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Detection of Chromosomal Rearrangements Derived From Homeologous Recombination in Four Mapping Populations of *Brassica napus* L.

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ABSTRACT

Genetic maps of *Brassica napus* were constructed from four segregating populations of doubled haploid lines. Each mapping population had the same male parent and used the same set of RFLP probes, facilitating the construction of a consensus map. Chromosomal rearrangements were identified in each population by molecular marker analysis and were classified as *de novo* homeologous nonreciprocal transpositions (HNRTs), preexisting HNRTs, and homeologous reciprocal transpositions (HRTs). Ninety-nine *de novo* HNRTs were identified by the presence of a few lines having duplication of a chromosomal region and loss of the corresponding homeologous region. These *de novo* HNRTs were more prevalent in one population that had a resynthesized *B. napus* as a parent. Preexisting HNRTs were identified by fragment duplication or fragment loss in many DH lines due to the segregation of HNRTs preexisting in one of the parents. Nine preexisting HNRTs were identified in the three populations involving natural *B. napus* parents, which likely originated from previous homeologous exchanges. The male parent had a previously described HRT between N7 and N16, which segregated in each population. These data suggest that chromosomal rearrangements caused by homeologous recombination are widespread in *B. napus*. The effects of these rearrangements on allelic and phenotypic diversity are discussed.

CHROMOSOMAL rearrangements have played an important role in our understanding of genetics, evolution, and speciation of many organisms (see Stebbins 1971, Rieseberg 2001, and Levin 2002 for examples in plants; Navarro and Barton 2003 and Bailey *et al.* 2004 for examples in humans). Chromosomal rearrangements derived from nonhomologous chromosome exchanges were first identified in corn (*Zea mays*) using traditional cytogenetic techniques (McClintock 1929) and have been commonly termed translocations or interchanges (Burnham 1962). In diploid organisms, translocation heterozygotes produce duplication-deletion meiotic products resulting in semisterility (Griffiths *et al.* 2000). In allopolyploids, gene redundancy can buffer the phenotypic effects of duplications and deficiencies caused by segregating translocations, especially for translocations that result from homeologous chromosomal exchanges. Stebbins (1947) referred to the chromosomal pairings that could lead to such exchanges as heterogenetic associations. Substitutions of entire homeologous chromosomes from such associations was recognized as a potential cause of occasional off-types in cultivated oats (*Avena sativa*) and in wheat (*Triticum aestivum*; reviewed by Huskins 1946). Osborn *et al.* (2003) recently described an exchange between segments of homeologous chromosomes in some genotypes of the allotetraploid *Brassica napus* and referred to this rearrangement as an interstitial homeologous reciprocal transposition.

B. napus is an amphidiploid species composed of homeologous A and C genomes, which are thought to have derived from the recent progenitors of extant *B. rapa* and *B. oleracea*, respectively (U 1935). In a resynthesized *B. napus* amphihaploid, meiotic chromosome pairing was observed, suggesting that the A and C homeologs have retained sufficient homology to form syndetic structures in the absence of true homologous chromosome pairs (Attia and Röbbelen 1986a). After chromosome doubling the amphihaploid, meiotic chromosome pairing between homologs was most prevalent, but some multivalent structures were observed (Attia and Röbbelen 1986b). Multivalent structures were also observed in natural allotetraploid *B. napus*, although at lower frequencies than observed in resynthesized *B. napus* (Newell *et al.* 1984; Attia and Röbbelen 1986b). These cytological observations suggest that exchanges could occur between homeologous chromosomes of *B. napus*.

Evidence for homeologous exchanges also has been obtained by analyzing segregating populations of *B. napus* with molecular markers. Sharpe *et al.* (1995) and Parkin *et al.* (1995) first documented the occurrence of homeologous nonreciprocal transpositions (HNRTs, termed nonreciprocal translocations in these articles)

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in *B. napus*. They used RFLP markers to generate genetic maps of three segregating populations of doubled haploid (DH) lines derived from reciprocal crosses between a winter and a spring *B. napus* cultivar (Sharpe *et al.* 1995) and from a resynthesized *B. napus* crossed to the same winter parent (Parkin *et al.* 1995). Unusual RFLP patterns of a few DH lines suggested that homeologous exchanges occurred during meiosis of F_1 plants at low frequencies and generated *de novo* HNRTs. Another type of rearrangement, a homeologous reciprocal transposition (HRT), was also characterized in these three mapping populations and in three additional *B. napus* mapping populations (Osborn *et al.* 2003). All six mapping populations were derived from crosses between spring and winter parents. The HRT was identified as an exchange between homeologous regions of N7 and N16 that was inherited from the spring parent and segregated in the progeny. Molecular marker evidence for HRTs was further supported by pachytene spreads of meiotic cells from anthers from a winter × spring F_1 hybrid, which illustrated possible exchange points between chromosomes.

In this study, we have generated four genetic maps of *B. napus* from four segregating populations of DH lines using RFLP markers. The four populations shared a common male parent, facilitating the assembly of a *B. napus* consensus map. The female parent of one population was a *B. napus* line resynthesized from *B. rapa* and *B. oleracea*, which allowed unambiguous identification of RFLP alleles from the A and C subgenomes of *B. napus*. Rearrangements among homeologous chromosomes (*de novo* HNRTs, preexisting HNRTs, and an HRT) were identified and classified on the basis of irregular segregation patterns of RFLP alleles and by matching RFLP alleles to the diploid progenitor species. The role of these rearrangements in polyploid genome evolution and their effects on allelic and phenotypic diversity of *B. napus* are discussed.

MATERIALS AND METHODS

Population development: Four DH populations were developed by crossing the RV289, TO1147, MF216, and RV128 lines with a male-fertility restorer line, P1804, produced by Bayer CropScience, Saskatoon, Saskatchewan, Canada. RV289 is a single plant selection from Hua-dbl2, a Chinese open-pollinated winter cultivar kindly provided by Jinling Meng, Huazhong Agricultural University, Wuhan, China. TO1147 is a resynthesized *B. napus* line created by crossing a *B. rapa* plant (as the female), grown from a yellow seed of cultivar Reward (Scarth *et al.* 1992), to an inbred *B. oleracea* plant, TO1000, a S_5 inbred line from the rapid-cycling *B. oleracea* stock CrGC3-3 developed by the Crucifer Genetics Cooperative (Madison, WI). An F_1 embryo from this interspecific cross, TO1147, was rescued and chromosome doubled by immersing its roots in a 0.34% colchicine solution for 1.5 hr (Song *et al.* 1993). MF216 is a noncanola quality DH line derived from a cross between the French winter cultivar Major and the Canadian spring canola cultivar Stellar (Ferreira *et al.* 1994) and has ~60% of its genome derived from Major. RV128 was developed by backcrossing alleles for spring growth habit from Westar (a Canadian spring canola cultivar) into Samourai (a French winter canola cultivar) using RFLP marker loci to select for Westar alleles at the major vernalization-requiring flowering-time QTL *VFN1* (Osborn *et al.* 1997) and for Samourai alleles at ~85% of the remaining marker loci scattered throughout the genome (R. D. Vogelzang and T. C. Osborn, unpublished data). Each of the four female parents, RV289, TO1147, MF216, and RV128, were crossed with P1804. Two different F_1 plants were used as the sources for microspores for the (RV289 × P1804) population and a single F_1 plant was used for the other three populations.

Haploid plants of each population were created from their respective F_1 through microspore culture (Chuong and Beversdorf 1985). They were selected to contain the male-fertility restorer gene *Rf*, required for the Seedlink hybrid seed production system of Bayer CropScience, by selecting for resistance to glufosinate herbicide conferred by a linked resistance gene included in the *Rf* construct (Mariani *et al.* 1992). Approximately 1000 haploid plants were treated with colchicine and male-fertile plants were self-pollinated to establish DH lines. From 162 to 170 DH lines that produced sufficient seed (>100) for future testing were genotyped for each population (Table 1). The F_1-derived DH populations are referred to as HUA (RV289 × P1804), MF (MF216 × P1804), and RV (RV128 × P1804), SYN (TO1147 × P1804).

Genetic markers: The majority of the RFLP probes included in this study had been used in previous mapping studies (Ferreira *et al.* 1994; Teutonico and Osborn 1994; Parkin *et al.* 1995; Sharpe *et al.* 1995; Thormann *et al.* 1996; Kole *et al.* 1997; Butruille *et al.* 1999) and came from three libraries: a *Pst*I genomic DNA library and a cDNA library from *B. napus* cv Westar, originally designated as "WG" and "EC" probes, respectively (Thormann *et al.* 1994), and an *Eco*RI genomic DNA library from *B. rapa* cv Tobin ("TG" probes) that was constructed and screened as described for the Westar genomic library (Ferreira *et al.* 1994). Clones from the *Pst*I and *Eco*RI genomic DNA libraries were labeled "pW" probes and those from the cDNA library were labeled "pX" probes, according to the nomenclature of Parkin *et al.* (1995) and Sharpe *et al.* (1995). This nomenclature was used due to its greater simplicity and ease of cross-referencing with other studies that used this nomenclature. Additional information about these probes, including DNA sequences, is available at http://osbornlab.agronomy.wisc.edu/research.html. Four cloned genes that were homologs of the *Arabidopsis thaliana FLOWERING LOCUS C* (*FLC*) gene from *B. rapa* (Schranz *et al.* 2002), and a clone of the *A. thaliana FLOWERING LOCUS T* gene (Kardailsky *et al.* 1999) were also used as probes.

The probes (120 pWs, 53 pXs, and the cloned genes) were screened by hybridization to small blots containing genomic DNA from the *B. napus* parental lines, *B. oleracea* (TO1000), and three *B. rapa* cultivars (Reward, Per, and R500) separately digested with *Hin*dIII and *Eco*RI, the enzymes used in previous studies (Ferreira *et al.* 1994; Thormann *et al.* 1996; Butruille *et al.* 1999). Polymorphisms observed between P1804 and the other parents were genetically mapped by hybridizing probes to mapping blots containing appropriately digested samples of the DH lines. Loci were named using the probe name with a two-letter suffix. The first letter distinguished multiple loci detected by a probe. Fragments detected in the P1804 parent were labeled alphabetically from the highest to lowest molecular weight. The second letter indicated the enzyme used to detect the RFLP locus (E, *Eco*RI; H, *Hin*dIII).

Map construction: JoinMap 3.0 was used to create the genetic linkage maps (van Ooijen and Voorrips 2001). Linked loci were grouped on the basis of pairwise LOD values of 5 or 6. Locus order within the LOD groupings was determined

through an optimized algorithm using three rounds of marker placement with the Kosambi mapping function (Kosambi 1944; Stam 1993). Starting with the most informative pair in the first round, markers were added in each possible position one by one. The best order was selected by goodness of fit. Markers with negative recombination fraction or large contributions to lack of fit (parameter value of 5.00) were held for the second round. Each marker not placed in the first round was tried again using the same fitting criteria, but with a more complete linkage group. Finally, the remaining markers were placed into the most likely position by minimizing the lack-of-fit parameter even though it may have exceeded the initial threshold. After positioning new loci, the ripple command with a three-locus window was used to reestimate locus orders and distances. After map construction, the data files were searched for putative double recombinants, which were verified or corrected by reexamining the RFLP autorads. Generally, after a few iterations between map construction and data verification, the third round of marker placement was not needed when reconstructing the linkage groups.

Consensus map construction: A consensus map was constructed using individual maps from all four DH populations. The four maps were aligned by common loci using the same set of RFLP probes and previous published maps (Parkin *et al.* 1995; Sharpe *et al.* 1995; Butruille *et al.* 1999). Since each population had a common parent (P1804), individual linkage groups could be aligned using common alleles from P1804. In cases where different enzymes were used, common loci were inferred on the basis of the position of common flanking loci and the enzyme designation was omitted from the locus name. The consensus map was created using JoinMap 3.0 (van Ooijen and Voorrips 2001), which utilized the mean recombination frequencies and combined LOD scores to position common loci mapped in more than one population. JoinMap also was used to evaluate significant differences in recombination between common markers in each of the individual maps. MapChart (Voorrips 2002), PowerPoint, and Excel were used to draw the maps and graphically illustrate the homologous and homeologous relationships between linkage groups.

RESULTS

Genetic map characteristics: Individual genetic maps were constructed for four segregating populations of F_1-derived DH lines from crosses involving three natural and one resynthesized *B. napus* crossed with a single natural *B. napus* line (Table 1). Nineteen linkage groups that probably correspond to the 19 chromosomes of *B. napus* were identified in each mapping population (Figure 1). Each linkage group could be assigned to either the *B. rapa* (A) or the *B. oleracea* (C) genome on the basis of the identification of alleles as coming from either the A- or the C-genome progenitors of the SYN population. Linkage groups were designated as in previous studies (Parkin *et al.* 1995; Sharpe *et al.* 1995; Butruille *et al.* 1999) with N1–N10 corresponding to the A genome and N11–N19 corresponding to the C genome. The four individual genetic maps showed a high level of collinearity. Some inconsistencies found in locus order among the individual genetic maps could be due to chromosomal rearrangements (*e.g.*, N11 and N16; see below). The largest total recombinational map was detected for the SYN population, perhaps because it included more polymorphic loci and thus had more extensive genome coverage.

A considerable proportion of the polymorphic loci detected within the individual maps (26% to 41%) had allelic segregation that deviated significantly ($P < 0.01$) from the 1:1 ratio expected for a population of DH lines derived from an F_1 (Table 1). All four DH populations were selected for glufosinate resistance via the microspore culture media to utilize the Seedlink hybrid production system (Goldberg *et al.* 1993) in subsequent experiments. The bottom of linkage group N7 was completely skewed toward the P1804 allele, indicating this as the most likely position for the glufosinate resistance transgene. The top of N3 also was skewed significantly toward the P1804 allele in all populations, perhaps because of inadvertent selection of early flowering alleles at *BrFLC3* and *BrFLC5* on N3. *BrFLC3* and *BrFLC5* are homologs of the *A. thaliana* flowering time gene *FLC* and allelic variation at *BrFLC5* has been associated with flowering time in *B. rapa* (Schranz *et al.* 2002). This and other regions that had significant deviations from a 1:1 ratio also may have segregated for various genetic factors that affected the generation or selection of the doubled haploid lines used in the mapping populations (see Xu *et al.* 1997). In some populations skewed segregation occurred in regions that segregated for chromosomal rearrangements. These rearrangements affected the amount of intergenome heterozygosity and may have had subtle effects on the amount of seed produced (see discussion).

A consensus map was constructed from the four segregating populations of DH lines (Figure 1). Each individual map contributed complementary sets of polymorphic loci providing thorough coverage of the genome in the consensus map (Figure 1 and Table 1). On average, each DH map contributed six loci per linkage group to the consensus map. Most of the RFLP probes (88%) detected more than one segregating locus, with an average of 1.65–2.23 loci/probe detected in the individual maps and 3.02 loci/probe in the consensus map (Table 1). These multiple loci often had collinear linkage arrangements between linkage groups from the A and C genomes (Figure 2), reflecting the homeologous relationships reported previously (Parkin *et al.* 1995; see below).

Chromosomal rearrangements: Several factors facilitated the detection of chromosomal rearrangements in this study. First, the DH lines used for mapping were completely homozygous, each being derived from single gametes, and their use prevented the potential misclassification of some marker genotypes as heterozygous in lines containing rearrangements. Second, by comparing four mapping populations sharing one parent we could more easily identify unusual patterns of segregation that indicated the presence of chromosomal rearrangements. Third, inclusion of a resynthesized *B. napus* line

TABLE 1

Characteristics of four genetic linkage maps and their consensus map constructed using DH lines of *B. napus*, genotyped with RFLP and SSR markers

	HUA	MF	RV	SYN	Consensus
No. of DH lines	162	170	164	162	NA
No. of RFLP probes	132	127	124	140	167
No. of RFLP loci detected	243	218	205	312	508
*Eco*RI	89	96	90	119	NA
*Hin*dIII	154	122	115	193	NA
No. of RFLP loci/probe	1.84	1.70	1.65	2.23	3.02
No. of RFLP loci with a null allele	12	16	12	12	37
No. of SSR loci	0	0	0	2	2
No. of unlinked loci	5	0	0	3	NA
No. of linkage groups	19	19	19	19	19
Average linkage group length (cM)	77	74	77	88	100
Total map length (cM)	1460	1398	1453	1668	1823
Average distance between loci (cM)	7.3	7.6	7.5	6.0	3.8
No. of loci with segregation distortion					
$0.01 < P < 0.05$	19	15	12	43	NA
$0.001 < P < 0.01$	13	23	21	44	NA
$P < 0.001$	49	39	48	83	NA

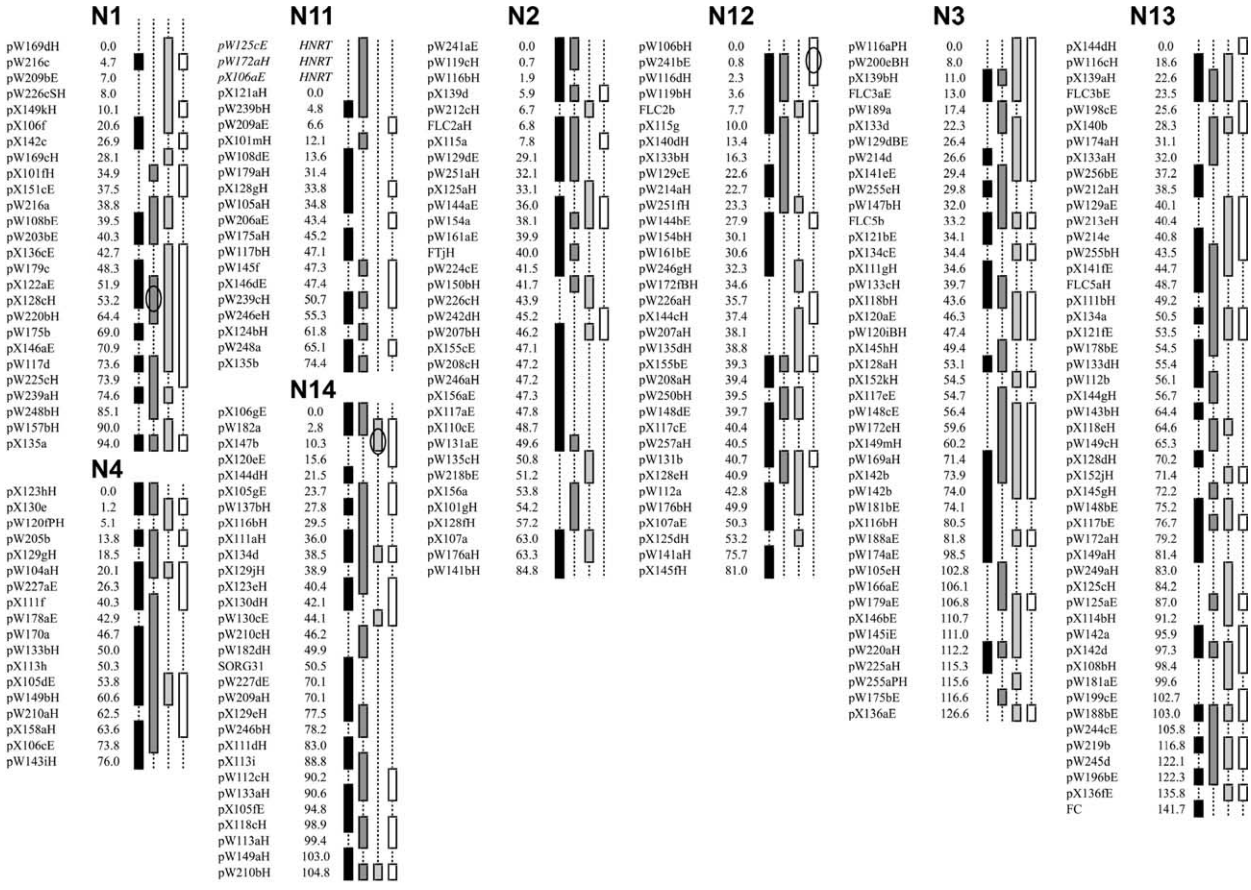
NA, not applicable.

as one of the mapping parents allowed accurate assignment of loci to either the A or the C genome. Fourth, use of RFLP probes that hybridized to multiple, codominant markers allowed simultaneous detection of alleles at homeologous loci.

Three types of chromosomal rearrangements were detected in the four mapping populations. The first type was *de novo* HNRTs, which apparently were derived from occasional homeologous exchanges during meioses in the F_1 plants. The second type was preexisting HNRTs within one of the mapping parents, which were inherited by the F_1 plant and segregated among the DH progenies. The third type of rearrangement was a preexisting HRT between homeologous segments of linkage groups N7 and N16 in the male parent (P1804) that segregated in the four DH populations. *De novo* HRTs may have occurred in some DH lines that inherited both transposed chromosomal segments from a homeologous exchange, but these could not be distinguished from DH lines inheriting unrearranged homeologous chromosomes.

***De novo* homeologous nonreciprocal transpositions:** These HNRTs were the simplest type of chromosomal rearrangement to recognize. Individual DH lines with *de novo* HNRTs were missing both alleles for a given locus and had allele duplication at their homeologous loci, containing either four doses of one allele or two doses of each of two alleles (Figure 3). These distinct allelic banding patterns were most readily recognized when a single probe hybridized to, and was polymorphic for, both homeologous loci. During construction of the individual maps, it became apparent that these losses and/or duplications within individual DH lines were among the linked loci of two homeologous genomic regions and that pairing and exchange of homeologous chromosomes was the most likely explanation for these *de novo* HNRTs. The population involving the resynthesized *B. napus* parent had the highest frequency of *de novo* HNRTs. As a percentage of total chromosomal exchanges detected in each map, homeologous exchanges represented 1.60, 1.09, 0.49, and 0.43% in the SYN, HUA, MF, and RV populations, respectively. The high-

FIGURE 1.—A consensus genetic linkage map of molecular markers compiled from individual maps constructed for four segregating populations of *B. napus* doubled haploid lines. Marker locus names and map positions (in centimorgans) are in the first two columns of each linkage group. Individual maps contributed complementary sets of polymorphic loci to the consensus map, as shown by the bars in the four columns (SYN, HUA, MF, and RV, respectively) that are aligned with loci in each linkage group. Linkage groups are numbered N1–N10 (A genome) and N11–N19 (C genome). Ovals identify loci that had different orders (>2 cM) in the individual DH maps compared to the consensus map. Linkage group N11 of MF map was not included in the consensus map due to a very different locus order. HNRT indicates loci that were part of a homeologous nonreciprocal transposition on N11 for which genetic distances could not be estimated. Loci on N7 and N16 having P1804 alleles the same size as fragments found in *B. rapa* are in italics. Loci on N7 and N16 that had segregating monomorphic loci in the SYN population are underlined.


N1
pW169dH 0.0
pW216c 4.7
pW209bE 7.0
pW226cSH 8.0
pX149kH 10.1
pX106f 20.6
pX142c 26.9
pW169cH 28.1
pX101fH 34.9
pX151cE 37.5
pW216a 38.8
pW108bE 39.5
pW203bE 40.3
pX136cE 42.7
pW179c 48.3
pX122aE 51.9
pX128cH 53.2
pW220bH 64.4
pW175b 69.0
pX146aE 70.9
pW117d 73.6
pW225cH 73.9
pW239aH 74.6
pW248bH 85.1
pW157bH 90.0
pX135a 94.0
N4
pX123hH 0.0
pX130e 1.2
pW120fPH 5.1
pW205b 13.8
pX129gH 18.5
pW104aH 20.1
pW227aE 26.3
pX111f 40.3
pW178aE 42.9
pW170a 46.7
pW133bH 50.0
pX113h 50.3
pX105dE 53.8
pW149bH 60.6
pW210aH 62.5
pX158aH 63.6
pX106cE 73.8
pW143iH 76.0
N11
pW125cE HNRT
pW172aH HNRT
pX106aE HNRT
pX121aH 0.0
pW239bH 4.8
pW209aE 6.6
pX101mH 12.1
pW108dE 13.6
pW179aH 31.4
pX128gH 33.8
pW105aH 34.8
pW206aE 43.4
pW175aH 45.2
pW117bH 47.1
pW145f 47.3
pX146dE 47.4
pW239cH 50.7
pW246eH 55.3
pX124bH 61.8
pW248a 65.1
pX135b 74.4
N14
pX106gE 0.0
pW182a 2.8
pX147b 10.3
pX120eE 15.6
pX144dH 21.5
pX105gE 23.7
pW137bH 27.8
pX116bH 29.5
pX111aH 36.0
pX134d 38.5
pX129jH 38.9
pX123eH 40.4
pX130dH 42.1
pW130cE 44.1
pW210cH 46.2
pW182dH 49.9
SORG31 50.5
pW227dE 70.1
pW209aH 70.1
pX129eH 77.5
pW246bH 78.2
pX111dH 83.0
pX113i 88.8
pW112cH 90.2
pW133aH 90.6
pX105fE 94.8
pX118cH 98.9
pW113aH 99.4
pW149aH 103.0
pW210bH 104.8
N2
pW241aE 0.0
pW119cH 0.7
pW116bH 1.9
pX139d 5.9
pW212cH 6.7
FLC2aH 6.8
pX115a 7.8
pW129dE 29.1
pW251aH 32.1
pX125aH 33.1
pW144aE 36.0
pW154a 38.1
pW161aE 39.9
FTjH 40.0
pW224cE 41.5
pW150bH 41.7
pW226cH 43.9
pW242dH 45.2
pW207bH 46.2
pX155cE 47.1
pW208cH 47.2
pW246aH 47.2
pX156aE 47.3
pW117aE 47.8
pX110cE 48.7
pW131aE 49.6
pW135cH 50.8
pW218bE 51.2
pX156a 53.8
pX101gH 54.2
pX128fH 57.2
pX107a 63.0
pW176aH 63.3
pW141bH 84.8
N12
pW106bH 0.0
pW241bE 0.8
pW116dH 2.3
pW119bH 3.6
FLC2b 7.7
pX115g 10.0
pX140dH 13.4
pX133bH 16.3
pW129cE 22.6
pW214aH 22.7
pW251fH 23.3
pW144bE 27.9
pW154bH 30.1
pW161bE 30.6
pW246gH 32.3
pW172fBH 34.6
pW226aH 35.7
pX144cH 37.4
pW207aH 38.1
pW135dH 38.8
pX155bE 39.3
pW208aH 39.4
pW250bH 39.5
pW148dE 39.7
pX117cE 40.4
pW257aH 40.5
pW131b 40.7
pX128eH 40.9
pW112a 42.8
pW176bH 49.9
pX107aE 50.3
pX125dH 53.2
pW141aH 74.7
pX145fH 81.0
N3
pW116aPH 0.0
pW200eBH 8.0
pX139bH 11.0
FLC3aE 13.0
pW189a 17.4
pX133d 22.3
pW129dBE 26.4
pW214d 26.6
pX141eE 29.4
pW255eH 29.8
pW147bH 32.0
FLC5b 33.2
pX121bE 34.1
pX134cE 34.4
pX111gH 34.6
pW133cH 39.7
pX118bH 43.6
pX120aE 46.3
pW120iBH 47.4
pX145hH 49.4
pX128aH 53.1
pX152kH 54.5
pX117eE 54.7
pW148cE 56.4
pW172eH 59.6
pX149mH 60.2
pW169aH 71.4
pX142b 73.9
pW142b 74.0
pW181bE 74.1
pX116bH 80.5
pW188aE 81.8
pW174aE 98.5
pW105eH 102.8
pW166aE 106.1
pW179aE 106.8
pX146bE 110.7
pW145iE 111.0
pW220aH 112.2
pW225aH 115.3
pW255aPH 115.6
pW175bE 116.6
pX136aE 126.6
N13
pX144dH 0.0
pW116cH 18.6
pX139aH 22.6
FLC3bE 23.5
pW198cE 25.6
pX140b 28.3
pW174aH 31.1
pX133aH 32.0
pW256bE 37.2
pW212aH 38.5
pW129aE 40.1
pW213eH 40.4
pW214e 40.8
pW255bH 43.5
pX141fE 44.7
FLC5aH 48.7
pX111bH 49.2
pX134a 50.5
pX121fE 53.5
pW178bE 54.5
pW133dH 55.4
pW112b 56.1
pX144gH 56.7
pW143bH 64.4
pX118eH 64.6
pW149cH 65.3
pX128dH 70.2
pX152jH 71.4
pX145gH 72.2
pW148bE 75.2
pX117bE 76.7
pW172aH 79.2
pX149aH 81.4
pW249aH 83.0
pX125cH 84.2
pW125aE 87.0
pX114bH 91.2
pW142a 95.9
pX142d 97.3
pX108bH 98.4
pW181aE 99.6
pW199cE 102.7
pW188bE 103.0
pW244cE 105.8
pW219b 116.8
pW245d 122.1
pW196bE 122.3
pX136fE 135.8
FC 141.7
N5
pX149hH 0.0
pW216dE 17.8
pX157hE 22.0
pX103aPE 28.0
pW125fE 28.4
pW247aH 31.0
pW126aH 38.9
pW232aE 39.3
pW209gH 42.5
pX123gH 45.1
pX142bE 46.3
pW108aH 48.1
pX101lH 51.7
pX102bH 54.1
pX151aE 54.8
pW114aE 55.8
pW217a 56.2
pW221eH 57.5
pX133e 58.7
pX129fH 59.6
pX123dE 59.7
pX134cH 60.3
pX121eE 63.3
pW137a 69.2
pX105e 73.8
pX118dH 77.2
pX116cH 91.0
N8
pW148aE 0.0
pW177bH 5.0
pW184dH 13.8
pW199dE 15.5
pW213dH 18.2
pW245bH 24.0
pX126eH 26.1
pW231cH 28.1
pW138aH 29.4
pW146aH 40.7
pX102aE 51.0
pW225cE 60.1
pX146fE 60.1
pW117bE 60.4
N15
pW249bH 0.0
pW125eE 4.4
pX149p 9.6
pW247b 10.0
pW232bE 11.2
pW126a 11.3
pW238bH 13.1
pW127bH 16.6
pW114bE 20.4
pX102dE 24.5
pX133iE 25.2
pX123cPH 25.2
pW146cH 33.3
pX140bH 33.9
pW246aH 34.3
pW237aE 43.0
pW138dH 46.0
pW164b 47.9
pW123aE 54.2
pW127eH 55.3
pW199bE 67.0
pW213aH 71.0
pX119fE 89.8
N18
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pW160dH 3.2
pW164eE 6.1
pX150eE 8.6
pW222bH 13.0
pW209cH 13.6
pW227bE 15.1
pX130bH 19.1
pW243aE 21.5
pW205a 25.5
pW188cE 27.8
pX150bE 32.5
pW104c 33.6
pW231aH 43.8
pW138bH 47.7
pW245aE 53.0
pX126dE 61.6
pW184bH 66.3
pW213cH 66.3
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pW244fE 73.7
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pW147bE 85.0
N6
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pW213bH 20.1
pW184aH 21.8
pW199aE 23.8
pW246dH 29.7
pW145cE 35.8
pW160eH 40.5
pW237bE 43.0
pW127cH 47.0
pX119eE 49.4
pW161bE 49.4
pW196aH 50.2
SN2426 50.4
pW219c 50.4
pX108bH 51.0
pW200dBH 52.6
pW258a 58.0
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pW137iH 62.2
pX104eH 71.5
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pW218aE 112.8
N9
pW243aE 0.0
pW123bE 24.7
pX109aH 25.4
pW160cH 33.2
pW222c 35.6
pW118aH 36.5
pW227cE 37.6
pW209dH 37.7
pX123iH 46.7
pW194bE 64.4
pW145gH 66.3
pW146bH 72.9
pW162kE 75.4
pX121dE 78.0
pX119dE 80.9
pW233aH 81.1
pX109b 82.1
pW203aE 84.5
pW122bH 90.4
pW128aE 95.3
pW200bH 111.1
pW167fE 119.6
pW137dH 129.6
pW157aH 133.8
pX117eE 142.5
N16
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pW221d 18.0
pW170cE 20.8
pX133aE 21.4
pW221bH 26.5
pW103bH 31.8
pX101eH 35.0
pW154a 35.8
pW130fE 36.4
pX130aH 45.7
pW228aH 46.6
FTaH 48.4
pW202aH 49.2
pW150cH 50.3
pW224bE 50.6
pW134cH 50.8
pW242cH 51.1
pX127aH 51.2
pW208c 53.7
pW226bBH 56.0
pX156bE 57.1
FTlH 65.3
pW170aH 74.7
N19
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pX144eH 22.9
pW119fH 24.6
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pX139eH 35.1
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pW128bH 35.7
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FLC1aH 40.0
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pW240bE 67.7
pW129cE 68.3
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pW194aH 97.5
pW114cE 109.8
pW137jH 121.3
pW157dH 133.1
N7
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pW186c 8.0
pW108aE 17.0
pW162eE 18.0
pX126fH 28.9
pX115fH 37.2
pX145aH 39.4
pX104aH 40.9
pW130dE 60.5
pW103aH 61.1
pX123d 65.4
pW228bH 65.8
pW150dH 68.8
pW206d 69.5
pX101jH 70.0
pX149cH 70.2
pX144aH 70.4
pW127fH 71.5
pW202bH 73.0
pX101dH 80.0
pX156fE 91.1
pX114eH 92.7
N10
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pW120g 1.0
pW147a 8.4
pX147hH 8.7
pW240a 12.7
pW196c 13.0
pW129b 13.5
pW201aE 21.3
pW212fH 21.7
pW256a 22.5
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pW255cH 25.8
pW198bE 26.4
pW189aE 27.3
pW155cH 28.3
pW107oH 44.1
FLC1fH 44.8
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pW200bE 56.2
pX144b 61.0
pW119aH 67.1
N17
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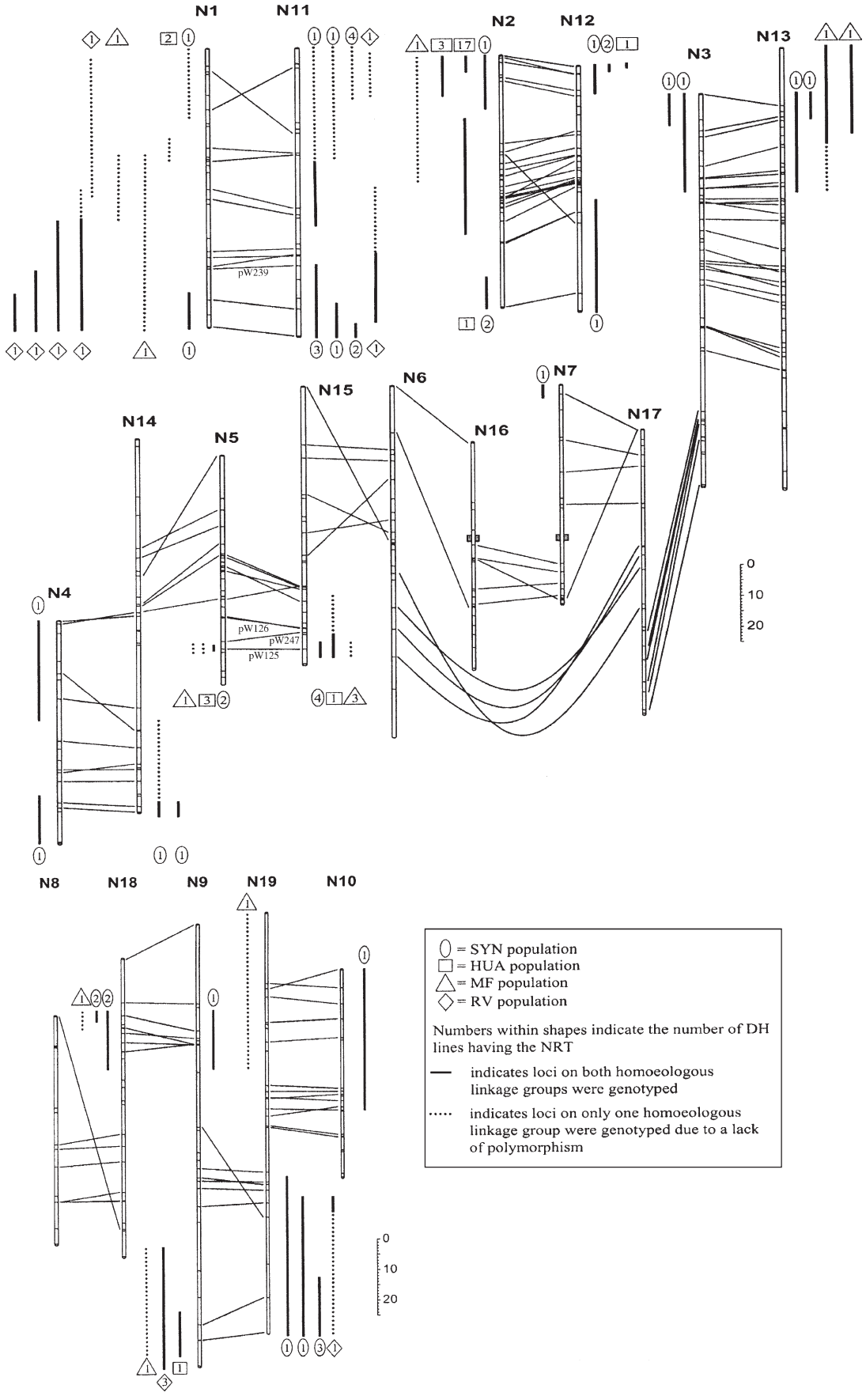
pW194bE 0.0
pW128bE 0.0
pX116g 3.1
pW192cE 5.2
pW162cE 8.1
pX126aH 11.9
pX145eH 23.6
pW192hE 25.2
pX110aE 37.8
pW257bH 38.3
pW112gH 40.4
pX104dH 45.7
pW167eE 56.8
pX105aE 58.0
pW104dH 65.2
pW174dE 65.2
pW175eH 68.6
pW166bE 76.9
pW179bE 78.0
pX136bE 84.6
pW120aH 85.4
pW220eH 90.7


N1
N11
N2
N12
N3
N13
N14
N5
N15
N6
N16
N7
N17
N4
N8
N18
N9
N19
N10
pW239
pW126
pW247
pW125
0
10
20
= SYN population
= HUA population
= MF population
= RV population
Numbers within shapes indicate the number of DH lines having the NRT
indicates loci on both homoeologous linkage groups were genotyped
indicates loci on only one homoeologous linkage group were genotyped due to a lack of polymorphism

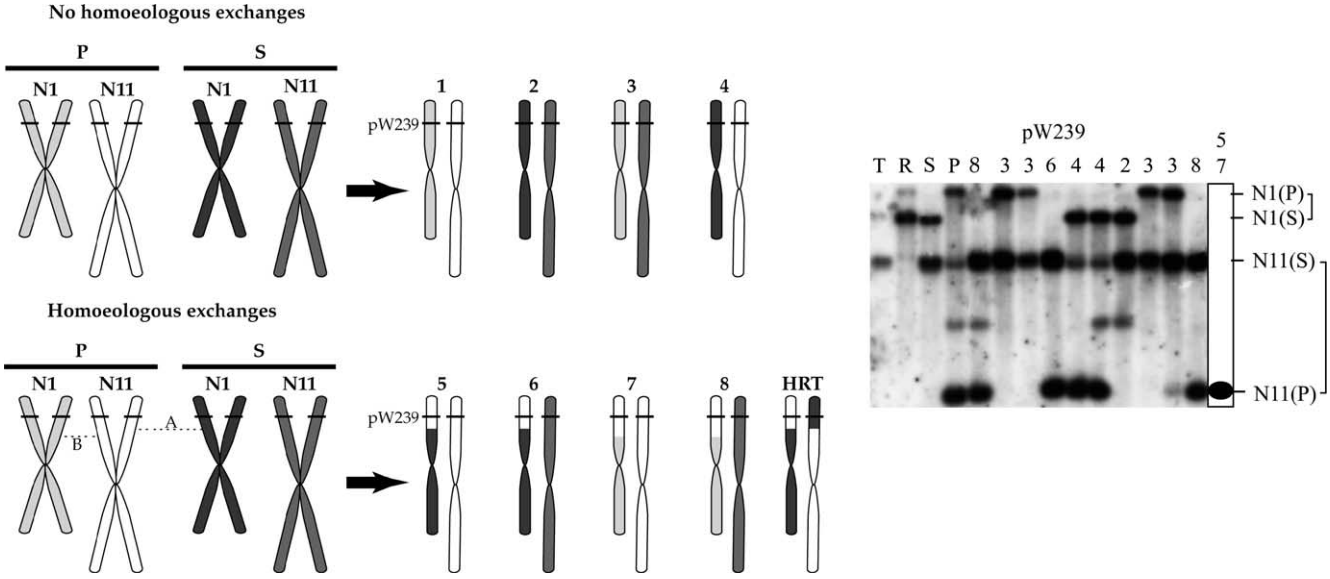


FIGURE 3.—Chromatid model of homeologous exchange between N1 and N11 creating *de novo* HNRTs, and segregation of DH lines for RFLP loci with the exchanged region. The smaller chromosome represents N1 (A genome) and the larger chromosome represents N11 (C genome). Chromosomes of the F_1 from a cross of the resynthesized *B. napus* parent (S) and the P1804 *B. napus* parent (P) are shown on the left side of each arrow prior to meiosis. On the right side of each arrow are some of the possible gametic products of meiosis. (Top) Sorting of the chromatids with no homeologous association or exchange. (Bottom) Some of the gametes from two homeologous exchanges (dashed lines A and B). (Right) RFLP loci detected by hybridizing probe pW239 to blots containing diploid parents of S (T, TO1000, *B. oleracea*; R, Reward, *B. rapa*), parents S and P, and several DH lines derived from the (S × P) F_1 (numbers above the lanes correspond to gametes shown at the left). This probe identified homeologous loci on N1 and N11 at positions shown by the P and S allele designations. Three DH lines inherited N11 alleles from both parents and are missing both alleles of N1 (lanes labeled 8, 6, 8). These DH lines are intragenomic heterozygotes and were derived from two distinct homeologous exchanges (dashed lines A and B, respectively) as determined by linked marker genotypes. Gametes 5 and 7 are intragenomic homozygotes and could also be derived from these exchange events, but were not found with this probe (the last lane was drawn to indicate how these genotypes would appear). HRT represents a gamete containing both products of a homeologous exchange (homeologous reciprocal transposition) and could not be distinguished from gamete type 4, which had no homeologous exchange.

est frequencies of *de novo* HNRTs involved exchanges between N1 and N11, perhaps due to a greater degree of homology between these homeologous linkage groups. There was also a higher frequency of C-genome duplications than of A-genome duplications among the *de novo* HNRTs of the SYN population, especially for linkage groups N11 *vs.* N1, N18 *vs.* N8, and N19 *vs.* bottom of N9 (Figure 2).

Preexisting homeologous nonreciprocal transpositions: Molecular marker data suggested that each of the natural *B. napus* parents had preexisting HNRTs that segregated among the DH lines: MF216 had two HNRTs, one composed of N11 containing a transposed homeologous section of N1 [N11.N1(T)] and one composed of N13.N3(T); RV289 had two composed of N12.N2(T) and N13.N3(T); RV128 had one composed of N13.N3(T); and P1804 had four composed of N11.N1(T), N12.N2(T), N15.N5(T), and N10.N19(T). These rearrangements were more difficult to identify than *de novo* HNRT. The clearest evidence for preexisting HNRTs was the presence of RFLP alleles with identical fragment sizes at homeologous loci within the parental genotype (*i.e.*, four doses of the same allele). In some cases, the identical-size fragments matched fragments from either a *B. rapa* or a *B. oleracea* genotype included on the screening blot, suggesting that they originated from the same diploid genome; however, one allele mapped to an A-genome homolog, and the other mapped to a C-genome homolog. For example, pW126 and pW247 each detected two loci, one on N5 and one on N15, which had alleles with identical frag-

FIGURE 2.—Collinearity among homeologous linkage groups in the *B. napus* consensus map. Linkage groups are numbered N1–N10 (A genome) and N11–N19 (C genome). Lines connect RFLP loci detected with the same probe and indicate intergenomic homeologous relationships. Loci duplicated within the A and C genomes have not been connected by lines to emphasize homeologous relationships of the A and C genomes. Vertical lines displayed parallel to the linkage groups indicate specific HNRTs between homeologous linkage groups. Not all *de novo* HNRTs drawn extend to the end of the linkage group because the DH populations in which they were found were not polymorphic for those distal loci. The vertical lines are placed adjacent to the duplicated homeolog. The possible breakpoints of the reciprocal translocation on N7 and N16 are indicated by shaded boxes. The names and positions of loci described in other figures are shown.

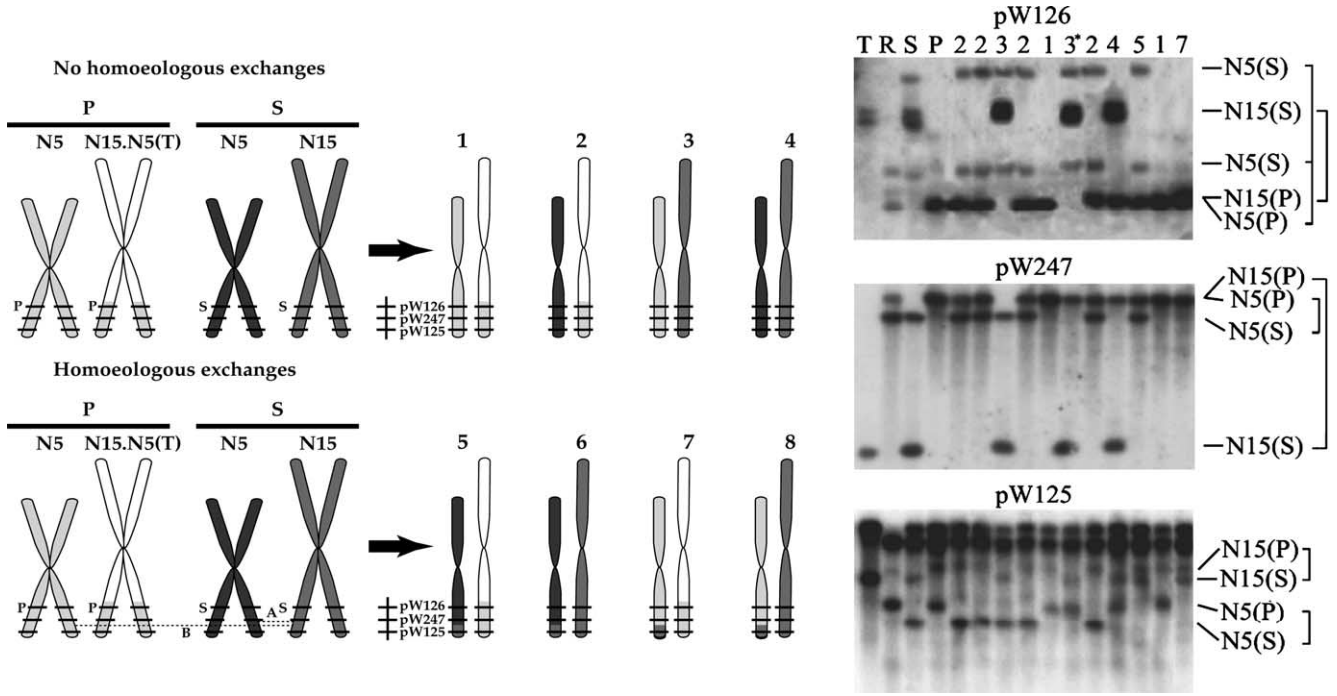


FIGURE 4.—Chromatid model of a region containing a preexisting HNRT between N5 and N15 and segregation of DH lines for RFLP loci in this region. The smaller chromosome represents N5 (A genome) and the larger chromosome represents N15 (C genome). Chromosomes of the F_1 from a cross of the resynthesized *B. napus* parent (S) and the P1804 *B. napus* parent (P) are shown on the left of each arrow prior to meiosis. The bottom region of the N15 chromosome of P contains a transposed segment from N15 [N5.N15(T)]. On the right of each arrow are some of the possible gametic products of meiosis. (Top) Sorting of the chromatids with no homeologous exchange. (Bottom) Some of the gametes after two distinct homeologous exchanges that result in *de novo* HNRTs (dashed lines A and B, resulting in gametes 5/6 and 7/8, respectively). Only gametes with exchanges involving N5 are pictured. (Right) RFLP loci detected by hybridizing three probes (pW126, pW127, and pW125) to identical blots containing DNA from diploid parents of S (T, TO1000, *B. oleracea*; R, Reward, *B. rapa*), parents S and P, and several DH lines derived from the (S × P) F_1 (numbers above the lanes correspond to gametes shown at the left). For all three probes, the fragment sizes of the P alleles are similar to those of the R alleles. For probes pW126 and pW247, P has a single band containing identical alleles at two homeologous loci, indicating a preexisting HNRT [N15.N5(T)]. The asterisk denotes a line with homologous recombination on N5.

ment sizes in P1804, and these matched the fragment sizes of alleles in *B. rapa* cv. Reward (A genome); however, one P1804 allele mapped to N5 (A genome), and the other mapped to N15 (C genome; Figure 4). Thus, P1804 appears to have a preexisting HNRT involving a segment of N5 transposed onto N15 [N15.N5(T)]. Preexisting HNRTs were difficult to delimit because homeologous loci having identical-size alleles were interspersed with those having polymorphism at only one locus or those having different-size alleles at both homologous loci. An example of the later case are loci detected by pW125 on the transposed N5 segment of N15 and the normal N5 chromosome of P1804 (Figure 4). This and other such loci may delimit the transposed segment, having originated by a subsequent round of homeologous chromosome exchange to reintroduce a smaller homologous segment. However, in the case of pW125 the fragment sizes of both loci in P1804 matched the fragment sizes of two of the *B. rapa* genotypes included on our screening blots (data not shown). Thus, the HNRT on N15 may include pW125; however, its allele could be different from the N5 allele because of outcrossing subsequent to the transposition.

Segregating loci on N1 and N11.N1(T) were particularly difficult to characterize in the MF and HUA maps. For both parents, some probes hybridized to alleles with identical fragment sizes on N1 and N11 (pW239, pW172, and pW125 in P1804; pW216 in MF216), and the fragment sizes matched those of *B. rapa* genotypes, further suggesting that N11 contained a segment of N1 in both parents. The preexisting HNRT segments of MF216 and P1804 may have had different origins since loci detected by pW239, pW172, and pW125 on N11.N1(T) were polymorphic between MF216 and P1804. Differences in the sizes or arrangement of these two transposed segments may have affected recombination in the region, because N11 of the MF map consisted of a cluster of tightly linked loci that was not detected in the other three genetic maps. This linkage group probably comprised the transposed N1 segment of N11 and a small portion of N11 that was not polymorphic in the other three maps. Thus, the locus order of N11 in the MF genetic map was distinct enough to exclude it during assembly of the consensus map (Figure 1). In the HUA map, we could not account for allelic relationships of several markers on N11. When P1804 alleles of these loci were

each scored as a single "dominant locus, they mapped together at the top of N11, although their linkage distances could not be determined due to segregation of identical-size alleles that were probably on N1. Since these loci also were found in the MF map, they were included in the consensus map to document their presence on linkage group N11, but linkage distances are not reported (Figure 1).

Many of the *de novo* HNRTs occurred in genomic regions for which there was evidence of preexisting HNRTs. This may have been due to pairing and exchange of homologous segments on homeologous chromosomes. The largest number of *de novo* HNRTs were detected on N2 in the HUA population (Figure 2) where there was evidence of preexisting HNRTs in both parents, RV289 and P1804 [N12.N2(T)]. Only one of the F_1 plants contained the N12.N2(T) from RV289, which apparently was heterozygous. All of these *de novo* HNRT lines originated from this F_1, as determined by three probes that detected loci in the transposed region. Thus, the presence of N2 segments on both N12 chromosomes in this F_1 appears to have caused frequent pairing and exchanges with the N2 chromosomes, resulting in *de novo* HNRTs that actually were between homologous N2 segments.

Molecular markers also provided evidence that multivalents had formed during the meioses that resulted in *de novo* HNRTs. Linkage groups of DH lines missing both alleles due to *de novo* HNRTs were inspected for additional recombination events within the same linkage group. Nearly half of the homeologous syndeses resulting in *de novo* HNRTs had additional exchanges with homologous chromosomes. This indicated that the chromosome with the preexisting HNRT had associated with both homologous and homeologous chromosomes in a trivalent or quadrivalent configuration during meiosis. Evidence for multivalent formation was found more frequently in the SYN F_1 than in the F_1's between the natural *B. napus* parents.

Homeologous reciprocal transposition: Fragment sizes and marker segregation patterns provided evidence that a HRT between N7 and N16 was segregating in each of the four populations. Fragment sizes of the resynthesized parent were most useful for identifying the transposed regions because the allelic origins of TO1147 fragments were known (*i.e.*, A or C genome). In the resynthesized parent, loci with a *B. rapa* (Reward; A genome) allele mapped to the N7 linkage group and loci with a *B. oleracea* (TO1000; C genome) allele mapped to the N16 linkage group. In P1804, many of the loci (*e.g.*, pW150cH and pW228aH) on N16 had alleles with fragment sizes that matched those in *B. rapa* genotypes included on the screening blots, suggesting that the common parent, P1804, contained a *B. rapa* segment on N16. However, none of the N7 loci had P1804 alleles with fragment sizes matching those in TO1000. Several probes detecting loci on N7 and N16 (pW150, pW228, pW130, pW208, and pX156) hybridized to identical-size fragments in TO1147 and P1804 (*i.e.*, monomorphic), and yet they segregated in the SYN population. For several of these loci, their genome origin was known (A genome) but they were allelic with fragments on N16 (Figure 1). In all four populations, loci on N16 showed balanced segregation for both alleles, while the N7 loci were nearly fixed for the P1804 allele, probably due to selection for the transgene conferring herbicide resistance. This fortunate coincidence greatly facilitated correct linkage group assignment of N7 and N16 loci within the four DH maps. Segregating "monomorphic" fragments were observed previously using some of the same probes in three other segregating populations of *B. napus* and were attributed to an interstitial homeologous reciprocal transposition between N7 and N16 in the spring-type parent of the crosses (Osborn *et al.* 2003).

DISCUSSION

Genetic linkage maps: A consensus genetic linkage map of *B. napus* was developed on the basis of data from four segregating populations of DH lines using publicly available RFLP probes. The 19 linkage groups found in each of the maps probably correspond to the 19 chromosomes of *B. napus*, but only the *B. oleracea* linkage groups have been associated cytogenetically with chromosomes (Howell *et al.* 2002). The locus order of the consensus map was generally in agreement with previous maps of *B. napus*, which used some of these same probes for RFLP detection (Ferreira *et al.* 1994; Parkin *et al.* 1995; Sharpe *et al.* 1995; Uzunova *et al.* 1995; Howell *et al.* 1996; Fray *et al.* 1997; Kelly *et al.* 1997; Butruille *et al.* 1999).

Use of a resynthesized *B. napus* as a parent in one of the populations allowed us to unambiguously assign each of the linkage groups to the A or C genome in each of the four genetic maps. Our results, in agreement with Parkin *et al.* (1995), suggest that the process of chromosome recombination in natural *B. napus* has remained relatively unchanged compared to the diploids *B. rapa* and *B. oleracea*, even though they have evolved separately since the formation of the allopolyploid. Of the 167 probes used to construct the consensus map, 147 detected more than one locus, revealing a high level of intergenomic (between A and C) duplication and intragenomic (within A and C) duplication, as has been observed in other Brassica genome studies (Kianian and Quiros 1992; Kowalski *et al.* 1994; Lagercrantz and Lydiate 1996; Cheung *et al.* 1997; Lan *et al.* 2000; Babula *et al.* 2003; Lukens *et al.* 2003). Large stretches of collinear marker loci, some including entire linkage groups, were found between the A- and C-genome components of *B. napus*. These stretches were similar to those reported previously (Lydiate *et al.* 1995; Parkin *et al.* 1995; Sharpe *et al.* 1995; Parkin *et al.* 2003) and probably reflect homeologous relationships between the A and C genomes. Similar to Kelly *et al.* (1997) but

unlike Sharpe *et al.* (1995) and Howell *et al.* (1996), we did not detect significant differences in the number of polymorphic loci between the A and C genomes in any of the DH populations.

Chromosomal rearrangements: The majority of DH lines appeared to derive from meiotic events in which recombination occurred only between homologous chromosomes. However, some DH lines appeared to derive from meiotic events that included some exchanges between homeologous chromosomes, resulting in *de novo* HNRTs. These *de novo* HNRTs were identified as duplications and losses of the respective homeologous RFLP loci. While they occurred throughout the genome, chromosome pairs with extensive regions of homeology had the highest frequencies of *de novo* HNRTs (N1–N11, N2–N12, N3–N13). A total of 99 homeologous transpositions were detected in the four DH populations, representing ~0.90% of the total recombination events. Here, and in other studies (Parkin *et al.* 1995; Sharpe *et al.* 1995; Uzunova *et al.* 1995), N1–N11 had the most homeologous exchange events, suggesting that these are the least divergent homeologs in the A and C genomes.

The SYN population had a higher frequency of *de novo* HNRTs than the populations derived from two natural *B. napus* parents. The frequencies observed for the MF and RV populations (0.49% and 0.43%, respectively) are similar to those detected in a natural × natural *B. napus* DH population examined by Sharpe *et al.* (1995). These results suggest that the natural *B. napus* subgenomes have become partially stabilized or "diploidized," resulting in a lower rate of homeologous exchange. It is possible that homeologous recombination occurred in the natural *B. napus* F_1's at a frequency equal to the F_1 involving the SYN parent, but some of the individuals derived from these gametes did not survive or were selected against due to the uncovering of deleterious recessives masked by intergenomic heterozygosity in natural *B. napus*.

The observation of *de novo* HNRTs in DH lines of the mapping populations used in this study suggests that HNRTs also may exist in *B. napus* cultivars. Indeed, we found evidence of nine preexisting HNRTs in the four natural *B. napus* mapping parents used in our study, which were derived from oilseed cultivars. HNRTs were identified previously between N1 and N11 (Sharpe *et al.* 1995; Butruille *et al.* 1999) and between N2 and N12 (Parkin *et al.* 1995) in the parents used for other mapping studies. We also observed preexisting HNRTs between N1 and N11 (in MF216 and P1804) and between N2 and N12 (in P1804 and RV289), and we found evidence for other preexisting HNRTs between homeologous linkage groups N3 and N13 (MF216, RV128 and RV289), N5 and N15 (P1804), and N10 and N19 (P1804). The actual number of preexisting HNRTs may have been underestimated because we had limited numbers of diploid genotypes on our screening blots and some genomic regions were not polymorphic in the natural × natural populations.

Preexisting HNRTs could have led to incorrect assembly of linkage groups. For example, the two loci detected by each of pW126 and pW247 on N5(P) and N15(P) (Figure 4) could have been scored as single dominant loci having 3:1 segregation ratios, and this may have resulted in pseudolinkage of loci on N5 and N15 during map construction. Pseudolinkage was previously described between loci on N1 and N11 due to the presence of an HNRT (Sharpe *et al.* 1995). In this study, pseudolinkages were detected during assembly of three of the DH genetic maps (between loci on N1 and N11 for the MF and SYN populations and between loci on N2 and N12 for the HUA population). These loci, which initially created fused pseudolinkage groups, either were corrected to account for their true allelic relationships or were removed from the data set.

Preexisting HNRTs also could contribute to genetic variation for chromosome pairing behavior among *B. napus* genotypes. Jenczewski *et al.* (2003) recently obtained evidence for genetic control of chromosome pairing in *B. napus* by examining segregation for pairing behavior in haploids produced from F_1 hybrids between natural *B. napus* lines with high and low pairing of haploids. They observed that the parental pairing patterns were inherited in a Mendelian fashion, supporting the presence of a single major gene that affects homeologous chromosomal pairing in haploids. Differences in the amount of pairing between the parental and F_1 haploids indicated that minor genetic factors could segregate in addition to this putative major gene. These minor factors may include preexisting HNRTs that differed between the parents and segregated among the haploids examined by Jenczewski *et al.* (2003).

Many of the *de novo* HNRTs that we detected were in regions containing possible preexisting HNRTs in one of the parents. This observation suggests that chromosome pairing may frequently initiate between preexisting HNRTs and their original homologs and then extend from the paired homologous segments into homeologous regions of the two chromatids where recombination could occur. For example, the homology at the top of N2 and N12.N2(T) in the HUA population may have mediated the formation of *de novo* HNRTs, which appeared to have extended beyond the length of the preexisting HNRT (Figure 2). Thus, HNRTs that initially involved only small segments may have been extended farther into the homeologous linkage groups via subsequent rounds of meiosis. By bringing other homeologous chromatids into closer proximity during pachytene, such pairings could also facilitate exchanges between completely homeologous chromatids, creating additional *de novo* NRTs.

Our observation of frequent homeologous exchanges in some regions of the *B. napus* genome raises the ques-

tion of whether this species should be classified as a segmental allopolyploid. Stebbins (1950) defined a segmental allopolyploid as "a polyploid containing two pairs of genomes which possess in common a considerable number of homologous chromosomal segments or even whole chromosomes, but differ from each other in respect to a sufficiently large number of genes or chromosome segments, so that the different genomes produce sterility when present together at the diploid level." Previous observations of sterility and of chromosome pairing in natural and resynthesized amphihaploids of *B. napus* (Attia and Röbbelen 1986a) are consistent with this definition, as are the observations reported here and elsewhere (Parkin *et al.* 1995; Sharpe *et al.* 1995) of frequent *de novo* HNRTs in some genomic regions. However, the frequency of *de novo* HNRTs is low enough to indicate that homologous chromosome pairing is greatly preferred over homeologous chromosome pairing, and thus, these homeologous associations create only slight deviations from disomic inheritance. We observed a higher frequency of *de novo* HNRTs when one parent had a preexisting HNRT, indicating that this condition leads to more frequent association of homeologs, and thus, greater deviation from disomic inheritance. In the HUA population, both parents had a preexisting HNRT on N12 (N12.N2), and we observed segregation approaching tetrasomic inheritance for N2 and N12.N2. This type of inheritance also was observed for these chromosomes in a different mapping population where one parent had preexisting HNRTs (Parkin *et al.* 1995). However, we did not observe genotypic classes having alleles only from the two parental N2 chromosomes or from the two parental N12.N2 chromosomes, which would be expected if these chromosomes exhibited complete tetrasomic inheritance. This may be due to the presence of different gene deficiencies on each homeolog that are severe enough to prevent survival when they are exposed in these genotypes.

Thus, our results indicate that some *B. napus* genotypes have become more homologous for certain chromosomal regions due to previous homeologous exchanges, and that progeny from matings between genotypes that have the same homogenized regions segregate in a fashion more typical of autopolyploids (polysomic inheritance) than of allopolyploids (disomic inheritance) for these regions. It is possible that *B. napus* will eventually become homogeneous for these rearrangements, leading to a well-defined segmental allopolyploid. This has not yet occurred on the basis of the polymorphism we observed for rearrangements among *B. napus* genotypes, and it may not occur if there is a selective advantage to having more intergenome heterozygosity (see below). It is also possible that some pairs of homeologous chromosomes may become more homogenized due to the cumulative effects of homeologous and homologous exchanges that alternate over many generations and create a mosaic of chromatin from both progenitor genomes. Evidence that this may occur comes from our observation that not all loci in HNRTs contained alleles with sizes identical to those in homeologous loci (*e.g.*, pW125 on N5 and N15) and from our previous analysis of the N7 and N16 HRT, which exists as an interstitial homeologous exchange (Osborn *et al.* 2003). Because these rearrangements between homeologous chromosomes may be constantly evolving and difficult to delineate, we have chosen to use the more general term transpositions, which can include small interstitial segments of chromosomes, as opposed to translocations, which generally refer to larger distal segments of chromosomes.

Effects of chromosomal rearrangements on allelic and phenotypic diversity: The homeologous chromosomal rearrangements detected in this study did not have large, obvious effects on phenotypes. Some HNRTs preexisted in cultivars that had been previously selected for seed yield and other desirable traits, and the DH lines with *de novo* HNRTs had no obvious defects. However, the genome homogenization caused by HNRTs could have subtle quantitative effects on phenotypes. This might occur through the effects of dosage-regulated genes that have additive effects between homeologs and convert to all one or the other homeolog in HNRT genotypes. Pires *et al.* (2004) reported the effects of such an exchange between N3 and N13 on variation for flowering time in resynthesized *B. napus*. Genome homogenization caused by HNRTs also affects the level of intergenome heterozygosity, and thus it could affect traits that show heterosis and respond to changes in heterozygosity, such as seed yield. Osborn *et al.* (2003) previously analyzed three populations derived from N7–N16 HRT heterozygotes and showed that the two nonparental genotypic classes (equivalent to HNRTs) had significantly lower seed yields than the parental genotypic classes in replicated field trials. Presumably, these nonparental genotypic classes had lost intergenomic heterozygosity due to chromosome assortment and contained four doses of a single allele (intragenomic homozygote) in the transposed segment. In our study, segregation distortion was observed in regions segregating for HNRTs, and in each case there was an excess of genotypes without the HNRT (*i.e.*, those having more intergenome heterozygosity). The overall importance of genomic heterozygosity also was suggested by the bias toward intragenomic heterozygotes (*i.e.*, two doses of two alleles) *vs.* intragenomic homozygotes found within *de novo* HNRTs throughout the genome, implying that intragenomic homozygotes may have had a lower fitness. In addition, a bias toward C-genome duplication in *de novo* HNRTs was also found in the SYN population. This may have been due to a higher frequency of deleterious recessive alleles derived from the open-pollinated *B. rapa* parent Reward than from the highly inbred *B. oleracea* parent TO1000.

Homeologous chromosomal transpositions could have a large impact on the genetic improvement of oilseed *B. napus*. If elite alleles (*e.g.*, those controlling low erucic acid, low glucosinolates, or transgenes) are linked to or located in a transposed segment, selection for the elite allele would maintain the rearrangement and its corresponding effects (both favorable and unfavorable) in the selected lines. Such elite allele configurations may be widespread in germplasm used for cultivar development. For example, the restorer of fertility gene utilized in this study is located on an N7 chromosome containing a segment transposed from N16. Homeologous chromosomal rearrangements also could alter quantitative traits of interest through the effects of genome homogenization, as described above. Evidence for these effects could be obtained by mapping QTL in populations segregating for rearrangements. It remains to be seen to what extent the phenotypic variation in *B. napus* is due to effects of chromosomal rearrangements derived from homeologous recombination.

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