A genome in flux: homoeologous exchanges, subgenome dominance, and gene dosage balance constraints in resynthesized allopolyploid *Brassica napus*

Kevin A. Bird[1,2], J. Chris Pires[3], Robert VanBuren[1,4], Zhiyong Xiong[5], Patrick P. Edger[1,2]

1. Department of Horticulture, Michigan State University,East Lansing, MI, 48824 USA
2. Ecology, Evolution, and Behavior Program, Michigan State University,East Lansing, MI, 48824 USA
3. Division of Biological Sciences, University of Missouri, Columbia, MO, 65211 USA
4. Plant Resilience Institute, Michigan State University, East Lansing, MI, 48824 USA
5. Key Laboratory of Herbage and Endemic Crop Biotechnology, Inner Mongolia University, Hohhot, 010070 China

Abstract:

Allopolyploidy involves the hybridization of two evolutionary diverged species and the doubling of genomic material. Frequently, allopolyploids exhibit genomic rearrangements that recombine, duplicate, or delete homoeologous regions of the newly formed genome. While decades of investigation have focused on how genome duplication leads to systematic differences in the retention and expression of duplicate genes, the impact of genomic rearrangements on genome evolution has received less attention. We used genomic and transcriptomic data for six independently resynthesized, isogenic *Brassica napus* lines in the first, fifth, and tenth generation to identify genomic rearrangements and assess their impact on gene expression dynamics related to subgenome dominance and gene dosage constraint. We find that dosage constraints on the gene expression response to polyploidy begin to loosen within the first ten generations of evolution and systematically differ between dominant and non-dominant subgenomes. We also show that genomic rearrangements can bias estimation of homoeolog expression bias, but fail to fully obscure which subgenome is dominantly expressed. Finally, we demonstrate that dosage-sensitive genes exhibit the same kind of coordinated response to homoeologous exchange as they do for genome duplication, suggesting constraint on dosage balance also acts on these changes to gene dosage.

Introduction:

The genome of a newly formed allopolyploid is a genome in flux. Upon the merger of evolutionarily diverged genomes, epigenetic markers like DNA methylation are frequently remodeled over early generations (Madlung et al., 2001; Edger et al., 2017; Bird et al., 2021) which can lead to major alterations in gene regulation (Chen, 2007) and activation of transposable elements (Vicient and Casacuberta, 2012). Polyploid genomes also must accommodate inherited and novel expression differences in homoeologous genes, resulting in subgenome dominance (Bird et al. 2018;2021, Wendel et al. 2018). Studies in resynthesized polyploids have shown that from the first meiosis in new polyploid genomes, major reorganizations occur in the form of homoeologous recombination, partial or complete chromosomal duplications, and deletions (Szadowski et al. 2010; Xiong et al. 2011; Nicolas et al. 2012; Mason and Wendel 2020). Rearrangements continue to accumulate over time, producing extensive genomic diversity in early polyploids (Xiong et al. 2011; Mason and Wendel, 2020). These genomic rearrangements are often destructive to the organism and meiotic stability is more frequently observed in natural polyploids compared to resynthesized (Gaete and Pires, 2010; Pele et al. 2018; Xiong et al. 2020). It is likely meiotic stability is under strong selection in natural polyploid populations (Gaete and Pires, 2010; Pele et al. 2018; Xiong et al. 2020; Gonzalo et al. 2019; Gaebelein et al. 2019; Ferreira de Carvalho et al. 2021). At the same time, genomic rearrangements generate novel diversity in resynthesized polyploids (Pires et al. 2004; Gaeta et al. 2007; Wu et al. 2021) and are frequently observed in natural polyploids (Chalhoub et al. 2014; Lloyd et al. 2018; Edger et al. 2019 He et al. 2017). These rearrangements often underlie gene presence/absence variation and agronomically valuable quantitative trait loci in *Brassica napus* (Stein et al. 2017; Samans et al. 2017; Hurgobin et al. 2017; Bayer et al. 2021) and generate novel, chimeric transcripts in multiple polyploid species (Zhang et al, 2020).

The precise impact of these rearrangements on global gene expression and subsequent genome evolution is still a topic of intense study. Studies investigating the effect of aneuploidy and whole-genome duplication on phenotypic variation and genome evolution have been numerous. It's been long established that the duplication or deletion of individual chromosomes produces larger phenotypic impacts than whole-genome duplications (Blakeslee, 1921). Advances in molecular genetics allowed for the direct investigation of the effect of these rearrangements on gene expression and protein abundance. These investigations found that gene expression responses to aneuploidy were varied. In some cases, aneuploidy produced dosage effects, where expression increased proportionally to changes in gene dosage. In others, it produced dosage compensation, where expression remained unchanged despite changed gene dosage (Birchler and Newton, 1981; Guo and Birchler, 1994). They also observed that expression of the non-duplicated regions frequently changed inversely to expression changes on the altered chromosome. (Birchler and Newton, 1981; Guo and Birchler, 1994). Meanwhile, the expression differences caused by polyploidy were much smaller (Birchler and Newton, 1981). The genes identified as causing many of these dosage responses were observed to be enriched for transcription factors and genes involved in signaling cascades and multimeric protein complexes led to the creation of the Gene Balance Hypothesis (GBH) to synthesize and explain these phenomena. The core of the GBH argues that changing the

stoichiometry of members of networks and protein complexes involved in multicomponent interactions affects their kinetics, assembly, and function of the whole, which causes negative fitness consequences (Birchler et al., 2005; Birchler and Veitia, 2007, 2010, 2012). Subsequent comparative genomic studies have reinforced the GBH showing that the retention of duplicate genes shows biased patterns depending on whether a gene is duplicated by whole-genome duplication or by small scale duplications (Maere, 2005; Freeling, 2009; Edger and Pires, 2009; De Smet et al., 2013; Li et al., 2016; Tasdighian et al., 2018).

Since these early studies, multiple experiments using next-generation sequencing data have investigated the expression responses caused by aneuploidy and polyploidy (Coate et al. 2016; Hou et al. 2018; Song et al. 2020; Shi et al. 2021; Yang et al. 2021). Coate et al. (2016) and Song et al. (2020), in particular attempt to connect observed patterns of long-term duplicate gene retention and short-term duplicate gene expression. They use tenets of the GBH to predict two patterns in short-term expression response. First, genes that are reciprocally retained after whole-genome duplication (e.g. highly connected in gene networks, involved in multicomponent protein complexes, etc.) show expression changes in response to genome duplication. Second, these changes are similar for all genes in the network, what they call a “coordinated response”. Coate et al. (2016) address this question using natural soybean (*Glycine* L.) allopolyploids with an origin ~500,000 years ago and known diploid progenitors, while Song et al. (2020) use three *Arabidopsis thaliana* autopolyploid/diploid pairs. Both studies determined that genes that are highly reciprocally retained post-WGD showed a more coordinated gene expression response to polyploidy (Coate et al. 2016; Song et al. 2020). While greatly informative, these investigations leave unanswered the extent to which immediate transcription response to allopolyploidy resembles and differs from the response to autopolyploidy.

Unlike aneuploidy and polyploidy, the impact of homoeologous exchanges on gene expression is largely unexplored. Early studies in multiple resynthesized *Brassica napus* lines used low-resolution techniques like cDNA-AFLP markers to identify changes in the transcriptome caused by non-reciprocal homoeologous recombination, arguing these transcriptional changes caused observed phenotypic diversity of the resynthesized lines (Gaeta et al. 2007). In allopolyploids, there are reasons to believe homoeologous exchange can alter the global transcriptome and expression levels of homoeologous gene pairs due to subgenome dominance. The main effect of subgenome dominance in early polyploids is an unequal expression of homoeologous copies. For most gene pairs, the homoeolog on the dominant subgenome is expressed more than the homoeolog on the submissive genome (Woodhouse et al. 2014; Edger et al. 2017; Bird et al. 2018; Bird et al. 2021). Therefore, when homoeologous exchange alters the ratio of dominant and submissive homoeologs, the combined expression for a homoeologous gene pair would differ from the balanced 2:2 tetraploid state. For example, a study in natural *B. napus* demonstrated that homoeologous exchanges caused dosage-dependent gene expression changes and showed signs of weakening over time (Lloyd et al. 2018). Bird et al. (2018) and Edger et al. (2019) have hypothesized from this observation that the expression changes from homoeologous exchange can alter the global transcriptome in a way that can obscure or exaggerate the extent of subgenome dominance. Often studies do not have paired whole-genome sequencing (WGS) and RNAseq data to identify homoeologous exchanges and subgenome dominance at the same time. Bird et al. (2021) analyzed

subgenome dominance in resynthesized *B. napus* but only investigated genes identified as 2:2 using WGS data, but did not assess the effect of homoeologous exchange on subgenome dominance inference. These predictions from Bird et al. (2018) and Edger et al. (2019) have yet to be tested. Furthermore, the dosage-dependent expression changes from homoeologous exchanges (HEs) greatly resemble the gene-dosage effects seen in aneuploid and polyploid organisms (Birchler and Newton, 1981). Edger et al. (2019) proposed that constraints on stoichiometric balance and altered gene dosage explained subgenome biased HE patterns in the octoploid strawberry genome. However, it is unknown whether there are also dosage compensation responses to HEs in other regions of the genome and if the gene expression response to homoeologous exchange follows predictions from the Gene Balance Hypothesis.

We analyzed paired WGS and RNASeq data for six independently resynthesized and isogenic *Brassica napus* (CCAA) lines sampled at three generations to determine if the immediate gene expression responses to allopolyploidy are consistent with the Gene Balance Hypothesis using the approaches of Coate et al. (2016) and Song et al. (2020). Next, we investigated the presence and variability of genomic rearrangements and gene dosage changes in the resynthesized polyploids. We used the identified genomic rearrangements (homoeologous exchanges and chromosomal duplications and deletions) to test if they bias inferences of global subgenome expression dominance. Finally, we extended the investigation of gene expression response to dosage changes used by Coate et al. (2016) and Song et al. (2020) to determine if changes in gene expression from homoeologous exchanges also follow predictions of the Gene Balance Hypothesis. Using plants from first, fifth, and tenth generations, we further tested if the gene expression response to both polyploidy and homoeologous exchange changes over time and if it differs based on subgenome dominance of a homoeologous gene pair. Our findings provide novel insights into the alteration of global expression by homoeologous exchanges and extend our understanding of how the Gene Balance Hypothesis constrains gene expression and genome evolution across various modes of gene dosage changes.

Results:

Assessing Early Gene Expression Response to Dosage Changes From Allopolyploidy

Over a century of work has highlighted gene dosage changes as a powerful and important aspect of gene expression abundance, quantitative trait variation, and the evolution of genomes (see reviews by Birchler and Veitia 2007,2010,2012). Gene dosage changes can lead to large phenotypic changes and can be highly deleterious for certain classes of genes, especially those involved in highly connected regulatory networks and multimeric protein complexes (Birchler and Newton, 1981; Birchler et al., 2001; Makino and McLysaght, 2010; Birchler and Veitia, 2012). The need to maintain the stoichiometric balance of gene products in

the face of change in gene dosage from both small-scale and whole-genome duplication influences genome evolution in important and predictable ways. For example driving biased duplicate gene retention of certain classes of genes after whole-genome duplications (Blanc and Wolfe, 2004; Maere et al. 2005; Thomas and Freeling, 2006). Many of these studies have focused on meso- or paleopolyploids, where genomes have returned to a diploid-like state. Recently, studies have also employed resynthesized autopolyploids and aneuploid series to study the immediate dosage response to whole-genome duplications, contributing greatly to our understanding of how genomes respond to duplication (Hou et al. 2018; Song et al. 2020; Shi et al. 2021; Yang et al. 2021). How or if this response may differ in allopolyploids, which involve interspecific hybridization in addition to genome duplication, is not well understood. For example, previous studies using allopolyploids relied on natural soybean polyploids which formed approximately 500kya, leaving early aspects of allopolyploid dosage response unexplored. Similarly, there has also been little opportunity to observe gene dosage response over early generations rather than only immediately after whole-genome duplication. Here, we interrogated these resynthesized allopolyploid *B. napus* lines for systematic expression response to the dosage change induced by whole-genome duplication over the ten generations of selfing.

We investigated the relative gene expression change (change in transcripts per gene pair) for individual homoeologous gene pairs in 2:2 dosage by taking the fold change of the summed transcript count for homoeologous gene pairs in the allopolyploid individuals and the mid parent value of the transcript count of the gene copies in the progenitors (gene pair expression in polyploids / mid parent gene expression). It should be noted, this approach did not normalize RNA with exogenous spike-in as other studies have, meaning values reported are relative gene expression levels and their response to genome doubling rather than the absolute expression response. While this will introduce some biases to our measures because the increase in transcriptome size of polyploids does not scale perfectly with the increase in genome size, our ability to detect broad patterns consistent with the Gene Balance Hypothesis should still remain. For this study, a ratio of 1 represents dosage compensation, resulting in no change in expression between polyploid and mid parent expression and a ratio of 2 represents a 1:1 expression response to dosage change e.g. doubled expression. Looking at all 16 individuals together, we observed high levels of variation in expression response to polyploidy. The median relative expression response to allopolyploidy was 1.86, just below a 1:1 expression response (Fig 1a). However, extreme values ranging from a very strong negative dosage response of 0.02 (essentially silenced) to 147 fold increase in expression in response to allopolyploidy were observed. Many genes also exhibited patterns consistent with dosage compensation, with ~8.8% of gene pairs less than or equal to a ratio of 1. These results mirror observed gene expression changes in autotetraploid/diploid maize comparisons (CITE Birchler paper).

When broken down by generation, we observed a progressive change in dosage response. Earlier generations (one and five), show median relative dosage responses of 1.84 and 1.78, respectively. Ten generations after polyploidy, however, the median relative dosage

response rises to 2.04 (Fig 1b). This change in the median is largely driven by increased variance in expression dosage response. In generations one and five, there are 8.8% and 7.6% of gene pairs with a dosage response less than or equal to 1, respectively, while generation ten showed 11% of gene pairs less than or equal to 1. Likewise, 41.2% and 37.2% of gene pairs had dosage responses greater than 2 in generations one and five, while 51.5% of gene pairs show such a dosage response in generation 10. This increased spread of dosage response in the higher and lower ranges in the tenth generation may suggest that dosage constraint progressively weakens over time in these resynthesized lines. It is likely that dosage constraint exists on a spectrum, where the weakening of constraint is most prominent for dosage-insensitive genes while dosage-sensitive genes remain relatively unchanged over time.

Figure 1. Expression response to polyploid induced dosage changes

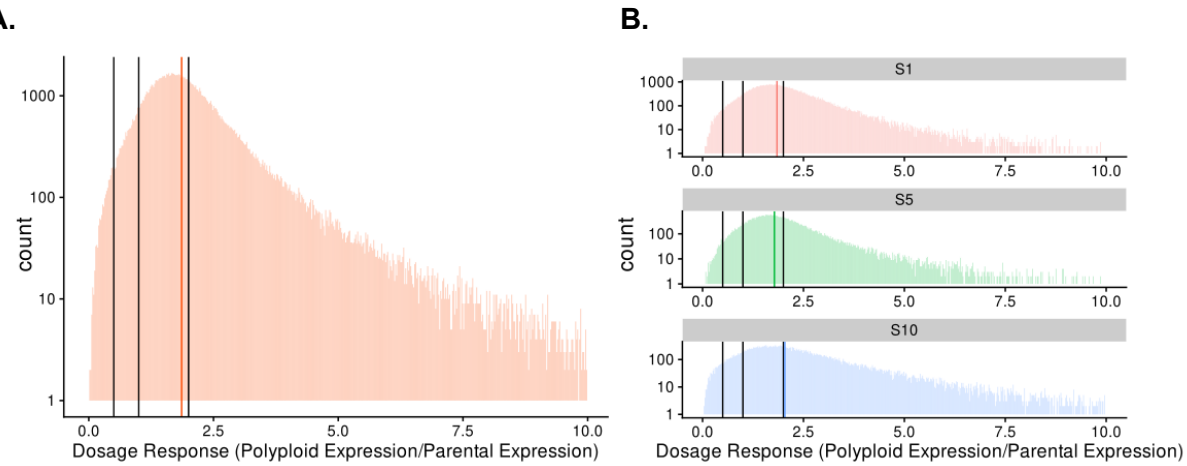

Fig 1. **A)** Dosage response to allopolyploidy by comparing summed gene expression of 2:2 homoeologous gene pairs in all 16 isogenic polyploid plants **(A)** combined or **(B)** grouped by generation to the summed expression of orthologs in the parental lines. Ratios of 1 represent dosage compensation, and a ratio of 2 represents expression change equal to the genomic dosage increase. Black lines represent dosage ratios of 0.5, 1, and 2 and colored lines represent median dosage response.

To further assess how the dosage sensitivity of genes affects their response to gene dosage changes from allopolyploidy, we used the dosage-balance-sensitivity gene class assignments for *Arabidopsis thaliana* from Song et al. (2020). As per Song et al. (2020), Class I Gene Ontology (GO) categories are putatively dosage-insensitive and Class II are putatively dosage-sensitive based on the observed reciprocal retention of genes from the investigated GO categories following polyploidy across the Angiosperms. The assigned classes of *Arabidopsis* genes were used to assign *B. rapa* and *B. oleracea* orthologs to dosage-sensitivity GO classes and assess how dosage response differs between classes in the resynthesized allopolyploids. We also used the polyploid response variance (the coefficient of variance of the relative dosage response) measure from Song et al. (2020) to assess how coordinated the response to polyploidy is in the different gene classes.

As observed previously in resynthesized autopolyploids and natural *Glycine* allopolyploids, the polyploid response variance was significantly lower in genes from the putatively dosage-sensitive GO categories compared to the dosage-insensitive categories (Kruskal-Wallis test, p=0.0024; Fig 2a). Using an allopolyploid gave us the opportunity to observe if gene pairs with different homoeolog expression biases respond differently to whole-genome duplication. We compared the dosage-sensitive and dosage-insensitive GO categories broken down by homoeolog expression bias relationships and found that while *B. napus* C subgenome (BnC) biased and unbiased homoeolog pairs show the same significant difference between dosage-sensitive and dosage-insensitive polyploid response variance as above (Kruskal-Wallis test, p=0.0037; 0.0158), the *B. napus* A subgenome (BnA) biased homoeolog pairs show no significant difference between GO classes (Kruskal-Wallis test, p=0.2933; Fig 2b). This result suggests that homoeolog expression bias somehow constrains gene dosage response. When broken down by generation, we observe the same increase in variance over time, with both dosage-sensitive and dosage-insensitive showing higher polyploid response variance in generation ten than in the first generation (Fig 2c). In fact, in generation ten the dosage-sensitive GO categories show higher mean polyploidy response variance than dosage-insensitive GO categories in the first generation.

Gene dosage changes are highly variable and do not show signs of subgenome bias

This study utilized a previously generated population of independently resynthesized *B. napus* lines, produced by hybridizing *B. oleracea* acc. TO1000DH and *B. rapa* acc. IMB-218DH. Importantly, because these lines were created from two doubled haploid parental lines all individuals started completely isogenic. An individual from six resynthesized lines was sequenced at the first (S1), fifth (S5), and tenth selfing generation (S10) and we analyzed the genomes of these 18 individuals to examine if changes in gene (homoeolog) dosage due to homoeologous exchanges are biased towards a particular subgenome. The genome sequencing data was aligned to an *in silica* allopolyploid genome made by combining the reference genomes of the *B. oleracea* double-haploid parent line TO1000 (Parkin et al. 2014) and a parental-SNP corrected *B. rapa* R500 reference genome (Lou et al. 2020). We identified 26,114 homoeologs between the BnA and BnC subgenomes by identifying syntenic orthologs between the progenitor *B. oleracea* and *B. rapa* genomes. Shifts in read depth coverage between these gene pairs allowed us to pinpoint changes in gene dosage from genomic rearrangements across each of the six lines and over the ten generations. Genomic rearrangements occur through homoeologous exchanges, where non-reciprocal homoeologous recombination between syntenic regions of the parental subgenomes replaces one homoeolog with another chromosomal deletions and duplications, or gene conversion events. Previous studies of this resynthesized *B. napus* population using a handful of DNA or cytogenetic markers identified extensive chromosomal duplications and deletions and homoeologous exchanges that resulted in immense phenotypic variation in both plant height and pollen count (Xiong et al. 2011).

Figure 2. Expression changes from allopolyploidy reflect predictions from the dosage balance hypothesis

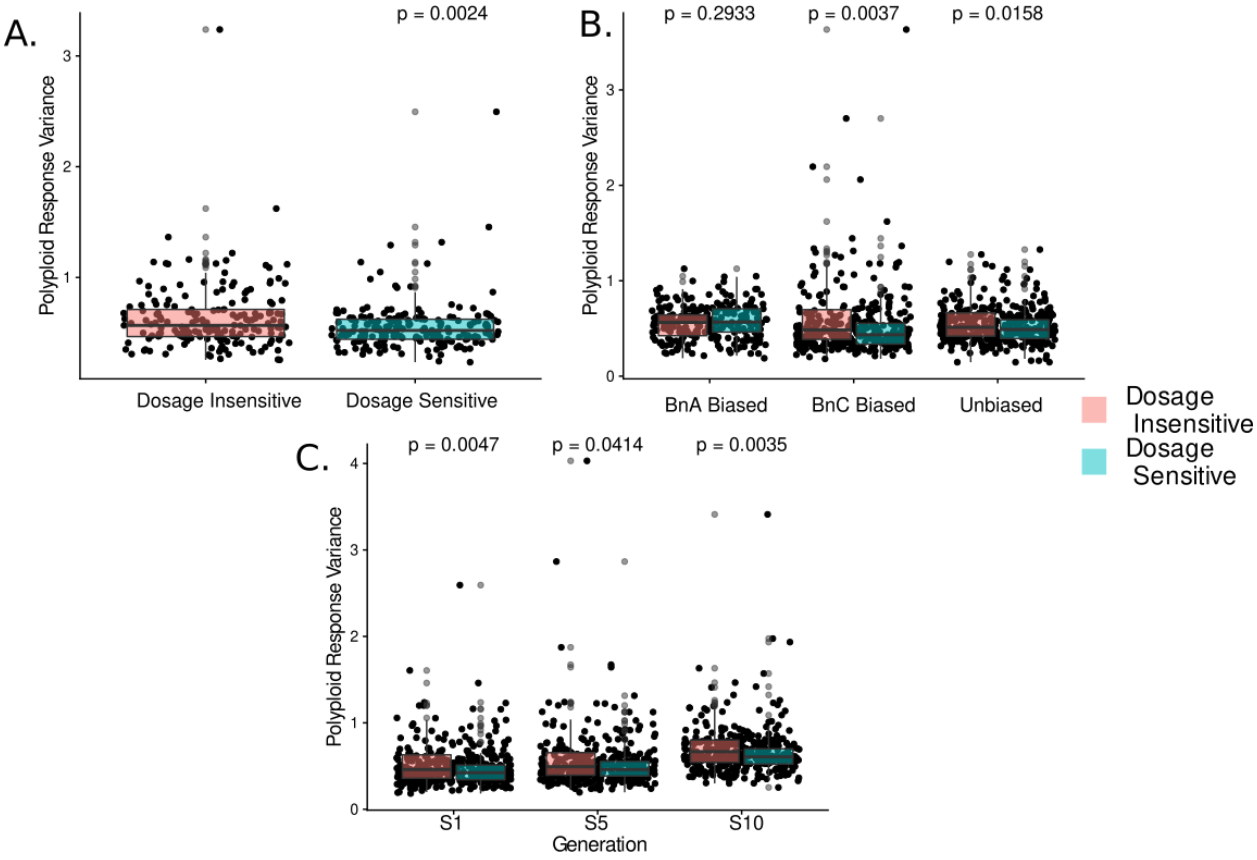

Fig 2. Polyploid response variance (coefficient of variation of dosage response) for all 2:2 balanced homoeologs in all 16 isogenic polyploid plants broken by **A)** only putatively dosage-insensitive (Class I) and dosage-sensitive (Class II) GO categories from Song et al. 2020, **B)** GO Dosage categories and subgenome dominance relationship in parental lines, **C)** GO Dosage categories and generation. P-values represent results of Kruskal-Wallis test of polyploid response variance between Class I vs Class II dosage categories.

However, the previous set of markers had limited resolution and small-scale exchanges were not identifiable. We used a whole-genome resequencing approach to identify at higher resolution genomic rearrangements that altered the relative dosage of homoeologs among individuals across this population.

The direction of dosage changes and proportion of regions with changed dosage varied greatly between lines and generations with no consistent pattern significantly favoring the BnA or BnC subgenome (Fig 3a). Individual lines ranged in the number of exchanged homoeolog pairs from 114 to 10,231 (Table 1).Overall, nine of 18 plants had significantly more genomic

rearrangements increasing BnC copy number than expected, while 8 out of 18 had significantly more rearrangements increasing BnA copy number than expected. Only two lines, EL-300 and EL-1100 showed the bias in genomic rearrangements in the same direction for each generation, while the other four lines showed a change in the direction of bias across generations.

Figure 3. Variability of gene dosage changes and hotspots in resynthesized B. napus

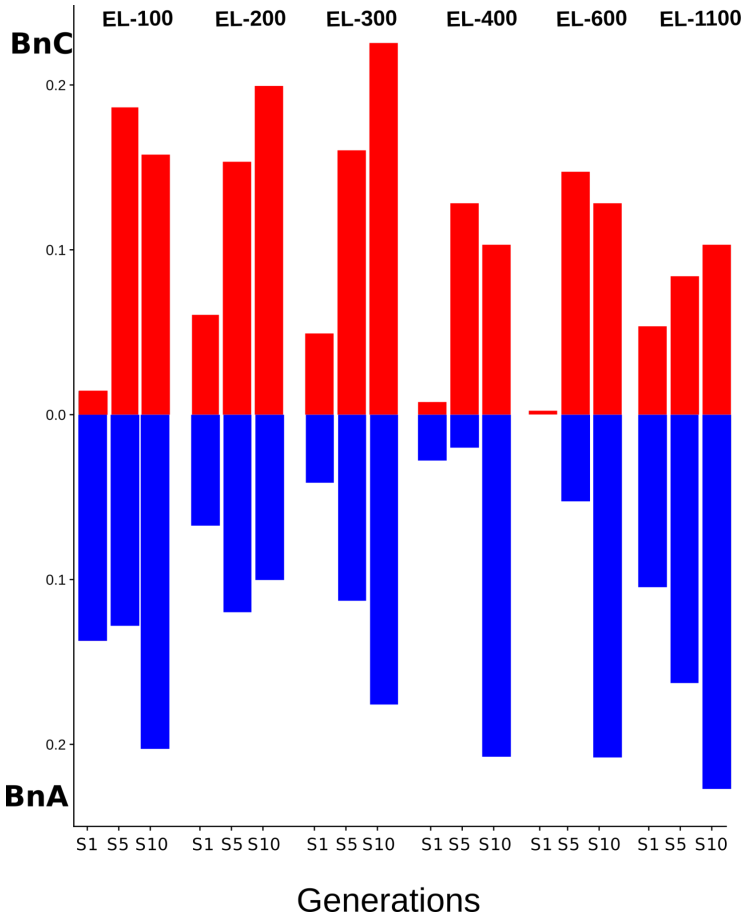

Fig 3. Proportion of homoeolog pairs showing ACCC or CCCC dosage (red) and AAAC or AAAA dosage (blue) for each resynthesized polyploid line (EL-100, EL-200, EL-300, EL-400, EL-600 and EL-1100) in the S1, S5 and S10 generations.

Impact of dosage changes on homoeologous expression bias

In certain allopolyploids, such as *B. napus*, homoeologous genes from a particular subgenome are often more highly expressed, it is unclear if gene dosage alterations from aneuploidy and homoeologous exchange exaggerate or obscure homoeolog expression bias. We take advantage of paired genomic and transcriptomic sequencing data to compare homoeologous expression bias when only analyzing genes inferred as in 2:2 dosage and when including all genes regardless of gene dosage alterations. In the first generation, before most

Table 1.

Homoeologous Exchange Bias Chi Squared table

Sample	*BnC. Observed*	*BnC. Expected*	*BnA. Observed*	*BnA. Expected*	*Chi. Squared*	*P.value*
EL-100S1	368	1931	3494	1931	2530.26	0
EL-100S5	4749	4024.5	3300	4024.5	260.85	1.12e-58
EL-100S10	4007	4573	5139	4573	140.11	2.52e-32
EL-200S1	1535	1620.5	1706	1620.5	9.02	0.003
EL-200S5	3875	3459	3043	3459	100.06	1.48e-23
EL-200S10	5049	3803.5	2558	3803.5	815.71	2.08e-179
EL-300S1	1255	1156	1057	1156	16.96	3.82e-05
EL-300S5	4082	3473	2864	3473	213.58	2.27e-48
EL-300S10	5725	5107.5	4490	5107.5	149.31	2.45e-34
EL-400S1	201	452.5	704	452.5	279.57	9.33e-63
EL-400S5	3207	1855	503	1855	1970.79	0
EL-400S10	2633	3953.5	5274	3953.5	882.11	7.58e-194
EL-600S1	53	26.5	0	26.5	53	3.34e-13
EL-600S5	3748	2542.5	1337	2542.5	1143.15	1.38e-250
EL-600S10	3267	4294	5321	4294	491.26	7.59e-109
EL-1100S1	1366	2019	2672	2019	422.4	7.34e-94
EL1100S5	2133	3171.5	4210	3171.5	680.11	6.33e-150
EL-1100S10	2590	4197	5804	4197	1230.62	1.35e-269

gene dosage changes occur, the distribution of the log2 expression ratio of homoeologous gene pairs when including and excluding gene dosage alterations broadly overlap (Fig 4) and the ratio of BnC to BnA biased gene pairs is not significantly different for 4/6 lines (χ^2-test, $p > 0.05$). In the fifth and tenth generations, after more gene dosage events accumulate, the distributions visibly begin to diverge (Fig 4). Only one of ten of these individuals have ratios of BnC and BnA biased homoeolog pairs that are not significantly different between analyses that include only 2:2 dosage balanced homoeologs compared to all homoeologous pairs (Table 2). In six of ten cases, the gene dosage cases reduced the proportion of BnC biased gene pairs and increased the proportion of BnA biased gene pairs. The other four of ten cases showed an increased proportion of BnC biased gene pairs and decreased the proportion of BnA biased gene pairs (Table 2).

These results demonstrate that gene dosage changes from aneuploidy and homoeologous exchange do alter the distribution of homoeolog expression bias and the ratio of biased gene pairs in statistically significant ways. Importantly, however, gene dosage changes never completely reversed the dominance relationship of the subgenomes. In other words, gene dosage events never led to the non-dominant BnA subgenome becoming the dominantly expressed subgenome by having more biased homoeolog pairs compared to the BnC subgenome. Because gene dosage changes in this study were not biased with respect to subgenome, it is unclear if it would be possible to completely reverse subgenome expression dominance relationships if dosage changes occurred in a biased fashion. However, among the 6 lines, there was variation in HE bias. Some lines, like EL1100, tended to have more HEs that increased BnA dosage, and lines like EL300 tended to have more HEs that increased BnC dosage (Fig 3a). Even in line EL1100 there was never a case where HEs resulted in BnA being the dominant subgenome (Table 2).

Expression changes from homoeologous exchanges appear to behave according to the gene-balance hypothesis

The defining feature that distinguishes allopolyploidy from autopolyploidy is the diverged evolutionary history of homoeologous regions in allopolyploids. This evolutionary divergence has been shown to frequently produce differences in the expression of homoeologs, with a dominant subgenome being more highly expressed than the submissive (recessive) subgenome. A feature of subgenome dominance is that altered ratios of homoeologs change total expression from the 2:2 state. Additionally, because allopolyploid subgenomes result from independent evolutionary histories, they often develop species-specific coevolved protein complexes prior to interspecific hybridization. This may result in functional differences between homoeologous gene products and their interactions with diverged protein complexes. Therefore, an unexplored aspect of allopolyploid genome evolution is that gene dosage changes from homoeologous exchanges may lead to gene expression responses in accordance with the gene-balance hypothesis.

Figure 4. Impact of homoeologous exchange on subgenome dominance

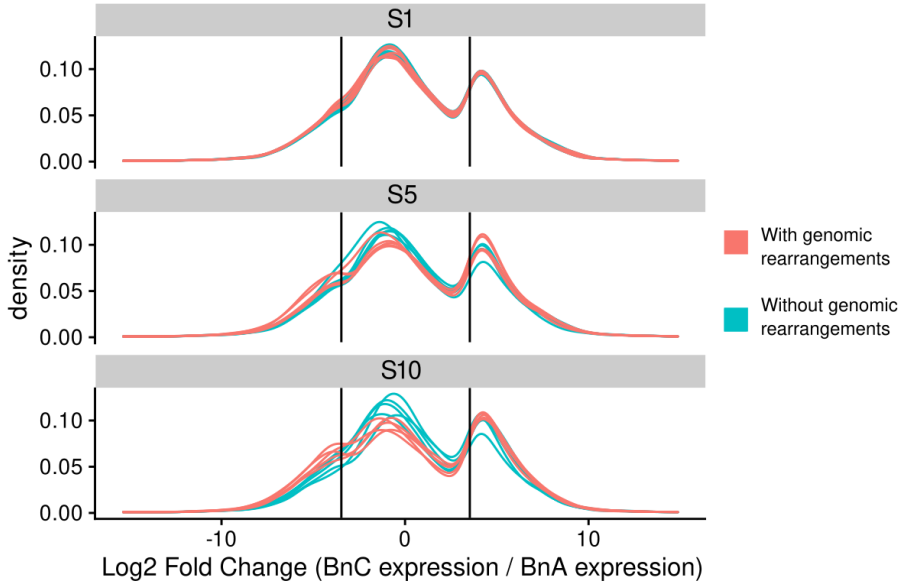

Fig 4. Distribution of Log2 Fold Change ($Expr_{BnC}/Expr_{BnA}$) for six lines across all three sampled generations. Lines in blue represent expression excluding dosage changes and homoeologous exchange by considering only genes which are in 2:2 dosage balance, lines in red represent expression including all dosage changes and homoeologous exchange.

Table 2.

Homeolog Expression Bias with and without Homoeologous Exchanges Chi Squared table

Sample	*BnC biased pairs with HEs*	*BnC biased pairs without HEs*	*BnA biased pairs with HEs*	*BnA biased pairs without HEs*	*Chi. Squared*	*P.value*
RS-100S1	3423 (0.67)	3581.29 0.70)	1698 (0.33)	1539.71 (0.30)	23.270	1.41e-06
RS-100S5	3355 (0.60)	3886.38 (0.69)	2278 (0.40)	1746.62 (0.31)	234.320	6.81e-53
RS-100S10	4108 (0.66)	4528.96 (0.72)	2162 (0.34)	1741.04 (0.28)	140.910	1.68e-32
RS-200S1	3571 (0.68)	3698.61 (0.70)	1692 (0.32)	1564.39 (0.30)	14.810	1.19e-04
RS-200S5	3879 (0.67)	3866.63 (0.67)	1868 (0.33)	1880.37 (0.33)	0.120	0.73
RS-200S10	4234 (0.68)	3955.51 (0.64)	1984 (0.32)	2262.49 (0.36)	53.890	2.12e-13
RS-300S1	3421 (0.67)	3442.93 (0.68)	1672 (0.33)	1650.07 (0.32)	0.430	0.51
RS-300S10	3979 (0.62)	4317.57 (0.67)	2479 (0.38)	2140.43 (0.33)	80.100	3.55e-19
RS-400S1	3555 (0.67)	3616.74 (0.68)	1739 (0.33)	1677.26 (0.32)	3.330	0.068
RS-400S5	3987 (0.69)	3808.79 (0.66)	1803 (0.31)	1981.21 (0.34)	24.370	7.96e-07
RS-600S1	3625 (0.68)	3588.89 (0.68)	1685 (0.32)	1721.11 (0.32)	1.120	0.290
RS-600S5	3528 (0.66)	3246.27 (0.60)	1851 (0.34)	2132.73 (0.40)	61.670	4.07e-15

RS-600S10	4327 (0.65)	4571.01 (0.69)	2280 (0.35)	2035.99 (0.31)	42.270	7.96e-11
RS-1100S1	3581 (0.68)	3626.07 (0.68)	1717 (0.32)	1671.93 (0.32)	1.780	0.183
RS-1100S5	3362 (0.60)	3931.43 (0.70)	2256 (0.40)	1686.57 (0.30)	274.730	1.06e-61
RS-1100S10	4067 (0.63)	4761.25 (0.73)	2424 (0.37)	1729.75 (0.27)	379.870	1.32e-84

The observed extensive homoeologous exchange and presence of homoeologous expression bias in these resynthesized *B. napus* lines provide an opportunity to test for the first time whether homoeologous exchanges cause gene expression responses that are predicted by the gene balance hypothesis. Using resequencing data, we focused on regions not identified as 2:2, representing homoeologous exchanges with 0:4, 1:3, 3:1, and 4:0 dosage ratios (BnC:BnA), and compared their expression to the summed expression of the progenitor genomes. To avoid contamination from likely aneuploidy events, chromosomes that frequently showed dosage changes for the entire length of the chromosome are excluded. Plotting the expression response to homoeologous exchange shows a skewed distribution with a median of 0.99, almost equivalent to 1, which represents compensated expression. However, the distribution shows high variability in expression responses (Fig 5). Since each gene pair will have different expression fold change differences between homoeologs it is impossible to know precisely which ratio represents a proportional dosage increase. Still, over 25% of homoeologous exchange gene pairs are either twice as expressed or half as expressed as when in a 2:2 dosage state (Fig 5).

Next, we investigated the extent that expression responses from homoeologous exchanges systematically differ among the identified dosage-sensitive and dosage-insensitive GO categories. We again used the coefficient of variation, this time termed Homoeologous Exchange Response Variance (HERV), to assess how coordinated the expression response was for dosage-sensitive and insensitive genes. Across all lines, genes belonging to putatively dosage-sensitive GO terms again showed significantly lower homoeologous exchange response variance, indicating a more coordinated expression response than for genes belonging to putatively dosage-insensitive GO terms (Fig 6a, Kruskal-Wallis test, p=0.00011). When broken down by direction of homoeolog expression bias we again see that homoeologous gene pairs with expression biased toward the dominant BnC subgenome (Kruskal-Wallis test, p=0.00093) and unbiased gene pairs (Kruskal-Wallis test, p=0.00041) show significantly lower homoeologous exchange expression variance in dosage-sensitive GO terms than in

Figure 5. Expression response to non-reciprocal homoeologous exchange induced dosage changes

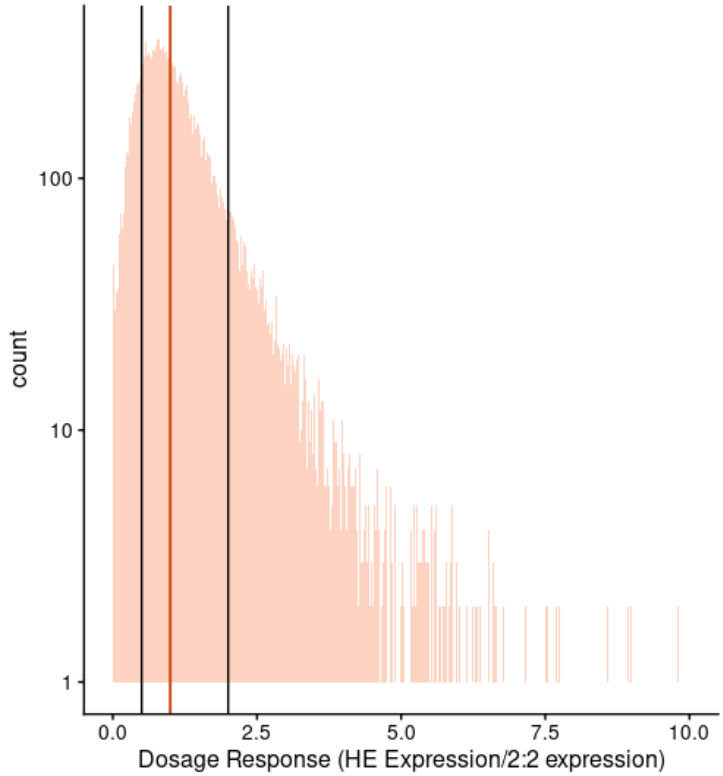

Fig 5. Dosage response to non-reciprocal homoeologous exchange by comparing summed gene expression of a dosage imbalance homoeologous gene pairs in all 16 isogenic polyploid plants combined to the summed expression of orthologs in the parental lines. Black lines represent dosage ratios of 0.5, 1, and 2. Dosage ratio of 1 represents dosage compensated expression. The colored line represents the median of the distribution.

dosage-insensitive GO terms (Fig 6b). Again we see that homoeologous gene pairs with expression biased toward the submissive BnA subgenome do not show a difference in homoeologous exchange response variance between dosage-sensitive and insensitive GO terms (Fig 6b, Kruskal-Wallis test, p=0.83926). Furthermore, we found that there was not a significant difference in homoeologous exchange response variance between dosage-sensitive and dosage-insensitive GO terms in the first generation (Fig 6c, Kruskal-Wallis test, p=0.79), but dosage-sensitive and insensitive GO terms did show different homoeologous exchange response variance in generations five and ten (Fig 6c, Kruskal-Wallis test, $p=9.5x10^{-5}$, p=0.04). We also found that homoeologous exchange response variance increased over time with dosage-sensitive and dosage-insensitive GO terms showing mean HERV of 0.547 and 0.540, respectively, in generation one and increasing to 0.789 and 0.860, respectively, in generation ten.

Expression changes from homoeologous exchanges are distinct from the effect of polyploidy.

While our findings suggest that dosage changes caused by homoeologous exchanges increase the copy number of one homoeolog over the other, it is possible these results are an artifact of our analysis also picking up the effects of dosage changes caused by allopolyploidy or aneuploidy. To determine if the results obtained for homoeologous exchanges are distinct from the effect of polyploidy, we directly compared the coefficient of variation for the expression response of the two dosage change conditions (Fig 7a-d).

First, we compared the proportion of gene pairs belonging to dosage-sensitive and dosage-insensitive GO terms in all 16 individuals for the polyploidy and homoeologous exchange analysis. For the polyploid analysis, the mean proportion of genes belonging to dosage-insensitive GO terms is 0.554, while it is 0.541 for the homoeologous exchange analysis, a significant difference (t-test, p=0.021). However, a greater proportion of gene pairs having dosage-insensitive GO terms would be predicted to result in a higher coefficient of variation, instead we found a significantly higher coefficient of variance from homoeologous exchanges (Fig 7a, Kruskal-Wallis test, $p<2x10^{-16}$), which had a lower proportion of genes belonging to dosage-insensitive GO terms. Both allopolyploidy and homoeologous exchange dosage changes produced significantly different expression responses from genes belonging to dosage-sensitive and insensitive GO terms (Fig 7b), and we determined that the coefficient of variation was significantly different between polyploidy and homoeologous exchange dosage changes for gene pairs from both dosage-sensitive (Kruskal-Wallis test, $p = 3.56x10^{-14}$) and dosage insensitive (Kruskal-Wallis test, $p=1.153x10^{-12}$). Likewise, for both homoelogous exchange and polyploidy induced dosage changes, the difference in expression response between genes belonging to dosage-sensitive and insensitive GO terms was significantly different (Fig 7c).

Our results also showed that the coefficient of variance from homoeologous exchange induced dosage changes was significantly higher than for polyploidy induced dosage changes for gene pairs belonging to both dosage-sensitive and insensitive for all homoeolog expression bias relationships (Table 3). In generational comparisons, homoeologous exchange and polyploidy induced dosage changes showed the same patterns for differences in coefficient of variation in generations five and ten, but not generation one where the coefficient of variation did not significantly differ by dosage sensitivity for homoeologous exchange induced dosage changes (Fig 7d). We also found that the coefficient of variance for homoeologous exchange induced dosage changes was significantly higher than for dosage changes induced by polyploidy for both dosage-sensitive and insensitive GO terms, but only for generations five and ten (Table 4).

Figure 6. Expression changes from non-reciprocal homoeologous exchange reflect predictions from the dosage balance hypothesis

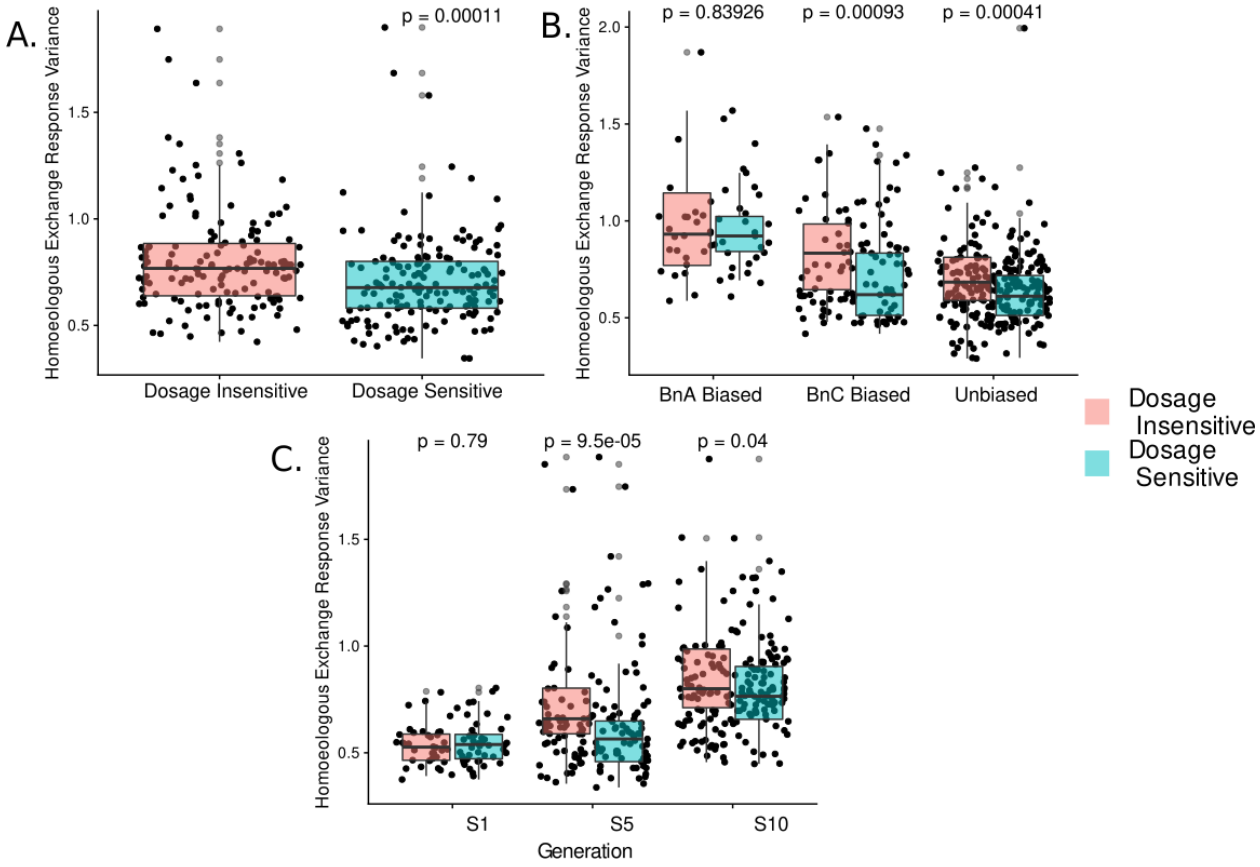

Fig 6. Homoeologous Exchange response variance (coefficient of variation of dosage response from homoeologous exchange) for all dosage imbalanced homoeologs in all 16 isogenic polyploid plants broken down by **A)** only putatively dosage-insensitive (Class I) and dosage-sensitive (Class II) GO categories from Song et al. 2020, **B)** GO Dosage categories and subgenome dominance relationship in parental lines, **C)** GO Dosage categories and generation. P-values represent results of Kruskal-Wallis test of polyploid response variance between Class I vs Class II dosage categories.

That the expression response to homoeologous exchanges and polyploidy induced dosage changes are significantly different overall, and among several comparisons is strong evidence that the patterns observed for homoeologous exchange induced dosage changes are distinct from the effects of polyploidy induced dosage change. Furthermore, it is likely that dosage constraint is weaker for dosage changes from homoeologous exchange, leading to a less coordinated expression response compared to polyploidy. This is because the coefficient of variation for the expression response to homoeologous exchange dosage changes was higher than that for polyploidy induced dosage changes for both dosage-sensitive and dosage-insensitive GO terms.

Figure 7. Expression responses from allopolyploidy and homoeologous exchange appear to be distinct

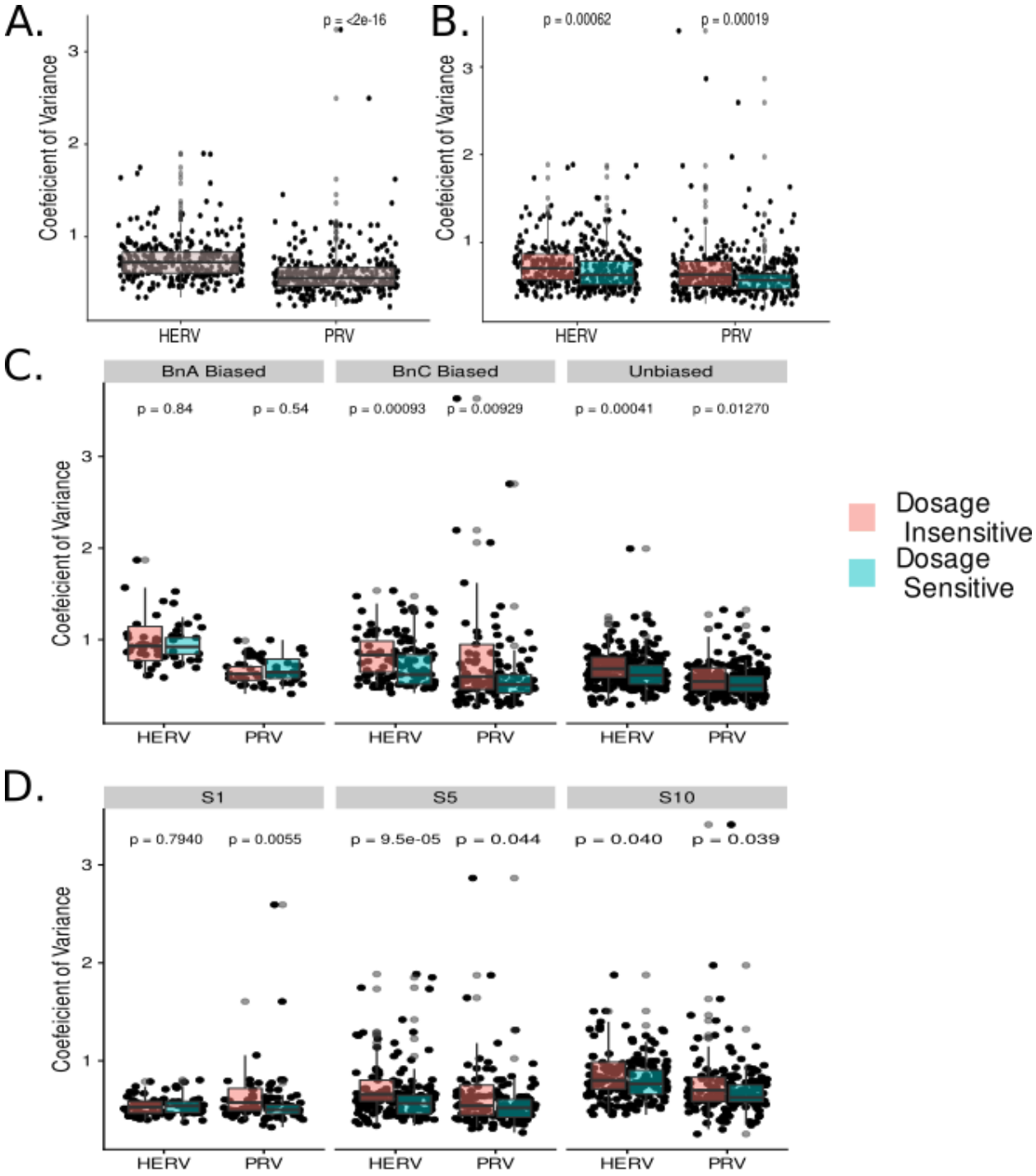

Fig 7. Comparison of expression response variance for non-reciprocal homoeologous exchanges (HERV) and allopolyploidy (PRV) for **A)** all lines and gene groups combined, **B)** all lines grouped by dosage class from Song et al. 2020, **C)** GO Dosage categories and subgenome dominance relationship in parental lines and **D)** GO Dosage categories and generation. For **A)** and **B)** P-values represent results of Kruskal-Wallis test of expression response

variance between HERV and PRV and for **C)** and **D)** P-values represent results of Kruskal-Wallis test of expression response variance for Class I vs Class II dosage categories.

Table 3: Kruskal-Wallis test exploring the difference in expression coefficient of variation from homoeologous exchange and allopolyploidy induced dosage changes broken down by dosage sensitivity and subgenome bias.

GO Class	Subgenome Bias	HERV mean (SD)	PRV mean (SD)	X2	df	p-value
Dosage Insensitive	BnC Biased	0.846 (0.240)	0.792 (0.585)	7.428	1	**0.0064**
Dosage Insensitive	BnA Biased	0.997 (0.313)	0.656 (0.141)	22.948	1	**9.90×10^{-7}**
Dosage Insensitive	Unbiased	0.708 (0.183)	0.585 (0.183)	26.173	1	**3.12×10^{-7}**
Dosage Sensitive	BnC Biased	0.721 (0.269	0.569 (0.331)	17.342	1	**3.122×10^{-5}**
Dosage Sensitive	BnA Biased	0.930 (0.142)	0.681 (0.141)	22.69	1	**1.90×10^{-6}**
Dosage Sensitive	Unbiased	0.634 (0.193)	0.525 (0.150)	34.658	1	**3.93×10^{-9}**

Table 4: Kruskal-Wallis test exploring the difference in expression coefficient of variation from homoeologous exchange and allopolyploidy induced dosage changes broken down by dosage sensitivity and generation.

GO Class	Generation	HERV mean (SD)	PRV mean (SD)	X2	df	p-value
Dosage Insensitive	S1	0.540 (0.0989)	0.629 (0.225)	2.9305	1	0.086
Dosage Insensitive	S5	0.747 (0.298)	0.634 (0.282)	8.6133	1	**0.0033**
Dosage Insensitive	S10	0.860 (0.231)	0.766 (0.381)	14.394	1	**0.0015**
Dosage Sensitive	S1	0.547 (0.0985)	0.551 (0.326)	2.6211	1	0.105
Dosage Sensitive	S5	0.615 (0.259)	0.555 (0.297)	5.4126	1	**0.0199**
Dosage Sensitive	S10	0.789 (0.214)	0.666 (0.198)	25.114	1	**5.4×10^{-7}**

Discussion:

The gene balance hypothesis has garnered extensive empirical support and has guided understanding of many aspects of genome evolution. For example, many lines of evidence support the idea that biased retention of dosage sensitive gene duplicates is related to selection to maintain balanced dosage of dosage sensitive genes (Maere et al. 2005; Freeling, 2009; Tasdhigian et al. 2018). Two recent investigations have helped demonstrate the connection between gene expression responses to dosage changes and dosage sensitivity (Coate et al. 2016; Song et al. 2020). By comparing the expression of synthetic *Arabidopsis* autopolyploids and natural *Glycine* allopolyploids to the diploid level expression, it was shown that expression response in dosage-sensitive genes was more coordinated than for dosage-insensitive genes. Dosage constraints producing coordinated expression of dosage-sensitive genes provide a mechanism by which selection for dosage balance can impact long-term gene retention. The present study fills in gaps in knowledge by investigating multiple resynthesized *B. napus* allopolyploids across the first ten generations of self-fertilization. This allowed a direct test of how observations from resynthesized autopolyploids carry over to newly formed allopolyploids, the investigation of how subgenome dominance interacts with dosage balance constraints, and the investigation of how dosage changes from homoeologous exchange are affected by dosage balance constraints. However, there are some limitations to this study that warrant future follow-up. These resynthesized lines experienced aneuploidy and homoeologous recombination in various combinations and so even when excluding regions affected by aneuploidy it isn't possible to eliminate confounding from trans effects of aneuploidy. Additionally, due to the small number of genes generally affected by homoeologous recombination we combined all dosage combinations (AAAA, AAAC, ACCC, CCCC) when performing these analyses which makes it difficult to ascertain the specific direction of expression changes or to isolate particular kinds of homoeologous exchanges. If there were ways to generate or introduce homoeologous exchanges of a specific dosage in a controlled genetic background a more precise investigation of the effect of these dosage changes would be possible. Despite these shortcomings, this study has provided new insight into the role of dosage constraint and the balanced gene drive in affecting gene expression changes from genomic rearrangements and opened up new avenues of investigation.

Evolutionary dynamics of early expression response to allopolyploidy

We first followed the general protocol of Coate et al. (2016) and Song et al. (2020). Our analysis of relative expression response to polyploidy showed a similar pattern, where genes exhibited a wide variety of expression responses ranging from dosage-dependent expression changes to dosage compensation. We also identified the same pattern where dosage-sensitive genes (those belonging to GO ontologies that show over-retention) have more coordinated expression compared to dosage-insensitive genes. The magnitude of expression coordination and differences between gene classes appears to be similar to what was previously observed. Overall these results suggest that the global response to polyploidy is not different between newly formed auto- or allopolyploids, as expected if dosage constraint was a general evolutionary force acting on all polyploids immediately upon genome duplication.

Dosage constraint and selection on relative gene dosage is not the only evolutionary force that leads to biases in gene loss and retention following WGD. An additional bias only experienced by some allopolyploid species is subgenome dominance, the biased retention of duplicate genes from one subgenome in allopolyploid genomes. Current hypotheses for subgenome dominance hold that lower expression of non-dominant subgenomes produces biased genome fractionation and that parental differences in transposable elements lead to greater epigenetic silencing of homoeologs from one subgenome which creates these expression differences (Bird et al. 2018; Alger et al. 2020; Edger et al. 2017). Importantly, because subgenome dominance only occurs in allopolyploid species, previous work on resynthesized autopolyploids (e.g. Song et al. 2020) could not investigate the interplay of dosage constraint and subgenome dominance. Both dosage constraints and subgenome dominance impact short and long-term genome dynamics. Dosage constraint predicts more coordinated expression responses to gene dosage changes for dosage sensitive genes, while subgenome dominance produces systematic expression differences between gene copies. Schnable et al. (2012) observed that biased retention of dosage sensitive genes broke down over time, with only 50% of genes retained from a genome duplication event being retained in duplicate after a second duplication event. They observed that the lower expressed copy was more likely to be lost, and proposed the lower expressed copies contribute less to overall gene product dosage, and so experience less purifying selection and weaker dosage constraint (Schnable et al. 2012). Similarly, when subgenome dominance was first described in *Arabidopsis* it was also associated with the production of clusters of dosage sensitive genes (Thomas et al. 2006).

These observations provide an account for how subgenome dominance and dosage balance constraints may interact to govern long-term evolutionary processes of gene loss and retention, but whether there is any short-term interaction between these processes and what they may look like is still largely unknown. Our results suggest that from the moment of genome duplication, subgenome dominance interacts with dosage constraints to produce systematic differences in the kinds of expression responses. Analysis of expression response to polyploidy showed that gene pairs where the homoeolog on the non-dominant BnA subgenome was more highly expressed did not show more coordinated expression response to dosage changes. Previous analysis of homoeologs in these resynthesized lines showed that gene pairs where the non-dominant copy was more highly expressed exhibited no significant connectivity in the *Arabidopsis* protein-protein interaction network (Bird et al. 2021). This lack of connectivity may explain why even putatively dosage-sensitive genes do not show coordinated expression since they are not highly connected in gene networks. Additionally, because the non-dominant subgenome is more lowly expressed and lowly expressed genes are under reduced dosage constraint this may also explain why only the BnA subgenome biased gene pairs show no difference between expression responses of dosage-sensitive and dosage-insensitive.

Selective constraints due to dosage sensitivity act immediately on duplicate genes and previous work suggests dosage constraint remains for long evolutionary periods, though is not permanent (Conant et al., 2014; Schnable et al., 2012). Although previous analysis of synthetic and natural *Arabidopsis* autopolyploids did not show marked differences in coordination of gene

expression, we observed a general increase in polyploid response variance for both dosage-sensitive and -insensitive genes over the ten generations observed, suggesting a decrease in coordination over a short period of time. Indeed, by the tenth generation, the dosage-sensitive genes showed less expression coordination than the dosage insensitive genes in the first generation. This potentially suggests that the strength of dosage constraint starts to change earlier in polyploid evolution than previously thought. However, it is known that dosage constraint is not a binary condition. Li et al. (2016) analyzed duplicate gene retention across angiosperms and described three broad groups: those with a strong preference for single copy, those with duplicates retained in most or all species, and those that are retained as duplicates for a prolonged period of time and then return to single copy (Li et al. 2016). It is possible our results reflect the start of dosage constraint loosening on some of these intermediately retained dosage sensitive genes. Alternatively, it is known that dosage changes localized to a chromosome also induce trans-expression effects on chromosomes that did not have their dosage altered. In our plants, several genomic rearrangements occurred simultaneously with lines exhibiting aneuploidy and homoeologous exchanges and rearrangements occurring on multiple chromosomes. Later generations also accumulated more genomic rearrangements than earlier ones. Therefore, we were unable to control or measure these kinds of trans dosage effects and they could potentially drive these changes in expression coordination observed between earlier and later generations.

Homoeologous exchange and early polyploid genome evolution

Homoeologous exchanges have long been recognized as an engine of phenotypic diversity and novelty in newly formed polyploids (Pires et al. 2004; Gaeta et al. 2007). Our analysis of genomic rearrangements and homoeologous exchanges in resynthesized *B. napus* confirmed at higher resolution the extensive rearrangements in these lines (Gaeta et al. 2007; Xiong et al. 2011). Although individual lines showed significant subgenome biases, there was no consistent subgenome bias for homoeologous exchanges. This is at odds with observed biases favoring replacing BnC segments with BnA segments in the reference accession Darmor-bzh (Chalhoub et al. 2014) and a population of field-grown natural and synthetic *B. napus* (Samans et al. 2017). A likely explanation is that although the mechanism for homoeologous recombination is largely a random process of meiosis there are fitness costs in natural environments of the field that select against homoeologous exchanges in a certain direction. Gaebelein et al. (2019) noted reduced fertility when C-genome regions replaced A-genome regions in a *Brassica* allohexaploid (AABBCC), supporting this idea. This population of resynthesized lines was grown in the more hospitable greenhouse and growth chamber conditions and hand-pollinated, which likely offsets the fitness costs identified by other studies and prevented the formation of systematic bias in homoeologous exchange.

Recent work has shown that homoeologous exchanges change gene expression in a dosage-dependent fashion (Lloyd et al. 2017). This is similar to a resynthesized allotetraploid *Arabidopsis suecica* series (TTTT, ATTT, AATT, AAAT, AAAA) showing dosage-dependent and independent expression changes with changing parental dosage (Shi et al. 2015). In light of this, Bird et al. (2018) suggested that, in the presence of extensive and biased genomic rearrangement, subgenome dominance analysis may be potentially obscured if they are not accounted for in the analysis. Comparing analysis of subgenome dominance that excluded or

included genomic rearrangements showed that although the precise proportion of biased homoeologs substantially changed the qualitative direction of the bias did not, even when a line showed strong subgenome bias in direction of homoeologous exchange. Therefore, although not accounting for homoeologous exchanges may lead to imprecise estimates of subgenome dominance dynamics they may likely still provide a reliable estimate of the direction of subgenome dominance. Considering these resynthesized lines accumulate more genomic rearrangements than natural *B. napus* it is less likely that subgenome dominance estimates are severely biased.

Investigations of genome imbalance and dosage sensitivity have often focused on polyploidy and aneuploidy as the sources of gene dosage alteration (Hou et al. 2018; Yang et al. 2021; Shi et al. 2021). These studies have greatly increased our understanding of how changes in dosage affect cis and trans gene expression, and subsequent analysis has connected these kinds of expression changes to long-term evolutionary patterns of gene retention (Song et al. 2020). However, aneuploidy and polyploidy are not the only mechanisms that alter gene dosage or gene expression. Homoeologous exchanges, which alter the ratio of parental chromosomes, produce dosage-dependent expression (Lloyd et al. 2017). These dosage changes from homoeologous exchanges have not been investigated for dosage constraints or general patterns of expression response expected from the gene balance hypothesis. Our results show that expression response to homoeologous exchanges exhibits a variety of behavior with expression sometimes staying equal to the 2:2 expression level but other times increasing or decreasing far beyond that baseline. Because these HE events represent multiple dosage changes and directions, and the homoeolog specific expression levels change between gene pairs it's not clear what proportion is changing in a dosage-dependent or independent manner or being dosage compensated but previous results from the *Arabidopsis* ploidy series showed that the majority of genes (54%) changed expression in a dosage-dependent manner for both homoeologs. The variation in expression response from homoeologous exchanges appears to be broadly similar to the response to polyploidy.

We further find that dosage changes resulting from homoeologous exchanges produce the same patterns of more coordinated expression response of dosage sensitive genes. We also saw similar patterns of lower expression coordination in later generations and lack of different expression responses in BnA dominant homoeolog pairs we observed when investigating expression response to polyploidy. Such results have not been reported before, to our knowledge, and suggest that homoeologous exchanges will also experience dosage constraint and selection for balanced dosage, like genes affected by polyploidy or aneuploidy. If homoeologous exchanges evolve in ways predicted by the gene balance hypothesis then we might expect biased retention of HEs containing dosage sensitive genes, or of dosage sensitive genes within ancient HE regions. Our comparison of homoeologous exchange and polyploidy response variance showed that overall gene expression was less coordinated in response to homoeologous exchange compared to polyploidy. This may mean that genes affected by homoeologous exchange experience less dosage constraint. Although the patterns observed for homoeologous exchanges could be an artifact of the effect of polyploidy, the fact that the patterns for response to homoeologous exchange are significantly different than the polyploidy

response suggests this is a distinct phenomenon. This could be a promising avenue for future comparative and evolutionary genomic studies to investigate.

Methods:

Data generation

This study used the whole genome resequencing (WGS) and RNASeq data from Bird et al. (2021). An abbreviated description follows but see Bird et al. (2021) for full methods description.

Plant growth, tissue collection, library prep

The resynthesized B. napus allopolyploid lines (CCAA) were grown under 23°C: 20°C, 16 h: 8 h, day: night cycles in a growth chamber. True leaf three was collected from all plants within 1 h, starting at 10 am (4 h into the day), and immediately flash-frozen in liquid nitrogen. Leaves were split in half for RNA and DNA isolation. Total RNA and DNA was isolated using the respective KingFisher Pure Plant kits (Thermo Fisher Scientific, Waltham, MA, USA) and quantified using a Qubit 3 fluorometer (Thermo Fisher Scientific). DNA and RNA libraries were prepared using the KAPA HyperPrep and mRNA HyperPrep kit protocols, respectively (KAPA Biosystems, Roche, USA). Libraries were submitted to a genomics facility (Beijing Nuohe Zhiyuan Technology Corp., Beijing, China) and sequenced with paired-end 150-bp reads on an Illumina HiSeq 4000 system.

In silico reference genome construction

We SNP corrected the *Brassica rapa* R500 (Lou et al. 2020) reference genome with paired-end 150-bp Illumina reads for the doubled haploid Brassica rapa accession IMB-218, using Bowtie2 v.2.3.4.1 (Langmead & Salzberg, 2012), Picardtools v.2.8.1. and GATK v.3.5.0. A new fasta reference was made using GATK FastaAlternativeReferenceMaker. This IMB-218 reference genome was concatenated to the B. oleracea TO1000 reference genome to create an in silico reference genome for B. napus matching the two progenitors used in our study.

WGS analysis

Paired-end 150-bp genomic Illumina reads were filtered with Trimmomatic v.0.33 (Bolger et al., 2014) to remove Illumina TruSeq3 adapters. Trimmed reads were aligned to the in silico B. napus reference genome with Bowtie2 v.2.3.4.1(Langmead & Salzberg, 2012) on default settings with the flag '--very-sensitive-local'. BAM files were sorted using Bamtools (Barnett et al., 2011) for use in downstream analyses.

We identified syntenic homologous genes (syntelogs) between *Brassica rapa* (IMB218 SNP-corrected, R500 reference genome; Lou et al. 2020) and *Brassica oleracea* (TO1000; Parkin et al. 2014) reference genomes with the MCScan toolkit (Tang et al., 2008). For the synthetic polyploid these represent as syntenic homoeologs. BED files based on the chromosome and start/stop position information for each subgenome were generated. Read

depths for the A subgenome (BnA) syntenic homoeologs were determined in Bedtools (Quinlan & Hall, 2010) with Bedcov using the R500 syntelog BED file and for the C subgenome (BnC) using the TO1000 syntelog BED file. Read depths for each syntenic homoeolog were normalised by gene length and reads per million for subgenome of origin.

RNA-seq analysis

Raw RNA-seq reads were filtered using Trimmomatic v.0.33 (Bolger et al., 2014) to remove Illumina TruSeq3 adapters and mapped to the in silico reference using Star v.2.6.0 (Dobin et al., 2013) on default settings. Transcripts were quantified in transcripts per million (TPM) from RNA-seq alignments using Stringtie v.1.3.5 (Pertea et al., 2015). We kept only syntenic gene pairs where the total TPM of the pair was > 10 to remove lowly expressed gene pairs while allowing a homoeolog to have no expression.

Homoeologous Exchange analysis:

We took the read depth results from BEDTools and averaged the proportion of reads mapping to a syntenic homoeolog compared to the overall read mapping for a syntenic homoeolog pair over a window of 50 genes with a step size of one gene. We identified homoeologous exchanged regions by calculating the average proportion of reads mapping to the BnC subgenome along a sliding window of 170 (85 upstream and downstream) genes and step size of one. If 10 or more consecutive genes had read depths within a preselected range it was called a homoeologous exchange. Regions 0 ≤ read depth < 0.2 were predicted to be in a 0:4 dosage ratio (BnC:BnA), for 0.2 ≤ read depth < 0.4 a dosage ratio of 1:3, for 0.4 ≤ read depth < 0.6 a dosage ratio of 2:2, for read depth between 0.6 ≤ read depth < 0.8 a 3:1 dosage ratio, and for read depth between 0.8 ≤ read depth < 1 a dosage ratio of 4:0. We calculated the proportion of genes for a given line that showed more BnA than BnC copies (ratios < 0.4) and more BnC than BnA copies (ratios > 0.6) and plotted the values in a stacked barplot using R v3.6.3. (R core team, 2020) A Chi-squared test was used to determine if the proportion of genomic rearrangements diverged from an expected 50/50 ratio. Significant deviations were considered to be biases in genomic rearrangements, either favoring more BnA than BnC copies or vice-versa.

Homoeolog Expression Bias:

We used the log2 fold change ($Expression_{BnC}/Expression_{BnA}$) to identify homoeolog expression bias. Syntenic homoeolog pairs with log2 fold change (FC) greater than 3.5 were called BnC biased, and less than -3.5 were called BnA biased. This cutoff follows the practice of Woodhouse et al. (2014) who used a log2 fold change cutoff of 2 to determine homoeolog expression bias, however, to more confidently reduce false positives a higher FC cutoff of 3.5 was used. We calculated the number of biased homoeolog pairs on all syntenic homoeolog pairs, including those with homoeologous exchange, and on a data set including only

homoeologous pairs with a 2:2 dosage ratio. We used a Chi-squared test to see if genomic rearrangements significantly altered the proportion of biased homoeologs compared to observed proportions when only analyzing homoeologous pairs with 2:2 dosage.

Dosage Response:

When investigating the dosage response to polyploidy, we limited our analysis to genes identified as 2:2 dosage. We calculated expression response to polyploidy as the ratio of the total expression of all 2:2 syntenic homoeologous pairs in the polyploids and the mid parent expression (summed expression of syntenic orthologs from progenitors divided by two). We used the dosage response classifications for *Arabidopsis thaliana* genes based on gene retention patterns of GO terms from Song et al. (2020) to classify syntenic orthologs between *A. thaliana* and *Brassica oleracea* identified from Synmap (Lyons et al. 2008) on CoGe (Lyons and Freeling, 2008). Classifications for *B. oleracea* genes were then used to classify the syntenic homoeologous pairs used in this study. We applied the same approach as Coate et al. (2016) and Song et al. (2020) and focused on the coefficient of variance of expression response (SD divided by mean), which we similarly term polyploid response variance (PRV). We calculated PRV only for GO terms that contained more than 20 genes. Statistical analysis was done through a Kruskal-Wallis test applied by the function stat_compare_means() in the R package ggpubr v.0.04.0 (R core team, 2020; Kassambara, 2020). When analyzing the response to polyploidy among different homoeolog expression biases, the expression bias of progenitor orthologs was used. Previous analysis showed that for over 70% of homoeologs, all six resynthesized *B. napus* lines shared the same homoeolog expression bias as the parents (Bird et al. 2021).

We included only homoeologous pairs that diverged from 2:2 dosage to investigate the effects. We also included chromosomes with inferred aneuploidy events based on contiguous altered dosage across the entire or majority of a chromosome. We defined the expression response to homoeologous exchange as the summed expression of the homoeologous pair divided by the summed expression of the orthologs in the progenitor genomes. We calculated the coefficient of variance of this expression response and termed it the homoeologous exchange response variance (HERV). The Kruskal-Wallis implementation from ggpubr (Kassambara, 2020) was used again for statistical analysis. Like for the polyploidy analysis, we only included GO terms with 20 or more genes and defined homoeolog expression bias in terms of expression bias in parental orthologs.

References:

Alger, E. I., & Edger, P. P. (2020). One subgenome to rule them all: underlying mechanisms of subgenome dominance. *Current opinion in plant biology*, *54*, 108-113.

Barnett, D. W., Garrison, E. K., Quinlan, A. R., Strömberg, M. P., & Marth, G. T. (2011). BamTools: a C++ API and toolkit for analyzing and managing BAM files. *Bioinformatics*, *27*(12), 1691-1692.

Bayer, P. E., Scheben, A., Golicz, A. A., Yuan, Y., Faure, S., Lee, H., ... & Edwards, D. (2021). Modelling of gene loss propensity in the pangenomes of three Brassica species suggests different mechanisms between polyploids and diploids. *Plant Biotechnology Journal*.

Birchler, J. A., Bhadra, U., Bhadra, M. P., & Auger, D. L. (2001). Dosage-dependent gene regulation in multicellular eukaryotes: implications for dosage compensation, aneuploid syndromes, and quantitative traits. *Developmental biology*, *234*(2), 275-288.

Birchler, J. A., & Newton, K. J. (1981). Modulation of protein levels in chromosomal dosage series of maize: the biochemical basis of aneuploid syndromes. *Genetics*, *99*(2), 247-266.

Birchler, J. A., & Veitia, R. A. (2007). The gene balance hypothesis: from classical genetics to modern genomics. *The Plant Cell*, *19*(2), 395-402.

Birchler, J. A., & Veitia, R. A. (2010). The gene balance hypothesis: implications for gene regulation, quantitative traits and evolution. *New Phytologist*, *186*(1), 54-62.

Birchler, J. A., & Veitia, R. A. (2012). Gene balance hypothesis: connecting issues of dosage sensitivity across biological disciplines. *Proceedings of the National Academy of Sciences*, *109*(37), 14746-14753.

Bird, K. A., Niederhuth, C. E., Ou, S., Gehan, M., Pires, J. C., Xiong, Z., ... & Edger, P. P. (2021). Replaying the evolutionary tape to investigate subgenome dominance in allopolyploid Brassica napus. *New Phytologist*, 230(1), 354-371.

Bird, K. A., VanBuren, R., Puzey, J. R., & Edger, P. P. (2018). The causes and consequences of subgenome dominance in hybrids and recent polyploids. *New Phytologist*, *220*(1), 87-93.

Blanc, G., & Wolfe, K. H. (2004). Functional divergence of duplicated genes formed by polyploidy during Arabidopsis evolution. *The Plant Cell*, *16*(7), 1679-1691.

Blakeslee, A. F. (1921). Types of mutations and their possible significance in evolution. *The American Naturalist*, *55*(638), 254-267.

Bolger AM, Lohse M, Usadel B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114– 2120.

Chalhoub, B., Denoeud, F., Liu, S., Parkin, I. A., Tang, H., Wang, X., ... & Wincker, P. (2014). Early allopolyploid evolution in the post-Neolithic Brassica napus oilseed genome. *science*, *345*(6199), 950-953.

Chen, Z. J. (2007). Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids. *Annu. Rev. Plant Biol.*, *58*, 377-406.

Coate, J. E., Song, M. J., Bombarely, A., & Doyle, J. J. (2016). Expression-level support for gene dosage sensitivity in three Glycine subgenus Glycine polyploids and their diploid progenitors. *New Phytologist*, 212(4), 1083-1093.

Conant, G. C., Birchler, J. A., & Pires, J. C. (2014). Dosage, duplication, and diploidization: clarifying the interplay of multiple models for duplicate gene evolution over time. *Current opinion in plant biology*, *19*, 91-98.

De Smet, R., Adams, K. L., Vandepoele, K., Van Montagu, M. C., Maere, S., & Van de Peer, Y. (2013). Convergent gene loss following gene and genome duplications creates single-copy families in flowering plants. *Proceedings of the National Academy of Sciences*, *110*(8), 2898-2903.

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., ... & Gingeras, T. R. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*, 29(1), 15-21.

Edger, P. P., & Pires, J. C. (2009). Gene and genome duplications: the impact of dosage-sensitivity on the fate of nuclear genes. *Chromosome Research*, *17*(5), 699-717.

Edger, P. P., Poorten, T. J., VanBuren, R., Hardigan, M. A., Colle, M., McKain, M. R., ... & Knapp, S. J. (2019). Origin and evolution of the octoploid strawberry genome. *Nature genetics*, *51*(3), 541-547.

Edger, P. P., Smith, R., McKain, M. R., Cooley, A. M., Vallejo-Marin, M., Yuan, Y., ... & Puzey, J. R. (2017). Subgenome dominance in an interspecific hybrid, synthetic allopolyploid, and a 140-year-old naturally established neo-allopolyploid monkeyflower. *The Plant Cell*, *29*(9), 2150-2167.

Ferreira de Carvalho, J., Stoeckel, S., Eber, F., Lodé-Taburel, M., Gilet, M. M., Trotoux, G., ... & Rousseau-Gueutin, M. (2021). Untangling structural factors driving genome stabilization in nascent Brassica napus allopolyploids. *New Phytologist*, *230*(5), 2072-2084.

Freeling, M. (2009). Bias in plant gene content following different sorts of duplication: tandem, whole-genome, segmental, or by transposition. *Annual review of plant biology*, *60*, 433-453.

Freeling, M., & Thomas, B. C. (2006). Gene-balanced duplications, like tetraploidy, provide predictable drive to increase morphological complexity. *Genome research*, *16*(7), 805-814.

Gaeta, R. T., Pires, J. C., Iniguez-Luy, F., Leon, E., & Osborn, T. C. (2007). Genomic changes in resynthesized Brassica napus and their effect on gene expression and phenotype. *The Plant Cell*, *19*(11), 3403-3417.

Gaeta, R. T., & Pires, J. C. (2010). Homoeologous recombination in allopolyploids: the polyploid ratchet. *New Phytologist*, *186*(1), 18-28.

Gaebelein, R., Schiessl, S. V., Samans, B., Batley, J., & Mason, A. S. (2019). Inherited allelic variants and novel karyotype changes influence fertility and genome stability in Brassica allohexaploids. *New Phytologist*, *223*(2), 965-978.

Gonzalo, A., Lucas, M. O., Charpentier, C., Sandmann, G., Lloyd, A., & Jenczewski, E. (2019). Reducing MSH4 copy number prevents meiotic crossovers between non-homologous chromosomes in Brassica napus. *Nature communications*, *10*(1), 1-9.

Guo, M., & Birchler, J. A. (1994). Trans-acting dosage effects on the expression of model gene systems in maize aneuploids. *Science*, *266*(5193), 1999-2002.

He, Z., Wang, L., Harper, A. L., Havlickova, L., Pradhan, A. K., Parkin, I. A., & Bancroft, I. (2017). Extensive homoeologous genome exchanges in allopolyploid crops revealed by mRNA seq-based visualization. *Plant biotechnology journal*, *15*(5), 594-604.

Hou, J., Shi, X., Chen, C., Islam, M. S., Johnson, A. F., Kanno, T., ... & Birchler, J. A. (2018). Global impacts of chromosomal imbalance on gene expression in Arabidopsis and other taxa. *Proceedings of the National Academy of Sciences*, 115(48), E11321-E11330.

Hurgobin, B., Golicz, A. A., Bayer, P. E., Chan, C. K. K., Tirnaz, S., Dolatabadian, A., ... & Edwards, D. (2018). Homoeologous exchange is a major cause of gene presence/absence variation in the amphidiploid Brassica napus. *Plant biotechnology journal*, *16*(7), 1265-1274.

Kassambara, Alboukadel (2020). ggpubr: 'ggplot2' Based Publication Ready Plots. R package version 0.4.0. https://CRAN.R-project.org/package=ggpubr

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature methods*, *9*(4), 357-359.

Li, Z., Defoort, J., Tasdighian, S., Maere, S., Van de Peer, Y., & De Smet, R. (2016). Gene duplicability of core genes is highly consistent across all angiosperms. *The Plant Cell*, *28*(2), 326-344.

Lloyd, A., Blary, A., Charif, D., Charpentier, C., Tran, J., Balzergue, S., ... & Jenczewski, E. (2018). Homoeologous exchanges cause extensive dosage‐dependent gene expression changes in an allopolyploid crop. *New Phytologist*, *217*(1), 367-377.

Lou, P., Woody, S., Greenham, K., VanBuren, R., Colle, M., Edger, P. P., ... & McClung, C. R. (2020). Genetic and genomic resources to study natural variation in Brassica rapa. *Plant direct*, 4(12), e00285.

Lyons, E., & Freeling, M. (2008). How to usefully compare homologous plant genes and chromosomes as DNA sequences. *The Plant Journal*, *53*(4), 661-673.

Lyons, E., Pedersen, B., Kane, J., Alam, M., Ming, R., Tang, H., ... & Freeling, M. (2008). Finding and comparing syntenic regions among Arabidopsis and the outgroups papaya, poplar, and grape: CoGe with rosids. *Plant physiology*, *148*(4), 1772-1781.

Madlung, A., Tyagi, A. P., Watson, B., Jiang, H., Kagochi, T., Doerge, R. W., ... & Comai, L. (2005). Genomic changes in synthetic Arabidopsis polyploids. *The Plant Journal*, *41*(2), 221-230.

Maere, S., De Bodt, S., Raes, J., Casneuf, T., Van Montagu, M., Kuiper, M., & Van de Peer, Y. (2005). Modeling gene and genome duplications in eukaryotes. *Proceedings of the National Academy of Sciences*, *102*(15), 5454-5459.

Makino, T., & McLysaght, A. (2010). Ohnologs in the human genome are dosage balanced and frequently associated with disease. *Proceedings of the National Academy of Sciences*, *107*(20), 9270-9274.

Mason, A. S., & Wendel, J. F. (2020). Homoeologous exchanges, segmental allopolyploidy, and polyploid genome evolution. *Frontiers in Genetics*, *11*, 1014.

Parkin, I. A., Koh, C., Tang, H., Robinson, S. J., Kagale, S., Clarke, W. E., ... & Sharpe, A. G. (2014). Transcriptome and methylome profiling reveals relics of genome dominance in the mesopolyploid Brassica oleracea. *Genome biology*, 15(6), 1-18.

Pelé, A., Rousseau-Gueutin, M., & Chèvre, A. M. (2018). Speciation success of polyploid plants closely relates to the regulation of meiotic recombination. *Frontiers in plant science*, *9*, 907.

Pertea, M., Pertea, G. M., Antonescu, C. M., Chang, T. C., Mendell, J. T., & Salzberg, S. L. (2015). StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nature biotechnology*, 33(3), 290-295.

Pires, J. C., Zhao, J., Schranz, M. E., Leon, E. J., Quijada, P. A., Lukens, L. N., & Osborn, T. C. (2004). Flowering time divergence and genomic rearrangements in resynthesized Brassica polyploids (Brassicaceae). *Biological Journal of the Linnean Society*, *82*(4), 675-688.
Samans, B., Chalhoub, B., & Snowdon, R. J. (2017). Surviving a genome collision: genomic signatures of allopolyploidization in the recent crop species Brassica napus. *Plant Genome*, *10*(3), 1-15.

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Schnable, J. C., Wang, X., Pires, J. C., & Freeling, M. (2012). Escape from preferential retention following repeated whole genome duplications in plants. *Frontiers in plant science*, *3*, 94.

Shi, X., Zhang, C., Ko, D. K., & Chen, Z. J. (2015). Genome-wide dosage-dependent and independent regulation contributes to gene expression and evolutionary novelty in plant polyploids. *Molecular Biology and Evolution*, *32*(9), 2351-2366.

Shi, X., Yang, H., Chen, C., Hou, J., Hanson, K. M., Albert, P. S., ... & Birchler, J. A. (2021). Genomic imbalance determines positive and negative modulation of gene expression in diploid maize. *The Plant Cell*, 33(4), 917-939.

Song, M. J., Potter, B. I., Doyle, J. J., & Coate, J. E. (2020). Gene balance predicts transcriptional responses immediately following ploidy change in Arabidopsis thaliana. *The Plant Cell*, 32(5), 1434-1448.

Stein, A., Coriton, O., Rousseau‐Gueutin, M., Samans, B., Schiessl, S. V., Obermeier, C., ... & Snowdon, R. J. (2017). Mapping of homoeologous chromosome exchanges influencing quantitative trait variation in Brassica napus. *Plant biotechnology journal*, *15*(11), 1478-1489.

Szadkowski, E., Eber, F., Huteau, V., Lode, M., Huneau, C., Belcram, H., ... & Chèvre, A. M. (2010). The first meiosis of resynthesized Brassica napus, a genome blender. *New Phytologist*, *186*(1), 102-112.

Tang, H., Bowers, J. E., Wang, X., Ming, R., Alam, M., & Paterson, A. H. (2008). Synteny and collinearity in plant genomes. *Science*, 320(5875), 486-488.

Tasdighian, S., Van Bel, M., Li, Z., Van de Peer, Y., Carretero-Paulet, L., & Maere, S. (2017). Reciprocally retained genes in the angiosperm lineage show the hallmarks of dosage balance sensitivity. *The Plant Cell*, *29*(11), 2766-2785.

Thomas, B. C., Pedersen, B., & Freeling, M. (2006). Following tetraploidy in an Arabidopsis ancestor, genes were removed preferentially from one homeolog leaving clusters enriched in dose-sensitive genes. *Genome research*, *16*(7), 934-946.

Vicient, C. M., & Casacuberta, J. M. (2017). Impact of transposable elements on polyploid plant genomes. *Annals of Botany*.

Wendel, J. F., Lisch, D., Hu, G., & Mason, A. S. (2018). The long and short of doubling down: polyploidy, epigenetics, and the temporal dynamics of genome fractionation. *Current opinion in genetics & development*, *49*, 1-7.

Woodhouse, M. R., Cheng, F., Pires, J. C., Lisch, D., Freeling, M., & Wang, X. (2014). Origin, inheritance, and gene regulatory consequences of genome dominance in polyploids. *Proceedings of the National Academy of Sciences*, 111(14), 5283-5288.

Wu, Y., Lin, F., Zhou, Y., Wang, J., Sun, S., Wang, B., ... & Liu, B. (2021). Genomic mosaicism due to homoeologous exchange generates extensive phenotypic diversity in nascent allopolyploids. *National Science Review*, *8*(5), nwaa277.

Xiong, Z., Gaeta, R. T., & Pires, J. C. (2011). Homoeologous shuffling and chromosome compensation maintain genome balance in resynthesized allopolyploid Brassica napus. *Proceedings of the National Academy of Sciences*, 108(19), 7908-7913.

Yang, H., Shi, X., Chen, C., Hou, J., Ji, T., Cheng, J., & Birchler, J. A. (2021). Predominantly inverse modulation of gene expression in genomically unbalanced disomic haploid maize. The Plant Cell, 33(4), 901-916.

Zhang, Z., Gou, X., Xun, H., Bian, Y., Ma, X., Li, J., ... & Levy, A. A. (2020). Homoeologous exchanges occur through intragenic recombination generating novel transcripts and proteins in

wheat and other polyploids. *Proceedings of the National Academy of Sciences*, *117*(25), 14561-14571.