2n); or 2:1, paternal (2n) to maternal (n), using chi-square

| Total | Hybrid-1 | | | | Hybrid-2 | | | |
|---|---|---|---|---|---|---|---|---|
| | Shared polymorphisms (number of markers) | | | Chi-square | Shared polymorphisms (number of markers) | | | Chi-square |
| | Baron paternal | NJE p-59 maternal | Total | | America paternal | Mystic maternal | Total | |
| Observed | 119 | 109 | 228 | | 74 | 168 | 242 | |
| Expected | | | | | | | | |
| 1:1 | 114 | 114 | 228 | 0.44 ns | 121 | 121 | 242 | 36.51*** |
| 1:2 | 76 | 152 | 228 | 36.49*** | 81 | 161 | 242 | 0.91 ns |
| 2:1 | 152 | 76 | 228 | 21.49*** | 161 | 81 | 242 | 140.46*** |

ns, Nonsignificant at the $P<0.05$ level of probability
*** Significantly different from expected at the $P<0.001$ level of probability

242 polymorphic markers was highly significantly different ($P<0.001$) from both 1:1 and 2:1 expectations but was not significantly different ($P>0.05$) from a 1:2 expected ratio. Thus, one-third of the parental polymorphisms were derived from the paternal parent and two-thirds were derived from the maternal parent. This result indicates that Hybrid-2 was a maternal $B_{III}$ hybrid derived from the union of an unreduced egg from Mystic with a reduced pollen from America.

Marker polymorphisms for each of the 20 individual primers were subsequently tested for goodness of fit with 1:1 expectations for Hybrid-1 and with 1:2 expectations for Hybrid-2. Inherited ssRAPD markers, from each of the 40 hybrid × primer combinations, did not show a significant departure ($P>0.05$) from these expectations, with the single exception of Hybrid-1 and primer OPA-5 with an observed ratio of 0 paternal markers to 6 maternal markers ($\chi^2=4.17$ $P<0.05$) which was attributed to a chance event.

## Discussion

This is the first demonstration of an ability to determine the genetic origins of aberrant plants derived from facultative, aposporous apomixis using either flow-cytometry or DNA marker data. The DNA marker technique we have presented is a modification of the original Williams et al. (1990) RAPD procedure. From our past experience, we found that the original RAPD technique was neither a reliable nor an entirely reproducible method of producing DNA markers for Kentucky bluegrass. However, after modifying the thermocycling ramp times and utilizing silver-stained acrylamide gels, rather than ethidium bromide-stained agarose gels, we believe our results to be highly reproducible between different laboratories. In order to distinguish our modifications from the original technique, we have renamed our method silver-stained RAPD markers, or ssRAPD. ssRAPD analysis continues to require replications of PCR runs in order to minimize mis-scoring of markers due to poor resolution, pipetting errors, or sporadic products that occasionally occur for a few samples. Flow cytometry was found to be more informative than ssRAPD markers for analyzing self-fertilized progeny; whereas, ssRAPD markers were more informative than flow cytometry for identifying and analyzing cross-fertilized progeny. Depending on the questions being addressed, most genetic studies will probably require combined information from flow cytometry and ssRAPD markers for determining the genetic origins of aberrant individuals.

Our results suggest that silver-stained ssRAPD markers and values of nuclear 2C DNA content determined by flow cytometry were inherited in a manner that fit expectations based on the model of facultative apospory described in Table 1. Although the present study did not identify any progeny derived from an unreduced pollen nucleus (paternal-$B_{III}$ and $B_{IV}$ progeny), the occurrence of $B_{IV}$ progeny has been reported for Kentucky bluegrass (Grazi et al. 1961). We can envision no inherent reason why flow cytometry and ssRAPD marker techniques would not be able to detect the genetic origins of such progeny when they occur. Thus, Table 1 has been constructed to take account of these individuals for future detection efforts. In general, most ssRAPDs are inherited as dominant markers (Welsh and McClelland 1990; Williams et al. 1990). Segregation of heterozygous ssRAPD marker loci would tend to distort the observed polymorphism inheritance ratios of the present study. However, the segregation of recessive ssRAPD phenotypes was most likely buffered by the confounding influence of high levels of polyploidy. Polyploidy further complicates genetic analysis in Kentucky bluegrass. The polyhaploids had the least complex genomes examined,

but are nevertheless considered to be highly polyploid at the hexaploid level (2n = 6x = approximately 42). Polyhaploids are particularly interesting because they provide a genetic system that is equivalent to a gamete; each contains one-half the amount of nuclear DNA of the polydiploid parent. In the future, the frequency and copy number of dominant ssRAPD alleles might be deduced from the frequency of loss of dominant ssRAPD phenotypes in polyhaploids; provided, of course, that the polyhaploids under investigation have a simple genomic structure. Polyhaploids of Kentucky bluegrass have been identified which possess, or are near to, the diploid number of chromosomes (2n = 14, 16, and 18; Nielsen 1946). Given a suitable number of such "dihaploid" individuals might allow for a more detailed genetic analysis of the genomic composition and inheritance of both ssRAPD markers and economically important traits from highly-polyploid apomictic species.

The present study also indicates that ssRAPD marker analysis may be useful as a measure of genetic distance in Kentucky bluegrass. For example, the genetic distance of Hybrid-1 was half-way between the parental varieties Baron and NJE P-59; while, the genetic distance of Hybrid-2 was two-thirds the genetic distance of the maternal parent variety Mystic from the paternal variety America. Calculating genetic distance among varieties and breeding populations, using ssRAPD markers, may be useful for strain identification and germplasm monitoring (Huff et al. 1993).

We have found flow cytometry determination of nuclear 2C DNA content and ssRAPD fingerprints to be highly repeatable, accurate, and easy to perform. By contrast, our past experience with more classical techniques were labor intensive, tedious, and extremely time consuming. The most efficient method of detecting aberrant progeny in large apomictic populations is through spaced-planted progeny test nurseries (Tinney and Embedded 1940). The added ability to distinguish the genetic origin of aberrant progeny, using flow-cytometry and ssRAPD markers, has powerful applications for more accurate descriptions of commercial varieties, for detecting hybridization events during early stages of a breeding program, and for genetic studies analyzing variation in apomixis pathway components and the frequency of different genetic origins.

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