Genetic factors inherited from both diploid parents interact to affect genome stability and fertility in resynthesized allotetraploid B. napus

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Research Article

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Genetic factors inherited from both diploid parents interact to affect genome stability and fertility in resynthesized allotetraploid *Brassica napus*

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Abstract

Established allopolyploids are known to be genomically stable and fertile. However, by contrast, most newly resynthesized allopolyploids are infertile and meiotically unstable. Identifying the genetic factors responsible for genome stability in newly formed allopolyploid is key to understanding how two genomes come together to form a species. One hypothesis is that established allopolyploids may have inherited specific alleles from their diploid progenitors which conferred meiotic stability. Resynthesized *B. napus* lines are often unstable and infertile, unlike *B. napus* cultivars. We tested this hypothesis by characterizing 41 resynthesized *B. napus* lines produced by crosses between eight *B. rapa* and eight *B. oleracea* lines for copy number variation resulting from non-homologous recombination events, and fertility. We resequenced eight *B. rapa* and five *B. oleracea* parent accessions, and analyzed 19 resynthesized lines for allelic variation in a list of meiosis gene homologs. SNP genotyping was performed using the Illumina Infinium *Brassica* 60K array for three individuals per line. Self-pollinated seed-set and genome stability (number of copy number variants) were significantly affected by the interaction between both *B. rapa* and *B. oleracea* parental genotypes. We identified thirteen putative meiosis gene candidates which were significantly associated with frequency of copy number variants and which contained putatively harmful mutations in meiosis gene haplotypes for further investigation. Our results support the hypothesis that allelic variants inherited from parental genotypes affect genome stability and fertility in resynthesized rapeseed.

Keywords: Copy number variation, fertility, genome stability, meiosis, resynthesized *Brassica napus*, Single nucleotide polymorphism

Introduction

Polyploidy is the heritable condition of possessing more than two sets of chromosomes (Comai 2005). The extra set/s of chromosomes may originate from the same individual or from within the same species, which is referred to as autopolyploidy, or from hybridization between two different species, known as allopolyploidy (Otto 2007). Polyploidy confers a number of evolutionary advantages (Soltis and Soltis 2000), and many crop species are allo- or autopolyploids. One important consequence for allopolyploids is that disomic inheritance of each parental locus may be upset, and genetic heterozygosity between the parental species may not remain fixed after whole genome duplication (Ramsey and Schemske 2002).

The *Brassica* genus is one of 51 genera in the tribe *Brassiceae* belonging to the crucifer family (*Brassicaceae*) and is the most economically important genus within this tribe (Rakow 2004). It is an interesting model for allopolyploid formation in agricultural crops as six agriculturally significant species share a genomic interrelationship (NU 1935). *Brassica napus* (genome $A_nA_nC_nC_n$) was spontaneously formed by recent allopolyploidy between ancestors of *B. oleracea* (Mediterranean cabbage, genome C_oC_o) and *B. rapa* (Asian cabbage or turnip, genome A_rA_r) in the last 7500 years, and is thought to be polyphyletic in origin (Allender and King 2010; Chalhoub *et al.* 2014). Therefore, the *Brassica* genus and most especially *B. napus* are increasingly receiving attention as a model for regulation of meiosis in a young polyploid crop species (Mason and Snowdon 2016).

Synthetic polyploids can be produced through genome doubling of diploid plants or hybrids via methods such as chemical treatment with colchicine (Spoelhof *et al.* 2017). However, most synthetic polyploids remain largely unstable in terms of meiosis and genome inheritance (reviewed by Pelé *et al.,* 2018). Meiotic aberrations are common in newly formed autopolyploid and allopolyploid plants, which negatively affects their fertility and early demographic success (Ramsey and Schemske 2002; Gaeta and Pires 2010). Synthetic allopolyploids lack the phenotypic and genomic stability of established allopolyploids (Soltis and Soltis 1995; Pikaard 1999; Comai *et al.* 2000). Abnormal phenotypes and frequent failure of pollen and embryo development (Schranz and Osborn 2000; Comai *et al.* 2003) as well as widespread changes in gene expression have been observed in other synthetic allopolyploids (Kashkush *et al.* 2002). Although not all newly resynthesized allopolyploids are genomically unstable (Comai *et al.* 2000; Wang *et al.* 2006; Novikova *et al.* 2017; Chen *et al.* 2020), most are, including synthetic *Brassica* allotetraploids (Song *et al.* 1995), and this has been attributed

to abnormal meiosis (Szadkowski *et al.* 2010). This meiotic instability involves homoeologous pairing or interactions between the closely related A and C genome chromosomes during meiosis (Comai 2000; Nicolas *et al.* 2007, 2012; Leflon *et al.* 2010). Although several studies have produced and investigated synthetic *Brassica* e.g. (Abel *et al.* 2005; Mason *et al.* 2010; Szadkowski *et al.* 2010; Girke *et al.* 2012; Jesske *et al.* 2013; Karim *et al.* 2014), almost all synthetic *Brassica* lines investigated so far appear to be meiotically unstable (Chen *et al.* 2011; Gaebelein and Mason 2018).

The question then is why the established *B. napus* species is stable and the resynthesized lines are unstable. One hypothesis is that *B. napus* may have gained genetic control via the inheritance of specific alleles from its diploid progenitor species (Mason *et al.* 2015a). A few studies have been conducted to explain genome instability in resynthesized *Brassica napus* allotetraploids (Gaeta *et al.* 2007; Szadkowski *et al.* 2010; Xiong *et al.* 2010), and recently several quantitative trait loci were identified to be present in natural *Brassica napus* that confer reduced homoeologous recombination rates (Higgins *et al.* 2020). However, no study to date has investigated multiple genotypes of resynthesized *B. napus* in order to test the idea that allelic variation inherited from the progenitor species conferred meiotic stability to natural *B. napus*. In this study, we aimed to test the hypothesis that allelic variants inherited from diploid progenitor species *B. rapa* and *B. oleracea* affect the frequency of homoeologous recombination in resynthesized *B. napus*, and hence that inherited allelic variation may have conferred genomic stability to resynthesized *B. napus* lines.

Materials and Methods

Description of plant material

The materials used in this study comprise resynthesized *B. napus* seeds derived from crosses between homozygous *B. rapa* and *B. oleracea* parents as described in (Abel et al. 2005), where these are referred to as “spring-type domesticated lines”. The parental genotypes are either doubled haploid or highly inbred lines. C- genome genotypes are either cauliflower (*B. oleracea* var. *botrytis*) and Chinese Kale (*B. oleracea* var. *alboglabra*), A- genome genotypes are yellow sarson (*B. rapa* ssp. *trilocularis*), oilseed turnip (*B. rapa* ssp. *oleifera*), and Chinese cabbage (*B. rapa* ssp. *Pekinensis*) (Supplementary File 1). Abel et al. (2005) produced seeds from 336 cross-combinations between twenty-one *B. rapa* (maternal parent) and sixteen *B.*

oleracea lines including a core set of 64 cross-combinations between eight *B. rapa* and eight *B. oleracea* lines**.** Seeds from 41 resynthesized *B. napus* genotypes produced from crosses between eight *B. rapa* (A4, A6, A7, A8, A9, A13, A16 and A19), and eight *B. oleracea* parent genotypes (C34, C36, C37, C38, C42, C46, C47 and C49) via embryo rescue, chromosome doubling and self-pollination (S1 generation), as well as their *B. rapa* and *B. oleracea* parent genotypes, were used in this study. Established *Brassica* cultivars were used as controls for fertility in our experiment: winter-type *B. napus* 'Darmor', spring-type *B. napus* 'Argyle', and semi-winter type *B. napus* 'Ningyou7', as well as *B. rapa* var. *oleifera* (unknown accession) and *B. oleracea* var. *botrytis* 'NGB 1810.2'.

The resynthesized *B. napus* lines are represented by codes in the form "A1C1", where "A1" is the *B. rapa* parent genotype and "C1" is the *B. oleracea* parent genotype. In the present study, three seeds from each of 41 resynthesized genotypes and cultivars of established *B. napus*, *B. rapa*, and *B. oleracea* used as controls were sown in quick-pots and seedlings transferred to small pots without vernalization between September and November 2017 under glasshouse conditions at Justus Liebig University (JLU). Eight *B. rapa* parent genotypes (A4, A6, A7, A8, A9, A13, A16, and A19) as well as six *B. oleracea* lines (C34, C36, C37, C38, C46, and C47) used to produce the resynthesized *B. napus* lines were likewise sown on the 12th of September 2019 under the same glasshouse conditions at JLU as the resynthesized lines . All lines except C38 successfully germinated and produced plants. Three plant replicates from each genotype were then isolated in bags at flowering to ensure self-pollination.

Assessment of purity in resynthesized *B. napus* lines

The purity of 41 resynthesized *B. napus* genotypes with SNP genotyping information was assessed. Eight parent *B. rapa*; five DH lines (A6, A7, A8, A9, and A13) and three inbred lines (A4, A16, A19) as well as eight *B. oleracea* genotypes; five DH lines (C34, C36, C37, C38, C42) and three inbred lines (C46, C47, and C49) were used to produce the resynthesized lines (Abel et al 2005). Resynthesized lines produced by DH parental crosses between *B. rapa* and *B. oleracea* (AA × CC) are expected to be completely homozygous. Therefore, individuals of the same progeny sets having the same maternal *B. rapa* (AA) or paternal *B. oleracea* (CC) parents are expected to be non-segregating. We assessed purity using two criteria: 1) the absence of segregation pattern among progeny sets; and 2) the absence of continuous blocks of heterozygous (AB) calls across the A and the C genome in all individuals.

Fertility assessment in resynthesized *B. napus* lines

Three parameters were scored to describe fertility in resynthesized lines: pollen viability, total number of seeds produced per plant, and number of seeds produced per ten pods. Pollen viability was assessed for two freshly opened flowers per plant, and pollen grains stained with 1-2% acetocarmine solution (Leflon *et al.* 2006). At least 600 pollen grains per plant were counted and pollen viability was assessed using a light microscope (Leica DMR, Leica Microsystems), assuming darkly stained (red) pollen grains were viable, and weakly stained or shrivelled pollen grains were non-viable. The total number of self-pollinated seeds produced per plant and the number of seeds produced per ten pods were counted for each plant after harvesting.

DNA extraction and genotyping using the Illumina Infinium *Brassica* 60K SNP array

Young leaf samples were collected in 2 ml micro-centrifuge tubes at the 4 to 6 leaf stage of plant development. DNA was extracted for 41 resynthesized lines (123 plants) using the BioSprint 96 plant work station (Qiagen, Hilden, Germany) according to the manufacturer's instructions (http://qiagen.com/). Single nucleotide polymorphism (SNP) genotyping was carried out using the high-throughput Illumina Infinium 60K *Brassica* SNP array for the resynthesized lines. Hybridization protocols were performed according to the manufacturer's instructions for all samples.

SNP data was analysed, visualized, and exported into text files using Genome Studio v2.0.4 software (Illumina Inc., San Diego CA, USA). All 52 149 SNPs were exported for the A and C genome after application of the recommended "brassica60K" cluster file (Clarke *et al.* 2016). Top BLAST alignment hits for the SNP probes against the A and C genomes of the reference genome sequence of Darmor-*bzh* version 8.1 (Bayer *et al.* 2017) were used for genome position information. Hits to unplaced contigs were removed from further analyses. Data from samples of each *Brassica* species sourced from Mason et al. (2015b) were used as controls for filtering SNPs. SNPs which mapped to the A genome but which amplified in *Brassica carinata* (BBCC) and *Brassica oleracea* (CC) genotype controls as well as SNPs which mapped to the C genome but amplified in *Brassica juncea* (AABB) and *Brassica rapa* (AA) genotype controls in > 50% of the controls were filtered out. SNP markers with > 50% heterozygous AB calls in all five *B. napus* homozygous control cultivars (Boomer, Monty_028DH, Surpass400_024DH, Trilogy and Westar_10DH) as well as SNPs which showed > 99% missing calls (NC) across all lines were removed. Further filtering steps included the removal of SNPs with ≥ 80% AB calls across individuals. After filtering, 21 938 SNPs were retained: 8369 SNPs in the A genome and 13569

SNPs in the C genome (Supplementary File 2). Genotype calls were then converted to homozygous/heterozygous calls (0 and 2 for homozygous and 1 for heterozygous) and incidence of missing calls represented by NA. Since the resynthesized lines were produced by chromosome doubling of parent F_1 AC hybrids using colchicine, the allotetraploid hybrids are expected to be homozygous. After quality filtering of SNPs, we used the filtered SNPs to plot dendrograms separately for both the A and C genome parents (Supplementary Figs. 1and 2).

Detection of copy number variation (CNV) in resynthesized *B. napus* lines

A copy number pipeline was developed in R (Schiessl et al., unpublished). The pipeline uses the logR ratios (Supplementary File 3) to make plots for every individual line based on estimated cut off values to score copy number variants. Log R ratios estimate relative fluorescence intensity for each SNP marker, and are an output metric of Illumina GenomeStudio, the program used to call SNPs from raw data. For every SNP, we screened a diverse population representative for the diversity among natural *B. napus* (ASSYST) to get the expected log R ratio (Bus *et al.* 2012; Körber *et al.* 2012) distribution for this specific SNP. We then use quantiles to determine if the log R ratio of the SNP in our test population is unexpectedly low or high. The quantiles used for "deletion" were 10, for "missing copy" were 25 and for "extra copy" were 75: If SNP x in line y had a log R ratio value lower than the expected 10th quantile, SNP x was marked as a threshold SNP in line y. Windows with more than five threshold SNPs were kept and merged in case of physical overlap. The merged regions were re-evaluated for the threshold SNP content, and regions with more than 50% threshold SNPs were retained. Regions with the same direction of copy number variation (extra copy/copies, or missing regions/deleted regions) that were very close together (< 5 Mb) were merged (declared as deletion in the case that a deletion and a missing copy region were merged), and regions < 2 Mb were also filtered out, so as not to overestimate CNV numbers based on noise in the data, especially since we only expect a limited number of non-homologous recombination events per chromosome and hence smaller CNVs are less likely in this population (two close-together non-homologous recombination events are required for a small, non-telomeric CNV). Therefore, using the above-mentioned criteria, we assessed copy number variation in the resynthesized *B. napus* lines as deletion, missing/reduced copy, and extra/higher copy, with two copies as the expected copy number (Fig. 1). We also screened the LogR ratio data of the resynthesized *B. napus* lines to check whether the same CNVs (inherited) are present in all progeny set derived from the *B. rapa* or *B. oleracea* parents.

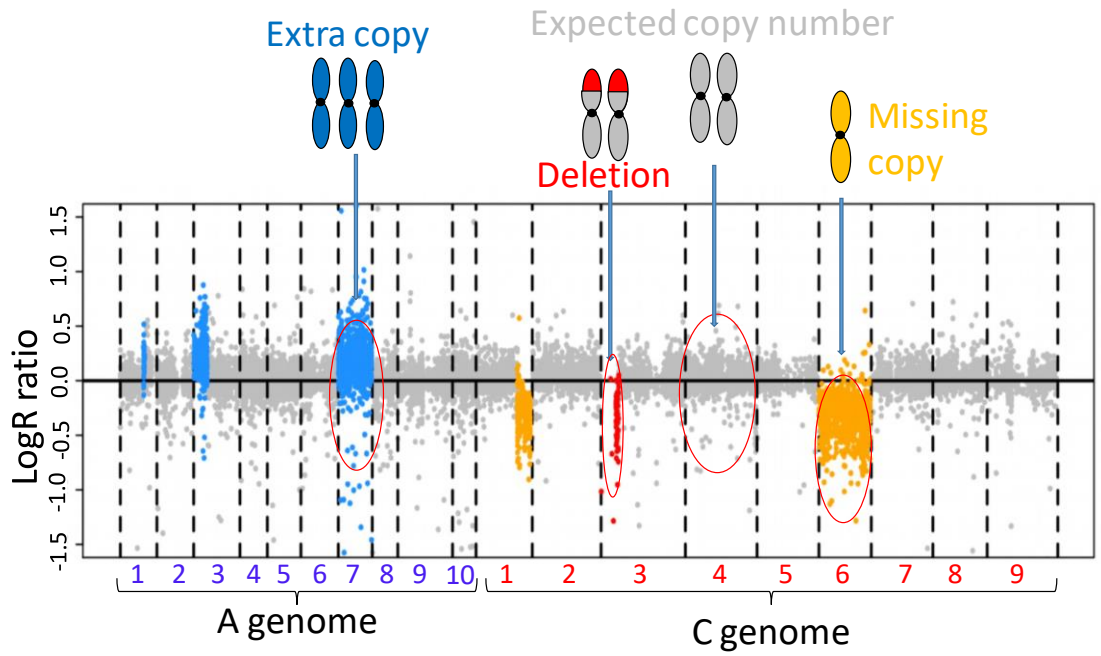

Fig. 1 An example of copy number variant calling in resynthesized *B. napus* lines showing regions of extra copy number (blue), no copies / deletion (red), expected copy (grey), and single / reduced copy (orange).

DNA extraction, sequencing and sequence analysis of parental lines

DNA was extracted from young leaf samples of eight *B. rapa* genotypes (A4, A6, A7, A8, A9, A13, A16, and A19) as well as five *B.oleracea* genotypes (C34, C36, C37, C46, and C47) using the Doyle and Doyle DNA extraction protocol (Doyle and Doyle 1987). Illumina paired-end sequencing was performed at Novogene Company Limited, United Kingdom on a Illumina HiSeq machine to produce reads of 150 bp length. Quality control was performed using FASTQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), no further processing was found to be necessary. The parental reference genomes *B. rapa* cv. Chiifu version 3.0 (Zhang *et al.* 2018) and *B. oleracea* cv. JZS version 2.0 (Cai *et al.* 2020) were downloaded from the BRAD database (Chen *et al.* 2022). Reads were trimmed by removing low-quality reads and unpaired reads using TRIMMOMATIC version 0.39 (Bolger *et al.* 2014). Subsequently, reads were mapped to their respective reference genomes using bwa-mem (Li and Durbin 2010). Uniquely and high-quality mapping reads were selected by "samtools view -q 20" (-b: output is bam files, -q 20, mapping quality of phred score gives a 99% probability

the mapping is correct) (Danecek *et al.* 2021). The depth of each base pair was obtained by "samtools depth -a" (-a all sites). SNP calling was performed using bcftools mpileup and filtered for a minimum quality of 30 and a minimum read depth of 10 using vcftools (Danecek *et al.* 2011), restricted to meiosis gene positions (Supplementary File 4). SNP annotation was performed using CooVar (Vergara *et al.* 2012).

Detection of copy number variation in parental *B. rapa* and *B. oleracea* lines

Since protein coding genes are more conserved and less repetitive than other parts of the genome, the detection of copy number variation was carried out only for gene coding regions. The median sequencing depth of each gene (based on gene annotation) was calculated. These gene depths were then normalized by dividing by the mean depth of all genes. As our lines are not the same genotypes as the reference genome, there was probably some mapping bias. If all lines from one parent species showed low (<0.5 fold relative to mean coverage) or high (>1.5 fold relative to mean coverage) mapping rates, these genes were excluded from the analysis. To avoid uneven distribution of sequencing depth along the genome, a sliding window was calculated for the median depth of 40 genes. Relative read coverage for the median depth of 40 genes was carried out for each of the sequenced *B. rapa* and *B. oleracea* genotypes (Supplementary Figs. 3 and 4).

Identification of meiosis genes candidates in resynthesized *Brassica napus*

SNP information from the five *B.oleracea* genotypes (C34, C36, C37, C46, and C47), and eight *B. rapa* genotypes (A4, A8, A9, A13, A16, and A19) excluding A6 and A7 were analyzed separately in order to detect putative meiosis genes candidates using the following steps. Firstly, the SNP data of *B. oleracea /B. rapa* parents were read in. In the next step, all non-informative SNPs were removed: if one line had "missing information" this SNP was excluded. Gene haplotypes were obtained from the SNP information. We then inferred the allelic state of the S_1 resynthesized *B. napus* lines by combining the respective parents, and subsequently matched these to the phenotype data (fertility, CNV). Next, one-way ANOVA was used to test for significant differences between haplotypes and total CNVs as well as total seed set. The p-values were corrected using the False Discovery Rate (FDR) test. Subsequently, Fisher's exact test for count data was used to check for significant differences between putatively "stable" and "unstable" lines.

Statistical analysis

Genotypic effects on fertility (self-pollinated seeds and seeds per ten pods) and genome stability (as measured by number of CNVs) were tested for associations with alleles inherited from either *B. rapa* or *B. oleracea* parent or with the interaction between the two in the resynthesized lines. One-way ANOVA was used to test for significant differences in means followed by Tukey's HSD to test for differences between parent *B. rapa* and *B. oleracea* genotype groups using R v. 3.6.3 (The R Team for Statistical Computing).

Results

Purity of S_1 resynthesized *B. napus* lines

SNP genotyping was carried out for all 41 resynthesized *B. napus* genotypes (123 individuals). All individual lines were homozygous and identical in allele inheritance to other individuals in the same progeny set, as expected. However, we observed unexpected differences in allele inheritance between progeny sets with the same parental lines C46, C49, and A19. For progeny sets sharing *B. oleracea* parent line C46 segregating regions were observed on chromosome C06 (~1.4 Mb) and C08 (2.4 Mb). Progeny sets with parent C49 also showed segregation on chromosome C01 (~1.1 Mb) and C04 (0.3 Mb). A 1 Mb region on chromosome A04 was also segregating between progeny sets A19C37, A19C47 and A19C49. Each of C46, C49 and A19 were homozygous inbred lines, rather than doubled-haploid.

We also observed large differences in A genome allele inheritance between progeny sets of resynthesized lines with *B. rapa* A6 and A7 as A-genome parents. Hence, parent genotypes A6 and A7 were likely actually heterozygous instead of homozygous as expected; explaining why the A genomes of different resynthesized genotype combinations with A6 and A7 *B. rapa* parents were not all the same (Supplementary Fig. 1). Consequently, we renamed all resynthesized *B. napus* lines which had A6 or A7 *B. rapa* parents with codes represented in the form "A6aC1" or "A7aC1", where "A6a" or "A7a" represents different progeny sets (S_0 plants) resulting from the heterozygous A6 or A7 *B. rapa* parent genotype and "C1" represents the *B. oleracea* parent genotype. Other progeny sets with *B. rapa* parental genotypes A4, A8, A9, A13, and A16 as well as *B. oleracea* genotypes C34, C36, C37, C38, and C42 (Supplementary File 2) were completely homozygous, as no continuous blocks of heterozygosity and/or allele segregation between progeny sets were observed in these lines.

Resynthesized *B. napus* lines show comparable fertility to parent *Brassica rapa* and *Brassica oleracea* genotypes

Total self-pollinated seeds per single plant in the resynthesized lines ranged from 1 to 2067 (mean 445) (Supplementary Fig. 5a, Supplementary File 5), with a mean of 45 seeds per ten pods (range 0 – 148) (Supplementary Fig. 5b). Resynthesized lines also showed average pollen viability of 81% (Supplementary Fig. 6). Average number of self-pollinated seeds in resynthesized lines was higher compared to *B. rapa* and *B. oleracea* parental genotypes (Fig. 2). Average number of seeds per 10 pods and average number of self-pollinated seeds were moderately highly correlated (r = 0.68) (Supplementary Fig. 7), although there was no significant correlation between pollen viability and either of the seed fertility measures (Supplementary Fig. 8, a and b).

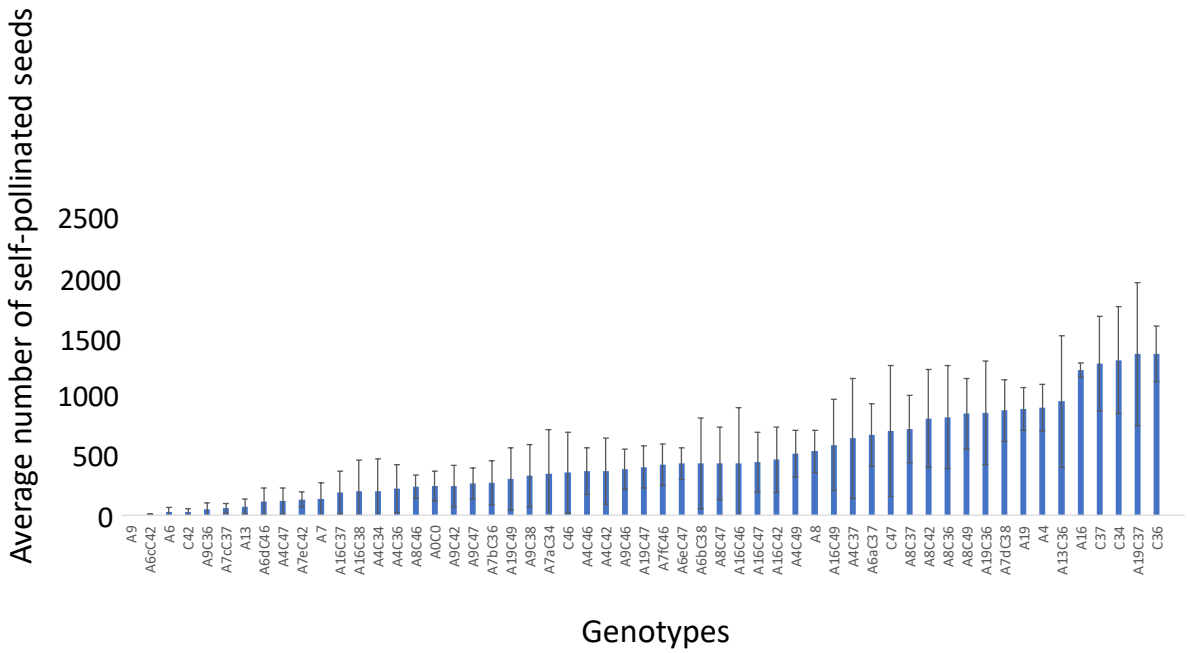

Fig. 2 Fertility of resynthesized lines (genotype combinations given as AXCX) measured by the average number of self-pollinated seeds was compared to progenitor *B. rapa* (A4, A6, A7, A8, A9, A13, A16 and A19), and *B. oleracea* (C34, C36, C37, C42, C46 and C47) genotypes.

Interactions between *B. rapa* and *B. oleracea* parent genotypes affected fertility

Resynthesized *B. napus* lines were assessed in order to detect whether maternal (*B. rapa*) or paternal (*B. oleracea*) genotypes independently influence fertility (total number of self-pollinated seeds and seeds per ten pod). The total number of self-pollinated seeds produced was significantly affected by *B. rapa* parent genotype (ANOVA, p = 0.000539) (Supplementary Fig. 9a) but not by *B. oleracea* parent genotype (Supplementary Fig. 9b). Neither *B. rapa* nor *B. oleracea* parent genotype independently affected seeds per ten pods (Supplementary Figs. 10a, b). However, a significant interaction effect (one-way ANOVA, p = 5.97e-05, Tukey's HSD $p < 0.05$) was observed for the combination of *B. rapa* and *B. oleracea* parent genotypes on the total number of self-pollinated seeds produced in *B. napus* resynthesized lines based on our linear model (Supplementary Table 1).

Frequent copy number variation detected in resynthesized *B. napus* lines

Copy number variants (deletions, reduced copy numbers, and higher copy numbers) were detected at a high frequency across the A and C genomes in the resynthesized *B. napus* lines (Fig. 3). The total number of CNVs detected varied widely between resynthesized *B. napus* lines (Supplementary Fig. 11, Supplementary File 5). We also observed no inherited CNVs (> 0.5Mb) between progeny sets of resynthesized *B. napus* lines with either the same A or C genome parental genotypes.

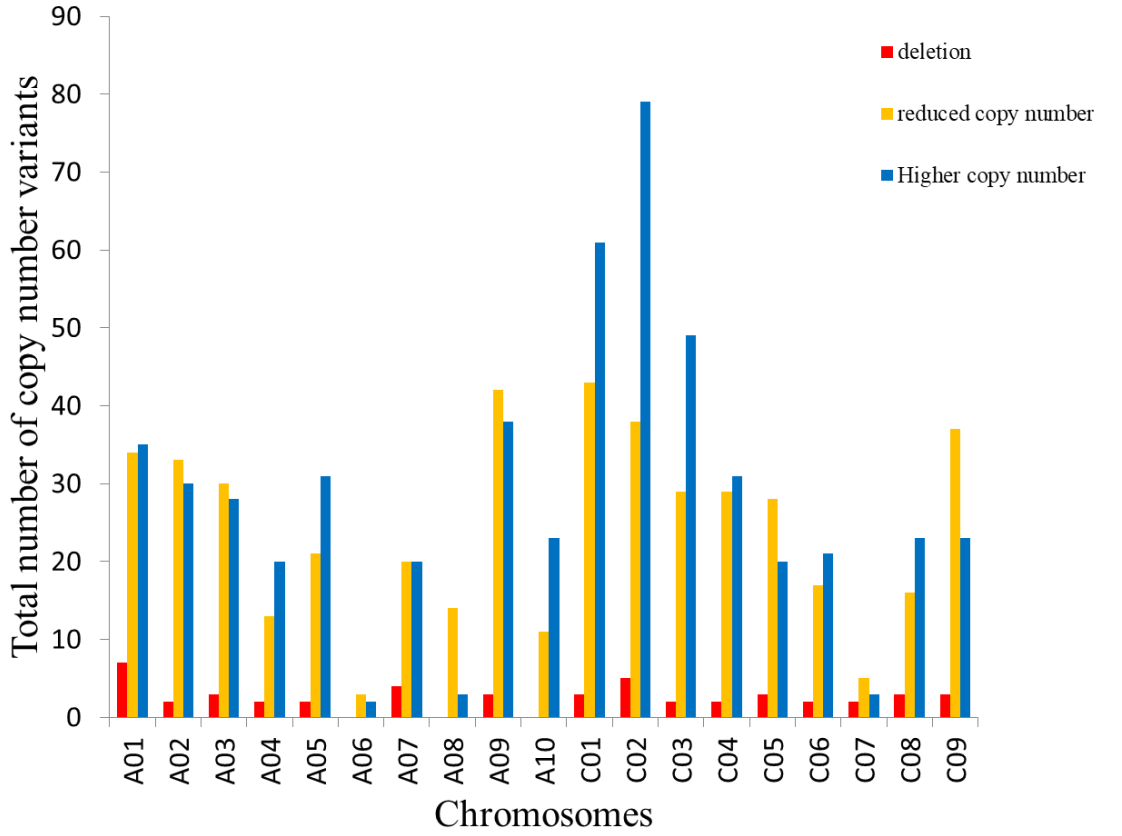

Fig. 3 Genome-wide copy number variation was detected in resynthesized *B. napus* lines. Deletions (blue), reduced copy (red), and higher copy number (green)

B. rapa and *B. oleracea* parent genotypes interact to affect genome stability (number of copy number variants) in resynthesized rapeseed lines

Although parent *B. rapa* and *B. oleracea* genotypes independently had no significant effect on the number of CNVs, the interaction between the two parent genotypes was significant, such that there were significant differences between resynthesized lines (ANOVA, $p = 3.15e\text{-}06$; Fig. 4, Tukey's HSD $p < 0.05$ Supplementary Table 2). Hence, the number of CNVs detected was affected by different cross combinations of *B. rapa* and *B. oleracea* parent genotypes based on our linear model.

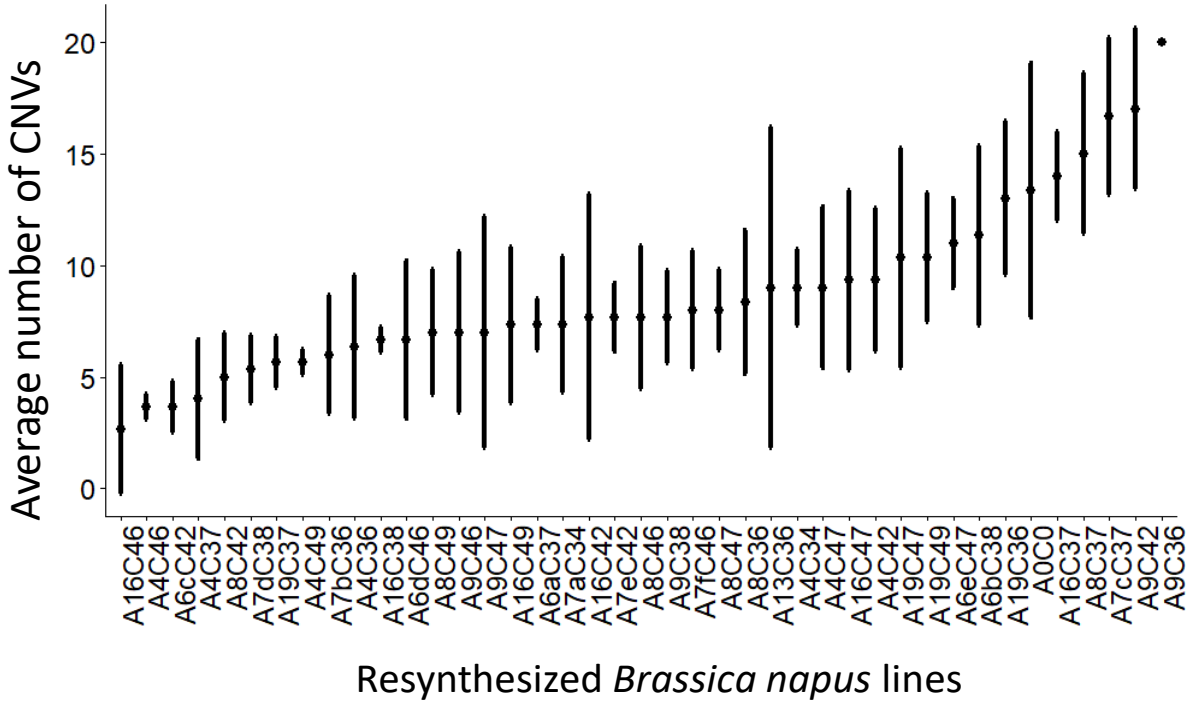

Fig. 4 Average number of CNVs between resynthesized *Brassica napus* (rapeseed) lines from different genotype combinations . Parent genotypes of *B. rapa* are A4, A6, A7, A8, A9, A13, A16 and A19, parent genotypes of *B. oleracea* are C34, C36, C37, C38, C42, C46, C47 and C49, and synthetic rapeseed lines are indicated by the parent combination in the form AXCX or AXaCX.

The relationship between genome stability (as measured by copy number variation) and fertility was assessed in the resynthesized *B. napus* lines. Genome stability was negatively correlated with fertility (r = - 0.22) in resynthesized *B. napus* lines (Supplementary Fig. 12). This relatively weak relationship indicates that other factors apart from genome stability might also contribute to fertility in resynthesized lines.

Allelic state of resynthesized S_1 *B. napus* lines predicted by estimating CNVs

Nineteen resynthesized *B. napus* lines, excluding cross combinations with heterozygous A6 and A7 parents, were classified into stable, intermediate, and unstable using the following criteria and process. Firstly, we undertook pairwise comparisons of CNV data to determine which resynthesized lines were significantly different from each other (Tukey's HSD $p < 0.05$,

Supplementary Table 2). Two groups were established which were significantly different from each other in numbers of CNVs: these lines were classified as either putatively "stable" (low numbers of CNVs) or putatively "unstable" (high numbers of CNVs) while lines which were not significantly different from any other line were classified as "intermediate". Based on this, resynthesized lines with average number of CNVs below 6 were classified into putatively "stable" (4 combinations), from 6 to 10 putatively "intermediate" (11 combinations), and above 10 as putatively "unstable" (4 combinations) (Table 1).

Table 1 Classification of resynthesized *B. napus* cross combinations into putatively "stable", putatively "unstable", and putatively "intermediate" by pairwise comparisons and estimation of average CNVs trait data. Genotypes without data (no plants) are indicated with "-"

	C34 var bot dh	**C36 var bot dh**	**C37 var bot dh**	**C46 var alb il**	**C47 var alb il**
A4 var tri il	Intermediate	Intermediate	Stable	Stable	Intermediate
A16 var tri il	-	-	Unstable	Stable	Intermediate
A19 var tri il	-	Unstable	Stable	-	Intermediate
A8 var tri dh	-	Intermediate	Unstable	Intermediate	Intermediate
A9 var olei dh	-	Unstable	-	Intermediate	Intermediate
A13 var pek dh	-	Intermediate	-	-	-

*var olei represents *B. rapa var. oleifera*, var tri represents *B. rapa var. trilocularis*, var bot represents *B. oleracea var. botrytis*, and var alb represents *B. oleracea var. albogabra*. *dh means double haploid while il means inbred lines

Allelic variation in meiosis genes is associated with number of CNVs

Eight parent accessions of *B. rapa* (A4, A6, A7, A8, A9, A13, A16, A19) and five parent accessions of *B. oleracea* (C34, C36, C37, C46, C47) were resequenced. However, *B. rapa* A6 and A7 accessions was found to be heterozygous, and was subsequently taken out of the analysis. In the next step, the allelic variation was analysed in a list of meiosis gene homologs. 3689 SNPs in *B. rapa* meiosis genes were detected, of which 832 were non-synonymous and no splice variants were detected (Supplementary File 6a). In *B. oleracea* meiosis genes, 2549 SNPs were detected, of which 729 were non-synonymous, 4 were splice variants and 3 were stop codon gains (Supplementary File 6b). Moreover, CNVs in meiosis genes were detected by analysing coverage. It was found that 96 of the 197 *B. rapa* meiosis gene copies carried a deletion in at least one accession, out of which two were deleted in all 8 accessions, and 90 gene copies carried a duplication (Supplementary File 6c). In *B. oleracea*, 33 gene copies out

386 of 193 were deleted in at least one accession, one of them in all accessions, and 57 were
387 duplicated (Supplementary File 6d).

388 From these data, the allelic state of the S1 *B. napus* resynthesized lines by combining the
389 respective parents was inferred (Supplementary File 6, e and f) from which the list of putative
390 meiosis genes candidates were pulled (Supplementary File 7, a-d) . Phenotypic data for 19
391 cross combinations (excluding combinations with heterozygous A6 and A7 parents) which
392 could be tested in the greenhouse was used, and CNV counts classified lines into putatively
393 "stable" (4 combinations), "intermediate" (11 combinations), "unstable" (4 combinations) and
394 "missing" (11 combinations) **(Table 1).** In the next step, meiosis candidate genes were selected
395 using the following criteria (Supplementary File 7, a-e). Firstly, significant associations of
396 number of CNVs with meiosis gene haplotypes after FDR correction (Supplementary File 7, b
397 and e). Secondly, presence of putatively harmful mutations in meiosis genes haplotypes which
398 fulfil criterion 1 (non-conservative missense codon, stop codon gain variants and/or splice
399 variants) (Supplementary File 7, b and e). Thirdly, putative gene function in meiosis related to
400 DNA or double strand break repair, effects on meiotic crossover or effects on homoeologous
401 recombination (Supplementary File 7e).

402 Using these three criteria, we identified thirteen putative meiosis genes from the *B. oleracea* C
403 genome parents used to produce the resynthesized *B. napus* lines (Table 2). Of these, *RPA1C*,
404 *MSH2*, and *RECQ4B* also showed presence of either a stop codon or a splice variant in at least
405 one gene copy. *RPA1C* gene copies carried two non-conservative missense codons and one
406 stop codon gain. *MSH2* carried one stop codon gain and one splice donor variant, while
407 *RECQ4B* showed 4 missense codons, one stop codon gain variant, and one splice acceptor
408 variant. Other putative candidate genes with significant CNV association with haplotypes and
409 carrying at least one non-conservative missense codon were *BRCA*, *ATR*, *RAD51C*, *MLH3*,
410 *RECQ4A*, *SDS*, *RAD51*, *BLAP75/RMI1*, and *SYN1/DIF1/REC8*, *AtGR1/COM1* (Table 2). In
411 the *Brassica rapa* parents, none of the gene haplotypes fulfilled criterion 1, which is our most
412 important criterion for the selection of putative meiosis gene candidates. So these genes from
413 *Brassica rapa* parents (Supplementary File 7, c-d) were not considered as interesting meiosis
414 gene candidates.

415

416

Table 2 Meiosis gene haplotypes associated with copy number variants (CNVs) in resynthesized *B. napus* derived from crosses between *B. rapa* and *B. oleracea* parents

Candidate genes involved	chromosome	Location of other copies	*B. oleracea* copies	*Arabidopsis thaliana* homologue	Type of mutation	Significant CNV association with haplotypes after FDR correction ($p < 0.05$)
RPA1C	C02	C09	*Bo2g127130.1, Bo9g061490.1*	*AT5G45400.1, AT5G45400.1*	Non-conservative missense codon, stop codon gain	0.04, 0.04
MSH2	C06	C06, C03	*Bo6g030570.1, Bo3g071550.1, Bo6g003510.1*	*AT3G18524.1, AT3G18524.1, AT3G18524.1*	Stop codon gain, Splice variant donor	P > 0.05, 0.03, P > 0.05
RECQ4B	C09	-	*Bo9g043460.1*	*AT1G60930.1*	Non-conservative missense codon, stop codon gain, splice acceptor variant	0.04
AtGR1/COM1	C04	-	*Bo4g125630.1*	*AT3G52115.1*	Non-conservative missense codon	0.03
ATR	C04	-	*Bo4g145640.1*	*AT5G40820.1*	Non-conservative missense codon	0.03
BRCA	C01	C01	*Bo1g023820.1, Bo3g001340.1*	*AT4G21070.1, AT5G01630.1*	Non-conservative missense codon	0.03, 0.04
RAD51C	C04	-	*Bo4g038470.1*	*AT2G45280.1*	Non-conservative missense codon	0.04
MLH3	C07	-	*Bo7g117460.1*	*AT4G35520.1*	Non-conservative missense codon	0.03
RECQ4A	C08	C08	*Bo8g059730.1, Bo8g109200.1*	*AT1G10930.1, AT1G10930.1*	Non-conservative missense codon	0.03, 0.03
SDS	C08	C05	*Bo8g066230.1, Bo8g106580.1, Bo5g019980.1*	*AT1G14750.1, AT1G14750.1, AT1G14750.1*	Non-conservative missense codon	0.04, 0.03, 0.03
RAD51	C09	C03	*Bo9g151450.1, Bo3g015380.1*	*AT5G20850.1, AT5G20850.1*	Non-conservative missense codon	0.04, 0.03
BLAP75/RMI1	C09	-	*Bo9g017740.1*	*AT5G63540.1*	Non-conservative missense codon	0.03
SYN1/DIF1/REC8	C09	-	*Bo9g177100.1*	*AT5G05490.1*	Non-conservative missense codon	0.04

Genetic analyses of resynthesized *Brassica napus* lines

Analysis of the genetic background of the parental lines used in this study showed that maternal *B. rapa* var. *trilocularis* was the parent for all stable resynthesized lines (4 out of 4 considered as putatively stable), although this subspecies also contributed to intermediate (8 out of 11) and

unstable lines (3 out of 4) (Table 1). Resynthesized cross combinations with C46 (*B. oleracea var. alboglabra*) as paternal *B. oleracea* parent were also either putatively stable or intermediate (Table 1). Although it is not possible to draw statistically significant conclusions from these results in the current study, further investigation of this association may be warranted in future.

Discussion

In this study, we analyzed relative genome stability (as measured by copy number variants) and measured fertility using self-pollinated seed set, seeds per ten pods, and pollen viability in resynthesized *Brassica napus* after one generation of self-pollination as well as screened for variants of meiosis candidate genes possibly affecting genome stability in the lines. Our results show that allelic variants inherited from both diploid *B. rapa* and *B. oleracea* parents interact to affect genome stability and fertility in resynthesized *B. napus* lines. Resynthesized rapeseed lines from different genetic backgrounds also vary significantly in both fertility and genome stability. We identified thirteen putative meiosis candidate genes which were significantly associated with frequency of copy number variants and which contained putatively harmful mutations in meiosis gene haplotypes for further investigation.

Our results show a negative correlation between genome stability (CNVs) and fertility (Spearman correlation r = -0.22) in resynthesized *B. napus*. In natural *B. napus,* inheritance of unbalanced translocation events in mapping populations was also associated with a fertility penalty (Osborn *et al.* 2003). Negative correlations between chromosome rearrangements and fertility have also been observed in both natural and resynthesized *B. napus* populations (Samans *et al.* 2017). These results support the present study where copy number variation was significantly negatively associated with fertility (self-pollinated seed set). However, the detected correlation was low, indicating that other factors apart from genome stability might be contributing to fertility in resynthesized *B. napus* lines.

We observed a wide range of genotype-dependent fertility across the resynthesized *B. napus* lines, and some resynthesized lines showed higher fertility than *B. rapa* and *B. oleracea* parental genotypes in our study. Malek et al. (2012) detected higher fertility in synthetic *B. napus* compared to its parental *B. rapa* and *B. oleracea* genotypes in terms of the number of seeds per silique, 1000-seed weight and seed yield per plant. In support of the hypothesis that

polyploid crops (interspecific hybrids) often show higher yield levels and outperform their diploid relatives (Sattler *et al.* 2016), highlighting the heterotic potential of resynthesized *B. napus* lines (Abel et al. 2005). Rousseau-Gueutin et al. (2017) assessed fertility (number of seeds/50 pollinated flowers and number of seeds/50 pods) in both open-pollinated and manually-self-pollinated resynthesized *B. napus* populations, and found very low fertility compared to natural *B. napus* using both fertility measures. Rousseau-Gueutin et al. (2017) hypothesized that self-incompatibility alleles carried by the parental diploid species might have affected the fertility of their hybrids, since different subspecies of *B. rapa* and *B. oleracea* parents had been used to produce the resynthesized *B. napus* population. Many genotypes of *B. rapa* and *B. oleracea* are self-incompatible, a trait genetically controlled by the self incompatibility S-locus (Camargo *et al.* 1997; Kimura *et al.* 2002; Kitashiba and Nasrallah 2014), which prevents self- seeds. Self-pollinated seed set varied greatly across our *B. rapa* and *B. oleracea* parents and their progeny, with possible self-incompatibility issues in a few parental genotypes used to produce the resynthesized lines (Fig. 2). One genotype, *B. rapa* A9, was most likely self-incompatible, setting no self-pollinated seed. Interestingly, none of the synthetic combinations with A9 were completely sterile, and some were highly fertile, suggesting the synthetic *B. napus* mostly overcame self- fertilization via recognition in the stigma and failed germination of pollen with the same S-haplotype as the parent plant. However, self-incompatibility alleles present in some parent genotypes used to produce our resynthesized lines might be responsible for low fertility in a few lines (9 genotypes produced <15 seeds). Further investigation would be needed to confirm this.

In this study, we detected copy number variation at a high frequency across the A and C genome. We also found fewer deletions (5.6%) than either reduced (one missing copy) or higher copy number (one or two extra copies as predicted by the pipeline) variants. Several studies have shown that chromosomal rearrangements occur frequently in both resynthesized and natural *B. napus* (Pires *et al.* 2004; Udall *et al.* 2005; Leflon *et al.* 2006; Liu *et al.* 2006; Nicolas *et al.* 2007; Chalhoub *et al.* 2014; Guo *et al.* 2016). Homoeologous exchanges have been detected in translocated regions (deletion-duplication events) between A and C homoeologous chromosomes in both resynthesized and natural *B. napus* (Chalhoub *et al.* 2014; Samans *et al.* 2017; Mason *et al.* 2018). Samans et al. (2017) analyzed 52 highly diversed *B. napus* genotypes including 32 natural and 20 synthetic *B. napus* accessions using whole genome sequencing, and detected a greater number and size of genomic rearrangements in synthetic *B. napus* compared to natural accessions as well as more areas with deletions than

duplications. In contrast to the above mentioned studies, which were all on established lines of *B. napus* or synthetic *B. napus* which had been through many generations of self-pollination, our resynthesized *B. napus* material has undergone only one self-pollination event (two meiosis). Hence, any novel variants which have arisen are unlikely to be "fixed" (homozygous, present in both homologous chromosomes), as the products of a single homoeologous crossover event during meiosis rarely segregate together: two recombinant chromatids are produced from one homoeologous crossover, but these are usually separated into different gametes in the first meiosis. Subsequently, selection against unbalanced translocations may "fix" these events in self-pollinated progeny, resulting in balanced duplication/deletions.

Our copy number pipeline was not robust enough to efficiently discriminate whether three copies of an allele or more copies (4+) were present, leading us to use a combined "higher copy number" category. Our copy number pipeline may also be over-estimating reduced and higher copy number CNV calls. Failed SNP calls from the 60K *Brassica* SNP chip used possibly lowered the average copy numbers across specific regions, leading to high false positive error rates (Mason *et al.* 2017). In addition, lower signal detection may result from other types of sequence polymorphism other than CNVs, which may also result in false positives as reported by Zmieńko et al. (2014). Although both hybridization-based arrays and NGS approaches used for the detection of CNVs have different limitations (Zmieńko *et al.* 2014); high-coverage sequencing likely provides a more robust method of calling CNVs (Yoon *et al.* 2009). However, obtaining sufficient read depth is still factorially more expensive than calling CNVs from array data (Mason *et al.* 2017).

We observed that different cross combinations of *B. rapa* and *B. oleracea* genotypes significantly affect genome stability (as measured by number of CNVs after self-pollination; two meiosis events) based on our linear model. This observation is likely due to the selection of specific allelic variants from the parent genotypes which influenced genome stability in the resynthesized lines. Gaeta et al. (2007) suggested that *B. napus* might have initially been unstable, but that alleles responsible for genetic control of meiosis inherited from one or both diploid progenitors may have been selected for over time, possibly by conferring improved seedset. In *Arabidopsis arenosa*, selection of specific alleles of meiosis genes seems to be responsible for reduced crossover frequency, resulting in meiotic stability in the polyploid (Yant *et al.* 2013). Recently, allelic variants of *ASY1* and *ASY3* in particular were found to reduce multivalent frequencies and help regulate meiosis in polyploid *Arabidopsis arenosa* (Morgan *et al.* 2020), and introgression of meiosis gene alleles from *A. arenosa* was found to

help stabilize tetraploid *A. lyrata* (Marburger *et al.* 2019). Similarly, selection of genetic variants at pre-existing loci may have contributed to form stable meiosis in ancient polyploid *Brassica* (Lloyd *et al.* 2014).

We presented a summary of thirteen putative meiosis gene candidates which show significant CNV association and presence of putatively harmful mutation in meiosis genes haplotypes as well as putative gene function in meiosis related to DNA or double strand break repair, effects on meiotic crossover or suppression of homoeologous recombination. Of the thirteen genes, three are of special interest due to the presence of stop codons or splice variants in at least one copy: *RPA1C*, *MSH2*, and *RECQ4B*.

Replication protein A(*RPA*) is a eukaryotic, singe stranded DNA binding protein made up of three subunits *RPA1*, *RPA2*, and *RPA3*, and plays important roles in almost all DNA metabolic pathways including S-phase genome replication, DNA recombination, and DNA excision repair (Aklilu *et al.* 2014). *RPA1C* has been shown to promote homologous recombination in early meiosis, and interactions between *RPA1C* and *RPA1E* are primarily responsible for DNA repair in *Arabidopsis thaliana* (Aklilu *et al.* 2014; Aklilu and Culligan 2016). In rice, *RPA1C* is shown to be required for ~79 % of chiasma formation, and the *RPA* complex comprising *RPA1C* and *RPA2C* is required to promote meiotic crossovers (Li *et al.* 2013). In *B. napus*, *RPA1C* was found within the *BnaA9* QTL region responsible for the prevention of homoeologous chromosome pairing (Higgins *et al.* 2020). In the present study, we found two copies of *RPA1C* on chromosomes C02 and C09 from the *B. oleracea* parent of resynthesized *B. napus.* The two copies significantly associated with CNVs and fertility, and radical SNP mutations were observed in both C02 and C09 copies while a stop codon gene variant was predicted in the C02 copy.

MutS is an ATPase involved in mismatch recognition, with four MutS homologues identified in *Arabidopsis* (*AtMSH2*, *AtMSH3*, *AtMSH6*, and *AtMSH7*) on the basis of their sequence conservation (Emmanuel *et al.* 2006). The *MSH2* protein regulates meiotic recombination during Prophase 1, thereby functioning in a pro-crossover role in regions of higher sequence diversity in *A. thaliana*. *AtMSH2* has also been shown to have an anti-recombination meiotic effect in *A. thaliana* (Emmanuel *et al.* 2006). *MSH2* was found in the QTL interval underlying fertility on chromosome C3 in *Brassica* allohexaploids derived from a cross (*B. napus* × *B. carinata*) × *B. juncea* (Gaebelein et al. 2019). Here, we found three gene copies of *MSH2*. Two copies of this gene were found in the *B.* o*leracea* parent genome, two on chromosome C06 and

one copy on C03. Although one of the gene copies on C06 showed no significance association with CNV number, a stop codon gain variant as well as a splice variant donor were observed as allelic variants of this gene copy. However, the other C06 copy was not significantly associated with fertility or CNV traits, with no SNP mutations observed. Another gene copy on chromosome C03 was significantly associated with CNV and total seed set and contained a missense codon.

RecQ helicases are involved in the processing of DNA structures arising during replication, recombination, and repair throughout all kingdoms of life (Hartung *et al.* 2007). Seven different *RecQ* genes are present in *Arabidopsis*. Among them are two paralogs, *RECQ4A* and *RECQ4B*, which arose a a result of a recent duplication, and which are nearly 70% identical on a protein level (Hartung *et al.* 2007; Schröpfer *et al.* 2014). In *Arabidopsis*, *RECQ4A* and *RECQ4B* have both been shown to limit crossovers (Fernandes *et al.* 2018; Serra *et al.* 2018). However, an earlier study showed that *AtRECQ4B* is specifically required to promote but not to limit crossovers, a role which is different from all other known eukaryotic *RecQ* homologues (Hartung *et al.* 2007). de Maagd et al. (2020) investigated the role of tomato *RecQ4* on crossover formation in an interspecific cross between cultivated tomato and its wild relatives, and observed a 1.53 fold increase of ring bivalents, suggesting a less important role in limiting crossover compared to *Arabidopsis*. Here, we found *RECQ4B* on chromosome C09 in *B. oleracea* used to produce our resynthesized *B. napus* interspecific cross. *RECQ4B* was significantly associated with CNV numbers, and showed predicted radical SNP mutations with a potential harmful effect on protein function, as observed by a stop codon gain variant, as well as a splice acceptor variant. Two copies of *RECQ4A*, which is the other paralog of *RECQ4B*, were found on C08. Both copies were also significantly associated with CNVs and seed-set, with radical SNP mutations as indicated by the presence of non-conservative missense codons.

Genetic variation in meiosis genes in general may cause large effects on genome stability in different plant lineages (Addo Nyarko and Mason 2022). Although *B. napus* is not thought to have undergone detectable gene fractionation since formation (Chalhoub *et al.* 2014), knock-out of one existing *MSH4* gene copy was shown to help prevent non-homologous chromosome pairing in *B. napus* (Gonzalo *et al.* 2019), supporting the idea that loss of functional meiosis gene copies in mesopolyploids *B. rapa* and *B. oleracea* may also then contribute to formation of allopolyploids with higher meiotic stability. We could not identify any interesting meiosis gene candidates from the *B. rapa* parent genotypes based on our analysis, most likely due to the small numbers of genotypes in our study. We have identified putative meiosis genes present

586 in the diploid *B.* o*leracea* progenitor genotypes used to produce our resynthesized *B. napus*
587 lines, some of which were present in more than one copy. Meiosis gene copies have been shown
588 to be under strict control, with most genes returning rapidly to single copies (Lloyd *et al.* 2014),
589 presumably to avoid meiotic abnormalities caused by the retention of several gene copies
590 following polyploidization. However, allelic variants of meiosis genes which are only present
591 in a few copies could potentially have an impact on genome stability and /or fertility of
592 resynthesized *B. napus*. Hence, the respective allelic variants of the putative meiosis genes
593 identified are putatively good candidates for the variation in copy number observed in our
594 study. *Brassica napus* itself appears to be too young (<10 000 years) to have undergone any
595 major gene fractionation: almost all (if not all) A and C genome gene copies in *B. napus* are
596 still intact (and expressed) relative to progenitor *B. rapa* and *B. oleracea* subgenomes
597 (Chalhoub *et al.* 2014). However, *B. rapa* and *B. oleracea* are themselves mesopolyploids, and
598 not all meiosis genes have been reduced to single copy in these species (Lloyd *et al.* 2014).
599 Hence, these genomes may contain allelic variants including loss-of-function mutations which
600 could conceivably affect meiosis in resynthesized *B. napus.*

601 Our results show that some resynthesized lines are more genomically stable and fertile than
602 others, and suggest that allelic variation present in both of the diploid parents interacts to affect
603 the chance of chromosome rearrangement and copy number variation events, but that the
604 presence of such events may not always be detrimental to fertility in resynthesized *B. napus*
605 lines. Our study suggests meiotic stability in *B. napus* arose via selection of allelic variants
606 from its diploid progenitor species, and provides information that will be useful for breeders
607 aiming to use resynthesized lines in breeding programs. The production of genomically stable
608 resynthesized *B. napus* lines might be useful in future as a germplasm resource to broaden the
609 limited genetic diversity of established *B. napus* cultivars or for hybrid breeding (Abel et al.
610 2005).

Data Availability

The data that supports the findings of this study are available in the supplementary material of this article. Sequencing data generated in this project is available under Bioproject accession code PRJNA724876 from the National Center for Biotechnology Information (NCBI).

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Conflict of interest

The authors declare no conflict of interest.

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Figure legends

Figure 1 An example of copy number variant calling in resynthesized *B. napus* lines showing regions of extra copy number (blue), no copies / deletion (red), expected copy (grey), and single / reduced copy (yellow).

Figure 2 Fertility of resynthesized lines (genotype combinations given as AXCX) measured by the average number of self-pollinated seeds was compared to progenitor *B. rapa* (A4, A6, A7, A8, A9, A13, A16 and A19), and *B. oleracea* (C34, C36, C37, C42, C46 and C47) genotypes.

Figure 3 Genome-wide copy number variation was detected in resynthesized *B. napus* lines. Deletions (blue), reduced copy (red), and higher copy number (green).

Figure 4 Average number of CNVs between resynthesized *Brassica napus* (rapeseed) lines from different genotype combinations . Parent genotypes of *B. rapa* are A4, A6, A7, A8, A9, A13, A16 and A19, parent genotypes of *B. oleracea* are C34, C36, C37, C38, C42, C46, C47 and C49, and synthetic rapeseed lines are indicated by the parent combination in the form AXCX.

Table legends

Table 1 Table 1 Classification of resynthesized *B. napus* cross combinations into putatively "stable", putatively "unstable", and putatively "intermediate" by pairwise comparisons and estimation of average CNVs trait data. Genotypes without data (no plants) are indicated with "-"

Table 2 Meiosis gene haplotypes associated with copy number variants (CNVs) in resynthesized *B. napus* derived from crosses between *B. rapa* and *B. oleracea* parents.

Supplementary information

Supplementary Figures

Figure S1 Phylogenetic relationships in the A genomes of resynthesized *B. napus* lines showed heterozygosity in genotypes with A6 and A7 *B. rapa* cross combinations.

Figure S2 Phylogenetic relationship in the C genome of resynthesized *B. napus* lines showed homozygosity in *B. oleracea* parental cross combination

Figure S3 Relative read coverage of sequenced *B. rapa* parent genotypes (A4, A6, A7, A8, A9, A13, A16 and A19) calculated for a median depth of 40 genes showing regions of the chromosomes with expected copies as well as copy number variation

Figure S4 Relative read coverage of sequenced *B. oleracea* parent genotypes (C34, C36, C37, C46, and C47) calculated for a median depth of 40 genes showing regions of the chromosomes with expected copies as well as copy number variation

Figure S5 Fertility of resynthesized *B. napus* lines was measured by the total number of self-pollinated seeds produced, as well as the number of seeds per ten pods a) total number of self pollinated seeds produced in resynthesized lines and b) number of seeds per ten pods in resynthesized lines

Figure S6 Percent pollen viability across individuals in resynthesized *B. napus* lines

Figure S7 Moderate positive correlation between average number of seeds per ten pods and average self-pollinated seeds ($r = 0.68$)

Figure S8a Correlation between average percent pollen viability and average self-pollinated seed set ($r = 0.06$)

Figure S8b Correlation between average percent pollen viability and average seeds per ten pods ($r = 0.22$)

Figure S9a. *B. rapa* maternal genotype significantly affected the total number of self-pollinated seeds produced (ANOVA, $p = 0.000539$, Tukey's HSD, $p < 0.05$) in resynthesized *B. napus* lines produced from different combinations of *B. rapa* crossed with *B. oleracea* genotypes represented by "CX". Letters "ab" on boxplots represent non significant association

while “a” and b represent significant differences between genotypes based on Tukey pairwise comparison

Figure S9b. *Brassica oleracea* paternal genotypes show no significant association with total number of self-pollinated seeds (ANOVA, p= 0.068) in resynthesized *B. napus* lines produced from different *B. rapa* genotypes represented by “AX” crossed with *B. oleracea* genotypes. Letters “a” on boxplots represent no significant association between genotypes.

Figure S10a. *Brassica rapa* maternal genotype show no significant effect on the number of seeds per ten pods (ANOVA, p = 0.658) in resynthesized *B. napus* lines produced from different combinations of *B. rapa* genotypes crossed with different *B. oleracea* genotypes represented by “CX”. Letters “a” on boxplots represent no significant association between genotypes.

Figure S10b. *Brassica oleracea* paternal genotype show no significant effect on the number of seeds per ten pods (ANOVA, p = 0.0982) in resynthesized *B. napus* lines produced from different combinations of *B. rapa* represented by “AX” crossed with *B. oleracea* genotypes. Letters “a” on boxplots represent no significant association between genotypes.

Figure S11 Number of copy number variants present varied widely between different synthetic *B. napus* lines

Figure S12 Negative correlation between average number of self-pollinated seeds and average number of copy number variants in 41 resynthesized *B. napus* lines (r = -0.2)

Supplementary Tables

Table S1 Pairwise comparison (Tukey’s HSD) in total number of self-pollinated seeds between different resynthesized *B. napus* genotypes produced

Table S2 Pairwise comparisons (Tukey’s HSD) in copy number variation between different resynthesized *B. napus* genotypes produced

Supplementary Files

File S1 Description of S_1 resynthesized *Brassica napus* lines

File S2 SNP genotyping data for all resynthesized *Brassica napus* S_1 individual lines across the A and C genomes after quality control

File S3 LogR ratio values of S_1 resynthesized *Brassica napus* lines

File S4 Meiosis gene positions determined by BLAST in *Brassica rapa* and *B. oleracea* genotypes

File S5 Phenotype information for all resynthesized *Brassica napus* S_1 lines

File S6a SNP variation and meiosis gene positions in *Brassica rapa* parents

File S6b Copy number variation and meiosis gene positions in *Brassica rapa* parents

File S6c SNP variation and meiosis gene positions in *Brassica oleracea* parents

File S6d Copy number variation and meiosis gene positions in *Brassica oleracea* parents

File S6e SNP variation and meiosis gene positions in resynthesized *Brassica napus* lines

File S6f Copy number variation and meiosis gene positions in resynthesized *Brassica napus* lines

File S7a List of meiosis gene candidates in *Brassica oleracea* parents and putative effect mutations (p-values before FDR correction)

Figure S7b List of meiosis gene candidates in *Brassica oleracea* parents (p-values after FDR correction)

File S7c List of meiosis genes candidates in *Brassica rapa* parents and putative effect mutations (p-values before FDR correction)

File S7d List of meiosis gene candidates in *Brassica rapa* parents (p-values after FDR correction)

File S7e Selection of meiosis candidate genes of interest from *Brassica oleracea* parents

Figures

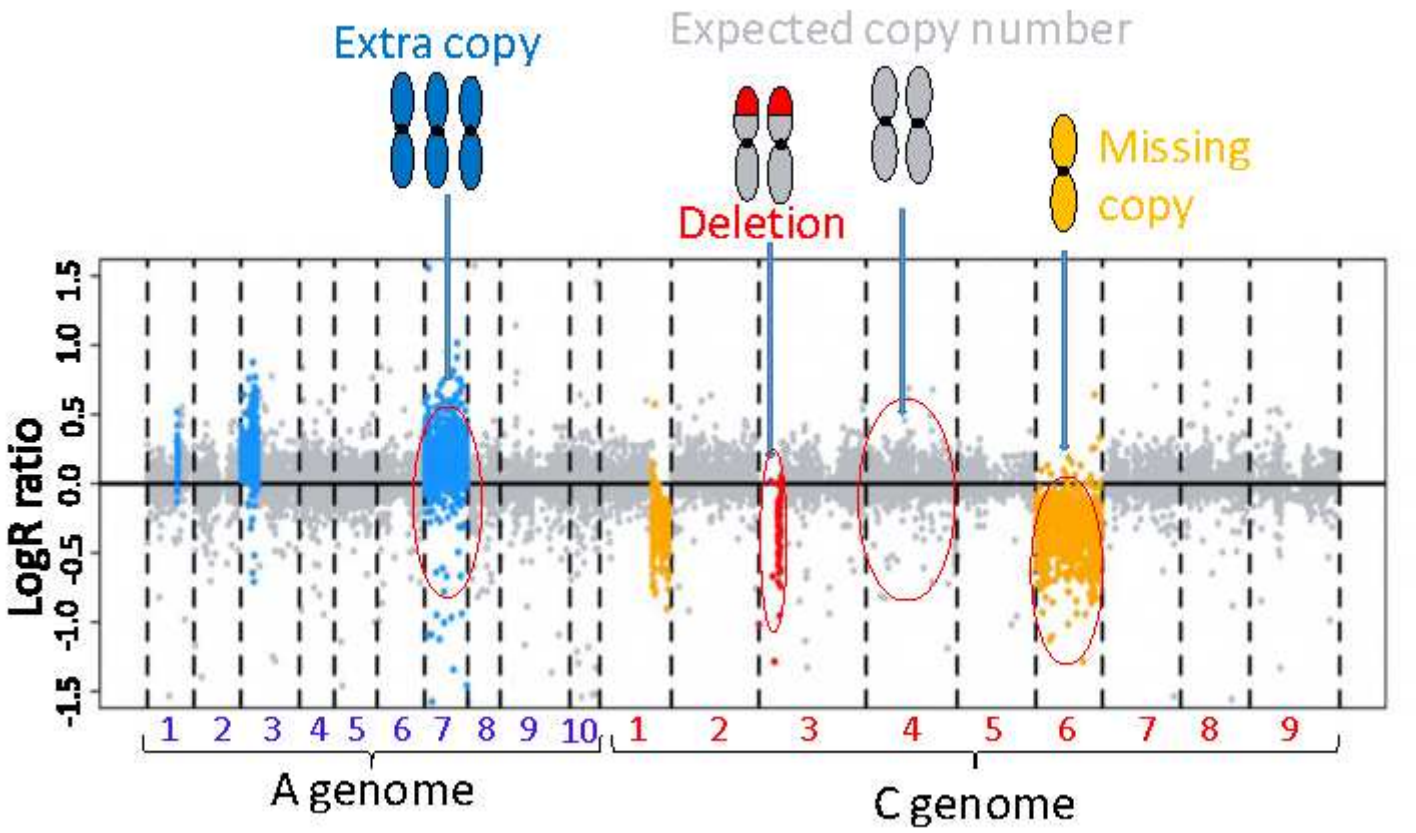

Figure 1

Fig. 1 An example of copy number variant calling in resynthesized *B. napus* lines showing regions of extra copy number (blue), no copies / deletion (red), expected copy (grey), and single / reduced copy (yellow)

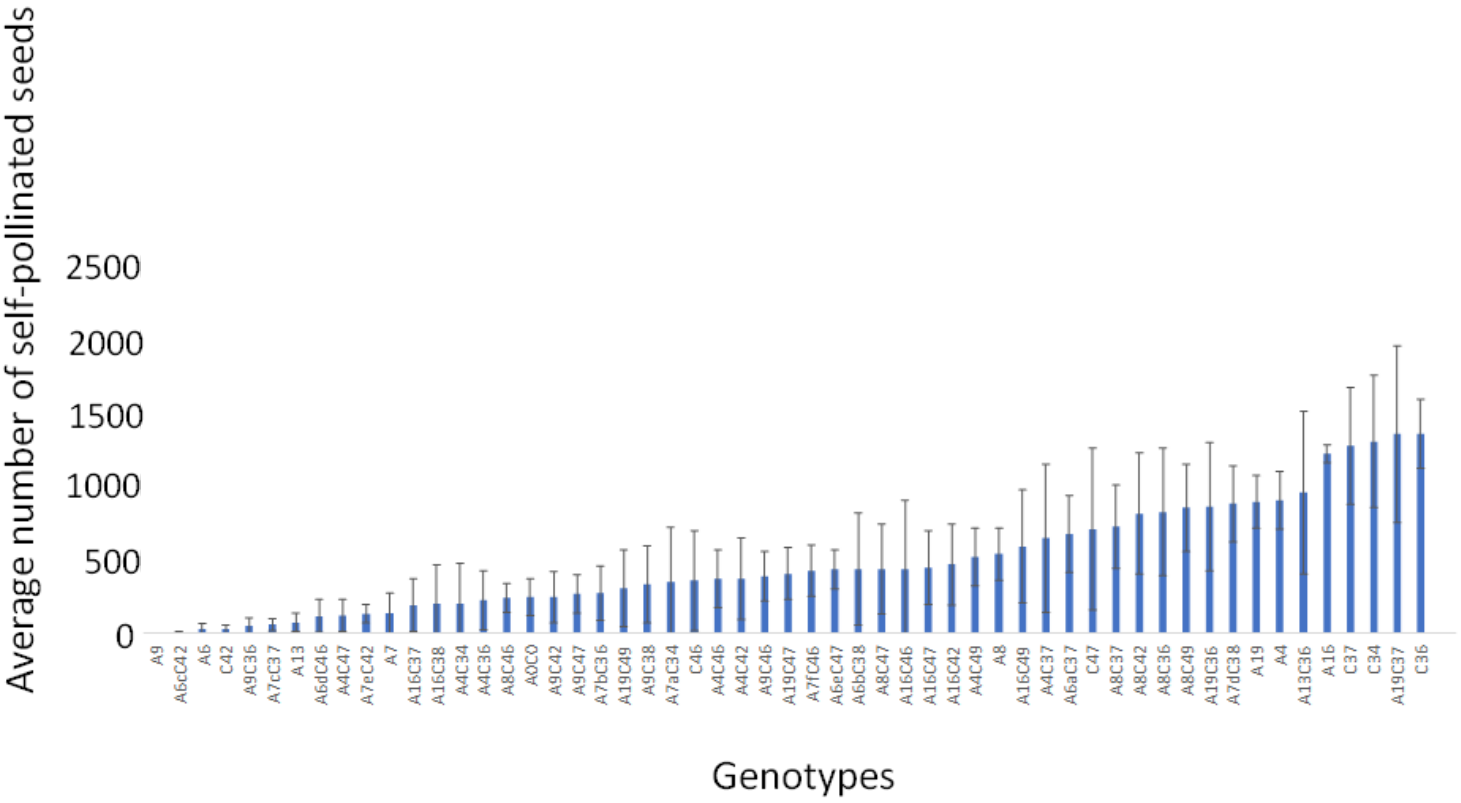

Figure 2

Fig. 2 Fertility of resynthesized lines (genotype combinations given as AXCX) measured by the average number of self-pollinated seeds was compared to progenitor *B. rapa* (A4, A6, A7, A8, A9, A13, A16 and A19), and *B. oleracea* (C34, C36, C37, C42, C46 and C47) genotypes.

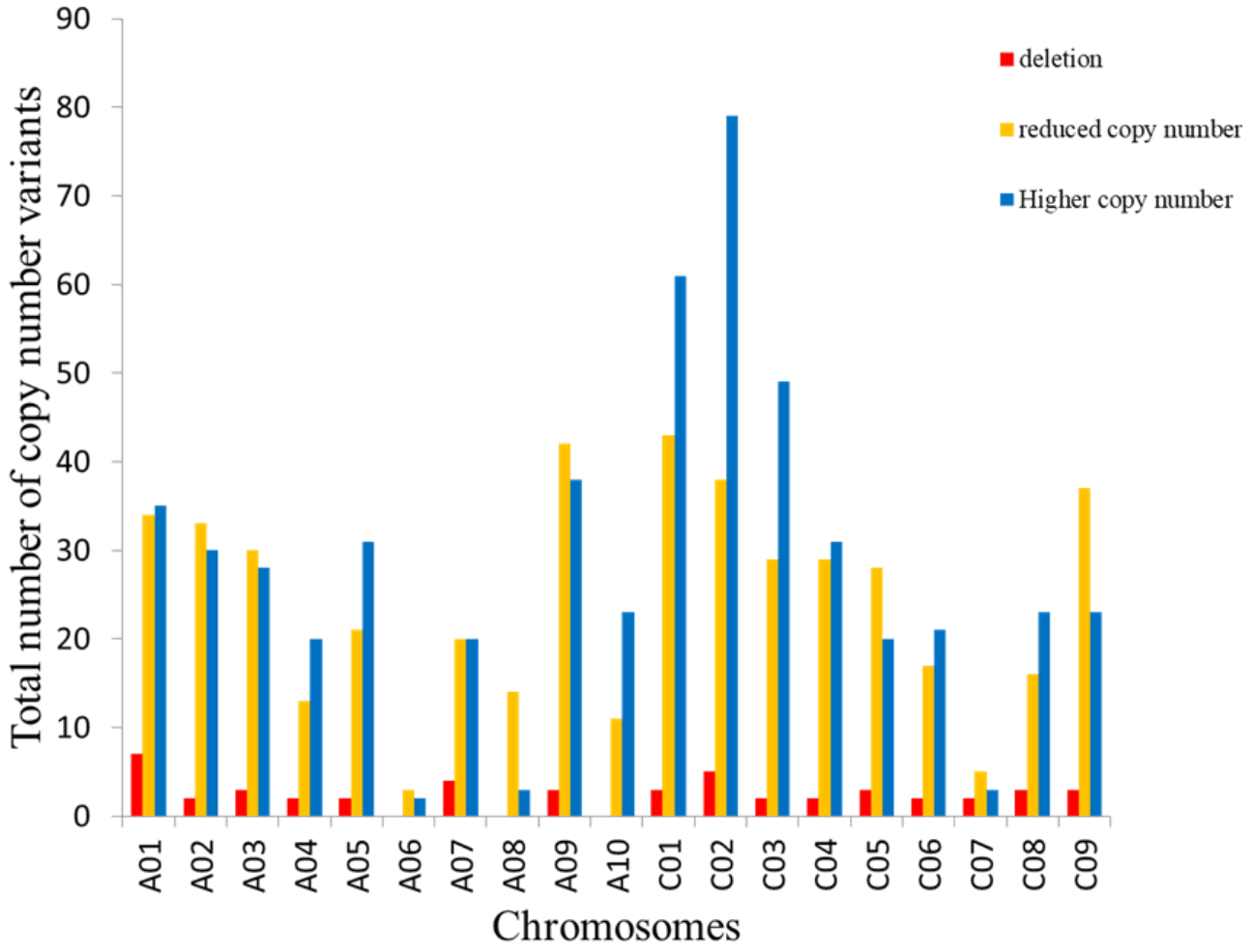

Figure 3

Fig. 3 Genome-wide copy number variation was detected in resynthesized *B. napus* lines. Deletions (blue), reduced copy (red), and higher copy number (green)

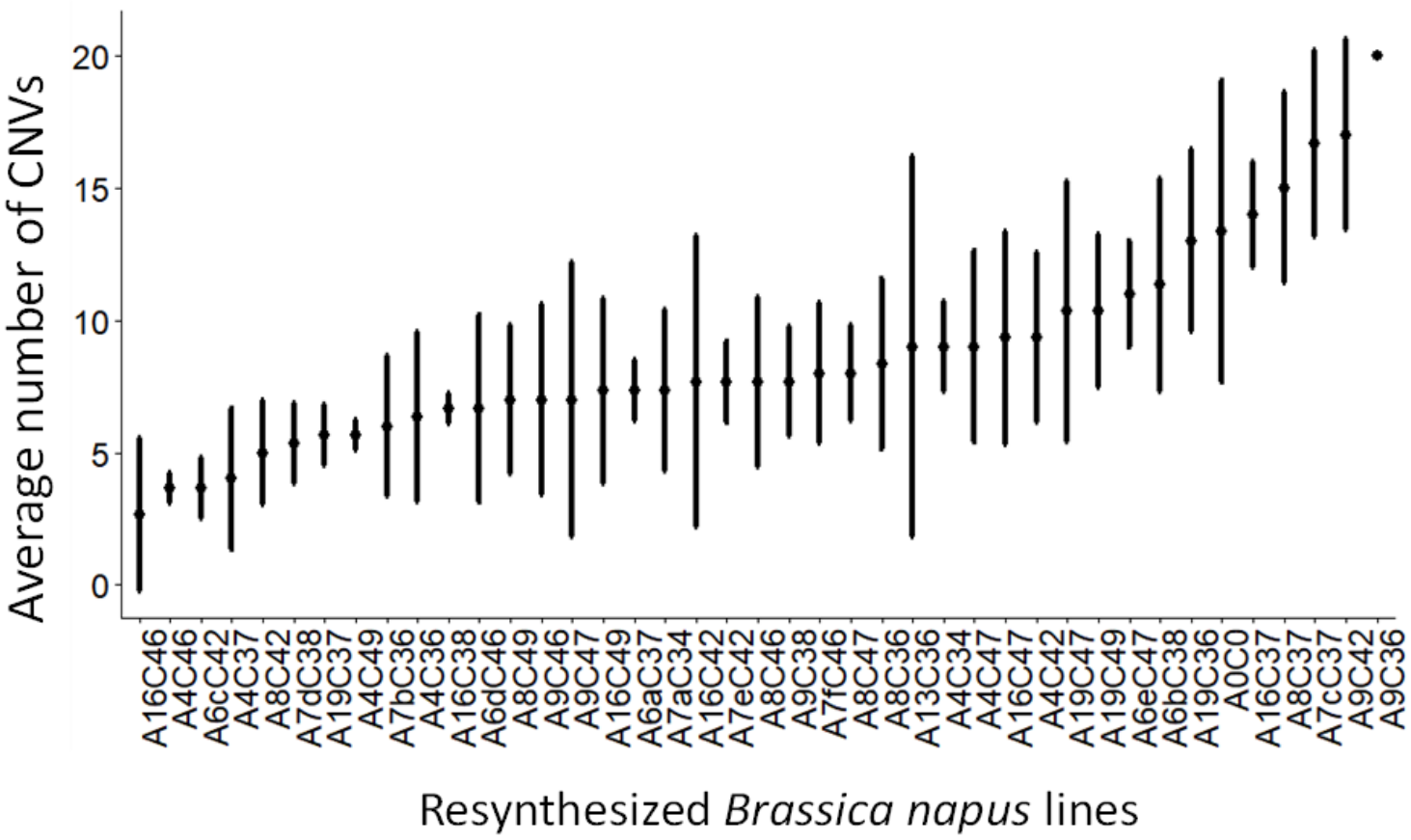

Figure 4

Fig. 4 Average number of CNVs between resynthesized *Brassica napus* (rapeseed) lines from different genotype combinations. Parent genotypes of *B. rapa* are A4, A6, A7, A8, A9, A13, A16 and A19, parent genotypes of *B. oleracea* are C34, C36, C37, C38, C42, C46, C47 and C49, and synthetic rapeseed lines are indicated by the parent combination in the form AXCX or AXaCX.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 20221206SupplementaryFiguresandTables.pdf
- FileS3logRratioS1resynthesizedBrassicanapus.xlsx
- FileS4meiosisgenesposition.xlsx
- FileS5phenotypeinformation.xlsx
- FileS6SNPsCNVsparentresynthesizedlines.xlsx
- FileS7summaryselectionofmeiosisgenes.xlsx
- FileS1DescriptionofresynthesizedS1Bnapuslines.xlsx
- FileS2SNPdataresynthesizedBrassicanapus.xlsx