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The first meiosis of resynthesized *Brassica napus*, a genome blender

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Summary

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- Polyploidy promotes the restructuring of merged genomes within initial generations of resynthesized *Brassica napus*, possibly caused by homoeologous recombination at meiosis. However, little is known about the impact of the first confrontation of two genomes at the first meiosis which could lead to genome exchanges in progeny. Here, we assessed the role of the first meiosis in the genome instability of synthetic *B. napus*.
- We used three different newly resynthesized *B. napus* plants and established meiotic pairing frequencies for the A and C genomes. We genotyped the three corresponding progenies in a cross to a natural *B. napus* on the two homoeologous A1 and C1 chromosomes. Pairing at meiosis in a set of progenies with various rearrangements was scored.
- Here, we confirmed that the very first meiosis of resynthesized plants of *B. napus* acts as a genome blender, with many of the meiotic-driven genetic changes transmitted to the progenies, in proportions that depend significantly on the cytoplasm background inherited from the progenitors.
- We conclude that the first meiosis generates rearrangements on both genomes and promotes subsequent restructuring in further generations. Our study advances the knowledge on the timing of genetic changes and the mechanisms that may bias their transmission.

Introduction

Compelling evidence has accumulated for ancient and/or recent genome-wide polyploidy among angiosperms (Doyle *et al.*, 2008; Soltis *et al.*, 2009; Van de Peer *et al.*, 2009). Studies of natural allopolyploids (i.e. hybrids possessing two or more different sets of chromosomes) have revealed their divergence from the simple addition of progenitor genomes. Numerous genetic and epigenetic mechanisms have been shown to generate the wide range of structural and functional genome modifications associated with merged genomes arising from polyploidy events (Comai, 2005; Chen & Ni, 2006; Leitch & Leitch, 2008).

Some newly formed or resynthesized allopolyploids seem to be quiescent with few changes in genome organization, such as cotton and *Spartina* (Liu *et al.*, 2001; Baumel *et al.*, 2002), whereas most others are extensively remodelled within a few generations. Pairing between nonhomologous chromosomes at meiosis is thought to be responsible for many genomic restructuring events, such as homoeologous translocations and aneuploidy, as chiasmatic pairs between homoeologous chromosomes and univalent formation lead to abnormal genetic composition in gametes (Pikaard, 2001; Leitch & Leitch, 2008).

Resynthesized *Brassica* provides a very good example of meiosis-driven genome reshuffling in allopolyploids. The seminal work of Song *et al.* (1995) on resynthesized *Brassica* species was among the first to show that major genomic changes occur as a result of meiotic irregularities. *Brassica napus*, an amphidiploid polyploid originating from different spontaneous crosses between *B. rapa* (*n* = 10) and *B. oleracea* (*n* = 9) (Song & Osborn, 1992), has since become one of the most widely used models to address this issue further (Osborn *et al.*, 2003; Pires *et al.*, 2004; Udall *et al.*,

2005; Albertin *et al.*, 2006; Lukens *et al.*, 2006; Gaeta *et al.*, 2007). However the timing of genetic changes remains unclear. For instance, Lukens *et al.* (2006) observed few rearrangements in the S0 generation of resynthesized *B. napus* polyploids, but advocated that undetected rearrangements after S0 meiosis (Parkin *et al.*, 1995; Udall *et al.*, 2005) may become visible in further generations. Song *et al.* (1995) confirmed that genomic restructuring was frequently observed at the fourth generation of selfing. By contrast, Gaeta *et al.* (2007) detected this phenomenon in some progeny plants of selfed pollinated S0 individuals, rearrangements becoming homozygous in the bulking process at the third generation of selfing for most collinear chromosomes, and at the fifth generation for almost all chromosomal pairs. In this latter study, distortion in the transmission of rearrangement was detected, with the A genome being more frequently retained than the C genome (Gaeta *et al.*, 2007). All these observations do not necessarily indicate that genome merging in S0 plants creates a burst of rearrangements at the first meiosis, which could then freely segregate and become homozygous in the latter generations. Indeed, Udall *et al.* (2005) observed that *de novo* chromosomal rearrangements occurred frequently in the vicinity of pre-existing translocations, which may suggest that only a few rearrangements produced during the meiosis of S0 plants give rise to an increasing number of genome changes in the following generations. Additional complexity comes from the fact that resynthesized *B. napus* plants produced with various progenitors display different levels of meiotic irregularities (univalent and multivalent formation), suggesting that the extent of homoeologous recombination is influenced by the progenitor genotype and/or their combination (Prakash & Hinata, 1980; Attia & Robbelen, 1986).

In order to obtain knowledge on the timing of genetic changes that occurred among *B. napus* synthetics, we investigated the role of the first meiosis in generating rearrangements between homoeologous chromosomes, and determined the importance of selection for viable gametic configurations.

We focused our study on the homoeologous chromosomes A1 and C1, which are the most collinear within the haplotype, and the most frequently rearranged pair in synthetic *B. napus* (Gaeta *et al.*, 2007). To take into account the effect of the genome progenitor on the nature and frequency of rearrangements, three different S0 synthetic amphidiploids were used. To determine the precise transmission frequencies associated with the duplication and deletion of genetic regions from A1 and C1, we derived large populations from crosses between the three synthetic amphidiploids and a natural *B. napus*.

We established, through molecular analysis and cytological observations, that the first meiosis generates the genetic changes observed in the progeny. This study also sheds light on the timing of rearrangements, their consequences and

the possible mechanisms responsible for the stabilization of *B. napus* synthetics.

Materials and Methods

Production of synthetic S0 material and F₁ crosses with a natural *B. napus*

Three synthetic lines were produced by A-M. Chèvre *et al.* (unpublished) by crossing *B. rapa* L. and *B. oleracea* L., plants belonging to different cultigroups. Parental plants were the selfed progeny of a *B. rapa* plant 'C1.3' belonging to a fodder variety named 'chicon' var. *rapifera* and three homozygous doubled haploid lines: 'Z1', a *B. rapa* var. *trilocularis* oilseed variety (provided by K. C. Falk, Agriculture and Agri-Food Canada, Ottawa, ON, available on request), 'HDEM', a *B. oleracea* var. *botrytis italica*, and 'RC34', a *B. oleracea* var. *alboglabra*. Interspecific hybridization between HDEM (maternal parent) and Z1 led to a haploid synthetic called BoEMZ (*B. oleracea* as maternal parent, prefixed Bo). Reciprocal hybridization between RC and C1.3 led to the haploid synthetics BoRCC (*B. oleracea* as maternal parent) and BrCRC (*B. rapa* as maternal parent, prefixed Br). The corresponding doubled hybrids (BoEMZ38, BoRCC38 and BrCRC38, respectively) were obtained after colchicine treatment, as described by Chevre *et al.* (1989). Three populations of 87, 91 and 92 plants were obtained by crossing the three S0 synthetic plants as maternal parent to a natural *B. napus* cv. Darmor (an oilseed rape winter type line) as male parent, and were called BoEMZ38 × Darmor, BoRCC38 × Darmor and BrCRC38 × Darmor, respectively.

Meiotic behaviour and chromosome counting of a panel of F₁ plants

Floral buds were fixed in Carnoy's solution (ethanol–chloroform–acetic acid, 6 : 3 : 1) for 24 h and stored in 50% ethanol. Observations on pollen mother cells (PMCs) were performed on anthers squashed and stained in a drop of 1% acetocarmine solution; 18–43 PMCs per plant were observed at metaphase I to establish the meiotic behaviour of a panel of individuals from BoRCC × Darmor or BrCRC × Darmor populations.

Fluorescence *in situ* hybridization (FISH) to detect A–C pairs at meiosis and translocation in S0 × Darmor progenies

Bacterial artificial chromosome (BAC) FISH analyses were carried out on meiotic chromosomes for synthetic *B. napus* BrCRC38, BoRCC38 and BoEMZ38 at metaphase I in PMCs. We used a repeated sequence within a BAC (BoB014O06 from *B. oleracea)* as a probe for the C genome

sequences (Alix *et al.*, 2008). This 'genomic *in situ* hybridization (GISH)-like' BAC labelling hybridized specifically to all C genome chromosomes in previous *B. napus* FISH experiments (Leflon *et al.*, 2006; Nicolas *et al.*, 2007, 2009). Briefly, BAC clone BoB14O06 was provided by Warwick Horticultural Research International (Wellesbourne, Warwick, UK) (Howell *et al.*, 2002), labelled by random priming with biotin-14-deoxyuridine triphosphate according to the manufacturer's protocol (Invitrogen, San Diego, CA, USA; Life Technologies) and used as a probe at 100 ng per slide. Fluorescence images were captured using a CoolSnap HQ camera (Photometrics, Tucson, AZ, USA) on an Axioplan 2 microscope (Zeiss, Oberkochen, Germany) and analysed using MetaVue (Universal Imaging, Downington, PA, USA).

Mapping translocation in F_1 plants on A1 and C1 chromosomes

DNA extraction was performed as described by Lombard & Delourme (2001). In order to establish a genetic mapping position for all A1 and C1 markers used (some loci that mapped to one homoeologue were unable to be mapped on the other because of a lack of polymorphism in all our populations), we integrated the A1 and C1 linkage groups from the progenitor lines (C1.3 × Z1 F2 and C10 × HDEM F2 populations, respectively) with those from the *B. napus* DH Darmor-bzh × Yudal population (Lombard & Delourme, 2001; Rocherieux *et al.*, 2004; Delourme *et al.*, 2006) using Biomercator (Arcade *et al.*, 2004). The two linkage groups A1 and C1 are shown for each population (see Supporting Information Fig. S1). All microsatellite markers are publicly available and originated either from the Biotechnology and Biological Sciences Research Council, Swindon, Wiltshire, UK (prefixed with Na, Ol or Ra; Lowe *et al.*, 2004), CELERA Company, Alameda, California, USA (prefixed with CB, BRAS or MR; Piquemal *et al.* (2005)) or Agriculture and Agri-Food Canada (prefixed with sN). Markers prefixed with the letters CZ have been developed from coding sequences of *Arabidopsis* and *Brassica* (H. Belcram & B. Chalhoub, unpublished; primer sequences are available on request to Genoplante). PCR assays were conducted essentially as described in Delourme *et al.* (2006). Forward primers were tailed with an M13 extension to be revealed by fluorescent technology (Schuelke, 2000). PCR products were analysed on a 16-capillary ABIPrism 3130xl, as described by Esselink *et al.* (2004). We scored the frequency of rearrangements in gametes by surveying the disappearance of parental bands from synthetic *B. napus* (i.e. A1 loci from *B. rapa*; C1 loci from *B. oleracea*). To consider small- and large-scale rearrangements at our locus density, one marker loss per chromosome was considered to represent a rearrangement.

Determination of the allele copy number based on the allele dosage on a fluorescent capillary genetic analyser in an S0 synthetic crossed to a natural *B. napus*

Fluorescent PCR analysis was used to determine the allele copy number of A1 and C1 chromosomes from the synthetic parents BoEMZ38, BoRCC38 and BrCRC38 in the crosses BoEMZ38 × Darmor, BoRCC38 × Darmor and BrCRC38 × Darmor. Data collection and statistical analysis were performed as described in Nicolas *et al.* (2007). Briefly, the peak area of the A1 or C1 marker from the synthetic plant was compared with the peak area of the recurrent parent in S0 × Darmor progenies. Linear relationships could be visually identified for six loci (five markers), allowing the distinction of three lines, one with the loss of the synthetic allele, one with a single dose of the synthetic allele and the last with a double dose of the synthetic allele. To adjust the quality of the data, individuals with a peak area lower than 600 for either the synthetic peak or the recurrent parent peak were considered as missing values. We performed statistical analysis according to Nicolas *et al.* (2007) and repeated the molecular assay for four loci that confirmed previous observations.

Statistical analysis to detect the effect of rearrangements on S0 × Darmor meiosis

Statistical analyses were performed on the number of univalents, multivalents and the frequency of cells with 19 bivalents, reflecting global pairing, aberrant pairing frequencies and normal gamete formation, respectively.

The effects of complete and partial nullisomy/trisomy, A1 or C1 rearrangements and A1 rearrangement sizes were tested for all the variables by ANOVA with the procedure GLM from SAS software (SAS Institute Inc., Cary, North Carolina, USA). The normality of the distribution of residuals was tested using Shapiro–Wilk's test. The homogeneity of variances was checked with Bartlett's test. We compared the classes of plants using the Student–Newman–Keuls' test ($\alpha = 0.05$).

Results

Cytological evidence for A–C recombination at first meiosis of synthetic *B. napus*

We evaluated cytogenetically the extent to which A and C chromosomes pair at meiosis (metaphase I) to assess the effect of the first meiosis on the genesis of genetic changes. Metaphase I observations in PMCs for the three S0 plants BoEMZ38, BoRCC38 and BrCRC38 showed between 37.5% and 60% of cells with 10 AA bivalents and nine CC bivalents, whereas 30–47.5% of cells had at least one pair involving A–C chromosomes (Table 1, Fig. 1).

Table 1 Frequencies of metaphase I in pollen mother cells (PMCs) involving A and C pairing configuration at first meiosis of euploid S0 synthetic *Brassica napus*

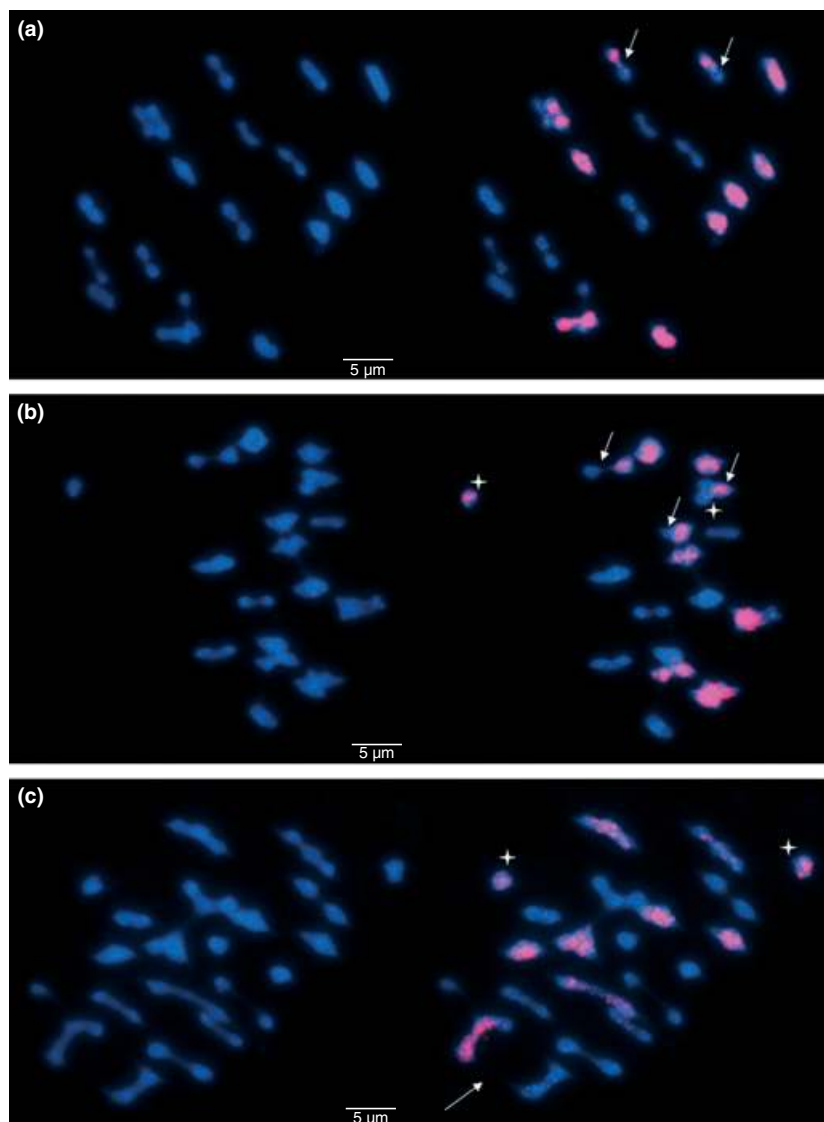| Synthetic S0 plant	No. obs. cells	10 A–A + 9 C–C bivalents (%)	Cells with univalent A and/or C (%)	Cells with one A–C bivalent (%)	Cells with two or more A–C bivalents (%)	Multivalent involv. A–C chr. (%)	Cells with A–C pairs (%)
BoEMZ38	40	40	33	7.50	35	10	47.50
BoRCC38	20	60	15	5	15	10	30
BrCRC38	16	37.50	37.50	6.25	18.75	12.50	31.25

Fig. 1 A–C bivalents and multivalents at metaphase I of first meiosis of euploid S0 synthetic *Brassica napus*. Unlabelled chromosomes (A genome) are in 4′,6-diamidino-2-phenylindole (blue, left) and Bob14O06 (GISH-like signal on the C genome) is stained in red by avidin–Texas red antibody (right). (a) EMZ.38 plant with two A–C bivalents (white arrows); (b) EMZ.38 plant with three A–C bivalents (white arrows) plus two univalents, one A and one C (white stars); (c) CRC.38 plant with one quadrivalent (AACC) (white arrow) plus two A univalents (white stars).

Owing to the frequent occurrence of A–C bivalents and/or multivalents, gametes with unbalanced chromosomal composition and/or carrying chromosomal rearrangements were both expected, unless they were eliminated by selection. Molecular markers were used to decipher which of the two situations occurred for A1 and C1.

Detection of trisomics/monosomics in the progeny of S0 synthetic *B. napus*

We assayed the parental marker transmission in the three populations produced by crossing the three S0 synthetic allotetraploids with the same natural *B. napus* genotype. Eight markers located on A1 and 11 markers located on C1
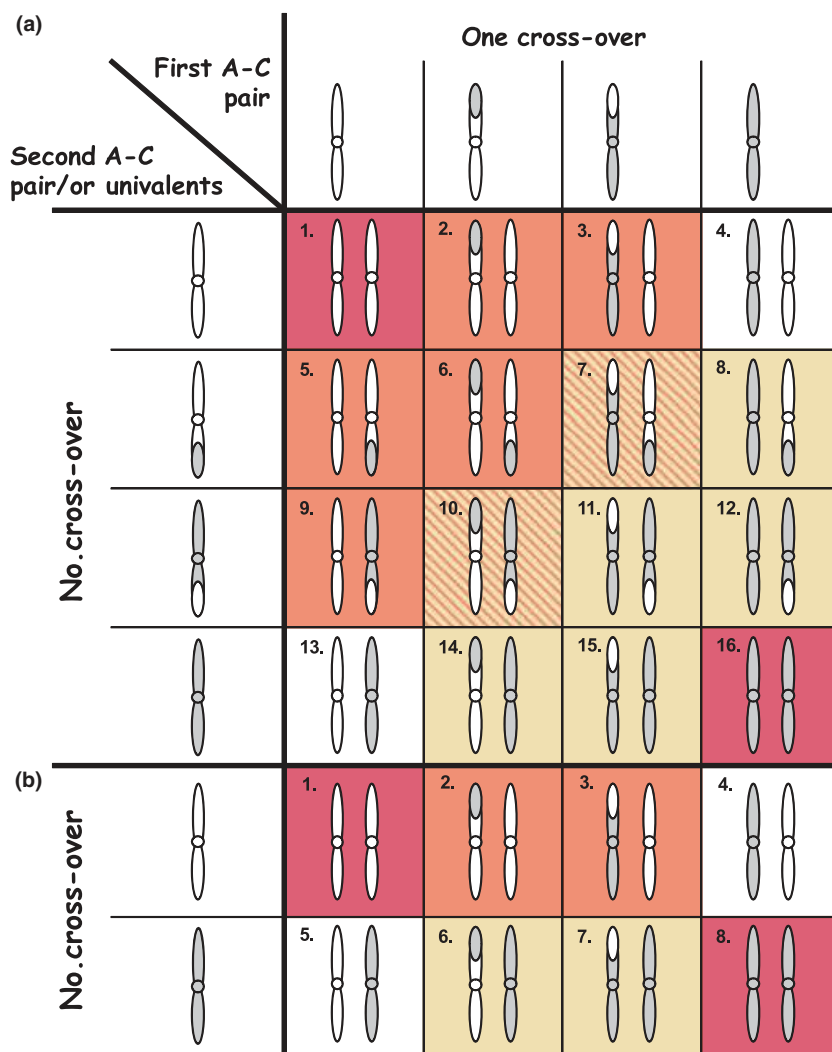
Fig. 2 Expected genomic structure in gametes (n = 19) resulting from cross-over in the presence of one or two A1–C1 bivalents at first meiosis of synthetic *Brassica napus*. Chromosome in white represents the A1 chromosome, and chromosome in grey represents the C1 chromosome. Translocations resulting from homoeologous pairing are represented by ellipses in the respective colour to their chromosomal origin. Two cases of meiosis are represented, in which one A1–C1 pair is formed (one cross-over) and for the remaining A1 and C1 chromosomes: (a) a second A1–C1 pair occurs with a cross-over at a different position, or (b) two univalents segregate in the gametes. Noncoloured cells [cells 4 and 13 for (a), cells 4 and 5 for (b)] represent parental-type gametes without translocations and chromosome loss. Cells in dark red [cells 1 and 16 for (a), cells 1 and 8 for (b)] represent gametes with two copies of the homoeologous chromosome and the loss of the reciprocal one. Orange cells [cells 2, 3, 5, 6 and 9 for (a), cells 2 and 3 for (b)] represent gametes carrying C1 region loss, whereas light-brown cells [cells 8, 11, 12, 14 and 15 for (a), cells 6 and 7 for (b)] represent gametes carrying A1 region loss. Striped orange and light brown cells [cells 10 and 7 for (a)] represent gametes carrying both A1 and C1 marker losses.

(Fig. S1) were used to genotype these populations, with six A1 and four C1 markers being common to all populations. These markers allowed the integration of at least 80% of the A1 and C1 linkage groups.

Over the 270 plants from the three populations, 5.2% of plants (4.6%, 5.4% and 5.5% for BoEMZ38 × D, BoRCC38 × D and BrCRC38 × D, respectively) lacked all the markers from one parental linkage group (either A1 or C1), whereas markers of the homoeologous linkage group were present. Cytological counting of chromosomes on these plants indicated that they all had 38 chromosomes. Among the 15 plants that could be analysed for the dosage of synthetic alleles, six plants (out of seven) showed the concurrent loss of markers from A1 and the duplication of markers from C1, whereas eight plants (out of eight) showed the concurrent loss of markers from C1 and duplication of markers from A1. Cytological counting indicated that all 14 plants had 38

chromosomes per PMC, which suggests that they were trisomic for one chromosome and monosomic for its homoeologue (carrying an A1 and a C1 from the natural *B. napus* cv. Darmor paternal gamete). Fig. 2 provides a model showing that gametes with a normal chromosome number, i.e. 19 chromosomes, can carry two copies of A1 (with no C1; Fig. 2(a,b), cell 1) or two copies of C1 (with no A1; Fig. 2(a), cell 16; Fig. 2(b), cell 8) when one or two A1–C1 bivalents are formed, therefore called nullisomic/disomic gametes.

As expected from the meiotic behaviour of S0 resynthesized *B. napus*, monosomics/trisomics for A1 and C1 were detected in the progeny of these synthetics. These plants were discarded from further analysis to avoid biasing the deletion frequency per locus. Therefore, populations were reduced to 82, 87 and 86 individuals for BoEMZ38 × Darmor, BoRCC38 × Darmor and BrCRC38 × Darmor, respectively (255 plants).
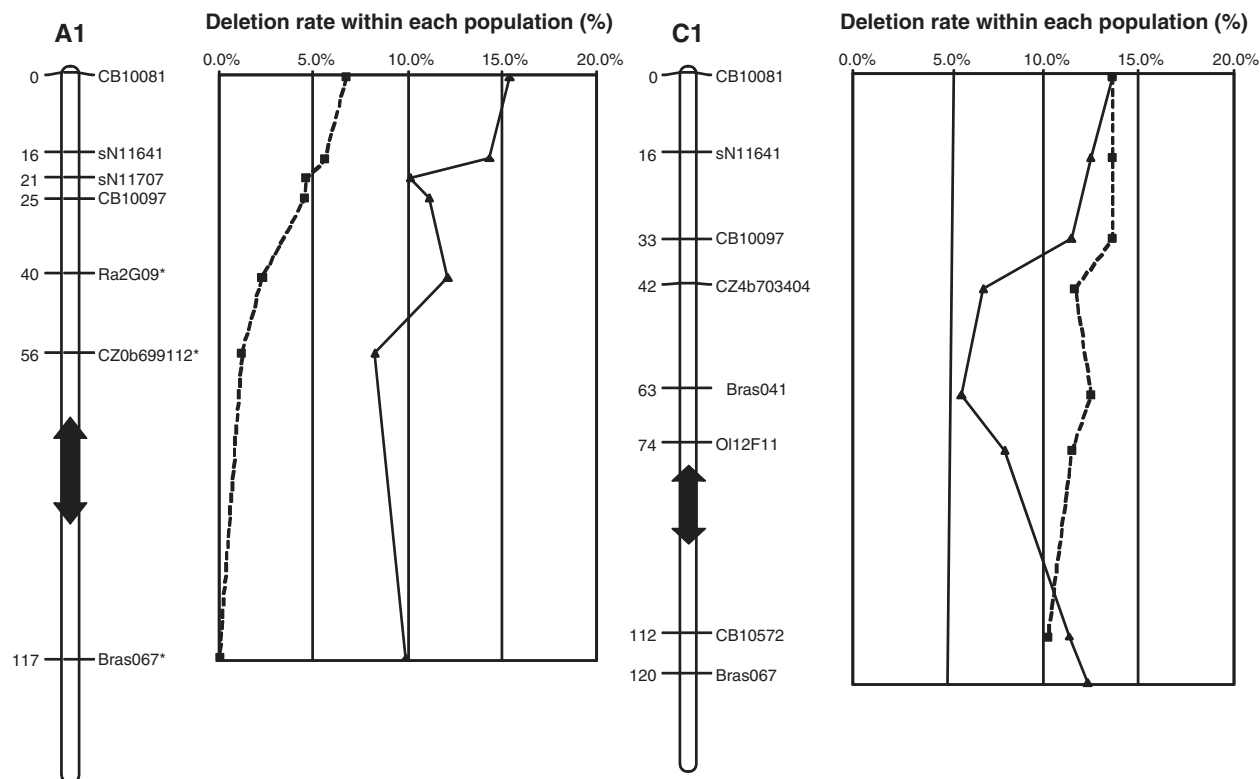
Fig. 3 Observed deletion rate of alleles from A1 or C1 linkage groups following first meiosis of two S0 synthetic *Brassica napus* in the *B. rapa* cytoplasm population BrCRC38 × Darmor (squares and broken line) or the *B. oleracea* cytoplasm population BoRCC38 × Darmor (triangles and full line). Significant differences between the deletion rates of the two populations are indicated with an asterisk. Black arrows represent the presumed position of centromeres according to Pouilly *et al.* (2008). Cumulated genetic distances (Kosambi) are indicated on the left side of the linkage groups in centimorgans.

Detection of chromosomal rearrangements in the progeny of S0 synthetic *B. napus*

We genotyped the progeny of synthetic *B. napus* to detect chromosomal rearrangements, resulting in the loss of some but not all markers of a given linkage group. Plants carrying rearrangements on A1 and/or C1 represented 40.4% of the 255 plants observed. Plants with rearrangements on both A1 and C1 chromosomes accounted for 10.6%. None of the 255 plants surveyed displayed a concurrent loss of both homoeologous loci on A1 and C1 chromosomes. Considering 510 total A1 and C1 chromosomes in the 255 plants, 74.3% were not rearranged, 19.4% had a single breakpoint and 6.3% had multiple breakpoints. Single breakpoints along the chromatid explain the difference between the frequencies of marker losses along A1 and C1 chromosomes, with distal markers tending to be more frequently lost than pericentromeric ones (see Fig. 3 for an example on BoRCC38 × Darmor and BrCRC38 × Darmor populations).

To estimate the rate of marker loss as a result of cross-overs between A1 and C1 at meiosis, we determined whether any loss of marker on A1 was associated with the duplication of markers from the C1 homoeologous region (and vice versa). Concurrent loss and duplication were examined for six homoeologous loci (see Materials and Methods). Among 24 loss events on A1, where duplicated regions could be scored, 18 (75%) were associated with duplication of the C1 allele, whereas, for 48 loss events on C1, where duplicated regions could be detected, 44 (91.7%) individuals showed duplication of A1. Among the 255 plants studied, seven (2.7%) presented a double dose of A1 without any marker loss on both chromosomes, whereas seven others (2.7%) showed the reciprocal situation with a double dose of C1. One plant (0.4%) had both A1 and C1 doubled without any deletions.

Our marker data confirmed that the first meiosis of resynthesized *B. napus* generates extensive genetic changes (plants with unbalanced chromosome composition, chromosomal rearrangements) that are transmitted to the progenies. This indicated that all genetic changes were not discarded by selection. We then examined whether differences could be detected among the three populations.

Table 2 Percentages of plants carrying rearrangements on A1 and/or C1 chromosomes in the progeny of each S0 synthetic crossed to a natural *Brassica napus*

Population	BoEMZ38 × Darmor (%)	BoRCC38 × Darmor (%)	BrCRC38 × Darmor (%)
A1 total	30.50	26.40	7
C1 total	37.80	26.40	25.60
Total	50.00	41.40	30.20

Populations were composed of 82, 87 and 86 individuals for BoEMZ38 × Darmor, BoRCC38 × Darmor and BrCRC38 × Darmor, respectively.

Some rearrangements are differentially counter-selected in a population-dependent and chromosome-dependent manner

We compared the frequencies of rearrangements between homoeologous chromosomes, the distribution of rearrangements along the chromosomes and the type of rearrangements (using breakpoint number per plant and chromosome as an estimator) among the three populations.

Significant differences were observed in the progenies of the reciprocal synthetics (BrCRC38 vs BoRCC38) that shared a common nuclear genetic background but different maternally donated cytoplasms. BrCRC38 × Darmor showed an excess of plants without rearrangement (69.8% vs 58.6%, respectively, $P < 0.01$, χ^2 test) and a lower frequency of plants carrying A1 marker loss (7% vs 26.4%, respectively, $P < 0.05$, χ^2 test) than BoRCC38 × Darmor (Table 2). Multiple breakpoints were less frequent on A1 in the BrCRC38 × Darmor population compared with BoRCC38 × Darmor ($P < 0.05$, χ^2 test between the distribution for the two populations). By contrast, no difference was found between C1 marker loss frequencies in the progenies of BoRCC38 and BrCRC38.

Conversely, populations BoRCC38 × Darmor and BoEMZ38 × Darmor with a *B. oleracea* cytoplasm were not significantly different for: (1) the frequency of plants carrying marker losses; (2) the frequency of losses along A1 and C1 chromosomes; (3) the breakpoint number per chromosome (Table 3). These data showed that the genetic backgrounds on *B. oleracea* cytoplasm did not influence the frequency of rearrangements.

Our results suggest that the frequency and size of chromosomal translocations are biased in a population-dependent manner in the progenies of S0 *B. napus* synthetics. We therefore investigated whether these rearrangements would also affect the regularity of meiosis in offspring carrying genetic changes.

Rearrangements and their 'size' condition the normal meiotic behaviour of S0 × Darmor progenies

We established the meiotic behaviour for 17 plants from BoRCC38 × Darmor and BrCRC38 × Darmor populations (Table 4), selected on the basis of the size of their rearrangements, ranging from intact A1 and C1 chromosomes to complete exchange of a chromosome. To represent the meiotic behaviour with synthetic variables, we scored univalent and multivalent frequencies to reflect the pairing ability between chromosomes, and the frequency of cells with 19 bivalents to reflect the formation of normal gametes.

We first tested the impact of complete and partial monosomic/trisomic plants on the meiotic behaviour, and found that plants monosomic for A1 and trisomic for C1 showed significantly more univalents and a lower ratio of cells with 19 bivalents ($P < 0.01$, ANOVA test) than controls that had a balanced (one A1 and one C1 inherited from the re-synthesized parent) composition. By contrast, plants with the reciprocal genetic structure (complete or partial monosomic plants for C1 and trisomic for A1) were not significantly different from controls.

We then tested the impact of chromosomal rearrangements on A1 or C1 on the regularity of meiosis. Plants rearranged for A1 or C1 showed significantly more univalents than nonrearranged plants ($P < 0.05$, ANOVA test), but the accumulation of both A1 and C1 rearrangements did not induce more univalents than expected.

To assess the effect of rearrangement size further, we focused on the A1 chromosome and contrasted plants without rearrangements (BoRCC38 × Darmor024, BrCRC38 × Darmor068 and BrCRC38 × Darmor004 in Table 4), plants with subtelomeric rearrangements (BrCRC38 × Darmor027 and BoRCC38 × Darmor077 in Table 4) and plants with a half-arm loss (BoRCC38 × Darmor073 and BoRCC38 × Darmor011 in Table 4). The latter plants

Table 3 Repartition of plants according to the number of breakpoints per rebuilt linkage group (LG) A1 and C1 in the progeny of a three-synthetic-S0 *Brassica napus* crossed with cv. Darmor

Breakpoints per LG		0	1	2	3	4	Total LG
BoEMZ38 × Darmor	A1	58	13	10	0	1	82
B. oleracea cytoplasm	C1	51	22	8	1	0	82
BoRCC38 × Darmor	A1	62	19	4	2	0	87
B. oleracea cytoplasm	C1	64	18	4	0	1	87
BrCRC38 × Darmor	A1	80	6	0	0	0	86
B. rapa cytoplasm	C1	64	21	1	0	0	86

Table 4 Meiotic behaviour from pollen mother cells (PMCs) of S0 × Darmor individuals carrying various chromosomal rearrangements on A1 and/or C1 chromosomes

Plant	A1 chromosome						C1 chromosome									Mean meiotic behaviour					
	CB 10081*	sN 11641	sN 11707	CB 10097	Ra 2G09*	CZ 0b699112 (Bras 067 →)	Bras 067	CB 10081*	sN 11641	CB 10097	CZ 4b703404	OI 12F11	Bras 041	CB 10572* (Bras 067 →)	Bras 067	Obs. PMC	I	II	III	IV	V
BrCRC38 × Darmor027					1			1						1	–	20	1.6	17.7		0.3	
BoRCC38 × Darmor077					1			1						1		21	0.7	18.4	0.2		
BoRCC38 × Darmor006								1						1		20	0.7	17.5	0.3	0.4	
BrCRC38 × Darmor034								2						2		20	0.8	17.2	0.6	0.3	
BoRCC38 × Darmor009	1							1						1		21	1.5	17.8	0.3		
BoRCC38 × Darmor015	1				2			1								43	2.6	16.9	0.1	0.3	
BrCRC38 × Darmor049	1				2			–						1	–	20	0.6	18		0.4	
BoRCC38 × Darmor084	1				2			1						1		21	0.9	18	0.4		
BoRCC38 × Darmor007	–				2											23	1.1	16.9	0.2	0.6	0.04
BoRCC38 × Darmor046	2				2											20	0.7	18	0.05	0.3	
BrCRC38 × Darmor030	2				2											18	0.72	18.1	0.4		
BoRCC38 × Darmor073								2								21	0.7	17.4	0.5	0.2	
BoRCC38 × Darmor011								1								20	1.1	17.5	0.1	0.4	
BoRCC38 × Darmor008	1				1			1						1		22	0.9	16.8	0.1	0.8	
BoRCC38 × Darmor024					1			1						1		21	0.5	18.3	0.1	0.1	
BrCRC38 × Darmor068	1				1			1						1		23	0.4	18.5	0.1	0.1	
BrCRC38 × Darmor004	1							1						1		22		18.5	0.3		

Meiotic behaviour is established by the mean number of univalents (I), bivalents (II), trivalents (III), quadrivalents (IV) and pentavalents (V). Rearrangements on A1 and/or C1 chromosomes for each plant are characterized by molecular markers: dark grey represents marker loss; light grey represents marker presence. Markers with asterisk are used to detect allele duplication, and the number of copies according to dosage intensity is reported in their respective column. Marker intervals containing centromeres are indicated with a black arrow (According to Pouilly et al., (2008)). Missing data are represented with (–).
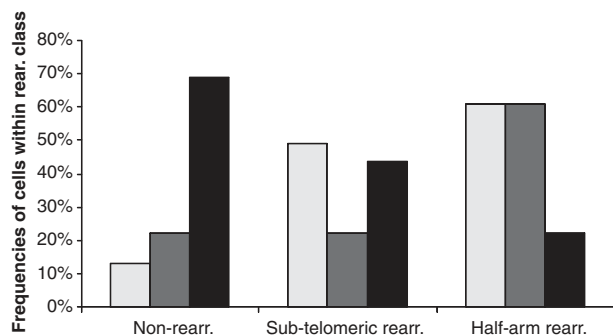
Fig. 4 Effect of genetic size of rearrangement on meiotic behaviour in S0 × Darmor progenies. Percentage of cells with univalent(s) (light grey), multivalent(s) (dark grey) and 19 bivalents (black) on plants without rearrangements (percentage based on three plants), with subtelomeric rearrangements (percentage based on two plants) and half-arm rearrangement (percentage based on two plants).

showed more univalents and multivalents than nonrearranged plants (Fig. 4), and therefore had fewer cells carrying 19 bivalents ($P < 0.05$, ANOVA test). By contrast, plants with subtelomeric rearrangements had intermediate frequencies, but were not significantly different from both classes of plants.

All these results suggest that viable genetic changes produced during the meiosis of S0 synthetics may have an effect on the occurrence of new genetic changes in later generations.

Discussion

Meiosis drives extensive genetic changes that are transmitted to the progenies of S0 *B. napus* synthetics

Our results showed that the meiosis of resynthesized *B. napus* acts as a genome blender.

Cytological observations first showed that recombination frequently occurred between homoeologous chromosomes. For the three synthetics analysed, metaphase I A–C bivalents and multivalents were present in 30–47.5% of PMCs, and up to 10% of total cells had more than three A–C chromosome associations. These findings extend the observations of multivalents by Attia & Robbelen (1986) and Prakash & Hinata (1980), thanks to the use of BAC FISH with a 'GISH like' pattern that allows the discrimination of A and C chromosomes.

We then demonstrated that extensive genetic changes were transmitted to the progenies of resynthesized *B. napus*. Over all populations, 5.2% of plants had an abnormal chromosomal composition [whilst carrying 38 chromosomes; see Lim *et al.* (2008) for a similar example in natural *Tragopogon* populations] and 40.4% of plants carried rearrangements on A1 and/or C1 chromosomes, at a higher rate compared with Udall *et al.* (2005). These genetic changes were expected as a result of chromosome mis-segregation and/or cross-overs

between homoeologous chromosomes during the meiosis of S0 synthetics (see Pikaard, 2001; Leitch & Leitch, 2008).

Finally, our molecular data confirmed that most chromosomal rearrangements (81.8% on A1 and 94.4% on C1) were likely to be the products of cross-overs between homoeologous A1 and C1 chromosomes, because they resulted in the concurrent loss and duplication of the homoeologous region (Gaeta *et al.*, 2007; Nicolas *et al.*, 2007). This rate is slightly higher than that obtained in the progenies of haploids from natural *B. napus* on A1–C1 (73%; Nicolas *et al.*, 2007, 2009). In addition, the occurrence of multiple breakpoints for 6.3% of the rearranged chromosomes suggests that more than one cross-over per homoeologous A1–C1 chromosome would occur at first meiosis, although part of these rearrangements could also originate from one single breakpoint on each pair of A–C bivalents (Fig. 2(a), cells 6 and 11). Our results are coherent with those from natural *B. napus* that confirm pre-existing A1 and C1 translocations in varieties and the ability of this homoeologous pair to recombine *de novo* (Udall *et al.*, 2005), with a similar increase in deletion frequencies towards telomeres for haploids of natural *B. napus* (Nicolas *et al.*, 2007).

According to our results, we would expect to detect rearrangements in the first selfing progeny of S0 synthetic *B. napus*, which have already been detected before the bulking process for a few loci and plants in previous work on *B. napus* synthetics with a *B. oleracea* cytoplasm (Gaeta *et al.*, 2007). However, large selfing populations would be required to detect accurately such genetic changes, because similar rearrangements must be generated and transmitted through male and female meioses to the same S1. Several successive generations, up to third or fifth according to Song *et al.* (1995) and Gaeta *et al.* (2007), are usually required for a rearrangement to become homozygous in a selfed plant (Gaeta & Pires, 2010).

Different genome changes are observed in the progenies of reciprocal synthetics

Although our results have indicated that many of the meiosis-driven genetic changes are transmitted, comparisons of the progenies of the reciprocal synthetics suggest that cytoplasm-dependent selective effects may also bias the transmission of some rearrangements.

Comparison of the BoRCC38 × Darmor and BrCRC38 × Darmor populations revealed a bias in favour of A1 chromosome retention in BrCRC38 × Darmor progeny, without any effect on the frequency of C1 rearrangements. As the two synthetic S0 parents (BoRCC38 and BrCRC38) shared the same nuclear genetic background and displayed almost the same proportion of cells showing A–C chiasmatic associations (Table 1), we would hypothesize that some rearrangements resulting in the loss of DNA from A1 were counter-selected and discarded from the progeny of S0 synthetic

BrCRC38 (gametic or zygotic selection) that carried a *B. rapa* cytoplasm. Presumably, the preferential retention of A1 DNA in the progeny of BrCRC38 could thus be the result of nucleocytoplasmic interactions that were not detected with the *B. oleracea* cytoplasm (Tsunewaki, 1993). It is noteworthy that Song *et al.* (1995) did not detect any significant cytoplasmic effect on the transmission of rearrangements between (advanced) reciprocal synthetic *B. napus*, but a significantly biased loss of B-genome restriction fragment length polymorphisms among F5 individuals derived from an initial AABB synthetic allopolyploid generated with the A cytoplasm. Our hypothesis thus needs to be evaluated by further studies.

By contrast with the comparison of BoRCC38 × Darmor and BrCRC38 × Darmor populations, we did not detect any difference in genetic changes on A1–C1 in the progenies of BoEMZ38 and BoRCC38 that both carried the *B. oleracea* cytoplasm (Table 3). At first sight, this result is in contrast with the difference in meiotic behaviour observed between the two plants, BoEMZ38 showing more cells with A–C associations than BoRCC38. However, this difference mostly originated from a larger number of cells showing two or more A–C bivalents in BoEMZ38 compared with BoRCC38 (Table 1), suggesting that another pair of homoeologues rather than A1–C1 may recombine in BoEMZ38 but not in BoRCC38. Notwithstanding the validity of this hypothesis, cytoplasm-specific and chromosome-specific differences in rearrangement frequency can be expected among the progenies of different S0 synthetics. Our results suggest that this would affect the regularity of meiosis in plants of the subsequent generations, especially if they carry rearrangements of different size.

Chromosomal restructuring at the heterozygous stage from the first meiosis disturbs the meiosis of S0 × Darmor progenies

We observed that the larger the rearrangements, the less regular the meiosis. This sounds like a truism at first sight, but specific comparisons provide considerably more information. Notably, we observed that the regularity of meiosis was more severely affected by the loss of chromosome A1 than the loss of C1. Although we do not know what is responsible for this difference, this result provides a potential explanation of why more frequent and larger C genomic regions (compared with A genomic regions) are lost in subsequent selfing generations (Gaeta *et al.*, 2007). In addition, rearrangements were shown to hamper normal chromosome pairing and recombination during meiosis; thus, they are likely to increase the frequency of restructuring in subsequent generations, certainly much further than at the fifth generation of selfing (A-M. Chèvre, unpublished), and are responsible for the polyploid ratchet described by Gaeta *et al.* (see detailed explanations in Gaeta & Pires, 2010). It

is probable that the establishment of a genetic control of homoeologous pairing (described in *B. napus* by Jenczewski *et al.*, 2003), as well as illegitimate recombination (Wang *et al.*, 2009), contribute to stabilize the genomes after several successive generations and under purifying selection.

Our results indicate that, in *B. napus* synthetics, genome restructuring by homoeologous recombination at first meiosis is influenced by cytoplasmic interactions. This process is ongoing over generations, creating novel ratios of homoeologous regions that modulate the plant phenotype (see Pires *et al.*, 2004 for flowering time), and should be addressed in terms of evolutionary consequences.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 A1 and C1 linkage groups used for the three S0 × Darmor populations.

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