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A consensus linkage map for rapeseed (*Brassica napus* L.): construction and integration of three individual maps from DH populations

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Abstract A framework consensus map for rapeseed (*Brassica napus* L.) was constructed from the integration of three DH mapping populations derived from crosses between or within spring- and winter-type parents. Several sources of genetic markers were used: isozymes, RFLPs, RAPDs, and AFLPs. A total of 992 different markers were mapped to at least one population, of which 540 were included in the consensus map and 253 were common to at least two populations. Markers were distributed over 19 linkage groups, thus reflecting the basic chromosome number of rapeseed and covered 2,429 cM, which was in the mean confidence-interval estimates of genome length (2,127–2,480) cM. Markers were evenly spaced on the entire genome even if, for several linkage groups, both RAPD and AFLP markers were not uniformly distributed. In the population resulting from a cross between two spring lines, a higher recombination rate was observed and a translocation was identified. The consensus approach allowed to map a larger number of markers, to obtain a near-complete coverage of the rapeseed genome, to fill the number of gaps, and to consolidate the linkage groups of the individual maps.

Keywords Rapeseed · Linkage map · Consensus map · RAPD · AFLP

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Introduction

Genetic linkage maps have been constructed for most cultivated plant species. They offer the possibility to gain a better knowledge of genome organization and evolution through comparative mapping, to develop genetic studies on various agronomic traits through the localization of major genes and QTLs (quantitative trait loci), and to help breeding programs with the development of marker-assisted selection. The knowledge of the localization of molecular markers is also useful to estimate precisely the genetic relationships between cultivars in the context of plant registration and protection (Dillmann et al. 1997). For all these applications, there is a need to obtain a saturated map which could be of nearly universal use for different genetic backgrounds. To achieve this objective, the construction of consensus maps synthesizing the information provided by multiple segregating populations has many advantages over mapping based on a single population. A larger number of loci are mapped, thus increasing the number of potential useful markers in various genetic backgrounds and providing a greater genome coverage. Loci order and map distances can be assessed more precisely, and possible chromosomal rearrangements between the different parents used can be identified.

In *Brassica napus*, many genetic maps have been reported (Landry and Hubert 1991; Ferreira et al. 1994; Parkin et al. 1995; Sharpe et al. 1995; Uzunova et al. 1995; Foisset et al. 1996; Cheung et al. 1997). To-date, no saturated or integrated genetic map has been published for rapeseed. Only one study (Parkin and Lydiate 1997) reported on the alignment of two genetic maps. We have developed a *B. napus* genetic map on a highly polymorphic DH population derived from the cross between a French winter rapeseed cultivar, 'Darmor-*bzh*', and a Korean spring rapeseed line, 'Yudal' (Foisset et al. 1996). This map has been used to localize genes and QTLs involved in various agronomic traits (Foisset et al. 1995; Jourdren et al. 1996a; Pilet et al. 1998a, b; Manzanares-Dauleux et al. 2000) as well as transgenes (Baranger et al. 1997) or interspecific introgressions

(Barret et al. 1998; Delourme et al. 1998). To complete the genetic analysis of some agronomic traits, two other genetic maps were elaborated for crosses involving either two spring (Jourdren et al. 1996b) or two winter rapeseed cultivars (Pilet 1999).

Here, we report on the comparison of the genetic maps obtained on these three independent populations and on the integration of these individual maps into a consensus linkage map. Various types of markers, such as isozyme, RFLP, RAPD (random amplified polymorphic DNA) and AFLP (amplified fragment length polymorphism) markers, were mapped. The objectives were: (1) to consolidate each linkage group and to detect possible chromosomal rearrangements between parental lines, (2) to gain a better genome coverage, and (3) to obtain the general order and the map distances among a greater number of markers through the construction of a saturated, framework consensus map which could serve as a reference.

Material and methods

Mapping populations

Three doubled-haploid (DH) populations derived from isolated microspore cultures [as described by Polsoni et al. (1988)] were used. The first one was obtained from the cross 'Darmor-*bzh*'×'Yudal' (referred to as the DY cross). 'Darmor-*bzh*' is a dwarf near-isogenic line resulting from the introduction of the dwarf *Bzh* gene in a French winter-type cultivar, 'Darmor'. 'Yudal' is a Korean line that behaves as a spring-type in a temperate climate. A total of 152 DH lines from the DY cross were previously used to establish a genetic map with 266 segregating markers (Foisset et al. 1996). The second population was derived from the cross 'Darmor'×'Samourai' (DS cross). 'Samourai' is a French winter-type line; 134 DH lines from DS cross were used for mapping (Pilet et al. 1999). The third population (94 DH lines) was obtained from the cross 'Stellar'×'Drakkar' (SD cross) (Jourdren et al. 1996b). 'Stellar' (Canadian) and 'Drakkar' (French) are two spring-type lines.

Genetic markers

The *Bzh* gene was previously mapped in the DY progeny (Foisset et al. 1995). Isozyme, RAPD and RFLP procedures were as described in Foisset et al. (1996). Isozyme nomenclature was that of Chèvre et al. (1995). RAPD primers were from Operon Technologies Inc. with the exception of the primer RA1275 (Kuginuki et al. 1997). RAPD markers were named using the kit letter and the primer number, followed by the molecular weight of the fragment (in base pairs). For DY progeny, 15 in-house RFLP probes were selected in addition to the 56 ones used in Foisset et al. (1996). For DS progeny, 171 probes corresponding to anonymous clones from a *B. napus* embryo cDNA library were provided by DNA Landmarks (Canada). Some of these were previously used for the DY map construction (Foisset et al. 1996) and the others, encoded with the 'BN' prefix, were new ones. These 171 probes were used in combination with six restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Hind*III, *Kpn*I and *Xba*I). RFLP markers were coded by the name of their derived probe, followed by a letter. Five PCR-specific markers (FAD3.A, FAE1.A, FAE1.C, SLG and AG.3), described in Foisset et al. (1996), Jourdren et al. (1996c), Fourmann et al. (1998) and Brunel et al. (1999), were mapped. Seven microsatellite loci [NGA162, (Bell and Ecker 1994); Bn9A, Bn35D, Bn92A, Bn59A, Bn12a and Bn40C1, (Szewc-McFadden et al. 1996)] were used.

Table 1 Coding of the AFLP primer combinations employed

Primer combination[a]	Code
E-AAC+M-CAA	E1M1
E-AAC+M-CAG	E1M3
E-AAC+M-CAT	E1M4
E-AAC+M-CTA	E1M5
E-AAC+M-CTT	E1M7
E-AAG+M-CAA	E2M1
E-AAG+M-CAC	E2M2
E-AAG+M-CAT	E2M4
E-AAG+M-CTC	E2M6
E-AAG+M-CTT	E2M7
E-ACC+M-CAA	E3M1
E-ACC+M-CAG	E3M3
E-ACC+M-CTC	E3M6
E-ACT+M-CAT	E4M4
E-ACT+M-CTC	E4M6
E-ACT+M-CTT	E4M7
E-AGG+M-CAT	E5M4

[a] The core sequences of primers for the selective amplification were: E-=5´-GACTGCGTACCAATTTC-3´ for *Eco*RI primers; M-=5´-GATGAGTCCTGAGTAA-3´ for *Mse*I primers. Each primer contained three selective nucleotides at the 3´ end

Sixty individuals per population were characterized with AFLP markers. DNA extraction and AFLP analyses were performed as described in Lombard et al. (2000). Seventeen primer combinations involving five *Eco*RI and seven *Mse*I primers, were used. AFLP fragments were scored as dominant, i.e. presence versus absence of bands. AFLP markers were named using a code for each *Eco*RI and *Mse*I primer (e.g. E2M4) (Table 1), followed by numbers in ascending molecular-weight order as described in Lombard et al. (2000). For several markers, a letter followed the name of the primer pair instead of a number, because there were additional polymorphic bands in comparison with those previously scored in Lombard et al. (2000) on a collection of rapeseed cultivars.

Construction of the individual maps

The goodness of fit to expected Mendelian ratios for each individual locus was tested by chi-square analysis (α=1%). Linkage analyses for each mapping population were performed using MAPMAKER/EXP version 3.0 (Lincoln et al. 1992). Linkage groups (LGs) were first established with a minimum LOD threshold of 4.0 for the DY map and 3.0 for the DS and SD maps, and a maximum recombination frequency of 0.3. The constitution of the linkage groups with distorted segregation ratios were confirmed with a chi-square (Mather 1957) test for the independence of two segregations, conditional on their marginal frequencies (α=1%). LOD scores were then successively increased to 5.0 and 6.0 to separate the groups associated through skewed segregating markers. The loci order was first estimated using a LOD threshold of 3.0 and remaining loci were placed at a LOD≥2.0. The original scorings for single loci flanked by double crossovers were re-examined. Centimorgan distances were expressed by the Kosambi function (Kosambi 1944).

Comparison of recombination rates among individual maps

A test for a global difference in recombination rates in the three mapping populations was performed on the basis of common markers. For a given pair of mapping populations, a *t*-test was applied on the mean of the differences in distance between adjacent common markers. Unilateral and bilateral tests were performed for each pair of mapping populations (DY/SD, DY/DS and DS/SD) at the 5% level.

Construction of the consensus linkage map

Linkage analyses for each consensus linkage group were performed using the mapping software CARTHAGENE (Schiex and Gaspin 1997) by pooling data from the three populations in a single data set. Because our objective was to obtain a framework consensus map, we chose all the common markers and then, for each LG of each individual map, we chose markers spaced by a minimum of 3 cM.

CARTHAGENE combines an optimized version of the expectation maximization (EM) algorithm with local search techniques (Aarts and Lenstra 1997) originating from artificial intelligence and operation research. It is able to directly handle data coming from multiple populations which may be either F_2 backcross, recombinant inbred lines, F_2 intercross, phase-known outbreds and/or radiation hybrids (Schiex et al. 2000). CARTHAGENE permits the ordering of a large set of markers using a true multipoint maximum-likelihood criterion which is computed using an extended EM algorithm that performs order of magnitudes faster than MAPMAKER without any loss of precision. It has been found to provide significantly better results than JOINMAP (Stam 1993) software (Schiex and Gaspin 1997).

The local-search algorithm chosen to build consensus linkage groups was the simulated-annealing procedure. The values of the parameters were as followed: the initial temperature was 4°C, the final temperature was 0.1°C, and the number of permutations at a given temperature was $n(n\text{-}1)/2$ where n is the number of markers in the consensus group. All four-locus permutations of marker order within each linkage group were compared in CARTHAGENE using the "flip" command (equivalent to the "ripple" command in MAPMAKER) to evaluate LOD support for the order. Finally, the best position of each individual marker was checked using the "polish" command: the marker is removed from the group and its best position is determined by testing each position along the linkage group made up of the remaining markers.

For linkage groups in individual maps where the most-likely locus order was not consistent with the consensus map, the log-likelihood of the most-likely order in each progeny was compared with the log-likelihood of the most-likely order from the consensus map. If the difference was not significant (difference in LOD≤3.0) then the locus order of the consensus map was used for the individual maps (Beavis and Grant 1991). The same comparisons were then applied between individual maps when inconsistent orders remained.

Marker distribution

Marker distribution along each linkage group was evaluated by comparing the difference between the expected positions of markers under a uniform distribution and the observed ones with the critical D value of the Kolmogorov-Smirnov statistic (α=5%). The observed distribution on each linkage group was built as follows: let L_i and N_i be the length and the number of markers of LGi, respectively; P_{ij} is the position of marker j along LGi; the observed cumulative distribution is made up of each P_{ij}/L_i value; the expected cumulative distribution is made up of each j/N_i value.

Estimation of genome length and map coverage

From the total data set of each mapping population, estimations of genome length, E(G), were calculated using the method of Hulbert et al. (1988), as modified in method 3 of Chakravarti et al. (1991) from pairwise segregation data for marker pairs above LOD thresholds of 3, 4 and 5. The confidence interval for these estimations, $I_\alpha(G)$, was calculated as described in Gerber and Rodolphe (1994). The observed genome map coverage, E(Co), was calculated with the equation of Echt and Nelson (1997), from the results of map length calculated as the sum of the linkage-group lengths obtained with MAPMAKER. All the procedures for the calculation of genome length and map coverage are detailed in Echt and Nelson (1997).

Results

Polymorphism and single-locus segregation analyses

From the nine isozyme systems tested, eight, three and six were polymorphic, and provided ten, four and six markers in the DY, DS and SD populations, respectively. Out of the seven microsatellites mapped in the DY population, only one was polymorphic in the SD population and none in the DS population. From the 56 RFLP probes directly screened on 'Darmor-*bzh*' and 'Yudal' DNA, digested only with one enzyme (*Hind*III),

Table 2 Basic characteristics of the three individual maps

Item	DY	DS	SD
Markers			
Bzh	1	0	0
Isozymes	10	3	6
PCR-specific markers	3	1	3
Microsatellites	7	0	1
RFLP	79	68	0
RAPD	271	150	167
AFLP	219	122	163
Total number of markers	590	344	340
Number of individuals	152	134	94
Number of linkage groups	20	24	23
Number of pairs	1	1	1
Number of unlinked markers	5	4	8
Map length (cM)	2,023.5	1,574.3	1,911.8
Marker density (marker/cM)	3.6	5.0	6.2
(Standard deviation)	(4.8)	(6.6)	(7.2)
Total number of skewed markers	145 (24.6%)	48 (14.0%)	30 (8.8%)
Skewed RFLPs	20 (25.3%)	12 (17.6%)	0
Skewed RAPDs	95 (35.0%)	28 (18.7%)	16 (9.6%)
Skewed AFLPs	22 (10.0%)	8 (6.5%)	14 (8.8%)

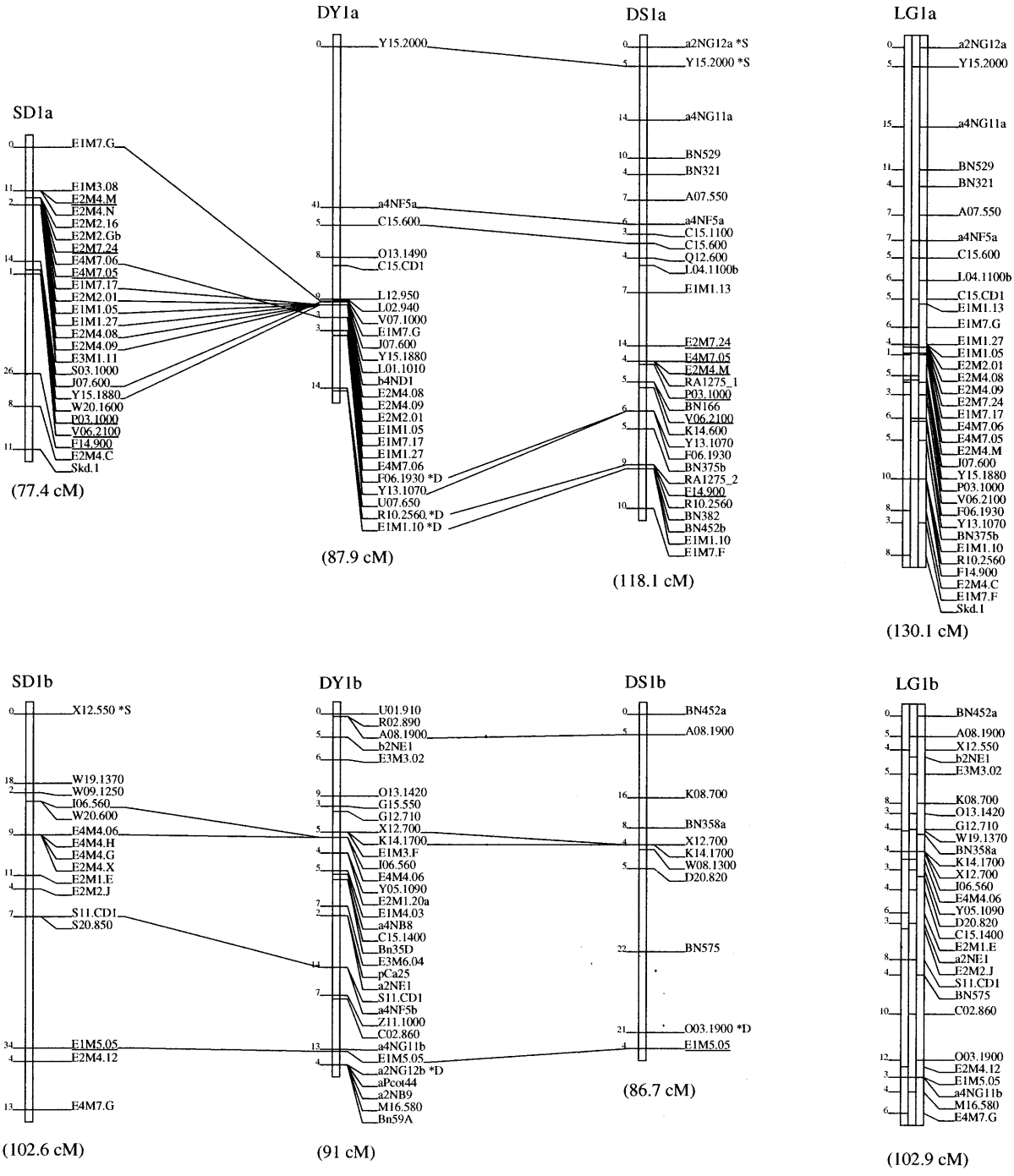
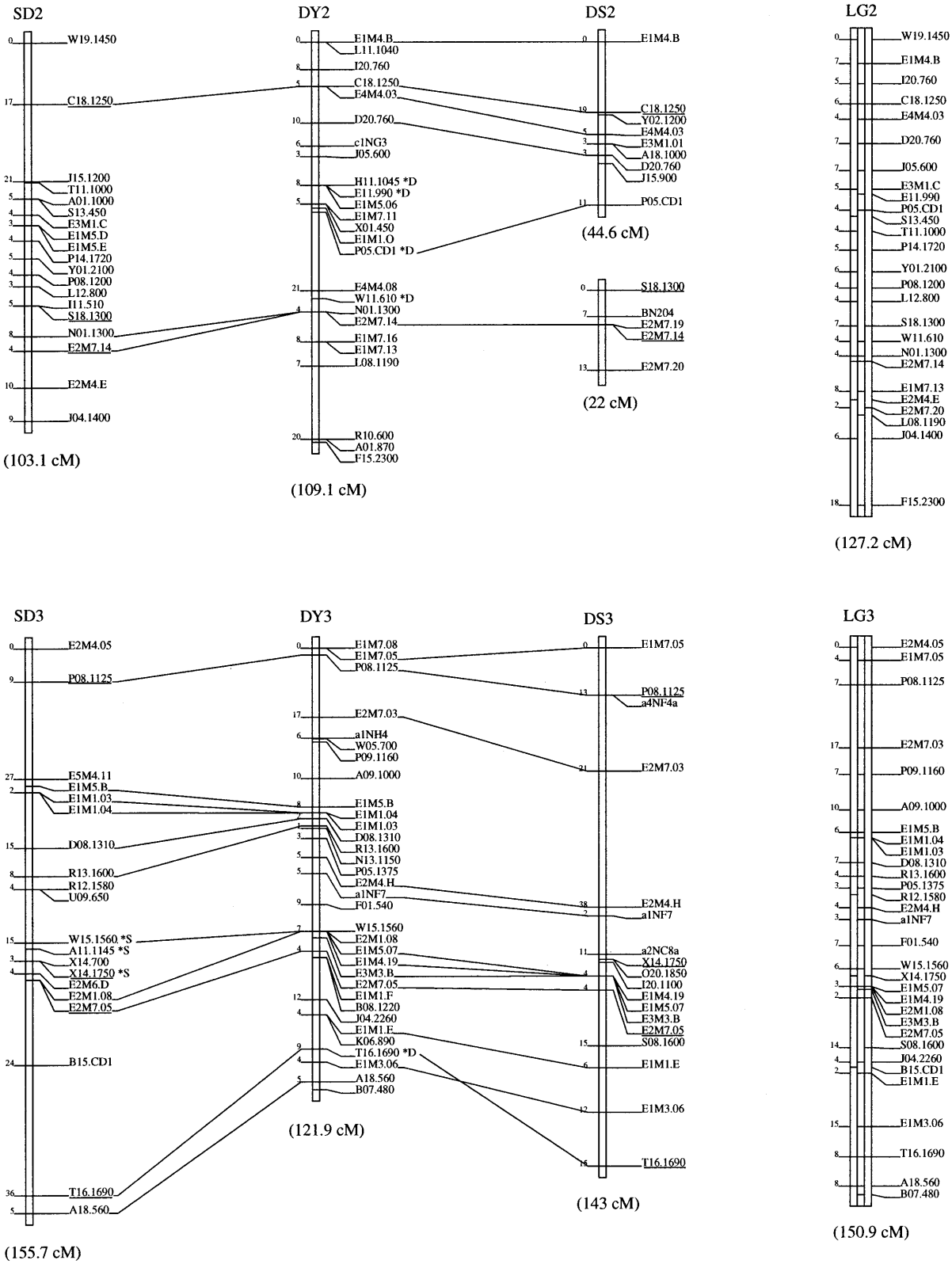


Fig. 1 Individual maps and the integrated map of 19 rapeseed linkage groups. SD, DY, DS and LG refer to the populations of 'Stellar'×'Drakkar', 'Darmor-*bzh*'×'Yudal', 'Darmor'×'Samourai' and to the consensus map. For each consensus LG, *horizontal bars* show the loci mapped in the SD, DY and DS populations, from left to right. *Lines* between groups connect markers common to the SD and DY maps and to the DY and DS maps. Markers common to the SD and DS maps are *underlined*. Values *in parentheses* correspond to linkage group lengths in centimorgans (Kosambi mapping function). Loci showing segregation distorsion ($P<0.01$) are indicated with a *star*, followed by a *letter* indicating the favored parental allele

84% were polymorphic (Foisset et al. 1996). One hundred and seventy three probes were screened on 'Darmor' and 'Samourai' using six restriction enzymes. Only 55 probes (32%) were polymorphic. Most of the 266 RAPD primers first analysed on 'Darmor-*bzh*' and 'Yudal' (89.5%) were polymorphic (Foisset et al. 1996). Then, 130 primers were used in this study and revealed 271 markers in the DY population. Of these, 125 and 123 primers were screened on 'Darmor' and 'Samourai', and on 'Stellar' and 'Drakkar', respectively. Ninety (72%) and 84 (68%) were polymorphic and revealed 150 and 167 markers in the DS and SD popula-



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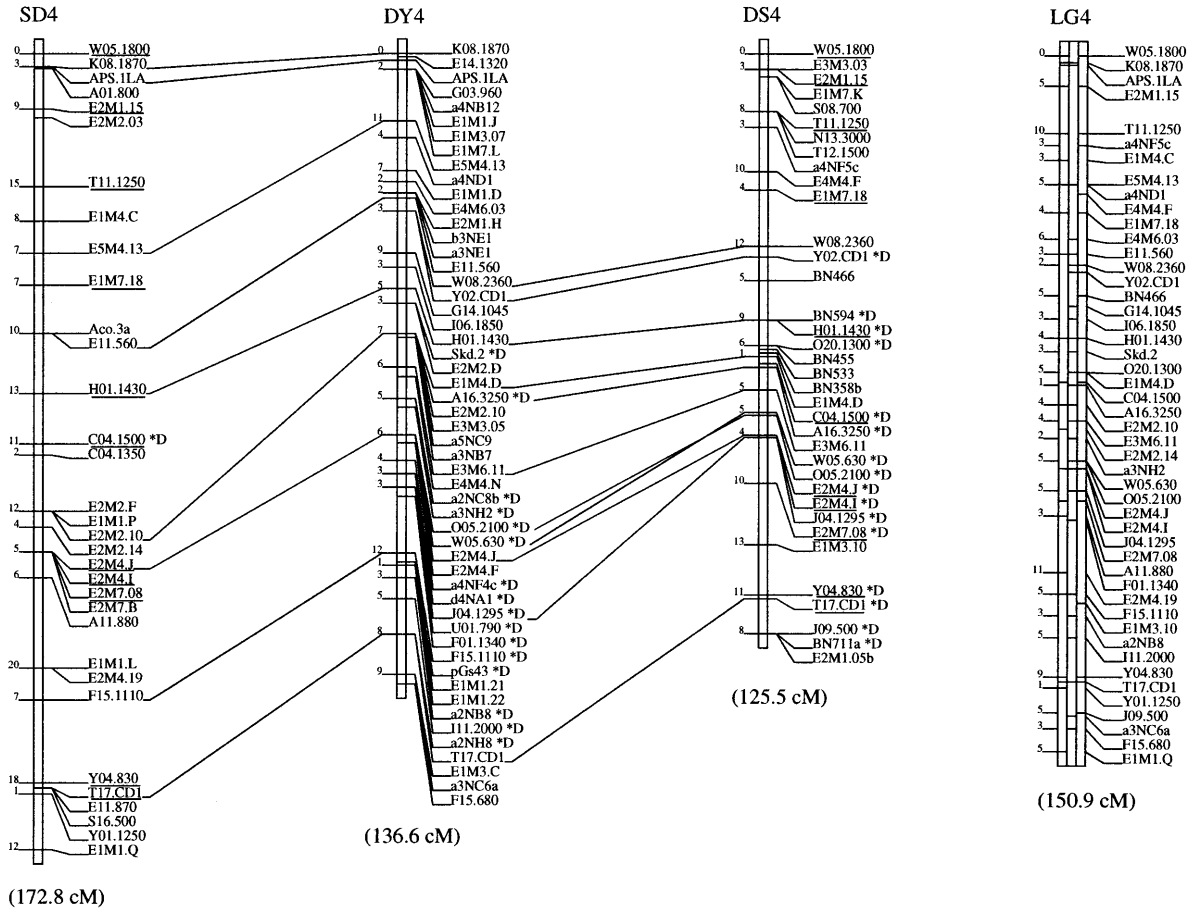

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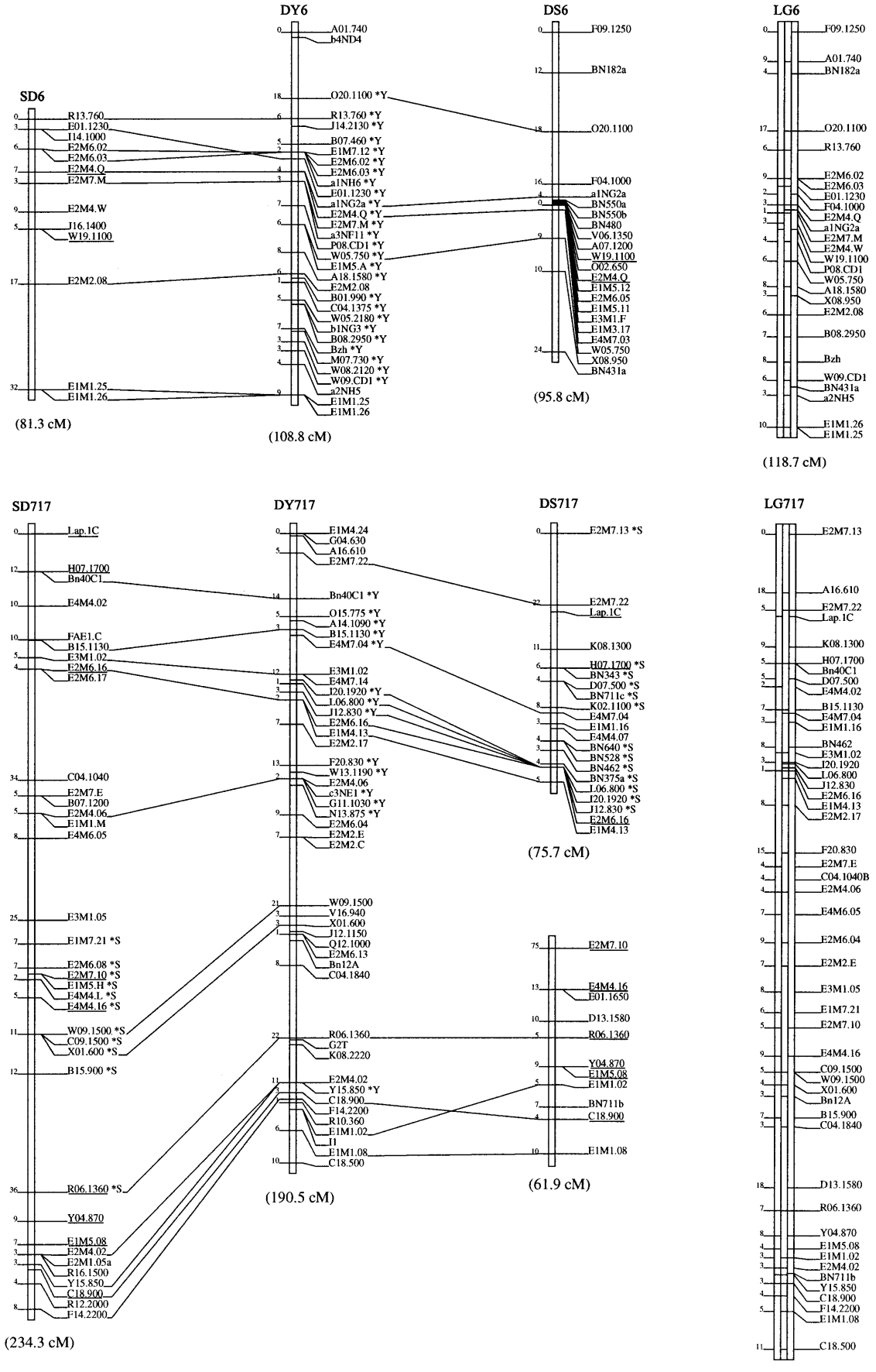

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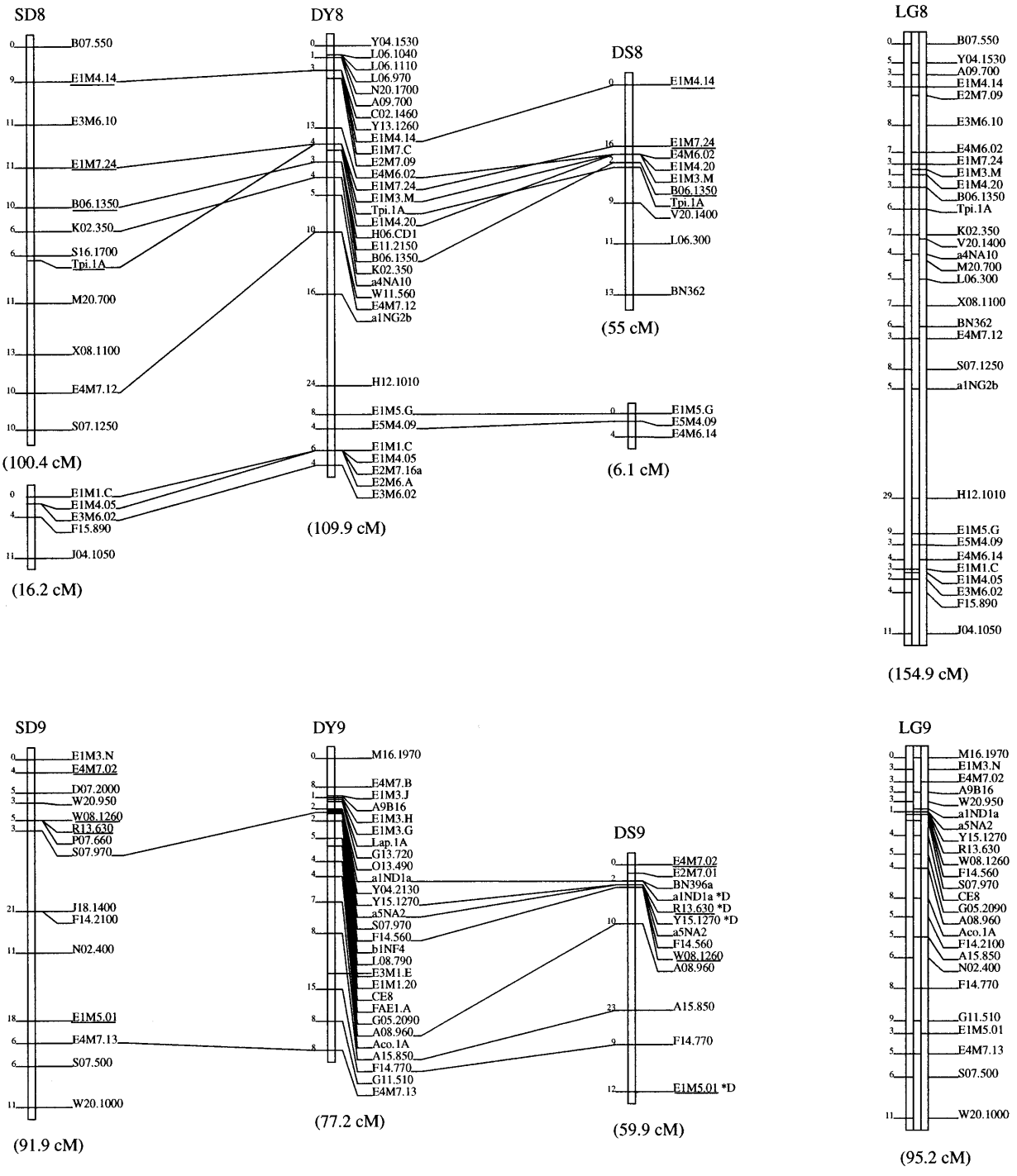

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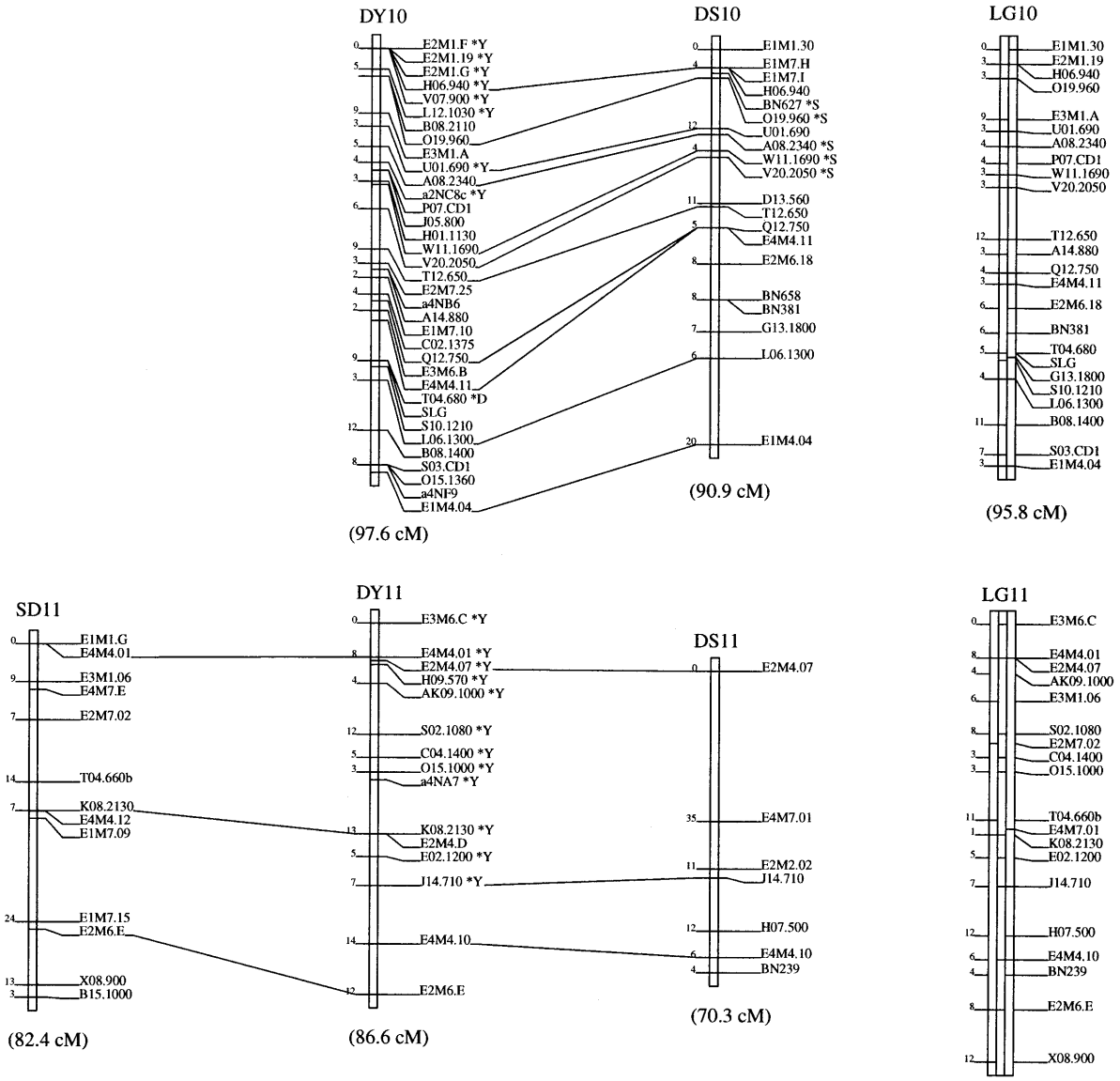
tions, respectively. Each primer gave on average 2.1, 1.6 and 2.0 markers on the DY, DS and SD populations, respectively. With the 17 AFLP primer combinations (PCs) used, 377 markers were polymorphic on at least one population with an average of 22.2 markers per PC (with a range from 7 to 39 according to the PCs). Out of these 377 AFLP markers, 219 (58.1%), 122 (32.4%) and 163 (43.2%) were polymorphic in the DY, DS and SD populations, respectively.

Construction of the individual maps

The basic characteristics of the three individual maps are given in Table 2. The number of mapped markers was 590, 344 and 340 for the DY, DS and SD maps, respectively, which represented a total number of 992 different loci. The maps generated by MAPMAKER covered 2,023.5, 1,574.3 and 1,911.8 cM for the DY, DS and SD populations, respectively. The most complete and the densest map was



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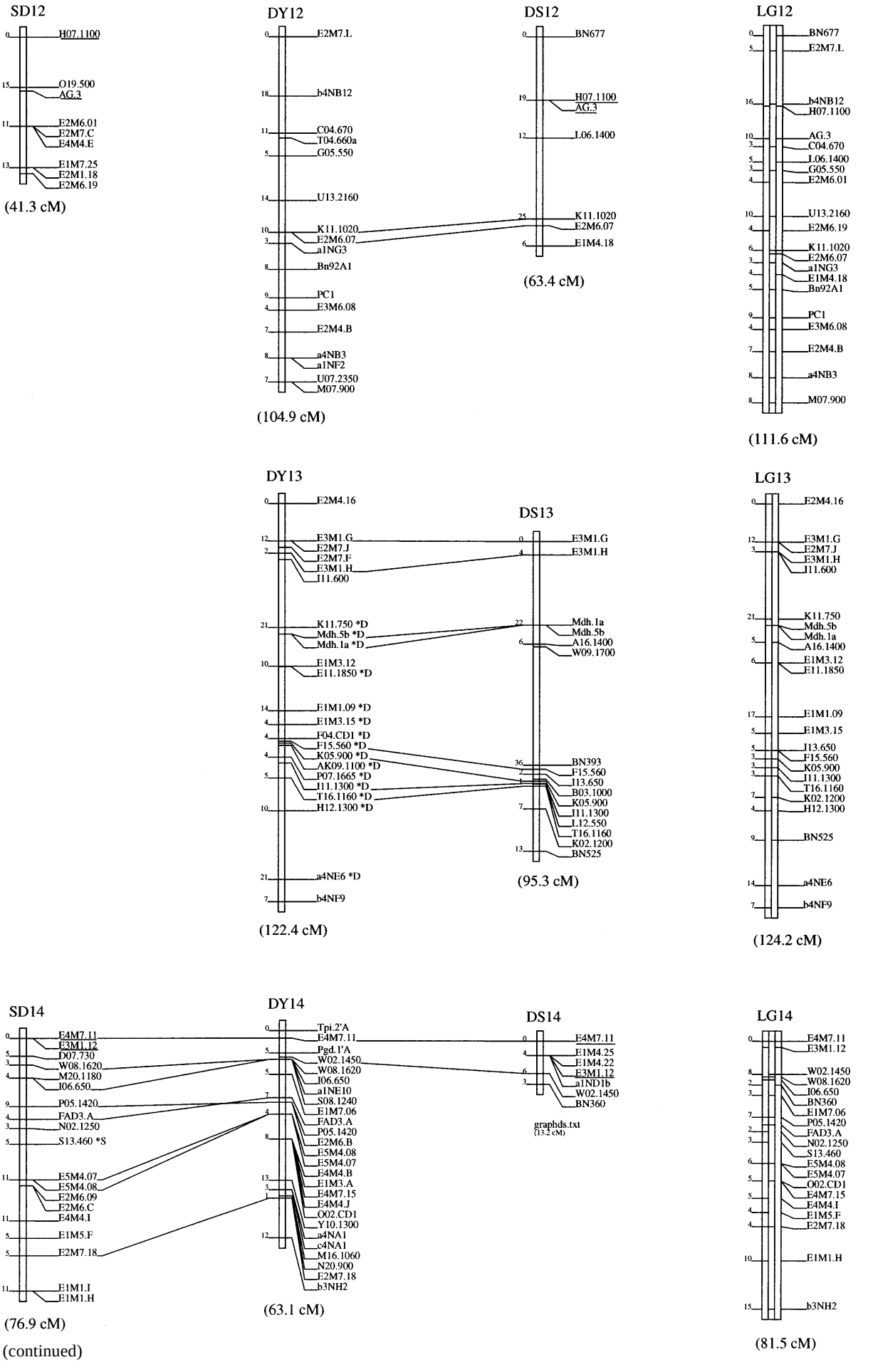
obtained on the DY population with, on average, one marker every 3.6 cM, whereas the marker density was one marker every 5.0 and 6.2 cM for DS and SD, respectively.

The LGs were numbered as in the earlier published map of the DY population (Foisset et al. 1996) except for two modifications. The earlier DY1 group was split into two LGs (DY1a and DY1b) when the marker W02.1110 was discarded due its aberrant segregation. The two LGs DY7 and DY17 were joined into a single one, DY717, through the addition of new markers. This group was consolidated through the alignment of our individual maps with the one built on the cross 'Mansholt's Hamburger Raps'×'Samourai' (Uzunova et al. 1995), by using a common set of AFLP markers (Delourme, Lombard and Ecke, unpublished data).

Out of the 590, 344 and 340 markers mapped on the DY, DS and SD populations, 24.6%, 14% and 8.8% did not segregate according to the expected Mendelian ratio (Table 2). Skewed segregations were observed for the different types of markers but the percentage of skewed AFLP loci was the lowest one (Table 2). Markers with skewed segregation tended to cluster on eight and four LGs on the DY and DS maps, respectively, each cluster comprising loci exclusively favoring the alleles of the same parental line (Fig. 1). A few loci did not fit the expected Mendelian ratios on the SD population. The linkage group 717 showed skewed segregation in the three populations, but the skewed region on SD717 did not correspond exactly to those on DY717 and DS717 (Fig. 1). On LGs 4 and 10, the same regions carried skewed loci in the DY and DS populations, and some skewed loci were located in the same region of SD19 and DY19.



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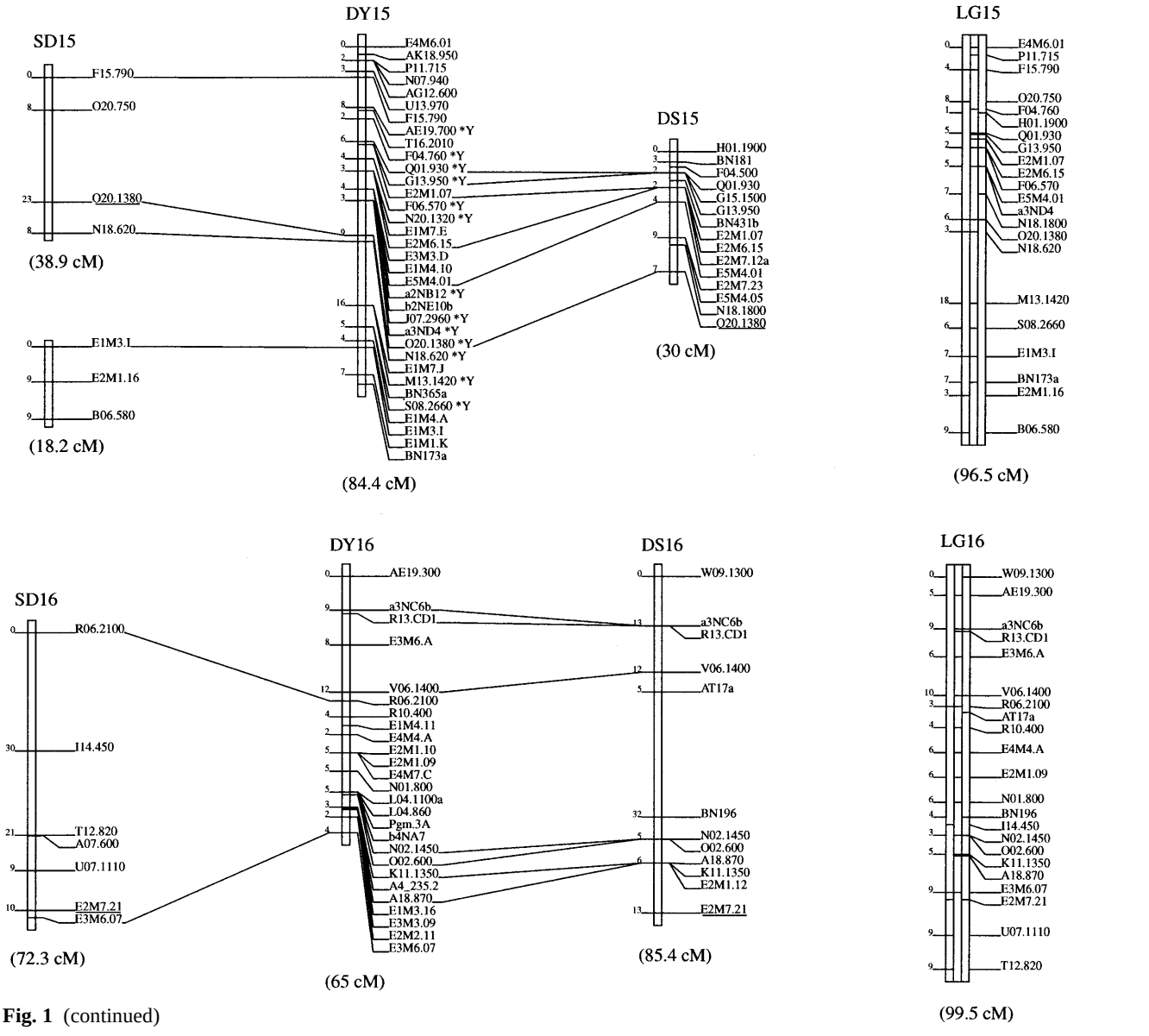

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Marker distribution

The correlation between the number of markers per LG and the length of the LGs gave an indication of the distribution of the markers over the whole genome. The correlation coefficients were high for the three individual maps, with 0.73, 0.80 and 0.90 ($P<0.001$) for DY, DS and SD, respectively, which indicated a random distribution of the markers between LGs. The distribution of markers was uniform within most LGs in each mapping population, with the exception of six LGs for DY, seven LGs for DS and two LGs for SD. Evidence of clustering was found for DY1a, DY9, DY16, DY19, DS6 and SD1a (Fig. 1). For the other LGs, either small clusters and gaps caused the rejection of the hypothesis of a uniform distribution of markers. The results of markers distribution for LGs with less than six markers were not analyzed because it seemed difficult to infer anything from such a low number of markers.

Comparison of individual maps

Common markers among homologous LGs allowed for a comparison of the marker order between the three mapping populations. A total number of 253 markers were common to at least two populations, of which 29 were common to the three mapping populations (Table 3). Ten significant differences in marker order between homologous LGs of the three mapping populations were found for LGs 3, 717, 8, 18 and 19 between DY and DS, for LGs 1a, 6, 8 and 14 between DY and SD, and for LG10 between DS and SD (Figs. 1 and 2). Eight out of ten differences involved markers spaced by less than 5 cM in at

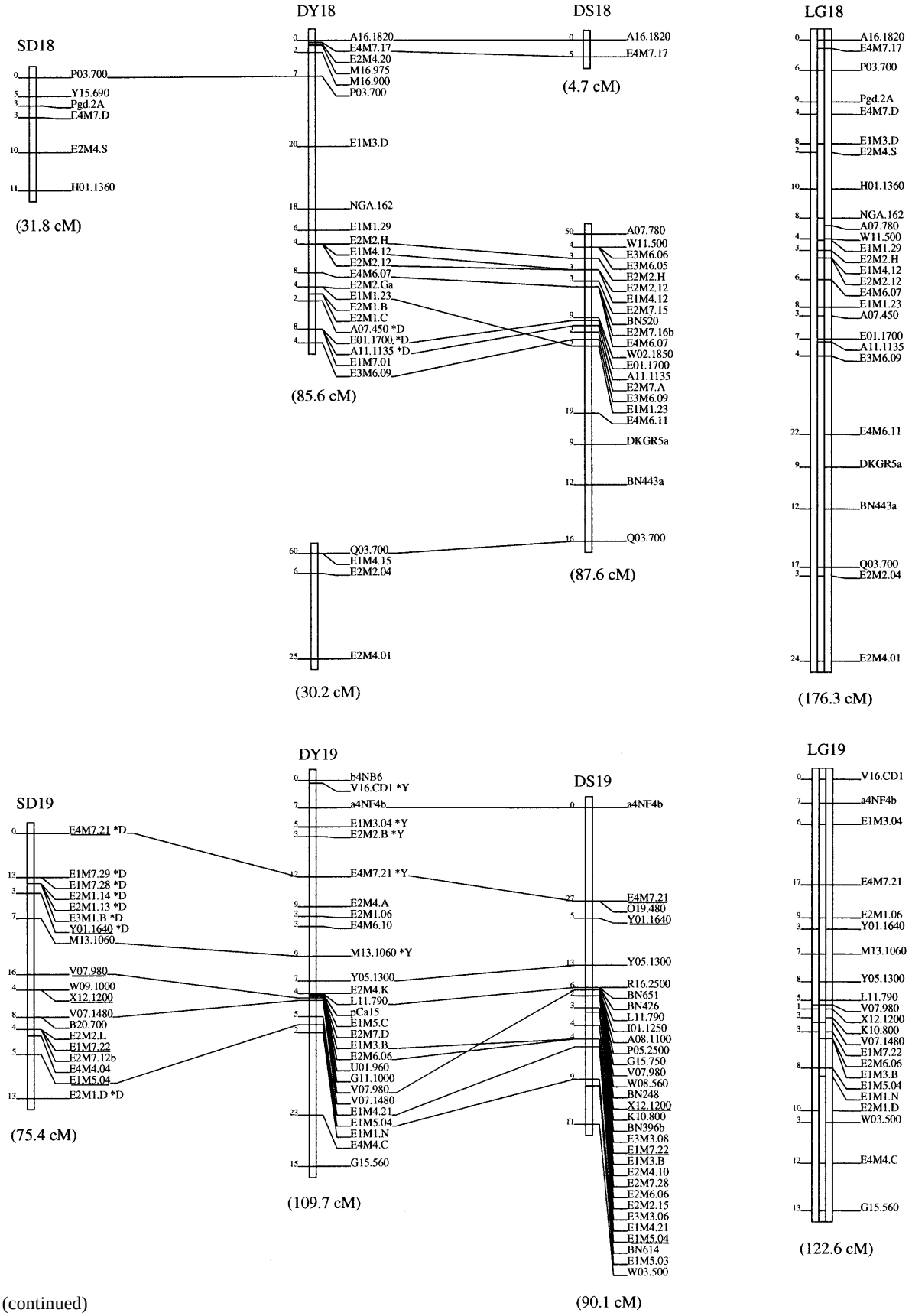

Fig. 1 (continued)

Table 3 Number and type of common markers used for map integration

Markers common to	Total	Isozyme	Microsatellite	Specific PCR	RFLP	RAPD	AFLP
DY/DS	109	1	0	0	7	44	57
DY/SD	77	1	1	1	0	37	37
DS/SD	38	1	0	1	0	18	18
DY/DS/SD	29	1	0	0	0	13	15
Total	253	4	1	2	7	112	127

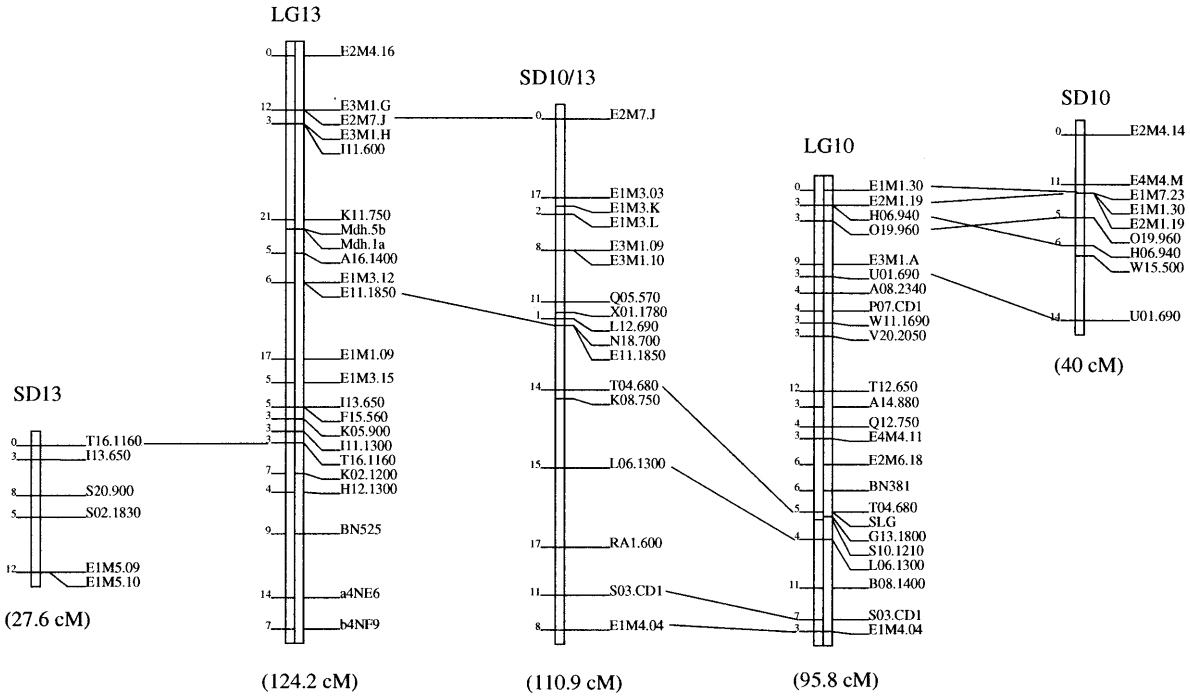

Fig. 2 The translocation event illustrated by a comparison of consensus LG10, consensus LG13, SD10, SD13 and SD10/13. Consensus LG10 and LG13 are formed by the integration of markers from DY10 and DS10, and from DY13 and DS13, respectively. SD10/13 is formed by the association of markers that mapped on LG10 and LG13. *Lines* between groups connect identical markers. *Values in parentheses* correspond to linkage group lengths in centimorgans (Kosambi mapping function)

least one of the two compared populations, and the two remaining differences involved markers spaced by less than 8 cM.

However, a major departure from collinearity, which can be attributed to a translocation, was observed between the SD map and the two other maps. In the SD population, a linkage group was formed by the association of markers that mapped on LG10 and LG13 on the DY and DS maps (Fig. 2). This group was called SD10/13. The regions corresponding to the upper part of LG10 and to the lower part of LG13 formed two small groups (SD10 and SD13) which remained unlinked in the SD population.

The mean distance between pairs of common adjacent markers was significantly greater in SD than in DY (P=6.8×10^{-5}) and DS (P=4.4×10^{-3}), whereas the mean difference in map distance between DY and DS was not statistically different from zero (P=0.63). Orientations of the lines that joined common markers in Fig. 1 gave some information about the LGs that were most affected by the difference of recombination rate between SD and the two other mapping populations. The most evident differences between DY and SD were for LGs 1a, 3, 4, 717, 8, 16 and 19. These seven LGs may have caused the rejection of the hypothesis of a global equality of the recombination rate between SD and DY, and between SD and DS.

Estimates of genome length were calculated for a range of Z from pairwise segregation data for each mapping population (Table 4). The average estimates of genome length were 2,986.3, 2,223.7 and 2,352.5 cM from the DY, DS and SD maps, respectively. The estimated genome coverage E(Co) was 2,312.6 cM, 1,924.2 cM and 2,298.1 cM, and the framework maps covered 87.5%, 81.8% and 81.6% of these estimations for DY, DS and SD, respectively. As expected, the most-complete coverage was provided by the DY map.

Construction of the consensus map

Because of the translocation, the markers from SD10, SD13 and SD10/13 were not used for the construction of

Table 4 Estimated genome length and coverage

Mapping population	No. loci	G[a]	E(G)[b]	95% Confidence interval I_{95} (G)	Co[c]	%Co[d]
DY	590	2,023.5	2,986.3	(2,898.4–3,079.6)	2,312.6	87.5
DS	344	1,574.3	2,223.7	(2,126.7–2,329.9)	1,924.2	81.8
SD	340	1,911.8	2,352.5	(2,237.2–2,480.2)	2,298.1	81.6

[a] Map length based on linkage analyses
[b] Average estimated genome length in centimorgans using the Kosambi mapping function with three LOD treshold values (3, 4 and 5)
[c] Average observed genome coverage calculated from linkage analyses on three LOD treshold values (3, 4 and 5)
[d] Percentage of genome coverage calculated as G divided by Co

the consensus map. The resulting consensus map contained 540 markers, of which 253 were common to at least two populations, and it covered 2,429 cM. Of these 540 markers, most were RFLP (58), RAPD (269) and AFLP (195) markers. In addition, nine isozyme, three specific PCR, five microsatellites markers and the *Bzh* gene were mapped.

The combination of the three individual maps into a consensus map allowed us to extend the DY map (our most-complete individual map) towards distal parts in 15 out of its 19 LGs, and to fill the gap between the two subgroups of DY18. The total extension of the DY map accounted for 237.3 cM, which resulted in a total map length of 2,261 cM versus 2,429 cM for the consensus map. The difference in length between the consensus LGs, and the LGs made up of DY and the DS/SD extensions, was pronounced for eight LGs (1a, 2, 3, 717, 8, 14, 16 and 19). The addition of DS and SD markers in the consensus map allowed us to fill 13 out of the 35 gaps of more than 10 cM, and eight out of ten gaps of more than 20 cM, in the DY map (Fig. 1).

There were significant differences in the marker order between the consensus and the individual maps. These are for LG18 with the DS map, for LGs 3, 5, 717, 8, 14 and 19 with the DY map, and for LGs 1a, 6 and 16 with the SD map. With the exception of the inversion for LG16 in SD, six out of ten inversions spanned less than 5 cM and three spanned less than 8 cM.

Discussion

The major objective of this study was the construction of a saturated map for rapeseed by using a consensus-mapping approach. Rather than a high-density map with a fine order of markers, our purpose was to develop a framework consensus map with a general order of markers that could be used as a reference for more-precise genetic studies. The consensus map consists of 540 markers distributed over 19 linkage groups and covers 2,429 cM. The parents of our crosses have different genetic origins, with either winter-type or spring-type genotypes. A total of 992 different loci could be mapped, whereas only 590, 344 and 340 markers were mapped on the DY, DS and SD maps, respectively. Then, the integration of three individual maps derived from three distinct DH mapping populations allowed us to map a larger number of markers, to obtain a more complete coverage of the rapeseed genome, and to fill a number of gaps for the individual maps.

Segregation distortions were observed with varying degrees in each population and for each type of marker. The overall percentage of loci showing deviation from expected Mendelian ratios may be underestimated because only 60 individuals were genotyped for AFLP markers, which limited the power of detection of distorted loci for these markers. Segregation distortions and clustering of the skewed loci are a common feature of microspore-derived DH populations in various species [(Foisset and Delourme 1996) for a review], including rapeseed. Some regions are biased in two of the mapped populations and could contain common loci interfering with in vitro androgenesis. Foisset et al. (1996) reported that the linkage group DY4 could correspond to a region involved in microspore-culture responsiveness identified by Cloutier et al. (1997). The fact that, in this region, segregation bias is towards the responsive parental lines 'Darmor-*bzh*' and 'Darmor' in both the DY and DS populations reinforces this hypothesis. The identification of LGs containing skewed loci in only one population might reflect different alleles in the parental lines of the three populations at the putative factors influencing in vitro androgenesis. The hypothesis of a gametic selection in some of the biased regions cannot be excluded, as suggested by Ferreira et al. (1994) and Foisset et al. (1996). Segregation distortions might be due to chromosomal rearrangements, such as translocations, as suggested by Fauré et al. (1993) and Barzen et al. (1995). This hypothesis does not seem to be valid in our case since segregation distortions were never observed for the same region in the three populations, and a good alignment of markers was obtained between LGs showing segregation distortion in only one population. This conserved order of markers between biased and non-biased LGs also indicates that the distortions did not disturb the map elaboration, after LGs containing biased loci have been separated using an increased LOD threshold.

A significant higher recombination rate was found in SD in comparison with the two other maps. Such differences in recombination frequency have already been reported between male and female parents in loblolly pine (Sewell et al. 1999), apple (Maliepaard et al. 1998), or

rubber tree (Lespinasse et al. 2000), between maize F_2 populations (Beavis and Grant 1991), and between DH populations of *Brassica oleacera* (Sebastian et al. 2000). However, Parkin and Lydiate (1997) did not report any difference of recombination frequency between maps from two DH populations of *B. napus*, although one was derived from a cross between a re-synthetized rapeseed and a winter rapeseed. No apparent explanation may be offered to interpret the difference in our case.

A translocation was identified between the SD map and the two others. In the SD map, there is an association between the LG10 and LG13 markers, as defined in the DY and DS maps. However, there is no obvious crossover suppression on SD10/13, which suggests that both lines 'Stellar' and 'Drakkar' carry the same translocation. If the translocation was only present in one of the parental lines, crossover suppression and difficulty in ordering the markers would have been expected, as was observed in pea (Ellis et al. 1992). If the translocation is reciprocal, we would expect an association between the upper part of LG10 and the lower part of LG13 in a unique LG, whereas these two regions remained unlinked in the SD population. This could be due to the lack of polymorphic markers in this region to join these two subgroups. A reciprocal translocation involving two partially homoeologous LGs has previously been reported in rapeseed (Parkin et al. 1995; Osborn et al. 2000). Osborn et al. (2000) identified this translocation in DH progenies deriving from crosses of winter and spring rapeseed cultivars. This corresponds to our findings since the SD map was derived from a cross between two spring cultivars and the DS map from a cross between two winter cultivars. 'Yudal' is classified as a spring-type but it does not seem that it carries the chromosomal arrangements of 'Stellar' and 'Drakkar' because a complete collinearity was obtained between the DY and DS maps on the LGs involved in the translocation. Some non-reciprocal translocations resulting from homoeologous pairing have also been reported in rapeseed (Sharpe et al. 1995).

The high and significant correlation between the length of the LGs and the number of markers in each mapping population indicates that markers are randomly distributed among LGs. However, non-uniform marker distributions were observed for six DS LGs, seven DY LGs and two SD LGs, either due to clusters or gaps of markers. Non-uniformity has been reported in rapeseed by Uzunova et al. (1995), Foisset et al. (1996) and Parkin and Lydiate (1997), and in other crops such as tomato (Tanksley et al. 1992). This non-uniformity may be caused by a non-random sampling of the genome, by an uneven distribution of the recombination rate along the LGs (Tanksley et al. 1992), or by a clustering tendency of some markers due to their preferential targetting of some genomic regions (Castiglioni et al. 1999). A comparison between the maps gives some ideas about what phenomena may prevail. For LGs 1a, 6, 13 and 19, the presence of gaps in one population and the absence in another suggests a non-random sampling of the genome. For LGs 1a, 8, 15 and 19 either gaps or clusters containing different types of markers in the three populations may indicate an uneven distribution of the recombination rate.

By integrating the individual maps into a consensus map, the length of the DY map increased from 2,023.5 to 2,429 cM. Fifty eight percent of this increase was due to the addition of molecular markers to the distal parts of the LGs and the filling of the gap between the two subgroups of LG18. A major part of the remainder was probably due to recombination-frequency differences between DY and SD. The validity of the construction of a consensus map based on individual populations where a difference occurs in recombination frequency was questioned by Beavis and Grant (1991). However, if the marker order between individual maps and the consensus map is conserved, the composite map remains valuable (Lespinasse et al. 2000). In our study, only small changes in marker order were observed between individual maps, with the exception of the translocation, and between individual and consensus maps, with the exception of LG16. Small differences might be due to mapping-imprecisions rather than real rearrangements. For LG16, the distance between the two common markers R06.2100 and E3M6.07 was very different in DY and SD: 33.4 cM in DY and 72.3 cM in SD. The difference was so high that the log-likelihood of LG16 was better by inverting the order of the segment made up of T12.820, U07.1110 and E2M7.21 (Fig. 1). This is the only case in our consensus map where the difference in recombination frequency leads to an inaccurate marker order in the consensus map. In apple, Maliepaard et al. (1998) observed such a difference of marker order in the integrated LG1, caused by a large difference in recombination rate between the populations.

Our consensus linkage map covers 2,429 cM and should correspond to the 19 basic chromosomes of the haploid *B. napus* genome. Among the published maps of rapeseed, the longest one was developed by Cheung et al. (1997), covering 1,954.7 cM for 19 major LGs and 2,124.9 cM by including ten unassigned segments of less than four markers. Three rather complete maps were published by Parkin et al. (1995) and Sharpe et al. (1995). Their size ranged from 1,606 cM to 1,741 cM. Based on our individual maps, the estimated genome length E(G) ranged from 2,223.7 cM for DS to 2,986.3 cM for DY maps. Foisset et al. (1996) estimated the genome length of rapeseed, from the results of a less-complete DY map, to be 2,486 cM, which is smaller than the estimate computed from the more-complete actual DY map. The estimates of genome length depend on the number of markers and the maximum distance among pairs above a LOD threshold. It is likely that the formula of Hulbert et al. (1988) overestimates the genome length in the case of the DY map due to the presence of gaps in the map despite the large number of markers. The estimate given by the former DY map (Foisset et al. 1996) is close to the ones provided by the DS and SD maps. Then, the genome length of rapeseed probably ranges

from 2,127 cM to 2,480 cM, the average values of DS and SD 95% confidence-interval estimates. The size of our consensus linkage map is 2,429 cM, which seems to indicate a near-complete genome coverage.

The integration of three distinct genetic maps into a consensus map made it possible to obtain a general order and distances for a greater number of markers, and to get a near-complete coverage of the rapeseed genome. The obtained framework consensus map can be used as a reference map to develop genetic studies in different genetic backgrounds. For this purpose, the DH lines from the DY population, which produced the most-complete genetic map, and the corresponding genotyping data will be made publicly available. These genetic maps are being progressively enriched by adding new markers such as ACGM (Amplified Consensus Genetic Markers) which have been developed (Fourmann et al. 1998; Brunel et al. 1999), and should enable the localization of genes of known biological function through the use of *Arabidopsis*-known sequences.

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References

Aarts E, Lenstra JK (1997) Interscience series in discrete mathematics and iptimization. John Wiley and sons, New York, USA

Baranger A, Delourme R, Foisset N, Barret P, Dupuy P, Renard M, Chèvre A (1997) Wide mapping of a T-DNA insertion site in oilseed rape using bulked segregant analysis and comparative mapping. Plant Breed 116:553–560

Barret P, Guérif J, Reynoird J, Delourme R, Eber F, Renard M, Chèvre A (1998) Selection of stable *Brassica napus – Brassica juncea* recombinant lines resistant to blackleg (*Leptosphaeria maculans*). 2. A 'to and fro' strategy to localise and characterise interspecific introgressions on the *B. napus* genome. Theor Appl Genet 96:1097–1103

Barzen E, Mechelke W, Ritter E, Schulte-Kappert E, Salamini F (1995) An extended map of the sugar beet genome containing RFLP and RAPD loci. Theor Appl Genet 90:189–193

Beavis WD, Grant D (1991) A linkage map based on information from four F_2 populations of maize (*Zea mays* L.). Theor Appl Genet 82:636–644

Bell C, Ecker J (1994) Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis*. Genomics 19:137–144

Brunel D, Froger N, Pelletier G (1999) Development of amplified consensus genetic markers (A.C.G.M.) in *Brassica napus* from *Arabidopsis thaliana* sequences of known biological function. Genome 42:387–402

Castiglioni P, Ajmone P, van Wijk R, Motto M (1999) AFLP markers in a molecular linkage map of maize: codominant scoring and linkage group distribution. Theor Appl Genet 99: 425–431

Chakravarti A, Lasher LK, Reefer JE (1991) A maximum-likelihood method for estimating genome length using genetic linkage data. Genetics 128:175–182

Cheung WY, Champagne G, Hubert N, Landry BS (1997) Comparison of the genetic maps of *Brassica napus* and *Brassica oleracea*. Theor Appl Genet 94:569–582

Chèvre A, Delourme R, Eber F, Margale E, Quiros C, Arus P (1995) Genetic analysis and nomenclature for seven isozyme systems in *Brassica nigra*, *B. oleracea* and *B. campestris*. Plant Breed 114:473–480

Cloutier S, Cappadocia M, Landry B (1997) Study of microspore-culture responsiveness in oilseed rape (*Brassica napus* L.) by comparative mapping of a F_2 population and two microspore-derived populations. Theor Appl Genet 91:841–847

Delourme R, Foisset N, Horvais R, Barret P, Champagne G, Cheung W, Landry B, Renard M (1998) Characterisation of the radish introgression carrying the *Rfo* restorer gene for the Ogu-INRA cytoplasmic male sterility in rapeseed (*Brassica napus* L.). Theor Appl Genet 97:129–134

Dillmann C, Charcosset A, Goffinet B, Smith JSC, Dattée Y (1997) Best linear unbiased estimator of the molecular genetic distance between inbred lines. In: Krajewski P and Kaczmarek Z (eds) 10th Meeting EUCARPIA Section Biometrics in Plant Breeding. Poznan, Poland, pp 105–110

Echt CS, Nelson CD (1997) Linkage mapping and genome length in eastern white pine (*Pinus strobus* L.). Theor Appl Genet 94:1031–1037

Ellis T, Turner L, Hellens R, Lee D, Harker C, Enard C, Domoney C, Davies D (1992) Linkage maps in pea. Genetics 130:649–663

Fauré S, Noyer J, Horry J, Bakry F, Lanaud C, Gonzalez de Leon D (1993) A molecular marker-based linkage map of diploid bananas (*Musa acuminata*). Theor Appl Genet 87:517–526

Ferreira ME, Williams PH, Osborn TC (1994) RFLP mapping of *Brassica napus* using doubled-haploid lines. Theor Appl Genet 89:615–621

Foisset N, Delourme R (1996) Segregation distortion in androgenic plants. In: Mohan Jain S, Sopory S, Veilleux R (eds) In vitro haploid production in higher plants. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 189–201

Foisset N, Delourme R, Barret P, Renard M (1995) Molecular tagging of the dwarf Breizh (*Bzh*) gene in *Brassica napus*. Theor Appl Genet 91:756–761

Foisset N, Delourme R, Barret P, Hubert N, Landry BS, Renard M (1996) Molecular-mapping analysis in *Brassica napus* using isozyme, RAPD and RFLP markers on a doubled-haploid progeny. Theor Appl Genet 93:1017–1025

Fourmann M, Barret P, Renard M, Pelletier G, Delourme R, Brunel D (1998) The two genes homologous to *Arabidopsis FAE1* co-segregate with the two loci governing erucic acid content in *Brassica napus*. Theor Appl Genet 96:852–858

Gerber S, Rodolphe F (1994) An estimation of the genome length of maritime pine (*Pinus pinaster* Ait.). Theor Appl Genet 88:289–292

Hulbert SH, Ilott TW, Legg EJ, Lincoln SE, Lander ES, Michelmore RW (1988) Genetic analysis of the fungus, *Bremia lactucae*, using restriction fragment length polymorphisms. Genetics 120:947–958

Jourdren C, Barret P, Horvais R, Foisset N, Delourme R, Renard M (1996a) Identification of RAPD markers linked to the loci controlling erucic acid level in rapeseed. Mol Breed 2:61–71

Jourdren C, Barret P, Horvais R, Delourme R, Renard M (1996b) Identification of RAPD markers linked to linolenic acid genes in rapeseed. Euphytica 90:351–357

Jourdren C, Barret P, Brunel D, Delourme R, Renard M (1996c) Specific molecular marker of the genes controlling linolenic acid content in rapeseed. Theor Appl Genet 93:512–518

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

Kuginuki Y, Ajisaka H, Yui M, Yoshikawa H, Hida K, Hirai M (1997) RAPD markers linked to a clubroot-resistance locus in *Brassica rapa* L. Euphytica 98:149–154

Landry BS, Hubert N (1991) A genetic map for *Brassica napus* based on restriction fragment length polymorphisms detected with expressed DNA sequences. Genome 34:543–552

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 linkage maps with Mapmaker/exp 3.0: a tutorial and reference manual. 3rd edn, Whitehead Institute Technical Report

Lombard V, Baril CP, Dubreuil P, Blouet F, Zhang D (2000) Genetic relationships and fingerprinting of rapeseed cultivars using AFLP: consequences for varietal registration. Crop Sci 40:1417–1425

Maliepaard C, Alston FH, van Arkel G, Brown LM, Chevreau E, Dunemann F, Evans KM, Gardinier S, Guilford P, van Heusden AW, Janse J, Laurens F, Lynn JR, Manganaris AG, den Nijs APM, Periam N, Rikkerink E, Roche P, Ryder C, Sansavini S, Schmidt H, Tartarini S, Verhaegh JJ, Vrielink-van Ginkel M, King GJ (1998) Aligning male and female linkage maps of apple (*Malus pumila* Mill.) using multi-allelic markers. Theor Appl Genet 97:60–73

Manzanares-Dauleux M, Delourme R, Baron F, Thomas G (2000) Mapping of one major gene and of QTLs involved in resistance to clubroot in *Brassica napus*. Theor Appl Genet 101:885–891

Mather K (1957) The measurement of linkage in heredity. Methuen and Co, London

Osborn T, Butruille D, Sharpe A, Lydiate D, Hall K, Parker J (2000) Translocation in linkage mapping and QTL analysis. In: Plant and animal genome VIII conf. San Diego, California (http://www.intl-pag.org/pag/)

Parkin IAP, Lydiate DJ (1997) Conserved patterns of chromosome pairing and recombination in *Brassica napus* crosses. Genome 40:496–504

Parkin IAP, Sharpe AG, Keith DJ, Lydiate DJ (1995) Identification of the A and C genomes of amphidiploid *Brassica napus* (oilseed rape). Genome 38:1122–1131

Pilet M (1999) Analyse génétique de la résistance du colza à la Nécrose du collet et à la cylindrosporiose à l'aide de marqueurs moléculaires. PhD thesis, INA Paris-Grignon

Pilet M, Duplan G, Barret P, Renard M, Delourme R (1999) Genetics of blackleg resistance in winter rapeseed. In: Proc 10th Int Rapeseed Congr, Canberra

Pilet ML, Delourme R, Foisset N, Renard M (1998a) Identification of loci contributing to quantitative field resistance to blackleg disease, causal agent *Leptosphaeria maculans* (Desm.) Ces. et de Not., in winter rapeseed (*Brassica napus* L.). Theor Appl Genet 96:23–30

Pilet ML, Delourme R, Foisset N, Renard M (1998b) Identification of QTLs involved in field resistance to light leaf spot (*Pyrenopeziza brassicae*) and blackleg resistance (*Leptosphaeria maculans*) in winter rapeseed (*Brassica napus* L.). Theor Appl Genet 97:398–406

Polsoni L, Kott S, Beversdorf W (1988) Large-scale microspore culture technique for mutation-selection studies in *Brassica napus*. Can J Bot 66:1681–1685

Schiex T, Gaspin C (1997) CARTHAGENE: constructing and joining maximum-likelihood genetic maps. In: Proc 5th Int Conf Intelligent Systems for Molecular Biology, Porto Caras, Halkidiki, Greece, pp 258–267

Schiex T, Chabrier P, Gaspin C (2000) CarthaGene: a maximum-likelihood multiple population genetic/radiated hybrid mapping software. In: Plant and animal genome VIII conference, San Diego, California (http://www.intl-pag.org/pag/)

Sebastian RL, Howell EC, King GJ, Marshall DF, Kearsey MJ (2000) An integrated AFLP and RFLP *Brassica oleracea* linkage map from two morphologically distinct doubled-haploid mapping populations. Theor Appl Genet 100:75–81

Sewell MM, Sherman BK, Neale DB (1999) A consensus map for loblolly pine (*Pinus taeda* L.). 1. Construction and integration of individual linkage maps from two outbred three-generation pedigrees. Genetics 151:321–330

Sharpe AG, Parkin IAP, Keith DJ, Lydiate DJ (1995) Frequent nonreciprocal translocations in the amphidiploid genome of oilseed rape (*Brassica napus*). Genome 38:1112–1121

Stam P (1993) Construction of integrated linkage maps by mean of a new computer package: JoinMap. Plant J 3:739–744

Szewc-McFadden A, Kresovich S, Bliek S, Mitchell S, McFerson J (1996) Identification of polymorphic, conserved simple sequence repeats (SSRs) in cultivated *Brassica* species. Theor Appl Genet 93:534–538

Tanksley SD, Ganal MW, Prince JP, de Vincente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Röder MS, Wing RA, Wu W, Young ND (1992) High-density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

Uzunova M, Ecke W, Weissleder K, Röbbelen G (1995) Mapping the genome of rapeseed (*Brassica napus* L.). 1. Construction of an RFLP linkage map and localization of QTLs for seed glucosinolate content. Theor Appl Genet 90:194–204