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Linkage arrangement of restriction fragment length polymorphism loci in *Brassica oleracea*

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Summary. A detailed genetic linkage map of *Brassica oleracea* was constructed based on the segregation of 258 restriction fragment length polymorphism loci in a broccoli × cabbage F_2 population. The genetic markers defined nine linkage groups, covering 820 recombination units. A majority of the informative genomic DNA probes hybridized to more than two restriction fragments in the F_2 population. "Duplicate" sequences having restriction fragment length polymorphism were generally found to be unlinked for any given probe. Many of these duplicated loci were clustered non-randomly on certain pairs of linkage groups, and conservation of the relative linkage arrangement of the loci between linkage groups was observed. While these data support previous cytological evidence for the existence of duplicated regions and the evolution of *B. oleracea* from a lower chromosome number progenitor, no evidence was provided for the current existence of blocks of homoeology spanning entire pairs of linkage groups. The arrangement of the analyzed duplicated loci suggests that a fairly high degree of genetic rearrangement has occurred in the evolution of *B. oleracea*. Several probes used in this study were useful in detecting rearrangements between the *B. oleracea* accessions used as parents, indicating that genetic rearrangements have occurred in the relatively recent evolution of this species.

Key words: *Brassica oleracea* – Linkage map – Restriction fragment length polymorphism – Duplicate loci

Introduction

The genus *Brassica* includes both diploid and amphidiploid species with numerous morphotypes serving as sources of vegetables, oil, and fodder throughout the world. Several important vegetable crops, including cabbage, broccoli, cauliflower, brussels sprouts, kale and, kohlrabi belong to the diploid species *B. oleracea*. The chromosomal relationships and evolution of this and other *Brassica* species have been studied extensively using cytogenetic techniques (reviewed by Prakash and Hinata 1980). Based on some of these studies, researchers have proposed that the diploid *Brassica* species *B. nigra*, *B. oleracea*, and *B. campestris* (n = 8, 9, and 10, respectively) have evolved as an ascending aneuploid series from a progenitor species with six chromosomes, and that the present diploid species exist as secondary polyploids (Prakash and Hinata 1980). Genetic formulae of AABBCCDEF (Haga 1938) and ABBCCDEEF (Robbelen 1960) have been proposed as the basic chromosome complement of *B. oleracea* from observations of secondary chromosome pairing and pachytene chromosome structure, respectively. Based on chromosome pairing in haploids, Armstrong and Keller (1982) proposed that the 2n chromosome complement of *B. oleracea* includes one set of hexasomic and one set of tetrasomic chromosomes.

To date, cytogenetic studies have provided most of the information on genome structure in *Brassica*. Very little information is available regarding the organization of genetic markers. Many morphological markers have been described in the literature, but only seven linkage groups containing a few markers each have been recognized (Sampson 1978; Wills 1977). The genetics of very few morphological markers have been described and most are associated with complex patterns of inheritance (Yarnell 1956). Several isozyme markers have been analyzed in segregating populations of *B. oleracea* (Arus and Orton 1983) and in *B. campestris-oleracea* addition lines (Quiros et al. 1987). Linkage among isozyme loci was not observed and they have not been tested for linkage to

morphological markers, although one isozyme locus, *6-PGD-2*, was localized to the same chromosome as a ribosomal DNA restriction fragment (Quiros et al. 1987). Duplicated isozyme loci have been observed, supporting the hypothesis that *B. oleracea* is a secondary polyploid, but the chromosomal organization of such duplicated sequences has not been described (Quiros et al. 1987, 1988).

The technique of Southern hybridization permits the detection and analysis of DNA restriction fragments in genomes. Restriction fragment length polymorphisms (RFLPs) have been used as genetic markers to construct linkage maps in several plant species, including corn, tomato, lettuce, pepper, potato, Arabidopsis, rice, and lentil (Bernatsky and Tanksley 1986, Helentjaris et al. 1986; Landry et al. 1987; Bonierbale et al. 1988; Chang et al. 1988; McCouch et al. 1988; Tanksley et al. 1988; Havey and Muehlbauer 1989). RFLPs can be detected easily in *Brassica,* even among cultivars within morphotypes (Figdore et al. 1988; Song et al. 1988 a, b). The majority of the probes tested in diploid sources of *Brassica* hybridized to multiple restriction fragments, consistent with the previous evidence of sequence duplication within *Brassica* diploid species. Information on the linkage arrangement of these markers would be useful for future genetic studies and for applications in plant breeding (Burr et al. 1983), and this information might also provide further insight on genome structure and evolution in *Brassica.* In the study reported here, we determined the linkage arrangement of 258 RFLP markers in *B. oleracea.* We report on the first detailed linkage map of this species and on the genome organization of duplicated RFLP loci.

Materials and methods

Plant materials and population generation

Genomic DNA libraries were prepared from lyophilized leaf tissue of three *Brassica* sources, "Early White" cauliflower (*B. oleracea* ssp. *botrytis*), "Wisconsin Golden Acres" cabbage (*B. oleracea* ssp. *capitata*), and "WR 70 Days" chinese cabbage (*B. campestris* ssp. *pekinensis*). Genomic DNA clones derived from three different subspecies of *Brassica* were used as probes to enhance the likelihood of detecting genetic rearrangements such as deletions. Genomic DNA was digested with the endonuclease restriction enzyme PstI (BRL), and fragments of 500–2000 base pairs were subcloned into the plasmid pUC19 or pTZ18R; the resulting recombinants were selected based on hybridization to low-copy-number sequences in the *Brassica* genome, as previously described (Figdore et al. 1988).

Plant DNA isolation, blot preparation, and hybridization conditions

Procedures for the preparation of plant total genomic DNA, digestion by endonuclease restriction enzymes, agarose gel electrophoresis, Southern hybridization, nick translation of probes and for the prehybridization, hybridization, washing, and autoradiography of blots have been previously described (Helentjaris and Gesteland 1983; Helentjaris et al. 1985, 1986; Osborn et al. 1987). Nylon membrane, obtained from MSI and Schleicher and Schuell, was used as the blotting matrix. Hybridizations with "Early White"-derived probe sequences were conducted at 60 °C, and final washes were carried out at stringency conditions of 0.25 × SSC (0.037 *M* NaCl, 3.75 m*M* Na-Citrate), 0.1% sodium dodecyl sulfate, 60 °C. Hybridizations with "Wisconsin Golden Acres" and "WR 70 Days" clones were at 65 °C, and final washes were in 0.1% sodium dodecyl sulfate and 0.1 × SSC at 65 °C.

Plant materials, identification of informative probes, nomenclature, and linkage analysis

The population used to map cloned sequences consisted of 96 F_2 individuals, which were the bud self-pollinated progeny of a single F_1 individual derived from a cross of "Packman" broccoli (*B. oleracea* var. *italica*) and "Wisconsin Golden Acres" cabbage (*B. oleracea* var. *capitata*). Plant DNA digested with EcoRI was used predominantly in the detection and mapping of RFLP loci, although HindIII-digested DNA was occasionally used. Probe-enzyme combinations which revealed polymorphisms between "Packman" and "Wisconsin Golden Acres" were identified as previously described (Figdore et al. 1988). A sample of DNA from the F_1 plant used to generate the segregating F_2 population was included in the screening process, as the parental materials were often observed to be heterozygous for similar or identical alleles that subsequently segregated in an informative fashion. Most of the cloned sequences from the "Wisconsin Golden Acres" and "WR70 Days" libraries were selectively chosen for inclusion in this mapping effort, based upon the relative simplicity of resulting hybridization patterns and the anticipated ease of future analyses of the detected loci in a wide range of *Brassica* germ plasm. One hundred and ninety-seven informative probes were hybridized to a set of 96 F_2 individuals, and frequency data for the homozygous "Packman", heterozygous, and homozygous "Wisconsin Golden Acres" genotypic classes were compiled for each RFLP locus. Goodness of fit to a 1:2:1 ratio was calculated for each locus by Chi-square analysis, and linkage relationships for all pairs of loci were determined by maximum likelihood using a computer program provided by D. Hoisington (University of Missouri). RFLP loci were ordered in linkage groups based on recombination values and comparison of genotype scores. In a number of cases, the segregation patterns of different clones could not be distinguished by any recombination events within the population of 96 F_2 individuals, and these RFLP loci were placed at identical positions on the linkage map. Multiple loci detected by a common probe share a common numerical designation, followed by a single letter designation (a, b, c, d, etc.).

Results

Description of the linkage map

Segregation data were collected and analyzed for 258 RFLP loci detected by hybridization with 197 cloned sequences. Allelic segregation for most of the loci (93.5%, $p > 0.05$) fit the 1:2:1 ratio expected for an F_2 population (Fig. 1). Based on recombination frequencies, all 258 loci were arranged into 9 linkage groups. The total linkage distance for this map is 820 recombination units, with an average interval of 3.5 units between adjacent loci and a maximum interval of 19 units.

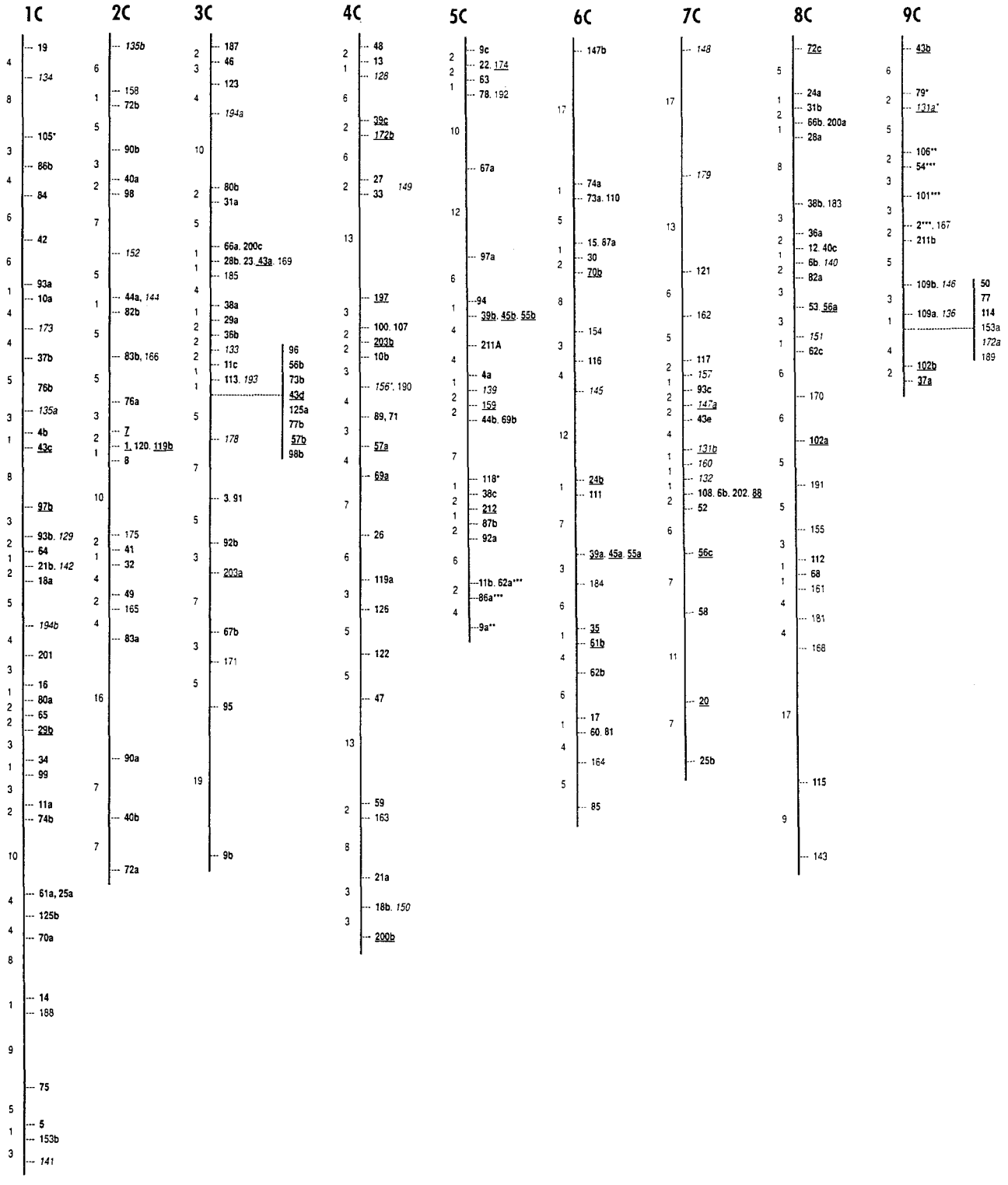

Fig. 1. RFLP linkage map of *B. oleracea*. *Vertical lines* denote the linkage group with percentage recombination indicated to the left. RFLP loci nomenclature is as described in the "Materials and methods". Loci detected by clones from "Early White", "Wisconsin Golden Acres", and "WR 70 Days" libraries are indicated with bold, italicized, and plain face type, respectively. Loci that were mapped based on the segregation pattern of a single band are *underlined*. Loci with segregation ratios that deviated from the expected 1:2:1 ratio are indicated with * ($0.05 < p < 0.02$), ** ($0.02 < p < 0.01$), and *** ($p < 0.01$)

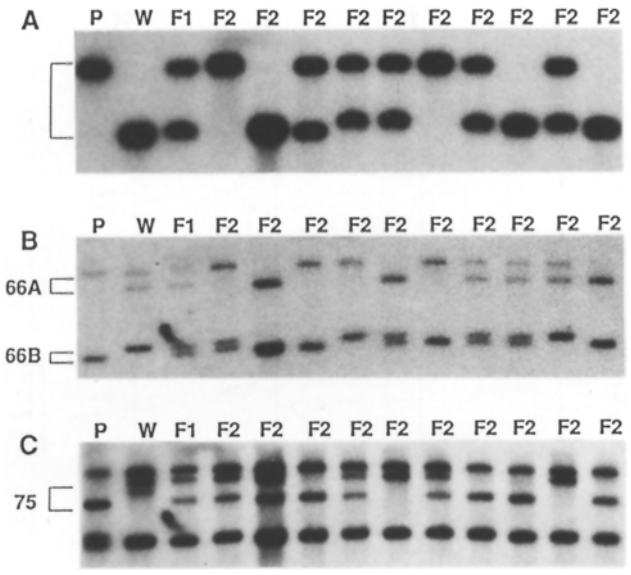

Fig. 2A–C. Hybridization patterns of three types of probes, as detected by autoradiography. Southern blots of EcoR1-digested total genomic DNA from the parental accessions "Packman" (P), "Wisconsin Golden Acres" (W), the F_1 offspring, and single F_2 plants were probed with cloned sequences hybridizing to **A** a single locus, *3*; **B** two unlinked loci, *66a* nd *66b*; and **C** to locus *75* and additional monomorphic bands

Several classes of hybridization patterns were observed in the F_2 population. These patterns could be summarized best for the subset of "Early White" cloned sequences used in this study. A minority of the informative clones from the "Early White" library (10/133, 7.5%) hybridized to single parental fragments that were polymorphic and segregated as single loci in the F_2 population (Fig. 2A). Typically, clones hybridized to more than two restriction fragments in the F_2 population. There were 76 clones (56%) for which segregation of a single locus was followed, although additional monomorphic fragments were observed in the F_2 population (Fig. 2C). Some of these monomorphic fragments were polymorphic when the genomic DNA was digested with an alternate enzyme. However, in this study single probe-enzyme combinations were used to map loci; thus, the level of mapped duplication underestimates the total level of sequence duplication. Segregation patterns of fragments corresponding to more than one locus could be analyzed for 47 of the 133 clones (35%), and the location of all the hybridizing multiple loci could be determined for 28 of these 47 clones (21% of the total clones) (Fig. 2B). We observed differences in hybridization signal intensity among nonallelic bands within some of the complex hybridization patterns, possibly due to

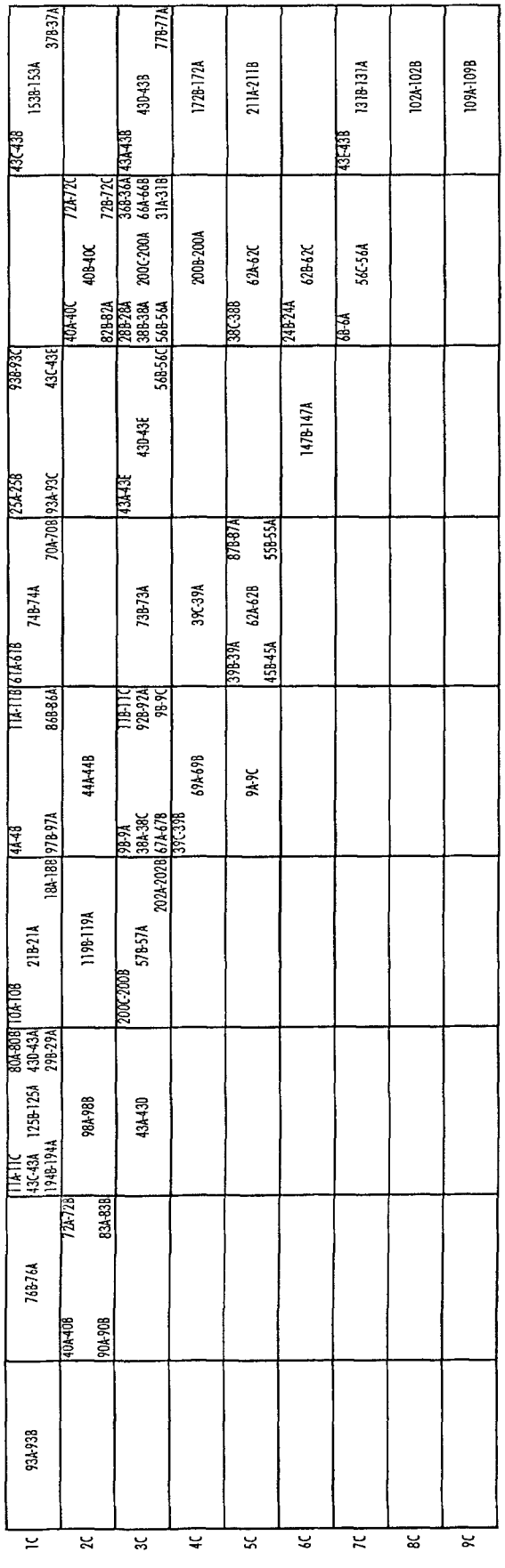

Fig. 3. The distribution of duplicated loci among the *B. oleracea* linkage groups. Pairwise comparisons of the linkage group assignments of mapped, duplicate loci are shown

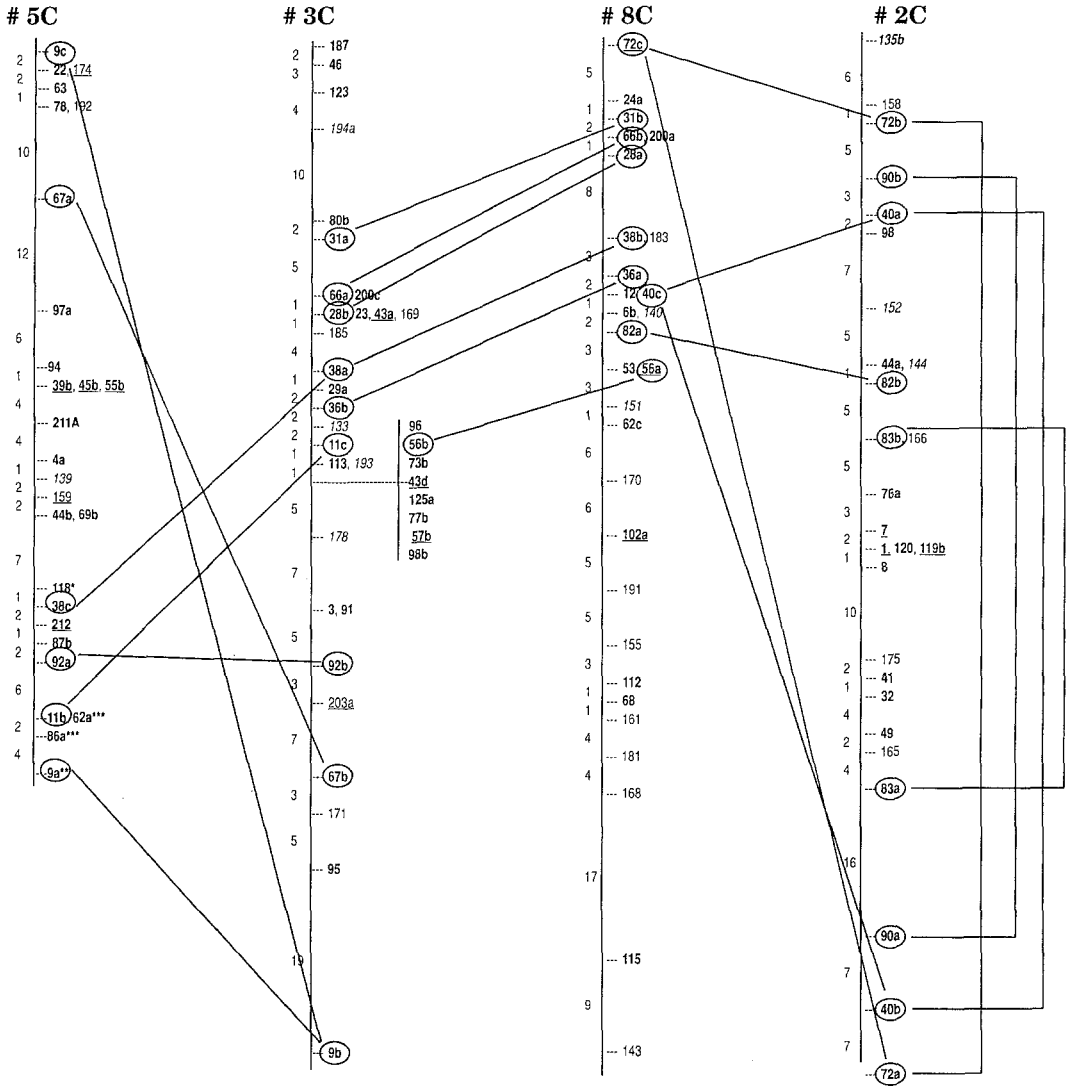

Fig. 4. A comparison of the linkage arrangement of some duplicated loci on linkage groups 2C, 3C, 5C, and 8C. *Solid lines* are drawn between duplicated loci having some degree of conserved linkage arrangement among these linkage groups

divergent copies of the cloned sequences at different sites in the genome. Multiple loci detected by a single clone will be referred to as duplicated loci, although more than two loci were detected with some clones.

Linkage arrangement of duplicated loci

Duplicated loci detected by eight clones were mapped as non-adjacent loci within linkage groups, and four of these pairs of duplicated loci were mapped within linkage group 2C (Fig. 3). Most duplicated loci (91%) were mapped to different linkage groups. These duplicated loci were detected between most pairs of linkage groups, although more duplicated loci were observed for some pairs of linkage groups than others (Fig. 3). Most notably, loci in linkage group 3C were frequently detected by cloned sequences that also hybridized to polymorphic loci in other linkage groups, often 1C, 5C, or 8C. Conservation, as well as rearrangement, of the linkage order of duplicated loci was observed among linkage groups. For example, the relative arrangement of six loci was conserved between linkage groups 3C and 8C (Fig. 4). Five loci in linkage group 3C were detected by the same clones as loci in linkage group 5C, but the linkage order of these markers differed (Fig. 4). Differences in recombination frequencies were sometimes observed in regions of linkage groups having conserved orders of duplicated loci (Fig. 4), and single copy loci were interspersed among duplicated loci. These data indicate that the *B. oleracea* genome includes large regions of duplications, although rearrangements may have occurred within some of these regions after they were duplicated.

Genome rearrangement among the parental accessions

The hybridization patterns and linkage arrangement of some clones suggested the presence of insertion or deletion rearrangements among the two parental accessions used in this study. Forty-one loci were scored as null for either a "Packman" or a "Wisconsin Golden Acres" allele (Fig. 1). Signal intensity differences in the hybridization patterns permitted discrimination between the hemizygous and hybridizing homozygous classes. The presence of monomorphic bands in the hybridization patterns frequently made it difficult to eliminate the possible existence of allelic restriction fragments for these loci. Null allele hybridization patterns could be explained by small undetected restriction fragments or weak hybridization of probes to diverged sequences. However, loci scored as having null alleles from the same parent often mapped in clusters, and these loci could not be differentiated by recombination within the F_2 population

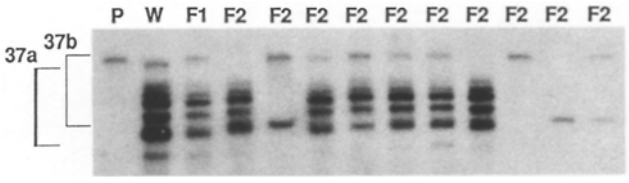

Fig. 5. Segregation analysis of a clone that detects genetic rearrangement between *B. oleracea* accessions. An "Early White" clone was hybridized to genomic DNA, as in Fig. 2. Segregation of the two parental restriction fragments was scored to map locus *37b*. Multiple cosegregating restriction fragments were observed in the "Wisconsin Golden Acres" parent, but were absent in the "Packman parent, and locus *37a* was mapped based on the segregation of signal intensity differences in F_2 plants

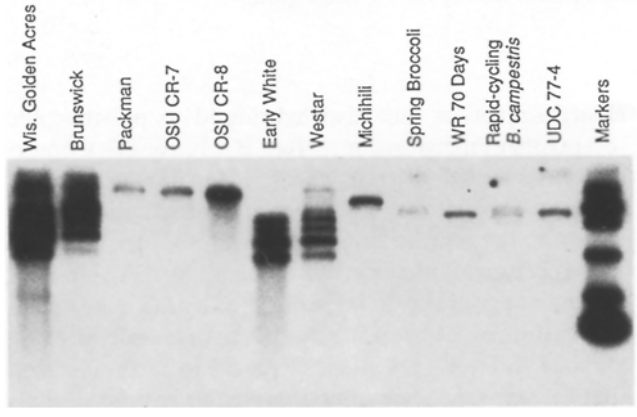

Fig. 6. Evidence of the polymorphic nature of a sequence rearrangement in various *Brassica* accessions. Polymorphism for the presence and absence of multiple alleles previously identified as locus *37a* is observed in an autoradiograph, following hybridization of the corresponding probe to a Southern blot of EcoR1-digested total genomic DNA from several *B. oleracea* accessions (*lanes 1–6*), *B. napus* (*lane 7*), and *B. campestris* (*lanes 8–12*)

(2C, 3C, 5C, 6C) (Fig. 1), suggesting the possible existence of large deletion or insertion rearrangements in these regions.

Evidence of genetic rearrangement among the parental accessions was supplied by the hybridization pattern of the clone corresponding to loci *37a* and *37b*. Two restriction fragments segregated as alleles of locus *37b* (1C) (Fig. 5). Multiple restriction fragments present in the "Wisconsin Golden Acres" parent, but absent in the "Packman" parent, cosegregated (Fig. 5) and were mapped as locus *37a* at the terminus of linkage group 9C (Fig. 1). Polymorphism for the presence or absence of this repeated array of restriction fragments was observed in a wide range of *Brassica* accessions (Fig. 6). Broccoli accessions varied for the presence or absence of this repeated structure, but this sequence was not detected in five different *B. campestris* accessions examined. This array of sequences on 9C may have evolved by duplication of a sequence from the site on 1C, followed by amplification. Possible amplification-deletion events resulting from recombination among sequences at *37a* were not detected in F_2 individuals under our conditions. Locus *37a* was the only example we found of closely linked duplicated sequences. However, some of the other loci we have mapped to single locations may represent tandemly oriented sequences having the same restriction fragment size.

Discussion

Despite the importance of *B. oleracea* and the existence of many morphological and isozyme markers, there has not been a concerted effort to develop a detailed linkage map of this species. We have taken advantage of the high degree of restriction fragment length polymorphism to develop the first detailed linkage map of *B. oleracea*. All 258 loci analyzed were arranged into nine linkage groups, which probably represent the nine haploid chromosomes of *B. oleracea*. The population we analyzed did not segregate for single gene morphological markers, nor were isozymes scored in this study. The *B. oleracea* linkage map would be enhanced by future efforts to map these types of markers with respect to RFLP markers and to assign markers to chromosomes using chromosome addition lines that are being developed (Quiros et al. 1987). Even without this information, RFLP markers have many potential applications in genetic studies and breeding, and examination of the linkage arrangement of these loci has provided additional information on the genome organization of this species.

Hybridization of cloned DNAs to multiple restriction fragments provided evidence that a high degree of sequence duplication exists in *B. oleracea*, with only a portion of this present as RFLPs amenable to the segrega-

tion analyses conducted in this study. Using a similarly constructed and screened library of clones, duplicated sequences were detected in maize, but the extent of sequence duplication was lower than that detected in *B. oleracea* (Helentjaris et al. 1988). Lower levels of sequence duplication have also been reported in RFLP analyses of other plant species, including rice, tomato, pepper, potato, lettuce, lentil, and Arabidopsis (Bernatsky and Tanksley 1986; Helentjaris et al. 1986; Landry et al. 1987; Bonierbale et al. 1988; Chang et al. 1988; McCouch et al. 1988; Tanksley et al. 1988; Havey and Muehlbauer 1989). These different findings might in some cases reflect technical differences in the analyses, but more likely they reflect differences in the genome structure of these species as compared to *B. oleracea*.

A variety of linkage arrangements of duplicated loci was observed in this study. We obtained evidence for tandem duplication of a sequence, and some loci were duplicated in non-adjacent positions within linkage groups. In several regions of the genome, we detected strong conservation of the linkage arrangement of duplicated loci on different linkage groups. Higher levels of replication of linked loci also were detected, with linkage of some loci being detected in triplicate [*40a–72b* (2C), *40b–72a* (2C), and *40c–72c* (8C)]. In many regions of the map, conservation of the linkage arrangement of duplicated loci was not detected. Many loci did not appear to be part of larger duplicated regions, although such linkage arrangements might exist and may not have been mapped in this population due to the lack of polymorphic restriction fragments. Lack of conservation of the linkage arrangement of duplicated loci was observed for several clones that lacks monomorphic bands [loci *92a* and *62a* (5C), *92b* (3C), *62b* (6C)].

Unlinked duplications have been detected in other plant species, and the mechanism for their generation is unknown (Tanksley and Pichersky 1988). The dispersed duplicated arrangement of some *B. oleracea* loci pairs seems to reflect the genetic rearrangement of relatively large blocks of sequence. Loci *80a, 80b*, and *66a* and *66b* were mapped as duplicated loci from hybridization patterns lacking monomorphic bands. Although the linkage arrangement of *80b* and *66a* (3C) is not conserved for the duplicated loci *80a* (1C) and *66b* (8C), the linkage arrangement of flanking duplicated loci is conserved [*80a–194b* (1C) and *80b–194a* (3C); *31a–66a* (3C), and *31b–66b* (8C)].

While our observations of blocks of duplicated RFLP loci are consistent with the existence of a lower chromosome number progenitor in the evolution of the *B. oleracea,* we did not find evidence of duplication throughout entire pairs of linkage groups. The overall arrangement of duplicated loci suggests that extensive genome rearrangement and chromosome restructuring has occurred during the evolution of *B. oleracea,* making it difficult to identify the possible structure of a lower chromosome number progenitor or to determine if *B. oleracea* evolved initially by duplication of entire linkage groups. Duplicated segments of chromosomes also could have evolved through the mechanism described by Gottlieb (1983), which involves crosses of individuals with overlapping reciprocal translocations followed by mating of individuals from similar crosses. Reciprocal translocations have been observed in some accessions of *B. oleracea* (Gustafsson et al. 1976; Quiros et al. 1988) and they may be widespread in this species. Repeated crosses of reciprocal translocation lines over time could have resulted in some of the duplicated regions observed in *B. oleracea.* Other mechanisms also may have given rise to some of the duplications observed in this study. For example, within linkage group 2C, four loci duplicated in nearly reverse order at opposite ends of the linkage group may represent the remnants of an isochromosome that has evolved to include a paracentric inversion. This arrangement also could have arisen by a recombination or translocation event involving sequences in 8C.

The regions of duplicated loci reported in this study could in part explain previous observations of multivalents during meiosis in *B. oleracea* (Gustafsson et al. 1976; Sampson 1970; Wills 1966). Variation in the frequency of observed multivalent might be due to some small regions of duplications that cause weak and variable associations in different genetic backgrounds. The types of insertion-deletion rearrangements we have proposed among the broccoli and cabbage parents used in this study might be widespread within *B. oleracea,* possibly accounting for variable frequencies of multivalents observed in different genetic stocks (Gustafsson et al. 1976; Sampson 1970). In addition to duplicated regions, other genetic factors may play a role in autosyndetic pairing.

Genetic duplication, in combination with mutational changes, has been regarded as a mechanism for gene evolution and differential gene expression (Ohno 1970). Differential hybridization of some clones to bands within complex hybridization patterns support the existence of divergent duplicated sequences within the *B. oleracea* genome. The presence of some degree of functional duplication, however, would be consistent with the observation of complex inheritance patterns associated with many traits in *Brassica* (Yarnell 1956). Additional mapping using cDNA clones and examination of trait-linkage associations in relation to duplicated regions should provide more information on the possible functionality of duplicated regions.

The high level of restriction fragment length polymorphism that exists between various accessions of *Brassica* will permit the use of many of the probes from this study in comparative examinations of linkage data and genome structure in other accessions, subspecies, and

species of *Brassica,* and perhaps more distantly related species. The ability to form fertile hybrids between many members of the genus and observations of chromosome pairing in interspecific hybrids suggest that similarity in chromosome structure must exist among germ plasm sources (Yarnell 1956; Armstrong and Keller 1982; Quiros et al. 1988). There are also obvious differences in genome structure, such as differences in chromosome number, which exist between species. Further studies using these molecular markers will help define the structural similarities and differences.

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References

Armstrong KC, Keller WK (1982) Chromosome pairing in haploids of *Brassica oleracea.* Can J Genet Cytol 24:735–739

Arus P, Orton TJ (1983) Inheritance and linkage relationships of isozyme loci in *Brassica oleracea.* J Hered 74:405–412

Bernatsky R, Tanksley S (1986) Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics 112:887–898

Bonierbale MW, Plaisted RL, Tanksley SD (1988) RFLP maps based on a common set of clones reveals modes of chromosomal evolution in potato and tomato. Genetics 120:1095–1103

Burr B, Evola SV, Burr FA, Beckmann JS (1983) The application of restriction fragment length polymorphism to plant breeding. In: Setlow J, Hollaender A (eds) Genetic engineering: principles and methods, Vol 5. Plenum Press, New York, pp 45–59

Chang C, Bowman J, DeJohn J, Lander E, Meyerowitz E (1988) Restriction fragment length polymorphism linkage map for *Arabidopsis thaliana.* Proc Natl Acad Sci USA 85:6856–6860

Figdore SS, Kennard WC, Song KM, Slocum MK, Osborn TC (1988) Assessment of the degree of restriction fragment length polymorphism in *Brassica.* Theor Appl Genet 75:833–940

Gottlieb LD (1983) Isozyme number and phylogeny. In: Jensen U, Fairbrothers DE (eds) Proteins and nucleic acids in plant systemics. Springer, Berlin Heidelberg New York pp 209–221 (Proceedings life sciences)

Gustafsson M, Bentzer B, Bothmer R von, Snogerup S (1976) Meiosis in Greek *Brassica* of the *oleracea* group. Bot Not 129:73–84

Haga T (1938) Relationships of genome to secondary pairing in *Brassica.* Jpn J Genet 13:277–284

Havey MJ, Muehlbauer FJ (1989) Linkages between restriction fragment length, isozyme, and morphological markers in lentil. Theor Appl Genet 77:395–401

Helentjaris T, Gesteland R (1983) Evaluation of random cDNA clones as probes for human restriction fragment polymorphisms. J Mol Appl Genet 2:237–247

Helentjaris T, King G, Slocum M, Siedenstrang C, Wegman S (1985) Restriction fragment polymorphisms as probes for plant diversity and their development as tools for applied plant breeding. Plant Mol Biol 5:109–118

Helentjaris T, Slocum M, Wright S, Schaefer A, Nienhuis J (1986) Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theor Appl Genet 72:761–766

Helentjaris T, Weber D, Wright S (1988) Identification of the genomic locations of duplicated sequences in maize by analysis of restriction fragment length polymorphisms. Genetics 118:353–363

Landry BS, Kesseli RV, Farrara B, Michelmore R (1987) A genetic map of lettuce (*Lactuca sativa* L.) with restriction fragment length polymorphism, isozyme, disease resistance and morphological markers. Genetics 116:331–337

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

Ohno S (1970) Evolution by gene duplication. Springer, Berlin Heidelberg New York

Osborn TC, Alexander DC, Fobes JF (1987) Identification of restriction fragment length polymorphisms linked to genes controlling soluble solids content in tomato fruit. Theor Appl Genet 73:350–356

Prakash S, Hinata K (1980) Taxonomy, cytogenetics, and origin of crop *Brassica,* a review. Opera Bot 55:1–59

Quiros CF, Ochoa O, Kianian SF, Douches D (1987) Analysis of the *Brassica oleracea* genome by the generation of *B. campestris-oleracea* chromosome addition lines: characterization by isozyme and rDNA genes. Theor Appl Genet 74:758–766

Quiros CF, Ochoa O. Douches D (1988) Exploring the role of x=7 species in *Brassica* evolution: Hybridization with *B. nigra* and *B. oleracea.* J Hered 79:351–358

Robbelen G (1960) Beiträge zur Analyse des *Brassica*-Genomes. Chromosoma 2:205–228

Sampson DR (1978) A second gene for hairs in *B. oleracea* and its tentative location in linkage group four. Can J Genet Cytol 20:101–109

Song KM, Osborn TC, Williams PH (1988a) *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). 1. Genome evolution of the diploid and amphidiploid species. Theor Appl Genet 75:784–794

Song KM, Osborn TC, Williams PH (1988b) *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). 2. Preliminary analysis of subspecies within *B. rapa* (syn. *campestris*) and *B. oleracea.* Theor Appl Genet 76:593–600

Tanksley SD, Pichersky E (1988) Organization and evolution of sequence in the plant nuclear genome. In: Gottlieb LD, Jain SK (eds) Plant evolutionary biology. Chapman and Hall, New York, pp 55–83

Tanksley SD, Bernatsky R, Lapitan NL, Prince JP (1988) Conservation of gene repertoire but not gene order in pepper and tomato. Proc Natl Acad Sci USA 85:6419–6423

Wills AB (1977) A preliminary gene list in *Brassica oleracea.* Eucarpia Cruciferae News 2:22–24

Yarnell SH (1956) Cytogenetics of the vegetable crops. II. Crucifers. Bot Rev 22:81–166