# Genetic linkage map of *Coffea canephora*: effect of segregation distortion and analysis of recombination rate in male and female meioses

P. Lashermes, M.C. Combes, N.S. Prakash, P. Trouslot, M. Lorieux and A. Charrier

**Abstract:** Two complementary segregating plant populations of *Coffea canephora* were produced from the same clone. One population (DH) comprised 92 doubled haploids derived from female gametes, while the other population (TC) was a test cross consisting of 44 individuals derived from male gametes. Based on the DH population, a genetic linkage map comprising 160 loci was constructed. Eleven linkage groups that putatively correspond to the 11 gametic chromosomes of *C. canephora* were identified. The mapped loci included more than 40 specific sequence-tagged site markers, either single-copy RFLP probes or microsatellites, that could serve as standard landmarks in coffee-genome analyses. Furthermore, comparisons for segregation distortion and recombination frequency between the two populations were performed. Although segregation distortions were observed in both populations, the frequency of loci exhibiting a very pronounced degree of distortion was especially high in the DH population. This observation is consistent with the hypothesis of strong zygotic selection among the DH population. The recombination frequencies in both populations were found to be almost indistinguishable. These results offer evidence in favour of the lack of significant sex differences in recombination in *C. canephora*.

*Key words*: coffee, mapping, sex differences, segregation distortion, recombination frequency.

**Résumé** : Deux populations complémentaires de plantes en ségrégation ont été produites à partir d'un même clone de *Coffea canephora*. Une population (DH) comprend 92 haploïde-doublés dérivés des gamètes femelles, tandis que l'autre (TC) consiste en 44 individus issus des gamètes mâles. Sur la base de la population DH, une carte génétique de liaison, comprenant 160 loci, a été construite. Onze groupes de liaison correspondant très vraisemblablement aux 11 chromosomes de *C. canephora* ont été identifiés. Parmi les loci cartographiés sont inclus 40 marqueurs « séquence-spécifiques », constitués par des sondes associées à du polymorphisme de fragments de restriction ou par des microsatellites, qui pourraient servir de balises de référence dans les études portant sur le génome des caféiers. Par ailleurs, une comparaison, entre les deux populations, des distorsions de ségrégation et des fréquences de recombinaison a été réalisée. Bien que des distorsions de ségrégation aient été observées au sein des deux populations, la fréquence des loci montrant un fort degré de distorsion est particulièrement élevée chez la population DH. Cette observation est cohérente avec l'hypothèse d'une forte sélection zygotique au sein de la population DH. Les fréquences de recombinaison apparaissent globalement similaires au sein des deux populations. Ces résultats suggèrent l'absence de différences sexuelles significatives pour la recombinaison chez *C. canephora*.

*Mots clés* : caféier, cartographie, différences sexuelles, distorsion de ségrégation, fréquence de recombinaison.

## Introduction

Genetic progress in a breeding program depends on the generation and selection of new recombinant genotypes. In this respect, the amount of genetic variation released in the segregating generations of a particular cross depends on the random reassortment of chromosomes and the amount of recombination within chromosomes. Several parameters, including environmental (e.g., temperature, stress conditions), physiological (e.g., age), and genetic effects, are known to influence the frequency and distribution of crossover events. One such factor is the relative recombination rates recovered in male and female gametes (Burt et al. 1991). In plants, a number of studies, for example, of *Zea mays* (Robertson

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1984), *Arabidopsis thaliana* (Zhuchenko et al. 1989), tomato (Ganal and Tanksley 1996), and *Brassica oleracea* (Kearsey et al. 1996), showed sex differences in overall recombination frequency, whereas other studies, for example, of pearl millet (Busso et al. 1995), wheat (Wang et al. 1995), and *Brassica napus* (Kelly et al. 1997), demonstrated no significant differences or differences specific to certain chromosome segments. Furthermore, it has been shown that the estimation of recombination fractions may be biased by deviations of single-locus segregation ratios from expected frequencies (Bailey 1949). A typical source of deviation is the upsets in the formation or function of gametes or zygotes, owing to the selection of one or more selected genes on the chromosomes. Several authors have discussed methods to test for linkage or to estimate recombination frequencies between genes showing segregation distortion (Bailey 1949; Garcia-Dorado and Gallego 1992; Lorieux et al. 1995).

Coffee production relies mainly on two tropical tree species—*Coffea arabica* L. and *C. canephora* Pierre. Although contributing to more than 70% of world coffee production, *C. arabica* is characterised by very low genetic diversity, which is attributable to its allotetraploid origin, reproductive biology, and evolution. In contrast, considerable diversity has been reported in the diploid species *C. canephora*, which is not only a species of economic importance but also one of the progenitor species of *C. arabica* (Lashermes et al. 1999). *Coffea canephora* constitutes the main source of disease resistances used so far in *C. arabica* breeding (Carvalho 1988). Observation of sex differences in recombination of *C. canephora* could have important consequences for genetic studies and breeding strategies.

*Coffea canephora* is a strictly allogamous species with a long juvenile period, consisting of polymorphic populations and strongly heterozygous individuals. Conventional segregating populations are therefore somehow difficult to generate and analyse. However, the ability to produce doubled-haploid (DH) populations in *C. canephora* offers an attractive alternative approach. The method of DH production is based on haploid embryos of maternal origin occurring spontaneously in association with polyembryony (Couturon 1982). Although characterised by low vigour, the potential value of DH in genetic research and coffee breeding has been shown by earlier studies (Lashermes et al. 1994*a*, 1994*b*). Using a DH population, a partial genetic linkage map has already been constructed based mostly on RAPD (random amplified polymorphic DNA) markers (Paillard et al. 1996).

The objectives of the study presented here were (*i*) to develop a complete linkage map of *C. canephora* and (*ii*) to compare two complementary populations produced from the same clone of *C. canephora*: one DH population derived from female gametes and the other (i.e., a test cross) derived from the male gametes. In particular, the deviation from Mendelian segregation ratios and the frequency and distribution of crossovers estimated through these two populations were investigated.

## Materials and methods

### Plant material

The plant material consisted of two segregating populations derived from the same hybrid clone, IF 200, of *C. canephora*. One population (DH) comprised 92 doubled haploids. Haploid plantlets were identified, and chromosome doubling was achieved by colchicine treatment, as previously described (Couturon 1982). The second population (TC) of 44 individuals resulted from a test cross between IF 200 (male parent) and a doubled haploid genotype (DH 160-02) derived from the clone IF 160 as female parent. Genomic DNA was isolated from lyophilised leaves through a nuclei-isolation step, as described by Agwanda et al. (1997).

### Molecular-marker assay

#### *AFLP protocol*

The AFLP (amplified length polymorphism) procedure was performed essentially as described by Vos et al. (1995), with minor adaptations for coffee DNA (Lashermes et al. 2000*a*). An aliquot of 500 ng of genomic DNA was digested with the restriction enzymes *Eco*RI and *Mse*I. Restriction fragments were ligated with double-strand *Eco*RI and *Mse*I adapters. A preamplification was done using the appropriate primers (designated E and M, respectively) in combination with one added selective nucleotide: E+A/M+C. The code following E or M corresponds to the selective nucleotides at the 3′ end of the *Eco*RI and *Mse*I primers, respectively. The reaction mixture was diluted 1/30, and 10 µL was used for the final amplification with two primers, each of which had three selective nucleotides. The primer combinations used were E+ACA/M+CAA; E+AAC with M+CTC, M+CTT, or M+CAT; and E+ACT with M+CTT or M+CAA.

#### *Microsatellite markers*

Eighteen microsatellite loci (Sat12, Sat20, Sat21, Sat27, Sat41, Sat42, Sat47, Sat154, Sat157, Sat158, Sat160, Sat167, Sat171, Sat177, Sat181, Sat189, Sat193, and Sat194), previously identified as polymorphic, were analysed using PCR. The specific primer pairs, amplification conditions, radioactive labelling, and polyacrylamide gel electrophoresis were as described elsewhere (Combes et al. 2000).

#### *RAPD*

Over 90 random decamer primers were used for PCR in conditions similar to those described by Paillard et al. (1996). Primers were obtained from Operon Technologies (Alameda, Calif., U.S.A.). Only 11 primers (B18, G6, H3, H4, H7, H19, K14, Q6, N14, Y13, and Z14) that showed clear and reproducible polymorphism were used for mapping.

#### *Restriction fragment length polymorphic (RFLP) markers*

Restriction enzyme digestion, gel electrophoresis, alkaline transfer, nonradioactive digoxigenin labelling of DNA probes, and Southern hybridisation were carried out as previously reported (Lashermes et al. 2000*b*). Three sources of probes were used: (1) genomic clones (gA) from a *Pst*I-fragment library of *C. arabica* (cv. N39) in pUC18, (2) genomic, and (3) cDNA clones (gR and cR, respectively) obtained from an "Arabusta" plant (i.e., interspecific hybrid between *C. arabica* and *C. canephora*), as described previously (Paillard et al. 1996). Four restriction enzymes, *Eco*RI, *Dra*I, *Hin*dIII, and *Eco*RV, were tested to detect polymorphism in a preliminary screening performed on blots containing DNAs of IF200 and four randomly chosen DH genotypes.

### Data scoring and marker nomenclature

Only those AFLP and RAPD fragments showing a clear polymorphism were scored as presence versus absence of bands. The AFLP products were designated by the name of the primer combination followed by a number reflecting the fragment position on the gel. The RAPD products were identified by "R," followed by the primer code. Only one band could be scored for a particular

primer. The RFLPs were designated by the probe codes followed by the letter A, B, C, or D, according to the restriction enzyme used, viz. *Eco*RI, *Dra*I, *Hin*dIII, or *Eco*RV, respectively. When more than one locus could be scored for a particular probe–enzyme combination, a small letter was appended to identify each locus. All RFLP and microsatellite markers were scored as di-allelic loci, with the exception of gA5Bc, gA5Bd, Sat167, and Sat194, which showed a presence–absence of polymorphism.

### Data and linkage analysis

The segregation in the two populations of each marker was tested for goodness of fit to the expected 1:1 Mendelian segregation ratio by $\chi^2$ analysis. Linkage analysis and map construction were performed separately for the DH and TC data, using the computer programme Mapmaker version 3.0b (Lander et al. 1987). Both populations, DH and TC, were treated as backcross populations, with an expected segregation of 1:1 for the two alternative alleles present in the clone IF 200. Linkage groups were established by two-point analysis using a minimum LOD (logarithmic odds) score of 5.0 and a maximal recombination frequency of 0.35. Three-point and multipoint analyses were then performed, to find the most probable locus order within each linkage group. The final locus order of each linkage group was tested by removing one locus at a time and checking for inconsistencies. The Kosambi function (Kosambi 1944) was used for converting recombination frequencies into map distances or centimorgan (cM) values.

To verify the impact of segregation distortion, two-point estimates were calculated in a manner similar to that in Lorieux et al. (1995), using the computer programme Map-disto (available via http://www.mpl.ird.fr/~lorieux). An appropriate model of selection based on observation of the frequencies of genotype classes generated by segregation analysis of each pair of adjacent markers was defined to derive the estimates.

To compare recombination frequencies estimated in the DH and TC populations for each pair of flanking marker loci in equivalent linkage intervals (i.e., a map segment showing a common set of ordered markers), the observed proportions of parental and recombinant lines in each of the two populations were compared using 2 × 2 contingency $\chi^2$ tests.

## Results

### Level of polymorphism

In the AFLP assay, six different combinations were used (Table 1). A total of 97 AFLP products were found to be polymorphic among the DH population. The efficiency of the method in terms of the effective multiplex ratio, that is, the total number of informative bands per gel lane, was 17.3, on average. The proportion of heterozygous loci in IF 200, estimated by the ratio between the number of segregating bands and the total number of DNA bands, was 33%.

Eighteen microsatellite markers were used in this study. Each generated only a single marker locus.

Eleven of the 90 tested RAPD primers produced consistent polymorphic fragments among the DH population. A total of 11 RAPD markers were generated by the 11 selected primers; each primer generated only one scorable polymorphism. In our conditions, RAPD reactions amplified an average of 3.4 clear prominent bands on ethidium bromide stained agarose gel. The proportion of heterozygous RAPD loci in IF 200 was, therefore, 4%.

Thirty-five DNA clones detected RFLP polymorphisms in both populations for at least one restriction enzyme – probe combination. These probes consisted of 20 nuclear genomic clones from *C. arabica* and 15 clones (12 genomic and three cDNA clones) from the interspecific hybrid (*C. arabica* × *C. canephora*). Most of the probes appeared to be single copy and only one, gA5, revealed more than one RFLP locus. Slight differences in detecting RFLPs were observed among the four restriction enzymes used; *Dra*I was the most efficient.

**Table 1.** Number of polymorphic AFLP amplification products generated with six different primer combinations.

| Primer combination[a] | | Total no. of amplified DNA bands | No. of segregating polymorphic bands |
|---|---|---|---|
| $E^{+3}$ | $M^{+3}$ | | |
| AAC | CTC | 47 | 12 |
| AAC | CTT | 69 | 25 |
| AAC | CAT | 53 | 16 |
| ACA | CAA | 67 | 18 |
| ACT | CTT | 48 | 12 |
| ACT | CAA | 49 | 14 |

[a] $E^{+3}$ and $M^{+3}$ are 3′-end selective nucleotides of the primers complementary to the *Eco*RI and *Mse*I adapters, respectively.

### Linkage analysis

Genetic segregation in the DH population was recorded for a total of 162 loci divided as follows: 97 AFLP loci, 11 RAPD loci, 18 microsatellite loci, and 36 RFLP loci.

Owing to heterozygosity and the presence of band-alleles inherited from the DH 160-02 parent, the number of scorable segregating markers was significantly reduced in the TC population. Variations in banding intensity were not considered, and only 53 of the 97 segregating AFLP markers were scored. Similarly, only 6 of the 11 RAPD markers appeared polymorphic in the TC population.

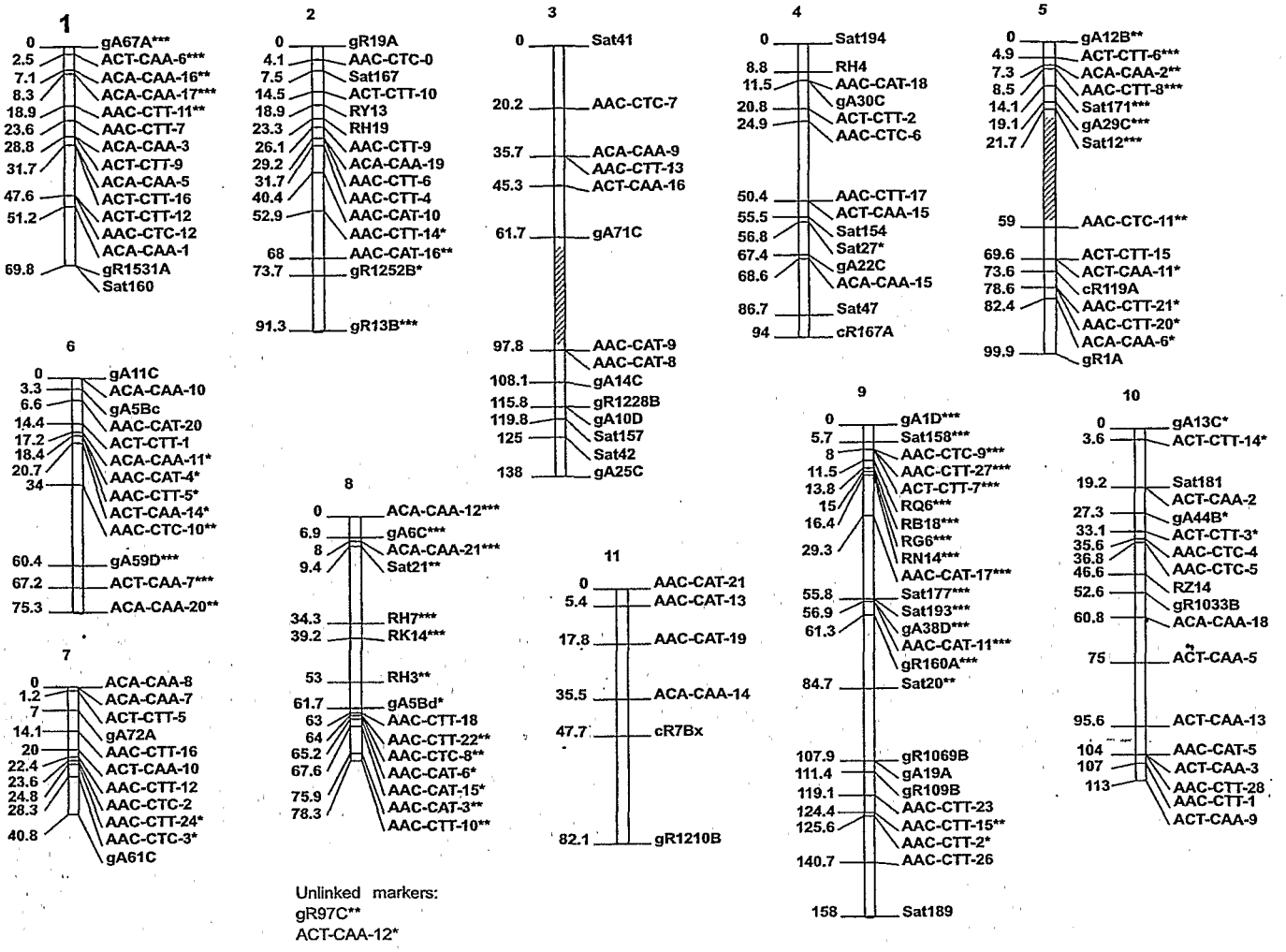
Linkage analysis in the DH population of the 162 marker loci using a LOD threshold of 5.0 and a maximum $r$ of 0.35 resulted in 13 linkage groups. Only two marker loci (i.e., gR97C and ACT-CAA-12) remained unlinked. As the LOD threshold was decreased to 3.5, linkage groups were associated, resulting in 11 linkage groups (Fig. 1). This equals the haploid number of chromosomes in the genome of *C. canephora*. With the exception of group 11, all linkage groups comprised more than 10 markers. The total map length was 1041 cM, with an average distance of 6.5 cM between markers. However, the AFLP loci and, to a lesser extent, the RAPD loci showed some level of clustering. In contrast, the RFLP as well as the microsatellite loci appeared more evenly distributed along the linkage groups. Correspondence between these 11 linkage groups and the 15 linkage groups of the incomplete map previously reported (Paillard et al. 1996) are indicated in Table 2.

Of the 162 loci, 109 were used to develop a partial map based on the TC population. These loci were organised into linkage groups corresponding to those identified in the DH-derived linkage map for further comparison.

### Segregation distortion in the DH and TC populations

In the DH population, segregation of 72 marker loci (i.e., 44%) deviated significantly ($P < 0.05$) from the expected Mendelian ratio. The loci showing distorted segregation were widely distributed on 9 of the 11 linkage groups (Fig. 1). In particular, the loci exhibiting a pronounced

**Fig. 1.** Linkage map of *Coffea canephora*. Cumulative distances in centimorgans (Kosambi (1944) function) are indicated on the left side of the linkage groups. All linkage groups were constituted with a LOD > 5, except for two associations (indicated by hatching) (LOD = 3.5). Loci marked with *, **, and *** deviated significantly from a 1:1 ratio at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.




degree of segregation distortion ($P < 0.001$) were numerous (i.e., 19%) and appeared clustered on five different linkage groups (namely, 1, 5, 6, 8, and 9). In contrast, in the TC population, only 13% of the marker loci displayed significant distorted segregation ($P < 0.05$). Most of the distorted markers observed in the DH population did not show significant skewed segregation ratios in the TC population (Table 3). On the other hand, the five loci associated with linkage group 7 in the TC-derived linkage map showed distorted segregation ratios ($P < 0.01$), although none of these loci exhibited significant segregation distortion in the DH population.

The influence of locus distortion on distance estimation in the DH population was investigated for two representative linkage groups (i.e., 5 and 9) composed of severely distorted marker loci (Fig. 2). Use of the two-point map distance estimate corrected for segregation distortion and resulted in a reduction in length of linkage groups 5 and 9 of 29 and 25%, respectively. The reduction of map distances appeared particularly significant for the segment carrying the most

**Table 2.** Correspondence between the linkage groups of the present map of *Coffea canephora* and the previously identified groups (Paillard et al. 1996).

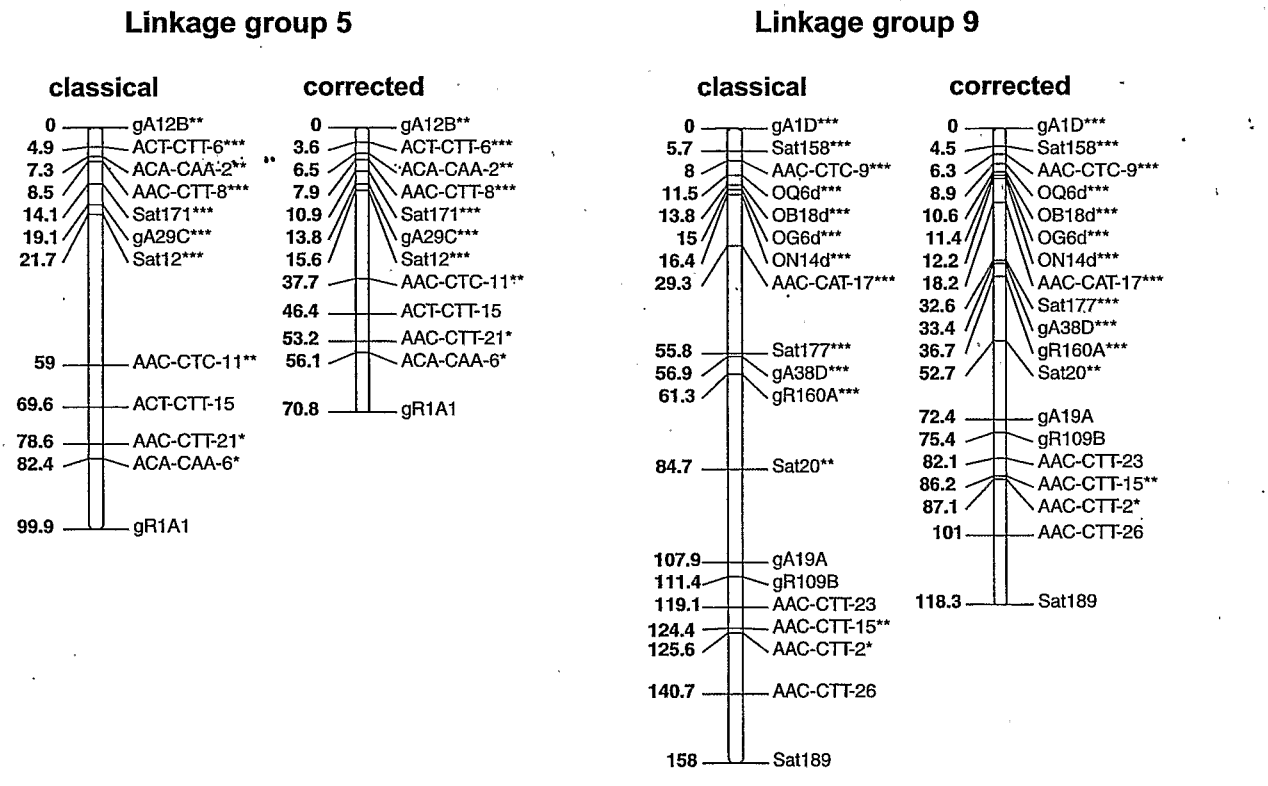
| Linkage group/ chromosome | Linkage groups defined in Paillard et al. (1996) |
|---|---|
| 1 | 1 |
| 2 | 2 |
| 3 | 3 |
| 4 | 4 |
| 5 | 5 |
| 6 | 6 + 10 |
| 7 | 7 |
| 8 | 8 |
| 9 | 9 + 15 |
| 10 | 12 + 14 |
| 11 | 11 + 13 |

**Table 3.** Comparison of marker-locus distortion and recombination frequencies in doubled-haploid (DH) and test-cross (TC) progenies.

| Chromosome | | | Segregation distortion[a] | | Recombination frequency[b] | | | |
|---|---|---|---|---|---|---|---|---|
| No. | Intervals | No. of common markers | DH | TC | DH | TC | $\chi^2$ | *P* |
| 1 | gA67A/ACT-CTT-12 | 7 | ** | ns | 0.23 | 0.24 | 0.08 | 0.77 |
| 2 | g19A /AAC-CAT-10 | 6 | ns | ns | 0.23 | 0.19 | 0.36 | 0.55 |
| 3 | AAC-CAT-9/gA10D | 4 | ns | ns | 0.16 | 0.17 | 0 | 1.00 |
| 4 | AAC-CTT-17/gA22C | 3 | * | ns | 0.12 | 0.21 | 1.97 | 0.16 |
| 5 | c119A1/ACA-CAA-6 | 3 | * | ns | 0.11 | 0.09 | 0.04 | 0.84 |
| 6 | gA11C/AAC-CTT-5 | 4 | ns | ns | 0.11 | 0.16 | 0.54 | 0.46 |
| 7 | ACA-CAA-7/AAC-CTT-24 | 5 | ns | ** | 0.15 | 0.22 | 0.92 | 0.34 |
| 8 | ACA-CAA-12/ACA-CAA-21 | 3 | ** | * | 0.09 | 0.09 | 0 | 1.00 |
| 8 | gA5Bd/AAC-CAT-3 | 4 | * | ns | 0.12 | 0.20 | 1.97 | 0.16 |
| 9 | AAC-CTC-9 /AAC-CAT-17 | 3 | ** | ns | 0.15 | 0.15 | 0.03 | 0.86 |
| 9 | gA19A/AAC-CTT-23 | 3 | ns | ns | 0.10 | 0.12 | 0.04 | 0.84 |
| 10 | gA13C/g1033B | 7 | * | ns | 0.34 | 0.24 | 1.79 | 0.18 |
| 11 | AAC-CAT-21/ACA-CAA-14 | 3 | ns | ns | 0.26 | 0.03 | 11.23 | 0.00 |

[a]Occurrence of segregation distortion determined by $\chi^2$ test: ns, not significant; *, significant at $P = 0.05$; **, significant at $P = 0.01$.
[b]Contingency $\chi^2$ test to determine whether the proportion of parental and recombinant gametes is the same in the two populations.

**Fig. 2.** Classical distances and distances corrected for segregation distortion in linkage groups 5 and 9 of *Coffea canephora*. Marker distances in centimorgans (Kosambi (1944) function) are indicated on the left side of the linkage groups.




distorted markers. Standard deviations of the recombination estimates obtained with both models, classical and corrected, were very similar.

## Recombination frequencies in the DH and TC populations

Scoring data from the two populations were tested to identify possible differences in the estimated recombination frequencies. Because of the relatively small size and limited number of markers in the TC population, the comparison was not performed on the whole genome but was restricted to short equivalent intervals (Table 3). These intervals, representing 31% of the total DH-derived map length, were distributed on the 11 linkage groups. The frequencies of recombination detected in both populations were very similar at each interval. Only 1 of the 13 intervals analysed exhibited a significant ($P < 0.001$) difference in recombination frequency between the two populations. This interval, positioned on linkage group 11, showed a higher recombination rate in the DH population, although the marker loci consid-

ered displayed similar Mendelian unbiased segregation ratios in both populations.

## Discussion

A genetic linkage map of *C. canephora* was constructed with 160 DNA markers spanning 1041 cM of the genome. While the previously reported map (Paillard et al. 1996) displayed an excessive number of linkage groups, the total number of linkage groups obtained in the present study was equivalent to the haploid number of chromosomes in *C. canephora*. Most likely it can be assumed that the 11 linkage groups correspond to the 11 gametic chromosomes of coffee. Therefore, the present map provides much better genome coverage, as reflected by the extremely low number of unlinked markers (i.e., two). Based on the procedure proposed by Hulbert et al. (1988), the estimated total length of the genetic map of the *C. canephora* genome is 1400 cM. Since the haploid nuclear DNA size of *C. canephora* is reported to be 800 Mb (Cros et al. 1995), 1 cM can be estimated to be equivalent to approximately 570 kb. However, the relationship between physical and genetic distances is usually nonlinear, even within a single chromosome (Werner et al. 1992), and substantial variation over very short distance can be anticipated.

In addition, this genetic linkage map comprises more than 40 specific sequence-tagged site markers, either single-copy RFLP probes or microsatellites, that are distributed on the 11 linkage groups. These markers constitute an initial set of standard landmarks for the coffee genome that could be used as anchor points for further map comparison. Moreover, it has been shown that these markers can be used in other coffee diploid species, as well as in the allotetraploid species *C. arabica* (Lashermes et al. 1999; Combes et al. 2000; Ky et al. 2000). Determination of the linkage relationships of these loci in other species will therefore allow the investigation of genome organisation and evolution in the genus. In particular, the linkage map of *C. canephora* will be very useful for studies relating to the mode of inheritance in *C. arabica* and interspecific hybrids, and the possibility of gene exchange between *C. arabica* and diploid relatives (Lashermes et al. 2000*b*).

The level of polymorphism observed in the DH population appeared high enough to envisage the development of an extensive linkage map for *C. canephora*, which is indispensable for isolating genes by, for example, chromosome landing (Rafalski et al. 1996). The AFLP technique used appeared very efficient in generating a large number of marker loci in a single assay. However, the nonrandom distribution of both AFLP and RAPD markers due to the occurrence of regions in which the markers are clustered needs to be taken into consideration. Such clusters are known to characterise centromeric and surrounding heterochromatic regions (Keim et al. 1997; Vuylstecke et al. 1999), and an AFLP-based mapping strategy combining methylation-sensitive and -insensitive enzymes has been suggested to overcome this limitation (Young et al. 1999).

The segregation of alleles transmitted by a hybrid parent via both female and male gametes is expected to follow a 1:1 ratio in the absence of selection. Although segregation distortions were observed in both populations of *C. canephora* analysed, the frequency of loci exhibiting a very pronounced degree of segregation distortion was especially high in the DH population. There are several possible causes for the unequal segregation of alleles, such as the abortion of male or female gametes or the selective exclusion of a particular gametic genotype from fertilization, owing to incompatibility, incongruity, certation, or zygote selection (Kreike and Stiekema 1997).

*Coffea canephora* is assumed to carry a high level of deleterious recessive alleles in relation to its outcrossing nature, owing to a self-incompatibility system (Berthaud 1986). In consequence, the strict homozygosity of DHs is likely to lead to the frequent expression of lethal genes. Furthermore, the lack of similar patterns of segregation distortion in the TC population is consistent with the hypothesis of strong zygotic selection among the DH population. However, the occurrence of gametic selection to some extent cannot be ruled out. In particular, the distorted loci observed on linkage group 7 in the TC population are likely to be due to such events. The *S* locus controlling the gametophytic self-incompatibility system in *C. canephora* could cause distorted segregation of closely linked markers. However, this locus has been previously mapped on linkage group 9 (Lashermes et al. 1996) and further work is required to determine the origin of the segregation distortion observed in the TC population.

In the DH population, the map lengths of linkage groups 5 and 9 were significantly reduced when corrected for segregation distortion, suggesting that the proposed estimates of recombination fractions were appropriate for these linkage groups. Indeed, our findings generally tend to indicate that segregation distortion results in overestimation of the recombination fraction, related to selection in favour of recombinant genotypes and (or) in disfavour of parental genotypes. In contrast, Cloutier et al. (1997), comparing maps in *Brassica napus* L., conclude that a small but significant segregation distortion results in reduced estimates of recombination fractions.

Independently of segregation-distortion effects, the recombination frequencies were found to be almost indistinguishable in the populations derived from the female and male meioses of IF 200, the parental clone of *C. canephora*. These results offer evidence in favour of the lack of significant sex differences in recombination in *C. canephora*. However, the compared equivalent intervals, though widely distributed, do not cover the whole genome, and significant sex differences in recombination frequency may exist in certain chromosomal segments. For instance, contrasting results have been observed in *Brassica nigra* (Lagercrantz and Lydiate 1995), where increased male recombination was found in proterminal regions and increased female recombination occurred in the centres of linkage groups.

In conclusion, the greatest significance of these results is that there is likely to be little difference in recombination rates recovered from either male or female gametes for the coffee breeder to exploit. These equivalent recombination rates suggest that crossing programmes can be carried out in the most convenient manner. Nevertheless, the possibility of strong segregation distortion has to be taken into consideration. If Mendelian segregation ratios are being sought, DH populations should obviously be avoided. On the other hand,

test-cross progenies would give more predictable allelic segregation, although the risk of distorted segregation could not be discarded.

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## References

Agwanda, C., Lashermes, P., Trouslot, P., Combes, M.C., and Charrier, A. 1997. Identification of RAPD markers for resistance to coffee berry disease, *Colletotrichum kahawae*, in arabica coffee. Euphytica, **97**: 241–248.

Bailey, N.T.J. 1949. The estimation of linkage with differential viability, II and III. Heredity, **3**: 220–228.

Berthaud, J. 1986. Les ressources génétiques pour l'amélioration des caféiers africains diploïdes. No. 188 in Collection Travaux et Documents de l'Institut Français pour la Recherche et le Développement en Coopération (ORSTOM), Paris.

Burt, A., Bell, G., and Harvey, P.H. 1991. Sex differences in recombination. J. Ecol. Biol. **4**: 259–277.

Busso, C.S., Liu, C.J., Hash, C.T., Witcombe, J.R., Devos, K.M., de Wet, J.M.J., and Gale, M.D. 1995. Analysis of recombination rate in female and male gametogenesis in pearl millet (*Pennisetum glaucum*) using RFLP markers. Theor. Appl. Genet. **90**: 242–246.

Carvalho, A. 1988. Principles and practices of coffee plant breeding for productivity and quality factors: *Coffea arabica*. *In* Coffee. Vol. 4. Agronomy. *Edited by* R.J. Clarke and R. Macrae. Elsevier Applied Science, London. pp. 129–165.

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

Combes, M.C., Andrzejewski, S., Anthony, F., Bertrand, B., Rovelli, P., Graziosi, G., and Lashermes, P. 2000. Characterisation of microsatellite loci in *Coffea arabica* and related coffee species. Mol. Ecol. **9**: 1178–1180.

Couturon, E. 1982. Obtention d'haploïde spontanés de *Coffea canephora* Pierre par l'utilisation du greffage d'embryons. Cafe Cacao The, **26**(3): 155–160.

Cros, J., Combes, M.C., Chabrillange, N., Duperray, C., Monnot des Angles, A., and Hamon, S. 1995. Nuclear DNA content in the subgenus *Coffea* (Rubiaceae): inter- and intra-specific variation in African species. Can. J. Bot. **73**: 14–20.

Ganal, M.W., and Tanksley, S.D. 1996. Recombination around the *Tm*2a and *Mi* resistance genes in different crosses of *Lycopersicon peruvianum*. Theor. Appl. Genet. **92**: 101–108.

Garcia-Dorado, A., and Gallego, A. 1992. On the use of the classical tests for detecting linkage. J. Hered. **83**: 143–146.

Hulbert, S.H., Ilott, T.W., Legg, E.J., Lincoln, S.E., Lander, E.S., and Michelmore, R.W. 1988. Genetic analysis of the fungus, *Bremia lactucae*, using restriction fragment length polymorphisms. Genetics, **120**: 947–958.

Kearsey, M.J., Ramsay, L.D., Jennings, D., Lydiate, D.J., Bohuon, E., and Marshall, D.F. 1996. Higher recombination frequencies in female compared to male meioses in *B. oleracea*. Theor. Appl. Genet. **92**: 363–367.

Keim, P., Schupp, J.M., Travis, S.E., Clayton, K., Zhu, T., Shi, L., Ferreria, A., and Webb, D.M. 1997. A high-density soybean genetic map based on AFLP markers. Crop Sci. **37**: 537–543.

Kelly, A.L., Sharpe, A.G., Nixon, J.H., Evans, E.J., and Lydiate, D.J. 1997. Indistinguishable patterns of recombination resulting from male and female meioses in *Brassica napus* (oilseed rape). Genome, **40**: 49–56.

Kosambi, D.D. 1944. The estimation of map distances from recombination values. Ann. Eugen. **12**: 172–175.

Kreike, C.M., and Stiekema, W.J. 1997. Reduced recombination and distorted segregation in a *Solanum tuberosum* (2*x*) × *S. spegazzinii* (2*x*) hybrid. Genome, **40**: 180–187.

Ky, C.L., Barre, P., Lorieux, M., Trouslot, P., Akaffou, S., Louarn, J., Charrier, A., Hamon, S., and Noirot, M. 2000. Interspecific genetic linkage map, segregation distortion and genetic conversion in coffee (*Coffea* sp.). Theor. Appl. Genet. **101**: 669–676.

Lagercrantz, U., and Lydiate, D.J. 1995. RFLP mapping in *Brassica nigra* indicates differing recombination rates in male and female meioses. Genome, **38**: 255–264.

Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J., Lincoln, S.E., and Newburg, L. 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics, **1**: 174–181.

Lashermes, P., Couturon, E., and Charrier, A. 1994*a*. Doubled haploids of *Coffea canephora*: development, fertility and agronomic characteristics. Euphytica, **74**: 149–157.

Lashermes, P., Couturon, E., and Charrier, A. 1994*b*. Combining ability of doubled haploid of *Coffea canephora*. Plant Breed. **112**: 330–337.

Lashermes, P., Couturon, E., Moreau, N., Paillard, M., and Louarn, J. 1996. Inheritance and genetic mapping of self-incompatibility in *Coffea canephora* Pierre. Theor. Appl. Genet. **93**: 458–462.

Lashermes, P., Combes, M.C., Robert, J., Trouslot, P., D'hont, A., Anthony, F., and Charrier, A. 1999. Molecular characterisation and origin of the *Coffea arabica* L. genome. Mol. Gen. Genet. **261**: 259–266.

Lashermes, P., Andrzejewski, S., Bertrand, B., Combes, M.C., Dussert, S., Grasiozi, G., Trouslot, P., and Anthony, F. 2000*a*. Molecular analysis of introgressive breeding in coffee (*C. arabica*). Theor. Appl. Genet. **100**: 139–146.

Lashermes, P., Paczek, V., Trouslot, P., Combes, M.C., Couturon, E., and Charrier, A. 2000*b*. Single-locus inheritance in the allotetraploid *Coffea arabica* L., and interspecific *hybrid C. arabica* × *C. canephora*. J. Hered. **91**: 81–85.

Lorieux, M., Goffinet, B., Perrier, X., Gonzalez de Leon, D., and Lanaud, C. 1995. Maximum-likelihood models for mapping genetic markers showing segregation distortion. 1. Backcross populations. Theor. Appl. Genet. **90**: 73–80.

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

Rafalski, J.A., Vogel, J.M., Morgante, M., Powell, W., Andre, C., and Tingey, S.V. 1996. Generating and using DNA markers in plants. *In* Non-mammalian genomic analysis: a practical guide. Academic Press, New York. pp. 75–134.

Robertson, D.S. 1984. Different frequency in the recovery of crossover products from male and female gametes of plant hypoploid for B–A translocations in maize. Genetics, **107**: 117–130.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and

Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. **23**: 4407–4414.

Vuylstecke, M., Mank, R., Antonise, R., Bastiaans, E., Senior, M.L., Stuber, C.W., Melchinger, A.E., Lübberstedt, T., Xia, X.C., Stam, P., Zabeau, M., and 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.

Wang, G., Hyne, V., Chao, S., Henry, Y., De Buyser, J., Gale, M.D., and Snape, J.W. 1995. A comparison of male and female recombination frequency in wheat using RFLP maps of homoeologous group 6 and 7 chromosomes. Theor. Appl. Genet. **91**: 744–746.

Werner, J.E., Endo, T.R., and Gill, B.S. 1992. Toward a cytogenetically based physical map of the wheat genome. Proc. Natl. Acad. Sci. U.S.A. **89**: 11 307 – 11 311.

Young, W.P., Schupp, J.M., and Keim, P. 1999. DNA methylation and AFLP marker distribution in the soybean genome. Theor. Appl. Genet. **99**: 785–790.

Zhuchenko, A.A., Korol, A.B., Vizir, I.Y., Bocharnikova, N.I., and Zamorzaeva, N.I. 1989. Sex differences in crossover frequency for tomato and thale cress (*Arabidopsis thaliana*). Sov. Genet. **24**: 1104–1110.

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