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Comparative Genome Mapping in Brassica

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Manuscript received May 23, 1996
Accepted for publication September 9, 1996

ABSTRACT

A *Brassica nigra* genetic linkage map was developed from a highly polymorphic cross analyzed with a set of low copy number Brassica RFLP probes. The Brassica genome is extensively duplicated with eight distinct sets of chromosomal segments, each present in three copies, covering virtually the whole genome. Thus, *B. nigra* could be descended from a hexaploid ancestor. A comparative analysis of *B. nigra*, *B. oleracea* and *B. rapa* genomes, based on maps developed using a common set of RFLP probes, was also performed. The three genomes have distinct chromosomal structures differentiated by a large number of rearrangements, but collinear regions involving virtually the whole of each the three genomes were identified. The genic contents of *B. nigra*, *B. oleracea* and *B. rapa* were basically equivalent and differences in chromosome number (8, 9 and 10, respectively) are probably the result of chromsome fusions and/or fissions. The strong conservation of overall genic content across the three Brassica genomes mirrors the conservation of genic content observed over a much longer evolutionary span in cereals. However, the rate of chromosomal rearrangement in crucifers is much higher than that observed in cereal genomes.

THE availability of molecular markers has stimulated rapid progress in mapping animal and plant genomes. High-density genetic maps have become a potent tool in the study of genome evolution. Comparing genetic linkage maps based on a common set of markers allows direct identification of homoeologous loci and collinear chromosomal segments. This approach has proved very successful in both animals and plants (BONNIERBALE *et al.* 1988; O'BRIEN *et al.* 1988; TANKSLEY *et al.* 1988; WHITKUS *et al.* 1992; ZHANG and WOMAK 1992; AHN and TANKSLEY 1993; PRINCE *et al.* 1993). For example, comparative mapping studies have revealed a surprising level of conservation between cereal genomes (DUNFORD *et al.* 1995; MOORE *et al.* 1995; VAN DEYNZE *et al.* 1995), in spite of large differences in genome size. This is particularly significant because some of these species diverged >50 mya (STEBBINS 1987). Comparative genetic mapping has also allowed the genome organization of cryptic polyploids such as maize to be fully elucidated (HELENTJARIS *et al.* 1988; PERERIA *et al.* 1993; MOORE *et al.* 1995).

The Brassica genus contains a number of diploid species that exhibit a continuous range of haploid chromosome numbers from seven to 12. These species include *B. nigra* ($n = 8$), *B. oleracea* ($n = 9$), and *B. rapa* ($n = 10$), which have been developed into a diverse range of important crops. Results from karyotype analysis (RÖBBELEN 1960) and chromosome pairing affinities in haploids (ARMSTRONG and KELLER 1981, 1982) have been used to suggest that the primary chromosome number of the Brassica genus is $x = 6$ and that specific chromosomes are tetrasomic or hexasomic in some species. However, cytology offers limited resolution in Brassica species which have small and morphologically similar chromosomes. High-density genetic linkage maps of *B. oleracea* (SLOCUM *et al.* 1990) and *B. rapa* (SONG *et al.* 1991; CHYI *et al.* 1992) have established that a high proportion of each genome is duplicated and that complex arrangements of chromosomal segments, rather than whole chromosomes, are present in several copies.

In the current analysis a genetic linkage map derived from a highly polymorphic cross between *B. nigra* accessions has been used to further elucidate the pattern of intragenomic duplication in a diploid Brassica genome. Additional maps of *B. rapa* and *B. oleracea*, generated using the same set of RFLP probes, have allowed a comparative analysis of the genomes of the three diploid Brassica crop species. These investigations have yielded interesting insights into the evolution of the Brassica genome.

MATERIALS AND METHODS

The genome analysis of *B. nigra* was based on a genetic linkage map containing 288 RFLP defined loci arranged into eight linkage groups spanning a total of 778 cM (LAGERCRANTZ and LYDIATE 1995). The loci were detected using high-stringency Southern hybridization analysis (the washing stringency demanded a sequence homology between the probe and the target DNA of ≥80%; BELTZ *et al.* 1983) and a set of 158 low copy number Brassica RFLP probes (SHARPE *et al.* 1995). Genetic segregation was assayed in 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 (LA-

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TABLE 1

Distribution of the number of *B. nigra* loci detected per low copy Brassica probe

No. of loci per probe	No. of probes	
	Mapped loci[a]	Total loci[b]
0	2	—
1	62	17
2	51	57
3	35	56
4	6	22
5	2	5
6	0	1

[a] Estimates based on mapped polymorphic loci only.
[b] Revised estimate where one or more monomorphic RFLP bands were taken to represent a *single* additional polymorphic locus.

GERCRANTZ and LYDIATE 1995). 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 the computer program 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 relatively few problems in the current analysis because 55% of the *B. nigra* loci positioned on the map from the recurrent parent were also positioned on the F1 map and because the significance of apparent inversions interrupting chromosomal segments with otherwise collinear marker orders was tested independently.

The comparative analysis of the Brassica A, B and C genomes was based on the above map of *B. nigra* (the B genome) and integrated genetic linkage maps of the A (*B. rapa*) and C (*B. oleracea*) genomes based on genetic segregation in populations of doubled haploid lines (PARKIN *et al.* 1995; SHARPE *et al.* 1995; BOHUON *et al.* 1996). The maps of all three genomes were developed using a common set of Brassica RFLP probes (SHARPE *et al.* 1995).

RESULTS

Genome organization in *B. nigra*: At least 60% of the 158 Brassica probes used in the construction of the *B. nigra* map detected more than one polymorphic locus, with an average of 1.9 loci detected per probe (Table 1). Only polymorphic loci could be positioned on the *B. nigra* map and, despite the high levels of genotypic variation between the parents of the *B. nigra* mapping population (probably as many as 70% of all the loci detected by the RFLP probes were polymorphic), the residual monomorphism caused an underestimate of the true level of genome duplication. Incomplete polymorphism is the main factor limiting attempts to resolve the organization of intragenomic duplications. Even where the probability of detecting a polymorphism (p) is relatively high for each locus, the probability that two unlinked loci of a duplicate pair will both be polymorphic is considerably lower (p^2). If for example, the chance of detecting a polymorphism at a given locus is 70%, the probability of detecting a polymorphism for both loci in a duplicate pair is just 49% and the probability of mapping three loci of a triplicated set is even lower (34% in this example). An approximate estimate of the total number of loci detected per probe including also monomorphic bands resulted in an average of 2.6 loci per probe (Table 1). To derive this estimate, one or more monomorphic RFLP bands detected by a particular probe after *Eco*RI digestion were taken to represent a *single* additional polymorphic locus.

Different linkage groups of the *B. nigra* map often contained conserved arrays of duplicate loci, probably representing homoeologous chromosomal segments. Virtually the whole genome could be assigned to eight sets (A–H) of triplicated collinear chromosomal segments (Figures 1 and 2). The different copies of each set were almost always present on three distinct linkage groups; the only exception to this rule were the two copies of set C on linkage group seven (G7; Figure 2). The majority of duplicate segments was completely collinear at the level of resolution afforded by the 288 marker loci employed. The only detectable internal inversion involved the interval bounded by pR115 and pR64 in region B, which was inverted on G5 with respect to G2 and G8 (Figure 1, Group B). The genome organization described above strongly suggests that the *B. nigra* genome consists of three complete copies of a fundamental ancestral genome. However, the three copies of the unit genome are rearranged with respect to one another, and collinear chromosomal segments, corresponding to (on average) a third of a linkage group, have been conserved rather than whole chromosomes (Figure 2).

In contrast to the order of homoeologous markers, which was conserved between the duplicate copies in sets A–H (Figure 1), the recombination rates in equivalent intervals were often strikingly different. For example, the loci detected by pC2 and pR95 (Figure 1, Group A), were separated by >30 cM on G1 and G6 but by only 9 cM on G8.

Comparative mapping in *B. nigra*, *B. oleracea* and *B. rapa*: It was possible to develop a comparative map of the Brassica A, B and C genomes because the *B. nigra* (B genome) genetic map (LAGERCRANTZ and LYDIATE 1995) was constructed with RFLP probes that had been used previously to map the *B. oleracea* (C genome) and *B. rapa* (A genome) components of resynthesized *B. napus* (PARKIN *et al.* 1995). The resulting map is presented in Figure 3, where homoeologous segments of the linkage groups from *B. oleracea* and *B. rapa* are aligned with the linkage groups of the *B. nigra* map. Distinct homoeologous segments of the Brassica A and C genomes were identified for virtually every portion of the *B. nigra* genome. The conspicuous exception to this general rule was the duplicated portion of *B. nigra* G6 (Figure 3). Almost the whole of the Brassica A and C genomes were represented by the aligned segments displayed in Figure 3. The homoeologous regions at

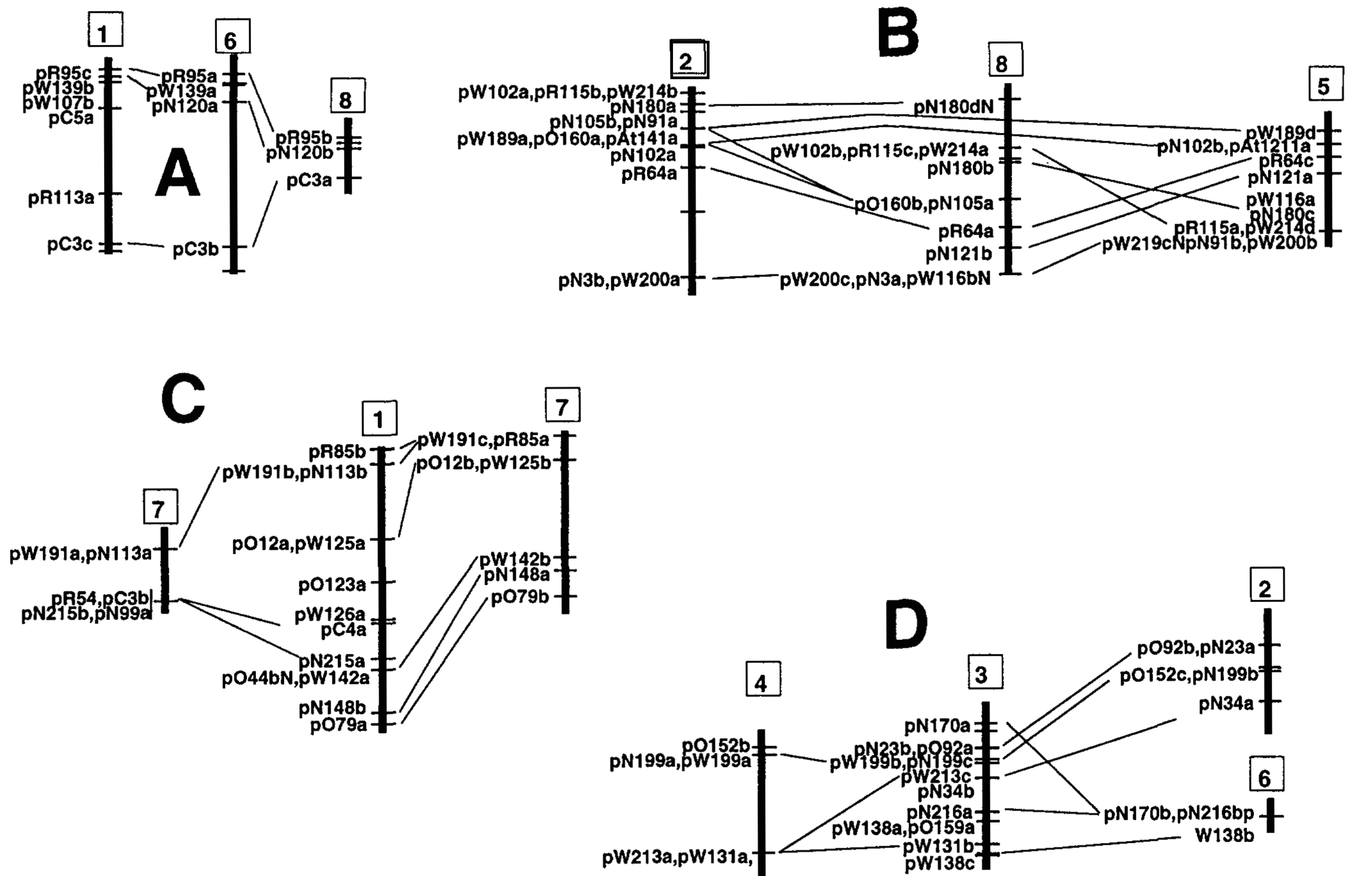

FIGURE 1.—The triplicated nature of the *B. nigra* genome. Detailed linkage maps of portions of the *B. nigra* genome are represented so as to emphasize the homoeologies between triplicated chromosomal segments. Each set of segments (A–H) has either three collinear copies (each linked by at least three pairs of homoeologous loci) or two collinear representations and a third related structure. RFLP-defined loci are encoded and positioned as described by LAGERCRANTZ and LYDIATE (1995), and the spacing between loci is proportional to centimorgan distances. The genomic distribution of the eight sets of related segments (A–H) is shown in Figure 2. Boxed numbers above chromosomal segments indicate the linkage groups from which they originate.

the bottoms of linkage groups R3 (N3) and O7 (N17) (PARKIN *et al.* 1995) were the only portions of the A and C genomes not represented by an identifiable equivalent region in the *B. nigra* genome. This suggests that the content of the Brassica A, B and C genomes is very similar but that the genomes are arranged into different numbers of structurally distinct chromosomes in each case. The only linkage groups that might represent complete homoeologues are G1 with R5 and G4 with R6. Conservation of overall content between the three Brassica genomes implies that the A and C genomes share the complete triplication of the hypothesized unit structure identified in *B. nigra.*

Two *B. nigra* linkage groups (G2 and G3) each consist of entire *B. rapa* linkage groups (R10 and R8, respectively) plus segments of additional *B. rapa* groups (R9 and R7, respectively) (Figure 3). The relative structures of these chromosomal regions in the A and B genomes suggest that chromosome fission and/or fusion has occurred during the divergence of the two genomes.

Comparative mapping of the Brassica A, B and C genomes suggests that new genome structures have also arisen via the translocation of large chromosomal segments. For example, the chromosome represented by R5/G1 in the A and B genomes, respectively, (Figure 3) has been disrupted in the C genome where the top half of this chromosome is associated with a chromosomal segment equivalent to the bottom of G4, and the bottom half of this chromosome is associated with a chromosomal segment equivalent to the bottom half of G6. It has been postulated that the points on G1 and G6 equivalent to the endpoints of the rearrangements in the C genome and are candidates for the centromeres (LAGERCRANTZ and LYDIATE, 1995), suggesting that whole chromosome arms have been translocated in the C genome.

The inversion of large chromosomal segments during the divergence of the Brassica genomes is also in evidence. For example, G5 of the B genome corresponds to two distinct segments of the A and C genomes; the bottom of G5 and homoeologous segments of O1/R1 have the same orientation with respect to the ends of the linkage groups, whereas, the top of G5 and homoeologous segments of O2/R2 have inverse orientations

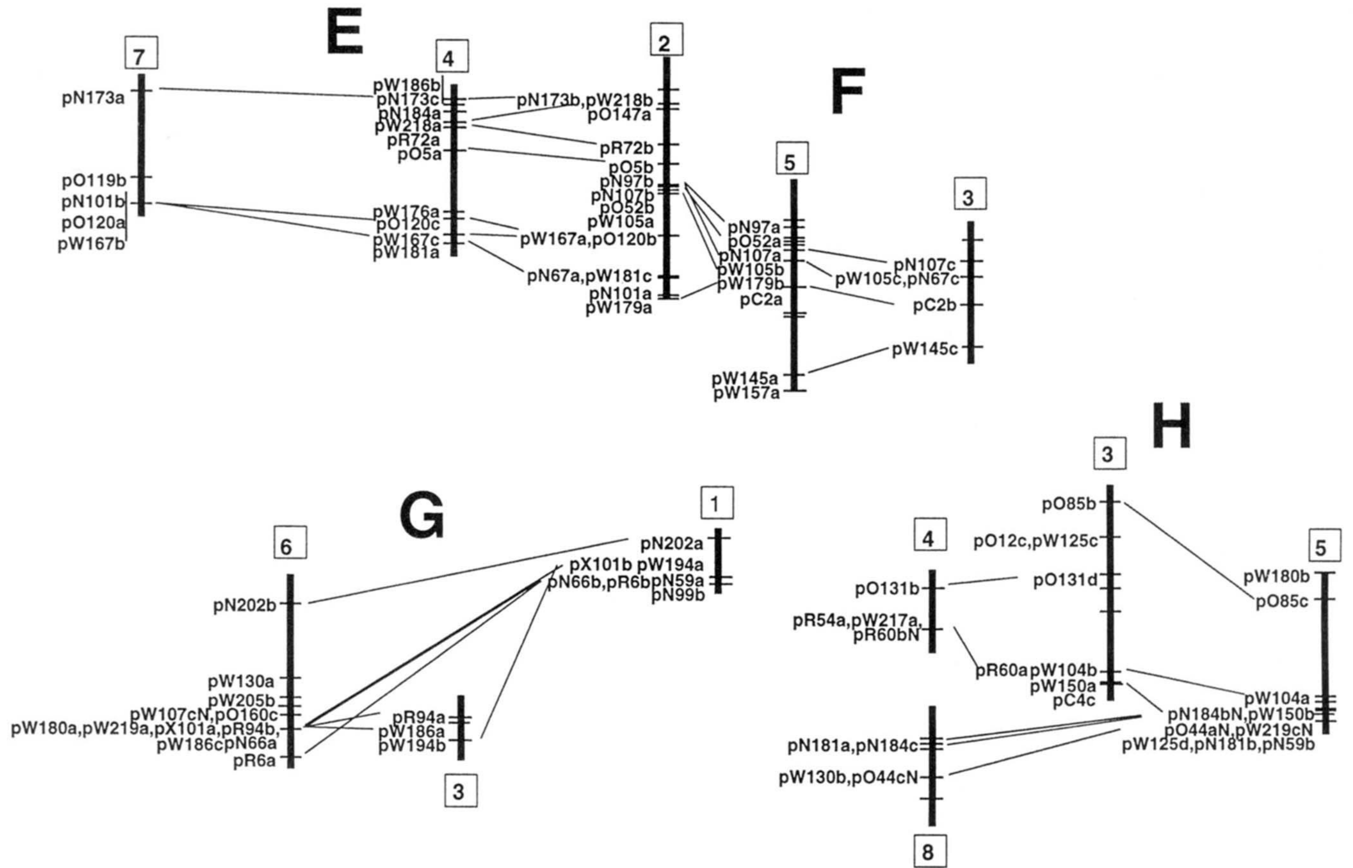

FIGURE 1.—*Continued*

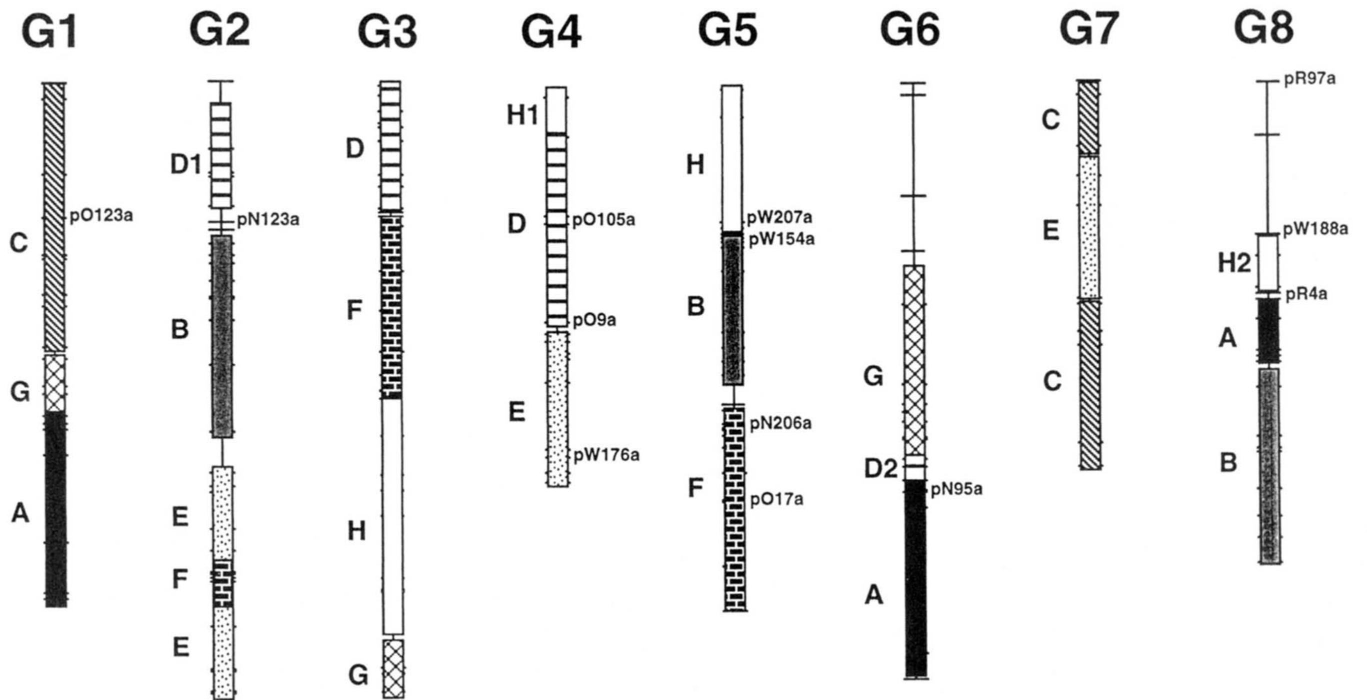

FIGURE 2.—Genetic map of *B. nigra* showing the distribution of the sets of triplicated chromosomal segments (A–H). Chromosomal segments with the same shading share common sets of homologous loci. Virtually the whole of the *B. nigra* genome has a recognizably triplicated structure. Detailed linkage maps of each of the eight set of related segments (A–H) are shown in Figure 1. The dispersed single copy loci are represented on the right-hand side of the linkage groups.

FIGURE 3.—Comparative map of the Brassica A, B and C genomes showing the alignment of *B. nigra* linkage groups (G1–G8, central) with the corresponding chromosome segments from *B. oleracea* (O1–O9, left) and *B. rapa* (R1–R10, right). Lines connecting the *B. nigra* linkage groups with chromosome segments from *B. oleracea* and *B. rapa* indicate the positions of homoeologous loci. Dotted lines indicate the positions of loci not detected in *B. nigra* but homoeologous in *B. oleracea* and *B. rapa*. Rounded ends on chromosome segments from *B. oleracea* and *B. rapa* indicate that they coincide with the termini of the corresponding linkage groups, while thick horizontal lines at the ends of chromosomal segments indicate that these are internal to the corresponding linkage groups. ×, inversions in *B. oleracea* with respect to *B. nigra*; □, inversions in *B. rapa* with respect to *B. nigra*; ⊠, inversions in both *B. oleracea* and *B. rapa* with respect to *B. nigra*.

TABLE 2

LOD scores supporting marker orders in areas where inversions are indicated from the comparative mapping of *B. nigra*, *B. oleacea* and *B. rapa* (FIGURE 3)

Linkage group	*B. oleracea*[a]	*B. nigra*[a]	*B. rapa*[a]
3	5	6	—
4	—	6	6
5	—	27	9
5	9	6	2
5	3	6	—
6	—	8	4
7	8	7	3

[a] LOD scores were calculated against the alternative order not resulting in an inversion.

with respect to the ends of the linkage groups (Figure 3). There are ≥12 instances where the ends of the linkage groups from *B. oleracea* and/or *B. rapa* have been brought into the internal regions of *B. nigra* linkage groups, or visa versa, during the differentiation of the three genomes (Figure 3). There are also at least seven inversions, internal to otherwise collinear regions, differentiating the A, B and C genomes. One inversion, namely that involving a segment of the upper portion of G3, differentiates the C genome from the A and B genomes (Figure 3). Two inversions, one involving a central segment of G5 and another involving a central segment of G7, differentiate the B genome from the A and C genomes (Figure 3). The LOD scores supporting marker orders in areas involved in apparent inversions are given in Table 2.

DISCUSSION

Analysis of the internal structure of the *B. nigra* genome has provided a novel insight into its organization and evolution. It seems that the diploid genome of *B. nigra* consists of three complete, but rearranged, copies of a fundamental ancestral genome. The most simple explanation for this genome organization is that *B. nigra* is descended from a hexaploid ancestor. The results are incompatible with the hypothesis proposed by RÖBBELEN (1960) that *B. nigra* ($n = 8$), *B. oleracea* ($n = 9$) and *B. rapa* ($n = 10$) are all descended from an $n = 6$ ancestor through the addition/duplication of individual, intact chromosomes. It has been noted previously that intragenomic duplication is a prominent feature of the *B. oleracea* and *B. rapa* genomes (SLOCUM *et al.* 1990; SONG *et al.* 1991; PARKIN *et al.* 1995), but previous analyses of the Brassica A and C genomes have stopped short of suggesting genome-wide triplication. However, the level of duplication in the A and C genomes has probably been systematically underestimated because the crosses used to analyze these genomes were less polymorphic than the *B. nigra* cross used in the current analysis of the B genome.

The amounts of nuclear DNA in *B. rapa, B. nigra* and *B. oleracea* are known to be similar (ARUMUGANATHAN and EARLE 1991). Comparative analysis of the Brassica A, B and C genomes also revealed a striking conservation of genome content, suggesting that all three Brassica genomes have inherited complete but rearranged copies of a duplicated (possibly hexaploid) ancestral genome. In spite of this conservation of genome content the three Brassica species have different chromosome numbers (8, 9, and 10). Comparative mapping indicated that chromosome fission/fusion had occurred during the divergence of the three genomes, and it is likely that the processes of fission and fusion brought about the changes in chromosome number rather than the duplication and/or elimination of whole chromosomes as suggested by RÖBBELEN (1960).

Modern phylogenies of cruciferous plants based on analyses of chloroplast genomes (WARWICK and BLACK 1991, 1994) indicate that *B. rapa* and *B. oleracea* (the A and C genomes) belong to a lineage of cruciferous species distinct from that containing *B. nigra* (the B genome). Furthermore, a wide range of species belonging to genera including Moricandia, Eruca, Diplotaxis, Raphanus and Sinapis are more closely related to either *B. nigra* or *B. oleracea* than these two species are to one another. Therefore, this wide range of species might all share the same hexaploid progenitor.

Although genome triplication is the general pattern of organization in the A, B and C genomes of Brassica species, additional events besides the hypothesized hexaploidization have clearly affected the degree of duplication of localized segments of the genomes. In the present study some probes detected more than three loci (Table 1), and these more highly duplicated loci might have arisen from single gene duplications or from segmental duplication of small genomic regions. One example of a possible segmental duplication is the tandemly duplicated portion of *B. nigra* G6 (Figure 3). No homoeologous segments corresponding to this region were found in either *B. oleracea* or *B. rapa,* suggesting that this duplication occurred after the divergence of the B genome from the other two Brassica genomes. Furthermore, 17 probes detected single copy loci in *B. nigra.* Polymorphic single copy loci were found on most linkage groups with no obvious clustering (Figure 2). It is possible that ancestral homoeologous copies of loci currently present in a single copy were deleted from the duplicate regions during evolution. According to this model, a large number of localized deletions, rather than a few relatively large events, would be required to explain the dispersed nature of single copy loci. Alternatively, the lack of hybridization to multiple loci might result from sequence divergence between the duplicate copies of the fundamental ancestral sequence.

The genome of *B. nigra* (0.97 pg DNA per diploid nucleus; ARUMUGANATHAN and EARLE 1991) is approxi-

mately three times larger than that of the related species *Arabidopsis thaliana* (0.3 pg, ARUMUGANATHAN and EARLE 1991). The genome of *A. thaliana* is generally considered to be free of large duplicated segments (CHANG *et al.* 1988; NAM *et al.* 1989) although recent data indicate that this might be an oversimplification (MCGRATH *et al.* 1993). These facts raise two fascinating possibilities, that the genome of Arabidopsis might be similar in content and perhaps organization to the fundamental unit genome of the hypothesized hexaploid Brassica ancestor and that gene spacing in diploid Brassica species might be similar to that in *A. thaliana.* Preliminary comparative investigations of the Brassica and Arabidopsis genomes reinforce these possibilities (LAGERCRANTZ *et al.* 1996).

The ancient units of the Brassica B genome are relatively well conserved because extensive collinear segments spanning most of the genome are still obvious (Figures 1 and 2). In contrast, the gross structure of the Brassica genome is relatively dynamic, with many rearrangements distinguishing the A, B and C genomes from one another. These two patterns of genome evolution are able to coexist if the endpoints of gross genome rearrangements coincide with the borders of the conserved segments of the unit genome, *i.e.*, that rearrangements preferentially occur at particular sites on the chromosomes. Such shuffling of conserved subchromosomal units has recently been used to explain genome evolution in the grasses (MOORE *et al.* 1995; VAN DEYNZE *et al.* 1995). In the current analysis, the probable positions of centromeres sometimes coincided with the endpoints of chromosome rearrangements that differentiate both the Brassica A, B and C genomes and the duplicated subchromosomal segments of the B genome. The precise mapping of centromeres would be a useful priority for the future analysis of Brassica genomes.

The comparative analysis of cereal genomes has suggested that large-scale chromosomal rearrangements are rare even over large evolutionary spans. The cereal genome has been divided into only 19 conserved linkage blocks even after 60 million years of evolution (MOORE *et al.* 1995). In contrast, comparative analysis of Brassica genomes has demonstrated that evolution from an ancestral polyploid genome has rearranged a minimum of ~24 (Figure 1) linkage blocks in less then 10 million years (MULLER 1981).

The hypothesized ancestral Brassica hexaploid would have exhibited extensive duplication of gene function. However, duplicated genes tend to acquire new functions or become silenced during evolution (OHNO 1970; LI and GRAUR 1991). The pace at which such events occur is still not well understood, and it will be interesting to investigate what fraction of the genes present in the hypothesized hexaploid are still active and what fraction of the active genes have acquired a new expression pattern in modern Brassica genomes. It will also be interesting to investigate the patterns of gene silencing and in particular whether linked genes are often silenced together. A degree of duplicate gene function probably still exists in modern diploid Brassica genomes because some instances of multiple genes controlling morphological traits follow the pattern of genome duplication in *B. oleracea* (KENNARD *et al.* 1994).

There are some ambiguities concerning the exact alignments of closely related homoeologous regions between the Brassica A, B and C genomes. The most likely alignments are represented in Figure 3. Alternative alignments require a larger number of rearrangements to explain the differences between the three genomes. There are mainly two avenues of research that will allow a more certain identification of primary homoeologies across the A, B and C genomes: identification of the most closely homologous pairs of DNA sequences derived from the different homoeologous chromosomal regions and charting of the pattern of intergenomic (homoeologous) recombination events in interspecific hybrids.

Understanding the relationships between the A, B and C genomes has important practical aspects. The degree of collinearity between the genomes of *B. nigra* and the more extensively cultivated *B. oleracea* and *B. rapa* will affect the possibilities for transfer of traits from *B. nigra* (and a large number of closely related species such as *Sinapis alba*) to the most important Brassica crops. Knowledge of the homoeologous regions of the genomes will increase the possibilities of transferring traits via homoeologous recombination in resynthesized hybrids and of selecting desirable hybrid chromosomes with the aid of genetic marker technology (LYDIATE *et al.* 1993).

The authors thank ISOBEL PARKIN for interesting discussions and ANDREW SHARPE for assistance with marker technology. This work was supported by grants from the Swedish Council for Agricultural and Forest Research and the Swedish Institute and by the UK Biotechnology and Biological Sciences Research Council.

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Communicating editor: J. CHORY