Genomic mosaicism due to homoeologous exchange generates extensive phenotypic diversity in nascent allopolyploids

Ying Wu1,2, Fan Lin 2, Yao Zhou1,2, Jie Wang1, Shuai Sun1, Bin Wang1, Zhibin Zhang1, Guo Li1, Xiuyun Lin1, Xutong Wang1, Yue Sun1, Qianli Dong1, Chunming Xu1, Lei Gong1,4,*, Jonathan F. Wendel4,*, Zhiwu Zhang 2, and Bao Liu1,∗

ABSTRACT

Allopolyploidy is an important process in plant speciation, yet newly formed allopolyploid species typically suffer from extreme genetic bottlenecks. One escape from this impasse might be homoeologous meiotic pairing, during which homoeologous exchanges (HEs) generate phenotypically variable progeny. However, the immediate genome-wide patterns and resulting phenotypic diversity generated by HEs remain largely unknown. Here, we analyzed the genome composition of 202 phenotypically euploid segmental allopolyploid individuals from the fourth selfed generation following chromosomal doubling of reciprocal F1 hybrids of crosses between rice subspecies, using whole-genome sequencing. We describe rampant occurrence of HEs that, by overcoming incompatibility or conferring superiority of hetero-cytonuclear interactions, generate extensive and individualized genomic mosaicism across the analyzed tetraploids. We show that the resulting homoeolog copy number alteration in tetraploids affects known-function genes and their complex genetic interactions, in the process creating extraordinary phenotypic diversity at the population level following a single initial hybridization. Our results illuminate the immediate genomic landscapes possible in a tetraploid genomic environment, and underscore HE as an important mechanism that fuels rapid phenotypic diversification accompanying the initial stages of allopolyploid evolution.

Keywords: nascent allopolyploidy, homoeologous recombination, phenotypic diversity, plant evolution, GWAS, dosage effects, epistasis

INTRODUCTION

Phylogenetic and phylogenomic studies have revealed that hybridization is widespread in all domains of life [1–4]. Merging of genomes from divergent lineages represents a potent evolutionary force that can facilitate adaptation, speciation and adaptive radiation [3–6]. There are two major forms of hybridization, one at the homoploid level and the second, allopolyploidization, entailing whole-genome duplication (WGD). Allopolyploidy is pervasive in the evolutionary history of higher plants, testifying to its creative role in adaptive evolution and species diversification of the plant kingdom [7–11]. Compared with newly formed homoploid hybrids that are often, though not always, sterile due to genic and/or chromosomal incompatibility [12], nascent allopolyploids often are partially to fully fertile, because of rapid establishment of diploid-like meiotic behavior [13–15]. It is established that many polyploids occur recurrently from different populations of their progenitors, whereby new genotypes are generated upon secondary contact [16], but some polyploids are of monophyletic origin. Regardless, in nascent allopolyploids, perfect homologous meiotic pairing often generates little variation, thus limiting evolvability [17]. This property of allopolyploidy constrains the generation of genetically variable progeny and also impedes purging of fixed
deleterious or slightly deleterious mutations due to genome merger. In addition, de novo recessive beneficial mutations that occur post-polyploidy will be masked by genetic redundancy [18]. Nonetheless, the near-ubiquity and prevalence of allopolyploidy across the angiosperm phylogenetic spectrum comprises prima facie evidence that there are solutions to the seemingly insurmountable constraints imposed by the foregoing population genetic considerations.

Apart from recurrent formation [16], another mechanism to mitigate allopolyploidy-associated genetic impoverishment is repeated introgression from diploid parental progenitors or related taxa [2,19,20], especially during niche expansion or human-mediated dissemination [15,21–23]. Yet, prior to these extrinsic sources of variation coming into play, how might nascent allopolyploids generate phenotypically relevant variation? At least a partial answer to this question is related to the multiple and diverse mechanisms of rapid changes in the genome, transcriptome and epigenome of allopolyploids [9,24–26]. It should be noted however that these immediate genomic responses due to genome merge and/or doubling turned out to be largely maladaptive in animals, which provides a novel explanation to the long-standing enigma, i.e. why polyploidy is rarer in animals but abundant in plants [27]. Intriguingly, in some lower vertebrates, such as certain fish, these allopolyploidization-incurred catastrophic genome consequences can be resolved by subgenome cooperation and balanced stabilization, and lead to re-diploidized lineages [28].

An important and frequent observation in many plant allopolyploids is that homologous chromosome meiotic pairing is not stringent, and that homoeologous exchanges (HEs) may arise that are transgenerationally cumulative and may be subject to natural and human selections [29–39]. It thus is evident that in many young plant allopolyploids, HEs provide a possible escape from pure homozygosity and that this may be an effective mechanism for generating rapid genetic variation [34]. Relatively little is understood, however, about the dynamics and pace of HE-mediated genomic diversification at the genomic and population levels, and even less is understood about its direct phenotypic consequences in the absence of confounding evolutionary forces.

Here, we focus on a segmental allotetraploid rice (Oryza sativa) population consisting of 202 sampled euploid individuals derived from inter-subspecies (japonica and indica) hybridization and chromosome doubling [40]. Previously, we used this system to assess the association between HEs and partitioning of homoeologous gene expression based on a subset of pre-selected genes [41], and effects of HEs on alternative splicing [42] and on DNA methylation stability [43] at individual plant levels. Here, we extend these analyses to genome-scale and at population levels, with the aims of (i) characterizing the immediate genomic landscape generated by HE-mediated admixture of two divergent genomes following WGD; (ii) determining the features and factors that constrain HE occurrence and/or perpetuation; and (iii) assessing the immediate impact of HE-mediated genomic mosaicism on phenotypic variation, as well as deciphering its underlying genetic basis. We show that (i) rampant HEs occurred in the tetraploids, generating widespread genomic mosaicism; (ii) cytonuclear interaction is an important intrinsic factor that constrains particular admixed patterns; and (iii) the extensive phenotypic diversity in the tetraploids is largely accounted for by HE-mediated homoeolog copy number alteration of known-function large-effect genes and their multiple interactions.

RESULTS

Extraordinary phenotypic diversity

We reported previously that the synthetic tetraploids (segmental allotetraploids) [44,45] of the rice subspecies japonica (cv. Nipponbare) and indica (cv. 93-11) manifested considerable phenotypic novelty compared with their parental cultivars and F1 hybrids. In addition, they displayed extensive changes in gene expression and alternative splicing as a result of the combined effects of hybridization and genome duplication [40–42]. Here we extend these previous results to describe the spectrum of phenotypic variation in progenies of the tetraploids at the population level, and explore their underlying genetic variation based on high-quality whole-genome analyses. We phenotyped 21 complex traits at the fourth selfed generation (S4) tetraploid populations of reciprocal origins (NN99 and 99NN) which contained a set of 202 euploid individuals (Supplementary Fig. S1 and Dataset S1). Populations of both parents and reciprocal F1 hybrids were phenotyped in parallel. Of these 21 traits, nine were related to vegetative growth and development, while the other 12 were related to reproduction and seed yield (Dataset S1–S2). Compared with the parents, F1 hybrids were uniformly heterotic in traits related to vegetative growth but inferior in traits related to seed production (Fig. 1A–D, Supplementary Fig. S2 and Dataset S2); the latter is expected given the inter-subspecific genic incompatibility that causes high hybrid infertility [46].
Figure 1. Illustration and quantification of phenotypic traits of the diploid parents (NPB and 93-11), reciprocal F1 hybrids (N9 and 9N) and reciprocal S4 tetraploids (NN99 and 99NN). (A) Overall plant status including plant height, tiller number and tiller angle. (B) Flag-leaf angle. (C) Flag-leaf length and width. (D) Grain length and width. (E) and (F) Quantification of plant height and grain length by boxplots and histograms, respectively. In (D), the aligned seeds depicting variations in grain length (horizontally arranged) and width (vertically arranged) are from different tetraploid lines (the 10 seeds arranged in each row are from one line). In (E), letters above each box denote statistically different phenotypic distributions in each comparison, with blue numbers above each box referring to the relevant standard deviations of the data from each box. In (F), the left ordinates are for the histograms and the right ordinates are for the density plots (the red and blue curves).

While the distribution of mean values of tetraploids, irrespective of cross direction, did not exceed either the diploid parents or F1 hybrids in 16 of the 21 traits, it was transgressive relative to the parents and F1 hybrids in the other five traits (Dataset S2). A striking feature of the tetraploids was the magnitude of variation in all 21 traits (Fig. 1A–E, Supplementary Fig. S2–S3 and Dataset S1). Standard deviation (SD), range (R, maximum minus minimum) and coefficient of variation (CV) analyses all confirmed that both tetraploid populations had substantially larger variation than those of the diploid parents and reciprocal F1 hybrids for all 21 traits (Fig. 1E, Supplementary Fig. S3 and Dataset S1). As expected, we found that although allopolyploidization (the combined effects of hybridity and polyploidy) itself contributed to phenotypic differences between the tetraploids and diploid parents and F1 hybrids, this effect cannot cause the rapidly emerged, dramatic variations in each trait among the tetraploids at population levels (Supplementary Fig. S4 and Supplementary Results and Analysis). Because there was no discernible difference in the phenotypic data distribution for reciprocal tetraploid populations (Fig. 1F and Supplementary Fig. S5), we do not differentiate them here unless indicated otherwise.

Notably, transgressive phenotypes were observed for all traits in at least some of the tetraploids (Fig. 1F, Supplementary Fig. S5 and Table S1). The number of individuals manifesting phenotypic over-transgressivity (greater than both parents) was significantly higher than those showing under-transgressivity (smaller than both parents) for 11 of the 21 traits, whereas the reverse was observed for nine traits, and one trait showed no significant difference (Supplementary Table S1).

Rampant homoeologous exchange

Given the close phylogenetic relatedness between the two rice subspecies, japonica and indica [47,48], it is expected that meiotic HE may occur in selfed progenies of the tetraploids, as indeed we showed in a pilot study involving four tetraploid individuals [43]. To further quantify the extent of HE at genome-wide scale in progenies of the tetraploids at a population level, we performed whole-genome re-sequencing (10X coverage) of 202 euploid tetraploids selected from a set of 340 individuals based on oligo-FISH (florescence in situ hybridization)-based karyotyping [49] (Supplementary Fig. S6). By using a customized pipeline, we verified the euploid identity of all 202 individuals, and determined the foci of HE breakpoints in each individual at a 5-kilobase (kb) resolution along each of the 12 rice chromosomes. Reliability of our pipeline was also validated by performing the same analysis on whole-genome re-sequencing data of the reciprocal F1 hybrids [43], and in which no recombinant tract (mimicking HE) was detected. Quantification across the 202 re-sequenced individuals (Supplementary Fig. S7) identified a total of 27 945 HEs after only four selfed generations, mapping to all 12 rice chromosomes (mean of 138.34 HEs per individual). This surprisingly large number of HEs may suggest they are not only transgenerationally cumulative but also likely arising in a ‘ratchet-like’ manner [34]. To test this, we analyzed HEs from an additional 45 individuals of the S5 (whole-genome sequencing data available) generation that are direct progenies of random individuals of the 202 S4 tetraploids (Supplementary Fig. S1 and Supplementary Results and Analysis). We found that HE rates per meiosis were 17.4 and 19.0 (or 0.72 and 0.79 per chromosome pair, n = 24) in S4 and S5, respectively (P = 0.0269, Student’s t-test), lending support to the ‘polyploid-ratchet-like’ metaphor [34]. However, the ratcheting process may hold only for a limited number of generations, i.e. before the segregating tetraploids reach a certain homozygosity threshold. HE frequencies varied markedly among chromosomes, with 1, 4 and 12 showing larger numbers of HEs while fewer were detected on chromosomes 6, 8 and 10 (Supplementary Table S2). However, when scaled by chromosome size, chromosomes 12 and 6 respectively showed an excess (27.24 cM/Mb, P < 0.05) and deficit (10.76 cM/Mb, P < 0.05) of HEs relative to expectations based on permutation-based Poisson tests (Supplementary Table S2). The inter-chromosome difference in HE frequency is interesting given that homoeologous recombination is likely under the control of the same machinery as homologous recombination (HR) [50], which primarily acts in trans [51]. Nonetheless, similar observations
Figure 2. Heatmaps depicting the genomic landscapes of 36 randomly selected S4 euploid tetraploid individuals from 36 selfed lines (Supplementary Fig. S1). Different colors denote different homoeolog compositions, where orange representing NPB homoeolog percentage at a given locus is 25%, i.e. the homoeolog ratio between NPB and 93-11 is 1 : 3. Each row represents one tetraploid individual, the 12 columns represent the 12 chromosomes in the rice genome, and the light blue dots denote centromeres.

were made in synthetic allotetraploids of *Brassica* [33] and wheat [50], suggesting generality of the phenomenon.

With respect to within-chromosome distribution, a general feature is lower density of HEs in pericentromeric regions (defined as three consecutive 500 kb bins harboring the centromere), while subtelomeric regions (defined as four consecutive 500 kb bins from the end of each chromosomal arm) showed the opposite trend (Supplementary Fig. S7 and Table S3); this observation is consistent with patterns of HR in plants [50], and again, suggests the same recombination machinery is at work [43]. Exceptions to this generality are apparent, however. For example, chromosome 1 experienced more HEs in the pericentromeric region and chromosome 9 showed fewer HEs in the subtelomeric regions (Supplementary Fig. S7 and Table S3). This chromosome-specific peculiarity of HEs was not found for the distribution of homologous recombination in rice [51,53,54], suggesting it is a unique property of HE.

For any given locus in an S4 individual, the ratio of homoeologs from the two parents, Nipponbare and 93-11, may fall into one of five types, i.e. Nipponbare:93-11 = 4:0, 3:1, 2:2, 1:3 or 0:4. We analyzed the genomic composition of all 202 S4 tetraploids and depicted their genomic composition either on a per-individual basis (one random individual for each of the 36 lines; Fig. 2) or on a per-line basis (all five or six individuals of a given line together; Supplementary Fig. S8). Genome-wide, the proportions of each of the five Nipponbare vs. 93-11 homoeolog ratios of all 202 individuals together were 17.4% (4:0), 13.0% (3:1), 24.8% (2:2), 16.2% (1:3) and 28.5% (0:4), respectively; notably, proportions between both the homologous ratios (4:0 vs. 0:4) and the heterozygous ratios (3:1 vs. 1:3) were asymmetric with respect to the null assumption of 50%:50% (P < 3.4E-16, exact binomial test; Supplementary Table S4). Overall, the genomic proportion of 93-11 homoeologs (averaged 56.34%) was significantly higher than that of Nipponbare (NPB) homoeologs (averaged 43.66%) in the tetraploids (P < 0.05, exact binomial test), and this trend holds in both cross directions (P = 0.077, chi-square test). Relative proportions of the five homoeolog ratios were also not equal among the 12 chromosomes. When considering together the homozygous (4:0 and 0:4) and heterozygous (3:1, 2:2 and 1:3) homoeolog ratios each as a group, chromosome 6 showed the highest proportions (average = 80.7%) of homozygous ratios, which were mainly contributed by the 93-11 homoeologs (average = 70.0%) (Supplementary Table S4), while chromosome 10 showed the highest proportion of heterozygous ratios, on average 70.9% (Supplementary Table S4). Chromosomes 6
Figure 3. Heatmaps showing the genomic compositions of 31 cytonuclear compatibility-related chromosomal segments being strongly selected for from all of the 202 genome-re-sequenced S4 tetraploid individuals. The different colors show the different genomic types, e.g. the orange color represents that the homoeologous ratio between NPB:93-11 in this given locus is 1:3. Each row represents one tetraploid individual, the 31 columns indicate 31 chromosomal segments. Cross direction and line names are labeled on the left of the rows. Information of segment ID, chromosome, genomic location, segment size and the number of cytonuclear molecular interaction genes is given in the lower panel. The framed box in Seg. 7 denotes a 1787 kb genomic region retaining at least one maternal copy in all (100%) individuals.

| Seg. 1, Chr. 1: 190001 - 1220000, 1030kb, 3; | Seg. 12, Chr. 4: 27095001 - 27375000, 280kb, 1; | Seg. 22, Chr. 12: 20645001 - 22060000, 1415kb, 5; |
| Seg. 2, Chr. 1: 40750001 - 41350000, 600kb, 4; | Seg. 13, Chr. 4: 34730001 - 35460000, 755kb, 2; | Seg. 23, Chr. 12: 22335001 - 23645000, 1310kb, 4; |
| Seg. 3, Chr. 2: 29250001 - 35700000, 645kb, 2; | Seg. 14, Chr. 7: 84650001 - 12040000, 3575kb, 4; | Seg. 24, Chr. 1: 7315001 - 8210000, 895kb, 5; |
| Seg. 4, Chr. 3: 13125001 - 13460000, 335kb, 3; | Seg. 15, Chr. 7: 12135001 - 13585000, 1270kb, 3; | Seg. 25, Chr. 4: 30030001 - 30860000, 830kb, 4; |
| Seg. 5, Chr. 3: 13545001 - 13755000, 210kb, 1; | Seg. 16, Chr. 10: 19630001 - 20280000, 650kb, 2; | Seg. 26, Chr. 4: 31470001 - 34730000, 3290kb, 15; |
| Seg. 6, Chr. 3: 33365001 - 33650000, 420kb, 2; | Seg. 17, Chr. 10: 21970001 - 22845000, 870kb, 4; | Seg. 27, Chr. 8: 21200001 - 25390000, 4185kb, 14; |
| Seg. 7, Chr. 6: 25770001 - 30590000, 4820kb, 13; | Seg. 18, Chr. 12: 18950001 - 29550000, 1060kb, 1; | Seg. 28, Chr. 8: 25475001 - 26305000, 560kb, 1; |
| Seg. 8, Chr. 6: 30755001 - 31220000, 465kb, 1; | Seg. 19, Chr. 12: 45800001 - 51750000, 595kb, 1; | Seg. 29, Chr. 9: 10810001 - 17540000, 6730kb, 17; |
| Seg. 9, Chr. 7: 21990001 - 22300000, 310kb, 1; | Seg. 20, Chr. 12: 53450001 - 58900000, 545kb, 1; | Seg. 30, Chr. 9: 18055001 - 22345000, 4890kb, 20; |
| Seg. 10, Chr. 11: 22050001 - 224450000, 385kb, 1; | Seg. 21, Chr. 12: 18780001 - 201850000, 1405kb, 4; | Seg. 31, Chr. 12: 76650001 - 82600000, 595kb, 3; |
| Seg. 11, Chr. 11: 22650001 - 232100000, 565kb, 1; |

**Figure 3.** Heatmaps showing the genomic compositions of 31 cytonuclear compatibility-related chromosomal segments being strongly selected for from all of the 202 genome-re-sequenced S4 tetraploid individuals. The different colors show the different genomic types, e.g. the orange color represents that the homoeologous ratio between NPB:93-11 in this given locus is 1:3. Each row represents one tetraploid individual, the 31 columns indicate 31 chromosomal segments. Cross direction and line names are labeled on the left of the rows. Information of segment ID, chromosome, genomic location, segment size and the number of cytonuclear molecular interaction genes is given in the lower panel. The framed box in Seg. 7 denotes a 1787 kb genomic region retaining at least one maternal copy in all (100%) individuals.

and 8 were overrepresented by homo-93-11 (NPB: 93-11 = 0:4) across the 202 individuals (Fig. 2 and Supplementary Fig. S8). We suspect this biased parental legacy is likely due to selection for early flowering in the northeast region of China where the plants were grown, consistent with enrichment of genes controlling heading date in chromosomes 6 and 8 (https://shigen.nig.ac.jp/rice/oryzabase/ and http://www.ricedata.cn/index.htm).

**Homoeologous exchange is constrained by cytonuclear interaction**

A salient observation is that some genomic regions manifested parental homoeolog composition patterns that are strikingly distinct between the tetraploid reciprocals, suggesting the possibility of hetero-cytonuclear incompatibility or superiority (Fig. 3). Genomic regions showing such features...
could be classified into three groups: Group I contained 11 segments (segments 1 to 11) that mapped to six chromosomes (1, 2, 3, 6, 7 and 11), with sizes ranging from 210 to 1030 kb, in which at least one copy of the maternal homolog was preferentially retained \( (P < 0.01, \text{chi-square test}) \), in a reciprocal manner, in >95% individuals, suggesting symmetric hetero-cytonuclear incompatibility. Group II contained 12 segments (segments 12 to 23) that mapped to four chromosomes (4, 7, 10 and 12) with sizes ranging from 280 to 1310 kb, in which at least one copy of paternal homolog was preferentially retained \( (P < 0.01, \text{chi-square test}) \), in a reciprocal manner, in >95% individuals, suggesting symmetric hetero-cytonuclear superiority. Group III contained eight segments (segments 24 to 31) that mapped to five chromosomes (1, 4, 8, 9 and 12) with sizes ranging from 560 to 6730 kb, which showed preferential retention \( (P < 0.01, \text{chi-square test}) \) of at least one copy of the paternal homolog in all individuals of the NN99 but not in 99NN (Fig. 3), suggesting asymmetric hetero-cytonuclear superiority. Notably, although hetero-cytonuclear incompatibility (group I) did not involve 100% of the individuals, the 5% of plants that did not harbor homo-cytonuclear compositions showed significant loss in reproductive fitness (i.e. fecundity) compared to their respective siblings of the 95%, reflected by reduced fertility (36.4% vs. 76.0%, \( P = 1.13E-05 \), Student’s t-test) and grain number per panicle (52.3 vs. 107.3, \( P = 0.0096 \), Student’s t-test) (Supplementary Table S5).

There were 1529, 2029 and 3517 genes that mapped to the segments of Groups I, II and III, respectively (Dataset S3A–B). Although gene ontology (GO) enrichment analysis of all three sets of genes, either together or separately, showed no specific functional relevance (Dataset S3C), we noted that each of the 31 segments harbored at least one gene that was functionally categorized as participating in cytonuclear (plastid- or mitochondrion-nuclear) molecular interactions, and in total, 147 such genes were identified (Fig. 3 and Dataset S3A and D). Specifically, groups I, II and III contained 32, 36 and 79 such cytonuclear interacting genes, respectively, which are significantly more than expected from the genome-wide average (Pearson’s Chi-squared test: \( P = 1.02E-4, 5.74E-3 \) and 1.32E-11, for groups I, II and III, separately; and \( P = 3.32E-14 \) for all three groups in aggregate) (Supplementary Table S6). Of these 147 genes, 21 participate in cytonuclear co-encoding enzyme complexes (CCECs), in which different subunits of organellar protein complexes are encoded by organellar (mitochondrion or plastid) and nuclear genes, while 126 encode cytonuclear enzyme complexes (CECs; organelle-targeting proteins without organellar interacting partners) (Dataset S3A and D). Notably, 103 of the 147 genes (collectively on all 31 segments) showed predicted functional divergence between the parental alleles (Dataset S3D). This suggests that biased retention might be functionally consequential. Taken together, these results suggest that both hetero-cytonuclear incompatibility and superiority likely are constraints that have contributed to homoeologous composition of the tetraploids, either symmetrically (both cross directions are affected) or asymmetrically (only one direction is affected).

**Homoeologous expression is predominantly copy number-dependent**

One immediate genetic consequence of HE is disruption of homoeologous expression ratios determined by parental legacy. Conceivably, for homoeologs that are functionally diverged or sub-functionalized between the parents, this outcome of HEs may have physiological and phenotypic consequences if expression levels correlate with copy number [55]. To address this, we performed transcriptome profiling using two tissues (leaf and root) sampled from 12 randomly selected S4 tetraploid individuals. From 11761 to 13800 and from 13892 to 15511 expressed genes were identified in leaf and root, respectively, across the 12 tetraploid individuals (Supplementary Table S7). Most genes (ca. 90%) showed a strong correlation between ratios of homoeolog transcript abundance and ratios of DNA homoeolog copy number, in both leaf and root (adjusted \( P < 0.05 \) by chi-square test; Supplementary Fig. S9 and Table S7). This indicates homoeologous expression levels for most genes in the rice tetraploids are dosage-sensitive and homoeolog copy number-dependent, likely due to constraint for total expression level to maintain gene balance [56,57]. This is also consistent with the recently documented regulatory evolutionary features of the rice genome, and their important fitness consequences [58].

**HEs include characterized large-effect genes that underpin trait variation**

To pinpoint the specific HE-mediated copy number variants that may be responsible for phenotypic diversity, we performed a genome-wide association study (GWAS) between variation in genome-wide homoeolog ratio and variation in each of the 21 quantified traits. The fixed and random model circulating probability unification (FarmCPU) method of GWAS was used to maximize statistical
power and robustness [59]. We used both additive and dominance models of GWAS and encoded the five types of homoeolog ratios (N : 9 of 0 : 4, 1 : 3, 2 : 2, 3 : 1 and 4 : 0) as 0, 1, 2, 3 and 4, respectively for the additive model. For the dominance model, we used three types of coding for the five homoeolog ratios corresponding to N : 9 of 0 : 4, 1 : 3, 2 : 2, 3 : 1 and 4 : 0, namely, (i) 0, 1, 1, 1, 0; (ii) 0, 2, 2, 1, 1; and (iii) 1, 2, 2, 2, 0. This was to reflect differential effects of the parental homoeologs when they had alternative homozygous (4 : 0 and 0 : 4) states, i.e. (i), (ii) and (iii) reflect equivalence, transgressive N and transgressive 9, respectively, while the three heterozygous homoeolog ratios (1 : 3, 2 : 2, 3 : 1) were coded as 1 or 2 to reflect transgressive phenotypic values in both directions. To eliminate potential false positives, we used the most conservative threshold, Bonferroni corrections; the threshold for significant association calling was determined to be $P < 1.3395\times 10^{-7}$.

There were 22 and 63 distinct signals passing the statistical threshold in 14 and 21 traits using the additive and dominance models, respectively (Supporting Material and Dataset S4A–V). Notably, some signals were detected in both models, suggesting they have both additive and dominant effects on the target traits. For each GWAS-reported signal, sizes of linked segments were decided by Pearson correlation analysis (correlation coefficient $r > 0.9$; Dataset S4B–V). Next, we scrutinized known genes located within the identified segments. In total, we identified 29 known genes in these segments, which were previously shown as causally linked to the traits (Dataset S4A–V). These include large-effect genes such as GS3 for grain length [60], TAC1 for tiller angle [61,62], NAL1 for flag-leaf width [63,64] and DTH7 for days to flowering [65]. Notably, these four genes are also responsible for trait divergence between Nipponbare and 93-11 (Supplementary Table S8, Supplementary Results and Analysis and Dataset S4B, E, G and S). Although no information regarding functional divergence between the two rice subspecies is available for the other 25 genes, we found 21 bear non-synonymous coding differences between the parents, suggestive of protein-level functional diversification (Dataset S4–V).

As an illustration, we show the associations between homoeolog ratios of three genes (GS3, TAC1 and NAL1) with their corresponding traits (grain length, tiller angle and flag-leaf width) revealed by the additive model in GWAS, using both Manhattan (Fig. 4A, D and G), Quantile-Quantile (QQ) plots (Fig. 4B, E and H) and box plots (Fig. 4C, F and I). For the TAC1-containing locus, if the balanced heterozygous state N : 9 = 2 : 1 is the starting point, tiller angle becomes larger as NPB homoeolog copy number decreases (hence proportional increase of the 93-11 homoeolog copy number) and vice versa (Fig. 4C). This mirroring phenotypic response indicates a negative linear correlation ($r = -0.63$, $P < 2.2E-16$, Pearson correlation test) between NPB homoeolog copy number of TAC1 and tiller angle in the tetraploids, and hence additive effects of the gene. A similar negative linear correlation ($r = -0.71$, $P < 2.2E-16$ by Pearson correlation test) was revealed between NPB homoeolog copy number of the GS3-containing locus and grain length, although in this case there was a slight deviation from the expected result when the NPB homoeolog copy number was 4, suggesting, in addition to its major additive effect, that there also existed a moderate dominant and/or epistatic effect (Fig. 4F) consistent with the result that GS3 was identified by both additive and dominant models. For the NAL1-containing locus, in principle the NPB homoeolog copy number should be negatively correlated with flag-leaf width since 93-11 has a wider flag-leaf than NPB; unexpectedly, however, we found the correlation was positive in the tetraploids when the copy number of the NPB homoeolog of this locus was in the range of 0 to 3 but not when it reached 4 ($r = 0.47$, $P = 3.79E-1$, Pearson correlation test; Fig. 4I). This again indicates that NAL1 has additive, dominant and/or epistatic effects, consistent with its detection under both models.

Under the dominance model, we selected three loci (DTH8, OsBZR1 or OsSIZ1) as examples to show the associations between homoeolog ratios and the three corresponding traits (days to flowering, thousand kernel weight and tiller number, respectively). These associations are illustrated using Manhattan plots (Supplementary Fig. S10A, D and G), QQ plots (Supplementary Fig. S10B, E and H) and box plots (Supplementary Fig. S10C, F and I). For the DTH8-containing locus identified in the 0-1-1-1-0 dominance model, tetraploids with heterozygous homoeolog ratios showed significantly fewer days to flowering than those with homozygous homoeolog ratios; however, no difference in the trait was evident among the three heterozygous homoeolog ratios, suggesting dosage-independent interaction (Supplementary Fig. S10C). This strong curvilinear association ($r = -0.29$, $P = 6.8E-05$, Pearson correlation test) points to a negative dominant effect of the DTH8-containing locus on days to flowering. A similar negative dominant association ($r = -0.29$, $P = 4.8E-05$, Pearson correlation test) between the OsBZR1-containing locus fitting the 1-2-2-2-0 model of NPB homoeolog copy number and thousand kernel weight was identified, although in this case it appeared the 2 : 2 heterozygous ratio had a
Figure 4. GWAS of tiller angle, grain length and flag-leaf width with the additive model by FarmCPU R scripts. (A), (D) and (G) are Manhattan plots, wherein the green lines represent thresholds based on Bonferroni tests, with genes controlling the given trait labeled in blue below the corresponding locus. (B), (E) and (H) are Quantile-Quantile plots of p-values. (C), (F) and (I) are boxplots showing the additive relationship between phenotype and NPB copy number in each of the three genes.

stronger effect than the 3:1 or 1:3 heterozygous ratio, suggesting dosage-dependent interaction (Supplementary Fig. S10F). In contrast, a positive dominant association ($r = 0.34$, $P = 1.4E-06$, Pearson correlation test) between the OsSIZ1-containing locus and tiller number was detected, i.e. plants bearing all three types of heterozygous homoeolog ratios showed significantly more tiller numbers than those with either homozygous homoeologs, which also represents dosage-independent interaction (Supplementary Fig. S10H). Pairwise sequence comparison indicated that all three known genes, DH8, OsBZR1 and OsSIZ1, contain non-synonymous coding differences between the Nipponbare and 93-11 parental alleles (Dataset S4B), suggesting their potential functional divergence.

Epistasis between different HEs is common

Epistasis, i.e. non-additive interactions between non-allelic genes is widespread in diverse organisms [66–68] (Supplementary Results and Analysis). Whether different parental homoeolog ratio alterations in a polyploid epistatically interact for a given phenotypic trait has not been reported before. Here we used trait-associated HEs by GWAS in the rice tetraploids and identified 2489 interacting locus pairs for the 21 traits, of which 816 (32.8%) showed significant epistatic effects (Dataset S5A,C–W). The epistatic effects fell into one or more of the four models: additive by additive (A by A), additive by dominant (A by D), dominant by additive (D by A) and dominant by dominant (D by D) (Supplementary Results and Analysis). Frequencies of trait-associated pairwise loci manifesting epistasis were unequal among the traits. For example, flag-leaf length showed the highest percentage of epistatic locus pairs (53.6%), while yield showed the lowest (4.4%) (Supplementary Table S9). Also, not all of the four models of epistasis occurred equally; frequencies of A by A, D by A, A by D and D by D were 10.4%, 9.6%, 7.2% and 5.6% ($P < 0.05$, chi-square test; Supplementary Table S9). A further pathway (KEGG) analysis for
Figure 5. Patterns of interactions of the four epistasis types displayed by various two-locus combinations. $P$ value in each panel represents the statistical probability for relative epistasis. The vertical axis represents phenotypic value, and genotypic types of 0–4 represent N:9 homoeolog ratios of 0:4, 1:3, 2:2, 3:1 and 4:0, respectively.

Figure 5. Patterns of interactions of the four epistasis types displayed by various two-locus combinations. $P$ value in each panel represents the statistical probability for relative epistasis. The vertical axis represents phenotypic value, and genotypic types of 0–4 represent N:9 homoeolog ratios of 0:4, 1:3, 2:2, 3:1 and 4:0, respectively.

Allopolyploidy is widely recognized as a driving force in evolution, most notoriously in plants but also in many other eukaryotic lineages [8,9,69–72]. In many allopolyploids, homoeologous pairing, a prerequisite for HE, is suppressed due to intrinsic parental genome divergence and/or by genetic controls, e.g. $Phl$ in polyploid wheat [73] and $PrBn$ in Brassica [74], resulting in near exclusive homologous chromosome pairing. This diploid-like meiotic behavior exhibited by many allopolyploids confers genome stability and organismal fertility, yet it may constrain evolvability due to the chromosomal homogeneity of offspring, especially during the initial stages of nascent allopolyploidy. HE was first systematically studied in Brassica synthetic allotetraploids using DNA markers [33,75], and proposed as the root cause directly or indirectly underlying rapid genomic and gene expression changes, as well as phenotypic novelty widely reported in nascent plant allopolyploids [34]. Many established allopolyploid species have genomes that have undergone HES, as evidenced by a large number of recently studied allopolyploid crops and wild species [29,31,35–38,76–79]. In all cases in which the consequences of HES have been studied, they have been found to alter gene expression and/or phenotypes, suggesting that HE is a powerful force for generating diversity in allopolyploidy [34]. Notably, however, all prior studies at genome-scale are on established species, thus the direct effects of HE cannot be de-convoluted from confounding effects of additional evolutionary forces. Here, by genome re-sequencing of 202 newly synthesized rice tetraploids of pure parental lines of subspecies, which were only four generations old, we unequivocally show that the process of HE can rapidly generate an enormous amount of genomic variation due to HE among derived lines, each of which is unique and carries homoeologs from the two parents that either have become fixed (0:4 or 4:0) or which will continue to segregate in progenies until they ultimately reach fixation. Thus, HE is a mutagenic mechanism with dual properties, one that generates a massive amount of potentially relevant phenotypic variation following the reunion of two diverged genomes in a common nucleus, but which also will subside (via selfing) as derivative lineages become homozygous for alternative and highly variable suites of homoeologs.
We show that the HEs are genomically widespread but heterogeneous within and among chromosomes, largely but not wholly in line with previous work on the distribution of homologous meiotic recombination [51,52]. Using copy number-dependent homoeolog expression as a foundation, a GWAS identified outlier loci that harbor large-effect known-function genes that are causally associated with the phenotypic traits. We further uncovered that these genes exerted their phenotypic impacts via all possible effects, additive, dominant and epistatic, suggesting their functional connectivity in determining quantitative traits [55]. Most importantly, we demonstrate that the genomic diversity among the S4 lineages has numerous phenotypic consequences, some of which involve traits that could be highly visible to natural (such as flowering time) and human (such as seed size) selections [33,75]. An added dimension to this discovery is that segregating tetraploid plants often exhibited phenotypes that are transgressive relative to the two parents, further increasing the net phenotypic space that might be ‘genomically explored’ during the early stages of an allopolyploid radiation. One can readily envision how selection might shape this diversity in response to varying ecological conditions or changing environments, leading to HE-mediated phenotypic and ultimately taxonomic diversification in allopolyploid lineages as they spread in time and space. HEs thus comprise one powerful means by which allopolyploidy is a creative force for generating biodiversity [34].

One novel aspect of our results concerns the demonstration that cytonuclear co-evolutionary divergence among progenitor diploids may have evolutionary consequences in their derivative allopolyploids, thus adding to our growing appreciation of the cytonuclear dimension of allopolyploid evolution [80]. We show that HE-mediated changes in homo-cytonuclear combinations are preferentially retained reciprocal in the tetraploids, as expected. Unexpectedly, however, large numbers of hetero-cytonuclear combinations were also favorably retained either reciprocally or unidirectionally. While the full scope of the phenotypic consequences of this novel form of cytonuclear selection remain to be studied, it seems clear that this form of interaction may also be important to the evolution of young allopolyploid lineages. Exploring the functional nature of these HE-mediated cytonuclear combinations will likely be a fruitful avenue of future exploration.

In sum, our study documents rapid transgenerational precipitation of extraordinary population genomic heterogeneity subsequent to genome admixture mediated by HE in synthetic plant tetraploids. The extensive yet individualized genomic mosaicism generates wide-ranging population-level phenotypic diversity. Remarkably, much of the phenotypic variation can be readily explained by HE-mediated homoeolog copy number alteration and interaction of large-effect known-function genes. We reveal that cytonuclear interaction, including both homo- and hetero-combinations, is an important constraint underpinning genomic composition of the tetraploids. Our genome-scale and sequence level results demonstrate how HE can be a potent mechanism to rapidly augment the genotypic and phenotypic space of newly formed allopolyploids even parented by pure lines, which provides novel insights into evolvability of nascent allopolyploidy, and bears implications for the rapid generation of genetic and biological diversity of potential contemporary utility.

CONCLUSION

Classical genetic theory predicts that a solo allopolyploidization event may lead to genetic depauperation due to founder-effect and diploid-like meiotic behavior, and hence is likely maladaptive. This tenet has been refuted by the vast genomic data and our enhanced understanding of polyploid genome evolution. The present study shows that when hybridizing parents are of moderate genetic divergence, allopolyploidization represents a highly permissive arena to enable HE as a major player that catalyzes rampant reshuffling of parental genomes whereby both genotypic and phenotypic space can be massively enlarged. This study provides novel insights with respect to how evolvability of nascent allopolyploidy can be boosted by HE, which also bears implications for translational evolutionary biology for rapid generation of potentially useful biodiversity.

METHODS

Plant materials and phenotyping

The rice allotetraploids (NN99 and 99NN) were generated by colchicine treatment of tillers of the reciprocal F1 hybrids (N9 and 9N) between pure line cultivars Nipponbare and 93-11, representing japonica and indica subspecies of O. sativa L. [40]. We term these segmental allopolyploids because of their patterns of chromosomal pairing and divergence, using multiple criteria [39,45,81]. The reciprocal tetraploids used in this study were from colchicine-doubled S0 tetraploids from one tiller of one F1 hybrid individual of each crossing direction, and then selfed for four successive generations, which contained 202 euploid individuals (Supplementary Fig. S1 and Dataset S1). In total, 21 quantitative traits were phenotyped on plants grown in
season under paddy-field conditions following standard methods [82]. Details of the morphological data comparisons and statistics are described in Supplementary Materials and Methods.

**Dual-color Oligo-FISH**

Two sets of rice oligo libraries were labeled with FAM-green and Texas red using a direct labeling protocol [49]. FISH was performed as reported [83]. Slides were examined under an Olympus fluorescence microscope and digitally photographed.

**DNA and RNA extraction, sequencing and data analyses**

Leaves were used for DNA extraction and whole-genome re-sequencing, and both leaves and roots were used for RNA extraction and RNA-seq. Library construction and sequencing were performed by standard Illumina protocols. Detailed information of nucleic acid extraction, sequencing procedure and preliminary data analyses are described in Supplementary Materials and Methods.

**Bioinformatic analysis**

Single nucleotide polymorphism (SNP)-based methods were used to determine the genomic compositions and HE loci in all S4 (n = 202) and S5 (n = 45) tetraploids. HE differences among and within chromosomes were tested by using corresponding statistical approaches. Homoeologous transcript ratios between Nipponbare and 93-11 for each gene in two tissues were quantified for homoeologous expression analysis. TargetP [84] (version 2.0) and LOCALIZER [85] (version 1.0.4) were used for genome-wide identification of cytonuclear molecular interaction genes. The online PANTHER 15.0 platform (http://www.pantherdb.org/) was used for GO analysis. KEGG analysis was conducted by the Clusterprofiler package [86] in R program (version 3.4.3, 13). Detailed analysis procedures are described in Supplementary Materials and Methods.

**Genome-wide association study**

Existing GWAS pipelines are primarily designed for diploid populations with genome-wide SNPs as the genetic variable to identify the causal locus or loci for a given phenotypic trait. By contrast, in the GWAS of our tetraploid population, the genetic variable is the HE-mediated homoeolog copy number variation (HCNV) of chromosomal segments that harbor large-effect genes with known functions. As a segregating, self-propagating population (S4), there exist five states of HCNV (N : 9 = 0 : 4, 1 : 3, 2 : 2, 3 : 1 and 4 : 0 for a given locus) in the rice allotetraploid populations. Accordingly, the FarmCPU method of GWAS was used [59]. Detailed analysis procedures for GWAS are described in Supplementary Materials and Methods.

**Analysis of epistasis**

Pairwise interaction between loci containing trait-determining genes were analyzed by F∞ model [87]. Detailed analysis procedures for epistasis analysis are described in Supplementary Materials and Methods.

**Statistics**

All statistical tests in this study were performed using basic packages in R (Version 3.6.1, https://www.r-project.org).

**DATA AVAILABILITY**

Clean data for all genome re-sequencing and RNA-seq generated in this study have been deposited in the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) with the accession code PRJNA678613, and the scripts used for data analysis are available at https://github.com/wuying003/HE-identification-for-allotetraploid-rice.

**SUPPLEMENTARY DATA**

Supplementary data are available at NSR online.

**ACKNOWLEDGEMENTS**

We thank Drs Bin Han and Xuehui Huang (Biological Institutes, Chinese Academy of Sciences) for constructive advice in the GWAS, and Dr Zhiyun Gong (Yangzhou University) for providing the oligo-FISH probes.

**FUNDING**

This work was supported by the National Key Research and Development Program of China (2016YFD0102003 to B.L.), the National Natural Science Foundation of China (91131000 to B.L and 31900197 to Y.W.), the China Postdoctoral Science Foundation (2018M631853 and 2019T120228 to Y.W.) and the US National Science Foundation (to J.F.W.).
AUTHOR CONTRIBUTIONS

B.L., Z.W.Z., L.G. and J.F.W. conceived and designed the study. Y.W., B.L. and J.F.W. wrote the manuscript, with input from all authors. Y.W., J.W., S.S., B.W., G.L., X.Y.L. and Y.S. performed field work and laboratory experiments. Y.W., F.L., Y.Z., Z.B.Z., X.T.W., Q.L.D., C.M.X., L.G. and Z.W.Z. performed the analyses. All authors read and approved the final manuscript.

Conflict of interest statement. None declared.

REFERENCES

1. Runemark A, Vallejomarin M and Meier Ji et al. Eukaryote hybrid genomes. PLoS Genet 2019; 15: e1008404.
2. Soltis PS and Soltis DE. The role of hybridization in plant speciation. Annu Rev Plant Biol 2009; 60: 561–88.
3. Mallet J. Hybrid speciation. Nature 2007; 446: 279–83.
4. Abbott RJ, Albach DC and Ansell SW et al. Hybridization and speciation. J Evol Biol 2013; 26: 229–46.
5. Lamichhaney S, Han F and Webster MT et al. Rapid hybrid speciation in Darwin’s finches. Science 2018; 359: 224–8.
6. Marques DA, Meier Ji and Seehausen O et al. A combinatorial view on speciation and adaptive radiation. Trends Ecol Evol 2019; 34: 531–44.
7. Jiao Y, Wickett NJ and Ayasampalayam S et al. Ancestral polyploidy in seed plants and angiosperms. Nature 2011; 473: 97–100.
8. De Peer YV, Mizrahi E and Marchal K et al. The evolutionary significance of polyploidy. Nat Rev Genet 2017; 18: 411–24.
9. Wendel JF. The wondrous cycles of polyploidy in plants. Am J Bot 2015; 102: 1753–6.
10. Baniaga AE, Marx HE and Arrigo N et al. Polyploid plants have faster rates of multivariate niche differentiation than their diploid relatives. Ecol Lett 2020; 23: 68–78.
11. Huang HR, Liu JJ and Xu Y et al. Homoeologue-specific expression divergence in the recently formed tetraploid Capsella bursa-pastoris (Brassicaceae). New Phytol 2018; 220: 624–35.
12. Wu CI. The genic view of the process of speciation. J Evol Biol 2001; 14: 851–65.
13. Bombles K, Higgins JD and Yant L et al. Meiosis evolves: adaptation to internal and external environments. New Phytol 2015; 206: 306–23.
14. Gonzalo A, Lucas M and Charpentier C et al. Reducing MSH4 copy number prevents meiotic crossovers between non-homologous chromosomes in Brassica napus. Nat Commun 2019; 10: 1–9.
15. Marburger S, Monnahan PJ and Seear PJ et al. Interspecific introgression mediates adaptation to whole genome duplication. Nat Commun 2019; 10: 2354.
16. Soltis DE and Soltis PS. Polyploidy: recurrent formation and genome evolution. Trend Ecol Evol 1999; 14: 348–52.
17. Pigliucci M. Is evolvability evolvable? Nat Rev Genet 2008; 9: 75–82.
18. Comai L. The advantages and disadvantages of being polyploid. Nat Rev Genet 2005; 6: 836–46.
19. Levin DA and Soltis DE. Factors promoting polyploid persistence and diversification and limiting diploid speciation during the K-Pg interlude. Curr Opin Plant Biol 2018; 42: 1–7.
20. He Z, Li X and Yang M et al. Speciation with gene flow via cycles of isolation and migration: insights from multiple mangrove taxa. Nat Sci Rev 2019; 6: 275–88.
21. Cheng H, Liu J and Wen J et al. Frequent intra-and inter-species introgression shapes the landscape of genetic variation in bread wheat. Genome Biol 2019; 20: 1–16.
22. Han T-S, Wu Q and Hou H-X et al. Frequent introgressions from diploid species contribute to the adaptation of the tetraploid Shepherd’s purse (Capsella bursa-pastoria). Mol Plant 2015; 8: 427–38.
23. He F, Pasam RK and Shi F et al. Exome sequencing highlights the role of wild-relative introgression in shaping the adaptive landscape of the wheat genome. Nat Genet 2019; 51: 896–904.
24. Doyle JJ and Coate JE. Polyploidy, the nucletype, and novelty: the impact of genome doubling on the biology of the cell. Int J Plant Sci 2019; 180: 1–52.
25. Song Q and Chen ZJ. Epigenetic and developmental regulation in plant polyploids. Curr Opin Plant Biol 2015; 24: 101–9.
26. Yoo M, Liu X and Pires JC et al. Nonadditive gene expression in polyploids. Annu Rev Genet 2014; 48: 485–517.
27. Liu S, Luo J and Chai J et al. Genomic incompatibilities in the diploid and tetraploid offspring of the goldfish × common carp cross. Proc Natl Acad Sci USA 2016; 113: 1327–32.
28. Luo J, Chai J and Chai J et al. From asymmetrical to balanced genomic diversification during rediploidization: subgenomic evolution in allotetraploid fish. Sci Adv 2020; 6: eAAZ7677.
29. Chester M, Gallagher JP and Symonds VV et al. Extensive chromosomal variation in a recently formed natural allotetraploid species, Tragopogon miscellus (Asteraceae). Proc Natl Acad Sci USA 2012; 109: 1176–81.
30. Edger PP, Poorten TJ and Vanburen R et al. Origin and evolution of the octoploid strawberry genome. Nat Genet 2019; 51: 541–7.
31. Hurbolin B, Golitz AA and Bayer PE et al. Homoeologous exchange is a major cause of gene presence/absence variation in the amphiploid Brassica napus. Plant Biotechnol J 2018; 16: 1265–74.
32. Lloyd A, Blary A and Charif D et al. Homoeologous exchanges cause extensive dosage-dependent gene expression changes in an allopolyploid crop. New Phytol 2018; 221: 367–77.
33. Gaeta RT, Pires JC and Iniguezuy FL et al. Genetic changes in resynthesized Brassica napus and their effect on gene expression and phenotype. Plant Cell 2007; 19: 3403–17.
34. Gaeta RT and Pires JC. Homoeologous recombination in allopolyploids: the polyploid ratchet. New Phytol 2010; 186: 18–28.
35. Bertoloni DJ, Jenkins J and Clevenger J et al. The genome sequence of segmental allotetraploid peanut Arachis hypogaea. Nat Genet 2019; 51: 877–84.
36. Chalhoub B, Liu S and Parkin IA et al. Early allopolyploid evolution in the post-Neolithic Brassica napus oilseed genome. Science 2014; 345: 950–3.
37. Novikova PY, Tsuchimatsu T and Simon S et al. Genome sequencing reveals the origin of the allotetraploid *Arabidopsis suecica*. *Mol Biol Evol* 2017; 34: 957–68.

38. Wang Z, Miao H and Liu J et al. *Musa balbisiana* genome reveals subgenome evolution and functional divergence. *Nat Plant* 2019; 5: 810–21.

39. Mason AS and Wendel JF. Homoeologous exchanges, segmental allopolyploidy, and polyploid genome evolution. *Front Genet* 2020; 11: 1014.

40. Xu CM, Bai Y and Lin XY et al. Genome-wide disruption of gene expression in allopolyploids but not hybrids of rice subspecies. *Mol Biol Evol* 2014; 31: 1066–76.

41. Sun Y, Wu Y and Yang CW et al. Segmental allotetraploidy generates extensive homoeologous expression rewiring and phenotypic diversity at the population level in rice. *Mol Ecol* 2017; 26: 5451–66.

42. Zhang ZB, Fu TS and Liu ZJ et al. Extensive changes in gene expression and alternative splicing due to homoeologous exchange in rice segmental allopolyploids. *Theor Appl Genet* 2019; 139: 2295–308.

43. Li N, Xu CM and Zhang A et al. DNA methylation repatterning accompanying hybridization, whole genome doubling and homoeolog exchange in nascent segmental rice allotetraploids. *New Phytol* 2019; 223: 979–92.

44. Spoelhof JP, Soltis PS and Soltis DE. Pure polyploidy: closing the gaps in autopolyploid research. *J Syst Evol* 2017; 55: 340–52.

45. Stebbins GL. Types of polyploids: their classification and significance. *Adv Genet* 1947; 1: 403–29.

46. Ouyang Y, Liu Y-G and Zhang Q. Hybrid sterility in plant: stories from rice. *Curr Opin Plant Biol* 2010; 13: 186–92.

47. Zhu Q and Ge S. Phylogenetic relationships among A-genome species of the genus *Oryza* revealed by intron sequences of four nuclear genes. *New Phytol* 2005; 167: 249–65.

48. Choi JY, Platts AE and Fuller DQ et al. The rice paradox: multiple origins but single domestication in Asian Rice. *Mol Biol Evol* 2017; 34: 969–79.

49. Liu XY, Sun S and Wu Y et al. Dual-color oligo-FISH can reveal chromosomal variations and evolution in *Oryza* species. *Plant J* 2020; 101: 112–21.

50. Zhang ZB, Gou XW and Xun HW et al. Homoeologous exchanges occur through intragenic recombination generating novel transcripts and proteins in wheat and other polyploids. *Proc Natl Acad Sci USA* 2020; 117: 14561–71.

51. Wang Y and Copenhagen GP. Meiotic recombination: mixing it up in plants. *Annu Rev Plant Biol* 2018; 69: 577–609.

52. Zielkowski M, Olson MA and Wang M et al. Diversity and determinants of meiotic recombination landscapes. *Trends Genet* 2019; 35: 359–70.

53. Chen M, Presting GG and Barbazuk WB et al. An integrated physical and genetic map of the rice genome. *Cell* 2002; 113: 457–95.

54. Si W, Yuan Y and Huang J et al. Widely distributed hot and cold spots in meiotic recombination as shown by the sequencing of rice F2 plants. *New Phytol* 2015; 206: 1491–502.

55. Birchler JA and Veitia RA. The gene balance hypothesis: implications for gene regulation, quantitative traits and evolution. *New Phytol* 2010; 186: 54–62.

56. Birchler JA and Veitia RA. Genomic balance and speciation. *Epigenet Insight* 2019; 12: 2518685718840291.

57. Hou J, Shi X and Chen C et al. Global impacts of chromosomal imbalance on gene expression in *Arabidopsis* and other taxa. *Proc Natl Acad Sci USA* 2018; 115: 11321–30.

58. Jolylopez Z, Platts AE and Gulko B et al. An inferred fitness consequence map of the rice genome. *Nat Plants* 2020; 6: 119–30.

59. Liu X, Huang M and Fan B et al. Iterative usage of fixed and random effect models for powerful and efficient genome-wide association studies. *PLoS Genet* 2016; 12: e1005767.

60. Mao H, Sun S and Yao J et al. Linking differential domain functions of the GS3 protein to natural variation of grain size in rice. *Proc Natl Acad Sci USA* 2010; 107: 9579–84.

61. Jiang J, Tan L and Zhu Z et al. Molecular evolution of the TAC1 gene from rice (*Oryza sativa* L.). *J Genet Genomics* 2012; 39: 551–60.

62. Yu B, Lin Z and Li H et al. TAC1, a major quantitative trait locus controlling tiller angle in rice. *Plant J* 2007; 52: 891–8.

63. Qi J, Qian Q and Bu Q et al. Mutation of the rice Narrow leaf1 gene, which encodes a novel protein, affects vein patterning and polar auxin transport. *Plant Physiol* 2008; 147: 1947–59.

64. Zhang G-H, Li S and Wang L et al. *LSCHL4* from japonica cultivar, which is allelic to *NAL1*, increases yield of indica super rice 93-11. *Mol Plant* 2014; 7: 1350–64.

65. Koo B, Yoo S and Park J et al. Natural variation in *OsPRA37* regulates heading date and contributes to rice cultivation at a wide range of latitudes. *Mol Plant* 2013; 6: 1877–88.

66. Khan A, Dinakar M and Schneider D et al. Cooper, negative epistasis between beneficial mutations in an evolving bacterial population. *Science* 2011; 332: 1193–6.

67. Phillips PC. Epistasis-the essential role of gene interactions in the structure and evolution of genetic systems. *Nat Rev Genet* 2008; 9: 855–67.

68. Sanjuan R and Elena S. Epistasis correlates to genomic complexity. *Proc Natl Acad Sci USA* 2006; 103: 14402–5.

69. Blischak PD, Mabry ME and Conant GC et al. Integrating networks, phylogenomics, and population genomics for the study of polyploidy. *Annu Rev Ecol Evol Syst* 2018; 49: 253–78.

70. Schwager EE, Sharma PP and Clarke TH et al. The house spider genome reveals an ancient whole-genome duplication during arachnid evolution. *BMC Biol* 2017; 15: 1–27.

71. Spoelhof JP, Kefefe R and McDaniel SF. Does reproductive assurance explain the incidence of polyploidy in plants and animals? *New Phytol* 2019; 227: 14–21.

72. Leitch A and Leitch I. Genomic plasticity and the diversity of polyploid plants. *Science* 2008; 320: 481–3.

73. Griffiths S, Sharp R and Foote T et al. Molecular characterization of *Ph* as a major chromosome pairing locus in polyploid wheat. *Nature* 2006; 439: 749–52.

74. Jenczewski E, Eber F and Grimaud A et al. *LSCHL4* encodes a novel protein, affects vein patterning and polar auxin transport. *Mol Genet Genomics* 2013; 288: 163–76.

75. Keller WP, Kieffer K and Wang L et al. Characterization of an Acrl homoeologous gene family in Arabidopsis. *Science* 2007; 315: 481–3.

76. Mogami H et al. The genome sequence of the allotetraploid *Arabidopsis suecica*. *Nat Genet* 2012; 44: 1287–9.

77. Xiong Z, Gaeta RT and Pires JC. Homoeologous shuffling and chromosomal rearrangements in Brassica polyploids. *Mol Gen Genomics* 2007; 277: 585–91.

78. Schwager EE, Sharma PP and Clarke TH et al. The house spider genome reveals an ancient whole-genome duplication during arachnid evolution. *BMC Biol* 2017; 15: 1–27.

79. Bertioli DJ, Jenkins J and Clevenger J et al. The role of genomic structural rearrangements derived from homoeologous recombination following allopolyploidy speciation in coffee. *Plant J* 2014; 78: 674–85.

80. Xiong Z, Gaeta RT and Pires JC. Homoeologous shuffling and chromosome compensation maintain genome balance in resynthesized allopolyploid *Brassica napus*. *Proc Natl Acad Sci USA* 2011; 108: 7906–13.

81. Schiestl R, Katcher E and Thien E et al. The role of genomic structural variation in the genetic improvement of polyploid crops. *Crop J* 2017; 1: 127–40.

82. Bertoli DJ, Jenkins J and Cleveingar J et al. The genome sequence of segmental allotetraploid peanut *Arachis hypogaea*. *Nat Genet* 2019; 51: 877–84.
80. Sharbrough J, Conover JL and Tate JA et al. Cytonuclear responses to genome doubling. *Am J Bot* 2017; **104**: 1277–80.

81. Koide Y, Kuniyoshi D and Kishima Y. Fertile tetraploids: new resources for future rice breeding? *Front Genet* 2020; **11**: 1231.

82. Wu Y, Sun Y and Sun S et al. Aneuploidization under segmental allotetraploidy in rice and its phenotypic manifestation. *Theor Appl Genet* 2018; **131**: 1273–85.

83. Zhang HK, Bian Y and Gou XW et al. Persistent whole-chromosome aneuploidy is generally associated with nascent allohexaploid wheat. *Proc Natl Acad Sci USA* 2013; **110**: 3447–52.

84. Armenteros JA, Salvatore M and Emanuelsson O et al. Detecting sequence signals in targeting peptides using deep learning. *Life Sci* 2019; **2**: e201900429.

85. Sperschneider J, Catanzariti A and Deboer KD et al. LOCALIZER: subcellular localization prediction of both plant and effector proteins in the plant cell. *Sci Rep* 2017; **7**: 1–14.

86. Yu G, Wang LG and Han Y. ClusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS* 2012; **16**: 284–7.

87. Zeng Z, Wang T and Zou W. Modeling quantitative trait loci and interpretation of models. *Genetics* 2005; **169**: 1711–25.