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A molecular marker-based linkage map of diploid bananas (*Musa acuminata*)

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Abstract. A partial molecular linkage map of the *Musa acuminata* diploid genome is presented. This map is based on 58 RFLP, four isozyme and 28 RAPD markers segregating in an F_2 population of 92 individuals. A total of 90 loci was detected, 77 of which were placed on 15 linkage groups while 13 segregated independently. Segregation distortions were shown by 36% of all loci, mostly favoring the male parent. Chromosome structural rearrangements were believed to be one of the main causes of these distortions. The use of genetic linkage data to further the genetic and evolutionary knowledge of the genus *Musa*, as well as to help improve the design of breeding strategies, is discussed.

Key words: *Musa acuminata* – RFLPs – RAPDs – Linkage map – Segregation distortion

Introduction

Dessert and cooking bananas represent one of the most important fruit crops in the world. Dessert bananas are intensively cultivated for export to Europe and North America, while cooking bananas and some dessert bananas are cultivated most often in backyard gardens for local consumption.

Bananas are monocotyledons belonging to the *Musaceae*. All wild bananas are diploid (with $2n = 2x = 22$) while cultivated ones may be diploid, triploid or sometimes tetraploid. Two wild species, *Musa acuminata* (A genome) and *Musa balbisiana* (B genome) are thought to have given rise to exiting edible bananas (Simmonds and Shepherd 1955). The typically seedles edible banana appears to be the product of two essential evolutionary processes accompanying domestication: parthenocarpy and sterility (Simmonds 1962). Sterility may be genetically controlled (Dodds and Simmonds 1948) or may arise from chromosome structural hybridity. Additionally, the fusion of reduced and non-reduced gametes following nuclear restitution gave rise to the triploid, fully-sterile cultivated forms (Simmonds 1962). Domestication has therefore resulted in the vegetative propagation of sexually-sterile clones. Thus, it is not surprising that breeding new triploid cultivars has not been an easy task and that success has been rare (Rowe 1984; Stover and Buddenhagen 1986).

There is, however, an urgent need to find new ways for developing cultivars that are resistant to diseases such as Panama disease (caused by *Fusarium oxysporum* f. *cubense*), Sigatoka disease (caused by *Mycosphaerella musicola*), or Black Leaf Streak disease (caused by *Mycosphaerella fijiensis*), among others. For example, until the first half of this century, export banana plantations were monocultures of cv 'Gros-Michel'. Panama disease destroyed most of the Central American and Caribbean plantations between 1910 and 1955 (Stover and Simmonds 1987) and 'Gros-Michel' was then replaced by a 'Cavendish' cultivar. Today, monocultural practices still prevail and the bananas are thus seriously threatened by Black Leaf Streak disease.

The classical breeding schemes involve crossing a male-fertile diploid, showing some desirable trait, onto a selected triploid cultivar haveing good female restitution. The choice of the diploid donor is difficult and

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often the agronomic characteristics of the new clones are not as good as those of widely-grown triploid varieties. The use of improved diploids developed from both cultivated clones and wild accessions has, therefore, become an important preliminary strategy for cultivar improvement (Shepherd 1987; Bakry et al. 1990). Indeed, diploid banana germplasm is genetically highly diverse (Jarret and Litz 1986; Shepherd 1987; Simmonds and Weatherup 1988; Horry 1989; Tezenas du Montcel 1990; Gawel and Jarret 1991) and constitutes an important source of genotypes resistant to many diseases (Rowe 1984; Shepherd 1987). However, there is practically no genetic knowledge concerning any of the important traits that breeders are attempting to transfer from diploids, such as disease resistances, or traits that are, as in the case of parthenocarpy, essential for the success of the final cultivars.

New genetic marker technologies, such as restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNAs (RAPDs), have now become standard tools for expediting the genetic dissection of hitherto little studies species. As was argued elsewhere (González de León and Fauré 1993), increased knowledge of the structure and function of the banana genome will facilitate its manipulation and help develop novel improvement strategies, especially at the diploid level. The present paper presents the first molecular marker-based genetic linkage map of diploid bananas (*M. acuminata*) and its possible contributions to the understanding of banana genome organization.

Materials and methods

Plant material

The mapping work was conducted on an F_2 progeny of a selfed F_1 hybrid plant, SFB5, derived from the cross SF265 × Banksii (Table 1). These materials were produced at the Guadeloupe CIRAD-IRFA center. SF265 and Banksii differ from each other in traits such as hand number, bunch position, and parthenocarpy. For the preliminary survey, leaves were harvested and dried in Guadeloupe (F.W.I.), then sent to the CIRAD-BIOTROP laboratory for DNA extraction and analyses. The F_2 population was propagated in vitro in Guadeloupe and sent to the CIRAD-BIOTROP laboratory where it was grown in a greenhouse. Probe libraries were constructed using either one of the two clones 'Petite Naine' or 'Gobusik' (Table 1).

RFLP methods

For the construction of nuclear genomic libraries, nuclear-enriched DNA was isolated from 40 g of young fresh leaf tissue. This was cut in small pieces, soaked for 2 min in cold diethyl ether, and ground with a Waring blender homogenizer (5 × 3 s, low speed) in 100 ml of extraction buffer [0.4 M sucrose, 0.05 M Tris-HCl pH 8, 2 mM $CaCl_2$, 0.03% β mercaptoethanol (v/v)]. The resulting suspension was filtered through a 150-µm-mesh nylon net, then through a 48 µm-mesh nylon net, and finally centrifuged for 10 min at 400 g. The pellet was gently resuspended in 10 ml of buffer A (0.25 M sucrose, 0.05 M Tris-HCl pH 8, 2 mM $CaCl_2$). The solution was carefully layered over 3 vol of a sucrose buffer (2 M sucrose, 0.05 M Tris-HCl pH 8, 2 mM $CaCl_2$) contained in a soft Teflon tube. Ultracentrifugation was performed in a swing rotor for 45 min at 30,000 g. The resulting pellet was dispersed in 10 ml of buffer A with a smooth brush, and the suspension was centrifuged for 10 min at 1,600 g. All the previous steps were performed at 4 °C. Nucleus enrichment was checked by microscopic examination after DAPI (4′,6-diamidino-2-phenyl-indole) staining. For nuclear lysis, the pellet was resuspended in 10 ml of buffer B (150 mM NaCl, 10 mM EDTA, 50 mM Tris-HCl pH 7.5, 2% SDS) and incubated for 30 min at 65 °C. Nuclear debris was pelleted by centrifugation for 30 min at 4000 g. NaCl was added to the supernatant to a final concentration of 1.4 M. A 1/10 vol of CTAB (mixed alkyltrimethylammonium bromide) solution (10% CTAB, 500 mM Tris-HCl pH 8, 100 mM EDTA) was then added and the mixture was incubated with gentle stirring for 10 min at 65 °C. One volume of chloroform:isoamylalcohol (24:1) was added and, after mixing, the solution was centrifuged for 5 min at 3,500 g at 20 °C. The supernatant was mixed with 2.5 vol of ethanol to precipitate high-molecular-weight DNA which was then washed in 76% ethanol-0.2 M NaOAc and resuspended in TE (10 mM Tris-HCl pH 8, 1 mM EDTA pH 8). RNase digestion was performed for 45 min at 37 °C. A final standard phenol:chloroform:isoamylalcohol (25:24:1) extraction was carried out to further purify the DNA. Nuclear DNA was digested with either *Eco*RI or *Pst*I and was then size-separated on a 10–40% sucrose gradient (Sambrook et al. 1989). Two ligation reactions were performed according to insert size range (0.7–2 kb, 2.5–5 kb). Fragments were ligated into pBluescript or pUC18 plasmids (insert 3 : plasmid 1) following the manufacturer's instructions for T4 DNA ligase (BRL). DH5α bacterial cells were then transformed with ligated plasmids (Chung and Miller 1988) and

Table 1. Plant material

Name	Classification	Geomic constitution	CIRAD-IRFA code number
SF265 (NBB11/SF265)	*Musa acuminata* (cultivar)	AA	II.04.20.004.020
Banksii	*Musa acuminata* ssp. *banksii* type 'Banksii' (wild)	AA	II.04.01.004.001
SFB5 (SF265 × Banksii)	–	AA	–
'Petite Naine'	'Cavendish'	AAA	II.04.30.002.012
'Gobusik'		AAB	ITC 1189 (INIBAP code)

selection of transformed colonies was performed following X-Gal and IPTG screening procedures. Plasmid minipreparations were according to Birnboim and Doly (1979). The libraries were screened for copy number by probing total DNA onto Southern blots of the probes. A wheat rDNA unit repeat clone (Gerlach and Bedbrook 1979) and pea *Adh* clone (Llewellyn et al. 1987) were used as intensity controls. The probes were classified into two categories based on the relative intensity of the detected signal. Clones were named pMaCIR#. Numbers greater than 1000 corresponded to the *Eco*RI library probes. Insert size was determined relative to a Raoul (Appligene) molecular weight marker using a computer-driven autoradiograph digitizer (Hoisington and González de León, in preparation).

For Southern and PCR analyses, total DNA was extracted according to Dellaporta et al. (1983), with modifications by Cordesse et al. (1990). The DNAs of nine banana clones (SF265, Banksii, and seven individuals representative of *M. acuminata* diversity) cut with one of ten restriction enzymes, were surveyed for restriction polymorphism using 13 genomic probes (data not shown). *Eco*RV and *Dra*I were selected for the mapping work. Digestions were performed according to supplier's recommendations (BRL), but with 2.5 units/μg DNA of restriction enzyme. Restricted DNA (10 μg/lane) was separated in 0.8%-TAE agarose gels at 1.04 V/cm for 9 h. The gels were depurinated in 0.25 N HCl for 10 min, denatured in 0.4 N NaOH for 30 min, and then blotted onto Hybond N^+ membranes (Amersham).

Probes were labeled with ^{32}P-αdCTP using the random primer labelling method of Feinberg and Vogelstein (1983). Incorporation of radioactive nucleotides was checked by chromatography on PEI-cellulose F. Prehybridization, hybridization and washes were performed in a hybridization oven (Appligène). One or two membranes were placed in a glass tube and the following buffer was added: 6 × SSPE, 0.5% SDS, 5 × Denhart, and 25 μg/ml of sheared herring sperm DNA. For hybridization, this buffer was supplemented with dextran sulphate to a final concentration of 8% (w/v). Membranes were washed at 68 °C for 30 min in each of the following buffers: 2 × SSPE, 2 × SSPE-0.1% SDS, 0.1 × SSPE-0.1 SDS. They were then exposed to X-ray film (Kodak X-OMAT) at −80 °C for 4 days with one intensifying screen. Blots were stripped in 1% SDS at 68 °C for 30 min and could be re-used up to 12 times.

Clones of known genes

The origin and source of the clones of known genes used in this study are given in Table 2.

Isozyme analysis

The F_2 population was assessed for allozymic segregation in the following enzyme systems: malate dehydrogenase (MDH, E.C. 1.1.1.37), cathodic peroxidase (POX, E.C. 1.11.17), phosphoglucomutase (PGM, E.C. 2.7.5.1), phosphoglucoisomerase (PGI, E.C. 5.3.1.9), shikimate dehydrogenase (SKDH, E.C. 1.1.1.25), acid phosphatase (ACP, E.C. 3.1.3.2), and esterase (EST, E.C. 3.1.1.2). Extraction buffer, electrophoresis and staining were according to Horry (1989).

RAPD analysis

A modified version of the protocol of Williams et al. (1990) was applied. Amplification reaction were performed in 25 μl volumes containing magnesium-free 1 × *Taq* DNA buffer (Promega), 0.2 mM dNTP mix (Pharmacia), 0.2 μM primer (Operon Technologies, Inc.; kits D, K, L, M, O, P and S), two units of *Taq* DNA polymerase (Promega), 2 mM $MgCl_2$ and 115 ng of genomic DNA, overlaid with one drop of pure mineral oil. Amplification were carried out in a Perkin Elmer- Cetus DNA thermal cycler programmed for one predenaturation cycle of 1 min at 94 °C and 40 cycles of 1 min denaturation at 94 °C, 1 min annealing at 36 °C and 2 min elongation at 72 °C. Amplification products were analyzed by electrophoresis in 2%-TBE agarose gels at 3.12 V/cm for 3 h. RAPD loci were named rOPX#, where X is the primer kit letter and # the number of the primer in the kit. When more than one band could be scored for a particular primer, a number was added to identify each locus.

Linkage analysis

Segregation was studied in 82 individuals for RFLP markers, 92 individuals for isozymes, and 89 individuals for RAPD markers. These discrepancies were due to the loss of some individuals during the period of study. All data were scored at least twice by two people.

The segregation of each marker was tested first for goodness-of-fit to expected Mendelian segregation ratios with a χ^2 test. Linkage analyses were performed on F_2 segregation data using Mapmaker V 1.0 (Macintosh) (Lander et al. 1987; Tingey personal communication). Linkage groups were constructed by two-point analysis using a minimum LOD score of 4.0 and a maximal recombination fraction of 0.40. Three-point and multipoint analyses were then performed using a minimum LOD score of 3.0 and a maximal recombination fraction of 0.40 to determine the most likely marker orders inside groups. Recombination fractions were converted to map distances with the Haldane mapping function (Haldane 1919).

Results

Library characterization and RFLP analyses

Sixty-eight percent of single-copy sequences were detected in the *Eco*RI library. By contrast, the *Pst*I library was more highly enriched in low-copy sequences since

Table 2. Clones of known genes

Clones	Genes	Species	References
H3C4	Histone	Maize	Chaubet et al. 1986
H4Cl4	Histone	Maize	Philipps et al. 1986
H4A748	Histone	*Arabidopsis thaliana*	Chaboute et al. 1987
AdhPG8	Alcohol dehydrogenase	Pea	Llewellyn et al. 1987
pTA71	rRNA gene	Wheat	Gerlach and Bedbrook 1979
C-II	Protease inhibitor	Soybean	Joudrier et al. 1987

only 10% of the probes revealed a signal corresponding to multiple-copy sequences. These findings were further substantiated by the RFLP patterns revealed by these probes in the parental accessions. In effect, these RFLP patterns could be classified into two groups: a single fragment per homozygous profile (Fig. 1a) was revealed by 49% of the probes, while 51% hybridized to two or more fragments per homozygous profile (Fig. 1b). These fragments were always revealed together by a single probe and always cosegregated. It is highly likely that they may be due to the presence of at least one *Eco*RV (or *Dra*I) restriction site inside the probe sequence. In all cases but one, each pattern revealed by any of the selected probes on each of the parental accessions seemed therefore to correspond to a single genetic locus.

Across both libraries taken together, polymorphism between SF265 and Banksii was revealed by 42% of the probes with at least one enzyme. Both *Eco*RV and *Dra*I revealed similar levels of RFLPs between the parental accessions (respectively 31% and 28%). However, some 50% of the probes detecting polymorphism revealed it with only one of the two enzymes. Among the heterologous probes, only the histone gene probe H4C14 revealed polymorphism between SF265 and Banksii.

The RFLP patterns of SF265, Banksii, and their hybrid, SFB5, suggested that Banksii was mostly homozygous, but that SF265 was heterozygous at many loci polymorphic between both accessions (Fig. 1b). SF265 and Banksii shared a common band for most probes for which SF265 was heterozygous. Consequently, about half of the loci revealed by such probes did not segregate in the F_2 population, and thus only 21% of all probes could be used to construct a linkage map from the cross SF265 × Banksii.

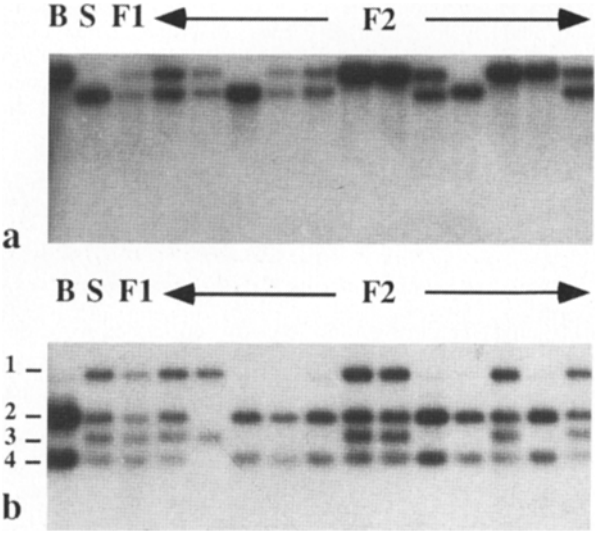

Fig. 1a, b. RFLP patterns for probes hybridized to DNAs from SF265 (*S*), Banksii (*B*), SFB5 (*F1*) and segregating F_2 progeny of the cross SF265 × Banksii (*F2*). **a** Probe pMaCIR214 showing one single fragment per homozygous profile (e.g., *lanes 1 and 2*) using *Eco*RV-digested DNA. **b** Probe pMaCIR1108 showing two fragments per homozygous profile (e.g., *lanes 1, 5, 6*), using *Dra*I-digested DNA. This probe revealed SF265 heterozygosity (*lane 2*). Bands 1 and 3 (*respectively 2 and 4*) always cosegregated

Isozyme and RAPD analyses

Of the seven isozyme systems investigated, four (MDH, POX, PGM, and EST) gave clear banding patterns and showed polymorphisms within the mapping population. Locus nomenclature was according to Horry (1989). Loci revealed by the POX, PGM, and EST systems coded for monomeric enzymes, while that revealed by MDH showed a dimeric enzyme phenotype.

Pattern differences between SF265 and Banksii were evident in 62 out of the 130 tested RAPD primers. Owing to the complexity of some of the patterns, and the partial heterozygosity of SF265, only 20 primers could be effectively used for mapping. The patterns obtained showed 1–13 bands per individual. The segregation of up to three bands per primer could be scored in terms of the presence or absence of each band in each individual in the F_2 population.

Construction of a partial molecular genetic linkage map of diploid bananas (M. acuminata)

Genetic segregation was recorded for a total of 90 loci divided as follows: 58 RFLP loci, four isozyme loci, and 28 RAPD loci. Twelve out of the fifty eight RFLP loci behaved as dominant markers. Skewed segregation was shown by 32 loci (36%), and distortion was generally in favor of the Banksii alleles (Table 3).

Two-point linkage analysis of the 90 loci segregating in the F_2 population using Mapmaker (LOD $\geq$ 4.0, $\theta \leq 0.4$), resulted in 15 linkage groups containing 77 loci while 13 others remained unassigned to any linkage group (Fig. 2). The probe pMaCIR160 was apparently the only one detecting two loci. These were mapped to two independent linkage groups (pMaCIR160a and pMaCIR160b respectively on group 7 and group 15). Linkage group size ranged from 4 cM to 80 cM. Eight clusters of three or more loci linked within an interval of less than 10 cM contributed to the skewed distribution of marker pair distances; these ranged from 0 to 38 cM with a mean of 10 cM (Fig. 3). The total length of the map was 606 cM.

In order to ascertain whether some of the smaller groups might correspond to the same chromosome and if some of the independent markers could be assigned to a linkage group, a second two-point analysis was done allowing the LOD score to be between 3.0 and 4.0. Linkage groups 3, 12, 13, and 15 then appeared

Table 3. Loci showing segregation distortion

Locus	Marker type	Group	F_2 genotypes			χ^2 for 1:2:1 (3:1) ratios
			S/S°	B/S°	B/B°	
pMaCIR1024	RFLP	1	19	21	30	14.7***
pMaCIR1033	RFLP	1	50		29	5.78*
rOPD16	RAPD	1	47		38	17.6***
pMaCIR49	RFLP	2	11	44	27	6.68*
pMaCIR137	RFLP	2	10	47	25	7.24*
pMaCIR429	RFLP	2	9	48	24	8.33*
MDH	Isozyme	3	17	42	33	6.26*
rOPK01	RAPD	3	41		42	29.01***
pMaCIR232	RFLP	4	17	52	13	6.29*
rOPK13	RAPD	6	34	54		8.73**
pMaCIR160a	RFLP	7	37		23	5.69*
pMaCIR1012	RFLP	8	12	37	31	9.48**
pMaCIR1050	RFLP	9	32	26	17	13.1**
rOPO02.3	RAPD	9	35	51		11.3***
rOPD20.2	RAPD	10	54		33	7.76**
pMaCIR23	RFLP	11	9	73		8.6**
pMaCIR560	RFLP	11	9	52	20	9.52**
H4C14	RFLP	12	41		40	25.68***
pMaCIR70	RFLP	12	25		44	55.31***
pMaCIR181	RFLP	12	24		45	59.52***
pMaCIR245	RFLP	12	37		44	37.14***
pMaCIR417	RFLP	12	39		42	31.15***
pMaCIR1001	RFLP	12	23		46	63.89***
rOPM07.2	RAPD	12	35		49	49.78***
rOPM20	RAPD	12	9	80		10.52**
pMaCIR31	RFLP	13	8	50	23	10**
pMaCIR411	RFLP	13	5	23	36	35.1***
rOPD02	RAPD	14	77		10	8.46**
pMaCIR94	RFLP	15	9	48	23	8.1*
pMaCIR1111	RFLP	15	10	46	24	6.7*
pMaCIR746	RFLP	–	6	46	27	13.3**
POX	Isozyme	–	10	48	14	8.44*

° S/S = homozygous for SF265 alleles, B/B = homozygous for Banksii alleles, B/S = heterozygous
* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

to form one longer cluster (see Fig. 4), as also did linkage groups 6 and 14.

Discussion

Polymorphism analysis

The *Pst*I library was far more enriched in low-copy sequences than the one constructed with *Eco*RI. *Pst*I is a methylation-sensitive restriction endonuclease (Gruenbaum et al. 1981) that tends to cut in or near coding regions (Burr et al. 1988). In other plant species, libraries constructed with *Pst*I have also been found to be enriched in single-copy probes (Tanksley et al. 1987; Helentjaris et al. 1988). There are exceptions however. In the case of *Triticum tauschii*, Gill et al. (1991) suggest that the very low fraction of single-copy sequences found in its genome after *Pst*I digestion is due to the fact that these sequences may be dispersed among repeated sequences. McCouch et al. (1988), on the other hand, suppose that the absence of methylation in a significant fraction of repeated DNA sequences explains the percentage of such sequences found in the rice *Pst*I library.

The screening of the *M. acuminata* parental accessions with probes from the two libraries revealed that these two individuals often shared a common band. These similarities corroborate the hypothesis that *M. acuminata* ssp. *banksii* could be among the ancestors of SF265 (Tezenas du Montcel, personal communication), and may explain the relatively low level of polymorphism detected between the parental clones. A much higher level of RFLPs is typically prevalent among diploid banana clones (Carreel et al., unpublished results in our laboratory). New mapping populations are being developed at CIRAD-IRFA, using pairs of highly-polymorphic parental accessions.

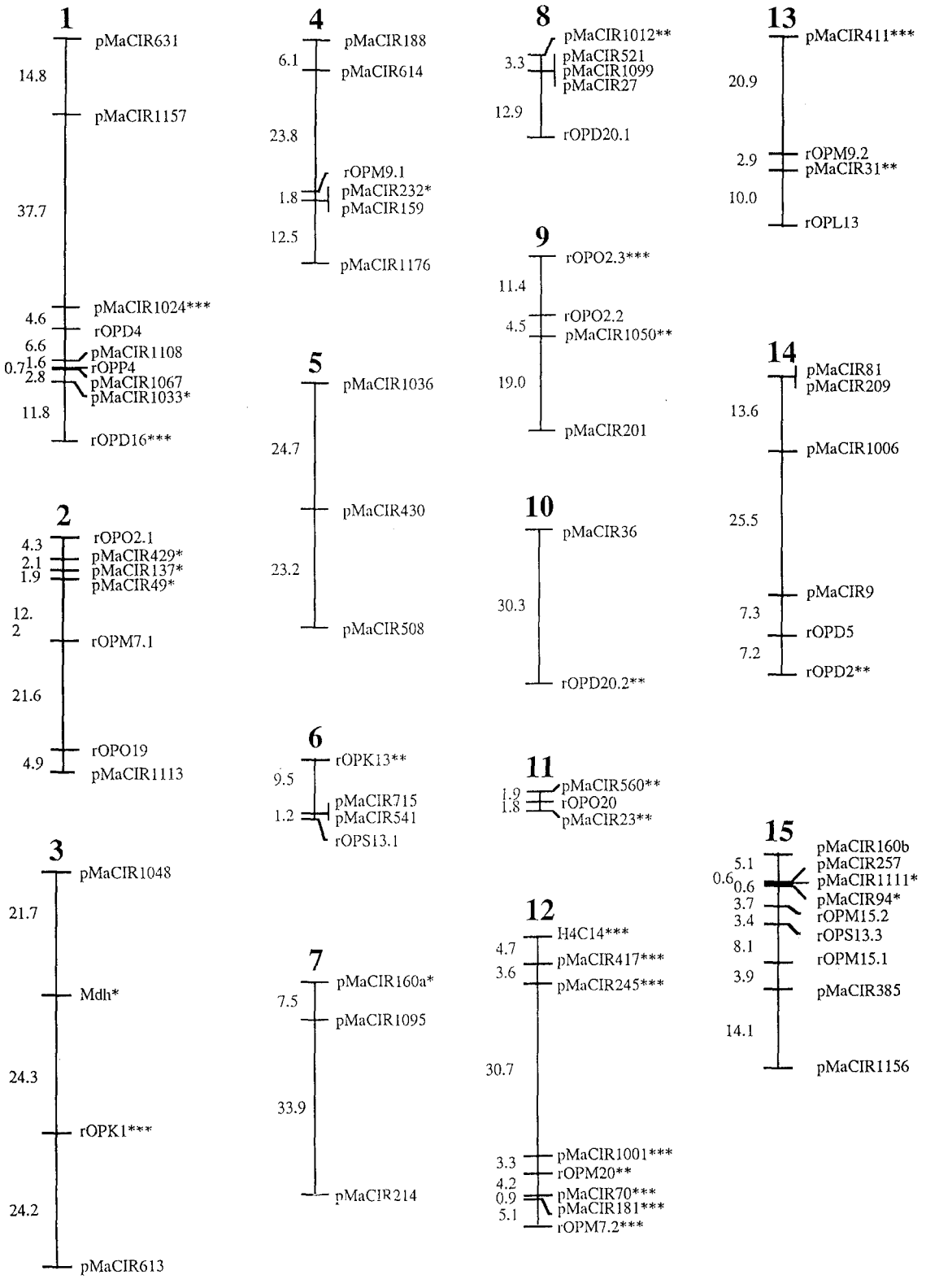

Fig. 2. Molecular linkage map of diploid bananas (*M. acuminata*) as derived from the cross SF265 × Banksii. *Numbers* at top indicate linkage groups. Loci are listed on the right of the vertical lines and distances on the left. Distances are in cM (Haldane 1919). *Asterisks* indicate loci showing segregation distortion. Unlinked markers are pMaCIR 108, pMaCIR124, pMaCIR227, pMaCIR462, pMaCIR746*, pMaCIR1064, Est, Pgm, Pox*, rOPM18, rOPS18.1, rOPS18.2, and rOPS20

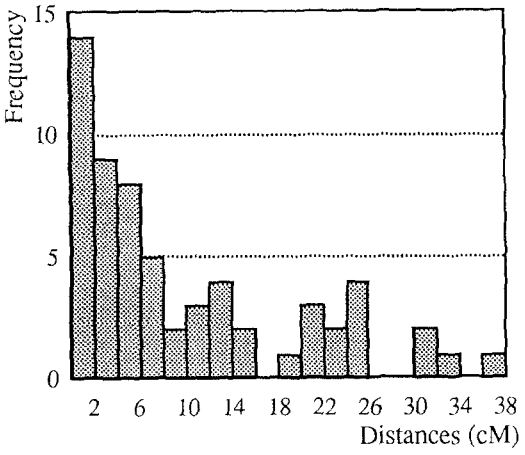

Fig. 3. Distribution of distances on the linkage map of diploid bananas (*M. acuminata*) as derived from the cross SF265 × Banksii

Three types of markers were used in this study: isozymes, RFLPs and RAPDs. Polymorphism detected by isozyme markers was high but the number of available systems is limited. RAPD markers differentiated parental accessions more often than did RFLP markers. But even if they are more rapidly implemented, difficulties in reading RAPD patterns make them less reliable. Their dominant nature implies that they are less informative in the case of an F_2 analysis than codominant RFLP markers. RFLP markers should therefore be preferred to RAPD markers in further molecular studies of bananas.

Restriction fragment length polymorphism may have arisen by point mutations or rearrangements such as deletions/insertions, inversions or translocations. A point mutation affects only a single enzyme recongnition sequence (on the hypothesis that restriction sites of different enzymes do not overlap), whereas a rearrangement may modify the recognition sequence of several enzymes. Analysis of polymorphism patterns obtained in this study suggested that both point mutations and rearrangements may be involved since about half of the probes differentiated parental accessions with only one of the two restriction enzymes. As has been argued for *Citrus* (Jarrell et al. 1992), the relative importance of point mutations could be explained by asexual propagation which may have allowed recessive mutations, and thus a substantial number of point mutations, to accumulate.

Segregation distortions

There were 32 loci that showed more-or-less distorted segregation ratios. In two cases, a locus showing distorted segregation was found to be tightly linked to one or two other loci with normal Mendelian ratios (e.g., pMaCIR159 and pMaCIR232 respectively); in both cases, the inconsistencies were due to sampling error arising from missing data for one or both loci.

Skewed segregation ratios have been frequently reported in the literature for morphological, isozyme, or RFLP markers. The percentage of loci showing segregation distortion in RFLP mapping studies of plant genomes may be high: 25.5% in potato (Gebhart et al. 1989), 12–59% in *Brassica oleracea* (Kianian and Quiros 1992). These data include both intraspecific and interspecific crosses, and involve both coding and non-coding sequences. Explanations for distortions most commonly encountered are certation (Wall and Whitaker 1971; Rick 1972; Nakagahra et al. 1974) and the occurrence of structural rearrangements (Stephens 1949, 1950; Tanksley 1984; González de León 1986). Cytogenetic studies have shown that SFB5, the F_1 hybrid that gave rise to the F_2 population, was heterozygous for two reciprocal interchanges involving three pairs of chromosomes (Fauré et al. 1993). Even if other explanations for segregaytion distortion cannot be totally excluded, chromosomal translocations may, therefore, be responsible for some or all of the distortions observed.

Map structure and chromosome structural rearrangements

The map obtained form the analysis using a LOD score greater than 4.0 was composed of 15 linkage groups and 13 independent markers. Given that the number of linkage groups is higher than the number of chromosomes in *M. acuminata* ($x = 11$), and that there are unassigned loci, it follows that the genome is not fully covered.

Allowing a LOD score of between 3.0 and 4.0, four of the fifteen linkage groups formed a longer group (Fig. 4). It is of interest to consider this in the light of cytogenetic studies. In an individual heterozygous for two reciprocal interchanges involving three pairs of chromosomes, pairing of homologous regions at meiosis may lead to the formation of eight bivalents and one hexavalent. As a result, marker segregation would reflect the interchromosomal linkages entailed by such a structure, and a longer, disproportionate group would be detected. That is, loci showing genetic linkage to an interchange breakpoint will appear to be linked among themselves irrespective of whether they are located on the same or a different parental chromosome. Concomitantly, markers on such a structure should exhibit varying degrees of segregation distortion according to how often they end up in unbalanced, less functional, or sterile gametes. Additionally, markers in those regions may also show reduced recombination (Burnham 1956). The existence of two translocations in SFB5 may explain the appearance of

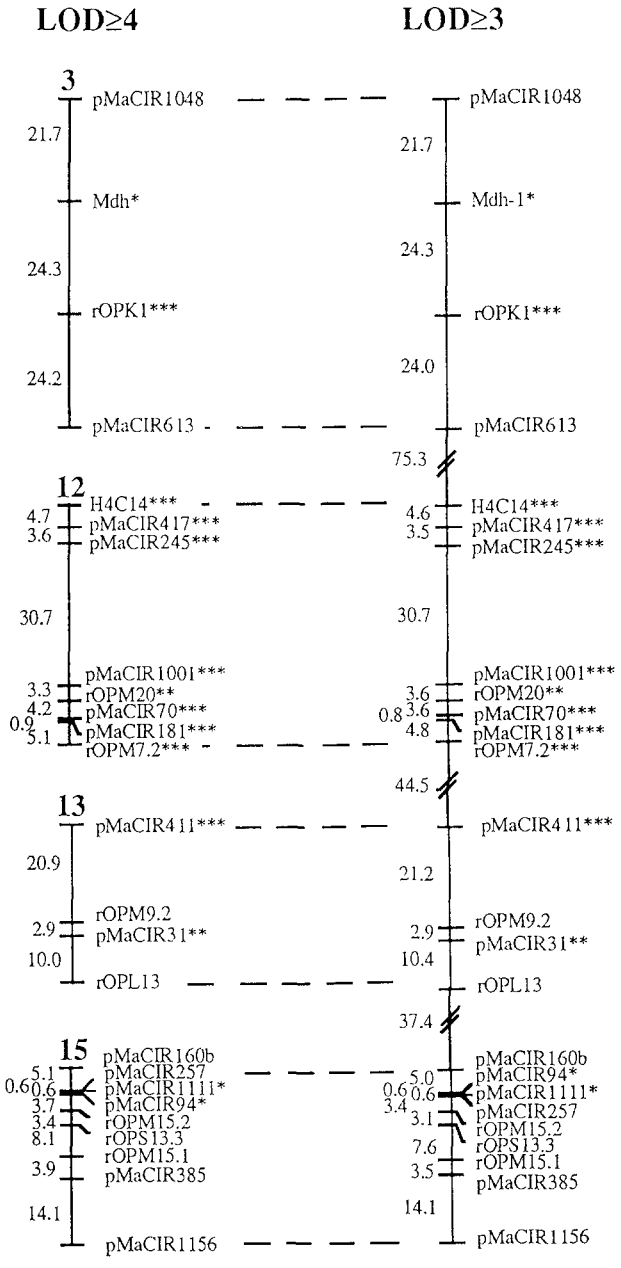

Fig. 4. Behaviour of groups 3, 12, 13, and 15 of the linkage map derived from the cross SF265 × Banksii when the LOD score is greater than 4.0 vs greater than 3.0 in two-point analysis

a longer group when lowering the threshold value of the LOD score. Fifteen out of the thirty-two loci deviating from Mendelian expectations, as well as two out of the eight clusters of closely-linked loci, were on the longer group made by the association of the groups 3, 12, 13, and 15. Whether these clusters correspond to a reduction of recombination in the vicinity of a translocation breakpoint cannot be concluded without further mapping and cytogenetic experiments. The demonstration of linkage groups corresponding to more than one pair of homologous chromosomes has been reported in species such as maize (reviewed by Burnham 1956), *Capsicum* (González de León 1986), and pearl millet (Liu personal communication). In contrast to the data for the former two genera, no reduction of recombination was observed for markers present on the interchromosomal linkage group of pearl millet (Liu personal communication).

It is important to emphasize that the linkage groups and the marker orders determined in this study are specific to SFB5. They reflect the relative arrangement of chromosomal segments present in the parental accessions. In individuals differing from either of these by structural rearrangements, some linkages may be present or absent according to the presence and/or the location of translocation breakpoints. Cytogenetic studies have shown that structural rearrangements such as inversions and translocations are frequent phenomena in diploid bananas (Dessauw 1988a, b; Fauré et al. 1993; K. Shepherd, unpublished). Construction and comparison of maps from various segregating populations derived from parents having different putative structural rearrangements should provide new data on chromosomal evolution and repatterning in *Musa* and may help locate breakpoints as has been done for pearl millet (Liu personal communication) or *B. oleracea* (Kianian and Quiros 1992). It will also lead to a better understanding of the consequences of interchanges on segregation and linkage map structure. Finally, crossing strategies based on this knowledge should help avoid or favor specific genic combinations in breeding programs using diploids.

The present work demonstrates the feasibility and possible applications of a molecular marker-based genetic linkage map of the diploid bananas. The map presented provides a starting point for the further molecular study of banana genetics. Although additional markers are needed to cover the genome more fully, it is now clear that mapping should be carried out in several different populations in order to unravel the complexities of banana chromosomal differentiation and its consequences in breeding. Moreover, this should provide information on the real specificity of RAPD markers and on the possibility of transferring RAPD loci from one population to another. In the next few years, it will become possible to dissect traits such as parthenocarpy, bunch position, or resistance to pathogens, that are most important for the development of successful edible cultivars. This should improve the efficiency of breeding, particularly for the screening of diploids involved in breeding strategies. The ability to identify the markers linked to an agronomic characteristic will be most useful for assisting the introgression of chromosome segments from germplasm to germplasm, both within between *Musa* species. Finally, such a linkage map constitutes a helpful tool for diversity and

phylogeny studies. Mapped probes evenly distributed throughout the banana genome are useful molecular tools both to clarify the relation between taxa and to describe the diversity existing in the genus *Musa*.

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