

Genomic Asymmetry for Morphology in Allopolyploids Within and out of *Brassica*

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Abstract: The genomic asymmetry in the nonrandom retention and expression of controlling genes for some traits from one parental diploid is obvious in some natural and synthetic allopolyploids, and has the evolutionary implications. Here we review the genomic asymmetry for the morphological performance in three cultivated *Brassica* allotetraploids and some intergeneric allopolyploids within *Brassicaceae* species. For the phenotypic biases of *Brassica* allotetraploids, *Brassica oleracea* (genomes CC) is dominant over *B. nigra* (BB) and *B. rapa* (AA) in *B. carinata* (CCBB) and *B. napus* (CCAA), respectively, and *B. nigra* is dominant over *B. rapa* in *B. juncea* (BBAA), showing the C>B>A dominance hierarchy. However, the morphology of several *Brassica* species including *B. oleracea* at top dominance is largely recessive in their intergeneric hybrids and allopolyploids with other crucifers, such as *Raphanus sativus*, *Orychophragmus violaceus*. The morphology of *Arabidopsis thaliana* is also recessive in its two allotetraploids. Among the dominant features, the leaf serration is expressed consistently in these intergeneric and *Arabidopsis* allopolyploids. The phenotype expression of the recessive diploid is subject to the euploidy or aneuploidy state of its genome, and the dominant traits are still mostly expressed in the aneuploidy state of their genome. The morphological biases in these allopolyploids are discussed in the contexts of the genomic structure and interplay.

Keywords: Genomic Asymmetry, Allopolyploids, *Brassicaceae*, Morphology, Phenotypic Dominance

1. Introduction

The genomic asymmetry refers to the nonrandom retention of controlling genes for some traits, favoring one genome over the other(s) in interspecific hybrids and allopolyploids [1]. The classical example of genomic asymmetry for gene transcription is the uniparental expression of ribosomal RNA genes and the subsequent formation of a nucleolus or nucleoli at the nucleolar organizer regions (NORs) of only one species, which is so known as nucleolar dominance, while the rRNA genes from other species are silenced and no nucleoli formed at the original NORs where rDNA loci are still located [2]. The genomic asymmetry in the allopolyploid wheat is obvious for the control of a variety of morphological, physiological, and molecular traits, including nucleolar

dominance, i.e., complete or predominant control of certain traits by one of the constituent genomes [1]. Such genomic asymmetry, together with build-up and maintenance of enduring and favorable inter-genomic genetic combinations, is considered to contribute to the evolutionary success of allopolyploids. Otherwise, the missing of some allopolyploids in nature between or among the extant diploid species might be caused by the lack of their adaptive ability to establish genomic asymmetry after the genome fusion, though the synthetics are obtained.

Three cultivated *Brassica* allotetraploids, *Brassica carinata* Braun (2n = 34, BBCC), *B. juncea* (L.) Czern. (2n = 36, AABB), and *B. napus* L. (2n = 38, AACC) are ideal for revealing the genomic asymmetry and the effect of different genomic contexts, because any two of them share one same subgenome of three diploids, *B. nigra* (L.) Koch (2n = 16, BB),

B. oleracea L. ($2n = 18$, CC), and *B. rapa* L. ($2n = 20$, AA). The genomic asymmetry in these *Brassica* allotetraploids is manifested for nucleolar dominance with a hierarchy of B subgenome > A subgenome > C subgenome [3], and also for their phenotype biased to one diploid parent. In particular, the genomic asymmetry constantly appears in the intergeneric hybrids and allopolyploids of *Brassica* species with *Raphanus sativus* L. and *Orychophragmus violaceus* (L.) O. E. Schulz, with the latter being largely dominant for the morphology, especially the constant expression of leaf serration. Here we review the genomic asymmetry and its occurrence hierarchy for the control of the morphological traits, with the emphasis on the leaf margin, in hybrids and allopolyploids of *Brassica* and other genera in *Brassicaceae* (Table 1), and discuss possible mechanisms in the context of genomic structure.

2. Morphological Bias in *Brassica* Allotetraploids

The natural and synthetic allotetraploid *B. carinata* gives a plant exterior similar to the diploid *B. oleracea*, while the characteristic morphology of the diploid *B. nigra* is largely masked [4]. Remarkably, *B. carinata* possesses stronger resistance to biotic and abiotic stresses than other *Brassica* species, which is ascribed to the combined genetic contributions of its two parental diploids showing also good resistance. The plant phenotype of the black mustard, *B. nigra* is dominant over that of *B. rapa* in oilseed type of the mustard *B. juncea*, which is pictographically named as mustard-type oilseed in China. *B. nigra* also contributes the good resistance to biotic and abiotic stresses for *B. juncea*. The whole exterior of the synthetic and natural *B. napus* was more biased to the parent *B. oleracea*, particularly by expressing its trait of the deeply green leaves covered with a thicker layer of waxy powder [4]. So *B. napus* was called cabbage-type rapeseed in China, and replaced the native *B. rapa* and *B. juncea* for its higher seed yield and stronger resistance to biotic and abiotic stresses, after its introduction into China in 1930s-1940s. The better resistance of *B. napus* was also largely contributed by *B. oleracea*.

Intriguingly, the progenitor *B. rapa* was restituted from the natural *B. napus* through inducing the preferential elimination of C-subgenome chromosomes in intertribal crosses (Zhu et al., 2016) [5]. The novel *B. rapa* should reflect the proximal image of the actual *B. rapa* progenitor, in consideration of the short history of its hybridization with *B. oleracea* ~7500 yr ago [6], and expressed a phenotype resembling some type of *B. rapa* but hidden in *B. napus*. This result provides the direct evidence for the phenotypic dominance of *B. oleracea* over *B. rapa* in *B. napus*. Similarly, by inducing the preferential elimination of C-subgenome chromosomes in the artificially synthesized *Brassica* allohexaploids ($2n=54$, BBCC.AA) from *B. carinata* × *B. rapa* crosses [7], the novel *B. juncea* genotypes (BB.AA) with the B subgenome from natural *B. carinata* and A subgenome from extant *B. rapa* are obtained and show the typical features of *B. nigra* which were masked

in *B. carinata* but reactivated and still were dominant over those of *B. rapa*.

Totally, the genomic asymmetry for plant morphology in three *Brassica* allotetraploids is obvious and exhibits the dominance hierarchy of C subgenome > B subgenome > A subgenome, with the morphology of *B. oleracea* being dominant over *B. nigra* in *B. carinata* and over *B. rapa* in *B. napus*, and that of *B. nigra* being dominant over *B. rapa* in *B. juncea* (Figure 1) (Table 1). So the dominance hierarchy of the morphology (C>B>A) is inconsistent to that of nucleolar dominance (B>A>C) [3]. Then, the morphology dominance and nucleolar dominance are consistent and are in the control by the same B subgenome only in *B. juncea* (AABB), but are under control by different subgenomes in *B. carinata* (BBCC) and *B. napus* (AACC) (Table 1). Reversely, such genomic asymmetry for the control of the phenotypic and agronomic traits, and the resistance to biotic and abiotic stresses sustains further for the proper hybridization combinations of three diploids and the evolutionary success of three allotetraploids in nature and by human selection.

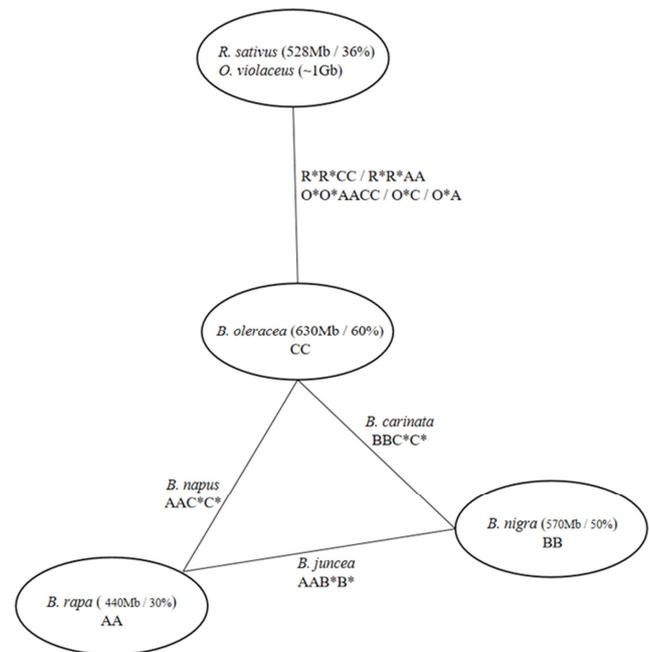


Figure 1. The summary of phenotypic dominance in the hybrids and allopolyploids produced among three *Brassica* diploids, radish and *Orychophragmus*. The positions of parental species indicate the hierarchy of phenotypic dominance is *R. sativus* / *O. violaceus* > *B. oleracea* > *B. nigra* > *B. rapa*. The dominant genome is marked by the asterisk. The genome sizes and the percentages of the repeats for parents available are given.

3. Morphological Bias in Synthetic *Brassica* Allohexaploids

Despite the evolutionary success of three *Brassica* allotetraploid species, no trigenomic allohexaploid *Brassica* species ($2n=54$, AABBCC) has been found so far in nature or in agriculture. However, such allohexaploids have been artificially synthesized by several possible cross combinations

among the six *Brassica* species, in order to provide the new germplasm for genetic studies and breeding or even to develop a new allohexaploid crop [8]. From the sequential crosses between three *Brassica* diploids, *B. rapa*, *B. oleracea* var. *alboglabra* and *B. nigra*, followed by chromosome doubling, the two allohexaploids (AA.CC.BB, CC.AA.BB) obtained which contained the same genome complement but the different types of the cytoplasm showed the similar morphology biased more to the parental *B. oleracea* var. *alboglabra* by expressing its thick and flesh leaves and stems, while the characteristic features of *B. nigra* was covered mostly [4, 9]. The allohexaploid (AACC.BB) between *B. napus* and *B. nigra* was mainly morphologically biased to *B. napus* and expressed a few phenotypic traits from *B. nigra* [10]. The allohexaploid (AABB.CC) between *B. juncea* and *B. oleracea* var. *alboglabra* also showed the obvious bias to the morphology of *B. oleracea* var. *alboglabra* and the suppression of the *B. juncea* phenotype to some extents, in spite of the latter combination of the C genome [9]. As expectedly, the allohexaploid (BBCC.AA) between *B. carinata* and *B. rapa* resembled *B. carinata* for the phenotype [7, 9]. So the phenotypic dominance of *B. oleracea* is still maintained in these synthetic allohexaploids with various genome combinations from six extant *Brassica* species (Table 1), which hints some inherent elements for the genome asymmetry for morphological control. Similarly, the nucleolar dominance of B subgenome from *B. nigra* is also reserved in these allohexaploids [7], again revealing the inconsistency between nucleolar dominance and phenotypic dominance.

4. Morphological Bias in Intergeneric Allopolyploids Between *Brassica* and *Raphanus*

The genomic asymmetry for plant morphology is also obvious in some intergeneric hybrids and allopolyploids produced through hybridizations between *brassicaceae* and other species (Figure 2). The allotetraploid *Raphanobrassica* ($2n=36$, RRCC) between *Raphanus sativus* L. ($2n=18$, RR) and *B. oleracea* is morphologically biased to radish (Table 1), by expressing its half pinnately divided leaves, silique shape and large white flowers, except for the enlarged roots. Besides the thick layer of waxen powder, the phenotype of *B. oleracea* remains largely hidden (Figure 2), despite its top dominance over other two *Brassica* diploids (*B. rapa*, *B. nigra*). The morphological feature of the radish is also largely maintained in the intergeneric hybrids (RACC) between *Raphanobrassica* and *B. napus* (AACC) (Figure 2), despite the two copies of C genome and the single copy of R genome. Another allotetraploid between *R. sativus* and *B. rapa* subsp. *pekinensis* (\times *Brassicoraphanus*, $2n=38$, RRAA) also produces the radish-like morphology (Table 1), particularly with the enlarged roots but at lesser extent than the radish [11]. Intertribal somatic hybrids ($2n=32$, RRII) between radish and the Chinese woad (*Isatis indigotica* Fort., $2n=14$, II) obtained by Tu *et al.* also resembles the radish in pinnately divided

leaves, semi-hollow pith and little enlarged roots [12]. So the phenotype of radish is dominant over that of these species in their allotetraploids.



Figure 2. The phenotypic dominance of radish over *Brassica* species in their intergeneric hybrids and allopolyploids. The young plant of the allotetraploid *Raphanobrassica* (C) between *Brassica oleracea* var. *alboglabra* (A) and radish (B) gives a phenotype biased to radish, and the hybrid (D) with the RACC genomes between *Raphanobrassica* and *B. napus* also expresses obviously the plant feature of radish.

5. Morphological Bias in Intergeneric Hybrids and Allopolyploids Between *Brassica* and *Orychophragmus*

The morphology of *O. violaceus* is dominant over several *Brassica* species in their hybrids at different extents depending on the genome combinations (Figure 1) (Table 1). The somatic hybrids between *B. napus* and *O. violaceus* ($2n=62$, AACCOO) were morphologically biased towards *O. violaceus* in all growth stages, as they expressed its characteristic traits, including deep green and oval serrated and hairy leaves, basic clustering stems, more primary, secondary and even tertiary and quaternary branches, and purple color on petals, as reported by Zhao *et al.* [13]. The classical hybrids ($2n=21$, CO) with *B. oleracea* produced by Li and Heneen also shows the phenotypic attributes of the serrated leaves and branching, and purple petals of *O. violaceus* origin [14]. Though the hybrid with *B. rapa* L. ssp. *chinensis* L. was a mixoploid with the loss of the variable chromosomes from *O. violaceus*, it still expressed its typical phenotype of the serrated leaves and basal branching, which was quite different from the phenotype of the Chinese cabbage [14]. So the trait of the serrated leaves from *O. violaceus* is consistently expressed in these intergeneric hybrids. The phenotypic dominance is in accordance with the nucleolar dominance in the somatic hybrids between *B. napus* and *O. violaceus*, for only the transcripts of rRNA genes from *O. violaceus*, but not from *B. rapa* were detected [7]. The result from addition lines (with *B. napus* genomes plus one of three *O. violaceus* chromosome pairs carrying NORs) revealed that the rDNA loci on three *O. violaceus* chromosomes were active and gave differential amounts of rRNA transcripts in the *B. napus* background, showing the variable degrees of nucleolar dominance [15].

6. Morphological Bias in *Arabidopsis* Allotetraploids

Phenotypic dominance also occurs in two *Arabidopsis* allotetraploids with the preferential repression of the model plant *A. thaliana* phenotype (Table 1). The natural and

synthetic allotetraploids *A. suecica* ($2n=4x=26$, AtAtAaAa) between *A. thaliana* ($2n=10$, AtAt) and *A. arenosa* ($2n=16$, AaAa) resembled the *A. arenosa* parent in these morphological characteristics including long leaves, tall stature, many branches, deeply serrated rosette leaves, and large rosettes and flowers. The pink flower color of *A. arenosa* appeared in the early generations of the synthetic allotetraploid and changed to white after several generations [16]. The synthetic allopolyploid ($2n=4x=26$, AtAtAlAl) between *A. thaliana* and *A. lyrata* subsp. *petraea* ($2n=2x=16$, AlAl) is phenotypically more similar to *A. lyrata* in several growth characteristics such as plant stature, the tendency to produce aerial rosettes, the vernalization requirement and the long-lived habit [17]. Phenotypic dominance also occurs coincidentally with the nucleolar dominance in these two allotetraploids, as the *A. thaliana* rDNA loci were always repressed. Furthermore, the species-specific bias against *thaliana* gene expression is not restricted to rRNA genes, as the genome-wide analysis of gene expression reveals global down-regulation of the *A. thaliana* genome in favor of the *A. arenosa* genome [16] (Table 1).

7. Phenotypic Dominance and Genome Contexts in Allopolyploids

As shown in these allopolyploids above, the exhibition extent and then the recognizability of the phenotypic dominance biased to one parent seem to correlated with the genomic relationships and the morphological difference between parents. If two parents are more distantly related and show more distinct traits, it is probable that their hybrids and allotetraploids should present more characteristics which are identifiable for the parental origin and then classifiable into the expression dominance. As *B. napus* has two progenitors *B. rapa* and *B. oleracea* which are most closely related among three *Brassica* diploids and show least difference in morphology, it gives fewest traits distinguishable between two parents among the three allotetraploids. Because *B. nigra* is characterized by its morphology much distinct from both *B. rapa* and *B. oleracea*, *B. juncea* and *B. carinata* show more traits assignable to parents, obviously to *B. nigra* in *B. juncea*. The fact that the distinct phenotype from *B. nigra* is largely covered in *B. carinata* is quite evident for the strong expression of the *B. oleracea* morphology.

Interestingly, the characteristic phenotypic traits from the other genera including *R. sativus* and *O. violaceus* are preferred for expression in their intergeneric hybrids and allopolyploids with *Brassica* species, particularly the leaf serration [13-15] (Table 1) (Figures 1, 2). As morphological traits from these species out of *Brassica* are more divergent than those in *Brassica* species, the phenotypic dominance is more easily identifiable when they are expressed, including the invariable leaf serration. The more obvious performance of phenotypic dominance in intergeneric allopolyploids than *Brassica* ones likely results from the more distant relationship and more distinct traits, while the high homoeologous genomes in

Brassica nullify the effect of the other genome. Notably, the leaf serration is dominantly expressed in these intergeneric allopolyploids, *B. juncea*, and *Arabidopsis* allotetraploids.

The expression and dominance of phenotypic traits are subject to the genome structure and interplay in the allopolyploids. In the first situation, the genes controlling the traits of the recessive parent is re-activated in the aneuploidy state of its genome. Though the morphology of *B. nigra* is largely masked in synthetic *B. carinata*, its hidden traits including the distinct leaf serration are expressed in the monosomic alien addition lines (MAALs) carrying individual chromosome of B subgenome and all chromosomes of C subgenome [18], which reveals that the aneuploidy state of B subgenome must but its euploidy state suppress the gene expressions, suggesting the differential interaction mechanisms for the euploidy and aneuploidy backgrounds. In the second situation, the traits of the dominant parent are still expressed in the aneuploidy state of its genome. Those MAALs carrying individual chromosome of B subgenome and all chromosomes of A subgenome expressed the distinct traits of *B. nigra*, as observed in the synthetic *B. juncea* and *B. oleracea-nigra* MAALs. Some *B. rapa-nigra* MAALs sharing the same B subgenome chromosome as *B. oleracea-nigra* MAALs presents the more obvious expression of the same trait (unpublished), showing that A subgenome is more motivative or less suppressive for the gene expression of B subgenome than C subgenome. Similarly, nearly all the dominant traits from *O. violaceus* in the somatic hybrids with *B. napus* are expressed by different MAALs with its different chromosomes in the *B. napus* background [15]. This indicates that the expressions of the traits from the dominant parent in the allopolyploids are mostly maintained in the aneuploidy state of MAALs only carrying single chromosome related. Additionally, these MAALs also show some nonparental traits, likely caused by the interspecific aneuploidy [15].

From genome sequencing and transcriptome analysis of all six *Brassica* species, no significant colinear homoeologous gene expression dominance in the two subgenomes are detected in three natural allotetraploids [6, 19, 20]. In the six tissues of *B. carinata* with the high-quality genome sequence, the colinear gene pairs showing expression dominance account for only 6.50% of whole-genome genes, and the dominance between the two subgenomes is insignificant. Among only 1.50% of genes displaying homoeolog expression dominance in all tissues, 647 (0.66%) and 815 (0.84%) genes are dominant toward B and C subgenomes, respectively [20]. So the correlation between phenotypic dominance and gene expression dominance at genome-wide level is low and not causal at least in these allotetraploids, though their co-occurrence is revealed in some allopolyploids [21] (Table 1). The genomic structures of three *Brassica* diploids seem not to agree with the prevailing explanation for the preferential expression of homoeologs regarding the parental differences in the number and distribution of transposable elements (TEs) and their adjacency to genes [22], as the genome of *B. oleracea* [23] has the higher percentage of repetitive sequences ~60% than

B. nigra (~50%) [24], and *B. rapa* (30%) [25] (Table 2) (Figure 1). Accordingly, the dominant *R. sativus* (~36%) [26] has a genome with the size and rate of repetitive sequences similar to the recessive *B. rapa*, but smaller in size and less repetitive than the recessive *B. oleracea* (Table 2). Similarly,

the genome of the dominant *O. violaceus* (~1Mb genome size) is also characterized by the large size comparable to *B. napus* and the even distribution of repetitive sequences along the whole chromosomes [27].

Table 1. Phenotypic, nucleolar and transcriptomic dominance in natural and synthetic allopolyploids from sexual and somatic hybridizations between species of *Brassicaceae*.

Allopolyploids	Parental combinations	Dominant genome in		
		Phenotype	Nucleolus	Transcriptome
<i>B. carinata</i> (BBCC)	<i>B. nigra</i> (BB) × <i>B. oleracea</i> (CC)	C	B	NO
<i>B. juncea</i> (AABB)	<i>B. rapa</i> (AA) × <i>B. nigra</i> (BB)	B with leaf serration	B	NO
<i>B. napus</i> (AACC)	<i>B. rapa</i> (AA) × <i>B. oleracea</i> (CC)	C	A	NO
AABBCC	Six <i>Brassica</i> species	C	B	
RRCC	<i>R. sativus</i> (RR) × <i>B. oleracea</i> (CC)	R with leaf serration	RR	-
RRAA	<i>B. rapa</i> (AA) × <i>R. sativus</i> (RR)	R with leaf serration	-	-
RRII	<i>R. sativus</i> (RR) + <i>I. indigotica</i> (II)	R with leaf serration	-	-
AACCOO	<i>B. napus</i> (AACC) + <i>O. violaceus</i> (OO)	O with leaf serration	O	-
<i>A. suecica</i> (AtAtAaAa)	<i>A. thaliana</i> (AtAt) × <i>A. arenosa</i> (AaAa)	Aa with leaf serration	Aa	Aa
AtAtAIAI	<i>A. thaliana</i> (AtAt) × <i>A. lyrata</i> (AIAI)	AI	AI	-

“-”: Data unavailable.

Table 2. Genome data of three *Brassica* diploids and radish which present the $R > C > B > A$ hierarchy of phenotypic dominance in their allopolyploids.

Species / Genome	<i>R. sativus</i> / RR	<i>B. oleracea</i> / CC	<i>B. nigra</i> / BB	<i>B. rapa</i> / AA
Genome size (Mb)	547	630	570~608	443
Assembly %*	70.0	60.0	78.4~88.8	79.7
Repeat and TE %	36.7	38.8	33~54	37.5

Raphanus sativus var. *hortensis* genome was assembled using ≥ 500 -bp and ≥ 2 -kb scaffolds, with a total of 121.8× sequence data [26]. The draft genome assembly of *Brassica oleracea* var. *capitata* line 02–12 was produced by interleaving Illumina, Roche 454 and Sanger sequence data [23]. The *de novo* assemblies for the *B. nigra* genome were generated by a combination of nanopore sequencing, Illumina error correction, Hi-C sequencing and genetic mapping [24]. An improved assembly of the *B. rapa* genome (v3.0) for the Chinese cabbage (accession Chiifu-401-42) was obtained by using single-molecule sequencing, optical mapping, and chromosome conformation capture technologies (Hi-C) [25].

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8. Conclusions

The phenotypic dominance and particularly its hierarchy among these hybrids and allopolyploids involving the species within and out of *Brassica* stimulate many questions on the regulation mechanisms of subgenome dominance for further studies, including the “outstanding questions” proposed [22]. The allopolyploids and the MAALs which express some easily identifiable traits (such as leaf serration) provide the suitable systems for solving these questions, and for connecting the biased homeolog expression and subgenome dominance to their genomic and individual chromosome contexts, with the availability of the genome data for the species involved.

The formation of leaf margin which can be categorized into smooth, serrated or lobed is under the complex regulation of numerous factors including phytohormones, transcription factors (TFs), and microRNAs (miRNAs) [28–30]. The elucidation of the mechanism behind the dominant phenotype of the leaf serration in these allopolyploids should shed new insights not only into the leaf development, but also into the differential homeolog expression.

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