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Interspecific genetic linkage map, segregation distortion and genetic conversion in coffee (*Coffea* sp.)

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Abstract An interspecific partial genetic linkage map of *Coffea* sp. based on 62 backcross hybrids is presented. F_1 hybrids were generated by a cross between the wild *C. pseudozanguebariae* and the anciently cultivated *C. liberica* var. *dewevrei* (DEW); progeny were then derived from a backcross between F_1 hybrid and DEW. The map construction consisted of a two-step strategy using 5.5 and 3.1 LOD scores revealed by simulation file. The map consisted of 181 loci: 167 amplified fragment length polymorphism (AFLP) and 13 random fragment length polymorphism (RFLP) loci. The markers were assembled into 14 linkage groups, each with 4–31 markers covering 1,144 cM. Segregation distortion was observed for 30% of all loci, in particular 3:1 and 1:3 ratios equally favouring each of the two parents. The existence of such ratios suggests genetic conversion events. This map also represents an initial step towards the detection of quantitative trait loci.

Key words *Coffea* sp. · AFLPs · RFLPs · Linkage map · Segregation distortion · Genetic conversion

Introduction

Polymerase chain reaction (PCR)-based methods reduce both costs and time when screening large numbers of genetic markers, such as amplified fragment length polymorphism markers (AFLP, Vos *et al*. 1995).The AFLP technology has two main advantages: (1) more reliability and consistency than random amplified polymorphic DNAs (RAPDs); (2) a large number of markers on a single high-resolution sequencing gel. Consequently, a high-density map can be obtained from a small number of primers and minute amounts of DNA. AFLP markers are typically dominant, and most fragments correspond to unique positions on the chromosome and can be exploited in genetic and physical mapping (Thomas et al. 1995; Meksem et al. 1995). RFLP (restriction fragment length polymorphism) markers do not have such advantages; the number of markers obtained from a gel is lower and higher amounts of DNA are required. In contrast, they are co-dominant, which allows between-map comparison.

Coffee trees belong to the genus *Coffea* sub-genus *Coffea*, family *Rubiaceae* and are mostly present in tropical and subtropical regions of the world. Most species (up to 80 taxa) are diploid (2n=22), except *C. arabica*, which is tetraploid (Leroy 1980; Bridson 1987). Two species are currently cultivated world-wide: *C. arabica* and *C. canephora*. As wild species constitute the base of genetic resources for breeding, interspecific crosses have been realised to test breeding possibilities by introgression (Louarn 1992). Those that include East African species and a West African species constitute an extreme situation, which is illustrated by the cross between *C. pseudozanguebariae*, native to Kenya and Tanzania, and *C. liberica* var. *dewevrei*, native to Central Africa. In the investigation reported here we used the *C. pseudozanguebariae*×*C. liberica* dewevrei cross for genetic mapping for two main reasons: (1) the availability of F_1 hybrids as well as backcrossed hybrids and (2) the low genetic distance between *C. liberica* dewevrei and *C. canephora* (Carvalho and Monaco 1967; Charrier 1978; Louarn 1992), which facilitates relating the results to cultivated species.

Distorted marker segregation has been reported in several interspecific linkage analyses (Grant 1975). This systematic deviation from an equal representation of al-

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leles among functional gametes involves all chromosomes and could be caused by selection processes at the gamete or zygote stage (Gadish and Zamir 1986; Zamir and Tadmor 1986). Our study was also designed to obtain a quantitative estimation of the proportion of unequally segregating loci in the interspecific cross and to provide a possible explanation for its presence in *Coffea* genera. The main purposes of this paper are: (1) to present the first interspecific AFLP marker-based genetic linkage map of a cross in *Coffea*; (2) to identify sets of markers showing important segregation distortion.

Materials and methods

Plant material

Plant material is maintained at the Agricultural Station IRD (Man, Ivory Coast). The cross between C. *pseudozanguebariae* Bridson (PSE; genotype 8044 used as female parent) and C. *liberica* var. *dewevrei* (DEW; genotype 5851; used as male parent) was made by hand pollination under bags according to Louarn (1992). Twenty-five F_1 hybrids were obtained and grew on the same plot of the station. Sixty-two backcross hybrids on DEW (BCDEW) were obtained by open pollination of 13 F_1 hybrids (used as female parent). Backcross hybrids were identified using RFLP and AFLP molecular markers (180 PSE and 30 DEW specific markers). Twelve trees of both species were used to identify species-specific AFLP markers.

Before being sent to France, fresh coffee leaves were packed in wet newspaper in an isotherm box. They were lyophilised and then stored in a cold room (4°C, 20% relative humidity).

DNA extraction

Coffee leaves of PSE and the hybrids were recalcitrant to DNA extraction due to the presence of polysaccharides and other secondary metabolites. A direct extraction with CTAB (Doyle and Doyle 1987) or MATAB resulted in DNA in a gelatinous substance (polysaccharide), consequently limiting PCR amplification and enzymatic digestion. A two-step, large-scale DNA extraction (nuclei isolation followed by MATAB lysis) ultimately constituted the protocol followed for DNA extraction in the coffee plant:

Step 1 – Nuclei extraction:

- crushing of lyophilised leaves for 4 min in a ball mill (Dangoumill) to obtain a fine powder;
- mixing of powder (1 g) with 100 mL of nuclei extraction buffer in an Erlenmeyer flask; the slightly modified lysis buffer of Dolezel (1989) was used: 15 m*M* TRIS, 2 m*M* EDTA, 80 m*M* KCl, 20 m*M* NaCl, 0.5% Triton X-100, 0.1% β-mercaptoethanol, pH 9;
- shaking of Erlenmeyer flask for 2 h on a stirring table at 125 rpm;
- transfer of the suspension into two 50 ml capped tubes (Sarstedt);
- centrifugation for 20 min at 3000 *g*;
- pooling of pellets and re-suspensions in 20 ml lysis buffer, which was composed of 4% MATAB, 0.1 *M* TRIS, 1.25 *M* NaCl, 0.02 *M* EDTA, pH 8.0;
- storage of samples –20°C.

Step 2 – Nuclei lysis and DNA extraction:

- incubation of capped tubes at 65°C for 4 h;
- centrifugation at 3000 *g* for 10 min;
- transfer of supernatant into a new 50 mL tube;
- addition of 20 ml of chloroform/isoamyl alcohol (24/1:v/v);
- after a brief agitation, centrifugation of the emulsion for 10 min at 3000 *g*; washing with chloroform/ isoamyl alcohol was carried out twice;
- RNA suppression by incubation for 30 min at 37°C with 200 µl RNAase solution (10 mg/ml RNAase A from bovine pancreas, Boehringer);
- DNA precipitation with 20 ml isopropyl alcohol;
- centrifugation at 3,000 *g* for 10 min;
- re-suspension of pellet in 0.7 ml TE (10 m*M* TRIS-HCl, 1 m*M* EDTA, pH 8.0);
- transfer into 1.5-ml tubes;
- DNA precipitation with 0.6 ml isopropyl alcohol and 70 µl 3 *M* sodium acetate (pH 5.2);
- centrifugation at 12,000 *g* for 10 min;
- washing of the pellet in 70% ethanol;
- centrifugation at 12,000 *g* for 10 min;
- drying of pellet;
- re-suspension in TE.

DNA content was evaluated on 1% agarose gel to compare the sample to the λDNA/*Eco*RI+*Hin*dIII marker (Promega). DNA yield depended considerably on the genotypes and varied from 125 µg to 1,000 µg per 1 g of lyophilised leaves.

RFLP markers

DNA probes came from two genomic libraries. Probes for which the name begins with a G or AR were derived from a *Bam*HI library of the Arabusta genotype (hybrid issue from a cross between *C. arabica* and *C. canephora*). The probes designed CA were obtained from a *C. arabica Pst*I library. Preparation and characteristics of the libraries have been previously described (Paillard et al. 1996). DNA digestion, separation by electrophoresis, blotting and hybridisation were performed following the manufacturer's instructions.

AFLP markers

The AFLP protocol by Zabeau and Vos (1993) and Vos *et al.* (1995) was carried out with minor modifications. Total genomic DNA (50 ng/ µl) was restricted with two enzyme combinations, 10 U *Eco*RI and 5 U *Mse*I (from Gibco BRL), in 5×T4 ligase buffer for 2 h at 37°C. Twenty-five microlitres of a mixture containing 100 ng/µl of *Eco*RI adapter, 1 µg/µl of *Mse*I adapter, 1 U T4 DNA ligase (from Gibco BRL) and AFLP water-grade was added to the restricted DNA. The ligation reaction was incubated at 37°C for 3 h.

The pre-amplification profile by PCR was performed with 5 µl of template DNA using a primer pair based on the sequences of the *Eco*RI and *Mse*I adapters. These had one additional selective nucleotide at the 3' end (*Eco*RI+1 primer and *Mse*I+1 primer) (Vos et al. 1995). This first round of amplification consisted of: 20 cycles: 30 s at 94°C, 1 min at 56°C and 1 min at 72°C. The pre-amplification products were then diluted 30-fold with AFLP water-grade. The second and final round of amplification was performed using primers based on the same sequence but with two additional selective nucleotides at the 3' end (*Eco*RI+3 primers and *Mse*I+3 primers). The *Eco*RI+3 primers were labelled by phosphorylating the 5' end with [γ-[^{33}P]-ATP (Amersham). The amplification profile consisted of 13 cycles of 30 s at 94°C, 30 s at 65°C and 1 min at 72°C and 33 cycles of 30 s at 94°C, 30 s at 56°C and 1 min at 72°C. The PCR was performed on a MJ Research PTC 200 thermocycler.

The PCR products were mixed with an equal volume of 98% formamide, 10 m*M* EDTA, 0.05% bromophenol blue and xylene cyanol as tracking dyes. They were denaturated at 90°C (3 min) and maintained at 70°C. Aliquots (5 µl) were loaded onto a denaturating 8% polyacrylamide gel which consisted of 40 g urea, 12 ml acrylamide, 8 ml 10×TBE (108.0 g TRIS, 55.0 g boric acid, 40.0 ml 0.5 *M* EDTA, pH 8 in 1 l) and 28 ml distilled water. The electrophoresis buffer was 1×TBE. Gels were run at constant pow-

er (50 W for 3 h 40 min), fixed, dried as for the sequencing gels and then exposed to X-ray film (Kodak Biomax film) for 48–72 h.

Morphological marker

Only one morphological and monogenic marker was used for mapping: the fruit colour at maturity (purple in PSE and red in DEW, with purple being dominant).

Data analysis

AFLP and RFLP data were obtained by two readers visually scoring the autoradiograms independently. Only unambiguous bands specific to PSE were scored (present=1, absent=0). Marker segregations were checked for deviation from the expected Mendelian segregation (1:1) by chi-squared analysis.

Map construction strategies

Linkage analysis was assessed using the MAPMAKER/EXP version 3.0b computer software (Lander et al. 1987; Lincoln et al. 1992). Map units (cM, centiMorgans) were derived using the Kosambi (1944) mapping function. The marker order was confirmed with the ripple command.

A two-step procedure was carried out to manage the two statistical error risks (I and II). In the first step, MAPMAKER was separately applied to distorted markers and non-distorted markers. The LOD score was defined to obtain highly likely linkage groups. In the second step, MAPMAKER was applied to regroup previously defined linkage groups that can be linked. The LOD score was then defined to avoid pseudo-linkage.

A simulation was carried out to define the two LOD score values. The simulated table of 62 individuals and 80 markers was structured as follows:

1) four independent linkage groups A, B, C, D of 10 non-distorted markers. The first marker of each linkage group was obtained using a binomial distribution (n=2, P=0.5). The second marker arose from the first marker by random swapping (0 became 1 and vice-versa) for 10% of the 62 individuals. The same process was used to obtain the third marker from the second one, and so on, up to the tenth marker. Markers were approximately equally spaced;
2) an independent linkage group E of 10 distorted markers (segregation 3:1). Obtaining differed only by the initial use of a binomial distribution with P=0.75;
3) a second distorted linkage group, F, related to the group A of non-distorted markers. This group F also included of 10 markers, but its first marker arose from the first marker of the group A in which 50% of 0 (selected at random) became 1. Thus, A and F should be linked after mapping;
4) ten distorted and independent markers, obtained using a binomial distribution (n=2, P=0.75);
5) ten distorted markers simulating band superposition. Each marker "M3" resulted from 2 markers (M1 and M2) randomly selected among the groups A, B, C and D. The algorithm was: if M1=1 or M2=1 then M3=1, otherwise M3=0.
6) The two LOD scores we defined from the simulation were 5.5 for the first step and 3.1 for the second one.

Results

A total of 192 PSE-specific AFLP markers (present in PSE and F_1 hybrids and absent in DEW) were scored on 60 gels. Table 1 gives the 30 primer combinations used. The number of selected bands varied from 1 to 12, with an average of 6 bands per combination. Sixteen RFLP markers were also scored; corresponding data can be found in Barre (1997).

Table 1 Number of AFLP amplification products generated with 30 primer combinations. *Eco* +3:3' end selective nucleotides of the primers are complementary to the *Eco* and *Mse* adapters, respectively

Primer combinations		Number of polymorphic bands
Eco+3	*Mse*+3	
AAC	CAA	3
AAC	CAC	10
AAC	CAG	3
AAC	CAT	10
AAC	CTA	10
AAC	CTC	4
AAC	CTG	10
AAC	CTT	7
AAG	CAT	11
AAG	CTA	8
AAG	CTC	1
AAG	CTG	6
AAG	CTT	12
ACA	CAA	5
ACA	CAC	4
ACA	CAT	3
ACA	CTA	10
ACA	CTC	5
ACA	CTG	8
ACA	CTT	6
ACC	CAA	7
ACC	CAC	9
ACC	CAT	4
ACC	CTA	5
ACC	CTG	4
ACC	CTT	6
ACT	CAA	5
ACT	CAT	7
ACT	CTC	5
ACT	CTT	7

In our mapping strategies, segregation distortions were tested for each marker, leading to two groups of markers being defined: distorted and non-distorted.

Genetic linkage map

The first step of the mapping strategy (using a LOD score of 5.5) defined 19 linkage segments of non-distorted markers and 6 linkage segments of distorted markers (black segments on Fig. 1). Twelve non-distorted markers and 16 distorted markers were found independently by MAPMAKER and were not mapped. The second step (LOD score value of 3.1) led to a final genetic map with 14 linkage groups (4–31 loci per linkage group) (Fig. 1).

Table 2 summarises the main statistics of each linkage group. Linkage groups could be classified into three categories: (1) large-sized linkage groups (150–225 cM) with 31–21 loci (groups A and B); 2) medium-sized linkage groups (48–113 cM) with 11–19 loci (groups C–J); and (3) small-sized linkage groups (18–27 cM) with 4–6

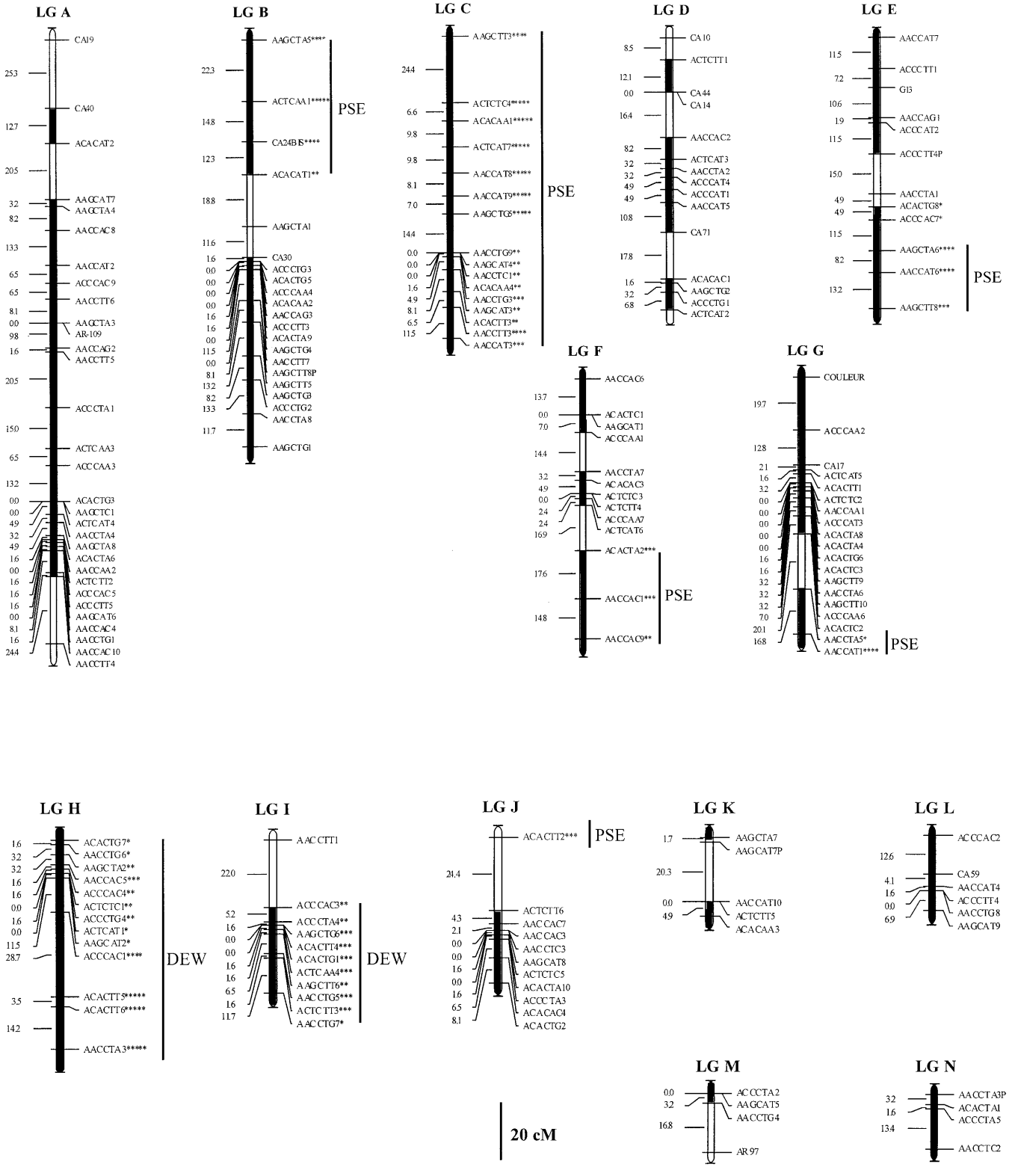

Fig. 1 Genetic linkage map constructed from 62 backcross hybrids derived from the interspecific cross [(PSE×DEW)×DEW] showing the location of 168 AFLP and 12 RFLP. AFLP markers are described in terms of the three selective nucleotides used; for example, AACCTG8 – the *Eco*RI primer is given to the *left* (*AAC*), the *Mse*I primer to the *right* (*CTG*). RFLP markers are *prefixed* with G or AR for loci detected by the Arabusta cDNA clone and CA for loci detected by random genomic clones of *C. arabica*. Recombination distances are given in centiMorgans (cM), Kosambi units on the *left side* of each linkage group (*LG*) and markers names are given to the *right* of each LG. Fourteen LG covering 1,144 cM are indicated by *letters A* through *N*, ordered by their length. Loci marked *, **, ***, **** and ***** deviated significantly from a 1:1 ratio at $P<0.01$, $P<0.001$, $P<0.0001$, $P<0.00001$ and $P<0.000001$, respectively. The *lines* specified by *PSE* or *DEW* correspond to distortion in favor of PSE and DEW, respectively

Table 2 Main characteristics of the linkage groups (size, number of markers and distances) in the genetic map resulting from the 62 BCDEW hybrid progeny

Linkage groups	Length (cM)	Number of markers	Means distances (cM)	Min-max (cM) distances
A	224.4	31	7.5	0.0–25.3
B	150.6	21	7.5	0.0–22.3
C	112.7	16	7.5	0.0–24.4
D	101.6	15	7.2	0.0–17.8
E	100.4	12	9.1	1.9–15.0
F	97.3	13	8.1	0.0–17.6
G	96.1	19	5.3	0.0–20.1
H	70.7	13	5.9	0.0–28.7
I	51.8	11	5.2	0.0–22.0
J	48.6	11	4.9	0.0–24.4
K	26.9	5	6.7	0.0–20.3
L	25.2	6	5.0	0.0–12.6
M	19.7	4	6.7	0.0–16.8
N	18.2	4	6.1	1.6–13.4

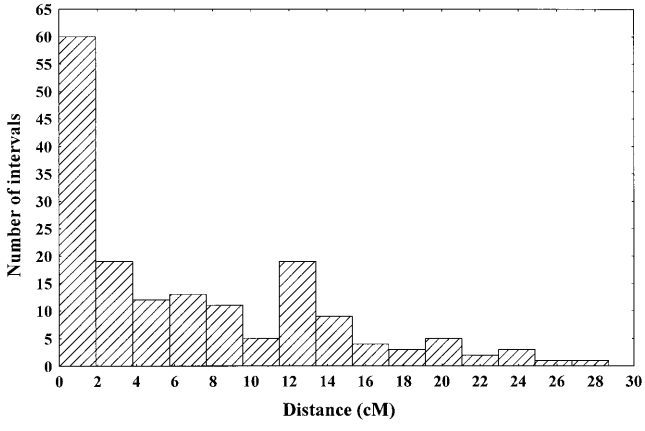

Fig. 2 Distribution of the distance between two successive markers

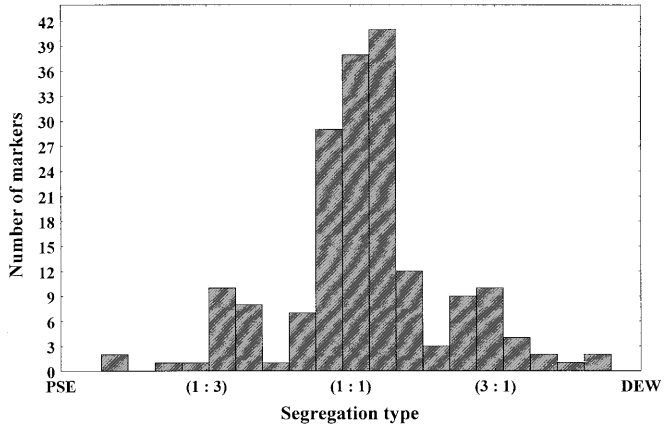

Fig. 3 Distribution of the 181 markers mapped in the BCDEW progeny in relation to the relative contribution of PSE. The figure clearly shows a predominant fraction of markers presenting a Mendelian segregation of 1:1 and two categories of segregation distorsion following a 3:1 ratio in favor to PSE and a 1:3 ratio in favor to DEW

Table 3 Average number of segregation types of the 181 markers in the BCDEW progeny

Total bands scored (181)	Segregation ratio			Other segregation Distortion ratio*
	1:1*	1:3*	3:1*	
100%	70%	14%	12%	4%

* Significant at α=0.05

markers (groups K–N). The genetic linkage map covered 1,144 cM.

The distances between 2 consecutive markers varied from 0 to 29 cM, with a mean of 7 cM. This distance distribution, shown on Fig. 2, reveals a strong skewness. Among the 167 intervals, 60 were smaller than 2 cM (36%), and 12 were larger than 20 cM (7%). The small intervals were gathered together and eight clusters could be easily observed in the large- and medium-sized linkage groups (except for groups D and E on Fig. 1). The density on these clusters varied from 6 to 15 markers for an interval segment of 20 cM.

Segregation distortion

Segregation distortion were observed for 30% of the loci (1 RFLP and 53 AFLP markers). The distribution of segregation ratio corresponded with three modes centred on three different ratios: (1:3, 1:1 and 3:1; Fig. 3). We assume that there are mainly three segregation types resulting in three binomial distributions, with the *P* parameter equal to 0.25, 0.5 and 0.75, respectively. Tested with chi-square, the distorted loci were classified as: (1) 26 loci (14% of the 181 markers) segregating according to a 1:3 ratio in favour of PSE; (2) 21 loci (12%) segregating as a 3:1 ratio in favour of DEW; (3) 7 loci presenting stronger distortion of segregation (Table 3). Proportions of 1:3 and 3:1 loci were similar.

The distribution of the 54 distorted markers on the genetic map is illustrated in Fig. 1. They were grouped at

the end of long and intermediate linkage groups and were not found on the 4 smaller groups. The segments which were distorted in favour of DEW constituted the whole of the H group and a part of the I group. The distorted fragments in favour of PSE were located on 6 linkage groups: all of C, half of each of the B, E and F groups and, for 1 and 2 markers, on the J and G groups, respectively.

Discussion

The PSE×DEW interspecific coffee map consisted of 181 markers covering 1,144 cM distributed over 14 linkage groups. AFLP clusters were observed, suggesting the location of centromeric regions. This map also revealed a relative important portion of distorted loci (30%), which were symetrically distributed following 3:1 and 1:3 ratios in favour of PSE (14%) and DEW (12%), respectively.

Genetic linkage map

PSE and DEW species have a strictly allogamous reproductive behaviour. Genitors are highly heterozygous and alleles segregate in the F_1 generation. The segregation of 2–4 alleles per loci and the impossibility in parents to distinguish between coupling and repulsion phase makes genetic mapping more complicated than for autogamous species. A double pseudo-test cross is then recommended (Grattapaglia and Sederoff, 1994). Our approach constituted an alternative: we selected specific PSE markers so DEW gametes could be considered to be genetically homozygous (double absence) for these markers. In contrast, the F_1 hybrids generated different gametes segregating in BCDEW hybrids. Consequently, the loci from parental or recombinant could be distinguished.

Fourteen linkage groups were identified for 11 chromosomes, an indication that the genome was not fully covered. Seven percent of 2 successive marker intervals were larger than 20 cM, confirming that additional markers are needed to cover all of the genome. The strong skewness of the distance distribution shows that this number should be high enough to fill all the large intervals.

Eight AFLP clusters were observed. Such clusters are known to characterise centromeric regions (Qi et al. 1998; Vuylstecke et al. 1999), and Alonsa-Blanco et al. (1998) used this property to locate the centromeric region on chromosome 3 of *Arabidopsis thaliana*. In addition, our markers emphasise (1) the very conserved parts of the PSE genome (no within-PSE polymorphism) and (2) the interspecific differentiation of the two genomes. Consequently, centromeric markers should be markers of speciation, thereby involving repeated sequences and implicated in chromosome pairing. Their presence on several centromers should also allow specific markers to be defined for chromosome in situ hybridisation studies.

The size difference between intraspecific (1,402 cM) and interspecific (1,144 cM) maps would result from the lower genome homology between parents in an interspecific cross, reducing recombination and map size. For example, in potatoes, the map size of an interspecific cross was found to be 65% smaller than the intraspecific one (Bonierbale et al. 1988; Gebhardt et al. 1991). In coffee, the map size of the interspecific cross is 18% smaller than the intraspecific one on *C. canephora*: 1,144 cM (Paillard et al. 1996). The smaller difference between map sizes in coffee trees could reflect a higher genome homology in the genus *Coffea* than in potatoes.

Among the 12 RFLP markers mapped, only 1 (CA 30) is common with the intraspecific map developed by Paillard et al. (1996), the other RFLP markers being homozygous in *C. canephora*.

Segregation distortion

With AFLP markers, co-migration and superimposition of non-allelic amplification products is possible (Cloutier et al. 1997) leading to pseudo-distortion of (3:1) ratio. With our two-step mapping method, the mapping of pseudo-distorted markers is not likely. Nevertheless, the symmetric situation between the 1:3 and 3:1 ratios (Fig. 3) constitutes the main argument in favour of true distorted markers.

Skewed segregation ratios have been reported frequently in interspecific cross for all types of markers (morphological, isozyme, RFLP or AFLP). The percentage of loci showing segregation distortions was highly variable: 69% in *Cryptomeria japonica* (Nikaido et al. 1999), 37% in *Citrus* (Luro et al. 1994), 36% in *Oryza* (Xu et al. 1997; Virk et al. 1998), 33% in *Prunus* (Foolad et al. 1995), 23% in *Helianthus* (Quillet et al. 1995), 8.4% in *Lens* sp. (Eujayl et al. 1998) and only 1.4% in *Hevea* spp. (Lespinasse et al. 2000). Compared to these data, our distorted frequency appears to be intermediate, 30%.

In the genus *Lycopersicum*, based on a wild species crossed with the cultivated *L. esculentum*, interspecific crosses led to distortion rates varying from 51% to 80% (Paterson et al. 1988, 1991; de Vincente and Tanksley 1993). Our cross included two species among the most differentiated of the genus (Lashermes et al. 1997), it should therefore be interesting to observe the variation of this parameter when the genetic distance between parental species is lower.

The explanations given for such distortions are mostly gametic, zygotic or/and post-zygotic selection, but these are not plausible in explaining a symmetric and polymodal distribution, especially 3:1 and 1:3 ratios. In contrast, gene conversion implying DNA heteroduplex formation is known to give such ratios (Leblon and Rossignol 1973; Nicolas and Rossignol 1983; Nag et al. 1989). Post-meiotic segregation is another process leading to distortion segregation, but with 5:3 and 3:5 ratios. A high rate of conversion and low rate of post-meiotic segregation reflect a high level of heteroduplex forma-

tion and efficient repair of the resulting mismatch (Nag et al. 1989). This explanation fits well with our results. A high rate of heteroduplex formation and mismatch are expected during meiosis pairing in interspecific hybrids. This phenomenum could be due to differences in DNA amount among parents as the DEW genome size is 25% higher than PSE one (Barre et al. 1996). The intergeneric cross *Citrus reticulata*×*Poncirus trifoliata* (tangerine tree×grapefruit tree) studied by Luro et al. (1994) showed 37% of distorted loci (for both RFLPs and RAPDs). Although not emphasised by authors, the data clearly show two types of distorted markers: 9 markers in favour of *Poncirus* with *P*=0.30 and 29 markers in favour of *Citrus* with *P*=0.75. Again the *P* values fit well with the 1:3 and 3:1 ratio, respectively. Obviously, gene conversion seems to characterise a interspecific cross and does not depend on the type of marker (AFLP, RFLP and RAPD). These results led us to develop software to test such ratios. MAPDISTO (the software) is available on the website: http://www. mpl.ird.fr/~lorieux.

Perspectives

The present work demonstrates the feasibility of an AFLP marker-based genetic linkage map of the diploid coffee genome. This map is a starting point for further molecular studies in coffee genetics.

Firstly, this map could be used to identify quantitative trait loci (QTL) for highly discriminant and specific traits. Biochemical traits implied in the coffee cup quality will be interesting because of the differences between species. This is the case for caffeine with 0% in PSE and 1% in DEW (Barre et al. 1998), the chlorogenic acids with 1.2% in PSE and 5.5% in DEW (Ky et al. 1999) and sucrose (8% in PSE and 3% in DEW (Ky et al. 2000). Identification and location of interspecific QTL will lead to the use of bordering molecular markers as tools for assisting in the introgression of this interesting characteristic from wild coffee to cultivated coffee species (molecular assisted selection).

Secondly, this map could be used to select AFLP markers for sequencing and obtaining co-dominant PCR markers. These markers will be used to compare our results with other interspecific maps currently in progress, such as these three from crosses under study: *C. pseudozanguebariae*×*C. canephora*, *C. heterocalyx*×*C. canephora* and *C. eugenioides*×*C. canephora*. And, finally, sequenced AFLP of either distorted loci or clustered loci will permit meiotic pairing mismatching using *in situ* hybridisation.

References

Alonso-Blanco C, Peeters AJM, Koornneef M, Lister C, Dean C, Van den Bosch N, Pot J Kuiper MTR (1998) Development of an AFLP based map of Ler, Col and Cvi *Arabidopsis thaliana* ecotypes and construction of a Ler/ Cvi recombinant inbred lines population. Plant J 14:259–271

Barre P (1997) Les transferts de gènes entre espèces de caféiers diplo: étude des hybrides F_1 et BC_1 entre *C. pseudozanguebariae* Bridson et *C. liberica* Bull ex Hiern (*C. dewevrei* De Wild et Th. Dur). PhD thesis, Ecole Nationale Supérieure Agronomique de Montpellier, France

Barre P, Noirot M, Louarn J, Duperray C, Hamon S (1996) Reliable flow cytometric estimation of nuclear DNA content in coffee trees. Cytometry 24:32–38

Barre P, Akaffou S, Louarn J, Charrier A, Hamon S, Noirot M (1998) Inheritance of caffeine and heteroside contents in an interspecific cross between a cultivated coffee species *Coffea liberica* var '*dewevrei*' and a wild species caffeine free *C. pseudozanguabariae*. Theor Appl Genet: 96:306–311

Bonierbale MW, Plaisted RL, Tanksley SD (1988) RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics 120:1095–1103

Bridson DM (1987) Nomenclatural notes on *Psilanthus*, including *Coffea* sect. *Paracoffea* (*Rubiaceae* tribe *Coffeeae*). Kew Bull 36:817–860

Carvalho A, Monaco LC (1967) Genetic relationships of selected *Coffea* species. Cienc Cult 19:151–165

Charrier A (1978) La structure génétique des caféiers spontanés de la région malgache et leurs relations avec les caféiers d'origine africaine (Eucoffea). PhD thesis, Mémoire ORSTOM, Paris, no. 87

Cloutier S, Cappadocia M, Landry BS (1997) Analysis of RFLP mapping inaccuracy in *Brassica napus* L. Theor Appl Genet 95:83–91

De Vicente MC, Tanksley SD (1993) QTL analysis of transgressive segregation in an interspecific tomato cross. Genetics 134: 585–596

Dolezel J, Binarova P, Lucretti S (1989) Analysis of nuclear DNA content in plant cells by flow cytometry. Biol Plant 32:113–120

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15

Eujayl I, Baum M, Powell W, Erskine W, Pehu E (1998) A genetic linkage map of lentil (*Lens* sp.) based on RAPD and AFLP markers using recombinant inbred lines. Theor Appl Genet 97:83–89

Foolad MR, Arulsekar S, Becerra V, Bliss FA (1995) A genetic map of *Prunus* based on an interspecific cross between peach and almond. Theor Appl Genet 91:262–269

Gadish I, Zamir D (1986) Differential zygotic abortion in an interspecific *Lycopersicum* cross. Genome 29:156–159

Gebhardt C, Ritter E, Barone A, Debener T, Walkemeier B, Schachtschabel U, Kaufmann H, Thompson RD, Bonierbale MW, Ganal MW, Tanksley SD (1991) RFLP maps of potato and their alignment with the homologous tomato genome. Theor Appl Genet 83:49–57

Grant V (1975) Genetics of flowering plants. Columbia University Press, New York

Grattapaglia D, Sederoff R (1994) Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross mapping strategy and RAPD markers. Genetics 137: 1121–1137

Kosambi DD (1944) The estimation of map distances recombination values. Ann Eugen 12:172–175

Ky C-L, Louarn J, Guyot B, Charrier A, Hamon S, Noirot M (1999) Relations between and inheritance of chlorogenic acids contents in an interspecific cross between *C. pseudozanguabariae* and *Coffea liberica* var '*dewevrei*'. Theor Appl Genet 98:628–637

Ky C-L, Doulbeau S, Guyot B, Akkafou S, Charrier A, Hamon S, Louarn J, Noirot M (2000) Inheritance of coffee bean sucrose content in the interspecific cross: *Coffea pseudozanguebariae*×*Coffea liberica 'dewevrei'*. Plant Breed 119:165–168

Lashermes P, Combes MC, Trouslot P, Charrier A (1997) Phylogenetic relationships of coffee-tree species (*Coffea* L.) as inferred from ITS sequences of nuclear ribosomal DNA. Theor Appl Genet 94:947–955

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Leblon G, Rossignol JL (1973) Mechanism of gene conversion in *Ascobolus immersus*. The interaction of heteroalleles in the conversion process. Mol Gen Genet 122:165–182

Leroy JF (1980) Evolution et taxogénèse chez les caféiers (*Coffea* L., *Psilanthus* Hook. f. et *Nostolachma* Durand). Hypothèse sur leur origine. C R Acad Sci 291:593–596

Lespinasse D, Rodier-Goud M, Grivet L, Leconte A, Legnate H, Seguin M (2000) A saturated genetic linkage map of rubber tree (*Hevea* spp.) based on RFLP, AFLP, microsatellite, and isozyme markers. Theor Appl Genet 100:127–138

Lincoln S, Daly M, Lander E (1992) Constructing genetic maps with MAPMAKER/EXP version 3.0. Whitehead Institute Technical Report, 3rd edn., Cambridge, Mass.

Louarn J (1992) La fertilité des hybrides interspécifiques et les relations génomiques entre les caféiers diplo d'origines africaines (Genre *Coffea* L. sous–genre *Coffea*), PhD thesis, University of Orsay, France

Luro F, Lorieux M, Laigret F, Bové JM, Ollitrault P (1994) Cartographie du génome des agrumes à l'aide des marqueurs moléculaires et distorsions de ségrégation. In: Berville and Tersac (eds) Techniques et utilisations des marqueurs moléculaires, vol. 72. INRA, Montpellier, France, pp 72:69–82

Meksem K, Leister D, Peleman J, Zabeau M, Slamini F, Gebhardt C (1995) A high-resolution map of the vinicity of the R1 locus on chromosome V of potato based on RFLP and AFLP markers. Mol Gen Genet 249:74–81

Nag DK, White MA, Petes TD (1989) Palindromic sequences in heteroduplex DNA inhibit mismatch repair in yeast. Nature 340:318–320

Nicolas A, Rossignol JL (1983) Gene conversion: point-mutation heterozygosities lower heteroduplex formation. EMBO J 2: 2265–2270

Nikaido A, Yoshimaru H, Tsumura Y, Suyama Y, Murai M, Nagasaka K (1999) Segregation distortion for AFLP markers in *Cryptomeria japonica*. Genes Genet Systems 74:55–59

Paillard M, Lashermes P, Pétiard V (1996) Construction of a linkage map in coffee. Theor Appl Genet 93:41–47

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721–726

Paterson AH, Damon S, Hewitt JD, Zamir D, Rabinowitch HD, Lincoln SE, Lander ES, Tanksley SD (1991) Mendelian factors underlying quantitative traits in tomato: comparison across species, generations, and environments. Genetics 127: 181–197

Qi X, Stam P, Lindhout P (1998) Use of locus-specific AFLP markers to construct a high density molecular map in barley. Theor Appl Genet 96:376–384

Quillet MC, Madjidan N, Griveau Y, Serieys H, Tersac M, Lorieux M, Bervillé A (1995) Mapping genetic factors controlling pollen viability in an interspecific cross in *Helianthus* sect. *Helianthus*. Theor Appl Genet 91:1195–1202

Thomas CM, Vos P, Zabeau M, Jones DA, Norcott KA, Chadwick BP, Jones JDG (1995) Identification of amplified restriction fragment polymorphism (AFLP) markers tightly linked to the tomato *Cf-9* gene for resistance to *Cladosporium fulvum*. Plant J 8:785–794

Virk PS, Ford-Lloyd BV, Newbury HJ (1998) Mapping AFLP markers associated with subspecific differentiation of *Oryza sativa* (rice) and an investigation of segregation distortion. Heredity 81:613–620

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Vuylsteke M, Mank R, Antonise R, Bastiaans E, Senior ML, Stuber CW, Melchinger AE, Lübberstedt T, Xia XC, Stam P, Zabeau M, Kuiper M (1999) Two high-density AFLP linkage maps of Zea mays L.: analysis of distribution of AFLP markers. Theor Appl Genet 99:921–935

Xu Y, Zhu L, Xiao J, Huang N, McCouch SR (1997) Chromosomal regions associated with segregation distortion of molecular markers in F_2, backcross, double haploids, and recombinant inbred populations in rice (*Oryza sativa* L.). Mol Gen Genet 253:535–545

Zabeau M, Vos P (1993) Selective restriction fragment amplification: a general method for DNA fingerprinting. European Patent Application number: 92402629.7, Publ. no. 0 534 858 A1

Zamir D, Tadmor Y (1986) Unequal segregation of nuclear genes in plants. Bot Gaz 147:355–358