Variation in cytonuclear expression accommodation among allopolyploid plants

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Abstract

Cytonuclear coevolution is a common feature among plants, which coordinates gene expression and protein products between the nucleus and organelles. Consequently, lineage-specific differences may result in incompatibilities between the nucleus and cytoplasm in hybrid taxa. Allopolyploidy is also a common phenomenon in plant evolution. The hybrid nature of allopolyploids may result in cytonuclear incompatibilities, but the massive nuclear redundancy created during polyploidy affords additional avenues for resolving cytonuclear conflict (*i.e.,* cytonuclear accommodation). Here we evaluate expression changes in organelle-targeted nuclear genes for six allopolyploid lineages that represent four genera (*i.e., Arabidopsis, Arachis, Chenopodium*, and *Gossypium*) and encompass a range in polyploid ages. Because incompatibilities between the nucleus and cytoplasm could potentially result in biases toward the maternal homoeolog and/or maternal expression level, we evaluate patterns of homoeolog usage, expression bias, and expression level dominance in cytonuclear genes relative to the background of non-cytonuclear expression changes and to the diploid parents. Although we find subsets of cytonuclear genes in most lineages that match our expectations of maternal preference, these observations are not consistent among either allopolyploids or categories of organelle-targeted genes. Our results indicate that cytonuclear expression accommodation may be a subtle and/or variable phenomenon that does not capture the full range of mechanisms by which allopolyploid plants resolve nuclear-cytoplasmic incompatibilities.

Introduction

Intergenomic coevolution between the nucleus and organelle(s) is a common feature among eukaryotes. Gene loss and transfers to the nucleus have greatly reduced the coding regions of modern mitochondrial and plastid genomes to a limited number of essential genes (Greiner and Bock 2013; Budar and Mireau 2018; Giannakis *et al.* 2021). Consequently, these organelles must coordinate transcripts and protein products from two or more different genomic compartments to carry out essential cellular functions. Over time, this functional interdependence results in coadaptation between the nucleus and each organelle; however, differences in mode of inheritance (i.e., biparental for the nucleus and cytoplasmic for the organelles) can lead to incompatibilities between nuclear and organellar alleles, particularly in hybrid lineages. These cytonuclear incompatibilities are widespread among species and can have dramatic consequences for fitness (Fishman and Willis 2006; Hill 2017; Fishman and Sweigart 2018; Postel and Touzet 2020), even leading to hybrid breakdown in some cases (Burke and Arnold 2001; Greiner *et al.* 2011; Burton and Barreto 2012; Burton *et al.* 2013; Budar and Mireau 2018).

Cytonuclear incompatibilities arising when evolutionarily distinct lineages merge to form allopolyploids may experience additional complex fates compared to incompatibilities in homoploid lineages (Sharbrough *et al.* 2017). The combined effects of genome merger and doubling have generally been associated with a diverse array of genomic and transcriptional

changes, including nonrandom gene loss, intergenomic gene conversion, and epigenetic/regulatory changes leading to (sometimes biased) alterations in gene expression (Chen 2007; Doyle *et al.* 2008; Freeling 2009; Gaeta and Pires 2010; Jackson and Chen 2010; Salmon *et al.* 2010; Grover *et al.* 2012; Madlung and Wendel 2013; Yoo *et al.* 2014; Song and Chen 2015; Bao *et al.* 2019; Gallagher *et al.* 2020). While often evaluated on an individual gene basis, many genes are sensitive to the abundance of interacting partners, particularly those involved in multi-subunit complexes (Birchler and Veitia 2010, 2014, 2021). In allopolyploid lineages, coordination of gene products becomes more complicated when interactions between previously isolated genomes occur and redundancy affords the possibility of gene loss or divergence (Adams and Wendel 2005; Conant and Wolfe 2008; Buggs *et al.* 2011; Conant *et al.* 2014; Gout and Lynch 2015; Panchy *et al.* 2016; Cheng *et al.* 2018; Nieto Feliner *et al.* 2020).

While cytonuclear incompatibilities arising in homoploid hybrid species and their roles in homoploid hybrid speciation have been described for many species (Levin 2003; Greiner *et al.* 2011; Burton and Barreto 2012; Burton *et al.* 2013; Sloan *et al.* 2017), the problem of maintaining coordinated expression after genome merger coupled with whole genome duplication has only recently been considered and may be particularly acute for nuclear-encoded organelle-targeted proteins whose organelle-encoded interacting partners derive from only one of the two parents (Sharbrough *et al.* 2017). In addition to issues surrounding parental divergence and potential copy number variability in some organelle-interacting genes, allopolyploid species both face additional challenges relating to their massive duplication, including nucleotypic effects (Doyle and Coate 2019), and harbor additional mechanisms for resolving conflict, such as homoeologous exchange (Gaeta and Pires 2010; Bird *et al.* 2018; Mason and Wendel 2020). Consequently, a number of co-evolutionary processes might operate to balance the interaction between the nucleus and organelles, including copy number changes in organelle-interacting nuclear genes, increased organellar biogenesis, up-regulation of maternal and/or organellar genes with concomitant paternal down-regulation, selection for gene conversion or other mutations favoring maternal-like sequences, and pseudogenization of incompatible paternal copies (Sharbrough *et al.* 2017; Doyle and Coate 2019).

Recent research has begun to shed light on the extent and consequences of cytonuclear incompatibility in polyploid species. One of the first examples came from the genus *Gossypium*, in which the Rubisco complex exhibits maternally biased homoeolog retention, expression levels, and asymmetric gene conversion (Gong *et al.* 2012), and these observations were extended for Rubisco in phylogenetically disparate allopolyploids including *Arabidopsis*, *Arachis*, *Brassica*, and *Nicotiana* (Gong *et al.* 2014). Similar results were seen for the organelle-interacting gene *MS1* in allohexaploid wheat (ABD genomes in a B cytoplasm), where only B-homoeologs exhibited expression, and homoeologs from the non-matching (AD) genomes were epigenetically silenced (Wang *et al.* 2017b). The recently formed allotetraploid *Tragopogon miscellus* also exhibited maternal bias for cytonuclear related genes, but only for a subset of the

naturally occurring *T. miscellus* individuals surveyed and none of the synthetic individuals (Sehrish *et al.* 2015; Shan *et al.* 2020). Similar observations were made for synthetic allopolyploids from *Cucumis* (Zhai *et al.* 2019), rice (Wang *et al.* 2017a), and in both the recent natural and newly synthesized forms of allopolyploid *Brassica* (Ferreira de Carvalho *et al.* 2019), suggesting that cytonuclear coordination may not occur immediately in nascent polyploid species.

Here we examine the evolutionary consequences of genome merger and doubling on the expression of nuclear-encoded genes whose products are targeted to the mitochondria or plastids and interact with mitochondrial and/or plastid gene products (*i.e*., cytonuclear genes). Using five independent polyploid events in four genera that encompass a range of polyploid ages and diploid divergence times, we quantify patterns of homoeolog usage in cytonuclear genes and patterns of total expression. We look for evidence of cytonuclear accommodation by testing the hypotheses that cytonuclear genes of allopolyploid taxa exhibit (1) maternally biased homoeolog expression and/or (2) maternal expression level dominance (*i.e.,* expression patterns that more closely resemble maternal diploids than paternal diploids), reflecting a response to the historical coevolution between the maternal subgenome and the maternally inherited organelles.

Methods

Plant Materials and sequencing.

Five plants were grown for each diploid and polyploid representative from four genera: *Arabidopsis, Arachis, Chenopodium*, and *Gossypium* (Supplementary Table 1). Growth conditions for each genus are listed below.

Arabidopsis. Allopolyploid *Arabidopsis suecica* (*Arabidopsis thaliana* x *Arabidopsis arenosa*) accession CS22505 seeds were acquired from Andreas Madlung (University of Puget Sound, Washington USA). These were grown in a common incubator with representatives of the parental species, *Arabidopsis arenosa* (paternal, accession CS3901xKB3) and *Arabidopsis thaliana* Landsberg *erecta* (maternal) whose seeds were provided by Roswitha Shmickl (Charles University, Prague) and Andreas Madlung, respectively. Seeds were surface sterilized using 70% v/v ethanol and placed on Murashige and Skoog (MS) plates for vernalization and germination. After the vernalization period (*i.e.*, two weeks at 4 ºC), plates were moved to their growing conditions (20ºC, 16/8 hours light/dark). Once germinated, seeds were moved to 6-inch diameter pots with potting soil (Sungro SUN52128CFLP). After several weeks of growth, plants were winterized (8ºC, 10/14 hours light/dark) to induce flowering. Once plants were mature, leaves were harvested from each plant at a uniform time of day (midday) and flash frozen for RNA extraction.

Arachis. *Arachis* was represented by two allopolyploid genotypes, *i.e.*, *Arachis hypogea* cv. Tifrunner (Holbrook and Culbreath 2007) and the synthetic (*Arachis ipaensis* x *Arachis duranensis*)^{4x} known as IpaDur1 ((Fávero *et al.* 2006; Leal-Bertioli *et al.* 2018); hereafter

Arachis IpaDur1), as well as their two model diploid progenitors, *Arachis duranensis* (accession V14167) and *Arachis ipaensis* (accession K30076). Notably, these two allopolyploid species have opposite parentage; *Arachis duranensis* is maternal for *Arachis hypogea* but paternal for *Arachis* IpaDur1. All species were grown in an environmentally-controlled greenhouse at the University of Georgia. The first expanded leaves were collected from eight-week-old plants; these were flash frozen in liquid nitrogen and shipped on dry ice to Iowa State University for RNA extraction.

Chenopodium. The allopolyploid species *Chenopodium quinoa* accession QQ74 was grown along with the model progenitor species *Chenopodium pallidicaule* (maternal; PI 478407) and *Chenopodium suecicum* (paternal) by David Brenner in the United States Department of Agriculture (USDA, Ames, Iowa) greenhouse at Iowa State University and provided as living material. Samples were harvested directly from the greenhouse at a uniform time of day and flash frozen in liquid nitrogen for RNA extraction.

Gossypium. Gossypium was represented by two allopolyploid species, *i.e.*, *Gossypium hirsutum* cultivar TM1 and *Gossypium barbadense* accession GB379, and their two model diploid progenitors, *Gossypium arboreum* (maternal) and *Gossypium raimondii* (paternal). Samples were grown from seed in a common environment in the Pohl Conservatory at Iowa State University. Seeds were planted in 2 gallon pots with a custom potting mixture of 4:2:2:1 Sungro soil : perlite : bark : chicken grit. *Gossypium* was grown to maturity (minimum of 6 months) under typical greenhouse conditions, collected at a uniform time of day, and flash frozen in liquid nitrogen for RNA extraction.

All plants: A minimum of five replicates (leaf tissue) were collected for each species. RNA was extracted from the *Arabidopsis*, *Arachis*, and *Chenopodium* samples using the Direct-zol RNA kit (Zymo Research), including 600ul of Trizol. For *Arachis,* an additional grind step in 600ul of Trizol using ⅛ inch diameter steel beads (1-2 minutes of vortexing) immediately followed the initial grind in liquid nitrogen, and 400ul of additional Trizol was added for extraction. All other steps follow the manufacturer protocol. *Gossypium* samples were extracted with the Spectrum Total Plant RNA kit (Sigma) following the manufacturer protocol. In total, 17 *Arabidopsis*, 20 *Arachis*, 15 *Chenopodium*, and 20 *Gossypium* samples were extracted for RNAseq (Supplementary Table 1). RNA was quantified using the Agilent 2100 BioAnalyzer and sent to the Yale Center for Genome Analysis (YCGA) for library construction and sequencing. Illumina libraries were constructed using the TruSeq Stranded Total RNA kit with Ribo-Zero Plant and sequenced on a NovaSeq 6000 S4 flow cell. A minimum of 40 million read pairs (2 x 150 nt) was generated for each sample. Raw sequencing reads are available through the Short Read Archive (SRA) under PRJNA726938.

Reference preparation and RNA-seq processing

Reference sequences for each genus were prepared by concatenating primary transcripts for each polyploid species with transcripts for each organelle (Supplementary Table 2). Primary transcripts were derived from recent genome sequences published for *Arabidopsis suecica* (Novikova et al. 2017), *Arachis hypogea* (Bertioli *et al.* 2019), *Chenopodium quinoa* (Jarvis *et al.* 2017), and *Gossypium hirsutum* (Chen *et al.* 2020). RepeatMasker (Smit *et al.* 2015) was used to mask each set of nuclear primary transcripts with both the organellar genomes and transcriptomes (Supplementary Table 2, and see below) for each species, and any transcript with fewer than 75 nucleotides of non-organelle derived sequence was discarded. Mitochondrial and plastid transcripts for each genus were derived from publicly available organelle genome annotations for a single representative species from each genus (Supplementary Table 2), with the exception of *Arachis* mitochondrial genes (see below). Each protein-coding gene set was manually curated to (1) add genes that were absent from the GenBank annotations (via BLAST identification; (Camacho *et al.* 2009)), (2) remove duplicate gene copies from the plastid inverted repeat, (3) remove non-conserved hypothetical genes, and (4) standardize gene naming conventions. Because there is no complete mitochondrial genome published for any *Arachis* species, we used available transcriptomic and genomic resources to extract protein-coding sequences for *Arachis* mitochondrial genes. Most genes were recovered by performing tBLASTN of *Arabidopsis* protein sequences against an unpublished dataset of *Arachis hypogaea* full-length cDNAs generated with PacBio Iso-Seq technology (NCBI Sequence Read Archive accession SRR14414925), and the remaining mitochondrial genes were extracted by searching against *Arachis hypogaea* genomic contigs in PeanutBase (Dash *et al.* 2016). Our curated mitochondrial and plastid protein-coding reference sequences for each taxon are available via https://github.com/Wendellab/CytonuclearExpression.

RNA-seq reads for each species were processed via Kallisto v0.46.1 (Bray *et al.* 2016) (i.e., *kallisto quant*) to assign orthologs and/or homoeologs to genes and quantify transcripts. Following Kallisto quantification, a principal component analysis (PCA) was generated for each genus using SNPRelate (Zheng *et al.* 2012) in R/4.0.2 (R Core Team 2020) to verify sample identity and generate an overview of the count data. PCA plots were visualized using ggplot2 (Wickham 2016) in R. Clustering heatmaps were generated using pheatmap (Kolde 2012). Code pertaining to this project can be accessed at https://github.com/Wendellab/CytonuclearExpression.

Ortholog identification and targeting inference

We followed the methods of (Sharbrough *et al.* 2021) to identify orthologous genes arising from allopolyploidy (*i.e.*, 'quartets' consisting of one homolog from each diploid parent and two homoeologs from the allopolyploid). Briefly, we used Orthofinder (v2.3.8) (Emms and Kelly 2019) to cluster protein coding genes into homologous gene families. We retained orthogroups containing three or more homologs, extracted coding sequences (CDS) for those proteins, and

aligned each using the L-INS-i algorithm in MAFFT (v7.480) (Katoh and Standley 2013). Model selection was done using jModelTest2 v2.1.10 (Darriba *et al.* 2012) and phylogenetic inference was performed in PhyML v3.3.20211021 (Guindon and Gascuel 2003), as previously described (Sharbrough *et al.* 2021). Because these gene trees often contain multiple orthologous groups resulting from ancient duplications, we extracted subtrees containing potential quartets (*i.e.* subtrees with the expected number of genes from each species) using *subTreeIterator.py* (Sharbrough *et al.* 2021). We merged these phylogenetically-based quartet predictions with independent synteny-based quartet predictions (generated via pSONIC; (Conover *et al.* 2021)) to identify high-confidence quartets. Quartets that were predicted by at least one method and were not in conflict with the second method were retained for analysis. Each quartet was analyzed for organelle targeting information using combined information from (1) CyMIRA (Forsythe *et al.* 2019); (2) *de novo* targeting software, including iPSORT v0.94 (Bannai *et al.* 2002), LOCALIZER v1.0.4 (Sperschneider *et al.* 2017), Predotar v1.03 (Small *et al.* 2004), and TargetP v1.1b (Emanuelsson *et al.* 2007); and (3) Orthofinder-based homology to the *Arabidopsis thaliana* Araport 11 proteome. Full details can be found in (Sharbrough *et al.* 2021), and relevant scripts can be found at https://github.com/jsharbrough/CyMIRA_gene_classification and https://github.com/jsharbrough/allopolyploidCytonuclearEvolutionaryRate/blob/master/scripts/subTreeIterator.py, as well as https://github.com/Wendellab/CytonuclearExpression.

Differential gene expression

Differential gene expression analyses were conducted in R/4.0.2 (R Core Team 2020) using DESeq2 (Love *et al.* 2014) with the design `~species` and with the reference transcriptomes detailed above. Genes with a Benjamini–Hochberg (Benjamini and Hochberg 1995) adjusted p-value <0.05 (as implemented in DESeq2) were considered differentially expressed. Expression PCA and pheatmaps were made in R using the base R package and pheatmap v1.0.12. Differential expression (DE) was evaluated three ways: (1) DE between diploid progenitor and corresponding polyploid subgenome, (2) DE between each diploid progenitor and the total polyploid expression (*i.e.,* summed homoeolog expression), and (3) DE between maternal and paternal homoeologs. Enrichment of differential expression (DE) genes in cytonuclear gene categories was conducted using Fisher's Exact Test (*fisher.test*) relative to the not-organelle-targeted (NOT) category.

We employed a mixed-effects modeling approach to test whether differences in expression across homoeologs were related to cytonuclear targeting category (inferred from CyMIRA), legacy effects of diploid progenitors (estimated here as the difference in expression across diploid relatives), and the interaction between targeting category and legacy effects. Expression modeling was conducted in R/4.1.1 and considered the two models: (1) $\Delta rlog \sim$ Targeting, and (2) $\Delta rlog \sim$ Targeting + ΔrlogDiploid + Targeting × $\Delta rlog_{\text{Diploid}}$, where $\Delta rlog$ represents the difference in DESeq2-derived rlog normalized counts (maternal - paternal homoeolog),

ΔrlogDiploid represents the difference in DESeq2-derived rlog normalized counts between the model diploid progenitors, Targeting represents the CyMIRA identified targeting category, and Targeting × $\Delta rlog_{\text{Diploid}}$ represents the interaction between category and diploid expression levels. Fixed effects for each model were evaluated using emmeans v1.7.0 and the analysis of variance (ANOVA) was evaluated using car v3.0-11, with a type II computation of the sums-of-squares. Because model 1 is nested within model 2, we compared these two models for each species using lrtest from lmtest v0.9-39 in R/4.1.1.

Functional enrichment tests

CyMIRA-based results were verified for *Arabidopsis suecica, Gossypium hirsutum,* and *Gossypium barbadense* using FUNC-E in conjunction with existing functional annotations from INTERPRO (Jones *et al.* 2014), GO ontology (The Gene Ontology Consortium 2019), and Plant Ontology (available for *Arabidopsis* only; (Avraham *et al.* 2008)). *Arabidopsis* functional annotations were downloaded from TAIR (Cheng *et al.* 2017), and the *Gossypium* functional annotations were downloaded from CottonFGD (Zhu *et al.* 2017), both accessed in January 2022. These custom ontology lists were used to generate vocabulary terms for each FUNC-E analysis (one per species). Two sets of genes were used as queries in functional enrichment analyses, both of which are restricted to ortholog-homoeolog quartets with statistically significant differential expression between homoeologs (DESeq2 p-value < 0.05) that was also greater than fourfold. An additional criterion for the second query gene set required that the difference in fold change (FC) between homoeologs and FC between parental orthologs also had to be greater than four (*i.e.*, $|\Delta FC| > 4$). In both cases, the reference (*i.e.*, background) set was composed of quartets regardless of p-value and/or fold-change; these comprised 11,307 for *Arabidopsis suecica*, 18,669 for *Gossypium hirsutum*, and 18,099 for *Gossypium barbadense*. Functional enrichment was determined in FUNC-E via a one-sided Fisher's Exact Test for each comparison, and multiple tests were subjected to Benjamini correction; significance was determined as adjusted $p < 0.05$. By default, upregulated and downregulated genes were tested separately.

Results

Generation and categorization of reference sequences

Representative transcriptomes for each genus were downloaded along with both organellar genomes and transcriptomes (Supplementary Table 2). In the case of *Arachis*, only putative transcripts were available for the mitochondria (see methods). Because reference genomes frequently have nuclear insertions of organellar genes that can be included in predicted transcripts, we first masked each nuclear transcriptome (primary transcripts only) with both the matching organellar genomes and transcriptomes, and we subsequently removed transcripts with fewer than 75 surviving nucleotides. Between 206 and 2,510 nuclear transcripts were filtered from each reference, leaving between 44,175 and 73,595 non-organellar nuclear transcripts. These were combined with the curated organellar transcriptomes, consisting of 108 - 112 genes

in total (see methods), resulting in polyploid reference transcriptomes ranging from 44,283 to 73,707 genes (Table 1).

A curated set of high-confidence homoeologs was generated for each reference genome using a combination of phylogenetics and synteny (see methods), which were subsequently characterized by their potential to interact with either/both organelles (Table 1). The number of homoeologous pairs in each genome ranged from 9,231 in *Chenopodium quinoa* to 20,124 in *Gossypium hirsutum*, representing twice that number of genes (18,462 and 40,248 homoeologs, respectively). As expected, most genes (80-87%) were not predicted to be targeted to either organelle, with an average of 2-3% of genes placed in the six organelle-related categories (*i.e.*, mitochondria-/plastid-/dual-targeted, interacting/non-interacting genes; range = 0 - 11%; Table 1), as determined by CyMIRA (see methods). Of those genes exhibiting signatures of organelle targeting, homoeolog pairs that function in the organelle but do not have direct interactions with organellar-encoded proteins were generally more abundant, with the exception of mitochondria-targeted interacting genes, which were 1.5 - 2 times more abundant in most species (except *Arabidopsis thaliana*; Table 1) than the non-interacting mitochondrial genes. These targeting predictions were subsequently applied to the reference transcriptome generated for each genus (Table 1 and see methods).

We also evaluated the degree of homoeolog loss between the maternal and paternal genome for genes where orthologs were recovered from both model progenitors but only one polyploid subgenome (Table 2). If there is a general cytonuclear incompatibility between the diploid progenitors, then we would expect an excess in paternal homoeolog loss for genes involved in cytonuclear categories, *i.e.*, dual-targeted interacting (DI), dual-targeted non-interacting (DNI), mitochondria-targeted interacting (MI), mitochondrial-targeted non-interacting (MNI), plastid-targeted interacting (PI), and plastid-targeted non-interacting (PNI). Because the *Chenopodium quinoa* has a large number of genes not assigned to maternal/paternal subgenome, and the *Arachis hypogea* genome exhibits a high degree of homoeologous exchange (thereby reducing the number of reliable quartets), we restricted our analysis of putative homoeolog loss to *Arabidopsis suecica* and *Gossypium hirsutum* (Table 2). For most categories, there was no significant difference in paternal versus maternal homoeolog loss relative to background (*i.e.,* genes whose products are not targeted to either organelle (NOT); Fisher's Exact $p > 0.05$). Only one cytonuclear category from the two genomes (*i.e.,* DNI from *Arabidopsis*) exhibited biased homoeolog loss, and the distribution of loss was contrary to what is expected given maternal inheritance of organelles.

Sequencing yields and general gene expression

Because the aim of this study was to characterize cytonuclear accommodation at the level of gene expression in polyploid species, total RNA was extracted for each accession and

Table 1: Composition of the mapping reference for each genus. Primary transcripts from each nuclear transcriptome were masked using the organellar transcriptomes and genomes, and nuclear transcripts matching organellar sequences were removed. Gene quartets composed of a single gene for each diploid species and two paired homoeologs from the polyploid reference were identified. Each quartet was classified with respect to putative organellar targeting. "Dual-targeted" transcripts are those that have targeting information for both organelles. "Interacting" transcripts code for products that interact with organellar gene products, whereas "non-interacting" transcripts are those which function in one or both organelles but do not physically interact with an organellar gene product.

	Arabidopsis	***Arachis***	***Chenopodium***	***Gossypium***
mitochondrial transcripts	32	32	30	35
chloroplast transcripts	78	76	78	77
nuclear transcripts	44,625	67,150	44,770	74,902
nuclear transcripts, excluding norgs	44,419	64,640	44,175	73,595
total transcripts	44,735	67,258	44,878	75,014
removed genes	206	2,510	595	1,307
total transcripts, excluding norgs	44,529	64,748	44,283	73,707
Homoeologous pairs (genome)	12,254	11,671	9,231	20,124
Not Targeted	9,830	10,121	7,575	17,606
Dual-targeted, interacting	45	52	62	76
Dual-targeted, non-interacting	185	746	771	1,103
Mitochondria-targeted, interacting	263	156	169	326
Mitochondria-targeted, non-interacting	467	94	84	135
Plastid-targeted, interacting	168	133	159	246
Plastid-targeted, non-interacting	1,296	369	411	632

Table 2. The number of paternal or maternal homoeologs lost from *Arabidopsis* and *Gossypium* for each category, and the proportion that represent maternal losses. If broad cytonuclear incompatibilities exist, we expect that the number of maternal homoeologs lost should be fewer in cytonuclear gene categories than for the rest of the genome, represented by low numbers in the %maternal columns. Cytonuclear categories that are statistically different in distribution from Non-organelle-targeted (NOT) genes are marked with an * in the column where loss is greater than expected by the NOT category (Fisher's exact p <0.05).

	Arabidopsis			*Gossypium*		
	paternal loss	maternal loss	% maternal	paternal loss	maternal loss	% maternal
Not-organelle-targeted	673	439	39%	342	542	61%
All Cytonuclear	106	81	46%	21	30	59%
Dual-targeted_Interacting	2	3	60%	0	2	100%
Dual-targeted_Non-interacting	2	8*	80%	9	13	59%
Mitochondria-targeted_Interacting	19	10	34%	4	4	50%
Mitochondria-targeted_Non-interacting	20	16	44%	1	2	67%
Plastid-targeted_Interacting	12	7	37%	1	2	67%
Plastid-targeted_Non-interacting	51	45	47%	6	7	54%

ribodepletion was used to remove ribosomal RNAs, circumventing the bias of polyA-selection protocols that exclude some organellar transcripts (Slomovic *et al.* 2006, 2008; Smith 2013). As expected, transcripts from the organelles were abundant Supplementary 3; however, sufficient nuclear transcriptome coverage was achieved, ranging from 26 to 90 M reads per sample (averages are *Arabidopsis* = 61 M, *Arachis* = 36 M, *Chenopodium* = 44 M, *Gossypium* = 61 M). One replicate each for *Arachis hypogea* and *Arachis* IpaDur1 was removed due to low mapping rates (i.e., < 25% of reads mapped; averages without outliers are *Arabidopsis* = 79%, *Arachis* = 82%, *Chenopodium* = 62%, and *Gossypium* = 72% of reads mapped). PCA and hierarchical clustering of the gene expression data exhibit clustering of replicates for each species within a genus, with one exception. *Chenopodium suecicum* replicate #1 was placed intermediate among all *Chenopodium* species via PCA (Supplementary Figure 1), and it clustered with *Chenopodium quinoa* via hierarchical clustering. Because this sample may represent a contaminated hybrid, it was excluded from subsequent analyses.

In general, the polyploid species exhibited more up-regulated genes than down-regulated genes relative to their diploid counterparts, both with respect to homoeolog-progenitor comparisons and total polyploid expression (Table 3). This pattern was most prominent in cotton, where all comparisons exhibited more up-regulated than down-regulated genes in polyploids (chi^2 $p<0.05$), followed by *Arabidopsis suecica*, where all maternal comparisons exhibited more up-regulation. Conversely, *Chenopodium quinoa* only exhibited more up-regulation of the total polyploid expression (*i.e.*, the summed expression of homoeologs), and the natural peanut polyploid, *Arachis hypogea*, only exhibited more up-regulation of maternal homoeologs relative to expression in the model maternal diploid progenitor, *Arachis duranensis* (Table 3). Interestingly, the synthetic allotetraploid, *Arachis* IpaDur1 also exhibits more up-regulation of *Arachis duranensis* homoeologs, here functioning as the paternal diploid progenitor, with concomitant down-regulation in expression of homoeologs from the maternal diploid parent, *Arachis ipaensis*, potentially indicating a general bias toward *Arachis duranensis* expression.

Expression level dominance in cytonuclear genes

Expression level dominance (ELD) is a phenomenon whereby the combined expression of homoeologs in a polyploid is statistically similar to one diploid parent and statistically dissimilar from the other parent. In the context of cytonuclear compatibility, we might expect a bias toward the maternal diploid expression level (*i.e.*, ELD) for the combined expression of both homoeologs in cytonuclear gene categories. When we consider expression level dominance of nuclear genes within each species, irrespective of category (*i.e.,* NOT or any cytonuclear category), we see a general bias towards maternal ELD for *Arachis hypogea* and both species of *Gossypium* (binomial test, $p < 0.05$), but not for *Arabidopsis suecica* or *Chenopodium quinoa* (binomial test, $p > 0.05$; Table 4; Supplementary Table 4). These results are also reflected in the NOT category itself, where *Arachis hypogea* and both *Gossypium* species exhibit bias toward maternal ELD. Interestingly, however, when we compare patterns of ELD for all organelle

Table 3. The total number of genes passing filter (see methods), and the number that are differentially expressed (parsed as up or down regulated). Cells that are highlighted are significantly different from equal (up-regulation vs down-regulation); chi2 < 0.05). Note that the different number of genes in the diploid-polyploid comparison (parsed as homoeologs) reflect differences in survivability in the DE analysis.

		Gossypium hirsutum		*Gossypium barbadense*		*Chenopodium quinoa*		*Arabidopsis suecica*		*Arachis hypogea*		*Arachis* IpaDur1	
diploid divergence		5 - 10 mya				11 mya		6 - 8 mya		2.2 mya			
polyploid origin		1 - 2 mya				2.5 - 3 mya		16 kya		9,400 ya		synthetic	
		A	D	A	D	A	B	T	A	D	I	I	D
diploid-polyploid, parsed as homoeologs	Total genes	18,197	18,355	18,197	18,355	8,603	8,616	11,154	11,225	10,154	10,404	10,404	10,154
	down-regulated	2,803 (15%)	3,912 (21%)	2,617 (14%)	3,461 (19%)	2,390 (28%)	1,984 (23%)	1,358 (12%)	1,888 (17%)	328 (3%)	1,778 (17%)	2,290 (22%)	28 (0%)
	up-regulated	3,166 (17%)	4,390 (24%)	2,908 (16%)	4,052 (22%)	2,519 (29%)	1,965 (23%)	1,478 (13%),	1,987 (18%)	593 (6%)	1,716 (16%)	1,868 (18%)	166 (2%)
diploid-polyploid, total expression in polyploids versus one or the other diploid	Total genes	18,792	18,792	18,792	18,792	8,813	8,813	11,610	11,610	10,645	10,645	10,645	10,645
	down-regulated	3,387 (18%)	4,012 (21%)	3,133 (17%)	3,529 (19%)	2,322 (26%)	2,435 (27%)	1,828 (16%)	2,040 (18%)	669 (6%)	1,722 (16%)	1,617 (15%)	90 (1%)
	up-regulated	4,085 (22%)	4,691 (25%)	3,944 (21%)	4,351 (23%)	2,551 (29%)	2,398 (27%)	2,160 (19%)	2,086 (18%)	609 (6%)	1,862 (18%)	1,893 (18%)	108 (1%)

Table 4. Number of genes exhibiting expression level dominance (ELD) toward each parental expression level, parsed by cytonuclear category. Categories are dual-targeted interacting (DI), dual-targeted non-interacting (DNI), mitochondria-targeted interacting (MI), mitochondrial-targeted non-interacting (MNI), plastid-targeted interacting (PI), and plastid-targeted non-interacting (PNI).

	Arabidopsis suecica		*Arachis hypogea*		*Arachis hypogea*		*Chenopodium quinoa*		*Gossypium barbadense*		*Gossypium hirsutum*	
Category	Maternal ELD	Paternal ELD	Maternal ELD†	Paternal ELD	Maternal ELD	Paternal ELD†	Maternal ELD	Paternal ELD	Maternal ELD†	Paternal ELD	Maternal ELD†	Paternal ELD
Not-organelle-tar	1481	1486	1659	365	41	2026	1294	1293	2961	2421	3063	2273
All cytonuclear	419	409	361*	54	1	420	361*	288	430	345	462	325
DI	8	8	11	1	0	11	15	14	9	12	12	15
DNI	31	30	184*	25	0	203	184*	126	194	156	213	156
MI	51	39	23	8	0	39	42*	21	47	46	54	33
MNI	85	82	26	4	1	28	10	14	23	26	29	19
PI	22	37	32	2	0	38	31	29	48*	21	46	30
PNI	222	213	85	14	0	101	79	84	109	84	108	72
† parental ELD is significantly different from 1:1 and biased toward the noted parent												
* cytonuclear category distribution (maternal versus paternal) is significantly different from the distribution in the NOT category and overrepresented by the noted parent												

targeted genes versus those in the NOT category, we find that *Arachis hypogea* and *Chenopodium quinoa* have significantly more genes (Fisher's exact, $p<0.05$) exhibiting ELD in maternally-biased categories (*i.e.,* categories IV and IX; Supplementary Table 4) than expected from the overall distribution of maternal and paternal ELD, whereas both species of *Gossypium* exhibited similar patterns of ELD for cytonuclear genes as NOT genes. Notably, *Arachis* IpaDur1 exhibited an excess of paternal ELD, which is in contrast to the maternal ELD exhibited by *Arachis hypogea* but biased toward the same diploid parent (*i.e.,* biased toward *Arachis duranensis* in both cases). On the level of individual categories, four categories in three species exhibit an excess of ELD (Fisher's exact, $p < 0.05$), all maternally biased: *Arachis hypogea*, DNI; *Chenopodium quinoa*, DNI and MI; and *Gossypium barbadense*, PI. All other individual categories exhibited similar ELD bias as displayed by the NOT genes for that species (Table 4).

We also identified some genes in these polyploids with expression levels that fell outside the range of the two parental diploid models (*i.e.*, transgressive expression), which may be associated with organelle copy number in a cytonuclear context. When considering all genes, regardless of targeting, *Arabidopsis suecica* and both *Arachis* species have statistically similar numbers of genes that are transgressive down-regulated (categories III, VII, and X in Supplementary Table 4) as transgressive up-regulated (categories V, VI, and VIII), whereas *Chenopodium quinoa* and both species of *Gossypium* have ~20-35% more genes exhibiting transgressive up-regulation (versus down-regulation; Supplementary Table 4). Accounting for these global patterns, we find no species-category combinations exhibiting transgressive expression patterns in cytonuclear genes that are statistically different from NOT genes (after Benjamini-Hochberg *p*-value correction for multiple testing), although we note that many of these cytonuclear categories had very few genes (Supplementary Table 4) and are therefore difficult to statistically characterize.

Homoeolog expression in cytonuclear genes

We evaluated homoeolog expression for each polyploid species in the context of the six cytonuclear categories with the biological expectation that maternal homoeologs should be preferentially up-regulated relative to paternal homoeologs (Figure 1 and Supplementary Tables 5-8). Figure 1 summarizes the results of the homoeolog comparisons for each homoeolog in 2 x 2 grids for each species-category, where maternal (left) and paternal (right) expression is measured relative to the model diploid progenitor and over-/under-representation is determined relative to the pattern observed in NOT genes (*i.e.*, background). Because cytonuclear incompatibility predicts upregulation of the co-evolved maternal cytonuclear homoeologs and down-regulation of the evolutionarily more distant paternal homoeologs, we expect a combination of the following patterns (Figure 1): (1) overrepresentation (depicted in red) for maternal homoeolog up-regulation (upper left square), (2) overrepresentation (red) for paternal homoeolog down-regulation (lower right square), (3) underrepresentation (depicted in blue) for

maternal homoeolog down-regulation (lower left square), and/or (4) underrepresentation (blue) for paternal homoeolog up-regulation (upper right square). In general, fewer than half of the categories per polyploid species are consistent with cytonuclear incompatibility expectations, and, in both *Arachis* IpaDur1 and *Gossypium barbadense*, we do not observe any categories whose patterns are consistent with our biological expectations. None of the categories were consistent with cytonuclear expectations in more than two species, although each category was significant in at least one. Interestingly, the most frequently observed patterns were contrary to cytonuclear expectations (Figure 1); that is, 12 species-category comparisons contradict cytonuclear expectations (versus 7 consistent species-categories), although these contradictory patterns were also observed in no more than half of the categories per species.

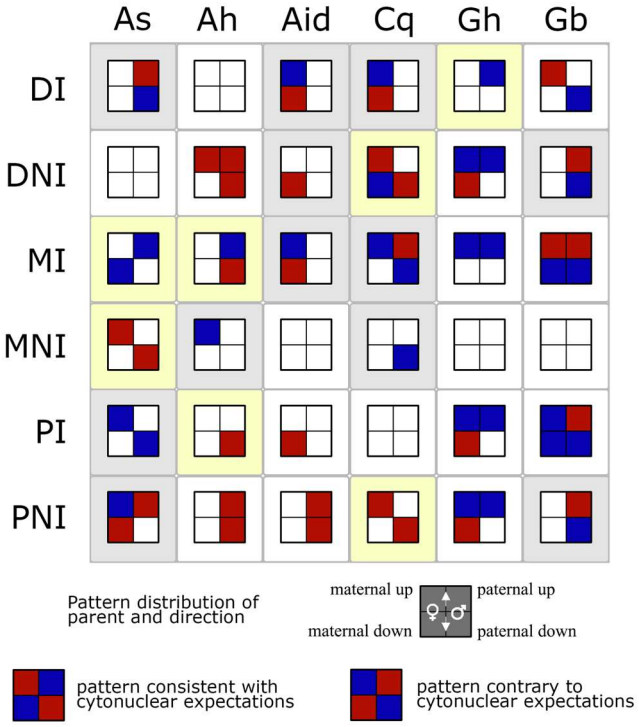

Figure 1. Summary of differential gene expression in cytonuclear categories for each polyploid species relative to each model diploid progenitor, partitioned as homoeologs. This pictogram displays the statistically significant (Fisher's exact $p < 0.05$) overrepresentation (red) or underrepresentation (blue) of up- or down-regulated genes for each category, relative to non-cytonuclear genes. Each species/category is represented by a four-square grid, where the rows specify regulation (up or down) and columns specify the homoeolog comparison (i.e., maternal homoeolog vs maternal progenitor and paternal homoeolog versus paternal progenitor, respectively). In each quadrant, red indicates that there were more genes statistically significant in that parent-category combination than was expected based on the NOT distribution, whereas blue indicates there were fewer statistically significant genes in that parent-category combination. Example color patterns consistent with and contrary to cytonuclear expectations are shown on the bottom. Species-category combinations highlighted in yellow are consistent with the hypothesis that cytonuclear accommodation in polyploid species favors expression from the "more compatible" maternal genome (via up-regulation) and/or diminishes expression from the potentially "less favorable" paternal genome (via down-regulation), whereas species-category highlighted in grey specifically contradict cytonuclear expectations. Species include *Arabidopsis suecica* (As), *Arachis hypogea* (Ah), *Arachis* IpaDur1 (Aid), *Chenopodium quinoa* (Cq), *Gossypium hirsutum* (Gh), and *Gossypium barbadense* (Ah). Categories include Dual-Targeted Interacting (DI), Dual-

Targeted Non-Interacting (DNI), Mitochondria-Targeted Interacting (MI), Mitochondria-Targeted Non-Interacting (MNI), Plastid-Targeted Interacting (PI), Plastid-Targeted Non-Interacting (PNI). All comparisons are relative to the Non-Organelle Targeted (NOT) genes.

We also directly compared expression between homoeologs to ascertain the extent (or lack) of maternal expression bias, both in general and with respect to cytonuclear categories (Figure 2). Homoeolog expression bias (HEB) is distinct from expression level dominance (ELD) in that HEB reports statistically different expression levels *between* homoeologs, whereas ELD (see above) refers to instances where the *total* gene expression (of both homoeologs) is similar to only one parent. We find that most of the polyploids (except the synthetic *Arachis* IpaDur1) exhibit more genes with paternal HEB versus maternal, for all paired homoeologs regardless of category (Table 5). When these genes are partitioned into cytonuclear categories, however, we detect maternal bias for some individual categories, most notably *Arabidopsis suecica* and *Chenopodium quinoa*, where four of the six cytonuclear categories have more genes with maternal bias than paternal. In most cases, this directional shift toward maternal bias is not statistically significant from the NOT distribution (Fisher's Exact Test, $p > 0.05$) and may either represent a lack of biological relevance or a lack of statistical power due to the small numbers in many of these categories (Table 5). The only categories that did exhibit statistically significant higher numbers of maternally HEB were the PI and PNI categories from *Arabidopsis suecica* and DI from *Gossypium hirsutum*. The latter may be somewhat surprising not only because this is the sole maternally biased category from either *Gossypium* species, but also because the closely related species *Gossypium barbadense* exhibits three cytonuclear categories with bias in the opposite direction (more paternal HEB than is expected from the NOT distribution, *i.e.,* DNI, PI, and PNI; Table 5).

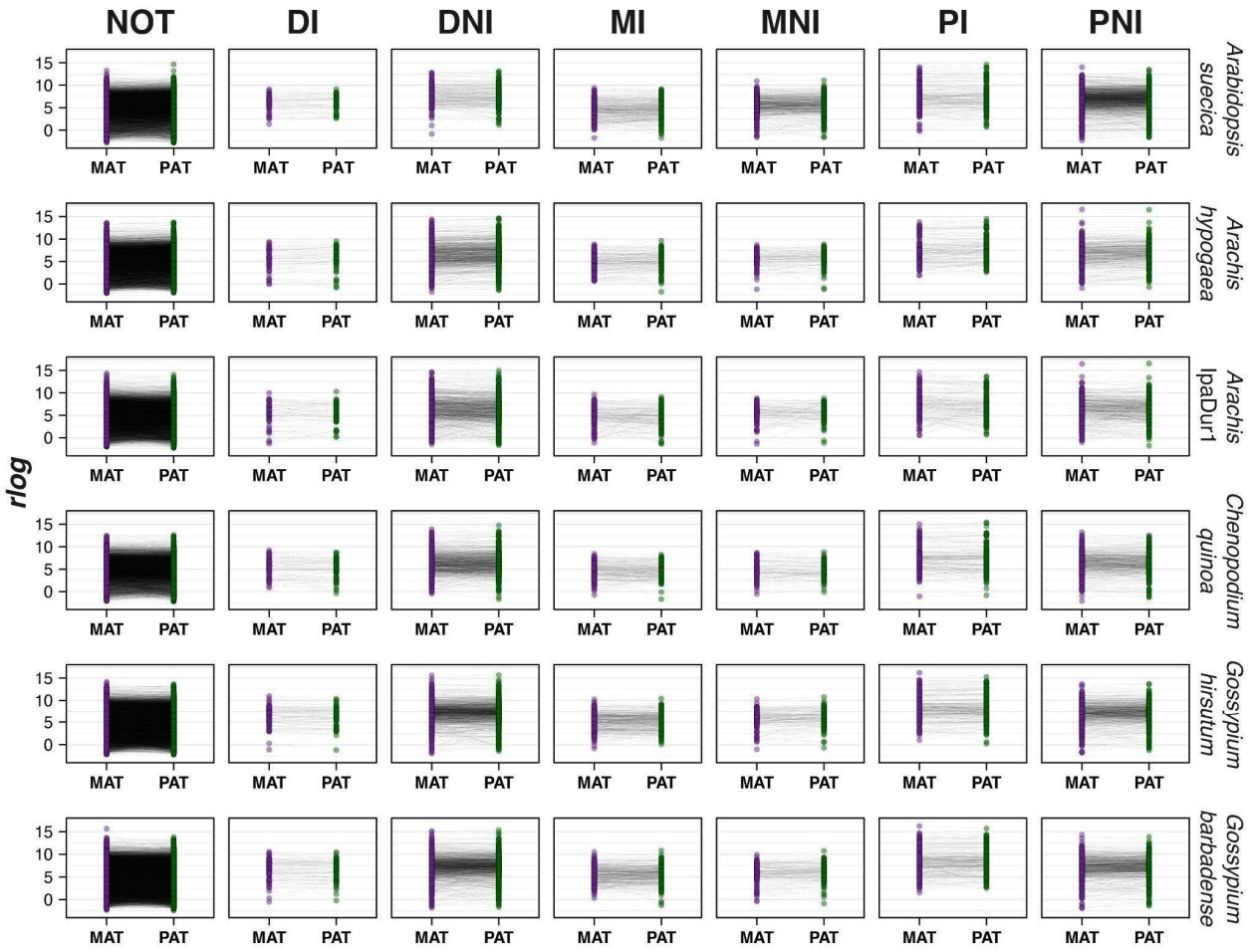

Figure 2. Mean normalized gene expression across homoeologs of six allotetraploids. Mean rlog values (circles) from 4-5 biological replicates each are depicted for maternal (left, purple) and paternal (right, green) homoeologs, partitioned into seven functional categories: Non-organelle-targeted (NOT), Dual-targeted Non-Interacting (DNI), Mitochondria-targeted Non-Interacting (MNI), Plastid-targeted Non-Interacting (PNI), Dual-targeted Interacting (DI), Mitochondria-targeted Interacting (MI), and Plastid-targeted Interacting (PI). Semi-transparent lines connect maternal and paternal homoeologs.

Table 5. Homoeolog expression biases for each polyploid, partitioned as maternal and paternal bias. Bias is considered when homoeolog expression is statistically significant (adjusted p< 0.05), regardless of the magnitude of the change. The distribution of maternally-paternally biased genes for each cytonuclear category was evaluated relative to the NOT category using a Fisher's Exact Test. Significant deviations (p<0.05) from the NOT distribution are noted by an asterisk, and the column (maternal or paternal) designates the parental bias that is overrepresented for that category.

	Arabidopsis suecica		***Arachis hypogea***		***Arachis* IpaDur**		***Chenopodium quinoa***		***Gossypium hirsutum***		***Gossypium barbadense***	
	Maternal bias	**Paternal bias**	**Maternal bias**	**Paternal bias**	**Maternal bias**	**Paternal bias**	**Maternal bias**	**Paternal bias**	**Maternal bias**	**Paternal bias**	**Maternal bias**	**Paternal bias**
Total	**1634**	**1887†**	**757**	**836†**	**2282†**	**1678**	**1376**	**1613†**	**3282**	**3690†**	**2536**	**2734†**
NOT	1251	1527	628	689	1878	1397	1088	1310	2836	3151	2262	2345
DI	5	4	3	3	11	12	10	9	22*	13	7	8
DNI	33	29	64	74	198	136	125	142	184	231	127	172*
MI	53	53	6	11	36	24	16	35*	64	73	24	36
MNI	67	75	8	9	24	20	18	14	23	29	14	24
PI	28*	17	13	18	46	26	37*	22	41	55	27	47*
PNI	197*	182	35	32	89	63	82	81	112	138	75	102*

† parental HEB is significantly different from 1:1 and biased toward the noted parent

* cytonuclear category distribution (maternal versus paternal) is significantly different from the distribution in the NOT category and overrepresented by the noted parent

We further evaluated the possible effects of cytonuclear category membership on homoeolog expression using linear modeling. We began with a model that asked if the difference in observed expression between maternal and paternal homoeologs was a function of where it was targeted ($\Delta rlog$ ~ Targeting) using the six aforementioned categories. For this model, we evaluated expression in each polyploid as a difference in *rlog* normalized counts (derived from DESeq2) between the maternal homoeolog and the paternal homoeolog (as $\Delta rlog = rlog_{Maternal} - rlog_{Paternal}$). The results of this model (Table 6) suggest that membership in a cytonuclear category (*i.e.,* Targeting) does have an effect on the difference between homoeolog expression levels for *Arabidopsis suecica*, *Arachis* IpaDur1, *Chenopodium quinoa*, *Gossypium hirsutum,* and *Gossypium barbadense*, but it is not significant for *Arachis hypogea* (ANOVA, $p < 0.05$). The number and identities of categories with fixed effects significantly different from NOT vary between polyploids (Table 6; Supplementary Figure 2), with the MNI category exhibiting significant fixed effects most frequently (3 of 5 significant polyploids) while MI is not significant for any polyploid. Contrasts among categories are even less suggestive of expression differences due to targeting for most species, although in *Arabidopsis suecica* most categories (except DI and MI) exhibited significantly greater expression differences between homoeologs than the NOT category ($p < 0.05$) and in the expected direction (*i.e.,* expression differences between homoeologs in those cytonuclear categories are more maternally biased than NOT). In the remaining species, only PI in *Chenopodium quinoa* and DNI in *Gossypium barbadense* were significantly different from the NOT category, the latter of which contradicted our expectations (*i.e.,* NOT in *Gossypium barbadense* is more maternally biased than is DNI; Table 6).

Importantly, this first model fails to account for the effects of parental legacy on expression levels in the polyploid and how deviations from parental expression levels may occur within the polyploid, the latter of which may be important depending on functional category (Supplementary Figure 3). Therefore, we repeated the analysis with a second model that also considered the difference in diploid expression as an explanatory term for the observed difference in homoeolog expression (*i.e.*, $\Delta rlog$ ~ Targeting + $\Delta rlog_{Diploid}$ + Targeting × $\Delta rlog_{Diploid}$). We find that both targeting category and legacy expression differences ($\Delta rlog_{Diploid}$, representing the difference in the *rlog* values for the maternal and paternal diploid model species) both affect homoeolog expression differences and strongly interact (Targeting × $\Delta rlog_{Diploid}$) in all comparisons (ANOVA, $p < 0.05$; Table 7). Unlike the previous model, all of the targeting categories are significant predictors of $\Delta rlog$ in at least two polyploid species (Table 7). Additionally, contrasts in all species (except *Arachis hypogea*) suggest that two to four targeting categories per species are significant predictors of differences in homoeolog expression beyond that predicted by non-organelle-targeted genes (Table 7). Interestingly, however, the direction of these differences is not consistent and in some cases are contrary to the biological expectation that homoeolog expression differences will be more maternally biased in categories that interact with the maternally-inherited organelles. Here we find few instances of greater expression divergence between homoeologs in targeting categories (versus NOT), which are

Table 6. Type III ANOVA-based p-value for the *Targeting* category. Estimated Marginal Means effect size for individual contrasts between *Targeting* categories are listed below, with significant categories ($p<0.05$) marked with an *.

	Arabidopsis suecica	*Arachis hypogea*	*Arachis ipadur*	*Chenopodium quinoa*	*Gossypium hirsutum*	*Gossypium barbadense*
Targeting (ANOVA p-value)	1.13E-26*	0.061	0.021*	0.007*	0.031*	1.13e-05*
Fixed Effects						
DI	0.1006	0.1147	-0.2537	0.1750	0.1351	0.1086
DNI	0.2202*	-0.0425	-0.0151	-0.0017	-0.0041	-0.0854*
MI	0.1190*	-0.0713	0.0537	-0.0500	-0.0453	-0.0451
MNI	0.0996*	0.1271	-0.3053*	0.0756	-0.1532*	-0.1351*
PI	0.2209*	-0.1035	0.1383	0.1862*	-0.0459	-0.1316*
PNI	0.1871*	-0.0318	-0.0401	0.0566	-0.0237	-0.0335
Contrasts						
NOT vs DI	-0.1006	-0.1147	0.2537	-0.1750	-0.1351	-0.1086
NOT vs DNI	-0.2202*	0.0425	0.0151	0.0017	0.0041	0.0854*
NOT vs MI	-0.1190	0.0713	-0.0537	0.0500	0.0453	0.0451
NOT vs MNI	-0.0996*	-0.1271	0.3053	-0.0756	0.1532	0.1351
NOT vs PI	-0.2209*	0.1035	-0.1383	-0.1862*	0.0459	0.1316
NOT vs PNI	-0.1871*	0.0318	0.0401	-0.0566	0.0237	0.0335
DI vs DNI	-0.1196	0.1572	-0.2386	0.1767	0.1392	0.1940
MI vs MNI	0.0194	-0.1983	0.3590	-0.1256	0.1079	0.0900
PI vs PNI	0.0338	-0.0717	0.1784	0.1297	-0.0222	-0.0982
DI vs MI	-0.0184	0.1860	-0.3074	0.2249	0.1804	0.1537
DI vs PI	-0.1203	0.2182	-0.3920	-0.0113	0.1810	0.2403
MI vs PI	-0.1019	0.0322	-0.0846	-0.2362	0.0006	0.0865
DNI vs MNI	0.1206	-0.1696	0.2902	-0.0773	0.1492	0.0497
DNI vs PNI	0.0331	-0.0107	0.0250	-0.0583	0.0196	-0.0519
MNI vs PNI	-0.0875	0.1589	-0.2652	0.0190	-0.1296	-0.1017

Table 7. Type III ANOVA-based p-values for the categories *Targeting* and *dipDelta,* and their interaction term. Estimated Marginal Means effect size for individual contrasts between *Targeting* categories are listed below, with significant categories ($p<0.05$) marked with an *.

	Arabidopsis suecica	*Arachis hypogea*	*Arachis ipadur*	*Chenopodium quinoa*	*Gossypium hirsutum*	*Gossypium barbadense*
Targeting	6.35E-31*	1.45E-04*	1.53E-10*	1.52E-10*	1.63E-08*	3.34E-14*
dipDelta	0*	0*	0*	0*	0*	0*
Targeting:dipDelta	3.58E-17*	7.17E-29*	1.86E-32*	4.89E-14*	7.97E-05*	6.98E-07*
Fixed Effects						
DI	0.2006	0.0326	-0.4834*	-0.3167*	0.1080	0.1288
DNI	0.3069*	-0.0800*	-0.1315*	0.0344	-0.0643*	-0.1225*
MI	0.6809*	0.0952	-0.3517*	-0.3461*	0.0674	0.0857*
MNI	0.2744*	0.1327	-0.1239	-0.1988*	-0.0095	0.0541
PI	0.4127*	-0.1174	-0.1389	0.1174*	-0.1570*	-0.1893*
PNI	0.1076*	-0.1208*	-0.0847	-0.0398	-0.0996*	-0.0883*
dipDelta	0.3809*	0.1800*	0.6092*	0.3879*	0.4879*	0.5021*
DI:dipDelta	0.0556	-0.1028*	-0.0122	0.1130*	-0.0330	0.0290
DNI:dipDelta	0.0765*	-0.0656*	0.1965*	0.0665*	-0.0226	0.0136
MI:dipDelta	0.1815*	0.0675*	0.3203*	0.0686*	0.1072*	0.1285*
MNI:dipDelta	0.0530*	0.0482	0.2568*	0.0626	0.0891*	0.1377*
PI:dipDelta	0.1723*	-0.0764*	0.1347*	0.0345	-0.0486	0.0445
PNI:dipDelta	0.0066	-0.0984*	0.0136	0.1420*	-0.0023	0.0356*
Contrasts						
NOT vs DI	-0.0742	-0.1483	0.4824*	0.2485	-0.1329	-0.1068
NOT vs DNI	-0.1330*	0.0062	0.1480*	-0.0748*	0.0472	0.1327*
NOT vs MI	-0.2681*	-0.0192	0.3785*	0.3047*	0.0136	0.0113
NOT vs MNI	-0.1538*	-0.0784	0.1454	0.1610	0.0768	0.0500
NOT vs PI	-0.0209	0.0315	0.1502	-0.1383	0.1202*	0.2229*
NOT vs PNI	-0.0926*	0.0101	0.0859	-0.0464	0.0978*	0.1152*
DI vs DNI	-0.0588	0.1545	-0.3344	-0.3233*	0.1801	0.2396
MI vs MNI	0.1142	-0.0592	-0.2331	-0.1437	0.0632	0.0386
PI vs PNI	-0.0717	-0.0214	-0.0644	0.0919	-0.0224	-0.1078
DI vs MI	-0.1939	0.1290	-0.1038	0.0562	0.1465	0.1182
DI vs PI	0.0533	0.1797	-0.3322	-0.3868*	0.2531*	0.3298*
MI vs PI	0.2472*	0.0507	-0.2283	-0.4430*	0.1067	0.2116*
DNI vs MNI	-0.0208	-0.0846	-0.0026	0.2358*	0.0296	-0.0828
DNI vs PNI	0.0404	0.0039	-0.0621	0.0284	0.0507	-0.0176
MNI vs PNI	0.0612	0.0885	-0.0596	-0.2074	0.0210	0.0652

limited to most categories for *Arabidopsis suecica* and the DNI category in *Chenopodium quinoa* (Table 7). Conversely, three categories each in *Arachis* IpaDur1, *Gossypium hirsutum*, and *Gossypium barbadense* and one in *Chenopodium quinoa* (MI) exhibit a greater difference between homoeologs for the NOT category, which contradicts the assumption that organelle-targeted homoeologs should preferentially up-regulate maternal homoeologs and/or down-regulate paternal homoeologs, both of which increase the difference in expression between homoeologs.

Tests of functional enrichment

Functional enrichment analyses were conducted for *Arabidopsis* and *Gossypium* to further assess whether the lack of clear cytonuclear patterns were also observable through broad functional categories (versus the heretofore used CyMIRA categorizations) for those species where suitable information was available. Using a list of species-specific vocabulary terms from existing resources (i.e., TAIR (Cheng *et al.* 2017) and CottonFGD (Zhu *et al.* 2017)) to annotate our gene sets, we compared the suite of genes with greater than four-fold differences in homoeolog expression (maternal *vs.* paternal) with those that exhibited any difference in homoeolog expression (regardless of fold-change or significance). More functional annotations are available in the model genus *Arabidopsis*, so it is unsurprisingly that a greater number of terms were enriched for *Arabidopsis* (126 terms; Supplementary Table 9) compared to *Gossypium* (75 and 52 terms for *Gossypium hirsutum* and *Gossypium barbadense*, respectively; Supplementary Table 10). Enriched terms in both *Arabidopsis suecica* and *Gossypium barbadense* were nearly evenly split with respect to parental bias, contrary to the general bias toward paternal homoeolog expression. In *Arabidopsis suecica*, 65 (out 126) terms exhibited paternal expression bias; likewise, 24 (out of 52) enriched terms exhibited paternal bias in *Gossypium barbadense*. Conversely, *Gossypium hirsutum* exhibited a clear maternal bias in enriched terms, *i.e.,* 53 maternally-biased terms versus 22 paternally-biased (Supplementary Table 10). Of those terms exhibiting enrichment in DE genes (>fourfold change, relative to background), only *G. barbadense* contained organelle relevant terms (*i.e.*, GO:0009523, photosystem II; GO:0009654, photosystem II oxygen evolving complex; IPR002683, PsbP C-terminal; and GO:0015979, photosynthesis) all of which exhibited a general bias towards maternal expression (Supplementary Table 10). Because a given gene can have multiple Gene Ontology (GO) and/or InterPro (IPR) terms associated with it, these four vocabulary terms represent only 5 genes in *G. barbadense* with an average 4.9-fold difference between homoeologs. Notably, all four organelle relevant terms exhibited a general bias towards maternal expression (Supplementary Table 10), consistent with the biological expectation of preference for maternal cytonuclear genes.

Interestingly, although *Gossypium barbadense* had the only organelle-relevant terms, none of these remain enriched when the analysis is restricted to only those genes exhibiting more than a fourfold difference in expression between homoeologs *and* whose fold-change between homoeologs compared to fold-change between diploids was at least 4.0 (*i.e.,* $\Delta FC_{Homoeolog} > 4$ & $\Delta FC_{Diploid} > 4$; see methods; Supplementary Table 10), possibly indicating that some of the

observed differences are best explained by the diploid progenitors. Conversely, while no organelle related terms were enriched in *Arabidopsis suecica* when only homoeolog fold change ($\Delta FC_{\text{Homoeolog}} > 4$) was thresholded, a different functional term (*i.e.*, GO:0009941; "chloroplast envelope") did show enrichment in the restricted set ($\Delta FC_{Homoeolog} > 4$ & $\Delta FC_{Diploid} > 4$). Chloroplast envelope is associated with six pairs of maternally-biased, DE homoeologs whose average 10.6-fold difference in expression is substantially different from the average 0.6-fold difference in expression between parental orthologs. Interestingly, while chloroplast envelope alone is enriched here (and maternally-biased) for *Arabidopsis suecica*, the expression patterns in plastid-related CyMIRA categories (relative to the diploid parents) generally contrast our expectation of maternal up-regulation and/or paternal down-regulation (Figure 1).

Expression accommodation in Rubisco

Previous analyses of the Rubisco small subunit (*rbcS*) cytonuclear gene family in multiple polyploid species reported patterns of maternally-biased gene conversion and preferential expression of maternally-converted paternal homoeologs (Gong *et al.* 2012, 2014); therefore, we specifically extracted expression patterns for rbcS from the current data. Consistent with previous results (Gong *et al.* 2012, 2014), we found that *rbcS* is composed of a small gene family in each polyploid species (Table Rubisco). Because our analyses are based on available genomic and/or transcriptomic reference sequences, which are far less developed for *Arachis hypogea*, we were unable to assign subgenomes (nor assess expression) for the six *rbcS* copies detected in either *Arachis* polyploid. For the remaining polyploids, the number of copies assigned to subgenome and/or paired as homoeologs varied depending on available information. In most cases where strict homoeologs could not be identified, it was due to copy number variation in the annotation. For example, the *Arabidopsis suecica* genome is divided into *Arachis thaliana* (maternal) and *Arabidopsis suecica* (paternal) contigs; however, seven of the nine *rbcS* copies are in two tandem arrays (AsAa_g20535-37 and AsAt_g19714-17), making orthology difficult to determine; the unpaired copies of *rbcS* in *Chenopodium* and *Gossypium* were also a result of tandem duplications complicating orthology assignment. In general, comparisons of *rbcS* between subgenome and diploid progenitor suggest upregulation of *rbcS* in the *Arabidopsis* and *Chenopodium*, but not in either *Gossypium* species (Table 8). Notably, the *Chenopodium rbcS* genes assigned to subgenome (*i.e.,* paternal: AUR62042566 and maternal: AUR62018154) follow our biological expectations in that the maternal homoeolog exhibits upregulation relative to the diploid state; however, a comparison of expression between these homoeologs suggests that the paternal homoeolog is expressed 1.4-fold greater than the maternal homoeolog, contrary to the expectation that the maternal homoeolog would be preferentially expressed. We also note that seven copies of *rbcS* were omitted from the *Chenopodium* analysis because they were not assignable to a subgenome, which may contribute to an overall bias that cannot be determined here. *Gossypium hirsutum*, on the other hand, exhibits a slight, but statistically significant, maternal homoeolog bias (Table 8), congruent with biological expectations; however, a similar limitation resulting in the omission of four *rbcS* copies may also affect our inferences in the present analysis.

Table 8. For *Arachis* and *Chenopodium*, incomplete information prohibited assignment of individual rbcS copies to subgenome (*i.e.,* n.d., or not determined). The average fold-change between each polyploid subgenome and it's model diploid progenitor (e.g., Maternal/Paternal FC) is listed for each other species. Comparisons that did not achieve statistical significance are marked as NS. Differences in homoeolog expression for the single pair in each genome is listed (Homoeolog FC) and reported as paternal versus maternal.

	A. suecica	***A. hypogea***	***A. ipadur***	***C. quinoa***	***G. hirsutum***	***G. barbadense***
Copies	9	6		9	6	
maternal	5	n.d.		1+	3	
paternal	4	n.d.		1+	3	
paired	1 (2 homoeologs)	0		1 (2 homoeologs)	1 (2 homoeologs)	
Maternal FC	2.7	n.d.	n.d.	5.2	NS	NS
Paternal FC	3.3	n.d.	n.d.	NS	NS	NS
Homoeolog FC	NS	n.d.	n.d.	1.4	-0.9	NS

Discussion

Allopolyploids face a complex array of challenges stemming both from whole genome duplication and from hybridization of divergent genomes. These challenges include maintaining stoichiometric balance among interacting molecules (Birchler and Veitia 2010, 2012, 2014, 2021), which may be even more problematic for interactions between the biparentally-inherited, organelle-targeted genes and those occurring in the maternally-coevolved organelles (Wolf and Hager 2006; Sharbrough *et al.* 2017). These potential cytonuclear incompatibilities may underlie observations of rapid and repeated return to single copy for organelle-targeted genes in polyploid species (De Smet *et al.* 2013; Li *et al.* 2016) and the expectation that any paternal cytonuclear homoeologs that exhibit deleterious interactions should evolve rapidly when not immediately lost (Rand *et al.* 2004; Sloan *et al.* 2014; Bock *et al.* 2014). Evidence from homoploid hybrids (Turelli and Moyle 2007; Greiner *et al.* 2011; Bock *et al.* 2014) suggests that stabilizing cytonuclear interactions is key to establishing a successful lineage, and surveys of Rubisco in diverse plant lineages (Gong *et al.* 2012, 2014) report differential homoeolog retention, biased expression, and asymmetric gene conversion favoring maternal homoeologs, although exceptions exist (Wang *et al.* 2017a; Zhai *et al.* 2019).

Emerging research into cytonuclear accommodation in allopolyploid species both supports and contradicts *a priori* cytonuclear expectations of maternal bias (Gong *et al.* 2012, 2014; Sehrish *et al.* 2015; Wang *et al.* 2017a, 2017b; Ferreira de Carvalho *et al.* 2019; Zhai *et al.* 2019; Shan *et al.* 2020; Sharbrough *et al.* 2021), meaning that only some allopolyploids exhibited maternal bias in some cytonuclear genes whereas others did not. Against this backdrop of observations and expectations, we surveyed global gene expression for five allopolyploid species and one synthetic representing four different genera encompassing a wide range of divergence times to evaluate the extent to which gene expression patterns change in accordance with cytonuclear expectations. While we analyze these data here for the purpose of evaluating gene expression changes in allopolyploids, we also note that because these data include ncRNAs and organellar reads, they represent a valuable resource for the allopolyploid community.

Total gene expression exhibits limited evidence of cytonuclear maternal expression level dominance

Cytonuclear imbalance in polyploids could potentially arise due to the changes in dosage balance between organellar and nuclear genomes that accompany polyploidy. In response, the nascent polyploid might be expected to experience selection to mitigate any dosage-related detrimental effects by altering total gene expression in either the organelle or nucleus. Changes in organelle copy number and/or genome copy per organelle have been associated with polyploidy (Bingham 1968; Beversdorf 1979; Dean and Leech 1982; Butterfass 1987; Murti *et al.* 2012; Oberprieler *et al.* 2019; Coate *et al.* 2020; He *et al.* 2021; Fernandes Gyorfy *et al.* 2021), and these have been

associated with cytonuclear compensation at the expression level (Doyle and Coate 2019; Coate *et al.* 2020). On the other hand, it is common for polyploids to undergo rapid changes in nuclear expression (Chen 2007; Doyle *et al.* 2008; Freeling 2009; Gaeta and Pires 2010; Jackson and Chen 2010; Salmon *et al.* 2010; Grover *et al.* 2012; Madlung and Wendel 2013; Yoo *et al.* 2014; Song and Chen 2015; Bao *et al.* 2019; Gallagher *et al.* 2020), which could include changes that compensate for deleterious cytonuclear stoichiometric imbalances.

In the present study, we characterized how total expression of nuclear-encoded cytonuclear genes changes relative to the rest of the transcriptome and whether those changes are biased toward the maternal parent. We evaluated each polyploid for evidence of maternally-biased expression level dominance (ELD) in cytonuclear genes that is statistically different from any global, or background, bias exhibited by genes not involved in cytonuclear processes. Our expectation was that we would observe some degree of ELD for cytonuclear genes that might provide evidence of cytonuclear compensation to coordinate expression with the maternally co-evolved organelles. While three of the five polyploids (*i.e., Arachis hypogea*, *Gossypium hirsutum*, and *Gossypium barbadense*) exhibited a general bias toward maternal ELD, only *Arachis hypogea* and *Chenopodium quinoa* exhibited an excess of maternal ELD in cytonuclear genes (in general) relative to the remaining transcriptome (*i.e.,* NOT; Table 4), with only 1-2 categories exhibiting evidence of significant ELD (DNI in both species and MI in *Chenopodium quinoa*). Interestingly, however, *Gossypium barbadense*, while not exhibiting a general parental bias in cytonuclear ELD, did exhibit maternally-biased ELD in the PI cytonuclear category alone. While these results suggest that global ELD in cytonuclear genes is not a *general* consequence of cytonuclear accommodation, it is noteworthy that in many cases, and for all species, the number of genes exhibiting maternally-biased ELD in cytonuclear categories does exceed the expected number (although not significantly so). This may be a function of the limited numbers of genes in each category, trends of partial yet non-ubiquitous maternally-biased ELD in cytonuclear categories, and/or both. While we also evaluated patterns of transgressive expression in cytonuclear categories relative to non-organelle-targeted genes, we did not find evidence of biased transgressive expression that would indicate a global up- or down-regulation of cytonuclear genes to compensate for the number of organelles/organelle genomes; however, we again note that most categories were limited in membership, leading to low statistical power.

Variability in cytonuclear homoeolog expression patterns

Cytonuclear imbalance in allopolyploid species can also arise from incompatibilities between the organellar genomes and the more divergent paternal cytonuclear genes, and we expect these to become more common as the divergence time between progenitor genomes increases. Reconciliation of potentially maladaptive mutations is possible through a variety of mechanisms, as previously noted (Gong *et al.* 2012, 2014; Sharbrough *et al.* 2017). For example, at the genomic level, gene loss and maternally-biased gene conversion could either remove or "correct"

maladaptive mutations acquired by the paternal genome since its divergence from a common ancestor, minimizing their deleterious potential (Sharbrough *et al.* 2021).

With respect to expression, compensation for maladaptive paternal mutations could present as a combination of up-regulated maternal homoeologs and/or down-regulated paternal homoeologs. This, however, does not appear to be a global reaction to allopolyploidy in the species surveyed. When we compared homoeolog expression for each of the six allopolyploid species with their respective diploid progenitor genomes, we observed no clear and consistent pattern of homoeolog up-/down-regulation within polyploids and/or for any of the cytonuclear categories. At most, any given polyploid displayed two cytonuclear categories consistent with our biological expectations of excess maternal up-regulation and/or paternal down-regulation (Figure 1), and concomitantly have as many or more categories that directly contradict our cytonuclear predictions (*i.e.,* enrichment of maternal down-regulation and/or paternal up-regulation). Individual cytonuclear categories were no more consistent, with the MI category being most frequently consistent (*i.e.,* agreed with expectations in two species, *Arabidopsis suecica* and *Arachis hypogea*), while also being contradictory in the same number of species (*Arachis* IpaDur1 and *Chenopodium quinoa*).

Maternal homoeolog expression bias (*i.e.,* genes where maternal expression outweighs paternal, irrespective of diploid expression) was similarly intermittent in cytonuclear categories. When compared to any global HEB exhibited by each species, few cytonuclear categories exhibited an excess of maternal HEB (*i.e., Arabidopsis suecica* PI/PNI and *Gossypium hirsutum* DI only; Table 5). Interestingly, a single category in *Chenopodium quinoa* (MI) and several in *Gossypium barbadense* (DNI/PI/PNI) exhibited an excess of *paternal* HEB, which is contrary to cytonuclear expectations. We do note, however, that these relative expression biases are often parentally inherited, as noted by the previous analysis.

Importantly, our analytical methodology was designed to disentangle parental or progenitor legacy effects (*i.e.*, differences at the diploid level vertically inherited in the polyploids at formation) from evolved cytonuclear responses subsequent to polyploid formation. When we combined our assessment of homoeolog expression differences with legacy parental effects (Table 7), we find that not only do targeting (*i.e.*, cytonuclear category) and legacy diploid expression influence the difference in homoeolog expression, but there is also an interactive effect between targeting and legacy expression differences. Interestingly, however, many of the fixed effects are not congruent with our expectations under the cytonuclear hypotheses, *i.e.*, that the difference in *rlog* counts between maternal and paternal homoeologs should be greater in the cytonuclear categories (or positive relative to the intercept established by NOT genes). Contrasts between each cytonuclear category and NOT genes also exhibited sporadic significance and were frequently incongruent with expectations (*i.e.,* that the cytonuclear categories would exhibit greater HEB when accounting for diploid legacy) for most species. Only *Arabidopsis suecica*

showed significant, congruent cytonuclear effects for most categories, suggesting that DNI, MI/MNI, and PNI were generally composed of genes whose maternal HEB was greater than expected by NOT and diploid legacy.

In light of previous research that both supports and contrasts the results presented here, we speculate that cytonuclear accommodation is variable among lineages, among cytonuclear categories, and among genes within categories themselves. It also may be that for most genes (and especially those in the organellar genomes, which experience low mutation rates), the rates of molecular evolution are too low to permit signals of cytonuclear selection to become evident on the divergence scales studied here. It is possible, for example, that cytonuclear selection is ongoing and even pervasive, but that for the most part it is subtle, involving expression level changes or genomic signatures that simply do not rise to the level of statistical significance given the timescales encompassed by the allopolyploids studied here. Some polyploids, such as *Arabidopsis suecica*, provide a modest level of support for our *a priori* expectations for cytonuclear accommodation vis-á-vis gene expression, whereas others, such as *Gossypium barbadense*, contradict expectations more frequently than not. The variability in our observations may suggest that species with fewer cytonuclear-congruent expression changes either have fewer detrimental cytonuclear incompatibilities and/or have other methods for resolving deleterious conflict between the co-evolved maternal subgenome, the potentially detrimental paternal homoeologs, and the cytoplasmically inherited organelles.

Data Availability Statement

All sequence data used in the analysis are available from NCBI under PRJNA726938, and all scripts used to analyze the data are available from Github under https://github.com/Wendellab/CytonuclearExpression commit XXXXXXXX.

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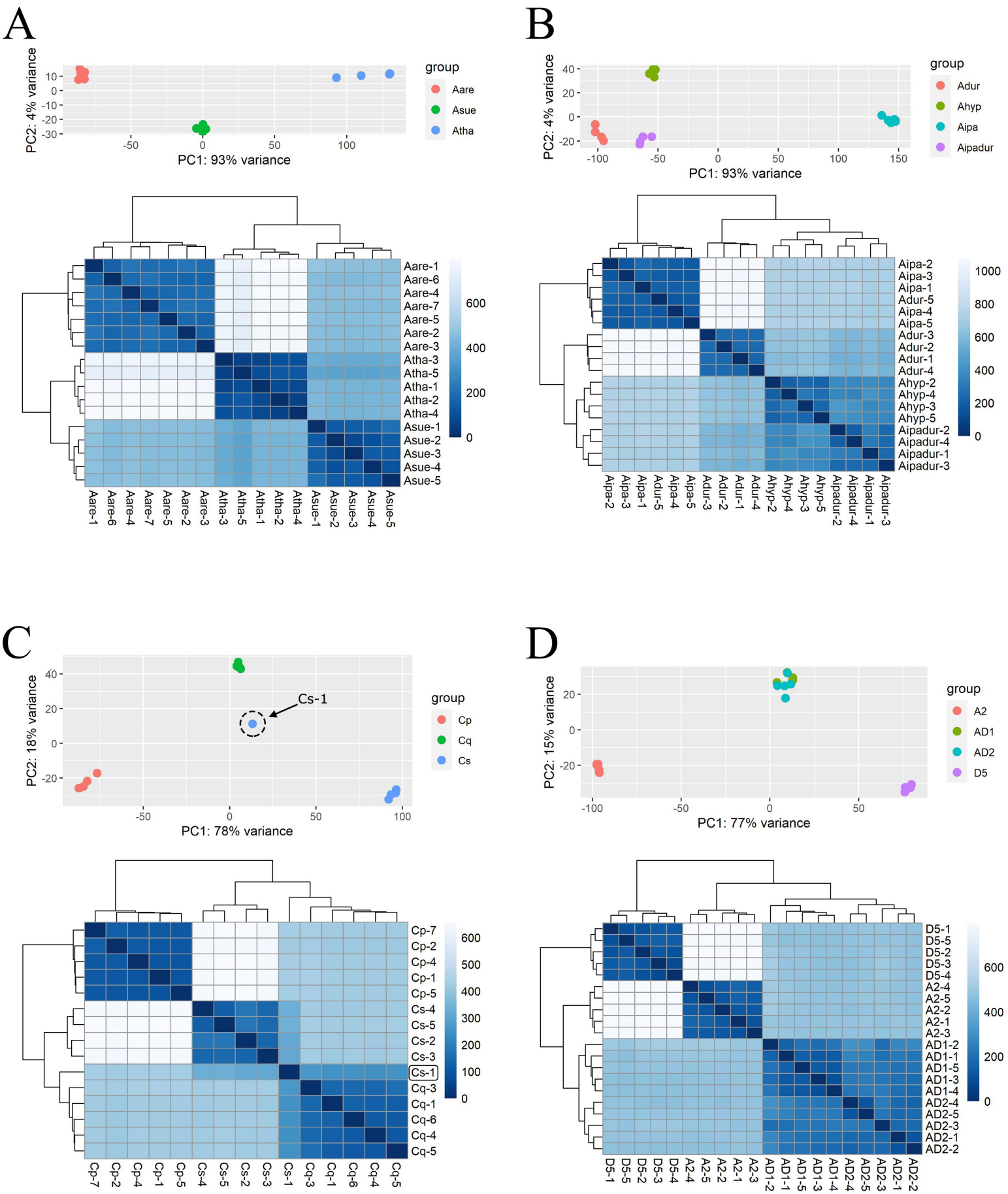
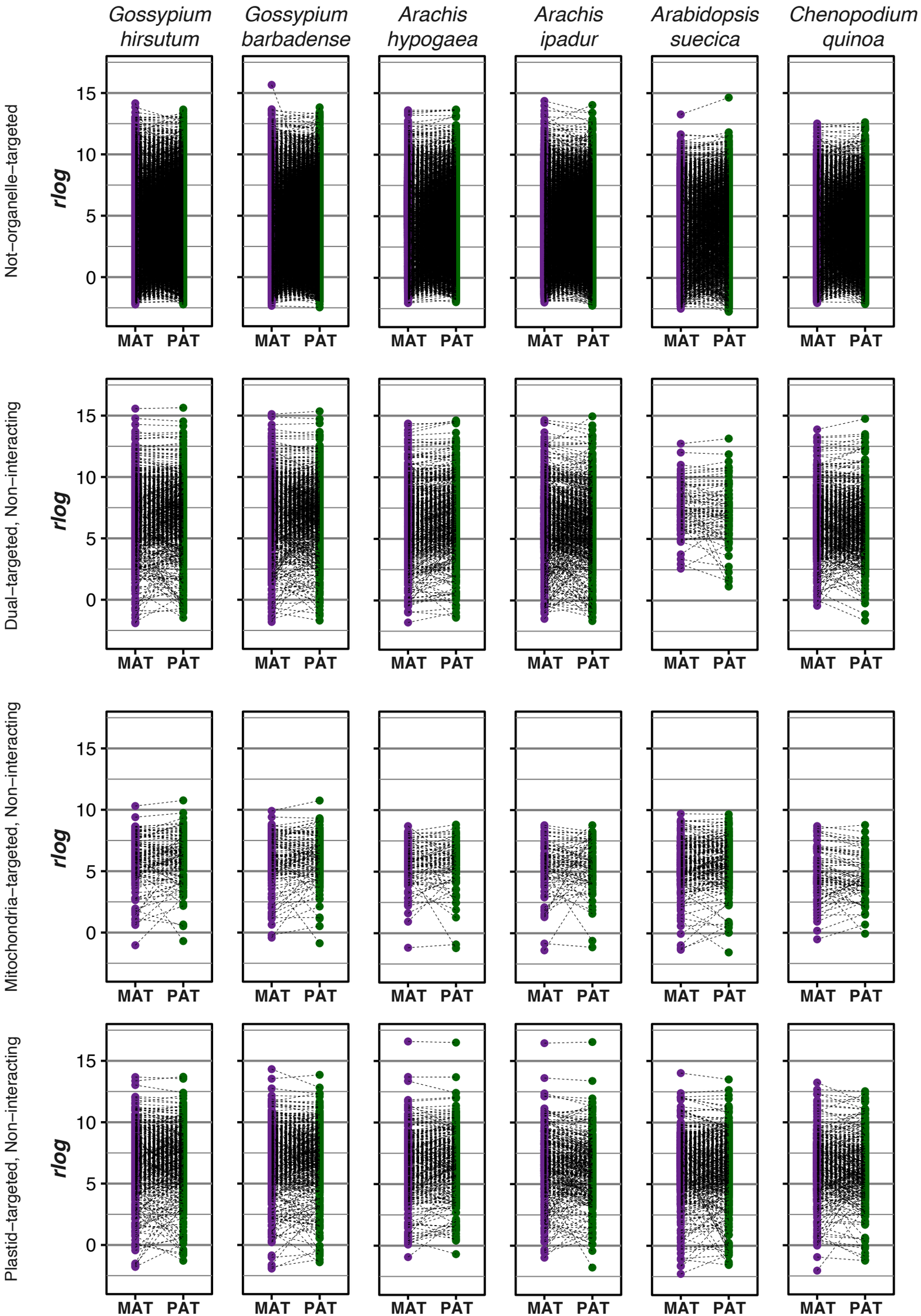
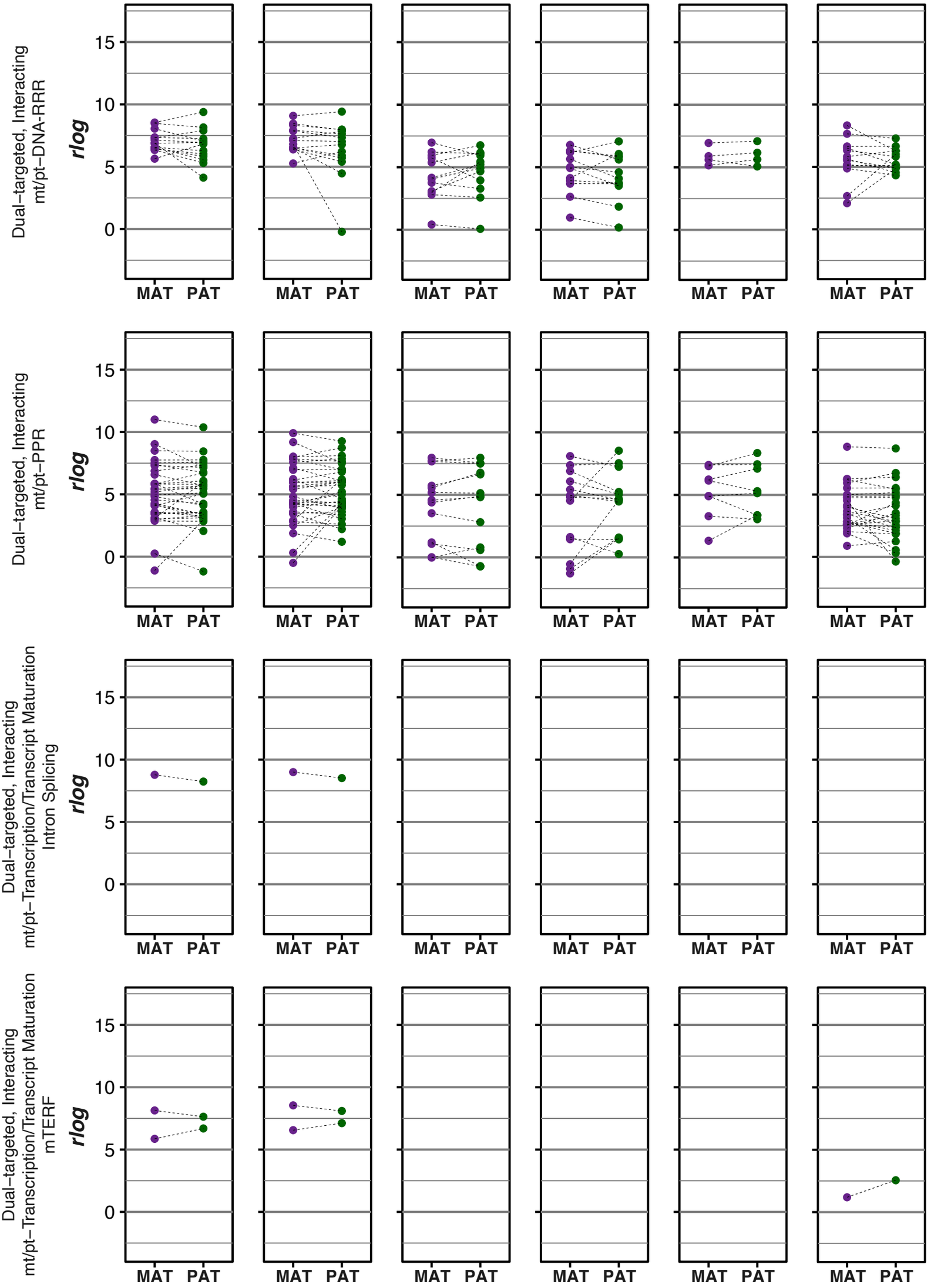



Gossypium hirsutum
Gossypium barbadense
Arachis hypogaea
Arachis ipadur
Arabidopsis suecica
Chenopodium quinoa
Not−organelle−targeted
Dual−targeted, Non−interacting
Mitochondria−targeted, Non−interacting
Plastid−targeted, Non−interacting
rlog
15
10
5
0
MAT
PAT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mt/pt−DNA-RRR
Dual−targeted, Interacting
mt/pt−PPR
Dual−targeted, Interacting
mt/pt−Transcription/Transcript Maturation
Intron Splicing
Dual−targeted, Interacting
mt/pt−Transcription/Transcript Maturation
mTERF
rlog
15
10
5
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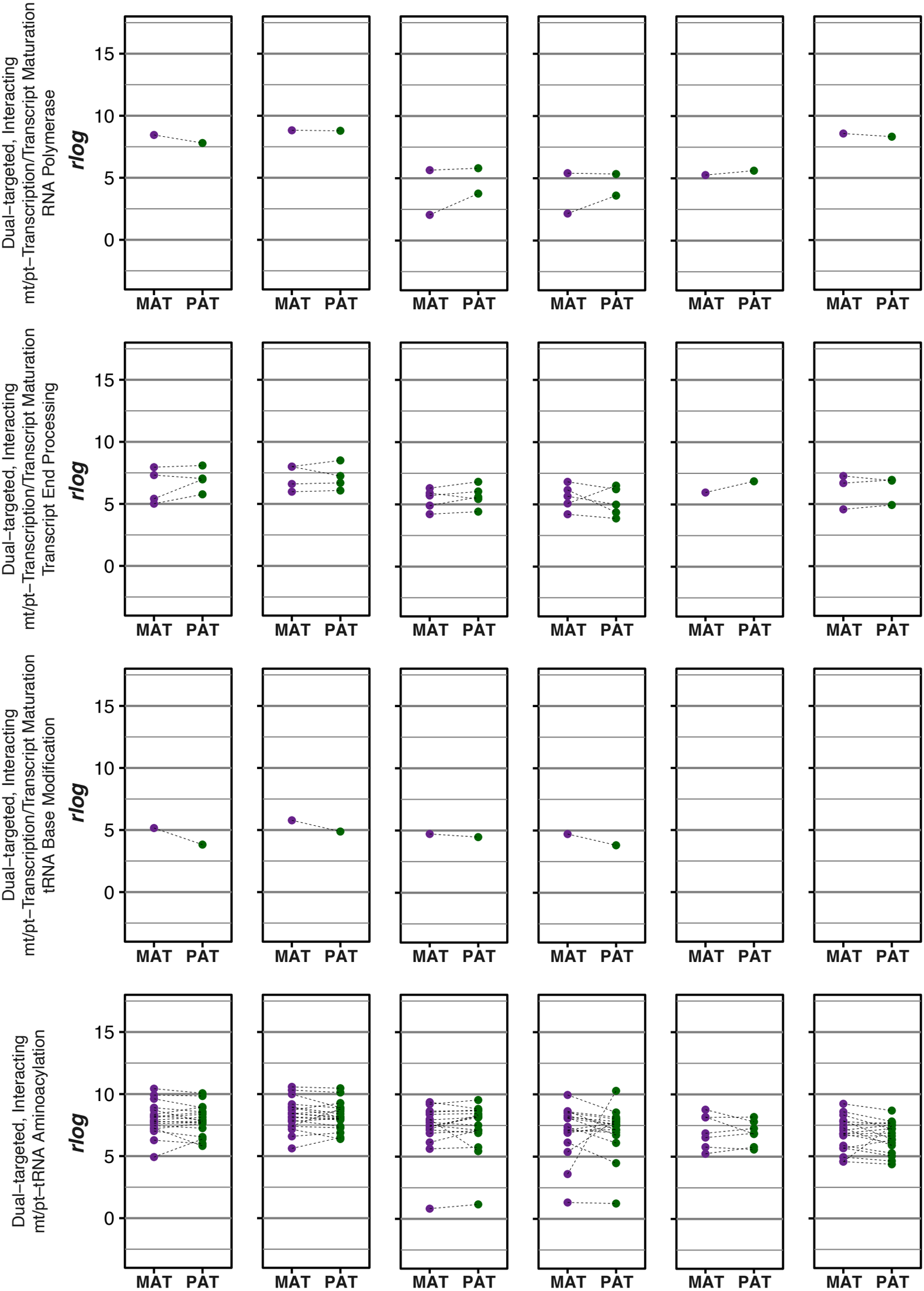
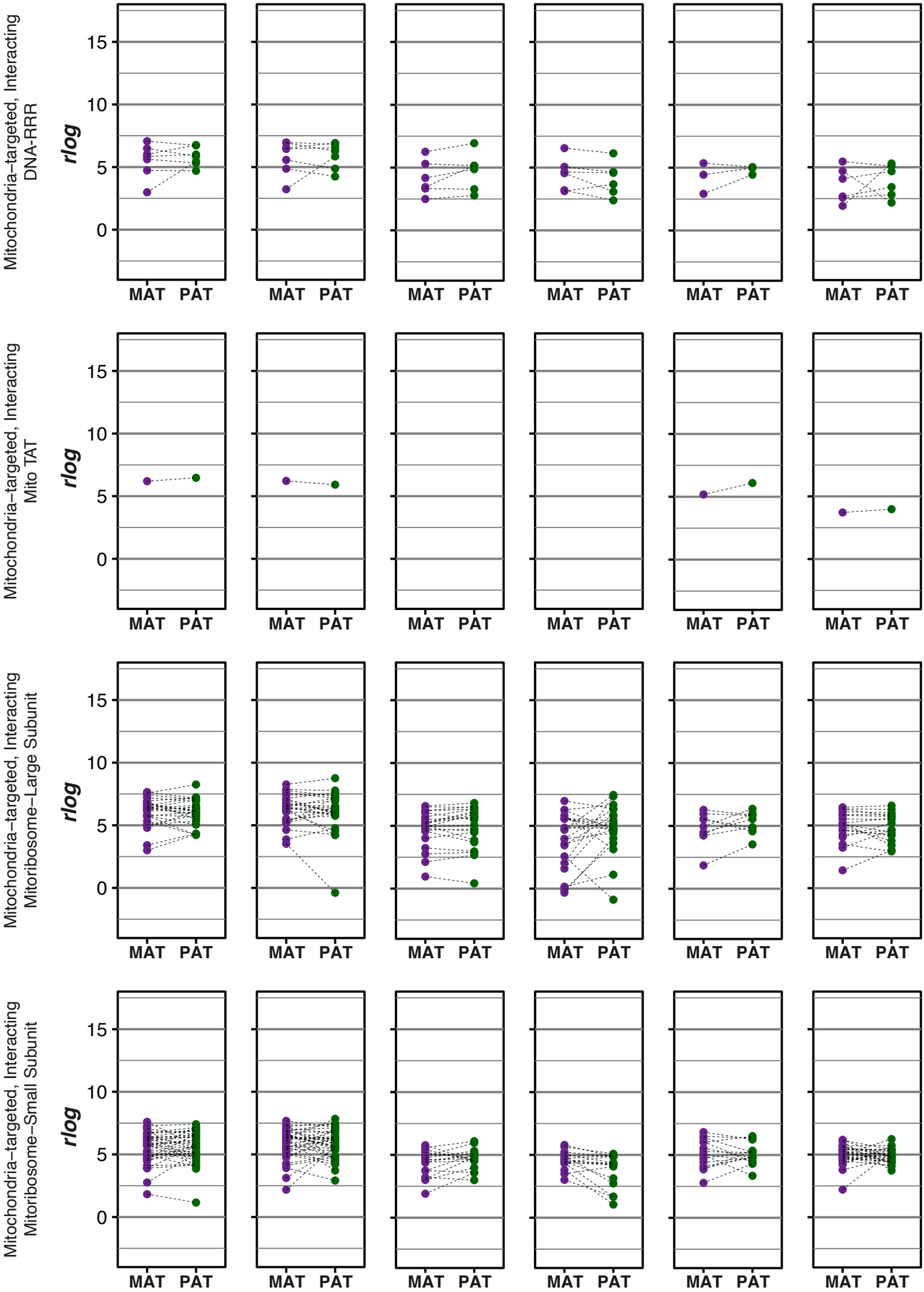
MAT
PAT


Dual−targeted, Interacting
mt/pt−Transcription/Transcript Maturation
RNA Polymerase
Dual−targeted, Interacting
mt/pt−Transcription/Transcript Maturation
Transcript End Processing
Dual−targeted, Interacting
mt/pt−Transcription/Transcript Maturation
tRNA Base Modification
Dual−targeted, Interacting
mt/pt−tRNA Aminoacylation
rlog
15
10
5
0
MAT
PAT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DNA-RRR
Mitochondria−targeted, Interacting
Mito TAT
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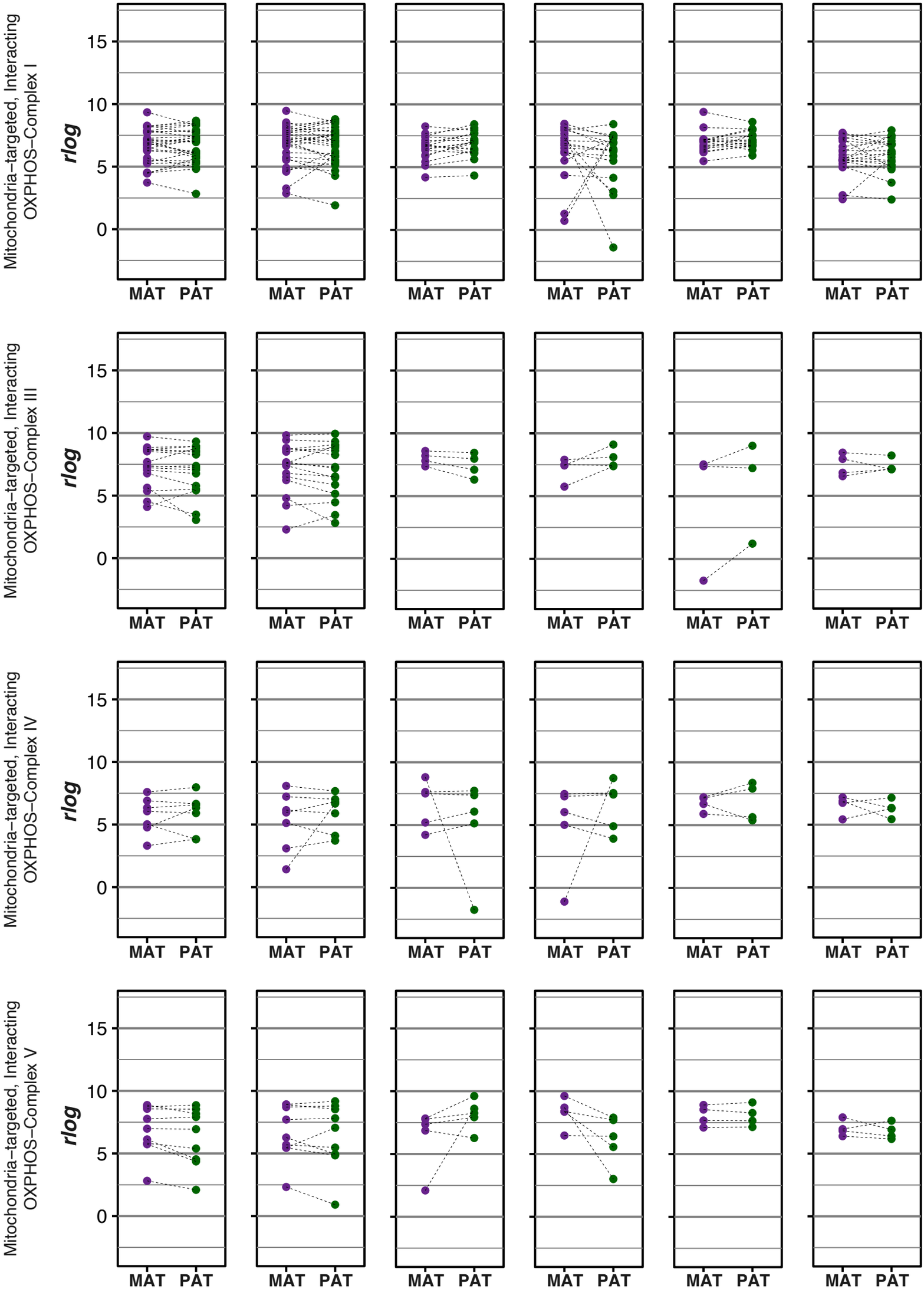
Mitochondria−targeted, Interacting
Mitoribosome−Small Subunit
rlog
15
10
5
0
MAT
PAT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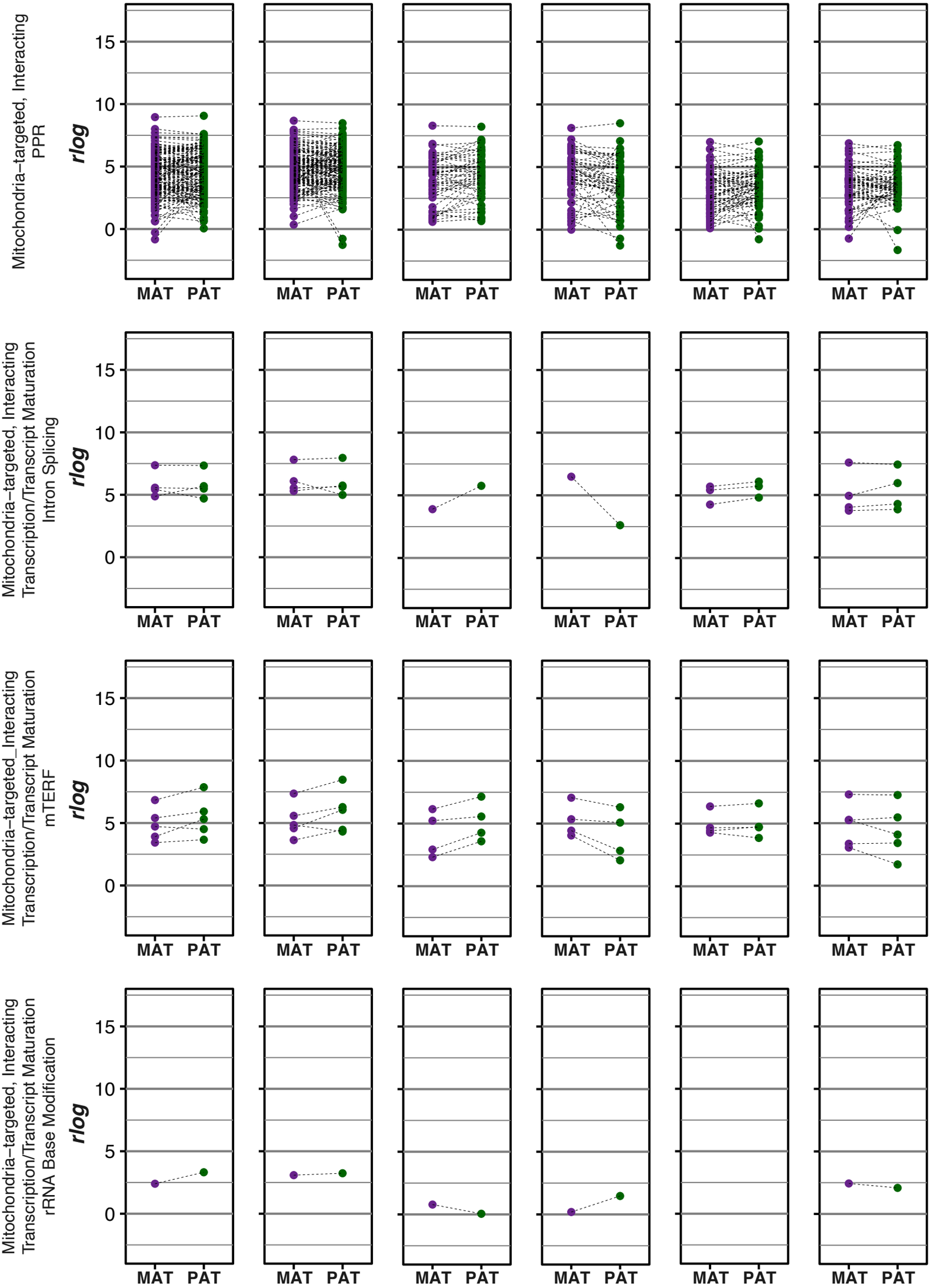
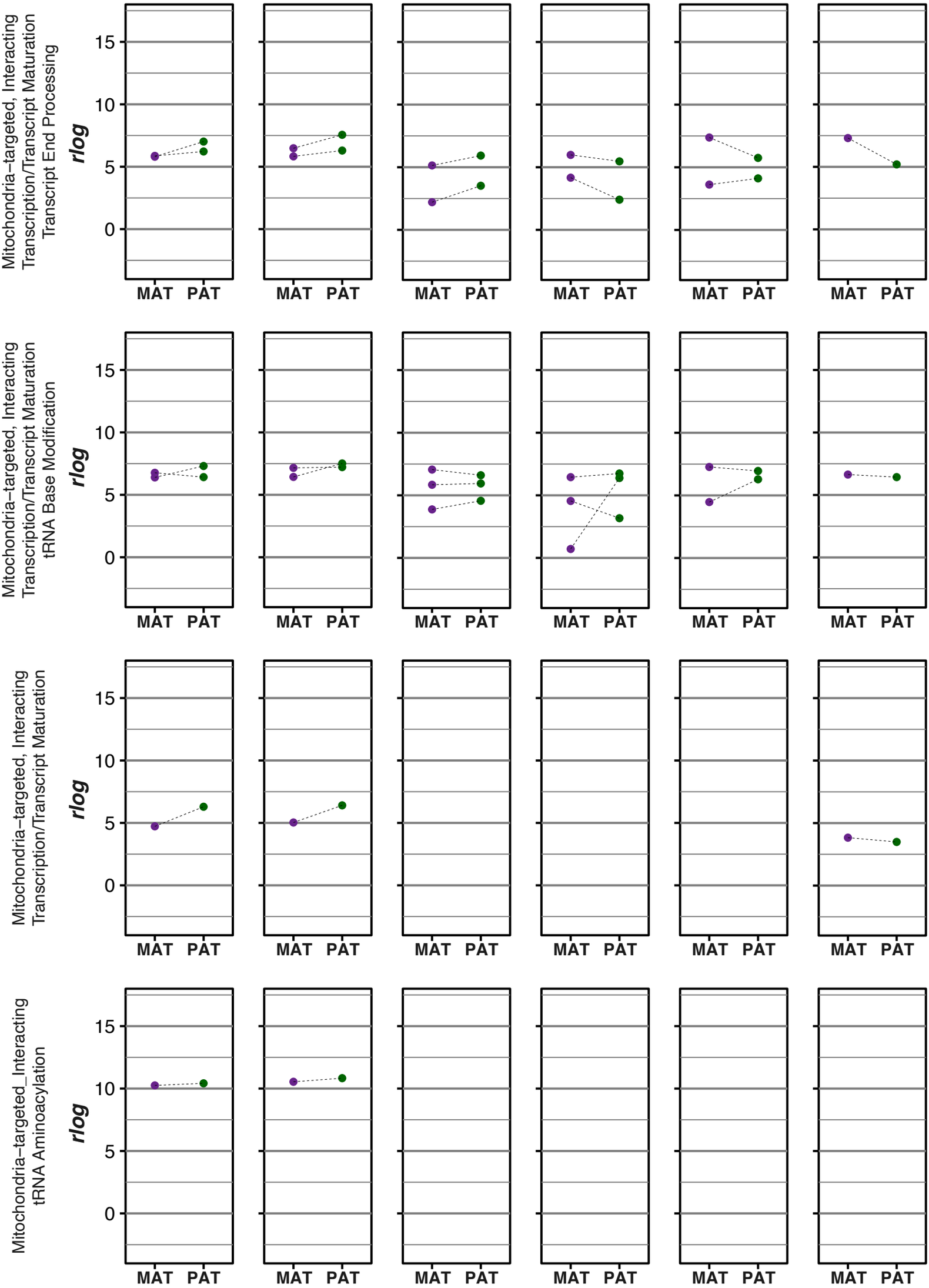
Mitochondria−targeted, Interacting OXPHOS−Complex V
rlog
15
10
5
0
MAT
PAT


Mitochondria–targeted, Interacting
PPR
Mitochondria–targeted, Interacting
Transcription/Transcript Maturation
Intron Splicing
Mitochondria–targeted_Interacting
Transcription/Transcript Maturation
mTERF
Mitochondria–targeted, Interacting
Transcription/Transcript Maturation
rRNA Base Modification
rlog
15
10
5
0
MAT
PAT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tRNA Base Modification
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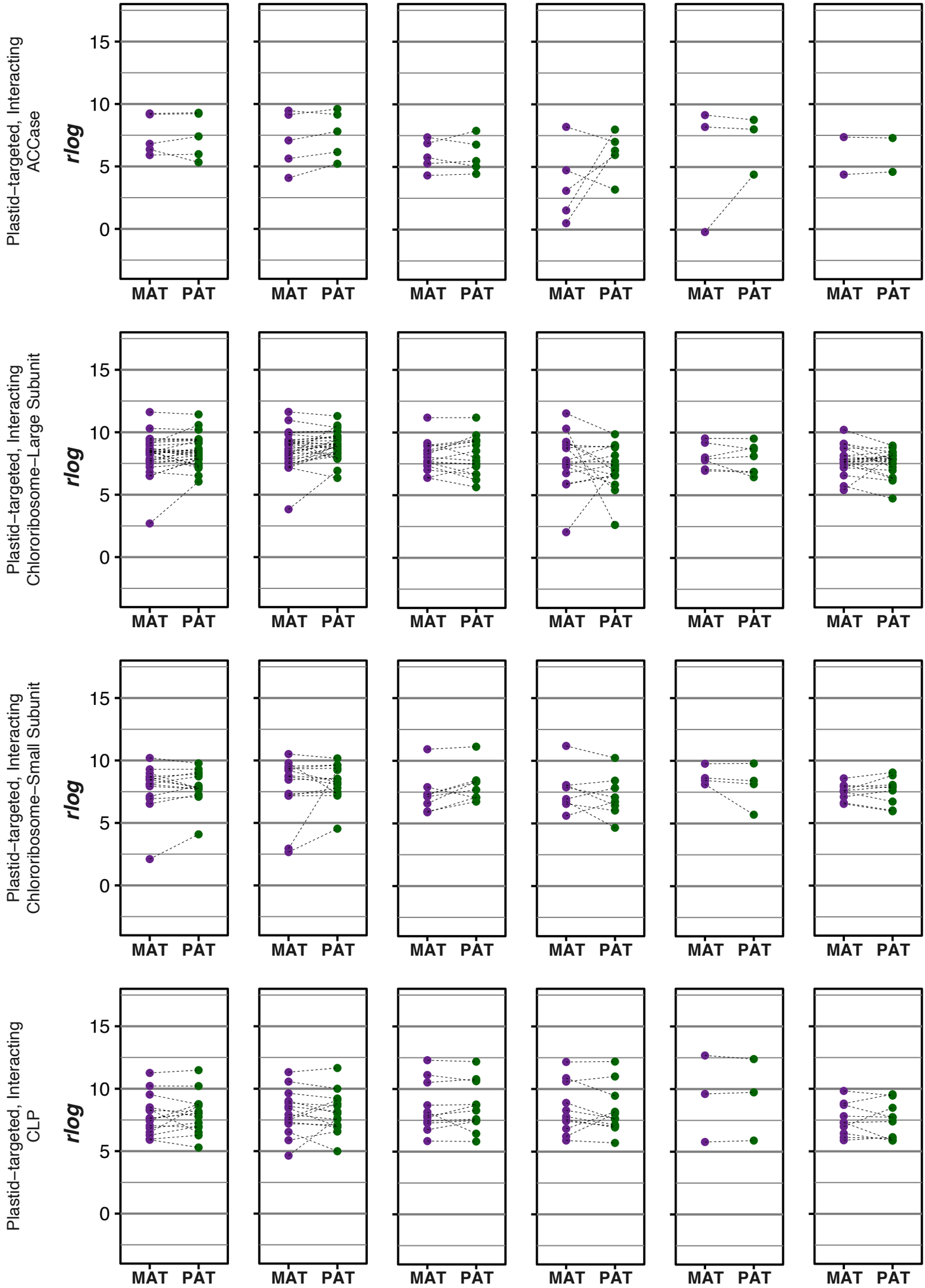
Transcription/Transcript Maturation
Mitochondria–targeted_Interacting
tRNA Aminoacylation
rlog
15
10
5
0
MAT
PAT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Plastid−targeted, Interacting Chlororibosome−Large Subunit
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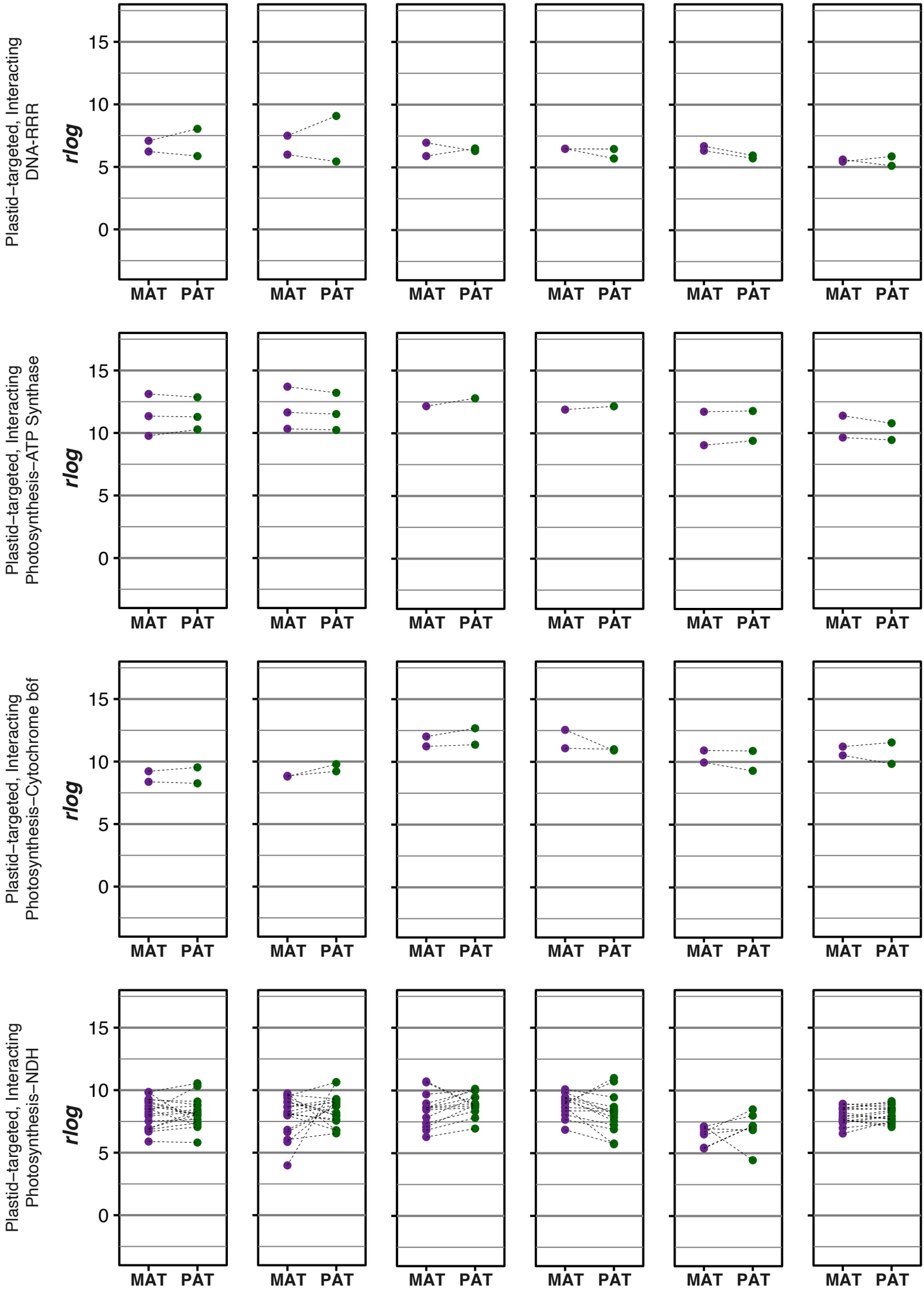
rlog
15
10
5
0
MAT
PAT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10
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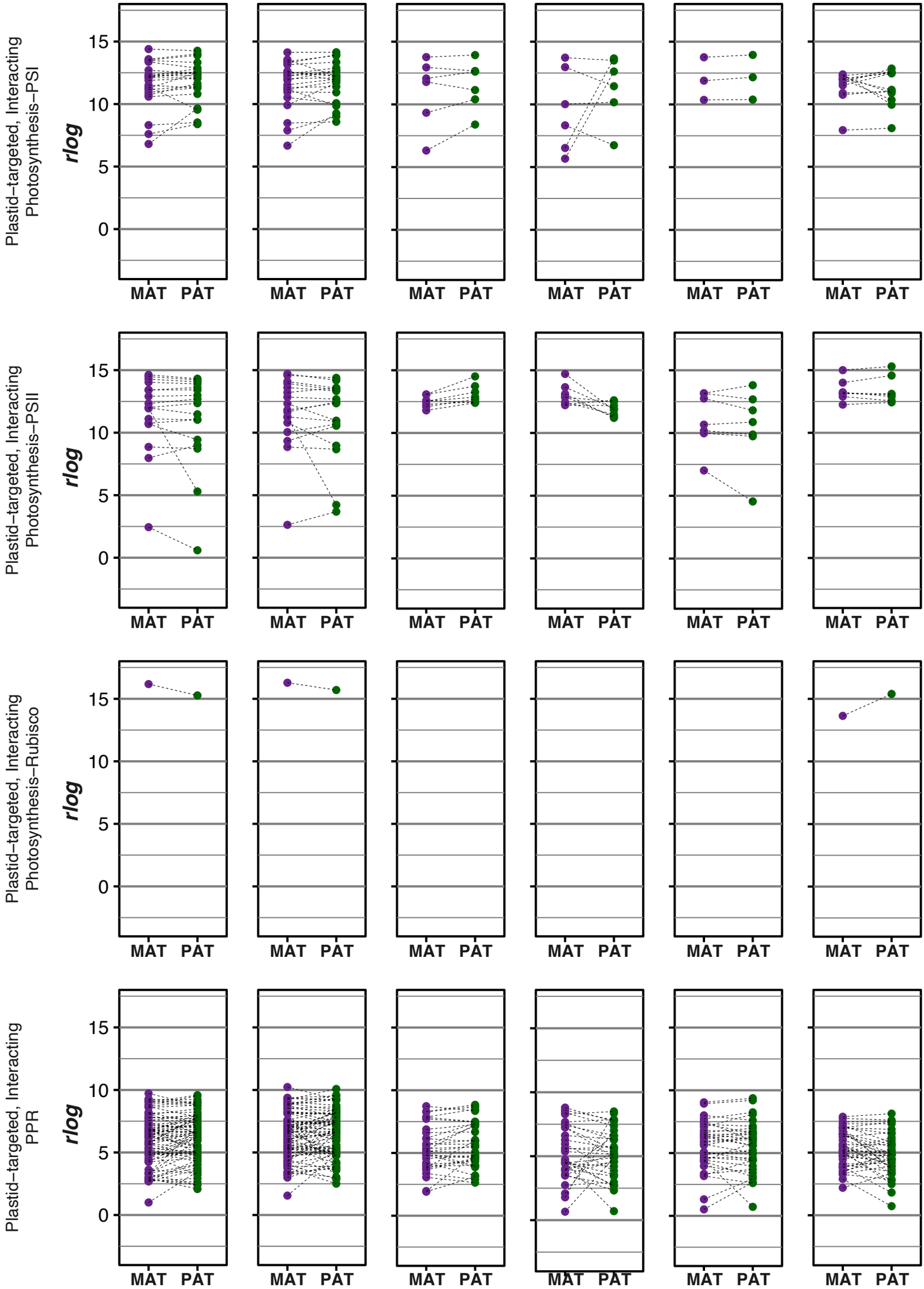
MAT
PAT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Photosynthesis−PSI
Plastid−targeted, Interacting
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Photosynthesis−Rubisco
Plastid−targeted, Interacting
PPR
rlog
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10
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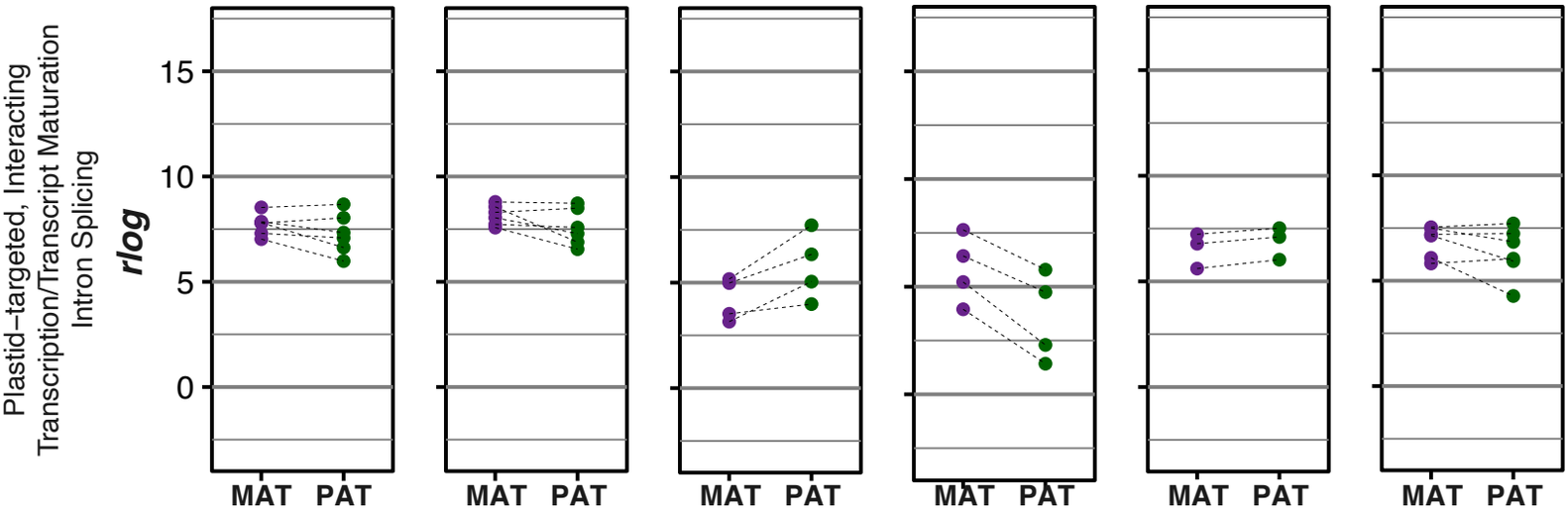
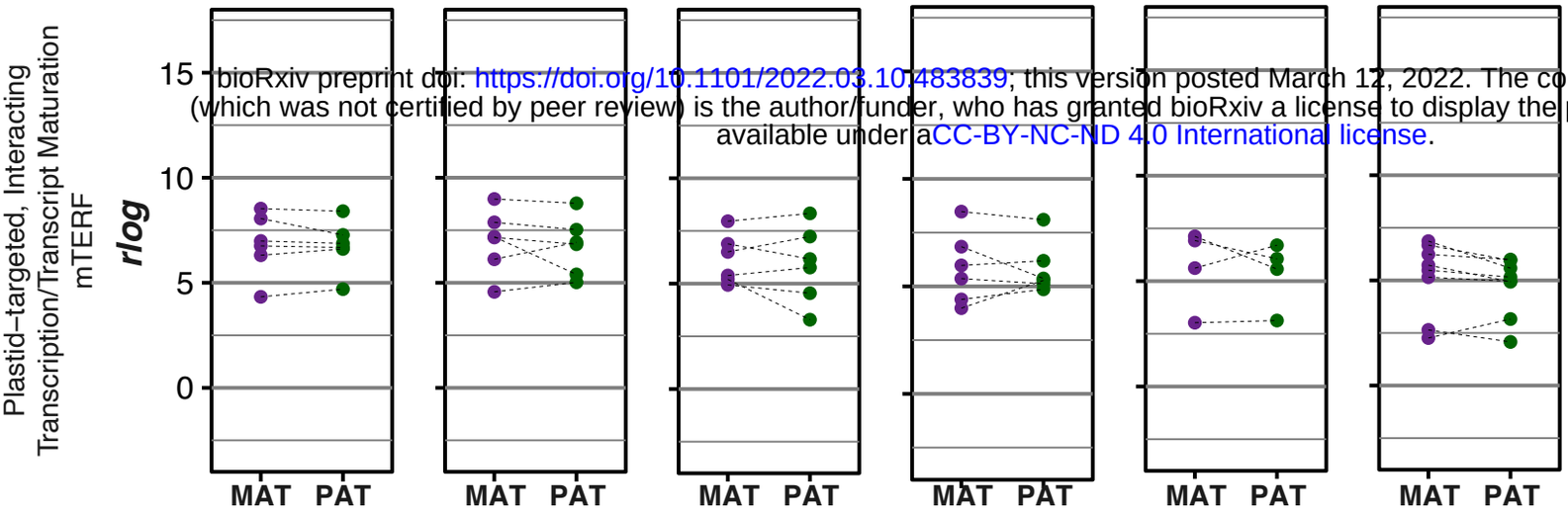
MAT
PAT





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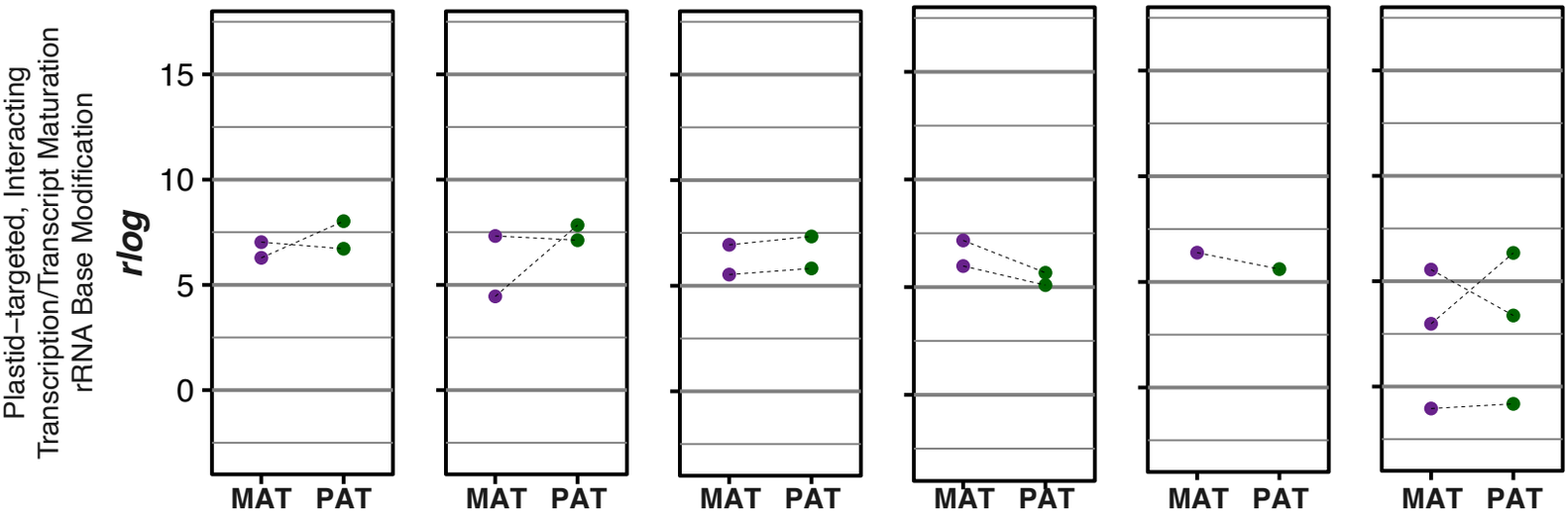

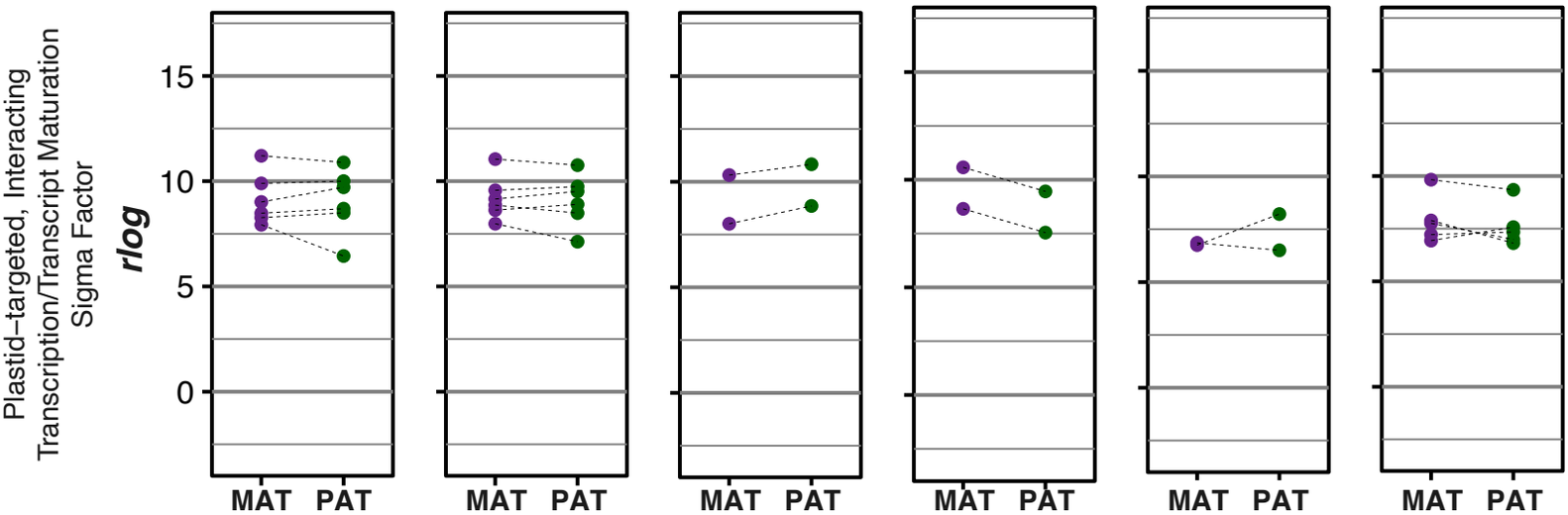

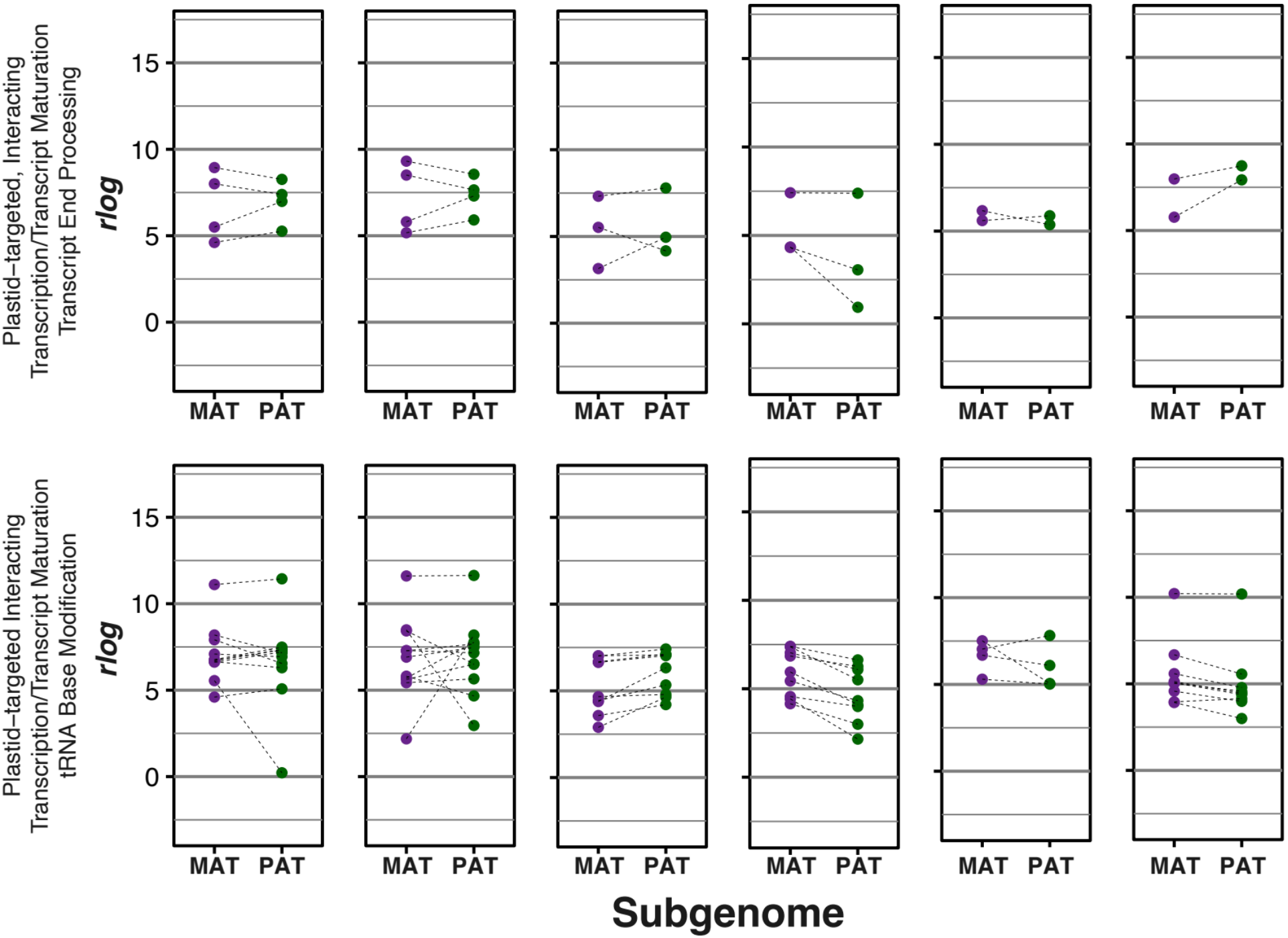
Plastid−targeted, Interacting
Transcription/Transcript Maturation
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Transcription/Transcript Maturation
tRNA Base Modification
rlog
15
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Subgenome

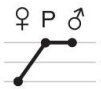
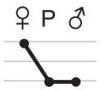
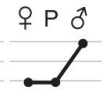
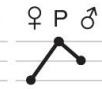
Supplementary Table 1. Species and accession used, with ploidy levels.

Genus	Species	Accession	Ploidy
Chenopodium			
	C. quinoa	QQ74	tetraploid
	C. pallidicaule	PI 478407	diploid
	C. suecicum	Not Available	diploid
Gossypium			
	G. hirsutum	TM1	tetraploid
	G. barbadense	GB 3-79	tetraploid
	G. arboreum	A2 101	diploid
	G. raimondii	JFW	diploid
Arabidopsis			
	A. suecica	CS22505	tetraploid
	A. thaliana	Landsberg CS69111	diploid
	A. arenosa	900118	diploid
Arachis			
	A. hypogea	Tifrunner	tetraploid
	A. ipaensis x A. duranensis	Bertioli et al. 2019	tetraploid
	A. ipaensis	GK30076	diploid
	A. duranensis	V14167	diploid

Supplementary Table 2. Genomic and transcriptomic references used in reference transcriptome curation.			
Genus	chloroplast	mitochondria	nuclear
Arabidopsis	NC_000932	NC_037304	Novikova et al. 2017
Arachis	NC_037358	NCBI SRA SRR14414925 and PeanutBase	Bertioli et al. 2019
Chenopodium	MK159176	MK182703	Jarvis et al. 2017
Gossypium	NC_007944	JX065074	Chen et al. 2020

Supplementary Table 3. Average sequencing and mapping results for RNA-seq libraries, by species.					
	replicates	# fragments sequenced (in millions)	% fragments mapped	% fragments mapped to chloroplast	% fragments mapped to mitochondria
Arabidopsis thaliana	5	65.6 (50.4 - 81.4)	85% (83 - 87%)	73% (69 - 75%)	1% (1 - 2%)
Arabidopsis suecica	5	55.1 (37.3 - 66.5)	76% (67 - 81%)	67% (63 - 70%)	2% (2 - 3%)
Arabidopsis arenosa	7	61.6 (53.5 - 83.0)	66% (57 - 75%)	66% (56 - 71%)	3% (2 - 3%)
Arachis duranensis	5	38.7 (33.1 - 43.3)	82% (80 - 85%)	67% (56 - 76%)	1% (1 - 2%)
Arachis ipaensis	5	40.5 (34.3 - 45.0)	81% (80 - 82%)	57% (53 - 59%)	1% (1 - 2%)
Arachis hypogea	4	31.3 (26.1 - 43.5)	85% (83 - 86%)	66% (58 - 74%)	1%
Arachis IpaDur1	4	33.9 (30.1 - 41.2)	79% (75 - 83%)	62% (56 - 71%)	2% (1 - 2%)
Chenopodium pallidicaule	5	44.5 (41.0 - 50.4)	61% (55 - 68%)	73% (72 - 75%)	2% (2 - 3%)
Chenopodium suecicum	4	41.2 (37.9 - 45.3)	57% (40 - 69%)	74% (73 - 76%)	1% (1 - 2%)
Chenopodium quinoa	5	47.0 (33.8 - 59.9)	68% (56 - 80%)	74% (72 - 76%)	1% (1 - 2%)
Gossypium arboreum	5	51.0 (44.6 - 57.7)	74% (65 - 79%)	56% (46 - 62%)	2%
Gossypium raimondii	5	68.7 (52.0 - 89.9)	59% (50 - 67%)	43% (38 - 50%)	3% (2 - 4%)
Gossypium hirsutum	5	59.8 (53.7 - 71.7)	78% (67 - 85%)	52% (47 - 58%)	2% (2 - 3%)
Gossypium barbadense	5	55.5 (49.3 - 68.5)	79% (76 - 81%)	53% (43 -63%)	2% (1 - 2%)

Supplementary Table 4. Expression level dominance in six allotetraploids.

					Intermediate expression		Paternal expression level dominance		Maternal expression level dominance		Transgressive down-regulation			Transgressive up-regulation			No change	Total
					I	XII	II	XI	IV	IX	III	VII	X	V	VIII	VI		
		Mom > Dad	Dad > Mom	Mom = Dad														
Arabidopsis suecica	All genes	**2337**	**2528**	**4518**	**436**	**353**	**1024**	**887**	**981**	**939**	**56**	**299**	**64**	**73**	**354**	**52**	**3865**	**9383**
	Not-organelle-targeted	**1737**	**2015**	**3605**	346	235	828	658	743	738	44	230	56	59	302	45	3073	7357
	Dual-targeted_Interacting	**13**	**9**	**14**	0	6	7	1	6	2	0	0	0	0	1	0	13	36
	Dual-targeted_Non-interacting	**42**	**36**	**66**	5	12	18	12	18	13	0	2	0	0	3	0	61	144
	Mitochondria-targeted_Interacting	**43**	**77**	**101**	19	10	29	10	23	28	0	3	0	1	2	0	96	221
	Mitochondria-targeted_Non-interacting	**87**	**133**	**156**	32	10	42	40	32	53	3	16	4	3	9	1	131	376
	Plastid-targeted_Interacting	**51**	**16**	**68**	2	4	8	29	16	6	0	5	0	0	2	2	61	135
	Plastid-targeted_Non-interacting	**350**	**217**	**458**	31	76	86	127	139	83	8	36	4	9	34	4	388	1025
	chloroplast	**13**	**5**	**45**	0	0	0	10	3	5	0	5	0	0	0	0	40	63
	mitochondria	**1**	**20**	**5**	1	0	6	0	1	11	1	2	0	1	1	0	2	26
Arachis IpaDur1	All genes	**1031**	**1527**	**5007**	**1**	**0**	**1506**	**972**	**31**	**13**	**1**	**52**	**21**	**6**	**59**	**7**	**4896**	**5042**
	Not-organelle-targeted	**835**	**1255**	**4292**	0	0	1238	788	29	12	1	32	12	4	46	6	4214	4315
	Dual-targeted_Interacting	**7**	**5**	**23**	0	0	5	6	0	0	0	1	1	0	0	0	22	24
	Dual-targeted_Non-interacting	**89**	**121**	**311**	0	0	121	82	0	0	0	11	6	0	5	1	295	318
	Mitochondria-targeted_Interacting	**23**	**17**	**65**	0	0	17	22	0	0	0	2	1	0	0	0	63	66
	Mitochondria-targeted_Non-interacting	**13**	**17**	**37**	0	0	15	13	0	1	0	0	0	1	0	0	37	38
	Plastid-targeted_Interacting	**21**	**17**	**65**	0	0	17	21	0	0	0	1	0	0	0	0	64	65
	Plastid-targeted_Non-interacting	**41**	**63**	**167**	1	0	61	40	0	0	0	5	1	1	5	0	157	169
	chloroplast	**0**	**23**	**33**	0	0	23	0	0	0	0	0	0	0	0	0	33	33
	mitochondria	**2**	**9**	**14**	0	0	9	0	2	0	0	0	0	0	3	0	11	14
Arachis hypogea	All genes	**1563**	**1097**	**5532**	**2**	**15**	**170**	**255**	**1204**	**827**	**74**	**255**	**20**	**24**	**250**	**69**	**5027**	**8192**
	Not-organelle-targeted	**1288**	**901**	**4767**	2	13	145	220	982	677	56	203	18	21	204	55	4360	6956
	Dual-targeted_Interacting	**5**	**8**	**25**	0	0	0	1	4	7	1	3	0	0	1	0	21	38
	Dual-targeted_Non-interacting	**137**	**89**	**329**	0	0	12	13	115	69	7	25	0	1	18	9	286	555
	Mitochondria-targeted_Interacting	**16**	**23**	**71**	0	1	3	5	10	13	7	4	0	0	3	0	64	110
	Mitochondria-targeted_Non-interacting	**16**	**15**	**41**	0	0	1	3	13	13	1	1	0	0	1	0	39	72
	Plastid-targeted_Interacting	**17**	**19**	**67**	0	0	1	1	15	17	1	3	0	0	3	1	61	103
	Plastid-targeted_Non-interacting	**68**	**40**	**180**	0	1	7	7	54	31	1	13	2	1	18	4	149	288
	chloroplast	**11**	**0**	**38**	0	0	0	2	9	0	0	0	0	0	0	0	38	49
	mitochondria	**5**	**2**	**14**	0	0	1	3	2	0	0	3	0	1	2	0	9	21
Chenopodium quinoa	All genes	**2487**	**2663**	**2579**	**607**	**541**	**804**	**795**	**800**	**891**	**178**	**442**	**136**	**183**	**519**	**215**	**1618**	**7729**
	Not-organelle-targeted	**1968**	**2065**	**2129**	439	419	644	649	617	677	143	363	114	162	438	169	1328	6162
	Dual-targeted_Interacting	**36**	**6**	**13**	2	8	1	13	12	3	0	2	0	0	1	3	10	55
	Dual-targeted_Non-interacting	**196**	**300**	**194**	92	42	77	49	75	109	12	31	7	10	43	23	120	690
	Mitochondria-targeted_Interacting	**79**	**33**	**39**	8	27	6	15	31	11	4	8	3	4	9	3	22	151
	Mitochondria-targeted_Non-interacting	**33**	**12**	**29**	2	10	4	10	8	2	3	4	2	1	9	3	16	74
	Plastid-targeted_Interacting	**45**	**44**	**54**	13	10	18	11	20	11	1	7	0	1	3	4	44	143
	Plastid-targeted_Non-interacting	**116**	**139**	**101**	39	22	40	44	35	44	11	24	5	5	13	10	64	356
	chloroplast	**0**	**60**	**14**	11	0	13	0	0	34	2	1	0	0	2	0	11	74
	mitochondria	**14**	**4**	**6**	1	3	1	4	2	0	2	2	5	0	1	0	3	24
Gossypium hirsutum	All genes	**4192**	**4172**	**7285**	**625**	**654**	**1475**	**1154**	**1941**	**1588**	**205**	**861**	**153**	**279**	**995**	**290**	**5429**	**15649**
	Not-organelle-targeted	**3575**	**3710**	**6240**	569	552	1344	929	1695	1368	173	699	135	256	931	264	4610	13525
	Dual-targeted_Interacting	**20**	**11**	**32**	0	3	4	11	6	6	0	8	0	1	4	0	20	63
	Dual-targeted_Non-interacting	**272**	**209**	**429**	24	48	63	93	111	102	15	50	10	5	23	10	356	910
	Mitochondria-targeted_Interacting	**49**	**68**	**128**	11	8	18	15	24	30	2	18	1	7	9	1	101	245
	Mitochondria-targeted_Non-interacting	**29**	**34**	**44**	5	2	10	9	16	13	1	8	0	5	6	2	30	107
	Plastid-targeted_Interacting	**57**	**33**	**104**	2	7	8	22	24	22	1	18	1	0	2	3	84	194
	Plastid-targeted_Non-interacting	**151**	**106**	**267**	14	30	28	44	62	46	13	43	5	5	19	10	205	524
	chloroplast	**27**	**0**	**25**	0	3	0	22	2	0	0	11	0	0	0	0	14	52
	mitochondria	**12**	**1**	**16**	0	1	0	9	1	1	0	6	1	0	1	0	9	29
adense	All genes	**4087**	**4107**	**7576**	**569**	**630**	**1600**	**1177**	**1891**	**1520**	**186**	**672**	**156**	**232**	**891**	**233**	**6013**	**15770**
	Not-organelle-targeted	**3494**	**3660**	**6466**	516	554	1380	1041	1563	1398	166	596	137	200	742	199	5128	13620
	Dual-targeted_Interacting	**17**	**10**	**32**	0	2	6	6	8	1	0	1	0	3	3	1	28	59
	Dual-targeted_Non-interacting	**257**	**203**	**451**	24	38	93	63	134	60	14	29	9	12	58	13	364	911
	Mitochondria-targeted_Interacting	**51**	**67**	**148**	9	4	38	8	34	13	0	7	1	7	25	4	116	266

Gossypium barbadense	Mitochondria-targeted_Non-interacting	**26**	**36**	**44**	3	4	21	5	16	7	1	5	0	4	8	1	31	106
	Plastid-targeted_Interacting	**56**	**25**	**112**	0	7	18	3	41	7	0	4	1	0	9	4	99	193
	Plastid-targeted_Non-interacting	**150**	**105**	**273**	17	19	44	40	75	34	4	21	5	6	46	11	206	528
	chloroplast	**25**	**0**	**35**	0	1	0	5	19	0	0	0	0	0	0	0	35	60
	mitochondria	**11**	**1**	**15**	0	1	0	6	1	0	1	9	3	0	0	0	6	27

Supplementary Table 5. Genes exhibiting differential expression (DE) relative to those not exhibiting differential expression (nDE) relative to the diploid parents. The homoeolog-based comparison refers to DE between maternal parent-maternal homoeolog or the paternal parent-paternal homoeolog comparison. Total expression evalutes DE between the indicated diploid parent and the total gene expression in the polyploid (represented by the sum of both homoeologs). For *Arabidopsis*, AsAt denotes the maternal parent and AsAa denotes the paternal.

	Homoeolog comparions					Total expression comparisons				
	Arabidopsis suecica					*Arabidopsis suecica*				
	AsAt-DE	AsAt-nDE	AsAa-DE	AsAa-nDE		AsAt-DE	AsAt-nDE	AsAa-DE	AsAa-nDE	
Dual-targeted, interacting	9	36	18	27	under-rep'd	17	28	18	27	Dual-targeted, interacting
up-regulated	7	38	15	30		10	35	16	29	up-regulated
down-regulated	2	43	3	42	over-rep'd	7	38	2	43	down-regulated
Dual-targeted, non-interacting	42	143	68	116		55	130	59	126	Dual-targeted, non-interacting
up-regulated	22	163	34	150		27	158	34	151	up-regulated
down-regulated	20	165	34	150		28	157	25	160	down-regulated
Mitochondria-targeted, interacting	50	212	78	185		77	186	99	164	Mitochondria-targeted, interacting
up-regulated	42	220	34	229		54	209	42	221	up-regulated
down-regulated	8	254	44	219		23	240	57	206	down-regulated
Mitochondria-targeted, non-interacti	122	329	182	272		174	280	190	264	Mitochondria-targeted, non-interacting
up-regulated	77	374	71	383		93	361	74	380	up-regulated
down-regulated	45	406	111	343		81	373	116	338	down-regulated
Plastid-targeted, interacting	37	130	43	125		58	110	40	128	Plastid-targeted, interacting
up-regulated	13	154	26	142		16	152	24	144	up-regulated
down-regulated	24	143	17	151		42	126	16	152	down-regulated
Plastid-targeted, non-interacting	325	938	466	804		459	817	470	806	Plastid-targeted, non-interacting
up-regulated	131	1132	273	997		190	1,086	285	991	up-regulated
down-regulated	194	1069	193	1077		269	1,007	185	1,091	down-regulated
						16	62	15	63	Chloroplast
						0	78	4	74	up-regulated
						16	62	11	67	down-regulated
						12	19	21	10	Mitochondria
						9	22	5	26	up-regulated
						3	28	16	15	down-regulated
not-targeted	2,251	6,530	3,020	5,822		3,120	5,922	16	15	not-targeted
up-regulated	1,186	7,595	1,534	7,308		1,761	7,281	3,214	5,828	up-regulated
down-regulated	1,065	7,716	1,486	7,356		1,359	7,683	1,602	7,440	down-regulated
Differentially expressed, total	**2,836**	**8,318**	**3,875**	**7,351**		**3,960**	**7,473**	**1,612**	**7,430**	**Differentially expressed, total**
up-regulated	**1,478**		**1,987**			**2,151**		**3,689**		**up-regulated**
down-regulated	**1,358**		**1,888**			**1,809**		**2,003**		**down-regulated**

Supplementary Table 6. Genes exhibiting differential expression (DE) relative to those not exhibiting differential expression (nDE) relative to the diploid parents. The homoeolog-based comparison refers to DE between maternal parent-maternal homoeolog or the paternal parent-paternal homoeolog comparison. Total expression evalutes DE between the indicated diploid parent and the total gene expression in the polyploid (represented by the sum of both homoeologs). For *Arachis*, Ad denotes the *Arachis duranensis* (maternal to *A. hypogea* and paternal to IpaDur1) and Ai denotes *Arachis ipaensis* (maternal to IpaDur1 and paternal to *A. hypogea*).

	Homoeolog comparions									Total expression comparisons								
	Arachis hypogea				*Arachis* IpaDur1					*Arachis hypogea*				*Arachis* IpaDur1				
	Ad-DE	Ad-nDE	Ai-DE	Ai-nDE	Ai-DE	Ai-nDE	Ad-DE	Ad-nDE		Ad-DE	Ad-nDE	Ai-DE	Ai-nDE	Ai-DE	Ai-nDE	Ad-DE	Ad-nDE	
Dual-targeted, interacting	3	48	29	22	27	24	0	51	under-rep'd	6	45	25	26	24	27	2	49	Dual-targeted, interacting
up-regulated	2		5		3		0			1		5		5		0		up-regulated
down-regulated	1		24		24		0		over-rep'd	5		20		19		2		down-regulated
Dual-targeted, non-interacting	81	648	347	387	376	358	13	716		93	642	318	417	310	425	26	709	Dual-targeted, non-interacting
up-regulated	64		158		133		11			46		166		144		9		up-regulated
down-regulated	17		189		243		2			47		152		166		17		down-regulated
Mitochondria-targeted, interacting	18	126	49	99	72	76	5	139		23	126	54	95	60	89	4	145	Mitochondria-targeted, interacting
up-regulated	10		15		16		4			6		17		18		1		up-regulated
down-regulated	8		34		56		1			17		37		42		3		down-regulated
Mitochondria-targeted, non-interacti	5	85	29	63	32	60	4	86		7	86	36	57	37	56	2	91	Mitochondria-targeted, non-interacting
up-regulated	1		14		13		4			2		18		21		1		up-regulated
down-regulated	4		15		19		0			5		18		16		1		down-regulated
Plastid-targeted, interacting	13	118	58	73	67	64	2	129		13	118	48	83	52	79	1	130	Plastid-targeted, interacting
up-regulated	12		26		23		2			8		23		20		0		up-regulated
down-regulated	1		32		44		0			5		25		32		1		down-regulated
Plastid-targeted, non-interacting	35	323	163	195	198	160	4	354		59	300	155	204	161	198	13	346	Plastid-targeted, non-interacting
up-regulated	26		89		81		3			34		88		79		6		up-regulated
down-regulated	9		74		117		1			25		67		82		7		down-regulated
										5	71	13	63	35	41	0	76	Chloroplast
										0		13		35		0		up-regulated
										5		0		0		0		down-regulated
										12	20	11	21	16	16	6	26	Mitochondria
										5		8		16		5		up-regulated
										7		3		0		1		down-regulated
not-targeted	766	7,885	2,819	6,071	3,386	5,504	166	8,485		1,077	8,050	2,949	6,178	2,866	6,261	150	8,977	not-targeted
up-regulated	478		1,409		1,599		142			512		1,546		1,606		91		up-regulated
down-regulated	288		1,410		1,787		24			565		1,403		1,260		59		down-regulated
Differentially expressed, total	**921**	**9,233**	**3,494**	**6,910**	**4,158**	**6,246**	**194**	**9,960**		**1,278**	**9,367**	**3,585**	**7,060**	**3,510**	**7,135**	**198**	**10,447**	**Differentially expressed, total**
up-regulated	**593**		**1,716**		**1,868**		**166**			**609**		**1,863**		**1,893**		**108**		**up-regulated**
down-regulated	**328**		**1,778**		**2,290**		**28**			**669**		**1,722**		**1,617**		**90**		**down-regulated**

Supplementary Table 7. Genes exhibiting differential expression (DE) relative to those not exhibiting differential expression (nDE) relative to the diploid parents. The homoeolog-based comparison refers to DE between maternal parent-maternal homoeolog or the paternal parent-paternal homoeolog comparison. Total expression evalutes DE between the indicated diploid parent and the total gene expression in the polyploid (represented by the sum of both homoeologs). For *Chenopodium*, A denotes the maternal parent and B denotes the paternal.

	Homoeolog comparions					Total expression comparisons				
	Chenopodium quinoa					*Chenopodium quinoa*				
	A-DE	A-nDE	B-DE	B-nDE		A-DE	A-nDE	B-DE	B-nDE	
Dual-targeted, interacting	33	29	26	36	under-rep'd	33	29	33	29	Dual-targeted, interacting
up-regulated	8		17			8		24		up-regulated
down-regulated	25		9		over-rep'd	25		9		down-regulated
Dual-targeted, non-interacting	436	332	393	372		424	345	462	307	Dual-targeted, non-interacting
up-regulated	270		171			261		197		up-regulated
down-regulated	166		222			163		265		down-regulated
Mitochondria-targeted, interacting	86	82	91	77		90	78	114	54	Mitochondria-targeted, interacting
up-regulated	30		65			31		78		up-regulated
down-regulated	56		26			59		36		down-regulated
Mitochondria-targeted, non-interacti	48	34	34	47		52	30	46	36	Mitochondria-targeted, non-interacting
up-regulated	23		25			22		31		up-regulated
down-regulated	25		9			30		15		down-regulated
Plastid-targeted, interacting	86	73	65	94		73	86	77	82	Plastid-targeted, interacting
up-regulated	49		27			41		39		up-regulated
down-regulated	37		38			32		38		down-regulated
Plastid-targeted, non-interacting	260	145	195	209		228	177	226	179	Plastid-targeted, non-interacting
up-regulated	138		82			112		93		up-regulated
down-regulated	122		113			116		133		down-regulated
						29	49	52	26	Chloroplast
						26		2		up-regulated
						3		50		down-regulated
						20	10	18	12	Mitochondria
						3		7		up-regulated
						17		11		down-regulated
not-targeted	3,960	2,999	3,145	3,832		3,924	3,244	3,805	3,363	not-targeted
up-regulated	2,001		1,578			2,047		1,927		up-regulated
down-regulated	1,959		1,567			1,877		1,878		down-regulated
Differentially expressed, total	**4,909**	**3,694**	**3,949**	**4,667**		**4,824**	**3,989**	**4,763**	**4,050**	**Differentially expressed, total**
up-regulated	**2,519**		**1,965**			**2,522**		**2,389**		**up-regulated**
down-regulated	**2,390**		**1,984**			**2,302**		**2,374**		**down-regulated**

Supplementary Table 8. Genes exhibiting differential expression (DE) relative to those not exhibiting differential expression (nDE) relative to the diploid parents. The homoeolog-based comparison refers to DE between maternal parent-matern

	Homoeolog comparions									Total expression comparisons								
	Gossypium hirsutum				*Gossypium barbadense*					*Gossypium hirsutum*				*Gossypium barbadense*				
	A-DE	A-nDE	D-DE	D-nDE	A-DE	A-nDE	D-DE	D-nDE		A-DE	A-nDE	D-DE	D-nDE	A-DE	A-nDE	D-DE	D-nDE	
Dual-targeted, interacting	22	54	33	43	25	51	28	48	under-rep'd	34	42	33	43	25	51	24	52	Dual-targeted, interacting
up-regulated	9		11		19		20			9		14		16		21		up-regulated
down-regulated	13		22		6		8		over-rep'd	25		19		9		3		down-regulated
Dual-targeted, non-interacting	327	758	486	602	336	749	450	638		390	701	461	630	397	694	437	654	Dual-targeted, non-interacting
up-regulated	108		236		167		297			144		221		234		291		up-regulated
down-regulated	219		250		169		153			246		240		163		146		down-regulated
Mitochondria-targeted, interacting	92	232	132	190	103	221	121	201		116	208	139	185	117	207	124	200	Mitochondria-targeted, interacting
up-regulated	43		57		85		92			51		56		96		91		up-regulated
down-regulated	49		75		18		29			65		83		21		33		down-regulated
Mitochondria-targeted, non-interacti	53	79	63	71	45	87	57	77		55	79	68	66	59	75	59	75	Mitochondria-targeted, non-interacting
up-regulated	30		33		27		35			31		34		41		36		up-regulated
down-regulated	23		30		18		22			24		34		18		23		down-regulated
Plastid-targeted, interacting	68	178	92	153	49	197	92	153		80	166	100	146	59	187	88	158	Plastid-targeted, interacting
up-regulated	17		47		25		77			20		44		40		74		up-regulated
down-regulated	51		45		24		15			60		56		19		14		down-regulated
Plastid-targeted, non-interacting	204	422	265	359	202	424	281	343		236	391	285	342	243	384	265	362	Plastid-targeted, non-interacting
up-regulated	63		132		103		192			83		144		143		178		up-regulated
down-regulated	141		133		99		89			153		141		100		87		down-regulated
										50	27	16	61	6	71	24	53	Chloroplast
										0		5		0		24		up-regulated
										50		11		6		0		down-regulated
										20	15	15	20	22	13	20	15	Mitochondria
										1		4		0		5		up-regulated
										19		11		22		15		down-regulated
not-targeted	5,203	10,505	7,231	8,635	4,765	10,943	6,484	9,382		6,561	9,733	7,617	8,677	6,177	10,117	6,883	9,411	not-targeted
up-regulated	2,896		3,874		2,482		3,339			3,747		4,178		3,374		3,660		up-regulated
down-regulated	2,307		3,357		2,283		3,145			2,814		3,439		2,803		3,223		down-regulated
Differentially expressed, total	**5,969**	**12,228**	**8,302**	**10,053**	**5,525**	**12,672**	**7,513**	**10,842**		**7,472**	**11,320**	**8,703**	**10,089**	**7,077**	**11,715**	**7,880**	**10,912**	**Differentially expressed, total**
up-regulated	**3,166**		**4,390**		**2,908**		**4,052**			**4,085**		**4,691**		**3,944**		**4,351**		**up-regulated**
down-regulated	**2,803**		**3,912**		**2,617**		**3,461**			**3,387**		**4,012**		**3,133**		**3,529**		**down-regulated**

Supplementary Table 9. Enriched ontology terms (Benjamini corrected p-value < 0.05) for modules comprised of differentially expressed homoeologs with greater than fourfold difference in expression. The homoeolog bias columns indicates general bias for that functional module. The bottom half of the table contains enriched terms from the set of DE genes whose difference in fold-change between the subgenomes is greater than fourfold compared to the diploid genomes. Modules containing only one DE gene were excluded as unreliable.

Homoeolog Bias	Term	Name	Number of DE genes	Number in background	Fishers pvalue	Benjamini corrected p-value	DE set
Paternal	GO:0001558	regulation of cell growth	3	14	1.8E-03	0.045	any DE
Paternal	GO:0003674	molecular_function	73	2729	8.4E-06	0.000	any DE
Paternal	GO:0004144	diacylglycerol O-acyltransferase activity	2	7	8.3E-03	0.046	any DE
Paternal	GO:0005575	cellular_component	16	466	1.0E-03	0.037	any DE
Paternal	GO:0005576	extracellular region	20	822	1.2E-02	0.046	any DE
Paternal	GO:0005634	nucleus	88	4983	7.0E-03	0.046	any DE
Paternal	GO:0005813	centrosome	2	16	2.4E-02	0.046	any DE
Paternal	GO:0005886	plasma membrane	27	1346	3.5E-02	0.046	any DE
Paternal	GO:0006633	fatty acid biosynthetic process	3	53	4.8E-02	0.049	any DE
Paternal	GO:0006886	intracellular protein transport	5	101	2.0E-02	0.046	any DE
Paternal	GO:0006890	retrograde vesicle-mediated transport, Golgi to endoplasmic reticulum	3	19	3.9E-03	0.045	any DE
Paternal	GO:0006891	intra-Golgi vesicle-mediated transport	3	19	3.9E-03	0.045	any DE
Paternal	GO:0008150	biological_process	38	1069	1.3E-06	0.000	any DE
Paternal	GO:0008865	fructokinase activity	2	5	5.0E-03	0.046	any DE
Paternal	GO:0009404	toxin metabolic process	2	14	2.2E-02	0.046	any DE
Paternal	GO:0016595	glutamate binding	2	4	3.6E-03	0.045	any DE
Paternal	GO:0019432	triglyceride biosynthetic process	3	16	2.5E-03	0.045	any DE
Paternal	GO:0019748	secondary metabolic process	7	153	9.3E-03	0.046	any DE
Paternal	GO:0020037	heme binding	5	101	2.6E-02	0.046	any DE
Paternal	GO:0030126	COPI vesicle coat	2	7	6.2E-03	0.046	any DE
Paternal	GO:0031491	nucleosome binding	2	12	2.0E-02	0.046	any DE
Paternal	GO:0034620	cellular response to unfolded protein	2	7	7.1E-03	0.046	any DE
Paternal	GO:0044550	secondary metabolite biosynthetic process	2	10	1.3E-02	0.046	any DE
Paternal	GO:0045492	xylan biosynthetic process	2	22	4.7E-02	0.048	any DE
Paternal	GO:0047196	long-chain-alcohol O-fatty-acyltransferase activity	2	3	2.4E-03	0.045	any DE
Paternal	GO:0048364	root development	11	223	7.0E-04	0.033	any DE
Paternal	GO:0048588	developmental cell growth	2	21	4.4E-02	0.047	any DE
Paternal	GO:0048638	regulation of developmental growth	4	37	2.9E-03	0.045	any DE
Paternal	GO:0051213	dioxygenase activity	2	19	4.3E-02	0.047	any DE
Paternal	GO:0080030	methyl indole-3-acetate esterase activity	3	13	1.9E-03	0.045	any DE
Paternal	GO:0080092	regulation of pollen tube growth	3	16	2.5E-03	0.045	any DE
Paternal	GO:0102966	arachidoyl-CoA:1-dodecanol O-acyltransferase activity	2	3	2.4E-03	0.045	any DE
Paternal	GO:2000241	regulation of reproductive process	4	82	3.7E-02	0.046	any DE
Paternal	IPR000008	C2 domain	3	43	4.3E-02	0.047	any DE
Paternal	IPR004255	O-acyltransferase, WSD1, N-terminal	2	3	2.8E-03	0.045	any DE
Paternal	IPR005123	Oxoglutarate/iron-dependent dioxygenase	4	38	5.5E-03	0.046	any DE
Paternal	IPR005225	Small GTP-binding protein domain	4	58	2.1E-02	0.046	any DE
Paternal	IPR006214	Bax inhibitor 1-related	2	5	5.7E-03	0.046	any DE
Paternal	IPR009721	O-acyltransferase WSD1, C-terminal	2	3	2.8E-03	0.045	any DE
Paternal	IPR012337	Ribonuclease H-like superfamily	3	43	4.3E-02	0.047	any DE
Paternal	IPR013094	Alpha/beta hydrolase fold-3	2	11	2.0E-02	0.046	any DE
Paternal	IPR022775	AP complex, mu/sigma subunit	2	6	7.5E-03	0.046	any DE

	Paternal	IPR026992	Non-haem dioxygenase N-terminal domain	3	24	1.1E-02	0.046	any DE
	Paternal	IPR027443	Isopenicillin N synthase-like	3	30	1.8E-02	0.046	any DE
	Paternal	IPR033140	Lipase, GDXG, putative serine active site	2	6	7.5E-03	0.046	any DE
	Paternal	IPR035892	C2 domain superfamily	3	45	4.8E-02	0.049	any DE
	Paternal	IPR039652	Coatomer subunit zeta	2	4	4.1E-03	0.045	any DE
	Paternal	PO:0000054	petal vascular system	2	14	3.9E-03	0.045	any DE
	Paternal	PO:0000191	synergid	2	48	3.5E-02	0.046	any DE
	Paternal	PO:0000262	trichoblast	2	51	3.9E-02	0.046	any DE
	Paternal	PO:0000263	non-hair root epidermal cell	3	39	1.9E-03	0.045	any DE
	Paternal	PO:0001016	L mature pollen stage	23	2176	8.6E-04	0.036	any DE
	Paternal	PO:0001017	M germinated pollen stage	24	2623	4.2E-03	0.045	any DE
	Paternal	PO:0002000	stomatal complex	2	18	6.0E-03	0.046	any DE
	Paternal	PO:0004723	sepal vascular system	2	14	3.9E-03	0.045	any DE
	Paternal	PO:0006504	leaf trichome	2	38	2.3E-02	0.046	any DE
	Paternal	PO:0007611	petal differentiation and expansion stage	85	9150	3.7E-08	0.000	any DE
	Paternal	PO:0007616	4 anthesis stage	86	9024	9.6E-09	0.000	any DE
	Paternal	PO:0009005	root	68	8436	6.6E-03	0.046	any DE
	Paternal	PO:0009031	sepal	64	8760	4.8E-02	0.049	any DE
	Paternal	PO:0009046	flower	93	9211	5.7E-07	0.000	any DE
	Paternal	PO:0020090	central cell	2	36	2.1E-02	0.046	any DE
	Paternal	PO:0020094	plant egg cell	6	75	8.8E-06	0.000	any DE
	Paternal	PO:0025022	collective leaf structure	71	8878	6.6E-03	0.046	any DE
	Paternal	PO:0025195	pollen tube cell	26	2691	1.1E-02	0.046	any DE
	Maternal	GO:0000118	histone deacetylase complex	2	16	2.4E-02	0.046	any DE
	Maternal	GO:0003714	transcription corepressor activity	2	14	1.8E-02	0.046	any DE
	Maternal	GO:0003724	RNA helicase activity	3	41	1.9E-02	0.046	any DE
	Maternal	GO:0005575	cellular_component	12	466	2.8E-02	0.046	any DE
	Maternal	GO:0005615	extracellular space	3	29	8.8E-03	0.046	any DE
	Maternal	GO:0005774	vacuolar membrane	3	45	2.6E-02	0.046	any DE
	Maternal	GO:0005811	lipid droplet	2	12	1.5E-02	0.046	any DE
	Maternal	GO:0005829	cytosol	27	1343	2.9E-02	0.046	any DE
	Maternal	GO:0006306	DNA methylation	2	12	1.0E-02	0.046	any DE
	Maternal	GO:0006817	phosphate ion transport	2	8	5.3E-03	0.046	any DE
	Maternal	GO:0006829	zinc ion transport	2	8	5.3E-03	0.046	any DE
	Maternal	GO:0008150	biological_process	21	1069	1.2E-02	0.046	any DE
	Maternal	GO:0008324	cation transmembrane transporter activity	2	10	1.0E-02	0.046	any DE
	Maternal	GO:0008757	S-adenosylmethionine-dependent methyltransferase activity	2	18	2.7E-02	0.046	any DE
	Maternal	GO:0009624	response to nematode	3	45	1.7E-02	0.046	any DE
	Maternal	GO:0009686	gibberellin biosynthetic process	2	12	1.0E-02	0.046	any DE
	Maternal	GO:0009694	jasmonic acid metabolic process	2	9	6.4E-03	0.046	any DE
	Maternal	GO:0009696	salicylic acid metabolic process	2	6	3.3E-03	0.045	any DE
	Maternal	GO:0010043	response to zinc ion	2	20	2.5E-02	0.046	any DE
	Maternal	GO:0010114	response to red light	3	52	2.4E-02	0.046	any DE
	Maternal	GO:0010218	response to far red light	2	30	5.0E-02	0.050	any DE
	Maternal	GO:0010286	heat acclimation	2	25	3.6E-02	0.046	any DE
	Maternal	GO:0015103	inorganic anion transmembrane transporter activity	2	6	4.4E-03	0.045	any DE
	Maternal	GO:0015144	carbohydrate transmembrane transporter activity	2	23	4.1E-02	0.047	any DE
	Maternal	GO:0030001	metal ion transport	2	9	6.4E-03	0.046	any DE

	Maternal	GO:0031625	ubiquitin protein ligase binding	2	21	3.5E-02	0.046	any DE
	Maternal	GO:0034605	cellular response to heat	2	28	4.4E-02	0.047	any DE
	Maternal	GO:0042542	response to hydrogen peroxide	3	34	8.2E-03	0.046	any DE
	Maternal	GO:0048868	pollen tube development	2	18	2.1E-02	0.046	any DE
	Maternal	GO:0051119	sugar transmembrane transporter activity	2	13	1.6E-02	0.046	any DE
	Maternal	GO:0051213	dioxygenase activity	2	19	3.0E-02	0.046	any DE
	Maternal	GO:0051259	protein complex oligomerization	2	9	6.4E-03	0.046	any DE
	Maternal	GO:0071456	cellular response to hypoxia	6	120	3.0E-03	0.045	any DE
	Maternal	GO:0080030	methyl indole-3-acetate esterase activity	2	13	1.6E-02	0.046	any DE
	Maternal	GO:0080031	methyl salicylate esterase activity	2	6	4.4E-03	0.045	any DE
	Maternal	GO:0080032	methyl jasmonate esterase activity	2	6	4.4E-03	0.045	any DE
	Maternal	GO:0099503	secretory vesicle	4	59	1.0E-02	0.046	any DE
	Maternal	IPR000073	Alpha/beta hydrolase fold-1	3	41	2.1E-02	0.046	any DE
	Maternal	IPR000232	Heat shock factor (HSF)-type, DNA-binding	2	14	1.9E-02	0.046	any DE
	Maternal	IPR000315	B-box-type zinc finger	2	21	3.7E-02	0.046	any DE
	Maternal	IPR000782	FAS1 domain	2	13	1.6E-02	0.046	any DE
	Maternal	IPR000953	Chromo/chromo shadow domain	2	9	8.9E-03	0.046	any DE
	Maternal	IPR002068	Alpha crystallin/Hsp20 domain	2	17	2.6E-02	0.046	any DE
	Maternal	IPR002213	UDP-glucuronosyl/UDP-glucosyltransferase	2	25	4.9E-02	0.050	any DE
	Maternal	IPR002524	Cation efflux protein	2	8	7.4E-03	0.046	any DE
	Maternal	IPR003663	Sugar/inositol transporter	2	25	4.9E-02	0.050	any DE
	Maternal	IPR005829	Sugar transporter, conserved site	2	24	4.6E-02	0.048	any DE
	Maternal	IPR011013	Galactose mutarotase-like domain superfamily	2	18	2.8E-02	0.046	any DE
	Maternal	IPR011042	Six-bladed beta-propeller, TolB-like	2	10	1.1E-02	0.046	any DE
	Maternal	IPR011545	DEAD/DEAH box helicase domain	3	45	2.6E-02	0.046	any DE
	Maternal	IPR011684	Protein Networked (NET), actin-binding (NAB) domain	2	10	1.1E-02	0.046	any DE
	Maternal	IPR016197	Chromo-like domain superfamily	2	9	8.9E-03	0.046	any DE
	Maternal	IPR023780	Chromo domain	2	6	4.7E-03	0.046	any DE
	Maternal	IPR026992	Non-haem dioxygenase N-terminal domain	2	24	4.6E-02	0.048	any DE
	Maternal	IPR027469	Cation efflux transmembrane domain superfamily	2	8	7.4E-03	0.046	any DE
	Maternal	IPR027725	Heat shock transcription factor family	2	14	1.9E-02	0.046	any DE
	Maternal	IPR029058	Alpha/Beta hydrolase fold	6	143	1.5E-02	0.046	any DE
	Maternal	IPR031107	Small heat shock protein HSP20	2	8	7.4E-03	0.046	any DE
	Maternal	IPR036378	FAS1 domain superfamily	2	13	1.6E-02	0.046	any DE
	Maternal	IPR036837	Cation efflux protein, cytoplasmic domain superfamily	2	5	3.5E-03	0.045	any DE
	Maternal	PO:0000293	guard cell	137	10112	1.7E-02	0.046	any DE
*	Maternal	GO:0009941	chloroplast envelope	6	347	4.6E-02	0.048	DE >4fold
	Paternal	GO:0005575	cellular_component	7	466	8.5E-05	0.025	DE >4fold
	Paternal	IPR033140	Lipase, GDXG, putative serine active site	2	6	4.2E-04	0.039	DE >4fold
	Paternal	GO:0009404	toxin metabolic process	2	14	9.5E-04	0.039	DE >4fold
	Paternal	IPR013094	Alpha/beta hydrolase fold-3	2	11	1.2E-03	0.039	DE >4fold
	Paternal	GO:0080030	methyl indole-3-acetate esterase activity	2	13	1.2E-03	0.039	DE >4fold
	Paternal	GO:0016787	hydrolase activity	2	31	5.7E-03	0.039	DE >4fold
	Paternal	PO:0007616	4 anthesis stage	19	9024	7.6E-03	0.039	DE >4fold
	Paternal	GO:0008150	biological_process	8	1069	1.2E-02	0.039	DE >4fold
	Paternal	IPR000008	C2 domain	2	43	1.4E-02	0.039	DE >4fold
	Paternal	IPR035892	C2 domain superfamily	2	45	1.5E-02	0.039	DE >4fold

	Paternal	PO:0007611	petal differentiation and expansion stage	18	9150	1.8E-02	0.039	DE >4fold
	Paternal	IPR013766	Thioredoxin domain	2	52	1.9E-02	0.039	DE >4fold
	Paternal	IPR029058	Alpha/Beta hydrolase fold	3	143	2.0E-02	0.039	DE >4fold
	Paternal	GO:0005634	nucleus	18	4983	2.1E-02	0.040	DE >4fold
	Paternal	GO:0010150	leaf senescence	2	95	3.2E-02	0.042	DE >4fold
	Paternal	GO:0003676	nucleic acid binding	2	96	4.5E-02	0.047	DE >4fold
	Paternal	PO:0009001	fruit	3	534	4.6E-02	0.048	DE >4fold
	Paternal	GO:0020037	heme binding	2	101	4.9E-02	0.049	DE >4fold
	Maternal	IPR023780	Chromo domain	2	6	1.3E-03	0.039	DE >4fold
	Maternal	GO:0006829	zinc ion transport	2	8	1.7E-03	0.039	DE >4fold
	Maternal	GO:0005829	cytosol	20	1343	2.5E-03	0.039	DE >4fold
	Maternal	IPR000953	Chromo/chromo shadow domain	2	9	2.5E-03	0.039	DE >4fold
	Maternal	IPR016197	Chromo-like domain superfamily	2	9	2.5E-03	0.039	DE >4fold
	Maternal	GO:0003724	RNA helicase activity	3	41	2.6E-03	0.039	DE >4fold
	Maternal	IPR011042	Six-bladed beta-propeller, TolB-like	2	10	3.0E-03	0.039	DE >4fold
	Maternal	IPR011545	DEAD/DEAH box helicase domain	3	45	4.5E-03	0.039	DE >4fold
	Maternal	GO:0005774	vacuolar membrane	3	45	5.2E-03	0.039	DE >4fold
	Maternal	GO:0048868	pollen tube development	2	18	6.8E-03	0.039	DE >4fold
	Maternal	GO:0031625	ubiquitin protein ligase binding	2	21	9.0E-03	0.039	DE >4fold
	Maternal	GO:0015144	carbohydrate transmembrane transporter activity	2	23	1.1E-02	0.039	DE >4fold
	Maternal	GO:0005622	intracellular anatomical structure	3	63	1.3E-02	0.039	DE >4fold
	Maternal	IPR005829	Sugar transporter, conserved site	2	24	1.4E-02	0.039	DE >4fold
	Maternal	IPR003663	Sugar/inositol transporter	2	25	1.5E-02	0.039	DE >4fold
	Maternal	GO:0010218	response to far red light	2	30	1.7E-02	0.039	DE >4fold
	Maternal	IPR001650	Helicase, C-terminal	3	76	1.7E-02	0.039	DE >4fold
	Maternal	IPR005828	Major facilitator, sugar transporter-like	2	28	1.8E-02	0.039	DE >4fold
	Maternal	IPR029058	Alpha/Beta hydrolase fold	4	143	1.9E-02	0.039	DE >4fold
	Maternal	IPR014001	Helicase superfamily 1/2, ATP-binding domain	3	80	2.0E-02	0.039	DE >4fold
	Maternal	IPR036855	Zinc finger, CCCH-type superfamily	2	32	2.3E-02	0.041	DE >4fold
	Maternal	IPR036259	MFS transporter superfamily	3	92	2.8E-02	0.041	DE >4fold
	Maternal	GO:0009416	response to light stimulus	8	585	3.3E-02	0.043	DE >4fold
	Maternal	IPR020846	Major facilitator superfamily domain	2	40	3.4E-02	0.043	DE >4fold
	Maternal	GO:0009624	response to nematode	2	45	3.5E-02	0.043	DE >4fold
	Maternal	GO:0048827	phyllome development	2	45	3.5E-02	0.043	DE >4fold
	Maternal	IPR000073	Alpha/beta hydrolase fold-1	2	41	3.6E-02	0.043	DE >4fold
	Maternal	GO:0004252	serine-type endopeptidase activity	2	47	3.8E-02	0.044	DE >4fold
	Maternal	IPR003657	WRKY domain	2	44	4.0E-02	0.045	DE >4fold
	Maternal	IPR036576	WRKY domain superfamily	2	44	4.0E-02	0.045	DE >4fold
	Maternal	GO:0071456	cellular response to hypoxia	3	120	4.2E-02	0.046	DE >4fold
	Maternal	GO:0010114	response to red light	2	52	4.5E-02	0.047	DE >4fold
	Maternal	IPR000571	Zinc finger, CCCH-type	2	49	4.8E-02	0.049	DE >4fold
	Maternal	GO:0009553	embryo sac development	2	55	4.9E-02	0.049	DE >4fold

Supplementary Table 10. Enriched ontology terms (Benjamini corrected p-value < 0.05) for modules comprised of differentially expressed homoeologs with greater than fourfold difference in expression. The bottom half of the table contains enriched terms from the set of DE genes whose difference in fold-change between the subgenomes is greater than fourfold compared to the diploid genomes. Modules containing only one DE gene were excluded as unreliable.

Species	Homoeolog Bias	Term	Name	Number of DE genes	Number in background	Fishers p-value	Benjamini corrected p-value	DE set
G. hirsutum	Paternal bias	GO:0005634	nucleus	20	410	0.001	0.046	any DE
G. hirsutum	Paternal bias	IPR006689	Small GTPase superfamily, ARF/SAR type	4	25	0.003	0.046	any DE
G. hirsutum	Paternal bias	GO:0006357	regulation of transcription by RNA polymerase II	3	15	0.005	0.046	any DE
G. hirsutum	Paternal bias	GO:0032784	regulation of DNA-templated transcription, elongation	2	6	0.01	0.046	any DE
G. hirsutum	Paternal bias	IPR020904	Short-chain dehydrogenase/reductase, conserved site	3	21	0.012	0.046	any DE
G. hirsutum	Paternal bias	IPR002119	Histone H2A	2	7	0.014	0.046	any DE
G. hirsutum	Paternal bias	IPR032454	Histone H2A, C-terminal domain	2	7	0.014	0.046	any DE
G. hirsutum	Paternal bias	GO:0046982	protein heterodimerization activity	4	48	0.021	0.046	any DE
G. hirsutum	Paternal bias	IPR009072	Histone-fold	4	48	0.021	0.046	any DE
G. hirsutum	Paternal bias	IPR023214	HAD superfamily	6	100	0.022	0.046	any DE
G. hirsutum	Paternal bias	GO:0006508	proteolysis	10	237	0.022	0.046	any DE
G. hirsutum	Paternal bias	GO:0003684	damaged DNA binding	2	10	0.024	0.046	any DE
G. hirsutum	Paternal bias	GO:0005992	trehalose biosynthetic process	2	11	0.025	0.046	any DE
G. hirsutum	Paternal bias	GO:0006289	nucleotide-excision repair	2	11	0.025	0.046	any DE
G. hirsutum	Paternal bias	IPR001965	Zinc finger, PHD-type	5	77	0.026	0.046	any DE
G. hirsutum	Paternal bias	IPR003337	Trehalose-phosphatase	2	11	0.028	0.046	any DE
G. hirsutum	Paternal bias	IPR006379	HAD-superfamily hydrolase, subfamily IIB	2	11	0.028	0.046	any DE
G. hirsutum	Paternal bias	IPR014756	Immunoglobulin E-set	3	30	0.029	0.046	any DE
G. hirsutum	Paternal bias	GO:0007049	cell cycle	2	13	0.033	0.046	any DE
G. hirsutum	Paternal bias	GO:0003677	DNA binding	27	895	0.036	0.046	any DE
G. hirsutum	Paternal bias	GO:0000786	nucleosome	3	32	0.046	0.046	any DE
G. hirsutum	Paternal bias	GO:0004252	serine-type endopeptidase activity	4	64	0.049	0.049	any DE
G. hirsutum	Maternal bias	IPR014001	Helicase superfamily 1/2, ATP-binding domain	6	64	0.001	0.046	any DE
G. hirsutum	Maternal bias	IPR001650	Helicase, C-terminal	6	65	0.001	0.046	any DE
G. hirsutum	Maternal bias	IPR019821	Kinesin motor domain, conserved site	4	28	0.001	0.046	any DE
G. hirsutum	Maternal bias	IPR008847	Suppressor of forked	2	3	0.002	0.046	any DE
G. hirsutum	Maternal bias	GO:0005871	kinesin complex	4	41	0.002	0.046	any DE
G. hirsutum	Maternal bias	IPR000629	ATP-dependent RNA helicase DEAD-box, conserved site	3	15	0.003	0.046	any DE
G. hirsutum	Maternal bias	GO:0051082	unfolded protein binding	5	56	0.004	0.046	any DE
G. hirsutum	Maternal bias	GO:0007018	microtubule-based movement	4	44	0.004	0.046	any DE
G. hirsutum	Maternal bias	IPR001752	Kinesin motor domain	4	41	0.005	0.046	any DE
G. hirsutum	Maternal bias	IPR011545	DEAD/DEAH box helicase domain	4	41	0.005	0.046	any DE
G. hirsutum	Maternal bias	IPR027640	Kinesin-like protein	4	41	0.005	0.046	any DE
G. hirsutum	Maternal bias	IPR002194	Chaperonin TCP-1, conserved site	2	6	0.006	0.046	any DE
G. hirsutum	Maternal bias	IPR017998	Chaperone tailless complex polypeptide 1 (TCP-1)	2	6	0.006	0.046	any DE
G. hirsutum	Maternal bias	IPR008480	Protein of unknown function DUF761, plant	3	23	0.007	0.046	any DE
G. hirsutum	Maternal bias	IPR027410	TCP-1-like chaperonin intermediate domain superfamily	2	7	0.008	0.046	any DE
G. hirsutum	Maternal bias	GO:0009116	nucleoside metabolic process	2	8	0.008	0.046	any DE
G. hirsutum	Maternal bias	IPR025836	Zinc knuckle CX2CX4HX4C	3	24	0.008	0.046	any DE
G. hirsutum	Maternal bias	IPR008972	Cupredoxin	5	76	0.008	0.046	any DE
G. hirsutum	Maternal bias	GO:0003777	microtubule motor activity	4	44	0.009	0.046	any DE
G. hirsutum	Maternal bias	IPR002022	Pectate lyase	3	26	0.01	0.046	any DE
G. hirsutum	Maternal bias	IPR014014	RNA helicase, DEAD-box type, Q motif	3	26	0.01	0.046	any DE
G. hirsutum	Maternal bias	IPR018082	AmbAllergen	3	26	0.01	0.046	any DE
G. hirsutum	Maternal bias	IPR025558	Domain of unknown function DUF4283	3	28	0.012	0.046	any DE
G. hirsutum	Maternal bias	IPR000198	Rho GTPase-activating protein domain	2	9	0.012	0.046	any DE
G. hirsutum	Maternal bias	IPR003107	HAT (Half-A-TPR) repeat	2	9	0.012	0.046	any DE

G. hirsutum	Maternal bias	IPR008936	Rho GTPase activation protein	2	9	0.012	0.046	any DE
G. hirsutum	Maternal bias	IPR003653	Ulp1 protease family, C-terminal catalytic domain	2	10	0.014	0.046	any DE
G. hirsutum	Maternal bias	GO:0008017	microtubule binding	4	53	0.016	0.046	any DE
G. hirsutum	Maternal bias	IPR007524	Pectate lyase, N-terminal	2	11	0.017	0.046	any DE
G. hirsutum	Maternal bias	IPR002173	Carbohydrate/purine kinase, PfkB, conserved site	2	12	0.019	0.046	any DE
G. hirsutum	Maternal bias	IPR002453	Beta tubulin	2	12	0.019	0.046	any DE
G. hirsutum	Maternal bias	GO:0030570	pectate lyase activity	2	11	0.02	0.046	any DE
G. hirsutum	Maternal bias	IPR000095	CRIB domain	2	13	0.022	0.046	any DE
G. hirsutum	Maternal bias	IPR013838	Beta tubulin, autoregulation binding site	2	13	0.022	0.046	any DE
G. hirsutum	Maternal bias	GO:0006396	RNA processing	3	41	0.023	0.046	any DE
G. hirsutum	Maternal bias	IPR027413	GroEL-like equatorial domain superfamily	2	15	0.028	0.046	any DE
G. hirsutum	Maternal bias	IPR029044	Nucleotide-diphospho-sugar transferases	5	105	0.028	0.046	any DE
G. hirsutum	Maternal bias	GO:0006397	mRNA processing	2	17	0.028	0.046	any DE
G. hirsutum	Maternal bias	IPR011707	Multicopper oxidase, type 3	3	41	0.03	0.046	any DE
G. hirsutum	Maternal bias	IPR011611	Carbohydrate kinase PfkB	2	16	0.031	0.046	any DE
G. hirsutum	Maternal bias	IPR001117	Multicopper oxidase, type 1	3	42	0.032	0.046	any DE
G. hirsutum	Maternal bias	IPR027409	GroEL-like apical domain superfamily	2	17	0.034	0.046	any DE
G. hirsutum	Maternal bias	IPR029056	Ribokinase-like	2	17	0.034	0.046	any DE
G. hirsutum	Maternal bias	IPR001296	Glycosyl transferase, family 1	2	18	0.038	0.046	any DE
G. hirsutum	Maternal bias	IPR001305	Heat shock protein DnaJ, cysteine-rich domain	2	18	0.038	0.046	any DE
G. hirsutum	Maternal bias	IPR002423	Chaperonin Cpn60/TCP-1 family	2	18	0.038	0.046	any DE
G. hirsutum	Maternal bias	IPR024709	Putative O-fucosyltransferase, plant	2	18	0.038	0.046	any DE
G. hirsutum	Maternal bias	IPR027356	NPH3 domain	2	18	0.038	0.046	any DE
G. hirsutum	Maternal bias	GO:0006950	response to stress	3	52	0.041	0.046	any DE
G. hirsutum	Maternal bias	IPR017975	Tubulin, conserved site	2	19	0.041	0.046	any DE
G. hirsutum	Maternal bias	GO:0006457	protein folding	4	92	0.044	0.046	any DE
G. hirsutum	Maternal bias	IPR000330	SNF2-related, N-terminal domain	2	20	0.045	0.046	any DE
G. hirsutum	Maternal bias	GO:0031072	heat shock protein binding	2	18	0.045	0.046	any DE
G. hirsutum	Paternal bias	IPR006689	Small GTPase superfamily, ARF/SAR type	3	25	0.001	0.037	DE >4fold
G. hirsutum	Paternal bias	IPR023214	HAD superfamily	4	100	0.006	0.037	DE >4fold
G. hirsutum	Paternal bias	GO:0006357	regulation of transcription by RNA polymerase II	2	15	0.008	0.037	DE >4fold
G. hirsutum	Paternal bias	IPR002659	Glycosyl transferase, family 31	2	19	0.009	0.037	DE >4fold
G. hirsutum	Paternal bias	GO:0008378	galactosyltransferase activity	2	19	0.012	0.037	DE >4fold
G. hirsutum	Paternal bias	GO:0006260	DNA replication	2	27	0.022	0.037	DE >4fold
G. hirsutum	Paternal bias	IPR032675	Leucine-rich repeat domain superfamily	8	489	0.024	0.037	DE >4fold
G. hirsutum	Paternal bias	IPR026992	Non-haem dioxygenase N-terminal domain	3	86	0.024	0.037	DE >4fold
G. hirsutum	Paternal bias	GO:0006486	protein glycosylation	2	30	0.026	0.038	DE >4fold
G. hirsutum	Paternal bias	IPR027443	Isopenicillin N synthase-like	3	95	0.031	0.04	DE >4fold
G. hirsutum	Paternal bias	IPR005123	Oxoglutarate/iron-dependent dioxygenase	3	105	0.04	0.044	DE >4fold
G. hirsutum	Paternal bias	GO:0006351	transcription, DNA-templated	3	93	0.04	0.044	DE >4fold
G. hirsutum	Maternal bias	IPR002194	Chaperonin TCP-1, conserved site	2	6	0.001	0.037	DE >4fold
G. hirsutum	Maternal bias	IPR017998	Chaperone tailless complex polypeptide 1 (TCP-1)	2	6	0.001	0.037	DE >4fold
G. hirsutum	Maternal bias	IPR027410	TCP-1-like chaperonin intermediate domain superfamily	2	7	0.001	0.037	DE >4fold
G. hirsutum	Maternal bias	GO:0030570	pectate lyase activity	2	11	0.001	0.037	DE >4fold
G. hirsutum	Maternal bias	IPR007524	Pectate lyase, N-terminal	2	11	0.002	0.037	DE >4fold
G. hirsutum	Maternal bias	GO:0051082	unfolded protein binding	3	56	0.002	0.037	DE >4fold
G. hirsutum	Maternal bias	IPR027413	GroEL-like equatorial domain superfamily	2	15	0.003	0.037	DE >4fold
G. hirsutum	Maternal bias	IPR027409	GroEL-like apical domain superfamily	2	17	0.004	0.037	DE >4fold
G. hirsutum	Maternal bias	IPR002423	Chaperonin Cpn60/TCP-1 family	2	18	0.004	0.037	DE >4fold
G. hirsutum	Maternal bias	IPR008480	Protein of unknown function DUF761, plant	2	23	0.006	0.037	DE >4fold
G. hirsutum	Maternal bias	IPR008972	Cupredoxin	3	76	0.006	0.037	DE >4fold

	G. hirsutum	Maternal bias	IPR025836	Zinc knuckle CX2CX4HX4C	2	24	0.007	0.037	DE >4fold
	G. hirsutum	Maternal bias	GO:0005524	ATP binding	12	1264	0.007	0.037	DE >4fold
	G. hirsutum	Maternal bias	IPR002022	Pectate lyase	2	26	0.008	0.037	DE >4fold
	G. hirsutum	Maternal bias	IPR018082	AmbAllergen	2	26	0.008	0.037	DE >4fold
	G. hirsutum	Maternal bias	IPR017451	F-box associated interaction domain	2	27	0.008	0.037	DE >4fold
	G. hirsutum	Maternal bias	GO:0006457	protein folding	3	92	0.008	0.037	DE >4fold
	G. hirsutum	Maternal bias	IPR019821	Kinesin motor domain, conserved site	2	28	0.009	0.037	DE >4fold
	G. hirsutum	Maternal bias	IPR025558	Domain of unknown function DUF4283	2	28	0.009	0.037	DE >4fold
	G. hirsutum	Maternal bias	GO:0005871	kinesin complex	2	41	0.01	0.037	DE >4fold
	G. hirsutum	Maternal bias	GO:0003777	microtubule motor activity	2	44	0.016	0.037	DE >4fold
	G. hirsutum	Maternal bias	IPR001752	Kinesin motor domain	2	41	0.017	0.037	DE >4fold
	G. hirsutum	Maternal bias	IPR011707	Multicopper oxidase, type 3	2	41	0.017	0.037	DE >4fold
	G. hirsutum	Maternal bias	IPR027640	Kinesin-like protein	2	41	0.017	0.037	DE >4fold
	G. hirsutum	Maternal bias	GO:0007018	microtubule-based movement	2	44	0.018	0.037	DE >4fold
	G. hirsutum	Maternal bias	IPR001117	Multicopper oxidase, type 1	2	42	0.018	0.037	DE >4fold
	G. hirsutum	Maternal bias	IPR013763	Cyclin-like	2	43	0.019	0.037	DE >4fold
	G. hirsutum	Maternal bias	GO:0005507	copper ion binding	2	52	0.022	0.037	DE >4fold
	G. hirsutum	Maternal bias	GO:0008017	microtubule binding	2	53	0.023	0.037	DE >4fold
	G. hirsutum	Maternal bias	GO:0006950	response to stress	2	52	0.024	0.037	DE >4fold
	G. hirsutum	Maternal bias	GO:0004672	protein kinase activity	7	754	0.041	0.044	DE >4fold
	G. hirsutum	Maternal bias	GO:0006468	protein phosphorylation	7	754	0.047	0.047	DE >4fold
	G. barbadense	Paternal bias	IPR012340	Nucleic acid-binding, OB-fold	7	62	0.001	0.049	any DE
	G. barbadense	Paternal bias	IPR003851	Zinc finger, Dof-type	5	35	0.002	0.049	any DE
	G. barbadense	Paternal bias	IPR004853	Sugar phosphate transporter domain	4	29	0.006	0.049	any DE
	G. barbadense	Paternal bias	IPR023404	Radical SAM, alpha/beta horseshoe	2	4	0.007	0.049	any DE
	G. barbadense	Paternal bias	GO:0016620	oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor	3	16	0.01	0.049	any DE
	G. barbadense	Paternal bias	IPR012394	Aldehyde dehydrogenase NAD(P)-dependent	2	5	0.01	0.049	any DE
	G. barbadense	Paternal bias	IPR029979	Protein ESKIMO 1	2	5	0.01	0.049	any DE
	G. barbadense	Paternal bias	GO:0006081	cellular aldehyde metabolic process	2	5	0.011	0.049	any DE
	G. barbadense	Paternal bias	GO:0015780	nucleotide-sugar transmembrane transport	2	5	0.011	0.049	any DE
	G. barbadense	Paternal bias	GO:0050826	response to freezing	2	5	0.011	0.049	any DE
	G. barbadense	Paternal bias	IPR006638	Elp3/MiaB/NifB	2	6	0.013	0.049	any DE
	G. barbadense	Paternal bias	IPR016162	Aldehyde dehydrogenase, N-terminal	2	8	0.02	0.049	any DE
	G. barbadense	Paternal bias	GO:0006281	DNA repair	5	62	0.023	0.049	any DE
	G. barbadense	Paternal bias	IPR007197	Radical SAM	2	9	0.025	0.049	any DE
	G. barbadense	Paternal bias	IPR015590	Aldehyde dehydrogenase domain	2	9	0.025	0.049	any DE
	G. barbadense	Paternal bias	IPR016161	Aldehyde/histidinol dehydrogenase	2	9	0.025	0.049	any DE
	G. barbadense	Paternal bias	IPR016163	Aldehyde dehydrogenase, C-terminal	2	9	0.025	0.049	any DE
	G. barbadense	Paternal bias	IPR006689	Small GTPase superfamily, ARF/SAR type	3	25	0.025	0.049	any DE
	G. barbadense	Paternal bias	GO:0003684	damaged DNA binding	2	10	0.032	0.049	any DE
	G. barbadense	Paternal bias	IPR004146	DC1	3	28	0.032	0.049	any DE
	G. barbadense	Paternal bias	GO:0006289	nucleotide-excision repair	2	11	0.038	0.049	any DE
	G. barbadense	Paternal bias	IPR012946	X8 domain	3	31	0.041	0.049	any DE
	G. barbadense	Paternal bias	IPR006068	Cation-transporting P-type ATPase, C-terminal	2	13	0.044	0.049	any DE
	G. barbadense	Paternal bias	IPR004000	Actin family	2	14	0.05	0.05	any DE
	G. barbadense	Maternal bias	IPR014001	Helicase superfamily 1/2, ATP-binding domain	5	61	0.003	0.049	any DE
	G. barbadense	Maternal bias	IPR001650	Helicase, C-terminal	5	62	0.003	0.049	any DE
*	*G. barbadense*	Maternal bias	GO:0009523	photosystem II	3	26	0.007	0.049	any DE
	G. barbadense	Maternal bias	IPR013922	Cyclin PHO80-like	2	7	0.008	0.049	any DE
	G. barbadense	Maternal bias	IPR014014	RNA helicase, DEAD-box type, Q motif	3	24	0.008	0.049	any DE
	G. barbadense	Maternal bias	GO:0000079	regulation of cyclin-dependent protein serine/threonine kinase activity	2	8	0.011	0.049	any DE

	G. barbadense	Maternal bias	GO:0019901	protein kinase binding	2	8	0.011	0.049	any DE
	G. barbadense	Maternal bias	IPR000198	Rho GTPase-activating protein domain	2	9	0.011	0.049	any DE
*	*G. barbadense*	Maternal bias	IPR002683	PsbP, C-terminal	2	9	0.011	0.049	any DE
	G. barbadense	Maternal bias	IPR008936	Rho GTPase activation protein	2	9	0.011	0.049	any DE
*	*G. barbadense*	Maternal bias	GO:0009654	photosystem II oxygen evolving complex	2	12	0.015	0.049	any DE
	G. barbadense	Maternal bias	GO:0019898	extrinsic component of membrane	2	12	0.015	0.049	any DE
	G. barbadense	Maternal bias	IPR002173	Carbohydrate/purine kinase, PfkB, conserved site	2	11	0.016	0.049	any DE
	G. barbadense	Maternal bias	IPR016123	Mog1/PsbP, alpha/beta/alpha sandwich	2	11	0.016	0.049	any DE
	G. barbadense	Maternal bias	IPR000629	ATP-dependent RNA helicase DEAD-box, conserved site	2	13	0.021	0.049	any DE
	G. barbadense	Maternal bias	GO:0003950	NAD+ ADP-ribosyltransferase activity	2	12	0.022	0.049	any DE
	G. barbadense	Maternal bias	IPR027806	Harbinger transposase-derived nuclease domain	3	37	0.022	0.049	any DE
	G. barbadense	Maternal bias	IPR011545	DEAD/DEAH box helicase domain	3	39	0.026	0.049	any DE
	G. barbadense	Maternal bias	IPR011611	Carbohydrate kinase PfkB	2	15	0.027	0.049	any DE
	G. barbadense	Maternal bias	IPR013763	Cyclin-like	3	41	0.029	0.049	any DE
	G. barbadense	Maternal bias	IPR029056	Ribokinase-like	2	16	0.03	0.049	any DE
	G. barbadense	Maternal bias	IPR019794	Peroxidase, active site	3	42	0.031	0.049	any DE
*	*G. barbadense*	Maternal bias	GO:0015979	photosynthesis	3	40	0.032	0.049	any DE
	G. barbadense	Maternal bias	IPR024709	Putative O-fucosyltransferase, plant	2	18	0.036	0.049	any DE
	G. barbadense	Maternal bias	IPR025525	hAT-like transposase, RNase-H fold	2	18	0.036	0.049	any DE
	G. barbadense	Maternal bias	IPR021720	Malectin domain	2	19	0.04	0.049	any DE
	G. barbadense	Maternal bias	GO:0046983	protein dimerization activity	8	225	0.043	0.049	any DE
	G. barbadense	Maternal bias	IPR000823	Plant peroxidase	3	49	0.044	0.049	any DE
	G. barbadense	Paternal bias	GO:0006281	DNA repair	5	62	0	0.014	DE >4fold
	G. barbadense	Paternal bias	IPR003851	Zinc finger, Dof-type	3	35	0.003	0.036	DE >4fold
	G. barbadense	Paternal bias	GO:0003684	damaged DNA binding	2	10	0.004	0.036	DE >4fold
	G. barbadense	Paternal bias	GO:0006289	nucleotide-excision repair	2	11	0.007	0.036	DE >4fold
	G. barbadense	Paternal bias	IPR006626	Parallel beta-helix repeat	2	28	0.021	0.036	DE >4fold
	G. barbadense	Paternal bias	IPR008928	Six-hairpin glycosidase superfamily	2	29	0.022	0.036	DE >4fold
	G. barbadense	Paternal bias	IPR000743	Glycoside hydrolase, family 28	2	35	0.03	0.037	DE >4fold
	G. barbadense	Paternal bias	GO:0004650	polygalacturonase activity	2	35	0.036	0.04	DE >4fold
	G. barbadense	Maternal bias	IPR013922	Cyclin PHO80-like	2	7	0	0.014	DE >4fold
	G. barbadense	Maternal bias	IPR013763	Cyclin-like	3	41	0	0.014	DE >4fold
	G. barbadense	Maternal bias	GO:0000079	regulation of cyclin-dependent protein serine/threonine kinase activity	2	8	0	0.014	DE >4fold
	G. barbadense	Maternal bias	GO:0019901	protein kinase binding	2	8	0	0.014	DE >4fold
	G. barbadense	Maternal bias	IPR027806	Harbinger transposase-derived nuclease domain	2	37	0.006	0.036	DE >4fold