Comparative Mapping of *Arabidopsis thaliana* and *Brassica oleracea* Chromosomes Reveals Islands of Conserved Organization

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ABSTRACT

The chromosomes of *Arabidopsis thaliana* and *Brassica oleracea* have been extensively rearranged since the divergence of these species; however, conserved regions are evident. Eleven regions of conserved organization were detected, ranging from 3.7 to 49.6 cM in *A. thaliana*, spanning 158.2 cM (24.6%) of the *A. thaliana* genome, and 245 cM (29.9%) of the *B. oleracea* genome. At least 17 translocations and 9 inversions distinguish the genomes of *A. thaliana* and *B. oleracea*. In one case *B. oleracea* homoeologs show a common marker order, which is distinguished from the *A. thaliana* order by a rearrangement, indicating that the lineages of *A. thaliana* and *B. oleracea* diverged prior to chromosomal duplication in the Brassica lineage (for at least this chromosome). Some chromosomal segments in *B. oleracea* appear to be triplicated, indicating the need for reevaluation of a classical model for Brassica chromosome evolution by duplication. The distribution of duplicated loci mapped for about 13% of the DNA probes studied in *A. thaliana* suggests that ancient duplications may also have occurred in Arabidopsis. The degree of chromosomal divergence between *A. thaliana* and *B. oleracea* appears greater than that found in other confamilial species for which comparative maps are available.

THE family Cruciferae comprises 360 genera, organized into 13 tribes (SHULTZ 1936; ROLLINS 1942; AL-SHEHBAZ 1973). Basic diploid cytodeme numbers range from $n = 7$ (*Diplotaxis acris*) to $n = 13$ (*Diplotaxis harra*), a cytodeme being defined as a group of taxa sharing a common chromosome complement (HARBERD 1976). A number of allotetraploid cytodemes also exist, *e.g.*, *Brassica carinata* ($2n = 34$), *Brassica juncea* ($2n = 36$), *Brassica napus* ($2n = 38$) (U 1935). Nuclear DNA content ranges from 145 million basepairs (Mbp) per haploid complement (c) in *Arabidopsis thaliana* to 1235 Mbp/c in *B. napus* (ARUMUGANATHAN and EARLE 1991).

In the Cruciferae, numerous challenges complicate accurate botanical classification. Traditional classification based on morphology, although voluminous, has been difficult (HEDGE 1976). In some cases tribal and generic classifications may not accurately reflect true evolutionary relationships (WARWICK and BLACK 1991; HEDGE 1976). Only the classification of the tribes Brassiceae and Lepidieae can confidently be viewed as natural, *i.e.*, with clearly defined morphological boundaries which are easily recognized. The remaining 11 tribes have varying degrees of artificial classification, *e.g.*, the tribe Euclidideae, wherein the members seem to have little in common phylogenetically, being recognized by only negligible morphological features of the fruit pods (HEDGE 1976).

A. thaliana, $n = 5$ (tribe Sisymbrieae), an extensively utilized model system in plant biochemistry, physiology, and classical and molecular genetics (MEYEROWITZ 1989), is often referred to as a close relative of plants within the genus *Brassica* (tribe Brassiceae). This relationship is further suggested by extensive conservation of coding sequences between *Brassica* and *Arabidopsis* (LYDIATE *et al.* 1993). However, the degree of chromosomal divergence between these two genera has not previously been determined.

Genetic linkage maps have been constructed for *A. thaliana* (KOORNNEEF *et al.* 1983; CHANG *et al.* 1988; NAM *et al.* 1989; REITER *et al.* 1992; HAUGE *et al.* 1993; LISTER and DEAN 1993; MCGRATH *et al.* 1993), *Brassica oleracea* (SLOCUM *et al.* 1990; KIANIAN and QUIROS 1992; LANDRY *et al.* 1992; KENNARD *et al.* 1994), and *Brassica rapa* (CHYI *et al.* 1992). The high degree of molecular polymorphism found among the *Brassica* has facilitated restriction fragment length polymorphism (RFLP) mapping within this genus (FIGDORE *et al.* 1988). Comparative mapping of gene order on the chromosomes of both closely and distantly related species within the Cruciferae could help to clarify the degree of similarity between these various genomes, shed light on macroevolutionary events associated with divergence of these species, and facilitate cross-utilization of genetic resources and molecular tools. Although some comparative analyses between species within the genus *Brassica* are available (*B. oleracea* and *Brassica campestris*, now known as *B. rapa*; SLOCUM 1989; MCGRATH and QUIROS 1991), global comparisons of genomic structure and organization between more distantly related crucifers, *i.e.*,

TABLE 1

Total number of DNA probes surveyed/mapped in two populations of *A. thaliana*

Loci	WS × HM population				WS × M13 population			
	cDNA	*Pst*I	"M"	Total	cDNA	*Pst*I	"M"	Total
No data	0	14	0	14	0	14	0	14
0 loci	30	134	38	201	26	133	24	183
1 locus	13	25	17	57	15	23	30	68
2 loci[a]	1	1	0	1	2	4	1	7
3 loci	0	0	0	0	1	0	0	1
Total	44	174	55	273	44	174	55	273

Probe designations are: "cDNA," *A. thaliana* cDNA, *i.e.*, "AC" (25 AC clones have been mapped. The map locations of 12 are presented in Figure 1. The remainder will be presented elsewhere.); "*Pst*I" *Brassica* genomic clones "EW," "WG" and "WR" (SLOCUM *et al.* 1990); "M," *A. thaliana* genomic clones previously mapped (CHANG *et al.* 1988); see MATERIALS AND METHODS section for additional information.

[a] In three cases (*AC71*, *AC155* and *AC184*), a marker mapped to a single locus in each population, however the respective loci differed. This yielded three additional duplicated loci (see Figure 1, Table 5).

Brassica and *Arabidopsis*, have not previously been done.

By applying previously mapped *B. oleracea* DNA probes (SLOCUM *et al.* 1990) to *A. thaliana* populations, we have analyzed the relative organization of the chromosomes of *B. oleracea* and *A. thaliana*. Extensive rearrangement distinguishes the chromosomes of *B. oleracea* and *A. thaliana*, although numerous regions of locally conserved linkage and/or homoeology were also apparent. To a lesser extent than *B. oleracea* (SLOCUM *et al.* 1990), *A. thaliana* shows evidence of sequence duplication. Our results, together with other results previously published (MCGRATH *et al.* 1993) suggest that this may have involved ancient duplication of chromosomes or chromosome segments, in an ancestor of *A. thaliana*.

MATERIALS AND METHODS

Population development: Two F_2 mapping populations of *A. thaliana* were used in this study: Wassilewskija (WS) × Hannover/Mūnden (HM), comprised of 118 individuals, and Wassilewskija (WS) × mutant stock M13 (biological ecotype Landsberg, carrying *an*, leaf/silique phenotype, *dis*1, trichome phenotype, and *er*, inflorescence phenotype), comprised of 111 individuals (KRANZ and KIRCHHEIM 1987). Individual plants of the indicated ecotypes were hybridized by hand-crossing, hybridity of the F_1 verified by RFLP analysis, and the F_1 selfed to generate F_2 seed. F_1 and F_2 generations were grown in a 16-hr photoperiod and 22°, in a growth chamber.

Molecular markers: DNA markers used were derived from four sources; prefixes are defined as follows, AC: anonymous newly mapped Arabidopsis cDNAs; BC: an anonymous newly mapped DNA probe kindly provided by J. BRAAM (Rice University); M: genomic clones previously mapped (CHANG *et al.* 1988) generously provided by E. MEYEROWITZ; EW, WG, WR: Brassica *Pst*I genomic DNA clones, 138 of which have been previously mapped (SLOCUM *et al.* 1990), generously provided by Pioneer Hi-Bred Production Ltd. A total of 44 AC, 174 Brassica *Pst*I genomic DNA, and 55 M DNA clones were surveyed for polymorphisms (Table 1) among the three parents (WS, M13 and HM) used to generate the two F_2 mapping populations.

Genetic mapping: DNA extraction, electrophoresis, blotting, probe labeling and autoradiography were as described previously (KOWALSKI *et al.* 1994).

Stringency washes for Brassica *Pst*I genomic DNA clones applied to *A. thaliana* genomic DNA (survey hybridizations) were initially at 1 × SSC, 65°, with 111 of the clones treated in this manner. However, this tended to result in high background, so stringency was subsequently increased to 0.5 × SSC for the remaining 63 Brassica *Pst*I genomic DNA clones. Clones washed at 1 × SSC hybridized to an average of 4.83 (±0.40) genomic fragments, while clones washed at 0.5 × SSC hybridized to an average of 4.33 (±0.47) genomic fragments, a nonsignificant difference. Consequently, DNA probes treated by these slightly different procedures were pooled in our analyses of the degree of low copy sequence duplication (Table 2).

Eighteen of the 63 Brassica *Pst*I genomic DNA clones washed to a stringency of 0.5 × SSC, and 30 of the 111 of Brassica *Pst*I genomic DNA clones washed to a stringency of 1 × SSC were subsequently placed on the Arabidopsis map. All other hybridizations (survey and mapping) were washed to 0.5 × SSC.

Data analysis: Determination of recombination fractions utilized MapMaker (LANDER *et al.* 1987, provided by S. TINGEY, DuPont), on a Macintosh Quadra 700. Since some of the DNA markers provided informative polymorphisms in only one of the two populations (HM × WS or M13 × WS; Table 1), it was necessary to assemble a composite map of the Arabidopsis genome from their respective F_2 maps, as described elsewhere (BEAVIS and GRANT 1991). Specifically, "anchor loci" segregating in both populations are used to infer the relative order of loci segregating in only one of the two populations.

The extent of conservation between the genomes of *A. thaliana* and *B. oleracea* was estimated by previously published methods (NADEAU and TAYLOR 1984). These methods assume that (1) synteny of two or more markers is evidence of linkage, (2) chromosomal rearrangements fixed during evolution are randomly distributed throughout the genome, (3) crossovers are randomly distributed and (4) the distribution of homologous markers is random and independent. In performing the calculation two adjustments are necessary. Markers generally do not occur at the boundaries of chromosomal segments, so the apparent length of conserved segments on the genetic map (see Figure 1) is an underestimate. Therefore, a statistical correction is done; the expected range (*i.e.*, the expected length of a conserved segment) of a random sample taken from a uniform distribution (*i.e.*, the observed markers in a conserved segment) is determined. The second adjustment accounts for the bias toward longer conserved segments, since segments identified by one marker, or unidentified segments, are omitted. Therefore, a correction is made using a truncated Poisson distribution, which determines the probability that a

TABLE 2

Hybridization of homologous and heterologous DNA probes to *Eco*RI-digested genomic DNA from *A. thaliana* and *B. oleracea*

	Fragments strongly hybridizing[a]	Fragments weakly hybridizing	Total no. of probes tested
Brassica *Pst*I genomic DNA clones			
B. oleracea	130	120	86
A. thaliana	165	202	174
Arabidopsis cDNA clones			
A. thaliana	58	49	44

[a] "Strongly" and "weakly" hybridizing fragments are defined in RESULTS.

segment contains two or more markers, and should thus be included.

RESULTS

DNA sequence organization in *B. oleracea* and *A. thaliana*: Low copy DNA sequence repertoire was largely conserved between *A. thaliana* and *B. oleracea*. A subset of 80 *Pst*I-digested Brassica genomic DNA clones (which had been previously mapped; SLOCUM *et al.* 1990) were examined for hybridization to *Eco*RI-digested genomic DNA from both *A. thaliana* (WS ecotype) and *B. oleracea* (rapid cycling, self-compatible). Of these, 71 (89%) hybridized to *A. thaliana*, of which 22 (28%) were placed on the Arabidopsis composite map. Many additional clones were screened only on Arabidopsis, providing 26 additional mapped markers (Figure 1).

Brassica *Pst*I genomic DNA clones and Arabidopsis cDNAs hybridized to similar numbers of Arabidopsis genomic restriction fragments. Forty-four random cDNAs (isolated from an *A. thaliana* cDNA library) hybridized to an average of 2.43 *Eco*RI genomic fragments in Arabidopsis, not significantly different from the 2.11 fragments which hybridized to the Brassica *Pst*I genomic DNA clones (Table 2). This reemphasizes the extent of DNA sequence conservation between Arabidopsis and Brassica, at least in hypomethylated regions of the genome, *i.e.*, the regions in which cDNAs and *Pst*I genomic clones occur.

The degree of low copy DNA sequence duplication in the genomes of *A. thaliana* and *B. oleracea* was assessed by counting the number of *Eco*RI genomic fragments hybridizing to each Brassica *Pst*I genomic DNA clone, for WS (*A. thaliana*) and for a plant derived from several generations of selfing of a self-compatible population of *B. oleracea*. Brassica *Pst*I genomic DNA clones hybridized to an average of 2.91 genomic DNA fragments in *B. oleracea*, and 2.11 fragments in *A. thaliana* (Table 2). *Eco*RI genomic fragments were designated as "weakly" or "strongly" hybridizing, with "weakly hybridizing" fragments representing less than 10% of signal in a lane (based on visual assessment). On average, 52% of the 250 Brassica genomic fragments were "strongly hybridizing" (approximately 1.51 fragments per probe), and 45% of the 367 Arabidopsis genomic fragments were strongly hybridizing (approximately 0.95 fragments per probe) (Table 2).

An additional measure of the extent of DNA sequence duplication was provided by estimating the number of undoubtedly "single-copy" DNA clones in each genome. Clones were designated single copy when each of the five restriction enzyme digests tested showed only one genomic fragment which hybridized to the clone. In *B. oleracea*, only two (2.5%) of the 80 Brassica *Pst*I genomic DNA clones tested were deemed single copy, by this criterion. One of these (*EW7G06*) detected an RFLP, and was mapped to chromosome *2* of *A. thaliana* (Figure 1). In *A. thaliana*, 24 (14%) of 174 Brassica *Pst*I genomic DNA clones tested were deemed single copy. Six of these detected RFLPs, four mapping to chromosome *1* (*EW7E08, EW7D03, EW8E09, EW9A05*), one to chromosome *3* (*EW6G12*), and one to chromosome *5* (*EW5H06*) of *A. thaliana* (Figure 1). Six (14%) of 44 cDNAs tested were deemed "single copy". One of these (*AC97*) detected an RFLP which mapped to chromosome *2* of *A. thaliana* (Figure 1). We acknowledge that this underestimates the frequency of single-copy clones in each genome, as the occurrence of restriction sites within the genomic region spanned by the probe will, in some cases, generate two restriction fragments from a single locus.

Genetic maps of *A. thaliana* and *B. oleracea*: The composite RFLP linkage map of *A. thaliana* is presented in Figure 1. The map spans 187.5 cM on chromosome *1*, 92.2 cM on chromosome *2*, 91.6 cM on chromosome *3*, 118.8 cM on chromosome *4* and 151.4 cM on chromosome *5*, for a total recombinational length of 641.5 cM, with an average spacing of 6.1 cM between loci. The map is slightly longer than some previously reported Arabidopsis maps [501 cM, CHANG *et al.* (1988); 493 cM, NAM *et al.* (1989)], although not significantly different from the longest [630.4 cM, REITER *et al.* (1992)]. We note that WS was a common parent in the present map and that of REITER *et al.* (1992), suggesting that WS may be more recombinogenic than the more commonly used Landsberg and Columbia ecotypes. The aggregate length of intervals between anchor loci in the composite map was 331.1 cM, approximately intermediate between the two component maps, 402.1 cM (WS × M13) and 296.3 cM (WS × HM).

A total of 110 loci (including 11 duplicated loci and one triplicated locus) corresponding to 97 DNA probes were mapped (Figure 1): 22 loci are "anchors" (mapped in both populations); 28 loci were mapped in the HM × WS F_2 population only; 60 loci were mapped in the M13 × WS F_2 population only. The map shows the linkage arrangement of 49 loci detected by 44 previously mapped Brassica *Pst*I genomic DNA clones (SLOCUM *et al.* 1990). Also, four Brassica *Pst*I genomic DNA

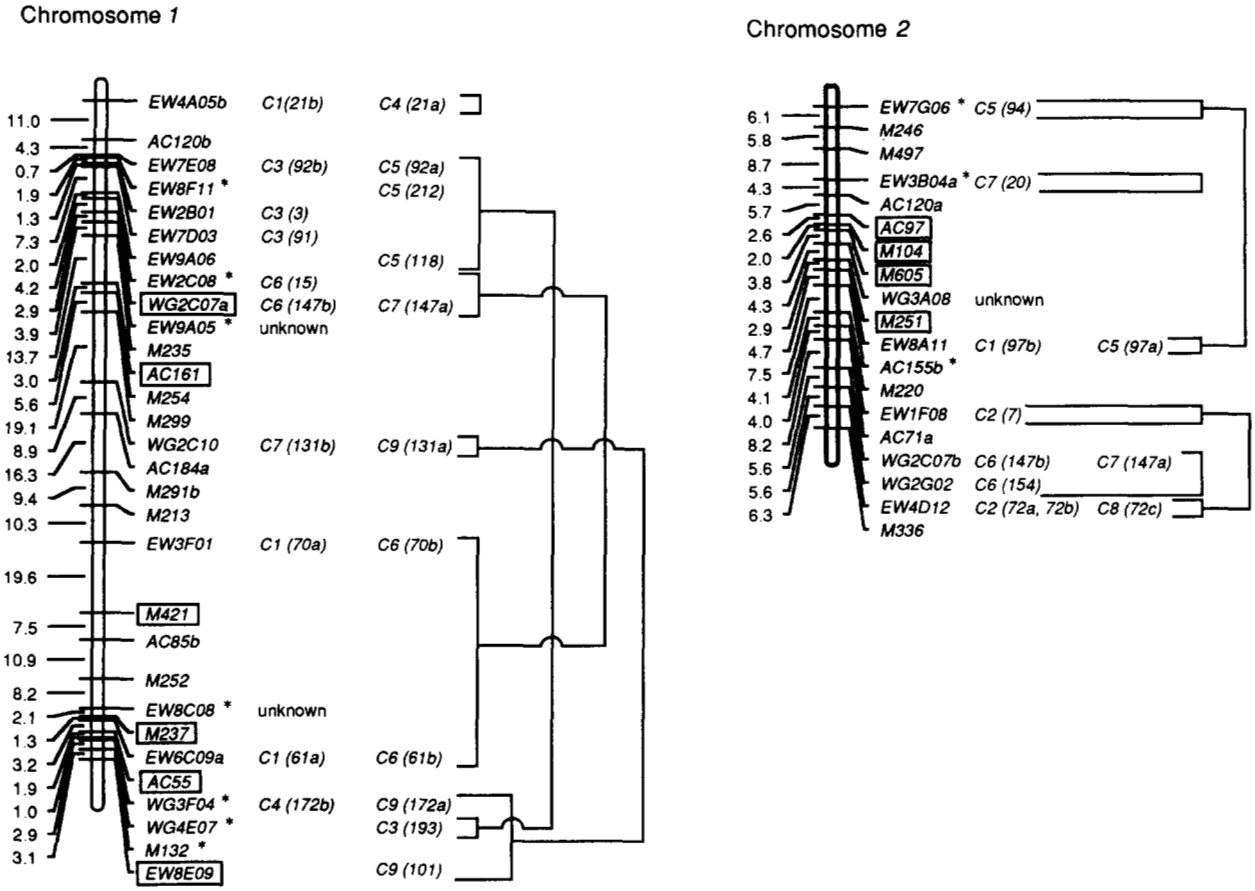

FIGURE 1.—Composite RFLP linkage map of *A. thaliana* HM × WS and M13 × WS F_2 populations. The map was generated using MAPMAKER (LANDER *et al.* 1987), and the composite map assembled as described in MATERIALS AND METHODS. Map distances are in centiMorgans, utilizing the KOSAMBI (1944) mapping function. Markers in boxes are anchor loci, common to both populations. Markers mapped in the HM × WS F_2 population are indicated by an asterisk (*). Remaining markers were mapped in the M13 × WS F_2 population only. Corresponding chromosome(s) and loci in *B. oleracea* (SLOCUM *et al.* 1990) are indicated next to markers. Regions of conserved chromosomal organization between the genomes of *A. thaliana* and *B. oleracea* are indicated by lines, with a first level of organization indicating regions of linkage conservation and/or homoeology, and subsequent levels of organization indicating possible inversions (involving progressively fewer markers). Groups of loci which retain common order in *A. thaliana* and *B. oleracea* are indicated by vertical lines adjacent to locus names. Groups of loci syntenic, *i.e.*, on common chromosomes, in *A. thaliana* and *B. oleracea* but separated by markers on different chromosomes, are connected by brackets, and are inferred to have become separated by inversions (as described in text and Table 4). Putative synteny (and inversions) supported by progressively smaller numbers of loci, are drawn progressively further from the chromosome.

clones not previously mapped are presented ("unknown" designation, Figure 1).

Three criteria were employed in assessing conservation of chromosome organization between *A. thaliana* and *B. oleracea*, and in developing a model (Figure 1, Tables 3–5) which attempts to account for rearrangements which distinguish the two species:

Criterion 1. Linkage: Linkage is defined as two or more markers which are linked in *A. thaliana* and also linked in *B. oleracea* and are uninterrupted by markers mapping to other *B. oleracea* chromosomes.

Criterion 2. Homoeology: Homoeology is defined as two or more markers linked in *A. thaliana*, which fall on homoeologous regions of *B. oleracea*, and are uninterrupted by markers mapping to non-homoeologous regions of *B. oleracea* chromosomes. Within a region showing evidence of homoeology (*e.g.*, upper end of *A. thaliana* chromosome *3*, with *B. oleracea* C*3* and C*8*), some probes mapped to only one of the homoeologous regions. We infer that such cases are explained by lack of an RFLP at the corresponding homoeologous locus (rather than lack of a corresponding locus).

Criterion 3. Synteny: Synteny is defined as two or more markers from a particular *B. oleracea* chromosome mapping to a common *A. thaliana* chromosome. Syntenic markers in Arabidopsis were often separated by intervening markers from other *B. oleracea* chromosomes or linkage groups. It was inferred that a single inversion, rather than two translocations, was more likely to be the means by which syntenic markers became separated. This assumption is based on the general observation that closely related taxa more frequently differ by inversions than by translocations [*Lycopersicon esculentum* and *Solanum tuberosum* (BONIERBALE *et al.*

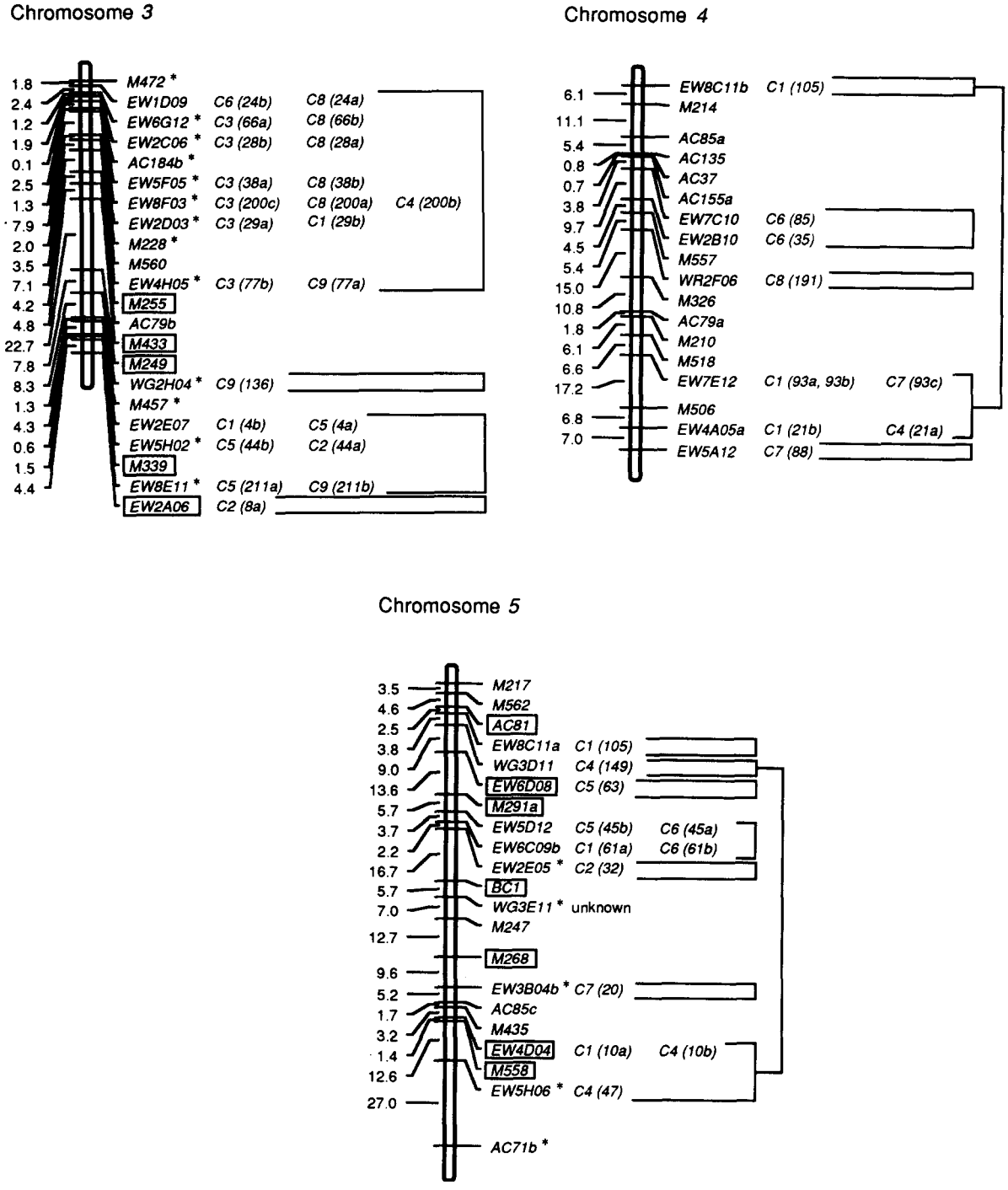

FIGURE 1.—Continued

1988; TANKSLEY *et al.* 1992); *B. oleracea* subspecies (KIANIAN and QUIROS 1992); homoeologous chromosomes of Gossypium (REINISCH *et al.* 1994) and our observation that Brassica probes which are syntenic in *A. thaliana* tend to be closely linked in *B. oleracea* (Table 5), also see below]. In cases where *A. thaliana* chromosomes were associated with two or more putatively non-homoeologous regions of the *B. oleracea* chromosomes, stronger conservation was assumed to be with the *B. oleracea* chromosome (or homoeologous group) showing a greater number of syntenic loci. The model for proposed rearrangements reflects this conservation, in that lines indicating progressively weaker levels of conservation are drawn progressively further from the chromosome (Figure 1). In cases involving duplicated loci at distal sites on a linkage group (all relevant duplications were on Brassica chromosomes), we inferred conservation (or synteny) to be over the shortest possible distance.

Using these three criteria, we have proposed a model which minimizes the number of rearrangements neces-

TABLE 3

Conserved chromosomal segments between the genomes of *A. thaliana* and *B. oleracea*

A. thaliana Chr.:loci[b]	*A. thaliana* length (cM)	*B. oleracea*[a] Chr.:loci[b]	*B. oleracea*[a] length (cM)
1:EW7E08-EW9A06	11.2	*3:92b-3,91*	5
		5:92a-118	6
1:EW2C08-WG2C07a	4.2	*6:15-147b*	23
1:EW3F01a-EW6C09a	49.6	*1:61a-70a*	8
		6:61b-70b	45
1:WG3F04-EW8E09	7.0	*9:101-172a*	14
2:WG2C07b-WG2G02	5.6	*6:147b-154*	34
3:EW1D09-EW4H05	22.8	*3:66a-77b*	15
		8:24a-38b	12
3:EW2E07-EW8E11	6.4	*5:44b-211a*	9
4:EW7C10-EW2B10	9.7	*6:35-85*	21
4:EW7E12-EW4A05a	24.0	*1:21b-93b*	3
5:EW5D12-EW6C09b	3.7	*6:45a-61b*	10
5:EW4D04-EW5H06	14.0	*4:10b-47*	40

[a] Data for *B. oleracea* are from SLOCUM *et al.* (1990).
[b] For both *A. thaliana* and *B. oleracea*, loci demarcate the chromosomal segments conserved.

TABLE 4

Summary statistics for conserved chromosomal regions of *A. thaliana* and *B. oleracea*

	A. thaliana Chr:					
	1	2	3	4	5	Total
Translocations	3	3	3	3	5	17
Inversions	3	2	2	1	1	9
cM in *A. thaliana*	72.0	5.6	29.2	33.7	17.7	158.2
cM in *B. oleracea*	101	34	36	24	50	245

Conserved regions are defined as either regions of linkage or homoeology (see RESULTS).

TABLE 5

Comparison of recombinational distances between syntenic markers in *A. thaliana* and *B. oleracea*

A. thaliana Chr.:loci[b]	*A. thaliana* length (cM)	*B. oleracea*[a] Chr.:loci[b]	*B. oleracea*[a] length 2 (cM)
1:EW7E08-WG4E07	166.2	*3:92b-193*	18
1:EW2C08-EW6C09a	146.9	*6:61b-147b*	71
1:WG2C10-EW8E09	106.6	*9:131a-172a*	24
2:EW7G06-EW8A11	46.2	*5:5-94-97a*	6
2:EW1F08-EW4D12	23.4	*2:7-72a*	56
4:EW8C11-EW4A05a	111.8	*1:21b-105*	51
5:WG3D11-EW5H06	110.0	*4:47-149*	62

[a] Data for *B. oleracea* are from SLOCUM *et al.* (1990).
[b] Distances are those between most distal loci in relevant regions.

sary to account for differences in marker order in the respective chromosomes of *A. thaliana* and *B. oleracea*. In the model, we have assumed that inversion occurs more readily than translocation (as described above). When two alternate marker orders were equally likely for *A. thaliana*, the marker order most parsimonious with that in *B. oleracea* was used.

Figures 1 and 2 illustrate, and Tables 3 and 4 summarize, the degree of conservation between the linkage maps of *A. thaliana* and *B. oleracea*. Note that C*1*-C*9* designate *B. oleracea* linkage groups (chromosomes) as per SLOCUM *et al.* (1990). The organization of individual chromosomes of *A. thaliana* relative to *B. oleracea* is described below. Chromosome numbers separated by a slash are deemed homoeologous in the relevant regions (based on mapping of duplicated loci), *e.g.*, C*3/8* refers to a homoeologous region of C*3* and C*8*.

Chromosome *1* of *A. thaliana* shows association with parts of C*1/6*, C*3/5*, C*4* and C*9* of *B. oleracea*, implying that a minimum of three translocations differentiate the *A. thaliana* and *B. oleracea* chromosomes.

A region spanning the five markers *EW7E08*, *EW8F11*, *EW2B01*, *EW7D03* and *EW9A06*, corresponds to a region of C*3* and C*5* of *B. oleracea*. Conservation in this region is inferred based on both linkage and homoeology (criteria 1 and 2).

Linkage (criterion 1) is observed between markers *EW2C08* and *WG2C07a* (C*6* in *B. oleracea*), between markers *EW3F01a* and *EW6C09a* (C*1/6* in *B. oleracea*), and tentatively between markers *WG3F04* and *EW8E09* (C*9* in *B. oleracea*), although this region may be interrupted by a marker (*WG4E07*) which maps to C*3* of *B. oleracea*. An alternate order placing *WG3F04* adjacent to *EW8E09* was less likely, but could not be ruled out at a statistically significant level. Since the order shown is preferred by LOD 1.08 we have tentatively accounted for this event with an inversion.

Three inversions are proposed, involving regions corresponding to C*3/5*, C*6* and C*9* of *B. oleracea* (Figure 1).

Chromosome *2* of *A. thaliana* shows association with parts of C2, C5, C*6* and C7 of *B. oleracea*, implying that a minimum of three translocations differentiate the *A. thaliana* and *B. oleracea* chromosomes.

Linkage was detected (criterion 1) between markers *WG2C07* and *WG2G02*, corresponding to C*6* of *B. oleracea*. Although *WG2C07* also maps to C7 of *B. oleracea*, conservation was assumed to be with the more numerous *B. oleracea* C*6* markers (criterion 3).

Two inversions are proposed, involving regions corresponding to C2 and C5 of *B. oleracea*, as illustrated (Figure 1).

Chromosome *3* of *A. thaliana* shows association with parts of C2, C*3/8*, C5 and C*9* of *B. oleracea*, implying that a minimum of three translocations differentiate the *A. thaliana* and *B. oleracea* chromosomes.

Linkage (criterion 1) was detected between markers *EW2E07*, *EW5H02* and *EW8E11*, but differing from their order in *B. oleracea* by an inversion between *EW2E07* and *EW5H02* (Figure 2). For each of the three markers involved, an additional locus was mapped in *B. oleracea* but to three different regions,

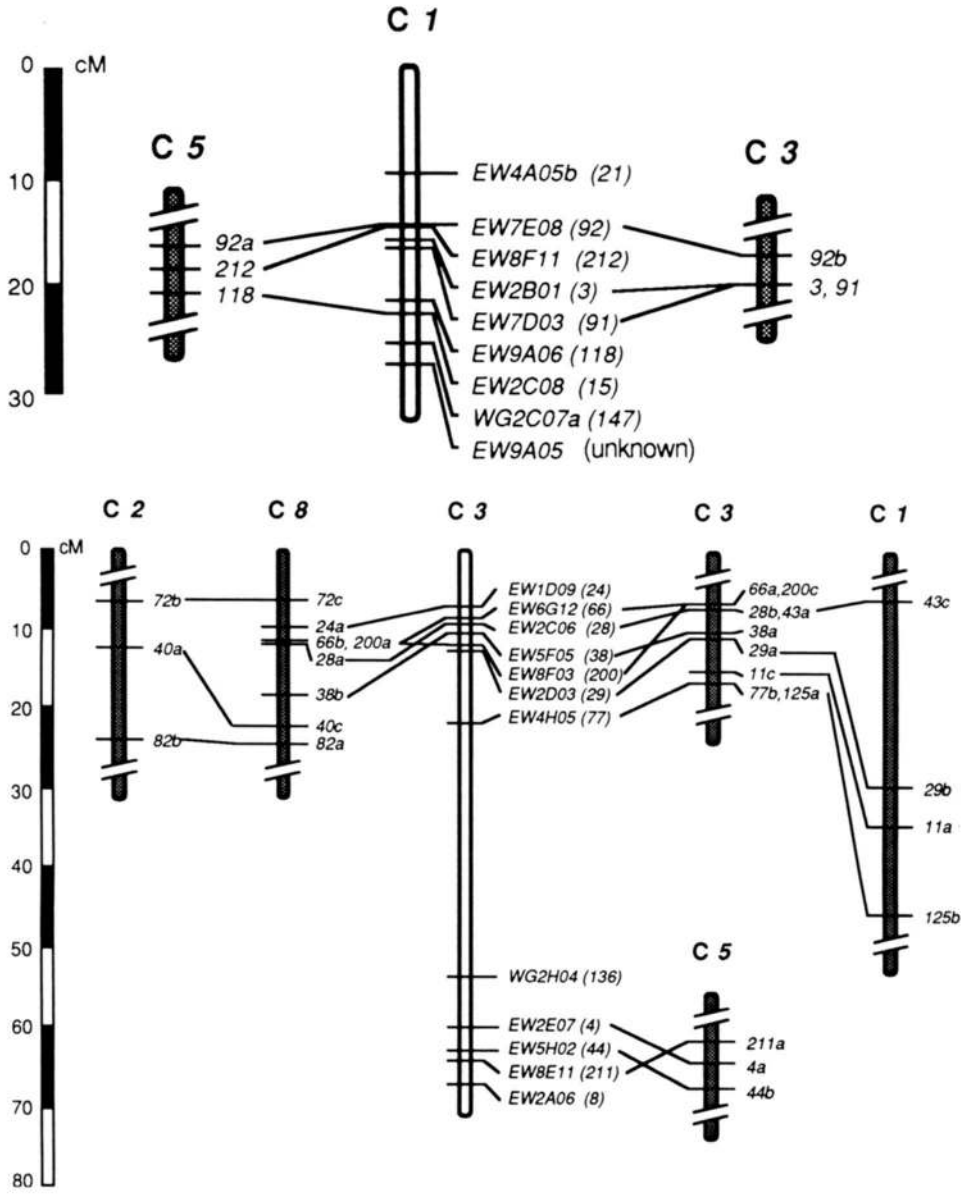

FIGURE 2.—Conserved linkages between *A. thaliana* chromosome *3* (open bar) and *B. oleracea* C*1*, C*3* and C*8* (speckled bars), and *A. thaliana* chromosome *1* (open bar) and *B. oleracea* C*3* and C*5* (speckled bars). Distances are in cM. Loci connected by a line are detected by the same DNA marker.

possibly representing independent duplications (criterion 3).

Both linkage and homoeology (criteria 1 and 2) were detected for markers *EW1D09*, *EW6G12*, *EW2C06*, *EW5F05*, *EW8F03*, *EW2D02* and *EW4H05*, which map to homoeologous regions of *B. oleracea* C*3* and C*8*. The order of these markers along both *B. oleracea* homoeologs is conserved, but differs in *A. thaliana* in that *EW8F03* (*B. oleracea* loci *200 a,c*) and *EW6G12* (*B. oleracea* loci *66a,b*) co-segregate in *B. oleracea* (on both homoeologs), but are separated by an interval of 5.7 cM, which includes markers *EW2C06* and *EW5F05*, in *A. thaliana*. Thus, marker order in these regions is conserved between *B. oleracea* homoeologs, but different between *B. oleracea* and *A. thaliana* (Figure 2). All relevant markers in this region were mapped in a common population (WS × HM), and alternate orders placing *EW8F03* (*B. oleracea* loci *200 a,c*) adjacent to *EW6G12* (*B. oleracea* loci *66a,b*) were rejected by a LOD of 7.44 or greater. For four of the markers in this region (*EW1D09*, *EW8F03*, *EW2D03*, *EW4H05*), additional loci were mapped in *B. oleracea*, but to four different regions, possibly representing independent duplications (criterion 3).

The two cases described above were the only inversions detected.

Chromosome *4* of *A. thaliana* appears to differ between HM and WS by a reciprocal translocation (KOWALSKI *et al.* 1994), therefore the map of chromosome *4* is not a composite but from the M13 × WS F_2 population (Figure 1). Chromosome *4* shows association with parts of C*1*, C*6* and C*7* of *B. oleracea*, implying that a minimum of three translocations differentiate the *A. thaliana* and *B. oleracea* chromosomes.

Linkage (criterion 1) was detected between markers *EW7C10* and *EW2B10*, corresponding to C*6* of *B. oleracea*,

TABLE 6

Distribution of duplicated loci among the chromosomes of *A. thaliana*

	Chr.			
Chr.	*2*	*3*	*4*	*5*
1	*AC120b-AC120a* *WG2C07a-WG2C07b*	*AC184a-AC184b*	*EW4A05b-EW4A05a* *AC85b-AC85a*	*M291b-M291a* *AC85b-AC85c* *EW6C09a-EW6C09b*
2			*AC155b-AC155a*	*AC71a-AC71b* *EW3B04a-EW3B04b*
3			*AC79b-AC79a*	
4				*EW8C11b-EW8C11a*

and between markers *EW7E12* and *EW4A05a*, corresponding to C*1* of *B. oleracea*. Although *EW7E12* also maps to C7 of *B. oleracea*, conservation was assumed to be with the more prevalent *B. oleracea* C*1* markers (criterion 3). In addition, conservation was assumed to be with locus 21b of *B. oleracea*, based on the proximity of loci (*21b-93a* spans 32 cM, *21b-93b* spans 3 cM; criterion 3).

One inversion is proposed, corresponding to C*1* of *B. oleracea* (Figure 1).

Chromosome *5* of *A. thaliana* shows association with parts of C*1*, C*2*, C*4*, C*5*, C*6* and C7 of *B. oleracea*, implying that a minimum of five translocations differentiate the *A. thaliana* and *B. oleracea* chromosomes.

Linkage (criterion 1) was detected between markers *EW5D12* and *EW6C09b*, corresponding to C*6* of *B. oleracea*. Although *EW5D12* and *EW6C09b* also map to C*5* and C*1* (respectively) of *B. oleracea*, conservation was assumed to be with the more prevalent *B. oleracea* C*6* markers (criterion 3). A second region of linkage was also detected, between *EW4D04* and *EW5H06*, corresponding to C*4* of *B. oleracea*. Although *EW4D04* also maps to C*1* of *B. oleracea*, conservation was assumed to be with the more prevalent *B. oleracea* C*4* markers (criterion 3).

One inversion is proposed, corresponding to C*4* of *B. oleracea* (Figure 1).

The total recombinational length of conserved regions between the genomes of *A. thaliana* and *B. oleracea* are 158.2 and 245 cM, respectively (Table 4). This represents 24.6% of the genome of *A. thaliana* (based on our map length of 641.5 cM) and 29.9% of the genome of *B. oleracea* [based on the published length of 820 cM; SLOCUM *et al.* (1990)].

Chromosomal inversions appear to account for synteny of unlinked markers: We have inferred (see above) that synteny, where two or more markers mapping to different regions of a particular *A. thaliana* chromosome also map to a common *B. oleracea* chromosome, does not occur simply by chance. Rather, we have proposed that inversion is the most likely means by which such markers have become separated (or joined). Previous studies have also suggested this (BONIERBALE *et al.* 1988; TANKSLEY *et al.* 1992; KIANIAN and QUIROS 1992; REINISCH *et al.* 1994). If markers syntenic in *A. thaliana* were conserved with regions of *B. oleracea* chromosomes, one would expect such markers to be close together in *B. oleracea*. Syntenic markers on distal regions of *A. thaliana* chromosomes were much closer together on *B. oleracea*, as measured by recombination (Figure 1, Table 5). This further supports the inference that such markers reflect localized regions of conservation between *A. thaliana* and *B. oleracea*, and that these regions are distinguished by inversions.

Mapping of multiple genetic loci in *A. thaliana*: Of the 97 DNA probes, 12 (12.5%) mapped to more than one locus in *A. thaliana*: 11 to two loci and one to three loci (Table 6). Three DNA probes detect genetically linked duplicated sites on regions spanning 69.3 cM of chromosome *1* and 68.5 cM of chromosome *5* (respectively), and differing in order by an inversion (Figure 3).

DISCUSSION

Chromosomal organization of *A. thaliana* and *B. oleracea*: Although extensive chromosomal rearrangements have occurred since the divergence of *B. oleracea* and *A. thaliana*, islands of conserved organization are discernible. At least one conserved region was detected on each of the five chromosomes (Figure 1, Table 3). In total, we have identified 11 regions spanning 24.6% of the *A. thaliana* genetic map which are closely conserved with 29.9% of the *B. oleracea* genetic map.

Using previously published methods (NADEAU and TAYLOR 1984), we estimate that chromosomal segments with an average length of 21.3 cM in *A. thaliana* are uninterrupted by rearrangements distinguishing them from their order in *B. oleracea*. This calculation predicts that approximately 25 chromosomal rearrangements have occurred since divergence of these two species, at a rate of 2.5 rearrangements per million years. This estimate is in close agreement with our proposed model (26 rearrangements: 17 translocations and nine inversions; Figure 1, Table 4). We conservatively assume that the divergence of *A. thaliana* and *B. oleracea* occurred 10 million years ago [paleopalynological evidence indicates that the plant order Capparales, including the families Capparaceae, Resedaceae and Cruciferae, first appeared during the upper Miocene, approximately 10 million years ago (MULLER 1981, 1984)]. By the same

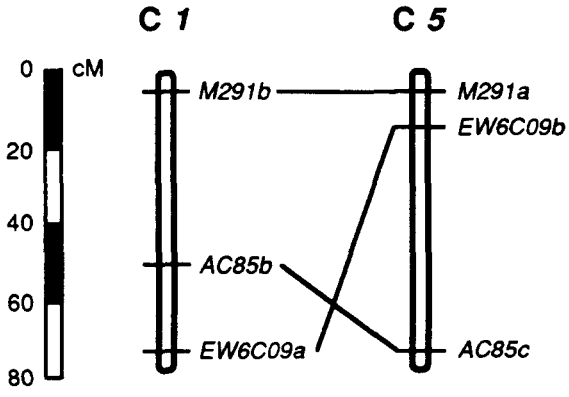

FIGURE 3.—Segmental duplication of chromosomes *1* and *5* in *A. thaliana*. Distances are in centiMorgans. Loci connected by a line are detected by the same DNA marker.

method, although with a much lower density of genetic markers, NADEAU and TAYLOR (1984) estimated that chromosomal segments with an average length of 8 cM had been conserved between the mouse and human genomes, and that 180 rearrangements had occurred since divergence of the human and mouse genomes (approximately 70 million years ago).

Despite concordance between the number of rearrangements observed herein and the number predicted by the method of NADEAU and TAYLOR (1984), it is likely that mapping of additional DNA probes will define a few more rearrangements. The relationship between the number of homologous markers mapped and the number of conserved segments identified approximately follows an asymptotic regression $[W = A - B(e^{-\alpha})]$ (NADEAU 1989; SNEDECOR and COCHRAN 1989). With continued mapping of homologous markers between *A. thaliana* and *B. oleracea*, it should be possible to clearly determine the actual number of rearrangements which distinguish the chromosomes of these organisms.

Relative to other plant species for which equivalent comparisons can be made, the chromosomes of *B. oleracea* and *A. thaliana* appear to have diverged relatively rapidly. The genomes of rice and maize, which diverged approximately 50 million years ago (BENNETZEN and FREELING 1993) have 32 conserved linkage segments along their 12 and 10 chromosomes (respectively), comprising 70% of the rice genome (AHN and TANKSLEY 1993), based on genetic mapping at moderately higher density of common markers than that reported here (markers averaging 8.5 cM apart, *vs.* 14.6 cM in our study). The A and D genomes of cotton, which diverged approximately 6–11 million years ago (WENDEL 1989) are distinguished by one translocation and six inversions along 11 (of an expected 13) homoeologous chromosome pairs for which sufficient data is available (REINISCH *et al.* 1994). The genomes of maize and sorghum, which diverged approximately 20 million years ago (BENNETZEN and FREELING 1993) are distinguished by at least nine inversions along the 10 chromosomes, although analysis of several chromosomes is incomplete (WHITKUS *et al.* 1992). The minimum number of 26 rearrangements which we estimate to distinguish the five chromosomes of *A. thaliana* from the nine chromosomes of *B. oleracea* suggests a level of rearrangement paralleled only by the rice-maize comparison, although rice and maize diverged at least 40 million years (5×) earlier.

Ancestral duplication of Arabidopsis chromosome segments: Based on the degree of gene and sequence duplication, as well as evidence for duplication of chromosomal segments, *A. thaliana* appears to have undergone ancient duplication of chromosomes or chromosome segments. Considerable duplication of individual sequences in *A. thaliana* is revealed by both Brassica *Pst*I genomic DNA clones, and Arabidopsis cDNAs, with each showing an average of two *Eco*RI fragments (Table 2). A minimum estimate of the frequency of DNA sequence duplication in *A. thaliana* can be obtained from the frequency of DNA probes detecting RFLPs at two (or more) unlinked loci, which account for about 12.4% of the DNA probes we mapped. MCGRATH *et al.* (1993) previously reported "that more than 15% of the genes in the *A. thaliana* genome may be encoded by multiple loci." However, such estimates are confounded with levels of DNA polymorphism in particular mapping populations. If the likelihood of detecting an RFLP at one locus is x, the likelihood of detecting RFLPs at each of two unlinked loci is x^2. At low levels of x, x^2 will approach zero, and it will rarely be possible to map duplicated loci (CHITTENDEN *et al.* 1994) (Table 1). A maximum estimate for the frequency of duplicated loci can be obtained from the frequency of DNA probes which detect only one genomic fragment in digests with several different restriction enzymes–we estimate this frequency at 14% (see RESULTS). In our study, mapped duplicate loci are found on each chromosome, with no cases of proximal duplication found (Figure 1, Table 6).

Although modest levels of DNA polymorphism among Arabidopsis ecotypes (KING *et al.* 1993) make it difficult to study possible chromosomal duplications, we did find some evidence supporting at least one such event. Despite the suggestion that many DNA probes may be duplicated in *A. thaliana*, only a small fraction could be mapped to two loci (Table 1). Nevertheless, we identified one region of chromosome *1* which may be homoeologous with a region of chromosome *5* (Figure 3).

Close inspection of previously published results provides independent corroboration of an ancestral chromosome (or segment) duplication in Arabidopsis. MCGRATH *et al.* (1993) report that three DNA probes detect RFLP loci duplicated on Arabidopsis chromosome *1* (*515A*, *559B*, *711A*, spanning 9.7 cM), and chromosome *5* (*559A*, *515B*, *711B*, spanning 108.9 cM). Each of the duplicated regions are close to the respective homoeologous regions on our map, based on anchor loci common to chromosome *1* (*M235*, *M213*),

and tightly linked reference loci [separated by 1.5 cM on the CHANG *et al.* (1988) map, on chromosome *5* (*M331* on the MCGRATH *et al.* map; *M268* on our map)]. In the MCGRATH *et al.* (1993) map, putative homoeologous regions of the genome may also be present between regions on chromosomes *2* (*579B, 415E, 574B, 173A, 415A*; spanning 31.3 cM) and *3* (*574A, 173B, 415C, 579C*; spanning 53.1 cM), and between regions on chromosomes *3* (*415C, 579C, 713C*; spanning 58.4 cM) and *4* (*713B, 579A, 415F, 713D, 415B*; spanning 52.8 cM). It must be noted that duplicate loci which contradict both our evidence and that of MCGRATH *et al.* (1993) have also been reported, with duplications between chromosomes *1* (*m281a, g2488b*; spanning 18.7 cm) and *3* [*g2488a, m281a*; spanning 48.5 cM; HAUGE *et al.* (1993)]. However, the bulk of the evidence suggests that chromosome *1* contains a segment having undergone duplication, possibly including sequences which have been rearranged, or duplicated by other mechanisms such as replicative transposition (VOYTAS and AUSUBEL 1988). The proposal that *A. thaliana* is a "paleopolyploid" is consistent with like proposals for several other species which show strict bivalent pairing at meiosis [maize (HELENTJARIS *et al.* 1988), sorghum (CHITTENDEN *et al.* 1994) and diploid cotton (REINISCH *et al.* 1994)].

Based on analysis of fossil guard cells, MASTERSON (1994) has proposed $n = 7$–9 as the primitive chromosomal complement of angiosperms. STEBBINS (1966) suggested (based on cytological evidence) that the earliest angiosperms possessed a fundamental chromosome number of $x = 6$ or $x = 7$. He further speculated that chromosomal evolution proceeded in both ascending and descending progressions of basic chromosome numbers, *e.g.*, from 6 to 5 to 4, etc., and from 7 to 8, etc. However, our proposal that *A. thaliana* has undergone at least one segmental duplication suggests an original chromosome number less than 5, *e.g.*, $x = 3$ or 4 with subsequent duplication events required to account for the contemporary *A. thaliana* genome. An alternative, however convoluted, explanation for the evolution of the *A. thaliana* genome would be a reduction from $x = 6$ or 7 to a lower chromosome number, followed by (at least) segmental duplication.

Consequences of ancestral duplication of Arabidopsis chromatin for physical mapping and chromosome walking: Beyond its evolutionary consequences, duplication in the *A. thaliana* genome has ramifications for molecular manipulations of large DNA. Duplication of large genomic regions could dramatically complicate long range restriction mapping and physical mapping in these regions, as is the case in polyploids (REINISCH *et al.* 1994). Some cases of apparently chimeric YACs, *i.e.*, a YAC with distal ends mapping to unlinked regions of the genome, may really be a result of genomic duplication. Based on estimates that 15% (MCGRATH *et al.* 1993) to 12.5% (herein) of Arabidopsis low copy DNA may occur at two or more sites with discernible homology, a like fraction of corresponding YAC ends would be expected to detect RFLPs at different (putatively homoeologous) sites. While it is well established that megabase DNA cloning is subject to chimeras, estimates of chimera frequency based upon RFLP mapping may be inflated as a result of ancestral duplication of Arabidopsis chromatin. As (putative) regions of ancestral duplication in Arabidopsis are better delineated by additional mapping, it will be easier to distinguish "truly chimeric" YACs from artifacts resulting from ancient duplications.

Chromosomal divergence among the Cruciferae: *Arabidopsis* and *Brassica* diverged from a common ancestor with less chromosomal duplication than *B. oleracea*. The relative orders of DNA markers along homoeologous chromosomal regions permit us to infer whether specific chromosomal rearrangements predate, or postdate, duplication of Brassica chromosomes. The segment of chromosome *3* of *A. thaliana* spanning markers *EW1D09, EW6G12, EW2C06, EW5F05, EW8F03, EW2D03* and *EW4H05* displays nearly complete linkage conservation with homoeologous regions on C*8* and C*3* of *B. oleracea*, with the exception of *EW6G12* (*66a,b*) and *EW8F03* (*200a,c*). Although these markers co-segregate on both C*8* and C*3* of *B. oleracea* (C*8*: *66b/200a*; C*3* *66a/200c*), they are separated by a distance of 5.7 cM, and two other markers, in *A. thaliana*. The simplest explanation for this would be that the prototypical *B. oleracea* and *A. thaliana* chromosomes differed by a rearrangement in this region, and that chromosomal duplications then propagated this region in *B. oleracea*, *i.e.*, the rearrangement predates duplication of the Brassica chromosomes. SADOWSKI *et al.* (1994) reported a complex of three tightly linked genes in *A. thaliana*, mapping to a single locus. Each of these probes map to duplicated loci in *B. oleracea*, co-segregating on one homoeolog, but with duplicated sites dispersed over three chromosomes. The simplest explanation for this would be that the prototypical *B. oleracea* and *A. thaliana* chromosomes showed close linkage of these markers, and that one *B. oleracea* homoeolog has been rearranged subsequent to duplication.

Based on cytological evidence, the fundamental number of chromosomes in the genus *Brassica* has been suggested to be $n = 6$ (PRAKASH and HINATA 1980). Cytological (ROBBELEN 1960), and more recently molecular evidence (SONG *et al.* 1990), suggests that the evolution of *Brassica* and closely related genera has proceeded in ascending order of chromosome number, from the $n = 6$ ancestor to $n = 7$ (*Diplotaxis erucoides*), to $n = 8$ (hypothetical "bridge" species), to $n = 9$ (*B. oleracea*). This model proposes that *B. oleracea* is a secondary polyploid, with a basic chromosomal complement of AABBCCDEF or ABBCCDEEF (HAGA 1938; ROBBELEN 1960). Linkage mapping of *B. oleracea* has supported evidence for the existence of duplicated chromosomal

segments, with nearly half of the DNA probes mapping to two or more loci, many of the duplicated loci being clustered on specific pairs of linkage groups. However, homoeology spanning entire pairs of linkage blocks was not found, indicating the occurrence of translocations during the evolution of *B. oleracea* (SLOCUM *et al.* 1990). Comparative mapping of the genomes of *B. oleracea* ($n = 9$) and *B. campestris* (*rapa*; $n = 10$) revealed predominant conservation of gene order along the chromosomes (SLOCUM 1989), suggesting that most rearrangements differentiating *Arabidopsis* from *Brassica* occurred before these Brassicas diverged.

Our results, in conjunction with previously published results (SLOCUM *et al.* 1990), suggest that some regions of the *B. oleracea* genome have been triplicated. Chromosome *3* of *A. thaliana* (in the region spanning markers *EW1D09, EW6G12, EW2C06, EW5F05, EW8F03, EW2D03, EW4H05*) is clearly homoeologous to both C*8* and C*3* of *B. oleracea*; however, C*3* and C*1* of *B. oleracea* also appear to be homoeologous in the same region [Figure 2, also SLOCUM *et al.* (1990)]. If chromosomal segments of C*1*, C*3* and C*8* are homoeologous (*e.g.*, triplicated), the model which postulates *B. oleracea* as a secondary polyploid, with a basic chromosomal complement of AABBCCDEF or ABBCCDEEF (HAGA 1938; ROBBELEN 1960) becomes inadequate to explain the evolution of *B. oleracea* chromosomes. An alternate hypothesis to triplication would invoke paleopolyploidy, suggesting that the close association between Brassica C*3* and C*8* is the result of a recent duplication, and the association between Brassica C*1* and C*3* is ancient. Neither of these hypotheses satisfactorily explain why no loci were found with duplicated RFLPs on C*1* and C*8*, however this could occur just by chance failure to detect the appropriate RFLPs.

KIANIAN and QUIROS (1992) proposed that the high level of duplications and chromosomal rearrangements in the genome of Brassica might impart enhanced "flexibility to change and evolve." Our suggestion, that *Arabidopsis* and *Brassica* appear to have diverged at the chromosomal level relatively rapidly, support this proposal. We note that the Cruciferae has a center of diversity in the temperate zone of the northern hemisphere, with the main radiation center proposed to have been from the eastern portion of the Irano-Turanian phytogeographical region (HEDGE 1976). The appearance of the Cruciferae is coincident with the advent of glaciation and major cooling, approximately 10 million years ago (TRAVERSE 1988). Therefore, this family evolved in an environment characterized by rapid climatic changes, with alternating glacial-interglacial cycles. It is tantalizing to speculate that the apparent plasticity of the Cruciferae genome (numerous duplications and rearrangements) may have conferred a selective advantage in taxa subjected to dramatic (at least, on an evolutionary time scale) changes in climate (MCCLINTOCK 1984). Alternately, physical subdivision of populations as a result of glaciation may simply have facilitated fixation of rare chromosomal rearrangements in small local populations.

Utility of a comparative map of the Cruciferae: Comparative maps of *A. thaliana* and *B. oleracea* permit cross-utilization of tools and resources which have been developed for each, a particular boon to the Brassica research community. Map-based cloning of orthologous genes may be much easier in *A. thaliana* than in *Brassica* spp., due to the small genome size and low level of repetitive DNA present in its genome (MEYEROWITZ 1989). One case in which a Brassica cDNA was used to complement an Arabidopsis mutation is already published (ARONDEL *et al.* 1992). More detailed fine-scale comparative mapping of these related crucifers may facilitate use of *A. thaliana* YAC islands (SCHMIDT *et al.* 1992) for positional cloning in *Brassica*. Finally, additional mapping of heterologous markers between *A. thaliana* and *B. oleracea* will provide much new basic information, helping us to understand the process of evolution in greater detail.

Contemporary molecular tools now permit detailed studies of "chromosomal archaeology." STEBBINS (1966) had foreseen these advances nearly three decades ago, when he suggested that "The opportunities for profitable investigations of this sort are by no means at an end, and new techniques may extend them to degrees of clarity and certainty which at present can hardly be imagined."

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