A major quantitative trait locus on chromosome A9, BnaPh1, controls homoeologous recombination in Brassica napus

Erin E. Higgins1, Elaine C. Howell2, Susan J. Armstrong2 and Isobel A. P. Parkin1

1Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK S7N 0X2, Canada; 2School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK

Author for correspondence:
Isobel A. P. Parkin
Email: isobel.parkin@canada.ca

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Summary

- Ensuring faithful homologous recombination in allopolyploids is essential to maintain optimal fertility of the species. Variation in the ability to control aberrant pairing between homoeologous chromosomes in Brassica napus has been identified. The current study exploited the extremes of such variation to identify genetic factors that differentiate newly resynthesised B. napus, which is inherently unstable, and established B. napus, which has adapted to largely control homoeologous recombination.
- A segregating B. napus mapping population was analysed utilising both cytogenetic observations and high-throughput genotyping to quantify the levels of homoeologous recombination.
- Three quantitative trait loci (QTL) were identified that contributed to the control of homoeologous recombination in the important oilseed crop B. napus. One major QTL on BnaA9 contributed between 32 and 58% of the observed variation. This study is the first to assess homoeologous recombination and map associated QTLs resulting from deviations in normal pairing in allotetraploid B. napus.
- The identified QTL regions suggest candidate meiotic genes that could be manipulated in order to control this important trait and further allow the development of molecular markers to utilise this trait to exploit homoeologous recombination in a crop.

Introduction

The pervasiveness of polyploidy throughout the plant kingdom provides a clue to the importance of genome duplication, hybridisation and rearrangement in evolution. Over 70% of angiosperms are characterised as polyploids, although all plants are believed to have gone through whole genome duplication (WGD) at some point in their evolutionary history (Masterson, 1994; Alix et al., 2017). Plants can be broadly divided into three categories: diploids such as rice (Oryza sativa) in which, subsequent to ancient WGD events, chromosomes have fused and undergone further rearrangements to the extent that they appear identical and behave independently during meiosis; autopolyploids, such as alfalfa (Medicago sativa) in which all chromosomes have been duplicated at least once within a single nucleus; and allopolyploids, in which genomes from related species have hybridised within a single nucleus, including the important monocot and dicot crop species, wheat (Triticum aestivum) and canola (Brassica napus), respectively. Like all organisms, survival of a polyploid species depends on the ability to produce viable offspring. In the allotetraploid B. napus, each chromosome has an identical partner (homologue) and one or more closely related partners (homoeologue) making the process of pairing, recombination and separation more difficult. Brassica napus was formed from the fusion of a Brassica A (n = 10) and C (n = 9) genome diploid progenitor and marker and sequence analyses have highlighted the close similarity between these genomes; however, their homoeologous relationship is not straightforward (Chalhoub et al., 2014). Chromosomes such as BnaA1 and BnaC1 are aligned along their entire length while others have more than one potential pairing partner, for example BnaA9 shares homology with both BnaC8 and BnaC9. A summary of the primary homoeologous regions of B. napus is shown in Supporting information Fig. S1.

Meiosis is conserved amongst sexually reproducing organisms and is tightly controlled. Recombination during meiosis ensures reduction to a haploid set of chromosomes in each cell and creates genetic diversity (Mercier et al., 2015). Briefly, in most plants during prophase I of meiosis I, an axis forms along each replicated chromosome and double-strand breaks (DSB) occur. Through a process that is not fully understood, homologues find each other and the synaptonemal complex that holds them together is constructed while DSBs are repaired. If the repair of a DSB involves a nonsister chromatid, the result is a crossover (CO) or a noncrossover (NCO). A CO involves a reciprocal exchange between two homologous nonsister chromatids. The length of chromatid exchanged can be considerable, from the CO to the telomere or to the next CO, depending on which chromatids are involved. Subsequently chromosomes condense and the synaptonemal complex is dismantled, but homologues...
are held together as bivalents at COs, seen cytogenetically as chiasmata. A minimum of one chiasma per bivalent is essential for correct chromosome alignment at metaphase I (MI). Cohesion is then lost between chromatids, allowing the homologues to move to opposite poles using the spindle apparatus. During meiosis II, chromatids separate and four haploid cells are produced. Because this paper focuses on COs, the term ‘recombination’ is used in the context of COs solely, not NCOs.

Aberrant recombination between homoeologues can cause aneuploidy, as homoeologous bivalents and multivalents and unpaired univalents may not align correctly at MI. Additionally, the exchange can lead to gain/loss of homoeologous genome segments when chromatids segregate. Such rearrangements can be stably inherited and these changes can alter the balance of gene expression, impact chromosome conformation and facilitate further homoeologous mispairing during subsequent meioses (Gaeta et al., 2007; Gaeta & Pires, 2010). Thus, for long-term genomic stability and offspring fitness, neopolyploids must derive mechanisms to allow them to behave genetically as diploids during meiosis. In some cases these rearrangements can be advantageous in creating novel phenotypes, for instance a deleterious allele may be replaced with its functional homoeologue or vice versa. Some of the first genetic maps provided evidence of fixed and de novo homoeologous recombination events in B. napus (Parkin et al., 1995; Sharpe et al., 1995) and sequencing of the genome revealed several historical homoeologous non-reciprocal exchanges, including one responsible for the low glucosinolate seed content that was instrumental for the development of B. napus as a major oilseed crop worldwide (Chalhoub et al., 2014). Current molecular techniques have uncovered relatively high rates of de novo homoeologous recombination events in some modern cultivars (Higgins et al., 2018) indicating that it is still an important mechanism in B. napus evolution.

Knowledge of the mechanisms that regulate chromosome pairing, synapsis and homoeologous recombination is important to enable their manipulation to improve crop diversity and productivity of allopolyploids. The species studied most extensively is hexaploid wheat (Triticum aestivum), as a line with a large deletion on chromosome 5B showed aberrant chromosome pairing at meiosis (Riley & Chapman, 1958; Sears & Okamoto, 1958). The locus controlling this phenotype, Ph1 (Pairing homoeologous 1), continues to be the subject of research (see Martín et al. (2017); Rey et al. (2017) for recent work). Minor effect loci have also been identified, including Ph2 (Mello-Sampayo, 1971) and loci implicated in either suppressing homoeologous or promoting homologous chromosome pairing and recombination (reviewed in Jenczewski & Alix (2004)). Less information is known for B. napus, but one locus, Pairing regulator in B. napus (PnBr), which is involved in the control of homoeologous chromosome pairing in B. napus haploids (AC), was discovered by exploiting natural variation for high and low allo-syndetic pairing in haploids of two B. napus lines (Jenczewski et al., 2003). This locus was mapped to chromosome BnaC9, and several minor loci with additive or epistatic effects were also identified (Liu et al., 2006). However, both AACC parental lines had regular bivalent pairing (Jenczewski et al., 2003), making the role that this locus plays in homoeologous recombination in the allopolyploid unclear.

Resynthesised B. napus lines, created by crossing B. oleracea with B. rapa and doubling the chromosome complement, have much higher rates of homoeologous recombination compared to established lines (Parkin et al., 1995; Sharpe et al., 1995; Udall et al., 2005; Gaeta et al., 2007; Samans et al., 2017). Similarly, a high frequency of homoeologous bivalents and multivalents, identified by labelling the C genome with a BAC probe, was observed in resynthesised lines (Szadkowski et al., 2010). The current study exploits the difference between established and resynthesised lines of B. napus to map quantitative trait loci (QTL) controlling homoeologous chromosome recombination in a segregating doubled-haploid population. Using both cytogenetic assessment of homoeologous events and quantification of homoeologous recombination events with high density SNP genotyping, one major and two minor QTL loci were genetically mapped. Candidate meiosis-specific genes underlying these QTL were identified by searches of genome annotation and supplementary gene expression data.

Materials and Methods

Plant material
All experiments used individuals from a B. napus doubled-haploid (DH) population known to segregate for homoeologous recombination, hereafter referred to as the SGDH population (Clarke et al., 2016). This population was created from two F1 lines derived by reciprocal crossing of DH12075, a DH spring-type B. napus derived from a cross between Westar and Cresor, and PSA12, a resynthesised B. napus derived from a cross between B. oleracea line A12 and B. rapa line PS270. Individuals from this population were selfed for three or four generations before use for the cytogenetic analysis or testcross population development, respectively. Details of growth conditions, crossing strategy, tissue harvest and sample preparation of plant material is provided in the Supplementary Methods S1.

Cytogenetic analysis, SNP array analysis and QTL mapping
DH12075 and 43 SGDH lines were examined cytogenetically using fluorescence in situ hybridisation (FISH) followed by genomic in situ hybridisation (GISH) on slides prepared from anthers with meioocytes at late diakinesis/MI (Methods S1) (Fig. 1). The majority of lines was represented by one plant, except three lines (SG-201, SG-309, SG-324) that had two plants examined and two lines (SG-152, SG-159) that had three plants examined for a total of 50 SGDH plants. A total of 3125 meioocytes was assessed, averaging 61 per plant (Table S1). Using FISH with 45S rDNA and a BAC probe (BoB061G14) the A and C genome chromosomes were divided into three and four groups, respectively, and within these groups, some individual chromosomes could be identified by the size and position of the 45S rDNA signal (Table 1). Linkage groups were assigned by comparison with published karyotypes (Howell et al., 2002, 2008; Xiong & Pires, 2011). Homoeologous bivalents, trivalents, quadrivalents and higher order multivalents identified by FISH
and GISH were recorded for each meiocyte. The score for each configuration is effectively the number of chromosome pairs involved minus one, for example a quadrivalent of two homoeologous chromosome pairs is given a score of ‘1’ for ‘one synaptic partner switch (SPS) accompanied by at least one CO (SPSC)’ and a multivalent involving three pairs of chromosomes is scored as ‘2’, etc.

This is a conservative estimate of homoeologous recombination because accurate counting of chiasmata was not possible, so the number of chiasmata above one per SPS is not assessed. A detailed description of the method is provided in the Methods S1 and illustrated in Fig. 2. The number of SPSC in each meiocyte was calculated and the total for each plant divided by the number of meiocytes scored gave a mean number of SPSC per meiocyte for each plant (Table S1).

For SNP array analysis, testcross F1 populations were created by crossing lines from the *B. napus* SGDH population with the adapted *B. napus* line, Rainbow. Initially, 31 SG lines were selected at random from the 124 genotyped lines in the SGDH population and, following preliminary QTL analysis, an additional 17 lines with crossovers near putative QTL regions were added. DH12075, PSA12 and the 48 SGDH lines for the testcross populations and all testcross F1 lines were genotyped using the *Brassica* 60K Illumina Infinium array (Clarke *et al.*, 2016). These data confirmed that SGDH lines were replicates of the individuals used to generate the original genetic map but identified small exchanges in DH12075 and PSA12 compared with the individuals used as parents to generate the SGDH population. The testcross F1 lines were scored for gain and loss of alleles using the method described in Higgins *et al.* (2018), whereby a change in fluorescence ratio of the two fluorophores was used to determine changes in copy number at a particular SNP locus. Physical positions of the SNP loci were used to determine changes in copy number at a particular SNP locus.

# Table 1

Categorisation of the chromosomes of *Brassica napus* DH12075, *B. rapa* PS270 and *B. oleracea* A12 by the distribution of 45S rDNA (S) and BoB061G14 (G) FISH signals on the A and C genome chromosomes.

| Probe signal          | Brassica A genome | Brassica C genome |
|-----------------------|-------------------|-------------------|
| 45S rDNA (S)          | AS                | CS                |
|                       | A3 S large, terminal | C8 S terminal   |
|                       | A5 S near centromere |                  |
| 45S rDNA (S) and      | AGS               | CGS               |
| BoB061G14 (G)         |                   |                   |
|                       | A1 S large, near centromere |             |
|                       | A6 S near centromere | C7 S terminal   |
|                       | A9 S near centromere | C4 S near centromere |
| BoB061G14 (G)         | AG                | CG                |
|                       | A2 A4 A7 A8 A10   | C1 C2 C4* C5 C6  |
| No signals (N)        | –                 | CN                |
|                       | –                 | C3 C9             |

†A9 – S is medium strength in DH12075 but very faint in PS270.

*C4 – S is present in A12 but absent in DH12075.

Fig. 1 Examples of chromosome spreads from *Brassica napus* SGDH lines. Chromosome spreads of meiocytes at late diakinesis/MI examined by FISH (left image of each pair) with 45S rDNA (green) and BoB061G14 (red), and GISH (right image of each pair) with labelled C genome (red), DAPI-stained A genome (blue). Homoeologous bivalents and quadrivalents (arrows), multivalents (stars) and a univalent whose partner is in a multivalent (triangles) are highlighted. (a) SG-261, a single C9 in a bivalent with an extra A10; (b) SG-5, two AG CGS bivalents, one AG CG bivalent, one multivalent (A10 C9 A9 C8 C8 A9 C9 A10); (c) SG-235, two A1 C1 homoeologous bivalents orientated with both A1 facing the same pole and both C1 facing the other, one A3 A3 C3 C3 quadrivalent; (d) SG-25, one AG AG CG CG quadrivalent, one multivalent (C8 C8 A9 C9 C9) with univalent A9. The 45S rDNA signal on A9 in (b–d) is not visible at this exposure. Bar, 5 μm.

Fig. 2. The number of SPSC in each meiocyte was calculated and the total for each plant divided by the number of meiocytes scored gave a mean number of SPSC per meiocyte for each plant (Table S1). This mean (or the cumulative mean for the five lines in which more than one plant was analysed) was used as the trait value for QTL mapping.

For SNP array analysis, testcross F1 populations were created by crossing lines from the *B. napus* SGDH population with the adapted *B. napus* line, Rainbow. Initially, 31 SG lines were selected at random from the 124 genotyped lines in the SGDH population and, following preliminary QTL analysis, an additional 17 lines with crossovers near putative QTL regions were added. DH12075, PSA12 and the 48 SGDH lines for the testcross populations and all testcross F1 lines were genotyped using the *Brassica* 60K Illumina Infinium array (Clarke *et al.*, 2016). These data confirmed that SGDH lines were replicates of the individuals used to generate the original genetic map but identified small exchanges in DH12075 and PSA12 compared with the individuals used as parents to generate the SGDH population. The testcross F1 lines were scored for gain and loss of alleles using the method described in Higgins *et al.* (2018), whereby a change in fluorescence ratio of the two fluorophores was used to determine changes in copy number at a particular SNP locus.
were designated as homoeologous recombination (HeR) events if the reciprocal gain and loss of A and C genome loci could be detected and these events were annotated separately from those in which only the duplication or deletion of chromosome segments could be identified. Although chromosomal duplications and deletions could have arisen through homoeologous chromosome pairing it is possible that other forms of aberrant pairing or intrachromosomal associations created such anomalies, thus such events were counted separately. The average number of HeR events in the testcrosses for each SGDH line was used for QTL mapping (Table S3).

QTL mapping was carried out using the WinQTL Cartographer software v.2.5 (Basten et al., 1999). Summaries of all methods as they apply to this data set and parameters for QTL analysis are also provided in the Methods S1.

RNA-seq

Replicate RNA-seq libraries were prepared from meiocytes and leaf tissue of the natural *B. napus* parent of the SGDH population, DH12075. Sequence data were submitted to NCBI short read archive under the accession number PRJNA664521. Resulting reads were aligned to the *B. napus* spring-type DH12075 genome sequence (I. A. P. Parkin, unpublished; available at http://Cruciferseq.ca) using STAR (Dobin et al., 2013) and gene read counts were generated using FeatureCounts from the

Fig. 2 Diagrams illustrating meiotic configurations resulting from synaptic partner switches from homologues to homoeologues in *Brassica napus* SGDH lines. Synaptic partner switches (SPS) (upper diagram), MI configuration (lower diagram). A genome chromosomes (blue), C genome chromosomes (red), centromeres (solid circles). Chromosomes consist of two chromatids (not shown). The minimum number of crossovers (CO) (black crosses) required in each synapsed region is shown. The allocated score is in the adjacent text box. (a–g) Two pairs of chromosomes with homoeology in one arm and one SPS. (a) One SPS but no CO in a region of synapsis between homoeologues results in two homologous bivalents and this has no score. (b–g) One SPS accompanied by one CO (one SPSC) with the outcome at MI depending on the presence/absence of COs in the other switched/not switched regions. (b) AC bivalent with A and C univalents. (c) two AC bivalents. (d) AAC trivalent and C univalent. (e) ACC trivalent and A univalent. (f) chain quadrivalent AACC. (g) ring quadrivalent AACC. (h) An example of two SPSC between three homoeologous chromosome pairs with additional COs resulting in one multivalent and a univalent as in Fig. 1(d). (i) An example of three SPSC with additional COs between four chromosome pairs with homoeology as in Fig. 1(b).
Results

Estimating level of homoeologous events using cytogenetics

Chromosome spreads at meiotic MI, obtained from pollen mother cells of DH12075 and 43 of the 48 SGDH lines used in the SNP array analysis, were used to assess the variation in the level of homoeologous events in the SGDH population. A total of 64 male meiocytes of DH12075 was assessed to provide an estimate of HeR events in the natural B. napus parent of the SGDH population. There was one ring quadrivalent involving A and C chromosome pairs in 14 meiocytes, giving a mean number of SPSC per meiocyte of 0.22 (14 SPSC in 64 meiocytes). It was clear that these were not all formed from the same chromosome pairs, as seven of the 12 possible combinations between the A and C groups (Table 1) were recorded (Table S1). Their infrequent occurrence, the variety of combinations seen and the fact that the plant was DH indicated that these were newly formed homoeologous quadrivalents. No homoeologous bivalents were seen. In four other meiocytes it was noted that one pair of chromosomes had not formed a bivalent and it was not the same pair each time. The absence of the ‘obligate chiasma’ between homologues was of interest because it is unusual in plants and can cause aneuploidy, but it did not contribute to the SPSC score of this plant.

Of the 50 SGDH plants (representing 43 lines) examined, 36 had 38 chromosomes but four of these had numbers of A or C chromosomes differing from the expected 20A and 18C (Table S1). The other 14 plants had 35 to 40 chromosomes, with the complement of A ranging from 17 to 22 and C from 15 to 21. Of the five SGDH lines represented by two (three lines) or three (two lines) plants, three had plants with atypical numbers: SG-324 gained BnaA1, SG-324-a gained BnaA1 and lost BnaC1; SG-152 lost BnaC8, SG-152-a1 and a2 were normal; SG159-1 gained BnaA1 and lost BnaC1, SG159-2 was normal, SG159-3 lost BnaA1 (Table S1).

Examples of chromosome spreads of meiocytes from SGDH lines are shown in Fig. 1. The most frequent configurations between A and C genome chromosomes were quadrivalents. As far as the FISH signals allowed, the two A chromosomes appeared to be homologues, as were the two C chromosomes, as expected. Of the 12 possible combinations of the groups in Table 1, all but one, BnaA3 or BnaA5 with BnaC8, contained at least one set of A and C chromosomes with known homoeologous regions (Fig. S1; Table S1). The BnaA3 or BnaA5 with BnaC8 combination was observed only six times in 3125 chromosome spreads. Mean values of SPSC per meiocyte for each of the 12 observable combinations were calculated for each plant, as well as the overall mean per plant (Table S1).

One advantage of cytogenetics is its ability to detect chromosome configurations that may produce nonviable gametes due to mis-segregation. In some cases, the orientation of a pair of homoeologous bivalents (Fig. 1c) or a quadrivalent at MI was such that both A centromeres faced one pole and both C centromeres faced the other. The resulting pollen grains will have either two A chromosomes or two C chromosomes (those chromatids involved in the COs would be mainly A or C but have a section of the other genome). In some plants, multivalents involving more than two pairs (Fig. 1b,d) were seen, and for those in which specific chromosomes could be identified, the chromosomes that had chiasmata with two different chromosomes had known homoeology with both. The SG-261 plant (Fig. 1a) had an extra BnaA10 but only one BnaC9 and, in 15 of the 31 meiocytes scored, these formed a homoeologous bivalent that contributed to the total score. The plants with the highest means (SG-271, 3.7 and SG-5, 3.0), had high scores for at least two combinations and both had atypical numbers of A and C chromosomes. The scores of individual meiocytes from the SG-5 plant ranged from 0 (one cell) to 6 (one cell). The mean of SG-230 at 0.11 was similar to DH12075 (0.22), but even this plant was not normal as one chromosome arm of a C chromosome was shorter than its homologue. The maximum score for a meiocyte in SG-230 was 1 and the heteromorphic pair was not involved in any of the homoeologous quadrivalents. There was some variation between the means of the plants in the five lines represented by two or three plants, partly due to differences in chromosome numbers.

Estimating level and extent of homoeologous events using SNP markers

Testcross lines generated from crosses between DH12075 and PSA12, and B. napus line Rainbow indicated as expected that the resynthesised line PSA12 had higher levels of HeR and unpaired duplications/deletions. Forty-four testcross individuals from DH12075 were analysed, only one HeR event was observed (0.02 HeR events per individual). The first 2 Mb of chromosome BnaC2 was missing from all DH12075-derived testcross lines and two unique duplication and six unique deletion events were identified (on average 1.18 duplication/deletion events per individual). Chromosome BnaC2 was completely deleted in two of the DH12075 testcross lines, and one individual had an extra BnaA9 chromosome. By contrast, PSA12 testcross individuals had, on average, 3.85 reciprocal events per line and 5.13 deletion/duplication events. Of the unpaired events, five were whole chromosome deletions and, in three cases, an extra copy of an entire chromosome was present. Analysis of the testcross populations indicated that the PSA12 parent carried both fixed and heterozygous HeR events (Table S5). For example, the top 2 Mb of BnaC1 was missing in all PSA12 testcross lines, suggesting a fixed event, while an HeR event between BnaA1 and BnaC1 segregated in the F1 (present in 20 of 40 lines), although the size of this event varied from a 2 Mb exchange to reciprocal deletion of BnaA1 and duplication of BnaC1 in their entirety. This variation in length of event was due to the heterozygous HeR region being further modified in the meiosis that produced the testcross individuals, demonstrating that homoeologous chromosome pairing
and recombination continues with every generation and is further evidence of the instability of the A and C genomes in newly resynthesised *B. napus* lines. The parental and SGDH lines had been selfed for multiple generations before creating the testcross F1 populations and it was clear from the SNP data in some of the lines that homoeologous exchanges had occurred in previous generations. Although it cannot be known what effect a preexisting homoeologous exchange has on recombination, calculation of the number of HeR per testcross individual would include both inherited and new events as both are caused by meiotic instability. Informative SNP array data for all testcross lines is given in Table S2 and a summary of all quantified events is shown in Table S3.

Only one of the testcross families, SG-230, did not show evidence of a reciprocal HeR event, and all families had at least one event in which only the duplication or deletion of alleles was observed. The highest level of HeR was seen in the SG-5 family with an average of 9.9 events per individual. Similar to observations from the PSA12 F1 lines, the SG-5 F1 individuals had fixed events that had been inherited from the SG-5 parent and further modified in the testcrosses. The 'hyper-recombination' led to extreme values, particularly in the SG-5 and SG-235 testcross families, that skewed the distribution of HeR frequency (Fig. 3). The quantification of homoeologous events measured using the SNP array and cytogenetic analysis had a strong correlation (*r* = 0.721) and the lines with extreme phenotypes such as SG-5 (high HeR) and SG-230 (low HeR) were the same in both analyses, thus validating the complementarity of the two methods (Fig. 3).

In total, there were 2095 HeR events in the 803 lines analysed, with the most common exchanges being between the highly collinear chromosomes BnaA1 and BnaC1, which had 31% of all HeR events (Fig. 4; Table S3). Chromosome BnaA6 had the fewest events, only five in total (0.2%), two with BnaC5 and three exchanges with BnaC7. Chromosome BnaA5 had a clear preference for recombination with BnaC5 rather than its alternate homoeologue BnaC4, 65% and 35%, respectively. For others with more than one potential pairing partner, such as BnaA9, the exchanges were much more balanced between the two possible homoeologous partners BnaC8 and BnaC9, 45% and 55%, respectively. HeR events in which the A genome replaced the C genome were 2.2 times more frequent than those in which the C genome replaced the A genome.

Unpaired duplication and deletion events (with no apparent reciprocal exchange) were 50% more common than HeR events. Duplications were more common that deletions, three times more likely, although this ratio was exaggerated by several duplications that were inherited from the SGDH parents and were present in a large number of testcross individuals. Specifically, there is a duplication at the top of BnaC3 and another at the bottom of BnaA10 present in almost half of the testcross F1 individuals. The unpaired deletions and duplications were more common in the A genome than the C genome, 65% and 56%, respectively. Chromosome BnaA1 had the highest number of deletions (12% of all deletions) and BnaC7 had the fewest (0.4%). Duplications were most common on chromosomes BnaA10 and BnaC3 due to the inherited duplications common to a large number of testcross individuals and were least common on BnaA8 (0.1% of all duplications). Aneuploids accounted for 10% of all unpaired deletions and 6% of all unpaired duplications in the SGDH testcross lines.

**Genetic mapping of QTL controlling level of homoeologous exchange**

A genetic map of the SGDH population with 21 118 SNP markers had previously been generated (Clarke *et al.*, 2016), genotype...
data for the SGDH lines used in the current analyses were extracted and QTL mapping was performed with phenotype data from both the cytogenetic and molecular estimates of HeR. Cytogenetic analysis identified two significant QTL, one on BnaA3 (25.7–26.2 Mb) and the second on BnaA9 (11.1–23.9 Mb) (Table 2). Based on the SNP marker analysis, the average rate per individual of HeR and unpaired deletions and duplications for each SG testcross family were used independently to map QTL controlling homoeologous recombination. Significant QTL controlling the level of HeR exchange were identified on chromosomes BnaA3 (23.3–26.2 Mb), BnaA9 (11.1–23.9 Mb) and BnaC7 (42.2–43.4 Mb). In the analysis of unpaired duplications/deletions a QTL was identified on BnaA9 (10.3–23.9 Mb). The three independently mapped QTLs on chromosome BnaA9 were located in the same very large pericentromeric region (Fig. 5); and the two QTLs near the bottom of BnaA3 from the cytogenetic and HeR analysis, respectively also overlapped. The HeR BnaC7 locus was not verified, potentially because fewer lines were used for cytogenetic analysis.

Of the identified QTLs, at the BnaA9 and BnaC7 loci alleles from the resynthesised line PSA12 contributed to high levels of HeR, but at the BnaA3 locus DH12075 alleles were associated with elevated HeR. A previous study showed that even elite B. napus lines exhibited generally low but varying levels of HeR (Higgins et al., 2018) so it is perhaps not surprising to detect alleles for increased rates of HeR coming from the natural B. napus line. It was noted in the cytogenetic analysis that when lines were ranked according to their mean scores, the nine highest had inherited the faint 45S rDNA signal attributed to the PSA12 parent that is close to the pericentromeric region on BnaA9. The only other lines with this signal, namely SG-155, SG-309 and SG-318, were ranked among the next 10. In all cases the BnaA9 locus was the strongest QTL representing 34, 32 and 58% of the variation in the HeR, deletion/duplication and cytogenetic analyses, respectively. The BnaA3 locus in the HeR events accounted for 17%, the BnaC7 locus for 9%, and the BnaA3 locus from the cytogenetic analysis was responsible for 9% of the observed variation (Table 2).

**Distribution of meiotic genes across the Brassica napus genome**

In order to determine if any of the QTL loci could be associated with annotated meiosis-related genes, homologues of documented meiosis genes were identified in the B. napus genome. Meiosis has been studied extensively in Arabidopsis thaliana, such that 141 meiosis-related genes could be collated from various sources and are listed in Table S6 (Kaur et al., 2006; Ma, 2006; Geuting et al., 2009; Bauknecht & Kobbe, 2014; De et al., 2014; Oh et al., 2014; Lario et al., 2015; Mercier et al., 2015; Wright et al., 2015; Vrielynck et al., 2016; Bolanos-Villegas et al., 2018; Chambon et al., 2018; Fernandes et al., 2018; Rohrig et al., 2018). Sequence homology searches against available gene annotations identified 416 and 410 homologous gene copies in two B. napus reference genomes, that of DH12075 and Darmor, respectively (Table S6). There was no obvious change in gene copy number since the formation of B. napus, as only 19.6% of all A. thaliana genes have only two copies since the formation of B. napus, as the progenitor genomes of B. oleracea and B. rapa contained 204 and 207 annotated genes, respectively (Table S6). Over half (53.9%) of the A. thaliana genes were found in only two homoeologous copies in the allopolyploid B. rapa. This was significantly higher than expected, as only 19.6% of all A. thaliana genes have only two potential orthologues in B. napus. The result mirrors that found in a range of angiosperms that showed a biased loss of meiotic gene duplicates subsequent to WGD (Lloyd et al., 2014). The progenitor A and C genomes of B. napus are suggested to have evolved through a two-step process with the third ancestral genome, which maintained a higher number of gene copies over time (least fractionated), presumed to be hybridised last (Cheng et al., 2012). Interestingly this evolutionary path is not obvious from the meiosis genes as, of those genes maintained in two copies, only 30.3% are found in the least fractionated genome.

![Events per chromosome](image-url)
suggesting a much more balanced fractionation or preferential maintenance of gene copies from the first two genomes.

For each of the QTL regions the sequence underlying the QTL confidence interval was searched for potential meiosis-related genes and all possible candidates are listed in Table 2. In each instance between one (BnaA3_SPSC) and five genes were identified (BnaA9 loci), which did not necessarily reflect the physical size of the QTL region (Fig. 5). As a number of genes with common function were identified for QTL BnaA3_HeR and BnaC7_HeR, it suggests they are derived from orthologous regions. RNA-seq libraries were made from isolated meiocytes and leaf tissue from DH12075. Read counts for all libraries are given in Table S4 and expression of the meiosis genes underlying the QTL regions for leaf and meiocyte tissue in DH12075 is summarised in Table 2. As it is not clear that all homologous duplicated gene copies found in *B. napus* would maintain a common function as that predicted from *A. thaliana* gene annotation, the *B. napus* RNA-seq data was used to indicate whether the particular gene copy was being expressed in meiotically active tissue. Of the 12 candidates, three showed no observable expression in meiocyte tissue of the stable parental line. For BnaA3_HeR and its homoeologous region on BnaC7, two genes were relatively highly expressed in meiocyte tissue, orthologues of At4g22970, *MSH3* and *RPA1C*. For the strongest QTL region on BnaA9, mapped by all phenotypes, the most obvious difference in expression levels between leaf and meiocyte tissue was detected for an orthologue of At5g45400 (*RPA1C*).

### Table 2 QTL loci controlling homoeologous pairing in *Brassica napus*.

| Chromosome | QTL name | Map position (Mbp) | LOD score | $R^2$ | Additive effect | *B. napus* candidates | Arabidopsis thaliana homologue | Common name(s) | RNA-Seq data |
|------------|-----------|-------------------|-----------|-------|-----------------|------------------------|-----------------------------|----------------|--------------|
|            |           |                   |           |       |                 |                        |                             |                | Leaf (cpm)  |
| Reciprocal exchange | | | | | | | | | |
| A3 | BnA3_HeR | 23.3–26.2 | 6.8 | 0.17 | 1.65 | BnaN03g45420 AT4G20900 | MS5, TDM1, FZR2, CCS52A1 | RSW4 | 0 | 0 |
| | | | | | | BnaN03g46530 AT4G22910 | 0 | 0.9 |
| | | | | | | BnaN03g46570 AT4G22970 | 63.5 | 3.7 |
| | | | | | | BnaN03g48460 AT4G25540 | 38.5 | 2 |
| | | | | | | BnaN09g19560 AT5G45400 | 26 | 1.7 |
| | | | | | | BnaN09g22360 AT4G05190 | 16.5 | 21.7 |
| A9 | BnA9_HeR | 11.1–23.9 | 8 | 0.34 | –2.63 | BnaN09g23400 AT4G30870 | MUS81 | 14 | 9.7 |
| | | | | | | BnaN09g25570 AT4G11920 | 3.7 | 1.1 |
| | | | | | | BnaN17g45230 AT4G22910 | 0 | 0.5 |
| | | | | | | BnaN17g45270 AT1G34355 | 51.5 | 5 |
| | | | | | | BnaN17g47090 AT4G25540 | 49 | 5 |
| C7 | BnC7_HeR | 42.2–43.4 | 3.3 | 0.09 | –1.76 | BnaN09g26800 AT1G34355 | RPA1C | 0 | 0 |
| | | | | | | BnaN17g45230 AT4G22910 | 0.1 | 0.5 |
| | | | | | | BnaN17g45270 AT1G34355 | 51.5 | 5 |
| | | | | | | BnaN17g47090 AT4G25540 | 49 | 5 |
| Deletion/duplication | | | | | | | | | |
| A9 | BnA9_DD | 10.3–23.9 | 4.8 | 0.32 | –2.05 | BnaN09g19560 AT5G45400 | RPA1C | 26 | 1.7 |
| | | | | | | BnaN09g22360 AT4G05190 | 16.5 | 21.7 |
| | | | | | | BnaN09g23400 AT4G30870 | 14 | 9.7 |
| | | | | | | BnaN09g25570 AT4G11920 | 3.7 | 1.1 |
| | | | | | | BnaN09g26800 AT1G34355 | 0 | 0 |
| Synaptic partner switch | | | | | | | | | |
| A3 | BnA3_SPSC | 25.7–26.2 | 3.5 | 0.09 | 0.48 | BnaN03g48460 AT4G25540 | MUS81 | 14 | 9.7 |
| | | | | | | BnaN09g19560 AT5G45400 | 38.5 | 2 |
| | | | | | | BnaN09g22360 AT4G05190 | 26 | 1.7 |
| | | | | | | BnaN09g23400 AT4G30870 | 14 | 9.7 |
| | | | | | | BnaN09g25570 AT4G11920 | 3.7 | 1.1 |
| | | | | | | BnaN09g26800 AT1G34355 | 0 | 0 |

**Discussion**

Structural variation in the form of copy number or presence/absence variants and even major chromosomal exchanges is being increasingly identified as natural sources of important trait variation (Gabur *et al.*, 2019). The recent advances in whole genome sequencing have begun to expose the extent of such variation within many crop species and the importance of HeR as a mechanism causing such changes in allopolyploid crops is now evident (Chalhoub *et al.*, 2014; Stein *et al.*, 2017). Genetic control of
HeR has either been clearly mapped or suggested for several important crop species including wheat (Martín et al., 2018), canola (Jenczewski et al., 2003), coffee (Lashermes et al., 2016) and cotton (Flagel et al., 2012), and the ability to control and manipulate chromosome rearrangements could be an effective tool for crop improvement. In this study both cytogenetic analysis and molecular quantification of HeR were used to identify loci important for controlling mispairing and subsequent HeR in B. napus. One major QTL on chromosome BnaA9 (BnaPh1, B. napus Pairing homoeologous 1) was independently verified using each method and was by far the largest contributor to variation in each QTL analysis, explaining 32–58% of the total variation. These QTLs are the first to be mapped in B. napus using HeR rate in a segregating allotetraploid population.

![Fig. 5 Map positions of QTL controlling homoeologous pairing events in *Brassica napus*. The outer circle represents the physical length of the chromosomes (A genome in blue, C genome in red), the inner circle (green) the genetic linkage groups, the position of the markers on the physical chromosomes is shown by the linked grey lines. The positions of the QTL loci are shown by coloured blocks, with the colours representing the different phenotypes used to identify loci; purple – HeR only, blue – HeR and cytogenetics, and yellow – common to all phenotypes. The synteny between *B. napus* meiosis genes are shown as connecting lines across the centre of the circle, those genes with only two orthologues are shown in red.](image-url)
Before this study, the only major locus controlling chromosome pairing in *B. napus*, PrBn, was identified by measuring different levels of chromosome pairing between the *Brassica* A and C genomes in allohaploid *B. napus* (Jenczewski et al., 2003). The PrBn QTL was mapped to chromosome BnaC9 and an additional six minor QTL were further identified on chromosomes BnaA1, BnaC1, BnaC3 and BnaC6 in segregating allohaploid lines (Liu et al., 2006). None of these previously mapped minor QTL corresponded to any of the QTL mapped in the current study, although interestingly the BnaC9 locus appears to locate to a homoeologous region to that of the BnaA9 locus identified in this study. More recently, Gaebelien et al. (2019) studied synthetic *Brassica* allohexaploid plants (A, B and C genomes) and used GWAS to identify SNP markers on A3, A4, A10, C3 as well as three B genome loci that correlated with seed yield as a proxy for meiotic stability, but similarly none of these loci overlapped with the QTLs identified in this study. However, the authors note that in one of their allohexaploid populations there was evidence that the loss of both copies of chromosome A9 could not be tolerated, although other aneuploids persisted through multiple generations. In this study aneuploids were reasonably common and all annotated genes within the BnaA9 region are listed (Table 2). The most interesting of these genes is *RPA1C* that is a candidate meiosis-related gene that is the major controller of mismatch repair in *E. coli* (Kunkel & Erie, 2005). There are seven known *MutS* homologues in *A. thaliana*, of which MSH4 and MSH5 have clearly identified roles in meiotic recombination (Higgins et al., 2004; Higgins et al., 2005) while MSH2, MSH3, MSH6 and MSH7 are important for DNA repair in somatic cells of *A. thaliana* (Culligan & Hays, 2000). More recent studies have demonstrated a role for MSH2 in suppression of HeR during meiosis in tomato (Tam et al., 2011), similarly MSH6 in rice (Jiang et al., 2020), and in Arabidopsis disruption of MSH7 led to an increase in meiotic recombination (Lario et al., 2015). Additionally, in wheat, MSH7 has been identified as a candidate *Ph2* gene (Dong et al., 2002; Lloyd et al., 2007). In *B. napus*, one of the candidate meiotic instability loci identified by Gaebelien et al. (2019) was *MSH2* on chromosome C3. Gonzalo et al. (2019) demonstrated that when MSH4 was reduced to only one functional copy HeR was decreased in *B. napus* allohaploids, but homologous recombination in allohaploids was unaffected. The effect of MSH4 reduction on the rate of HeR in allohaploids could not be determined due to the low rate of naturally occurring HeR. This work suggested that reducing the number of functional gene copies for meiotic genes may be an important evolutionary adaptation for meiotic stability in polyploids.

The need for neo-allopolyploids to quickly minimise mispairing between chromosomes and restrict recombination to homologues is paramount for ensuring the survival and adaptation of the new species. *Brassica napus* is a relatively recent allopolyploid (<10 000 yr old) and yet it already shows control of pairing and recombination, specifically when compared with a newly synthesised line. The rapid adaptation of this phenotype would perhaps be suggestive of gene deletion, yet all the identified meiosis-related genes were present in the respective diploids, although it is possible that either the substantive progenitor for *B. napus* is no longer available or there is untapped natural variation for this trait within the diploid germplasm. Because diploidisation is essential for allopolyploid survival, it is not surprising that it has
evolved in other major crop species but, based on current knowledge, the mechanism for achieving this may vary. For example, in wheat recent evidence points to ZIP4, a homologue of yeast Spo22, as the major gene controlling homoeologous chromosome pairing (Martín et al., 2018), but this is unlikely to be the case in *Brassica*. The phenotype for *Ph1* is extreme and is only seen in deletion lines, no natural variation has been found, unlike *B. napus* which showed a low but measurable amount of HeR even in elite lines (Higgins et al., 2018). The current study exploited the difference in recombination rates in a segregating population to identify loci important for this trait and evaluated the recognised meiotic genes underlying those as possible candidates. Further analysis of these loci in other populations may provide a more precise position for the major QTL on BnaA9 and new long read sequencing technologies can further elucidate the genes in the pericentromeric region that will be underrepresented in current short read genome assemblies. Combined, these two approaches will help to determine if plant species have found a common solution to one of the challenges brought about by allopolyploidy or if species have developed unique ways to deal with this problem.

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**Author contributions**

EEH carried out the molecular characterisation of the mapping population, analysed the data and drafted the manuscript. ECH carried out the cytogenetic analyses of the mapping population and generation of the meiocyte RNA-seq data with the advice and assistance of SJA and both assisted with drafting the manuscript. IAPP assisted with the analyses of the molecular data and the drafting of the manuscript. All authors edited and approved the final manuscript. The project design was conceived and contributed to by all authors.

**ORCID**

Susan J. Armstrong 🌐 [https://orcid.org/0000-0002-7284-5702](https://orcid.org/0000-0002-7284-5702)  
Isobel A. P. Parkin 🌐 [https://orcid.org/0000-0002-5807-9466](https://orcid.org/0000-0002-5807-9466)

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Supporting Information Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Alignment of the A and C genomes of B. napus.

Methods S1 Cytogenetic analysis of SGDH lines, SNP array analysis of testcross lines, QTL mapping parameters and RNA-seq library preparation and analysis methods.
Table S1 Summary of cytogenetic scoring.

Table S2 Informative SNP array data for all testcross lines.

Table S3 Summary of HeR and deletion/duplication quantification scored using SNP data.

Table S4 RNA-seq read counts for all libraries.

Table S5 Fixed and heterozygous events in DH12075 and PSA12 testcross parents.

Table S6 List of all recognised meiotic genes in *Brassica napus*, *B. rapa* and *B. oleracea*.

Table S7 All annotated genes underlying BnaA9 QTL.

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