The characteristics of mRNA m^6A methylomes in allopolyploid 
*Brassica napus* and its diploid progenitors

The mRNA m^6A methylome in *Brassica napus*

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Abstract

Genome duplication events, comprising whole genome duplication and single-gene duplication, produce a complex genomic context leading to multiple levels of genetic changes. However, the characteristics of m^6A modification, the most widespread internal eukaryotic mRNA modification, in polyploid species are still poorly understood. This study revealed the characteristics of m^6A methylomes within the early formation and following evolution of allopolyploid *Brassica napus*. We found the complex relationship between m^6A modification abundance and gene expression level depending on degree of enrichment or presence/absence of m^6A modification. Overall, the m^6A genes had lower gene expression levels than the non-m^6A genes. Allopolyploidization may change the expression divergence of duplicated gene pairs with identical m^6A patterns and diverged m^6A patterns. Compared with duplicated genes, the singletons with a higher evolutionary rate exhibited higher m^6A modification. Five kinds of duplicated genes exhibited distinct distributions of m^6A modifications at the transcript and gene expression. In particular, tandem duplication-derived genes showed unique m^6A modification enrichment around the transcript start site. Active histone modifications (H3K27me3 and H3K4me3) but not DNA methylation were enriched around genes of m^6A peaks. These findings provide a new understanding of the features of m^6A modification and gene expression regulation in allopolyploid plants with sophisticated genomic architecture.

Introduction

More than 70% of angiosperms have experienced at least one polyploidization event in their evolutionary lineages [1]. Compared with diploid progenitors, polyploids have physiological and phenotypic adaptive advantage [2]. Polyploidization causes
multilevel genetic changes, including genetic composition and gene expression, increasing adaptability through the formation of new regulatory pathways [3]. Depending on whether the genetic progenitors are the same species, polyploidy gives rise to autopolyploid or allopolyploid [1]. As the most common form of polyploidy, allopolyploids played an essential role in the evolutionary adaptation of plant species [4]. Although whole-genome duplication (WGD) provides numerous raw materials for the evolution of plants, WGD is episodic, and successive WGD events are spaced dozens of millions of years apart [5]. Therefore, plants need several kinds of single-gene duplication, including tandem duplication (TD), transposed duplication (TRD), dispersed duplication (DSD) and proximal duplication (PD), to continuously supply evolutionary material for environmental adaptation [5]. Many duplicated genes generated from duplication events lost partners and remained as singletons during evolution [6]. How plants distinguish genes produced by different mechanisms within the same content is an intriguing question. There has been evidence that duplicated genes undergo different purifying selection, which is associated with gene expression, posttranscriptional regulation and DNA methylation [7, 8]. These duplication events and the high retention rate of existing duplicated gene pairs have contributed to the abundance of duplicated genes, enhancing plant adaptations, such as disease resistance and adaptability to adversity [6, 8]. The molecular mechanisms involved in plant adaptations, such as transcriptome changes, DNA methylation, histone modification, and the m^6^A epitranscriptome, have been reviewed [3, 9-12]. However, how m^6^A modifications of multiple duplicated genes change during the formation and evolution of polyploid plants has yet to be delineated.

RNA molecules, which may be modified by a variety of chemical modifications [9], act as genetic information carriers, linking DNA to proteins and regulating
multifarious biological processes [13]. N\(^6\)-methyladenosine (m\(^6\)A), which is the most widespread inside messenger RNA (mRNA) modification, has been found to account for 50% of methylated nucleotides in polyadenylated mRNA in eukaryotes [14]. The dynamic and reversible m\(^6\)A modification is regulated by writers (RNA methyltransferases, including METTL3, METTL4, WTAP, VIRMA, HAKAI and so on) [15-19], erasers (RNA demethylases, comprising FTO and ALKBH5) [20, 21] and readers (RNA-binding proteins that identify and combine m\(^6\)A markers, including YTH domain family proteins) [22]. The putative m\(^6\)A regulatory machineries were recently described in 22 plant species [9]. Loss of function of components of regulatory machineries leads to early embryonic lethality [18]. Due to advances in transcriptome-wide m\(^6\)A sequencing and mapping technology [23], m\(^6\)A modification has been demonstrated to have pivotal roles in biological and developmental processes of plants, such as embryo development, shoot apical meristem development, fruit ripening, enhanced resistance, and response to stresses [15, 18, 24-27]. Yu et al. found that transgenic expression of human FTO in rice and potato mediated substantial m\(^6\)A demethylation and increased yield and biomass by approximately 50%, which demonstrating the value of modulating m\(^6\)A modifications in plant breeding [28]. Cheng et al. revealed that m\(^6\)A modifications promote the biosynthesis of auxin to guarantee male meiosis and fertility in rice [29]. Duan et al. demonstrated that mRNA demethylation mediated by ALKBH20B affects floral transition in Arabidopsis [30]. A recent study established transcriptome-wide m\(^6\)A methylomes of 13 representative plants and revealed the conservation and diversity of m\(^6\)A modifications in plants [31]. In paleo-polyploid maize, genes carrying m\(^6\)A peaks in transcripts exhibit biased subgenome fractionation, which is related to multiple sequence features and asymmetric evolutionary rates [6]. However, the characteristics
of m\(^6\)A methylomes are still unknown in plant polyploidization and subsequent evolutionary processes.

As a prominent economic oil crop, *Brassica napus* L. (2n = 4x = 38, AACC) was shaped by the hybridization and subsequent WGD of *B. oleracea* (2n = 18, CC) and *B. rapa* (2n = 20, AA) approximately 7,500 years ago [32]. These species serve as an ideal system for revealing genomic features and gene expression associated with polyploidization [11, 33]. However, the features of posttranscriptional RNA modifications in the shaping and subsequent evolution of *B. napus* are still obscure. Here, transcriptome-wide RNA N\(^6\)-methyladenosine (m\(^6\)A) of natural *B. napus*, resynthesized *B. napus* and its parents, *B. oleracea* and *B. rapa*, were investigated. The relationship of distinct m\(^6\)A modifications and differences in gene expression, and the distribution features of four epigenetic markers (H3K4me3, H3K27ac, H3K27me3 and DNA methylation) and m\(^6\)A modification of different types of genes were comprehensively analyzed. Information on transcriptome-wide m\(^6\)A modification and gene expression will provide essential resources for studying the molecular regulatory basis in *B. napus* and other polyploid plants.

**Results**

**Distribution characteristics of m\(^6\)A in four genotypes**

Twenty-four m\(^6\)A-immunoprecipitation (IP) and the corresponding non-IP control (input) libraries were constructed and sequenced, comprising natural *B. napus* (NAC), resynthesized *B. napus* (RAC), *B. oleracea* (C) and *B. rapa* (A) (Table S1), with three independent biological replicates for each genotype. There were high Spearman’s correlation coefficients (no less than 0.89) among biological replicates (Supporting Information Supplemental Figure S1, S2). The libraries obtained 36–59 million raw
reads (Table S1), which is comparable with the sequencing depth in studies of m^6A in tomato (20–30 million reads) [25], and strawberry (24–37 million reads) [27]. Approximately 99.99% of raw reads were clean reads after removing adaptor trimming and low-quality reads, and 61.75% – 90.44% of clean reads were mapped to the B. napus genome, indicating high alignment quality (Table S1). We identified 5,860–16,194 m^6A peaks within 5,570–14,908 gene transcripts using the MACS peak-calling algorithm in leaf tissue in four genotypes (Table S1).

To investigate the distribution feature of m^6A modifications in the whole transcriptome, the transcript was divided into five contiguous segments: 3’ untranslated region (UTR), stop codon (200 bp centered on the translational stop sites, stopC), coding sequence (CDS), start codon (200 bp centered on the translational start codons, startC) and 5’ UTR. As shown in Figure 1a, m^6A modifications in all genotypes were highly enriched near the stopC. The pie charts show that stopC regions were enriched exceeding 60% of the m^6A peaks; approximately 20% of the m^6A peaks were located in the start codon; and 14.58% to 16.85% of the m^6A peaks were positioned in the CDS of the four genotypes (Figure 1b). The relative m^6A enrichment analysis showed that m^6A modifications were mainly enriched in the stopC (Figure 1c). Thus, the transcriptome-wide mapping of m^6A modifications showed a conserved m^6A distribution pattern in B. napus and diploid progenitors.
Figure 1 Dynamic distribution feature of m$^6$A modification in leaves of A, C, RAC, and NAC. (a) Distribution of m$^6$A peak along transcripts. (b) The distribution of m$^6$A peaks in different transcript segments. (c) Relative enrichment of m$^6$A peaks in different transcript segments. UTR, untranslated region; CDS, coding sequence; A, B. rapa; C, B. oleracea; RAC, resynthesized B. napus; NAC, natural B. napus.

To identify the relationship between gene sequence features and m$^6$A modification, we performed a statistical analysis using gene sequence features (length of gene, exon, intron and number of exon) from m$^6$A genes (genes carrying m$^6$A peaks on transcripts) and non-m$^6$A genes (genes without m$^6$A on transcripts). In the four genotypes, the
lengths of genes, exons and introns were significantly longer in m^6A genes than in non-m^6A genes (P < 0.05) (Supplemental Figure S4a-c, S5a-c, S6a-c and S7a-c), indicating that the transcripts from longer genes were more likely to be modified with m^6A. The same results were also observed in maize [6]. Moreover, m^6A genes had significantly more exons than non-m^6A genes in *Brassica* plants (P < 0.05) (Supplemental Figure S4d, S5d, S6d and S7d), as seen in maize [6]. In general, these observations indicated that m^6A modification is related to gene sequence characteristics comprising length and exon number in plants.

**m^6A modifications abundance and gene expression levels in the four genotypes**

To investigate the characteristics of m^6A modification in the shaping and subsequent evolution of *B. napus*, a differential methylation level analysis of transcripts among the genotypes was performed (Figure 2a). Compared with its progenitors, 5,688 m^6A peaks were hypomethylated, whereas only 2,864 m^6A peaks were hypermethylated in resynthesized *B. napus* (Figure 2b and e). Compared with resynthesized *B. napus*, 1,795 hypomethylated m^6A peaks and 4,512 hypermethylated m^6A peaks were identified in natural *B. napus* (Figure 2d and g). Compared with diploid progenitors, a total of 6,009 hypomethylated m^6A peaks and 4,956 hypermethylated m^6A peaks were found in natural *B. napus* (Figure 2c and f). These results indicated the remarkable differences of m^6A methylome in the four genotypes. Gene ontology (GO) enrichment analysis was performed to explore the potential roles of genes whose transcripts comprised differential m^6A modifications (DMGs). DMGs between resynthesized *B. napus* and its progenitors were related to ‘macromolecule modification’, ‘cellular process’, and ‘RNA processing’, whereas DMGs between natural *B. napus* and its progenitors were involved in ‘gene expression’, ‘translation’ and ‘cellular amide
metabolic process’ (Table S2). In the comparison between resynthesized B. napus and natural B. napus, DMGs were highly enriched in ‘metabolic process’, ‘small molecule biosynthetic process’ and ‘cytoplasmic translation’ (Table S2). Interestingly, hypomethylated mRNAs were more likely to be down-regulated, whereas more hypermethylated mRNAs appeared to be up-regulated (Figure 2b-g). Notably, GO analysis of these significantly differentially expressed genes showed that they were involved in the ‘cytoplasm’.
Figure 2 Statistic of differentially methylated peaks and relation between m^6A modification and gene expression level in B. napus and diploid progenitors. (a) Differentially methylated peaks in different comparisons. (b-g) Volcano plots showing ratios of gene expression level of transcripts comprising (b) Hypomethylated mRNA in RAC vs. A, C. (c) Hypomethylated mRNA in NAC vs. A, C. (d) Hypomethylated mRNA in NAC vs. RAC. (e) Hypermethylated mRNA in RAC vs. A, C. (f) Hypermethylated mRNA in NAC vs. A, C. (g) Hypermethylated mRNA in NAC vs. RAC. Red dots represented significantly down-regulated mRNA; Purple dots were significantly up-regulated mRNA; Green dots indicated mRNA with no significantly changed; A, B. rapa; C, B. oleracea; RAC, resynthesized B. napus; NAC, natural B. napus.
Combined with the transcriptome results, we performed a statistical analysis of the \( m^6A \) modification of genes expressed in the leaves of four genotypes. The percentage of the \( m^6A \) gene was significantly higher in \( B. \ rapa \) than in \( B. \ oleracea \) (Supplemental Figure S8a). Although the overall proportion of the \( m^6A \) gene in resynthesized \( B. \ napus \) did not change significantly compared with progenitors, the difference between the subgenomes \( A_n \) and \( C_n \) diminished. On the other hand, the proportion of the \( m^6A \) gene in each subgenome of natural \( B. \ napus \) was notably increased. These results suggested that hybridization and WGD reduced the difference in the proportion of \( m^6A \) genes between subgenomes of resynthesized \( B. \ napus \). Compared with resynthesized \( B. \ napus \), \( m^6A \) genes in both subgenomes increased in natural \( B. \ napus \).

To explore the potential relationship between \( m^6A \) modification and gene expression levels, the mRNA abundance of gene transcripts exhibiting differential \( m^6A \) modification was compared. Compared with non-\( m^6A \) genes, \( m^6A \) genes showed significantly lower gene expression levels in all genotypes, indicating that the lower mRNA abundance of transcripts may be related to \( m^6A \) modifications (Supplemental Figure S8b).

We then compared the evolutionary rates (\( \omega \)), calculated by dividing nonsynonymous substitution (\( Ka \)) by synonymous sites (\( Ks \)), of \( m^6A \) genes and non-\( m^6A \) genes of \( B. \ napus \) and diploid progenitors (Table S3). The evolutionary rates were analyzed employing interspecific putatively orthologous sequences between \( B. \ napus \) and Arabidopsis. The \( m^6A \) genes had notably higher \( \omega \) values than non-\( m^6A \) genes in progenitors, which is consistent with diploid maize [6]. However, there was no significant difference in the \( \omega \) value between the two categories of genes in resynthesized \( B. \ napus \). Surprisingly, there was a reversal in the natural \( B. \ napus \): the
non-\( m^6A \) genes had notably higher \( \omega \) values than \( m^6A \) genes. These results indicated that the \( m^6A \) genes had higher evolutionary rates than non-\( m^6A \) genes in progenitors, but they were neutralized after hybridization and polyploidization and reversed during subsequent evolution.

To explore the differences in \( m^6A \) modification in the early shaping and subsequent evolution of \( B. napus \), we divided genes into four patterns (Table 1). The gene whose transcript was modified by \( m^6A \) in both genotypes in a comparison (e.g., resynthesized \( B. napus \) vs. progenitors) was designated as pattern I, and the gene whose transcript was not modified by \( m^6A \) in both genotypes was designated as pattern II. The gene whose transcript was modified in one genotype but not in another genotype was designated as pattern III, and the modified pattern in reverse was designated as pattern IV. We found no differences in \( m^6A \) patterns in more than 75% of gene transcripts in the three comparisons. Compared with progenitors, there was little difference in the proportion of patterns III and IV in resynthesized \( B. napus \).

Compared with natural \( B. napus \), the proportion of transcripts in pattern IV was significantly higher than that in pattern III, which was consistent with the previously described observation of increased \( m^6A \) genes of natural \( B. napus \) (Supplemental Figure S8a). These observations indicated that most mRNA \( m^6A \) modification patterns were maintained in the shaping and evolution of \( B. napus \).
Table 1. Statistics of transcripts in four patterns of m$^6$A modification differences.

<table>
<thead>
<tr>
<th>Pattern</th>
<th>m$^6$A modification differences</th>
<th>Numbers of transcripts (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A, C vs. RAC</td>
</tr>
<tr>
<td>I</td>
<td>m$^6$A→m$^6$A</td>
<td>8397(35.9)</td>
</tr>
<tr>
<td>II</td>
<td>non-m$^6$A→non-m$^6$A</td>
<td>11123(47.5)</td>
</tr>
<tr>
<td>III</td>
<td>m$^6$A→non-m$^6$A</td>
<td>2002(8.6)</td>
</tr>
<tr>
<td>IV</td>
<td>non-m$^6$A→m$^6$A</td>
<td>1875(8)</td>
</tr>
</tbody>
</table>


To explore the relationship of the presence or absence of m$^6$A modification with mRNA abundance, we analyzed the mRNA abundance of gene transcripts in patterns III and IV among genotypes. More transcripts with m$^6$A modification in progenitors but free of m$^6$A modification in resynthesized B. napus were up-regulated, whereas more transcripts without m$^6$A modification in progenitors but with m$^6$A modification in resynthesized B. napus were down-regulated (Figure 3a and d). These results reflected the negative relationship of m$^6$A modification and gene expression level.

Similar observations were also found in the comparison between natural B. napus and diploid progenitors (Figure 3b and e). However, more transcripts with reversed m$^6$A modification between resynthesized and natural B. napus were down-regulated (Figure 3c and f).
Figure 3 Gene expression level analysis of transcripts in pattern III and IV. (a) Transcripts in pattern III of A, C vs. RAC. (b) Transcripts in pattern III of A, C vs. NAC. (c) Transcripts in pattern III of NAC vs. RAC. (d) Transcripts in pattern IV of A, C vs. RAC. (e) Transcripts in pattern IV of A, C vs. NAC. (f) Transcripts in pattern IV of NAC vs. RAC. Green dots represented significantly down-regulated mRNA; Blue dots represented significantly up-regulated mRNA; Grey dots represented mRNA that no significantly changed; A, *B. rapa*; C, *B. oleracea*; RAC, resynthesized *B. napus*; NAC, natural *B. napus*.

The distribution characteristics of epigenetic modifications around genes of m^6^A peaks in four genotypes

Previous studies revealed that histone modification (H3K36me3) guides m^6^A RNA modification deposition co-transcriptionally in human and mouse, and showed an intimate linkage between H3K36me2 modification and m^6^A modification in Arabidopsis [35, 36]. To explore the distribution features of epigenetic modifications around genes of m^6^A peaks in *B. napus*, we investigated the distribution of histone modifications (H3K4me3, H3K27ac and H3K27me3) and DNA methylation around
genes of the m\(^6\)A peaks. We found that active histone modifications (H3K27ac and H3K4me3) were enriched around genes of m\(^6\)A peak center, whereas the repressive histone modification H3K27me3 was only slightly enriched around genes of the m\(^6\)A peak center compared with both flanks in all genotypes (Figure 4), which reflected the cooccurrence of H3K27ac and H3K4me3 modifications and m\(^6\)A modification. Moreover, CG, CHG, and CHH DNA methylation was depleted around genes of the m\(^6\)A peak center, and DNA methylation levels increased gradually with the distance from genes of the m\(^6\)A peak center (Figure 4). These results demonstrate that genes of the m\(^6\)A peaks were enriched of active histone modifications (H3K27ac and H3K4me3) but depleted of DNA methylation in *B. napus* and diploid progenitors.
Figure 4 The association between four epigenetic modifications and m^6A modification. The distribution of H3K27ac, H3K27me3, H3K4me3, and DNA methylation around m^6A peaks in B. rapa (a), B. oleracea (b), resynthesized B. napus (c), natural B. napus (d). center, m^6A peak center; A, B. rapa; C, B. oleracea; RAC, resynthesized B. napus; NAC, natural B. napus.

The singletons exhibited distinct distribution features of m^6A modification compared with the duplicated genes

A previous study revealed that genome duplication is a propensity of Brassica, in which diploid B. rapa and B. oleracea experienced an aggregate 36 × multiplication (3 × 2 × 2 × 3), and allopolyploid B. napus experienced an aggregate 72 × multiplication (3 × 2 × 2 × 3 × 2) [32]. A member of the duplicated gene pair produced by WGD may be lost (fractionated) during evolution, leaving the other one known as singleton. To explore the distribution of m^6A modification of singletons and
duplicated genes, a statistical analysis of the duplication status of the existing genes was performed. The proportions of singletons/duplicated genes of each genome/subgenome were relatively small (8.4% – 8.7%) (Figure 5a), indicating that most of the genes in each genome/subgenome still existed as duplicated genes, and there was no obvious biased gene fractionation among genomes/subgenomes. These results are different from those observed in maize, in which the proportion of singletons was higher than that of duplicated genes, and there was a significant biased gene fractionation between the two subgenomes (maize1 > maize2) [6]. These differences may be due to the higher frequency of WGD events and the timing of the last WGD was much more recent in *B. napus* than in maize [6, 32]. The proportion of the m^6^A gene of singletons was notably greater than that of duplicated genes in *B. rapa* and subgenome A^n^ of resynthesized *B. napus*, but not in subgenome A^n^ of natural *B. napus* (Figure 5a). There was no significant difference between the proportion of the m^6^A gene of singletons and duplicated genes of *B. oleracea* and subgenome C^n^ of resynthesized *B. napus*, but the proportion of the m^6^A gene of duplicated genes of subgenome C^n^ of natural *B. napus* was significantly higher (Figure 5a). These results indicated that the m^6^A modification of singletons and duplicated genes was different between the two diploid progenitors, and this difference was inherited after hybridization and WGD, but changed in the subsequent evolution process.
Figure 5 Proportion, gene expression level, metagenomic profiles of singletons and duplicated genes. (a) Proportion of different m^6A gene modified singletons and duplicated genes. (b) Gene expression level of singletons and duplicated genes. (c) Metagenomic profiles of m^6A modification on transcripts of singletons and duplicated genes. (d) Metagenomic profiles of H3K4me3 modification on singletons and duplicated genes. (e) Metagenomic profiles of H3K27ac modification on singletons and duplicated genes. A, B. rapa; C, B. oleracea; RAC, resynthesized B. napus; NAC, natural B. napus; Wilcoxon rank sum test was performed for statistical analysis. * P < 0.05 and *** P < 0.001.

To compare the features of m^6A modifications and gene expression levels of singletons and duplicated genes, we analyzed m^6A modifications and mRNA abundance among these transcripts. Although the m^6A modification of transcripts of both singletons and duplicated genes was highly enriched around the transcript start
site (TSS), the transcripts of singletons exhibited higher m^6^A modification than the duplicated genes in all genotypes (Figure 5c). Moreover, compared with progenitors, a smaller difference in m^6^A modification around TES and TSS between singletons and duplicated genes was found in B. napus. Interestingly, the gene expression of singletons was significantly lower than that of duplicated genes in progenitors, but there was no obvious difference in B. napus (Figure 5b). Then, we analyzed three histone modifications and DNA methylation among singletons and duplicated genes of four genotypes. As shown in Figure 5d and e and Supplemental Figure S9a, the repressive histone marker H3K27me3 was primarily distributed along the gene body between TSS and TES, while active histone markers (H3K4me3 and H3K27ac) were primarily located around the TSS. The singletons showed higher active histone markers except around TSS (Figure 5d and e), and higher repressive histone markers (H3K27me3) (Supplemental Figure S9a), and DNA methylation (Supplemental Figure S9b-d). These results reflected distinct distribution features of DNA methylation, H3K4me3, H3K27ac, H3K27me3 modifications and m^6^A modification of singletons and duplicated genes.

We compared the evolutionary rates of singletons and duplicated genes of B. napus and diploid progenitors. The singletons had significantly higher ω values than duplicated genes in all genotypes (Supplemental Figure S10). As shown in Table 2, regardless of whether the transcripts of genes were modified by m^6^A, singletons had significantly higher ω values than duplicated genes in the genome/subgenomes in all genotypes, indicating that singletons have experienced stronger purifying selection than duplicated genes. These results suggested that singletons whose transcripts showed higher m^6^A modification have experienced faster sequence divergence than duplicated genes. The duplicated genes of genome/subgenome C had significantly
higher $\omega$ values than those of genome/subgenome A of *B. napus*, but there was no obvious difference between singletons of the two genomes/subgenomes. These results indicated that the duplicated genes of genome/subgenome C have experienced stronger purifying selection than those in genome/subgenome A.
Table 2. Evolutionary rates (ω) of m\(^6\)A, non-m\(^6\)A singletons and duplicated genes in genomes/subgenomes.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Genome/subgenome</th>
<th>Evolutionary rates (ω)</th>
<th>P value</th>
<th>Evolutionary rates (ω)</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>m(^6)A singletons</td>
<td>m(^6)A duplicated genes</td>
<td></td>
<td>Non-m(^6)A singletons</td>
<td>Non-m(^6)A duplicated genes</td>
</tr>
<tr>
<td>A, C</td>
<td>A</td>
<td>0.1813±0.1078</td>
<td>0.1493±0.0944</td>
<td>&lt;0.001</td>
<td>0.1817±0.0956</td>
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<tr>
<td></td>
<td>C</td>
<td>0.1839±0.1089</td>
<td>0.1549±0.1010</td>
<td>&lt;0.001</td>
<td>0.1855±0.0994</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.7353</td>
<td>0.008577</td>
<td>-</td>
<td>0.704</td>
</tr>
<tr>
<td>RAC</td>
<td>A(_n)</td>
<td>0.1743±0.1097</td>
<td>0.1414±0.0928</td>
<td>&lt;0.001</td>
<td>0.1845±0.0951</td>
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<tr>
<td></td>
<td>C(_n)</td>
<td>0.1769±0.1047</td>
<td>0.1467±0.0993</td>
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<td>0.1880±0.0997</td>
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<tr>
<td></td>
<td>P value</td>
<td>0.6922</td>
<td>0.01415</td>
<td>-</td>
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<tr>
<td>NAC</td>
<td>A(_n)</td>
<td>0.1757±0.1069</td>
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<td>0.1820±0.0916</td>
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<td></td>
<td>C(_n)</td>
<td>0.1760±0.0989</td>
<td>0.1424±0.0983</td>
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<td>0.1982±0.1077</td>
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<tr>
<td></td>
<td>P value</td>
<td>0.7983</td>
<td>0.007345</td>
<td>-</td>
<td>0.1714</td>
</tr>
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</table>

A, B. rapa; C, B. oleracea; RAC, resynthesized B. napus; NAC, natural B. napus.
The distribution features of m^6A modification in five types of duplicated genes

Given the high proportion of duplicated genes due to the complex WGD events both in B. napus and diploid progenitors, we divided the duplicated genes identified in our study into five types for in-depth analysis according to a previous study [5]. The number of genes from WGD, DSD, and TRD identified in this study was higher than that from TD and PD in all genotypes (Figure 6a). Then, we counted the proportion of the m^6A gene of each type of duplicated gene in each genotype and found that the pattern of change was different between the two subgenomes (Figure 6b). In resynthesized B. napus, the proportion of the m^6A gene in subgenome A_n decreased, but the proportion of m^6A gene in subgenome C_n increased compared with progenitors. The proportion of the m^6A gene in both subgenomes increased in natural B. napus compared with resynthesized B. napus. These results indicated that hybridization and WGD balanced the proportion of the m^6A gene between subgenomes of B. napus, which was reflected in all kinds of duplicated genes (Figure 6b; Supplemental Figure S8a).
Figure 6 The number, gene expression level, m^6A modifications distribution of five types of duplicated genes. (a) The number of five kinds of duplicated genes in B. napus and diploid progenitors. (b) Proportion of m^6A genes in five kinds of duplicated genes in each genome/subgenome. (c) Gene expression level of five types of duplicated genes in B. napus and diploid progenitors. (d) Metagenomic profiles of m^6A peak distribution of five types of duplicated genes in B. napus and diploid progenitors. A, B. rapa; C, B. oleracea; RAC, resynthesized B. napus; NAC, natural B. napus.

We analyzed m^6A modifications among five types of duplicated genes and found that transcripts of TD-derived genes exhibited a different distribution of m^6A modification, which was highly enriched around the TSS (Figure 6d). In contrast, m^6A modification was highly enriched around the TES in the gene transcripts of other duplicated genes around the TES. Around the TES, the transcripts from WGD-derived
genes exhibited the highest level of $m^6$A modification, followed by transcripts from TRD-, DSD- and TD-derived genes. Interestingly, only transcripts of PD- and TD-derived genes were highly enriched in $m^6$A modification of the body of gene transcripts. Then, we analyzed the three histone modifications and DNA methylation among the five types of duplicated genes and found that the WGD-derived genes exhibited the highest level of active histone markers (H3K4me3 and H3K27ac) (Supplemental Figure S11a and b) but the lowest level of DNA methylation (Supplemental Figure S12a-c), which was consistent with their highest gene expression level (Figure 6c). The WGD-, TRD- and DSD-derived genes showed opposite distribution trends in the histone markers and DNA methylation: WGD-derived genes had the highest level of histone markers but lowest DNA methylation, while DSD-derived genes had the highest level DNA methylation but the lowest level of histone markers (Supplemental Figure S11, Supplemental Figure S12). Surprisingly, the PD-derived genes exhibited the highest level of repressive histone markers (H3K27me3) and DNA methylation in the CG content but the lowest level of active histone markers. Interestingly, active histone markers of the PD- and TD-derived genes showed opposite trends in the gene bodies of progenitors, but there was little difference in *B. napus* (Supplemental Figure S11a and b). These results indicated that genes derived from various duplication events showed distinct distribution features of these epigenetic modifications and $m^6$A modification.

We compared the evolutionary rates of five types of duplicated genes. The evolutionary rates ($\omega$) were analyzed using putatively orthologous sequences within *B. napus*. We found that TD- and PD-derived genes had higher evolutionary rates than WGD-, TRD- and DSD-derived genes (Supplemental Figure S13a). To investigate the association between $m^6$A modification and evolutionary rates of genes, we analyzed
the \( \omega \) values of duplicated gene pairs with distinct m\(^6\)A modification patterns: the identical m\(^6\)A (IM) pattern (both gene transcripts of partners had m\(^6\)A peaks), diverged m\(^6\)A (DM) pattern (only a gene transcript of partners carrying m\(^6\)A peaks), and non-m\(^6\)A (NM) pattern (neither gene transcript of partners had m\(^6\)A peaks). As shown in Figure 7a, the \( \omega \) values of IM were significantly lower than those of DM and NM in all genotypes, which indicated that the transcripts of conserved duplicated gene pairs are more likely to carry m\(^6\)A peaks. Comparing the \( \omega \) values of the three patterns in the five types of duplicated genes (Supplemental Figure S13b), only the TD- and PD-derived genes in *B. rapa* did not possess this rule.

We investigated the association between m\(^6\)A modification and expression divergence (ED) of duplicated genes. The ED was calculated following the formula in a previous study with minor modification: ED = |(E1 - E2)/(E1+E2)|, where E1 and E2 represent the gene expression levels of two genes in a duplicated gene pair [6]. The ED of the IM pattern was significantly higher than that of the DM pattern in progenitors, but the opposite was observed in *B. napus*, which indicated that hybridization and WGD changed the gene divergence of duplicated genes with different m\(^6\)A modification patterns (Figure 7b). We found that the methylated partners of the DM pattern exhibited a lower mRNA abundance than the non-methylated partners (Supplemental Figure S14). These observations indicated that m\(^6\)A modification was negatively associated with mRNA abundance, but hybridization and WGD may change the connection between m\(^6\)A modification and the expression divergence of duplicated genes.
**Figure 7** $K_a/K_s$ of duplicated gene pairs with different $m^6A$ modification patterns and gene expression divergence of duplicated genes in IM and DM. (a) $K_a/K_s$ of duplicated gene pairs with different $m^6A$ modification patterns on the whole. (b) Gene expression divergence of duplicated genes in IM and DM. IM, identical $m^6A$ pattern; DM, diverged $m^6A$ pattern; NM, non-$m^6A$ pattern; A, *B. rapa*; C, *B. oleracea*; RAC, resynthesized *B. napus*; NAC, natural *B. napus*.

**The dosage-dependent and dosage-independent genes displayed different distributions of $m^6A$ modification and four epigenomic markers**

As an important evolutionary mechanism, gene dosage balance guarantees normal expression of duplicated genes. Altered gene dosage may lead to gene expression changes. Therefore, the expression and fate of duplicated genes are influenced by the evolutionary mechanism of gene dosage balance [37]. Tan *et al.* divided genes of *B.*
*napus* into two categories according to the coefficients of determination ($R^2$): dosage-dependent ($R^2 > 0.59$) genes in subgenome $A_n$ (Ad) and in subgenome $C_n$ (Cd) and dosage-independent ($R^2 < 0.59$) genes in subgenome $A_n$ (Ai) and in subgenome $C_n$ (Ci) [37]. We explored the distribution of $m^6A$ modification and four epigenetic markers of these two kinds of genes in four genotypes. The AdCd genes had higher levels of $m^6A$ modification around TSS and TES (Figure 8a), active histone markers around TSS (H3K4me3 and H3K27ac) (Figure 8b and c), DNA methylation of CG content in the gene body (Supplemental Figure S15a), and repressive histone markers downstream of TES (H3K27me3) (Figure 8d), whereas AiCi genes had higher repressive histone markers in the gene body (Figure 8d), and DNA methylation of CHG and CHH content in the gene body (Supplemental Figure S15b-c). These results suggested that the dosage-dependent and dosage-independent genes showed different distribution features of three histone markers, DNA methylation in genes and $m^6A$ modifications in transcripts, which may influence the differentiation of dosage-related duplicated genes in *B. napus* and its diploid progenitors.
Figure 8 The distribution of m^6A modifications and three histone modifications of dosage-dependent and independent genes in B. napus and diploid progenitors. (a) Distribution feature of m^6A modifications. (b) Distribution feature of H3K4me3 modifications. (c) Distribution feature of H3K27ac modifications. (d) Distribution feature of H3K27me3 modifications. A, B. rapa; C, B. oleracea; RAC, resynthesized B. napus; NAC, natural B. napus.

Discussion

The conservation and variation of the distribution of m^6A modification

As the most prevalent modification in transcripts, m^6A modification has been demonstrated to play an essential role in developmental and biological processes [38]. Depending on the m^6A distribution and the sequence contexts within transcripts, m^6A modification can promote the degradation of mRNAs or stabilize mRNAs [27]. Recent studies on m^6A have focused on biological development and the response to
adversity [15, 26]. However, the characteristics of m6A modification during the early formation and subsequent evolution of polyploid plants is still unclear. Therefore, transcriptome-wide m6A modifications were comprehensively analyzed using allopolyploid B. napus and diploid progenitors as an ideal system. Overall, m6A modification was notably enriched around the stopC region and in the 3' UTR in B. napus and diploid progenitors (Figure 1a), similar to the distribution observed in Arabidopsis [26, 39], tomato [25], strawberry [27], maize [6], human and mouse [35]. These observations reflected that the distribution of m6A modification was highly conserved in eukaryotes, including both plant and animal kingdoms. Additionally, there was a minor m6A modification enrichment around the 5' UTR near the start codon in B. napus and its progenitors (Figure 1a). A similar summit of m6A peaks was also found in Arabidopsis [26, 39], strawberry [27], and rice [40] but not in tomato [25], maize [6], human [35], or mouse [23, 35]. The diverse distribution among these species may be related to species-specific genomic organizations and m6A regulatory mechanisms, which reflect the diversity of m6A methylomes. More interestingly, we observed that species with higher m6A modification enrichment around the stop codon and 3' UTR also had higher m6A modification in the CDS but lower m6A modification within the 5' UTR in B. napus and diploid progenitors. The opposite trend of m6A modification enrichment has also been observed between the region around the startC and stopC of strawberry in the ripening process [27], and Arabidopsis under different concentrations of salt stress [26], callus and leaf of rice [40]. These phenomena suggest that there may be a conserved antagonistic m6A modification mechanism regulating m6A enrichment at both ends of the transcripts in these species.

m6A modification showed multifaceted relationship with gene expression level
As a reversible internal RNA modification in mRNA, m^6A modifications regulate gene expression as key posttranscriptional regulators [38]. In mammals, the m^6A modification level is generally negatively associated with the gene expression level [16]. During tomato ripening, the differential m^6A modification was mainly deposited near the stopC regions or in the 3’UTR, which was generally negatively correlated with the gene expression level [25]. In the callus and leaves of rice, the enrichment abundance of m^6A peaks was also negatively correlated with the gene expression level [40]. These observations suggested that m^6A modification was negatively correlated with the gene expression level overall. However, the overall positive relationship between m^6A modification and gene expression levels during tomato expansion was revealed by Hu et al. [25]. Here, we found a complex relationship between the abundance of m^6A modifications and gene expression level. The differential methylation level analysis and differential gene expression analysis of all transcripts showed that the enrichment abundance of m^6A modification was positively correlated with gene expression overall (Figure 2b-g). However, the presence of m^6A in transcripts originally free of m^6A modification was more likely to decrease gene expression (Figure 3d-f). The absence of m^6A transcripts tended to up-regulate gene expression (Figure 3a-c). Methylated partners of duplicated genes exhibited lower gene expression levels than the non-methylated partners (Supplemental Figure S14). These observations reflect the opposite roles in the gene expression level of the presence/absence and the differential degree of enrichment of m^6A modification in transcripts. In a study of strawberry ripening, m^6A modification was found to have diverse regulatory roles on mRNAs depending on the distribution of m^6A [27]. The m^6A modifications located in the 3’UTR or around stopC regions tended to be negatively correlated with gene expression, whereas those in CDS regions were more
likely to stabilize the mRNAs [27]. These findings suggest that m^6^A may play unique roles in mRNAs, possibly due to its degree of enrichment, presence or absence, and different localization.

**Duplicated genes produced by various mechanisms exhibited distinct distributions of m^6^A modification**

In plants, gene duplication events, including WGD events and single-gene duplication events, supply many evolutionary raw materials to adapt to changing conditions [41]. To adapt to rapidly changing environments, plants underwent a higher frequency of tandem duplication than WGD [42]. TD-derived genes were more likely to be related to stress-associated functions than others [43]. Therefore, the expansion of stress-related genes by TD in plants is considered to be a protective mechanism against harmful stresses [43]. Some ancient TD-derived genes were interrupted by other genes to form proximal duplicates [44]. Plants may need to constantly acquire new TDs and PDs to adapt to drastic environmental changes [5, 42]. In this study, we found that frequent WGDs resulted in a high proportion of duplicated genes in *B. napus* and diploid progenitors (Figure 5a). A previous study suggested that gene expression divergence between duplicated genes might make *B. napus* more flexible in responding to abiotic stress [45]. Our study suggested that there was a great difference in gene expression divergence between IM and DM duplicated genes (Figure 7b). Hybridization and WGD reduced the difference in the proportion of m^6^A genes of five types of duplicated genes between the two subgenomes of *B. napus* (Figure 6b). We wondered whether different types of genes derived from various duplication events faced the same regulation in the same genetic regulatory environment. The transcripts of singletons exhibited higher m^6^A modification than
that of the duplicated genes on the whole in *B. napus* and diploid progenitors (Figure 5c). When duplicated genes were divided into five types, we found that transcripts from these genes exhibited different distributions of m^6^A modification (Figure 6d). Only TD-derived genes have higher enrichment of m^6^A modifications around TSS than TES in transcripts. Transcripts of TD- and PD-derived genes have a minor enrichment of m^6^A modifications in the gene body. Transcripts of WGD-derived genes showed higher m^6^A modifications than DSD- and TRD-derived genes in the four genotypes (Figure 6d). A previous study revealed that H3K36me3 guides m^6^A deposition globally in animals [35]. Shim *et al.* revealed a high correlation between H3K36me2 and m^6^A modification in plants [36]. In this study, we found that the singletons had obviously higher repressive histone markers (H3K27me3) and DNA methylation not only in the gene body but also in the upstream and downstream regions of the gene (Supplemental Figure S9a), and displayed overall higher enrichment of m^6^A modifications in the transcripts. Interestingly, in contrast to the distribution of m^6^A modification, the singletons and the duplicated genes have greater active histone markers (H3K4me3 and H3K27ac) (Figure 5d and e) around TSS than TES. The PD-derived genes showed lower levels of active histone markers (H3K4me3 and H3K27ac) (Supplemental Figure S11a and b), higher levels of repressive histone markers (H3K27me3) (Supplemental Figure S11c), higher levels of DNA methylation in the gene body (Supplemental Figure S12a-c), and displayed unique m^6^A modifications between TSS and TES (Figure 6d). The TD-derived genes exhibited lower levels of active histone markers and repressive histone markers and showed additional higher enrichment of m^6^A modifications around the TSS (Figure 6d; Supplemental Figure S11). These observations demonstrated that different types of duplicated genes exhibited distinct distributions of epigenetic modifications and m^6^A
modifications in transcripts, and the specific distribution patterns of m\(^6\)A modification may be an important marker for distinguishing the transcripts of different types of genes. Additionally, we found that TDs and PDs have some common features compared with other types of duplicated genes, such as lower active histone markers (Supplemental Figure S11a and b), unique m\(^6\)A modification in the middle of transcripts (Figure 6d), higher evolutionary rates (Supplemental Figure S13a) and lower gene expression levels (Figure 6c). These findings suggest that TDs and PDs experience faster functional divergence, while epigenetic modifications (H3K4me3, H3K27ac, H3K27me3 and DNA methylation) and m\(^6\)A modifications appear to play a major part in this process.

Materials and methods

Plant materials

Seeds of natural B. napus L. (cv. Darmor), resynthesized B. napus L. (HC-2) and its progenitors, B. rapa L. (cv. 9J6002) and B. oleracea L. (cv. 3YS013) were provided by the Oil Crop Research Institute, Chinese Academy of Agricultural Sciences, China. All plants were cultivated in light incubators at 23 °C with a photoperiod of day/night for 16 h/8 h. Leaves of one-month old seedlings of four genotypes were harvested in triplicate, freezing promptly in liquid nitrogen and then reserved at -80 °C.

RNA-seq and data analysis

Total RNA of all samples was extracted by using TRIzol Kit (Promega, Madison, USA). Then, the rRNAs were eliminated by NEBNext rRNA Depletion Kit (New England Biolabs, Inc., Massachusetts, USA) following the manufacturer’s instructions. RNA libraries were constructed by NEBNext® Ultra II Directional RNA Library Prep
Kit (New England Biolabs, Inc., Massachusetts, USA) according to the manufacturer’s instructions. The quality and quantified of was controlled by the libraries BioAnalyzer 2100 system (Agilent Technologies, Inc., USA). An Illumina Novaseq 6000 instrument was used to perform library sequencing on with 150 bp paired end reads and Q30 was used to control quality. After removing 3’ adaptor-trimming and low-quality reads by cutadapt software (v1.9.3) [46], the high-quality clean reads were aligned to the reference *B. napus* genome v5 (http://www.genoscope.cns.fr/brassicanapus/data/) with hisat2 software (v2.0.4) [47]. HTSeq software (v0.9.1) was used to get raw count, and then normalization of gene expression levels in terms of FPKM (fragments per kilobase million) was performed by edgeR. Differentially expressed genes (DEGs) were detected according to the criteria of p-value ≤ 0.05 and |log2 fold change| ≥ 1 by edgeR. g:Profiler (https://biit.cs.ut.ee/gprofiler/gost) was used to perform Gene Ontology (GO) enrichment analysis [48].

**MeRIP-seq and data analysis**

MeRIP-seq was carried out based on the method reported by a previous study with slight modifications [23]. In brief, fragmented RNA was incubated with anti-m⁶A polyclonal antibody (Synaptic Systems, 202003) in IP buffer for 2 h at 4 °C. The mixture was incubated with protein-A beads (Thermo Fisher, Waltham, MA, USA) at 4 °C for 2 h for immunoprecipitation. The bound RNA was eluted from the beads in IP buffer using N⁶-methyladensine (BERRY & ASSOCIATES, PR3732) and then extracted with Trizol reagent (Thermo Fisher, Waltham, MA, USA) based on the manufacturer’s instruction. RNA-seq library was constructed by purified RNA with NEBNext® Ultra II Directional RNA Library Prep Kit (NEB). An Illumina NovaSeq
6000 instrument was used to conduct library sequencing with 150 bp paired-end reads and Q30 was used to control quality. After removing 3’ adaptor and low-quality reads by cutadapt software (v1.9.3), clean reads were aligned to the reference B. napus genome by Hisat2 software. MACS software was performed to identify methylated sites on RNAs (peaks), parameters are as follows:

-p 0.05 -f BED --tsize 150 --keep-dup=all --verbose 3 --nomodel [49]. Differentially methylated sites were detected by diffReps with a criterion of fold change in m^A enrichment ≥ 2 and P < 0.0001 [50]. m^A motifs were identified by DREME with a limited length of 6 nucleotides.

Data analysis of four epigenetic markers

The data of histone modifications (H3K4me3, H3K27me3, and H3K27ac) and DNA methylation were obtained from a previous study [11]. The data of histone modifications were analyzed by deeptools [51], while the data of DNA methylation were analyzed by BatMeth2 [52].

Evolutionary rates analysis of orthologous and paralogous genes

The evolutionary rates (\( \omega \)) were calculated by Ka/Ks. Ka and Ks was calculated using PAML 4.8 [53]. The \( \omega \), Ka, and Ks of the singletons and duplicated genes in B. napus were calculated by comparing putatively orthologous sequences between B. napus and Arabidopsis thaliana, as that of duplicated gene pairs were estimated by comparing paralogous sequences between gene pairs in B. napus.

Statistical analysis

The Wilcoxon rank sum test was carried out by wilcoxon.test function and the \( \chi^2 \) test
was accomplished by chisq.test function in R package.

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Author contributions

This study was designed by J.W. and Z.L. Z.L. analyzed data and wrote the manuscript. X.W. provided the experimental materials. M.L. was responsible for planting materials. M.L. provided data of histone modifications and DNA methylation. The manuscript was revised by J.W. and X.W. All authors review and approved the manuscript.

Data availability

MeRIP-seq and RNA-seq data in this research have been stored in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) with the accession numbers SRR16842417- SRR16842428 and SRR16842657- SRR16842668. The data of four epigenetic markers can be obtained in NCBI with the accession codes SRR13306925-SRR13306936 (WGBS) and SRR13318007-SRR13318030 (ChIP-seq).

Conflict of interest

The authors declare that they have no conflict of interest.
Supplementary information

Additional Supporting Information may be found in the online version of this article.

**Supplemental Table S1.** Reads statistics of RNA-seq and MeRIP-seq in leaves of A, C, RAC, and NAC.

**Supplemental Table S2.** GO enrichment analysis of genes whose transcripts comprising differential methylation peaks.

**Supplemental Table S3.** Evolutionary rates ($\omega$) of m$^6$A genes and non-m$^6$A genes.

**Supplemental Figure S1.** Spearman correlation analysis of MeRIP-seq data.

**Supplemental Figure S2.** Spearman correlation analysis of RNA-seq data.

**Supplemental Figure S3.** Sequence motif detected in the m$^6$A peaks by DREME.

**Supplemental Figure S4.** Comparisons of multiple gene features of m$^6$A genes and non-m$^6$A genes in *B. rapa*. (a) Gene length. (b) Exon length. (c) Intron length. (d) Exon number.

**Supplemental Figure S5.** Comparisons of multiple gene features of m$^6$A genes and non-m$^6$A genes in *B. oleracea*. (a) Gene length. (b) Exon length. (c) Intron length. (d) Exon number.

**Supplemental Figure S6.** Comparisons of multiple gene features of m$^6$A genes and non-m$^6$A genes in resynthesized *B. napus*. (a) Gene length. (b) Exon length. (c) Intron length. (d) Exon number.

**Supplemental Figure S7.** Comparisons of multiple gene features of m$^6$A genes and non-m$^6$A genes in natural *B. napus*. (a) Gene length. (b) Exon length. (c) Intron length. (d) Exon number.

**Supplemental Figure S8.** Proportion and gene expression level of m$^6$A genes and non-m$^6$A genes in *B. napus* and diploid progenitors. (a) Proportion of m$^6$A genes and non-m$^6$A genes. (b) Gene expression level of m$^6$A genes and non-m$^6$A genes.
Supplemental Figure S9. Metagenomic profiles of H3K27me3 and DNA methylation of singletons and duplicated genes. (a) Metagenomic profiles of H3K27me3 modification on singletons and duplicated genes. (b) Metagenomic profiles of DNA methylation of CG content on singletons and duplicated genes. (c) Metagenomic profiles of DNA methylation of CHG content on singletons and duplicated genes. (d) Metagenomic profiles of DNA methylation of CHH content on singletons and duplicated genes.

Supplemental Figure S10. Ka/Ks values of singletons and duplicated genes.

Supplemental Figure S11. Metagenomic profiles of histone modifications of five types of duplicated genes. (a) Metagenomic profiles of H3K4me3 modification on singletons and duplicated genes. (b) Metagenomic profiles of H3K27ac on singletons and duplicated genes. (c) Metagenomic profiles of H3K27me3 on singletons and duplicated genes.

Supplemental Figure S12. Metagenomic profiles of DNA methylation of five types of duplicated genes. (a) Metagenomic profiles of DNA methylation of CG content on singletons and duplicated genes. (b) Metagenomic profiles of DNA methylation of CHG content on singletons and duplicated genes. (c) Metagenomic profiles of DNA methylation of CHH content on singletons and duplicated genes.

Supplemental Figure S13. Ka/Ks values of duplicated gene pairs with different m^6A modification patterns. (a) Ka/Ks values of five types of duplicated gene pairs. (b) Ka/Ks values of duplicated gene pairs with different m^6A modification patterns of five types of duplicated genes.

Supplemental Figure S14. Gene expression level of the m^6A-methylated partners and the non-m^6A-methylated partners of DM pattern.

Supplemental Figure S15. Metagenomic profiles of DNA methylation of
dosage-dependent and dosage-independent expression genes. (a) Metagenomic profiles of DNA methylation of CG content. (b) Metagenomic profiles of DNA methylation of CHG content. (c) Metagenomic profiles of DNA methylation of CHH content.

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