BMC Genomics

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Alternative splicing perspective to prey preference of environmentally friendly biological agent *Cryptolaemus montrouzieri*

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Abstract

Background *Cryptolaemus montrouzieri* (Coccinellidae) is widely utilized as biological control agents in modern agriculture. A comprehensive understanding of its food preference can help guide mass rearing and safety management during field application of pest control. Although some studies have paid attentions to the impacts of prey shift on *C. montrouzieri*, little is known regarding the role of post-transcriptional regulations in its acclimation to unnatural preys.

Results We performed a genome-wide investigation on alternative splicing dynamics in *C. montrouzieri* in response to the predation transition from natural prey to unnatural ones. When feeding on undesired diets, 402–764 genes were differentially alternative spliced in *C. montrouzieri*. It is noteworthy that the majority of these genes (> 87%) were not differentially expressed, and these differentially spliced genes regulated distinct biological processes from differentially expressed genes, such as organ development and morphogenesis, locomotory behavior, and homeostasis processes. These suggested the functionally nonredendant role of alternative splicing in modulating physiological and metabolic responses of *C. montrouzieri* to the shift to undesired preys. In addition, the individuals feeding on aphids were subject to a lower level of changes in splicing than other alternative diets, which might be because of the similar chemical and microbial compositions. Our study further suggested a putative coupling of alternative splicing and nonsense-mediated decay (AS-NMD), which may play an important role in fine-tuning the protein repertoire of *C. montrouzieri*, and promoting its acclimation to predation changes.

Conclusion These findings highlight the key role of alternative splicing in modulating the acclimation of ladybirds to prey shift and provide new genetic clues for the future application of ladybirds in biocontrol.

Keywords Alternative splicing, Biological control, Food preference, Functional nonredundancy, Ladybirds, Post-transcirptional regulation

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Introduction

Biological control provides an environmentally friendly strategy for pest control via their natural enemies, and acts as a key part of green agriculture [1–3]. Compared to chemical control, biological control is a more cost effective strategy with low negative impacts on local environment and human health, and has achieved huge successes in some areas [2, 4]. Since 1880s, approximately 2000 biological control agents have been applied with the aim to manage agricultural, forestry or horticultral pests [5]. However, not every introduction achieved expected benefits, either economically or ecologically. Success of biological control largely depends on their predatory efficiency and specialization, survival capacity, and proliferative ability [5, 6]. The usage of polyphgous species may cause negative effects to environments and ecosystems [7]. For example, *Harmonia axyridis* was introduced to Europe for controlling aphids, however, it attacks many non-target species and seriously destroys the local biodiversity [8, 9]. Thus, a comprehensive understanding of the food preference of predators can provide useful guidance for their application in pest control.

Cryptolaemus montrouzieri Mulsant, which is also known as "mealybug ladybird", is a specialized coccidophagy of Coccinellidae naturally distributed in Australia [10]. Under natural conditions, *C. montrouzieri* is reported to be only able to finish the entire life cycle through the predation of coccid species [11]. Such a narrow food spectrum makes *C. montrouzieri* an ideal biocontrol agent with low risks of attacking non-target species and disrupting the stability and health of local ecosystem. Since its first introduction to California, US in 1891, *C. montrouzieri* has been worldwide used for controlling mealybugs and achieved huge success [12, 13]. In contrast to natural scenarios, *C. montrouzieri* individuals reared under laboratory conditions can also feed on alternative diets, such as aphids, whiteflies, or ladybird eggs, but exhibited substantial decreases in the performance of growth, development, and reproduction [14]. Recent genomic and transcriptomic researches have largely broadened our understanding of the molecular mechanisms underlying the physiological responses to alternative diets in *C. montrouzieri* [6, 10, 15]. Li et al. [6] identified 598 expanded gene families in the genome of *C. montrouzieri*, including those related to chemosensors, digestion, detoxification, and immune response, reflecting its adaptive evolution to coccidophagy. When feeding on unnatural preys, genes associated with protein translation, nutrient metabolism, and xenobiotics biodegradation were significantly upregulated, which can help *C. montrouzieri* combat nutritional deficiency and undesired intake of harmful toxins [6, 10]. Besides expression variation, post-transcriptional regulations have been demonstrated to play important roles in fine-tuning phenotypic plasticity of insects [16]. However, no current studies have evaluated the contribution of post-transcriptional mechanisms to acclimation to diet shift in *C. montrouzieri*, which leaves a knowledge gap in the evolution of predation preferences in ladybirds.

Alternative splicing is a ubiquitous post-transcriptional gene regulation mechanism in eukaryotic organisms [15, 17–19]. Alternative splicing produces distinct isoforms of different combinations of exons, and sometimes introns, from one single gene, thus largely promoting repertoire of both mRNAs and proteins without increase in gene number [20–22]. Compared to gene transcription, splicing generally acts a smaller role in regulating protein expression and diversity, and therefore is supposed to be less constrained by the selection pressure, and plays an important role in facilitating the rapid adaptation of eukaryotes [18, 23]. Over the recent decade, the contributions of differential splicing to phenotypic plasticity and adaptive potential to environmental stresses have been characterized in many invertebrates at genome scale [18, 24–28]. For instance, *Drosophila melanogaster* displayed one magnitude increase in the level of differential splicing when exposed to extreme temperature, compared to those in a suitable environment [24]. In *Caenorhabditis elegans*, 517 genes were identified to undergo differential splicing during dietary restriction, and these genes were functionally related to metabolism, cell division, and development and reproduction, which helps the physiological and metabolic remodeling in response to energy restriction [25]. However, the role of alternative splicing in acclimation to prey shift in ladybirds remains unknown.

Here, we investigated the alternative splicing dynamics in *C. montrouzieri* in response to predation shift from natural prey (citrus mealybugs (*Planococcus citri*)) to four types of alternative diets (pea aphid (*Megoura japonica*), larvae of black soldier fly (*Hermetia illucens*), eggs of brine shrimp (*Artemia salina*), and pollen of *Brassica campestris*) on a genome-wide scale. We hypothesized that alternative splicing played an important regulatory role in promoting acclimation to undesirable diets in *C. montrouzieri*, but in a different aspect from differential expression. The findings would provide new insights into the genetic basis underlying the obligate coccidophagy of *C. montrouzieri* and also broaden our understanding of the evolution of food preference in ladybirds.

Materials and methods

Alternative splicing analysis upon different feeding scenraios in *C. montrouzieri*

In this study, the analysis of alternative splicing were implemented for *C. montrouzieri* individuals under different feeding scenarios using our previously published RNA-seq data (BioProject ID of the National Center

for Biotechnology Information (NCBI) Sequence Read Archive (SRA): PRJNA509782) [6]. Data preprocessing was carried out following the descriptions in Li et al. [6]. For each alternative predation, the splicing profile was identified and compared to the feeding of natural prey using rMATS v. 4.0.2 [29]. The reference genome of *C. montrouzieri* used for the analysis was from our previous study [6], and the score of Benchmarking Universal Single-Copy Orthologs (BUSCO) [30] suggested a high assembly completeness of the reference genome (Figure S1). Five primary types of alternative splicing were analyzed by rMATS, including exon skipping (ES), alternative 3' splice site (A3SS), alternative 5' splice site (A5SS), intron retention (IR), and mutually exclusive exons (MXE) (Fig. 1A). For each tested event, *p*-value was calculated by likelihood-ratio test, and False Discovery Rate (FDR) was obtained by Benjamini-Hochberg procedure. The events with FDR < 0.05 were considered to be significantly different in splicing between the natural and alternative preys. The genes with significantly differential splicing events were denoted as "differentially spliced genes (DSGs)".

To assess the biological importance of the DSGs of interests, a custom annotation database package was constructed for *C. montrouzieri* using the makeOrgPackage function of AnnotationForge package [31], and Gene Ontology (GO) enrichment analysis was performed using the enrichGO function of clusterProfiler package [32]. The significance levels of GO terms were determined by hypergeometric distribution, and those with *p*-value < 0.05 were considered to be significantly enriched. Then, the overrepresented GO terms with high similarity were further grouped together using the treeplot program of enrichplot package [33].

Premature termination codons introduced by intron retention and alternative 3' and 5' splice site

Changes in fragment inclusion/exclusion during splicing may disrupt open reading frame and introduce premature termination codons (PTCs) to mRNA isoforms, which prematurely terminate translation process and lead to truncated product proteins that might be deficient in function [25]. In this study, the PTCs given rise by IR, A3SS and A5SS events and their influences on the gene function were investigated in *C. montrouzieri*. Specifically, for each gene undergoing IR, A3SS or A5SS, coding sequence of the alternative isoforms including the retained intron or excluding the 5' or 3' alternative regions were extracted from the reference genome using fastaFromBed of Bedtools [34]. For the isoforms with multiple terminations, the termination codon at the end of the sequence was referred to as "constitutive termination codon", while the one nearest to the start site of the isoform was considered as the PTC. A dot plot was used to visualize the location distribution of PTCs across all the tested isoforms. Here, the PTCs occurred at the first 80% length of isoforms, which caused > 20% amino acid loss in the product proteins, were supposed to pose a substantial impact on protein functions. The genes with a downregulated proportion of PTC-containing isoforms upon prey shift, which can reduce the production of truncated proteins, were considered to be important for

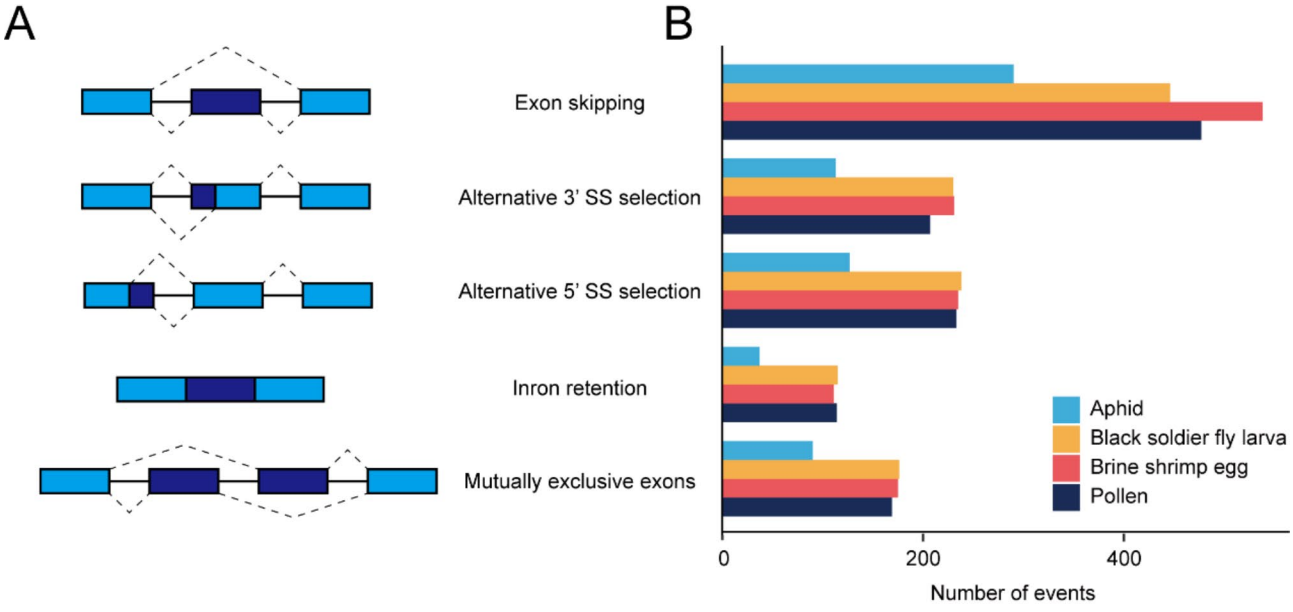

Fig. 1 Alternative splicing modes and number of differential splicing events in *C. montrouzieri* upon unnatural feeding scenarios. (**A**) Schematic diagram showing five primary types of alternative splicing we analyzed in this study. (**B**) Number of differential splicing events of each alternative splicing type when feeding on pea aphids, larvae of black soldier fly, eggs of brine shrimp, and pollen of *B. campestris*

survival and normal growth of *C. montrouzieri*, and GO enrichment analysis was carried out for these genes to assess their functional significance.

Comparison between alternative splicing and gene expression

Both transcriptional and post-transcriptional regulations make important contributions to animal's phenotypic plasticity [18, 23, 27, 35, 36]. To investigate the specificities between alternative splicing and gene expression, more exon-inclusive DSGs were compared to upregulated differentially expressed genes (DEGs) for each alternative feeding scenario. The upregulated DEGs were defined following the procedure of Li et al. [6]. According to the regulatory modes, the genes were assigned into three categories: (1) genes specifically under the regulation of differentially alternative splicing (DS-specific genes), (2) genes specifically under the regulation of differential expression (DE-specific genes), and (3) genes under the regulations of both differential expression and differentially alternative splicing (DE-DS genes). GO enrichment analysis was performed for each category of genes with a *p*-value cutoff of 0.05.

Results

Patterns of differential splicing in *C. montrouzieri* between natural and alternative predations

In a total, 657–1290 alternative splicing events from 402 to 764 genes were significantly differentiated in *C. montrouzieri* when feeding on alternative diets (Fig. 1B). Consistently across all four diets, ES events were of the highest abundance among the five alternative splicing modes, which accounted for 37.01–44.14% of the significantly differential splicing events, followed by A5SS (18.22–19.75%), A3SS (17.20–19.09%), and MXE (13.57–14.61%). IR was the rarest type, which only accounted for 5.63–9.54% (Fig. 1B). It is noteworthy that, for all the splicing modes, the individuals feeding on aphids were subject to the lowest level of differential splicing than those of the other feeding scenarios, which might be because of its highest similarity to mealybugs than other preys.

Among the ES events, 18.27–29.7% displayed a higher inclusion level of alternative exons (more exon-inclusive) upon unnatural predations, which were supposed to promote the expression of the proteins with complete structure. GO enrichment analysis highlighted their important roles in *C. montrouzieri* in responding to diet shift. When preying on pea aphids, the more exon-inclusive DSGs were mainly associated with circulatory system development, locomotory behavior, retinal cell programmed cell death, cellular response to nutrient levels and starvation, and cellular homeostasis (Fig. 2A). The genes with an increased exon inclusion level upon pollen feeding were overrepresented for the GO terms of wing disc development, dorsal closure, behavior regulation, R7 cell development, photoreceptor cell differentiation, and starvation responses (Fig. 2B), while those in response to the feeding of brine shrimp eggs were involved in growth, organ morphogenesis, sensory perception of mechanical stimulus, and locomotory behavior (Fig. 2C). When feeding on larvae of black soldier fly, the DSGs were enriched for circulatory and respiratory system development, response to starvation, developmental growth and morphogenesis, and photoreceptor cell axon guidance (Fig. 2D). The changes in these genes and biological processes mirrored the adverse impacts of diet transition on the normal growth, development, and cell homeostasis of *C. montrouzieri*, and highlighted the importance of splicing reprogramming in regulating the corresponding physiological and metabolic responses.

Specificities of differential splicing among different feeding scenarios

Our results moreover revealed ample specificities of differential splicing among different feeding scenarios, that only 35 out of the 389 more-exon inclusive DSGs were shared in all the four alternative diets (Fig. 3). These commonly shared DEGs mainly participated in cellular homeostasis, developmental growth and morphogenesis, and locomotory behavior of *C. montrouzieri*. Comparatively, 53, 43, 39, and 35 more-exon inclusive DSGs were specifically induced by the transitions from coccidophagy to feeding on pollen, black soldier fly larvae, brine shrimp eggs, and pea aphids, respectively. Of them, the DSGs specific to the predation of pollen were highly represented in starvation response, and development of circulatory system, wing disc and photoreceptor cells, while respiratory system development was specifically enriched upon the diet of black soldier fly larvae. When feeding on brine shrimp eggs, the genes involved in developmental maturation and organ development and morphogenesis were significantly differentially spliced. The DSGs related to neuromuscular process and retinal cell programmed cell death were specifically induced by the prey switch to aphids.

Premature termination codons introduced by alternative splicing and their functional impacts

In *C. montrouzieri*, IR, A3SS and A5SS events were observed to give rise to PTCs to alternative isoforms. When feeding on pea aphids, 60.0%, 18.3% and 11.8% of the differential IR, A3SS and A5SS events were found to produce PTC-containing transcripts. More importantly, for all four feeding scenarios, the vast majority (87.1 −90.1%) of the PTCs occurred at the first 80% length of isoforms, which led to truncation or even loss of function of product proteins (Fig. 4A). GO enrichment

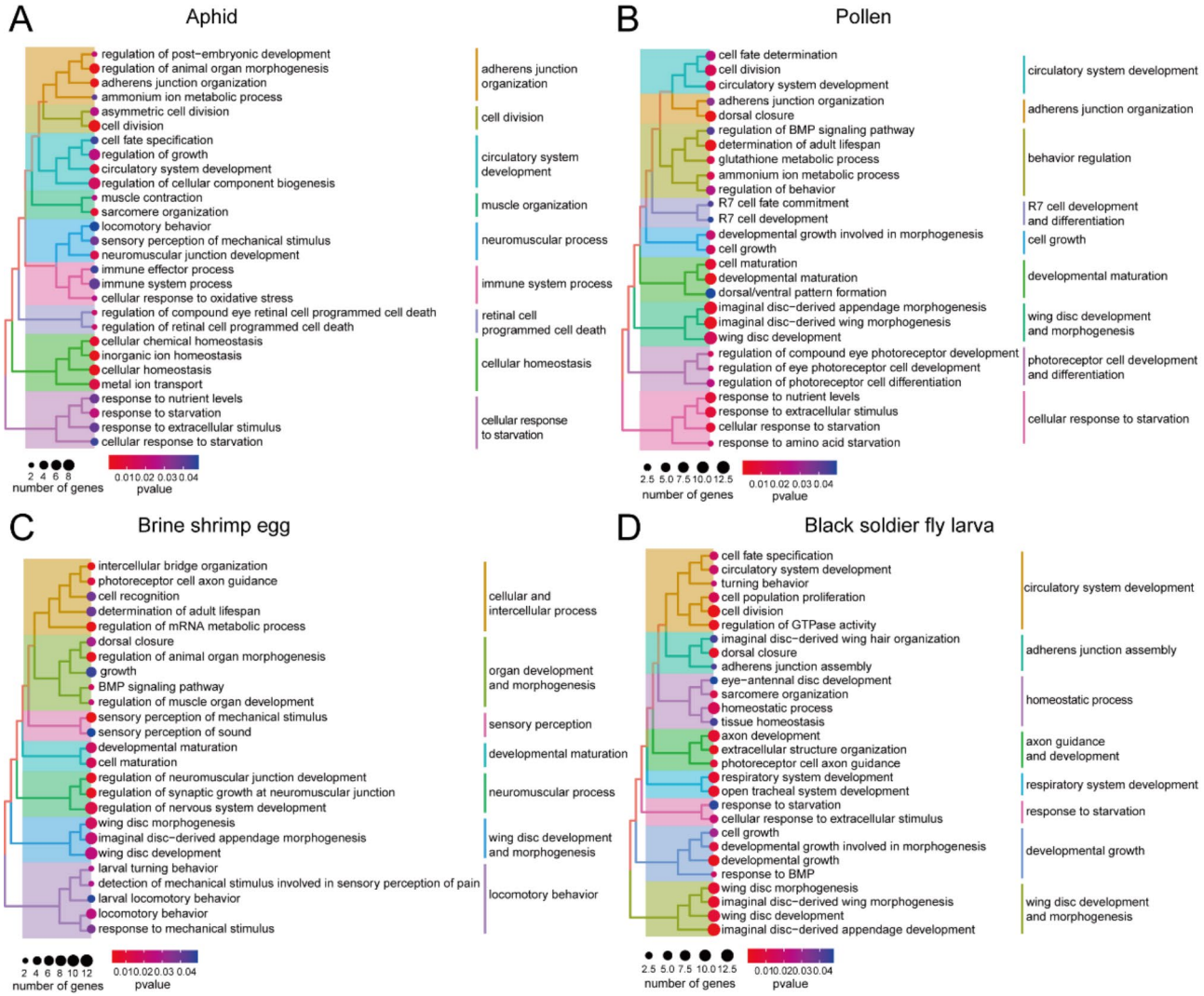

Fig. 2 Tree diagram showing the functional group of Gene Ontology (GO) terms enriched for more exon-inclusive differentially alternative spliced genes (DSGs) in *C. montrouzieri* feeding on alternative diets: pea aphids (**A**), pollen of *B. campestris* (**B**), eggs of brine shrimp (**C**), and larvae of black soldier fly (**D**)

analysis showed that, when feeding on aphids, these low intron-inclusive genes were mainly involved in regulation of synapse structure or activity, organic cation transport, growth regulation, wing disc and sensory organ development, and eye photoreceptor cell fate commitment (Fig. 4B). When switching to black soldier fly larvae, the DSGs were overrepresented for actomyosin structure organization, morphogenesis-related cellular component assembly, R7 cell differentiation regulation, and developmental maturation. Comparatively, dorsal trunk growth, mRNA metabolic process, neuron recognition, and feeding behavior regulation were specifically enriched in response to the predation of brine shrimp eggs, while those participating in organ growth and muscle cell development, energy reserve metabolic process, and flight behavior were highly represented upon pollen feeding.

Difference between the regulations of alternative splicing and gene transcription

The comparison between more-exon inclusive DSGs and upregualted DEGs revealed that only a small proportion of genes were overlapped under each feeding scenario (Fig. 5; Figure S2). For example, when feeding on pea aphids, only 15 out of the 142 more-exon inclusive DSGs (10.6%) were significantly differentially expressed as well (DE-DS genes) (Fig. 5). It indicated that alternative splicing plays a different role from gene transcription in modulating the responses to prey shift in *C. montrouzieri*. Functional enrichment analysis revealed that the aphid-induced DE-DS genes were overrepresented for ion transport and metabolic processes, such as carbohydrate catabolism and energy reserve metabolism, while the DE-specific genes were primarily invovled in the responses to starvation, toxic substances and bacterial challenge.

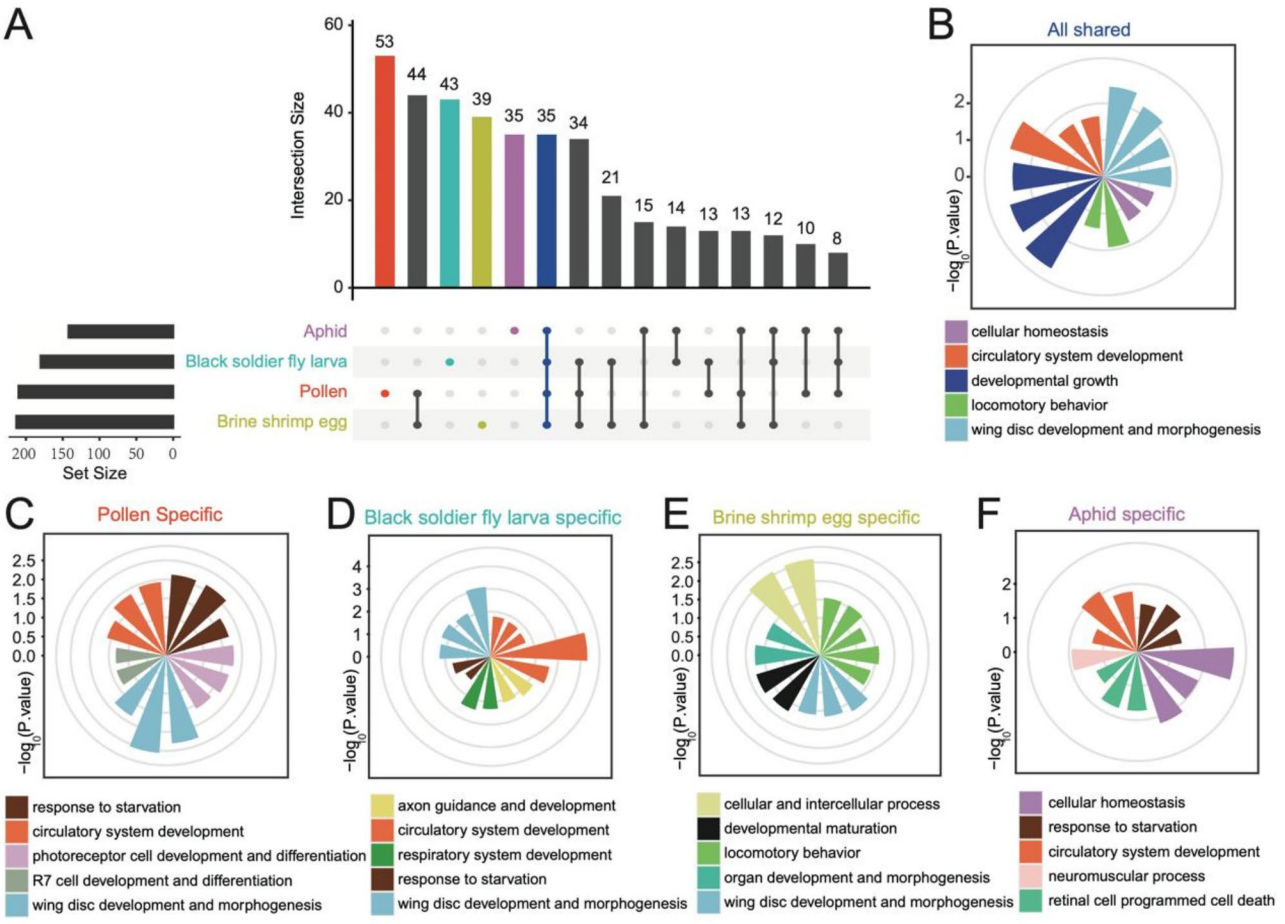

Fig. 3 Overlap of more exon-inclusive genes across four unnatural feeding scenarios. (**A**) UpSet plot illustrating the overlap of more exon-inclusive genes across four unnatural feeding scenarios. In the plot, rows of the matrix corresponds to the four feeding conditions, and columns shows the intersections among these scenarios. The number of genes of the four feeding conditions and the intersections are shown by the horizontal and ventrical bars, respectively. The intersections we mainly focused on are highlighted by different colors. (**B-F**) Bar charts showing the representative Gene Ontology (GO) terms significantly enriched for the differentially alternative spliced genes (DSGs) shared (**B**) or specific to a certain feeding scenario (**C**: Pollen; **D**: Black soldier fly larva; **E**: Brine shrimp egg; and **F**: Aphid). The GO terms of similar functions are presented in the same color. The circles in each bar chart represent the level of *p*-value in -$\log_{10}$ scale

The DG-specific genes were enriched in the GO terms of organ development and morphogenesis, behavior regulation, and homeostatic process.

Upon the diet of pollen, the 24 DE-DS genes were enriched for muscle structure development and organization, and intercellular bridge organization (Figure S2A). The 1455 DE-specific genes were mainly involved in cation transport, sensory perception, and synaptic signaling, while the 204 DS-specific genes were highly represented for appendage development, response to nutrient levels, adherens junction assembly, and dorsal closure (Figure S2A). For the DE-DS genes induced by the predation of brine shrimp eggs, the GO terms of cell population proliferation, muscle contraction, and neuron projection development were significantly enriched (Figures S2B). Ion transport, cellular carbohydrate metabolism, and muscle cell differentiation were primarily modulated by differential expression, while the genes of locomotory behavior, compound eye photoreceptor development, and adult lifespan determination were differentially spliced (Figure S2B). When feeding on black soldier fly larvae, the genes related to cell division, wing disc development, and circulatory system process specifically underwent alternative splicing regulations, while the processes of energy reserve metabolism, muscle structure development, and compound eye morphogenesis were under the modulation of gene transcription (Figure S2C). Taken together, our findings suggested that alternative splicing was functionally nonredundant with gene transcription and has an important bearing on the responses to prey shift in *C. montrouzieri*.

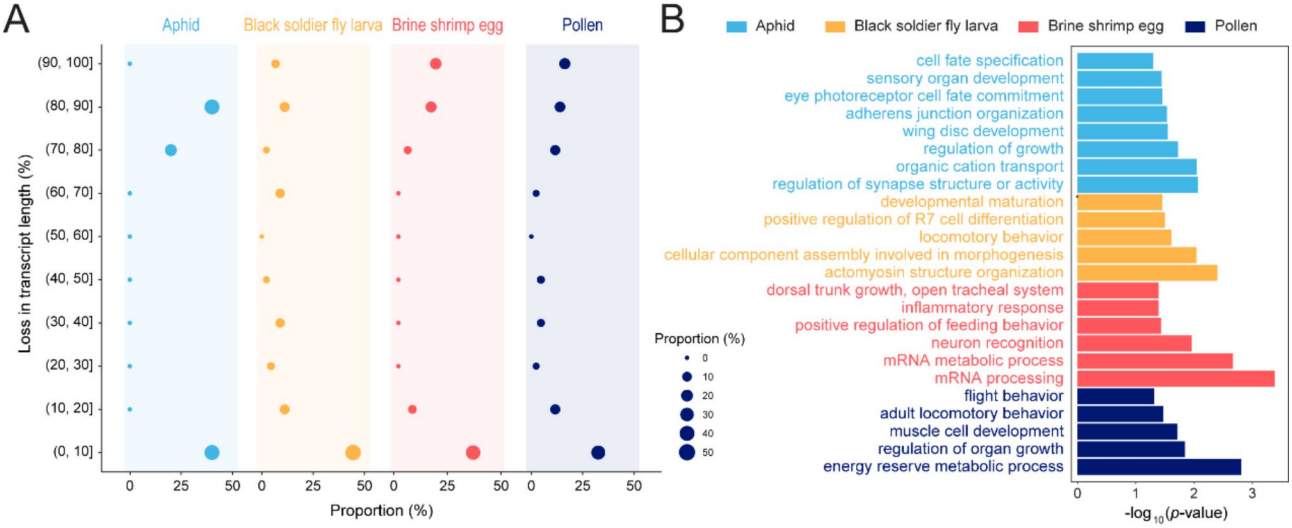

Fig. 4 Premature termination codons (PTCs) introduced by intron retention (IR), alternative 3′ splice sites (A3SS), and alternative 5′ splice sites (A5SS) in *C. montrouzieri* upon unnatural feeding scenarios. (**A**) Relative proportion of PTCs in different locations on transcripts upon each alternative feeding scenario. The location intervals of transcripts are listed along vertical axis, and dot sizes correspond to the relative proportion of the PTCs in each location interval. (**B**) Featured Gene Ontology (GO) terms enriched for less intron-inclusive genes in *C. montrouzieri* feeding on alternative diets

Discussion

Previous studies have revealed that large-scale differential gene expression was associated with *C. montrouzieri*'s acclimation to diet shifts [6, 10]. Compared to gene transcription, alternative splicing can fine-tune protein expression, and increase the functional diversity of proteome without changes in genome [37]. Alternative splicing has been demonstrated to act an important role in regulating growth, development, survival, reproduction, and sex determination of insects [38, 39]. In the current study, we investigated the splicing changes underlying predation plasticity of *C. montrouzieri* on a genome-wide scale. Compared to natural diet, feeding on alternative preys induced 657–1290 significantly differential splicing events (Fig. 1A). Of them, only a small proportion (~10.6–12.1%) of the DSGs were also differentially expressed (Fig. 5; Figure S2). Moreover, the DS-specific genes were found to participate in different biological processes from those DE-specific genes (Fig. 5; Figure S2). For instance, when feeding on aphids, the DE-specific genes were highly represented in the responses to nutrient level and toxic and xenobiotic stimuli, while the genes specifically modulated by alternative splicing were primarily involved in organ development and morphogenesis, behavior regulation, and homeostasis processes (Fig. 5). The findings indicated that alternative splicing is functionally nonredudant and acts a complementary role to gene transcription in *C. montrouzieri* in supporting physiological and biochemical remodeling to ensure somatic maintenance and survival during dietary changes.

Upon all the dietary changes, the splicing mode of 35 genes that were associated with growth and developmental processes substantially altered (Fig. 3), suggesting the adverse impacts of food transition on the development and flight ability of *C. montrouzieri*. Compared to natural prey, alternative diets have apparently distinct compositions of nutrients and chemical compounds; thus, a shift of prey may cause malnutrition in insects and impair their normal growth and development [40]. For instance, although pollen is a valuable food source for many insects, a strict pollen diet is insufficient to supply enough nutrients to their larvae to complete the entire growth and development [41]. To combat the adverse impacts, the genes related to energy metabolism and starvation responses displayed a significant increase in the inclusion level of alternative exons (Fig. 3), which would enhance the expression of the functional proteins and promote *C. montrouzieri*'s tolerance to nutritional deficiency. For example, the gene encoding the receptor of activated protein kinase C 1 (*rack1*) became more-exon inclusive upon the predation of alternative diets. In *Drosophila*, *rack1* participates in glycogen production and autophagic response to starvation [42]. Similarly, the activity of gene *p38b* was also enhanced in response to prey shift, which promotes energy supply and organ growth by positively regulating the nutrient-sensing Target of Rapamycin Complex 1 (TORC1) signaling pathway, as well as the cross-talk between TORC1 and insulin signaling pathways [43–45]. In addition to nutrient deficiency, undesired diets may also bring toxic substances and pathogens to ladybirds [6]. In the present study, we showed that alternative splicing played a substantial role

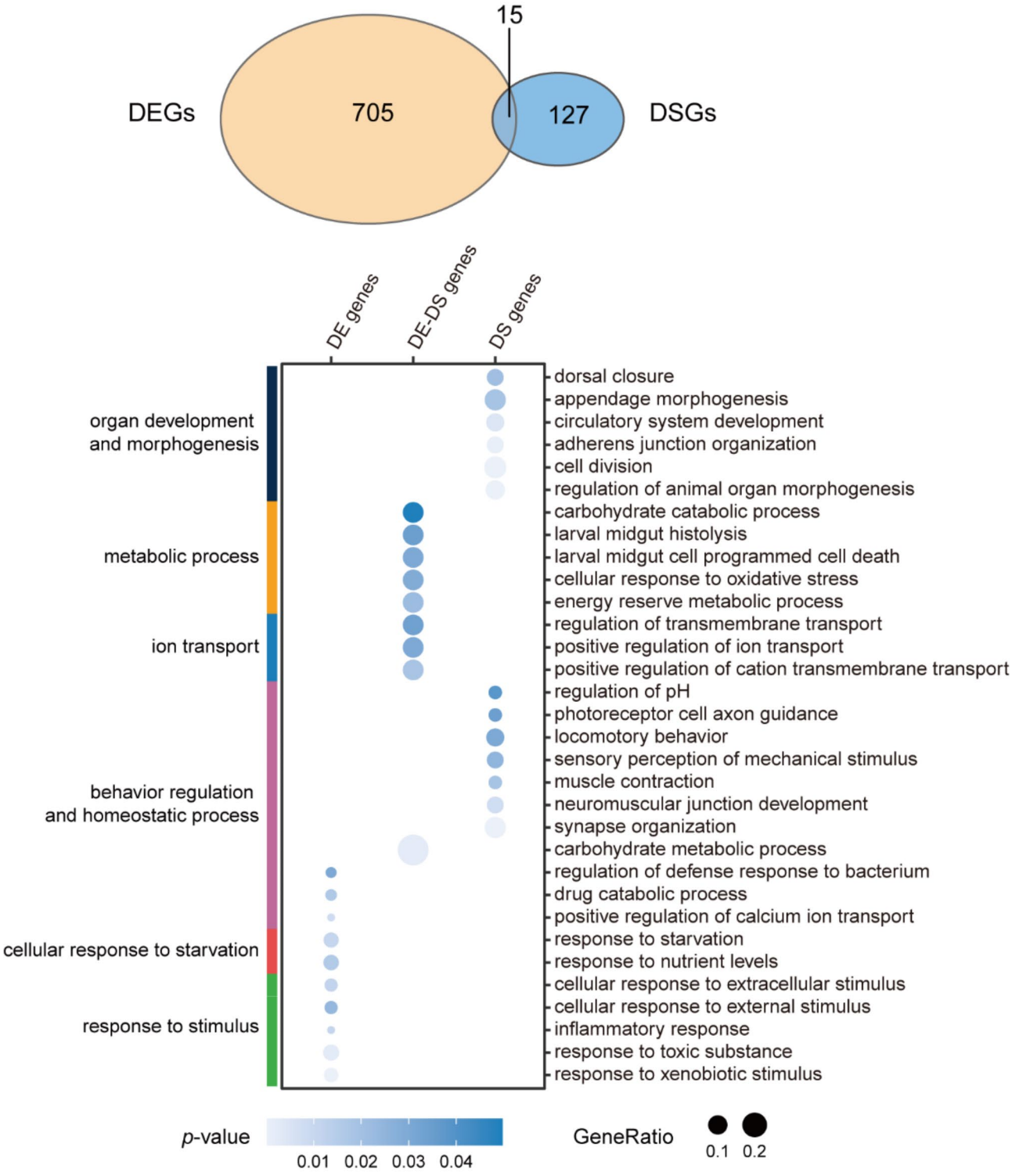

Fig. 5 Comparison between differential splicing and differential expression in the *C. montrouzieri* feeding on pea aphids. (**A**) Venn diagram showing the overlap between upregulated differentially expressed genes (DEGs) and more exon-inclusive differentially alternative spliced genes (DSGs). Circle sizes correspond to the total number of DEGs and DSGs. (**B**) Representative Gene Ontology (GO) terms enriched for the genes that were only differentially expressed (DE-specfic), both differentially expressed and differentially spliced (DE-DS), and only differentially spliced (DS-specific)

in modulating toxin metabolism and bacterium response of *C. montrouzieri* individuals upon prey shift. For example, gene *cyp6a9* of P450 CYP6 family was significantly differentially spliced when feeding on black soldier fly larvae (Figure S2C), which could enhance detoxification and exogenous substances metabolism of *C. montrouzieri* [46, 47]. In addition, *cyp6a9* was also found to participate in reactive oxygen species (ROS)-mediated immunological response and signal transmission in pathogen-infected gut of *Drosophila* [48]. Together, these results highlighted the indispensable contribution of splicing reprogramming in ladybirds to coping with the malnutrition and toxinic and bacterial challenges induced by prey shift.

In *C. montrouzieri*, IR, A3SS and A5SS events were found to give rise to PTCs in produced mRNAs (Fig. 4A). These aberrant PTC-containing transcripts are likely to be eliminated by nonsense-mediated decay (NMD) pathway, in order to reduce the expression of toxic truncated proteins [49]. In *Drosophila*, activation of NMD is determined by the distance between the PTC and the 3' end poly(A) tail of a transcript [50]. Terminating ribosome generally stops at the normal stop codon, and interacts with poly(A) binding proteins (PABPs) and peptide-release factors to terminate translation. However, appearance of PTC leads to an abnormally large distance between terminating ribosome and PABPs, which disturbs their normal interaction, triggers interaction between the ribosome and NMD factors instead, and activates NMD pathway [51]. In our case, most PTCs were identified at the first 10% length of the isoforms (Fig. 4A), and indeed the inclusion of PTC-harboring introns or exons in many genes was significantly decreased with the transition to unnatural diets. These genes were associated with insect development, behavior, and morphogenesis (Fig. 4B). Of them, *SRp55*, a member of the SR protein family, is one of the important trans-acting factors involved in intron recognition, splicing assembly, and alternative splicing regulation [52]. Its expression during the early stage of embryonic development is essential to larvae development, and an aberrant splicing conduces to developmental failure [53, 54]. Another SR protein-encoding gene *rbp1* was also significantly increased in mRNA level in response to all four different diets. In *Drosophila*, *rbp1* is broadly expressed in various tissues and developmental stages, and it has been reported to negatively autoregulate the splicing environment and affect homeostasis [53, 55]. In other instances, both *Mhc2* and *GPDH1* displayed decreased inclusion level of alternative introns upon prey shifts. *Mhc* genes act important roles in sarcomere formation in *Drosophila* muscle [56], and in *Tribolium castaneum*, RNAi of *Mhc2* disrupted wing development [57]. *GPDH1* encodes the key enzyme glycerol-3-phosphate dehydrogenase 1 for glycerol 3-phosphate (G3P) synthesis and cytoplasmic NADH transfer into mitochondria in the glycerol-3-phosphate shuttle, which is critical for carbohydrate metabolism and energy supply [58]. Given the high energy consumption of translation, degradation of non-functional PTC-containing transcripts can prevent energy waste on producing truncated proteins, especially under the scenario of nutrient depletion. Similarly, the coupling of alternative splicing and NMD (AS-NMD) was demonstrated to play an essential role in *C. elegans* in rationalizing energy usage during dietary restriction [25]. Together, these findings highlighted the biological significance of AS-NMD in fine-tuning the homeostatic expression of proteins, with a positive bearing on the acclimation to predation changes.

It is worthy to note that the *C. montrouzieri* feeding on aphids was subject to substantially smaller number of differential splicing events than other alternative diets (Fig. 1A). There is evidence that, despite not being well adapted, *C. montrouzieri* is able to complete the entire life cycle by simply relying on aphidophagy [10]. Individuals of *C. montrouzieri* that fed on aphids were found to lay an equivalent amount of eggs as those that fed on mealybugs [14]. Compared to other alternative preys (e.g., black soldier fly larvae, brine shrimp eggs, and *B. campestris* pollen tested in this study), aphids and mealybugs are both Hemiptera insects, and they may have a more similar composition in nutrients, metabolites, and microbes. An evolutionary approach has revealed that ladybirds of Coccinellidae experienced a complex switching in predation that Coccinellidea species primarily consume scale insects as basal diets, while some Coccinellini species, such as *Propylea japonica*, *Coccinella septempunctata* and *H. axyridis*, evolved new capacity to prey on aphids [41]. Compared to genome sequence variation or gene expression, alternative splicing is supposed to be less selectively constrained, and putatively associated with the evolution of phenotypic divergence in many species [21, 23, 59]. Our study emphasized the differences in mRNA splicing between coccidophagy and aphidophagy in *C. montrouzieri*, which broadened our understanding of the evolutionary history of predation transition in ladybirds.

In summary, our study suggests the important contribution of alternative splicing plasticity to the acclimation to diet shift in mealybug ladybird *C. montrouzieri*. Compared to gene transcription, splicing reprogramming plays a functionally nonredundant role in regulating physiological and metabolic responses to the shift to undesired preys. These findings thus give a novel mechanistic insight into the genetic basis of predatory specialization in ladybirds and also provide guidance for rationalizing their mass rearing and application in biological control.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-024-10870-6.

Supplementary Material 1

Acknowledgements
This work was supported by the National Natural Science Foundation of China (No. 32201420 to YY).

Author contributions
Y. Y. and H. P. designed and supervised the study; Y. L., X. X., W. R., X. H. and X. T. analyzed the data and visualized the results; Y. L. and Y. Y. wrote the initial manuscript. All the authors contributed critically to the writing of the final manuscript, and gave the approval for publication.

Data availability
Data were obtained from the BioProject ID of the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA): PRJNA509782.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 5 May 2024 / Accepted: 7 October 2024
Published online: 15 October 2024

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