Theor Appl Genet (1994) 89:998–1006

M. Gupta · Y.-S. Chyi · J. Romero-Severson · J. L. Owen

Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats

Received: 7. July 1994 / Accepted: 28. July 1994

Abstract The abundance and scattered distribution of simple-sequence repeats (SSR) in eukaryotic genomes prompted us to explore the use of SSR-based oligonucleotide primers in single primer amplification reactions. In a pilot experiment, 23 primers were used across a panel of evolutionarily diverse eukaryotic genomes, including grapes, lettuce, tomato, pine, maize, salmon, chicken, Holstein cows and humans. The primers were 16–20 bases in length and represented SSRs of di-, tri-, tetra-, and pentanucleotide repeats. The results showed that tetranucleotide repeat primers were most effective in amplifying polymorphic patterns. Of 11 such primers tested, 70% produced polymorphic patterns from the DNA of one or more species. Primers representing a combination of two tetranucleotide repeats, or compound microsatellites, were equally effective. The polymorphisms contained in such fingerprints were able to identify individuals of vertebrate species as well as lines or varieties of plants. Inheritance of the polymorphic bands was studied in a maize recombinant inbred population, DE811 × B73. Thirty-two polymorphic bands, derived from two amplification patterns, were mapped as dominant markers on an existing RFLP map of the same population. The bands were distributed across nine of the ten chromosomes.

Key words Simple-sequence repeats · Amplification DNA markers · Plants · Vertebrates

Introduction

The ease of obtaining and screening DNA markers is essential to efficient marker-assisted breeding programs. Restriction fragment length polymorphisms (RFLPs) have proven successful both in the construction of genetic linkage maps (e.g., Helenjaris 1987; Bernatsky and Tansley 1986; Burr et al. 1988; McCouch et al. 1988; Liu and Tsunewaki 1991) and in marker-assisted breeding (Murray et al. 1991). Amplifying genomic DNA in single primer amplification reactions (SPARs) is a new way of discovering DNA markers, which is fast and simple. Of the three technologies based on similar principles, RAPD (Williams et al. 1990), DAF (Caetano-Anolles et al. 1991) and AP–PCR (Welsh and McClelland 1990; Owen and Uyeda 1991), RAPDs have been most widely used for developing genetic markers and for the construction of genetic linkage maps (Reiter et al. 1992).

Simple-sequence repeats (SSRs), or microsatellites, occur as tandem repeats of di-, tri-, tetra-, and penta-nucleotides and are ubiquitous in eukaryotic genomes. These repeats have been found interspersed in various combinations of two or more SSRs (compound SSRs) as well as with the other single and multicopy sequences (Hamada et al. 1984 a, Shafer et al. 1986; Epplen 1988; Martinez-Soriano et al. 1991). Often, they were singled out from other sequences of interest because of their association with a high level of polymorphism (Luty et al. 1990; Morral et al. 1991). Of the most studied repeats, $(GT)_n$, $(GA)_n$, $(GATA)_n$ and $(GACA)_n$, the copy number of $(GT)_n$ per haploid genome varies from 100 copies in yeast to 100000 copies in the mouse genome (Stallings et al. 1991). The copy number of the tetranucleotide repeat, $(GATA)_n$, is appoximately 10000 in the mouse genome (Shafer et al. 1986). It has been suggested for the human genome that one SSR occurs on average every 10 kb of DNA with a fairly random distribution (Tautz 1989). Various functional roles have been attributed to SSRs; for example, hot spots of recombination (Kobori et al. 1986; Bullock et al. 1986); the regulation and expression of genes (Hamada et al. 1984b; Shafer et al. 1986; Murphy et al. 1989), and sex determination (Singh et al. 1980).

A high level of SSR-associated polymorphism has been observed both in plants and vertebrates (Poulsen et al. 1993; Schmidt et al. 1993; Ali et al. 1986). PCR has re-

Communicated by J. S. Beckmann

M. Gupta (✉) · Y.-S. Chyi · J. Romero Severson · J. L. Owen[1]
Mycogen Plant Sciences, Madison Laboratories,
5649 E. Buckeye Road, Madison, WI 53714, USA

Present address:
[1] Life Technologies Inc., 8717 Grovemont Circle, Gaithersburg, MD 20884, USA

cently been used to detect variation in the number of repeats found within a repeat motif of different SSRs by designing primers flanking the motif. This strategy can reveal a single base-pair polymorphism and has proven successful in distinguishing individuals of vertebrate species (Fries et al. 1990; Love et al. 1990; Weber 1990). However, this approach is cumbersome, as it requires sequence information from the DNA regions flanking the repeat motif in order to design primers. As such, it cannot be applied rapidly to a new species.

Given the attraction of SSRs because of their abundance and distribution in eukaryotic genomes, we have designed primers based on SSRs and used them in SPARs of evolutionarily diverse eukaryotic genomes. These experiments indicate the value of a systematic designing of a series of universal primers that can be tested on species of interest.

The purpose of the present study was to evaluate the potential of SSR-based primers in amplifying DNA markers from plant and vertebrate DNAs. We have used DNA of pine, grapes, lettuce, tomato, maize, salmon, chicken, cow and human to demonstrate that such primers produce polymorphic band patterns from complex eukaryotic genomes. These polymorphic bands were heritable and can be used as DNA markers. Additionally, the markers were found to be scattered throughout the maize genome. Thus the use of primers which mimic SSRs are demonstrated here to rapidly and easily identify DNA markers useful in breeding programs and other genetic experiments.

Materials and methods

Genomic DNA

DNAs from the plants (except pine) and blood samples were isolated by the procedures of Saghai-Maroof et al. (1984) and Signer et al. (1988) respectively. Pine DNA was isolated from the needles of individual loblolly pine trees and the procedure will be described elsewhere (Gupta, in preparation). The trees were from different regions in the U.S. Lettuce DNA was prepared from individual Romaine, Iceberg, and Greenleaf lettuce heads, which were purchased from the local supermarket. Grape DNA was isolated from two different plants of one variety of Thompson grapes. The third grape sample was from a distinct variety, Thompson's "perlette". Tomato DNA was isolated from individual plants of Sweet 100, Early Girl, and yellow pear. Human DNA was isolated from the blood donated by three staff members. The individuals were of caucasian, Asian and Eastern European origins. Chicken and cattle (Holstein) DNAs were prepared from the blood of individuals of separate flocks or herds respectively. Salmon DNA was obtained from the blood of random fish collected in the Connecticut river of Northeastern U.S.

Primers

Oligonucleotide primers were synthesized either on a Cyclone DNA synthesizer (Milligen Biosearch) by the standard procedure or were obtained from Genosys Inc. San Diego, Calif. The oligonucleotides were subjected to cartridge purification.

SPAR conditions

Genomic DNA samples were amplified in a final volume of 30 µl containing 10 mM Tris-HCl, pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.2 mM each of dATP, dCTP, dGTP and dTTP, 1.0 µM primer, 30 ng genomic DNA, and 0.75 units of *Taq* DNA Polymerase (Perkin Elmer Cetus). Amplifications were performed in a Perkin Elmer Thermocycler for 35 cycles. Each cycle consisted of the following steps: 1 min at 95°C, 1 min at the annealing temperature specified for each primer in Table 1, and 5 min at 70°C. The melting temperature used was in each case an estimate based on an approximation. It was calculated by adding 2°C for A and T, and 4°C for G and C in a primer sequence (Berger and Kimmel 1987). All products were analyzed on 1.8% agarose gels (Seakem, GTG) and electrophoresed in 1.5 × Tris-acetate buffer (Sambrook et al. 1989) unless otherwise specified.

When amplified-DNA was analyzed on a polyacrylamide gel (PAG), 10 µC of ^{32}P-dATP (3000 Ci/mmole, NEN DuPont) was added to each 30-µl reaction described above. Four microliters of the amplified products were mixed with 6 µl of loading dye (0.5% bromophenol blue, 0.5% xylene cyanole and 20 mM EDTA in formamide), heated at 95°C for 5 min, and 2–4 µl loaded in each lane of a 5% denaturing PAG. The PAGs were cast between 30 cm×40 cm glass plates and 0.5 mm–1.5 mm wedged spacers and 2 x 25 well sharkstooth combs. The gels were electrophoresed in SR2 sequencingunits (Bethesda Research Laboratories, Gaithersburg, Md.), fixed in 10% acetic acid and 10% methanol, then dried and exposed to X-ray films.

Recombinant inbred population

Two-hundred maize recombinant inbred lines (RI) were developed by single-seed descent from a F_2 ear of DE811 × B73. Forty F_6 RI lines from this population were chosen at random for this experiment.

Results

Tetranucleotide repeat primers were most effective

In a pilot experiment, 23 SSR primer were used in SPARs to amplify DNA of evolutionarily diverse plant and animal species (Table 1). The dinucleotide-based primers amplified smears or no patterns. Forty-five percent of the trinucleotide-based primers amplified multiband patterns from most species; however, only a few species exhibited polymorphism with each of those primers. The tetranucleotide repeat primers were most effective in amplifying the polymorphic patterns. Seventy percent of the primers were useful across the species. A typical polymorphic pattern had several common bands and a few polymorphic bands (Fig. 1). All three samples of lettuce, maize, pine and tomato were distinguishable using this primer. Two grape varieties were also identifiable due to the presence of two extra bands in the "perlette" variety (lane 3). No polymorphism was visible between two plants of the same variety (lanes 1 and 2), as expected. Polymorphisms were also evident among chicken, cattle and human DNA samples. Some of the polymorphisms were subtle (close bands) and beyond the resolution capacity of the agarose gels. Overall, fewer polymorphisms were discovered among the vertebrate samples when compared to the plant samples, except in the case of the chicken. Human DNA amplified with $(TGTC)_4$ produced the most polymorphic pattern (3 out of a total of 12 bands).

Table 1 A summary of DNA patterns amplified by SSR primers in SPARs from plant and vertebrate DNAs. M.T.=melting temperature of oligonucleotides. **weak band patterns. 1 and 2=patterns polymorphic at 48°C and 43°C annealing temperatures. MB=multiband pattern; FB=few band pattern; P=polymorphic; S=smear; N=No Pattern

Primer sequence	Annealing temp.(M.T.)[a]	*Pine*	*Lettuce*	*Grape*	*Tomato*	*Maize*	*Salmon*	*Chicken*	*Cattle*	*Human*
$(GA)_{10}$	55 (60)	S	S	S	S	S	S	MB, S	MB, S	S
$(CA)_{10}$	55 (60)	S	S	S	S	S	S	S	S	S
$(AT)_{10}$	35 (40)	N	N	N	N	N	N	N	N	N
$(AGG)_6$	55 (60)	MB, S, P	MB, S	MB, P	MB	MB, S	MB, S	MB, P	MB, S	MB, S
$(CGG)_6$	55 (72)	MB, S	S	MB	MB, S	S	MB	MB, P	MB, P	MB
$(TGG)_6$	55 (60)	MB, S	S	FB, S	S	S	S	S	S	S
$(CGT)_6$	55 (60)	S	S	S	S	S	S	S	FB, S	S
$(GTG)_6$	45 (50)	MB, S	S	MB, S	MB	MB	FB, S	S	S	S
$(TCC)_5$	45 (50)	MB, S	MB, S	MB, P	MB	MB, P	S	MB, S	FB, S	MB, S
$(CAC)_5$	45 (50)	S	S	S	S	S	S	S	S	S
$(CAT)_5$	35 (40)	S	S	MB, S, P	S	S	S	S	S	S
$(CAT)_6$	45 (48)	S	S	S	S	S	S	S	S	S
$(GACT)_5$	55 (60)	MB, P	FB	N	N	S	S	MB, P	S	S
$(TGGA)_5$	55 (60)	MB	MB, P	MB, P	MB**	MB, P	MB,P,S	MB,P,S	MB, P	MB,S
$(TCTA)_5$	45 (50)	S	S	S	S	S	S	S	S	S
$(TGTC)_4$	45 (48)	MB, P	FB	MB, P**	MB**	FB, P	S	MB, P	MB, P	MB, P
$(ACTG)_4$	45 (48)	MB, P	MB, P	FB, P	MB, P	MB, P	MB	MB, P	MB	MB
$(GACA)_4$	45 (48)	MB, P	MB, P	MB, P	MB	MB, P	S	MB, P	MB, S	MB, S, P[1]
$(TTTA)_5$	35 (40)	N	FB	MB, P	N	N	MB, P	MB, P	MB, P	MB, P
$(TATC)_4$	35 (40)	S	N	N	S	N	S	N	N	N
$(GTAT)_4$	35 (40)	S	S	S	S	S	S	S	S	S
$(GATA)_4$	35 (40)	MB, P	FB, P	FB	MB, P	FB, P	FB	FB, P	FB	MB, P[2]
$(GACAC)_4$	55 (64)	MB, P	S	FB, P	MB, P	MB, P	MB,S	MB, P	MB	MB

[a] All temperatures in °C

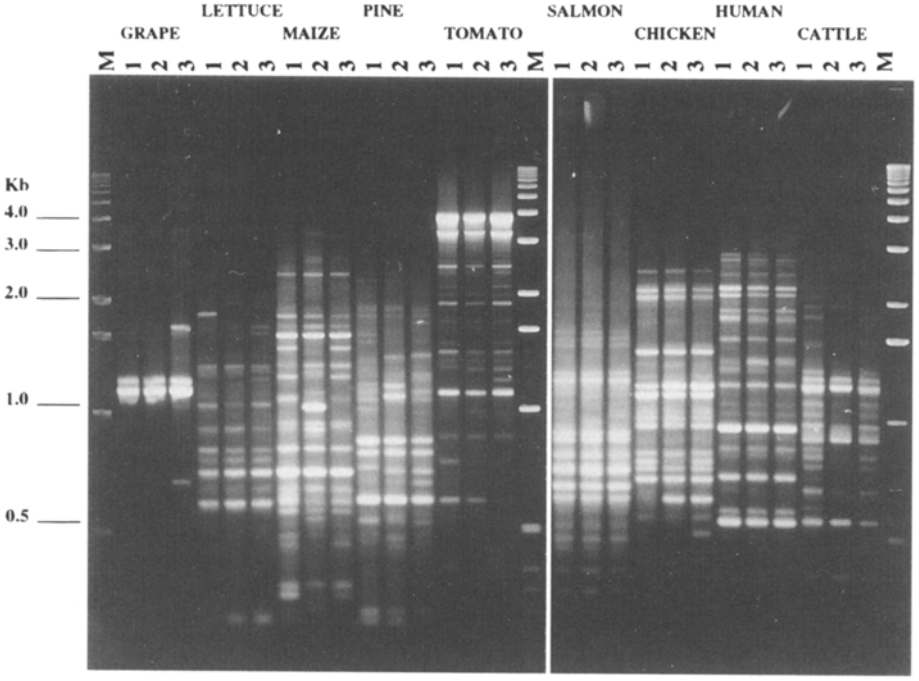

Fig. 1 An ethidium bromide-stained agarose gel showing DNA patterns amplified by primer $(ACTG)_4$ from the DNA of nine plant and vertebrate species. DNA samples in the numerical order for the each species were as follows. **Grape:** two vines of one variety of Thompson grapes and Thompson grape variety "perlette"; **Lettuce:** Romaine, Iceberg, and Greenleaf; **Maize:** A632, B73Htrhm, and A635; **Pine:** tree samples from different regions in the U.S; **Tomato:** Sweet 100, Early Girl, and yellow pear; **Salmon**, **chicken** and **cattle** (Holstein): three different individuals; **Human:** three individuals of caucasian, Asian and eastern European origins. *M* 1-kb ladder (BRL) as a DNA size standard; left side shows sizes of the standard DNA fragments

The patterns were reproducible among experiments and primer lots. Controls which did not contain genomic DNA were consistently clean. Polymorphic bands of low intensity were reproducible across samples. For example, in the tomato pattern (Fig. 1) the less intense band present in sample 3 above the 1-kb band was reproducible and included in the identification process.

The SPAR pattern varies with primer length

The length variants, 10-mer, 16-mer and 24-mer, of $(ACTG)_n$ and $(GACA)_n$ were designed to find the optimal primer length (Fig. 2). SPARs were performed at three annealing temperatures corresponding to three primer lengths. In experiments with $(ACTG)_n$, amplification with

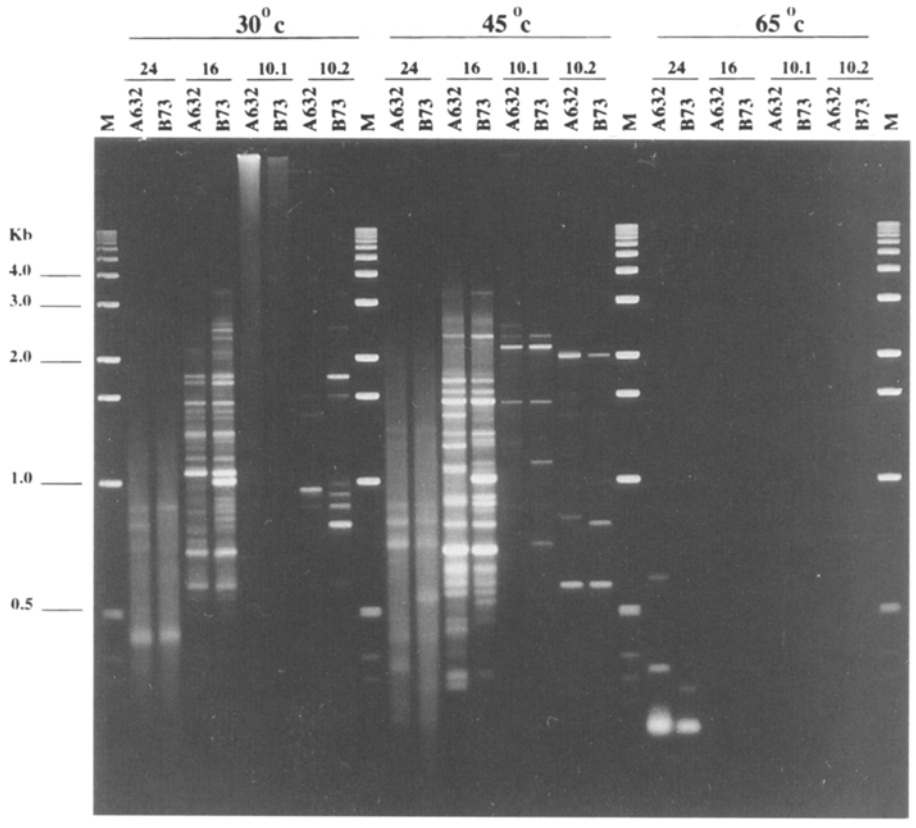

Fig. 2 Analysis of maize DNA patterns on an agarose gel amplified by length variants of primer $(ACTG)_n$. The maize samples, A632 and B73 are inbred lines. Primer 24; $(ACTG)_6$; Primer 16; $(ACTG)_4$; Primer 10.1; $(ACTG)_2AC$; Primer 10.2; $TG(ACTG)_2$. The primer annealing temperatures in the SPARs is shown on top of the figure. *M* 1-kb ladder (BRL) as a DNA size standard; left side shows sizes of the standard DNA fragments

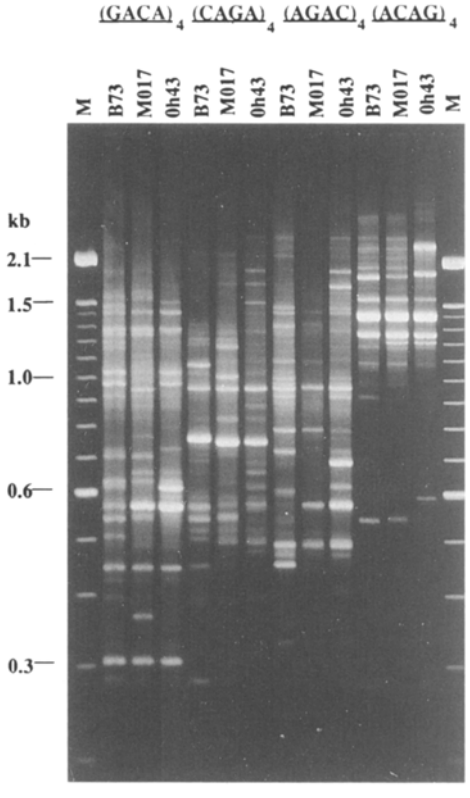

Fig. 3 Examples of DNA patterns obtained by sequence order-variants of SSR primers. DNA of the indicated maize inbred lines was amplified by the variants of primer $(GACA)_4$. The products were fractionated on a 3% agarose gel (NuSieve: BRL UltraPure agarose, 1:1) electrophoresed in TAE buffer as described in the Materials and methods. The amplification steps were 1 min at 95°C, 1 min at 50°C and 5 min at 70°C for 35 cycles. *M* 100 bp-ladder (BRL) as a DNA size standard. Fragment sizes are shown on the left side

the 16-mer length produced the most informative patterns at 45°C, the optimal annealing temperature for the length. Increased yields of DNA, as well as a greater number of polymorphisms, were observed at this temperature. A different and less intense pattern was obtained by the same primer at 30°C annealing, which could be due to non-specific annealing of the primer to the target sequences. The 24-mer produced a few bands at 60°C in the each sample. The two variants of 10-mers amplified some bands at 30 °C and 45 °C which although polymorphic were not easily discernible. The same result was obtained with a set of four $(GACA)_n$ variants (data not shown). Separate experiments with the length variants of two SSR primers proved that 16-mer is the optimal SPAR primer length for the tetranucleotide-based SSR primers.

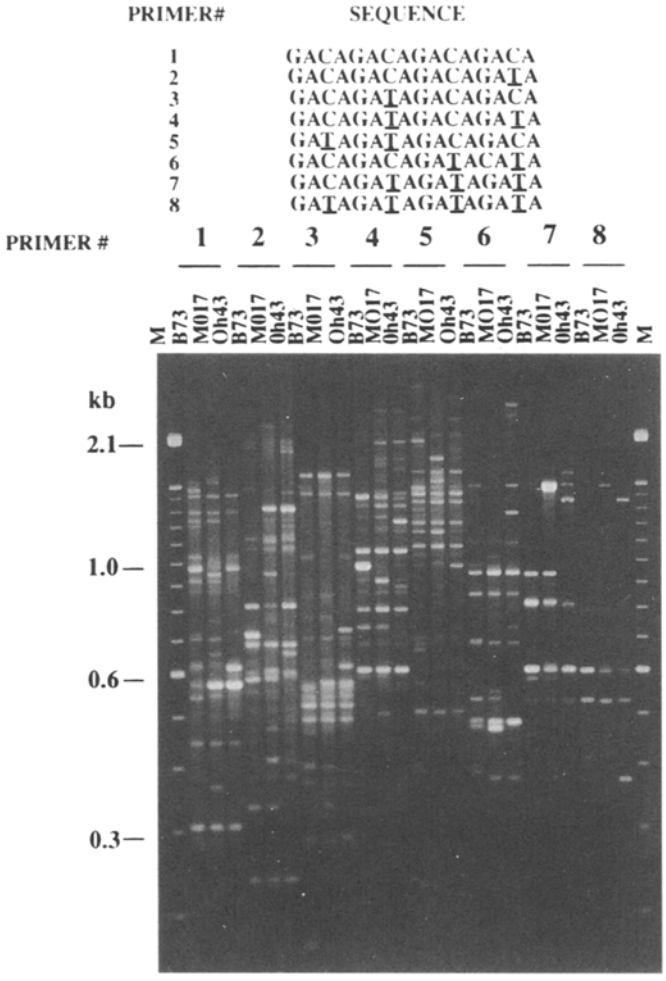

Fig. 4 Examples of maize DNA patterns amplified by compound SSR primers. Three maize inbred lines, B73, Mo17, and Oh43, used in the experiment, are shown. The amplification program was 35 cycles of 1 min at 95°C, 1 min at the annealing temperature described below and 5 min at 70°C. The annealing temperatures for the primers were as follows: 1 and 3, 48°C; 2,4 and 6, 44°C; and 5, 7 and 8, 43°C. The agarose-gel electrophoresis conditions are described in the legend of Fig. 3. The primers are listed on top of the photograph. The nucleotide T in the GATA sequence is *underlined*. *M* 100-bp ladder (BRL) as a DNA size standard

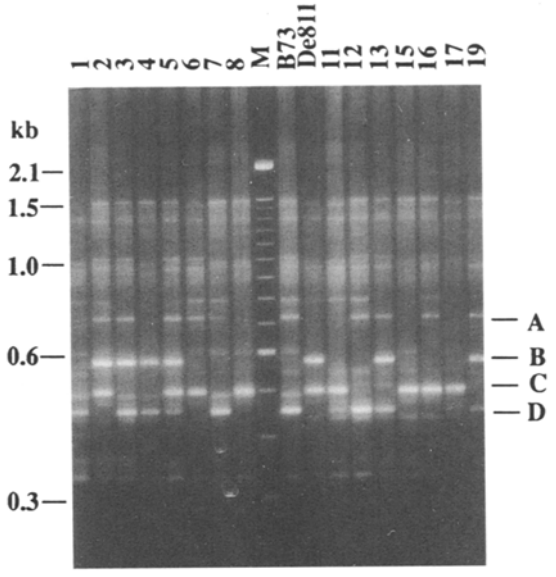

Fig. 5 Agarose-gel electrophoresis shows segregating bands in the samples of a recombinant inbred population (B73×De811) amplified by primer $(ACAG)_4$. *A–D* denotes bands that were unambiguously scorable from the gel. The gel electrophoresis conditions are described in the legend of Fig. 3. *M* 100-bp ladder as a DNA size standard

The SPAR pattern varies with the repeat sequence-order in primers

The possiblity of quickly obtaining polymorphisms for genetic mapping or fingerprinting was tested by designing three sequence-order variants of $(GACA)_4$ and $(GATA)_4$. All three variants of $(GACA)_4$ amplified polymorphic patterns from the maize DNA (Fig. 3). Each fingerprint was unique and equally polymorphic. No stutter bands were apparent. This confirmed that all primer variants were not priming from the same large genomic repeat. This was also true for fingerprints of the other species tested. Pine and tomato patterns exhibited polymorphisms with $(AGAC)_4$ and $(ACAG)_4$. Chicken and human DNAs amplified polymorphic patterns for all three variants. Similar results were obtained by using the repeat sequence-order variants of $(GATA)_4$ (data not shown). The results showed a high probability of amplifying polymorphic patterns from the sequence-order variants of a successful primer.

Compound SSR primers amplify polymorphic patterns

In one experiment a set of compound SSR primers were used in which $(GACA)_4$ was replaced with $(GATA)_4$ at various positions and with differing frequencies (Fig. 4). The DNA fingerprint amplified by each primer was unique. The primers were also tested on tomato, pine, and vertebrate DNAs. The useful primers were as follows: 3, 4 and 6 for tomato; 5, 6, 7 and 8 for pine; 2, 5, 6, 8, for human; and 1, 3, 4, 6, 7 and 8 for chicken. The results showed that compound SSR-based primers were useful. Other combinations of two or more SSRs may also be informative.

SSR-based SPAR bands are heritable markers

DNA of 40 maize RI from DE811 × B73 was amplified with SSR primers and the patterns were analyzed for the segregation of polymorphic bands. In the first experiment, primer $(ACAG)_4$ was used and the amplification products were fractionated on an agarose gel. A large number of bands were observed but few polymorphic bands were scorable. In order to test if better resolution of the same

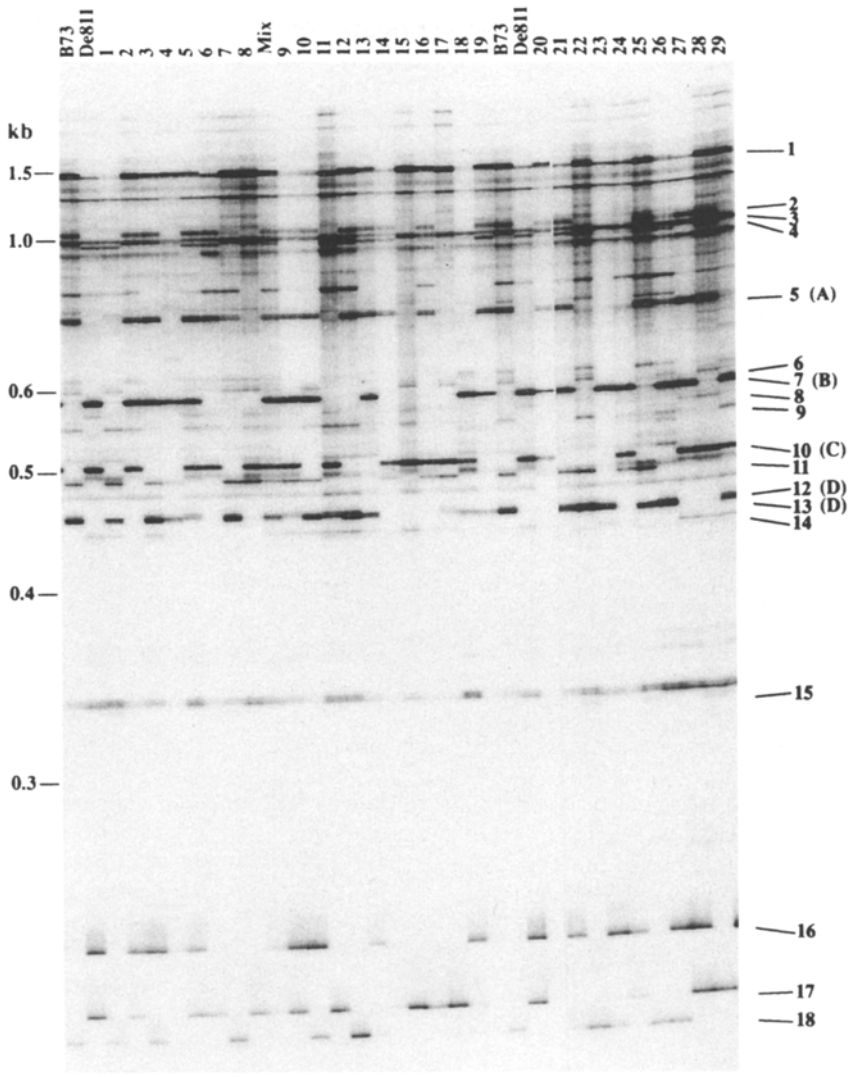

Fig. 6 Analysis of the segregating band patterns on a 5% polyacrylamide gel amplified by primer $(ACAG)_4$ from samples of a recombinant inbred population B73×De811. B73 and De811 are the parental sample lanes. "Mix" represents a 1:1 mixture of the amplified samples of the parental DNAs. Twenty-nine of the forty samples used in the study are shown. Sample numbers and bands *A–D* in Fig 5 correspond with the same in this figure. Left side: shows DNA molecular weights; right side: denotes *bands 1–18* that were scored for the mapping study

pattern would provide more polymorphic bands, amplification was performed in the presence of alpha-^{32}P (see Materials and methods) and the patterns were fractionated on a 3% agarose gel and a 5% polyacrylamide gel (PAG). Only four bands (A–D) were unambiguously scorable on the agarose gel (Fig. 5). However, the PAG showed many more bands equal in intensity to bands A–D (Fig. 6). Bands less than 300 bases in size that were undetectable on the agarose gel were clearly scorable on the PAG. A total of 18 unambiguous dominant bands segregating across the lines were scored. Eleven bands were from the B73 parent and seven from the DE811 parent. Comparing the resolution of bands on the PAG with that of agarose gel, bands A and D resolved into two and three bands respectively on the PAG. Band A is a doublet and both bands were segregating together. This is due to differential migration of the antiparallel strands of DNA in the denaturing PAG. Band D resolved into three bands. The top and bottom bands were scored; each was segregating in combination with the middle band. These two bands were mapped to two loci on chromosome 5 and were more than 10 map units apart (see below).

Amplification of the population DNA with a second primer, $(GATA)_2$ $(GACA)_2$, followed by separation of the products on a 5% PAG, yielded 15 scorable bands. Only three were scorable on an agarose gel (data not shown). Six bands were from the B73 parent and nine from the De811 parent. Again, some single bands on the agarose gels resolved into more than one band on the PAG.

Thirty-two of the thirty-three scored bands were mapped as dominant bands on a previously constructed De811 × B73 RFLP map containing 158 markers (unpublished, Fig. 7). One band, band 1 in Fig. 6, could not be mapped because it consisted of 2–3 closely spaced bands and needed further resolution. The mapped bands were scattered on nine out of ten maize chromosomes. (Fig. 7). Sixteen markers were distributed at a distance of more than 10 map units and 13 at less than 5 map units. Six were codominant markers. More precise map distances, and the al-

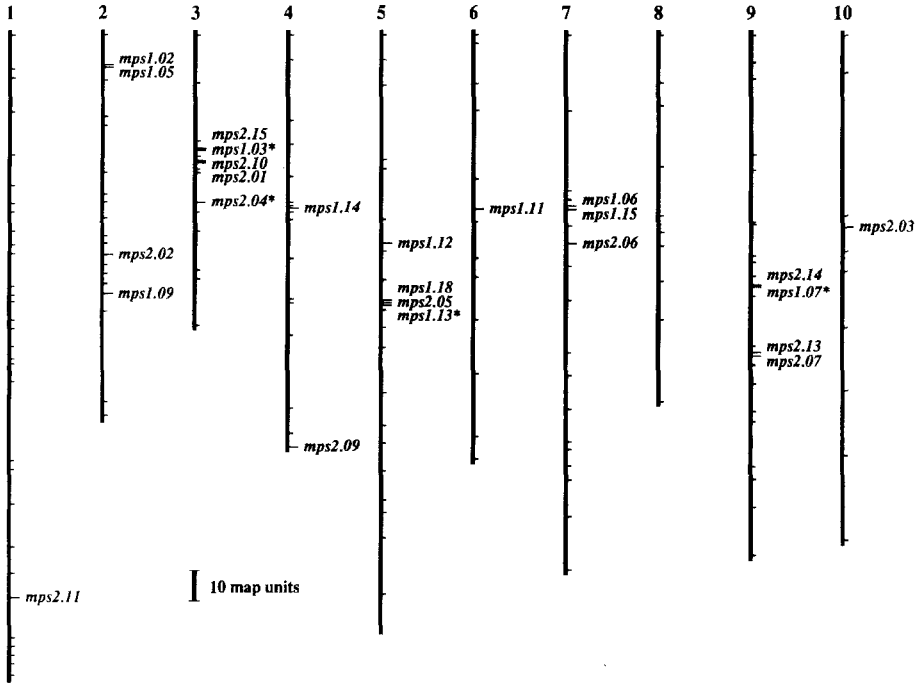

Fig. 7 A maize RFLP linkage map constructed with the recombinant inbred population from B73×DE811, displaying the distribution of the DNA markers, mps 1.01–mps 1.18, amplified by $(ACAG)_4$, and the DNA markers, mps 2.01–mps 2.15, amplified by $(GATA)_2$ $(GACA)_2$. *Short lines* on the chromosomes represent the locations of the 158 RFLP markers. An *asterisk* on the markers shows that either codominant marker was detected or else that other markers mapped at the same locus. These markers are described as follows. mps1.03: mps1.04; mps2.04: mps2.12; mps1.13=mps1.18: mps1.08=mps1.10=mps1.17; mps1.07=mps1.16=mps2.08. A *colon* between two markers represents codominant markers and an *equal sign* shows markers mapped to the same locations

lelic relationship between the markers, could be inferred by including more RI in the study. Overall, the ease of amplifying a large number of markers at once, makes SSR-SPAR useful for genetic experiments.

Discussion

The results demonstrated that single primers based on SSRs amplify reproducible and heritable polymorphic bands from evolutionarily diverse eukaryotic species. The observed level of polymorphism can be related to the genomic diversity within a species. Primers based on quadruplet repeats were most effective in amplifying DNA markers. No single primer showed polymorphisms in every species. However, there were certain primers, e.g., $(GACA)_4$ and $(ACTG)_4$, which amplified polymorphic fingerprints from all the plant species. Apparently, 16 bases is the length of tetranucleotide SSRs that occurs most frequently in a configuration required for SPAR. This may be either by chance or due to a functional significance which leads to genome rearrangements. Over half of the trinucleotide-based and all dinucleotide-based primers were ineffective and the smears obtained may reflect their reported abundance in the genomes. Such patterns may be the result of amplification when complementary strands of the repeats anneal to each other and form concatamers, as was observed in the amplification of minisatellite sequences (Jeffreys et al. 1988). Recently, it has been reported that specific patterns from dinucleotide repeat primers can be obtained in SPAR by anchoring 2–4 bases either at 3′ or 5′ termini of the primers (Zietkiewicz et al. 1994). This strategy reportedly works for most eukaryotes. However, in our experience, primers representing very high copy number sequences can amplify artifactual maize DNA fingerprints. Such fingerprints must be rigorously tested for reproducibility.

No constraints of%GC content were followed in designing the primers. Primers with no GC content, e.g., $(TTTA)_4$, and primers with 25% GC content, e.g., $(GATA)_4$, amplified patterns that showed discrete bands on ethidium bromide stained agarose gels. It was thought that plant genomes are proportionately higher in AT content, and that primers rich in AT content would specifically target AT-rich regions.

In the first experiments described here, amplified products were fractionated on agarose gels. However, agarose gels have limited resolution in the low-molecular-weight range in which most amplified bands fell (0.2–2.0 kb). Higher-percentage agarose gels (Nusieve agarose gels) improved resolution, but still proved unsatisfactory for the resolution of unrelated overlapping bands. Insufficient resolution could be one of the causes of reported failure to map a large percentage of bands (Reiter et al. 1992). Information was increased many fold by fractionation on PAG. Although selection of the resolution matrix remains a laboratory choice, it is clear that the ease of use of agarose carries a penalty in information.

An attractively large number of markers per reaction were amplified and mapped on the maize chromosomes. Both parents contributed to an equal number of markers (17 and 16) indicating no genotype-specific amplification by SSRs. This feature is indispensible for using DNA markers in mapping experiments. Despite the fact that

the majority of markers are fixed in a recombinant inbred population, amplification was able to detect heterozygous markers. This was expected and is not an artifact of SPAR.

The majority of the markers that were present at a distance of less than 10 map units were observed in clusters. By comparing cytogenetic and RFLP linkage maps (data not shown), the clusters on chromosomes 3, 5, 7 and 9 were found to be located close to centromeres. Whether such proximity was coincidental or real remains to be explored.

SSRs allow a systematic rationale for the designing of primers which can be used as a universal set across genomes of interest. There are $(4)^4$=256 potential motifs of quadruplet repeats in the genome. Likewise, there are $(4)^5$=1 024 possible combinations of penta-nucleotide repeats. Only one primer of this class was studied, and even that produced polymorphic DNA fingerprints. Moreover, combinations of various di-, tri-, tetra- and penta-nucleotide repeats in compound sequence primers offer an unlimited possibility for designing primers.

In summary, SPAR using SSR-based primers is a very useful system for efficient retrieval and analysis of genetic information in eukaryotes. The system has an advantage over conventional RAPDs in that more polymorphic bands per reaction are amplified.

Acknowledgements We are grateful to Dr. Mike Murray for encouragement and support during the course of this study. We thank Dale Park and Mary P. Hall for the photographic assistance.

References

Ali S, Muller CR, Epplen JT (1986): DNA fingerprinting by oligonucleotide probes specific for simple repeats. Hum Genet 74: 239–243

Berger SL, Kimmel AR (1987) Guide to molecular cloning techniques. Methods Enzymol 152:435–437

Bernatzky R and Tansley SD (1986) Toward a genetic map in tomato based on isozymes and random cDNA sequences. Genetics 112:887–898

Bullock P, Miller J, Botchan M (1986) Effects of poly[d(pGpT) . d (pApC)] and poly[d(pCpG) . (dpCpG)] repeats on homologous recombination in somatic cells. Mol Cell Biol 6:3948–3953

Burr B, Burr FA, Albertsen MC, Stuber CW (1988) Gene mapping with recombinant inbreds in maize. Genetics 118:519–526

Caetano-Anolles D, Bassam BJ, Gresshoff PM (1991) DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. Biotechnology 9:553–557

Epplen JT (1988) On simple repeated GA^TCA sequences in animal genomes a critical reappraisal. J Heredity 79:409–417

Fries R, Eggen A, Stranzinger G (1990) The bovine genome contains polymorphic microsatellites. Genomics 8:403–406

Hamada H, Petrino MG, Kakunaga T, Siedman M, Stroller, BD (1984 a) Characterization of genomic poly(dT-dG) . poly(dC-dA) sequences: structure, organization and conformation. Mol Cell Biol 4:2610–2621

Hamada H, Seidman M, Howard B, Gorman CM (1984 b) Enhanced gene expression by the poly(dT-dG) . poly(dC-dA) sequence. Mol Cell Biol 4:2622–2630

Helentjaris T (1987) A genetic linkage map for maize based upon RFLPs. Trends Genet 3:217–221

Jeffreys AJ, Wilson W, Neumann R, Keyte J (1988) Amplification of human minisatellites by the polymerase chain reaction towards DNA fingerprinting of single cells. Nucleic Acids Res 16: 10953–10971

Kobori JA, Strauss E, Minard K, Hood, L (1986) Molecular analysis of the hotspot of recombination in the murine major histocompatibility complex. Science 234:173–179

Liu Y-G, Tsunewaki K (1991) Restriction fragment length polymorphism (RFLP) analysis in wheat. II. Linkage maps of the RFLP sites in common wheat. Jpn J Genet 66:617–633

Love JM, Knight AM, McAleer MA, Todd JA (1990) Towards construction of a high-resolution map of the mouse genome using PCR-analyzed microsatellites. Nucleic Acids Res 18:4123–4130

Luty JA, Guo Z, Willard HF, Ledbetter DH, Ledbetter S, Litt M (1990) Five polymorphic microsatellite VNTRs on the human X chromosome. Am J Hum Genet 46:776–783

Martinez-Soriano JP, Wong WM, Van Ryk DI, Nazar RN (1991) A widely distributed "CAT" family of repetitive DNA sequences. J Mol Biol 217:629–635

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tansley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

Morral N, Nunes V, Casals T, Estivill X (1991) CA/GT microsatellite alleles within the cystic fibrosis transmembrane conductance regulator (CFTR) gene are not generated by unequal crossing-over. Genomics 10:692–698

Murphy GL, Connell TD, Barritt DS, Koomey M, Cannon JG (1989) Phase variation of gonococcal protein II. Regulation of gene expression by slipped-strand mispairing of a repetitive DNA sequence. Cell 56:539–547

Murray MG, Chyi Y-S, Cramer JH, DeMars S, Kirschman J, Ma Y, Pitas J, Romero-Severson J, Shoemaker J, West DP, Zaitlin D, (1991) In: Herrmann RG, Larkins B (eds) Application of restriction fragment length polymorphism to maize breeding. Plant Molecular Biology. Plenum Press, New York, pp 249–261

Owen JL, Uyeda CM (1991) Single primer amplification of avian genomic DNA detects polymorphic loci. Animal Biotech 2:107–122

Poulsen GB, Kahl G, Weising K (1993) Abundance and polymorphism of simple repetitive DNA sequences in *Brassica napus L.* Theor Appl Genet 85:994–1000

Reiter RS, Williams JGK, Feldman KA, Rafalski, A, Tingey SV, Scolnik PA (1992) Global and local genome mapping in *Arabidopsis thaliana* by using recombinant inbred lines and random amplified polymorphic DNAs. Proc Natl Acad Sci USA 89: 1477–1481

Saghai-Maroof MA, Soliman KM, Jergensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphism in barley Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci 81 USA: 8014–8018

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Schmidt T, Boblenz K, Metzlaff M, Kaemmer D, Weising K, Kahl G (1993) DNA fingerprinting in sugar beet (*Beta vulgaris*) – identification of double-haploid breeding lines. Theor Appl Genet 85: 653–657

Signer E, Kuenzle CC, Thomann PE, Hubscher U (1988) DNA fingerprinting: improved DNA extractions from small blood samples. Nuceic Acids Res 16:7738

Singh L, Purdom IF, Jones KW (1980) Sex chromosome-associated satellite DNA evolution and conservation. Chromosoma 79: 137–157

Shafer R, Boltz E, Becker A, Bartels F, Epplen JT (1986) The expression of the evolutionary conserved GATA/GACA repeats in mouse tissues. Chromosoma 93:496–501

Stallings RL, Ford AF, Nelson D, Torney DC, Hildebrand CE, Myozis RK (1991) Evolution and distribution of $(GT)_n$ repetitive sequences in the mammalian genome. Genomics 10: 807–815

Tautz D (1989) Hypervariability of simple-sequences as a general source for polymorphic DNA markers. Nucleic Acids Res 17: 6463–6461

Weber J (1990) Human DNA polymorphisms based on length variations in simple-sequence tandem repeats. Genome analysis vol. 1: genetic and physical mapping. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp 159–180

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18:7213–7218

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymophisms amplified by arbitrary primers are useful genetic markers. Nucl Acid Res 18:6531–6535

Zietkiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple-sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20:176–183