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Trait responses, nonconsumptive effects, and the physiological basis of *Helicoverpa armigera* to bat predation risk

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Predation reduces the population density of prey, affecting its fitness and population dynamics. Few studies have connected trait changes with fitness consequences in prey and the molecular basis and metabolic mechanisms of such changes in bat-insect systems. This study focuses on the responses of *Helicoverpa armigera* to different predation risks, focusing on echolocating bats and their calls. Substantial modifications were observed in the nocturnal and diurnal activities of *H. armigera* under predation risk, with enhanced evasion behaviors. Accelerated development and decreased fitness were observed under predation risks. Transcriptomic and metabolomic analyses indicated that exposure to bats induced the upregulation of amino acid metabolism- and antioxidant pathway-related genes, reflecting shifts in resource utilization in response to oxidative stress. Exposure to bat predation risks enhanced the activity of DNA damage repair pathways and suppressed energy metabolism, contributing to the observed trait changes and fitness decreases. The current results underscore the complex adaptive strategies that prey species evolve in response to predation risk, enhancing our understanding of the predator–prey dynamic and offering valuable insights for innovative and ecologically informed pest management strategies.

Predation risk plays a powerful part in shaping the behavior of prey, with consequences for the individual phenotypic, physiology, population dynamics, community interactions, and ecosystem functions[1,2]. Darwin (1839) hypothesized that prey escape responses cost considerable time and energy to maintain, and the adoption of various defensive tactics may rival or even exceed direct predation in terms of the impact on prey populations and ecosystems[3,4]. Recent studies confirm that predation risk can significantly influence prey fitness and behavior. Studies have shown that even without direct predation, the mere sound of predators can trigger widespread transcriptomic alterations in prey species, suggesting a high degree of phenotypic plasticity and response to predation risk[5]. Moreover, the evolutionary context of these interactions, where chronic stress responses may benefit prey survival and reproduction under high predation risk, challenges the traditional view that chronic stress is inherently harmful[6]. Boonstra's work further emphasizes that chronic stress can lead to adaptations that enhance survival and reproductive success in natural populations, underscoring the dynamic nature of predator–prey interactions and their broader ecological implications[6,7].

The predation risk effects involve several steps and processes. First, the morphology, behavior, physiology, or life history traits of prey may change[8,9] via the risk-induced trait response (RITR), which induces additional costs to prey species, directly or indirectly altering the fitness (e.g., growth rate, mortality, and fecundity) and abundance of prey via factors known as nonconsumptive effects (NCEs). Lastly, alterations in the traits of prey because of predation risk can indirectly affect other interacting species (resources, competitors, and other predators), resulting in significant ecological consequences. Ecologists refer to such changes as trait-mediated indirect effects (TMIEs), which are common in nature and form a basis for the construction of trophic relationships and community structures while also profoundly impacting the maintenance of ecosystem functions by affecting the material cycle and energy flow in food webs. Although the concept of TMIEs has been widely accepted, few studies have connected trait changes with fitness

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consequences, and knowledge concerning the molecular basis and metabolic mechanisms that prompt such changes is limited in bat-insect systems[7]. Therefore, understanding the gene expression and metabolic networks that underlie trait and fitness changes is expected to provide deeper insights into the mechanisms of TMIEs.

The effects of predation risk have been investigated across numerous taxa, especially insects and amphibians[10]. Insects have been intensively studied primarily because TMIEs have higher occurrence opportunities in herbivore-plant systems[11], and many animals feed on insects. Thus, information about insect predation has significant application value in terms of pest control[12]. However, owing to challenges in quantifying risk perception and the trait responses of insects, previous studies investigating the effects of predation risk on terrestrial insects are associated with several limitations. First, most studies focused only on changes in insect behavior, and there is insufficient evidence concerning the response traits related to insect morphology and life history. Second, insect behavioral traits allow a rapid response to predation risk, while the physiological and morphological responses of insects are relatively slow, and few studies have focused on the corresponding molecular mechanisms in bat-insect systems. Therefore, the molecular pathways that drive the insect stress response against predation risk and associated processes, such as RITR, NCEs, and TMIEs, remain unclear[13]. Third, meta-analyses have demonstrated that predation risk effects are stronger when predators and prey have a co-evolutionary relationship[12]. Lastly, our current understanding of the effects of predation risk on insect prey is primarily based on small-scale and short-term studies. Specifically, over 50% of studies have examined the impact of a single predation risk cue on insect prey within 24 h[13], while the predation risks of insects in the natural world are typically multifaceted and enduring.

Echolocating bats and nocturnal insects represent one of the classic textbook cases of co-evolution between predators and prey, rendering them an ideal system for studying TMIEs triggered by predation risks[5]. Bats are the only truly flying mammals, and most use echolocation to navigate and find food in dark environments. Echolocation calls can generally be classified into three types: constant frequency (CF), frequency modulation (FM), and quasi constant frequency[14]. The acoustic arms race between echolocating bats and nocturnal insects, primarily moths, has been ongoing for at least 50 million years[15]. In fact, ~85% of the ~115,000 currently described macromoths have evolved tympanal organs or similar structures[16]. Remarkably, the tuning of the tympanic organs in insect species is almost matched to the specific acoustic characteristics of the sympatric bat communities in which they evolved. Powerful flight and skilled echolocation not only enable bats to directly consume large numbers of insects but also create landscapes of fear in the dark[17,18]. The superior flying ability, skilled echolocation, and co-evolutionary relationship mean that the bat-moth system is ideal for studying the effects of predation risk.

Except for acoustic cues, prey can reduce their exposure to predators via sensory visual, olfactory, mechanosensory, and chemosensory modalities, allowing them to detect and evaluate predation risk. However, there is no consensus on whether prey can perceive these cues and assess the risk posed by predators. The threat-sensitivity hypothesis predicts that the intensity of prey defensive responses should scale with predation risk levels and respond in a graded manner. Therefore, evaluating whether prey can perceive predation risks and make reasonable defense countermeasures is important. In the case of eared moths, when compared with the playback of a single echolocation call, the presence of real bats may lead to the integration of multiple cues (chemical, visual, and other behaviors, such as flapping wings) as a response to danger[19].

The cotton bollworm (*Helicoverpa armigera*) is a global agricultural pest that inflicts significant economic damage across diverse crops including maize, cotton, and tomatoes[20]. It is notorious for its pesticide resistance and for causing annual losses amounting to billions of dollars worldwide[21]. Understanding its responses to natural predators like bats is crucial for developing sustainable pest management strategies. This study investigated the adaptive responses of *H. armigera* to predation risks posed by two distinct bat species: *Rhinolophus sinicus* and *Miniopterus fuliginosus*. Both species have widespread distributions and employ different echolocation calls and foraging strategies, which exemplify the diverse predation pressures encountered by *H. armigera* in various ecological settings. *R. sinicus* emits constant frequency–frequency modulation (CF-FM) calls, well-suited to the cluttered environments typical of many agricultural landscapes where *H. armigera* is prevalent. In contrast, *M. fuliginosus* utilizes FM calls, which are more effective in open areas, providing a comparative perspective on the predator–prey dynamics across different habitat types. We innovatively examined the comprehensive effects of bat predation risk on the entire life cycle of *H. armigera*. Transcriptomic and metabolomic analyses were integrated to elucidate the underlying molecular mechanisms. *R. sinicus* and *M. fuliginosus* and their echolocation calls during foraging were studied to investigate the effects of four different predation risks on larvae and moths over their life history. Additionally, we included a white noise group as a critical treatment, which covers a broad spectrum of frequencies, serving as a baseline to assess the general auditory sensitivity of moths. This group is crucial in ensuring that the observed behavioral and physiological changes are indeed responses to predator-specific signals, rather than general reactions to any auditory stimuli. Thus, the white noise group is essential for differentiating the moths' specific responses to bat echolocation calls from their general auditory responses to non-specific sounds.

Herein, we hypothesized that the predation risk from bats would induce a trait response with corresponding changes in the gene expression and metabolic processes, reducing the fitness of the prey. The following predictions were explored: (1) Larvae exposed to predation risk will display significant alterations in the behavior and metabolism, including reduced food intake and delayed growth, driven by changes in the genes related to stress response. (2) Predation risk will accelerate the larval-to-pupal transition, resulting in small, less viable pupae, with observable changes in genes related to development and stress responses affecting pupal survival and eclosion failure. (3) Adult *H. armigera* will alter their nocturnal activity to avoid peak predators, enhancing avoidance behavior at the expense of reproductive activities. This shift is expected to reduce fecundity and shorten the lifespan owing to increased energy devoted to evasion. In addition, altered metabolic rates and gene expression will reflect this trade-off between survival and reproductive investment.

Results

Trait responses of *Helicoverpa armigera* to bat predation risk

Investigation into the circadian rhythms of *H. armigera* exposed to bat predation risk revealed significant alterations in their activity patterns. The results indicated distinct modulation in the circadian rhythms of moth activity in response to the various predation risks, with diminished nocturnal activity observed across all treatments. The sharpest declines were documented in the MF-bat and RS-bat treatments, with the lowest levels of nocturnal activity in these groups indicating an acute response to the proximate threat of predators. In contrast, the control group maintained higher activity levels throughout the nocturnal hours, presumably due to the absence of predation stress. Additionally, a marked reduction in activity during daytime hours across all treatments when predation risk was not present suggests consistent behavioral adjustment to the decreased threat of predation during daylight (Fig. 1A). Examination of the avoidance behavior of *H. armigera* showed the lowest avoidance behavior in the control group, with higher levels of activity during both night and day. This behavior suggests lower risk perception when predatory cues are absent. Conversely, the groups that were directly exposed to bats demonstrated significantly higher avoidance behavior, with the MF-bat group exhibiting the most pronounced avoidance, particularly at night, suggesting that direct exposure to bat predation risk notably enhances the evasive strategies of moths (Fig. 1B).

Compared to the control group, all *H. armigera* larvae exposed to risk responded by accelerating their development, with larvae exposed to CF-call, FM-call, and bat predation risks (MF-bat and RS-bat) reaching peak mass earlier than those in the control group, albeit via varying growth strategies, indicating an adaptive response to the perceived threats.

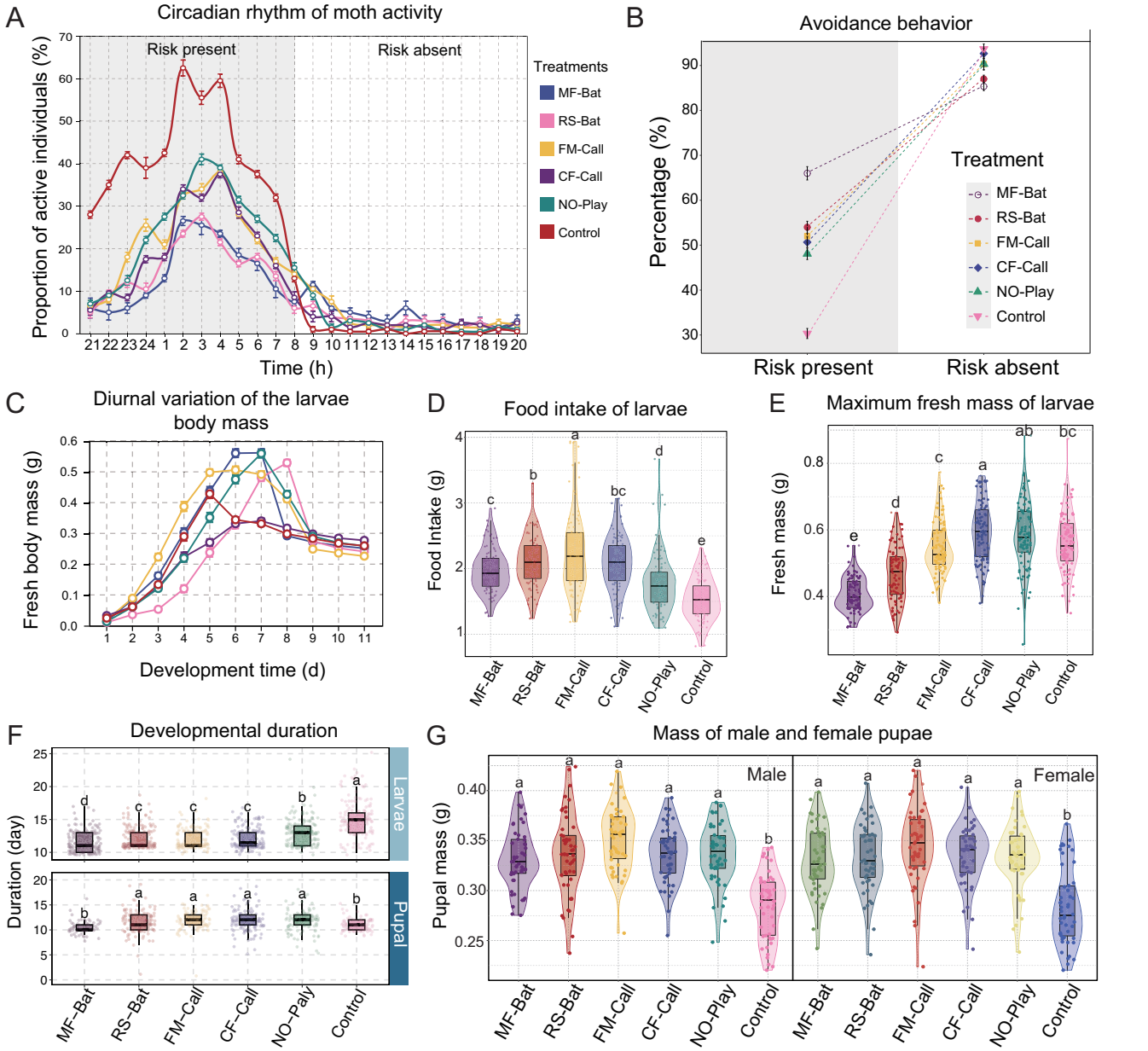

Fig. 1 | Behavioral and morphological trait responses of *Helicoverpa armigera* to different predation risks. A Circadian activity rhythms ($n = 10$ pairs per group, with 12 biological replicates); **B** Avoidance behavior ($n = 30$ biologically independent observations); **C** Diurnal body mass changes ($n = 300$ biologically independent samples); **D** Food intake by larvae ($n = 120$ biologically independent larvae); **E** Maximum fresh mass of larvae ($n = 120$ biologically independent larvae); **F** Developmental duration under predation risk ($n = 120$ for larvae and $n = 60$ for pupae biologically independent samples); and **G** Pupal mass of male and female *H. armigera* individuals ($n = 50$ biologically independent samples). All data are presented as mean ± SE, and statistical significance was assessed using Duncan's multiple range test for comparisons among groups. Different lowercase letters indicate statistically significant differences between groups ($P < 0.05$).

Discernible differences were observed in the growth patterns of treatment groups. The CF-call-exposed larvae exhibited the most rapid growth, peaking on d 6, while the FM-call and MF-bat groups reached their peak mass on d 7. Noise treatment resulted in the latest peak at d 8, suggesting that non-specific white noise may delay the growth response. The RS-bat group showed an early but lower peak mass than the CF-call group, which may reflect a specific adaptive strategy to the predation risk posed by RS-bats (Fig. 1C).

H. armigera larvae under predation risk also showed a significant increase in terms of food intake as compared to the control group ($P < 0.05$, Fig. 1D). Larvae that were exposed to bat predation treatment showed the highest food intake, while the NO-play treatment elicited a significant increase; however, the food intake was significantly lower than those in bat predation groups ($P < 0.05$, Fig. 1D). However, the increase in food intake did not translate to significant body mass gain ($P < 0.05$, Fig. 1E), especially for those in the MF-bat and RS-bat exposure groups, where the lowest fresh body mass was observed ($P < 0.001$, Fig. 1E).

The developmental duration for larvae under various conditions shows a clear trend indicating that the control group larvae experienced a significantly prolonged developmental period compared to other groups (Fig. 1F). However, the risk was observed to prolong the pupal stage of all *H. armigera*, except for those under MF-bat treatment (all $P < 0.05$, Fig. 1F). The pupal weight of both genders was significantly higher in all bat predation risk treatments and the NO-play group than the control. However, among the bat predation risk treatments and the NO-play group, differences in pupal weights were not statistically significant, indicating that while

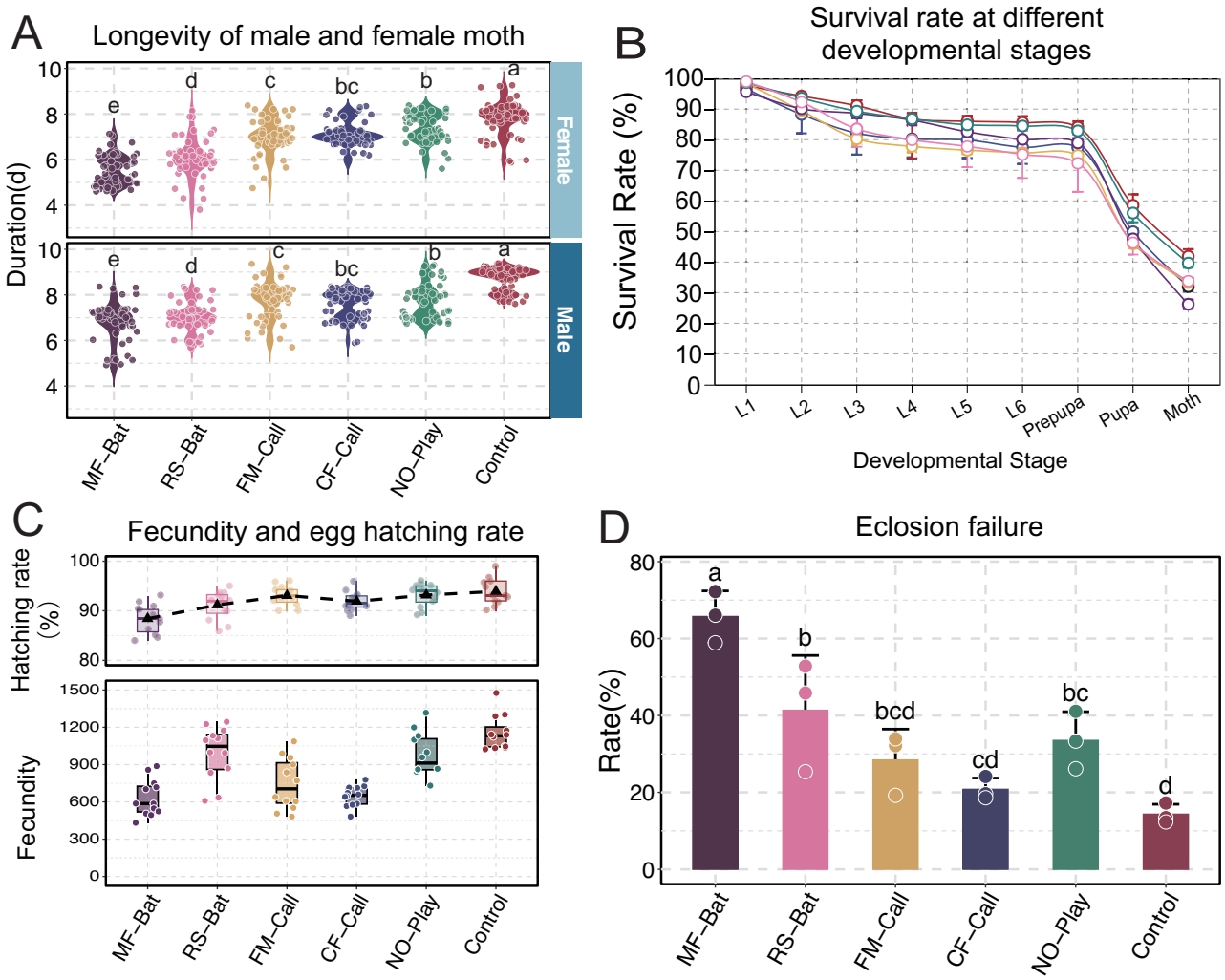

Fig. 2 | NCEs on *Helicoverpa armigera* under different predation risks. A Adult longevity of male and female adults exposed to different predation risks ($n = 50$ biologically independent samples per group for both male and female); **B** Survival rate at different developmental stages ($n = 300$ biologically independent samples); **C** Fecundity ($n = 12$ biologically independent individuals per group) and egg hatching rate ($n = 100$ randomly selected eggs per group); and **D** Eclosion failure, quantifying the proportion of pupae that do not successfully mature into healthy adult moths ($n = 3$ biologically independent groups, with 50 individuals per group). All data are presented as mean ± SE, and statistical significance was assessed using Duncan's multiple range test for comparisons among groups. Different lowercase letters indicate statistically significant differences between groups ($P < 0.05$).

exposure to bat predation risks and noise generally results in increased pupal mass, the specific type of cue does not differentially affect this outcome (Fig. 1G).

Non-consumptive effects of bat predation risks on *Helicoverpa armigera*

H. armigera was able to complete its entire generational survival under different risk treatments. However, bat predation risk and white noise led to the adoption of phenotypic and developmental strategies to cope with different predation risks, with various response levels observed (Fig. 2). A consistent trend was observed in adult moth longevity across the different treatments, with female moths outliving their male counterparts under all conditions. The control group presented with the greatest longevity for both genders, suggesting that optimal survival occurs without predation risk stress. The MF-bat treatment group exhibited the shortest lifespans for both females and males, indicating that the highest stress is associated with this specific predation risk condition. The lifespans of moths in the RS-bat, FM-call, CF-call, and NO-play groups fell between the extremes of the control and MF-bat groups (Fig. 2A), with a general declining trend observed in survival rates across the developmental stages from L1 to moth. While assessing the impact of various treatments on the survival rates of *H. armigera* across developmental stages, we observed distinct patterns. The highest survival rate was consistently maintained throughout all stages in the control group, indicating that the absence of both predation risks and noise stimuli results in better overall survival (Fig. 2B).

Significant differences were also observed in terms of oviposition. Overall, the total fecundity of *H. armigera* decreased in all treatment groups ($P < 0.05$, Fig. 2C). Moths in the MF-bat group produced the lowest number of eggs, which was significantly lower than in all other groups. Egg hatching rates between the groups differed minimally and not significantly (Fig. 2C). Complementing our analysis of survival rates, the assessment of eclosion failure focuses on observations during the final developmental stage. This measurement quantifies the proportion of pupae that fail to mature into healthy adult moths, detailing specific issues such as deformed wings (crippled or curled), inability to fly, incomplete shedding of the pupal case, and mortality at the eclosion phase. Except for the CF-call treatment, all treatments were associated with significantly higher eclosion failure rates as compared to the control group ($P < 0.001$, Fig. 2D), and the MF-bat treatment had the highest eclosion failure rate, followed by the RS-bat treatment, NO-play, and FM-call treatment (Fig. 2D).

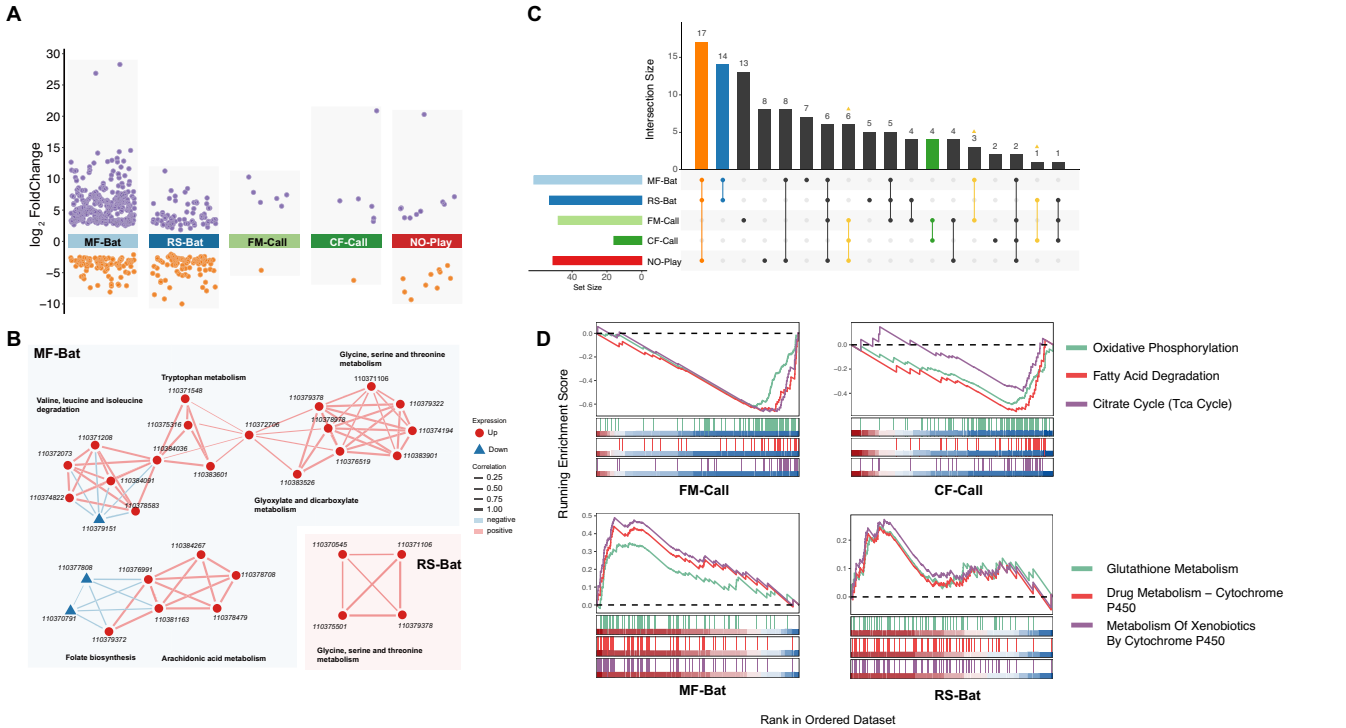

Fig. 3 | Transcriptomic responses of *Helicoverpa armigera* to different predation risks. **A** Differentially expressed genes (DEG) analysis across different treatments; **B** Network diagrams of enriched pathways in the MF-bat and RS-bat treatments; **C** Number of DEGs across different treatments; **D** Gene Set Enrichment Analysis (GSEA) for selected metabolic pathways. All data presented are based on three biologically independent samples for each treatment group ($n = 3$).

Transcriptomic responses to predation risk in *Helicoverpa armigera*

Examination of the transcriptomic changes in *H. armigera* under various predation risk treatments indicated distinct gene expression patterns. A total of 912,693,000 raw reads (137.82 Gb) were generated from different risk treatments. The Q20, Q30, and GC content of all samples are shown in Fig. 3. After filtering, 901,438,538 clean reads (133 Gb) were mapped to the reference genome, with a mapping rate of more than 90% for each sample. A total of 490 differentially expressed genes (DEGs) were identified in the risk treatment groups as compared to the control group (Fig. 3A), with the greatest number of DEGs identified in the MF-bat treatment, followed by the RS-bat treatment (Fig. 3C). Amino acid metabolism-related pathways were enriched by upregulated genes in both the MF-bat and RS-bat groups, with valine, leucine, and isoleucine degradation (haw00280); tryptophan metabolism (haw00380), glyoxylate and dicarboxylate metabolism (haw00630); glycine, serine, and threonine metabolism (haw00260); folate biosynthesis (haw00790); and arachidonic acid metabolism (haw00590) pathways significantly enriched in the MF-bat treatment group and only glycine, serine, and threonine metabolism (haw00260) being significantly enriched in the RS-bat group (Fig. 3B). In contrast, *H. armigera* in the echolocation call playback treatment groups exhibited fewer DEGs, and no amino acid metabolism-related pathway enrichment was observed in these groups (Fig. 3D).

Functional enrichment of the *Helicoverpa armigera* transcriptome under predation risk

Gene Set Enrichment Analysis (GSEA) was performed for global comprehension of the transcriptome profile (Fig. 3D). Upregulation was observed in DNA damage repair pathways such as DNA replication (haw03030) and mismatch repair (haw03430) in moths from both the FM-call and CF-call groups. FM-call treatment led to the upregulation of pathways involved in clearing misfolded proteins, such as the proteasome (haw03050); ubiquitin-mediated proteolysis (haw04120); and autophagy (haw04140). Downregulated pathways related to energy and glucogenic amino acid metabolism, including the citrate cycle (haw00020); oxidative phosphorylation (haw00190); tryptophan metabolism (haw00380), and arginine and proline metabolism (haw00330) were observed in both echolocation call playback groups (Fig. 3D). The exposure of *H. armigera* to live bats led to the upregulation of several antioxidant pathways, including glutathione metabolism (haw00480), drug metabolism-cytochrome P450 (haw00982), and xenobiotic metabolism by cytochrome P450 (haw00980), with upregulation of two glucogenic amino acid metabolism pathways: glycine, serine, and threonine metabolism (haw00260), in addition to phenylalanine metabolism (haw00360) (Fig. 3D).

Metabolic profiling of *Helicoverpa armigera* under predation risk

Changes in metabolite levels under different treatments were analyzed to better understand the response of *H. armigera* to various predation risks on a metabolic level. After preprocessing the raw metabolic data, a total of 7701 metabolic peaks were identified, with 3854 in the positive ion mode and 3847 in the negative ion mode. Successful annotation of 664 metabolite peaks was performed using public databases. Based on the OPLS-DA model, a total of 339 differentially abundant metabolites were identified in *H. armigera* under all five predation risk types, with more observed in the live bat and NO-play playback groups as compared to the echolocation call playback groups (Fig. 4A). Specifically, 124, 62, and 75 differentially abundant metabolites were observed in the RS-bat, MF-bat, and NO-play groups, respectively, with only 40 and 38 obtained for the CF-call and FM-call playback groups, respectively. The intersection between differential metabolites revealed that *H. armigera* exposed to different predation risks had differential metabolite compositions, which was consistent with the PLS-DA results (Fig. 4B). HMDB classification of the metabolites indicated that most lipid-related metabolites were differentially abundant, especially in the live bat treatment and NO-play playback groups, with glycerophospholipid metabolism (haw00564) pathways (Fig. 4C, D) also showing

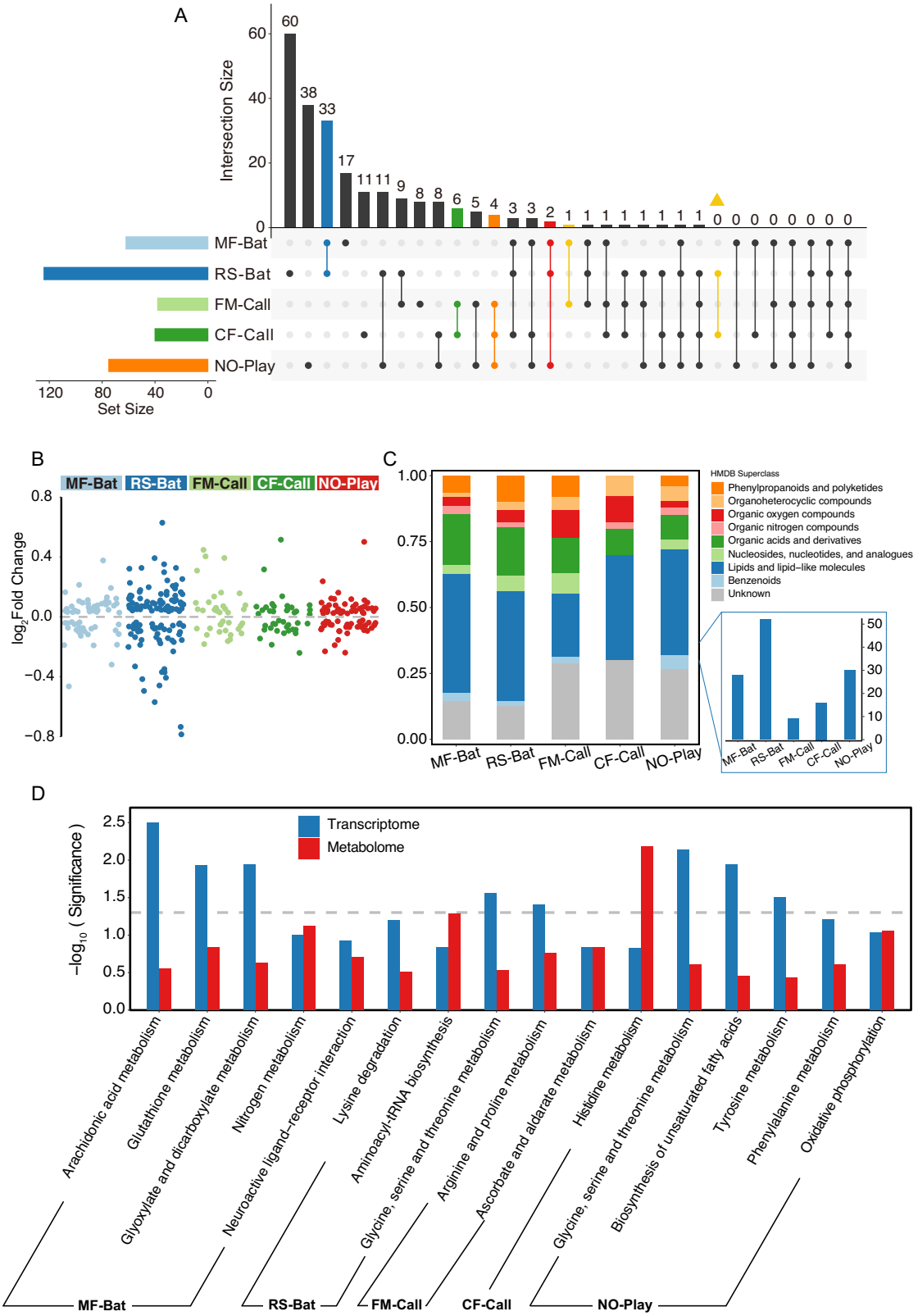

Fig. 4 | Metabolite analysis of *Helicoverpa armigera* under different predatory exposure treatments. **A** Overlap and unique distribution of differentially expressed metabolites; **B** Metabolites in each group; **C** Proportion of metabolite classes identified within each treatment according to HMDB superclass categorization; **D** Transcriptomes and metabolic pathways affected in *H. armigera* across treatments. All data presented are based on three biologically independent samples for each treatment group ($n = 3$).

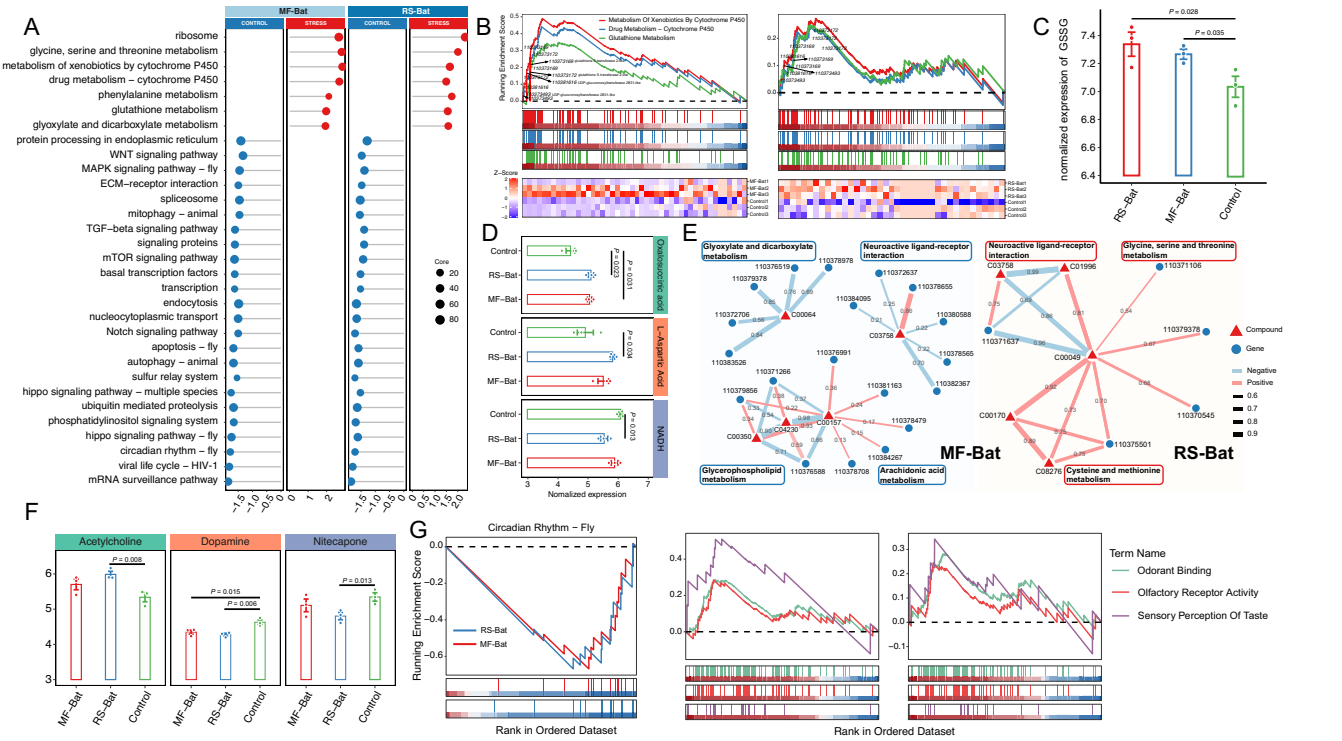

Fig. 5 | Transcriptomic and metabolic pathway alterations in *Helicoverpa armigera* when exposed to different predation risks. A Number of differentially expressed genes and pathways across treatments; **B** Variation in the expression of genes associated with key metabolic pathways in response to MF-bat and RS-bat treatments; **C** Proportion of metabolite superclass categories for each treatment group; **D** Significance levels of the metabolic and transcriptomic changes in key pathways; **E** Network of the interconnectedness of metabolic pathways and the impact of predation risk on metabolic processes in MF-bat and RS-bat groups; **F** Expression of neurotransmitters in *H. armigera* under predation risks; **G** GSEA for circadian rhythm and olfactory receptor activity pathways. All data presented are based on three biologically independent samples for each treatment group ($n = 3$).

enrichment in the MF-bat, RS-bat, and NO-play groups. This result highlights the importance of considering lipid metabolic pathways and their potential contribution to observed differences in metabolic activity. Additionally, D-Glutamine and D-glutamate metabolism (haw00471) and nitrogen metabolism (haw00910) pathways were enriched across all groups. Purine metabolism (haw00230) was enriched in the FM-call group, while ascorbate and aldarate metabolism (haw00053) as well as histidine metabolism (haw00340) were enriched in the CF-call group.

Integrative transcriptomic and metabolomic analysis of the *Helicoverpa armigera* response to bat predation risk

Significant pathway enrichments were observed in both the MF-bat and RS-bat treatment groups (Fig. 5A). Notable enrichment was observed in pathways related to cytochrome P450 and glutathione metabolism, with the core enrichment genes including members of the glutathione S-transferase (*GST*) gene and UDP-glucuronosyltransferase (*UGT*) gene families (Fig. 5B). Upregulation of the glutathione oxidation (GSSG) pathway was observed in both MF-bat and RS-bat groups (Fig. 5C). Additionally, differentially abundant intermediate metabolites, such as increased oxaloscuccinic acid, were observed in both the MF-bat and RS-bat treatment groups, with elevated nicotinamide adenine dinucleotide reduced (NADH) levels in the RS-bat group (Fig. 5D). GSEA also revealed significant upregulation in several amino acid metabolism-related pathways, including those associated with glycine, serine, and threonine metabolism, phenylalanine metabolism as well as glycolate and dicarboxylate metabolism, with network analysis indicating changes in the abundance of metabolites involved in glucogenic amino acid metabolism in the live bat treatment groups, including glutamine downregulation in the MF-bat treatment and aspartic acid upregulation in the RS-bat treatment groups (Fig. 5E).

Significant changes in the expression of genes and the abundance of metabolites related to the nervous system were also observed in the RS-bat and MF-bat treatment groups, with a distinct downregulation of dopamine and changes in the expression of acetylcholine and nitecapone, particularly prevalent in the RS-bat group (Fig. 5F). This was accompanied by a noticeable downregulation of circadian rhythms in both the MF-bat and RS-bat groups. GSEA of the Gene Ontology (GO) terms indicated significant upregulation in the expression of olfactory-related terms under both MF-bat and RS-bat treatment, including terms such as odorant binding, olfactory receptor activity, and the sensory perception of taste. Further analysis of the core enrichment genes within these terms revealed the upregulation of multiple genes that are associated with the olfactory receptor family or annotated with olfactory receptor-related domains. These observations point to changes in the olfactory abilities of *H. armigera* under predation risk (Fig. 5G).

Moreover, exposure to bat predation risk triggered the upregulation of pathways involved in antioxidant defense and amino acid metabolism, indicating an increase in the moth's metabolic and immune responses (Fig. 6). Pathways linked to glycine, serine, and threonine metabolism were notably enriched. Changes were also observed in lipid metabolism, which is crucial for energy storage and membrane structure. The immune response was also affected, suggesting a heightened state of defense readiness. Neurotoxic effects were indicated by alterations in dopamine and acetylcholine levels, potentially impacting neural functions and circadian rhythms. Lastly, there was an upregulation of olfactory receptor activities, highlighting the moth's enhanced sensory perception in response to bat predation risk (Fig. 6). Taken together, our findings reveal a complex interplay of physiological adjustments through which *H. armigera* copes with predation threats.

Discussion

Systematic investigation of the responses of *H. armigera* to the tested predation risks revealed significant behavioral, developmental, and molecular

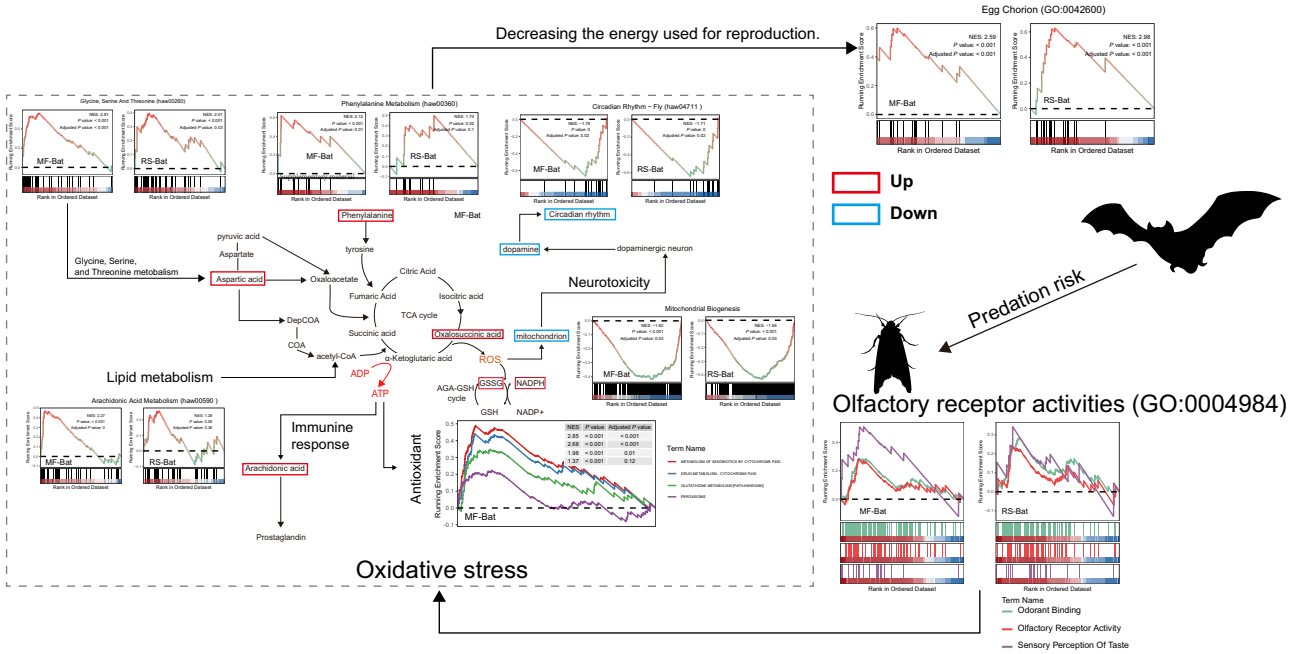

Fig. 6 | Transcriptomic and metabolic pathway responses to bat predation risk in *Helicoverpa armigera*. Red illustrates increased metabolites, while blue indicates decreased. The GSEA results illustrate the expression patterns associated with pathways implicated in metabolite alterations. The bat and moth illustrations were sourced from PhyloPic (https://www.phylopic.org). The bat illustration, created by Melissa Ingala, and the moth illustration, by Gareth Monger, are both used under the Attribution 3.0 Unported license.

adaptations. *H. armigera* exhibited a range of adaptive strategies in response to predation risk, evident from the altered circadian rhythms, avoidance behavior, and developmental patterns observed, ultimately affecting moth fitness. Notably, the distinct responses elicited in the presence of live bats and bat echolocation calls suggest that *H. armigera* can finely tune its physiological and behavioral mechanisms to different levels and types of predation risk, supporting our first and second hypotheses. Moreover, the observed upregulation of genes involved in DNA damage repair and oxidative stress pathways, especially under echolocation called stress, highlights a potential molecular mechanism through which *H. armigera* alleviates the cellular impact of predator-induced stress. The distinct transcriptomic and metabolomic responses identified provide valuable insights into the complex interplay between environmental stressors and the survival strategies of this species. These results supported our third hypothesis. The findings of this study not only enhance our understanding of ecological interactions between predator and prey but also offer potential avenues for pest management strategies that target the behavioral and physiological vulnerabilities of *H. armigera*.

Predation risk frequently induces behavioral or morphological plasticity in prey, which in turn causes physiological shifts. Behavioral shifts, commonly studied trait responses in arthropods, are typically rapid and reversible[10]. Morphological shifts generally appear more slowly and are more difficult to reverse than behavioral or physiological shifts[10]. Physiological shifts can occur as direct risk responses but are frequently the consequences of a preceding behavioral shift.

Predators can significantly influence prey population growth through the induction of behavioral changes[22]. The results of behavioral and molecular analyses of the *H. armigera* response to predation risk reveal a fascinating interplay between physiological stress and adaptive changes. Our observations indicate a strategic shift in activity patterns, with pronounced reduction in nocturnal activity following direct exposure to bat predators, implying an evasive adaptation to avoid the peak predation period. The behavioral alteration is underpinned by significant molecular changes, particularly in the regulation of circadian rhythms. Enrichment analysis of the transcriptomic data showed enhanced expression of pathways related to circadian regulation, which aligns with the observed behavioral modifications (Fig. 4). The behavioral shift likely represents a stress response to a perceived threat, enabling *H. armigera* to minimize encounters with predators during their active periods[23]. Such adaptations are essential for reducing predation risks and are indicative of plasticity in species survival. Another significant finding in our study, enhanced evasion behavior, was especially pronounced in *H. armigera* individuals who were exposed directly to bats. This heightened avoidance behavior serves as an effective defense mechanism, increasing the survival rate under predation pressure[10]. The degree and nature of these behavioral changes are likely correlated with the level of predation risk perceived by *H. armigera*, reflecting their ability to assess and respond to varying degrees of risk.

Furthermore, our research indicated that *H. armigera* adapts its feeding behavior in response to predation risk. An increase in food intake suggests a shift in energy allocation toward faster growth and development rather than increased body mass. This strategic resource allocation may be a response to reduce the duration of vulnerable life stages, thereby minimizing the risk of predation, with the rapid growth and development under predation pressure likely enabling *H. armigera* larvae to quickly reach a less vulnerable life stage, enhancing their overall chances of survival[24]. Additionally, the accelerated completion of the lifecycle observed under predation risk is a critical adaptation in *H. armigera*. This rapid lifecycle progression is a clear example of how morphological and developmental changes can be employed as survival strategies in response to environmental stress. For example, Gotthard[25] demonstrated that fast growth rates in butterflies may increase with predation risk, suggesting a delicate balance between growth and survival. Similarly, Urban[26] found that prey species might adjust their growth strategies in response to the size of the predation threat. In contrast to these findings, the uniform acceleration in our study suggests that *H. armigera* may employ a generalized response to predation threats, highlighting a less specific but potentially more robust adaptation strategy. By comparing these responses, we underscored the complexity of survival strategies in nature and their implications for understanding predator–prey dynamics across different ecological contexts.

Although *H. armigera* mounted a rapid response to predation risk, it appeared to come at a cost, as evidenced by the elevated eclosion failure rates observed, particularly in the MF-bat treatment group (Fig. 2D). The high

rate of eclosion failure in this group did not coincide with an extended pupal stage, contrary to that observed in the other predation risk treatment groups, suggesting that while the larvae in this group rushed their development to avoid the predation-prone larval stage, the hastened growth did not allow adequate preparation for the complex eclosion process. Consequently, this rapid development may have led to accumulated physiological deficits, which manifested as higher eclosion failure rates, curtailing the transition to successful adulthood[27]. The lower eclosion failure rate observed in the control group reinforces the notion that a more measured developmental pace, free from the urgency imposed by predation risk, allowed for thorough maturation, culminating in a higher success rate during eclosion. These results support ecological theories suggesting that the presence of predators can significantly influence the life history trajectories of prey species, affecting not just their immediate survival but also the quality of their subsequent life stages[28].

The increased pupal mass observed across all predation risk treatments, including the NO-play group, may be a compensatory response to developmental stress[29]. A larger pupal mass could imply a better-resourced individual, potentially offsetting the accelerated larval development and increasing the chances of successful eclosion (Fig. 1G). However, this increased mass was not enough to ensure higher eclosion success rates for moths in the MF-bat group, indicating that resource allocation strategies may be limited when facing intense predation pressure[4]. Moreover, we observed an overall increase in pupal weight in response to predation risks compared to the control, and the specific type of predation risk did not differentially affect the outcome. The results suggested that *H. armigera* may respond to a general threat of predation rather than to the specific characteristics of predator cues. This generalized response might indicate an evolutionary adaptation that simplifies the physiological stress response to diverse predatory threats. Additionally, adult longevity exhibited a clear predation risk effect, with females tending to outlive males, a pattern that persisted across all treatments (Fig. 2A). Individuals in the control group showed the highest longevity, likely due to the absence of predator-induced stress. These results suggest that energy that is otherwise expended in predator evasion or stress responses can be redirected toward maintenance and reproductive efforts. Conversely, the decreased longevity in the predation risk groups may indicate an increased allocation of energy toward antipredator defenses, which may detract from other physiological processes such as repair and reproduction, potentially reducing lifespan[30].

Survival rates remained high throughout the various developmental stages (Fig. 2B) but were notably higher in the control group, underscoring the impact that predation stress has on developmental success. Predation risk, even when not resulting in direct consumption, can still lead to increased mortality due to stress-related factors such as suboptimal feeding and growth conditions or heightened vulnerability to other environmental stressors. Fecundity and egg hatching rates (Fig. 2C) further reflect the NCEs. The lower fecundity observed in the predation risk groups compared to the control could stem from the diversion of resources toward survival at the cost of reproduction—a common trade-off under ecological stress[31]. Although the egg hatching rate does not vary significantly, the reduced number of eggs laid could lead to lower overall reproductive success, and, thus, population growth rates.

The comprehensive transcriptomic and metabolomic analyses that were performed in this study revealed the intricate molecular responses of *H. armigera* to bat predation risks, which comprise a cascade of molecular responses that form the foundation for observable behavioral adaptations. These responses are not merely reactionary but are a coordinated expression of genetic plasticity, allowing for rapid and dynamic responses to environmental challenges. Predation risk incites significant transcriptomic alterations in *H. armigera*, with the molecular interplay between genes and metabolites orchestrating moth survival strategies, thus forming a bridge between environmental cues and physiological adaptation[32]. Under the risks posed by live bats, *H. armigera* upregulated genes involved in amino acid metabolism, suggesting an adaptive mechanism to optimize resource utilization for survival[10]. Significant upregulation in the pathways associated with antioxidant defenses, particularly glutathione metabolism and cytochrome P450, were also observed, indicating a heightened oxidative stress response[32]. This is supported by the observed upregulation of *GST* and *UGT* gene family members, which are known for their role in detoxification[33,34]. Metabolomic profiling corroborated these findings, distinguishing the live bat exposure groups from controls based on marked variations in lipid metabolism, as evidenced by the differential expression of metabolites that are related to glycerophospholipid metabolism. This could suggest alterations in cell membrane composition or signaling as part of the organism's defensive strategy[35,36]. The increased levels of glucogenic amino acids and intermediates, such as oxalosuccinic acid, highlight a potential shift toward enhanced gluconeogenesis and energy provision during times of stress[37].

Concurrently with transcriptomic shifts, metabolomic profiling revealed distinct changes in *H. armigera* under various predation risks. Specifically, changes in lipid metabolism pathway activity suggest a significant role for energy storage and mobilization, which are crucial for meeting the energetic demands imposed by stress[35]. This underscores the importance of lipid reserves, not just as energy substrates, but also as integral components of an organism's rapid stress response[38]. Furthermore, the downregulation of NADH, along with increased oxalosuccinic acid, points to the acceleration of the tricarboxylic acid (TCA) cycle to meet the energy demands associated with stress[37]. Increased food intake may lead to elevated energy absorption, necessitating an enhanced TCA cycle to efficiently convert these energy resources into usable forms. This metabolic enhancement is essential for supporting faster growth rates, as it provides the necessary energy and biosynthetic precursors required for rapid development. Additionally, the downregulation of NADH may serve as a regulatory mechanism to optimize energy production and mitigate oxidative stress associated with heightened metabolic activity. These molecular responses reflect a complex physiological restructuring that is aimed at enhancing survival prospects under predation pressure, with energy metabolism and stress responses finely tuned[39,40].

The integrated analysis of transcriptome and metabolome data provides a holistic understanding of *H. armigera*'s response to predation risk. The dichotomy of increased energy metabolism in the face of live bat predation versus reduced energy metabolism under echolocation call exposure exemplifies a trade-off between the immediate survival tactics and other physiological processes, including reproduction and longevity. These results highlight the intricate balance act that organisms must maintain when faced with predation, allocating resources judiciously to optimize fitness across various life history stages[27]. These findings align with the genomic adaptations related to migratory flight activity in *H. armigera*, as reported by Jones et al., which underscore the genetic basis for complex behavioral and metabolic traits essential for long-distance migration and predator avoidance[41]. The overlap in metabolic and sensory pathways revealed by both studies suggests a potential common genetic framework that supports both rapid energy mobilization for immediate survival and sustained energy expenditure necessary for migration[41]. This integrative perspective proposes that *H. armigera* optimizes its fitness across various life history stages by leveraging these genetic pathways to efficiently manage energy and sensory inputs under diverse ecological pressures. Understanding these shared mechanisms not only provides insights into the moth's broader adaptive strategies but also offers implications for understanding its natural behavior and improving pest management approaches.

Prey utilizes sensory inputs across various modalities to perceive predation risks and consequently modify their behavior. In this study, *R. sinicus*, employing CF-FM calls, adapted to navigate the acoustically cluttered agricultural environments where *H. armigera* is prevalent, which necessitated the moth's specific auditory adaptations for survival. Conversely, *M. fuliginosus* uses FM calls and is a specialized moth predator, likely causing pronounced risk responses in *H. armigera*[42]. This specialization could explain heightened sensory adaptations in the moth, as these bats represent a significant threat in open environments. The distinction between the risk levels posed by actual bat predators versus isolated bat

echolocation calls is pivotal in understanding the adaptive responses of *H. armigera*. The holistic risks presented by live bats likely encompass a suite of multisensory cues that evoke a more pronounced stress response in the prey. This multimodal perception is in line with the threat-sensitivity hypothesis, which posits that as the perceived risk from predators escalates, so too does the magnitude of antipredator behaviors[43]. *H. armigera* demonstrated a nuanced ability to discern these varied risks and modulate responses such as morphological change to behavioral adjustments, particularly when confronted with live bats. This variation underscores an organism's capacity to tailor its defensive strategies to the intensity and nature of predation risk, optimizing survival[44].

Furthermore, our findings indicate upregulation in the expression of olfactory receptor genes when *H. armigera* is exposed to actual bats, suggesting the utilization of chemosensory cues in predation risk perception[45]. While the auditory detection of predator cues is a well-documented response, the heightened olfactory sensitivity reflects a complex multimodal defensive mechanism. Insect prey have previously been documented to modify their behavior based on chemical cues from predators, which is indicative of an advanced threat detection system[46]. The elevated expression of olfactory genes may also be reflective of the synergistic effects of cross-modal sensory input, where combined auditory and olfactory cues provide a comprehensive picture of the predation risk, leading to more effective evasion strategies[47]. However, the enhanced olfactory sensitivity comes with increased metabolic costs, suggesting resource allocation toward survival over other physiological processes[48]. In conclusion, the adaptive strategies of *H. armigera* in the face of predation risks are underscored by a complex interplay between multimodal sensory perception and the corresponding behavioral and physiological responses. The differential expression of olfactory receptors, in conjunction with changes in behavior and physiology, highlights the sophisticated risk perception capabilities of the organism, which are crucial for survival in environments laden with predators.

In our study, broad-spectrum white noise and predator-specific echolocation calls elicited distinct behavioral and physiological responses in *H armigera*, each of which may have significant implications for survival. White noise induces a general stress response characterized by heightened metabolic activity and increased sensory alertness. This response, mediated by a generalized auditory pathway, is evolutionarily tuned to detect potential threats across a wide range of frequencies. Such adaptability may confer an advantage in naturally noisy environments where the precise identification of predator-specific sounds may be challenging. Conversely, echolocation calls from predators elicit more targeted behavioral adaptations in *H. armigera*. These adaptations include tactical evasion maneuvers such as abrupt cessation of flight or rapid directional changes, crucial for escaping aerial predators. The capacity of *H. armigera* to perceive these specific sounds from ambient noise suggests a highly sophisticated sensory system, optimized to enhance survival during predator encounters. This differentiation in response underscores the complexity of the moth's sensory perception and highlights its evolutionary adaptations to distinct environmental challenges.

Our study has a few limitations. First, our study utilized transcriptomic and metabolomic analyses to delineate the responses of *H. armigera* to predation risk; however, it did not extend to experimental manipulations for functional validation. This approach was constrained by initial resource limitations, and the complex functional validation is beyond the scope of the study. To build on these foundational insights, future work will implement targeted experimental approaches, such as gene editing or pharmacological interventions, to rigorously test the functional implications of the observed molecular alterations and establish causative relationships between gene expression changes and phenotypic outcomes. Second, the present study was conducted under controlled laboratory conditions, which may not adequately replicate complex and unpredictable natural ecosystems. The intricate interactions of predators and prey within ecosystems, influenced various biotic and abiotic factors, may affect the applicability and scalability of our findings to real-world scenarios. Therefore, future studies should aim to expand our results through field experiments in diverse ecological settings to ensure the broad applicability and relevance of our conclusions.

Conclusions and perspectives

This study provides a comprehensive understanding of the adaptive responses of *H. armigera* to predation risks, delving into behavioral, morphological, developmental, and molecular changes. We observed complex trade-offs between developmental speed, resource allocation, and survival, revealing how *H. armigera* manages energy and sensory inputs to effectively mitigate predation risks. These adaptations, which include decreased nocturnal activity, pronounced evasion behaviors, and accelerated development to reduce the vulnerable larval period, underscore the ecological strategies and fitness impacts of the moth. While these adaptations help in immediate survival, they may adversely affect longevity and fecundity, highlighting the intricate balance between survival and reproductive success.

At the molecular level, changes in gene expression and metabolic profiles reflect the organism's robust response to predator threats. Notably, under direct bat exposure, *H. armigera* upregulates genes that are associated with antioxidant pathways and amino acid metabolism, enhancing the response to oxidative stress and regulating energy metabolism. Conversely, the downregulation of energy metabolism observed under echolocation call exposure reflects a trade-off between urgent survival and other physiological demands. These results indicate that *H. armigera* utilizes a suite of complex adaptive responses to predation, with broad implications for ecosystem dynamics. These findings not only shed light on the specific adaptive strategies of *H. armigera* but also suggest significant implications for pest management and ecosystem dynamics, as behavioral and morphological changes can alter feeding patterns, affecting plant herbivory rates and potentially cascading through trophic levels to influence the broader ecological community.

Our study highlighted actionable insights into the behavioral and physiological adaptations of *H. armigera* to predation risks, offering new avenues for developing integrated pest management strategies. Specifically, we proposed utilizing sound-based interventions that exploit the moth's responses to predation risks. By implementing devices that mimic bat echolocation calls, farmers may deter moths from critical agricultural zones, reducing reliance on chemical pesticides and enhancing crop protection in an environmentally friendly manner. Additionally, leveraging the moth's stress responses can optimize the timing and effectiveness of pest control measures. For example, introducing stress-inducing environmental cues during peak activity periods could disrupt moth behavior and reproduction, providing a targeted approach that minimizes ecological impacts. Such strategies not only align with sustainable agricultural practices but also open up new possibilities for managing pest populations by integrating behavioral ecology insights.

Future research should consider the long-term effects of predation pressures across different environments and *H. armigera* populations. Given that predation risks can induce changes in genetic structure at the population level, understanding the impact that these factors have on the population dynamics of *H. armigera* and ecosystem functions is crucial. Moreover, exploring how these ecological findings can be applied to more sustainable and eco-friendly pest management practices is expected to be an important direction for future research. Further experiments and observations are warranted to reveal the ecological significance of other potential NCEs, such as the secondary effects of predation risk on the behavior and metabolism of *H. armigera* in their natural settings.

Methods

Ethics statement

Experiment procedures were approved by the Ethics Committee of Science and Technology, Northeast Normal University (Approval number: 202301001). We have complied with all relevant ethical regulations for animal use. Eight adult *R. sinicus* (Chiroptera, Rhinolophidae, ♂4, ♀4) and eight *M. fuliginosus* (Chiroptera, Miniopteridae, ♂4, ♀4) were collected from caves in Loudi, Hunan Province, and Nanyang, Henan Province, China, in

August 2021 using a mist net. Captured adults were maintained in a 6.5 m long × 5.5 m wide × 2.1 m high husbandry room at 28 ± 1 °C, 60% ± 5% RH, and 12 h light/12 h dark conditions, mimicking the natural environment inside the caves. Bats were fed on *Tenebrio molitor* larvae and freshwater enriched with vitamins and minerals for the subsequent experiments. All bats were released back to their original habitat after the experiments were completed.

Experimental setup and predator cue preparation

To test whether the *H. armigera* can distinguish and respond to different levels of bat predation risk, five experimental treatments were set up, with two bat predator exposure groups: the CF-FM bat exposure group (*R. sinicus*, RS-bat) and FM bat exposure group (*M. fuliginosus*, MF-bat) and three acoustic stimulation groups: the CF-call playback group (CF-call), FM-call playback group (FM-call), and white noise playback group (NO-play). The echolocation calls of bats during forage were recorded, edited, and played back. The control group (Control) was set up with no bat predator exposure or acoustic playback.

Foraging echolocation calls of individual *R. sinicus* (CF-FM) and *M. fuliginosus* (FM) were recorded for using as predator acoustic cue stimulation for *H. armigera*, with moths exposed to CF-FM and FM groups hereinafter referred to as the CF-call and FM-call groups, respectively. Bats were placed in a large flight cage (4.4 m long × 1.5 m wide × 1.8 m high) with flying moths and hanging insects. The method used by Zhang et al.[49] was used to record and playback the sounds made by bats when foraging. Six playback files comprising ultrasonic white noise (30–90 kHz) were also produced using Avisoft-SASLab Pro (version 5.2.14, R. Specht, Berlin, Germany) to detect whether the moth response to bat calls was similar to that of ultrasonic white noise.

Insect acquisition and rearing

H. armigera larvae were sourced from were obtained from Henan Jiyuan Baiyun Industry Co., Ltd (Henan, China) and reared individually on artificial diets[50] in growth chambers maintained at a climate-controlled temperature of 28 ± 1 °C and relative humidity of 75 ± 5%; a 16 h light/8 h dark photoperiod was maintained until pupation was complete. Pupa were disinfected in a 3% formaldehyde solution for 30 min before transferred into a 200-mesh cage for emergence. Upon emergence, 10% fresh honey water was placed in the cage each day to supplement nutrition during mating and oviposition. The first-generation eggs were collected carefully and placed in small plastic tubes, and the pipe orifice was covered with nylon mesh to prevent larvae from escaping, while allowing predator detection. All larvae were reared under the same conditions until emergence as adults. *H. armigera* eggs and adult moths of the same age and in good condition were used in all experiments.

Upon hatching, larvae were randomly distributed into six distinct experimental treatments. For predator exposure, eight adult *R. sinicus* and *M. fuliginosus* bats were confined in two cages with *T. molitor* and fresh water. All bats were free to fly or climb around tubes containing *H. armigera* larvae but were unable to physically contact or prey on larvae until they emerged as adults. For the predator acoustic playback and NO-play groups, an SPL ~ 60 dB speaker (Ultrasonic Dynamic Speaker, Avisoft Bioacoustics, Glienicke, Germany) was placed 1 m from the tested insect in each chamber, and a file broadcast was played in a loop during stimulation. The control group was placed in an identical setup without sound. Larvae in the different treatment groups were thus continuously exposed to the different predation risks, and larvae in the control group were held in the same environment without exposure to predation stimuli. For each treatment, the stimulation was run from 20.00 to 08.00 the following day and then withdrawn to imitate the activity of bats and insects in nature.

Experimental test for plasticity in the development of *Helicoverpa armigera*

To test the effects of predation risk on the NCEs of *H. armigera*, newly hatched larvae were randomly assigned to one of the six treatments and placed in finger tubes (as above) for exposure to bat risk. Each treatment comprised six cohorts of 50 larvae, totaling 300. Larvae were fed with adequate artificial diets. Developmental progress was monitored to determine the duration of the larval and pupal stages, the pupal mass and pupation rate, and the emergence rate. Newly hatched larvae were divided into five groups of 24 to measure the daily food intake and the changing trend of larval mass. Only the food intake of surviving individuals was used for subsequent data analysis. Each artificial diet block was weighed on an analytical balance (accurate to 0.01 mg) before feeding, and 25 additional diet blocks were placed in empty finger tubes to account for humidity changes. All blocks were then removed each day for weighing, and fresh ones were added. The larval body masses were measured each day, and pupae were weighed three days after pupation. Finally, one way analysis of variance (ANOVA) and Duncan's post-hoc test were used to assess differences in the developmental duration, food intake and maximum fresh body mass, pupation rate, and pupal mass. Statistical analyses were performed using SPSS version 22.0 (IBM, Armonk, NY, USA).

Behavioral and reproduction responses of adult moths to predation risk

The behavioral plasticity of avoidance maneuvers exhibited by adult moths in response to the different treatments was first measured. Experiments were performed by placing 10 pairs of three-day-old male and female moths into the cages (35 cm × 35 cm × 35 cm) with wheat seedlings (height was 17 cm) provided as a refuge at the bottom of cages, allowing assessment of whether exposure to bat predation risk would increase the avoidance rate. An infrared camera (FDR-AX60; Sony Corp., Tokyo, Japan) was used to record the behavior of each pair of moths under different treatments. The movement of moths was recorded in terms of both position and time. To robustly assess the impact of exposure to bat predation risk on the avoidance behavior of moths, each treatment was replicated 12 times.

To assess the impact of different treatments on the egg production and hatching rates of *H. armigera*, a mating experiment was conducted in which newly emerged male and female moths were paired in a plastic mating cup (10.0 cm diameter x 10.0 cm height) containing 10% fresh honey water in cotton balls. The cups were sealed with breathable gauze, and a rubber band was used to secure them. This setup allowed the gauze to separate the moths from the different predatory risk cues while also acting as a surface upon which the female *H. armigera* moths could lay eggs. The gauze and mating cups were replaced daily, ensuring minimal disturbance to the moths. Eggs were collected and counted each day until the death of the female moth. A random sample of 100 eggs was used each day to monitor and record the incubation process, with 10 biological replicates. Adult moth longevity, fecundity (egg production), and egg hatching rates were compared using ANOVA followed by a Duncan's multiple range post-hoc test to determine any significant differences between the groups. Analysis was performed using SPSS version 22.0 (IBM, Armonk, NY, USA).

Sample preparation for transcriptome sequencing

Following the experiments, moths were placed into a 2 mL cryogenic vial (Corning, USA) and immediately immersed in liquid nitrogen for 45 s. Moths were then removed and transferred quickly to a clean Petri dish on dry ice located on a sterilized workbench. Moth heads were removed and descaled by scraping with a scalpel, and brain tissue was immersed in cryogenic vials with RNAlater solution (Life Technologies). Samples were stored at −80 °C until preparation was complete. Each sample was divided equally into two parts: one for transcriptome sequencing and the other for metabolomics sequencing.

RNA-sequencing and transcriptome analysis

Total RNA was isolated from brain tissue using TRIZOL reagent (Invitrogen, USA) according to the manufacturer's instructions (Invitrogen). Genomic DNA was removed using DNase I (TaKara). RNA quantity and purity were assessed using the 2100 Bioanalyser (Agilent) and quantified

using the ND-2000 (NanoDrop Technologies). Only qualified RNA samples were used in constructing the cDNA library, which was sequenced on the Illumina HiSeq2000 sequencing platform from Shanghai Majorbio Biopharm Biotechnology (Shanghai, China). Samples were individually assessed for quality using FastQC. Raw transcriptome sequences were then filtered using fastp (ver. 0.23.1)[51], and high-quality (Phred scores of ≥20) sequences were retained for downstream analysis. High-quality sequences were then mapped onto the *H. armigera* reference genome (NCBI RefSeq GCF_023701775.1) using HISAT2 (ver. 2.2.1)[52]. Following the pipeline, mapped reads were counted using featureCounts (Subread package ver. 2.0.3)[53,54]. DESeq2 (ver. 1.38.3) was then used to normalize the gene counts and identify DEGs[55]. A false discovery rate (FDR) less than 0.05 and an absolute value of $\log_2$ Fold Change ≥ 1 were used as criteria for determining significant DEGs. Functional information describing *H. armigera* was downloaded from the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) databases, and the gene set collection was manually compiled. Normalized gene expressions and gene sets were analyzed for enrichment using Gene Set Enrichment Analysis (GSEA, ver. 4.3.2) in the default mode[56]. Significant results were retained for further analysis by filtering the GSEA results using |NES| > 1, NOM p-value < 0.05, and FDR q-value < 0.25. Core enrichment genes were then extracted from the results, and significantly differentially expressed enrichment genes were denoted "core DEGs" for subsequent downstream analysis. The raw RNA-seq data and raw count matrix were deposited at the NCBI SRA (BioProject accession: PRJNA1084744, SRA: SRR28238515 ~ SRR28238550).

Sample preparation for metabonomic analysis

Sample preparation for metabonomic and data analyses were performed by Shanghai Majorbio Biopharm Biotechnology (Shanghai, China) using standard procedures, with three biological replicates of each treatment analyzed for each treatment. Tissue (100 mg) from each sample was powdered in liquid nitrogen, resuspended in pre-chilled 80% methanol and 0.1% formic acid, and incubated on ice for 5 min before the tissue homogenate was centrifuged at 15,000 rpm for 5 min at 4 °C. The supernatant was then diluted with LC-MS-grade water to obtain a final concentration of 60% methanol and transferred through a 0.22 μm filter into a fresh Eppendorf tube. Samples were then centrifuged at 15,000 rpm for 5 min at 4 °C, and the supernatant was analyzed using the LC-MS/MS system. Raw data were processed using Progenesis QI (Waters Corporation, Milford, USA). Peaks were then filtered, and duplicates, internal standards, and known false positive peaks were removed before pooling.

The KEGG, Human Metabolome Database (HMDB, ver. 4.0), and Majorbio (https://cloud.majorbio.com/) database were utilized for metabolite annotation, which was preformed using the Majorbio cloud platform. Principal component analysis and orthogonal partial least squares discriminant analysis (OPLS-DA) were conducted using ropls (ver. 1.6.2), and metabolites with VIP (OPLS-DA) > 1, $P < 0.05$, and $|\log_2 \text{FoldChange}| > 0$ were considered significantly differential expressed metabolites. Metabolic information for *H. armigera* was obtained from the KEGG Compound database. KEGG functional enrichment analysis was conducted using the "enricher" function in the clusterProfiler R package (version 4.6.2)[57]. To establish a correlation between DEGs and metabolites, DEGs and differentially abundant metabolites were merged. KEGG enrichment analysis was then performed, and DEGs and differentially abundant metabolites in the same pathway were utilized to calculate the correlations and develop the network plot.

Integrated transcriptomic and metabonomic analysis of *Helicoverpa armigera* in response to predation risk

Transcriptomic sequencing analysis was used to determine the trade-offs between growth and defense in *H. armigera* and understand how these trade-offs are coordinated with behavioral changes under bat predation risk. This step aimed to reveal changes in the gene expression patterns of *H. armigera* under the various predation risks, providing insights into their responses at a molecular level. Metabonomic analysis was then performed to investigate alterations in the metabolite profiles of *H. armigera* under the different treatments, allowing a better understanding of how *H. armigera* adjusts its physiological processes at the metabolic level to cope with predation stress. Finally, by integrating the data from both transcriptomic and metabonomic analyses, a comprehensive picture of how *H. armigera* responds to predation risk across multiple biological levels was constructed. This integrated approach not only offers insights into the interactions between gene expression and metabolic changes but also aids in our understanding of how predation risk affects the physiology and behavior of *H. armigera*, elucidating how these responses are coordinated and integrated across different biological levels.

Statistics and reproducibility

In analyzing the developmental and trait responses of *H. armigera*, one-way ANOVA was employed to evaluate overall group differences, followed by Duncan's multiple range test to determine significant differences between groups. All data were expressed as mean ± SE. Three statistical analyses were performed in both transcriptomic and untargeted metabolomic. For transcriptomic analysis, adjusted P values (FDR correction) were used to ensure control over false discovery rates. In metabolomic analysis, fold-change values of metabolites were log-transformed to normalize the data and highlight differential changes. The mean values for transcriptomic and metabonomic data from each group were compared by unpaired one-tailed t test. In all tests, P values of less than 0.05 were considered statistically significant. Statistical analyses were performed using SPSS version 22.0 (IBM, Armonk, NY, USA).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The raw sequence data have been submitted to NCBI SRA (BioProject accession: PRJNA1084744, SRA: SRR28238515 ~ SRR28238550), the raw metabolomics data have been deposited in the MetaboLights public repository under accession code MTBLS9691. The numerical source data for all graphs presented in the manuscript are provided in Supplementary Data 1. Information on differentially expressed genes across various predation risks is available in Supplementary Data 2, while differentially expressed metabolites under these predation conditions are detailed in Supplementary Data 3. All other data are available from the corresponding author (or other sources, as applicable) on reasonable request.

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Author contributions

T.J. and Y.L. conceived and designed the research; Y.L., M.S., D.Z., H.Y., and Z.H. conducted experiments; Y.L. and Y.G. analyzed the data and prepared the manuscript; T.J. and J.F. reviewed the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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