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An EST-enriched Comparative Map of *Brassica oleracea* and *Arabidopsis thaliana*

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A detailed comparative map of *Brassica oleracea* and *Arabidopsis thaliana* has been established based largely on mapping of *Arabidopsis* ESTs in two *Arabidopsis* and four *Brassica* populations. Based on conservative criteria for inferring synteny, “one to one correspondence” between *Brassica* and *Arabidopsis* chromosomes accounted for 57% of comparative loci. Based on 186 corresponding loci detected in *B. oleracea* and *A. thaliana*, at least 19 chromosome structural rearrangements differentiate *B. oleracea* and *A. thaliana* orthologs. Chromosomal duplication in the *B. oleracea* genome was strongly suggested by parallel arrangements of duplicated loci on different chromosomes, which accounted for 41% of loci mapped in *Brassica*. Based on 367 loci mapped, at least 22 chromosomal rearrangements differentiate *B. oleracea* homologs from one another. Triplication of some *Brassica* chromatin and duplication of some *Arabidopsis* chromatin were suggested by data that could not be accounted for by the one-to-one and duplication models, respectively. Twenty-seven probes detected three or more loci in *Brassica*, which represent 25.3% of the 367 loci mapped in *Brassica*. Thirty-one probes detected two or more loci in *Arabidopsis*, which represent 23.7% of the 262 loci mapped in *Arabidopsis*. Application of an EST-based, cross-species genomic framework to isolation of alleles conferring phenotypes unique to *Brassica*, as well as the challenges and opportunities in extrapolating genetic information from *Arabidopsis* to *Brassica* and to more distantly related crops, are discussed.

Arabidopsis thaliana, a weed-like member of the *Cruciferae* family (tribe Sisymbriaceae), offers many advantages for basic and applied plant research. These features include small stature, short life cycle, small genome size ($2n=10$, estimated physical genome size of 100–120 Mb), low frequency of repetitive sequences (~10% of the nuclear genome; Leutwiler et al. 1984), and prolific seed production. These features, combined with research of the past several decades yielding many mutants, efficient transformation systems, detailed genetic and physical maps, the availability of several P1, YAC, and BAC libraries, and 36,569 public ESTs (<http://www.cbc.umn.edu/ResearchProjects/Arabidopsis>), make *A. thaliana* an ideal model for further molecular and genetic study (Meyerowitz and Somerville 1994). A multinational genome research initiative aiming to completely sequence the *Arabidopsis* genome by year 2004 (The Multinational Science Steering Committee 1997) is ahead of schedule. Such an accomplishment will undoubtedly create new scientific challenges and

opportunities. One of the core issues will be how to apply the information obtained from the *Arabidopsis* genome project to the improvement of the world’s leading crops.

The genus *Brassica* (tribe Brassiceae), including many important crops, is in the same taxonomic family as *Arabidopsis thaliana*. Such a close relationship suggests that crop plants of the genus *Brassica* will be among the earliest beneficiaries of a complete sequence of *Arabidopsis*. Economically, *Brassica* can be loosely categorized into oilseed, vegetable, and condiment crops. *Brassica campestris*, *Brassica juncea*, *Brassica napus*, and *Brassica carinata* provide ~12% of the worldwide edible vegetable oil supplies (Labana and Gupta 1993) and generate >\$8 billion market value in North America and Europe. *Brassica oleracea* and *B. campestris*, the so-called “Cole crops,” comprise a large variety of vegetables in our daily diet. Many of these vegetables have extreme morphological characteristics of basic interest, such as the enlarged inflorescence of cauliflower (*B. oleracea* subsp. *botrytis*) and broccoli (*B. oleracea* subsp. *italica*); enlarged stem of kohlrabi (*B. oleracea* subsp. *gongyloides*) and marrowstem kale (*B. oleracea* subsp. *medullosa*); enlarged root of turnip (*B. campestris* subsp. *rapifera*); enlarged and twisted leaves of Pak-choi (*B. campestris* subsp. *chinesis*) and Chinese cabbage (*B. campestris* subsp. *pekinensis*); and enlarged single apical

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bud of cabbage (*B. oleracea* subsp. *capitata*) or many axillary buds of Brussels sprouts (*B. oleracea* subsp. *gemmifera*) (Kalloo and Bergh 1993). Notably, although *Arabidopsis* is considered a close relative to *Brassica*, none of these phenotypes occur in *Arabidopsis* to nearly the same degree. Finally, *Brassica nigra* is primarily used as a condiment (mustard seed).

Through cytological study, the species relationship of crop Brassicas was described by the "triangle of U" (U 1935). Three allotetraploids, *B. juncea* ($2n=36$, AABB), *B. napus* ($2n=38$, AACC), and *B. carinata* ($2n=34$, BBCC), originated through interspecific hybridization between different pairs of the three diploid species, *B. nigra* ($2n=16$, BB), *B. oleracea* ($2n=18$, CC), and *B. campestris* ($2n=20$, AA). Based on cytological examination and hybrid analysis, the haploid chromosome number of monogenomic species in the Brassiceae were found to range from 7 to 12 (Mizushima 1980). However, because of the available resolution of cytological techniques, detailed genomic relationships among monogenomic species were not fully revealed. Understanding the genomic relationship among monogenomic *Brassica* species will not only shed light on the evolution of the *Brassica* genome but also facilitate gene transfer among *Brassica* species. The rise of comparative mapping, the alignment of chromosomes based on common DNA markers, has provided the means to study in depth the parallels in genome structure and function of closely related species (Tanksley et al. 1988; Ahn and Tanksley 1993), and distantly related species (Paterson et al. 1996).

The present study aimed to better characterize the comparative genome organization of *Brassica* and *Arabidopsis*. Previous study of the genus *Brassica* showed that the proportion of low-copy DNA sequences was similar among diploid *Brassica* species, but a large number of rearrangements result in distinct chromosomal number and organization (Slocum et al. 1990; Landry et al. 1991, 1992; Song et al. 1991; Kianian and Quiros 1992; Lagercrantz and Lydiat 1996). Corresponding chromosomes in diploid and amphidiploid *Brassica* have been reported (Teutonico and Osborn 1994; Cheung et al. 1997a,b). Comparative mapping between *Arabidopsis* and *Brassica* revealed even more extensive chromosomal rearrangements (Kowalski et al. 1994a; Lagercrantz et al. 1996; Osborn et al. 1997). These studies, however, did not provide a complete scope of the genome comparison between *Brassica* and *Arabidopsis* because of the limited numbers of common markers. To address this issue, a larger number of markers were needed on the comparative maps. The present work, based on 186 corresponding loci detected, provides a much more detailed picture of the comparative genome organization of *B. oleracea* and *A. thaliana*. Furthermore, study of chromosomal duplication within the *B. oleracea* genome, based on 367 loci, illustrates

some of the complexities that will be faced both in extrapolating *Arabidopsis* information to *Brassica* and in assembly of sequence-ready contigs for crop genomes.

RESULTS

DNA Polymorphism

Table 1 summarizes the DNA polymorphism detected by 200 *Arabidopsis* EST clones and 123 *Brassica* PstI genomic clones. The relatively low level of polymorphism in the *B. oleracea* (RCB) × *B. oleracea* ssp. *alboglabra* var. Bugh Kana (BK) F₂ population was consistent with the origin of RCB from *B. oleracea* ssp. *alboglabra* types (Song and Osborn 1992). The chance of detecting polymorphic probes is low and similar in both *Arabidopsis* crosses. There is variation in polymorphism rate associated with different restriction enzymes in *Brassica*, but no particular pattern is clear. In *Arabidopsis*, the restriction enzyme *Cfo*I consistently detects more polymorphism than the other restriction enzymes, in both populations.

Establishing Composite Linkage Maps

B. oleracea Linkage Maps

Because many of the mapped polymorphisms were unique to one *B. oleracea* population, we constructed a composite linkage map for *B. oleracea* to more completely reflect all of the available comparative information. The assembly of the *B. oleracea* chromosome 1 composite map was illustrated in Figure 1 as an example, built according to the following rules: (1) Common loci detected in different populations could be identified based on the size of the restriction fragment from RCB, the common parent. These permitted the initial alignment of chromosomes of different populations. (2) The RCB × GC map was used as the primary linkage map because the largest number of loci were mapped in this population. Markers that did not detect

Table 1. Summary of the Polymorphism Detected by *Arabidopsis* EST Clones and *Brassica* PstI Genomic Clones

	<i>Cfo</i> I	<i>Dra</i> I	<i>Eco</i> RI	<i>Eco</i> RV	<i>Hind</i> III
<i>Arabidopsis</i> EST clones					
RCB × GC	–	67.3%	73.2%	75.0%	54.0%
WS × M13	24.5%	–	14.5%	18.0%	13.0%
WS × HM	21.5%	–	21.0%	21.0%	13.0%
<i>Brassica</i> PstI clones					
RCB × GC	–	62.7%	83.8%	71.6%	70.0%
RCB × PK	–	54.2%	73.0%	69.7%	44.7%
RCB × CAN	–	85.3%	69.1%	89.9%	93.5%
RCB × BK	–	36.2%	35.5%	36.8%	30.0%

Less than 80 EST clones were surveyed for RCB × BK, RCB × CAN, and RCB × PK and, thus were not included for comparison.

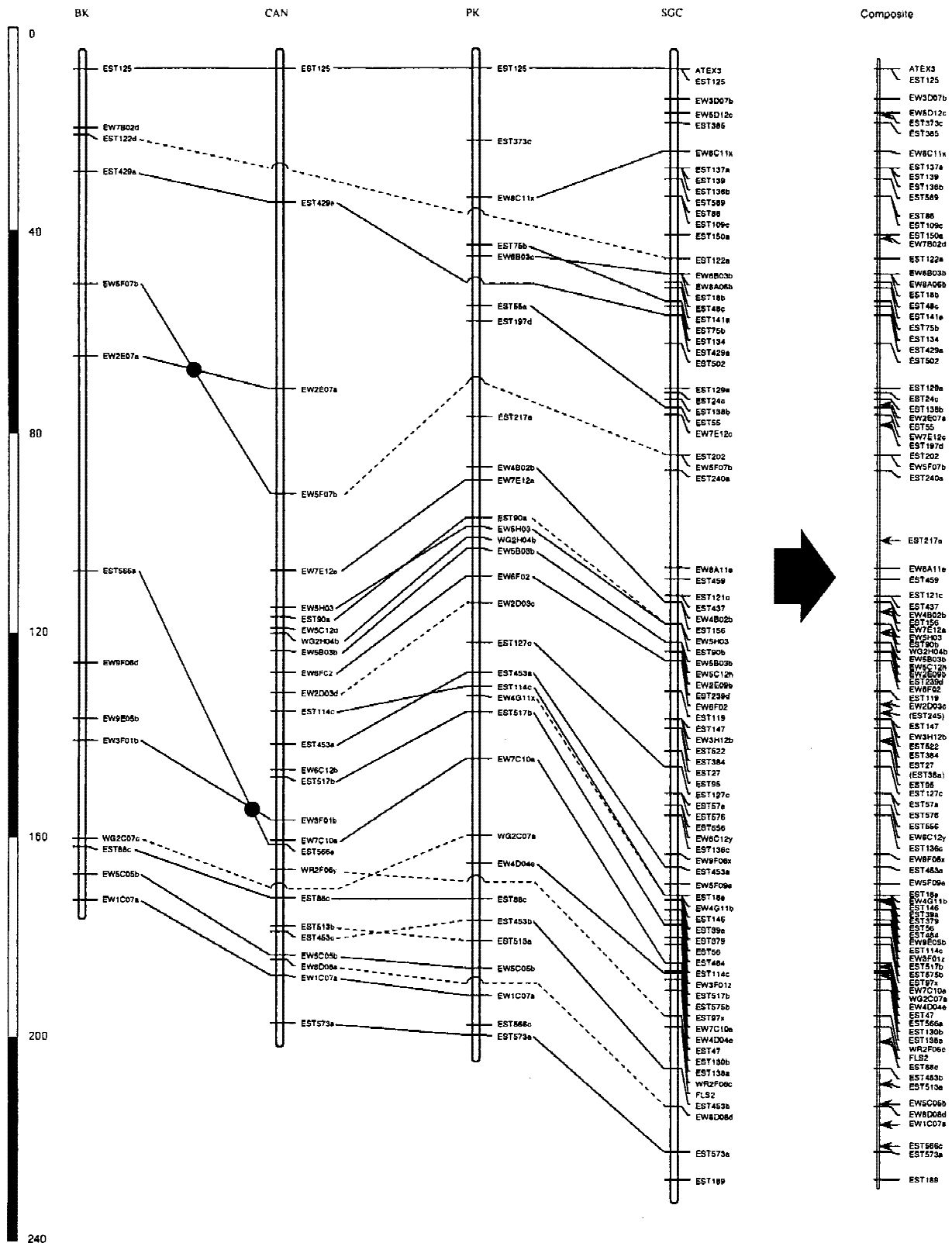


Figure 1 The assembly of the *Brassica* chromosome 1 composite map. Common loci (based on common restriction fragment sizes) were connected by solid lines, putatively homologous loci (with different restriction fragment sizes, but at corresponding sites) are connected by dashed lines. Filled circles placed on crossed lines indicate that respective orders of loci are statistically significantly different (\geq LOD 2.0) in the respective maps, suggesting possible chromosomal rearrangements. Arrows indicate the inferred locations of unique loci in the consensus map.

Table 2. Possible Chromosomal Rearrangements Detected in Different *Brassica* Varieties

Chromosome	Marker interval	<i>Brassica</i> variety
1	EW5F07b–EW2E07a	BK
	EST566a–EW3F01b	BK
2	EW9F06a–EW8D10a	CAN
7	EW7E01a–EW3A04b	PK
8	EST517d–EST22a	CAN
	EW1D09b–EW5C12b	CAN
9	EW5F07c–EW5H02	CAN
	EST195a–EW5F07c	CAN

polymorphism in RCB×GC population but did detect polymorphism in other populations were mapped in other populations accordingly. For chromosome 8, where the RCB×GC map exhibited few polymorphic markers, the RCB×PK linkage map was substituted. (3) The integration of unique loci was based on the closest common flanking loci, and the unique loci were positioned proportionally to their proximity to the flanking loci. (4) To test possible chromosomal rearrangements in different varieties, lod scores were calculated for the alternative (consensus) orders. Only if each possible consensus order in both populations could be ruled out by lod 2.0 was a rearrangement suggested.

The linkage maps span recombinational lengths of 743.0, 893.2, 947.1, and 871.3 cM across the *B. oleracea* genome in RCB×GC, PK, CAN, and BK populations, respectively, with an average length of 863.6 cM. The average recombinational lengths of *B. oleracea* chromosomes 1–9 are 189.2, 102.4, 91.7, 95.7, 97.4, 83.4, 77.2, 71.9, and 72.9 cM, respectively. A total of 367 loci were detected in the composite map with an average interval between loci of 2.35 cM. Based on an estimated DNA content of 660 Mb (Arumuganathan and Earle 1991), this corresponds to an average spacing of 1.8 Mb between markers and suggests that most genes are within 0.9 Mb of the nearest marker. Table 2 summarizes possible chromosomal rearrangements found among different *B. oleracea* varieties.

A. *thaliana* Linkage Maps

Construction of the *A. thaliana* composite map (Fig. 3, below) has been reported previously (Kowalski et al. 1994a), although this report includes 152 more loci. Specifically, common DNA polymorphisms detected on both populations served as “anchor loci” to infer the relative order of loci segregating in only one of the two populations. The map includes 262 loci across the *A. thaliana* genome. Thirty-one probes detect duplicated loci. Table 3 summarizes 20 duplicated loci newly detected by ESTs and cloned RAPD-amplified genomic DNA.

Patterns of Correspondence of *Brassica* Chromosomes with One Another and with the *Arabidopsis* Chromosomes

Figure 2 illustrates and Table 4 summarizes the composite linkage map of *B. oleracea*. We developed a model for the comparative organization of the chromosomes of *B. oleracea* and *A. thaliana* that assumes duplication of most *Brassica* chromosomes and one-to-one correspondence of *Brassica* chromosomes with *Arabidopsis* chromosomes. The extent to which the observed data cannot be explained by this “null hypothesis,” reflects the need for alternative hypotheses such as triplication of *Brassica* chromosomal segments or duplication of *Arabidopsis* chromosomal segments. The model was built based on the identification of SCEUS (smallest conserved evolutionary unit segments; O’Brien et al. 1993) of three or more loci that (1) maximize the number of corresponding DNA marker loci that are consistent with the model, (2) minimize the number of chromosomal rearrangements between duplicates (*Brassica*) or orthologs (*Arabidopsis*), (3) consider closely linked markers to be stronger evidence of synteny than distantly linked markers, and (4) consider a genetic distance of >5 cM to represent a true difference in locus order. This relatively large value was chosen to reflect not only the small size of the primary population but also the uncertainties associated with inference of loci mapped in other populations. Further constraints were imposed to evaluate the extent of du-

Table 3. Summary of Newly Detected Duplicated Loci in *Arabidopsis* Genome

	C1	C2	C3	C4	C5
C1	EST168a,b EST195a,b EST78a,b	EST46b,a R379a,b	EST36a,b R5342f,a	EST21a,b	EST150a,b EST371b,a
C2			EST30a,b EST95b,a	EST84c,a EST8b,a	EST151b,a R6191cd,ce
C3					EST138a,b R4392b,a
C5					EST240a,b EST68a,b

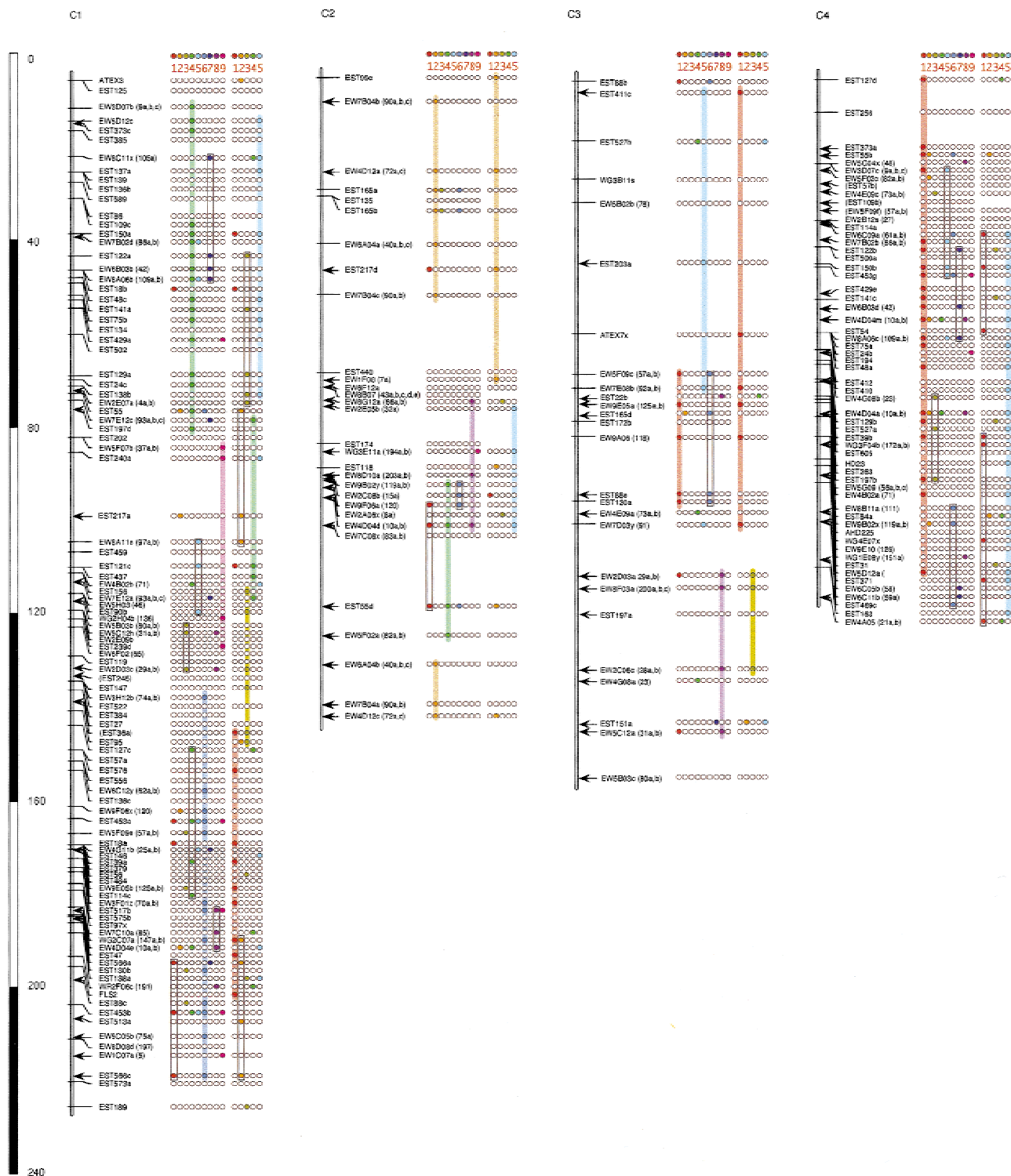


Figure 2 Composite RFLP linkage map of *Brassica oleracea* RCB x GC, RCB x CAN, RCB x PK and RCB x BK F2 Populations. Filled circles next to the loci indicate homoelogous *Brassica* loci (chromosomes 1-9, near right) or homologous *Arabidopsis* loci (chromosomes 1-5, far right) detected by the same probe. Open circles indicate that no polymorphism was detected for homoelogous (*Brassica*) and homologous (*Arabidopsis*) loci. A letter "R" next to the probe name indicates that the probe hybridizes to a repetitive DNA sequence in *Arabidopsis*. Specific colors are assigned to each homoelogous and homologous chromosome. Markers included in the duplication (*Brassica*) or one-to-one (*Arabidopsis*) models are connected by colored columns. Open columns indicate possible triplicated (*Brassica*) or duplicated (*Arabidopsis*) regions.



Figure 2 (Continued)

plication and triplication in *Brassica*. Specifically, possible regions of duplication along a chromosome were inferred first, in a manner that followed the above rules and did not allow different duplicated segments to overlap with each other by >5 cM (the threshold for inferring rearrangement). Finally, regions of possible triplication were inferred: These were allowed to overlap with duplicated segments but not with each other. From first principles, if the duplication process in *Bras-*

sica were random (not associated with large chromosomal region), the duplication model would explain 12.5% of data (given that *B. oleracea* has nine chromosomes). The extent to which the model improves on this reflects the strength of evidence for duplication and triplication. By the same rationale, one-to-one correspondence of *Brassica* to *Arabidopsis* must account for significantly more than the random expectation of 25% of data to be meaningful. Higher levels of corre-

Table 4. Summary of *Brassica-Brassica* and *Brassica-Arabidopsis* Correspondence

Chr.	No. of loci	Brassica			Arabidopsis		
		no. of corresponding loci	no. of probes with corresponding loci (avg/probe)	no. (%) of corresponding loci in model	no. (%) of corresponding loci	no. of probes with corresponding loci (avg/probe)	no. (%) of corresponding loci in model
1	101	86	57 (1.51)	34 (40%)	55	47 (1.17)	26 (47%)
2	30	29	19 (1.53)	16 (55%)	13	13 (1.00)	7 (54%)
3	26	27	20 (1.35)	12 (44%)	13	12 (1.08)	9 (69%)
4	58	54	34 (1.59)	21 (40%)	30	25 (1.20)	13 (43%)
5	33	24	16 (1.50)	8 (32%)	15	14 (1.07)	11 (73%)
6	29	35	22 (1.59)	15 (44%)	14	11 (1.27)	8 (57%)
7	28	19	15 (1.27)	8 (42%)	15	13 (1.15)	5 (33%)
8	20	23	17 (1.35)	7 (30%)	10	10 (1.00)	8 (80%)
9	42	41	25 (1.64)	19 (46%)	21	21 (1.00)	17 (81%)
Total	367	338	225 (1.50)	140 (41%)	186	166 (1.12)	106 (57%)

spondence in small chromosomal regions may be suggestive of duplication of chromosomal segments.

Brassica Chromosome 1

The “duplication” model, in which *Brassica* chromosome 1 corresponds to nonoverlapping segments of *Brassica* chromosomes 4, 9, and 6 (sequentially, moving down the chromosome), explains only 40% of the additional loci detected by probes for which at least one locus mapped to chromosome 1. Loci that are not included in the duplication model occur in several closely linked clusters that suggest higher order redundancy of chromatin. In particular, 20 loci suggest correspondence to regions of chromosomes 7 (near top), 5 and 3 (nonoverlapping regions near middle), 4 (parallel to upper part of chromosome 6 correspondence), and 8 and 1 (nonoverlapping regions parallel to lower part of chromosome 6 correspondence), which represent possible “triplicated” chromosomal segments and account for 23% of the corresponding loci. Eight additional loci corresponding to chromosomes 3 and 9 (near the bottom) are noted but could not be inferred to be syntenic by the rules of our model.

One-to-one correspondence to regions of *Arabidopsis* chromosomes 5, 4, 3, and 1 (moving down the *Brassica* chromosomes) accounts for 47% of corresponding loci. Possible duplication in *Arabidopsis* is suggested by five loci corresponding to *Arabidopsis* chromosomes 2 (parallel to chromosome 5 correspondence) and 8 (parallel to chromosome 4 and 1 correspondence), accounting for 26% of the corresponding loci.

Brassica Chromosome 2

One-to-one correspondence suggests an internal duplication where the upper part of the chromosome (EW7B04b–EW7B04c) corresponds to the lower part of the chromosome (EW6A04b–EW4D12c), based on

nine loci. The middle of chromosome 2 corresponds to chromosomes 8 and 4. Overall, these data explain 55% of the duplicated loci. Loci that are not included in this model suggest two possible segments corresponding to chromosomes 6 and 1 (parallel to chromosome 4 correspondence) and explain 21% of the corresponding loci.

One-to-one correspondence to regions of *Arabidopsis* chromosomes 2 and 5 accounts for 54% of corresponding loci. Three additional loci on *Arabidopsis* chromosome 2 partially overlap the correspondence of *Arabidopsis* chromosome 5.

Brassica Chromosome 3

One-to-one correspondence to segments of *Brassica* chromosomes 5, 1, and 8 explains 38% of the duplicated loci. Loci that are not included in this model suggest a triplicated segment corresponding to chromosome 6 (parallel to chromosome 1 correspondence) and explain 14% of the corresponding loci. Three isolated loci corresponding to chromosome 4 are noted but cannot be accommodated by the rules of the model.

One-to-one correspondence to regions of *Arabidopsis* chromosomes 1 and 3 accounts for 69% of the corresponding loci.

Brassica Chromosome 4

One-to-one correspondence to a segment of *Brassica* chromosome 1 explains 43% of the duplicated loci. Loci that are not included in this model suggest triplicated regions corresponding to chromosome 5, 3, 6, and 7, which explain 17% of corresponding loci. Additional loci corresponding to chromosome 6 and 8 are noted but cannot be accommodated by the rules of the model.

One-to-one correspondence of *Brassica* chromosome 4 to *Arabidopsis* chromosome 5 explains 43% of

the corresponding loci. Loci that are not included in this model suggest duplicated regions correspond to *Arabidopsis* chromosome 1 and explain 27% of corresponding loci. Additional loci corresponding to chromosome 3 are noted but cannot be accommodated by the rules of the model.

Brassica Chromosome 5

One-to-one correspondence to *Brassica* chromosome 1 explains 32% of the duplicated loci. Three loci not included in this model suggest a triplicated region corresponding to chromosome 9 explaining 12% of the corresponding loci. Four loci corresponding to chromosome 4 are noted but cannot be accommodated by the rules of the model.

One-to-one correspondence to regions of *Arabidopsis* chromosomes 1 and 2 explains 73% of the data.

Brassica Chromosome 6

One-to-one correspondence to segments of *Brassica* chromosomes 1 and 4 explains 44% of the duplicated loci. Loci that are not included in this model suggest a triplicated region corresponding to chromosome 2 and explain 9% of the corresponding loci. Isolated loci corresponding to chromosomes 2 and 8 are noted but cannot be accommodated by the rules of the model.

One-to-one correspondence to regions of *Arabidopsis* chromosomes 1 and 2 explains 57% of the corresponding loci. Three loci corresponding to chromosome 4 are noted.

Brassica Chromosome 7

One-to-one correspondence to segments of *Brassica* chromosomes 1 and 9 explains 42% of the duplicated loci. Loci that are not included in the model suggest a triplicated region corresponding to chromosome 4 and explain 16% of corresponding loci.

One-to-one correspondence to a region of *Arabidopsis* chromosome 5 explains 33% of the corresponding loci.

Brassica Chromosome 8

One-to-one correspondence to segments of *Brassica* chromosomes 4 and 3 explains 33% of the duplicated loci. Loci that are not included in this model suggest a triplicated region corresponding to chromosome 1 and explain 21% of corresponding loci. Four loci corresponding to chromosome 2 and three loci corresponding to chromosome 6 are noted.

One-to-one correspondence to regions of *Arabidopsis* chromosomes 4 and 3 explains 80% of the corresponding loci.

Brassica Chromosome 9

One-to-one correspondence suggests an internal duplication of chromosome 9 where the chromosomal segment EW8E09d–AKJ2c corresponds to the segment

AKJ2b–K457b, involving 10 loci. An intervening region corresponds to chromosome 7, and the lower part of chromosome 9 corresponds to chromosome 1. Overall, these regions explain 46% of the duplicated loci. Loci that are not included in this model suggest triplicated regions corresponding to chromosome 5 and explain 16% of corresponding loci. Four loci corresponding to chromosome 8 and three loci corresponding to chromosome 6 are noted but cannot be accommodated by the rules of the model.

One-to-one correspondence to regions of *Arabidopsis* chromosomes 1 and 5 accounts for 81% of the corresponding loci. Three loci correspond to *Arabidopsis* chromosome 3 are parallel to chromosome 5 correspondence and may reflect duplication in *Arabidopsis*.

Patterns of Correspondence of *Arabidopsis* Chromosomes with the *Brassica* Chromosomes

The “one-to-one correspondence” model and duplication model were also tested on the *Arabidopsis* linkage map as well, which was illustrated in Figure 3 and summarized in Table 5.

Arabidopsis Chromosome 1

The one-to-one model, in which *Arabidopsis* chromosome 1 corresponds to nonoverlapping segments of *Brassica* chromosomes 5, 1, 4, and 9, explains 46% of the loci detected by probes for which at least one locus mapped to chromosome 1. Loci that are not included in the one-to-one model suggest a duplicated region corresponding to *Brassica* chromosome 3 (parallel to chromosome 5 correspondence), explaining 10% of corresponding loci. Three loci corresponding to *Brassica* chromosome 6 are noted.

Arabidopsis Chromosome 2

The one-to-one model, in which *Arabidopsis* chromosome 2 corresponds to segments of *Brassica* chromosomes 1, 5, 1, and 2, explains 52% of the loci. Loci that are not included in the model suggest a duplicated region corresponding to chromosome 6, explaining 15% of corresponding loci.

Arabidopsis Chromosome 3

Our model suggests the correspondence of *Arabidopsis* chromosome 3 to nonoverlapping segments of *Brassica* chromosomes 1, 8, and 1 sequentially, explaining 49% of the duplicated loci. Loci that are not included in the model suggest duplicated segments of chromosome 4 (near top) and 9 (near bottom), explaining 17% of corresponding loci.

Arabidopsis Chromosome 4

The model suggests the correspondence of *Arabidopsis* chromosome 4 to *Brassica* chromosomes 8 and 4 and explains 36% of the loci. Loci that are not included in the model suggest a duplicated region corresponding

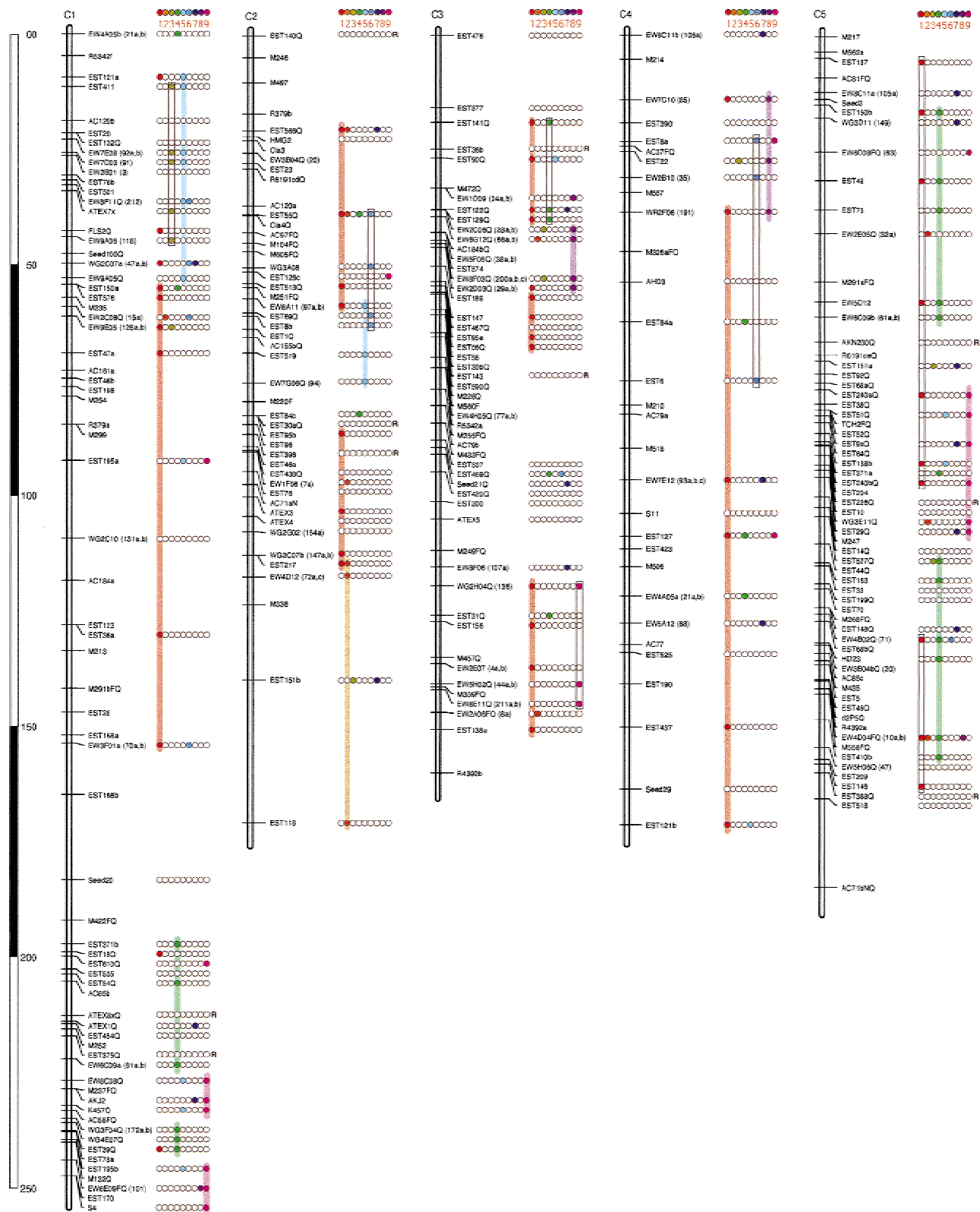


Figure 3 Composite RFLP linkage map of *Arabidopsis thaliana* HM x WS and M13 x WS F2 populations. Markers designated “FQ” are anchor loci, common to both populations. Markers mapped in the HM x WS population are designated “Q”. The remaining markers were mapped in M13 x WS only. The construction of data from two populations tends to inflate recombinational distance due to unequal recombination between populations. The filled circles next to the loci indicate homoeologous loci detected by the same probe on the *Brassica* composite map. Open circles indicate that no polymorphism was detected for homoeologous loci in RCB x GC *Brassica* populations. The letter “R” next to a probe name indicates that it hybridizes to a repetitive DNA sequence in the *Brassica* genome. Specific colors are assigned to each homoeologous chromosome. Markers included in the one-to-one model for *Arabidopsis-Brassica* correspondence are connected by filled columns. Open columns indicate possible duplicated regions in *Brassica*.

Table 5. Summary of *Arabidopsis*–*Brassica* Correspondence

Chr.	No. of loci	Arabidopsis		
		no. of corresponding loci	no. of probes with corresponding loci (avg/probe)	no. (%) of corresponding loci in model
1	70	52	34 (1.53)	24 (46%)
2	47	27	19 (1.42)	14 (52%)
3	51	35	24 (1.46)	17 (49%)
4	30	22	14 (1.57)	8 (36%)
5	64	44	27 (1.63)	17 (39%)
Total	262	180	118 (1.53)	80 (44%)

to chromosome 6, explaining 14% of corresponding loci. Three loci corresponding to chromosome 7 are noted.

Arabidopsis Chromosome 5

Our model suggests the correspondence of *Arabidopsis* chromosome 5 to segments of *Brassica* chromosomes 4, 9, and 4 and explains 39% of the corresponding loci. Loci that are not included in the model suggest duplicated regions corresponding to chromosome 1, explaining 23% of the corresponding loci. Six loci corresponding to chromosome 7 are noted.

DISCUSSION

It is timely to consider the challenges and opportunities in extrapolating structural genomic information from *Arabidopsis*, the first plant for which the genome will be completely sequenced, to *Brassica* and other more distantly related plants.

Our model (Fig. 2) suggests that at least 22 chromosomal rearrangements differentiate the *B. oleracea* homologs from one another and at least 19 rearrangements differentiate *A. thaliana* from *B. oleracea*. In several instances the locations of chromosomal rearrangement breakpoints between *Brassica* homologs approximately match the locations of the breakpoints between *Arabidopsis* and *Brassica*. Some such instances include (1) *Brassica* chromosome 2, where the correspondence with *Brassica* chromosomes 2 and 8 breaks between EW7B04c and EW6G12a and the correspondence with *Arabidopsis* chromosomes 2 and 5 breaks between EW1F08 and EW2E05b; (2) *Brassica* chromosome 3, where the correspondence with *Brassica* chromosomes 1 and 8 breaks between EST130a and EW2D03a and the correspondence of *Arabidopsis* chromosomes 1 and 3 breaks between EW7D03y and EW2D03a; (3) *Brassica* chromosome 8, where the correspondence with *Brassica* chromosome 4 and 3 homologs breaks between EW5G04b and EST517d and the correspondence of *Arabidopsis* chromosomes 4 and 3 breaks between EST22a and EW8F03b; (4) *Brassica* chromosome 9, where the correspondence of *Brassica* chromosomes 9

and 1 breaks between K457b and EST517g and the correspondence of *Arabidopsis* chromosomes 1 and 5 breaks between EW1G03a and EST9a. Such rearrangement breakpoints that appear to be common to *Brassica* and *Arabidopsis* may reflect cases where both *Arabidopsis* and one *Brassica* homolog retain the chromosome organization of their common ancestor, whereas a duplicated *Brassica* homolog has undergone rearrangement. Similarly, chromosomal regions in which *Arabidopsis* gene order corresponds to one but not both *Brassica* homoeologs may reflect rearrangement of one *Brassica* homoeolog since duplication. For example, on *Arabidopsis* chromosome 5, the order for marker EW5D12, EST075, and EST150 is EST150–EST75–EW5D12, and on *Brassica* chromosome 4, it follows the same order, but on *Brassica* chromosome 1, the order changes to EST75–EST150–EW5D12.

Comparative Organization of *Brassica* Homoeologous Chromosomes

The *Brassica* chromosomal duplication model explains 41% of the duplicated restriction fragment length polymorphism (RFLP) loci we mapped (Table 4). If there were no pattern to duplication, then the duplication would be expected to account for <12.5% (1 out of 8) of data, because there are nine pairs of chromosomes in *B. oleracea*. Our data clearly indicate that duplication has involved large chromosome segments in *Brassica*. In a similar manner, if triplication accounts for more than an additional 14.3% (1 out of 7) of data in *Brassica*, then it would be more common than expected to occur at random. Based on our model, triplication of *Brassica* chromosomal segments best explains 18% of the data, which is nominally greater than the expected value (14.3%). Although the case for triplication is much weaker than for duplication, the clustering of triplicated loci into linked groups does tend to support prior suggestions based on smaller numbers of probes and isolated genomic regions (Kowalski et al. 1994a; Lagercrantz et al. 1996; Osborn et al. 1997) that some regions of the genome of *B. oleracea* (as well as *B. rapa* and *B. nigra*) may be triplicated. A

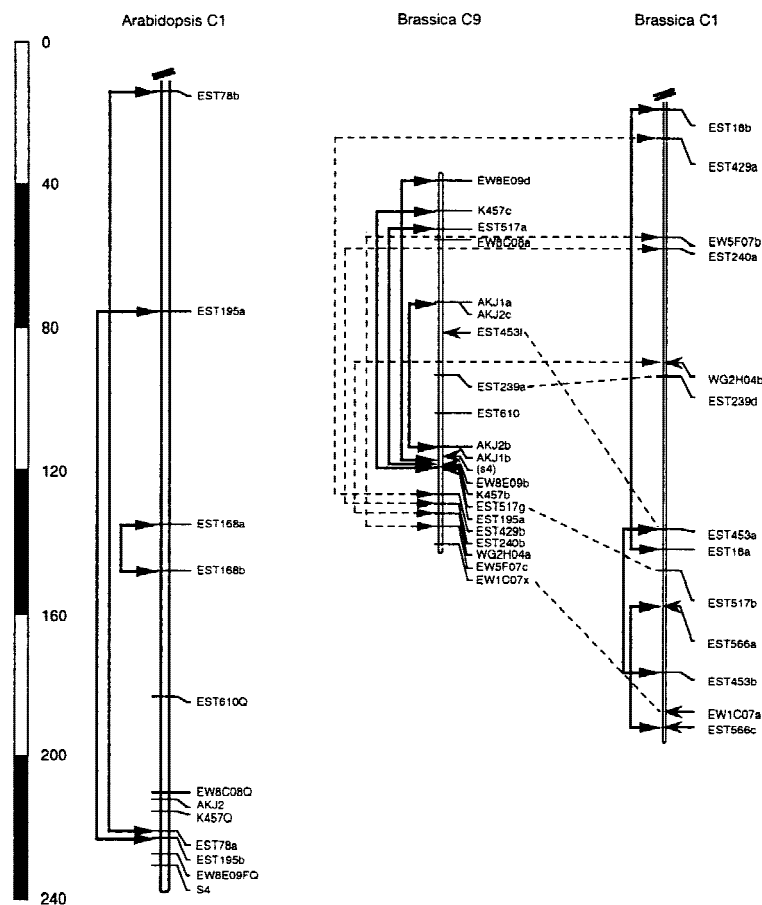


Figure 4 Intrachromosomal duplication of *Arabidopsis* chromosome 1, and an possible more-than-triplicated region of *Brassica* chromosome 1 and 9. Solid lines connect homoeologous loci (based on different restriction fragment sizes) located on the same chromosome. Dashed lines connect homoeologous loci located on different chromosomes.

fundamental problem in the use of genetic mapping data to evaluate duplication (and triplication) of chromatin is the need to detect DNA polymorphism. The assembly of physical maps for the *Brassica* genomes will alleviate this limitation but will require new methodology to efficiently determine the locus-specificity of BACs (or other large DNA clones) that hybridize to duplicated (or triplicated) probes.

Alignment of *Brassica* and *Arabidopsis* Chromosomes

The *Brassica/Arabidopsis* one-to-one correspondence model explain 57% of our observed data (Table 4). If the genomes of *Brassica* and *Arabidopsis* were randomly arranged with respect to one another, then one-to-one correspondence would account for < 20% (1 out of 5) of data in *Arabidopsis*. Our data clearly indicate extensive synteny of *Brassica* and *Arabidopsis*.

A total of 31 pairs of duplicated loci, including 20 pairs reported here for the first time (Table 3), mapped to *A. thaliana*, accounting for 23.7% of the loci de-

tected. These duplicated loci expand on the earlier suggestion (Kowalski et al. 1994a) that part of the *A. thaliana* genome may have undergone ancient duplication. These ancient duplications could complicate contig-map construction and also could reduce the subset of *Arabidopsis* genes that are susceptible to “knockout” experiments (Sundaresan et al. 1995; Kempin et al. 1997). Notably, an intrachromosomal duplication appears to occur in *A. thaliana* chromosome 1 (Fig. 4).

Intrachromosomal duplication was observed in chromosomes 1, 2, and 9 of *B. oleracea* (Fig. 5). Two independent studies on the genome of *B. nigra* reveal similar patterns on chromosome 5 (Truco and Quiros 1994) and chromosome 6 (Lagercrantz and Lydiat 1996), suggesting that such intrachromosomal duplications preceded the duplication/triplication of the ancestral *B. oleracea* genome, then even higher levels of duplication might be expected in modern *B. oleracea*. In our study, five probes did detect more than three segregating loci in *B. oleracea*, including EW4D04 (chromosomes 1, 2, 4, and 8), EW8A06 (chromosomes 1, 4, 5, and 7), EST55 (chromosomes 1, 2, 4, and 6), EST453 (chromosomes 1, 4, 5, 6, and 9), and EST517 (chromosomes 1, 6, 8, and 9). Although we cannot rule out the possibility that some of these more-than-triplicated loci might be the consequence of other du-

plication mechanisms, segments of *Brassica* chromosomes 1 and 9 did suggest the existence of such high-order chromosome segmental duplication (Fig. 5). More probes mapped in this region should provide further evidence.

Through comparative mapping, many powerful tools already created for *Arabidopsis* can now be applied to *Brassica*. For example, *Arabidopsis* cDNA sequences may be used to isolate homologous genes in *Brassica*, *Arabidopsis* BAC/YAC contigs may be used in *Brassica* for map-based cloning, and *Arabidopsis* high-resolution maps may help to resolve clustered markers in *Brassica* (Liu et al. 1996). *Arabidopsis* genomic tools may guide the isolation of *Brassica* alleles conferring unique phenotypes. *Brassica* and *Arabidopsis* may have diverged as little as 10 mya (Muller 1981), suggesting that ~90% of chromosomal segments <5 cM may remain colinear (Paterson et al. 1996). A comparative map with a density of <5 cM/marker makes it relatively easy to evaluate correspondence of *Brassica* quantitative trait loci (QTLs) to *Arabidopsis* mutations or can-

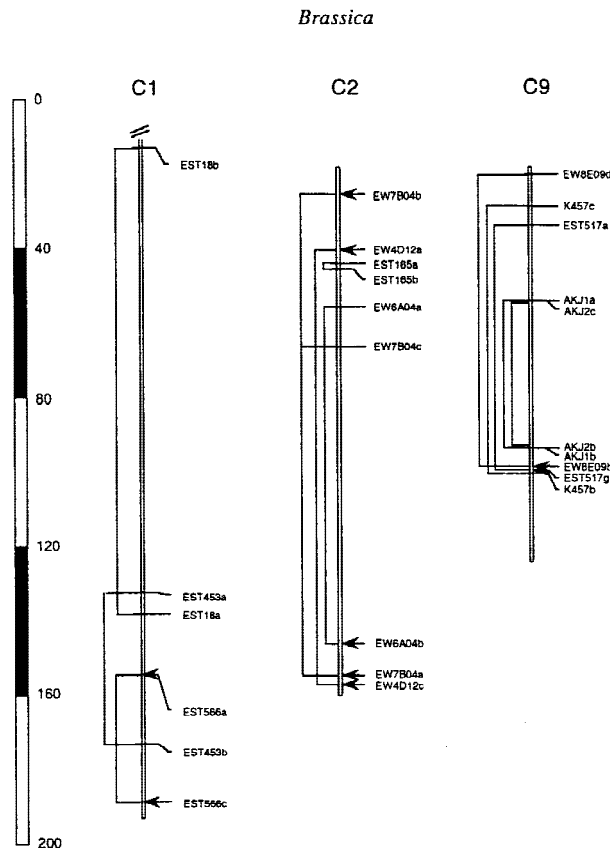


Figure 5 Intrachromosomal duplication detected by three or more duplicate loci in *Brassica*.

didate genes. Furthermore, a comparative map of *B. oleracea* (CC genome) and *A. thaliana* can be extended to an amphidiploid species of *Brassica* such as *B. napus* (AACC genome), where genome complexity is redoubled.

Genetic linkage maps based on ESTs (Berry et al. 1995) enable one to use sequence information to screen for conservation with distantly related taxa. For example, disease-resistance-like ESTs could be potentially useful in locating disease-resistance loci in a specifically designed segregating population other than *Arabidopsis* (Botella et al. 1997). Also, through selection of highly conserved ESTs, comparative organization of the chromosomes of even distantly related species such as *Arabidopsis*, *Gossypium* (cotton), and *Sorghum* can be studied using the same probes (Paterson et al. 1996). Thus, a cross-genome comparative map based on a common set of ESTs may eventually provide a direct comparison of macro- and microcolinearity across various species. The combination of ESTs and DNA microarray technology (Winzeler et al. 1998) could accelerate this process. Furthermore, mapping the common set of ESTs to *Arabidopsis* megabase DNA libraries (Schmidt et al. 1995; Zachgo et al. 1996; Agyare et al. 1997) will extend the *Arabidopsis* physical map and

DNA contigs to other plants. Thus, using *Arabidopsis* contigs to assist map-based cloning in cotton, sorghum, or other genomes may be more feasible. Such a cross-genome framework and toolbox could profoundly affect future genome sequencing projects in related taxa. It is of interest not only to elucidate the portions of genome that are conserved (common) among various species but also the portions that are divergent among species. Thus, the priority of subsequent crop genome sequencing projects might be focused on genomic regions that are poorly conserved, so that scarce financial resources are used more efficiently.

METHODS

Plant Materials

Two *A. thaliana* F₂ populations were used in this study: *A. thaliana* ecotype Wassilewskija (WS) × mutant stock M13 (Liu et al. 1996) and WS × Hannover/Münden (HM) (Kowalski et al. 1994b). Subsets of 78 individuals from each population were used for mapping *Arabidopsis* ESTs. Four *B. oleracea* F₂ mapping populations were used in this experiment: RCB (self-compatible) × *B. oleracea* var. Green Comet (USDA collection, accession no. G30771, from North America), RCB × *B. oleracea* var. Cantanese (USDA collection, accession no. PI462224, originally from Italy), RCB × *B. oleracea* var. Pusa Katki (USDA collection, accession no. PI274783, originally from India), and RCB × *B. oleracea* var. Bugh Kana (USDA collection, accession no. PI249556, originally from Thailand), composed of 56, 247, 250, and 246 individuals, respectively. *A. thaliana* seed were obtained from the *Arabidopsis* Biological Resources Center at Ohio State University, directed by Dr. R.L. Scholl. Rapid-cycling *Brassica* was from the Crucifer Genetics Cooperative, Madison, WI. Seed and pollen of other *B. oleracea* varieties were generously provided by Dr. J. McFerson and Dr. S. Kresovich, then at USDA-ARS, Geneva, NY.

Genotyping

DNA extraction, electrophoresis, Southern blotting and autoradiography were as described previously (Kowalski et al. 1994a). A total of 113 *Brassica* PstI genomic clones ("EW," "WG," and "WR," from Pioneer HiBred), 35 *Arabidopsis* genomic clones ("M," from Dr. E. Meyerowitz, Caltech), 23 *Arabidopsis* anonymous cDNA clones ("AC," "ATEX," and "TCH"), four cloned RAPD-PCR products ("R;" unpubl.), 198 *Arabidopsis* EST clones ("EST," from Dr. R.L. Scholl, the *Arabidopsis* Biological Resources Center, Ohio State University), and 19 putatively embryo-specific *Arabidopsis* EST clones ("AHD," "AKJ," "AKN," "Cla," "d2P," "FLS," "HD," "HMG," "K," "S," and "Seed," from Dr. Terry L. Thomas, Texas A&M University) were used in this study.

Data Analysis

RFLP linkage maps were constructed using MapMaker (Lander et al. 1987). Linkage groups were built at threshold of lod (logarithm of odds)=2.1 for *A. thaliana* and lod=2.5 for *B. oleracea*. Genetic distances (in centiMorgans) were calculated using the Kosambi mapping function.

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REFERENCES

- Agyare, F.D., D.A. Lashkari, A. Lagos, A.F. Namath, G. Lagos et al. 1997. Mapping expressed sequence tag sites on yeast artificial chromosome clones of *Arabidopsis thaliana* DNA. *Genome Res.* **7**: 1-9.
- Ahn, S. and S.D. Tanksley. 1993. Comparative linkage maps of the rice and maize genomes. *Proc Natl. Acad. Sci.* **90**: 7980-7984.
- Arumuganathan, K. and E.D. Earle. 1991. Estimation of nuclear DNA content of plants by flow cytometry. *Plant Mol. Biol. Rep.* **9**: 208-218.
- Berry, R., T.J. Stevens, N.A.R. Walter, A.S. Wilcox, T. Rubano et al. 1995. Gene-based sequence-tagged-sites (STSs) as the basis for a human gene map. *Nat. Genet.* **10**: 415-423.
- Botella, M.A., M.J. Coleman, D.E. Hughes, M.T. Nishimura, J.D.G. Jones et al. 1997. Map positions of 47 *Arabidopsis* sequences with sequence similarity to disease resistance genes. *Plant J.* **12**: 1197-1211.
- Cheung, W.Y., G. Champagne, N. Hubert, and B.S. Landry. 1997a. Comparison of the genetic maps of *Brassica napus* and *Brassica oleracea*. *Theor. Appl. Genet.* **94**: 569-582.
- Cheung, W.Y., L. Friesen, G.F.W. Rakow, G. Seguin Swartz, and B.S. Landry. 1997b. A RFLP-based linkage map of mustard (*Brassica juncea* (L.) Czern. and Coss.). *Theor. Appl. Genet.* **94**: 841-851.
- Kaloo, G. and B.O. Bergh. 1993. *Genetic improvement of vegetable crops*. Pergamon Press, Oxford, UK.
- Kempin, S.A., S.J. Liljegren, L.M. Block, S.D. Rounsley, M.F. Yanofsky et al. 1997. Targeted disruption in *Arabidopsis*. *Nature* **389**: 802-803.
- Kianian, S.F. and C.F. Quiros. 1992. Generation of a *Brassica oleracea* composite RFLP map: linkage arrangements among various populations and evolutionary implications. *Theor. Appl. Genet.* **84**: 544-554.
- Kowalski, S.P., T.-H. Lan, K.A. Feldmann, and A.H. Paterson. 1994a. Comparative mapping of *Arabidopsis thaliana* and *Brassica oleracea* chromosomes reveals islands of conserved organization. *Genetics* **138**: 499-510.
- . 1994b. QTL mapping of naturally-occurring variation in flowering time of *Arabidopsis thaliana*. *Mol. & Gen. Genet.* **245**: 548-555.
- Labana, K.S. and M.L. Gupta. 1993. Importance and origin. In *Breeding oilseed Brassicas* (eds. K.S. Labana, S.S. Banga, and S.K. Banga), pp. 1-20. Springer-Verlag Press, Berlin, Germany.
- Lagercrantz, U. and D.J. Lydiat. 1996. Comparative genome mapping in *Brassica*. *Genetics* **144**: 1903-1910.
- Lagercrantz, U., J. Putterill, G. Coupland, and D. Lydiat. 1996. Comparative mapping in *Arabidopsis* and *Brassica*, fine scale genome collinearity and congruence of genes controlling flowering time. *Plant J.* **9**: 13-20.
- Lander, E., P. Green, J. Abrahamson, A. Barlow, M. Daly et al. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* **1**: 174-181.
- Landry, B.S., N. Hubert, and T. Etoh. 1991. A genetic map for *Brassica napus* based on restriction fragment length polymorphisms detected with expressed DNA sequences. *Genome* **34**: 543-552.
- Landry, B.S., N. Hubert, and R. Crete. 1992. A genetic map of *Brassica oleracea* based on RFLP markers detected with expressed DNA sequences and mapping resistance genes to race 2 of *Plasmodiophora brassicae* (Woronin). *Genome* **35**: 409-419.
- Leutwiler, L., B. Hough-Evans, and E. Meyerowitz. 1984. The DNA of *Arabidopsis thaliana*. *Mol. & Gen. Genet.* **219**: 225-234.
- Liu, S.C., S.P. Kowalski, T.H. Lan, K.A. Feldmann, and A.H. Paterson. 1996. Genome-wide high-resolution mapping by recurrent intermingling using *Arabidopsis thaliana* as a model. *Genetics* **142**: 247-258.
- Meyerowitz, E.M. and C.R. Somerville. 1994. *Arabidopsis*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Mizushima, U. 1980. Genome analysis in *Brassica* and allied genera. In *Brassica crops and wild allies* (eds. S. Tsunoda, K. Hinata, and C. Gomez-Campo), pp. 89-106. Japan Scientific Societies Press, Tokyo, Japan.
- Muller, J. 1981. Fossil pollen records of extant angiosperms. *Bot. Rev.* **47**: 1-142.
- The Multinational Science Steering Committee. 1997. *The multinational coordinated Arabidopsis thaliana genome research project*. Progress report: Year six. National Science Foundation.
- O'Brien, S.J., J.E. Womack, L.A. Lyons, K.J. Moore, N.A. Jenkins et al. 1993. Anchored reference loci for comparative genome mapping in mammals. *Nat. Genet.* **3**: 103-112.
- Osborn, T.C., C. Kole, I.A.P. Parkin, A.G. Sharpe, M. Kuiper et al. 1997. Comparison of flowering time genes in *Brassica rapa*, *B. napus* and *Arabidopsis thaliana*. *Genetics* **146**: 1123-1129.
- Paterson, A.H., T.-H. Lan, K.P. Reischmann, C. Chang, Y.-R. Lin et al. 1996. Toward a unified map of higher plant chromosomes, transcending the monocot-dicot divergence. *Nature Genet.* **14**: 380-382.
- Schmidt, R., J. West, K. Love, Z. Lenehan, C. Lister et al. 1995. Physical map and organization of *Arabidopsis* chromosome 4. *Science* **270**: 480-483.
- Slocum, M.K., S.S. Figdore, and W.C. Kennard. 1990. Linkage arrangement of restriction fragment length polymorphism loci in *Brassica oleracea*. *Theor. Appl. Genet.* **80**: 57-64.
- Song, K. and T.C. Osborn. 1992. Polyphyletic origins of *Brassica napus*: New evidence based on organelle and nuclear RFLP analyses. *Genome* **35**: 992-1001.
- Song, K., J. Suzuki, M. Slocum, and T.C. Osborn. 1991. A linkage map of *Brassica rapa* (syn. *campestris*) based on restriction fragment length polymorphism loci. *Theor. Appl. Genet.* **82**: 296-304.
- Sundaresan, V., P. Springer, T. Volpe, S. Haward, J. Jones et al. 1995. Patterns of gene action in plant development revealed by enhancer trap and gene trap transposable elements. *Genes & Dev.* **9**: 1797-1810.
- Tanksley, S.D., R. Bernatzky, and N.L. Lapitan. 1988. Conservation of gene repertoire but not gene order in pepper and tomato. *Proc. Natl. Acad. Sci.* **85**: 6419-6423.
- Teutonico, R.A. and T.C. Osborn. 1994. Mapping of RFLP and qualitative trait loci in *Brassica rapa* and comparison to the linkage maps of *B. napus*, *B. oleracea*, and *Arabidopsis thaliana*. *Theor. Appl. Genet.* **89**: 885-894.
- Truco, M.J. and C.F. Quiros. 1994. Structure and organization of the B genome based on a linkage map in *Brassica nigra*. *Theor. Appl. Genet.* **89**: 590-598.
- U, N. 1935. Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. *Jpn. J. Bot.* **7**: 389-452.
- Winzeler, E.A., D.R. Richards, A.R. Conway, A.L. Goldstein, S. Kalman et al. 1998. Direct allelic variation scanning of the yeast genome. *Science* **21**: 1194-1197.
- Zachgo, E.A., M.L. Wang, J. Dewdney, D. Bouchez, C. Comilleri et al. 1996. A physical map of chromosome 2 of *Arabidopsis thaliana*. *Genome Res.* **6**: 19-25.

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An EST-enriched Comparative Map of *Brassica oleracea* and *Arabidopsis thaliana*

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