Homoeologous shuffling and chromosome compensation maintain genome balance in resynthesized allopolyploid *Brassica napus*

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Polyploidy has contributed to the evolution of eukaryotes, particularly flowering plants. The genomic consequences of polyploidy have been extensively studied, but the mechanisms for chromosome stability and diploidization in polyploids remain largely unknown. By using new cytogenetic tools to identify all of the homoeologous chromosomes, we conducted a cytological investigation of 50 resynthesized *Brassica napus* allopolyploids across generations $S_{0:1}$ to $S_{5:6}$ and in the $S_{10:11}$ generation. Changes in copy number of individual chromosomes were detected in the $S_{0:1}$ generation and increased in subsequent generations, despite the fact that the mean chromosome number among lines was approximately 38. The chromosome complement of individual plants (segregants) ranged from 36 to 42, with a bias toward the accumulation of extra chromosomes. Karyotype analysis of the $S_{10:11}$ generation detected aneuploidy and inter- and intragenomic rearrangements, chromosome breakage and fusion, rDNA changes, and loss of repeat sequences. Chromosome sets with extensive homoeology showed the greatest instability. Dosage balance requirements maintained chromosome numbers at or near the tetraploid level, and the loss and gain of chromosomes frequently involved homoeologous chromosome replacement and compensation. These data indicate that early generations of resynthesized *B. napus* involved aneuploidy and gross chromosomal rearrangements, and that dosage balance mechanisms enforced chromosome number stability. Seed yield and pollen viability were inversely correlated with increasing aneuploidy, and the greatest fertility was observed in two lines that were additive for parental chromosomes. These data on resynthesized *B. napus* and the correlation of fertility with additive karyotypes cast light on the origins and establishment of natural *B. napus*.

Polyploidy (i.e., whole genomic duplication) has played a significant role in the evolutionary history of all eukaryotes (1), and particularly in flowering plants (2). It is estimated that most flowering plants are polyploid, including most agricultural crops (3–7). Although all angiosperms have experienced at least one round of whole-genome duplication during their evolution, they have undergone diploidization (8–10). The structural evolution of genomes in ancient polyploids included reductions in chromosome number, chromosome fusions, and various types of chromosomal rearrangements (8, 11). Studies on newly resynthesized *Brassica napus* and recently formed polyploids of *Tragopogon* suggest that genomic changes occur rapidly following allopolyploidization in some plant species (12–14). Allopolyploidization can result in chromosomal rearrangements, DNA methylation changes, chromatin remodeling, changes in gene expression, and activation of transposable elements (13–22). Cytogenetic studies using FISH have reported chromosomal changes, including changes at ribosomal DNA loci, intergenomic translocations, aneuploidy, and loss of repeats (23–25). In *Arabidopsis,* synthetic allotetraploids are meiotically stable (26, 27), and the frequencies of aneuploidy and chromosome abnormalities are relatively low (27). Many previous studies have lacked the chromosome-specific markers necessary to identify all homoeologous chromosomes. Little is known about the stability of homoeologous chromosomes and the mechanisms for chromosome change in large populations of resynthesized allopolyploids.

B. napus affords an excellent opportunity for conducting cytogenetic investigations of evolution in a resynthesized allopolyploid. Natural *B. napus* (AACC; $2n = 38$) is thought to have formed 5,000 to 10,000 y ago by the hybridization of ancestors of *Brassica rapa* (AA; $2n = 20$) and *Brassica oleracea* (CC; $2n = 18$) (28, 29, 30). *B. rapa* and *B. oleracea* are also ancient polyploids, and large-scale chromosome rearrangements occurred in the A and C genomes following divergence from a common ancestor (31). Several studies have demonstrated that genetic changes caused by homoeologous chromosome rearrangement are common in newly resynthesized *B. napus* allotetraploids (12, 14); however, little is known about karyotype stability in resynthesized lineages. This is in part because of difficulties in distinguishing among the 38 small chromosomes of *B. napus*, and because of a lack of cytological markers. Mapping studies and BAC resources now permit rigorous evolutionary studies of homoeologous chromosomes in *B. napus*.

In this study, we conducted a cytological investigation of 50 resynthesized *B. napus* allopolyploids across generations $S_{0:1}$ to $S_{5:6}$ and in the $S_{10:11}$ generation. Previous studies of this population used mapped, genome-wide molecular markers and detected genome-wide homoeologous chromosome rearrangements and changes in homoeologous gene expression (14). As eight or more progenies of each line were pooled for analysis, these previous studies were only capable of detecting genetic changes that were fixed in each line. Furthermore, these studies did not include a cytological characterization of individual plants or their chromosome complement. By using a newly developed cytogenetic method to distinguish all 38 chromosomes present in *B. napus* (32), we report evidence for homoeolog pairing and chromosome rearrangements, aneuploidy, and homoeologous chromosome compensation.

Results

Karyotyping Resynthesized *B. napus* Using FISH with Repetitive DNA Sequences and BAC Clones. Previously we performed a cytogenetic analysis of natural *B. napus*, *B. rapa*, and *B. oleracea* by using 45S, 5S, CentBr1, CentBr2, and BAC clones containing repetitive sequences (32) (Fig. S1). In this study, we made a standardized karyotype of resynthesized allopolyploids of *B. napus* capable of distinguishing all parental chromosomes. Two BACs from each of the 10 chromosomes of *B. rapa* were used to identify the homoeologous chromosomes in the A and C genomes (Fig. S1). Chromosomes C1 and C5 were similar, but distinguishable by differences in centromere organization and the signal intensity of KBrB072L17.

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Aneuploidy in Resynthesized *B. napus*. Thirty-eight chromosomes are expected in resynthesized *B. napus*. This includes 13 pairs of chromosomes containing CentBr1 and six pairs of chromosomes with CentBr2 signals. However, the chromosome constitution of segregants within every line was variable across generations (range, $2n = 36$–42; Table 1 and Fig. S2). The frequency of extra or missing chromosomes (i.e., aneuploidy) among the five lineages analyzed increased from 24.1% in the $S_{0:1}$ to 71.4% in $S_{5:6}$ generation. The mean chromosome number remained approximately 38, and variation increased over successive generations (Table 1). Among the 50 $S_{5:6}$ lines analyzed, only one line (EL550) contained 38 chromosomes (26 CentBr1 and 12 CentBr2) in all segregants analyzed. The mean chromosome number among $S_{5:6}$ lines was 38.37, which was significantly different from 38 (one-sample *t* test, $P < 0.002$); however, the distribution of chromosome numbers was not normal, and was skewed toward the accumulation of more chromosomes (Shapiro–Wilk test, $W = 0.947$; $P < 0.05$; Fig. S2; skewness of 0.64). Sixty-five percent of $S_{10:11}$ plants were aneuploid based on centromere signals and total chromosome numbers (Table 1). The average number of chromosomes per line was 38.58, which was significantly greater than 38 (one-sample *t* test, $P < 0.05$); however, the distribution was not normal and was skewed toward the gain of chromosomes (Shapiro–Wilk test, $W = 0.885$; $P < 0.001$; Fig. S2; skewness of 0.865). Fifteen $S_{10:11}$ lines had 38 chromosomes; however, 13 of these were aneuploid for parental chromosomes, but had undergone homoeologous chromosome loss and replacement (as detailed later). Thus, whereas only 65% of $S_{10:11}$ lines were aneuploid based on centromere signals and chromosome numbers, 95% (36 of 38 lines) proved to be nonadditive for parental chromosomes following karyotype analysis (Table 1). The variance for chromosome number was significantly greater in the $S_{10:11}$ generation compared with the $S_{5:6}$ generation (Table 1 and Fig. S2; *F* test, $P < 0.05$). More than 10 samples of each of *B. rapa* (parent IMB218), *B. oleracea* (parent TO1000), and *B. napus* (cultivar DH12075) were analyzed for chromosome numbers (and karyotype analysis), and neither aneuploidy nor chromosome rearrangements were observed in these controls.

Chromosomal Instability Was Not Equal Across the Genome. Thirty-eight $S_{10:11}$ lines could be karyotyped, and only two lines (EL500 and EL2600) had the expected karyotype assuming additivity of parental chromosomes (i.e., A1–A10 plus C1–C9). Chromosome instability (gain and loss) was variable among chromosomes (Table S1). Chromosomes A1, A2, C1, and C2 were each aneuploid (deviated from two copies) in greater than 50% of lines. Chromosome A6 was the most stable, and was not lost or gained in any of the lines except EL1200, which lost the short arm of A6.

A total of 107 chromosomes were gained compared with 86 chromosomes lost among all 38 lines analyzed; however, this difference was not significant (one sample test of proportions; $P = 0.15$). The A genome did not show a strong bias for either loss or gain of chromosomes (48 lost and 52 gained; $P = 0.7642$). The C genome did not show a significant bias in the loss or gain of chromosomes (38 lost and 55 gained; $P = 0.0971$).

Table 1. Summary of chromosome counting in resynthesized *B. napus*

Generation	*n*	Mean* ± SD	Variance	Range	Aneuploid, %†
$S_{0:1}$	5	38.16 ± 0.34	0.12	37–40	24.14
$S_{1:2}$	5	38.12 ± 0.18	0.03	37–41	29.63
$S_{2:3}$	5	38.05 ± 0.15	0.02	37–40	63.16
$S_{3:4}$	5	38.04 ± 0.70	0.50	36–39	67.86
$S_{4:5}$	5	38.73 ± 0.75	0.56	37–42	62.07
$S_{5:6}$	50	38.37 ± 0.79	0.62	36–42	71.43
$S_{10:11}$	40	38.58 ± 1.43	2.05	36–42	94.74

*Mean chromosome number calculated for each generation by averaging subsampled plants within lines, and these values were averaged across all lines.
†Percentage of aneuploid plants in generations $S_{0:1}$ to $S_{5:6}$ was determined using FISH mixture 1 (*SI Materials and Methods*), and the percentage of aneuploid plants for the $S_{10:11}$ generation was determined using mixtures 1, 2, and 3.

Chromosomal Dosage Balance Among $S_{10:11}$ Resynthesized *B. napus*. In the $S_{10:11}$ generation, no chromosome exceeded four copies, and the total number of chromosomes in each homoeologous set (e.g., A1 + C1) was usually four. Lost chromosomes were often compensated by the gain of homoeologous chromosome, particularly for homoeologous chromosome sets A1/C1 and A2/C2 (Table 2, Fig. 1, and Fig. S3). The gain of two extra chromosomes usually occurred with the loss of one or both homoeologous chromosomes. Thirty-one of 38 $S_{10:11}$ lines (81.6%) were aneuploid for chromosomes within homoeologous sets A1/C1 or A2/C2, or in both homoeologous sets. Between 84.2% and 86.8% of these lines contained four chromosomes in homoeologous sets A1/C1 and A2/C2, respectively, and thus demonstrated compensating aneuploidy (total number of chromosome was four, but did not contain two copies of each homoeolog). A few lines contained three or five total chromosomes in these homoeologous sets (Table 2). No line had more than five chromosomes in a homoeologous set, and one line (EL7400) had two A2 chromosomes and was missing both C2 chromosomes. However, the missing C2 chromosomes were fused with C6, thus maintaining the dosage balance through a compensating translocation (as detailed later). We rejected the null hypothesis that counts (i.e., number of lines) in each category of dosage (Table 2) were random (χ^2 test, $P < 0.0001$).

Compensation between homoeologs A1/C1 and A2/C2 involved a high incidence of nullisomic–tetrasomic configurations (i.e., homoeologous chromosome substitution), as well as monosomy–trisomy (one homoeologous chromosome substituted; Table 2). A total of 24 cases of nullisomy were detected among these four chromosomes, and all were compensated by at least one homoeologous chromosome. Twenty-two (91.7%) of these cases were nullisomic–tetrasomic (A:C ratio of 4:0 or 0:4), and two cases (8.3%) involved nullisomic-trisomics (A:C ratio of 3:0 or 0:3). A total of 14 cases of monosomy among these four chromosomes were detected, and in 12 cases (85.7%), compensation by trisomy of a homoeologous chromosomes was observed (A:C ratio of 3:1 or 1:3). Overall, 23 lines were aneuploid for homoeologous chromosomes A1 and C1, and 19 of these lines (82.6%) showed homoeologous compensation between these two chromosomes.

Table 2. Chromosomal dosage balance within homoeologous chromosome sets A1/C1 and A2/C2

Chromosome	Dosage observed	Balance/ compensation	No. of lines*	Total chromosomes	Lines with this dosage, %
A1:C1	2:2	Balance	15	4	39.5
A1:C1	1:3	Both	2	4	5.3
A1:C1	3:1	Both	3	4	7.9
A1:C1	0:4	Both	9	4	23.7
A1:C1	4:0	Both	3	4	7.9
A1:C1	0:3	Compensation	1	3	2.6
A1:C1	2:1	Neither	2	3	5.3
A1:C1	1:4	Compensation	1	5	2.6
A1:C1	3:2	Neither	2	5	5.3
A2:C2	2:2	Balance	18	4	47.4
A2:C2	1:3	Both	3	4	7.9
A2:C2	3:1	Both	2	4	5.3
A2:C2	0:4	Both	6	4	15.8
A2:C2	4:0	Both	4	4	10.5
A2:C2	0:3	Compensation	1	3	2.6
A2:C2	2:0	Neither	1†	2	2.6
A2:C2	4:1	Compensation	1	5	2.6
A2:C2	3:2	Neither	2	5	5.3

*Distribution of lines among the categories of homoeolog dosage shown in column 2 was not random (χ^2 test, $P < 0.0001$) for homoeologous sets A1:C1 and A2:C2.
†This line, EL 7400, had chromosome fusion between C2 and C6 (Fig. 2 *C* and *D*).

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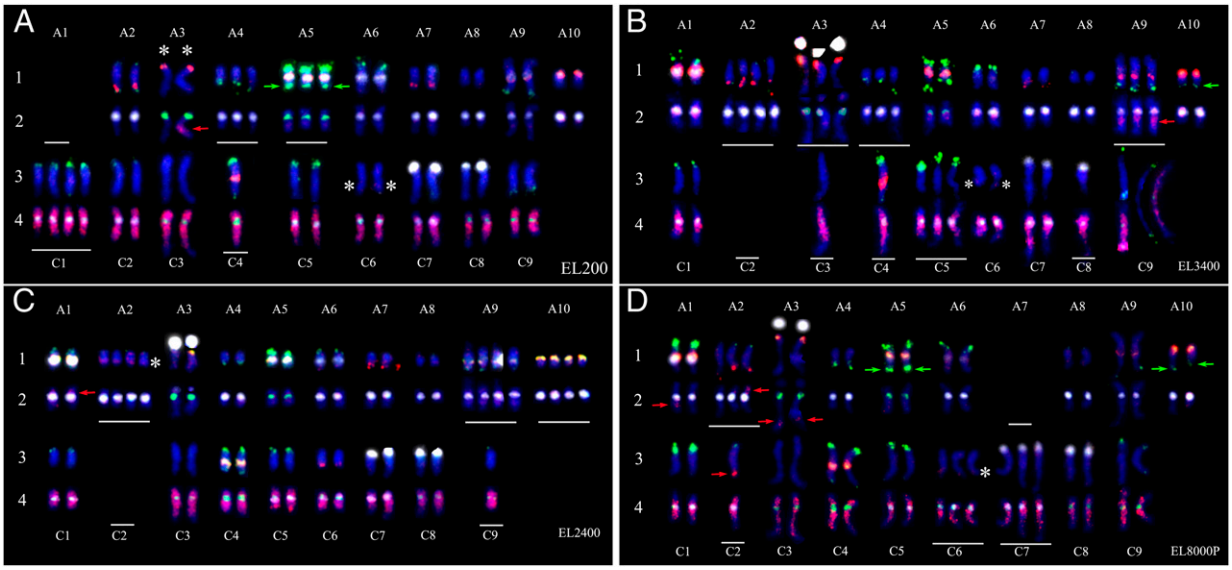

Fig. 1. Karyotype analyses of different lines in $S_{10:11}$ generation of resynthesized *B. napus*. The A genome is shown in lanes 1 and 2, and the C genome is shown in lanes 3 and 4. Hybridization results using mixture 2 (*SI Materials and Methods*) are shown in lanes 1 and 3, and included the following probes: 45S (white), 5S (yellow), BAC clone KBrB072L17 (green), and KBrH092N24 (red). Hybridization results using mixture 3 (*SI Materials and Methods*) are shown in lanes 2 and 4, and included the following probes: CentBr1 (red), CentBr2 (green), and BAC BNIH 123L05 (red) containing C genomic-specific repeated sequences. Karyotypes are shown for segregants of lines (*A*) EL200, (*B*) EL3400, (*C*) EL2400, and (*D*) EL8000P. Nullisomic, monosomic, trisomic, and tetrasomic chromosomes are underlined. Nullisomics of chromosomes A1 (EL200), C2 (EL 3400 and EL 2400), and A7 (EL 8000P) were compensated by homoeologous tetrasomics (C1 in EL200 and A2 in EL 3400 and EL 2400) or trisomics (C6 and C7 in EL 8000P). Monosomics of chromosomes C2 (EL 8000P), C3 (EL 3400), C4 (EL 200 and EL 3400), C8 (EL 3400), and C9 (EL 2400) were compensated by homoeologous tetrasomics or trisomics. Chromosome rearrangements are indicated with red or green arrows for red and green signal changes, respectively. Loss of interstitial chromosome fragments and rDNA changes are indicated with asterisks.

Seventeen of 19 lines (89.5%) that were aneuploid for A2 and C2 also showed homoeologous compensation.

Compensation among homoeologous chromosomes with segmental synteny was also observed; however, these chromosomes were relatively stable in comparison with A1/C1 and A2/C2. We found two cases of nullisomy at other chromosomes, and both were compensated by their homoeologs. For example, EL8000P lost two A7 chromosomes that were compensated by three copies of C6 and three copies of C7, which have homoeology with the long arm of A7 and with the short arm of A7, respectively (31). A total of 17 cases of monosomy were detected among other chromosomes, 13 (76.5%) of which were compensated by at least one corresponding homoeologous chromosome.

High Frequencies of Chromosome Rearrangements Were Detected by FISH in $S_{10:11}$ Resynthesized *B. napus*. Chromosomes that could be analyzed exhibited a high frequency of rearrangements (Table S2), regardless of whether chromosome complements were balanced or highly aneuploid (Fig. 1 and Fig. S3). We were unable to measure chromosome rearrangements on some chromosomes as a result of limited markers. The probe BNIH 123L05 was capable of detecting intergenomic translocation of C genome fragments onto A genome chromosomes (Table S2). By using this probe, intergenomic rearrangements involving large chromosome fragments from the C genome were detected on all A chromosomes except A6 and A8. Translocations of this kind were most frequently detected on chromosomes A1 and A2 [10 of 38 lines (26.3%) for each].

Chromosome A2 contains KBrH092N24 signals on its long arm in *B. rapa,* but these signals are absent on C2 in *B. oleracea*. Twelve lines of 38 (31.6%) gained KBrH092N24 signals on the long arm of C2, suggesting homoeologous translocation from chromosome A2 (Fig. 1, Fig. S3, and Table S2). Three lines showed loss of KBrH092N24 signals on chromosome A2. The long arm of C6 was homoeologous with A7 according to genetic maps (36) and our cytogenetic map (Fig. S1). Thirteen $S_{10:11}$ lines (34.2%) showed deletion of KBrH092N24 signals on C6, and seven lines (18.4%) showed deletions on A7.

The signals of BAC clone KBrB072L17 localized to multiple sites on homoeologous regions between the A and C genomes. Rearrangements could be detected easily at the homoeologous regions on the long arm of A5 and short arm of C4, which were polymorphic for KBrB072L17 signals (Fig. 1*B* and Table S2).

45S and 5S rDNA Loci Changes Among $S_{10:11}$ Generation. Cytogenetic analyses of synthetic *B. napus* revealed eight 45S and six 5S gene loci (Fig. S4). The signal patterns of 45S and 5S loci are different in size and location, allowing easy identification. Changes at nuclear organizing regions (NORs) were most frequent on chromosomes A3 and C7. Eight resynthesized lines in $S_{10:11}$ generation (21.1%) showed changes at 45S or 5S loci (Fig. S4). The most frequent change observed at NORs was partial loss of the 45S signals. Three lines lost most of 45S on A3, one line lost most of 45S and all 5S signals on A5, and four lines partially or totally lost 45S signal on C7 (Fig. S4). We never observed novel rDNA loci or significant enlargement of rDNA signals on existing loci. The loci carrying large 45S rDNA signals were prone to loss, whereas the small NOR loci on A4 and A6 were stable.

Chromosomal Breakage, Fusion, and Autosyndetic Chromosome Rearrangement. Chromosomal breakage and fragment loss were detected in three lines: EL1200, EL7300, and EL5910P. Line EL1200 contained one normal A6 chromosome and one with a large deletion (Fig. 2 *A* and *B*). The chromosome with the deletion contained a 45S locus similar to that on A6 in the middle of the long arm. BAC-FISH confirmed that there were two A6 chromosomes; however, one had broken at or near the centromere and was missing its short arm. Chromosomal breakage at the centromere was also observed on line EL7300. In line EL5910P, both A1 homologs were missing 45S signals and most of their long arms, and breakages had occurred between the positions of 45S and 5S loci (Fig. S5 *A* and *B*).

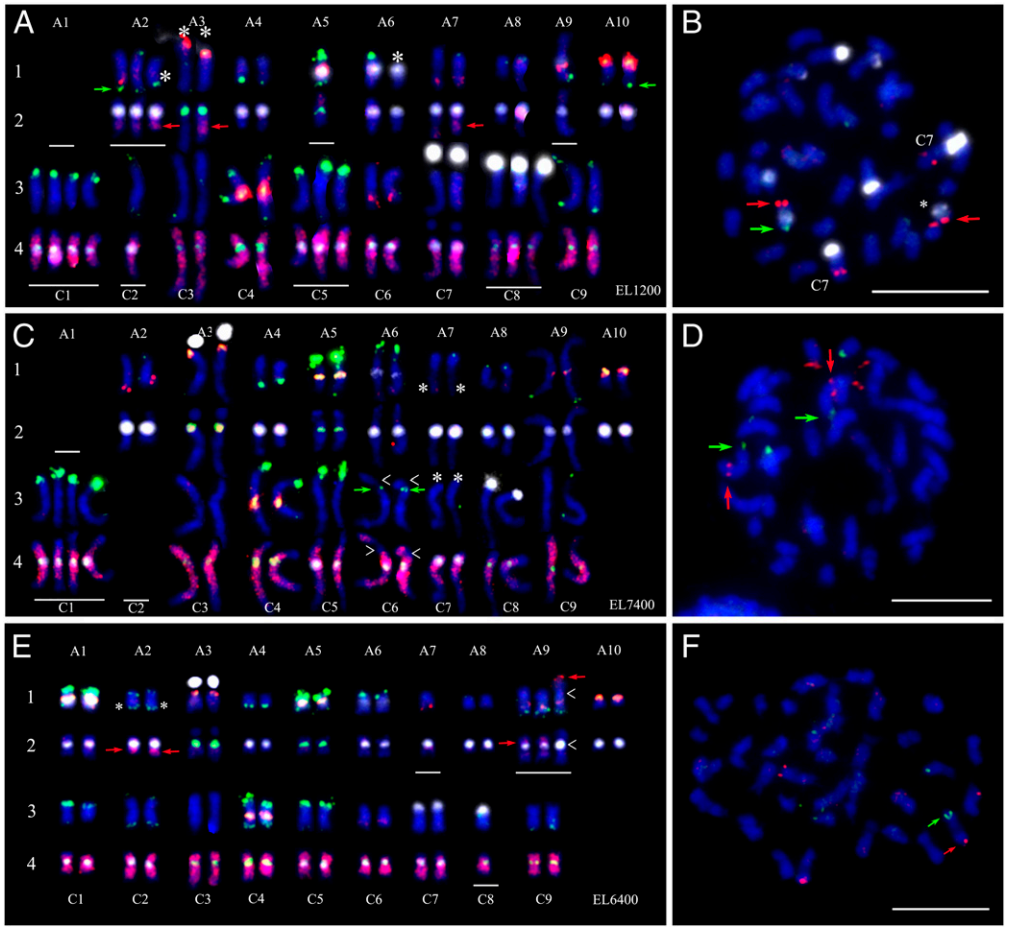

Fig. 2. Chromosomal breakage, fusion, and autosyndetic chromosome rearrangement in $S_{10:11}$-resynthesized *B. napus*. The probes used in karyotype analyses are the same as in Fig. 1. Chromosomes demonstrating aneuploidy are underlined. Chromosome rearrangements are shown with red or green arrows. Losses of signals show with asterisks. Chromosome fusions are shown with a "<" symbol. (*A*) Karyotype of EL1200 shows that one copy of A6 lost its short arm. (*B*) FISH with BAC clones KBrH003P24 (red signals) from the long arm of A6, KBrB022P06 (green signals) from short arm of A6, and 45S (white) confirmed that one copy of A6 lost its short arm (red arrow and asterisk). (*C*) Karyotype of EL7400 shows that it lost both copies of C2 and gained two fusion chromosomes (shown as C6). The fusion chromosomes contained green signals in the middle of the short arm (green arrows). (*D*) FISH using BAC clones KBrH004D11 (red signals) specific for the long arm of A2 and C2 and KBrH052E24 (green signals) specific for the long arm of A7 and C6, found that these two BACs localized to different arms of the fusion chromosome (red and green arrows), indicated autosyndetic chromosome fusion between C2 and C6. (*E*) Karyotype analysis of EL6400 showed that one of the three copies of chromosomes A9 gained KBrB092N24 signals on the short arm (red arrow), and had increased CentBr1 signals ("<" symbol). (*F*) FISH using BAC clones KBrB022L12 (red signals) specific for the long arm of A9 and C8 and KBrH052E24 (green signals) specific for the long arm of A7 and C7 found these signals on opposite arms of the fusion chromosome (green and red arrows).

Chromosome fusion between C2 and C6 was detected in line EL7400 (Fig. 2 *C* and *D*). BAC clones from the long arms of A2 and A7 also label homoeologous chromosomes C2 and C6, and FISH revealed signals on both the short and long arms of the fusion chromosomes (Fig. 2*D* and Fig. S1). The position of chromosome fusion occurred between the end of long arm of C2 and the end of short arm C6. The two novel chromosomes contained the intact chromosomes of C6 and the long arms of C2 (Table 2).

Two $S_{10:11}$ lines contained autosyndetic chromosome rearrangements. Line EL6400 lost one A7 and one C8 chromosome, and gained a novel chromosome that was a fusion between A7 and A9 (Fig. 2 *E* and *F*). Another autosyndetic chromosome rearrangement was detected in line EL5500 (Fig. S5 *C* and *D*). This chromosome also had chromosome rearrangements at the centromere position with an unknown chromosome from the C genome.

Chromosome Changes Correlate with Reduced Seed Yield and Pollen Viability Among $S_{10:11}$ Lines. We measured pollen viability and seed production in all of the lines of $S_{10:11}$ generation. With successive generations, resynthesized *B. napus* produced fewer seeds. Three lines (EL2700, EL8400, and EL9000) produced no seeds at the $S_{10:11}$ generation. The line with the highest pollen viability (96.2%) and seed set (1,709 seeds) was EL2600, which had 38 chromosomes, but contained several large chromosomal rearrangements (Fig. S3*A*). The line with the second highest pollen viability and third highest seed yield was EL500, and this line also contained 38 chromosomes and a single chromosome rearrangement. EL3400 had the lowest pollen viability and produced no seeds; it contained 39 chromosomes and had 11 chromosome changes, including four homoeologous chromosome substitutions (4A2/0C2, 3A3/1C3, 3A4/1C4, and 3A9/1C8; Fig. 1*B*). Aneuploids ($2n >$ or < 38) generally had lower seed yield.

Seed yield and pollen viability were not normally distributed among $S_{10:11}$ lines (Shapiro–Wilk test, $W = 0.835$, $P < 0.0001$ for seeds; and $W = 0.906$, $P < 0.01$ for pollen), and were positively correlated (Pearson correlation, $r = 0.53$, $P < 0.0004$; Spearman rank correlation, $r = 0.60$, $P < 0.0001$). Chromosome changes were defined as the total number of lost and gained chromosomes for each of the 19 parental chromosomes, for which two copies of each were expected. Chromosome changes were normally distributed among the lines (Shapiro–Wilk test, $W = 0.994$; $P > 0.05$), and were inversely correlated with seed yield (Pearson correlation, $r = -0.468$, $P < 0.01$; Spearman rank correlation, $r = -0.38$, $P < 0.05$). Chromosome changes were also inversely correlated with pollen viability (Pearson correlation, $r = -0.51$, $P < 0.001$; Spearman rank correlation, $r = -0.39$, $P < 0.05$). Thirty-five lines could be compared between this study and the molecular study conducted by Gaeta et al. (14). We detected weak evidence for a positive correlation between total $S_{10:11}$ chromosome changes detected by FISH and total $S_{5:6}$ genetic changes reported by Gaeta et al. (14) (Pearson correlation, $r = 0.35$, $P < 0.05$; Spearman rank correlation, $r = 0.24$, $P = 0.1662$).

Discussion

Aneuploidy Alters Gene Dosage and Affects Fertility in *B. napus* Allopolyploids. Phenotypic changes have been observed in resynthesized polyploids (14, 33, 34) and in natural polyploid species

(35, 36). Several mechanisms have been proposed to explain why nascent and resynthesized polyploids demonstrate novel phenotypic variation (8, 18, 37). It may be a result of novel combinations of gene regulatory factors (38), changes in gene regulation (38, 39), transposon activation (40, 41), dosage effects (38), chromosomal rearrangements (14, 33), and/or epigenetic changes (35).

In resynthesized *B. napus* polyploids, Schranz and Osborn (42) suggested that phenotypic variation might arise from few genetic changes. Pires et al. (33) described homoeologous rearrangements at FLC that affected flowering time. Gaeta et al. (14) investigated genetic, epigenetic, transcriptional, and phenotypic changes among nearly 50 independently resynthesized *B. napus* lines and found that homoeologous chromosomal rearrangements were a major mechanism creating novel phenotypic variation. By using the same set of materials as Gaeta et al. (14), we advanced the population of polyploids to the $S_{10:11}$ generation, and found further evidence of intergenomic rearrangements, deletion of rDNA loci, chromosome breakage and fusion, and aneuploidy among individual plants. The mechanisms whereby these genomic changes contribute to phenotypic variation among our *B. napus* polyploids remain unknown.

It is known that the addition or subtraction of single chromosomes to the karyotype can produce stronger phenotypes than whole-genome changes in both animals and plants (43–45). Many of these phenotypic changes may result from changes in the expression of dosage-sensitive genes because of changes in chromosomes (46). Aneuploidy and translocations are consequences of homoeologous pairing, and these chromosomal changes reduce viability because of the production of duplication and deficiency gametes (47). We observed reduced pollen viability and seed set with increasing numbers of chromosome dosage changes. Ninety-five percent of $S_{10:11}$-resynthesized *B. napus* lines were aneuploid; however, the average chromosome number deviated only slightly from the expected $2n$ of 38. Only two (5%) of our $S_{10:11}$ lines contained the "expected" additive karyotype of *B. napus*, and these lines had the highest fertility. Although many novel phenotypes were observed, high fertility and seed set give a plant a strong chance of contributing to future generations. Although our two most stable $S_{10:11}$ lines also showed evidence for intergenomic translocation, their karyotype was most like that observed in natural *B. napus* (32). Therefore, selection against aberrant individuals with low fertility and those lacking control over homoeologous pairing may have been an important factor in the origin and establishment of natural *B. napus*.

Chromosomal Instability and Rearrangement Were Not Randomly Distributed Across the Genome. Chromosome instability began as early as the $S_{0:1}$ generation, and aneuploidy increased with successive generations. Homoeologous linkage groups A1/C1 and A2/C2 are each syntenic along their entire chromosome length, whereas most remaining homoeologs share only segmental synteny (31, 48). The two most unstable homoeologous sets were A1/C1 and A2/C2. Changes in these homoeologous chromosomes occurred in more than 50% of lines, and included nullisomy, monosomy, trisomy, and tetrasomy (Table 2). These data are consistent with previous data on resynthesized *B. napus* allohaploids and allotetraploids, which have found that these homoeologous chromosomes show the highest rates of pairing and exchange (14, 49, 50, and Fig. S6).Chromosome 6 from the A genome had the lowest rate of chromosome change, and we observed a single loss of one chromosome arm of A6. Using a C genome-specific BAC clone as a probe, the frequency of chromosome translocations from the C genome to the A genome chromosomes was highest for chromosomes A1 and A2. These data demonstrated that chromosome changes (aneuploidy and translocations) occurred most frequently on homoeologous chromosome pairs that display the most extensive stretches of syntenic marker loci (14, 31, 49, 50).

Chromosomal Rearrangements in Resynthesized *B. napus* and Polyploid Establishment. Polyploidization, or whole genomic duplication, may increase genomic rearrangement rate in some species (7). Evidence for genomic rearrangements, including homoeologous exchange, fragment loss, and rDNA loci changes have been reported both in nascent polyploid taxa (12, 14, 24) and natural polyploid species (25). Our data supports previous observations that genomic rearrangements occur in synthetic polyploid *B. napus*. Rearrangements lead to genomic instability and can be deleterious to fitness (7). The novel variation caused by genomic changes could be important for niche exploitation and species formation in a new polyploid; however, there is no evidence that highly rearranged or unstable individuals were selected for during the establishment of natural cultivars of *B. napus* (32, 51, 52). Natural *B. napus* had strong control over homoeologous pairing, or natural selection quickly culled plants with low fertility and aberrant phenotypes from early populations, favoring those with pairing control, stable chromosome numbers, and few beneficial genome rearrangements. Natural accessions of *B. napus* vary significantly in meiotic pairing behavior, suggesting that genetic variation exists (53). Our replicated resynthesized allopolyploids were propagated and maintained for 10 generations of self-pollination, regardless of genome stability, pollen viability, and seed set. This allowed us to observe highly unstable lineages that might not have otherwise survived in nature. Natural selection against extreme individuals would be expected in early generations through reductions in seed set and pollen viability. This, combined with the age of the polyploid, may explain why analyses of natural *B. napus* have reported predominantly additive, stable genomes, which display few rearrangements (32, 51, 52).

Chromosomal Dosage Requirements Compensated for Chromosomal Instability. In mammals and plants, changes in gene dosage resulting from aneuploidy or chromosomal rearrangements can have deleterious effects (54). Compensating trisomy was first described by Blakeslee (55) to explain a situation in which the loss of a normal chromosome was compensated for by the presence of a secondary chromosome. Compensatory trisomy has been reported in wheat (56), tomato (57), Pearl millet (58), and *Tragopogon* (25). Recently, Lim et al. (25) observed a surprisingly high incidence of trisomy and monosomy in highly fertile plants with normal chromosome number in naturally occurring *Tragopogon* polyploids, and these polyploids represent the only example of this phenomenon from nature. However, the lack of chromosome-specific markers and other genetic information made it impossible to determine the homoeologous relationship of the trisomics and monosomics. In the present study, we observed trisomy and monosomy in newly synthetic polyploids of *B. napus*, and demonstrated that compensating aneuploidy occurred within homoeologous chromosome sets. Compensatory aneuploids were not frequent among chromosomes with segmental synteny; however, for the homoeologous chromosome pairs with the most extensive synteny, we observed frequent nullisomic–tetrasomic substitutions.

These observations provide an experimental explanation for the origin and existence of segmental allopolyploids (59, 60). In the absence of strong pairing control, homoeologs that are highly similar structurally and genetically can pair and recombine as in an autopolyploid, whereas sets that are highly diverged or only partially homologous do not pair, and behave as in a true allopolyploid. We never observed fewer than three or more than five copies of homoeologous chromosomes, suggesting that individuals with strong deviations from the chromosome balance (strongly deviated from four copies across the genome) had reduced fertility, and hence were selected against during advancement of the lines. For homoeologous chromosome pairs displaying synteny along their entire length, chromosomal substitution may have provided gene dosage compensation. This chromosome compensation may have decreased the deleterious effects of lost homoeologs and increased the survival of segmental allopolyploids (60). Homoeologous compensation may provide a buffering effect in the early generations of a polyploid, allowing for extended survival and seed production in individuals with aberrant genotypes. Chromosomal compensation and the frequent observation of four chromosomes in homoeologous chromosome sets demonstrated that chromosome dosage balance was main-

tained in newly resynthesized polyploids of *B. napus*, consistent with the predictions of the gene balance hypothesis (43).

Materials and Methods

Plant Materials. Fifty resynthesized *B. napus* allopolyploid lines (CCAA) were developed by hybridizing doubled haploid *B. oleracea* line TO1000 (egg donor; C genome) with doubled haploid *B. rapa* line IMB218 (pollen donor; A genome) as described previously (14). Details of plant materials are given in *SI Materials and Methods*.

Karyotyping by FISH. Chromosome-specific BACs were selected for chromosome identification and karyotyping as described previously (32) (Fig. S1). The processes of FISH and the determination of chromosome rearrangements are described in *SI Materials and Methods*.

Analysis of Pollen Viability. Pollen viability was tested by using MTT according to the method of Rodriguez-Riano and Dafni (61).

Statistical Analysis. Statistics were performed using data analysis functions in Microsoft Excel and SAS statistical software, version 9.2 (SAS). One sample tests of proportions were conducted using R statistical software (R development team, 2009). Details of statistical analysis are given in *SI Materials and Methods*.

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