Theor Appl Genet (1995) 91:582–588

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Template mixing: a method of enhancing detection and interpretation of codominant RAPD markers

Received: 5 October 1994 / Accepted: 10 February 1995

Abstract Ten codominant RAPD markers, ranging in size from about 300 to about 1350 bp, were identified in mapping populations of chickpea (*Cicer arietinum* L.) and diploid strawberry (*Fragaria vesca* L.). A distinguishing feature of all ten markers, and perhaps of codominant RAPD markers in general, was the presence in heterozygous individuals of a non-parental, heteroduplex band migrating more slowly than either of the respective parental bands. This non-parental band could also be generated by mixing parental DNAs before PCR (template mixing). As a means of identifying primers likely to detect codominant RAPD markers, parental and mixed-template (parent-parent) PCR-product gel lanes were compared for 20 previously untested RAPD primers (10-base oligomers). Four primers that produced a total of five non-parental, heteroduplex bands in mixed-template reactions were selected, and then used to detect a total of five segregating, codominant markers and nine dominant markers in the respective F_2 mapping population, a codominant marker frequency of 35.7%. When closely migrating fast and slow bands of codominant RAPDs were difficult to differentiate, parent-progeny template mixing was used to deliberately generate heteroduplex bands in fast- or slow-band F_2 homozygotes, respectively, allowing confirmation of marker phenotype.

Key words RAPD markers · Codominant · Template mixing · Heteroduplex DNA · Genome mapping

Introduction

RAPD (random amplified polymorphic DNA) markers are electrophoretic band polymorphisms generated by PCR amplification of genomic DNAs from different sources using short (usually 10-mer) oligonucleotide primers of arbitrary sequence (Welsh and McClelland 1990; Williams et al. 1990). Despite incomplete knowledge of the molecular basis of RAPD marker variation, the RAPD assay has become generally accepted as a powerful and economical tool for plant-genome mapping (Tingey and del Tufo 1993).

RAPDs are commonly inherited as dominant markers, where the presence of a particular band is dominant, and its absence is recessive (Tingey and del Tufo 1993). For most presence/absence banding polymorphisms, absence of a band can be attributed to the loss or alteration of one or both of the opposed pair of primer-binding sites needed to produce the PCR product. Alternatively, an insertion between the primer-binding sites may place them at a distance too great to allow amplification. In either case, the absence of a band effectively constitutes a recessive, null allele.

Codominant RAPD markers are comparatively rare. They have been identified when manifested as two bands of differing gel mobilities that exhibit complete, repulsion-phase linkage in a segregating population (e.g., Schulz et al. 1994). Such PCR-product length polymorphisms can be explained in terms of one or more insertions/deletions between the primer-binding sites in one DNA source as compared to another. As with other types of codominant markers, codominant RAPDs can be of particular value for purposes of linkage analysis because they provide maximum linkage information per individual in segregating populations (Allard 1956). The dominant inheritance of most RAPDs is considered a disadvantage of RAPDs as compared with isozymes and RFLPs, both of which are usually codominant in inheritance. Accordingly, it would be advantageous to be able to increase the detection of codominant RAPDs.

Dominant RAPD markers can sometimes be converted into codominant SCAR (sequence characterized amplified region) markers (Paran and Michelmore 1993). This method requires cloning and partial sequencing of the RAPD marker, allowing the design of

Communicated by J.S. Beckmann

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unique, elongated "SCAR primers" for each end of the marker. In some cases, the SCAR primers will produce a product of distinctive length (i.e., a product length polymorphism) in the crossing parent that carries the null RAPD allele (Paran and Michelmore 1993). Conversion of dominant RAPDs to codominant SCARs can be advantageous for specific purposes; however, SCAR markers require much more effort and expense to develop than do RAPDs.

Through our mapping experience with F_2 populations in chickpea (*Cicer arietinum* L.) and diploid strawberry (*Fragaria vesca* L.), we have encountered and characterized several codominant RAPD markers. A common feature associated with such markers has been the presence of a non-parental, heteroduplex band in heterozygous individuals (Davis et al., unpublished data[1]). Reports of similar observations have appeared (Hunt and Page 1992; Ayliffe et al. 1994). In the present report, we provide evidence that heteroduplex bands are a common, and perhaps diagnostic, feature of codominant RAPD markers. Furthermore, we describe methods for enhancing the identification and interpretation of codominant RAPDs by means of PCR template mixing.

Materials and methods

Genetic material

Mapping populations used to identify RAPD markers included F_2 populations of the cross *Cicer arietinum* (chickpea) accession ICC640 × *C. reticulatum* accession 489777, and *Fragaria vesca* cross 'Baron Solemacher' × WC6 (mapping results to be presented elsewhere). Where appropriate, segregating marker pairs were tested for linkage using the LINKAGE-1 computer program (Suiter et al. 1983).

DNA isolation/methodology

Genomic DNA was isolated from chickpea and strawberry using a mini-prep method, modified from Torres et al. (1993), and differing between the two species only in the initial tissue-extraction step. One-to-three unexpanded chickpea leaves or shoot buds were extracted by grinding for 1 min in 40 µl of CTAB buffer [100 mM Tris HCl, pH 8.0, 1.4 M NaCl, 20 mM Na_2-EDTA, 2% CTAB (hexadecyltrimethylammonium bromide), 0.4% β-mercaptoethanol (added immediately before use)] in a microfuge tube using a plastic pestle (Kontes), and then adding 460 µl of buffer and grinding for an additional 10 s. One or two unexpanded strawberry leaves were first ground to powder in liquid nitrogen using a ceramic mortar and pestle, then to a slurry upon addition of 1 ml of CTAB buffer. The slurry was transferred into a microfuge tube containing 100 µl of 24:1 chloroform:octanol. Tubes containing chickpea and strawberry tissue extracts were incubated for 1 h at 60 °C, then subjected to chloroform extraction as described (Torres et al. 1993), followed by ethanol precipitation. DNA was pelleted, dried, resuspended in 50 µl of TE (10 mM Tris HCl, pH 8.0, 1 mM Na_2-EDTA), diluted with water containing RNase A (10 µg/ml), incubated at 37 °C for 1h, quantified, diluted with water to a concentration of approximately 25 µg DNA/ml, then stored at 4 °C.

[1] A description of the CS47a/b codominant RAPD marker was presented in poster form (poster #33: use of RAPD tags to map chickpea root-nodulation genes) at the Plant Genome I conference, San Diego, Calif., Nov. 9–11, 1992

Selected RAPD bands were excised from the gel using either a GeneClean II kit (Bio 101, Inc.) or a Millipore Ultrafree -MC 0.45 µm filter unit, then cloned using the TA Cloning™ Kit (Invitrogen) according to manufacturer's specifications. Wizard™ Minipreps (Promega) were used to isolate recombinant plasmids from transformed *E. coli* cells. Inserts were sequenced by the University of New Hampshire core facility using an Applied Biosystems 373A Automated Sequencer. The NEBlot™ Phototope™ Kit (New England Biolabs) was used for Southern-blot analysis. DNA was transferred from a 2% TBE gel to an Immobilon™-S membrane (Millipore) via capillary action. The probed membrane was exposed to X-ray film for up to 4 h.

PCR conditions

PCR reactions (25 µl) contained reaction buffer, 100 µM of each deoxynucleotide, 0.75 U of *Taq* DNA polymerase, 3.0 mM of $MgCl_2$ (reagents supplied by Promega), 0.4 µM of primer, and 100 ng of template DNA. PCR was performed using either a Perkin-Elmer Cetus, Precision Scientific, or Thermolyne thermal cycler. The PCR profile consisted of 1-min denaturation at 94 °C, 2-min annealing at 34 °C, and 2-min extension at 72 °C. Each thermal cycler was programmed as necessary to achieve slow ramp times from the denaturation to the annealing temperature (approximately 2.5 min), and from the annealing to the extension temperature (approximately 1.0 min). After 40 cycles, the final extension period was extended by 7 min before terminating the PCR by slowly lowering the temperature to 5 °C. PCR products were electrophoresed on 2% TBE gels made with 1:1 standard:low-melt (NuSieve GTG) agarose.

DNA mixing experiments consisted of mixing equal amounts of template DNAs from two sources prior to PCR (template mixing), or of mixing equal volumes of separately generated PCR products (with or without 1 min of subsequent heating to 94 °C then gradually cooling, to achieve denaturation/renaturation) prior to electrophoresis (product mixing). In all product-mixing experiments, a subsample of each reaction product was electrophoresed separately prior to mixing to assure that the product was present.

RAPD primers were obtained from the University of British Columbia Biotechnology Center (Vancouver, B.C.) and from Operon Technologies (Alameda, Calif.). DNA sequence data from cloned RAPD marker BC194a/b was used to design SCAR (Paran and Michelmore 1993) primers for this marker by extending the 3′ end of the original RAPD primer as follows: forward, 5′-AggACgTgCCACCAATCCTAgT-3′; reverse, 5′-AggACgTgCCCATATATAgCTTg-3′ (original primer underlined). BC194a/b SCAR primers were synthesized by Kestone Labs (Boulder, Colo.).

Results

Characteristics of codominant RAPDs

In the course of two mapping projects (results to be presented elsewhere) we initially identified three codominant RAPD markers in chickpea and two in diploid strawberry (Table 1). Each of these markers could be categorized as one of two types, depending upon the marker banding pattern in heterozygotes (Fig. 1). A consistent characteristic of all five codominant RAPDs was the presence of a marker-associated non-parental band in heterozygous individuals (Fig. 1, arrows). In addition to the non-parental band, the parental slow (a) and fast (b) bands were sometimes of sufficiently different mobility as to be clearly distinguishable in heterozygotes (e.g., OPZ04a/b, Fig. 1, lane 5), resulting in a three-band marker phenotype (Table 1). In other cases the parental bands were of such similiar mobilities that they overlapped into a single band in

Table 1 Description of codominant RAPD markers identified in chickpea and diploid strawberry. The first five markers listed were encountered by chance in the course of mapping projects; the second five markers were identified in the template-mixing experiment. Note: the alternate alleles of marker OPZ14c/d were too close in size to distinguish between them.

Marker[a]	Species[b]	Size (bp)[c]		Heterozygote phenotype	RAPD primer sequence (5′-3′)
		Fast	slow		
CS47a/b	C	296	301	2 band	TTgCCgTgTT
OPZ04a/b	C	~360	~385	3 band	AggCTgTgCT
OPZ20a/b	C	~375	~380	2 band	ACTTTggCgg
BC89a/b	S	~375	~385	3 band	gggggCTTgg
BC194a/b	S	~610	~800	3 band	AggACgTgCC
OPZ14a/b	S	~650	~665	2 band	TCggAggTTC
OPZ04a/b	S	~660	~690	3 band	AggCTgTgCT
OPZ18a/b	S	~920	~930	2 band	AgggTCTgTg
OPZ07a/b	S	~980	~995	2 band	CCAggAggAC
OPZ14c/d	S	~1350	~1350	2 band	TCggAggTTC

[a] Two-letter prefixes indicates primer source:
CS = Charles W. Simon, Washington State University
BC = University of British Columbia Biotechnology Center
OP = Operon Technologies Incorporated

[b] C = Chickpea, S = strawberry

[c] Sizes estimated from comparison to a 123-bp ladder (Gibco-BRL), or determined directly from product sequence (CS47a/b only)

heterozygotes (e.g., OPZ20a/b, Fig. 1, lane 10; CS47a/b, Fig. 2), resulting in a two-band (doublet) phenotype (Table 1).

Insight into the nature of the non-parental heterozygote bands was gained via detailed characterization of marker CS47a/b, as follows. When parental (ICC640 and 489777) and F_1 (ICC640 × 489777) DNAs were first amplified with primer CS47 (Table 1), an interesting polymorphism was noted. A band (CS47a) from ICC-640 had a slightly slower electrophoretic mobility than a band (CS47b) from 489777 (Fig. 2a, lanes 1 and 2, respectively). The F_1 DNA produced a pair of bands (doublet): one, a non-parental band that was slower migrating than either parental band, and the other appearing intermediate between, or coincident with, the parental band positions (Fig. 2a, lane 3). In an ICC640 × 489777 F_2 population of size 56, no recom-

Fig. 1 Codominant RAPD markers as visualized on a 2% agarose, TBE gel. For each marker, a set of three lanes represents (left) the slow (*a*) band parent, (middle) the fast (*b*) band parent, (right) heterozygote. *Arrows* indicate heteroduplex bands. Markers are (left: *lanes 3–5*) OPZ04a/b and (right: *lanes 8–10*) OPZ20a/b, both from chickpea. Note that the *a* and *b* parental bands of marker OPZ04a/b are clearly distinguishable below the heteroduplex band in the heterozygote lane (*lane 5*); however, the similarly sized *a* and *b* parental bands of OPZ20a/b overlap into a single band in the heterozygote (*lane 10*). The molecular-weight marker (*lanes 1* and *12*) is a 123-bp ladder (Gibco-BRL)

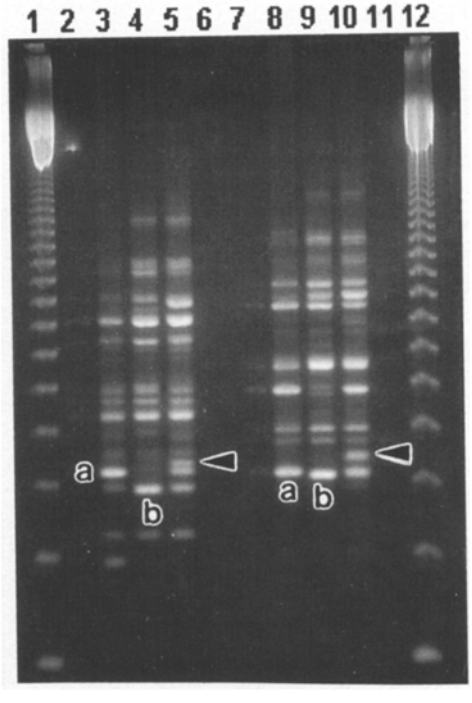
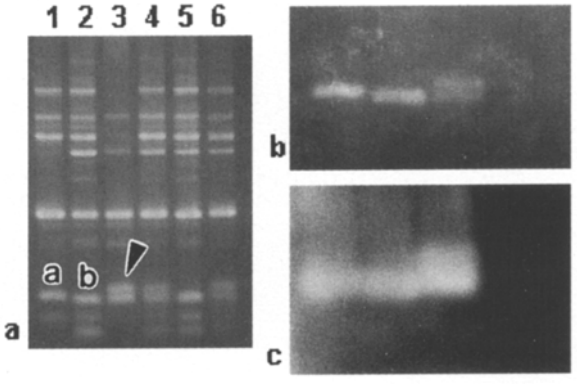


Fig. 2a–c Characteristics of CS47a/b codominant RAPD marker. **a** Primer CS47 RAPD products, and results of DNA-mixing experiments. (L – R) *lane 1*, ICC640 (CS47a band indicated); *lane 2*, 489777 (CS47b band indicated); *lane 3*, F_1 (ICC640 × 489777) (*arrow* indicates heteroduplex band); *lane 4*, parental DNAs mixed 1:1 before PCR; *lane 5*, separately amplified products of parental DNAs, mixed after PCR without subsequent heating; *lane 6*, separately amplified products of parental DNAs, mixed after PCR then heat-denatured/renatured before electrophoresis. **b** Ethidium bromide-stained gel used for Southern blot. (L-R) *lane 1*, ICC640; *lane 2*, 489777; *lane 3*, F_1 doublet. **c** Southern blot (negative image is of contact print made from X-ray film) showing hybridization of the CS47a probe to the parental bands and the two doublet bands



binant, double-null individuals were seen, and the CS47a, the doublet, and the CS47b bands segregated in a ratio of 11:27:18 [chi-square (1:2:1) = 1.86, n.s.] as would be expected for codominant alleles at a single locus, or for non-allelic markers tightly linked in repulsion phase.

To verify the apparent allelic relationship between the CS47 a and b bands, both products were cloned and sequenced. Because of the possibility of *Taq*-amplification error associated with sequencing only one clone of each allele, caution is indicated in the interpretation of the sequence data. However, alignment of sequences revealed complete identity (suggesting fidelity of amplification), with the exception of a 4-base deletion at position 201–204 and a 1-base deletion at position 65 in CS47b relative to the 301-bp sequence of CS47a (Table 2). When used to probe a PCR-product Southern blot (Fig. 2b, c), the CS47a clone hybridized to the CS47a and CS47b bands and to both of the doublet bands, demonstrating homology among the bands.

An explanation for the presence of the non-parental, doublet slow band in heterozygous individuals was sought via DNA mixing experiments. The doublet pattern was recovered, with both bands of equal intensity, when parental genomic DNAs were mixed 1:1 before PCR (Fig. 2a, lane 4), and when separately amplified ICC640 and 489777 PCR products were mixed after amplification, then heat-denatured/renatured before electrophoresis (Fig. 2a, lane 6). However, a single band co-migrating with the faster of the doublet bands was produced if the mixed PCR products were loaded directly into a gel without heating (Fig. 2a, lane 5).

We concluded that, under conditions causing denaturation/renaturation of co-amplified or mixed CS47a and CS47b PCR products (i.e., during PCR or post-PCR heating/cooling), renaturation of CS47a and CS47b homoduplex molecules was accompanied by the formation of two possible 'inter-allelic' heteroduplex species. These heteroduplexes, involving complementary CS47a and CS47b strands differing only by two small insertions/deletions, would have had abnormal secondary structures, and therefore probable alteration of their electrophoretic mobilities, compared to the CS47a and CS47b homoduplexes. The mixing data indicate that the doublet fast band, which could be mimicked by mixing (without subsequent heating) separately amplified CS47a and CS47b PCR products, consisted of the two possible types of homoduplex molecules, while the two possible heteroduplex species constituted the non-parental, doublet slow band.

As a final experiment, the fast and slow bands of the CS47a/b doublet were separately excised from a gel, purified, denatured/renatured, then run separately on a gel. The doublet banding pattern was recovered from both the fast- and slow-band DNAs (data not shown), indicating that the same PCR products were represented in both doublet bands, and that the mobility difference between the fast and the slow bands was determined by whether the single-stranded PCR products of the CS47a and CS47b alleles had renatured as 'inter-allelic' heteroduplexes (doublet slow band) or 'intra-allelic' homoduplexes (doublet fast band).

To further explore and document the phenomenon of RAPD marker heteroduplex formation, we cloned the fast (b) allele of the BC194a/b marker (Fig. 3, lane 3), sequenced the ends, and designed "SCAR" primers. These primers were used to generate the corresponding marker bands in the absence of the many other bands produced by the RAPD primer (Fig. 3). Although the heteroduplex band and the slow homoduplex band are hard to distinguish in RAPD-product lanes, they can be clearly distinguished in the corresponding SCAR-product lanes (e.g., Fig. 3, lane 6 versus lane 16). Two other, even slower migrating, non-parental bands were also faintly visible in SCAR marker heterozygotes (see Fig. 5, lanes 14, 16, 18 and 19), perhaps representing alternate secondary structures for the heteroduplexes.

Use of template mixing for primer selection and segregation analysis

The findings that (1) non-parental, heteroduplex bands were consistently associated with codominant RAPDs, and (2) that these bands could be deliberately generated by parent-parent template mixing, suggested that template mixing could be used to test RAPD primers for those likely to generate new codominant markers. For each of 20 arbitrarily chosen 10-mer primers, a set of four gel lanes was set up to allow comparison of PCR products of parent 1 ('Baron Solemacher'), parent 2 (WC6), parent 1 and parent 2 template DNA mixed 1:1 before PCR, and parent 1 and 2 individual PCR products mixed 1:1 after PCR (without denaturation/renaturation) (Fig. 4). A potential codominant marker was indicated by the presence of a non-parental band in the mixed-template lane (Fig. 4, lanes 4 and 8), as compared to the parental and mixed-product lanes. Four primers (OPZ04, OPZ07, OPZ14, and OPZ18) produced a total

Table 2 Sequence of the CS47a allele from ICC640. Underlined bases are missing in the CS47b allele from 489777. Lower case is used to clearly distinguish g from C. GenBank accession number is U07170

TTgCCgTgTT	ggCTATAgAA	ATTTAgTTAT	CTTTTCTATg	gACTTTTAAT	TTCATTTATT
TTTTTATTgg	gTTTgAACCC	TCTTgTTAAA	AACCTCATgT	TAATTCATgA	ATgTggAAAT
AAATggCACT	CAgATCTgAA	ATTTgTTggT	gAACCACACA	TgCATTTTCA	AAATTATTgg
AgACTgAACT	TgTTTgTATC	gTATAAgAAg	TgATTTtgAT	gAgACAACTA	TTAAgTtggg
TgTTTCAaAT	ggTCAAAATA	ATTAAACCCA	TTggTCCCTA	AAgATCTTAT	AAACACggCA
A					

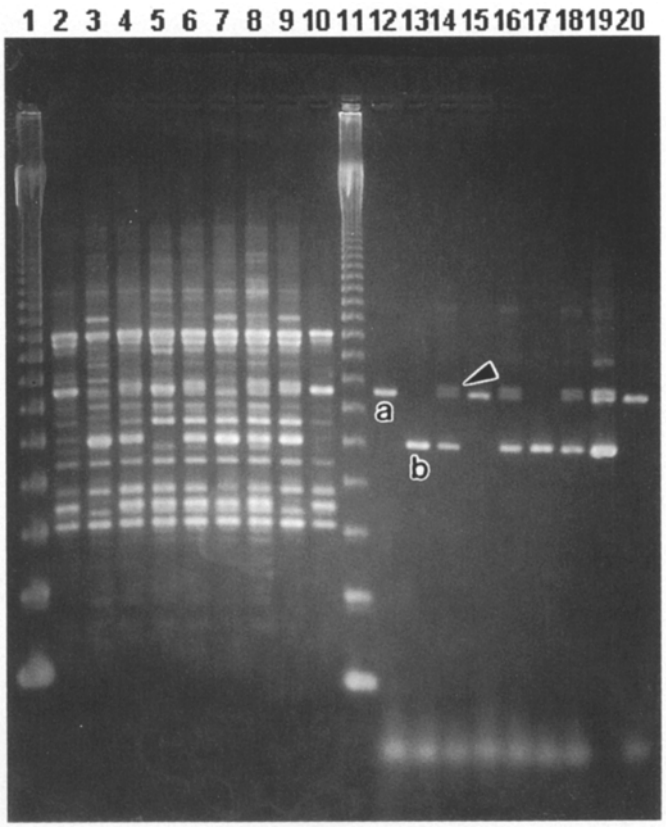


Fig. 3 Codominant RAPD marker BC194a/b (*lanes 2–10*) and corresponding SCAR marker, (*lanes 12–20*). Marker band positions are indicated in *lanes 12–14*. *Lane 2*, 'Baron Solemacher' (parent 1: band *a*); *lane 3*, WC6 (parent 2: band *b*); *lanes 4–10*, F_2 individuals of phenotypes (left to right) ab, a, ab, b, ab, ab, a. *Lanes 12–20*, same DNA sources as used in *lanes 2–10*, respectively. Note the diagnostic heteroduplex band, just above band *a*, in heterozygote lanes (e.g., *lane 14*, *arrow*). *Lanes 1* and *11*, 123-bp ladder

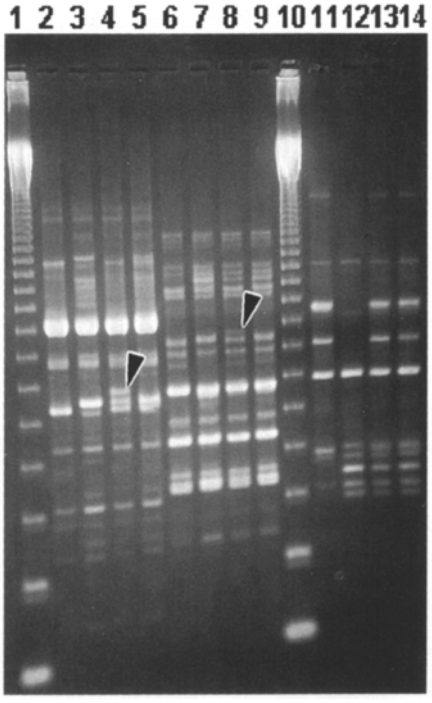


Fig. 4 Use of parent-parent template mixing to identify primers that detect codominant RAPD markers. Primers OPZ04 (*lanes 2–5*), and OPZ18 (*lanes 6–9*), BC200 (*lanes 11–14*). *Lanes 1* and *10* are 123-bp ladders. For each primer, a set of four lanes contains (left to right) PCR products of parent 1, parent 2, parent 1 and 2 template DNA mixed 1:1 before PCR, parent 1 and 2 individual PCR products mixed after PCR (without denaturation/renaturation). Note the presence of non-parental bands (*arrows*), diagnostic of codominant RAPD markers, generated by primers OPZ04 (*lane 4*) and OPZ18 (*lane 9*), but not by BC200

of five such bands, including two by primer OPZ14 (Table 1). The remaining 16 primers all amplified well, and revealed many polymorphisms, but no non-parental bands (e.g., Fig. 4, lanes 11–14). When the four primers producing non-parental bands were tested on an F_2 population ('Baron Solemacher' × WC6), all five putative codominant markers were confirmed by their segregation patterns (data not shown). A total of nine segregating, dominant RAPD markers was also detected by these four primers.

When alternate, codominant RAPD alleles were of similar band size (e.g., OPZ20a/b, Fig. 1), the two homozygous types (fast and slow, single bands) were often difficult to differentiate from each other in segregating populations, although heterozygotes could be easily recognized by the presence of the artifactual heteroduplex band (Fig. 5 a, lanes 3, 5, 8 and 9). We found that when genomic DNA from the slow-band crossing parent was mixed 1:1 with each F_2 template DNA prior to PCR, a heteroduplex band was produced for a subset of the homozygous F_2 individuals (Fig. 5b, lanes 1, 6 and 7) but not for the remaining homozygotes (Fig. 5b, lanes 2, 4 and 10). Thus, F_2 plants 1, 6, and 7 were fast-band homozygotes, while plants 2, 4 and 10 were slow-band homozygotes. These inferences were confirmed by similarly "spiking" a set of F_2 reactions with parental fast-allele DNA, with the result that heteroduplex formation was produced in the alternate subset of homozygous F_2 individuals (Fig. 5c, lanes 2, 4 and 10). Thus, the deliberate generation of heteroduplex bands by appropriate template mixing has allowed us to unequivocally confirm the RAPD marker genotype for each F_2 individual.

Discussion

Non-parental, heteroduplex bands are a distinguishing feature of codominant RAPDs

A feature common to the ten codominant RAPDs we have identified is the presence of a distinctive, non-parental band of slower gel migration than the parental bands in heterozygous individuals. For four of the ten markers described here, this band appeared as a third band in addition to the two distinguishable parental bands. The codominant RAPD described by Ayliffe et al. (1994), in which the slow allele (365 bp) was 38 bp

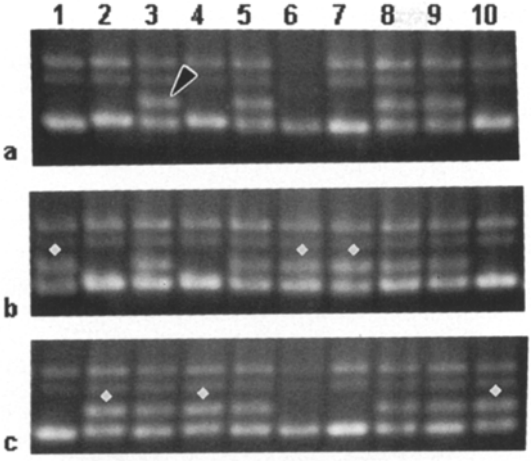


Fig. 5a–c Generation of heteroduplex bands by parent-progeny template mixing and amplification with primer OPZ20 (top portion of gel photographs not shown). **a** F_2 DNAs 1–10 (left to right), unmixed. Heterozygotes, *lanes 3, 5, 8* and *9*, have heteroduplex band (*arrow*). **b** F_2 DNAs 1–10 mixed 1:1 with slow parent DNA before PCR. Mixing-generated heteroduplex present in *lanes 1, 6* and *7* (*diamonds*). **c** F_2 DNAs 1–10 mixed 1:1 with fast parent DNA before PCR. Mixing-generated heteroduplex present in *lanes 2, 4* and *10* (*diamonds*)

longer than the fast allele, was also of this type. More often (six of ten markers) the non-parental band appeared as the slower migrating band of a distinctive doublet, in which the overlapping of the two, closely migrating parental bands constituted the faster band. Representing the latter marker type, the parental bands of marker CS47a/b were sequenced and found to differ in size by only five base pairs. Clearly, the relative size difference between the fast and slow parental bands accounts for whether heterozygotes have a three-band (larger difference, parental bands distinguishable) or two-band (smaller difference, parental bands overlap into a single band) pattern.

Through DNA-mixing experiments and Southern-blot analysis, we have provided evidence that the observed non-parental bands were the products of heteroduplex formation between complementary, single strands of the PCR products generated from alternate RAPD alleles. These bands were a consequence of heterozygosity, but could also be generated by mixing parental template DNAs (template mixing) in equal proportions prior to PCR, or by mixing individually amplified parental PCR products (product mixing) followed by heat denaturation/renaturation prior to electrophoresis. Thus, such bands were artifacts, in that they did not consist of discrete PCR products. Rather, they were generated by annealing of complementary single-stranded PCR products of alternate RAPD alleles.

Hunt and Page (1992) and Ayliffe et al. (1994) also attributed the formation of non-parental bands to heteroduplex formation. As in our experiments, they were able to generate the non-parental bands by PCR-template or product-mixing experiments. Ayliffe et al. (1994) further showed that mung-bean nuclease treatment could eliminate the non-parental band associated with their codominant RAPD marker, presumably by digesting a single-stranded loop in the heteroduplexes, while the parental bands were essentially unaffected.

Although not encountered here, heteroduplexes conceivably could also arise from interaction between PCR products of non-allelic, but homologous, sequences. However, when we converted one of our codominant RAPDs (BC194a/b) into a codominant SCAR, thereby eliminating all but the marker-associated bands, the heteroduplex band was still detected in heterozygotes. In this case the formation of the heteroduplex band was clearly attributable to an interactioin between the fast-and slow-allele PCR products, and was not dependent upon the presence of any other, non-allelic, PCR product.

Despite minimal documentation in the literature (e.g., Hunt and Page 1992; Ayliffe et al. 1994), the presence of an artifactual, heteroduplex band in heterozygotes may well be diagnostic of RAPD marker allelism/codominance in general. In previous reports of codominant markers, the allelic relationship of differently migrating bands has been inferred from linkage relationship – i.e., from the absence of any double-null recombinants for two repulsion-phase markers (Schulz et al. 1994). However, absence of recombinants between repulsion-phase markers can be explained by tight repulsion-phase (trans) linkage of non-allelic markers, as well as by allelism. In such cases, the detection of a heteroduplex band in heterozygotes could provide confirmation of the allelic relationship between markers.

Enhancing detection and interpretation of codominant RAPDs

Codominant RAPD markers are valuable because of their genetic information content, but are rare in comparison to dominant RAPDs. Template mixing is a technique with many possible applications for enhancing the detection and interpretation of codominant RAPD markers. As described here, parent-parent template mixing provides an effective and economical means of selecting primers likely to detect codominant RAPD markers in a given cross prior to conducting segregation analysis. Using this method, we tested 20 primers (requiring a total of 60 PCR reactions) against a pair of crossing parents and identified four primers that were then used to detect a total of five segregating, codominant markers and nine dominant markers in the respective F_2 mapping population; a codominant marker frequency of 35.7% for the four selected primers. By extension, use in segregation analysis of only those primers preselected to detect codominant markers could provide enough codominant RAPD markers to ensure inclusion of one or more in every linkage group. Enhanced detection and utilization of codominant RAPDs will be of particular value for mapping F_2 populations, as we are doing, because they can be used to tie together

pairs of repulsion-phase linkage groups, allowing dominant markers from both parents to be integrated together into a single map.

With marker OPZ20a/b, we have also demonstrated the use of parent-progeny template mixing to help classify segregating populations when closely migrating parental bands are difficult to distinguish. Our approach relied on mixing parental and F_2 template DNAs to intentionally generate diagnostic heteroduplex bands in F_2 individuals, thus allowing accurate determination of genotype. Template mixing should also be useful for confirming RAPD marker allelism in testcross progenies or recombinant inbred populations where two markers, each segregating 1:1 (presence:absence), are co-segregating in repulsion phase without evident recombination. The absence of heterozygotes in such populations would normally preclude observation of diagnostic heteroduplex bands; however, generation of such bands by appropriate parent-progeny or progeny-progeny template mixing could provide evidence of allelism.

In summary, we have confirmed and extended previous observations (Hunt and Page 1992; Ayliffe et al. 1994) about the nature and occurrence of non-parental, heteroduplex bands in RAPD PCR, and have demonstrated that such bands are a consistent and useful feature of codominant RAPD markers. Most importantly, we have exploited these observations to develop template-mixing methods that will greatly facilitate the identification and use of codominant RAPD markers in genome mapping and other genetic investigations.

Acknowledgements This research was supported in part by USDA Plant Genome Program Grant #92-27300-7442, and is submitted for publication as Scientific Contribution Number 1857 from the New Hampshire Agricultural Experiment Station. We thank Drs. N. Weeden, A. Klein and J. Collins for helpful comments on the manuscript.

References

Allard RW (1956) Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24:235–278

Ayliffe MA, Lawrence GJ, Ellis JG, Pryor AJ (1994) Heteroduplex molecules formed between allelic sequences cause nonparental RAPD bands. Nucleic Acids Res 22:1632–1636

Hunt GJ, Page, RE Jr (1992) Patterns of inheritance with RAPD molecular markers reveal novel types of polymorphism in the honey bee. Theor Appl Genet 85:15–20

Paran I, Michelmore RW (1993) Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet 85:985–993

Schulz B, Westphal L, Wricke G (1994) Linkage groups of isozymes, RFLP and RAPD markers in carrot (*Daucus carota* L. *sativus*). Euphytica 74:67–76

Suiter KA, Wendel JF, Case JS (1983) LINKAGE-1:a PASCAL computer program for the detection and analysis of genetic linkage. J Hered 74:203–204

Tingey SV, del Tufo JP (1993) Genetic analysis with random amplified polymorphic DNA markers. Plant Physiol 101:349–352

Torres AM, Weeden NF, Martin A (1993) Linkage among isozyme, RFLP, and RAPD markers in *Vicia faba*. Theor Appl Genet 85:937–945

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 polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535