Theor Appl Genet (1992) 84: 544–554

THEORETICAL AND APPLIED GENETICS

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Generation of a *Brassica oleracea* composite RFLP map: linkage arrangements among various populations and evolutionary implications

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Received November 21, 1991; Accepted January 10, 1992
Communicated by G. Wenzel

Summary. A composite linkage map of *Brassica oleracea* was developed from maps of four different populations, derived from 108 DNA, isozyme and morphological loci covering over 747 centimorgans in 11 linkage groups. Of these linkage groups, 8 were assigned to their respective chromosomes by alignment with gene synteny groups of *B. oleracea*. Distortions in segregation ratios increased with the level of divergence of the parents and were attributed to differentiation of parental chromosomes. Comparison of the individual maps demonstrates that the *B. oleracea* genome undergoes frequent chromosomal rearrangement, even at the subspecies level. Small inversions were the most frequent form of aberration followed by translocations. The former type of aberration could occur without a noticeable effect on meiotic behavior of chromosomes or on pollen fertility. The obvious deduction from the composite map is that a large fraction of the *B. oleracea* genome is duplicated, falling into three classes: randomly dispersed, linked-gene families, and blocks duplicated in non-homologous chromosomes. The genealogy of chromosomes sharing duplicated segments was formulated and indicates that *B. oleracea* is a secondary polyploid species derived from ancestral genome(s) of fewer chromosomes.

Key words: *Brassica oleracea* – Composite RFLP map – Multi-populational – Genetic distortions – Sequence duplications

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Introduction

Molecular linkage maps based on cloned DNA sequences and relying on variations in restriction fragment lengths have been developed for many crop species (Tanksley et al. 1987). In order to maximize polymorphisms, often it is necessary to create interspecific populations (Bernatzky and Tanksley 1986; Helentjaris et al. 1988; Havey and Muehlbauer 1989; Paterson et al. 1990). The inherent problems in such maps are segregation distortions and non-representative genetic distances and linkage relationships. Distortions from expected monogenic segregation ratios in progenies from intra- and interspecific crosses are well documented in several plant species (Grant 1975; Rick 1969). Similarly, reduced recombination is often present in these crosses (Rick 1972; Douches and Quiros 1988; Paterson et al. 1990). Altered linkage relationships may occur when chromosomal rearrangements are present in the species used (Burnham 1962). At the intraspecific level, distortions in linkage maps are also possible due to minor chromosomal rearrangements that have little effect on fertility or chromosome pairing in meiosis, such as those observed in a cross of broccoli by cabbage (Slocum et al. 1990). Ideally, to circumvent these problems, genetic maps should be developed in a cytogenetically well characterized species. Thus, before an attempt can be made to apply maps developed from a single cross to other intra- or interspecific progenies, their limitations should be established.

The inheritance of various morphological traits in *Brassica oleracea* has been described and some linkage associations established (Yarnell 1956; Sampson 1966). In recent years, molecular markers such as isozymes and DNA restriction fragment length polymorphism (RFLP) markers have revolutionized the mapping process. In conjunction with traditional cytogenetic techniques,

morphological, isozyme and RFLP markers were located on chromosomes of *B. oleracea* employing *B. campestris-oleracea* addition lines (Quiros et al. 1987; McGrath et al. 1990). These syntenic associations among markers provide important evolutionary information while expediting the mapping process. Recently, RFLP markers were used to generate a single-population linkage map of this species defining nine linkage groups and covering 820 recombination units (Slocum et al. 1990). The overall conclusion of these studies is that a major portion of the genome is duplicated, supporting the hypothesis that *B. oleracea* is a secondary polyploid (Quiros et al. 1987, 1988; McGrath et al. 1990; Slocum et al. 1990).

The present report addresses the problems associated with the generation of RFLP maps from a single population by comparing linkage maps generated from three intraspecific and one interspecific populations. Our objectives were to determine distortions in segregation ratios, linkage relationships and gene distances, as well as the extent of gene and genome duplication and its evolutionary implication. Finally, a composite RFLP map is generated by alignment of common markers from the individual maps.

Materials and methods

Plant material

Three intraspecific hybrids of *B. oleracea* were used to create the F_2 populations segregating for RFLP loci: collard (B115) × cauliflower (B265) (C × CA), collard (B115) × broccoli (B008) (C × B), and wild kale from USSR (B1661) × cauliflower (B265) (U × CA). A fourth F_2 family was derived from the interspecific hybrid *B. oleracea* kohlrabi (B255) × *B. insularis* (B364) (K × I). At least 60 plants per intraspecific, and 52 F_2 plants per interspecific cross were grown and scored for segregation of RFLP loci. The hybrids and their progenies were grown under standard greenhouse conditions.

Isozyme and morphological analysis

Horizontal starch gel electrophoresis was performed for the isozyme analysis (Quiros et al. 1987). The following enzymes were assayed: leucine amino peptidase (LAP), phosphoglucoisomerase (PGI), triose phosphate isomerase (TPI) and 6-phosphogluconate dehydrogenase (6-PGDH). Annual habit (An), presence of enlarged stem (bulb, B), anthocyanin pigmentation (purple color, P) and glossy foliage (gl-1) were the obvious morphological traits that could be scored as simply inherited characteristics (Yarnell 1956, Sampson 1966).

DNA extraction

Total cellular DNA was isolated from 5–10 g of fresh tissue by modification of the Fisher and Goldberg (1982) method. The tissue was ground to a fine powder by use of mortar and pestle with the aid of liquid nitrogen and sand as an abrasive. The powder was blended with 50 ml of ice-cold homogenization buffer (4 m*M* spermidine, 1 m*M* spermine, 10 m*M* EDTA, 10 m*M* Tris, 80 m*M* KCl, 500 m*M* sucrose, 0.2% 2-mercaptoethanol, pH 9.5) for 30–45 s at the highest speed. The homogenate was filtered through four layers of cheesecloth and one layer of Miracloth (Calbiochem) into a 50-ml centrifuge tube. Tubes were centrifuged at 3,000 g, 4 °C for 30 min. The pellet was resuspended in 7 ml of ice-cold lysis buffer (100 m*M* Tris, 400 m*M* EDTA, 6% sodium sarcosyl, pH 9.5) with a small paint brush. Immediately afterward 12.6 g CsCl was added, and the tubes were incubated at 60 °C for 30 min with occasional gentle swirling. After the CsCl had dissolved, tubes were centrifuged at 17,480 *g* for 1 h at room temperature. The supernatant was filtered through two layers of cheesecloth into a 13-ml Quickseal tube (Beckman) containing 150 µl of 10 mg/ml ethidium bromide. Tubes were brought up to volume with 1 × TE (10 m*M* TRIS HCl pH 8.0, 1 m*M* EDTA) giving a final CsCl concentration of 0.97 g/ml. After 18–24 h of ultracentrifugation at 330,624 *g* (60 krpm) in a 70.1 Ti rotor, the DNA band was extracted with a 16-gauge needle. DNA was recovered using standard procedures (Maniatis et al. 1982). Yields of 50 µg DNA/g of leaf were routine, and A_{260}/A_{280} ratios approximated 1.8. Size of the DNA was larger than 50 kb, compared with undigested lambda standards. DNA isolated by this method was essentially free of the contaminating polysaccharides that are troublesome in this group of species.

Detection of RFLP variants

Five to 7 µg of DNA was digested to completion with 40 units of *Eco*RI (BRL) for 16 h at 37 °C. All mapping utilized the restriction endonuclease *Eco*RI, since this enzyme is known to uncover useful levels of polymorphism within *B. oleracea* (Figdore et al. 1988). After electrophoresis on 1% agarose gels with 1 × TAE (40 m*M* TRIS-acetate, 1 m*M* EDTA) as the running buffer for 16 h at 30 volts, DNA was transferred onto Hybond membranes (Amersham) by the suggested alkaline blotting procedure. Hybridization with probes, labeled by the oligonucleotide labeling method of Feinberg and Vogelstein (1983), and subsequent washes were carried out as per Hosaka et al. (1990).

Probes

The majority of probes (50/61) were isolated from a cDNA library of *B. napus*, kindly provided by Dr. J. Harada (University of California, Davis, Dept of Botany). A number of these cDNA clones (11) have been shown to represent genes expressed during seed germination in a developmentally regulated manner (prefixed by pAX, pCOT, pCA, pGS and pLEA) (Harada et al. 1988). Randomly isolated clones, from the same library, prefixed p1 and p2 (6) were isolated by Dr. B. Landry (Agriculture Canada, Quebec), whereas those prefixed by pBN (33) were isolated in our laboratory. The latter clones were selected for insert size of greater than 500 base pairs (bp) corresponding to medium to low-copy number sequences as determined by dot-blot analysis (Maniatis et al. 1982). In addition, genome-specific probes (7) isolated from the *B. oleracea* and *B. napus* genomic libraries (prefixed pB) were utilized (Hosaka et al. 1990).

Linkage analysis

Linkage analysis was accomplished with the LINKAGE-1 program (Suiter et al. 1983) modified by Dr. R. Kesseli (University of California, Davis, Dept. of Vegetable Crops) to accept more loci. Segregation ratios different from expected values (significant at $P = 0.05$ or less) were classified as distorted. Recombination frequencies were converted to map distances by the mapping function described in Suzuki et al. (1989).

A composite map was generated by aligning common linkage groups identified in each population. This was possible because many loci were shared among segregating populations. Groups displaying altered gene orders were excluded, since such alterations are due to karyotypic differences in the parental lines.

Linkage distances in the composite map were formulated by averaging the values from the populations.

Results

Cytology of the F_1 hybrids

Pollen stainability as a measure of pollen fertility for the F_1 intraspecific hybrids ranged from 88% for U × CA to 92% for C × CA, while that of the interspecific hybrid (K × I) was 44.9%, a significant reduction ($\chi^2(1{:}1)=22.6$, $P<0.001$). The slight (non-significant) reduction in fertility of the U × CA population did not correlate with abnormal meiotic behavior of the chromosomes. Conversely, the reduced stainability in the K × I hybrid was associated with abnormal meiotic behavior, quadrivalents in diakinesis and metaphase I and laggards and bridges during anaphase I, in the hybrid (Fig. 1).

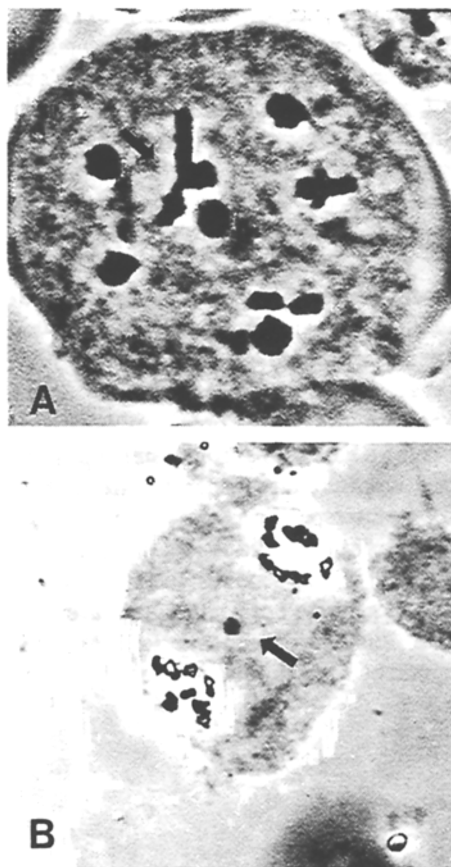

Fig. 1A, B. Diakinesis I (**A**) and anaphase I (**B**) in the intraspecific hybrid *B. oleracea* var 'kohlrabi' × *B. insularis*. *Arrows* indicate quadrivalents in diakinesis (**A**) and laggards in anaphase I (**B**) cells

Segregation of marker loci

A total of 92 loci including RFLP, isozyme and morphological characters were scored on the three intraspecific F_2 populations. A fraction of these loci were polymorphic in each family: 41 loci in C × CA (45%), 57 loci in U × CA (62%) and 58 in C × B (63%). Distorted segregation ratios were observed for 14 loci (15.2% of total), of which 7 belonged to the U × CA population (12.2% of total loci in that population), 4 to C × CA (9.7%) and 3 to C × B (5.2%). When mapped, the loci with distorted ratios in the U × CA population clustered together in 3 linkage groups while the others did not show any pattern of distribution (Fig. 2). Additionally, 2 of these loci from C × CA and 2 from C × B were unlinked. Only one of these loci, *pBN10-1* (on chromosome 7), had a distorted ratio in more than one population (C × CA and U × CA).

In the interspecific F_2 population (K × I) 71 loci were scored, of which 42 (59%) showed significant deviations from the expected Mendelian ratios. Among these distorted ratios, 30 (71.4%) were due to excess of kohlrabi alleles (maternal parent), 6 (14.3%) to an excess of *B. insularis* alleles and 6 (14.3%) were of the heterozygote type, a highly significant deviation in favor of kohlrabi, the cultivated parent, alleles ($\chi^2(1{:}1{:}1)=65.2$, $P=0.000$). These markers with distorted ratios mapped to every linkage group except for group 4 as shown in Fig. 2.

Of the 62 different probes tested only 9 (14.5%) did not detect segregation in any of the populations. The remaining 53 probes segregated in at least one of the four populations with the percentage of monomorphic probes varying for each population: 42.1% in C × B, 47.9% in C × CA, 43.9% in U × CA with an average of 44.6% in the intraspecific crosses and no significant differences among them ($\chi^2(1{:}1{:}1)=0.4$, $P>0.90$). The value of monomorphic probes dropped to 25.5% for the interspecific cross K × I, a highly significant difference when compared to the other populations ($\chi^2(1{:}1)=8.2$, $P=0.000$). The chromosomal distribution of loci for each of the four populations is presented in Table 1. Among the polymorphic probes, 20 (37.8%) disclosed RFLPs determined by a single locus, 13 by 2 loci (24.5%), 14 by 3 loci (26.4%) and 6 by 4 or more loci (11.3%). Based on the premise that these probes are a valid representation of the *B. oleracea* genome, it can be argued that about 14.5% (9/62) of the genome is highly conserved (monomorphic), 32.3% (20/62) single-copy and 53.2% (33/62) multi copy. Examples of the RFLP segregation pattern for each population are presented in Fig. 3.

Description of the linkage maps

On the basis of our population size, linkage was deemed significant if the recombination frequency was less than 20% and if this value plus the standard error was 30% or less. The linked loci could be arranged into 6 groups for the C × CA (244 cM), 7 groups for the C × B (350 cM), 8 groups for the U × CA (313 cM) and 9 groups for the

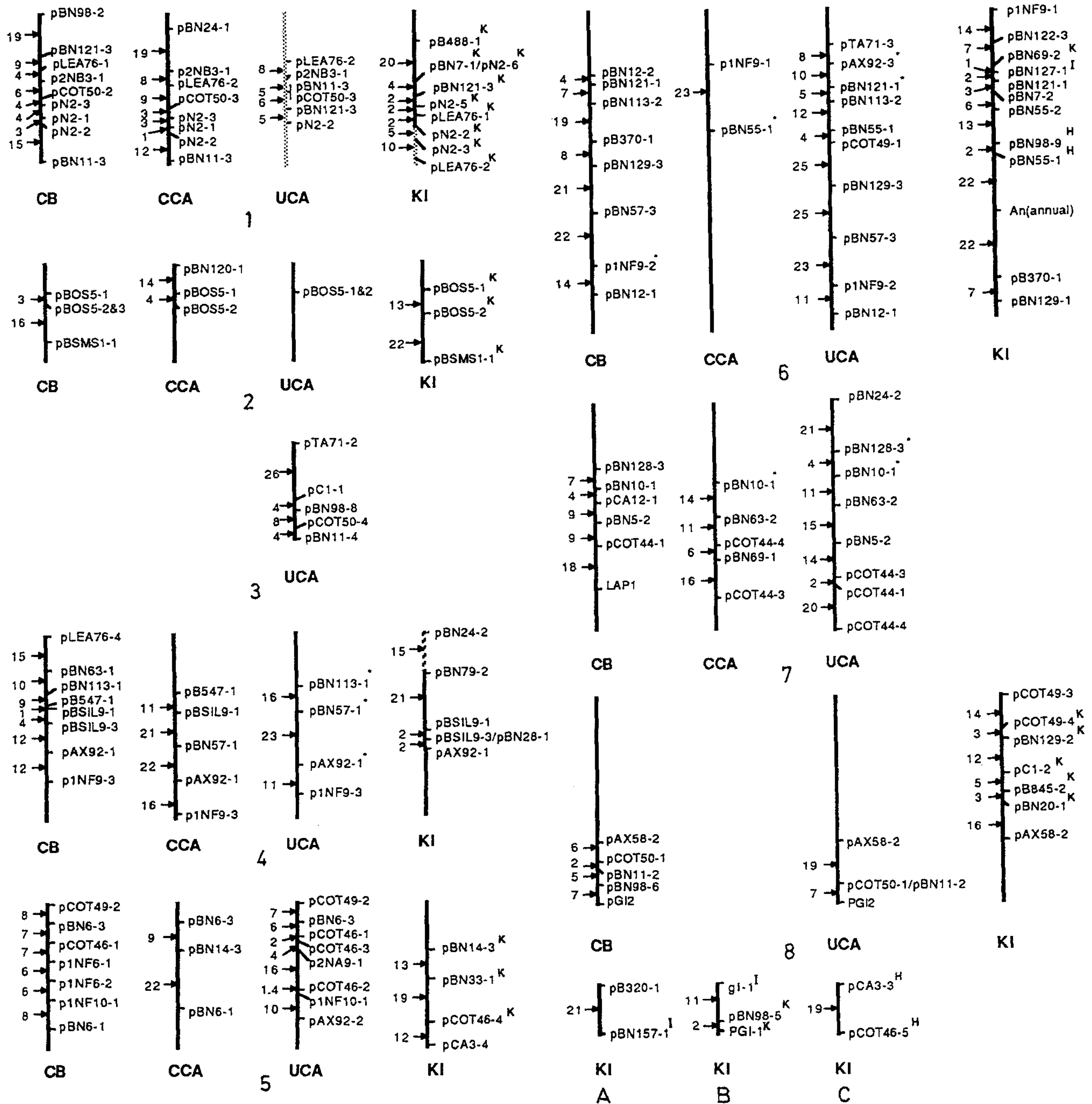

Fig. 2. Comparison of the linkage maps generated from the four populations. *CB B. oleracea* var 'collard' × broccoli, *CCA* collard × caulifower, *UCA* wild kale from USSR × cauliflower and *KI* kohlrabi × *B. insularis*. * indicates loci with distorted segregation ratios in the intraspecific population, while *K* and *I* indicate loci with preponderance of kohlrabi and *B. insularis* alleles in the *KI* population, respectively, and *H* indicates the abundance of heterozygote type. Linkage groups assigned to chromosomes are numbered accordingly (*1–8*), and unassigned groups are marked *A* through *C*. ⟶ indicates map distances in cM, ░░░ indicates inverted chromosome segments, //// indicates translocated chromosome segment

K × I (357 cM) population. These were integrated into 11 linkage groups by aligning common markers and assigned to their respective chromosomes by comparison with the previously described gene synteny groups of *B. campestris-oleracea* addition lines (McGrath et al. 1990, Fig. 2). The average interval between adjacent markers was 9.0 cM for C × CA, 7.5 cM for C × B, 6.8 cM for U × CA and 7.3 cM for K × I with ranges of 0.9–23 cM, 0–19 cM, 0–24 cM and 0–22 cM, respectively.

Genes controlling some simply inherited morphological traits of *B. oleracea* like the annual habit (*An*) and glossy foliage (*gl-1*) were located to linkage groups 6 and

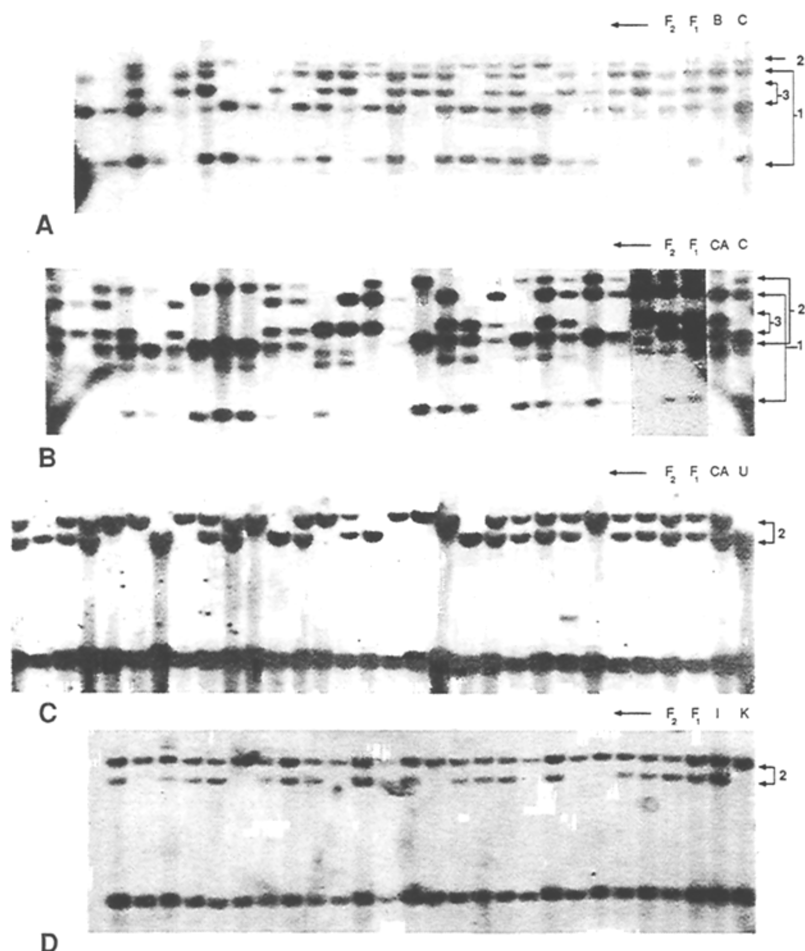

Fig. 3 A–D. Examples of complex (**A** and **B**) and simple (**C** and **D**) RFLP segregations in each of the four populations. **A, B** The restriction fragment pattern and loci assignment for probe pBN6 in the F_2 populations of **A** collard by broccoli (C × B) and **B** collard by cauliflower (C × CA). **C, D** The restriction fragment pattern and locus assignment for probe pAX58 in the F_2 populations of **C** wild kale from USSR by cauliflower (U × CA) and **D** kohlrabi by *B. insularis* (K × I). Note the distorted segregation in this latter population. The parental (*C* and *B* in **A**; *C* and *CA* in **B**; *U* and *CA* in **C**; *K* and *I* in **D**), F_1 and F_2 DNAs were digested with *Eco*RI and electrophoresed on 1% agarose gel. The parental F_1 and F_2 lanes are marked on the *top* and locus assignment is indicated on the *right side*

Table 1. Proportions of segregating loci disclosed by RFLP markers for seven chromosomes in the four populations

Chromosome	C × B	C × CA	U × CA	K × I	Total
1	9 (60%)	8 (53%)	6 (40%)	9 (60%)	15
2	4 (80%)	3 (60%)	2 (40%)	3 (60%)	5
4	8 (73%)	5 (45%)	4 (37%)	6 (54%)	11
5	7 (46%)	3 (20%)	8 (53%)	4 (27%)	15
6	8 (38%)	2 (10%)	10 (48%)	12 (57%)	21
7	6 (55%)	5 (45%)	8 (73%)	0 (0%)	11
8	5 (45%)	0 (0%)	4 (37%)	7 (64%)	11
Total	47 (53%)	26 (29%)	42 (47%)	41 (46%)	89

B, Broccoli; C, Collard; CA, cauliflower; I, *B. insularis*; K, kohlrabi

provisional group B, respectively. Two other traits, presence versus absence of bulb (*B*) and presence of anthocyanin pigmentation (*P*), did not show any significant linkage to other markers. Isozyme coding loci *Lap-1* and *Pgi-2* mapped to chromosomes 7 and 8, respectively.

Comparison of the linkage maps

Genetic maps derived from the C × B and C × CA populations were homosequential. They maintained the order of loci fully, and the differences in map units between the populations were not significant considering the standard error. On the other hand, the U × CA population displayed a rearrangement of several loci on chromosome 1 (Fig. 2). On this chromosome the order of the *pLEA76-2, p2NB3-1, pBN121-3* and *pBN11-3* loci was altered accompanied by a 50% reduction in the combined recombination frequency in that region (49 cM versus 24 cM, Fig. 2).

The major linkage rearrangements in the K × I population were a translocation of locus *pBN24-2* from chromosome 7 to chromosome 4 and an inversion involving the *pLEA76-1*, *pN2-2* and *pN2-3* loci on chromosome 1, which was accompanied by a 53% reduction in the recombination frequency (28 cM versus 15 cM, Fig. 2). Many of the markers present on chromosome 7 did not segregate in the K × I population, precluding the confirmation of reciprocity for the translocation.

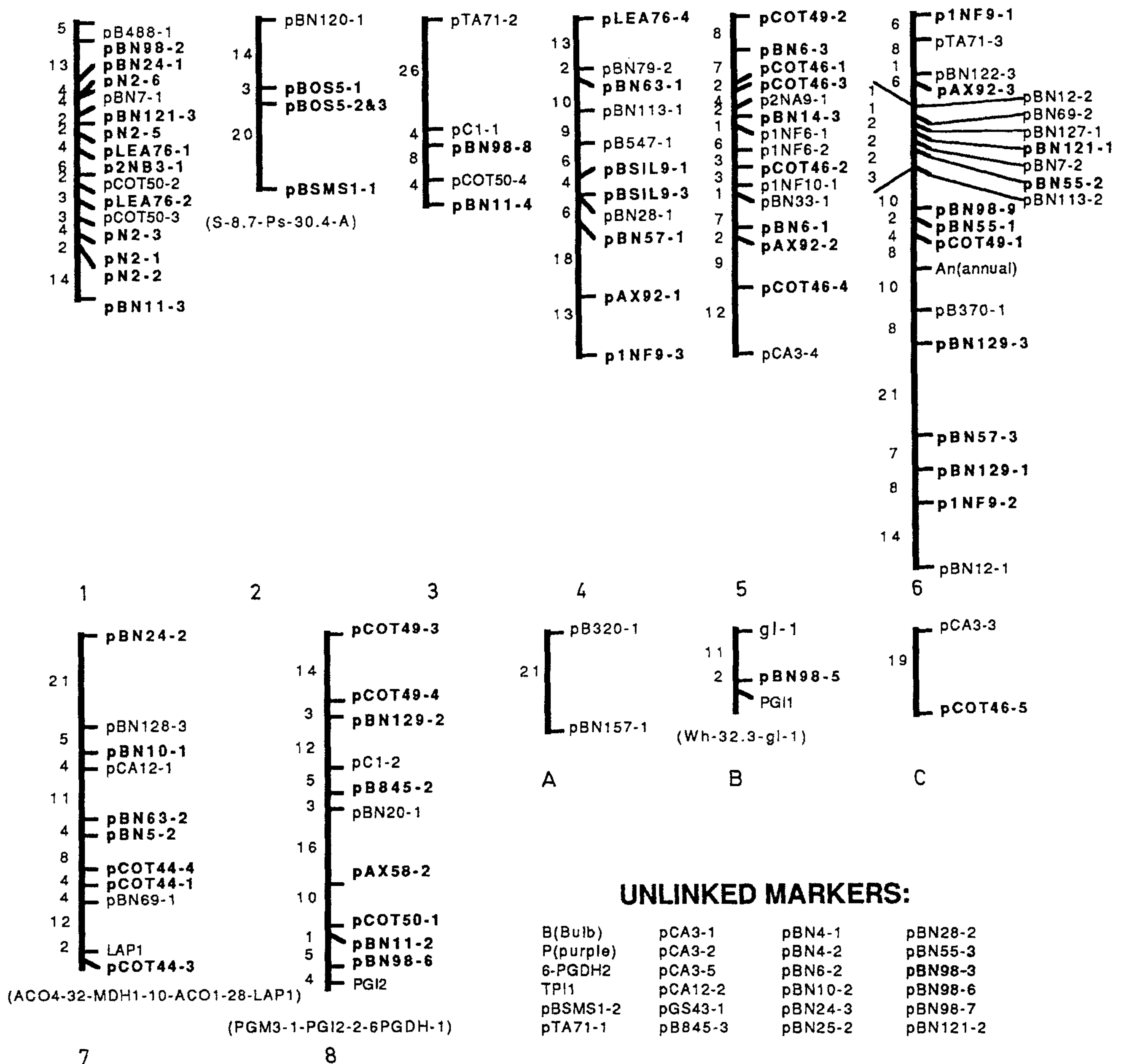

Fig. 4. The composite map of *Brassica oleracea* generated from four segregating populations (three intraspecific and one interspecific). *Bold letters* indicate markers that segregate in three or more populations. Letters *A*, *B* and *C* refer to linkage groups not assigned to chromosomes. Map units are in centiMorgans (cM)

Formulation of the composite map

Since the intraspecific populations each shared a parent (cauliflower in C × CA and U × CA; collard in C × CA and C × B) an alignment of common linkage groups was possible using shared loci (14 in C × B/C × CA, 27 in C × B/U × CA and 16 in C × CA/U × CA). Due to increased polymorphism in the interspecific population, close to 75% of the probes were polymorphic, which enabled the identification of shared loci with other populations. The comparative values for this population were 13 common loci with C × B, 10 with C × CA and 9 with U × CA.

The changes in gene order and map distances observed in the U × CA and K × I populations did not extend beyond the chromosomes mentioned. These changes could be readily rectified by comparison with other intraspecific crosses. Therefore, since there was in general a good agreement between the linkage maps generated from these four populations, they were combined to generate a fairly populated composite map of *B. oleracea* (Fig. 4). Map distances were formulated by averag-

Table 2. *Eco*RI restriction fragment sizes (in kb) for duplicated loci

Probe	Locus								
	1	2	3	4	5	6	7	8	9
pLEA76	1.6/null	2.4/3.8	–	5.5/8					
pC1	12.2/14	3.6/4.2							
pTA71	3.4/5.7	4.4/4.7	3.1/null						
pCA3	0.4/null	1.8/null	7.8/8.9	5.4/6.2	0.2/null				
pCOT46	1.8/2.5	3/5.5	1.2/null	4.4/4.7	5.9/6.1				
pCOT49	2.8/null	8/11	6.5/null	5.4/5.8					
pCOT50	1.9/3	14/null	3.4/4.8	1.1/null					
pAX92	0.2/1.6	1.9/2.8	15/18						
p1NF9	9/13	2.3/null	0.9/1.0						
pBN7	6.5/null	5.8/7.2							
pBN11	–	5.0/6.1	7.0/9.2	1.2/null					
pBN24	7.2/8	10/14	3.6/null						
pBN57	3.5/7	–	4.5/5.7	–	8/null				
pBN63	0.7/null	1.8/2.3							
pBN69	6.8/null	1.5/3.2							
pBN98	–	0.5/null	1.0/null	–	5/null	5.9/6.8	3.0/null	0.7/0.9	1.8/2.0
pBN113	0.6/7.9	8/11.5							
pBN121	1.5/3.0	9.5/null	6.0/9.0						
pBN129	0.9/null	0.6/null	1.2/6.0						

Table 3. Distribution of RFLP loci in *Brassica oleracea* genome

	Chromosome number								Unassigned groups			Total
	1	2	3	4	5	6	7	8	A	B	C	
Number of RFLP loci	16	5	5	11	15	20	10	10	2	1	2	97
Number of Duplicated RFLP loci	14	3	5	8	11	17	6	7	0	1	2	74
Duplicated RFLP loci/RFLP loci	0.88	0.60	1.00	0.73	0.73	0.85	0.60	0.70	0.0	1.00	1.00	0.76

Table 4. Distribution of duplicated loci among linkage groups

1–3	1–7	3–6	4–6	5–8	6–8
pBN98 pCOT50 pBN11	pBN24	pTA71 pBN98	p1NF9 pAX92 pBN113 pBN57	pCOT49	pCOT49 pBN129 pBN98
1–4	**1–8**	**3–8**	**4–7**	**5–C**	**6–B**
pLEA76	pBN98 pCOT50 pBN11	pC1 pBN98 pCOT50 pBN11	pBN63	pCOT46 pCA3	pBN98
1–6	**1–B**	**3–B**	**5–6**	**6–7**	**8–B**
pBN98 pBN7 pBN121	pBN98	pBN98	pAX92 pCOT49	pBN69	pBN98

Each pair of numbers or letters (i.e. 1–3 or 3–B) refer to linkage groups that share duplicated sequences. Only 18 of the 55 possible combinations shared duplicated loci

ing the values from the populations. This map contains over 108 DNA, isozyme and morphological markers covering over 747 cM in 11 linkage groups, and 26 unlinked markers. Of these linkage groups, 8 were assigned to their respective chromosomes by alignment with the previously described gene synteny groups of *B. campestris-oleracea* addition lines (McGrath et al. 1990). Certain DNA probes revealed RFLP markers that segregated in all four populations (Fig. 4).

Sequence duplications

Among the 48 mapped probes more than 27 (56%) detected sequences mapping to more than 1 locus (Table 2). The goodness of fit χ^2 test demonstrated that the number of duplicated RFLP loci per total number of RFLP loci on each chromosome showed no significant deviation from random distribution (Table 3). Therefore, the relative density of duplicated sequences is fairly uniform throughout the genome. On the other hand, the distribution of duplicated loci shared among non-homologous chromosomes is not random (Table 4). Only 18 of the 55 possible pairwise combinations of chromosomes had sequences in common and, of these, 9 shared more than one sequence; these involved linkage groups 1, 3, 4, 5, 6, 8, and C. For example, chromosomes 1, 3 and 8 shared sequences homologous to the pBN98, pCOT50 and pBN11 probes (Fig. 4).

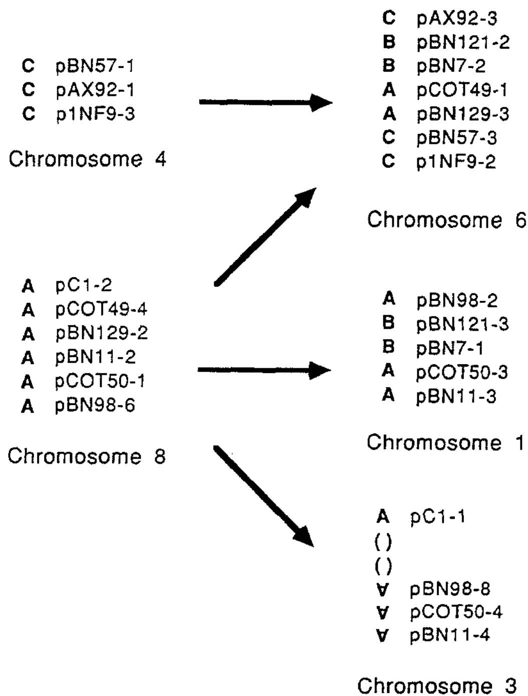

Fig. 5. Hypothetical evolutionary pathway for the five *Brassica oleracea* chromosomes based on duplicated loci. Sequences of the putative ancestral chromosomes are represented by letters *A*, *B* and *C*. ( ) represents deletions and ∀ represents inversions

Linked duplications, in the form of gene families, were seen on chromosomes 1, 2, 4, 6, 7 and 8 (Fig. 4). These include napin (pN2), pLEA76, pCOT50 on chromosome 1, self-incompatibility (pBOS5) on chromosome 2, isocitrate lyase (pBSIL9) on chromosome 4, pCOT46, p1NF6 and pBN6 on chromosome 5, pBN12, pBN129, pBN55, pBN12 and p1NF9 on chromosome 5, pBN12, pBN129, pBN55, pBN12 and p1NF9 on chromosome 6, pCOT44 on chromosome 7 and pCOT49 on chromosome 8 (Fig. 4).

Duplications in the form of chromosome segments were seen on linkage groups 1–6, 1-3-8, 6–8, 4–6 and 5–11 (Fig. 4). The markers for this type of analysis included five groups of duplicated segments: pBN7-pBN121 on chromosomes 1 and 6, pC1-pBN98-pCOT50-pBN11 on chromosomes 1, 3 and 8, pCOT49-pBN129 on chromosomes 6 and 8, pBN57-pAX92-p1NF9 on chromosomes 4 and 6 and pCOT46-pCA3 on linkage groups 5 and 11 (Fig. 5). These duplications might be the result of previous genome reshuffling events or polyploidization. Dispersed duplications with no apparent pattern were observed for sequences like pLEA76, pBN121, and pBN98 (Fig. 4).

Discussion

In many plant species it is often necessary to work with interspecific populations to disclose an adequate number of segregating loci (Bernatzky and Tanksley 1986; Helentjaris et al. 1990). In this study the amount of polymorphism observed for an intraspecific population (53%) agreed with the values reported previously (Figdore et al. 1988; Slocum et al. 1990; McGrath et al. 1990). As expected, the interspecific progenies disclosed a significantly higher level of polymorphism than any of the intraspecific ones. When all four lines were pooled together, more than 75% of the sequences probed were polymorphic in combination with a single restriction endonuclease, *Eco*RI. The distribution of mapped loci on the chromosomes of each population was fairly uniform except that the C × CA family chromosome 8 and the K × I family chromosome 7 lacked polymorphic loci. Thus, within *B. oleracea* the level of polymorphism is high enough to analyze intraspecific progenies, but the usefulness of specific probes as markers will depend on the population employed.

Mapping based on wide crosses carries the risk of distorted segregations and altered linkages due to gametic selection and/or chromosomal rearrangements. Distorted ratios are known to occur in interspecific crosses of rice (McCouch et al. 1988), potato (Bonierbale et al. 1988), lentil (Havey and Muehlbauer 1989), tomato (Paterson et al. 1990) and *Brassica* (Slocum et al. 1990). When such loci are localized on specific chromosomal segments and further information is lacking, their deviation is attributed to the fitness of that particular combination (Paterson et al. 1990; Slocum et al. 1990). As expected, the deviations observed increased with the level of divergence of the parents. The higher limit was for the interspecific cross, where 59% of the loci displayed distorted ratios with predominant skewing toward the kohlrabi alleles, followed by the U × CA population, which involved a wild kale from USSR crossed to cauliflower. In the other two intraspecific crosses involving different cultivated types only 7% of the segregations suffered distortion with no linkage or correspondence among the affected loci. These might indicate incipient genetic differentiation of the parents. Most of the affected loci for the interspecific and the U × CA cross were organized in specific linkage groups, perhaps indicating differentiation of specific chromosomal segments. This is supported by the observation of altered gene order and recombination frequencies for chromosomes 1 and 4 in these two crosses which, in the case of the interspecific cross, was accompanied by meiotic aberrations and reduced pollen stainability of the F_1 hybrid.

The major structural change observed in the chromosomes of the K × I hybrid was a translocation involving chromosomes 4 and 7 that may be responsible for the semi-sterile F_1 hybrids and the presence of a quadrivalent in diakinesis. Another important aberration was an inversion in chromosome 1 that resulted in an altered gene order and a reduced recombination expected from chro-

mosomal inversions (Burnham 1962). In the U × CA population the main structural change was also an inversion on chromosome 1 accompanied by reduced recombination with no apparent meiotic abnormality or significant effect on fertility. Either this is a small inversion or the deficiencies arising from crossing-over in the inverted region are buffered by the duplicated nature of the genome. In support of this latter hypothesis is the observation of viable and fertile interspecific aneuploids in *Brassica* species (Quiros et al. 1987; McGrath et al. 1990), which indicates tolerance to genetic imbalance.

It is apparent that mapping at the multi-population level has many advantages over that based on a single population. A larger number of loci are mapped, gene order and map distances are estimated more accurately, and alterations in these values, due to possible chromosomal rearrangements affecting the parents involved in the crosses, are easily detected. In addition, polymorphic loci common to all populations, which may have a higher chance of segregating in other crosses, are disclosed (Fig. 4). These loci may be used as consensus markers useful to align linkage groups identified in different crosses.

The map formulated from the present study has resolved 8 fairly populated linkage groups and 3 additional small groups (Fig. 4). More markers are needed to assign these smaller groups and to identify those that might belong to chromosome 9. On the basis of common markers, other linkage groups reported elsewhere for *B. oleracea* could be added to our map. The linkage groups for isozyme-encoding loci *Aco-4, Mdh-1, Aco-1* and *Lap-1* were positioned on chromosome 7 by the *Lap-1* locus, and *Pgm-3, Pgi-2,* leaf color (*g*) and *6pgdh-1* on chromosome 8 by the *Pgi-2* locus (Arus 1989). Additionally, the two linkage groups reported by Sampson (1966) for morphological markers were added to our map. The first group formed by *ps* (persistent sepals), *S* (self-incompatibility) and *A* (purple ovary) is probably located on chromosome 2, where the three S-linked loci mapped using the probe pBOS5 (Kianian and Quiros 1992). The second group, *Wh* (white petal) and *gl-1* (glossy foliage), was located on our provisional linkage group B containing *gl-1*. Even though the locations of these markers on the chromosomes are determined, their orientations could not be ascertained on the basis of a single common marker.

Our results demonstrate that 44% of the *B. oleracea* genome is duplicated. This is in agreement with the findings of Slocum et al. (1990), who reported 35% duplication for low-copy sequences, and McGrath et al. (1990), who found 42% sequence duplication. The duplicated sequences fall into three main classes: (1) dispersed sequences involving single loci with no apparent distribution pattern, (2) linked duplications in the form of gene families and (3) duplication of linkage blocks onto non-homologous chromosomes. The mechanism by which dispersed duplications could occur is unknown, but reverse transcription of mRNA or heterogeneous nuclear RNA (hnRNA) with subsequent reinsertion into the genome, formation of tandem duplication with subsequent dispersal (Helentjaris et al. 1988) and/or breakage and reintegration elsewhere in the genome of linear DNA fragments (Pichersky 1990) are possibilities.

The second class, linked duplications in the form of gene families, is seen on almost all the chromosomes. It is believed that genetic duplication in combination with mutational changes are responsible for evolution and differential expression of genes (Ohno 1970). The expression of many of the gene families, identified here with sequences prefixed pAX, pCOT, pCA, pGS and pLEA, are known to be under temporal and spatial regulation during seed germination and postgerminative development (Harada et al. 1988). It is possible that different gene family members are controlled by distinct regulatory factors and/or by developmental stage-specific factors that interact with the same sequence.

The third class of repeats, segmental duplication, is illustrated by chromosomes 1, 3, 4, 6 and 8 (Table 4). In general, the gene distances and the order of these markers on the different chromosomes were altered. However, some loci, like *pBN98-2, pCOT50-2 and pBN11-3* on chromosome 1 and *pBN98-8, pCOT50-4* and *pBN11-4* on chromosome 3, have maintained their order. A possible mechanism for the origin of segmental duplication is overlapping reciprocal translocation followed by hybridization and selfing (Gottlieb 1983). These may have been followed, in some cases, by small inversions altering the order and genetic distances of the loci. Such chromosomal aberrations are often observed in *Brassica* species (Gustafsson et al. 1976; Quiros et al. 1988; Kianian 1990). Another mechanism for the orgin of duplicated segments may be interspecific aneuploidy combining homoeologous chromosomes of related genomes after hybridization, amphiploidization and subsequent backcrossing to the parental diploid species. This could result in the recombination of homoeologous chromosomes altering further the order and syntenic relations of the loci. Moreover, deletions, inversions and translocations could follow, producing novel recombinant chromosomes. Evidence supporting the existence of such events have been observed in *Brassica* (Quiros et al. 1987; McGrath et al. 1990; McGrath and Quiros 1990; Hu and Quiros 1991).

The distribution of markers on more than one chromosome and the duplication of chromosomal segments agree with the hypothesis that *B. oleracea* is, at least partially, a secondary polyploid species (Prakash and Hinata 1980). However, it is obvious that the genome was not formed by the simple reiteration of whole chromosomes maintaining their integrity as postulated (Haga 1938; Richharia 1937a, b; Robbelen 1960). As a corol-

lary, it is possible that the genome of this species derives from one or more ancestral genomes that have suffered extensive rearrangements during or after the events responsible for their duplication. A comparison of shared homologous segments detected by duplicated loci allows the genealogy of five *B. oleracea* chromosomes to be traced to three ancestral chromosomes (sequences A, B and C in Figure 5).

RFLP analysis may provide an efficient method for measuring the evolutionary changes and structure of genomes. The comparison of different linkage maps presents a glimpse into the genome at both the species and subspecies level. From this view we can conclude that the *Brassica* genome in general and the *B. oleracea* genome in particular go through frequent chromosomal rearrangements. These changes are the likely cause of genome evolution and variation in the chromosome number of species in this genus. According to our composite map tandem and segmental duplications are prevalent. A high degree of morphological polymorphism is a prominent feature of the *B. oleracea* cytodeme, which might be a reflection of the variability that exists at the genomic level. The extra genetic material existing in the form of duplicated sequences provides the flexibility to change and evolve.

Acknowledgement. We are indebted to Drs. R. L. Phillips, H. W. Rines, J. M. McGrath and D. W. Davis for reviewing the manuscript. We are also grateful to V. D'Antonio, G. Huestis, and O. Ochoa for technical assistance. This research was financed by USDA competitive grant 88-37262-4018 and 90-37140-5425 to C. F. Quiros.

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