Detection and Effects of a Homeologous Reciprocal Transposition in *Brassica napus*

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ABSTRACT

A reciprocal chromosomal transposition was identified in several annual oilseed *Brassica napus* genotypes used as parents in crosses to biennial genotypes for genetic mapping studies. The transposition involved an exchange of interstitial homeologous regions on linkage groups N7 and N16, and its detection was made possible by the use of segregating populations of doubled haploid lines and codominant RFLP markers. RFLP probes detected pairs of homeologous loci on N7 and N16 for which the annual and biennial parents had identical alleles in regions expected to be homeologous. The existence of an interstitial reciprocal transposition was confirmed by cytological analysis of synaptonemal complexes of annual × biennial F_1 hybrids. Although it included approximately one-third of the physical length of the N7 and N16 chromosomes, few recombination events within the region were recovered in the progenies of the hybrids. Significantly higher seed yields were associated with the parental configurations of the rearrangement in segregating progenies. These progenies contained complete complements of homeologous chromosomes from the diploid progenitors of *B. napus*, and thus their higher seed yields provide evidence for the selective advantage of allopolyploidy through the fixation of intergenomic heterozygosity.

MOLECULAR markers are widely used for developing linkage maps and studying quantitative trait loci (Dudley 1993; Tanksley 1993; Lee 1995). Disomic chromosome behavior and adherence to Mendel's laws are generally assumed in these studies. Polysomic polyploids are a common exception among cultivated plants, and they present a challenge for building genetic linkage maps and analyzing quantitative traits (Wu *et al.* 1992). Many polyploids, however, have disomic chromosome behavior. Most of these probably arose as allopolyploids from hybridization of related species, and they contain two or more sets of homeologous chromosomes from the parent species. Differences between chromosomes from the parent species, along with the possibility of genetic control of chromosome pairing behavior (*e.g.*, *PH1* in hexaploid wheat; Riley and Chapman 1958), generally prevent pairing between homeologs and enforce disomic inheritance. However, occasional exceptions to strict homologous chromosome pairing can occur even in stable allopolyploids and sometimes homeologous chromosomes can pair and exchange.

Researchers often construct linkage maps using progenies derived from crosses of distantly related genotypes to increase the frequency of polymorphic loci or to introgress alleles from divergent germ plasm. For such parents, there is also an increased likelihood of fixation of nondeleterious chromosomal rearrangements, especially in self-pollinating allopolyploids, where exchanges between homeologous chromosomes may have occurred and become fixed in certain lineages. Evidence for chromosomal rearrangements has been found by comparing linkage maps of related species (for examples, see Nelson *et al.* 1995; Lagercrantz and Lydiate 1996; Brubaker *et al.* 1999; Livingstone *et al.* 1999) and by cytological examination of F_1 hybrids from wide crosses within several species (wheat, Riley *et al.* 1967; cotton, Stelly *et al.* 1990; oats, Morikawa and Leggett 1996). However, there is little evidence of chromosomal rearrangements between genotypes on the basis of aberrant segregation of molecular markers in mapping populations (but see Sharpe *et al.* 1995; Cloutier *et al.* 1997; Livingstone *et al.* 1999). This may be due, in part, to the difficulty of recognizing chromosomal rearrangements as the cause for aberrant segregation under the assumption of disomic inheritance. The use of dominant, mono-allelic markers, such as randomly amplified polymorphic DNAs (RAPDs; Williams *et al.* 1990) or amplified fragment length polymorphisms (AFLPs; Vos *et al.* 1995), also can make chromosomal rearrangements difficult to recognize because the alleles at homologous and homeologous loci are not detected.

Several linkage maps have been developed for the

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oilseed crop *Brassica napus* ($n = 19$), an allopolyploid that arose by hybridization of *B. rapa* ($n = 10$) and *B. oleracea* ($n = 9$; U 1935; Song *et al.* 1993; Parkin *et al.* 1995). Many of these maps rely on populations derived from wide crosses, especially crosses between European annual or biennial cultivars and Canadian annual cultivars. Some cases of unexpected marker segregation have been observed in these populations and attributed to chromosomal translocations (Sharpe *et al.* 1995; Cloutier *et al.* 1997). Sharpe *et al.* (1995) noted that they observed evidence for a reciprocal translocation between linkage groups N7 and N16 in one parent of their mapping population. Using a different population, Thormann (1995) reported a linkage group (LG6) having a pair of inverted duplicated markers. Once the two research groups had used common markers to build a consensus map (Osborn *et al.* 1997; Butruille *et al.* 1999; our unpublished data), it became clear that LG6 was actually two linkage groups corresponding to N7 and N16 and that both populations had a reciprocal transposition segregating in them.

In this article, we present a more thorough description of the N7-N16 reciprocal transposition and characterize its effects on genetic recombination and seed yield in progenies segregating for the rearrangement. We provide evidence that the rearrangement involves interstitial homeologous regions of N7 and N16, that it may be widespread in annual *B. napus* germ plasm, and that it could be an important determinant of seed yield in breeding programs using crosses among divergent cultivars. The effects of the segregating rearrangement on seed yield also provide evidence for the selective advantage of allopolyploidy through the fixation of intergenomic heterozygosity.

MATERIALS AND METHODS

Plant materials and RFLP analyses: Six segregating populations of oilseed *B. napus* were used for this study. Three were populations of doubled haploid (DH) lines, one derived from an F_1 of Major (a French biennial rapeseed cultivar) × a DH line derived from Stellar (a Canadian annual canola cultivar; Ferreira *et al.* 1994) and the other two derived from a pair of reciprocal F_1's (N72-8 and N69-8) of N-o-1 (a DH line derived from the Canadian annual canola cultivar Westar) × N-o-9 (a DH line derived from a British biennial breeding line; Sharpe *et al.* 1995). Two were populations composed of inbred backcross (IB) lines derived by backcrossing Ceres (a German biennial canola cultivar) as a donor to Westar or Marnoo (an Australian annual canola cultivar) as recurrent parents (Butruille *et al.* 1999). The sixth was a segregating population of DH lines derived from F_1 plant N61-13 from the resynthesized *B. napus* × N-o-9 population described by Parkin (1995).

The RFLP data for loci on N7 and N16 were collected as described previously (Major × Stellar, Ferreira *et al.* 1994; Thormann *et al.* 1996; N-o-1 × N-o-9, Sharpe *et al.* 1995; IB populations, Butruille *et al.* 1999; N61-13, Parkin *et al.* 1995). The probes used in this study were described by Ferreira *et al.* (1994), Sharpe *et al.* (1995), and Thormann *et al.* (1996). Some identical probes have different nomenclatures: TG5D9 = pW104, WG2A3 = pW127, WG5A1 = pW134, and WG7F5 = pW208. Fragment data were scored as described in the results. To determine if an RFLP fragment in *B. napus* was located on a *B. rapa* or *B. oleracea* homolog, we compared the size of the fragment to those detected on a screening blot containing DNAs from several *B. rapa* and *B. oleracea* accessions. The presence of an identical size fragment in one or more accessions of a progenitor species and absence in another species was taken as evidence of common ancestry. Also, the diploid origin of each chromosome in the N-o-9 parent had been unambiguously determined by marker analysis of a population derived from the resynthesized *B. napus* × N-o-9 (Parkin 1995; Parkin *et al.* 1995).

Cytology: Synaptonemal complexes were prepared from anthers of the N-o-1 and N-o-9 parental plants and the F_1 hybrids at the prophase I stage of meiosis using the protocol described in Hall *et al.* (1997).

Seed yield evaluations: Ninety-eight of the Major × Stellar DH lines and seven checks were evaluated for seed yield near Marshallville, Georgia, by Mycogen Plant Sciences. The lines were planted in October 1992 in 1.5-m-long plots containing six rows spaced 30 cm apart and plots spaced 1.5 m apart. The experimental design consisted of a balanced group design with four replications (blocks). The lines were separated into three groups of 35 entries according to their flowering times and planted in these groups to facilitate harvest. The entries were swathed at maturity and combined, and the yield (in kilograms/hectare) estimated from the harvested seed. The mean seed yield of each DH line was calculated and used to determine the effect of the segregating rearrangement on seed yield.

The two populations of IB lines were evaluated for seed yield as lines *per se* and as hybrids to Topas in two spring-planted, replicated trials near Madison, Wisconsin, in 1996 and 1997. The details of those trials are described in Butruille *et al.* (1999). We calculated least-square means of IB lines and their hybrids to determine the effect of the segregating rearrangement on seed yield.

RESULTS

Evidence for a homeologous reciprocal transposition based on marker segregation: Five populations used for mapping molecular markers provided evidence for segregation of a reciprocal transposition between homeologous regions of linkage groups N7 and N16. The clearest evidence came from three segregating populations of DH lines derived from an F_1 plant of Major (European biennial) × Stellar DH (Canadian annual; Ferreira *et al.* 1994) and from reciprocal F_1's of N-o-9 (European biennial) × N-o-1 (Canadian annual; Sharpe *et al.* 1995). These populations were derived from highly homozygous parents and they contain lines that were completely homozygous due to chromosome doubling of haploids. Most probes detected segregating loci for which the two parents had polymorphic allelic fragments and the progenies had either one or the other fragment. However, several probes detected segregating loci for which the two parents had identical size fragments (monomorphic). In these cases, the monomorphic fragment from each parent was allelic to a polymorphic fragment detected by the same probe in the other

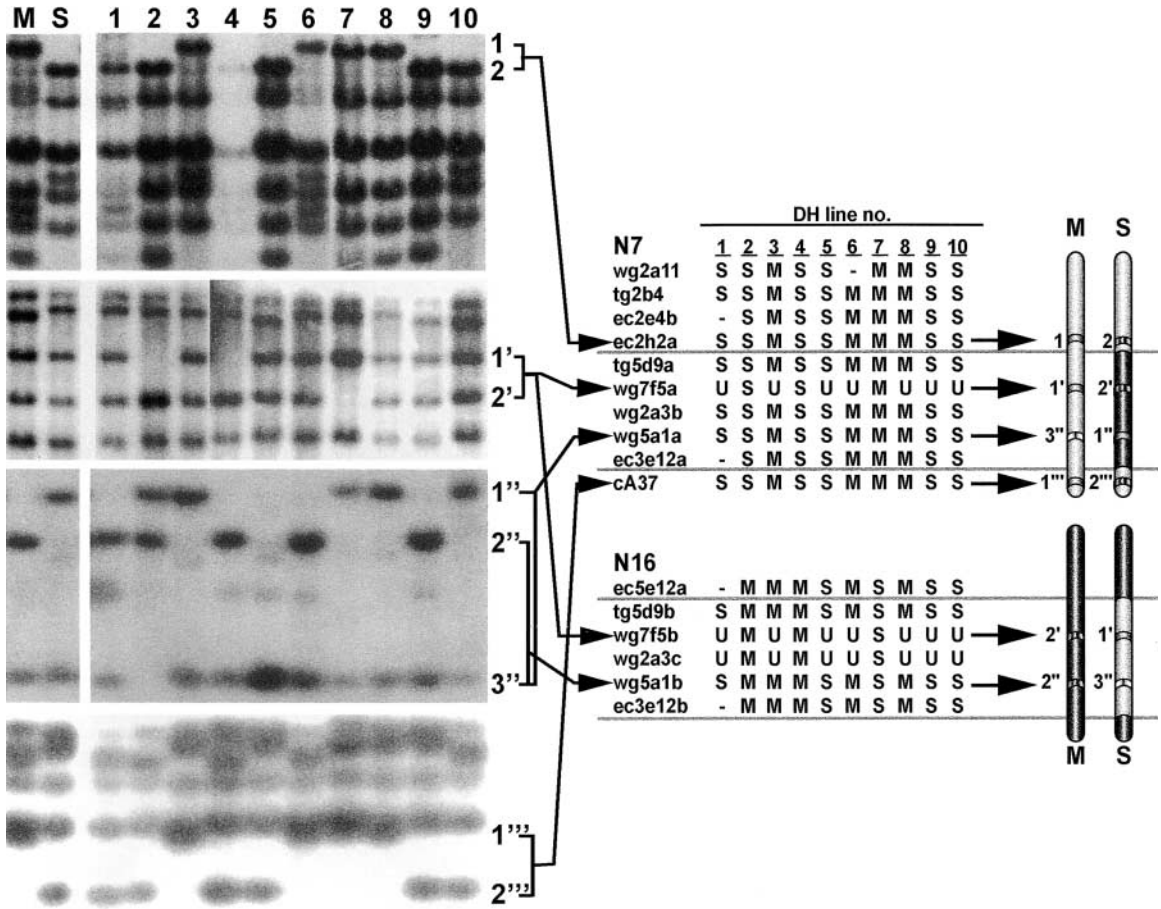

FIGURE 1.—Segregation of RFLP markers on linkage groups N7 and N16 of *B. napus*. RFLP patterns are shown for four probes, EC2H2, WG7F5, WG5A1, and cA37 (top to bottom on the left) hybridized to *Eco*RI- (for WG7F5), *Hin*dIII- (for EC2H2 and WG5A1), or *Dra*I- (for cA37) digested DNAs from the parents, Major (M) and Stellar (S), and 10 F_1-derived DH lines. Numbers assigned to each fragment and their allelic relationships are shown. Scoring of these loci and other linked loci in the DH lines are shown on the right: M, S, U, and — indicate homozygous Major, homozygous Stellar, and unknown and missing data, respectively. For the pair of homeologous loci in which Major and Stellar shared identical alleles (*e.g.*, wg7f5a and wg7f5b), the genotypes of DH lines missing both identical alleles (*e.g.*, DH line nos. 2, 4, and 7) were known to be M at one locus and S at the other and assignments of these genotypes were made to minimize crossovers. The positions of the exchange break points are shown with gray horizontal lines. Loci within the regions of the transposition (*e.g.*, wg7f5a and wg7f5b and wg5a1a and wg5a1b) had identical alleles at homeologous loci, resulting in unusual segregation patterns. Loci above (*e.g.*, ec2h2a on N7) and below (*e.g.*, cA37 on N7) the rearranged regions had normal segregation patterns. Genotypes of the parents for loci detected by these probes are shown on the far right. DH lines 1, 3, 5, 6, 8, 9, and 10 have parental configurations for the transposition; DH lines 2, 4, and 7 have nonparental configurations.

parent. This could be recognized by the absence of the monomorphic fragment when both polymorphic fragments were present and by the presence of two doses of the monomorphic fragment when both polymorphic fragments were absent (see WG5A1 in Figure 1). For one probe, the monomorphic fragments were allelic to a second set of monomorphic fragments, recognized by the presence of two doses of one monomorphic fragment when the other monomorphic fragment was absent (WG7F5 in Figure 1). The unusual loci detected by these probes were linked on homeologous regions of N7 and N16, with the monomorphic fragment at the locus from one parent mapping to N7 and that from the other parent mapping to N16 (Figure 1).

A reciprocal transposition in one of the two parents is the most likely explanation for the presence of identical alleles from each parent in regions that are expected to be homeologous (Figure 2). N7 derives from *B. rapa* and N16 from *B. oleracea* (PARKIN *et al.* 1995), and this expectation was confirmed for the entire N7 and N16 linkage groups in the biennial parents by matching fragment sizes of alleles to those in the diploid germ plasm (data not shown). In the annual parents, N7 and N16 loci outside the exchange region had normal segregation patterns (*e.g.*, ec2h2a and cA37 in Figure 1) and alleles with fragment sizes that matched their expected origins; however, loci within the exchange region had unusual segregation patterns (*e.g.*, wg7f5a and wg5a1a in Figure 1) and alleles with fragment sizes that matched the reverse of their expected origins (data not shown).

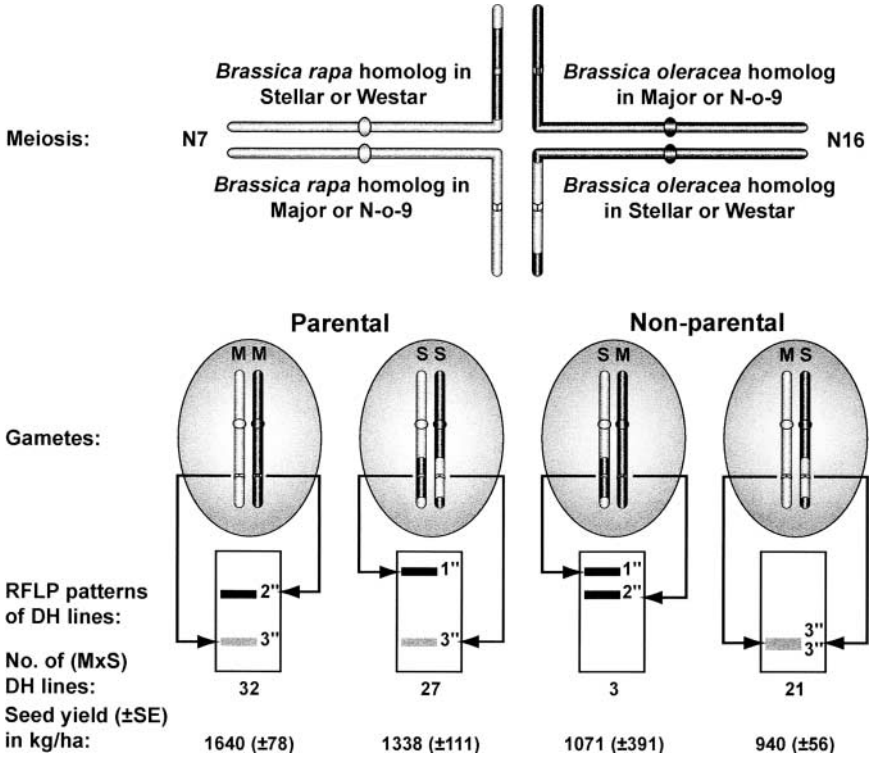

FIGURE 2.—Configuration of chromosomes corresponding to *B. napus* linkage groups N7 and N16 in meiosis of F_1 plants heterozygous for an interstitial reciprocal transposition and the resulting gametes, RFLP patterns, and seed yields of DH lines derived from these meioses. The species origin of the chromosomes or chromosome segments (dark shading for *B. oleracea* and light shading for *B. rapa*) and the cultivars used as parents having these chromosome constitutions are shown. Gametes resulting from these meioses contain parental or nonparental configurations for the rearrangement, resulting from alternate and adjacent-1 segregation, respectively. The RFLP patterns of DH lines derived from these gametes are shown for two homeologous loci and correspond to the patterns observed for probe WG5A1 in Figure 1. The number of DH lines derived from the (Major × Stellar) F_1 having each of the four configurations and that were evaluated for seed yield are shown along with their mean seed yields and standard errors (SE).

Thus, the reciprocal transposition appears to have occurred in the lineages of the Canadian annual parents.

The meiotic configuration of the F_1 between these parents, and the resulting gametes, can be visualized like those of a translocation heterozygote (Figure 2). The DH lines derived from these F_1's represent gamete genotypes. Ignoring recombination, these lines could be placed into four genotypic categories: two having parental configurations for the rearranged region (*B. rapa* for N7 and *B. oleracea* for N16 or the reverse), resulting from alternate segregation, and two having nonparental configurations (*B. rapa* for both N7 and N16 or *B. oleracea* for both N7 and N16), resulting from adjacent-1 segregation (Figure 2). We did not observe DH genotypes resulting from adjacent-2 segregation, which would have contained either two N7 homologs or two N16 homologs for nonexchanged portions of these chromosomes. In translocation heterozygotes of maize, adjacent-2 segregation produces nonviable gametes and can be detected by a high frequency of nonstaining pollen (BURNHAM 1962). In our study, all parents and F_1 hybrids tested (Stellar, Westar, Major, Ceres, Major × Stellar, and Westar × Ceres) had >95% stainable pollen.

Cytological evidence for a reciprocal transposition: Synaptonemal complexes were made of the pachytene stage of prophase I from meiotic cells of anthers from N-o-9 × N-o-1 F_1 hybrids. The six spreads with the most well-separated chromosomes were evaluated and showed the same characteristic quadrivalent structure during meiosis (Figure 3a). Equivalent spreads of the two parent genotypes did not exhibit a quadrivalent as in the F_1 hybrids.

The quadrivalent structure reveals two synapsis exchange points (Figure 3a). One of these points (top arrow in Figure 3a) probably corresponds to the beginning of the exchanged regions on N7 and N16 that contained RFLP loci with unusual segregation patterns (Figures 1 and 2). The bottom arrow probably corresponds to a synapsis exchange between homeologs near the ends of the chromosomes, perhaps due to a second exchange event that resulted in the terminal segments having their original centromere associations. A normal segregation pattern was observed for an RFLP locus at the end of N7 in the Major × Stellar population (cA37 in Figure 1) and at the end of both N7 and N16 (pO3 and pO10 in Figure 3) in the N72-8 population (SHARPE 1997), supporting the hypothesis that the reciprocal transposition involved only interstitial segments of the chromosomes. Equivalent synapsis exchanges also were present in the quadrivalents observed in the other five spreads from F_1 nuclei. Analysis of the synaptonemal complexes from the F_1 hybrids allowed the physical length of the exchanged region to be estimated at approximately one-third of the total length of the N7 and N16 chromosomes.

Effect of the reciprocal transposition on recombination frequency: The effect of the reciprocal transposition on recombination frequency was evaluated by comparing the genetic distances calculated for linkage group N16 in populations affected and unaffected by the rearrangement (Figure 3b). This demonstrated that the reciprocal transposition has a negative effect on recombination frequency in the affected N72-8 population (SHARPE *et al.* 1995) when compared to the unaffected N61-13 population (PARKIN 1995).

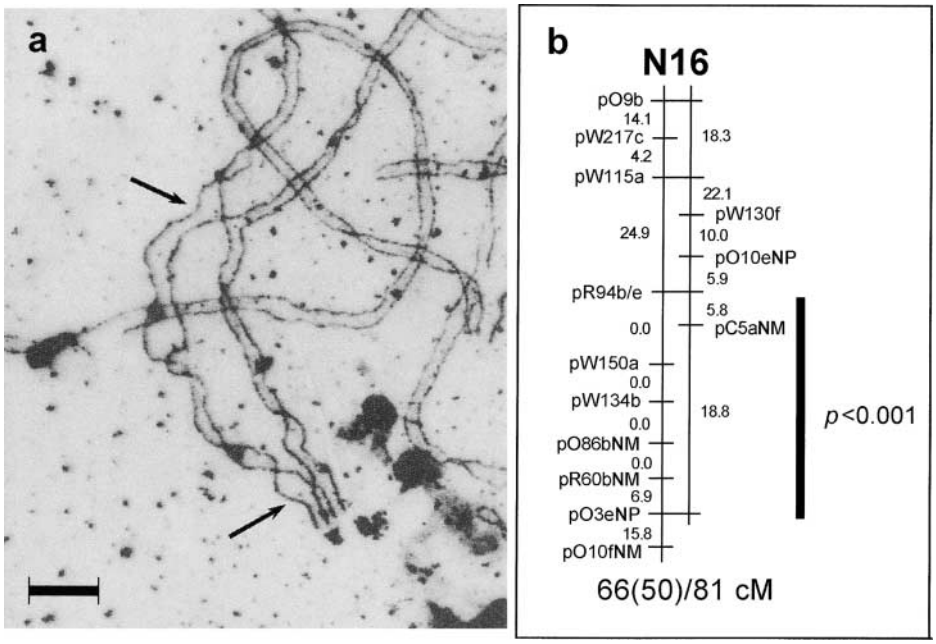

FIGURE 3.—Cytological and genetic mapping evidence for the N7-N16 reciprocal transposition. (a) Meiotic chromosome behavior in the F_1 hybrid N-o-9 × N-o-1. Part of a synaptonemal complex spread showing quadrivalent formation between two chromosome pairs. Two synapsis exchange points are indicated by arrows. Bar, 2 μm. (b) Integrated genetic linkage data for linkage group N16 based on segregation data in the N72-8 (SHARPE *et al.* 1995) and N61-13 (PARKIN 1995) populations of DH lines. Vertical lines represent the N16 linkage groups and locus codes as described in SHARPE *et al.* (1995). Loci common to both populations are represented by horizontal lines across both vertical lines. Map distances in centimorgans (cM) on the left and right side are those calculated for the N72-8 and N61-13 populations, respectively. Total map lengths are represented at the bottom of the linkage groups, with the length of the region where the two linkage groups can be compared in parentheses. All loci separated by recombination in either population are represented with even spacing to facilitate the comparison of recombination frequencies. The solid bar indicates the intervals on the linkage groups where the recombination frequencies differed to a degree expected to occur by chance with $P < 0.001$.

Effects of the reciprocal transposition on seed yield: The configuration of the N7-N16 rearrangement had a large and highly significant effect on seed yield of DH lines derived from Major × Stellar ($P < 0.00001$ for the effect due to N7-N16 configuration from analysis of variance). Lines with the parental configurations had the highest yields and lines with the nonparental configurations had significantly lower yields (Figure 2). Lines having recombination within the rearranged region were not included in the analysis. Of the two parental configurations, lines with the nonrearranged form of N7-N16 (Major type) had significantly higher yield than those with the rearranged form (Stellar type). Seed yields of lines having the two nonparental configurations did not differ significantly from each other. Only three lines having the nonparental configuration with *B. oleracea* segments in the rearranged region produced enough seed to be evaluated for yield in the field. Three additional lines with this configuration did not produce enough seed to be evaluated in the field. Inclusion of these lines might have further reduced the mean seed yield of this category.

Effects of the N7-N16 reciprocal transposition on seed yield also were evaluated for two populations of IB lines derived by crossing Ceres (European biennial, no rearrangement) as a donor to Westar and Marnoo (Canadian annual and Australian annual, respectively, both carrying the rearrangement) as recurrent parents. These lines were categorized for the number of *B. rapa* homologs using data for the five pairs of homeologous RFLP loci in the rearranged region (Figure 1). They were not categorized on the basis of which allele they contained (Ceres *vs.* Westar or Marnoo). Thus, the two parental configurations were combined into one group that contained two *B. rapa* homologs. The nonparental types had zero, one, three, or four copies of *B. rapa* homologs.

For each population (Westar as recurrent parent or Marnoo as recurrent parent), the configuration of the N7-N16 rearrangement had a large and highly significant effect on seed yield of the IB lines *per se* ($P < 0.001$). The effect was similar to that observed for the Major × Stellar DH lines: IB lines with the parental configurations (two *B. rapa* homologs) had the highest seed yield and lines with nonparental configurations (zero, one, three, or four *B. rapa* homologs) had reduced seed yields (Figure 4a). The configuration of the rearrangement also had a significant effect on seed yield of hybrids produced by crossing each IB line to Topas as a tester ($P < 0.05$). The effect was similar to that observed for the lines *per se*: hybrids derived from lines with the parental configurations had higher seed yields than those derived from lines with nonparental configurations, although the magnitude of this effect was much smaller than that for the IB lines *per se* (Figure 4b).

DISCUSSION

A reciprocal transposition involving interstitial homeologous segments of linkage groups N7 and N16 of *B. napus* was identified on the basis of the presence of identical alleles at two homeologous sets of linked RFLP loci in segregating populations of DH lines, and the presence of a reciprocal transposition was verified in

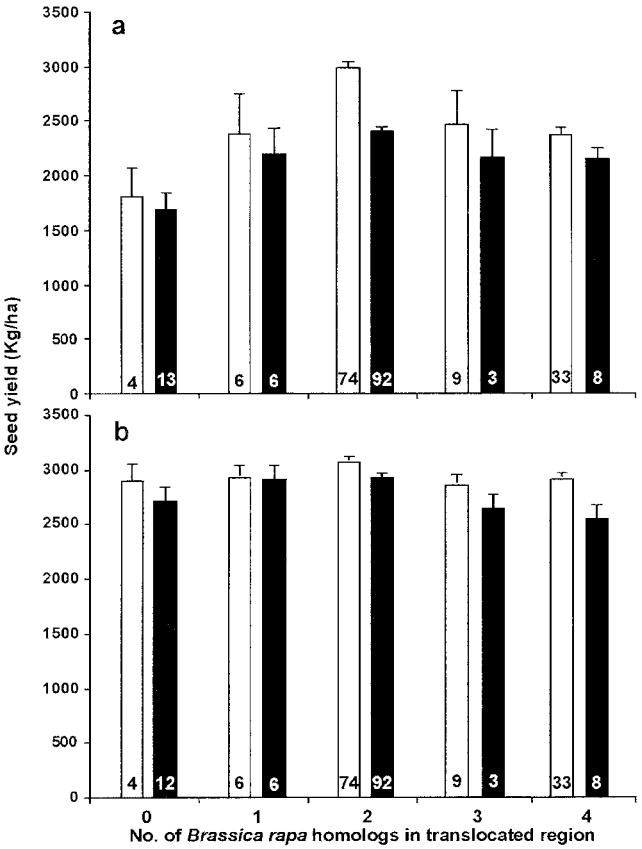

FIGURE 4.—Mean seed yields of IB lines *per se* (a) and in hybrid combination with Topas (b) divided into five categories for the N7-N16 rearrangement. IB lines from two populations with Marnoo or Westar as recurrent parents are shown by open and solid bars, respectively. The number of IB lines in each category is shown at the base of the bars. Standard errors for each category are indicated at the tops of the bars.

meiosis of the F_1 genotype for one of these populations. Four types of progenies were observed from F_1's that were heterozygous for the transposition: two representing the parental configurations resulting from alternate segregation and two representing the nonparental configurations resulting from adjacent-1 segregation. The nonparental progeny class with two copies of the *B. oleracea* region was underrepresented in the Major × Stellar population (Figure 2). Self-incompatibility loci have been mapped in *B. rapa* and *B. oleracea* to homologs of N7 (R7) and N16 (O6) in regions where the rearrangement occurs in *B. napus* (CAMARGO *et al.* 1997; EKUERE 1997). It is possible that self-incompatibility alleles at the *B. oleracea* locus are functional in *B. napus*, but normally superceded by the presence of self-compatibility alleles at the *B. rapa* locus. In our study, latent self-incompatibility may have been exposed in the underrepresented nonparental class, and this could have caused inadvertent selection against these genotypes during seed increases.

In diploid organisms, translocations occur between chromosomes that have little or no homeology, and adjacent-1 and adjacent-2 segregation results in nonviable gametes due to chromosomal deficiencies created by these segregation patterns (BURNHAM 1962). In *B. napus*, the N7-N16 reciprocal transposition is between homeologous regions of these chromosomes, and therefore severe gene deficiencies are not created by the loss of only one of the two homeologous regions resulting from adjacent-1 segregation. The products of adjacent-2 segregation also should not have severe gene deficiencies, since the nonrearranged portions of N7 and N16 have homeologous regions on N17 and N6, respectively (PARKIN *et al.* 1995), which would compensate for their loss in adjacent-2 segregation. This is consistent with our observation of >95% stainable pollen from F_1's that were heterozygous for the exchange; however, we did not observe progenies with genotypes that would result from adjacent-2 segregation. It is possible that adjacent-2 segregation occurred and the products developed into stainable pollen, but some minor deficiencies prevented their development into plants during microspore culture. Alternatively, adjacent-2 segregation may not have occurred. This type of segregation requires formation of a ring of four chromosomes in meiosis, and suppression of recombination in the exchanged region may have prevented formation of this meiotic configuration. Observation of these chromosome structures at diakinesis would confirm this; however, the small size of chromosomes N7 and N16 would limit this analysis. Chromosome O6 in *B. oleracea* is equivalent to N16 and has been characterized as one of the smallest chromosomes in the C genome (HOWELL *et al.* 2002).

The most likely reason for the greatly reduced recombination observed in linkage group N16 is chiasma suppression caused by the failure of intimate pairing around exchange break points in heterozygotes (SYBENGA 1975; PARKER *et al.* 1982). Alternatively, an additional chromosomal rearrangement, such as an inversion, could be present in the exchanged region in one of the parents of the N72-8 population. This would not be surprising considering the level of duplication detected within N16 in *B. napus* (PARKIN *et al.* 2003) and the equivalent O6 chromosome in *B. oleracea* (RYDER *et al.* 2001). The physical size of the rearranged region is difficult to estimate accurately from the RFLP and cytological evidence presented here; however, comparative mapping between *B. oleracea* and Arabidopsis using RFLP markers has revealed that this region is likely to be 5–10 Mbp (RYDER *et al.* 2001). The RFLP probes used in this study are being anchored to a *B. napus* bacterial artificial chromosome library, and this should allow for a more accurate estimation of the physical size of the rearrangement in the future.

The detection of this rearrangement through segregation analysis was simplified by the use of doubled haploid lines and RFLP markers. Doubled haploid lines are completely homozygous, whereas F_2 and backcross populations include heterozygotes, which could compli-

cate the assignment of alleles to loci in the rearranged region. RFLP markers simplified identification of the reciprocal transposition because they detect codominant alleles at sets of homeologous loci, unlike less informative AFLP or RAPD markers. A reciprocal transposition would not be easily recognized by scoring DNA fragments as dominant markers (presence or absence of fragments), and this type of scoring could lead to spurious linkage associations. Fragments at single loci within the rearrangement (present in one parent and absent in the other) would be mapped correctly as loci in either one or the other of the chromosomes involved in the rearrangement. Identical size fragments that occur at loci in both the rearranged and nonrearranged homeologs (present in both parents) also would segregate in the progenies, but they would produce a composite segregation pattern of a pair of homeologous loci. This pattern would exhibit linkage to loci in each of the homeologous regions and spuriously associate linkage groups representing separate but homeologous chromosomes. Livingstone *et al.* (2000) used simulated data sets to show how variances in recombination frequencies can be used to identify pseudolinkage caused by reciprocal translocations. However, they did not consider the potential for reciprocal transpositions of homeologous regions and the use of homeologous marker loci to aid in identifying these rearrangements.

Chromosomal rearrangements have been hypothesized as a major cause of reproductive isolation and speciation due to decreased fertility in hybrids of rearranged genotypes (White 1978). However, a recent study in yeast found more translocations between closely related species than between more distantly related species, suggesting that chromosomal rearrangements are not a prerequisite for speciation (Fischer *et al.* 2000). In *B. napus*, the N7-N16 reciprocal transposition appears to have very little effect on fertility of plants heterozygous for the rearrangement. F_1 hybrids between the parents used in this study were completely male and female fertile, and hybrids between all the IB lines and Topas (some of which must have been heterozygous for the transposition) produced very high seed yields, suggesting that they had no serious reduction in fertility.

The origin of the N7-N16 reciprocal transposition is not known, but we observed this rearrangement in several lines that have common parents in their pedigree. Westar and Stellar (both carrying the transposition) are Canadian cultivars derived from selections of Argentine (a cultivar introduced into Canada from Argentina) crossed to the sources of low erucic acid (Liho) and low glucosinolates (Bronowski) used to produce all canola quality cultivars (J. L. Sernyk, personal communication). Our data indicate that Marnoo has the reciprocal transposition and Kelly (1996) identified the transposition in Maluka. Both of these are Australian cultivars and both have Argentine, Liho, and Bronowski in their pedigrees (J. L. Sernyk, personal communication). Thus, the reciprocal transposition could have come from any one of these sources. If it came from Liho or Bronowski, it has not been maintained due to genetic linkage with canola quality factors because genes for low glucosinolates and low erucic acid map elsewhere in the genome (Toroser *et al.* 1995; Thormann *et al.* 1996), and canola quality cultivars without the N7-N16 transposition have been developed (Ceres and N-o-9 used in this study and Topas; see below). All of these potential sources of the N7-N16 reciprocal transposition are annual forms of *B. napus* and they could have been isolated reproductively from biennial forms since before domestication. The European annual germ plasm in general has different parents in its pedigree (except for the sources of canola quality) and the N7-N16 reciprocal transposition may not be widespread in this germ plasm. Indeed, Topas, a European annual cultivar, does not carry this rearrangement (Kelly 1996).

Plant breeders often make crosses between cultivars having different genetic backgrounds to identify new favorable gene combinations. The N7-N16 reciprocal transposition could segregate in some of these crosses and have a large effect on seed yield. Phenotypic selection would probably be effective in avoiding nonparental configurations of the rearrangement because our results show that the seed yields of these are greatly reduced. However, if there are smaller differences in seed yield between the two parental configurations, as we observed for the Major × Stellar progeny, then genotyping of the rearranged region and selection on the basis of molecular markers could be valuable. Markers also would be of value in determining if genes of interest are linked to this region, and if so, for selecting favorable alleles linked to the desired configuration of the rearrangement. A good example is *LEM*, a locus affecting resistance to black leg disease, which maps to N7 near the exchange point of the transposition (LG6 in Ferreira *et al.* 1995).

We do not know the genetic bases for the seed yield differences associated with different configurations of the N7-N16 rearrangement; however, some potential causes seem unlikely. The populations we analyzed were derived from crosses of annual and biennial cultivars, and it is possible that N7 and/or N16 segregated for genes affecting flowering time that also had an effect on seed yield. However, we previously analyzed three of these populations for QTL controlling flowering time and did not find any significant flowering-time effects that cosegregated with the N7-N16 rearrangement (Osborn *et al.* 1997; Butruille *et al.* 1999). It is also possible that some configurations of the rearrangement caused reduced seed yield because of reduced gametic fertility associated with aberrant meioses. For example, plants having two *B. rapa* or two *B. oleracea* homologs in the region of the exchange might have an increased frequency of multivalents in meiosis due to pairing between these homologous regions. However, we did not

observe reduced fertility associated with any of the configurations. Also, one would expect plants that are heterozygous for the exchange to experience the greatest frequency of aberrant meioses and reduction in fertility, but the seed yields of hybrids between IB lines and Topas, which would have included a high frequency of transposition heterozygotes, were the same as or greater than those of the IB lines *per se* (Figure 4, a and b). In a separate study, we found high levels of heterosis for F_1 hybrids, some of which were heterozygous for the N7-N16 transposition (Butruille *et al.* 1999). Apparently, *B. napus* is tolerant of any potential effects that the N7-N16 rearrangement might have on meiotic irregularities.

Our preferred explanation for the effects of different configurations of this rearrangement on seed yield is based on their effects on the level of intergenome heterozygosity. The success of disomic allopolyploids has often been attributed to heterosis associated with fixed heterozygosity in the form of different alleles contributed by each parental genome (Grant 1971; White 1978). This hypothesis is difficult to test and there is little empirical evidence to support it. Garcia *et al.* (1991) provided some support based on the relationship between genetic diversity and adaptedness for allozyme loci in diploid and tetraploid Avena species. Our results also are consistent with this hypothesis. Lines with the parental configurations for the rearrangement have alleles from both *B. rapa* and *B. oleracea* in homeologous regions of N7 and N16, whereas the nonparental configurations have only either *B. rapa* or *B. oleracea* alleles. This reduction in allelic diversity was associated with reduced seed yield.

At least two unresolved issues regarding intergenomic diversity pertain to this hypothesis. One is whether the homeologous gene differences on N7 and N16 are of a similar nature to allelic differences between inbred lines that show F_1 heterosis. If so, plants with the nonparental configuration of the rearrangement would be analogous to partial inbreds and the reduction in seed yield would be due to a reduced heterotic state. It is also possible that more extreme divergence exists between some homeologous genes, perhaps involving the absence or silencing of one homeolog. In this case, the reduced seed yield associated with nonparental configurations might be due to a gene-dosage imbalance that would not be analogous to inbreeding depression.

The second issue deals with the timing of divergence between homeologous genes. Gene differences that existed between diploid progenitors before formation of *B. napus* would have contributed to intergenome heterozygosity and perhaps to an advantageous heterotic effect. Alternatively, some homeologous gene differences may have arisen after polyploid formation. These differences would not have contributed to an initial heterotic effect, but any new variant with deleterious effects would be uncovered in nonparental segregants of the rearrangement and could have depressed seed yield. *B. napus* can be resynthesized from hybridization of *B. oleracea* and *B. rapa* (Song *et al.* 1993), and progenies from crosses of these new polyploids with natural *B. napus* appear to readily acquire rearrangements between homeologous chromosomes (Parkin *et al.* 1995). If progenies from self-pollination of resynthesized *B. napus* polyploids acquire reciprocal transpositions, segregation studies of these may help further our understanding of the importance of intergenome heterozygosity in newly formed allopolyploids.

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