# Gene dosage constraints affect the transcriptional response to allopolyploidy and homoeologous exchange in resynthesized *Brassica napus*

Short title: gene balance constrains homoeologous exchanges

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# Abstract

- Allopolyploidy involves the hybridization of two evolutionary diverged species and the doubling of genomic material. Allopolyploids also exhibit homoeologous exchange that recombines, duplicate, or delete homoeologous regions of the newly formed genome. These kinds of changes to gene dosage are hypothesized to be constrained by selection to maintain balanced gene dosage. The dynamics of this constraint immediately after allopolyploidy and in response to homoeologous exchange is unknown.

- 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 gene dosage constraint.

- Dosage-sensitive genes show a more coordinated expression response to polyploidy, consistent with selective constraint for balanced gene dosage. We also find that the expression response systematically differs for dosage-sensitive genes depending on whether homoeolog expression is biased toward the dominant or non-dominant subgenome. Expression coordination appears to change over early generations, possibly suggesting a weakening of dosage constraint. Dosage-sensitive genes also exhibit the same kind of coordinated expression response to homoeologous exchanges as they do to genome duplication.

- Constraint on gene dosage acts on gene expression for newly formed allopolyploids as it does for autopolyploids and exerts a detectable effect on homoeologous exchanges. These findings connect patterns of long- and short-term gene retention in polyploids and suggest novel patterns for the evolution of homoeologous exchanges.

# Introduction

Changes in gene dosage are known to be a powerful and important driver of gene expression abundance, quantitative trait variation, and the evolution of genomes (Birchler and Veitia 2007,2010,2012). The observation that imbalanced gene dosage changes can have a large phenotypic impact and can be highly deleterious for certain classes of genes, especially those involved in highly connected regulatory networks and multimeric protein complexes lead to the formulation of Gene Balance Hypothesis (Birchler and Newton, 1981; Birchler et al., 2001; Makino and McLysaght, 2010; Birchler and Veitia, 2012). 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). The need to maintain the stoichiometric balance of gene products in the face of changes in gene dosage from both small-scale and whole-genome duplication influences genome evolution in important and predictable ways. Comparative genomic studies have supported predictions from 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 (Blanc and Wolfe, 2004; Maere, 2005; Paterson et al. 2006; Thomas and Freeling, 2006; Freeling, 2009; Edger and Pires, 2009; De Smet et al., 2013; Li et al., 2016; Tasdighian et al., 2018).

Many of these studies have focused on meso- or paleopolyploids, where genomes have returned to a diploid-like state, leaving the immediate transcriptional impact of large-scale gene dosage changes less well understood. However, several authors have recently 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 to short-term duplicate gene expression responses. 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.) should experience a change in gene expression in response to genome duplication. Second, these changes should be 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).

These investigations have been greatly informative but were unable to address the extent that the immediate transcriptional response differs between a whole-genome duplication involving the hybridization of distinct progenitor genomes (allopolyploidy) and when a whole-genome duplication involves duplication of genetically similar chromosomes (autopolyploidy). While both result in a duplication of the genome, allopolyploidy also involves the merger of evolutionarily diverged genomes, which frequently results in remodeling of epigenetic markers (Madlung et al., 2001; Edger et al., 2017; Bird et al., 2021), 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 which often results in subgenome dominance, where expression is biased in favor of homoeologs from one progenitor genome over others. (Alger et al. 2021; 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). These rearrangements continue to accumulate over time, generating 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 (Gaeta and Pires, 2010; Pele et al. 2018; Xiong et al. 2020). It is likely that meiotic stability is under strong selection in natural polyploid populations (Gaeta 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 phenotypic novelty 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). Additionally, homoeologous exchanges 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).

Unlike aneuploidy and polyploidy, the impact of gene dosage constraint on gene expression changes from homoeologous exchanges is largely unexplored. There are reasons to believe homoeologous exchange can alter the dosage balance of gene products. Early studies in multiple resynthesized *Brassica napus* lines identified changes in the transcriptome caused by non-reciprocal homoeologous recombination, arguing these transcriptional changes produced phenotypic diversity among the lines (Gaeta et al. 2007). Furthermore, homoeologous exchanges (HEs) have been shown to alter expression in a dosage-dependent manner (Lloyd et al. 2017) that greatly resemble the gene dosage effects seen in aneuploid and polyploid organisms (Birchler and Newton, 1981). Finally, because the main effect of subgenome dominance is an unequal expression of homoeologous copies, altering the ratio of dominant and submissive homoeologs by homoeologous exchange has the potential to change the balance of gene products from the 2:2 tetraploid state. 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, which are known to accumulate large amounts of genomic rearrangement (Xiong et al. 2011), at three generations to determine if the immediate gene expression responses to allopolyploidy are consistent with 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. We further identified homoeologous exchange events to test if changes in gene expression from homoeologous exchanges exhibit patterns of dosage constrain consistent with the Gene Balance Hypothesis. 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.

# Methods

## Sequencing data

We downloaded the data and files for previously identified genomic rearrangements and transcript quantification from Bird et al. (2021) at the associated Data Dryad repository https://doi.org/10.5061/dryad.h18931zjr

## Dosage response to polyploidy

When investigating the dosage response to polyploidy, we limited our analysis to the syntenic homoeologous genes identified as being in a 2:2 dosage ratio. We combined data from all polyploid lines together and calculated expression response to polyploidy for each gene pair, defined as the fold change of polyploid expression for a 2:2 syntenic homoeolog pair and the mid-parent expression of the progenitor ortholog pair ($\frac{Exp_{B.oleracea} + Exp_{B.rapa}}{2}$). For both polyploid and diploid progenitor samples, Bird et al. (2021) mapped to the *in silico* polyploid reference genome and transcripts were quantified in the same way so normalization was consistent. The distribution of polypoid dosage response for all sampled gene pairs in all lines was plotted as a histogram, along with the median of the distribution, using ggplot (Wickham) in R v 3.6.3 (R core team, 2020).

## Dosage sensitivity assignment

To leverage the well-curated gene annotations of *Arabidopsis thaliana*, and the close phylogenetic relationship between *A. thaliana* and the *Brassica* genus, we assigned our *Brassica* gene pairs to the GO category of their *A. thaliana* ortholog. Orthologs between *A. thaliana* and *Brassica oleracea* were identified with Synmap (Lyons et al. 2008) on CoGe (Lyons and Freeling, 2008) and the *A. thaliana* GO annotations were directly assigned to the *B. oleracea* orthologs and from *B. oleracea* to the *B. rapa* syntelogs. Next, we used the GO category dosage response assignments (dosage-insensitive and dosage-sensitive) from Song

et al.'s (2020) analysis of gene retention patterns of *A. thaliana* genes to classify our syntenic homoeologs as belonging to dosage-sensitive and dosage-insensitive GO categories.

## Polyploid response variance

We applied the same approach as Coate et al. (2016) and Song et al. (2020) and focused on the coefficient of variation of expression response ($\frac{\sigma_{exp}}{\mu_{exp}}$), which we similarly termed the polyploid response variance (PRV). We calculated PRV only for GO terms that contained more than 20 genes. Statistical analysis was done with 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).

## Homoeologous exchange response variance

We included only syntenic homoeolog pairs that diverged from 2:2 dosage ratio (e.g. gene pairs with read-depth ratio less than 0.4 or greater than 0.6) to investigate the effects of gene dosage changes. To eliminate confounding effects from aneuploidy, we excluded chromosomes where we observed skewed read-depth ratios that spanned the entirety or majority of a chromosome. This resulted in the removal of syntenic homoeologs from chromosomes A1/C1, A2/C2, and A10 from all lines, and chromosome C4 only for line EL-1100 at generation 10. We defined the expression response to homoeologous exchange as $\frac{Exp_{BnC} + Exp_{BnA}}{Exp_{B.\,oleracea} + Exp_{B.\,rapa}}$ which is the fold change of the summed expression for a homoeologous pair in the polyploids and the summed expression of the progenitor orthologs when mapped to the *in silica* polyploid genome. We calculated the coefficient of variation 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. As for the previous analysis, we only included GO terms with 20 or more genes and defined homoeolog expression bias in terms of expression bias in parental orthologs.

# Results

## Assessing early gene expression response to dosage changes from allopolyploidy

We investigated the relative gene expression change 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 mid-parent value of the progenitors. 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 progenitor genomes, 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 (Fig 1). 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 (Shi et al. 2021).

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. *However, it should be noted that our design makes it difficult to distinguish the isolated effects of time against changes in inter-individual variation and concomitant trans-effects, which increases over time due to accumulating genomic rearrangement. As such the increase in the variance of expression response over time may be due to a change in dosage constraint itself, which allows more variance in expression, or a result of accumulating individual variation and trans-effects that increase variance. In either case, the results reveal a greater tolerance to expression variance than suggested by a single generation analysis.* 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**

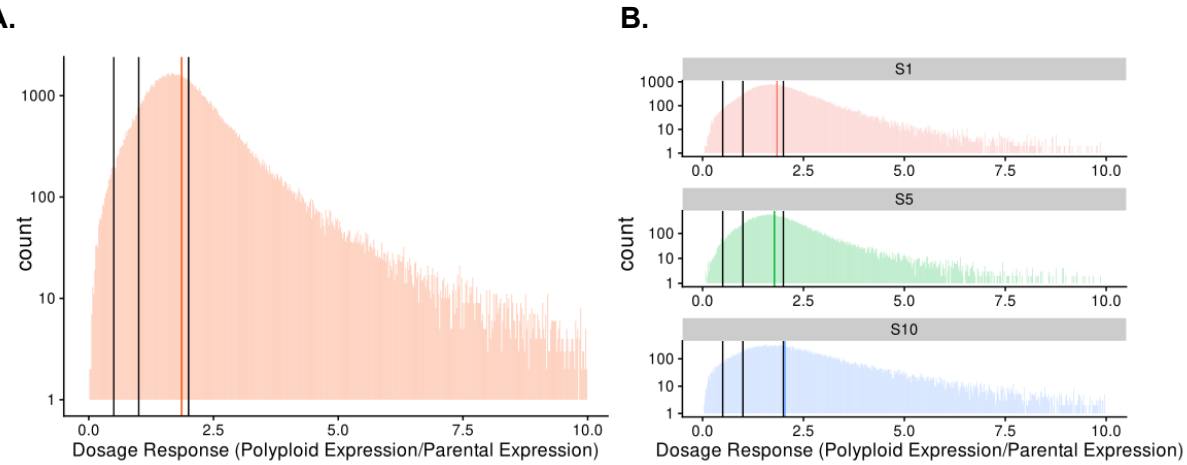


Fig 1. **A)** Dosage response to allopolyploidy by comparing summed gene expression of 2:2 homoeologous gene pairs in all 16 isogenic polyploid plants **(A)** combined or **(B)** grouped by generation to the summed expression of orthologs in the parental lines. Ratios of 1 represent dosage compensation, 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. To leverage the superior annotation quality of *A. thaliana*, *B. rapa* and *B. oleracea* orthologs were assigned to dosage-sensitivity GO classes based on their ortholog in *Arabidopsis*. These dosage-sensitivity assignments were used to assess how dosage response differs between classes in the resynthesized allopolyploids. We also used the polyploid response variance (PRV measure from Song et al. (2020) and Coate et al. (2016), defined as ) the coefficient of variation of the relative expression response, to assess how coordinated the expression response to polyploidy is in the different gene classes.

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

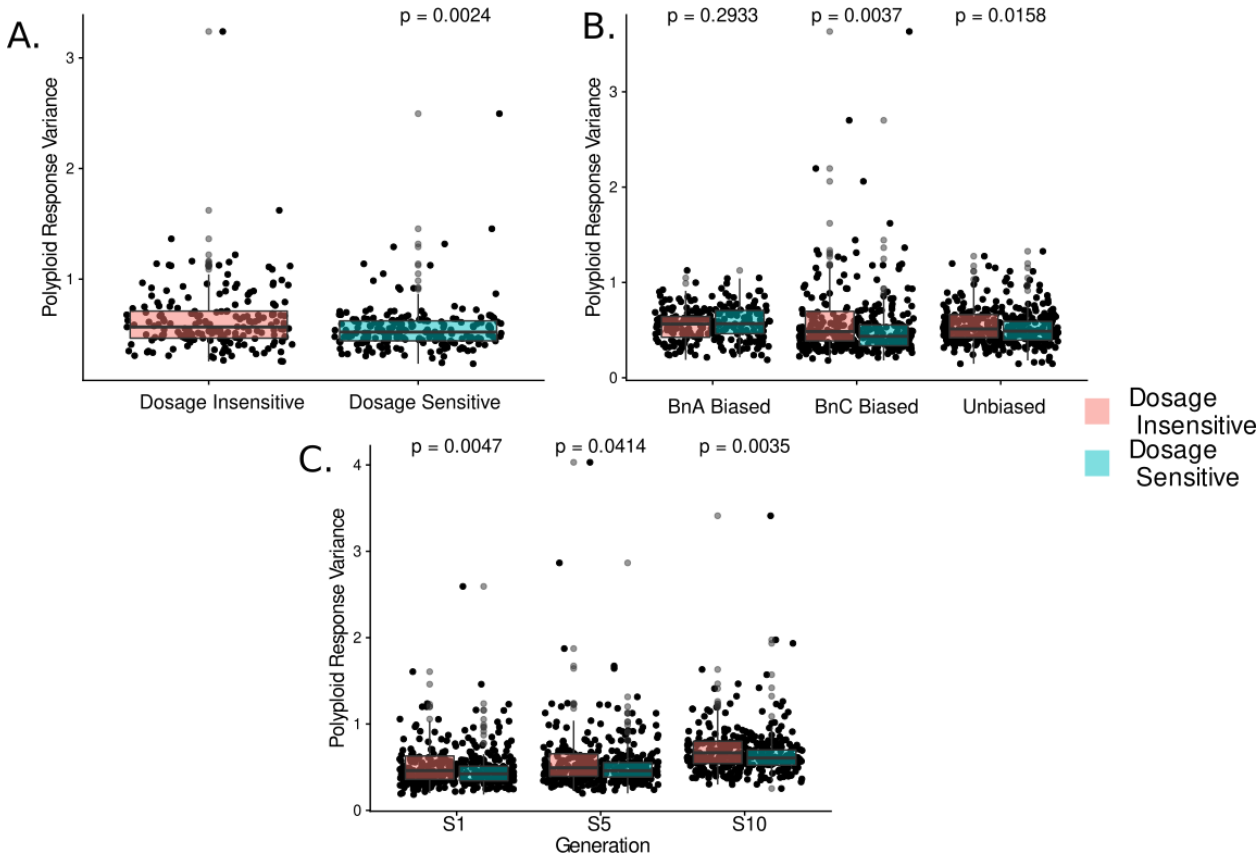


Fig 2. Polyploid response variance (coefficient of variation of dosage response) for all 2:2 balanced homoeologs in all 16 isogenic polyploid plants broken by **A)** only putatively dosage-insensitive (Class I) and dosage-sensitive (Class II) GO categories from Song et al. 2020, **B)** GO Dosage categories 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. In all plots, individual dots represent a GO term, restricted only to GO terms that were represented by 20 or more genes in our dataset.

As observed previously in resynthesized autopolyploids and natural *Glycine* allopolyploids, the polyploid response variance was significantly lower (i.e. the expression response was more coordinated) in genes from GO categories in the dosage-sensitive class compared to the dosage-insensitive class (Kruskal-Wallis test, p=0.0024; Fig 2; 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 of the gene pair and found that pairs with expression biased toward the *B. napus* C subgenome (BnC) biased and pairs with unbiased expression show the same significant difference between PRV of dosage-sensitive and dosage-insensitive GO categories as above (Kruskal-Wallis test, p=0.0037; 0.0158). However, gene pairs biased toward the *B. napus* A subgenome (BnA) showed no significant difference in PRV between dosage-sensitive and insensitive GO classes (Kruskal-Wallis test, p=0.2933; Fig 2b). This result suggests that constraint on the gene dosage response manifests differently depending on homoeolog expression bias. When broken down by generation, we observe the an increase in the coefficient of variation over time, with both dosage-sensitive and dosage-insensitive showing higher PRV in generation ten than in the first generation (Fig 2c). Notably, in generation ten the dosage-sensitive GO categories show higher mean polyploidy response variance than dosage-insensitive GO categories in the first generation.

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

The extensive genomic rearrangements observed in this population of resynthesized lines (Xiong et al. 2011; Bird et al. 2021) provide an opportunity to test for the first time whether gene expression changes from homoeologous exchange events experience dosage balance constraints as predicted by the gene balance hypothesis. Using the published results from Bird et al. (2021), we focused on genomic regions identified as not be in 2:2 dosage, representing homoeologous exchanges with 0:4, 1:3, 3:1, and 4:0 dosage ratios (BnC:BnA). To avoid the inclusion of likely aneuploidy events, genes on chromosomes that frequently showed dosage

changes for the entirety or majority of the chromosome were excluded. With this dataset of likely gene pairs affected by homoeologous exchange events, we compared their expression to the summed expression of the gene pair in the progenitor genomes. 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 3). 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 3). As before, our design prevents fully distinguishing the isolated effects of HEs from the impact of novel trans- regulation in the hybrid and allopolyploid genome.

**Figure 3. Expression response to non-reciprocal homoeologous exchange induced dosage changes**

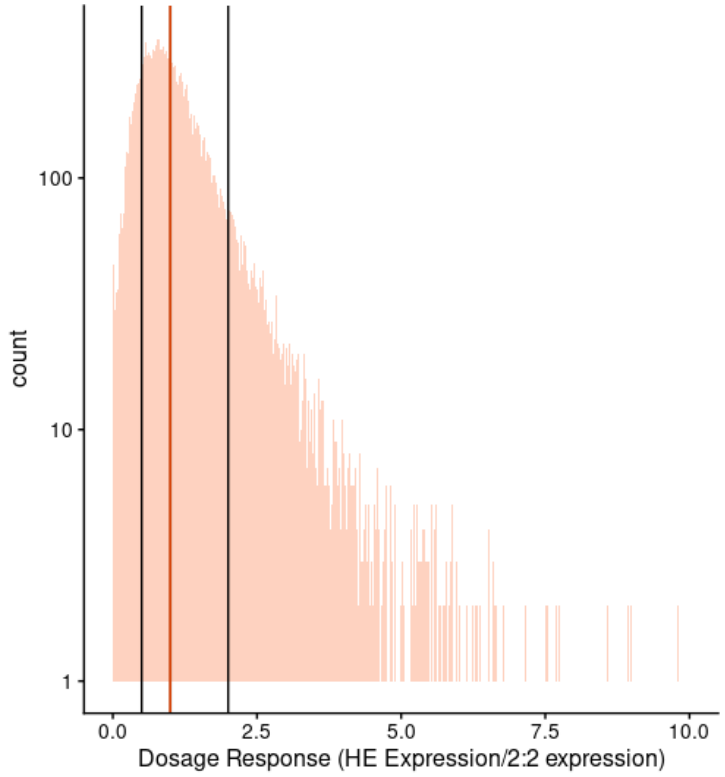


Fig 5. Dosage response to non-reciprocal homoeologous exchange by comparing summed gene expression of a dosage imbalance homoeologous gene pairs in all 16 isogenic polyploid plants combined to the summed expression of orthologs in the parental lines. Black lines represent dosage ratios of 0.5, 1, and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

Next, we investigated the extent that expression responses from homoeologous exchanges systematically differ among the identified dosage-sensitive and dosage-insensitive GO categories (Fig 4). We again used the coefficient of variation, this time termed Homoeologous Exchange Response Variance (HERV), to assess how coordinated the expression response was for genes from dosage-sensitive and insensitive GO categories. Across all lines, genes belonging to putatively dosage-sensitive GO categories again showed significantly lower HERV, indicating a more coordinated expression response than that for genes from putatively dosage-insensitive GO categories (Fig 4a, 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 HERV in dosage-sensitive GO terms than dosage-insensitive GO terms (Fig 4b). 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 4b, Kruskal-Wallis test, p=0.83926).

Furthermore, we found that there was not a significant difference in HERV between dosage-sensitive and dosage-insensitive GO terms in the first generation (Fig 4c, Kruskal-Wallis test, p=0.79), but dosage-sensitive and insensitive GO terms did show different HERV 4c, 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.

**Figure 4. Expression changes from non-reciprocal homoeologous exchange reflect predictions from the dosage balance hypothesis**

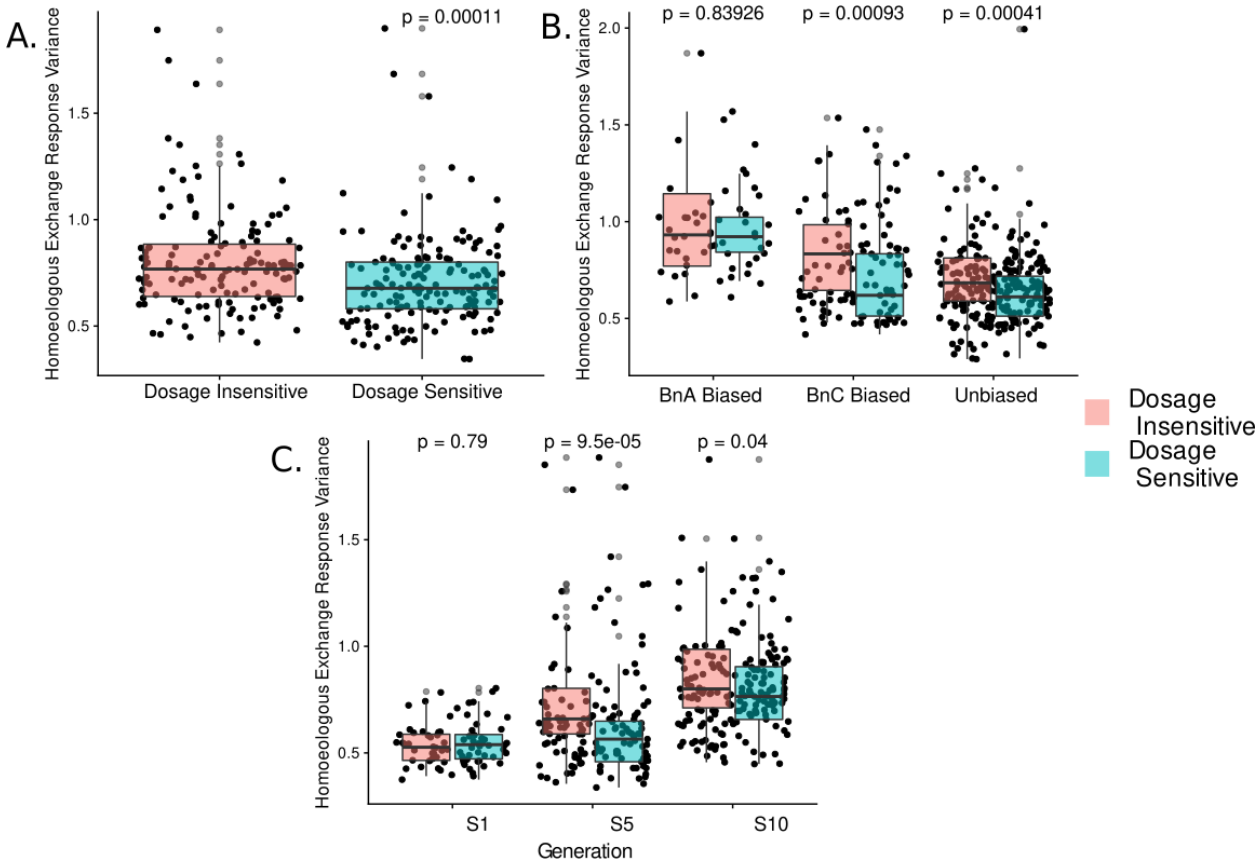


Fig 6. Homoeologous Exchange response variance (coefficient of variation of dosage response from homoeologous exchange) for all dosage imbalanced homoeologs in all 16 isogenic polyploid plants broken down by **A)** only putatively dosage-insensitive (Class I) and dosage-sensitive (Class II) GO categories 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. In all plots, individual dots represent a GO term, restricted only to GO terms that were represented by 20 or more genes in our dataset.

## 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 7).

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 variation from homoeologous exchanges (Fig 7a, Kruskal-Wallis test, $p<2x10^{-16}$), which had a lower proportion of genes belonging to dosage-insensitive GO categories. Both allopolyploidy and homoeologous exchange dosage changes produced significantly different expression responses from genes belonging to dosage-sensitive and insensitive GO categories (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, $p=1.153x10^{-12}$) GO categories.

Likewise, for both homoeologous exchange and polyploidy induced dosage changes, the difference in expression response between genes belonging to dosage-sensitive and insensitive GO terms was significantly different for BnC biased and unbiased homoeologous pairs, but not for BnA biased pairs (Fig 7c). Our results also showed that the coefficient of variation 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 1).

**Figure 5. Expression responses from allopolyploidy and homoeologous exchange appear to be distinct**

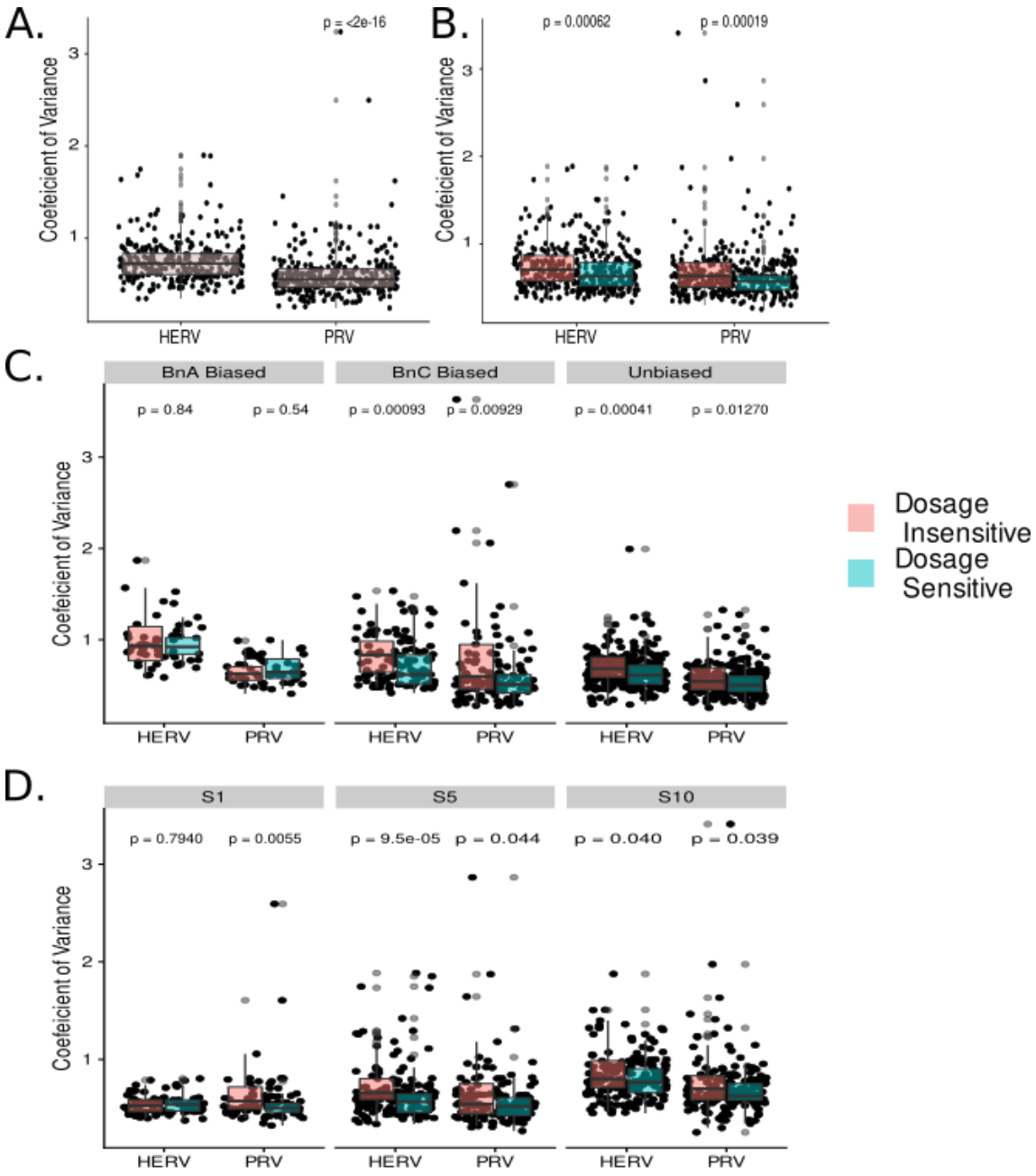


Fig 7. Comparison of expression response variance for non-reciprocal homoeologous exchanges (HERV) and allopolyploidy (PRV) for **A)** all lines and gene groups combined, **B)** all lines grouped by dosage class from Song et al. 2020, **C)** GO Dosage categories and subgenome dominance relationship in 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. In all plots, individual dots represent a GO term, restricted only to GO terms that were represented by 20 or more genes in our dataset.

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 variation 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 2).

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

**Table 1:** 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 | **$9.90 \times 10^{-7}$** |
| Dosage Insensitive | Unbiased | 0.708 (0.183) | 0.585 (0.183) | 26.173 | 1 | **$3.12 \times 10^{-7}$** |
| Dosage Sensitive | BnC Biased | 0.721 (0.269 | 0.569 (0.331) | 17.342 | 1 | **$3.122 \times 10^{-5}$** |
| Dosage Sensitive | BnA Biased | 0.930 (0.142) | 0.681 (0.141) | 22.69 | 1 | **$1.90 \times 10^{-6}$** |
| Dosage Sensitive | Unbiased | 0.634 (0.193) | 0.525 (0.150) | 34.658 | 1 | **$3.93 \times 10^{-9}$** |

**Table 2:** 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 | **$5.4 \times 10^{-7}$** |

## Discussion

The gene balance hypothesis has garnered extensive empirical support and has guided understanding of many aspects of genome evolution, such as biased retention of duplicate genes from particular functional categories (Maere et al. 2005; Paterson et al. 2006; Freeling, 2009; Tasdighian 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). These authors showed in synthetic *Arabidopsis* autopolyploids and natural *Glycine* allopolyploids that the expression response to WGD in dosage-sensitive genes was more coordinated than for dosage-insensitive genes. They concluded that dosage constraints produce a coordinated expression for dosage-sensitive genes and that this provides a proximal mechanism by which dosage constraint can impact long-term gene retention.

By leveraging our population of resynthesized allopolyploid *B. napus* lines, this study directly tested how similar auto- and allopolyploids immediately respond to WGD. The unique aspects of *B. napus* also allowed for a novel investigation of how subgenome dominance interacts with dosage balance constraints and how dosage changes from homoeologous exchanges are constrained to maintain dosage balance. However, there are some key

limitations to this study that warrant future follow-up. There are several trans-effects on expression, both from hybridization and aneuploidy experienced in these lines that could not be controlled for when assessing expression changes. As such, the expression responses we detect are an unknown combination of responses to WGD and homoeologous exchange in addition to these trans-effects. However, previous analysis of gene expression in these resynthesized lines over ten generations showed that over 70% of genes showed the same biased expression toward the dominant subgenome and over 50% showed the same biased expression toward the non-dominant subgenome across all six lines and between the progenitor genomes (Bird et al. 2021). This suggests that trans-effects from hybridization and unshared genomic rearrangements should not entirely alter expression in a way that invalidates comparisons of progenitor genomes and resynthesized allopolyploids.

Additionally, due to the small number of genes generally affected by homoeologous recombination we combined all dosage combinations (AAAA, AAAC, ACCC, CCCC), which makes it difficult to ascertain the specific direction of expression changes or to isolate particular kinds of homoeologous exchanges. As genomic rearrangements accumulate and diversity over time, merging these factors will increase inter-individual variation. This means the comparisons across generations will be confounded by changing inter-individual variation and interpretation is not straightforward. 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 provides new insight into the role of dosage constraint and gene balance in affecting gene expression changes from genomic rearrangements and opens up avenues for future investigation.

## Evolutionary dynamics of early expression response to allopolyploidy

Our analysis of the relative expression response to allopolyploidy reinforces the idea that a general response to dosage changes is expression changing in a variety of ways ranging from compensation to dosage-dependent, as previously observed an *Arabidopsis* aneuploid series (Hou et al. 2017), *Arabidopsis* autopolyploids (Song et al. 2020), and an *Arabidopsis* allopolyploid dosage series (Shi et al. 2015). We further identified similar patterns of more coordinated expression responses among putatively dosage-sensitive genes, similar to the

reports from synthetic autopolyploid *Arabidopsis* (Song et al. 2020) and wild allopolyploid *Glycine* that originated ~500,000 years ago (Coate et al. 2016). Overall, these results suggest that the effect of dosage constraint on the global expression response to polyploidy is similar between newly formed auto- and allopolyploids, as expected if dosage constraint was a general evolutionary force acting on all polyploid genomes immediately upon 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. Subgenome dominance also drives the biased retention of genes from one subgenome in allopolyploid genomes. This biased retention is hypothesized to be caused by higher expression of homoeologs from the dominant subgenome (Schnable et al. 2011; Woodhouse et al. 2014; Renny-Byfield et al. 2015; Renny-Byfield 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. Our results suggest novel interaction between subgenome dominance and dosage constraint such that dosage-sensitive genes show more coordinated expression when homoeolog expression is unbiased or biased toward the dominant subgenome, but not when biased toward the non-dominant subgenome.

Over the long term, this would be predicted to preserve more dosage-sensitive genes from the dominant subgenome than the non-dominant. In line with this, Schnable et al. (2012) observed that biased retention of dosage sensitive genes broke down over time, with only 50% of genes retained from one genome duplication event being retained in duplicate after a subsequent duplication event. They further 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*, the dominant subgenome was also associated with the production of clusters of dosage-sensitive genes (Thomas et al. 2006).

Our results provide a unified account for short-term and long-term interactions of subgenome dominance and dosage constraint. Upon duplication, a more coordinated expression response for homoeologs biased toward the dominant subgenome will produce

greater retention of dosage-sensitive genes from the dominant genome and concomitant under-retention from the non-dominant subgenome. Additionally, previous analysis of these resynthesized lines showed that homoeologous pairs biased toward the dominant subgenome were highly connected in a protein-protein interaction network, while pairs with expression biased toward the non-dominant subgenome showed no such connectivity (Bird et al. 2021). This lack of connectivity may explain why putatively dosage-sensitive genes with biased expression toward the non-dominant subgenome do not show coordinated expression; without high connectivity in gene networks, they do not experience strong dosage constraints.

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 (Song et al. 2020), 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. Alternatively, it is known that dosage changes 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. We were unable to control or measure these kinds of trans dosage effects and they could potentially create inter-individual variation and drive these observed changes in expression coordination between earlier and later generations.

Previous analysis of duplicate gene retention across angiosperms described three broad groups of genes: 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 genes. However, if our results were driven by inter-individual variation from trans effects, instead of showing a loosening of

dosage constraint, we would be revealing a greater tolerance for uncoordinated expression responses than one would infer from the levels of coordination in the first generation.

## 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). Investigations of genome imbalance and dosage sensitivity have predomiately 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, homoeologous exchanges, which alter the ratio of parental chromosomes, have also been shown to produce dosage-dependent expression changes (Lloyd et al. 2017). These dosage changes from homoeologous exchanges have not been investigated for dosage constraints or more 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. Previous results from an *Arabidopsis* allopolyploid dosage (AAAA, AAAT, AATT, ATTT, TTTT) series showed that the majority of genes (54%) changed expression in a dosage-dependent manner for both homoeologs (Shi et al. 2015). However, our results suggest a more varied response to homoeologous exchange than Lloyd et al. (2017), who determined over 95% of expression changes from homoeologous exchanges were dosage-dependent. Overall, 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 responses from dosage sensitive genes. We also saw similar patterns of lower expression coordination in later generations and a lack of differences in expression coordination from homoeolog pairs biased toward the non-dominant subgenome that we observed when investigating expression response to polyploidy. Such results have not been reported before, to our knowledge, and suggest that homoeologous exchanges also experience selective constraint for balanced gene dosage in the same way as genes affected by polyploidy or aneuploidy.

If homoeologous exchanges evolve in ways predicted by the gene balance hypothesis then we might expect selection to disfavor homoeologous exchanges containing dosage-sensitive genes, producing biases in gene functions surviving homoeologous exchanges to be similar to small-scale duplications. Following these predictions, Hurgobin et al. (2017) and Bayer et al. (2021) identified a significant degree of gene presence-absence variation in *B. napus* arising from homoeologous exchanges, and these genes were associated with membership in the protein-protein interaction network (Bayer et al. 2021) and GO terms related to plant defense and stress pathways (Hurgobin et al. 2017). They also observed several homoeologous exchanges generating presence-absence variation in paralogs of the large gene family *FLC,* which regulates flowering time. Analysis of expression dynamics of *FLC* paralogs in *B. napus* showed that while FLC paralogs are dosage-sensitive, dosage constraints act on overall *FLC* gene family expression allowing compensatory drift (Thompson et al. 2016) and expression divergence (Calderwood et al. 2020). This *FLC* example shows that the interplay of homoeologous exchange and dosage constraint may be highly dynamic depending on the gene family in question. Homoeologous exchange may also drive systematic subgenome biases in the direction of homoeologous exchange. For example, Edger et al. (2019) proposed that constraints on stoichiometric balance and altered gene dosage explained the overwhelming bias in direction of homoeologous exchange, favoring the dominant subgenome, in the octoploid strawberry genome.

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 weaker dosage constraints, although it may also simply be due to high levels of inter-individual variation among lines. While 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.

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## Author Contributions:

KAB contributed to conceptualization, formal analysis, investigation, methodology, validation, visualization, writing—original draft, and writing—review editing; JCP. contributed to resources, and writing—review editing; RV. contributed to supervision, methodology, and writing—review editing; ZX. contributed resources, experimental design, and writing—review editing; and PPE. contributed supervision, methodology, and writing – review editing.

## Data availability:

Raw data from this project are available on the NCBI Sequence Read Archive (SRA) Project PRJNA577908. Intermediate files can be found at https://doi.org/10.5061/dryad.h18931zjr and code to recreate main figures can be found at https://github.com/KevinABird/Bird_GenomeInFlux_BNapus

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