Comparative Mapping Between *Arabidopsis thaliana* and *Brassica nigra* Indicates That Brassica Genomes Have Evolved Through Extensive Genome Replication Accompanied by Chromosome Fusions and Frequent Rearrangements

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ABSTRACT

Chromosome organization and evolution in the Brassicaceae family was studied using comparative linkage mapping. A total of 160 mapped *Arabidopsis thaliana* DNA fragments identified 284 homologous loci covering 751 cM in *Brassica nigra*. The data support that modern diploid Brassica species are descended from a hexaploid ancestor, and that the *A. thaliana* genome is similar in structure and complexity to those of each of the hypothetical diploid progenitors of the proposed hexaploid. Thus, the Brassica lineage probably went through a triplication after the divergence of the lineages leading to *A. thaliana* and *B. nigra*. These duplications were also accompanied by an exceptionally high rate of chromosomal rearrangements. The average length of conserved segments between *A. thaliana* and *B. nigra* was estimated at 8 cM. This estimate corresponds to ~90 rearrangements since the divergence of the two species. The estimated rate of chromosomal rearrangements is higher than any previously reported data based on comparative mapping. Despite the large number of rearrangements, fine-scale comparative mapping between model plant *A. thaliana* and Brassica crops is likely to result in the identification of a large number of genes that affect important traits in Brassica crops.

ONE important aspect of genome evolution is changes in organization of the DNA caused by duplications and chromosomal rearrangements. Comparative linkage mapping has indicated that many animal and plant genomes have remained surprisingly conserved during evolution (O'Brien *et al.* 1988; Whitkus *et al.* 1992; Ahn and Tanksley 1993; Morizot 1994; Moore *et al.* 1995). However, a variation between different evolutionary lineages in the rate of chromosomal evolution is evident, both from cytogenetic work (Wilson *et al.* 1974, 1977; Bush *et al.* 1977; Eldridge and Close 1993) and comparative mapping (Nadeau and Taylor 1984; Barendse *et al.* 1994; Johansson *et al.* 1995; Graves 1996; Ehrlich *et al.* 1997). This pattern has led O'Brien *et al.* (1988) to suggest that karyotypes are generally conservative but occasionally undergo radiation or "shuffles." The cause of these sporadic shuffles is still under debate. In plants, conservation of surprisingly large chromosomal segments has been observed in the Graminae family (Moore *et al.* 1995), while tomato and chili pepper, in the Solanaceae family, often have been cited as a pair of species that differ by a relatively large number of rearrangements (Tanksley *et al.* 1988; Prince *et al.* 1993).

Polyploidy is particularly common among plants. It has been estimated that up to 80% of angiosperms are polyploid (Masterson 1994). Furthermore, recent genetic mapping has revealed cryptic polyploids indicating that the level of polyploidy has been underestimated in several species (Helentjaris *et al.* 1988; Reinisch *et al.* 1994; Moore *et al.* 1995; Lagercrantz and Lydiate 1996). Recent studies have also suggested that polyploidization may be accompanied by rapid genomic change (Song *et al.* 1995; Kenton *et al.* 1993; Chen and Armstrong 1994; Jellen *et al.* 1994; Lagercrantz and Lydiate 1996; Shoemaker *et al.* 1996; Leitch and Bennet 1997).

The family Brassicaceae (Cruciferae) is widely distributed and comprises more than 3000 species in approximately 350 genera. The family includes important crops such as *Brassica oleracea*, *B. napus*, and *B. rapa*, as well as the extensively studied model plant *Arabidopsis thaliana*. The family's major centers of diversity are southwestern and central Asia and the Mediterranean region. Secondary centers of diversity are in the arctic, western North America, and the mountains of South America (Price *et al.* 1994).

Species within the family exhibit a continuous range of haploid chromosome numbers from 5 to 15, excluding a large number of known polyploid species with higher chromosome numbers. Previous comparative mapping has indicated that present-day diploid species in the Brassica genus are derived from a hexaploid ancestor (Lagercrantz and Lydiate 1996). Furthermore, preliminary comparisons between Brassica species and *A. thaliana* have indicated that the *A. thaliana* genome

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has a complexity corresponding to one of the three subgenomes of modern diploid Brassica species (Lagercrantz *et al.* 1996; Scheffler *et al.* 1996; Cavell *et al.* 1998). Comparative mapping data have also indicated a relatively high rate of chromosomal rearrangements in the Brassicaceae family (Kowalski *et al.* 1994; Lagercrantz and Lydiate 1996; Osborn *et al.* 1997). With one exception, previous comparisons between the Brassica and Arabidopsis genomes have been limited to small genomic regions (Lagercrantz *et al.* 1996; Scheffler *et al.* 1996; Cavell *et al.* 1998). The only across-genome comparison published so far (Kowalski *et al.* 1994) was based on a low marker density (the 75 previously positioned *B. oleracea* loci had an average spacing of one locus per 13 cM; Slocum *et al.* 1990). Because of the limitations of incomplete polymorphism in the *B. oleracea* population, the degree of genome replication in *B. oleracea* was also underestimated in that study. Consequently, to elucidate the relationship between the uniquely small genome of *A. thaliana* and the highly replicated Brassica genomes, there is a need for additional comparative mapping data. The apparently high rate of chromosomal rearrangements in the Brassicaceae family also requires an exceptionally high density of markers.

The complete genome sequence of *A. thaliana* will be available within a few years (Ecker 1998), and the function of many of the identified genes will become elucidated. An understanding of the complex relationship between the small genome of *A. thaliana* and its highly replicated close relatives in the Brassica genus could have a great impact on the understanding of the biology and the improvement of the important Brassica crops.

To obtain a more comprehensive picture of the relationships between *A. thaliana* and the members of the Brassica genus, I applied a large number of previously mapped *A. thaliana* probes to a highly polymorphic mapping population of *B. nigra*. This strategy yielded a high marker density (an average spacing of one locus per 3 cM), and a high proportion of replicated loci mapped. Compared to earlier studies, these data allowed a much more detailed comparison comprising the entire *A. thaliana* genome. The data support that diploid Brassica genomes contain three copies of a basic genome similar in size to the *A. thaliana* genome, and that chromosome evolution in Brassicaceae seems to involve an exceptionally high rate of chromosomal rearrangements. The comparative data also have implications for the possibilities of transferring genetic resources and information between *A. thaliana* and Brassica species.

MATERIALS AND METHODS

The *B. nigra* genetic map was developed using a previously described mapping population (Lagercrantz and Lydiate 1995). The population was a highly polymorphic, first backcross population of 88 individuals in which it was possible to score segregation of markers from both the F_1 and (highly heterozygous) recurrent parents. Linkage maps from meioses in the F_1 and recurrent parents were initially developed separately using MAPMAKER 3.0 (Lander *et al.* 1987; Lincoln *et al.* 1992) and then integrated using JoinMap (Stam 1993). The integration of two linkage maps can introduce ordering errors, particularly if few markers are common to both maps. This potential source of inaccuracy caused few problems in the current analysis as a majority of the loci positioned on the map from the recurrent parent were also positioned on the F_1 map (Lagercrantz and Lydiate 1996). All loci were detected using Southern hybridization analysis as described by Lagercrantz and Lydiate (1995), except that washing was carried out at a lower stringency (2× SSC, 0.1% SDS at 65°). Hybridization probes were prepared by PCR or gel isolation of inserts and labeled with [^{32}P]dCTP.

A set of 160 DNA fragments from the *A. thaliana* genome were used as RFLP probes. The probes were derived from the following sources (prefix, reference, and source in parentheses): 69 genomic *Pst*I clones (mi, Liu *et al.* 1996, Ohio Stock Center); 21 anonymous cDNAs (ve, D. Bouchez, personal communication, Ohio Stock Center); 18 genomic clones from the ARMS set (m, Fabri and Schaeffner 1994, Ohio Stock Center); 20 anonymous cDNAs (um, McGrath *et al.* 1993, Ohio Stock Center); 8 anonymous cDNAs (y and tai224, unpublished data, Ohio Stock Center); and six YAC end probes and 3 anonymous cDNAs (L, R, and c, Lagercrantz *et al.* 1996, Dr. G. Coupland). In addition, the following characterized *A. thaliana* genes were used: Phytochrome A, B, and C (phy, Sharrock and Quail 1989, Dr. P. Quail); Athb3, Athb7, Athb13 (Athb, Söderman 1996, Dr. P. Engström); Chalcone synthase (chs, Feinbaum and Ausubel 1988, Dr. G. Coupland); Ara (Matsui *et al.* 1989, Dr. H. G. Nam); Rps2 (rps2, Mindrinos *et al.* 1994, Dr. F. Ausubel); Gpa1 (gpa1, Ma *et al.* 1990, Dr. H. Ma); GapC (gapC, Shih *et al.* 1991, Dr. M-C. Shih); Rns1 (rns1, Bariola *et al.* 1994, Dr. P. Green); Ag (ag, Yanofsky *et al.* 1990); Ga1 (ga1, Sun and Kamiya 1994); and Pg11 (pg11, Gallant and Goodman, unpublished data). Probes for these were derived from cDNA clones, except for Chs2 (genomic clone) and Ag, Ga1, and Pg11 (PCR amplified from genomic *A. thaliana* DNA; Konieczny and Ausubel 1993).

Most probes (88%) were mapped in *A. thaliana* using the recombinant inbred (RI) population of Lister and Dean (1993). The positions of the loci detected by a few of these probes were inferred from physical mapping data. Thirteen *A. thaliana* expressed sequence tags (ESTs) (ve29, ve30, ve45, ve50, ve53, ve57, y22, y29, y33, y34, y35, y36, and y37) were assigned to YAC clones (ve clones, D. Bouchez, personal communication; y clones, http://genome-www.stanford.edu/Arabidopsis/EST2YAC.html/), and the genetic map position was inferred from the position on the RI map of genetically mapped fragments from the same YAC clone. The genetic map positions of all loci on the RI map were extracted from the map published electronically (http://nasc.nott.ac.uk/new_ri_map.html/).

Nineteen probes were mapped in separate *A. thaliana* crosses (Ara and PhyB, Hauge *et al.* 1993; 17 of the um probes, McGrath *et al.* 1993). Loci segregating in two crosses were used to infer the relative order of loci mapped in one cross only. Several of the um probes also detect multiple loci in *A. thaliana*. This complicates the identification of the true orthologous/homeologous loci in *B. nigra*. In this study, um loci in *A. thaliana* and *B. nigra* were considered homeologous if they were positioned next to another homeologous locus pair detected with a non-um probe. Consequently, occasional erroneous classifications of true homeologous locus pairs

would result in an underestimate of the number of rearrangements that have occurred since the divergence of the lineages leading to *A. thaliana* and *B. nigra*. Data from the um probes were excluded in calculations of the degree of duplication in *B. nigra*.

Polymorphic loci detected using the *A. thaliana* probes were positioned on the *B. nigra* genetic map relative to 288 loci previously mapped using Brassica RFLP probes (Lagercrantz and Lydiate 1995).

RESULTS

A total of 160 mapped DNA fragments from the *A. thaliana* genome revealed 284 polymorphic loci that were incorporated into the existing *B. nigra* linkage map (Figure 1). With one exception, each *B. nigra* linkage group contained loci detected with probes from all five *A. thaliana* chromosomes (Figure 1, Table 1). This pattern indicates that substantial chromosomal rearrangements have occurred since the divergence of the ancestors to *A. thaliana* and *B. nigra*.

To be able to elucidate the true degree of collinearity between the genomes of *A. thaliana* and *B. nigra*, the highly duplicated nature of the *B. nigra* genome has to be fully appreciated. A majority of *A. thaliana* probes detect a single locus in *A. thaliana* (Cavell *et al.* 1998; Chang *et al.* 1988). In contrast, in *B. nigra*, the *A. thaliana* probes detected on average 1.8 polymorphic loci per probe, which is close to the 1.9 loci per probe detected in the same population using Brassica RFLP probes (Lagercrantz and Lydiate 1996). Both those estimates are clearly underestimates of the true level of duplication caused by residual monomorphism (Lagercrantz and Lydiate 1996). Even if the degree of polymorphism is as high as 70% (as estimated in the present study), the probability that all loci in a triplicate set are polymorphic is 34%. Thus, in a hexaploid where virtually every probe should identify three homeologous loci, about one-third of these triplicate loci would be mapped, even in this extremely polymorphic population.

Previous mapping using Brassica RFLP probes has suggested that the entire *B. nigra* genome appears to consist of large, duplicated segments, with the majority of RFLP loci detecting *three* dispersed, homeologous loci (Lagercrantz and Lydiate 1995, 1996). The present study supports these findings. The mapping population used by Lagercrantz and Lydiate (1995, 1996) was also used in the present study, and the additional 284 loci based on *A. thaliana* probes corroborated the previously identified triplicated segments comprising virtually the whole *B. nigra* genome (data not shown). These data support the hypothesis that *B. nigra* is descended from a hexaploid ancestor.

How does this triplicated nature of the *B. nigra* genome correspond to the structure of the *A. thaliana* genome? Because of the high frequency of rearrangements (see below), collinear segments are quite short in many cases. However, a few large chromosomal segments have remained largely intact since the divergence of Arabidopsis and Brassica, and for these segments, which occur as single copy in *A. thaliana*, there are strong indications that they occur in three homeologous copies in *B. nigra* (Figure 2). At least 40 cM of the top of *A. thaliana* chromosome *5* is present in three homeologous copies on linkage groups G2, G5, and G8 in *B. nigra*. Two of the *B. nigra* segments appear collinear with the *A. thaliana* segment, while the third segment on G5 contains a large inversion compared to that of *A. thaliana*.

Furthermore, large portions of *A. thaliana* chromosome *2* seem to correspond to three homeologous segments in *B. nigra* (Figure 2). The bottom 40 cM of *A. thaliana* chromosome *2* corresponds to a contiguous 50-cM tract on *B. nigra* G6, while the homeologous segments on *B. nigra* G1 and G8 are interrupted by segments from other *A. thaliana* chromosomes (Figures 1 and 2). Loci homologous to those from the top half of *A. thaliana* chromosome *2* are scattered mainly on the three linkage groups in *B. nigra*: G3–G5.

In addition, a 40-cM segment of *A. thaliana* chromosome *3* corresponds to three homeologous tracts in *B. nigra* (Figure 2). The three *B. nigra* tracts are all associated with tracts homeologous to *A. thaliana* chromosome *1* segments (depicted in gray in Figure 2). In two of the *B. nigra* tracts, the *A. thaliana* chromosome *3* homeologous segments are interrupted by the tract homeologous to chromosome *1*, and for the third tract, an inversion seems to have placed the chromosome *1* homeologous tract at the end of the linkage group. Triplicated homeologous copies are also discernible for more limited regions of the *A. thaliana* genome (data not shown), but as the homeologous regions get smaller, it becomes progressively more difficult to detect triplicated copies, if they exist, because of lack of polymorphic loci.

Although it is not possible to identify triplicated structures in *B. nigra* corresponding to the entire *A. thaliana* genome, probes from the different *A. thaliana* chromosomes detected a similar level of duplication, ranging from 1.4 to 1.9 loci per probe (Table 1; $F_{4137} = 1.8$, $P > 0.1$), indicating that none of the *A. thaliana* chromosomes are significantly over- or underrepresented in duplicate homeologous copies in *B. nigra*. To get a more complete description of replicated regions corresponding to different *A. thaliana* segments, an even higher density of markers is needed.

The scattered distribution of markers from different *A. thaliana* chromosomes on all *B. nigra* linkage groups suggests that a very large number of chromosomal rearrangements have occurred since the divergence from a common ancestor. Simply counting the number of breakpoints that are needed to account for markers from different *A. thaliana* chromosomes that are adjacent on *B. nigra* linkage groups yields 79 breakpoints

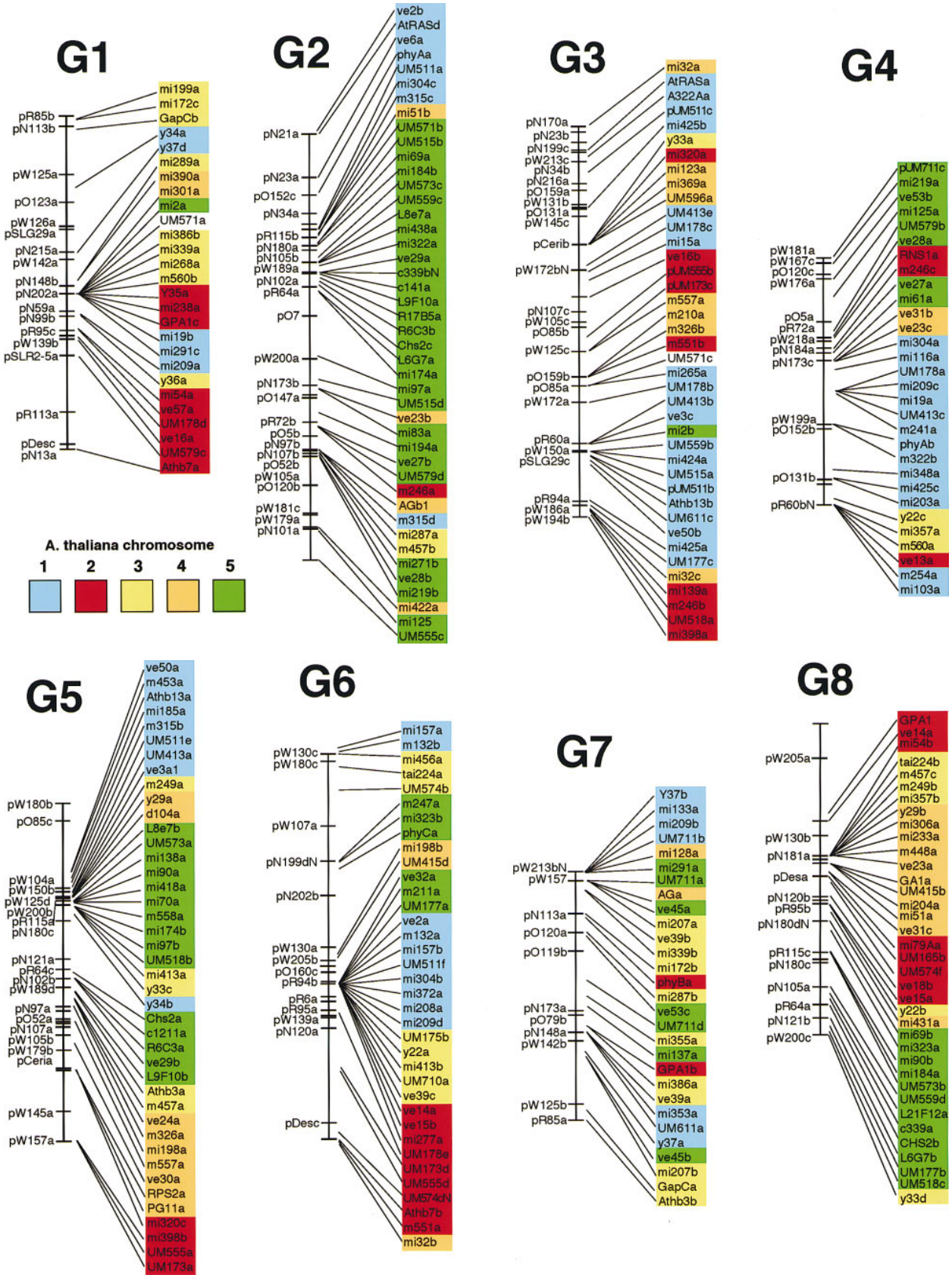

Figure 1.—Comparative map of *A. thaliana* and *B. nigra*. The eight linkage groups of *B. nigra* (G1–G8) are represented by vertical lines. Loci on the right of each linkage group were detected using probes previously mapped in *A. thaliana*. The chromosomal locations of *A. thaliana* loci detected using these probes are shown with different colors as indicated. Loci on the left of each linkage group were previously located using Brassica RFLP probes (Lagercrantz and Lydiate 1995). Recombination distances are given in Kosambi centimorgans.

TABLE 1

Number of polymorphic loci detected with RFLP probes from different *A. thaliana* chromosomes

A. thaliana chromosome	No. of probes	*B. nigra* linkage group								Total no. loci	Loci/probe
		1	2	3	4	5	6	7	8		
1	33	5	7	10	12	7	9	5	0	55	1.7
2	18	7	1	6	3	2	5	2	6	32	1.8
3	22	9	2	1	3	5	5	11	6	42	1.9
4	27	2	4	7	2	9	2	2	10	38	1.4
5	39	1	22	1	6	13	5	5	8	61	1.6
1-5	140	24	36	25	26	36	26	25	30	228	1.6
All probes	160	26	44	40	30	42	36	29	37	284	1.8

Probes known to detect more than one locus in *A. thaliana* are excluded except in the last row (all probes).

(Figure 1). This calculation does not account for the fact that several blocks of loci syntenic with particular *A. thaliana* chromosomes are not collinear. For example, on *B. nigra* G2, neither the block on top homeologus to *A. thaliana* chromosome *1* nor the large, contiguous block homeologous to *A. thaliana* chromosome *5* are collinear with their *A. thaliana* counterparts. In both cases, inversions either in the Arabidopsis or the Brassica lineage are needed to account for the different order of homeologous loci.

To obtain a more detailed picture of the number and types of rearrangements that have occurred during the evolution of Arabidopsis and Brassica from a common ancestor, an attempt was made to reconstruct ancestral

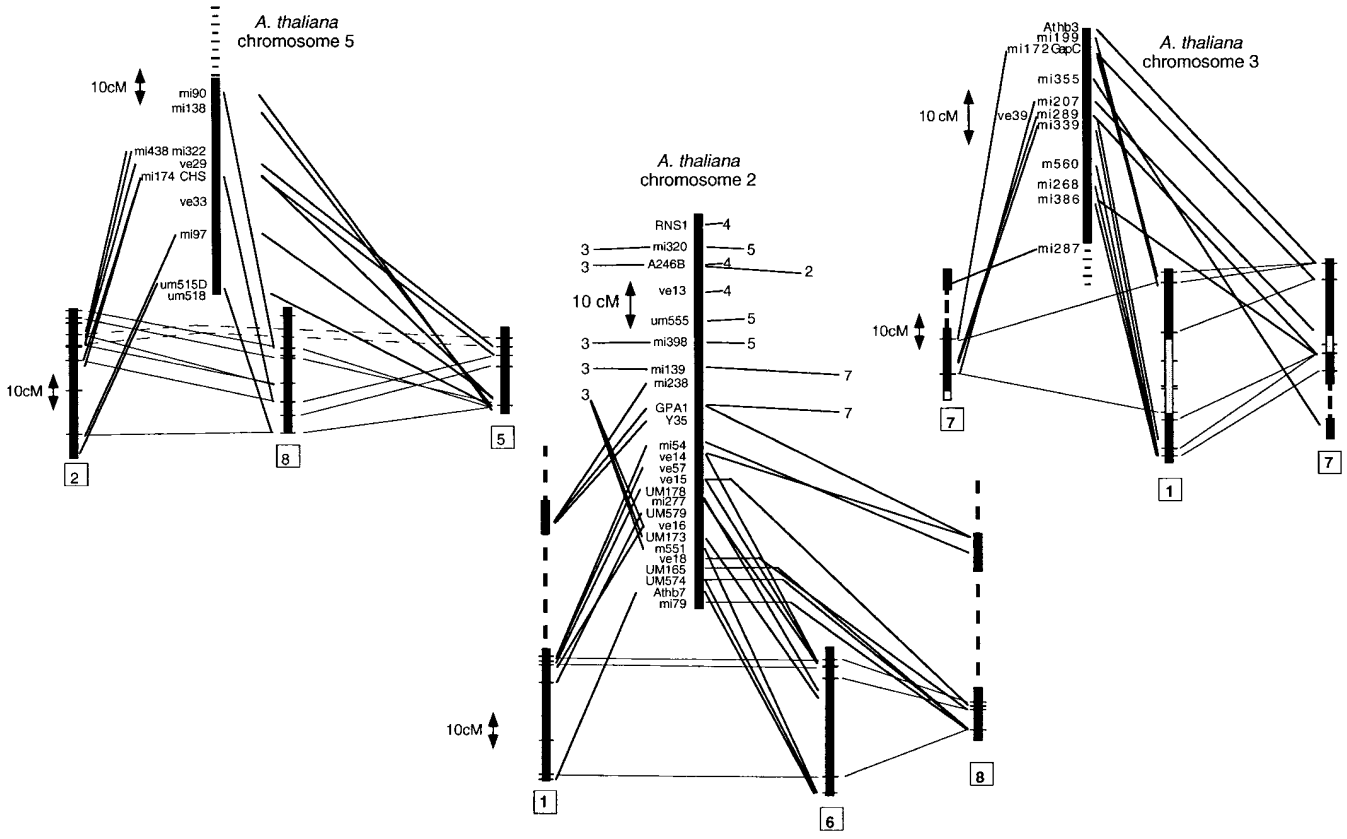

Figure 2.—Large *A. thaliana* chromosomal segments correspond to triplicated homeologous segments in *B. nigra*. Detailed comparative linkage maps of selected segments of the *A. thaliana* genome. A 40-centimorgan segment of *A. thaliana* chromosome *5* corresponds to three homeologous tracts on G2, G5, and G8 in *B. nigra*. The bottom half of *A. thaliana* chromosome *2* is homeologous to segments on *B. nigra* G1, G6, and G8. Forty centimorgans of the top of *A. thaliana* chromosome *3* corresponds to triplicated segments, one on *B. nigra* G1 and two on G7. The numbers connected with lines to loci on *A. thaliana* chromosome *2* indicate linkage group location of homologous loci in *B. nigra*. Those *B. nigra* loci are not adjacent on their respective linkage groups and, thus, are not connected by vertical lines (representing synthenic segments) in the figure.

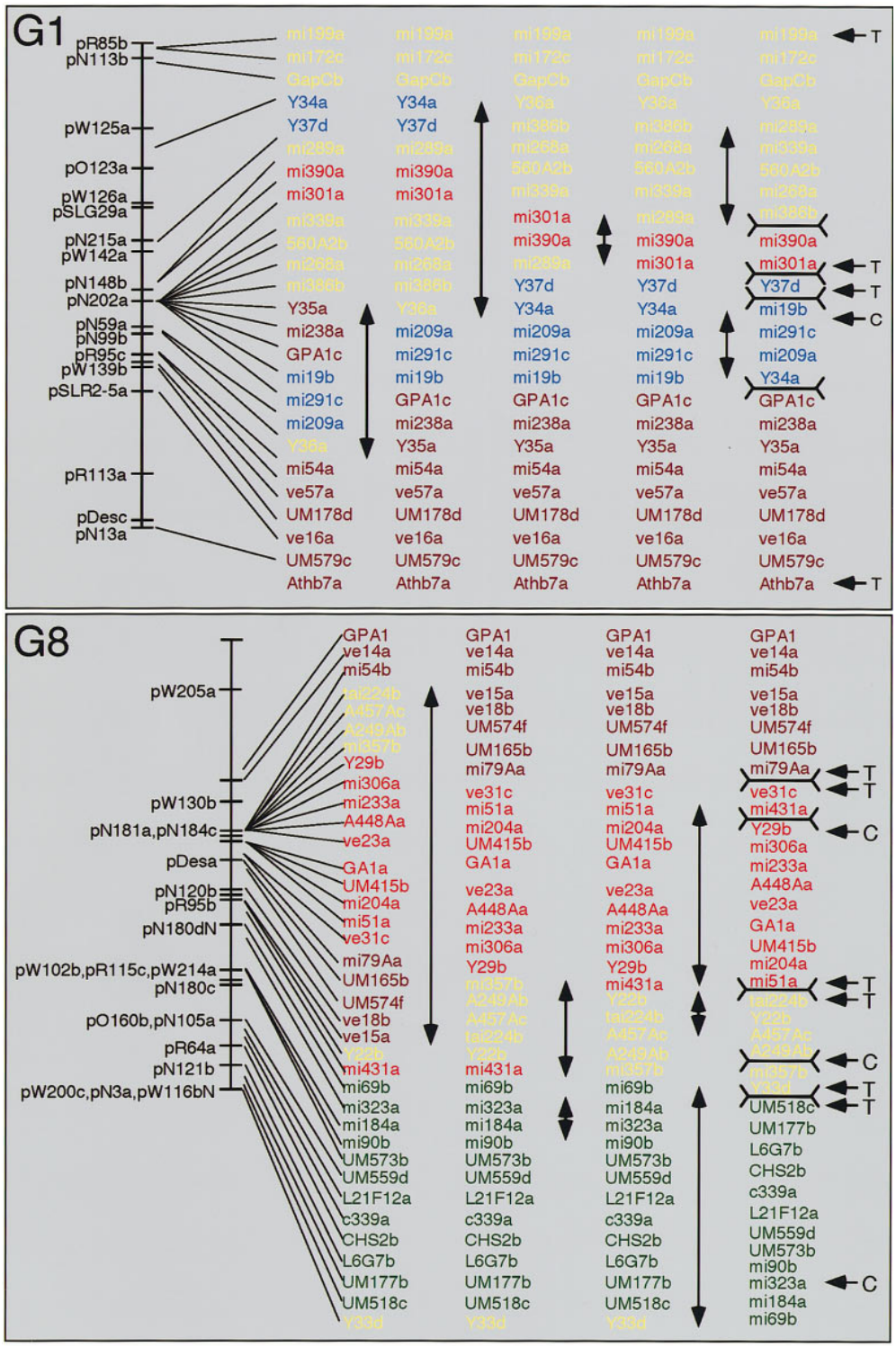

Figure 3.—Tentative models for the evolution of *B. nigra* G1 and G8 (see text for details). The present-day *B. nigra* linkage groups are shown on the left. ↔, inversions; ⟩—⟨, additional chromosome breaks (fissions of translocations) that are necessary to explain the differences between *A. thaliana* and *B. nigra* linkage groups; T, loci where the *A. thaliana* homologue is located close to a telomere; C, positions that in *A. thaliana* correspond to positions close to a centromere. The color of the different loci indicate the chromosomal location of the *A. thaliana* locus detected with the corresponding probe, as in Figure 1.

chromosomal segments by invoking a minimal number of rearrangements resulting in segments collinear with tracts in the *A. thaliana* genome. The *B. nigra* linkage groups show a typical pattern of relatively large blocks of markers from particular *A. thaliana* chromosomes interrupted by a few markers from one or more other *A. thaliana* chromosomes. This distribution can, to a large extent, be explained by a limited number of inversions, as illustrated for linkage groups G1 and G8 in Figure 3. Invoking six inversions on *B. nigra* G8 results in four syntenic blocks, two of which are apparently contiguous and collinear with *A. thaliana*.

The other two blocks (homeologous to segments from *A. thaliana* chromosomes *3* and *4*) are also collinear, but they probably lack an internal piece of the segment, as indicated by an additional breakpoint in

TABLE 2

Estimated number of inversion and translocations/ fusions since the divergence of *A. thaliana* and *B. nigra*

Linkage group	Inversions	Translocations/ fusions	Total
1	5	4	9
2	9	7	16
3	11	8	19
4	13	7	20
5	8	6	14
6	3	5	8
7	7	8	15
8	6	5	11
Total	62	50	112

Figure 3. This conclusion is based on the fact that the *A. thaliana* homologues to loci flanking the breakpoints are separated by a large segment, and that within this *A. thaliana* segment there are at least subsegments that correspond to three homeologues on other *B. nigra* linkage groups.

It should be pointed out that there are other possible scenarios for the chromosomal evolution than those presented in Figure 3, but the fact that a single inversion often places scattered makers, not only in a syntenic block, but also in a collinear position, makes the proposed scenario attractive. The estimates of the number of rearrangements are shown in Table 2. The estimates range from 8 to 20 rearrangements per *B. nigra* linkage group, resulting in a total of 112 rearrangements. These estimates should be regarded as maximum estimates based on the present data as all interruptions of synteny are not necessarily the result of chromosomal rearrangements. There are a number of segments in Figure 1 that are defined by a single locus. An alternative explanation to the occurrence of such single loci disrupting an otherwise syntenic segment could be the transposition of a duplicated small segment or even partial transcripts.

To reduce the potential bias caused by single deviant loci, the number of rearrangements and the length of conserved segments were also estimated from map distances between the outmost markers of conserved segments (Nadeau and Taylor 1984). Using this method, a single deviant locus will only affect the estimate if it disrupts an otherwise collinear segment. In the present data, however, the single deviant loci are always located at the breakpoint between different segments (Figure 1). Even when a single deviant locus is flanked by loci homologous to the same *A. thaliana* chromosome (*e.g.*, as for ve23b about two-thirds down on G2; Figure 1), this locus does not interrupt collinearity as it is located at an inversion breakpoint. The estimate of the conserved length using mapping data from *B. nigra* is 8 cM. This estimate is based on 41 conserved segments, including two or more markers. An estimate of the number of chromosomal rearrangements based on these data requires the number of chromosomes in the last common ancestor of *A. thaliana* and *B. nigra*. This number is not known, but it will only affect the estimate marginally. Assuming tentatively that the ancestor to *B. nigra* has 15 chromosomes (three copies of the *A. thaliana* genome) results in an estimate of 87 rearrangements. Assuming conservatively that the ancestor to *B. nigra* has 8 chromosomes results in a marginally higher estimate of 94 rearrangements. In comparisons of the number of rearrangements with other species (see discussion), an estimate of 90 rearrangements was used.

Several loci that map close to the telomeres on *A. thaliana* chromosomes have homologous loci mapping internally on *B. nigra* linkage groups. The positions of some of those loci in *B. nigra* suggest that direct telomere-telomere fusion might have been important in the restructuring of Brassica genomes. In the hypothesized scenario for G8 (Figure 3), there are at least three positions where adjacent blocks corresponding to two different *A. thaliana* chromosomes are joined by loci that have homologues close to the telomeres in *A. thaliana*.

The positions of centromeres on the genetic map of *A. thaliana* have been reported recently (Round *et al.* 1997). In Figure 3, homologous positions to some of the *A. thaliana* centromeres have been inferred through the positions of *B. nigra* homologues to centromere-linked loci in *A. thaliana*. The proposed locations of the regions that are homologous to *A. thaliana* centromeres are close to the breakpoints of chromosomal rearrangements in all cases (Figure 3). In at least two cases, however, the positions of flanking loci indicate that the breakpoint has not been in the centromere itself. On G1, mi19b and mi291c map in a cluster of loci from different *A. thaliana* chromosomes. The centromere is located between mi19 and mi291 in *A. thaliana*, suggesting that the chromosome break resulting in the different structures in *A. thaliana* and *B. nigra* took place outside these flanking markers. Similarly, on *B. nigra* G8, the homologous segment to *A. thaliana* chromosome *4* includes y29b and mi306a mapping closely together. Assuming that the mapping data in *A. thaliana* are correct, the homologues to these two loci flank the centromere.

DISCUSSION

Replication in Brassica genomes: The *A. thaliana* genome is one of the smallest among higher plants, with an estimate of 145 million bp (Mbp, Arumuganathan and Earle 1991) distributed on five chromosomes in the haploid complement. In the Brassicaceae family, chromosome numbers and DNA content show a large variation, from a low in *A. thaliana* to 19 chromosome pairs and 1235 million bp per haploid complement in

B. napus. It has been suggested that the small genome size of *A. thaliana* results from an exceptionally low amount of repetitive DNA and high gene density (Meyerowitz 1992; Bevan *et al.* 1998). The present study and other comparative mapping data now suggest that these attributes might be typical for most Brassicaceae species, and that the differences in genome content, to a large extent, are caused by different degrees of whole-genome replication. Comparative mapping among the three diploid species—*B. nigra*, *B. oleracea*, and *B. rapa*—supports the hypothesis that these genomes were descended from a hexaploid ancestor with three copies of a rearranged unit genome still discernible (Lagercrantz and Lydiate 1996). There is also good evidence that *B. napus* is an amphidiploid arisen from the hybridization of the two diploid species *B. oleracea* and *B. rapa* or close relatives (U 1935; Parkin *et al.* 1995).

In the present study, the few large segments that have remained largely intact since the divergence of the ancestors of Brassica and Arabidopsis are present in three homeologous copies in *B. nigra*, but in a single copy in *A. thaliana*. In addition, there are a number of smaller segments of the *A. thaliana* genome that have three homeologous copies in *B. nigra*. Although it was not possible to detect three homeologous copies of every single segment of the *A. thaliana* genome, the present data support that the *A. thaliana* genome is similar in complexity to the triplicated unit genome of the diploid Brassica species. Furthermore, the *B. nigra* genome (0.97 pg/diploid nucleus; Arumuganathan and Earle 1991) is approximately three times larger than that of *A. thaliana* (0.3 pg/diploid nucleus; Arumuganathan and Earle 1991). Parallel comparisons of limited portions of the *A. thaliana* and *B. napus* genomes (Scheffler *et al.* 1996; Osborn *et al.* 1997; Cavell *et al.* 1998) are congruent with the present data, showing an average of six homeologous copies of *A. thaliana* segments in the amphidiploid *B. napus*. Kowalski *et al.* (1994) also found evidence for triplication of some homeologous copies of *A. thaliana* segments in the *B. oleracea* genome, but a genome-wide triplication was not suggested. The lower marker density and limited polymorphism in this earlier study probably led to a systematic underestimation of genome replication in Brassica.

Assuming that the lineage leading to the present-day diploid Brassica species has indeed gone through a triplication of the genome, these replications must have been accompanied by a number of chromosome fusion events to reduce the chromosome number. It is not likely that the common ancestor of Arabidopsis and Brassica had a considerably lower number than *A. thaliana*. If the common ancestor also had 5 chromosomes and this genome was triplicated, the chromosome number had to be reduced from 15 to 8 in the lineage leading to *B. nigra*.

The present comparative mapping data support such a reduction in chromosome numbers through chromosome fusions. In *B. nigra*, homologues to loci that in *A. thaliana* are located close to a telomere often map internally on *B. nigra* linkage groups (Figure 3). *B. nigra* G8 comprises six such homologous loci, indicating six interstitial telomeric sites. Preliminary data (J. Fahleson, T. Axelsson and U. Lagercrantz, unpublished results) indicate that at least some of these sites actually contain sequences hybridizing to the telomeric repeat from *A. thaliana* (Richards and Ausubel 1988). Furthermore, comparative mapping of *B. oleracea* ($n = 9$), *B. nigra* ($n = 8$), and *B. rapa* ($n = 10$) support that changes in chromosome numbers caused by chromosome fusion or fission are frequent and also have occurred recently in the Brassicaceae family (Lagercrantz and Lydiate 1996).

Rapid chromosomal evolution in Brassica genomes: A number of comparative analyses of genomes within the animal and plant kingdoms have suggested that the rate of chromosomal rearrangements is surprisingly low in most cases (Nadeau and Taylor 1984; O'Brien *et al.* 1988; Moore *et al.* 1995). However, the present study indicates that the evolution of genomes in the Brassicaceae family involves an unusually high rate of chromosomal rearrangements. Different methods for estimating the amount of rearrangements since the divergence of Arabidopsis and Brassica lineages resulted in figures ranging from 79 to 112. To compare the *A. thaliana-B. nigra* data with previously published data, I used the estimate of 90 rearrangements obtained using the method of Nadeau and Taylor (1984). The number of rearrangements in other species was also recalculated from published data using the same method (Table 3, Figure 4). This method has shown to be remarkably good, even with small sample sizes; modest genetic maps and possible mapping errors do not seem to influence results dramatically (Ehrlich *et al.* 1997).

Estimates of the divergence times between different species and genera in the Brassicaceae family vary widely. Divergence time ranging from 10 million years, based on paleopalynological data (Muller 1981), to 35 million years, based on DNA sequence data from the rbcL gene (R. Price, personal communication), have been suggested for the lineages leading to Arabidopsis and Brassica. Even if we conservatively assume a divergence time of 35 million years, the *A. thaliana-B. nigra* comparison reveals an exceptionally high rate of chromosomal rearrangements (Table 3, Figure 4). Among previous comparisons, humans and mice are among those that have diverged most rapidly (Ehrlich *et al.* 1997). The estimate of the length of conserved segments from Copeland *et al.* (1993) of 8.8 cM results in an estimate of 144 rearrangements between man and mouse (Ehrlich *et al.* 1997). Assuming a divergence time of rodents and primates of 114 million years (Janke *et al.* 1994) yields an estimate of 0.63 rearrangements per million years. Even in comparison to the divergence of mouse and man, *A. thaliana-B. nigra*, with approximately 3 rearrangements per million years, stands out

TABLE 3

Estimated number of chromosomal rearrangements and approximate divergence times for various taxa

Species/genera	Divergence time (million yr)	Number of rearrangements[a]
B. rapa-B. oleracea	1[b]	5[h]
B. nigra-B. rapa	20[b]	10[h]
B. nigra-B. oleracea	20[b]	12[h]
A. thaliana-B. nigra	35[b]	90[i]
Lycopersicon-Solanum	10[c]	5[j]
Lycopersicon-Capsicum	40[c]	14[k]
Oryza-Zea	66[d]	35[l]
Sorghum-Zea	24[e]	15[m]
Gossypium spp.	10[f]	9[n]
Homo-Sus	93[g]	35[o]
Sus-Mus	114[g]	77[o]
Homo-Mus	114[g]	144[p]

[a] Estimates based on the method presented by Nadeau and Taylor (1984), except Lycopersicon-Solanum and Gossypium A/D genome comparisons, which were direct counts from published data (Tanksley *et al.* 1992; Reinisch *et al.* 1994). Recalculations from original publications were performed on some data (Whitkus *et al.* 1992; Ahn *et al.* 1993; Prince *et al.* 1993; Lagercrantz and Lydiate 1996).
[b] R. Price (personal communication).
[c] Paterson *et al.* (1996).
[d] E. Kellogg, (personal communication).
[e] Gaut and Doebley (1997).
[f] Wendel (1989).
[g] Janke *et al.* (1994).
[h] Lagercrantz and Lydiate (1996).
[i] This study.
[j] Tanksley *et al.* (1992).
[k] Prince *et al.* (1993).
[l] Ahn *et al.* (1993).
[m] Whitkus *et al.* (1992).
[n] Reinisch *et al.* (1994).
[o] Johansson *et al.* (1995).
[p] Copeland *et al.* (1993).

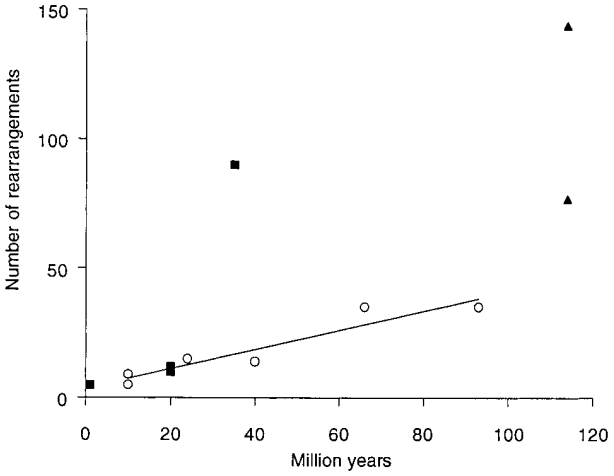

Figure 4.—Estimated number of chromosomal rearrangements differentiating various animal species pairs and plant species pairs *vs.* approximate divergence times. All estimates are based on comparative linkage mapping. ■, comparisons between species in the Brassicaceae family; ▲, comparisons including Mus; ○, all other comparisons. The straight line is estimated from linear regression excluding data from Brassicaceae (■) and Mus (▲).

as having experienced an extreme rate of chromosomal repatterning.

Kowalski *et al.* (1994) obtained an estimate of at least 26 rearrangements since the divergence of *A. thaliana* and *B. oleracea*. This estimate is considerably lower than that obtained in the present study. A considerable part of this difference most likely results from a lower marker density in the previous study. Comparative mapping data between limited chromosomal regions of *A. thaliana* and *B. napus* support an extremely high rate of rearrangements for some chromosomal regions (Osborn *et al.* 1997). A 14-cM tract on the *B. napus* linkage group N2 corresponds to segments from four different *A. thaliana* chromosomes. If such highly rearranged regions are common, as also indicated in the present study, a marker density of 15 cM [as for *B. oleracea* markers on the *A. thaliana* map of Kowalski *et al.* (1994)] is likely to underestimate the true level of rearrangements.

Because of the poor data on divergence times in Brassica, it is not possible to conclude if the high rate of chromosomal divergence is typical of Brassicaceae species that have diverged more recently than *A. thaliana* and *B. nigra*. Sequence data from the large subunit of rubisco suggest that *B. nigra* diverged from *B. rapa*/*oleracea* ~20 mya and *B. rapa* diverged from *B. oleracea* 1 mya (R. Price, personal communication). If these data are reasonably correct, the rate of chromosomal rearrangements in these lineages does not seem to be higher that in most other plant and animal species (Table 3, Figure 4).

Why has the rate of chromosomal repatterning been so high between *A. thaliana* and *B. nigra*? Population structure and recent polyploidization are probably important factors contributing to the rapid rearrangements of Brassicaceae chromosomes. The present study and other data (Lagercrantz and Lydiate 1996) suggest that modern diploid species of Brassica and related genera have descended from a common hexaploid ancestor and are, thus, degenerate hexaploids. It seems likely that the replications have occurred through amphidiploidization the same way as novel amphidiploids such as *B. napus*, *B. juncea*, and *B. carinata* are derived from hybridization between different diploids (U 1935; Parkin *et al.* 1995; Axelsson *et al.* 1998). Allopolyploidization is likely to result in an increase in aberrant meiotic pairing and translocations among homeologous chromosomes. Studies of resynthesized *B. napus* plants show that intergenomic translocations are surprisingly frequent between chromosomes from *B. oleracea* and *B. rapa* (Lydiate *et al.* 1993; U. Lagercrantz and D. Lydiate, unpublished results).

Chromosome fusions after duplication also might have resulted indirectly in an increase of the frequency

of rearrangements. As discussed above, polyploidization in the Brassica lineage has probably been followed by an extensive reduction in chromosome number through chromosome fusion events. These fusions have apparently resulted in interstitially located telomere repeats [ITRs $(TTTAGGG)_n$]. There are several independent data suggesting that such ITRs may be particularly prone to recombination, breakage, and fragility (Hastie and Allshire 1989; Meyne *et al.* 1990; Barnett *et al.* 1993; Bertoni *et al.* 1994; Slijepcevic *et al.* 1996). The position of ITRs in the *B. nigra* genome (J. Fahleson, T. Axelsson and U. Lagercrantz, unpublished results) supports their involvement in chromosomal rearrangements. The ITRs that have been positioned are almost exclusively located at the breakpoints between conserved blocks in the *B. nigra* genome.

Assuming no selective advantage of chromosomal rearrangements (Lande 1979), the rate of chromosomal rearrangements depends on mutation rate and random fixation rate. The effect of replication provides an explanation as to the generation of a relatively high number of new rearrangements in Brassicaceae genomes. However, a key step in karyotype evolution is the fixation of newly arisen chromosomal rearrangements. Translocations and inversions are generally deleterious when heterozygous, but have normal fitnesses when homozygous (White 1973). The fixation of such rearrangements requires small, isolated populations and is aided greatly by self-fertilization (Lande 1979). Many wild Brassicaceae species occupy marginal fragmentated habitats, such as maritime cliffs (Snogerup *et al.* 1990; Mithen *et al.* 1995). This distribution is likely to result in small deme sizes and high turnover rates of local populations. The exposed habitat is likely to lead to relatively frequent local extinction and recolonization. Local fixation is favored by small, reproductively isolated demes and selfing. Once established in a deme, a negatively heterotic gene arrangement can spread in homozygous form through a subdivided population by random local extinction and recolonization. Although the population structure of Brassicaceae species in the past is largely unknown, the present-day population structure of many species suggests an explanation as to the high rate of chromosomal rearrangements evident in some lineages of Brassicaceae. A combination of an enhanced level of chromosomal mutations caused by genome replication and a population structure characterized by small deme size may have contributed to the exceptionally high rate of rearrangements observed between *A. thaliana* and *B. nigra*.

Additional comparative mapping studies within the Brassicaceae, also including species closely related to *A. thaliana*, and more precise estimates of divergence times between species within Brassicaceae will shed more light on the rapid chromosomal evolution observed in the present study. Has rapid chromosome evolution been restricted mainly to the polyploid Brassica species, and were rearrangements mainly confined to a short period after polyploidization? Have rearrangement frequencies been higher in species where chromosome numbers have been reduced as a result of chromosomal fusions, or is the frequency of rearrangements mainly an effect of population structure?

Practical implications: Obviously, the highly replicated nature of Brassica genomes must be acknowledged. It is likely that many important traits in Brassica species are controlled by duplicated genes originating from previous whole-genome replications. Identification of such duplicate genes would facilitate the understanding of the genetics and the improvement of various agronomic traits.

There are also good prospects for utilization of the rich source of biological information and genetic resources emanating from *A. thaliana* research. Even though the rearrangements have been frequent since the divergence of Arabidopsis and Brassica, the average length of conserved segments between *A. thaliana* and *B. nigra* was estimated at 8 cM. Thus, mapping a Brassica gene to an interval of <10 cM is often likely to allow the identification of the homeologous collinear region in *A. thaliana*. It should be kept in mind that some regions of the genome are considerably more rearranged, which will require much more detailed mapping. There is also a lack of data on the amount of fine-scale rearrangements that are not detected using comparative linkage mapping data. Such local rearrangements could obviously complicate the identification of homologous genes in *A. thaliana* and Brassica solely on the basis of their map position.

Still, with the prospect that a large proportion of the genes in *A. thaliana* will soon be identified, further fine-scale comparative mapping in the Brassicaceae family is likely to result in the identification of a large number of genes that affect important traits in different Brassica crops.

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LITERATURE CITED

Ahn, S., and S. Tanksley, 1993 Comparative linkage maps of the rice and maize genomes. Proc. Natl. Acad. Sci. USA **90:** 7980–7984.

Ahn, S., J. Anderson, M. Sorrells and S. Tanksley, 1993 Homeologous relationships of rice, wheat and maize chromosomes. Mol. Gen. Genet. **241:** 483–490.

Arumuganathan, K., and E. D. Earle, 1991 Nuclear DNA content of some important plant species. Plant Mol. Biol. Rep. **9:** 208–218.

Axelsson, T., C. Bowman, D. Lydiate and U. Lagercrantz, 1998 RFLP mapping indicates conserved A and B genome linkage groups in Brassica juncea. Proceedings, Plant Genome VI, p. 133.

Barendse, W., S. Armitage, L. Kossarek, A. Shalom, B. Kirkpatrick *et al.*, 1994 A genetic linkage map of the bovine genome. Nat. Genet. **6:** 227–235.

Bariola, P., C. Howard, C. Taylor, M. Verburg, V. Jaglan *et al.*, 1994 The Arabidopsis ribonuclease gene RNS1 is tightly controlled in response to phosphate limitation. Plant J. **6:** 673–685.

Barnett, M., V. Buckle, E. Evans, A. Porter, D. Rout *et al.*, 1993 Telomere directed fragmentation of mammalian chromosomes. Nucleic Acids Res. **21:** 27–36.

Bertoni, L., C. Attolini, L. Tessera, E. Mucciolo and E. Giulotto, 1994 Telomeric and nontelomeric $(TTAGGG)_n$ sequences in gene amplification and chromosome stability. Genomics **24:** 53–62.

Bevan, M., I. Bancroft, E. Bent, K. Love, H. Goodman *et al.*, 1998 Analysis of 1.9 Mb of contiguous sequence from chromosome 4 of *Arabidopsis thaliana*. Nature **391:** 485–488.

Bush, G., S. Case, A. Wilson and J. Patton, 1977 Rapid speciation and chromosomal evolution in mammals. Proc. Natl. Acad. Sci. USA **74:** 3942–3946.

Cavell, A., D. Lydiate, I. Parkin, C. Dean and M. Trick, 1998 A 30 centimorgan segment of *Arabidopsis thaliana* chromosome 4 has six collinear homologues within the *Brassica napus* genome. Genome **41:** 62–69.

Chang, C., J. L. Bowman, A. W. De John, E. S. Lander and E. M. Meyerowitz, 1988 Restriction fragment length polymorphism linkage map for *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **85:** 6856–6860.

Chen, Q., and K. Armstrong, 1994 Genomic *in situ* hybridization in *Avena sativa*. Genome **37:** 607–612.

Copeland, N., N. Jenkins, D. Gilbert, J. Eppig, L. Maltais *et al.*, 1993 A linkage map of the mouse: current applications and future prospects. Science **262:** 57–66.

Ecker, J., 1998 Genes blossom from a weed. Nature **391:** 438–439.

Ehrlich, J., D. Sankoff and J. Nadeau, 1997 Synteny conservation and chromosome rearrangements during mammalian evolution. Genetics **147:** 289–296.

Eldridge, M., and R. Close, 1993 Radiation of chromosome shuffles. Curr. Opin. Genet. Dev. **3:** 915–922.

Fabri, C., and A. Schaeffner, 1994 An *Arabidopsis thaliana* RFLP mapping set to localize mutations to chromosomal regions. Plant J. **5:** 149–156.

Feinbaum, R., and F. Ausubel, 1988 Transcription regulation of the *Arabidopsis thaliana* chalcone synthase gene. Mol. Cell. Biol. **8:** 1985–1992.

Gaut, B., and J. Doebley, 1997 DNA sequence evidence for the segmental allotetraploid origin of maize. Proc. Natl. Acad. Sci. USA **94:** 6809–6814.

Graves, J., 1996 Mammalians that break the rules: genetics of marsupials and monotremes. Annu. Rev. Genet. **30:** 233–260.

Hastie, N., and R. Allshire, 1989 Human telomeres: fusion and interstitial sites. Trends Genet. **5:** 326–331.

Hauge, B., S. Hanley, S. Cartinhour, J. Cherry and H. Goodman, 1993 An integrated genetic/RFLP map of the *Arabidopsis thaliana* genome. Plant J. **3:** 745–754.

Helentjaris, T., D. Weber and S. Wright, 1988 Identification of the genomic locations of duplicate nucleotide sequences in maize by analysis of restriction fragment length polymorphims. Genetics **118:** 353–363.

Janke, A., G. Feldmaier-Fuchs, W. Kelley Tomas, A. von Haeseler and S. Pääbo, 1994 The marsupial mitochondrial genome and the evolution of the placental mammals. Genetics **137:** 243–256.

Jellen, E., B. Gill and T. Cox, 1994 Genomic *in situ* hybridization differentiates between A/D- and C-genome chromatin and detects intergenomic translocations in polyploid oat species (genus *Avena*). Genome **37:** 613–618.

Johansson, M., H. Ellegren and L. Andersson, 1995 Comparative mapping reveals extensive linkage conservation—but with gene order rearrangements—between the pig and the human genomes. Genomics **25:** 682–690.

Kenton, A., A. S. Parokonny, Y. Y. Gleba and M. D. Bennett, 1993 Characterization of the *Nicotiana tabacum* L. genome by molecular cytogenetics. Mol. Gen. Genet. **240:** 159–169.

Konieczny, A., and F. Ausubel, 1993 A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. Plant J. **4:** 403–410.

Kowalski, S., T. Lan, K. Feldmann and A. Paterson, 1994 Comparative mapping of *Arabidopsis thaliana* and *Brassica oleracea* chromosomes reveals islands of conserved organization. Genetics **138:** 499–510.

Lagercrantz, U., and D. Lydiate, 1995 RFLP mapping in *Brassica nigra* indicates differing recombination rates in male and female meiosis. Genome **38:** 255–264.

Lagercrantz, U., and D. Lydiate, 1996 Comparative genome mapping in Brassica. Genetics **144:** 1903–1910.

Lagercrantz, U., J. Putterill, G. Coupland and D. Lydiate, 1996 Comparative mapping in Arabidopsis and Brassica, fine scale genome collinearity and congruence of genes controlling flowering time. Plant J. **9:** 13–20.

Lande, R., 1979 Effective deme size during long-term evolution estimated from rates of chromosomal rearrangements. Evolution **33:** 234–251.

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.

Leitch, I., and M. Bennett, 1997 Polyploidy in angiosperms. Trends Plant Sci. **2:** 470–476.

Lincoln, S., M. Daly and E. Lander, 1992 *Mapping Genes Controlling Quantitative Traits with MAPMAKER/QTL 1.1*, Ed. 2. Whitehead Institute Technical Report, Cambridge, MA.

Lister, C., and C. Dean, 1993 Recombinant inbred lines for mapping RFLP and phenotypic markers in *Arabidopsis thaliana*. Plant J. **4:** 745–750.

Liu, Y., N. Mitsukawa, C. Lister, C. Dean and R. Whittier, 1996 Isolation and mapping of a new set of 129 RFLP markers in *Arabidopsis thaliana* using recombinant inbred lines. Plant J. **10:** 733–736.

Lydiate, D., A. Sharpe, U. Lagercrantz and I. Parkin, 1993 Mapping the *Brassica* genome. Outlook Agric. **22:** 85–92.

Ma, H., M. Yanofsky and E. Meyerowitz, 1990 Molecular cloning and characterization of GPA1, a G protein alpha subunit gene from *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **87:** 3821–3825.

Masterson, J., 1994 Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. Science **264:** 421–424.

Matsui, M., S. Sasamoto, T. Kunieda, N. Nomura and R. Ishizaki, 1989 Cloning of Ara, a putative *Arabidopsis thaliana* gene homologous to the Ras-related gene family. Gene **76:** 313–320.

McGrath, J. M., M. M. Jansco and E. Pichersky, 1993 Duplicate sequences with a similarity to expressed genes in the genome of *Arabidopsis thaliana*. Theor. Appl. Genet. **86:** 880–888.

Meyerowitz, E., 1992 Introduction to the *Arabidopsis* genome, pp. 102–118 in *Methods in Arabidopsis Research*, edited by C. Koncz, N. Chua and J. Schell. World Scientific, Singapore.

Meyne, J., R. Baker, H. Hobart, T. Hsu, O. Ryder *et al.*, 1990 Distribution of non-telomeric sites of the $(TTAGGG)_n$ telomeric sequence in vertebrate chromosomes. Chromosoma **99:** 3–10.

Mindrinos, M., F. Katagiri, G. Yu and F. Ausubel, 1994 The *A. thaliana* disease resistance gene RPS2 encodes a protein containing a nucleotide-binding site and leucine-rich repeats. Cell **78:** 1089–1099.

Mithen, R., A. F. Raybold and A. Giamoustaris, 1995 Divergent selection for secondary metabolites between wild populations of *Brassica oleracea* and its implications for plant-herbivore interactions. Heredity **75:** 472–484.

Moore, G., K. Devos, Z. Wang and M. Gale, 1995 Grasses line up and form a circle. Curr. Biol. **5:** 737–739.

Morizot, D., 1994 Reconstructing the gene map of the vertebrate ancestor. Anim. Biotech. **5:** 113–122.

Muller, J., 1981 Fossil pollen records of extant angiosperms. Bot. Rev. **47:** 1–142.

Nadeau, J., and B. Taylor, 1984 Lengths of chromosomal segments conserved since divergence of man and mouse. Proc. Natl. Acad. Sci. USA **81:** 814–818.

O'Brien, S., H. Seuánez and J. Womack, 1988 Mammalian genome organization: an evolutionary view. Annu. Rev. Genet. **22:** 323–351.

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.

Parkin, I., A. Sharpe, D. Keith and D. Lydiate, 1995 Identification of the A and C genomes of amphidiploid *Brassica napus* (oilseed rape). Genome **38:** 1122–1131.

Paterson, A., T. Lan, K. Reischmann, C. Chang, Y. Lin *et al.*, 1996 Toward a unified genetic map of higher plants, transcending the monocot-dicot divergence. Nat. Genet. **14:** 380–382.

Price, R., J. Palmer and I. Al-Shehbaz, 1994 Systematic relationships of *Arabidopsis*: a molecular and morphological perspective, pp. 7–19 in *Arabidopsis*, edited by E. Meyerowitz and C. Somerville. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Prince, J., E. Pochard and S. Tanksley, 1993 Construction of a molecular linkage map of pepper and a comparison of synteny with tomato. Genome **36:** 404–417.

Reinisch, A., J. Dong, C. Brubaker, D. Stelly, J. Wendel *et al.*, 1994 A detailed RFLP map of cotton, *Gossypium hirsutum* × *Gossypium barbadense*: chromosome organization and evolution in a disomic polyploid genome. Genetics **138:** 829–847.

Richards, E., and F. Ausubel, 1988 Isolation of a higher eukaryotic telomere from *Arabidopsis thaliana*. Cell **53:** 127–136.

Round, E., S. Flowers and E. Richards, 1997 *Arabidopsis thaliana* centromere regions: genetic map positions and repetitive DNA structure. Genome Res. **7:** 1045–1053.

Scheffler, J., A. Sharpe, H. Schmidt, P. Sperling, I. Parkin *et al.*, 1996 Desaturase multigene families of *Brassica napus* arose through genome duplication. Theor. Appl. Genet. **94:** 583–591.

Sharrock, R., and P. Quail, 1989 Novel phytochrome sequences in *Arabidopsis thaliana*: structure, evolution, and differential expression of a plant regulatory photoreceptor family. Genes Dev. **3:** 1745–1757.

Shih, M., P. Heinrich and H. Goodman, 1991 Cloning and chromosomal mapping of nuclear genes encoding chloroplast and cytosolic glyceraldehyde-3-phosphate dehydrogenase from *Arabidopsis thaliana*. Gene **104:** 133–138.

Shoemaker, R., T. Olson and V. Kanazin, 1996 Soybean genome organization: evolution of a legume genome, pp. 139–150 in *Genomes of Plants and Animals: 21st Stadler Genetics Symposium*, edited by J. P. Gustafson and R. Flavell. Plenum Press, New York.

Slijepcevic, P., Y. Xiao, I. Dominguez and A. Natarajan, 1996 Spontaneous and radiation-induced chromosomal breakage at interstitial telomeric sites. Chromosoma **104:** 596–604.

Slocum, M. K., S. S. Figdore, W. C. Kennard, J. Y. Suzuki and T. C. Osborn, 1990 Linkage arrangements of restriction fragment length polymorphism loci in *Brassica oleracca*. Theor. Appl. Genet. **80:** 57–64.

Snogerup, S., M. Gustafsson and R. von Bothmer, 1990 Brassica sect. Brassica (Brassicaceae). I. Taxonomy and variation. Willenowia **19:** 271–365.

Söderman, E. 1996 Genes encoding homeodomain-leucine zipper proteins in Arabidopsis thaliana. Ph.D. thesis, Uppsala University, Uppsala Sweden.

Song, K., P. Lu, K. Tang and T. Osborn, 1995 Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution. Proc. Natl. Acad. Sci. USA **92:** 7719–7723.

Stam, P., 1993 Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J. **3:** 739–744.

Sun, T., and Y. Kamiya, 1994 The *Arabidopsis* GA1 locus encodes the cyclase ent-kaurene synthetase A of gibberellin biosynthesis. Plant Cell **6:** 1509–1518.

Tanksley, S., M. Ganal, J. Prince, M. de Vicente, M. Bonierbale *et al.*, 1992 High density molecular maps of the tomato and potato genomes. Genetics **132:** 1141–1160.

Tanksley, S., R. Bernatzky, N. Lapitan and J. Prince, 1988 Conservation of gene repertoire but not gene order in pepper and tomato. Proc. Natl. Acad. Sci. USA **85:** 6419–6423.

U, N., 1935 Genomic analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jpn. J. Bot. **7:** 389–452.

Wendel, J., 1989 New world cottons contain old world cytoplasm. Proc. Natl. Acad. Sci. USA **86:** 4132–4136.

White, M., 1973 *Animal Cytology and Evolution*. William Clowes and Sons, London.

Whitkus, R., J. Doebley and M. Lee, 1992 Comparative genome mapping of sorghum and maize. Genetics **132:** 1119–1130.

Wilson, A., V. Sarich and L. Maxon, 1974 The importance of gene rearrangements in evolution: evidence from studies of rates of chromosomal, protein, and anatomical evolution. Proc. Natl. Acad. Sci. USA **71:** 3028–3030.

Wilson, A., T. White, S. Carlson and L. Cherry, 1977 Molecular evolution and cytogenetic evolution, pp. 375–393 in *Molecular Human Cytogenetics, ICN-UCLA Symposia on Molecular and Cellular Biology*, Vol. VII, edited by E. A. Sparkes. Academic Press, San Francisco.

Yanofsky, M., H. Ma, J. Bowman, G. Drews, K. Feldmann *et al.*, 1990 The protein encoded by the *Arabidopsis* homeotic gene agamous resembles transcription factors. Nature **346:** 35–39.

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