

## Supplementary Figure S1. Pearson correlations of relative expression differences (R<sub>FPKM</sub>) of WGD duplicates between different tissues.

**A.** Between leaf and petiole in *Nelumbo*. **B.** Between juvenile leaf and juvenile leafstalk in *Nymphaea*. **C.** Between young leaf and old leaf in *Acorus*. WGD, whole genome duplication; FPKM, fragments per kilobase of exon model per million mapped fragments; grey shading band, the 95% (default) confidence level interval for predictions from a linear model; red font, positive correlation; \*\*, *p*-value < 0.01.



Supplementary Figure S2. Pearson correlations between average relative expression differences ( $R_{FPKM}$ ) of WGD duplicates and gene features in *Nelumbo*. A-B. Average  $R_{FPKM}$  of WGD duplicates are significantly positively correlated with average synonymous substitutions per site (d*S*) after WGD (**A**) and average  $\omega$  (d*N*/d*S* ratio) after WGD (**B**). C-D. Average  $R_{FPKM}$  of WGD duplicates are significantly negatively correlated with average exon number (**C**) and average coding sequence (CDS) length (**D**). WGD, whole genome duplication; FPKM, fragments per kilobase of exon model per million mapped fragments; *r*, correlation coefficient of Pearson correlation test; log, log-transformed values of gene features in x-axis; grey shading band, the 95% (default) confidence level interval for predictions from a linear model; red font, positive correlation; blue font, negative correlation; \*\*, *p*-value < 0.01.









Supplementary Figure S3. Pearson correlations between average relative expression differences ( $R_{FPKM}$ ) of WGD duplicates and gene features in *Nymphaea*. A-D. Average  $R_{FPKM}$  of WGD duplicates are significantly positively correlated with average non-synonymous substitutions per site (d*N*) after WGD (**A**), average synonymous substitutions per site (d*S*) after WGD (**B**), average  $\omega$  (d*N*/d*S* ratio) after WGD (**C**), tissue specificity ( $\tau$ ) of gene expression (**D**). **E-J.** Average  $R_{FPKM}$  of WGD duplicates are significantly negatively correlated with average exon number (**E**), average coding sequence (CDS) length (**F**), average protein length (**G**), No. of Pfam domains genes (**H**), No. of protein-protein interactions (PPIs) of putative orthologs in *Arabidopsis* (**I**), lethal-phenotype score of *Arabidopsis* putative ortholog groups (**J**). WGD, whole genome duplication; FPKM, fragments per kilobase of exon model per million mapped fragments; *r*, correlation coefficient of Pearson correlation test; log, log-transformed values of gene features in x-axis; grey shading band, the 95% (default) confidence level interval for predictions from a linear model; red font, positive correlation; blue font, negative correlation; \*, *p*-value < 0.05; \*\*, *p*-value < 0.01.







0.75



Supplementary Figure S4. Pearson correlations between average relative expression differences ( $R_{FPKM}$ ) of WGD duplicates and gene features in *Acorus*. A-C. Average  $R_{FPKM}$  of WGD duplicates are significantly positively correlated with average non-synonymous substitutions per site (d*N*) after WGD (**A**), average synonymous substitutions per site (d*S*) after WGD (**B**), and tissue specificity ( $\tau$ ) of gene expression (**C**). D-J. Average  $R_{FPKM}$  of WGD duplicates are significantly negatively correlated with average  $\omega$  (d*N*/d*S* ratio) after WGD (**D**), average exon number (**E**), average coding sequence (CDS) length (**F**), average protein length (**G**), No. of Pfam domains genes (**H**), No. of protein-protein interactions (PPIs) of putative orthologs in *Arabidopsis* (**I**), lethal-phenotype score of *Arabidopsis* putative ortholog groups (J). WGD, whole genome duplication; FPKM, fragments per kilobase of exon model per million mapped fragments; *r*, correlation coefficient of Pearson correlation test; log, log-transformed values of gene features in x-axis; grey shading band, the 95% (default) confidence level interval for predictions from a linear model; red font, positive correlation; blue font, negative correlation; \*, *p*-value < 0.05; \*\*, *p*-value < 0.01.



Supplementary Figure S5. Pearson correlations of the correlation coefficients (*r*) of average relative expression ( $R_{\text{FPKM}}$ ) and gene features among three species. (A) *r* of average  $R_{\text{FPKM}}$  and gene features between Nymphaea and Nelumbo. (B) *r* of average  $R_{\text{FPKM}}$  and gene features between Nymphaea and Acorus. (C) *r* of average  $R_{\text{FPKM}}$  and gene features between Nymphaea and Acorus. FPKM, fragments per kilobase of exon model per million mapped fragments. Gene features were listed in Supplementary Table S6.



Supplementary Figure S6. Violin plot showing how the relative expression difference (*R*<sub>FPKM</sub>) for *Nymphaea* homoeologs varies among duplicate genes belonging to different Gene Ontology (GO) slim categories. FPKM, fragments per kilobase of exon model per million mapped fragments; red bar, median; pink bar, quartile. The sample sizes of the GO slim categories, from bottom to top in the figure, are as follows: 37, 14, 17, 14, 28, 81, 44, 103, 125, 139, 25, 32, 244, 172, 47, 206, 126, 13, 212, 85, 243, 268, 183, 660, 955, 19, 180, 40, 80, 41, 181, 78, 485, 153, 122, 35, 131, 42, 32, 213 and 48.



Supplementary Figure S7. Violin plot showing how the relative expression difference (*R*<sub>FPKM</sub>) of *Acorus* homoeologs varies among duplicate genes belonging to different Gene Ontology (GO) slim categories. FPKM, fragments per kilobase of exon model per million mapped fragments; red bar, median; pink bar, quartile. The sample sizes of the GO slim categories, from bottom to top in the figure, are as follows: 43, 30, 62, 16, 149, 106, 219, 192, 165, 16, 222, 353, 281, 54, 67, 66, 445, 426, 365, 463, 21, 17, 109, 222, 1559, 72, 218, 1110, 164, 248, 247, 847, 302, 360, 302, 52, 34, 39, 68, 50, 194, 24 and 21.



Supplementary Figure S8. Pearson correlations between average relative expression differences (*R*<sub>FPKM</sub>) of WGD duplicates and propensity of gene loss. A. *Nymphaea*. B. *Nelumbo*. C. *Acorus*. WGD, whole genome duplication; FPKM, fragments per kilobase of exon model per million mapped fragments; grey shading band, the 95% (default) confidence level interval for predictions from a linear model; red font, positive correlation; \*\*, *p*-value < 0.01.



Supplementary Figure S9. Distributions of the proportion of genes with premature stop codon mutations in *Nelumbo* populations according to relative expression difference between the two copies ( $R_{_{FPKM}}$ ). FPKM, fragments per kilobase of exon model per million mapped fragments.



Supplementary Figure S10. Differences between homoeologs with higher and lower gene expression in immature stamen of *Nelumbo*. Differences in synonymous substitutions per site (d*S*) after whole-genome duplication (WGD) (**A**),  $\omega$  (d*N*/d*S* ratio) after WGD (**B**), exon number (**C**), coding sequence (CDS) length (**D**), No. of Pfam domains of genes (**E**), *cis*- and *trans*-regulatory change magnitude (H8-S1) (**F**) between homoeologs with higher expression and lower expression in immature stamen of *Nelumbo* (paired *t* tests, \*\* *p*-value < 0.01, \* *p*-value < 0.05). Solid line in violin plot, median value; dashed line in violin plot, quantile; n, sample size.



Supplementary Figure S11. Differences between homoeologs with higher and lower gene expression in juvenile leaf of Nymphaea. Differences in non-synonymous substitutions per site (dN) after whole-genome duplication (WGD) (A), synonymous substitutions per site

(dS) after WGD (B),  $\omega$  (dN/dS ratio) after WGD (C), exon number (D), coding sequence (CDS) length (E), protein length (F), No. of Pfam domains of genes (G), tissue specificity ( $\tau$  index) of gene expression (H) between homoeologs with higher expression and lower expression in juvenile leaf of *Nymphaea* (paired *t* tests, \*\* *p*-value < 0.01). Solid line in violin plot, median value; dashed line in violin plot, quantile; n, sample size.



Supplementary Figure S12. Differences between homoeologs with higher and lower gene expression in young leaf of *Acorus*. Differences in non-synonymous substitutions per site (d*N*) after whole-genome duplication (WGD) (A), synonymous substitutions per site (d*S*) after WGD (B),  $\omega$  (d*N*/d*S* ratio) after WGD (C), exon number (D), coding sequence (CDS) length (E), protein length (F), No. of Pfam domains of genes (G),

tissue specificity ( $\tau$  index) of gene expression (**H**) between homoeologs with higher expression and lower expression in young leaf of *Acorus* (paired *t* tests, \*\* *p*-value < 0.01). Solid line in violin plot, median value; dashed line in violin plot, quantile; n, sample size.







(n = 4,937) (n = 4,937)









Proportion of genes (%)







Nelumbo

Rhizome elongation zone

D

(%)

Proportion of genes

Η

Proportion of





4.00-2.00-2.86% 0.00 High expression Low expression (n = 4,935) Nelumbo Ρ

3.71%

(n = 4,935)

Nelumbo

Nelumbo



Supplementary Figure S13. Differences in premature stop codon mutations in Nelumbo populations between homoeologs with higher expression and lower expression in 19 different tissues of *Nelumbo* .  $\chi^2$  test, \*\* *p*-value < 0.01, \* *p*-value < 0.05; n, sample size.