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Non-random distribution of extensive chromosome rearrangements in *Brassica napus* depends on genome organization

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SUMMARY

Chromosome rearrangements are common, but their dynamics over time, mechanisms of occurrence and the genomic features that shape their distribution and rate are still poorly understood. We used allohaploid *Brassica napus* (AC, $n = 19$) as a model to analyze the effect of genomic features on the formation and diversity of meiotically driven chromosome rearrangements. We showed that allohaploid *B. napus* meiosis leads to extensive new structural diversity. Almost every allohaploid offspring carried a unique combination of multiple rearrangements throughout the genome, and was thus structurally differentiated from both its haploid parent and its sister plants. This large amount of genome reshuffling was remarkably well-tolerated in the heterozygous state, as neither male nor female fertility were strongly reduced, and meiosis behavior was normal in most cases. We also used a quantitative statistical model, which accounted for 75% of the observed variation in rearrangement rates, to show that the distribution of meiotically driven chromosome rearrangements was not random but was shaped by three principal genomic features. In descending order of importance, the rate of marker loss increased strongly with genetic distance from the centromere, the degree of collinearity between chromosomes, and the genome of origin (A < C). Overall, our results demonstrate that *B. napus* accumulates a large number of genetic changes, but these rearrangements are not randomly distributed in the genome. The structural genetic diversity produced by the allohaploid pathway and its role in the evolution of polyploid species compared to diploid meiosis are discussed.

Keywords: genome rearrangement, genome evolution, meiotic recombination, polyploidy, structural variation, homeologous recombination.

INTRODUCTION

Chromosome rearrangements are a driving force in evolution. In contrast to the long-held view that chromosome rearrangements are strongly deleterious, counter-selected and rare within species (Stankiewicz and Lupski, 2002), an unexpectedly large amount of structural variation has been observed at the whole-genome level between healthy individuals within several diploid species (Redon *et al.*, 2006; Springer *et al.*, 2009) and between related species (Coghlan *et al.*, 2005; Lysak *et al.*, 2006; Mandakova *et al.*,

2010). Some of these rearrangements appear to correlate with phenotypic differences (Stranger *et al.*, 2007; Hollox, 2008), possibly conferring fitness advantages in various habitats (Rieseberg *et al.*, 2003). Others contribute to speciation by reducing recombination between genomes (Rieseberg, 2001; Navarro and Barton, 2003).

On an evolutionary time scale, the rate of chromosome rearrangement varies strongly between and within phyla, indicating that genome stability is not constant across

species or time (Coghlan *et al.*, 2005; Murphy *et al.*, 2005; Fischer *et al.*, 2006; Scannell *et al.*, 2006). For example, although near-complete genomic stasis has been reported in newly formed *Spartina anglica* or cotton (*Gossypium*) species (Liu *et al.*, 2001; Baumel *et al.*, 2002), genome changes arose rapidly following polyploid formation in other new allopolyploids formed by the merging and doubling of related genomes (Ozkan *et al.*, 2001; Gaeta *et al.*, 2007; Lim *et al.*, 2008; Szadkowski *et al.*, 2010; Xiong *et al.*, 2011). However, even in species with a highly dynamic genome, the timing of generation and fixation of genome-wide chromosome rearrangements is usually unclear (Dvorak *et al.*, 2004). It is not clear whether there is a burst of rearrangements that generates new genome combinations upon which selection then acts (Nicolas *et al.*, 2007; Szadkowski *et al.*, 2010), or conversely whether rearrangements are generated and accumulate gradually in a ratchet-like manner (Gaeta and Pires, 2010).

It is also unclear whether chromosome rearrangements can occur in any part of the genome (Rieseberg *et al.*, 1995; Rieseberg, 2001; Navarro and Barton, 2003; Nicolas *et al.*, 2007). In *Caenorhabditis elegans* and *Saccharomyces cerevisiae*, essential genes tend to be clustered in less-rearranged, low-recombinogenic regions of the genome (Fischer *et al.*, 2006). This suggests that selection acted to modify both the fine-scale intra-genomic variation in the recombination rate and the distribution of essential genes (Hurst *et al.*, 2004). A similar correlation between gene order and recombination rate has been reported in diploid and polyploid wheat. In this species, gene loss and duplication follow a positive gradient along the centromere–telomere axis that correlates with recombination rates (Akhunov *et al.*, 2003a,b, 2007; Dvorak and Akhunov, 2005). However, the mechanisms underpinning this correlation remain unknown.

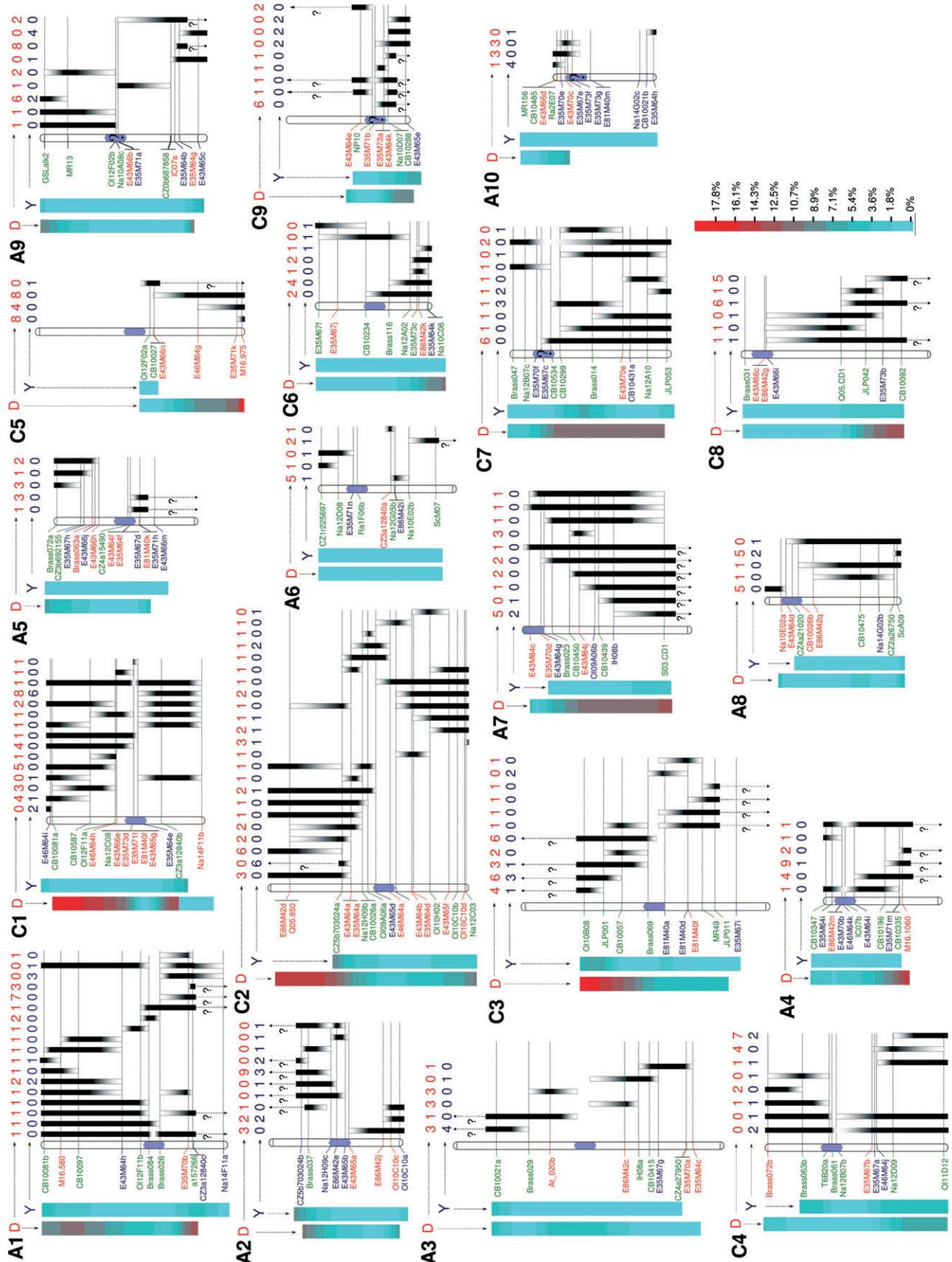
Brassica napus (AACC, $2n = 38$) is an excellent model for investigating the dynamics of meiotically driven chromosome rearrangements in polyploid genomes (reviewed by Gaeta and Pires, 2010; see also Szadkowski *et al.*, 2010, 2011 and Xiong *et al.*, 2011). *Brassica napus* is a young allopolyploid species that was formed by repeated inter-specific hybridization and genome doubling

between its ancestors *Brassica oleracea* (CC, $2n = 18$) and *Brassica rapa* (AA, $2n = 20$) (U, 1935; Palmer *et al.*, 1983; Song and Osborn, 1992). It is now clear that a whole-genome triplication event (i.e. paleohexaploidy event) occurred in these species soon after divergence between the Arabidopsis and *Brassica* lineages, 13–17 millions years ago (Wang *et al.*, 2011a). Studies in *B. napus* allohaploids (AC, 19 chromosomes; Nicolas *et al.*, 2007, 2009), *B. oleracea* × *B. rapa* F₁ inter-specific hybrids (Szadkowski *et al.*, 2011) and corresponding resynthesized S₀ amphidiploids (Song *et al.*, 1995; Gaeta *et al.*, 2007; Szadkowski *et al.*, 2011) have shown that extensive chromosome changes, commonly described as translocations or rearrangements, are the result of meiotic cross-overs between non-homologous chromosomes. These cross-overs most commonly, but not invariably, occur between homeologous chromosomes (Nicolas *et al.*, 2007). However, autosyndetic bivalents between pairs of A chromosomes or pairs of C chromosomes were nonetheless detected during meiosis of *B. napus* allohaploid plants (Nicolas *et al.*, 2007, 2009) or inter-specific A×C hybrids (Szadkowski *et al.*, 2011). The products of meiotic cross-overs between homeologous chromosomes were found to segregate naturally among *B. napus* accessions (e.g. Lombard and Delourme, 2001) and contribute to variation for some relevant agronomic traits (Osborn *et al.*, 2003; Pires *et al.*, 2004; Zhao *et al.*, 2006). Two studies reported that chromosome rearrangements were not randomly distributed across the genome (Gaeta *et al.*, 2007; Xiong *et al.*, 2011). However, the importance of chromosomal features, such as the position along the centromere–telomere axis, in determining this pattern has not yet been specifically addressed. In addition, neither of these studies provided a clear estimation or description of genome-wide structural variation in the progeny of *B. napus* synthetics or related plants.

The aim of this study was to investigate the structural dynamics of an allopolyploid species by (i) measuring the extent of genome-wide structural diversity in the progeny of *B. napus* allohaploids, and (ii) analyzing how this overall variation is affected by different levels of chromosome organization. Using appropriate statistical modeling, we examined whether chromosome rearrangements are

Figure 1. Distribution of chromosome rearrangements and variation in rearrangement rates throughout the *Brassica napus* genome in the progenies of *Darmor-bzh* and *Yudal* haploids.

The 19 linkage groups (open rectangles) of *B. napus* (A1–A10 for genome A, C1–C9 for genome C) are based on the frame genetic map established by Delourme *et al.* (2006). The purple areas indicate the most likely genetic position of the centromeres according to Pouilly *et al.* (2008). The genetic positions of genotyped markers are given on the left (green, co-dominant markers; red, dominant markers for *Darmor-bzh*; blue, dominant markers for *Yudal*). The estimated frequencies of marker loss by locus are indicated to the left of each linkage group for the two progenies (D for *Darmor-bzh* and Y for *Yudal*), and are represented by a smoothed color scale ranging from blue (no loss: marker additivity) to red (22% of marker loss). The scale is shown at the bottom right. Chromosome rearrangements are represented to the right of every linkage group. They were drawn based on the assumption that concurrent loss of linked loci (black rectangles) originated from a single rearrangement. The adjacent genomic regions harboring the corresponding breakpoint(s) are indicated in gray (where dark gray indicates that a marker is absent, and light gray indicates that a marker is present). The arrows with a question mark represent the probable extension of chromosome rearrangements up or down to the end of linkage groups (when no marker is available in the most distal parts of linkage groups). The occurrence of the various rearrangement types in the two progenies is given by the numbers above the corresponding graphical representation: red, *Darmor-bzh*; black, *Yudal*.



randomly distributed in the genome, and determined which chromosome features affect genomic susceptibility to chromosome rearrangements. This study is thus complementary to our previous reports in which we demonstrated that genetic changes are mainly driven by meiotic cross-overs between non-homologous chromosomes (Nicolas *et al.*, 2007), the frequency of which depended on the alleles present at a major locus, called *PrBn* (Nicolas *et al.*, 2009). However, further studies were required to fully characterize and model the structural diversity within the progeny of allohaploids, the structural genomic features that modulate the rate and distribution of chromosome rearrangements throughout the genome, and the consequences of this structural diversity on plant fitness. These goals are achieved and discussed here.

RESULTS

A total of 149 and 141 markers were previously mapped on a double allohaploid derived from the *Darmor-bzh* × *Yudal* cross (Figure 1; see Table S1 for details). In this study, these markers were used to genotype two F₁ populations obtained by crossing allohaploid plants ($n = 19$) with euploid plants ($2n = 38$) from genotypes *Darmor-bzh* and *Yudal* (see Nicolas *et al.*, 2009; for a detailed marker list, positions, and information on crosses). Non-additive transmission of allohaploid parental markers (PCR fragment loss), covering 75% of the genetic map, was used to detect *de novo* chromosome rearrangements that were generated during meiosis of the allohaploid parent and transmitted to their progeny by unreduced gametes (Nicolas *et al.*, 2007).

Mapping *de novo* rearrangements throughout the genome in the progeny of allohaploids

Analysis of marker additivity showed fragment losses for more than 75% of markers in at least one of the progenies, with 25% of loci being rearranged in more than 7.5% of plants. In more than 97% of cases, the missing alleles were from the allohaploid parents (Table S1), demonstrating that the vast majority of chromosome rearrangements were generated during meiosis of the allohaploid plants. In fact, the frequency of genetic changes per locus was highly variable throughout the genome (Figure 1), varying in a non-random manner from 0 to 22% depending on where the loci were localized in the genome or along chromosomes (see detailed analysis below).

Considering the simultaneous loss of adjacent parental markers in one plant as derived from the same rearrangement, we scored a total of 454 chromosome rearrangements in the two progenies (Figure 1). The type and size of chromosome rearrangements generated during allohaploid meiosis varied depending on the localization and number of rearrangement breakpoints on the chromosome (Figure 1). Five types of chromosome rearrangements were differenti-

ated (Figure 1 and Table S1): (i) distal (one breakpoint), (ii) interstitial (two breakpoints), (iii) double distal (two breakpoints), (iv) distal and interstitial (three breakpoints), and (v) double interstitial (four breakpoints). Simple distal rearrangements were the most frequent (73%), followed by interstitial (22%), distal and interstitial (3%) and double distal (3%) rearrangements. The last two categories, which led to concurrent removal of two regions on a single chromosome, were only observed for eight of the most rearranged linkage groups (A1, C1, A2, C2, A4, C3, C7 and C9; $z = 3.5$, P value <0.001; Figure 1). When the distribution of the linkage groups encompassing one, two, three or more breakpoints was compared to the distribution expected by chance, we found that multiple breakpoints occurred more frequently than expected by chance ($\chi^2 = 57$, P value <0.001). The genetic size of the removed regions was highly variable throughout the genome (Figure 1). Considering all rearrangements together in the two F₁ populations, only 15% of the genetic map was free of rearrangements (Figure 2).

Allohaploid meiosis generated extensive and transgressive genetic diversity

The allohaploid × euploid cross led to highly differentiated progenies. We used factorial analyses to analyze the overall structural diversity (Figure 3) and phylogenetic trees (Figure S1) to examine genomic similarities among individuals, and observed a continuous distribution among plants, with very little overlap in the first plane of the factorial anal-

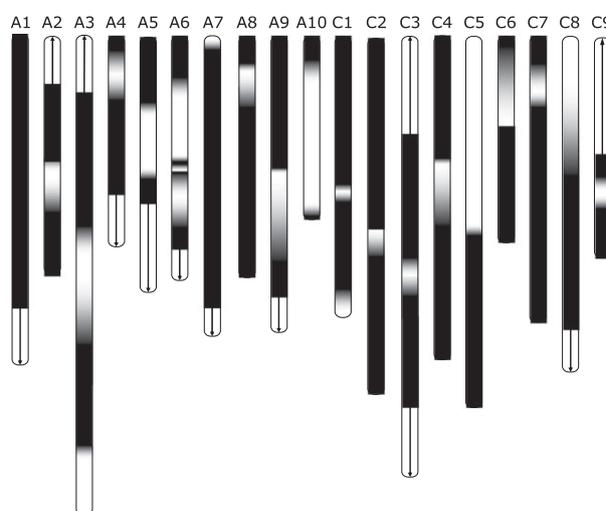


Figure 2. Graphical genotype obtained by aggregating chromosome rearrangements across the progenies of *Brassica napus* haploids.

The observed removed regions across the progenies of haploids were combined in one genetic map to show the total extent of genome reshuffling. On each linkage group, black areas represent the regions where at least one chromosome rearrangement was found in at least one progeny, while white areas represent the regions in which marker additivity was found across all progenies. Gray areas indicate genomic regions delineated by a rearranged locus (dark gray) or a non-rearranged locus (light gray). Other nomenclature is defined in Figure 1.

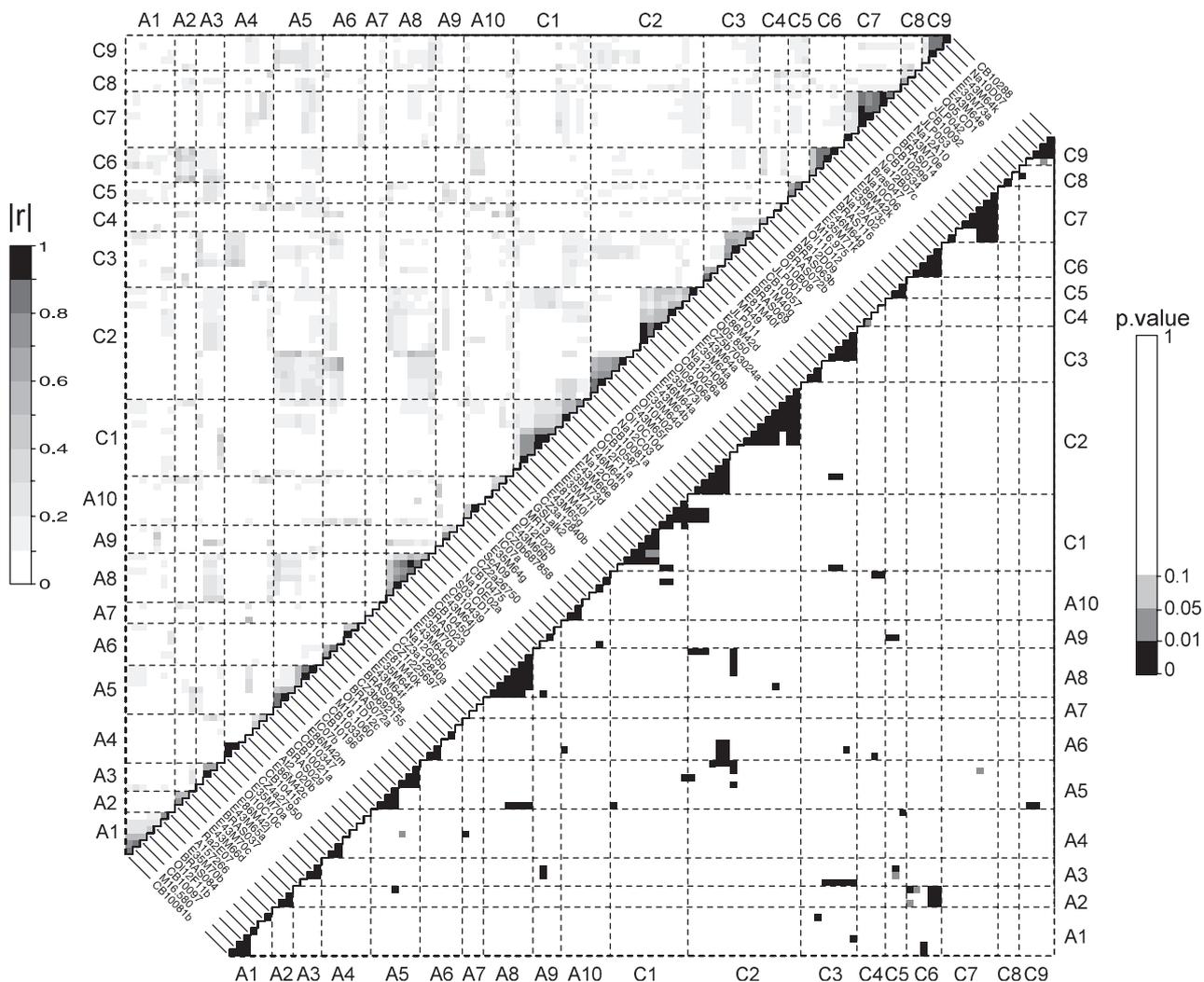


Figure 4. Linkage disequilibrium between all pairs of loci in the progeny of haploid *Darmor-bzh*. Estimated absolute square Pearson correlation coefficients $|r|$ for all pairs of markers (upper left triangle) are shown together with the corresponding P values from a Fisher test (lower right triangle). The strengths of correlations, as well as the significance level of P values after an False Discovery Rate correction, are indicated on a gray scale ranging from blank ($|r|$ close to 0 and the associated P value close to 1) to black ($|r|$ close to 1 and the associated P value close to 0). Loci indicated between the two triangles are ordered according to the map shown in Figure 1. Only polymorphic loci with <20% of data missing were plotted. The dotted lines indicate the separation between linkage groups.

We then tested whether rearrangements occurred or segregated independently from one another by analyzing correlations between the occurrence of rearrangements in two linkage groups (Figure S4) or at two loci (linkage disequilibrium; Figures 5 and S5). After correction for multiple comparisons, no or poorly significant correlations were found between linkage groups (corrected P value between 5 and 10%) that mostly involved A and C chromosomes (Figure S4). In contrast, strong and significant positive correlations were found between adjacent loci within a linkage group (Figures 4 and S5); these correlations were expected as a consequence of the concurrent loss of linked loci caused by proximal cross-overs. The significant correlations between locus plotted in Figures 4 and S5 show

that the frequency of PCR fragment loss at some loci changes based on the frequency of fragment loss at some other loci located on another chromosome. This indicates that the incidence of rearrangements occurred non-independently throughout the genome. However, the number of rearrangements observed in our two haploid progenies did not provide enough statistical power to identify and measure accurately the correlation between loci and linkage groups.

The distribution of rearrangements throughout the genome is not random; it depends on genome structure

Figure 1 showed that rearrangements are not randomly distributed among chromosomes. We used a generalized binomial log linear model to identify the main source of

Table 1 Deviance analysis of the reduced model for variation in the rearrangement rate obtained by selection based on the Bayesian information criterion

Effect	Kenward–Roger degree of freedom	Deviance	P value
Genotype of haploid parent	170	144.45	<0.0001
Genome	15.74	5.01	0.0253
Degree of collinearity	14.88	7.92	0.0049
Genetic distance from the centromere	134.1	76.36	<0.0001

variation among rearrangement rates. The only chromosome features that had a significant effect on the rearrangement rate were the genome origin, the degree of collinearity between homeologues, and the genetic distance from the centromere (Table 1). The effects of these genomic factors did not depend on the genotype or each other, as no significant interactions were detected between factors or genotype. To quantify the effects of these genomic features, we performed an analysis of variance on the probability of rearrangements occurring at each locus predicted by our model (Table S2).

The genetic distance from the centromere clearly had the most influence on the partitioning of rearrangements between loci. It explained 22% of variation in rearrangement rates throughout the genome (Table S2). Overall, the most distal loci were the most frequently rearranged (Figures 1 and S6). Only a small number of loci did not follow this rule (e.g. Na14F11b at the bottom of A1 in allohaploid *Darmor-bzh* progeny, Na14F11a at the bottom of C1 in allohaploid *Yudal* progeny).

To determine whether the non-random distribution of rearrangements within the chromosome originated from the non-random distribution of rearrangement breakpoints, we used a generalized binomial log linear model to study the factors influencing breakpoint distribution throughout the genome. The genetic distance from the centromere had no significant effect on the breakpoint rate if it was assumed that the breakpoints occurred in the middle of an interval delineated by the presence and absence of parental markers at two adjacent loci (Table S3). The main genomic factor explaining the distribution of breakpoints throughout the genome was the genetic size of the interval (Table S4). The breakpoint rate was positively and significantly correlated with the rate of homologous recombination between the two markers delineated this interval within the *Darmor-bzh* × *Yudal* reference genetic map ($r^2 = 0.3$, $P < 0.001$). Thus, the increase in rearrangement rate in the most distal chromosome regions stems mostly from the accumulation of distal rearrangements produced by single cross-overs distributed all along the centromere–telomere axis following a pattern similar to that of homologous cross-overs.

The second strongest genomic factor identified by our model was the degree of collinearity between homeologous chromosomes (Figure S6). This variable accounted for 7% of the rearrangement rate between loci and the breakpoint rate between intervals (Table S2). Totally collinear linkage groups (A1, C1, A2, C2, A3, A4 and A8) showed approximately twice as many rearrangements (Confidence Interval = 1.1–2.5) as partially collinear linkage groups (0.159 versus 0.086 missing regions per plant and per linkage group, $P < 0.0001$). This twofold difference is not simply due to the presence of simultaneous rearrangements on two chromosome arms as a significant difference remained even when only the simple distal rearrangements were considered and simultaneous rearrangement on both arms was excluded (0.105 versus 0.061 missing regions per plant and per linkage group, $P < 0.001$).

The genome (A or C) at which the locus is located had the least effect, but it was still significant, accounting for 4% of the rearrangement rate between loci (Table S2). Comparison of the rearrangement rate showed that allohaploid parental markers were lost more frequently than duplicated at several loci on the C genome (0.133 versus 0.094 missing regions per plant and per linkage group, $P < 0.0001$), whereas the opposite phenomenon was observed for corresponding homeologous A genome loci (Figure 1). Breakpoint rates were also significantly different between the two genomes (1.9 versus 2.4% in genomes A and C, respectively, $\chi^2 = 8.3$, $P < 0.001$). These differences cannot be explained by a difference in genome coverage or marker density as these were very similar for the A and C genomes (Table S5).

Finally, our very simple model, which included the genomic factors described above and the allohaploid genotype, was very good at predicting variation in the rearrangement rate, as the correlation between the expected and observed rearrangement rate was 0.7 (Table S2). Thus the genomic features identified here explain half of the variation in rearrangement rates between loci. The genetic distance from the centromere was the only genomic factor explaining the intra-chromosome variation, while the genome origin and degree of collinearity together explained the inter-chromosome variation. Finally our model predicts the variation in rearrangement rates with the genetic distance from the centromere for both the haploid parents and in relation to the degree of collinearity (Figure S6).

Relationship between chromosome rearrangements, fertility and meiotic behavior in the progeny of *B. napus* allohaploids

Comparison of meiotic behavior between the progenies of allohaploid and euploid plants showed that the chromosome rearrangements described above disrupt meiosis. Euploid plants showed 19 bivalents in all pollen mother cells, but the progeny of allohaploid *Darmor-bzh* and *Yudal* showed irregular meiosis, with only 69 and 80% of pollen mother cells

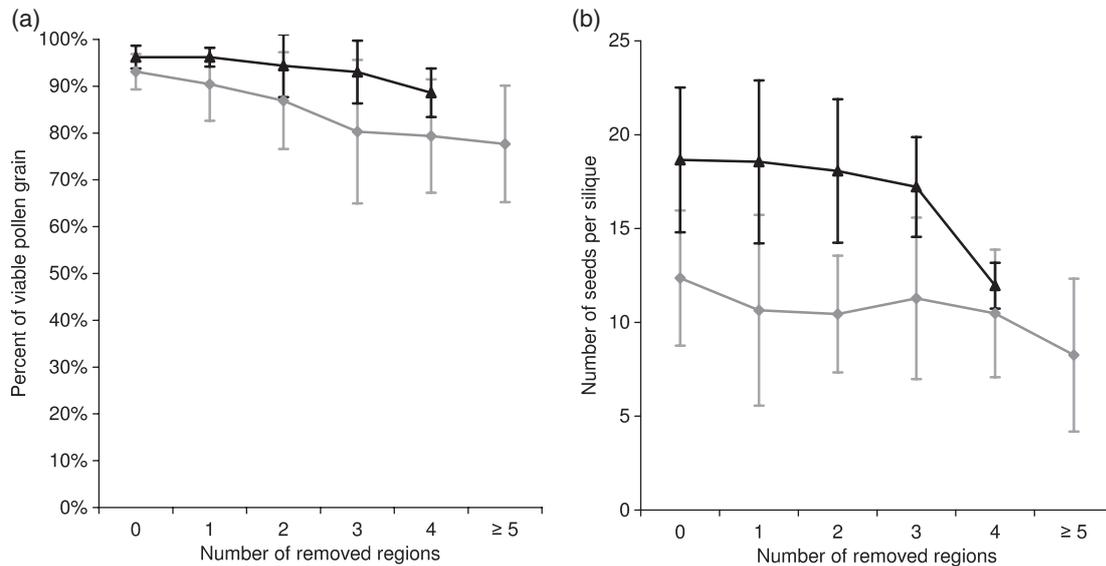


Figure 5. Variation in male and female fertility with the number of removed regions in the progeny of haploids.

(a) Male fertility: percentage of viable pollen grain estimated by counting grains stained with acetocarmine.

(b) Female fertility: number of seeds per silique.

Gray diamond, *Darmor-bzh*; black triangle, *Yudal*. Values are means \pm SE.

carrying 19 bivalents, respectively (out of 383 and 295 pollen mother cells observed, respectively). The meiotic behavior varied strongly between offspring within each progeny of allohaploids, as the proportion of pollen mother cells with 19 bivalents ranged from 25–100 to 55–95% in the progeny of allohaploid *Darmor-bzh* and *Yudal*, respectively (Table S6). Surprisingly, there was a weak relationship between variations in meiotic behavior and the number of removed regions carried by allohaploid offspring (Figure S7). We analyzed the effect of these disruptions on male and female fertility (Tables S7 and S8).

Male fertility, estimated by counting the number of viable pollen grain (Table S7), was significantly reduced in the progeny of the *Darmor-bzh* allohaploid compared to the progeny of euploid controls (82 versus 96% acetocarmine-stained pollen grains, $\chi^2 = 2043$, degree of freedom = 1, $P < 0.01\%$). Likewise, these plants showed reduced female fertility even though they produced a mean of 10.3 seeds per silique (Table S8). As expected, we observed that, on average, male and female fertility were increasingly affected as the number of removed regions in a plant increased (Figure 5). This relationship was illustrated by comparing the progeny of *Darmor-bzh* versus *Yudal* allohaploids. *Darmor-bzh* progeny showed more rearrangements and an increased number of less fertile plants than *Yudal* (82 versus 95% of acetocarmine-stained pollen grains, $\chi^2 = 9820$, degree of freedom = 1, $P < 0.1\%$; 10.3 versus 18.5 seeds per silique, $t = 14$, degree of freedom = 209, $P < 0.01\%$). However, the negative relationship between the number of rearrangements and fertility was weak; we observed some plants in which only one rearrangement was detected but

that showed much reduced male and female fertility, while others, which carried a minimum of five or six rearrangements, were almost as fertile as the controls (Figure 5). Thus, although it is clear that significant genome rearrangements decrease plant fitness, this is a weak effect that is difficult to predict based solely on marker additivity in the progeny of allohaploid plants.

DISCUSSION

Here we showed that meiosis in *B. napus* allohaploids generates a large amount of new genetic diversity, with multiple *de novo* chromosome rearrangements segregating in the progenies of these plants. These *de novo* chromosome rearrangements, which are remarkably tolerated by *B. napus* in the heterozygous state, are not randomly distributed throughout the genome. Their frequency largely depends on the genetic distance from the centromere, the degree of collinearity between homeologues, and the genome of origin. These effects are not genotype-dependent (*Darmor-bzh* versus *Yudal*) (see Nicolas *et al.*, 2009), and illustrate particular properties of polyploid plant genomes.

Allohaploid meiosis drives extraordinary new structural diversity

The rates of chromosome evolution are heterogeneous across taxa, suggesting that different aspects of their biology predispose some species to high rates of chromosome evolution while others remain quiescent. The results of our study demonstrate that non-homologous meiotic recombination can drive extraordinary new structural diversity (Figure 1). We observed that most progeny of *B. napus*

allohaploids displayed a original combination of distal and/or interstitial chromosome rearrangements. However, the overall structural diversity may still be under-estimated. A significant number of cryptic chromosome rearrangements remained undetected in the progenies, either because they occurred in regions that are poorly spanned by markers, or because individuals carrying them showed marker additivity in the case of reciprocal translocations (Nicolas *et al.*, 2007; Gaeta and Pires, 2010). Such reciprocal exchanges represent half of the genetic changes generated by meiotic cross-overs (Nicolas *et al.*, 2007); their widespread occurrence explains why the observed number of changes per plant was only poorly correlated with meiotic regularity. Because loss of the same region may correspond to various events (Nicolas *et al.*, 2007, 2009), it may be that almost all the plants derived from an allohaploid \times euploid cross are different from one another when the frequency of meiotic cross-overs is high. This extensive structural diversity is greater than that produced during meiosis of natural and synthetic euploid *B. napus* (Song *et al.*, 1995; Udall *et al.*, 2005; Lukens *et al.*, 2006). Multiple chromosome rearrangements were especially common in allohaploid progeny, with some plants showing up to ten chromosome rearrangements. We also observed a significant number of rearrangements on a series of chromosomes for which only a few or no rearrangements were detected in the progeny of natural and synthetic *B. napus* lines (e.g. A6, A7, A8, A10, C6 and C7; see Udall *et al.*, 2005; Gaeta *et al.*, 2007). Most of these rearrangements originated from cross-overs between homeologous chromosomes, although some resulted from cross-over formation between the duplicated regions retained from ancestral triplication of the Brassiceae (Nicolas *et al.*, 2007; discussed in Nicolas *et al.*, 2008). As a consequence, few, if any, regions of the genomes (15% of the genetic map) are protected from rearrangement in the progeny of *B. napus* allohaploids.

Our results provide interesting insights into the timing and forces that drive genome evolution across and within species, particularly allopolyploid species. We have shown that a burst of chromosome rearrangements can generate a wealth of structural variation in one go. This burst confirms, and extends at the whole-genome level, the conclusions of Szadkowski *et al.* (2011) who analyzed the progeny of *B. oleracea* \times *B. rapa* F₁ inter-specific hybrids on the A1–C1 chromosome pair. As there are slightly more cross-overs between non-homologous chromosomes in these hybrids than in *B. napus* allohaploids (Cifuentes *et al.*, 2010), an even greater structural diversity is expected in the progeny of these hybrids. Meiosis in inter-specific hybrids and allohaploids increases cross-over formation between chromosomes that would only rarely recombine otherwise (see discussion in Nicolas *et al.*, 2008). Allohaploid meiosis is also a powerful non-random mutational mechanism, as the rate of genetic change reported here (3.3% per locus and per generation

depending on the genotype) is far greater than the nucleotide substitution rate (approximately 10^{-6}) or the microsatellite mutation rate (approximately 10^{-3}) (Freeman *et al.*, 2006). All these results highlight the role that naturally occurring allohaploids (Ramsey and Schemske, 1998) may play in evolution; these plants are semi-fertile and generate progeny with transgressive traits when they are crossed with euploid forms (Sears, 1939; Lesins, 1952; Rao and Stokes, 1963; Jauhar, 2003). Thus, new phenotypic variation can result from the genetic changes described here. For example, Pires *et al.* (2004) reported that the chromosomal rearrangements originated from of cross-overs between homeologous chromosomes resulted in different flowering times.

In general, lines with extensive rearrangements also show low fertility (Gaeta and Pires, 2010). In this study, we observed that the male and female fertilities of *B. napus* allohaploid progeny were far from null. For example, an 'average' plant in the progeny of the *Darmor-bzh* allohaploid, which is expected to carry three inter-genomic exchanges (Nicolas *et al.*, 2009), has a pollen viability of 83% and produces a mean of 10.3 seeds per silique. This reduction in fertility is clearly not sufficient to prevent transmission of chromosome rearrangements to the next generations, opening up a window of opportunity for natural selection to purge the most deleterious rearrangements or promote some adaptive rearrangements to colonize new environments (Rieseberg *et al.*, 2003) and for genetic drift to fix some rearrangements in small populations.

Non-random pattern of *de novo* rearrangements throughout the genome

Nadeau and Sankoff (1998) raised the issue of 'whether the extent to which the location of rearrangement breakpoints is guided by natural selection to preserve particular gene combinations, by structural DNA features that promote or restrict chromosome breakage and repair, or by simply random processes'. In this study, we demonstrated that the distribution of chromosome exchanges was non-random (Figure 1), and identified a number of chromosome features that drive differential genomic permeability to chromosome rearrangements.

Using appropriate statistical modeling, we demonstrated that the position along the centromere–telomere axis was the main source of variation in the frequency of changes throughout the genome. Generally speaking, the more distal the locus, the higher the frequency of non-additivity in the progeny (Figures 1 and S5). This pattern is similar in wheat, in which the distance from the centromere is positively correlated with the rate of homologous recombination (Dvorak and Akhunov, 2005). We also observed that the genetic size of the interval, which measures the homologous recombination rate, was positively correlated with the frequency of breakpoints ($P < 0.001$). When more cross-overs occur in an interval between a pair of homologous chromosomes, the number of

cross-overs between non-homologous chromosomes in the same interval increases. The allocation of cross-overs between non-homologous chromosomes thus follows essentially the same rules as the distribution of homologous cross-overs. This indicates that the distribution of chromosome rearrangements is at least in part determined by the same 'DNA features that promote or restrict chromosome breakage and repair' between homologous chromosomes (Jones *et al.*, 2002). The low rearrangement rate observed within centromeric regions could be explained by the same genomic features that suppress homologous recombination in this region of chromosome in almost all species (see Drouaud *et al.*, 2006, for example). Thus, the observed pattern of genetic changes originated from the accumulation of distal chromosome rearrangements that were generated all along the chromosome in a pattern that mirrored the distribution of homologous cross-overs.

The observed pattern of structural change has several evolutionary implications. First, genes located in the distal region of the chromosomes have a greater chance of being deleted, duplicated or rearranged than more proximally located genes. In the long term, this could lead to chromosome structural stratification (Wang *et al.*, 2011a), with the distal regions evolving faster than more proximal regions. This is consistent with the theory that the gene distribution or order across the chromosome is under selective constraint (Pal and Hurst, 2003), as supported by the preferential presence of essential genes in low-recombination centromeric regions in yeast (Fischer *et al.*, 2006) and *C. elegans* (Johnsen *et al.*, 2000). Second, the genes located in the distal part of the chromosome have more chance of being exchanged or introgressed between related species than the proximally located genes. For instance, introgressive hybridization, which is common in plants, could drive a gradient of divergence between genes belonging to relatives from a same species complex along the centromere–telomere axis. Reticulation could therefore have a greater effect on distal than proximal genes.

The second most influential source of variation in the frequency of chromosome rearrangements in the genome is the level of collinearity between homeologous chromosomes, which explained 7% of the variation for both the breakpoint and rearrangement rates (Table S2). Pairs of totally collinear homeologues showed twice as many rearranged chromosomes as other less collinear chromosomes. The same trends were observed by Gaeta *et al.* (2007) and more recently by Xiong *et al.* (2011). The mechanisms by which the degree of collinearity changes the cross-over rate are not known, but it may be hypothesized that totally collinear homeologues have a higher propensity to have their two arms simultaneously bound by chiasmata (ring bivalents). This will increase the probability of detecting at least one rearrangement on either of these two arms, and is consistent with the fact that concurrent loss of two regions

on the same chromosome is rare and mainly observed for totally collinear homeologues (A1, C1, A2, C2, A4 and C3; Figure 1). Overall, our results confirm that chromosome structural differences result in different genomic permeability to genetic exchanges (Rieseberg *et al.*, 1995).

The last factor affecting the variation in rearrangements identified by statistical modeling was the genome of origin (A versus C). We observed that, on average, regions on genome C are lost more frequently than regions on genome A (Figure S6), whereas markers on genome A are duplicated more frequently than markers on genome C (data not shown). This effect is very weak at the genome-wide scale, and the extent of this bias is actually highly variable across the genome; some regions showed extensive asymmetry in the relative rate of A versus C rearrangements (e.g. A1/C1), while others showed no obvious trend. Very similar trends were observed in some newly synthesized *B. napus* (Udall *et al.*, 2005; Lukens *et al.*, 2006), but not in others (Szadkowski *et al.*, 2011). They cannot directly result from the molecular mechanisms that generate the rearrangements, because cross-overs lead to symmetrical exchanges (see Nicolas *et al.*, 2008; Gaeta and Pires, 2010) and thus an even number of genetic changes on both genomes. Instead, the greater maintenance of genome A integrity may indicate that rearrangements leading to the loss of some A genomic regions are counter-selected. This may be explained in a number of ways, including biased nucleo-cytoplasmic interactions (see Szadkowski *et al.*, 2010) or genomic dominance (Rapp *et al.*, 2009). As asymmetrical exchanges concerned localized regions, our results strongly suggest that this asymmetry depends on the genomic content and features of the exchanged region.

In conclusion, our study showed that the distribution of meiotically driven chromosome rearrangements is not simply guided by random processes but instead results from the combined effect of recombination and selection. We highlighted the role of three genomic features in the non-random allocation of non-homologous cross-overs throughout the genome, which are partially correlated with the distribution of homologous cross-overs. A comparison of cross-over distribution in various genomic contexts (allohaploid versus euploid) may help unravel the complex interactions between recombining genes and the genome structure, which controls the cross-over distribution (Nicolas *et al.*, 2008). The recent and ongoing assembly of the *B. napus*, *B. rapa* and *B. oleracea* genome sequences will help to address these questions (Wang *et al.*, 2011b, brassica info: <http://www.brassica.info/resource/sequencing.php>).

Within a broader context, our results also highlight the role that allohaploid meiosis plays in the evolution of polyploid species. Allohaploid meiosis generates a burst of new genome combinations that can be transmitted to euploid populations by unreduced gametes. Depending on the regions exchanged, some of these genetic changes may

lead to variations in gene contents (duplication/deletion), gene composition (replacement by related duplicated copy) or the gene environment (promoter regions), driving transgressive phenotypes that may be adaptive or not. Because allohaploids arise spontaneously in wild populations of several species, including *B. napus* (Thompson, 1969; Stringham and Downey, 1973), and because they are semi-fertile when they are crossed with euploid forms (Ramsey and Schemske, 1998), allohaploids could contribute to adaptation and speciation in a similar way as homoploid species (Rieseberg *et al.*, 1995, 2003; Livingstone and Rieseberg, 2004). It will be interesting to evaluate this scenario using *B. napus* as a model system.

EXPERIMENTAL PROCEDURES

Plant material

The plant material used in this study has been described previously (Nicolas *et al.*, 2009). Allohaploid plants (AC; 19 chromosomes) from *B. napus* cultivars *Darmor-bzh* and *Yudal* were used as female parents in crosses with male *Yudal* and *Darmor-bzh* euploids, respectively. A total of 117 and 103 plants carrying 38 chromosomes were sorted in the two progenies.

Cytological and fertility observations

Floral buds were first fixed in Carnoy's solution (ethanol/chloroform/acetic acid, 6:3:1) for 24 h. A minimum of ten pollen mother cells per plant were observed at metaphase I from anthers squashed and stained in a drop of 1% acetocarmine solution. A total of 34 and 19 F_1 plants were analyzed in the allohaploid progenies of *Darmor-bzh* and *Yudal*, respectively (Table S6).

Male fertility was estimated by counting the number of pollen grains stained by acetocarmine solution, a specific DNA stain, to give an estimation of pollen viability. A total of 600 and 800 pollen grains from at least two flowers were observed for every plant in the allohaploid progenies of *Darmor-bzh* and *Yudal*, respectively. Seven and eight euploid *Darmor-bzh* and *Yudal* plants were included as a control (Table S7). We used the number of seeds per silique after back-crossing to a euploid parent to estimate individual female fertility for a total of 111 and 100 progenies of allohaploid *Darmor-bzh* and *Yudal*, respectively (Table S8).

Molecular analysis

Molecular analyses and detailed information on the markers used have been described previously by Nicolas *et al.* (2009) and are summarized in Table S5. The products of meiotic cross-overs were detected by (i) scoring the presence/absence of allohaploid parental alleles at every locus independently of the others (Table S1), (ii) considering the concurrent loss of linked loci as a consequence of a single rearrangement, and (iii) considering the number of breakpoints on linkage groups that occurred when one marker was present but the marker located on the other side of the same interval was absent. Duplications were detected for a subset of loci using allele peak height as described previously (Nicolas *et al.*, 2007).

Statistical analysis

Statistical and molecular analysis were performed on 111 and 101 progenies of allohaploid *Darmor-bzh* and *Yudal*, respectively, after

removing allohaploid offspring that had more than 20% of data missing for all loci. Almost all statistical and graphical analyses were performed using R version 2.12.1 software (R Development Core Team, 2010, <http://www.R-project.org>), notably the `chisq.test` (for χ^2), `fisher.test` (for the exact Fisher test) and `t.test` (for Student's *t* test) functions.

Diversity analysis. Diversity analyses were performed on the basis of the absence and presence of the allohaploid parental allele at each locus in their two allohaploid progenies after removing loci that had more than 30% of data missing. To analyze the diversity in both F_1 populations, we first performed a factorial analysis based on a dissimilarity matrix produced by simple matching of allohaploid parental alleles between the F_1 offspring with 86 co-dominant markers (Figure 3). To explore the relationship between F_1 offspring in more depth, we analyzed the two allohaploid progenies separately, because approximately 40% of the markers were dominant and therefore impossible to genotype in one of the two haploid progenies.

The mean, median and distribution of dissimilarity between F_1 offspring were analyzed (Figure S2), and a phylogenetic tree was constructed using hierarchical clustering with ward methods for each F_1 population (Figure S1). Factorial and phylogenetic analysis were performed using DARwin software version 5.0.157 (Perrier and Jacquemoud-Collet, 2006, <http://darwin.cirad.fr>).

Pearson correlations and the χ^2 test were used to analyze the extent to which two linkage groups or two loci were independently rearranged (Figures 4, S4 and S5). To visualize the results, we used the `Corrplot` and `LDheatmap` packages in R for linkage groups and loci, respectively.

Genome-wide quantitative analysis of chromosome rearrangements and breakpoints. We used a generalized mixed log linear binomial model to test, quantify and classify how the overall variation in chromosome rearrangements and breakpoints can be partitioned into components representing the genotype of the haploid parent (*Darmor-bzh* or *Yudal*) (abbreviated as Gnt) and various levels of chromosome organization: genome origin (A or C; abbreviated as Gnm), degree of collinearity (partial or total; abbreviated as Col), the position along the centromere–telomere axis (Figure S6), i.e. the genetic distance from the centromere (DistCent), and the genetic size of the interval between adjacent polymorphic markers (Size). Genome-wide integration of *B. napus* maps (Wang *et al.*, 2011a) allowed assignment of all the *Darmor-bzh* \times *Yudal* linkage groups to the A and C genomes, respectively. Linkage groups were then classified as totally (across their whole length) or only partially collinear with their homeologues using information provided by Parkin *et al.* (2003, 2005). The genetic distance from the centromere (DistCent) for each locus was measured using the genetic position of the centromere identified in Pouilly *et al.* (2008). The information on these structural features is summarized in Table S2. Rearrangements and breakpoints were considered as binary variables (presence = 1/absence = 0) for each marker or each interval in each plant. They were assumed to follow binomial distributions with the probability parameter *P* depending on genomic features through the logit link function and through linear mixed model predictor formulae including the following terms:

- (i) for rearrangements:
 - fixed effects: Gnt + Gnm + Col + DisCent
 - random effects: LG + Locus
- (ii) for breakpoints:
 - fixed effects: Gnt + Col + Size
 - random effects: Interval

where LG, Locus and Interval denote qualitative factors defined by the linkage group, locus and interval of each observation, respectively. This generalized linear mixed model was fitted using the glimmix procedure with SAS 9.2[®] software (SAS Institute Inc., <http://www.sas.com/>). The selection procedure for both the fixed and random variables (Table S9 and S10), the reliability of the model (Figure S8) and the estimation of parameters in our final model are described in Appendix S1. We used the Pearson correlation between the rearrangement/breakpoint rate observed and the rate predicted by the final model to evaluate the prediction quality. The contributions of the variables to the rearrangement and breakpoint rates were quantified and compared by performing an analysis of variance on the least square means produced by the final models (Tables S2 and S4).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Phylogenetic tree of the progenies of haploid *Darmor-bzh*.

Figure S2. Distribution of the dissimilarity between pairs of plants from progeny of haploid *Darmor-bzh* and *Yudal*.

Figure S3. Diversity of chromosome rearrangements in 12 euploid plants derived from a haploid × euploid cross.

Figure S4. Correlation plot for the simultaneous occurrence of rearrangements between linkage groups in progeny of haploid *Darmor-bzh* and *Yudal*.

Figure S5. Linkage disequilibrium between all pairs of loci in progeny of haploid *Yudal*.

Figure S6. Variation in rearrangement rate with genetic distance from the centromere in the progeny of haploid *Darmor-bzh* and *Yudal*.

Figure S7. Relationship between meiotic behavior and the number of removed regions in progeny of haploid *Darmor-bzh* and *Yudal*.

Figure S8. Graphical analysis of the residuals in the reduced best model obtained using the Bayesian information criterion for the rearrangement and breakpoint rates.

Table S1. Raw genotyping data for the presence/absence of haploid parental alleles in the progenies of haploid *Darmor-bzh* and *Yudal*.

Table S2. Variance analysis on the mean probability of rearrangements at each locus predicted by the best model using the Bayesian information criterion.

Table S3. Deviance analysis of the reduced model for variation in the breakpoint rate obtained by selection based on the Bayesian information criterion.

Table S4. Variance analysis on the mean probability of breakpoints within each interval throughout the genome predicted by the best model using the Bayesian information criterion.

Table S5. Description of the genome structure and molecular analysis performed at the linkage group level.

Table S6. Raw data for cytological observations of the meiotic behavior in pollen mother cells in the progeny of haploid *Darmor-bzh* and *Yudal*.

Table S7. Raw data for male fertility in the two progenies.

Table S8. Raw data for female fertility in the two progenies.

Table S9. Selection of the best model for rearrangement rates using the Bayesian information criterion.

Table S10. Selection of the best model for breakpoint rates using the Bayesian information criterion.

Appendix S1. Selection procedure for the random and fixed variables in the rearrangement and breakpoint models.

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