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A RFLP-based linkage map of mustard [*Brassica juncea* (L.) Czern. and Coss.]

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Abstract A genetic linkage map of *Brassica juncea* was constructed based on restriction fragment length polymorphism (RFLP) detected by anonymous cDNA markers from *B. napus*, using a segregating F_1-derived doubled haploid (DH) progeny from a cross between a canola-quality mustard line (J90-4317) and a high-oil-content mustard line (J90-2733). The RFLP probes consisted of 229 cDNA probes from *B. napus* and a *B. napus* tandem repeat sequence, RDA2. The map consisted of 343 marker loci arranged in 18 major linkage groups plus five small segments with two to five marker loci, covering a total map distance of 2073 cM. Twenty-four percent of the markers were dominant in nature. Sixty-two percent of the marker loci were duplicated, and the majority were involved in inter-linkage group duplications, illustrating that complex duplications and subsequent rearrangements occurred after allopolyploidy. Deviation from the Mendelian segregation ratio for a DH population was observed for 27% of the markers. Two-thirds of these markers with a skewed segregation were clustered in 6 linkage groups and two unassigned segments. The overall average marker interval of the *B. juncea* map reported here was 6.6 cM, which would provide a marker density satisfactory for efficient use of the map in breeding applications, such as tagging of important agronomic traits and marker-assisted selection.

Key words *Brassica juncea* · Mustard · Linkage map · RFLP

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Introduction

The amphidiploid *Brassica juncea* ($2n = 4x = 36$, genome AABB) is grown as a source of condiment mustard. This species has many unique and valuable attributes, which include superior heat and drought tolerance, resistance to blackleg disease, early maturity, and resistance to pod shattering (Downey and Rakow 1987). The development of a canola-quality mustard as an oilseed crop has been of interest to breeders.

Genetic linkage maps are useful tools for studying genome structure and evolution, for identifying introgressions, and for comparing the genome organization and gene order of different species (Bonierbale et al. 1988; Tanksley et al. 1988; Hosaka et al. 1990). They are also used to identify molecular markers that can be used for marker-assisted selection in plant breeding. Genetic linkage maps of restriction fragment length polymorphism (RFLP) markers have been constructed for *B. oleracea* (Slocum et al. 1990; Kianian and Quiros 1992; Landry et al. 1992; Camargo 1994), *B. rapa* (Song et al. 1991; Chyi et al. 1992), *B. nigra* (Lagercrantz and Lydiate 1995), and *B. napus* (Landry et al. 1991;Ferreira et al. 1994; Uzunova et al. 1995). There is currently no detailed genetic or molecular linkage maps for *B. juncea* that can be used efficiently for the identification of molecular markers linked to important agronomic traits, and for marker-assisted selection in breeding programs. With the view that a detailed molecular linkage map for *B. juncea* can facilitate the possibility of mapping disease resistance and important

agronomic traits, which can assist breeders to make efficient selection of canola-quality *B. juncea* cultivars, we report here a RFLP-based linkage map constructed using cDNA markers from *B. napus* and a segregating F_1-derived doubled haploid (DH) progeny of 119 individuals from a cross between a canola-quality mustard breeding line (J90-4317) and a high-oil-content line (J90-2733), both from the AAFC Research Centre, Saskatoon. J90-4317 is a line with a low level of glucosinolates, a low oil and high protein content, and is susceptible to white rust. J90-2733 has high levels of glucosinolates, a high oil and low protein content, and is resistant to white rust.

Materials and methods

Plant materials

Seeds of both parental lines, the canola-quality mustard line (J90-4317) and the high-oil-content line (J90-2733), and the mapping population of 119 F_1-microspore-derived DH lines produced by the Saskatchewan Wheat Pool were obtained from the Agriculture and Agri-Food Canada Research Centre, Saskatoon. All plants were grown in 25-cm pots in the greenhouse at 18°–24°C with a photoperiod of 14–18 h. For each parental or DH line, tissues from four plants were pooled for DNA extraction.

Plant genomic DNA was isolated as described previously (Landry et al. 1991) with one modification – the replacement of the purification by CsCl gradient with two successive chloroform/isoamyl-alcohol (24 : 1 v/v) extractions.

Source of probes

A total of 319 cDNA probes were obtained from a library of *B. napus* embryo cDNA clones (Harada et al. 1988); markers identified by these probes are labelled with the prefix "X" on the map (Fig. 1). In addition, 15 characterized seedling-specific probes (Harada et al. 1988) were used. Markers identified by the latter type of probes are labelled with the prefix "P" on the map (Fig. 1). The cDNA inserts were isolated from *Pst*I digests of the clones by agarose gel electrophoresis and were further purified using the Sephaglas™ band Prep kit (Pharmacia). A genomic DNA probe (RDA2) containing a tandem repeat sequence from *B. napus* was obtained as a by-product of a representational difference analysis involving two *B. napus* lines (Cheung et al. unpublished). The insert of this clone was isolated from a *Bam*HI digest and purified in a similar fashion as the cDNA probes.

Southern blot hybridizations

DNA samples of the parental and the DH lines were digested with *Bam*HI, *Eco*RI, *Eco*RV and *Hin*dIII in separate reactions (5 U/μg DNA). Digested DNA (3 μg/lane) were electrophoresed in 0.8% agarose gels and transferred onto Hybond N^+ membranes (Amersham) by the alkaline transfer procedure recommended by the manufacturer. Probes were radioactively labelled with [^{32}P] using the T7 Quick Prime™ kit (Pharmacia). Conditions for hybridizations and autoradiography were as described by Landry et al. (1991).

Segregation and linkage analyses

Polymorphic cDNA probes were identified as differences in the banding patterns between the parental lines on autoradiographs. Segregation analysis was done by hybridizing each of the selected probes to the 119 F_1-derived DH samples digested with the selected restriction enzyme that displayed the maximum degree of polymorphism. Multi-point linkage analysis of the marker loci was performed with the computer program MAPMAKER Macintosh V2.0 (Dupont). RFLP loci were assigned and mapped to linkage groups (LGs) based on a threshold LOD score of 6.0 and a maximum recombination fraction (RF) of 0.4.

Results and discussion

Selection of probes and level of polymorphism between the parental lines J90-4317 and J90-2733

In this study, 334 anonymous *B. napus* cDNA probes and a tandem repeat probe were used to screen for RFLP between the parental lines J90-4317 and J90-2733. Four restriction enzymes, *Bam*HI, *Eco*RI, *Eco*RV, and *Hin*dIII were employed, and the percentages of useful probes for detection of polymorphisms with each restriction enzyme are summarized in Table 1a. The results showed that the average percentage of useful probes using one restriction enzyme was 57%, and there was no significant difference with the choice of enzymes. When the four enzymes were used simultaneously, 269 probes (81%) could detect a polymorphism with one or more enzymes. A subset of the probes used in this study had been employed previously in the construction of maps of *B. napus* and *B. oleracea* using the same four restriction enzymes in screening the parental lines. The proportion of useful clones identified by individual enzymes was higher with *B. oleracea* (71–74%) than with *B. juncea* (51–61%), but was lower with *B. napus* (23–27%). This observation can be considered merely to be a reflection of how closely related the parental DNA samples used in the screening are, where a wide cross was used in the case of *B. oleracea*, and a narrow cross was chosen in the case of *B. napus*. Even though less useful clones were selected by the use of a single enzyme with *B. juncea* than with *B. oleracea*, by combining the four enzymes, we were able to enhance the level of useful probes for detection of RFLP in *B. juncea* to a similar level (81%) as in *B. oleracea* (76%). Only for 38 probes (11%) was the polymorphism detected by a single restriction enzyme; while with 231 probes (70%), the polymorphism was simultaneously detected by two or more enzymes. These results indicate that the majority of the detected polymorphisms are caused by deletions, insertions, or rearrangements, rather than by point or small mutations. This observation also applies to *B. napus* and *B. oleracea* using the same set of probes (Landry et al. 1991, 1992).

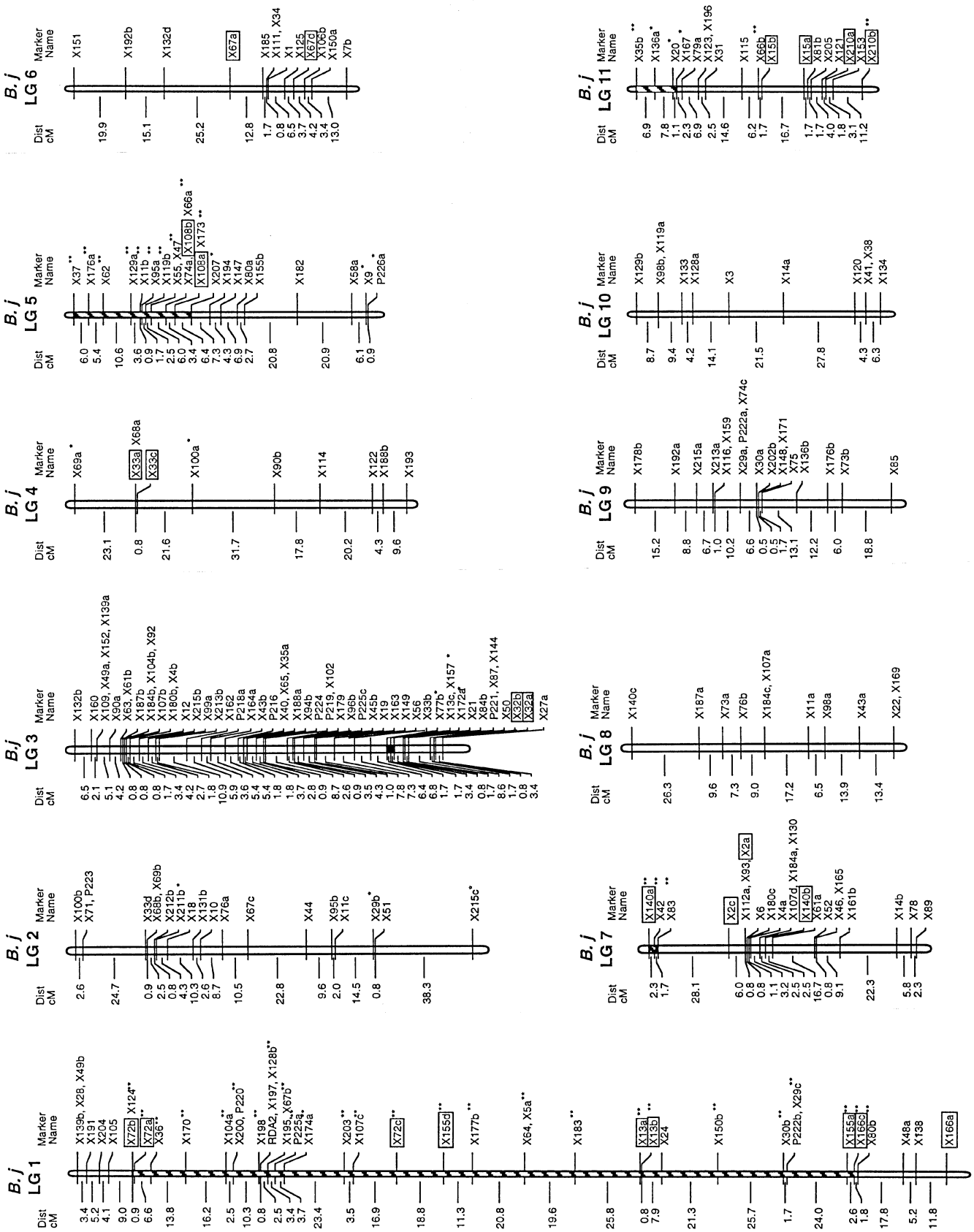
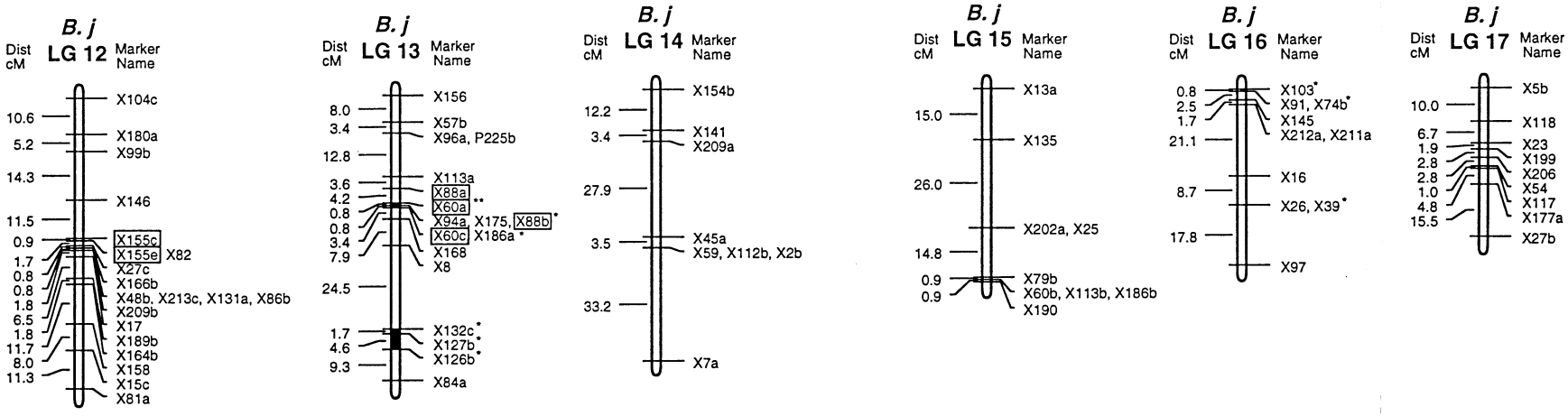
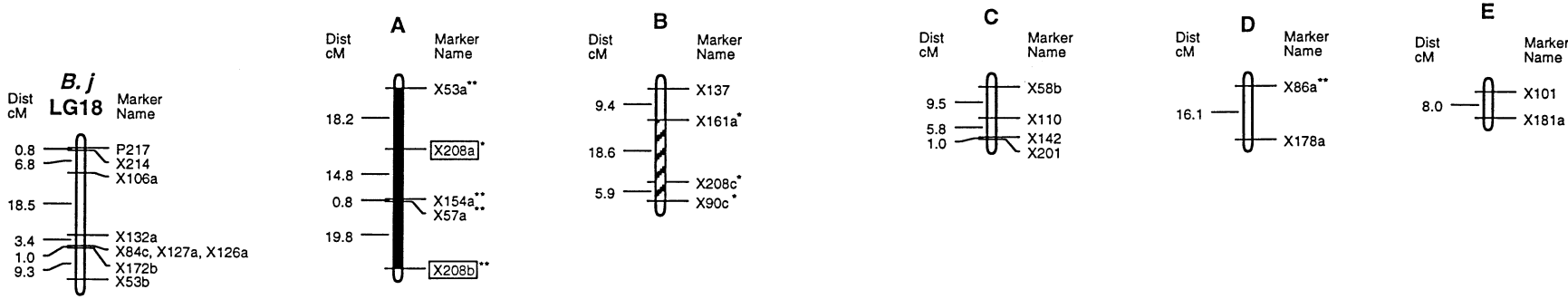


Fig. 1 See page 845 for legend



Unassigned segments:



Unlinked markers: X13e, X70, X77a, X143a, X143b, X113c, X166d, X174b, X177c, X181b, X189a, P218b, P226b

Table 1a Proportion of cDNA probes detecting polymorphisms between *Brassica juncea* lines J90-4317 and J90-2733 using four restriction enzymes

Restriction enzymes	Number of clones			Percentage useful clones
	No polymorphism	Polymorphism detected	Total	
*Bam*HI	145	189	334	57
*Eco*RI	145	189	334	57
*Eco*RV	129	205	334	61
*Hin*dIII	164	170	334	51

Table 1b Degree of DNA polymorphisms between *Brassica juncea* lines J90-4317 and J90-2733 detected by cDNA probes

Restriction enzymes	Number of monomorphic fragments	Number of polymorphic fragments	Total number of fragments	% Polymorphism
*Bam*HI	970	282	1252	23
*Eco*RI	945	281	1226	23
*Eco*RV	1016	315	1331	24
*Hin*dIII	1020	265	1285	21
Overall	3951	1143	5094	22

The majority of the *B. napus* cDNA probes cross-hybridized well with *B. juncea* DNA. Only 2% of the probes hybridized to significantly fewer fragments in *B. juncea* (genome AABB) than in *B. napus* (genome AACC), suggesting that few cDNA sequences were A-genome-specific probes. This result also indicated that there are substantial homologies between the B and C genomes of *Brassica* species.

The data on polymorphisms with the four restriction enzymes were used to estimate the level of polymorphisms between the parental lines (Table 1b). On average, each probe hybridized to four restriction fragments. Out of the 5094 hybridized fragments obtained using four restriction enzymes and 334 probes, 1143 polymorphic fragments and 3951 monomorphic fragments were detected, indicating an overall 22% polymorphism between the two parental lines.

Fig. 1 Linkage map of *Brassica juncea* (*B.j*). Linkage group (*LG*) *numbers* are indicated *above* the linkage groups. Recombination distances between markers are in Kosambi centiMorgans (*left*). Marker loci are named after the cDNA probes that detected the loci (*right*). Duplicated loci detected by the same probe are labelled with a different *lower-case suffix* following the name of the probe. RDA2 is the locus detected by a *B. napus* genomic probe containing a tandem repeat sequence. The 343 RFLP loci are assembled into 18 major groups assigned arbitrarily as group 1–18, and 5 smaller segments are labelled as unassigned segments A–E. Thirteen loci remained unlinked to any other marker and are listed at the *bottom* of the figure. Marker loci showing segregation distortion are indicated with * for $P < 0.05$, ** for $P < 0.01$. Segregation distortion towards the J90-4317 parental alleles are marked by ▨, and distortion towards the J90-2733 parental alleles are marked by ■. Intra-linkage group duplicated loci are indicated by a box around the name of the locus.

A total of 229 cDNA probes and a tandem repeat probe were chosen for segregation analyses of 119 F_1-derived DH progeny from the cross between J90-4317 and J90-2733. The choices were based on the maximum number of easily scored polymorphisms revealed by these probes.

Residual heterozygosity of parental lines detected by RFLP probes

Both J90-4317 and J90-2733 are not pure homozygous lines. J90-4317, the canola-quality parent, in particular has a complex pedigree, including an earlier interspecific cross with *B. rapa* and numerous backcrosses with different *B. juncea* strains and varieties (Love et al. 1990). Parental residual heterozygosity can manifest either as (1) polymorphism between the parents that does not segregate in the F_1-derived DHs, depending on the genotype of the F_1 plant used in microspore culture; or (2) as segregating polymorphic fragments among the DHs, which are not polymorphic between the parental lines. In this cross, 12 probes (out of 230 used for mapping) demonstrated the former case, and nine probes the latter. Altogether, 9% of the probes revealed residual heterozygosity, which was lower than the results of Cloutier et al. (1995) with *B. napus* using a subset of the probes used in this study. In each case, a single fragment was involved. Taking into account the 1033 hybridized fragments (both monomorphic and polymorphic) observed in the segregation analyses with the 230 probes, 21 fragments corresponded only to 2% of residual heterozygosity in the parental lines. J90-2733 was shown to have the major portion of the residual heterozygosity.

The RFLP map

A total of 230 probes used for mapping provided 356 segregating RFLP marker loci in the DH mapping population, which corresponded to 1.5 loci per probe. When the same source of probes was used, the *B. napus* and *B. oleracea* maps (Landry et al. 1991, 1992; Cloutier et al. 1995) revealed a similar efficiency of the polymorphic probes in detecting segregating RFLP loci. This level of efficiency does not differ significantly from polymorphic genomic probes used in the genetic mapping of *B. rapa* and *B. napus* (Chyi et al. 1992; Uzunova et al. 1995). Of the segregating marker loci, 87 (24%) were dominant in nature, while 269 (76%) were co-dominant.

All RFLP probes used detected duplicated loci (segregating and non-segregating loci). In this study, 93 probes (40%) used for segregation analyses detected duplicated segregating loci. The level of duplicated loci detected by polymorphic probes may correlate with the source of the probes (whether they are cDNA or genomic DNA in nature). So far, cDNA probes (40–90%) appeared to be capable of revealing more duplicated loci than genomic probes (20%) (Landry et al. 1991, 1992; Chyi et al. 1992; Uzunova et al. 1995) irrespective of whether the species is diploid or amphidiploid. This may be caused by the pre-selection of low- or single-copy clones for the genomic probes as well as the more unique and diversed nature of some of the low-copy non-coding sequences found among the genomic probes as compared to the more conserved coding sequences of the cDNA probes. However, the number of duplicated loci detected may vary with different species and the parents used in each case. This can be explained by the fact that the majority of non-duplicated loci result from one segregating locus among additional monomorphic fragments, which may be revealed as duplicated segregating loci in other germplasm.

Based on the frequency of recombination in the DH mapping population, 326 RFLP loci were arranged into 18 linkage groups (LGs) (maximum recombination fraction 40%, LOD 6.0) numbered 1–18 in descending order of their lengths in centiMorgans that cover a genetic distance of 1945 cM (Kosambi function), possibly corresponding to the 18 basic chromosomes of the haploid *B. juncea* genome (Fig. 1). Each of these LGs had eight or more marker loci. In addition, there were 17 RFLP loci located on five smaller, unassigned segments (A–E; each with two to five marker loci) (Fig. 1), spanning an additional 128 cM. The total map distance is therefore 2073 cM with 343 RFLP loci. Thirteen RFLP loci could not be linked significantly to the LGs of the map. The size and number of markers, density of markers, and average marker interval of each LG are summarized in Table 2. The sizes of the LGs varied substantially between the maximum of 343 cM (LG 1) and the minimum of 40 cM (LG 18). The number of markers located on each linkage group also varied greatly from eight (LGs 14) to 55 (LG 3). As a result, the marker density and the average marker interval of each group also differed more than four-fold between the maximum and minimum. LG 3 was the most densely populated LG, and it showed an average marker interval of 2.7 cM, while LG 4, the least densely populated group, had an average marker interval of over 10 cM. The overall marker interval of the *B. juncea* map was 6.6 cM, and 10 of the 18 LGs showed marker densities equal to or higher than the average value. These 10 LGs as a group showed an average marker interval of 4.7 cM; the remaining eight LGs showed a corresponding value of 9.0 cM. These findings raise the question of whether or not this result is a reflection of the recombination rates of the genomes that constitute the amphidiploid. Further comparative mapping between *B. rapa*, *B. nigra*, and *B. juncea* is necessary to test the hypothesis.

Clustering of marker loci was observed in 11 of 18 LGs. However, apart from LG 3, which had five

Table 2 *Brassica juncea* RFLP linkage map**:** Linkage group size, number of markers, marker density, and average marker interval per linkage group

Linkage group	Size (cM)	Number of markers	Density (marker/cM)	Average marker interval (cM)
1	343.2	43	0.13	8.0
2	156.0	19	0.12	8.2
3	149.4	55	0.37	2.7
4	129.1	10	0.08	12.9
5	116.3	23	0.20	5.1
6	106.3	13	0.12	8.2
7	106.1	22	0.21	4.8
8	103,2	11	0.11	9.4
9	101.3	18	0.18	5.6
10	96.4	11	0.11	8.8
11	90.2	18	0.20	5.0
12	87.1	20	0.23	4.4
13	85.1	18	0.21	4.7
14	80.3	8	0.10	10.0
15	57.5	9	0.16	6.4
16	52.7	10	0.19	5.3
17	45.3	9	0.20	5.0
18	39.9	9	0.23	4.4
Σ/mean	1945.4	326	0.18	6.6

Unassigned segment	Size (cM)	Number of markers
A	53.7	5
B	33.9	4
C	16.3	4
D	16.1	2
E	8.0	2
Σ	128.0	17

discernable clusters, all the other LGs showing the effect had only one cluster of loci each, leading to the speculation that these regions were close to the centromeres, where recombination was generally reduced. Pronounced clustering of RFLP markers had been observed in the RFLP maps of *B. oleracea* (Landry et al. 1992) and *B. nigra* (Lagercrantz and Lydiate 1995), but was not evident in the maps of *B. oleracea* (Slocum et al. 1990), *B. rapa* (Song et al. 1991; Chyi et al. 1992), and *B. napus* (Landry et al. 1991). Incidentally, the RFLP marker RDA2, corresponding to a tandem repeat, mapped as a single locus at one of such clusters. This same probe also mapped at a cluster of RFLP loci in *B. oleracea* (Cheung and Hubert, unpublished). Further investigation into the location of this tandem repeat on the *B. juncea*, *B. napus*, and *B. oleracea* chromosomes by in situ hybridization will ascertain the association of loci clustering with centromeric regions.

Segregation distortions

Chi-square analyses of each locus for deviation from the expected Mendelian segregation ratio of 1:1 for our DH population ($P < 0.05$) revealed that 27% (97/356) of the RFLP loci showed distorted segregation, which was comparable to the values observed in other *B. napus*, *B. rapa*, and *B. oleracea* DHs and F_2 populations used in genetic mapping (Landry et al. 1991, 1992; Song et al. 1991; Chyi et al. 1992; Cloutier et al. 1995; Uzunova et al. 1995). Twenty-three of the loci showing segregation bias exhibited an excess of J90-2733 alleles, while 74 exhibited an excess of J90-4317 alleles. The location and distribution of these markers with segregation bias are shown in Fig. 1. The majority of the marker loci with an excess of J90-2733 alleles were clustered on LG 3 and LG 13 and unassigned segment A; those with an excess of J90-4317 alleles were clustered on LGs 1, 5, 11, 16, and unassigned segment B. The occurrence of clusters of loci with biased segregation could be influenced by a combination of factors operating in gametic selection during in vitro androgenesis or the subsequent plant regeneration involved in deriving the DH population (Orton and Browers 1985; Guiderdoni et al. 1991; Thompson et al. 1991; Foisset et al. 1993), and could also be a reflection of the genetic distance between the parental lines (Bonierbale et al. 1988; Gebhardt et al. 1989; Havey and Muehlbauer 1989; Landry et al. 1987,1991; McCouch et al. 1988; Slocum et al. 1990; Song et al. 1991; Chyi et al. 1992). In *B. napus*, seven or eight clusters have been observed in different DH populations (Cloutier et al. 1995; Uzunova et al. 1995), and similar number of clusters are observed in *B. juncea*.

Intra-linkage group duplications

Among the 92 probes (40% of the total) that detected 221 duplicated segregating loci (62% of the total segregating loci) through sequence homology, only 15 probes and 33 loci were involved in intra-linkage group duplications. One of the 15 probes (X155) revealed intra-linkage group duplications in two LGs. Such duplications are summarized in Table 3 and shown in Fig. 1. They were found in nine major LGs (LGs 1, 3, 4, 5, 6, 7, 11, 12, 13) and one unassigned segment A (Table 3). Apart from the triplicated loci detected by the probe X72 on LG1, all of the other intra-linkage duplications involved only two duplicated loci. On five occasions with probes X32, X72, X88, X108, and X210, all the segregating loci revealed by each probe were located on the same LG, while in the other 10 cases, there were other additional duplicated loci located elsewhere in the genome apart from the intra-linkage duplications listed in Table 3. The distance between the duplicated loci varied from 0.8 cM to 158.9 cM. Duplications with greater intervals also tended to have a number of other loci located at the intervening distance. Probe X155 detected pairs of duplicated loci on LG 1 and LG 12, but the distances between the duplicated loci, 158.9 cM and 0.9 cM, respectively, varied more than hundredfold in the different LGs. In the case of the former, there were numerous other marker loci located in between the 2 duplicated loci indicating the occurrence of complex rearrangements. Another feature observed among the internal duplications was on LG 13 where the 4.2-cM X88a-X60a segment was duplicated to form the 0.8-cM X88b-X60c interval.

Table 3 *Brassica juncea* RFLP Linkage map: location and interval of intra-linkage group duplications

Linkage group	Duplicated marker loci	Distance apart (cM)	Number of duplicated loci in other linkage groups
1	X72b-X72a-X72c	0.9, 104.5	0
1	X13a-X13xb	0.8	1
1	X155d-X155a	158.9	3
1	X166c-x166a	36.6	1
3	X32b-X32a	0.8	0
4	X33a-X33c	0.8	2
5	X108a-X108b	3.4	0
6	X67a-X67d	25.5	2
7	X140a-X140b	46.5	1
7	X2c-X2a	6.0	1
11	X210a-X210b	14.3	0
11	X15b-X15a	16.7	1
12	X155c-X155e	0.9	3
13	X88a-X88b	5.0	0
13	X60a-X60c	1.6	1
A	X208a-X208b	35.4	1

Inter-linkage group duplications in the *B. juncea* genome

The majority of the probes (78; 34% of the total) that detected duplicated segregating loci were involved in inter-linkage group duplications. Inter-linkage rather than intra-linkage duplications have been found more frequently than expected among the amphidiploids, for example, *B. napus* (Landry et al. 1991; Cloutier et al. 1995) and *B. juncea*. The opposite is true for *B. oleracea* (Landry et al. 1992). All these maps were built from the same cDNA probe source. When genomic DNA probes were used, *B. oleracea*, *B. rapa*, *B. nigra*, and *B. napus* all have shown more inter- than intra-linkage group duplications (Song et al. 1990; Slocum et al. 1990; Chyi et al. 1992; Lagercrantz and Lydiate 1995; Uzunova et al. 1995). There were, however, no simple complete linkage group duplications where the synteny of the loci of a LG was entirely conserved. The overall situation is complex, but this has not been unusual among the *Brassica* species (Song et al. 1990; Slocum et al. 1990; Chyi et al. 1992; Lagercrantz and Lydiate 1995; Uzunova et al. 1995). Seven LGs (LG 6, LG10, LG11, LG 14, LG 15, LG16, LG 17) shared homologous loci with one other LG. Four LGs (LG 2, LG 4, LG 5, and LG 8) had loci duplicated in more than two LGs. The complexity increased with LG 7, LG 9, LG 13, and LG 12, which shared homologous loci with three other LGs. LG 1 and LG 3 represented the most complicated situation, with loci duplicated in nine and six other LGs, respectively. An example of the most extensive (involving the largest number of loci) inter-linkage

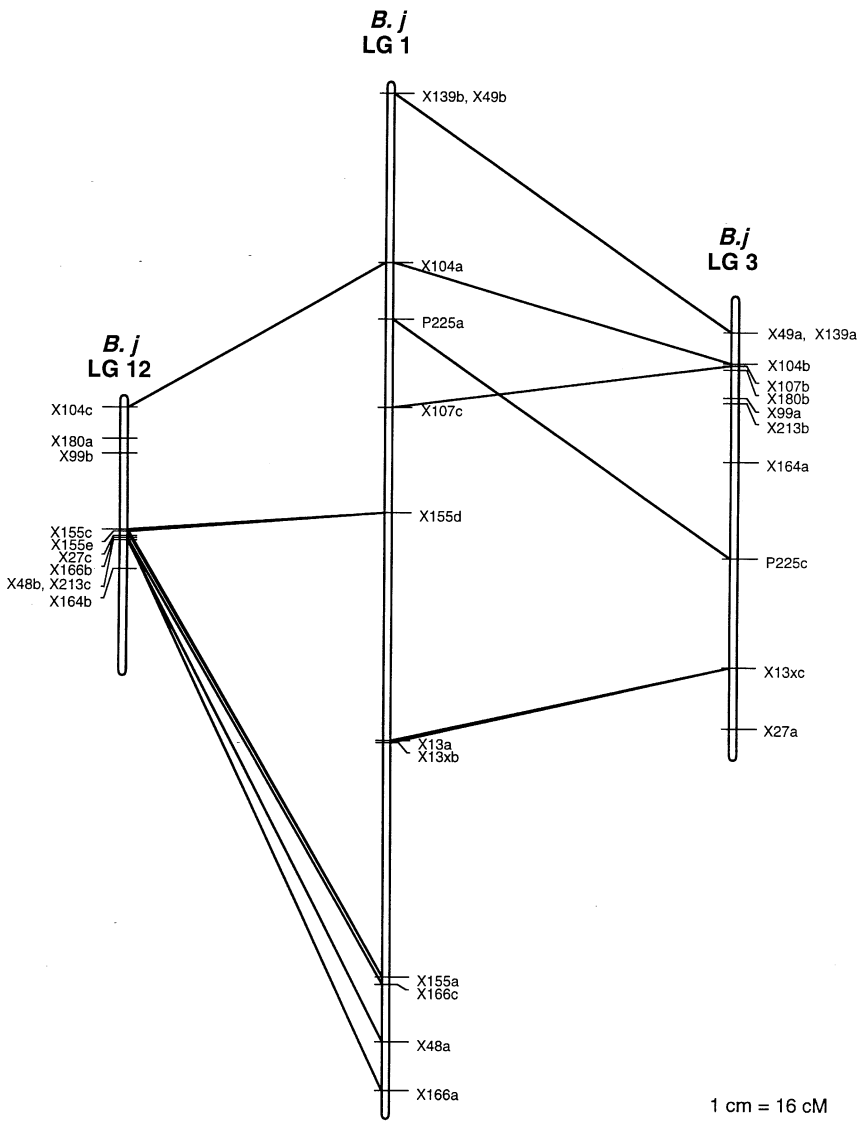


Fig. 2 An example of inter-linkage group duplications found between LG1, LG3, and LG12. Only the names and locations of the relevant duplicated loci are indicated, and these are joined by *solid lines* between the corresponding linkage groups

Table 4 *Brassica juncea* RFLP Linkage map: location and size of inter-linkage group duplications between LG 1, LG 3, and LG 12

Duplication 1			Duplication 2		
Markers	LG	Size (cM)	Marker	LG	Size (cM)
X139b	1	223.4	X139a	3	118.7
X49a			X49a		
X104b			X104b		
P225a			X107b		
X107c			P225c		
X13a			X13xc		
X13xb					
X104a	1	284.0	X104c	12	45.8
X155d			X155c		
X155a			X155e		
X166c			X166b		
X48a			X48b		
X166a					
X104c	12	56.1	X104b	3	129.9
X180a			X180b		
X99b			X99a		
X27c			X213b		
X213c			X164a		
X164b			X27a		

duplications can be seen with the duplication between LG 1, LG 3, and LG 12, as illustrated in Fig. 2, and summarized in Table 4. Among the duplicated regions shown in Fig. 2, most of the loci were in conserved order, but the corresponding distance between adjacent loci were different. There were also different single-copy markers interspersed among the duplicated homologous sequences in these regions. As mentioned earlier, the majority of non-duplicated loci resulted from one segregating locus among additional monomorphic loci; mapped duplications should therefore be regarded as the minimum in the genome concerned. This suggests that many mutations (insertions, deletions as well as point mutations) or rearrangements have accumulated since the initial formation of the allopolyploid species. Similar phenomena have been observed in both diploid and amphidiploid *Brassica* species where molecular linkage maps are available (Song et al. 1990; Slocum et al. 1990; Landry et al. 1991; 1992; Chyi et al. 1992; Lagercrantz and Lydiate 1995; Uzunova et al. 1995.). Duplications of homologous loci in a number of different LGs have been observed in *B. rapa* (Song et al. 1991; Chyi et al. 1992), which is the A-genome progenitor of *B. juncea*. It was therefore not surprising to see the complex duplications in *B. juncea*, where some of the duplications were superimposed on duplications both within and between the A (*B. rapa*) and the B (*B. nigra*) genomes followed by subsequent rearrangements during the evolution of the A and B genomes before and/or after amphidiploid formation. A clearer picture of the origin of different duplications may emerge when the same sets of probes are used to map *B. rapa* and *B. nigra*.

Homology between the *B. juncea* and the *B. napus* maps

B. juncea (genome AABB) and the economically important *B. napus* (genome AACC) are both amphidiploids that have a complement of ten pairs of chromosomes originating from the A-genome progenitor. Since in our laboratory, RFLP mapping of *B. napus* was carried out with the same set of RFLP probes (Cheung et al. 1997), it would be interesting to compare the two maps to reveal the extent of homologies between the LGs of the two amphidiploids. A summary of the results is presented here without the details of the comparison, which will be discussed elsewhere when we would compare the maps of *B. rapa* (genome AA), *B. napus* (genome AACC), and *B. juncea* (genome AABB) to address the extent of conservation of the A-genome in the two amphidiploids with respect to the A-genome progenitor.

Out of 230 RFLP probes used for the genetic mapping of *B. juncea* and the 186 RFLP probes used for *B. napus*, there were 110 common probes able to detect polymorphisms in both species. These probes revealed 179 and 175 marker loci in *B. juncea* and *B. napus*, respectively. On the basis of these marker loci revealed by the common RFLP probes, the two maps were compared. The results are illustrated in Fig. 3 where the common markers and the regions of the *B. juncea* genome showing homologies to the *B. napus* genome are indicated. Overall, there were 10 LGs revealing significant homologies to the *B. napus* LGs, suggesting the possibilities that these 10 LGs of *B. juncea* might correspond to the ten chromosomes from the A-genome. LG3 and LG11 of *B. juncea* showed the most extensive homologies between the *B. juncea* and the *B. napus* maps, which covered in each case almost the entire LG and had the most common markers. The regions of homology in the other eight LGs of *B. juncea* were less extensive and had fewer common markers. The overall synteny at the homologous area was well-maintained with usually one inversion or a small translocation. However, as discussed earlier, there are inter-linkage group duplications in the *B. juncea* and *B. napus* maps which might complicate the comparisons. Our results indeed provided evidence for such complications. With three *B. juncea* LGs, LG1, LG3, and LG12, each LG simultaneously showed homologies to two different *B. napus* LGs. There are also cases, for example, LG5 and LG12 of *B. juncea* in which two LGs showed homologies to the same region of the *B. napus* genome. Therefore, until extensive comparisons between the genomes of the A-genome and B-genome progenitors and these two amphidiploid species have been conducted, the origin of each LG in the *B. juncea* genome cannot be conclusively ascertained.

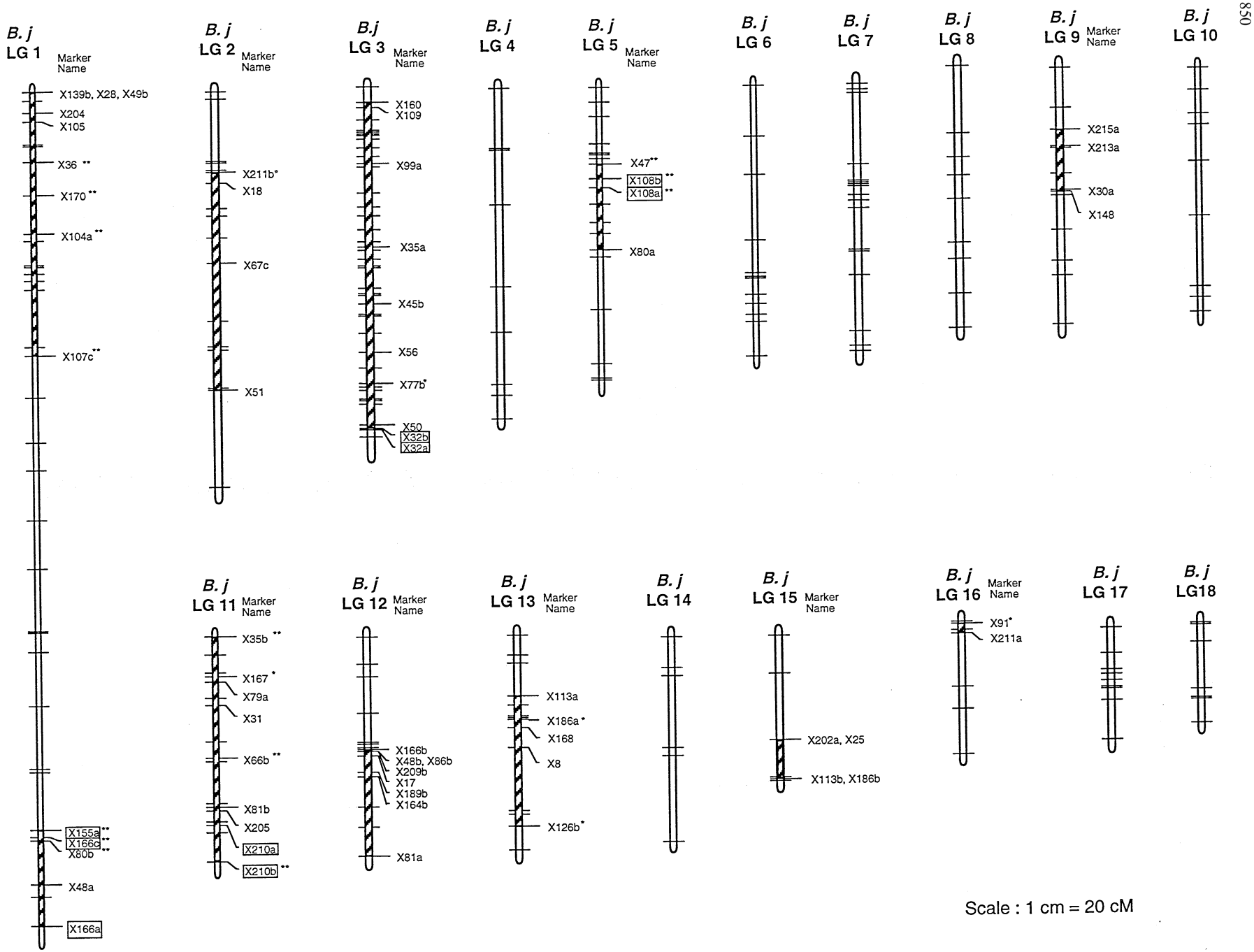

B. j LG 1
Marker Name
X139b, X28, X49b
X204
X105
X36 **
X170 **
X104a **
X107c **
X155a **
X166c **
X80b **
X48a
X166a
B. j LG 2
Marker Name
X211b*
X18
X67c
X51
B.j LG 3
Marker Name
X160
X109
X99a
X35a
X45b
X56
X77b*
X50
X32b
X32a
B. j LG 4
B. j LG 5
Marker Name
X47**
X108b **
X108a **
X80a
B. j LG 6
B. j LG 7
B. j LG 8
B. j LG 9
Marker Name
X215a
X213a
X30a
X148
B. j LG 10
B. j LG 11
Marker Name
X35b **
X167 *
X79a
X31
X66b **
X81b
X205
X210a
X210b **
B. j LG 12
Marker Name
X166b
X48b, X86b
X209b
X17
X189b
X164b
X81a
B. j LG 13
Marker Name
X113a
X186a *
X168
X8
X126b*
B. j LG 14
B. j LG 15
Marker Name
X202a, X25
X113b, X186b
B. j LG 16
Marker Name
X91*
X211a
B. j LG 17
B. j LG18
Scale : 1 cm = 20 cM

Fig. 3 Regions of the *B. juncea* (*B.j.*) genome homologous to the *B. napus* genome.The major regions in the *B. juncea* genome that are homologous to the *B. napus* genome are marked by ▨. Only the names of the relevant common loci are indicated. See Fig. 1 for clarification of symbols

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