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Inheritance of RAPDs in F_1 hybrids of corn

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Summary. Random amplified polymorphic DNA (RAPD) markers were analyzed in materials from a partial diallel, including 16 corn F_1 hybrids (with five reciprocals) and their five parental inbreds. Using 21 primers, we scored a total of 140 different fragments for their presence/absence and intensity variation, where appropriate. When all 21 genotypes were taken into consideration, 20.7% of these fragments were nonpolymorphic, 37.1% were unambiguously polymorphic, and 42.1% were quantitatively polymorphic. Unambiguous polymorphisms were distinguished by the simple presence or absence of a specific fragment in the inbred genotypes, whereas quantitative polymorphisms exhibited a variation in the intensity of a fragment. Of the F_1 patterns, 95.2% of the unambiguously polymorphic situations could be interpreted genetically by assuming complete dominance of the presence of the parental fragment, while 3.2% of the F_1 patterns exhibited a fragment intensity that was intermediate between the two parental patterns (partial dominance). For quantitative polymorphisms, values of 88.1% for complete dominance and 5.0% for partial dominance were obtained. The results suggest that specific types of errors can be detected in RAPD analysis, that uniparental inheritance is not common, and that RAPD analysis might be more prudently used for some applications than for others.

Key words: *Zea mays* L. – Molecular markers – Randomly amplified polymorphic DNAs

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Introduction

Restriction fragment length polymorphisms (RFLPs) have proven their utility as molecular markers in developing genetic maps of crops like maize (Helentjaris 1987), tomato (Bernatzky and Tanksley 1986), potato (Bonierbale et al. 1988; Gebhardt et al. 1989), soybean (Apuya et al. 1988), rice (McCouch et al. 1988), lettuce (Landry et al. 1987), barley (Heun et al. 1991), and wheat (Liu and Tsunewaki 1991). Recently, another type of molecular marker has been proposed by Welsh and McClelland (1990) and Williams et al. (1990). The advantages of these polymerase chain reaction (PCR)-based analyses, termed either AP-PCRs (arbitrarily primed-PCRs) or RAPDs (random amplified polymorphic DNA), include the ease and rapidity of analyses, the use of a general set of universal primers, and their requirement for minimal substrate DNA. First reports about the use of RAPDs in tomato (Martin et al. 1991; Klein-Lankhorst et al. 1991), lettuce (Michelmore et al. 1991), and *Brassica* (Quiros et al. 1991; Hu and Quiros 1991) have indeed demonstrated that RAPDs can efficiently generate both randomly dispersed markers as well as markers linked to specific genes.

Despite some obvious advantages, some problems have also been encountered with the use of RAPDs as molecular markers. Genetic studies with RAPDs in *Arabidopsis* implied that only 57% of the 392 polymorphic DNA fragments segregated in a Mendelian fashion in the mapping population (Reiter et al. 1992), whereas 76% of the 37 polymorphic fragments segregated as dominant Mendelian markers in diploid alfalfa (Echt et al. 1992). In their diallel analyses of F_1 progenies of conifers, Carlson et al. (1991) found that most but not all RAPDs were inherited in the expected

dominant way. Halward et al. (1992) found that normally dominant F_2 segregations occurred but that the banding patterns in peanut were often too complex to be used in genetic mapping. From these studies it would appear that there is a significant fraction of informative RAPD fragments that seemingly do not obey the simple dominant inheritance patterns expected for this type of marker.

The problems of RAPD analyses cited above might have a common basis. Since this technique utilizes low input substrate and many cycles of amplification, the fragments (detected on ethidium bromide-stained gels) that are amplified during RAPD analysis probably represent the most successful products among many more competing candidates for amplification. Hence, one might be concerned that the overall genetic background could determine which candidates are actually amplified and that marker results would not only reflect the sequence/annealing at a single locus but be influenced by other regions in the genome. In other words, one might expect that RAPDs would be subject to 'epistatic' effects not usually associated with other molecular markers. In this case a specific fragment, present in two individuals, could be amplified in one genetic background but not in another due to competition from other unlinked sites. Hence, the wrong conclusion would be drawn from these data concerning this specific locus in both backgrounds. This might be even more pronounced if intensity variations occur for specific fragments, particularly since even minor changes in the reaction conditions may alter the resulting pattern (Williams et al. 1991).

Most of the studies to date have utilized a single mapping population created from two parental backgrounds. In this article we present results from the analyses of 16 F_1 maize hybrids (including five reciprocal pairs) and their five inbred parents with 21 random primers. It was our intention to use this type of partial diallel design to determine how often polymorphic RAPD fragments were not observed to follow the expected mode of dominant inheritance; in this case by using several backgrounds as the variable instead of only two as occurs in most inheritance studies relying upon segregation in F_2 or BC populations. By determining informative (i.e., polymorphic) situations in the inbred parental lines, we could then assay whether the polymorphic fragments were in fact observed in every predicted F_1 situation and in both reciprocal crosses. By choosing parental lines that were known to be very diverse in their pedigree and by assuming a correlation with sequence diversity, we felt that we would be able to test the effect of drastically altered genetic background upon RAPD amplification of individual fragments.

Materials and methods

DNA extraction and PCR reaction

Seed of five parents (1 = B73, 2 = Z51, 3 = Mo17, 4 = F2, 5 = G28) and their 16 F_1 hybrids (1 × 3, 3 × 1, 1 × 4, 4 × 1, 1 × 2, 1 × 5, 5 × 1, 3 × 4, 4 × 3, 3 × 2, 2 × 3, 5 × 3, 4 × 2, 2 × 4, 5 × 4, and 5 × 2) were provided by S. Smith, Pioneer Hi-Bred Int., Johnston, Iowa. After these genotypes were grown for 18 days in a greenhouse, leaf tissue was harvested and lyophilized. The dried tissue was ground in a coffee grinder, and 0.3 g of that material was used to extract DNA using the CTAB procedure originally outlined by Saghai-Maroof et al. (1984).

Approximately 75 ng of genomic DNA in 1 µl TE was mixed with 49 µl of a reaction mix containing 10 m*M* TRIS-HCl, pH 8.3, 1.5 m*M* $MgCl_2$, 50 m*M* KCl, 0.1 mg/ml gelatin (all included with the 10 × buffer of Boehringer Mannheim), 0.1 m*M* each of dATP, dCTP, dGTP, and dTTP (from Pharmacia, diluted and mixed freshly), 0.25 µ*M* primer (from Operon), and 0.35 µl Taq polymerase (1.75 units, from Boehringer Mannheim). After this mixture was overlaid with mineral oil, the samples were exposed to the following temperature profiles using a Perkin Elmer DNA thermal cycler: One cycle of 95 °C for 0.5 min, 40 °C for 0.5 min, 70 °C for 1.0 min; followed by one cycle of 95 °C for 0.5 min, 92 °C for 2.5 min, 35 °C for 1.0 min, 72 °C for 2.0 min; followed by 44 cycles of 92 °C for 1.0 min, 35 °C for 1.0 min, 72 °C for 2.0 min; and finally one cycle of 72 °C for 10 min, and then kept at 4 °C till electrophoresis.

After completion of the PCR, the 50 µl were withdrawn into a new tube and mixed with 10 µl 5 × sample gel buffer (70% glycerol, 0.5 × NEB, 20 m*M* EDTA, 0.2% SDS, 0.6 mg/ml bromphenol blue). Of this mixture, 35 µl was loaded into a 1.2% agarose gel prepared with 1 × TBE buffer. The samples were electrophoresed for 20–24 h at low voltage. By this time the 506- and 517-bp fragments of the 1-kb ladder (from Gibco BRL) had migrated 8–9 cm, and the ethidium bromide-stained gel was photographed. The Polaroid film 55 produced both a positive and a negative (print area 9 × 11.5 cm); the negative was used to score the obtained bands because of its 6.5-fold higher print resolution (150–160 line pairs/mm).

Twenty-one primers from Operon, Alameda, Calif., were used. Their sequences read as follows from the 5' end:

A-01 = CAGGCCCTTC,	A-02 = TGCCGAGCTG,	A-03 = AGTCAGCCAC,
A-04 = AATCGGGCTG,	A-09 = GGGTAACGCC,	A-12 = TCGGCGATAG,
A-13 = CAGCACCCAC,	A-14 = TCTGTGCTGG,	A-16 = AGCCAGCGAA,
A-17 = GACCGCTTGT,	A-18 = AGGTGACCGT,	A-19 = CAAACGTCGG,
A-20 = GTTGCGATCC,	B-01 = GTTTCGCTCC,	B-02 = TGATCCCTGG,
B-03 = CATCCCCCTG,	B-04 = GGACTGGAGT,	B-05 = TGCGCCCTTC,
B-06 = TGCTCTGCCC,	B-07 = GGTGACGCAG,	B-08 = GTCCACACGG.

Data analyses

As many fragments as possible were scored visually for each of the 21 genotypes analyzed with the 21 primers. The scores were recorded as '+' for an intensely staining fragment, '(+)' for a less-visible fragment, and '−' for no visible fragment. These three classes accurately described more than 90% of the situations. Sometimes a fragment was considered just visible and recorded as '((+))', and sometimes no decision could be made about the existence of a minor product (due to fragments with close or overlapping migration values) and this was recorded as '?'. Missing data rarely occurred but was recorded as '0'. Very seldomly were products observed that possessed a signal with an extreme intensity; this was denoted as '*'. This initial scoring was completed for all genotypes irrespective of their pedigree. Subsequently, the scoring of each polymorphism (i.e., products of the same apparent molecular weight that are expressed differently between the five parental genotypes) was classified into one of two classes. 'Unambiguous' patterns possessed two very distinct results within the inbreds, termed either '+' versus '−', depending upon whether a fragment was observed or not. 'Quantitative' polymorphisms, on the other hand, displayed a variation in the intensity of the respective products in the parental inbreds. Most nonpolymorphic fragments were easy to score and thus not further classified.

Based upon this strategy, the entire data set (140 products detected and scored across 21 genotypes) fell into three categories: nonpolymorphic, unambiguously polymorphic, and quantitatively polymorphic. At this point the F_1 genotypes were compared with their respective parents, and their inheritance pattern was recorded as 'd', denoting a result of apparent complete dominance where the more intense fragment of the two parents was exhibited by the F_1, or 'i', denoting an F_1 product intermediate in intensity of the two parental bands, which might be genetically interpreted as partial dominance. For those F_1s where both parents are missing the relevant fragment, the absence of this fragment in the F_1s was considered to be in accordance with the hypothesis of complete dominance and was therefore also recorded as 'd'. All remaining cases were denoted as 'remaining'.

Results

The 21 genotypes (five parental inbred lines and 16 of their F_1s) were analyzed with 21 randomly chosen primers, each 10 nucleotides long and of arbitrary sequence. A total of 140 fragments were scored across all genotypes. Of the 140 fragments scored in the parental lines, 20.7% were classified as nonpolymorphic, 37.1% were denoted as unambiguously polymorphic, and 42.1% were quantitatively polymorphic. To illustrate our strategy for classifying the data, Fig. 1 presents the results obtained with primer B6.

It can be seen that different amplification fragments (visible as products of different molecular weight) are produced and that many of these vary in their intensity. The most obvious of these are distinguished by the presence versus absence of products (unambiguously polymorphic RAPDs) within the parental inbreds. For instance fragment no. 4 is present in all parental inbreds (lanes 18–22) but P1 (lane 18) and P5 (lane 22). Product no. 6 is absent in all of the parental lines but P2 (lane 19). Additionally there are some fragments in this sample that appear to exhibit a type of intensity polymorphism, which we have termed 'quantitative', such as fragment no. 8. The intensity of this product in parents P1 (lane 18) and P4 (lane 21) appears to be less than that in line P3 (lane 20). Only those situations that were found to be reproducible in their intensity differences were in fact classified as 'quantitative'.

The scores assigned to all of the product-genotype combinations obtained with primer B6 (shown in Fig. 1) are given in Table 1, part A. Examination of the F_1s reveals that for the most part, all of the polymorphic situations in the homozygous parental lines are appropriately reflected (assuming complete dominance) in both the homozygous and heterozygous F_1 situations. For instance, fragment no. 7 should be found in all F_1 combinations, both those presumed to be homozygous for the product and also those that should be heterozygous for its presence; and, in fact, it is. Similarly, fragment no. 4 should be found in those homozygous F_1s (2×3, 3×4, 2×4, etc.) and hetero-

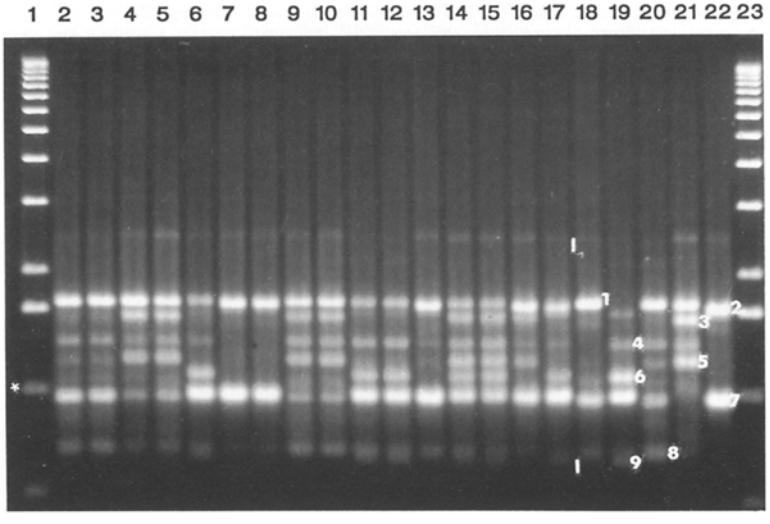

Fig. 1. RAPD analysis of 16 maize F_1 hybrids and their five inbred parents (arranged in the order given in Table 1; both marginal lanes contain molecular size markers) with primer B6. The 1018-bp fragment of the 1-kb DNA ladder is marked by *. The scored fragments are numbered 1–9; the scores are given in Table 1

Table 1. In part A, the scorings of the amplification products with primer B6 shown in Fig. 1 are given; in part B, a genetic interpretation of these F_1 scores is presented by comparing them with the parentals. The observed polymorphic fragments are classified into unambiguous and quantitative as outlined in Materials and methods, where the explanation of the symbols is also given in more detail

Fragment	F_1 hybrids																Parents				
	1×3	3×1	1×4	4×1	1×2	1×5	5×1	3×4	4×3	3×2	2×3	5×3	4×2	2×4	5×4	5×2	1	2	3	4	5
Part A[a]																					
Unambiguous																					
Fragment 2	−	−	−	−	−	+	+	−	−	−	−	+	−	−	+	+	−	−	−	−	+
Fragment 3	−	−	+	+	−	−	−	+	+	−	−	−	+	+	+	−	−	−	−	+	−
Fragment 4	+	+	+	+	+	−	−	+	+	+	+	(+)	+	+	(+)	(+)	−	+	+	+	−
Fragment 5	−	−	+	+	−	−	−	+	+	−	−	−	+	+	+	−	−	−	−	+	−
Fragment 6	−	−	−	−	+	−	−	−	−	+	+	−	+	+	−	+	−	+	−	−	−
Quantitative																					
Fragment 1	+	+	+	+	+	?	?	+	+	+	+	?	+	+	?	?	+	−	+	+	−
Fragment 7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Fragment 8	+	+	(+)	(+)	(+)	(+)	(+)	+	+	+	+	+	(+)	(+)	(+)	−	(+)	−	+	(+)	−
Fragment 9	−	−	−	−	+	−	−	−	−	+	+	−	+	+	−	+	−	+	−	−	−
Part B[b]																					
Unambiguous																					
Fragment 2	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d					
Fragment 3	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d					
Fragment 4	d	d	d	d	d	d	d	d	d	d	d	i	d	d	i	i					
Fragment 5	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d					
Fragment 6	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d					
Quantitative																					
Fragment 1	d	d	d	d	d	?	?	d	d	d	d	?	d	d	?	?					
Fragment 7	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d					
Fragment 8	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d					
Fragment 9	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d					

[a] +, Intensely staining fragment; (+), less-visible fragment; −, no visible fragment; ?, no decision could be made on presence or absence of a product

[b] See Materials and methods

zygous F_1s (1 × 2, 1 × 3, 1 × 4, 4 × 1, etc.), but not in the two situations predicted to be homozygous for its absence (1 × 5, 5 × 1). For the quantitative polymorphisms in this example, one can see that the intensity of the F_1 pattern reflects the parental fragment with the greatest intensity (see band no. 8).

The genetic interpretation of the F_1 patterns for primer B6 is shown in part B of Table 1. In almost all of the results with this specific primer, one can see a complete dominance relationship that is exhibited in the F_1. The most intense fragment of the two parents is observed in the F_1 progeny, no matter which direction the cross was made. Less often, F_1 patterns were found that seemed to reflect a situation where the F_1 fragment intensity was intermediate between the intensity of the two parental fragments. For instance, fragment no. 4 exhibits an intermediate pattern in those F_1s where one parent contained the product and parent P5 did not. On the whole, few intermediate reactions occurred within the entire data set among the unambiguous polymorphisms, although this percentage increased slightly when the quantitative polymorphisms were examined (Table 2).

Obviously, the vast majority of the F_1 reactions can be explained by dominant inheritance (either complete or partial dominance). It should be noted, that 10 instances of apparent inconsistency were obtained when the observed 555 polymorphic situations of the reciprocal crosses were examined, but none of these

Table 2. Percentages[a] for different genetic interpretations of F_1 patterns (completely dominant and partial dominant), analyzed within $16F_1$s in comparison to their five parents after classifying the observed 111 polymorphisms into unambiguous versus quantitative classes

Class	Completely dominant	Partially dominant	Remaining
Unambiguous	95.2%	3.2%	1.5%
Quantitative	88.0%	5.0%	6.9%

[a] Since 111 × 16 cases were analyzed, 1% equals approximately 18 cases

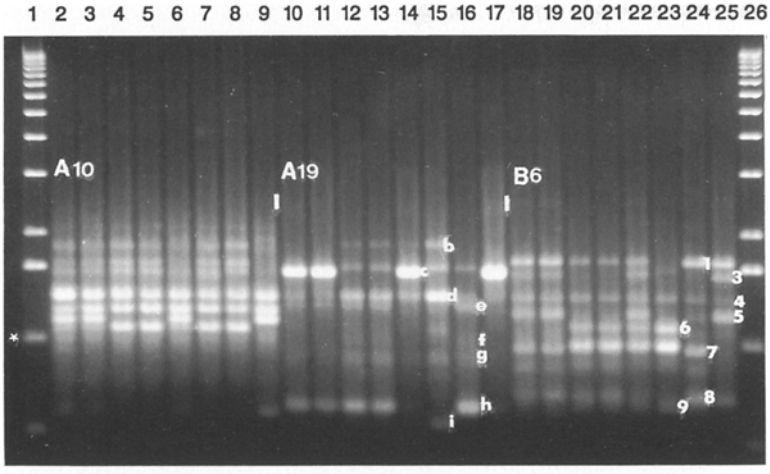

Fig. 2. RAPD analysis of 5 F_1s (3×4, 4×3, 3×2, 2×3, 4×2) and their three parents (2, 3 and 4) with primer A10 (*lane 2–9*), A19 (*lane 10–17*) and B6 (*lane 18–25*). The 1018-bp fragment of the 1-kb DNA ladder is marked by *. The scored fragments are denoted *1–9* for primer B6 and *a–i* for primer A19

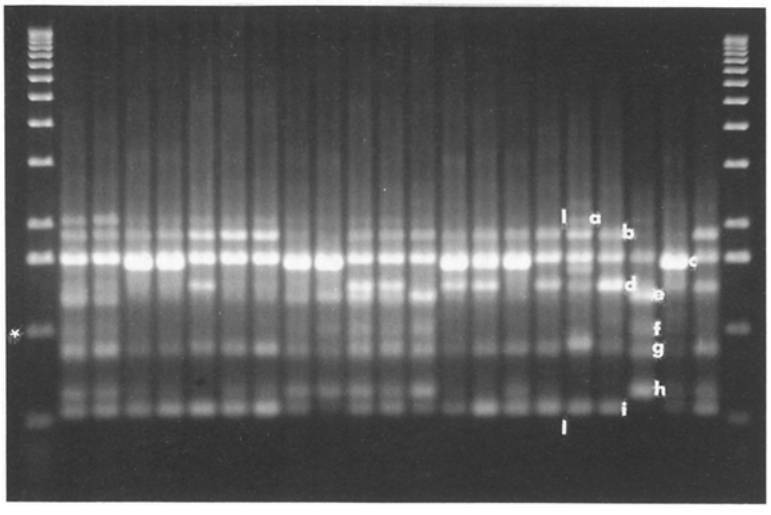

Fig. 3. RAPD analysis of 16 maize hybrids and their five inbred parents (arranged in the order given in Table 1) with primer A19. The 1018-bp fragment of the 1-kb DNA ladder is marked by *

were of the unambiguously polymorphic type. We were particularly interested in these, as we thought with this design we might detect cases of uniparental inheritance that might reflect the amplification of mitochondrial or plastid sequences. However, as none of these differences held true upon retesting, we eventually concluded that most of these reciprocal differences were due to the intrinsic problems associated with obtaining complete reproducibility with RAPD analysis.

Since 95.6% of all F_1 reactions were dominant (complete or partial) as expected and since 98.2% of the reciprocal F_1s (which might also be considered as a kind of internal standard) gave identical patterns, only a fraction of the whole data set was tested again. In Fig. 2 the results obtained with three primers (A10, not included in the data set, A19, and B6) and a partial set of five F_1s and the three parents involved (P2, P3, and P4) are shown to illustrate aspects of reproducibility with this type of analysis. It is evident that the results with primer B6 presented in Fig. 1 can also be seen in Fig. 2. This is true for both the unambiguous (see fragments 3, 5, and 6) as well as quantitative polymorphisms (see fragments 1 and 8). An interesting result is seen with A19, where in this case the strong fragment no. c of P4 seems to suppress the less pronounced fragments that are expected in the heterozygous F_1 situations.

Figure 3 shows the results obtained with all 21 genotypes analyzed with primer A19. The strong fragment of P4 again is very intense in this experiment, and the other fragments still appear to be affected but perhaps not as strongly in this particular experiment. The quantitative polymorphism of fragment no. i is quite visible in P1, P2, and P5 ('+'), whereas it is less intense in P4 ('(+)') and almost invisible in P3 ('((+))'). In Fig. 2, this fragment is barely detectable, although it is, as expected, visible in P2. The analysis of several

negatives with different exposure times helped to improve the reliability of the entire data set, but these faint amplification products are at the limits of RAPD analyses, and it is therefore reasonable to understand why the percentage of the 'remaining' category in Table 2 increased with the quantitative polymorphisms in relation to the unambiguous polymorphisms. We should add that this 'remaining' category contains a number of other instances where some products obviously 'out-complete' others in the amplification process.

Discussion

The purpose of this study was to develop data concerning the predictable expression of RAPDs in quite different genetic backgrounds. We did not concern ourselves with the intrinsic problems of achieving reproducible results with this type of analysis. Almost all investigators beginning work with this technology have encountered this issue but usually find that it can be solved with rigorous attention to detail. Here our concern was more one of attempting to understand why a particular fragment might not be expressed when the sequences which should insure its amplification are known to be present in the target. Our concern was not so much with the examination of maize F_1 hybrids in themselves, as to use this general diallel design to test how often one could expect fragments detected by this technique to follow the simple principles of inheritance. In order for a genetic marker to be useful, its expression must be predictable. The codominance of RFLPs and lack of epistatic interference in their expression have proven particularly advantageous in a number of applications. On the other hand, there seemed to be enough reasons to support concerns about the RAPDs' strategy, taken from the earlier observations that a significant percentage of them failed to segregate as expected. Most of these studies that raised questions concerning the reliability of RAPD markers were based upon their inheritance in single F_2 or BC populations that reflected no more than two genetic backgrounds. This type of observation has raised concerns amongst investigators who have also questioned whether a RAPD genetic map constructed in one population will have any relevance in any other population. Using the diallel type of design, one can specifically test for genetic background as a variable influencing inheritance of these types of polymorphisms. In particular, we wondered in a species as highly variable as maize how often patterns of amplification products would be recognizable across genetic backgrounds such that an investigator could use the data from one cross in beginning the analysis of a new cross. As this diallel was nearly complete, we were also able to ask questions as to whether any instances of uniparental inheritance were detected.

To identify enough polymorphisms, we used parental inbreds of different origin: B73, F2, and Mo13 are public lines with quite different pedigrees; G28 and Z51 are two unrelated, 'non-stiff stalk' inbreds developed by Pioneer. Additionally, this diversity meant that we were also able to test the amplification of all of the fragments in quite different genetic backgrounds, although at least 50% of the genetic complexity of any F_1 combination must still reflect the parental background where a fragment was first observed. As a mean value, 6.7 fragments were scored per primer and only 20.7% of them were nonpolymorphic, indicating that it is relatively easy to identify polymorphisms with this type of genetic marker in our material. This is not totally surprising as maize is high in sequence variation as determined by RFLP analysis and sequence comparison (Shattuck-Eidens et al. 1990). It appears from our studies and inferences from other investigations to date that RAPD analysis possesses about the same ability to detect sequence polymorphism as RFLP analysis, but the use of random primers and the rapidity of the analysis insure that RAPD analysis of unknown germ plasm can be a more efficient process for detecting polymorphic situations, particularly in species where no pre-existing set of RFLP clones is available.

Several observations from this study reflected quite positively on RAPDs as genetic markers. We never observed a fragment in any of the F_1s that was not observed in at least one of its parental inbreds, as was true of studies by Riedy et al. (1992). We also never detected the absence of any fragments in F_1s that were unpolymorphic within the parental inbred set. Of the polymorphic fragments, greater than 90% appeared to be inherited as expected in the F_1 generation. That we did not check the F_2 generation for any of these particular hybrid combinations means that our estimate of the percentage of 'reliable' RAPD fragments should probably be viewed as an upper estimate rather than a lower one. For instance, if a fragment was the summation of the amplification of a number of indentical but randomly-dispersed sequences found in one inbred but not another, then its presence or absence would not segregate as a single locus in the F_2-but this type of anomaly would not be detected within the F_1 generation. This might explain why our value of non-Mendelian inheritance (10%) was lower than that observed in some of the earlier cited studies in which it ranged as high as 40%.

One explanation for the aberrant inheritance that one might see in segregating populations would be the uniparental inheritance of a fragment amplified from one of the organellar genomes. Interestingly, we never

detected a single case of reproducible uniparental inheritance in this study, although there were numerous situations where this could have occurred. This could be due to the low contribution of mitochondrial or plastid genomes to the sequence complexity presented when using total DNA as the substrate. Alternatively, there may be fewer cases of the type of inverted repeat sequences apparently necessary for single primer amplifications in these genomes than in the nuclear genomes. The exact reason for the absence of this type of observation is still not clear from our studies but it can probably be assumed not to be a major factor in apparent non-Mendelian inheritance of RAPDs.

In general, patterns were generally recognizable across genetic backgrounds, and individual fragments that appeared to be inherited in Mendelian fashion in one F_1 also appeared to be inherited appropriately in all of the other F_1s. In a sense, the majority of fragments were found to act as expected, and those that did not seemed to repeatedly exhibit this aberrant behavior. This general result is a positive one for those using or contemplating using RAPD analysis, as it suggests some general rules for both the applications of this technology and the interpretation of its results. The most predictable types of RAPD fragments, as might be expected, were those of the greatest intensity in any one reaction. 'Minor' fragments seemed to possess the greatest propensity for irreproducibility. Using more than the 3–4 most intense fragments in any one reaction as a marker may be asking too much of this technology, where it appears that the end products represent a competition amongst many candidate sequences. The use of unambiguous or two-allele polymorphisms preferentially over 'quantitative' polymorphisms would also seem to improve the reliability of RAPD analysis. Nevertheless, since many of our unexpected results could be attributed to the 'competition' amongst fragments where one very intense product seemed to outcompete and overwhelm other expected fragments, even those of very high yields in inbred situations, one should use caution with certain applications. Once the Mendelian inheritance of a specific RAPD fragment has been verified within an F_2, its use in linkage analysis is probably quite reproducible. The use of RAPDs to 'target' particular areas of the genome by pooling strategies would also seem to be a very realistic use of this technology. On the other hand the use of RAPDs to define 'fingerprints' of individual genotypes should be viewed with some caution. Obviously, since the presence of some fragments can interfere with the amplification of others, the absence of these latter fragments from some genotypes cannot assure the absence of their sequences, which might 'reappear' in segregating situations if the primary fragment is allowed to segregate away. Studies of the relationship amongst isolates of germ plasm or, even more critically, the fingerprinting of isolates for patent protection purposes will be most sensitive to these types of RAPD anomalies, and further study is clearly warranted before relying too heavily upon this single strategy.

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