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Comparison of the genetic maps of *Brassica napus* and *Brassica oleracea*

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Abstract The genus *Brassica* consists of several hundreds of diploid and amphidiploid species. Most of the diploid species have eight, nine or ten pairs of chromosomes, known respectively as the B, C, and A genomes. Genetic maps were constructed for both *B. napus* and *B. oleracea* using mostly RFLP and RAPD markers. For the *B. napus* linkage map, 274 RFLPs, 66 RAPDs, and two STS loci were arranged in 19 major linkage groups and ten smaller unassigned segments, covering a genetic distance of 2125 cM. A genetic map of *B. oleracea* was constructed using the same set of RFLP probes and RAPD primers. The *B. oleracea* map consisted of 270 RFLPs, 31 RAPDs, one STS, three SCARs, one phenotypic and four isozyme marker loci, arranged into nine major linkage groups and four smaller unassigned segments, covering a genetic distance of 1606 cM. Comparison of the *B. napus* and *B. oleracea* linkage maps showed that eight out of nine *B. oleracea* linkage groups were conserved in the *B. napus* map. There were also regions in the *B. oleracea* map showing homoeologies with more than one linkage group in the *B. napus* map. These results provided molecular evidence for *B. oleracea*, or a closely related $2n = 18$ *Brassica* species, as the C-genome progenitor, and also reflected on the homoeology between the A and C genomes in *B. napus*.

Key words *Brassica napus* · *Brassica oleracea* · Genetic maps · RFLP · RAPD

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Introduction

The genus *Brassica* includes a diversity of economically important cultivated crops as well as a large number of wild species. It comprises both diploid and amphidiploid species. *B. oleracea* ($2n = 2x = 18$, genome CC), a diploid species, and *B. napus* ($2n = 2x = 38$, genome AACC), an amphidiploid, are among the most genetically studied species in this family. *B. oleracea* exhibits a wide variety of horticultural morphotypes; for example, cabbage, broccoli, cauliflower, kale, Brussel sprouts and Kohlrabi. *B. napus* is best known as oilseed rape and, to a lesser extent, also as the horticultural crop, rutabaga.

Molecular linkage maps based on RFLPs have been developed 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), *B. juncea* (Cheung et al. 1997) and *B. napus* (Landry et al. 1991; Ferreira et al. 1994; Uzunova et al. 1995). Comparative mapping has proven a useful tool for studying genome evolution in other species (Bonierbale et al. 1988; Hulbert et al. 1990; Ahn and Tanksley 1993; Ahn et al. 1993; Van Deynze et al. 1995). Comparisons of the linkage maps of *Brassica* species have been carried out for *B. oleracea* and *B. rapa* (McGrath and Quiros 1991), for *B. rapa* with *B. oleracea* and *B. napus* (Teutonico and Osborn 1994), and for synthetic *B. napus* with *B. oleracea* and *B. rapa* (Lydiate et al. 1993). Based on interspecific hybridization and cytogenetic data, *B. oleracea* and *B. rapa* were proposed as the progenitors of the amphidiploid *B. napus* (U 1935). Since molecular genetic mapping using RFLP and RAPD markers have been carried out on *B. oleracea* and *B. napus* in our laboratory, we have

the opportunity to examine the genome organization of these two species, and to substantiate the above hypothesis. The *B. oleracea* map used for this study was an updated, extended version of the map published by Landry et al. (1992), based on a F_2 population from a cross between a rapid cycling *Brassica* (CrGC-85) and a cabbage line (86-16-5), with 98 additional RFLPs, 31 RAPDs, one STS, three SCARs and four isozyme markers. The *B. napus* map was constructed using the same sets of RFLP probes and RAPD primers employed for the *B. oleracea* map. Genetic mapping was based on a segregating F_1-derived doubled-haploid (DH) progeny from a *B. napus* cross between a Winter (90-DHW-1855-4) and a Spring (87-DHS-002) breeding line. We report here the detailed characteristics of the linkage maps of *B. oleracea* and *B. napus* based on expressed DNA sequences and RAPD markers, and present a comparison of the genomes of these two species as revealed by these molecular markers.

Materials and methods

Plant materials

Seeds of the two *B. napus* parental lines (87-DHS-002 and 90-DHW-1855-4) and the mapping population of 95 F_1-derived DHs from the Cross 90-DHW-1855-4 × 87-DHS-002 were obtained from Pioneer Hi-Bred Production Ltd., Georgetown Research Station, Canada. The source of the *B. oleracea* parental lines (CrGC-85 and 86-16-5) and the F_2 mapping population used was as described previously (Landry et al. 1992). All plants were grown in the greenhouse at 18–24°C with a photoperiod of 14–18 h. For each parental, DH, or F_2 line, tissues from four plants were pooled for DNA extraction.

Isolation of genomic DNA

Plant genomic DNA used for RFLP analyses was isolated as described previously by Landry et al. (1991) for all the *B. oleracea* samples, and for the *B. napus* samples with one modification: the replacement of the purification by CsCl gradient with two successive chloroform/isoamyl-alcohol (24 : 1, v/v) extractions. The genomic DNA samples used for RAPD and specific PCR analyses were isolated from single leaf discs taken at the 2–4 leaf stage using the DNA micro-extraction method as described previously by Cheung et al. (1993).

Source of probes for RFLP mapping

The RFLP markers mapped in Figs. 1 and 2 were obtained with polymorphic probes which had been tested on the parental lines of each cross. These probes were selected from 341 random cDNA clones from a *B. napus* cDNA library (Harada et al. 1988) or from C.Quiros (University of California, Davis, USA), and as well as from *B. oleracea* genomic DNA (gDNA) probes obtained by representational difference analysis (RDA) (Landry et al., unpublished). Markers detected by the probes from C.Quiros were labelled with the prefix "pX" and the gDNA probes were identified by the prefix "DP" (Figs. 1 and 2). The cDNA inserts were isolated from *Pst*I digests of the clones by agarose-gel electrophoresis, and were purified using the Sephaglas™ BandPrep Kit (Pharmacia). The "DP" probes were isolated from *Eco*RI digests of the clones and further purified as for the cDNA inserts.

Southern-blot hybridizations

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

RAPD, STS and SCAR analyses

The RAPD markers labelled with the prefix "OP-" in Figs. 1 and 2 were obtained from screening 140 RAPD primers (Operon 10-mers, sets A to G) against the parental lines for polymorphisms, and subsequently mapped using the segregating populations onto the linkage groups shown in Figs. 1 and 2.

For each RAPD reaction, 25 ng of DNA (or 1/50 of the DNA sample from the micro-extraction of a single 5-mm leaf disc) was used in 25 μl of a reaction mixture containing 10 mM Tris-HCl (pH 8.2), 50 mM KCl, 2.5 mM $MgCl_2$, 200 μM of each dNTP (Pharmacia) with 0.2 μM of primer, and 0.5 U of Ampli*Taq*™ DNA polymerase (Perkin-Elmer Cetus). Amplification was carried out in a Hybaid thermal reactor programmed as follows: 30s at 94°C, and 1s at 42°C for one cycle; 1s at 50°C, 45s at 72°C, 5s at 94°C, 30s at 42°C for 45 cycles followed by 7 min at 72°C. The amplified products were analysed on a 1.4% agarose gel as described previously (Cheung et al. 1993).

The STS for self-incompatibility (SI) (labelled SIEFa and SIEFb in Fig. 1 and labelled STS-EF in Fig. 2) was obtained by a set of two *S*-locus-specific primers (CAGCATCTACTCGAGATTGAC and AAA(A/C/G)CCATCTCCACTGCAGCT; primers E and F, Brace et al. 1993) for *SLG* of *B. oleracea*. The reaction was carried out with 100 ng of DNA in 25 μl of the same reaction mixture described above for RAPD reactions, except for the addition of 1 U of Ampli*Taq*™ DNA polymerase. Amplification was carried out with a 'hot start' program as follows: 30 s at 94°C and 10 s at 58°C for one cycle, and the temperature was held at 58°C for the addition of Ampli*Taq*™ DNA polymerase; 1.5 min at 72°C, 20 s at 94°C, 2 min at 58°C for 30 cycles followed by 5 min at 72°C. The amplified products were digested with 1 U of the restriction endonuclease *Dde*I for 2 h, and then analysed on a 3% Nusieve 3 : 1 agarose gel.

The SCAR-G2 on the *B. oleracea* map (Fig. 2) was obtained with a set of two specific primers (CACGAAACCAAACTGAAG and AGCTCAATGCCGATGGTA). The reaction was carried out with 100 ng of DNA in 25 μl of the same reaction mixture described above for RAPD reactions, except for the addition of 1 U of Ampli *Taq*™ DNA polymerase.

Amplification was carried out with a 'hot start' program as follows: 30 s at 94°C and 10 s at 62°C for one cycle, and the temperature was held at 62°C for the addition of Ampli*Taq*™ DNA polymerase; 30 s at 72°C, 5 s at 94°C, 10 s at 46°C for 30 cycles followed by 5 min at 72°C. The amplified products were analysed on a 1.4% agarose gel. The polymorphic amplified fragment corresponding to the SCAR-G2 allele of *B. oleracea* 86-16-5 was used as a RFLP probe for detecting the marker locus G2-86 in the *B. napus* linkage map shown in Fig. 1, and the marker locus SCAR-G2c in the *B. oleracea* map shown in Fig. 2. Similarly, the polymorphic amplified fragment corresponding to the SCAR-G2 allele of *B. oleracea*

CrGC-85 when used as a RFLP probe revealed two marker loci, SCAR-G2a and SCAR-G2b, in *B. oleracea* (Fig. 2).

Morphological and isozyme markers in *B. oleracea*

LMOR, the marker for leaf morphology, was scored as described by Landry et al. (1992). The isozyme markers for triose-phosphate isomerase (TPI-1 and TPI-2), for phosphoglucose mutase (PGM-2), and for phosphoglucose isomerase (PGI-2), were analysed as described by Chevre et al. (1996).

Segregation and linkage analyses

Polymorphic cDNA probes were identified by differences in the banding patterns between the parental lines on autoradiographs. Segregation analysis was done by hybridizing each of the selected probes to the DNA samples from individuals of the mapping populations digested with the selected restriction enzyme that displayed the maximum degree of polymorphism. Similarly, RAPD primers, the SI-specific primers E and F that revealed polymorphic amplification profiles between the SI and self-compatible lines, and the SCAR-G2 primers, were used for amplification involving the whole segregating populations.

Multipoint linkage analysis of the marker loci was performed with the computer program MAPMAKER Macintosh V2.0 (Dupont). RFLPs, RAPDs, the SI-specific STS, the SCAR-G2, phenotypic and isozyme markers were assigned to linkage groups. In *B. napus*, the linkage criteria used were a LOD score of 5.0 and a maximum RF threshold of 0.4. With *B. oleracea*, the linkage criteria were a LOD score of 4.5 and a maximum RF of 0.3. All map distances were expressed in Kosambi cM.

Results and discussion

The *B. napus* map

A total of 186 RFLP probes used for mapping provided 290 segregating marker loci in the DH mapping population, which corresponded to 1.6 loci/probe. A similar efficiency of the polymorphic probes in detecting segregating RFLP loci has been observed with other *B. napus* crosses (Landry et al. 1991; Ferreira et al. 1994; Cloutier et al. 1995; Uzunova et al. 1995), as well as in another amphidiploid species, *B. juncea* (Cheung et al. 1997).

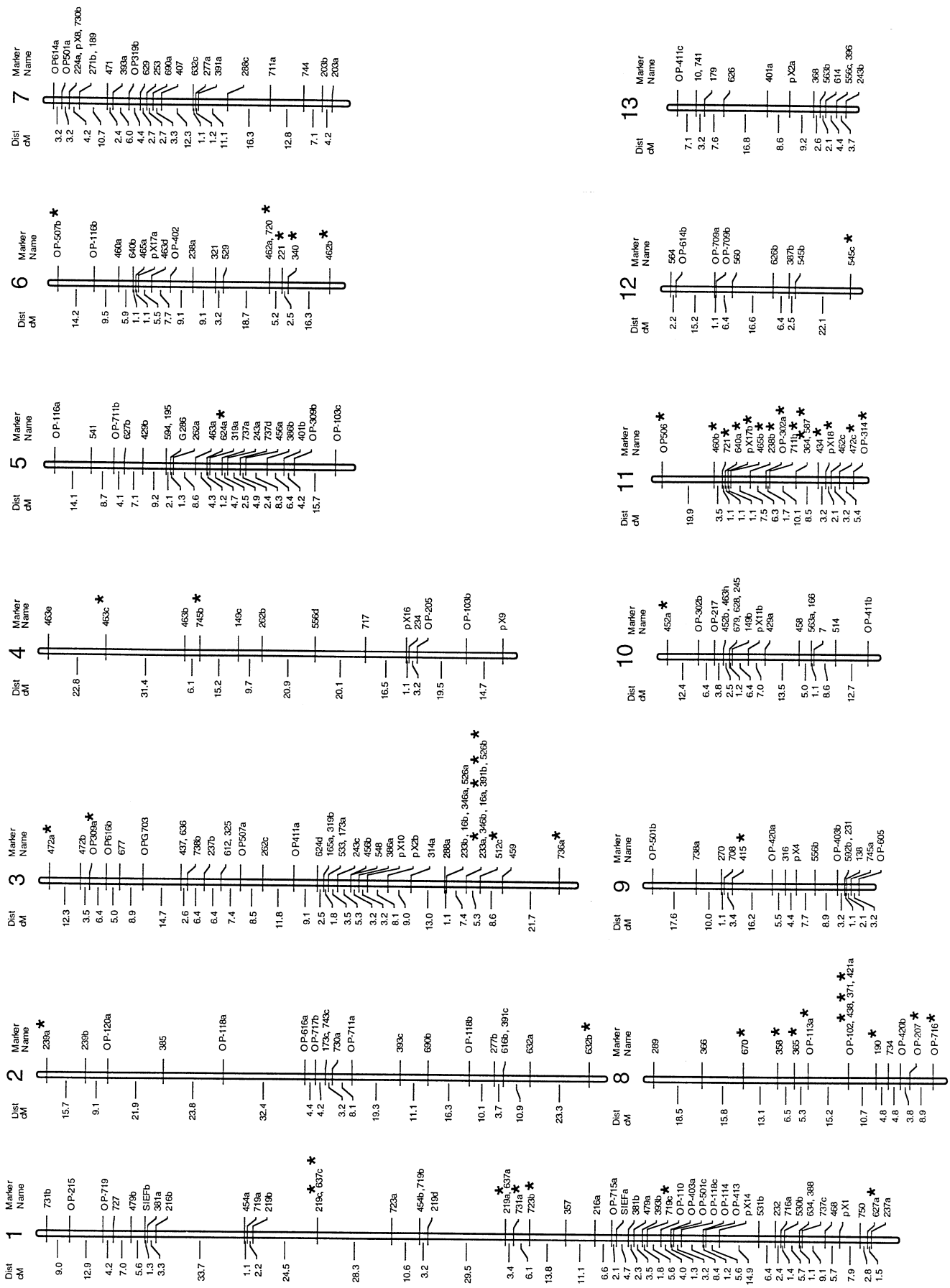
Fortytwo RAPD primers producing reproducible and easily scored polymorphisms were chosen out of 140 primers for segregation analyses. Seventy loci were obtained which corresponded to 1.7 loci/primer. This result indicated that polymorphic RAPD primers were as efficient as RFLP probes in the detection of reproducible segregating loci under our experimental conditions.

Based on the frequency of recombination in the DH mapping population, 251 RFLPs, 62 RAPDs and two *SLG*-specific STS loci were arranged into 19 linkage groups (LGs). The LGs were numbered 1 to 19 in descending order of their lengths in Kosambi cM, covering a genetic distance of 1954.7 cM, corresponding to 19 basic chromosomes of the haploid *B. napus* genome (Fig. 1). Each of the LGs had at least four loci and a total genetic distance greater than 20 cM. In addition, there were 27 loci (23 RFLP and 4 RAPD loci) located on ten smaller unassigned segments (A to J), each with less than four loci except for A which had four (Fig. 1). These segments spanned an additional genetic distance of 170.2 cM. Thus, the total map distance including these unassigned segments was 2124.9 cM with 342 loci. Twenty marker loci (16 RFLP and four RAPD loci) could not be linked significantly to these LGs on the map. The size, number of markers, and the average marker interval of each LG are summarized in Table 1. The sizes of the LGs varied substantially from 319.8 cM for LG 1 to 21.4 cM for the smallest group, LG 19. The number of loci located in each LG also differed greatly from 48 for LG 1 to four for LG 18. Although the average marker intervals also varied more than four-fold between the extreme values, all LGs except two had average marker intervals less than 10 cM. LG15 was the most densely populated LG with an average marker interval of 3 cM. The overall average marker interval for the map was 6.3 cM. This is by far the most densely populated published *B. napus* linkage map incorporating both RAPD and RFLP markers.

Segregation distortion was observed in 22% (10% biased towards 87-DHS-002 alleles; 12% biased towards 90-DHW-1855-4 alleles) of the segregating loci, which was within the average range observed in other *B. napus* crosses (Landry et al. 1991; Ferreira et al. 1994; Cloutier et al. 1995; Uzunova et al. 1995) as well as in *B. juncea* (Cheung et al. 1997). Clusters of loci with biased segregation were found in LGs 1, 3, 6, 8, 11, 17 and 18, and unassigned segments A, D and F (Fig. 1). Apart from LG1 and LG3, where there were clusters of loci with distorted segregation towards either parents, LG6, LG8 and LG17 showed segregation biased entirely towards 87-DHS-002 alleles; while LG 11, LG18 and unassigned segments A, D, F showed segregation bias towards 90-DHW-1844-5 alleles.

The *B. oleracea* map

A total of 218 RFLP probes used for segregation analyses provided 295 segregating RFLP loci in the F_2 mapping population, which corresponded to 1.4 loci/probe. The efficiency of the polymorphic probe in detecting RFLP loci was slightly lower than those observed with *B. napus* and *B. juncea* (Cheung et al. 1997). Only 18 RAPD primers were used for segregation analyses producing 34 segregating loci, which corresponded to 1.9 loci/primer. This, once again, supported the fact that polymorphic RAPD primers, once carefully selected, were as if not more efficient as RFLP probes in detecting reliable segregating loci.


1
Dist cM
Marker Name
731b
OP-215
OP-719
727
479b
SIEFb
381a
216b
454a
719a
219b
219c, 637c
723a
454b, 719b
219d
219a, 637a
731a
723b
357
216a
OP-715a
SIEFa
381b
479a
393b
719c
OP-110
OP-403a
OP-501c
OP-118c
OP-114
OP-413
p.X14
531b
232
716a
530b
634, 388
737c
468
p.X1
750
627a
237a
2
Dist cM
Marker Name
239a
239b
OP-120a
385
OP-118a
OP-616a
OP-717b
173c, 743c
730a
OP-711a
393c
690b
OP-118b
277b
616b, 391c
632a
632b
3
Dist cM
Marker Name
472a
472b
OP309a
OP616b
677
OPG703
437, 636
738b
237b
612, 325
OP507a
262c
OP411a
624d
168a, 319b
533, 173a
243c
456b
548
386a
pX10
pX2b
314a
288a
233b, 16b, 346a, 526a
233a, 346b, 16a, 391b, 526b
512c
459
736a
4
Dist cM
Marker Name
463e
463c
463b
745b
149c
262b
556d
717
pX16
234
OP-205
OP-103b
pX9
5
Dist cM
Marker Name
OP-116a
541
OP-711b
627b
426b
594, 195
G286
262a
463a
624a
319a
737a
243a
737d
456a
386b
401b
OP-309b
OP-103c
6
Dist cM
Marker Name
OP-507b
OP-116b
460a
640b
465a
pX17a
463d
OP-402
238a
321
529
462a, 720
221
340
462b
7
Dist cM
Marker Name
OP614a
OP501a
224a, pX8, 730b
271b, 189
471
393a
OP319b
629
253
690a
407
632c
277a
391a
288c
711a
744
203b
203a
8
Dist cM
Marker Name
289
366
670
358
365
OP-113a
OP-102, 438, 371, 421a
190
734
OP-420b
OP-207
OP-716
9
Dist cM
Marker Name
OP-501b
738a
270
708
415
OP-420a
316
pX4
556b
OP-403b
592b, 231
138
745a
OP-605
10
Dist cM
Marker Name
452a
OP-302b
OP-217
452b, 463h
679, 628, 245
149b
pX11b
429a
458
563a, 166
7
514
OP-411b
11
Dist cM
Marker Name
OP506
460b
721
640a
pX17b
465b
236b
OP-302a
711b
387
364
434
pX18
462c
472c
OP-314
12
Dist cM
Marker Name
564
OP-614b
OP-709a
OP-709b
560
626b
387b
545b
545c
13
Dist cM
Marker Name
OP-411c
10, 741
179
626
401a
pX2a
368
563b
614
556c, 396
243b

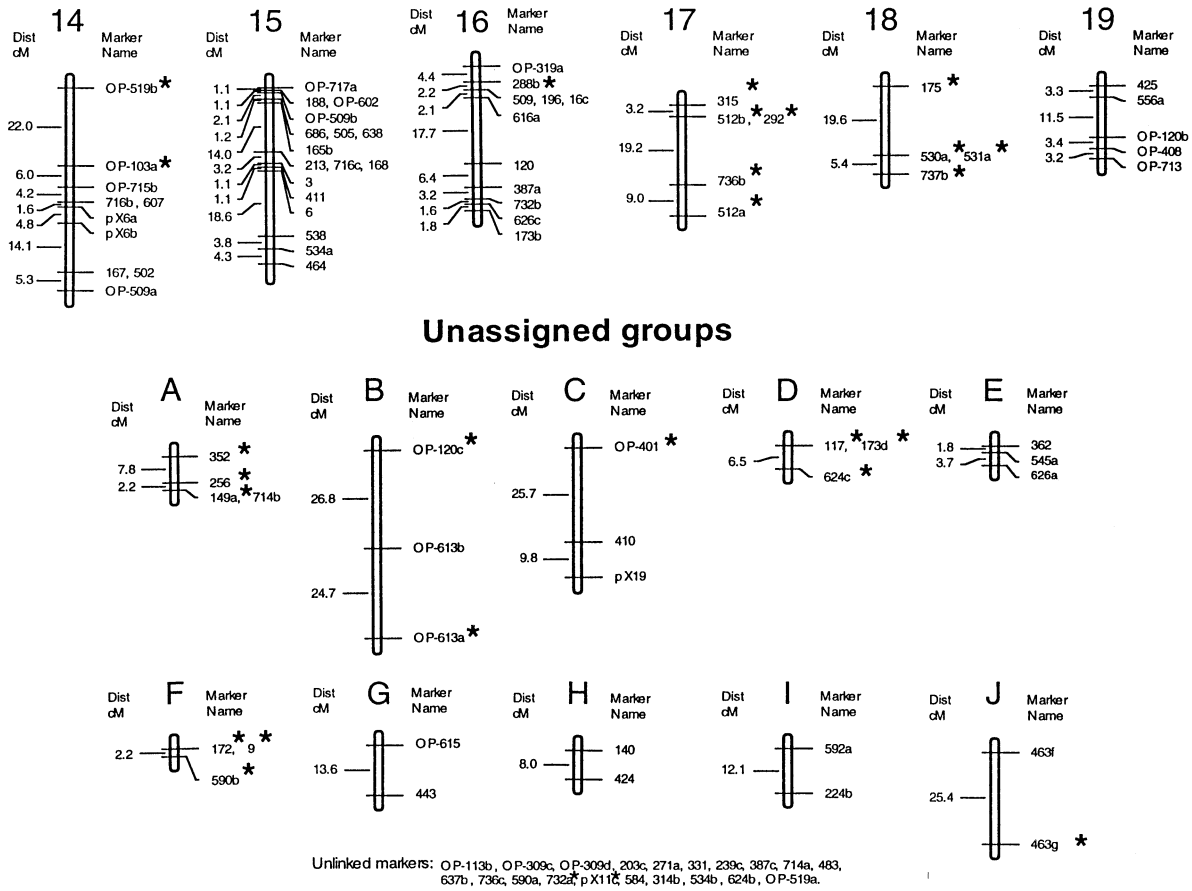
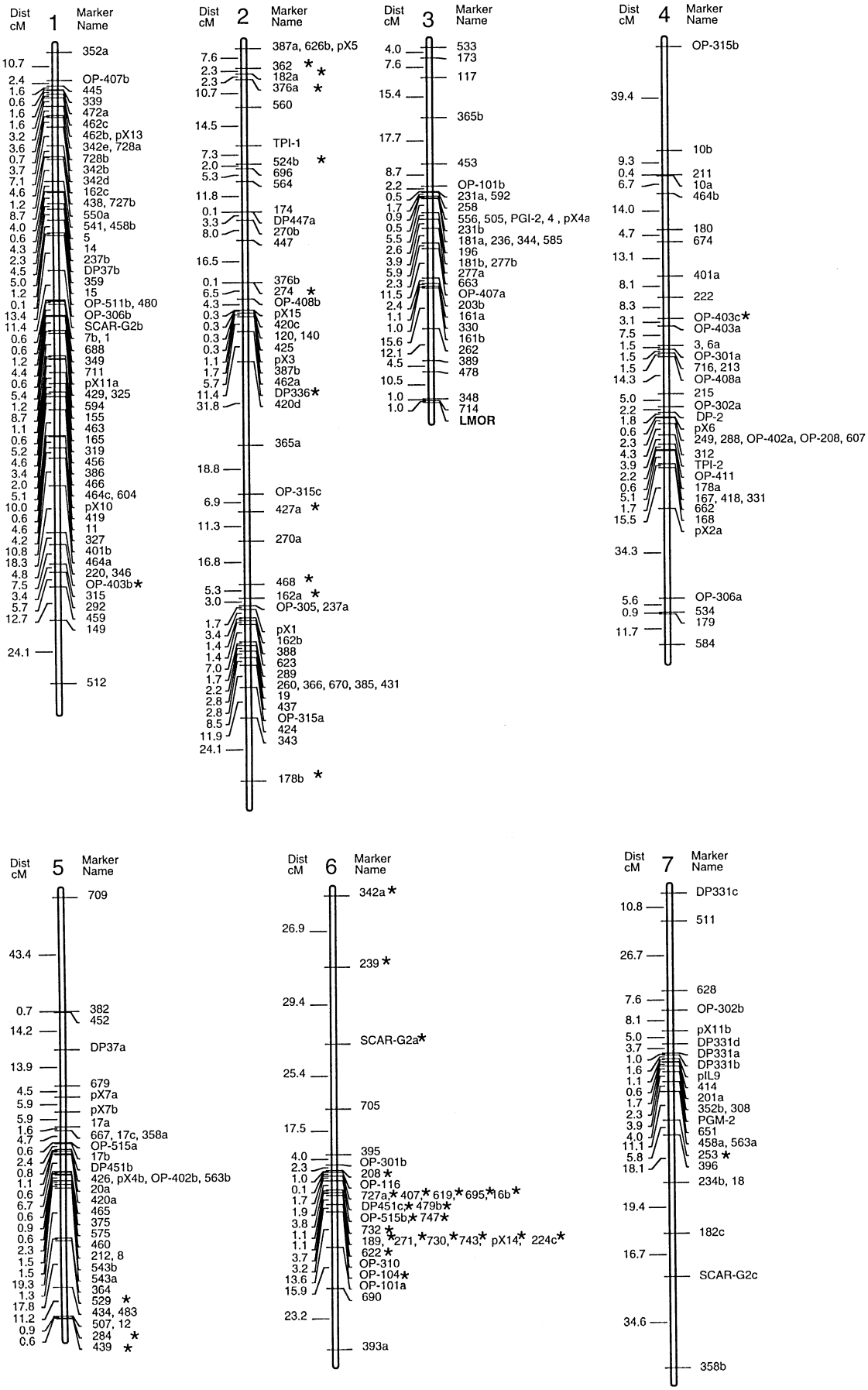


Fig. 1 Linkage map of *B. napus*. Linkage-group numbers are indicated above the linkage groups. Recombination distances between markers are in Kosambi centi-Morgans (*left*). RFLP loci are numbered after the codes of the DNA probes, and RAPD loci are named with the prefix "*OP*" and the code of the primer that detected the loci (*right*). Duplicated loci detected by the same probe or primer are labelled with a different *lower case suffix* following the name of the probe or primer. SIEFa and SIEFb are markers for the *S*-loci. The 342 loci are assembled into 19 major groups assigned arbitrarily as group 1 to 19, and ten smaller segments are labelled as unassigned segments A to J. Twenty loci (16 RFLPs and 4 RAPDs) remained unlinked to any other markers, and are listed at the bottom of the figure. Marker loci showing segregation distortion are indicated with *

Based on the frequency of recombination in the F_2 mapping population, 257 RFLP, 31 RAPD, three SCARs, one SLG-specific STS, one phenotypic and four isozyme markers were arranged into nine LGs. The LGs were numbered from 1 to 9 corresponding to the numbering system used in the earlier published version (Landry et al.1992). The total genetic distance covered by the nine LGs was 1546 cM (Fig. 2). Each of the LGs had at least four loci. In addition, there were 13 RFLP loci located on four smaller unassigned segments (A to D, each with two to four loci) (Fig. 2), covering an extra distance of 59.9 cM. Therefore the total map distance including these unassigned segments was 1606 cM with 310 marker loci. Twenty-seven marker loci (24 RFLPs and three RAPDs) remained unlinked to the map. The size, number of markers, and the average marker interval of each LG are summarized in Table 2. The sizes of the LGs in the *B. oleracea* map, like their counterparts in *B. napus*, also varied greatly from 286.5 cM for LG 2 to 34.6 cM for LG9. The number of loci in each LG also differed greatly from 4 for LG9 to 61 for LG 1. The average marker intervals varied just over two-fold between the extremes, but all LGs had average marker intervals less than 10 cM. LG3 was the most densely populated LG with an average marker interval of 4 cM. The overall average marker interval for the map was 5.7 cM. Compared to the other published *B. oleracea* maps, this map covered the greatest distance, and with more markers, than did the other maps.

Segregation distortion was observed only among 15% of the segregating loci, which was lower than observed with *B. napus*, but comparable to observations in other *B. oleracea* crosses (Kianian and Quiros 1992). A majority of the biased loci were found on LG2, LG5, LG6 and the unassigned segment A (Fig. 2). LG6 showed the most prominent cluster of loci with segregation biased towards alleles of CrGC-85.


Dist cM
1
Marker Name
352a
10.7
OP-407b
2.4
445
1.6
339
0.6
472a
1.6
462c
1.6
462b, pX13
3.2
342e, 728a
3.6
728b
0.7
342b
3.7
342d
7.1
162c
4.6
438, 727b
1.2
550a
8.7
541, 458b
4.0
5
0.6
14
4.3
237b
2.3
DP37b
4.5
359
5.0
15
1.2
OP-511b, 480
0.1
OP-306b
13.4
SCAR-G2b
11.4
7b, 1
0.6
688
0.6
349
1.2
711
4.4
pX11a
0.6
429, 325
5.4
594
1.2
155
8.7
463
1.1
165
0.6
319
5.2
456
4.6
386
3.4
466
2.0
464c, 604
5.1
pX10
10.0
419
0.6
11
4.6
327
4.2
401b
10.8
464a
18.3
220, 346
4.8
OP-403b*
7.5
315
3.4
292
5.7
459
12.7
149
24.1
512
Dist cM
2
Marker Name
387a, 626b, pX5
7.6
362 * *
2.3
182a
2.3
376a *
10.7
560
14.5
TPI-1
7.3
524b *
2.0
696
5.3
564
11.8
174
0.1
DP447a
3.3
270b
8.0
447
16.5
376b
0.1
274 *
6.5
OP-408b
4.3
pX15
0.3
420c
0.3
120, 140
0.3
425
0.3
pX3
1.1
387b
1.7
462a
5.7
DP336*
11.4
420d
31.8
365a
18.8
OP-315c
6.9
427a *
11.3
270a
16.8
468 *
5.3
162a *
3.0
OP-305, 237a
1.7
pX1
3.4
162b
1.4
388
1.4
623
7.0
289
1.7
260, 366, 670, 385, 431
2.2
19
2.8
437
2.8
OP-315a
8.5
424
11.9
343
24.1
178b *
Dist cM
3
Marker Name
4.0
533
7.6
173
117
15.4
365b
17.7
453
8.7
OP-101b
2.2
231a, 592
0.5
258
1.7
556, 505, PGI-2, 4 , pX4a
0.9
231b
0.5
181a, 236, 344, 585
5.5
196
2.6
181b, 277b
3.9
277a
5.9
663
2.3
OP-407a
11.5
203b
2.4
161a
1.1
330
1.0
161b
15.6
262
12.1
389
4.5
478
10.5
348
1.0
714
1.0
LMOR
Dist cM
4
Marker Name
OP-315b
39.4
10b
9.3
211
0.4
10a
6.7
464b
14.0
180
4.7
674
13.1
401a
8.1
222
8.3
OP-403c*
3.1
OP-403a
7.5
3, 6a
1.5
OP-301a
1.5
716, 213
1.5
OP-408a
14.3
215
5.0
OP-302a
2.2
DP-2
1.8
pX6
0.6
249, 288, OP-402a, OP-208, 607
2.3
312
4.3
TPI-2
3.9
OP-411
2.2
178a
0.6
167, 418, 331
5.1
662'
1.7
168
15.5
pX2a
34.3
OP-306a
5.6
534
0.9
179
11.7
584
Dist cM
5
Marker Name
709
43.4
382
0.7
452
14.2
DP37a
13.9
679
4.5
pX7a
5.9
pX7b
5.9
17a
1.6
667, 17c, 358a
4.7
OP-515a
0.6
17b
2.4
DP451b
0.8
426, pX4b, OP-402b, 563b
1.1
20a
0.6
420a
6.7
465
0.6
375
0.9
575
0.6
460
2.3
212, 8
1.5
543b
1.5
543a
19.3
364
1.3
529 *
17.8
434, 483
11.2
507, 12
0.9
284 *
0.6
439 *
Dist cM
6
Marker Name
342a*
26.9
239*
29.4
SCAR-G2a*
25.4
705
17.5
395
4.0
OP-301b
2.3
208*
1.0
OP-116
0.1
727a,* 407,* 619,* 695,* 16b*
1.7
DP451c,* 479b*
1.9
OP-515b,* 747*
3.8
732 *
1.1
189, *271, *730, *743,* pX14,* 224c*
1.1
622*
3.7
OP-310
3.2
OP-104*
13.6
OP-101a
15.9
690
23.2
393a
Dist cM
7
Marker Name
DP331c
10.8
511
26.7
628
7.6
OP-302b
8.1
pX11b
5.0
DP331d
3.7
DP331a
1.0
DP331b
1.6
pIL9
1.1
414
0.6
201a
1.7
352b, 308
2.3
PGM-2
3.9
651
4.0
458a, 563a
11.1
253 *
5.8
396
18.1
234b, 18
19.4
182c
16.7
SCAR-G2c
34.6
358b

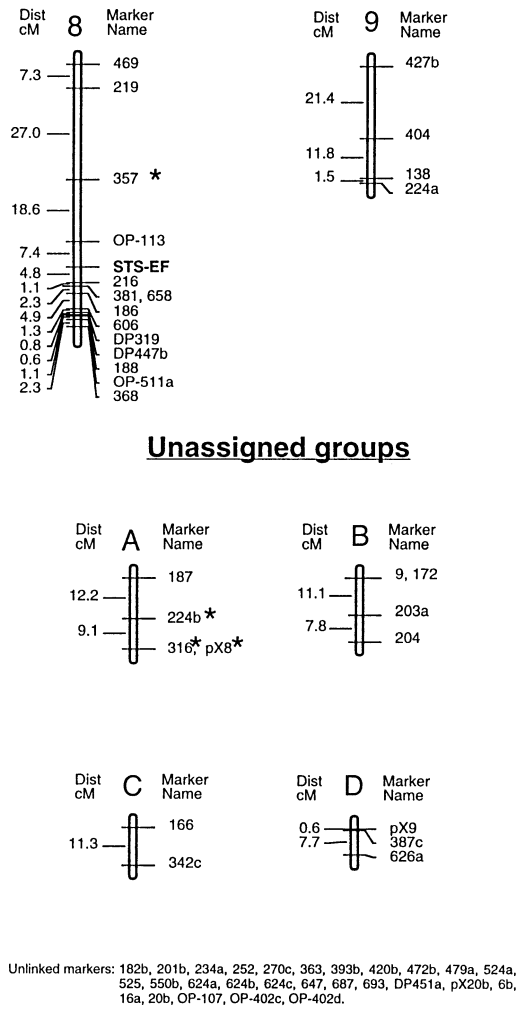


Fig. 2 Linkage map of *B. oleracea*. Linkage-group numbers are indicated above the linkage groups. Recombination distances between markers are in Kosambi centi-Morgans (*left*). RFLP loci are numbered after the codes of the DNA probes, and RAPD loci are named with the prefix "*OP*" and the code of the primer that detected the loci (*right*). Duplicated loci detected by the same probe or primer are labelled with a different *lower case suffix* following the name of the probe or primer. *LMOR* is the marker locus controlling leaf morphology in *B. oleracea*. *TPI-1*, *TPI-2*, *PGI-2* and *PGM-2* are isozyme markers. *STS-EF* is the marker for the *S*-locus in *B. oleracea*, and SCAR markers are labelled with the prefix "*SCAR*" The 310 loci are assembled into nine major groups, assigned groups 1 to 9 as numbered in the earlier published version (Landry et al. 1992), and four smaller segments are labelled as unassigned *segments A to D*. Twenty-seven loci (24 RFLPs and 3 RAPDs) remained unlinked to any other marker, and are listed at the bottom of the Figure. Marker loci showing segregation distortion are marked with ∗

Comparison of the *B. napus* map with the *B. oleracea* map.

Out of the 186 and 218 RFLP probes used, respectively, for the genetic mapping of *B. napus* and *B. oleracea* in this study, there were 117 common probes able to detect polymorphisms in both species. These probes revealed 154 and 190 marker loci, respectively, in the *B. oleracea* and the *B. napus* maps. Seven RAPD primers among the 18 used in *B. oleracea*, and the 42 used in *B. napus* mapping, were common. These seven RAPD primers revealed 14 polymorphic loci in *B. oleracea*, and 13 polymorphic loci in *B. napus*. In addition, the *SLG*-specific primers E and F provided a mapped locus (STS-EF) in *B. oleracea*, and two loci (SIEFa and SIEFb) in *B. napus*. Based on the marker loci revealed by the common RFLP probes and the RAPD and STS primers, the *B. napus* and *B. oleracea* maps were compared. The results are summarized in Table 3, and are illustrated in Fig. 3, where the homoeology between the *B. napus* LGs and the *B. oleracea* LGs are shown in relation to the *B. oleracea* map.

Three of the *B. oleracea* LGs (LG1, LG2 and LG5) were only partially homoeologous to three *B. napus* LGs, and three others (LG3, LG4 and LG6) each showed homoeology to two *B. napus* LGs simultaneously. Only

Table 1 Linkage-group size, number of markers, and the average marker interval per linkage group of the *B. napus* linkage map

Linkage group	Size (cM)	No. of markers	Average marker interval (cM)
1	319.8	48	6.7
2	217.5	19	11.4
3	196.8	40	4.9
4	181.1	13	13.9
5	109.9	20	5.5
6	109.2	16	6.8
7	108.9	22	5.0
8	107.3	15	7.2
9	84.3	15	5.6
10	80.6	17	4.7
11	74.7	16	4.7
12	72.5	9	8.1
13	65.3	13	5.0
14	58.1	10	5.8
15	51.5	17	3.0
16	39.5	11	3.6
17	31.3	5	6.3
18	25.0	4	6.3
19	21.4	5	4.3
Σ/mean	1954.7	315	6.3

Unassigned segment	Size (cM)	No. of markers
A	9.9	4
B	51.5	3
C	35.5	3
D	6.5	3
E	5.5	3
F	2.2	3
G	13.6	2
H	8.0	2
I	12.1	2
J	25.4	2
Σ	170.2	27

Table 2 Linkage-group size, number of markers, and the average marker interval per linkage group of the *B. oleracea* linkage map

Linkage group	Size (cm)	No. of markers	Average marker interval (cM)
1	249.4	61	4.1
2	286.5	52	5.5
3	140.0	35	4.0
4	231.0	40	5.8
5	165.4	36	4.6
6	175.9	30	5.9
7	183.8	24	7.7
8	79.5	15	5.3
9	34.6	4	8.7
Σ/mean	1546.1	297	5.7

Unassigned segment	Size (cM)	No. of markers
A	21.4	4
B	18.9	4
C	11.3	2
D	8.3	3
Σ	59.9	13

LG7 and LG8 of *B. oleracea* were each homoeologous to a corresponding single LG in *B. napus* (LG10 and LG1 of *B. napus* respectively). LG9, the smallest LG of *B. oleracea*, shared no noticeable common segments with any *B. napus* LG. All except five *B. napus* LGs (LG4, LG15, LG16, LG18 and LG19) were homoeologous to at least one *B. oleracea* LG. These results indicated major genome rearrangements between *B. oleracea* and *B. napus*, or else suggested the possibility that a different *Brassica* species with nine chromosomes might be the true progenitor of *B. napus*. The latter hypothesis has been supported by earlier phylogeny studies based on RFLP polymorphism in organelle genomes (Palmer et al. 1983; Song and Osborn 1992) in which a wild relative of *B. oleracea*, *B. montana*, was suggested to be the maternal ancestor of *B. napus*. *B. napus* was found to be distinct from modern *B. rapa* and *B. oleracea*. Therefore, RFLP mapping of the nuclear genome of *B. montana* might help to clarify this hypothesis.

In addition, most of the conserved regions in *B. oleracea* were shared by two *B. napus* LGs. These results might be partly explained by the homoeology between the A- and the C-genomes, as seen in an earlier comparison of the linkage maps of *B. rapa* and *B. oleracea* (McGrath and Quiros 1991; Teutonico and Osborn 1994). Furthermore, the inter-linkage-group segmental duplications in *B. oleracea* could also contribute to this observation. The explanation could be further clarified by comparative mapping of *B. rapa* with *B. napus* using the same sets of probes employed in this study.

The most extensive conservation was observed between *B. oleracea* LG1 and *B. napus* LG3, where nine *B. oleracea* loci corresponded to 11 *B. napus* loci due to intra-linkage-group duplications of the sequence detected by the probes 346 and 472 in *B. napus*. This synteny was largely maintained except for an inversion of the 36.8-cM (459–512) segment in *B. oleracea* at the distal end of the linkage groups (Fig. 3). The conserved region

Table 3 Conserved chromosomal segments between the genomes of *B. oleracea* and *B. napus*

B. oleracea			*B. napus*		
LG	Conserved marker loci	Genetic distance (cM)	LG	Conserved marker loci	Genetic distance (cM)
1	472a-237b-325-456-386-pX10-346-459-512	234.1	3	472a-472b-237b-325-456b-386a-pX10-346a-346b-512-459	175.1
1	541-SCARG2b-429-594-463-319-456-386-401b	110.9	5	541-429b-594-G286-463a-319a-456b-386b-401b	75.9
1	315-292-512	45.9	17	315-292-512b-512a	31.3
2	387a-626b-560-564-387b	104.9	12	387b-626b-560-564	47.9
2	365a-289-366-670	78.7	8	289-366-670-365	53.9
2	468-237a-pX1-388	14.8	1	388-468-pX1-237a	28.1
3	173-277b-277a	67.2	2	173c-277	68.1
3	231a-592-556-pX4a-231b	3.1	9	231-592b-556b-pX4	19.8
4	10b-10a-401a-OP411-179	179.9	13	OP411c-10-179-401a	34.7
4	pX6-607-167	13.9	14	607-pX6a-pX6b-167	20.5
5	452-679-563b	54.5	10	452a-452b-679-563a	58.2
5	OP402b-465-460-529	36.4	6	460a-465a-OP402-529	42.7
5	465-460-364-434	45.8	11	460b-465b-364-434	40.9
6	239-730-743-690-393a	149.0	2	239a-239b-743-730a-393c-690b	153.2
6	407-189-730-224c-271-690-393a	69.2	7	224a-730b-189-271b-393a-690a-407	36.4
7	628-OP302b-pX11b-7-458a-563a	40.6	10	OP302b-628-pX11b-458-563a-7	46.9
8	219-357-STSEF-216-381	58.9	1	219b-219c-219d-219a-357-216a-SIEFa-381b	143.9

covered 234.1 cM in the *B. oleracea* map and corresponds to 175.1 cM in the *B. napus* map. A large segment (110.9 cM) of *B. oleracea* LG1 also showed homoeology to almost the entire LG5 of *B. napus* (75.9 cM), with nine common marker loci on either LG and a common distal segment (45.9 cM) with *B. napus* LG17 (31.3 cM) (Fig. 3).

On three occasions, *B. napus* LG1, LG2 and LG10 revealed conserved segments in two *B. oleracea* LGs. For example, in LG1 of *B. napus*, the 219b-SIEFa-381b-216a-357 (143.9 cM) segment was homoeologous to 74% (58.9 cM) of LG8 of *B. oleracea*; while a non-overlapping distal part of *B. napus* LG1 (28.1 cM) was homoeologous to a small segment (14.8 cM) of *B. oleracea* LG2 (Fig. 3, Table 3). With *B. napus* LG10, the conserved areas with *B. oleracea* LG5 and LG7, respectively, were overlapping. A similar situation was observed with the homoeology between LG2 of *B. napus*, and LG3 and LG6 of *B. oleracea* (Fig. 3).

The distances of conserved regions between *B. oleracea* and *B. napus* were mostly comparable with less than two-fold differences (Table 3). Only three

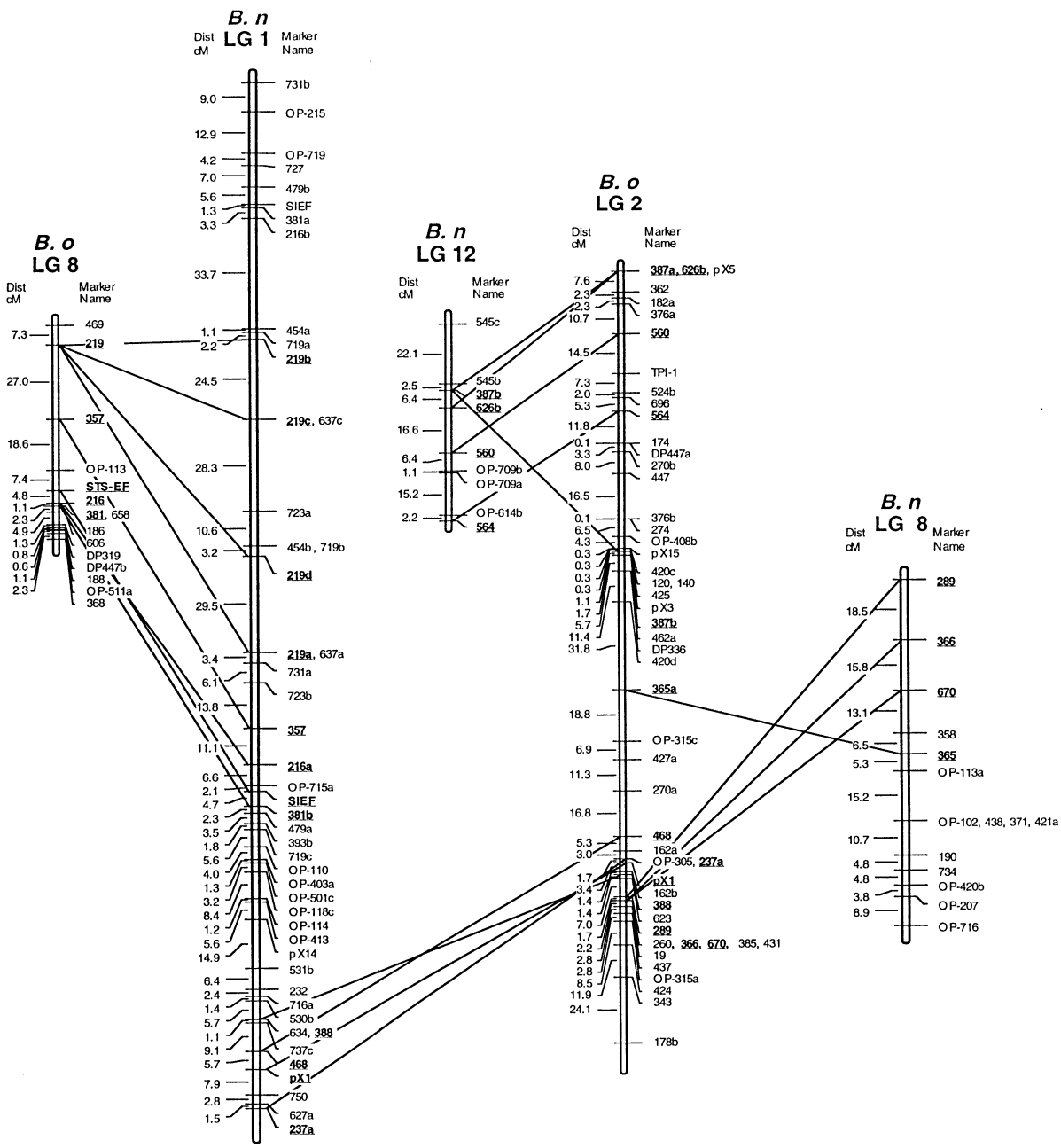


Fig. 3 See page 580 for legend

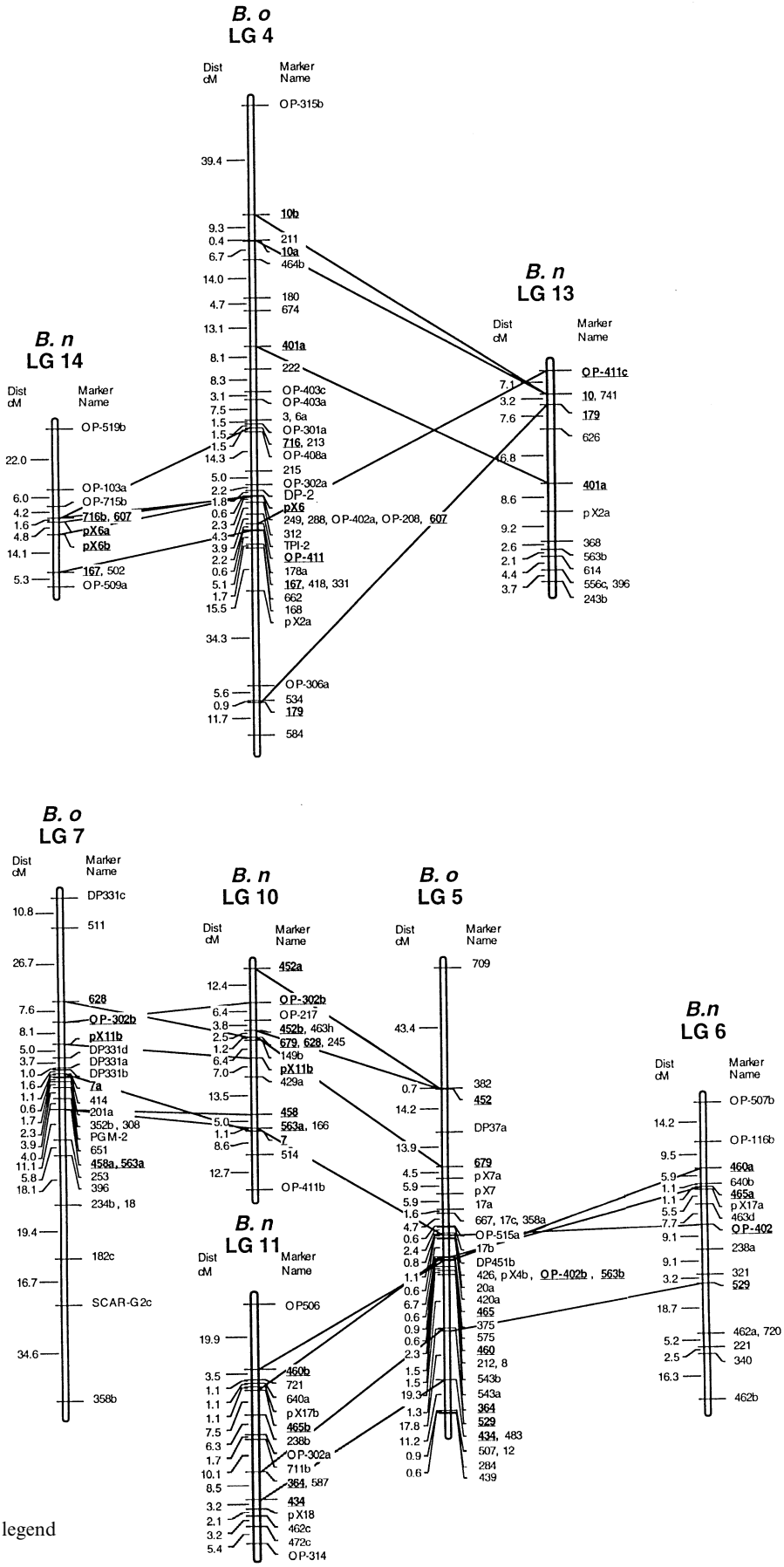


Fig. 3 See page 580 for legend

cases – the homoeologies (1) between LG3 of *B. oleracea* (3.1 cM) and LG9 of *B. napus* (19.8 cM), (2) between LG4 of *B. oleracea* (179.9 cM) and LG13 of *B. napus* (34.7 cM), and (3) between LG8 of *B. oleracea* (58.9 cM) with LG1 of *B. napus* (143.9 cM) – showed greatly different genetic distances covered by the common markers (Table 3). Based on the minimum total distances of the homoeologous regions identified here in the maps of both *B. napus* and *B. oleracea*, 85% of the *B. oleracea* genome had homoeology with the *B. napus* genome, and at least 48% of the *B. napus* genome possibly originated from *B. oleracea*.

Within the conserved regions, the orders of some of the conserved loci were altered by simple inversions or translocations. There were also numerous non-homoeologous marker loci interspersed with the homoeologous common anchor sequences. This may be due to markers polymorphic in the *B. napus* cross, but monomorphic in the *B. oleracea* cross, or vice versa. Similar results were obtained in the study of Parkin et al. (1995) where the genome origin of alleles for only 240 of the 399 polymorphic loci could be established, implying that some of the loci in the *B. napus* map were not polymorphic between the *B. oleracea* and the *B. rapa* progenitor used to re-synthesize the *B. napus* line used in the study. Alternately, this may be caused by insertions and deletions which occurred during interspecific hybridization of the A-genome and C-genome progenitors. Such chromosome aberrations have often been observed in *Brassica* species (Gustafsson et al. 1976; Quiros et al. 1988; Kianian 1990).

The extent and arrangements of the conserved regions between *B. oleracea* and *B. napus* suggested that, after the integration of the ancestral A and C genomes to form the *B. napus* genome during interspecific hybridization, the integrity of these ancestral chromosomes was not maintained. Frequent chromosomal rearrangements and mutations were most likely the cause of genome evolution. Similar phenomena have been observed in other *Brassica* genome comparisons by comparative mapping (Hoenecke and Chyi 1991; Teutonico and Osborn 1994).

All previous published comparisons of the linkage maps of *Brassica* species (McGrath and Quiros 1991; Lydiate et al. 1993; Teutonico and Osborn 1994; Parkin et al. 1995; Sharpe et al. 1995) were based on RFLP markers. Our current study has incorporated RAPD and STS markers in our maps as well as using

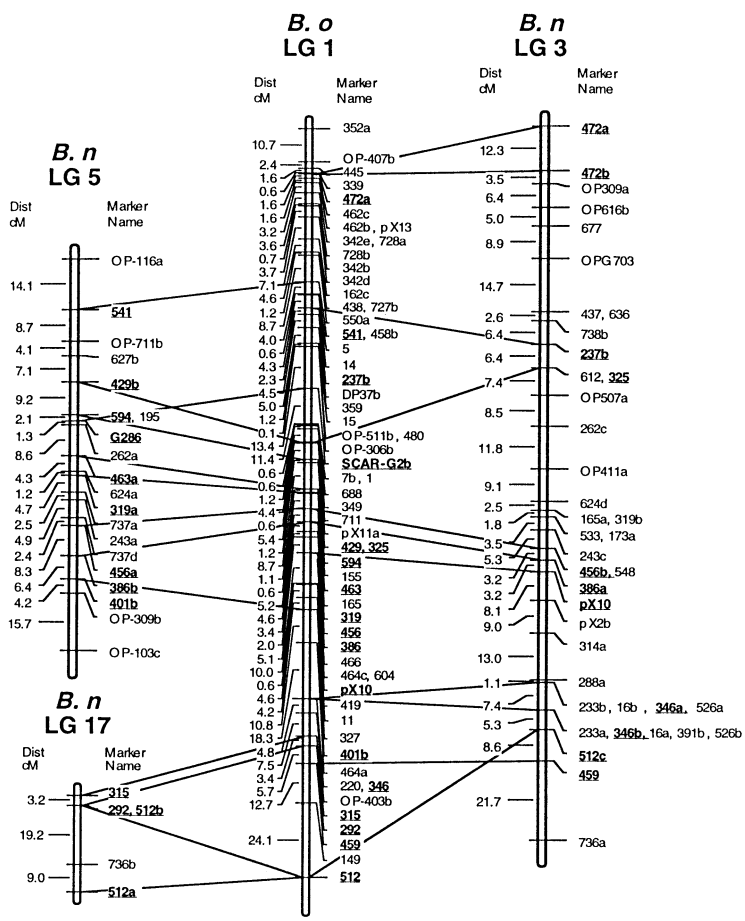


Fig. 3 See page 580 for legend

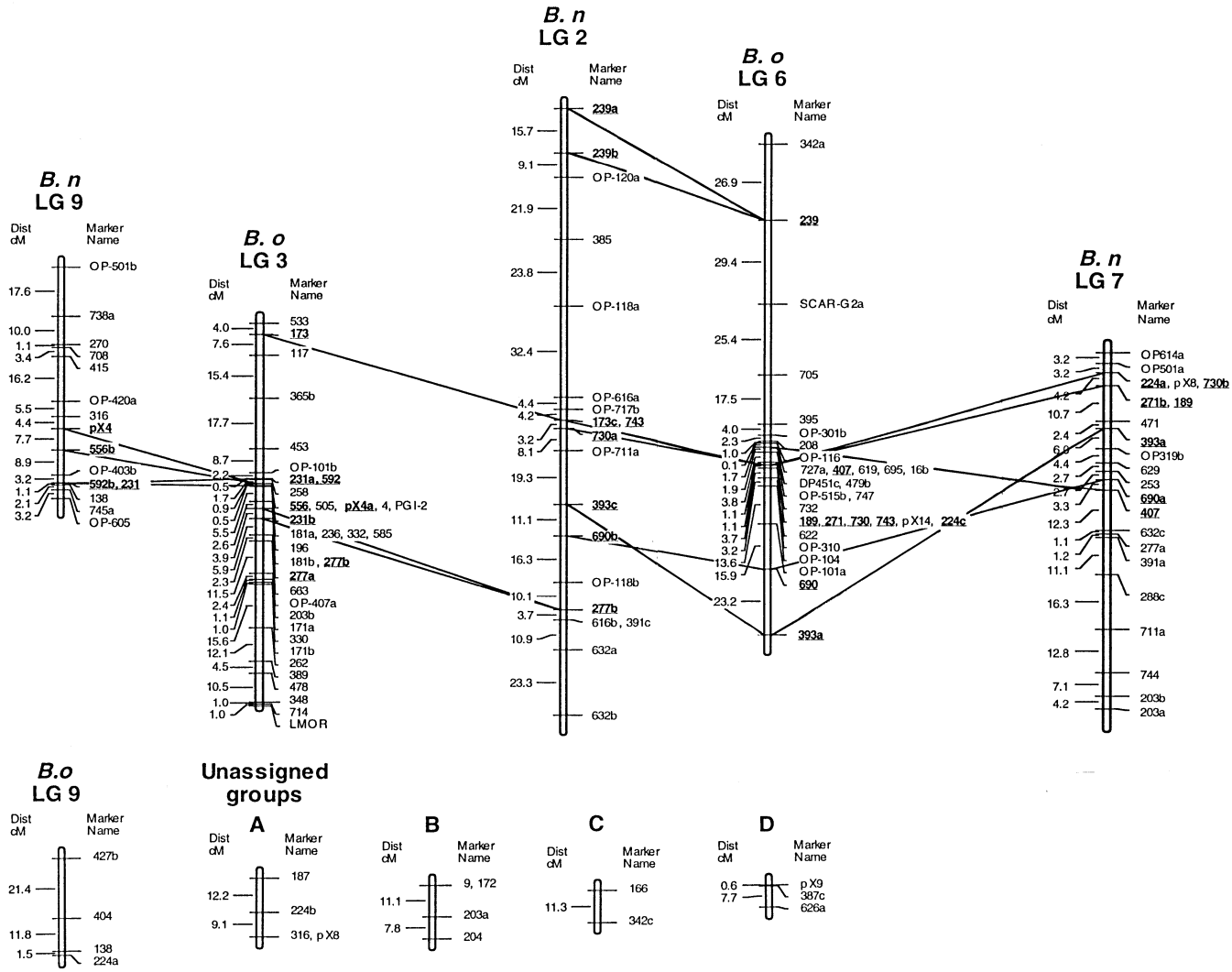


Fig. 3 Comparison of the genetic maps of *B. napus* and *B. oleracea*. Major conserved regions between the two genomes are shown with reference to the *B. oleracea* map. Common markers are *underlined* and joined by *solid lines* between the corresponding *B. oleracea* (*B.o*) and the *B. napus* (*B.n*) linkage groups

more densely populated, and greater genome coverage, maps in the comparison. The study of McGrath and Quiros (1991) involved a comparison between two diploid genomes of *B. rapa* and *B. oleracea* which showed that homoeology existed between these two genomes. The studies of Hoenecke and Chyi (1991) and Teutonico and Osborn (1994) were both comparisons of the *B. rapa* map with that of *B. napus* . The latter also addressed the homoeology between the A genome (*B. rapa*) and the C genome (*B. oleracea*). Neither of them involved a direct comparison of the diploid C genome (*B. oleracea*) with the amphidiploid AC genome (*B. napus*). Our study has directly addressed a comparison of the C genome (*B. oleracea*) map and the AC genome (*B. napus*) map based on *B. oleracea* and *B. napus* breeding lines.

The study of Lydiate et al. (1993), Parkin et al. (1995) and Sharpe et al. (1995) was a close parallel of the present study whereby the ancestral genome source of each LG of the *B. napus* map was defined, and, it was concluded that there were little rearrangement of the LGs within the amphidiploid. Their approach was different from ours and was specifically designed to investigate homoeologous pairing in *B. napus*. Their genetic maps of *B. napus* were constructed using two related crosses where a common winter *B. napus* line was crossed respectively to a spring *B. napus* line and to a synthetic *B. napus* (re-synthesized from *B. rapa* spp. *chinensis* and *B. oleracea* spp. *alboglabra*). Collinearity of the linkage maps based on both crosses was demonstrated supporting the claim that the *B. napus* genome had remained essentially unchanged since the initial hybridization of the two ancestral species. RFLP mapping using the synthetic *B. napus* enabled the identification of the A-genome and C-genome alleles at some of the marker loci, and hence determined the ancestral genome source of each LG of the *B. napus* map. Our study directly compared the *B. oleracea* and the *B.*

napus linkage maps with specific attention being paid to the common markers and the synteny maintained between the LGs of the two maps. Our observation was that there were frequent rearrangements in the C genome of *B. napus* when compared to the C genome of *B. oleracea*. Although our conclusions might seem different, they are not mutually exclusive. The results of Lydiate et al. (1993) could only demonstrate the co-existence of markers from the same progenitor genome in the LGs, but could not decide whether the order of these markers was being also maintained in the amphidiploid when compared to their order in the ancestral genomes. Moreover, a recent report of Sharpe et al. (1995) concluded that frequent non-reciprocal translocation occurred in the amphidiploid genome of *B. napus* based on the same study. Further comparison of the *B. napus* maps of Sharpe et al. (1995) and Parkin et al. (1995) with those of *B. oleracea* and *B. rapa* would provide information about the synteny of the markers, and it would be intriguing to see whether we could adopt the allele identification approach of Lydiate et al. (1993) in our comparison to obtain a more complete picture of C-genome evolution from the diploid *B. oleracea* to the amphidiploid *B. napus*.

Conclusion

In this study, a fairly densely populated *B. napus* map was constructed and the previously published *B. oleracea* map was updated with additional markers. These two linkage maps, of comparable marker density, were compared and the conserved regions between the *B. oleracea* and the *B. napus* genomes were identified. The extensive homoeology and the rearrangements observed between the two genomes suggested that *B. oleracea*, or more likely a closely related $2n = 18$ *Brassica* species, could be the C-genome progenitor of the amphidiploid *B. napus*. The results also indicated that, although wide segmental homoeologies exist between the two genomes, when breeding lines are used for the comparative mapping instead of synthetic *B. napus* compared with its A- and C-genome donors, the *B. oleracea* chromosomes have not been maintained intact in the amphidiploid. While a study with a synthetic *B. napus* has demonstrated the initial origins of chromosomes in *B. napus* after amphidiploidy, other comparisons similar to this study, using cultivars or breeding lines, reflect more the reality of the *Brassica* genome organization. Frequent chromosome rearrangements were most likely the cause of genome evolution and the variation in chromosome number of species in this genus.

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