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A linkage map of *Brassica rapa* (syn. *campestris*) based on restriction fragment length polymorphism loci

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Summary. A detailed linkage map of *B. rapa* (syn. *campestris*) was constructed based on segregation of 280 restriction fragment length polymorphism loci, detected by using 188 genomic DNA clones as probes on DNAs from a F_2 population of Chinese cabbage 'Michihili' × 'Spring broccoli.' These genetic markers covered 1,850 centiMorgans (cM) and defined ten linkage groups, which equals the haploid chromosome number of this species. Extensive sequence duplication was evident by the detection of two or more segregating loci with each of 69 clones (36.7% of the total). Although some duplicated loci were randomly distributed throughout the genome, many had linkage arrangements that were conserved on different linkage groups, suggesting that large chromosome fragments were present in multiple copies. However, conservation in the linkage arrangement of duplicate loci throughout entire pairs of linkage groups was not observed. Single-copy loci were often found to be located within conserved duplicated regions, and linkage distances between some loci having conserved duplicated arrangements were substantially different between the duplicated regions. Structural rearrangements, such as insertions, deletions, and inversions or combinations of these events, seemed to be related to the alternations of map distances between duplicated loci and to the dispersal of duplicated chromosome fragments. These results suggest that *B. rapa* has evolved in part by duplication of chromosomes or large chromosome fragments with subsequent structural rearrangements.

Key words: *Brassica rapa* – *Brassica campestris* – Restriction fragment length polymorphism – Linkage map – Duplicated loci

Introduction

Detailed genetic linkage maps in plants are very useful tools for studying genome structure and evolution, identifying introgression between different genomes, and localizing genes of interest (Beckmann and Soller 1986; Tanksley et al. 1989). However, in many important plant species, the lack of characterized genetic markers has hindered the construction and use of linkage maps. The development of methods to detect restriction fragment length polymorphisms (RFLPs) of nuclear DNAs has made it possible to construct detailed linkage maps for many species. RFLP markers are associated with simple genetic segregation patterns and they are potentially unlimited in number (Helentjaris et al. 1985; Burr et al. 1986). Detailed RFLP linkage maps have been constructed for several important crops, such as maize, tomato, lettuce, potato, and rice (Helentjaris et al. 1986; Bernatzky and Tanksley 1986; Landry et al. 1987; Bonierbale et al. 1988; McCouch et al. 1988).

The *Brassica* genus includes a diverse group of crops, such as cabbage, broccoli, cauliflower, Chinese cabbage, mustard greens, and rapeseed, which have great economic value worldwide. *B. rapa* (syn. *campestris*) (n = 10) is one of the most important species in *Brassica* and includes a variety of vegetables, such as Chinese cabbage and Pak choi, and oil seed crops. Over the past 60 years there have been many studies on morphology, cytogenetics, and breeding of *B. rapa* and other *Brassica* species, which have provided insight on genome relationships within and between species (reviewed by Prakash and Hinata 1980). For example, there have been reports of structural similarities between nonhomologous chromosomes observed in pachytene analyses (Robbelen 1960) and of chromosome pairing during meiosis in haploid *B. rapa* (Armstrong and Keller 1981). These and similar

observations in other *Brassica* species have led researchers to hypothesize that the diploid *Brassica* species (n = 7, 8, 9, 10, or 11) have evolved as an ascending aneuploid series from a prototype species of x = 6 (Prakash and Hinata 1980).

Additional information on the organization and evolution of *Brassica* genomes could be obtained from the linkage arrangement of genetic markers in *B. rapa*. Although the inheritance of some morphological markers has been reported in *B. rapa* (Williams and Hill 1985; Yarnell 1956), no genetic linkage map has been established. A high degree of polymorphism for restriction fragment lengths of nuclear DNAs has been observed within *Brassica* species (Figdore et al. 1988; Song et al. 1988 a, b, 1990). This has allowed the construction of a detailed linkage map of *B. oleracea* (Slocum et al. 1990), which provided new information on the genome structure of this species. In the study reported here, RFLP markers were used to construct a detailed genetic linkage map of *B. rapa*. The linkage arrangement of these markers has provided molecular evidence for extensive genome duplication and other structural rearrangements in this species.

Materials and methods

Preparation of DNA clones

Three genomic DNA libraries were constructed using 'Early White' (EW) cauliflower (*B. oleracea* ssp. *botrytis*), 'Wisconsin Golden Acre' (WGA) cabbage (*B. oleracea* ssp. *capitata*), and 'WR 70 days' (WR) Chinese cabbage (*B. rapa* ssp. *pekinensis*) as genomic DNA sources. These libraries were constructed by cloning gel-isolated *Pst*I fragments (500–2,000 bp in length) into plasmids pUC19 ('EW' clones) or pTZ18R ('WGA' and 'WR' clones), and by transforming recombinant plasmic into either JM83 or DH5α strains of *E. coli*. Recombinant DNA clones from these libraries were screened by colony lift or slot blot hybridization to total genomic, mitochondrial, and chloroplast DNAs in order to select for clones containing low-copy nuclear DNA sequences (Figdore et al. 1988).

Detection of RFLPs

Isolation of total genomic DNA, digestion with restriction endonucleases, gel electrophoresis, Southern blotting, and hybridization have been described previously (Helentjaris et al. 1986; Osborn et al. 1987). Nylon membrane, obtained from MSI or Schleicher and Schuell, was used as the blotting matrix. Probes were labeled by nick-translation. Hybridizations with 'EW'-derived probes were conducted at 60 °C and final washes were in 0.25 × SSC, 0.1% SDS at 60 °C. Hybridizations with 'WGA'- and 'WR'-derived probes were conducted at 65 °C and final washes were in 0.25 × SSC, 0.1% SDS at 65 °C.

Generation of mapping population, linkage analysis, and nomenclature

For the mapping population, a Chinese cabbage cultivar, Michihili (Olds Seed Co., Madison/WI), and an accession of Spring broccoli (Crucifer Genetic Cooperative, Madison/WI) were selected as the female parent and the male parent, respectively. These parents represent two diverse groups in *B. rapa* that are polymorphic for both morphological traits and restriction fragment lengths (Figdore et al. 1988; Song et al. 1988 b). Since both parents were from open-pollinated populations and were heterozygous for some marker loci, single plants were used in making the F_1 hybrid, and a single F_1 plant was selected and self-fertilized by bud pollination to produce an F_2 population. One F_2 population of 95 individuals was analyzed to generate the linkage map. Plant DNAs were digested with *Eco*RI for mapping of RFLP loci detected by most clones; however, *Hin*dIII was used for some probes. Probe-enzyme combinations which revealed polymorphism between Michihili and Spring broccoli in a previous study (Figdore et al. 1988) were used to detect segregating RFLP loci in the F_2 population. DNAs from the two parental plants and the single F_1 plant from which the mapping population was derived, or from bulk samples of the selfed progeny of parental plants and of the F_1 hybrids, were included on Southern blots used to determine the genotypes of F_2 individuals. Goodness of fit to a 1 : 2 : 1 ratio was determined by Chi-square analysis. Linkage data was analyzed using computer programs MAXLIKE 4 (1986 edition, by D. Hoisington, University of Missouri) and MAPMAKER II (1990 edition, by E. S. Lander, Whitehead Institute, Cambridge/MA). Recombination values were obtained for all possible pairs of loci. The locus arrangements and distances in the linkage map were based on the output results from MAPMAKER II using the 'map' function. RFLP loci detected by different probes were assigned different numbers, and multiple segregating loci detected by a single probe were assigned a common number followed by a letter (a, b, c, or d) to indicate each replicated locus.

Results

Description of linkage map and RFLP markers

Segregation data was collected for 280 RFLP loci detected by 188 clones. A sample of segregation data and Chi-square values for this data is shown in Table 1. Most of the segregating loci fitted the expected 1 : 2 : 1 Mendelian ratio (P-value > 0.05); however, 8 of 280 loci showed deviation from the expected ratio ($P < 0.05$) (Table 1). Loci with distorted segregation ratios were found to have fewer homozygous genotypic classes of either parent and more heterozygous individuals than expected, except locus *320*, which showed fewer heterozygous individuals than expected (Table 1).

Based on recombination values, two linkage maps were constructed using MAXLIKE 4 and MAPMAKER II. Comparison between these two maps indicated that, although they were very similar to each other for arrangement of loci and map distances, MAPMAKER II gave better resolution than the MAXLIKE 4 program, especially for tightly linked loci. For example, in the map constructed using MAXLIKE 4, the five loci *7c*, *12b*, *197c*, *323b*, and *329* all mapped to the same position on linkage group 3A, having two point values of 0 (data not shown), whereas in the map constructed by MAPMAKER II, these loci were resolved into four separate positions with tight linkages (Fig. 1). A similar situation was observed for the four loci *53b*, *73*, *51a*, and *74a* on

Table 1. Segregation data and Chi-square goodness of fit test for 35 RFLP loci from an F_2 population of 'Michihili' × 'Spring broccoli'

Locus name	Linkage group	No. of F_2 individuals				χ^{2a}
		Homozygous Michihili	Heterozygous	Homozygous Spring broccoli	Total	
19a	1	22	43	25	90	0.30
52	1	20	46	21	87	0.31
137	1	16	38	26	80	2.70
300	1	16	56	16	88	6.55*
72a	2	15	52	24	91	3.64
83b	2	28	49	14	91	4.85
61b	2	23	57	12	92	7.89**
312	3	22	46	19	87	0.49
133	3	29	36	24	89	3.81
144	3	23	38	19	80	0.60
27	4	22	53	20	95	1.36
22b	4	23	51	21	95	0.60
71	4	24	44	20	88	0.36
188	5	19	36	28	83	3.14
94	5	17	54	21	92	3.13
306	5	20	52	19	91	1.88
59a	5	29	49	17	95	3.13
202b	6	17	48	28	93	2.70
54b	6	23	41	31	95	3.13
51c	6	29	39	26	94	2.91
320	6	27	26	21	74	7.51**
148	7	16	50	28	94	3.45
79a	7	23	46	19	88	0.55
59b	7	19	43	23	85	0.39
186	8	18	46	21	85	0.79
29c	8	21	50	18	89	1.56
161	8	19	43	27	89	1.54
114	9	20	44	23	87	0.22
156	9	11	52	30	93	9.06**
87	9	14	54	26	94	5.15
315	9	11	51	24	86	6.91*
145	9	13	55	20	88	6.61*
9a	10	30	43	18	91	3.44
14	10	21	58	15	94	5.91*
141	10	18	54	12	84	7.71**

[a] Chi-square values are based on expected ratios of 1:2:1.
* and ** indicate that greater Chi-square value would be expected by chance at probabilites of $0.05 > P > 0.025$ and $0.025 > P > 0.01$, respectively

linkage group 6A (Fig. 1). Because of the advantages of multipoint linkage analysis using MAPMAKER II (Lander et al. 1987), only the results from analysis with this program will be presented.

The 280 segregating loci could be organized into ten linkage groups, corresponding to the haploid chromosome number of *B. rapa* (Fig. 1). The linkage groups were assigned designations 1A to 10A based on the linkage relationships of these loci compared to those detected by the same clones in the *B. oleracea* map (Slocum et al. 1990). These relationships between linkage groups in *B. rapa* and *B. oleracea* will be addressed in a separate paper. The total map distance of the *B. rapa* linkage groups was 1,850 cM, with an average distance of 6.6 cM and a maximum of 39 cM between adjacent loci. Some large linkage distances (>30 cM) were observed at the ends of linkage groups 1A, 3A, 6A, and 9A (Fig. 1). These regions were tested with the 'link' command of MAPMAKER II, and the differences in log likelihood for the test of linkage versus nonlinkage in these regions ranged from 3.98 to 8.78. For instance, a difference in log likelihood of 4.37 was found for loci *115* and *26d* on 1A. This indicates that *115* and *26d* are $10^{4.37}$ or about 23,000 times more likely to be linked than unlinked.

Various RFLP segregation patterns were observed in the *B. rapa* F_2 population for the 188 clones used for mapping (Fig. 2a–c). Based on a single restriction enzyme, 34 clones (18.1%) hybridized to single segregating RFLP loci, with no monomorphic bands detected (Fig. 2A and B); 85 clones (45.2%) hybridized to one segregating locus plus one to eight additional monomorphic fragments; 28 clones (14.9%) hybridized to multiple segregating loci with no monomorphic bands (Fig. 2C); and 41 clones (21.8%) hybridized to multiple segregating loci plus one to six monomorphic bands. Among the 69 clones that hybridized to multiple segregating RFLP loci, 51 clones hybridized to two segregating loci, 13 clones hybridized to three segregating loci, and 5 clones hybridized to four segregating loci.

Placement of clones into these four categories provided some information about the copy number of cloned sequences used as probes. However, the complexity of hybridization patterns observed depended in part on the restriction enzyme used to digest DNAs from the mapping population. For example, several clones that were hybridized to DNAs digested with *Eco*RI and *Hin*dIII revealed single segregating loci and no monomorphic bands with one enzyme, and single segregating loci plus monomorphic bands with another enzyme (data not shown). In other cases, monomorphic bands may represent undetected duplicated loci. For example, one probe used to map loci *18a* and *18b* hybridized to two polymorphic bands and two monomorphic bands with *Eco*RI-digested DNAs, allowing one locus to be mapped; however, with *Hin*dIII-digested DNAs, this probe hybridized to four polymorphic bands, allowing an additional locus to be mapped. Also, the simplicity of hybridization patterns varied with library source and corresponding hybridization conditions. Only 19% of the 'EW' probes, which were hybridized at lower stringency conditions, revealed single loci with no monomorphic bands, whereas 26% of the 'WGA' and 'WR' probes, which were hybridized at higher stringency conditions, revealed this type of pattern. Therefore, conclusions about the copy number of cloned DNA sequences we used to construct this map may be restricted to our experimental conditions.

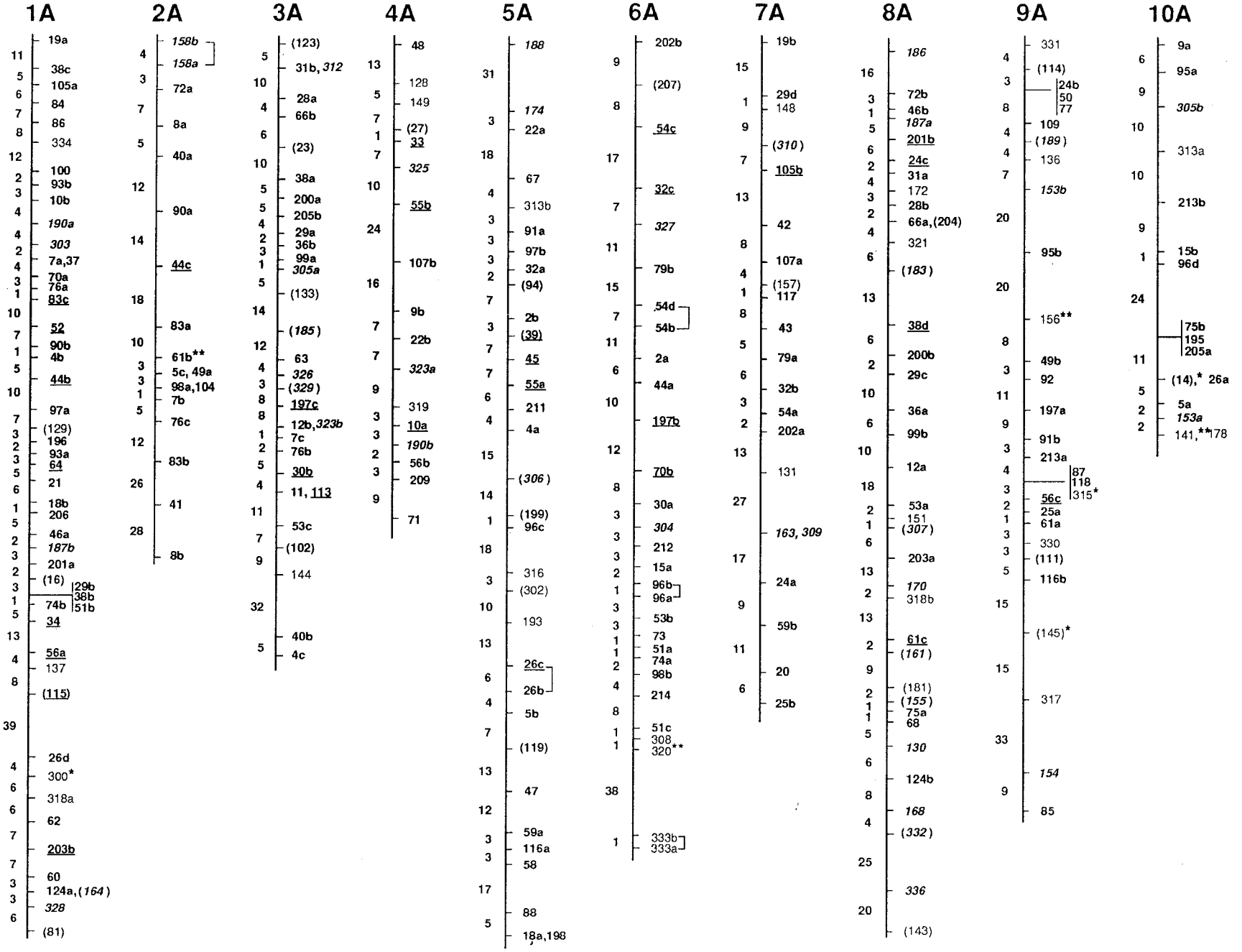

Fig. 1. *B. rapa* (syn. *campestris*) RFLP linkage map of 280 marker loci identified with 188 random genomic DNA clones. The ten linkage groups are listed at the top. The loci are listed on the *right* and the map distances in centiMorgans on the *left*. The nomenclature of RFLP loci is described in 'Materials and methods'. Loci detected by sequences from 'EW,' 'WGA,' and 'WR' libraries are indicated with *bold face, plain,* and *italicized* types, respectively. Clones that hybridized to single-copy loci (one segregating locus and no monomorphic bands) are in parentheses. Loci that were mapped based on segregation of a single band are *underlined*. Adjacent duplicated loci are marked with *brackets*. Loci with segregation ratios that deviated from the expected 1:2:1 ratio are indicated with * ($0.05 > P > 0.025$) and ** ($0.025 > P > 0.01$)

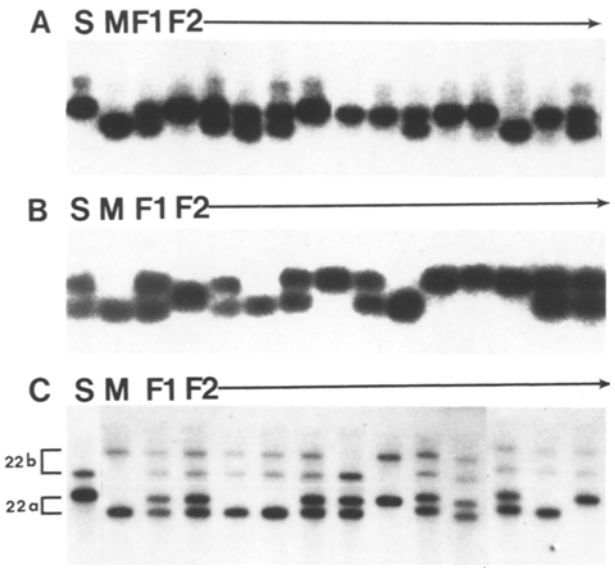

Fig. 2A–C. RFLP patterns for three probes hybridized to DNAs from Spring broccoli (S), Michihili (M), the F_1 of Michihili × Spring broccoli (F1), and different F_2 individuals (F2). **A** RFLP pattern for a probe that hybridized to a single segregating locus (*129*) using *Eco*-RI-digested DNAs. **B** RFLP pattern for a probe that hybridized to a single segregating locus (*306*) using *Eco*RI-digested DNAs; Spring broccoli is heterozygous, Michihili is homozygous, and the single F_1 plant contains the polymorphic allele combination. **C** RFLP pattern for a probe that hybridized to two segregating loci (*22a* and *b*) using *Eco*RI-digested DNAs

Twenty-nine loci were scored based on segregation of a single band (Fig. 1). The intensity of hybridization to the single segregating band relative to other bands in the pattern allowed differentiation between the homozygous present and hemizygous conditions (data not shown). The appearance of some null alleles could be explained by comigration of one allelic band with a monomorphic band, or by the lack of hybridization due to small homologous restriction fragments. However, the absence of hybridizing allelic bands for some loci could be due to deletion or insertion of these sequences in one of the parents.

Some probes that hybridized to polymorphic fragments between parental plants did not hybridize to RFLPs that segregated in our F_2 population. For these probes, one or both parental plants apparently were heterozygous and the two parents contained at least one RFLP allele in common which was transmitted by both parents to the F_1. F_2 segregation was observed only when the F_1 received polymorphic alleles from the parents (Fig. 2b).

Sequence duplication and arrangement of duplicated loci

Duplication in genomic DNA fragments of *B. rapa* was detected with 69 clones that hybridized to RFLPs at multiple segregating loci (Figs. 1 and 3). A majority (59) of these clones hybridized to multiple loci that were replicated on different linkage groups (Fig. 3). Ten clones hybridized to replicated loci within linkage groups (Fig. 3), among which five clones revealed adjacent loci with map distances ranging from 1 to 7 cM (Fig. 1). Replicated loci were assumed to represent homologous sequences derived by some mechanism of duplication and they will be referred to below as duplicated loci. There were 85 other clones that detected one segregating loci and additional monomorphic bands. The monomorphic bands sometimes could be detected as polymorphic when plant DNAs were digested with an alternate enzyme (e.g., loci *18a* and *18b* described above). However, in general only one restriction enzyme was used per probe and we did not pursue mapping of additional loci by examining segregation patterns with different enzymes. Thus, the level of sequence duplication revealed by our analysis probably underestimates the total level of duplication in the entire genome.

Pairwise comparisons were conducted among all linkage groups to identify duplicated loci in common to both linkage groups (Fig. 3). Comparisons between pairs of linkage groups having at least three duplicated loci in common are shown in Fig. 4a–d to illustrate the relative arrangement of duplicated loci between linkage groups. Many sets of duplicated loci were found to have the same order and similar map distances on different linkage groups. Conservation in organization of duplicated loci involved various linkage distances and encompassed three to ten duplicated loci (Fig. 4a–d). Strong conservation in the arrangement of duplicated loci was found among linkage groups 1A, 3A, and 8A (Fig. 4a). The highest degree of conservation was observed between 3A and 8A (sharing ten duplicated loci), whereas 1A and 8A shared nine duplicated loci, and 1A and 3A shared five duplicated loci (Fig. 4d). The levels of conserved duplication for other pairs of linkage groups were similar to each other and lower than those among 1A, 3A, and 8A. Some replicated loci also had conserved arrangements on three linkage groups (e.g., *7a-76a* on 1A, *7b-76c* on 2A, and *7c-76b* on 3A, Fig. 4d). In spite of the observed conservation in organization of many duplicated loci, nonconserved arrangements were observed for some duplicated loci on various linkage groups (Figs. 1 and 3), and no conserved arrangement of duplicated loci covering two entire linkage groups was found.

Although similar map distances often were observed for adjacent loci in conserved duplicated regions, substantial differences in map distances were observed between some loci. For example, loci *99b* and *12a* had a distance of 10 cM on linkage group 8A, whereas their duplicate loci *99a* and *12b* had a distance of 55 cM on linkage group 3A, and three single-copy loci (*133*, *185*, and *329*) were located between these duplicated loci

	1 A	2 A	3 A	4 A	5 A	6 A	7 A	8 A	9 A	10
1 A	38 C-B 93 B-A	7 A-B 44 B-C 76 A-C 83 C-A-B 90 B-C	4 B-C 7 A-C 29 B-A 38 C-A 76 A-B	10 B-A 56 A-B 190A-B	4 B-A 18 B-A 26 D-C-B 97 A-B	44 B-A 51 B-A-C 70 A-B 74 B-A	19 A-B 29 B-D 105 A-B	29 B-C 38 C-B-D 46 A-B 124 A-B 187 B-A 201 A-B 203 B-A 318 A-C	29 B-D 56 A-C	26 D-A
2 A		8 A-B 84 A-B 158 B-A	7 B-C 40 A-B 76 C-B		5 C-B	44 C-A 98 A-B		61 B-C 72 A-B	49 A-B 61 B-A	
3 A				323 B-A	4 C-A	30 B-A 53 C-B 197 C-B	29 A-D	12 B-A 28 A-B 29 A-C 31 B-A 36 B-A 38 A-D 53 C-A 66 B-A 99 A-B 200 A-B	197 C-A	205 B-A 305 A-B
4 A					22 B-A 55 B-A		107 B-A		56 B-C	9 B-A
5 A					26 C-B	2 B-A 32 A-C 96 C-B-A	32 A-B 59 A-B		91 A-B 116 A-B	5 B-A 26 C-B-A 96 C-D 313 B-A
6 A						51 A-C 54 C-D-B 96 B-A 333 B-A	32 C-B 54 C-D- B-A 79 B-A 202 B-A	53 B-A	197 B-A	15 A-B 96 B-A-D
7 A								24 A-C 29 D-C	24 A-B 25 B-A	
8 A									24 C-B 61 C-A	75 A-B
9 A										95 B-A 153 B-A 213 A-B
10 A										

Fig. 3. The distribution of duplicated loci among *B. rapa* linkage groups. The designations of duplicated loci are the same as in Fig. 1

(Fig. 4a). Similarly, the map distances between nine other pairs of duplicated loci (*201a-38b* versus *201b-38d*; *203b-124a* versus *203a-124b*; *32a-2b* versus *32c-2a*; *313a-96b* versus *313b-96c*; *95b-213a* versus *95a-213b*; *4a-26b* versus *4b-26d*; *97a-18b* versus *97b-18a*, *70b-51a* versus *70a-51b*; and *76a-4b* versus *76b-4c*) were substantially different on different linkage groups (linkages marked with '*' in Fig. 4a–d). Within seven of these nine duplicated regions, single-copy loci were interspersed among duplicated loci (Fig. 4). Deletions, insertions, and inversions or combinations of these events seemed to be associated with altered map distances between some duplicated regions. For example, the altered map distance between loci *99a-12b* (3A) and their duplicate loci *99b-12a* (8A) could be a result from an insertion of a chromosome fragment containing the three single-copy loci *133*, *185*, and *329* on 3A or a deletion of this fragment on 8A. Possible inversions also were observed for

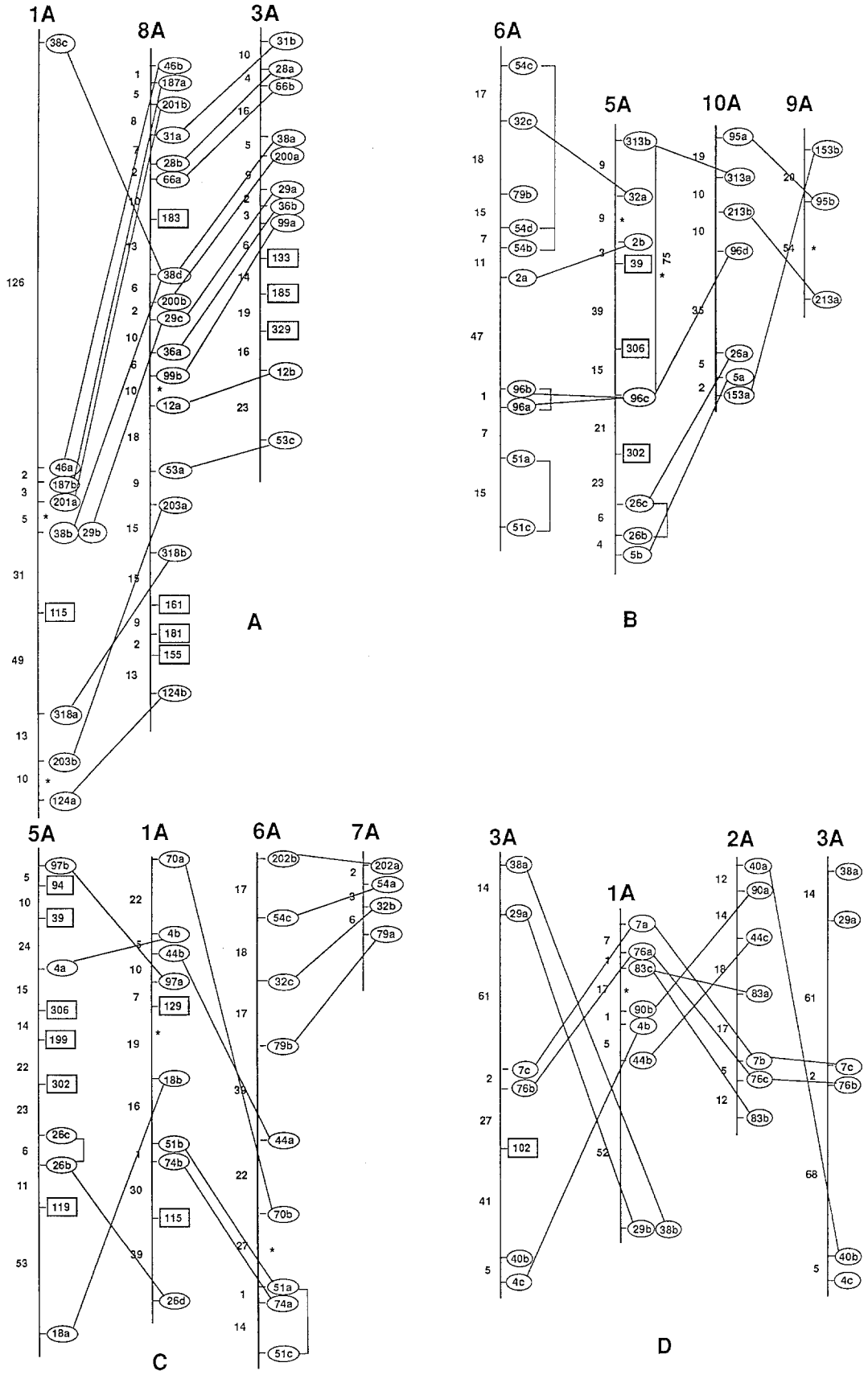

Fig. 4A–D. Arrangement of duplicated loci on portions of nine linkage groups of *B. rapa*. **A** Comparison of duplicated loci among linkage groups 1A, 3A, and 8A. These linkage groups showed strong conservation in the organization of duplicated loci which cover large chromosome fragments. **B** Comparison of duplicated loci among linkage groups 5A, 6A, 9A, and 10A, showing the conserved duplication regions between linkage groups and the duplication within linkage group 6A. **C** Arrangement of duplicated loci in linkage groups 1A, 5A, 6A, and 7A. The orientation of linkage group 7A is reversed compared to Fig. 1. **D** Arrangement of duplicated loci in linkage groups 1A, 2A, and 3A, showing the duplication of marker loci on three linkage groups (*7a–76a*, *7b–76c*, *7c–76b*). Linkage distances that are substantially different in two duplicated regions are indicated with *. The loci in *boxes* are single-copy loci which are interspersed among duplicated loci (see text for details)

some chromosome fragments encompassing duplicated loci. The relative positions of marker loci *318a-203b* on 1A and their duplicate loci *318b-203a* on 8A may reflect an inversion of a fragment containing these marker loci on either 1A or 8A (Fig. 4a). Another possible inversion event may have occurred in the duplicated region of linkage groups 1A and 6A (Fig. 4c).

Discussion

Analysis of segregating RFLP loci in an F_2 population has allowed us to develop the first genetic linkage map of *B. rapa*. This is a detailed map in which all of the 280 loci detected by 188 clones could be arranged into ten well-marked linkage groups. These ten linkage groups probably represent the ten haploid chromosomes of *B. rapa*. Although most marker loci in this linkage map were well dispersed with relatively small map distances, there were some regions on every linkage group with large distances between adjacent markers (>20 cM). These large map distances may represent 'hot spots' for recombination, or they could be due to low frequencies of DNA clones for sequences from these regions in the libraries used as sources of probes. Over one-third of the DNA clones used to construct this map detected multiple segregating loci. This level of sequence duplication is very high compared to that observed in previous studies in tomato (Bernatzky and Tanksley 1986), potato (Bonierbale et al. 1988), rice (McCouch et al. 1988), lettuce (Landry et al. 1987), and maize (Helentjaris et al. (1988), but it was similar to the level of duplication reported for *B. oleracea* (Slocum et al. 1990). The high level of duplication observed within the genome may reflect an important feature of genome evolution of *B. rapa*.

Based on cytogenetic evidence, previous researchers hypothesized that the genomes of diploid species in *Brassica* evolved as an ascending aneuploid series, with x=6 species as the prototype (Catcheside 1937; Mizushima 1980; Prakash and Hinata 1980; Attia and Robbelen 1986). Our recent phylogenic study also has provided some molecular evidence for possible ascending order in chromosome numbers during the evolution of *Brassica* species (Song et al. 1990). Robbelen (1960) proposed that the genome constitution of *B. rapa* is AABCDDEFFF based on analysis of pachytene chromosomes, and a similar result was obtained by karyotype analysis of mitotic chromosomes using an improved technique (K.L. Tang and P.H. Williams, unpublished data). Also, meiotic analysis has revealed frequent chromosome pairing in haploid *B. rapa* (Armstrong and Keller 1981). These and other studies suggest that *B. rapa* contains replicated chromosomes.

Comparisons of the map positions of duplicated loci detected in our study revealed regions with conserved patterns in the linkage arrangements and map distances of duplicated loci. The presence of large duplicated segments, such as the duplicated regions between linkage groups 8A and 3A, which covered almost the entire linkage group of 3A (127 cM), supports the previous cytogenic evidence that chromosome duplication has occurred during the evolution of *B. rapa*. However, if evolution of *B. rapa* has involved chromosome duplication, the lack of conservation in the arrangement of duplicated loci on entire pairs of linkage groups, as well as the presesence of some scattered duplicated loci, suggests that there has been considerable subsequent rearrangement within the genome. Large differences in map distances between pairs of duplicated regions indicated that structural rearrangements such as deletions, insertions, and inversions may have occurred frequently within *B. rapa*. For example, the linkage group 3A might represent a recent duplication of 8A with an insertion of a fragment containing three marker loci between *99a* and *12b*, whereas linkage groups 1A and 8A might be an earlier duplicated pair, differentiated by insertions between *201b* and *38d* and between *318b* and *124b* on 8A, or by deletions in these regions on 1A. In addition to chromosome duplication, other events, such as matings of individuals with overlapping reciprocal translocations, as described by Gottlieb (1983), could have caused some of the genome duplication we observed. Also, the five sets of adjacent duplication loci (Fig. 1) may have resulted from mechanisms involving localized intrachromosomal duplication. In specific regions, insertion/deletion rearrangements may differ among accessions, which could explain the appearance of null alleles for some loci.

Results from our study and previous cytogenetic studies suggest that the genome of *B. rapa* has evolved from a lower chromosome progenitor, in part by a mechanism of chromosome duplication followed by structural rearrangements. Chromosome duplication could have occurred by aneuploidy within the genome or by introgression from closely related genomes. In either case, meiotic instability, such as abnormal chromosome pairing, could occur in the newly formed aneuploid because of chromosome duplication, and thus structural rearrangement within the genome might take place. These arrangements would lead to differentiation of replicated chromosomes and to a decrease of meiotic irregularity by dispersing duplicated fragments among different chromosomes. Frequent and rapid rearrangement after duplication of chromosomes or large chromosome fragments provides a mechanism for stabilization of newly formed aneuploids, and this might play an important role in the genome evolution of *B. rapa* and other *Brassica* species.

Because of the high degree of RFLP observed within *B. rapa* (Figdore et al. 1988; Song et al. 1988b, 1990), the RFLP markers we have mapped will have many potential applications in future studies on genetics, evolution, and breeding of this species. Some of the markers in the

linkage map have been used to study taxonomy and genome evolution in *Brassica* and related genera (Song et al. 1990). The same set of probes can be applied to map different populations from various intraspecific crosses. The results from comparative mapping could be used to determine if the degree of genetic diversity between parental lines is related to map distances of RFLPs detected by using a common set of clones, and also if the level of structural rearrangement is the same among different crosses. Perhaps the most promising area of using RFLP mapping is to detect, map, and monitor genes controlling quantitative traits. RFLP markers in our map will be useful for analyzing genetic control of many traits in *Brassica*.

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