Insect-Plant Interactions Reveal Spatiotemporal Dynamics of Biodiversity in the Qinling Mountains

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Abstract: In recent years, the biodiversity and ecological functions of mountainous regions have been severely impacted by anthropogenic disturbances and climate change, particularly concerning insect diversity. Large-scale surveys conducted in Europe and North America indicate that approximately 40% of insect species in temperate countries may face extinction within the next few decades. The relationship between plants and insects is notably intimate, encompassing critical ecological functions such as pollination and energy flow. Therefore, understanding the interactions between plant and insect diversity is crucial for the conservation of mountainous ecosystems. Most biodiversity research has focused on tropical and temperate regions, while transitional zones—especially warm temperate forests—remain underexplored, particularly in the Qinling Mountains. As a region that has not been directly impacted by Quaternary glacial advances, the Qinling Mountains harbor an ancient and enigmatic biodiversity, rendering it of significant research value. In this study, we conducted intensive sampling using malaise traps at the Qinling Huangguan sample plot over the course of a year, with weekly collections of insects. By integrating DNA metabarcoding techniques with deep learning model, we obtained dynamic spatiotemporal insights into insect diversity. Our findings yielded a total of 12,716 operational taxonomic units (OTUs) across 20 insect orders, revealing distribution patterns and influencing factors at different spatial and temporal scales. Importantly, we accurately predicted insect species using plant diversity data, thus capturing a comprehensive overview of total insect numbers and deeply explored the mutual relationship between insects and plants. Furthermore, through whole-genome sequencing, we acquired historical demographic information on herbivorous insects and their shared host plants, enhancing our understanding of the interactions between insects and plants. Overall, this study represents the first comprehensive analysis of the distribution patterns and influencing factors of insect diversity in the Qinling Mountains across spatiotemporal scales. Notably, we employed plant models to accurately estimate total insect numbers and analyzed population dynamics from a historical perspective, shedding light on the evolutionary relationships between insects and their host plants. These findings provide new insights into the interactions between insects and plants, establishing a scientific foundation and offering a fresh perspective for future efforts to monitor insect diversity effectively and conserve forest ecosystems.

Keywords: Insects’ diversity;Plants’ diversity;Plant model;Metabarcoding;Population history;Deeplearning model

The evolution and mechanisms of genome gigantism in Caelifera insects

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Abstract: Caelifera (Insecta: Orthoptera) exhibits great variation in genome size (0.97-21.48Gb). Massive expansion of transposable elements (TEs) has been found in gigantic genomes. The results indicate that the radiation evolution of Acridoidea was accompanied by genome gigantism, with frequent chromosomal rearrangements observed during the same period, leading to an increase in chromosome numbers (n=6 to n=12). Although TE insertions can increase genome plasticity, they are largely deleterious. In gigantic genomes, a rapid reduction in the number of C2H2-ZNF domains (KRAB-ZFP host silencing factors) and slower TE loss rates were observed. For the PIWI-clade evolution, small genomes are subject to stronger purifying selection compared to gigantized genomes. In gigantic genomes, the ultra-long introns (>200 kb) in PIWI-clade genes caused by numerous TE insertions may represent a mechanism for TE anti-silencing. Moreover, Ago3 mRNA in the gigantic genome is much longer than in the small genome, primarily due to the elongation of the 3′ UTR. The proportion of TE silencing is lower in species with gigantized genomes compared to those with smaller genomes. Our results reveal that the arms race between TE transcripts and piRNAs is not consistent across different TE classes, and TE silencing within the genome is also uneven. Overall, host silencing shields and regulates TE transposition activity, driving genetic innovation through coevolution.

Keywords: genome gigantism;adaptive radiation;chromosomal rearrangement;transposable elements;host silencing

Ultrastructural Characterization of Developmental Stages and Head Sensilla in Phortica okadai, Vector of Thelazia callipaeda

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Abstract: *Phortica okadai*, a vector of *Thelazia callipaeda*, is associated with an increasing incidence of thelaziasis. The complex habitat and chemosensory system of *P. okadai* are critical for its proliferation and expansion. However, ultrastructural data across developmental stages remain limited. This study used scanning electron microscopy to examine the ultrastructure of *P. okadai* developmental stages, with a focus on head sensilla. The results showed that the eggs of *P. okadai* are dark brown and cylindro-oval. The larvae are vermiform, divided into 11 segments. The pupae are marked by a conspicuous respiratory tubercle, and the posterior spiracle contains three distinct spiracular slits. Among five types of sensilla (trichoid, intermediate, chaetica, coeloconic, and basiconic), coeloconic, intermediate, and trichoid sensilla were predominantly found on the antennae, while basiconic and chaetica sensilla were distributed on both the antennae and the maxillary palps of *P. okadai*. The analysis revealed that the absence of dorsal appendages on the eggs distinguishes *P. okadai* from *D. melanogaster*. Males have longer antennae and exhibit sexual dimorphism in the length of sensilla (ChII, TB, and LB). This study provides the first comprehensive ultrastructural characterization of *P. okadai* developmental stages and head sensilla, laying a foundation for species identification and olfactory system research.

Keywords: Phortica okadai;Thelazia callipaeda; sensilla;ultrastructure;antennae;maxillary palps

HR3 modulates metamorphosis and reproduction in Aedes aegypti mosquitoes enabling sustainable control strategies

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Abstract: The mosquito *Aedes aegypti* is a primary vector for several arboviruses, including Zika, dengue fever, and yellow fever virus, posing significant public health challenges globally. The traditional strategies primarily rely on chemical insecticides, which led to increasing resistance in mosquito populations. Thus, it’s urgent for innovative control strategies. In this study, we elucidated the role of 20-hydroxyecdysone (20E)-regulated *nuclear hormone receptor 3* (*AaHR3*) in pupal metamorphosis and adult reproductive progress of *Ae. aegypti* via double-strand RNA (dsRNA) treatment. Additionally, *AaHR3* has been shown to regulate lipid metabolism, as evidenced by increased lipid droplet accumulation and triglyceride levels in mosquitoes treated with ds*HR3*, ultimately culminating in ovarian dysgenesis. Building upon these findings, we investigated the efficacy of *Chlamydomonas reinhardtii* algae and chitosan-dsRNA delivery systems targeting *AaHR3* as sustainable mosquito control strategies, which induced larval mortality and impairing reproduction. Finally, the combination between the transgenic algae with chitosan-dsRNA complexes was confirmed via scanning electron microscope for further forming a dual delivery system. These results suggest that RNA interference (RNAi) targeting *AaHR3* could be a promising strategy for sustainable mosquito control, offering promising strategies for curbing *Ae. aegypti* and related disease vectors.

Keywords: metamorphosis; reproduction;HR3;chitosan-dsRNA;transgenic algae

miR-7-GABA influences female Aedes aegypti reproduction by modulating midgut homeostasis

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Abstract: The robust reproductive plasticity of female Aedes aegypti mosquitoes following blood-feeding exacerbates the transmission of mosquito-borne viruses, imposing a heavy burden on global public health. The blood-feeding-initiated post-blood meal (PBM) phase activates coordinated changes in the midgut-fat body-ovary axis, storing nutrients for female reproductive behaviors. MicroRNAs (miRNAs) precisely control gene expression through a post-transcriptional regulatory mechanism that targets mRNAs. To explore the impact of endogenous miRNAs on the digestion kinetics of exogenous hemoglobin (HGB), this study combined digestive physiology assays, sRNA-seq, CRISPR-Cas9, RNA interference (RNAi), in vitro neurotransmitter delivery to the digestive tract, and molecular biology assays. The results demonstrated that the miR-7-glutamate decarboxylase (GAD)-γ-aminobutyric acid (GABA) signaling axis mediates the reproductive cascade of the midgut-fat body-ovary axis by regulating midgut homeostasis. Through high-throughput sRNA-seq combined with digestion rate measurements, we identified miR-7 as a key regulator of the HGB digestion rate. Dual-luciferase reporter gene assays and fluorescence in situ hybridization (FISH) co-localization experiments confirmed that GAD is a target gene of miR-7. CRISPR-Cas9-mediated miR-7 knockout combined with GAD RNAi rescue experiments demonstrated that miR-7 deficiency disrupts the midgut glutamate-GABA homeostasis by derepressing GAD. High levels of GABA inhibit the secretion of digestive proteases by promoting midgut cell apoptosis. This endocrine imbalance is directly manifested as delayed blood-meal processing, secondary to impaired lipid storage and arrested ovarian development, ultimately reducing reproductive fitness. The in vitro experiment of delivering GABA to the digestive tract further verified the tissue-specificity of the above-mentioned view. Our study identified an important miRNA that affects reproductive output by regulating digestion, providing key targets for interventions aimed at blocking the transmission of arboviruses.

Keywords: miR-7;glutamate decarboxylase;gamma-aminobutyric acid;digestion;reproduction

A set of novel venom proteins induce apoptosis-mediated degradation in Drosophila imaginal discs for successful parasitism

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Abstract: Parasitoid wasps are well-known for their varied parasitic strategies, and their diverse venom components are crucial for successful parasitism. However, the precise mechanisms by which these venoms function have remained unclear. In this study, we investigated the *Drosophila* parasitoid wasp, *Asobara japonica*, and found that its venom induces the apoptosis-mediated degradation of host imaginal discs. This process elevates *dilp8* expression, causing a delay in host development that enables *A. japonica* to successfully parasitize the hosts. We further identified five DUF4803-domain genes that are involved in mediating this process. These findings significantly advance our understanding of parasitic strategies and offer valuable insights for potential applications in biological control.

Keywords: Parasitoid wasps;Venom proteins;Imaginal disc-degradation;Apoptosis;Development delay

Neonicotinoid insecticide imidacloprid induces chemosensory deficits in a nontarget parasitoid wasp

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Abstract: Chemical pesticides are widely used to manage the population of arthropod pests. Their increasing use in agriculture has raised concerns about their harmful effects on nontarget organisms, particularly some beneficial insects such as parasitoid wasps. To assess the potential risk and ecological safety of chemical pesticides, it is necessary to understand their impacts on the physiology and behaviour of those important natural enemies of arthropod pests. Here, we applied the *Drosophila* parasitoid *Leptopilina drosophilae* as a study model to investigate the effects of sublethal doses of imidacloprid, a widely used neonicotinoid insecticide. Our results demonstrated the detrimental effects of imidacloprid on the host-searching behaviour of *L. drosophilae* females and the courtship behaviour of *L. drosophilae* males. Comparative transcriptome and functional analysis provided further insights into the potential mechanisms underlying the impaired behaviours, with the downregulated expression of certain chemoreception genes in both female and male exposed wasps. Our findings thus emphasize the importance of understanding the risks associated with the use of chemical pesticides and the need to develop more eco-friendly pest management strategies for a sustainable balance between chemical and biological control.

Keywords: Parasitoid wasps;Imidacloprid;Host-searching behaviour;Courtship behaviour;Comparative transcriptome;Chemoreception genes

Chromosome-level genome assembly of the Asian eusocial hornet, Vespa velutina

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Abstract: The Asian hornet (Vespa velutina) serves as a predator, pollinator, and seed disperser, with its foraging and social behaviors critically dependent on the sensitive detection of chemical cues. The lack of genomic resources has impeded a deeper understanding of the molecular mechanisms underlying chemosensory perception and behavioral regulation. In this study, we present a chromosome-level reference genome for V. velutina, integrating Oxford Nanopore and Hi-C sequencing technologies. We assembled the scaffolds into 25 chromosomes, resulting in a chromosome-level genome of 224.93 Mb with an N50 value of 9.2 Mb. A total of 72.56 Mb (31.24%) of repetitive sequences were identified, and we predicted 11,863 protein-coding genes. We characterized the OBP, CSP, IR, OR, and GR gene families associated with host selection and adaptation, providing valuable resources for understanding insect host adaptation during evolution. The high-quality reference genome of V. velutina also establishes a foundation for studying Hymenoptera phylogeny and chemical ecological interactions.

Keywords: Vespa velutina;genome assembly;Chromosome-level genome;Odorant receptor

Gut bacteria-derived succinate promotes histone H3 acetylation and crotonylation to drive the growth of a weevil pest

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Abstract: Gut bacteria have profound effects on animal hosts' physiology by shaping their gene transcriptional network. However, the molecular mechanisms by which gut bacteria chemically interacts with host cells to reprogram the gene expression profile remain mostly elusive. Red palm weevil (RPW), Rynchophorus ferrugineus Olivier, is a notorious palm trees pest worldwide and has caused great economic loss. Here, we found gut bacteria can produce a wealth of metabolites, such as fatty acids, vitamins, amino acids and their derivates. Furthermore, the supplementation of succinate to RPW germfree larvae recapitulated their growth by altering host nutritional status. More interestingly, we demonstrated that succinate supplementation dramatically promote histone H3 acetylation and crotonylation to enhance the expression of the vital IIS-related genes. Therefore, our findings suggest that the RPW gut bacteria-derived metabolite, succinate, can exert on epigenetic events at the histone level to modulate host physiology, firstly establishing the roles of microbiota-nutritional physiology-epigenetics axis in the crosstalk between gut bacteria and animal hosts.

Keywords: Gut microbiota; Epigenetics; Rhynchophorus ferrugineus

Biocontrol characteristics of the fly pupal parasitoid Pachycrepoideus vindemmiae (Hymenoptera: Pteromalidae) reared from alternative hosts

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Abstract: *Pachycrepoideus vindemmiae* is an important pupal ectoparasitoid of many agricultural and economic pests such as fruit flies, houseflies, phorid flies and *Bactrocera dorsalis*. In this study, we compared the parasitic characteristics of *P. vindemmiae* reared in *D. melanogaster* (Pvdm) with those reared in *D. virilis*(Pvdv). We found that Pvdv had a larger size than Pvdm. The longevity of both female and male Pvdv wasps was significantly longer than that of Pvdm. Pvdv also survived longer than Pvdm at high temperatures, such as 35°C, although they both survived well at low temperatures. Furthermore, Pvdv showed significantly higher parasitism and emergence rates against phorid flies and *B. dorsalis*. Four days post-emergence, the number of mature eggs in the ovaries was significantly greater in Pvdv than in Pvdm. When parasitizing *D. suzukii* individually, female Pvdv produced significantly more female and male offspring than Pvdm, and also possessed a higher number of mature eggs under continuous parasitism conditions. Overall, Pvdv had an advantage in terms of body size, reproductive capacity, stress resistance, and parasitism efficiency. In summary, *P. vindemmiae* from *D. virilis* could improve biocontrol efficacy, thereby reducing public health risks and agricultural economic losses.

Keywords: Pachycrepoideus vindemmiae; fly pest; biocontrol; alternative host;parasitism

Male-specific lethal-3 gene is critical for survival and fecundity in rice brown planthopper, Nilaparvata lugens

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Abstract: Male-specific lethal-3 (MSL3) is a key component of the Drosophila dosage compensation complex, yet its role in hemipteran insects remains uncharacterized. Here, we cloned and functionally analyzed the MSL3 homolog (NlMSL3) in the brown planthopper (Nilaparvata lugens), a major rice pest. NlMSL3 encodes a 488-aa protein with a conserved chromo-barrel domain and exhibits sex- and tissue-specific expression, peaking in the testes. RNAi-mediated silencing of NlMSL3 caused significant mortality in later instar nymphs and adults. In females, knockdown impaired feeding, reduced body weight and honeydew production, suppressed vitellogenin expression, and disrupted ovarian development. Mating experiments revealed that NlMSL3-deficient males were sterile despite normal mating behavior. These findings indicate that NlMSL3 is essential for both survival and reproductive fitness in N. lugens.

Keywords: Male-specific lethal-3;Nilaparvata lugens;mortality;fecundity

Comparative transcriptome analysis highlights the role of NlABCG14 in the honeydew production of virulent brown planthoppers (Nilaparvata lugens Stål) to resistant rice varieties

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Abstract: Brown planthoppers (BPHs, Nilaparvata lugens Stål) are a major threat to rice cultivation in Asia, necessitating the development of pest resistant varieties for effective management. However, the adaptability of BPHs has resulted in the development of virulent populations, such as biotype Y BPHs, which exhibit significant virulence against the rice variety YHY15 that harbors the resistance gene Bph15. The various response mechanisms of BPH populations to resistant rice varieties are critical yet underexplored. Via through RNA sequencing, the present study identified distinct transcriptional profiles in avirulent (biotype 1) and virulent (biotype Y) BPH nymphs both before and after feeding on YHY15 rice. Our findings revealed differential expression patterns of gene clusters involved in protein synthesis, hydrolysis, fatty acid biosynthesis, metabolism, cuticle composition, and translocation. Further analysis elucidated changes in the expression of genes associated with longevity and structural components of cuticles, highlighting specific disruptions in both biotype 1 and biotype Y BPHs. Moreover, the two biotypes showed differences in the expression level of genes involved in ATP-binding cassette (ABC) transporters. A functional assessment of ABC transporter genes revealed a role of NlABCG14 in the honeydew production of biotype Y BPHs to YHY15 rice, without impacting their survival and developmental dynamics. These insights deepen our understanding of the mechanisms of virulent BPHs response to resistant rice varieties and highlight potential targets for improving pest management strategies.

Keywords: brown planthopper;resistant rice;response mechanism;RNA sequencing;ABC transporters

NlAgo3 and NlBiwi are essential for piRNAs biogenesis and female reproduction in Nilaparvata lugens

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Abstract: P-element-induced wimpy testis (PIWI) -interacting RNAs (piRNAs) are short non-coding RNAs crucial for silencing transposable elements (TEs) and maintaining genome stability, particularly in germ cells. While most research on piRNAs and PIWI proteins has traditionally focused on model organisms such as *Drosophila*, emerging studies are now extending to other insect orders. However, little is known about piRNAs in insects of the Hemiptera order. Here, we investigated the role of piRNAs in the brown planthopper (BPH), *Nilaparvata lugens*, a significant pest of rice that belongs to the Hemiptera order. We identified and characterized two PIWI subfamily protein genes in *N. lugens*: *NlAgo3* (a homolog of Ago3) and *NlBiwi* (the BPH Piwi). Both proteins contain Piwi/Argonaute/Zwille (PAZ) and PIWI domains. Sequence alignment and phylogenetic analysis demonstrated that NlAgo3 and NlBiwi are conserved across other insect orders. *NlAgo3 and NlBiwi* are highly expressed in female adults and their ovaries in *N. lugens*. Importantly, knockdown of *NlAgo3 and NlBiwi* significantly impaired eggs laying, leading to female sterility, suggesting that these proteins play a crucial role in female reproduction in *N. lugens*. Subsequently, through small RNA sequencing, we characterized piRNAs in *N. lugens* and identified a length peak in the range of 26-28 nucleotides, with differences in piRNA abundance between females and males. Additionally, we observed that piRNA clusters were distributed across each chromosome, with a higher density on the sex chromosomes. Moreover, RNAi-mediated knockdown of *NlAgo3 and NlBiwi* severely impaired piRNA production in BPH, highlighting that these proteins are essential for piRNA biogenesis in *N. lugens*, consistent with findings in other species. Overall, this study provides valuable insights into the role of piRNAs in the reproductive biology of *N. lugens*, a major pest of rice. By clucidating the characteristics of piRNAs in this insect species, this research enhances our understanding of piRNA-mediated gene regulation in Hemiptera insects. These findings have potential implications for the development of novel strategies for pest control strategies.

Keywords: NlAgo3;NlBiwi;Nilaparvata lugens;female reproduction; piRNAs

Emamectin benzoate reduces parasitic ability in wasps by downregulating juvenile hormone titers

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Abstract: The widespread use of chemical insecticides in agriculture is a key factor in the decline of invertebrate survival, including non-target beneficial insects like parasitoid wasps. Emamectin benzoate (EB) is regarded as a highly effective and environmentally compatible pesticide, yet its ecotoxicological risks to parasitoid wasps remain poorly understood. Here, we assessed the toxicity of EB on the Drosophila parasitoid Leptopilina drosophiliae and found that sublethal exposure (LC5) significantly impaired parasitic ability over a 14-day post-treatment period. Transcriptomic analysis in L. drosophiliae revealed that exposure to EB dramatically downregulated the farnesol dehydrogenase (FOSDR) gene, a crucial enzyme in juvenile hormone (JH) biosynthesis. This transcriptional suppression resulted in measurable reductions in JH titers. Notably, supplementation with the JH analog methoprene partially rescued the parasitism rate in EB-exposed L. drosophiliae. We further identified the vital roles of the FOSDR in JH synthesis and wasp parasitic ability through RNA interference (RNAi). Overall, these findings demonstrate that EB reduces parasitic ability in wasps by downregulating juvenile hormone titers, providing novel mechanistic evidence of sublethal neuroendocrine disruption induced by chemical insecticides in parasitoids, thus emphasizing the importance of judicious chemical insecticide use for integrated pest management.

Keywords: Parasitoid wasps;Emamectin benzoate;Parasitism ability;Transcriptomic analysis;Farnesol dehydrogenase;Juvenile hormone;Methoprene

Mechanisms maintaining the host-symbiont specificity within the bacterial pouch in Tetraponera ants

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Abstract: Animal gastrointestinal tracts typically harbor diverse and abundant symbiotic microbes that benefit their hosts in multiple ways. While host specificity, where certain microbes are restricted to particular host species, is widespread across animal taxa, the underlying mechanisms remain poorly understood. Here, we chose two species of *Tetraponera* nigra-group ants, which have been reported to evolve a specialized symbiotic organ named the bacterial pouch and stably harbor *Tokpelaia* symbiotic bacteria in it, to understand how hosts selectively maintain specific *Tokpelaia* symbionts while excluding non-symbiotic bacteria. Our inoculation experiments and behavior observations revealed a critical developmental window lasting ~72 h for newly emerged workers to acquire symbionts through trophallaxis, after which bacteria couldn't enter the bacterial pouch. Comparative transcriptomics identified significant downregulation of the peptidoglycan recognition protein PGRP-SC2 in the bacterial pouch, suggesting its role in modulating the symbiotic population. Furthermore, *Tokpelaia* expressed numerous stress-response genes (e.g., antioxidant defenses). These findings support a co-adaptive model where host development, behavior, immune system, and symbiont stress resistance mechanisms jointly maintain partnership specificity. A critical future direction will be to characterize the differential immune responses triggered by symbiotic versus non-symbiotic bacteria during host feeding, which could reveal key mechanisms underlying symbiont specificity maintenance.

Keywords: Host specificity;Symbiotic organ;Tetraponera ants;Immune regulation;Stress resistance

A vertically transmitted Sodalis symbiont coordinates cuticle development in Tetraponera sp.5 ant via tyrosine provisioning

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Abstract: Mutualistic interactions between insects and microbial symbionts play essential roles in host development. In the polygynous ant Tetraponera sp.5, we identified a novel Sodalis-related Enterobacterales symbiont that is vertically transmitted through the queen’ s ovaries and stably colonizes the fat bodies of non-reproductive workers. Localization using fluorescence in situ hybridization (FISH) and tissue-specific qPCR confirmed widespread presence of this symbiont in queen ovarian tissues and worker fat bodies.

Amplicon sequencing revealed that gut microbial communities remain highly stable across developmental stages, with Enterobacterales dominating (>95% relative abundance in over 81% of individuals). Phylogenetic analyses positioned this strain on a distinct branch separate from insect- and environment-derived Enterobacterales, indicating a high degree of host specificity likely shaped by long-term co-evolution.

Quantitative profiling showed a stage-specific “tick-shaped” abundance pattern: lowest in larvae, increasing during pupation, and peaking in adults and eggs. This dynamic may reflect metabolic demands related to cuticle remodeling during metamorphosis.

Metagenomic analysis revealed an extremely reduced genome (~821 kb) with selective retention of biosynthetic pathways for two essential aromatic amino acids: phenylalanine and tyrosine. These amino acids serve as critical precursors for insect cuticle formation, including melanization and sclerotization processes.

Although complete symbiont elimination via high-temperature or antibiotic treatment was impeded by host lethality (e.g., 100% larval mortality at 35°C), partial depletion altered symbiont load in workers. While phenotypic consequences are still under investigation, we hypothesize that reductions in symbiont abundance may lead to cuticle defects, consistent with a proposed role in maintaining cuticle integrity.

Our findings unveil a vertically transmitted mutualist that likely contributes to host cuticle development via aromatic amino acid provisioning, potentially supporting the ecological adaptation and success of T. sp.5 ants.

Keywords: Symbiosis;Vertical transmission;Enterobacterales;Aromatic amino acids;Cuticle development;Ant microbiome

Cross-kingdom RNA interference mediated by insect salivary microRNAs may suppress plant immunity

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Abstract: Communication between insects and plants relies on the exchange of bioactive molecules that traverse the species interface. Although proteinic effectors have been extensively studied, our knowledge of other molecules involved in this process remains limited. In this study, we investigate the role of salivary microRNAs (miRNAs) from the rice planthopper *Nilaparvata lugens* in suppressing plant immunity. A total of three miRNAs were confirmed to be secreted into host plants during insect feeding. Notably, the sequence-conserved miR-7-5P is specifically expressed in the salivary glands of *N. lugens* and is secreted into saliva, distinguishing it significantly from homologues found in other insects. Silencing miR-7-5P negatively affects *N. lugens* feeding on rice plants, but not on artificial diets. The impaired feeding performance of miR-7-5P-silenced insects can be rescued by transgenic plants overexpressing miR-7-5P. Through target prediction and experimental testing, we demonstrate that miR-7-5P targets multiple plant genes, including the immune-associated bZIP transcription factor 43 (OsbZIP43). Infestation of rice plants by miR-7-5P-silenced insects leads to the increased expression of OsbZIP43, while the presence of miR-7-5P counteracts this upregulation effect. Furthermore, overexpressing OsbZIP43 confers plant resistance against insects which can be subverted by miR-7-5P. Our findings suggest a mechanism by which herbivorous insects have evolved salivary miRNAs to suppress plant immunity, expanding our understanding of cross-kingdom RNA interference between interacting organisms.

Keywords: miRNA;saliva effector;Brown Planthopper

Circulation of symbiont-mediate dsRNA in Honey Bees and Varroa destructor

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Abstract: *Varroa destructo*r is an ectoparasite *V. destructor* of honey bees feeding on their hemolymph and fat body. The honey bee colony barely survive the infestation without a chemical treatment. Previous study applied RNA interference (RNAi) technology to target key genes in *V. destructor* through symbiont mediated dsRNA (double-stranded RNA) , effectively inducing *V. destructor* mortality. To further elucidate the circulatory of the symbiont expressed dsRNA, we engineered the honey bee gut symbiont *Snodgrassella alvi* to express dsRNA targeting genes of *V. destructor* and *Nosema ceranae*. We traced the designated dsRNA by 29 primer pairs spanning gene regions which naturally not available in bees, *V. destructor*s, and *N. cerana*. We found most of the long dsRNA bands in the hindgut and midgut of honey bees, while corresponding fragments were detected in the hemolymph. The data suggest that dsRNA secreted in the hindgut move retrograde to the midgut. In the rectum and hemolymph, we only detected short dsRNA, indicating a degradation of dsRNA. In *V. destructor* , all dsRNA fragments were shorter than 150 bp, supporting the short dsRNA in the hemolymph. Collectively, the symbiont expressed dsRNA fragmented and circulate through the hemolymph. This study partially elucidated the circulatory pathways of dsRNA in honey bees and *V. destructor*, shedding light on the mechanistic role of dsRNA in RNAi. Furthermore, by tracking the distribution and transmission characteristics of dsRNA, we confirmed that dsRNA enters *V. destructor* in fragmented forms and executes its gene-silencing function.

Keywords: dsRNA;RNAi;PCR;electrophoresis

Nanoparticle improves the efficiency of RNAi targeting CPR in Spodoptera frugiperda

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Abstract: The fall armyworm (Spodoptera frugiperda), as a major agricultural pest in China, causes severe crop damage. Extensive reliance on chemical insecticides for its control has led to the development of resistance, necessitating the exploration of eco-friendly and sustainable pest management strategies. RNA interference (RNAi), a gene-silencing mechanism that is highly specific and environmentally safe, has been emerged as a powerful tool for pest control.

Keywords: Spodoptera frugiperda; RNA interference (RNAi); Nanopesticide ; CS-TPP-dsRNA; Bio-derived pesticides

Effects of host plants on population fitness of Amphitetranychus viennensis (Acari: Tetranychidae)

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Abstract: [Aim] To clarify the development, reproduction, and population growth parameters of Amphitetranychus viennensis feeding on leaves of four host plant species: peach (Prunus persica), begonia (Begonia smperflorens), apple (Malus domestica), and apricot (Prunus armeniaca), thereby providing a theoretical basis for its scientific control. [Methods] Under controlled laboratory conditions (25 ± 1 °C, 75 ± 5% RH, photoperiod 16L : 8D), the effects of host plant species on the developmental period, fecundity, and life table parameters of A. viennensis were investigated using the leaf disc method with life-table analysis. [Results] The total developmental duration was shortest when A. viennensis fed on begonia leaves (10.13 d), followed by apple leaves (10.63 d), and significantly longest on peach (11.44 d) and apricot leaves (11.64 d). Both the mean oviposition period and total fecundity were highest on begonia leaves (12.30 d and 27.90 eggs/female, respectively), and lowest on apricot leaves (8.00 d and 17.75 eggs/female) ($P < 0.05$). Furthermore, when feeding on apple leaves, the net reproductive rate (R_0 = 22.09) and intrinsic rate of increase (rm = 0.21) of A. viennensis were signAificantly higher than those observed on begonia (R_0 = 17.16, rm = 0.18), peach (R_0 = 15.86, rm = 0.16), and apricot leaves (R_0 = 11.82, rm = 0.13) ($P < 0.05$). [Conclusion] Significant differences exist in the population fitness of A. viennensis on the four host plants. Apple was identified as the most suitable host, while apricot was the least suitable. However, A. viennensis successfully completed their life cycle on all tested hosts. Therefore, establishing mixed or adjacent orchards of apple, peach, and begonia should be avoided in orchard planning. Integrated control of T. viennensis across different host plants should also be implemented simultaneously to effectively manage their populations.

Keywords: Amphitetranychus viennensis; host plants; growth and development; reproduction; population parameters

Volatile cues of enhanced attractiveness to Parapanteles hyposidrae (Wilkinson) wasps mediated by jasmonic and salicylic acid pathways synergism in tea plant

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Abstract: BACKGROUND: The jasmonic acid (JA) and salicylic acid (SA) pathways are often thought to interact antagonistically in plants when mediating anti-herbivore resistance. However, we previously found that the two pathways in tea plant interact synergistically when treated with 1.5 mmol/L methyl jasmonate (MeJA) and 20 mmol/L SA at 12 h intervals (MeJA+SA treatment). Here, we investigated how and why JA-SA synergism in tea plants affected chemotaxis of *Parapanteles hyposidrae* (Wilkinson) wasps, the parasite of tea geometers.

RESULTS: Wasp attractiveness of MeJA+SA-treated plants was 1.3-fold higher than MeJA-treated plants, although SA-treated plants could not attract more wasps. Five attractive compounds, including (*Z*)-3-hexenyl acetate, linalool, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), indole and (*E*,*E*)-α-farnesene, were emitted more by MeJA+SA-treated than MeJA-treated plants. Based on the difference in volatiles induced by MeJA and MeJA+SA treatments, five attractive components were separately added to artificial MeJA-induced volatiles (MV) or taken out from artificial MeJA+SA-induced volatiles (MSV). The attractiveness of MV to wasps was enhanced when DMNT or indole was added, whereas the attractiveness of MSV decreased when DMNT, indole or linalool was taken out. When DMNT and indole were both added into MV, the attractiveness of volatile blend to wasps became similar to MSV.

CONCLUSION: Increased DMNT and indole emissions are the key cues causing volatiles mediated by JA-SA synergism to be more attractive to P. hyposidrae wasps than those mediated by JA pathway alone. This provides new insights into the phytohormone pathway networks in tea plants and the development of natural enemy attractants.

Keywords: Behavioral bioassay;exogenous induction;jasmonic acid;methyl jasmonate;salicylic acid;tea plant volatile

Enhanced volatile emissions and anti-herbivore functions mediated by the synergism between jasmonic acid and salicylic acid pathways in tea plants

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Abstract: The interaction between jasmonic acid (JA) and salicylic acid (SA) pathways, which affects plant stress resistance, is mainly considered to be antagonistic. Using an established theoretical model, we investigated how tea plant (*Camellia sinensis*) volatiles induced by exogenous elicitors of JA and SA pathways are affected by the sequence of the elicitor application, the elicitor identity, and the applied concentrations. We also examined the effects of the volatiles mediated by the JA–SA synergistic interaction on the behaviors of a tea leaf-chewing herbivore (*Ectropis grisescens*) and its parasitic wasp (*Apanteles* sp.). The JA and SA pathway interactions were almost always reciprocally synergistic when the two pathways were elicited at different times, except at high JA elicitor concentrations. However, the JA pathway antagonized the SA pathway when they were elicited simultaneously. The elicitor identity affected the degree of the JA–SA interaction. The volatiles induced by the JA pathway in the JA–SA reciprocal synergism treatments included up to 11 additional compounds and the total amount of volatiles was up to 7.9-fold higher. Similarly, the amount of emitted volatiles induced by the SA pathway in the reciprocal synergism treatments increased by up to 4.2-fold. Compared with the volatiles induced by either pathway, the enriched volatiles induced by the JA–SA reciprocal synergism similarly repelled *E. grisescens*, but attracted *Apanteles* sp. more strongly. Thus, non-simultaneous activation is important for optimizing the JA–SA reciprocal synergism. This reciprocal synergism enables plants to induce multifarious responses, leading to increased biotic stress resistance.

Keywords: JA–SA interaction;exogenous elicitation;tea plant;volatiles;synergism;anti-herbivore function

Development of a High-Efficiency RNA Pesticide Emulsion Delivery System and Its Application in Controlling Hawthorn Spider Mite (Amphitetranychus viennensis)

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Abstract: A mineral oil-based water-in-oil (W/O) nanoemulsion was developed to enhance the environmental stability and insecticidal efficacy of dsRNA targeting Amphitetranychus viennensis. The optimized formulation (HLB = 10; oil:water:surfactant = 2:1:2) produced ~89 nm droplets and significantly improved dsRNA delivery. Bioassays showed that dsRNA@W/O reduced the LT_{50} of larvae from 4.87 to 1.96 days and adults from 4.82 to 2.65 days. Under UVB exposure, the formulation preserved dsRNA activity with only 1.34% loss, compared to 48.67% in naked dsRNA. Field trials confirmed high control efficacy (85.75% at 20 days), surpassing naked dsRNA treatments. This simple and scalable delivery system offers a promising tool for RNAi-based pest management.

Keywords: Emulsion;Hawthorn Spider Mite;mineral oil;RNAi

Transboundary migration of Spodoptera litura between China and the South-Southeast Asian region

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Abstract: The common cutworm, Spodoptera litura (F.), feeds on a wide variety of food and cash crops and is one of the most widespread and destructive agricultural pests worldwide. Migration is the biological basis of its regional population outbreaks but the seasonal movement pattern of this pest between east and south-southeast Asia regions remains unknown. Here, Long-term aerial population monitoring, ovarian dissection, trajectory simulation, pollen tracing, gut content analysis, night wind fields analysis, and the distribution of specific plants were utilized to analyze the migration patterns of this pest. The results showed that migratory activity could occur throughout the year, with the main periods found in spring (April–May) and autumn (October–December). The ovarian development and mating status of the trapped females indicated that most individuals were in the middle or late stages of migration and that Ruili City was located in the transit area of the long-distance migration of the pest

Keywords: Spodoptera litura;Migration pattern;Regional management

Multi-omics analyses provide molecular insights into the interactions between migration and reproduction of noctuid moths

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Abstract: The fall armyworm (FAW), *Spodoptera frugiperda*, is a significant invasive pest with robust migratory and reproductive capacities, posing a global threat to agricultural security. However, the molecular mechanisms and physiological adaptations of FAW migrants remain poorly understood. Here, we employed high-altitude trap lamps to capture FAW migrants along the Yunnan border, revealing that females at various ovarian developmental levels can undertake long-distance migration. Therefore, we classified these migrants as LOM (Low ovarian-level migrants) or HOM (High ovarian-level migrants) based on their ovarian development status. Migrants exhibit significantly larger body sizes than non-migratory individuals (Laboratory population). Notably, HOM exhibits higher reproductive capacity than LAP, whereas LOM shows lower fertility than LAP. Additionally, juvenile hormone metabolic pathway exhibits more pronounced differences between LOM and HOM than the circadian rhythm pathway does. Metabolome–transcriptome analysis reveals reduced energy metabolism activity in HOM relative to LOM, enabling the accumulation of energy metabolites to support extensive egg production after migration. Our findings elucidate distinct energy allocation strategies and hormone regulation mechanisms in migrants at different ovarian development stages, advancing our understanding of the trade-offs between migration and reproduction.

Keywords: Spodoptera frugiperda;Multi-omics;Migration and reproduction;Trade-offs

The evaluation on control potential using X-ray to irradiate adult Spodoptera frugiperda (Lepidoptera: Noctuidae)

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Abstract: BACKGROUND: The sterile insect technique (SIT), involving the rearing of larvae for pupation followed by irradiating pupae, is employed for environmentally friendly invasive pest management. Despite its effectiveness, the cost of pupae production poses challenges for small farmers in developing countries. Alternatively, utilizing insect trapping techniques can capture abundant adult insects in their natural habitats, but application potential of adult SIT approach remains unclear.
RESULTS: We used the invasive pest *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to assess the effectiveness of X-ray irradiated moths on their sterility rates, mating competitiveness, flight ability, and larval control efficiency in the field. Our findings revealed that optimal X-ray doses for 1, 3, and 5-day-old adult *S. frugiperda* were 154, 173, and 180 Gy, respectively. These doses rendered males more than 80.0% infertile and females nearly completely infertile. Significantly, the flight ability of sterilized males remained unaffected by sub-sterilizing doses of irradiation. Furthermore, in a release ratio of 16:1:1 (irradiated males: unirradiated males: unirradiated females), irradiated males exhibited the highest mating competitiveness (0.79). In the field-cage experiments, the corrected leaf protection rate and the corrected population decline rate in the 16:1:1 release plot were 60.50% and 74.21%, respectively.
CONCLUSION: These findings suggest that radiation-induced sterility in adult *S. frugiperda* holds promise for practical applications, offering a conceptual framework and novel approaches for advancing radiation-based pest control technology.

Keywords: Spodoptera frugiperda; sterile insect technique (SIT); sub-sterilizing dose; flight ability; mating competitiveness; control effect

Integrated Management of Lepidopteran Pests in China: Bt Maize Control Efficacy and Synergistic Strategies

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Abstract: The invasive fall armyworm (FAW), *Spodoptera frugiperda*, and other lepidopteran pests such as *Agrotis ypsilon* pose significant threats to maize production in China. Chemical insecticides and Bt maize are the main control means, but the interaction between these two measures is also unclear. This study evaluates the efficacy of Bt maize expressing Cry1Ab (DBN9936), Vip3Aa (DBN9501), and their pyramided forms (DBN3601T and Bt11×MIR162) against these pests and explores synergistic strategies combining Bt maize with chemical insecticides for integrated pest management (IPM). Bioassays revealed that the susceptibility of FAW populations to different Bt maize proteins ranks as follows: Vip3Aa > Cry1Ab+Vip3Aa > Cry1Ab. Similarly, *A. ypsilon* showed higher sensitivity to Vip3Aa, with corrected mortalities exceeding 89.65% for larvae feeding on Vip3Aa-expressing maize, while Cry1Ab exhibited lower efficacy (16.46–76.13%). Behavioral tests indicated moths preferentially oviposited on undamaged Bt plants, suggesting potential for Bt maize as a trap crop. Further investigations highlighted synergistic effects between Bt maize and chemical insecticides (Emamectin benzoate and Chlorantraniliprole). Bt proteins inhibited detoxification enzymes (CarE, GSTs and MFO), resulting in an increase in the sensitivity of the FAW to insecticides. Field trials showed that combining pyramided Bt maize with low-dose EB or CAP increased control efficacy by 16.74% and 19.62%, respectively. These findings underscore the potential of pyramided Bt maize as a cornerstone for IPM, complemented by targeted insecticide use to delay resistance and enhance pest suppression. The study provides a scientific basis for optimizing Bt maize deployment and integrating chemical controls to sustainably manage lepidopteran pests in China.

Keywords: Lepidopteran pests;Bt maize;Control efficacy;Synergistic effect

Establishment of pupal color as a screening marker and activity analysis of six U6 promoters in Zeugodacus cucurbitae using white pupae gene

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Abstract: BACKGROUND: The genetic control method, which is environmentally friendly and species-specific, has effectively reduced or eliminated pests in many areas. One essential requirement to control a species is the identification of its genetic and molecular elements. Such elements, however, are rarely available in Zeugodacus cucurbitae, a very destructive insect pest worldwide.

RESULTS: In this study, we knocked out white pupae (wp) gene in Z. cucurbitae and generated a wp-strain, which has white pupae phenotype. And the white puparium color was successfully restored to brown by inserting the wp gene rescue allele into the genome of the wp- strain using piggyBac transgenic technology. The potential wp promoter were then truncated to drive the expression of the wp gene and the puparium color was rescued even using the 605 bp sequence upstream of its transcription initiation site. Further fertility test experiments showed that knocking out or rescuing the wp gene has no effect on the reproduction of adult flies. In addition, we identified six U6 promoters and tested their promoter activities in embryos of Z. cucurbitae. The ZcU6-2 and ZcU6-1 promoters exhibited significantly higher activity than others and are suitable for use in CRISPR technology-based genetic control methods.

CONCLUSION: Our work first shows the success of applying piggyBac transgenic technology in Z. cucurbitae. Our results demonstrate a highly efficient transgenic screening marker by puparium color and the promoter activity of multiple ZcU6 promoters, facilitating the construction of transgenic strains that are used for genetic control of tephritid species.

Keywords: Zeugodacus cucurbitae;genetic control;genetic screening marker;white pupae;transgenic technology;U6 promoter

Micro-CT data of complete metamorphosis process in Harmonia axyridis

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Abstract: Insect metamorphosis involves significant changes in insect internal structure and is thus a critical focus of entomological research. Investigating the morphological transformation of internal structures is vital to understanding the origins of adult insect organs. Beetles are among the most species-rich groups in insects, but the development and transformation of their internal organs have yet to be systematically documented. In this study, we have acquired a comprehensive dataset that includes 27 detailed whole-body tomographic image sets of *Harmonia axyridis*, spanning from the prepupal to the pupal stages. Utilizing this data, we have created intricate 3D models of key internal organs, encompassing the brain, ventral nerve cord, digestive and excretion systems, as well as the body wall muscles. These data documented the transformation process of these critical organs and correlations between the origin of adult and larval organs and can be used to enhance the understanding of holometabolous adult organ genesis and offers a valuable reference model for investigating complete metamorphosis in insects.

Keywords: insect 3D models;Micro-CT;Harmonia axyridis;Insect metamorphosis

Manipulation of host-plant preference by virus-induced changes to its insect vector’s olfactory system

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Abstract: Plant viruses are known to indirectly manipulate insect vector behavior by altering host-plant phenotypes, yet the mechanisms by which they directly regulate vector behavior to enhance transmission remain poorly understood. Here, we reveal how the southern rice black-streaked dwarf virus (SRBSDV) reprograms the host preference of its planthopper vector, *Sogatella furcifera*, from infected to healthy rice plants by disrupting immune-olfactory crosstalk. We demonstrate that the SRBSDV-encoded P8 protein competitively binds to the *S. furcifera* Pelle kinase, a core component of the Toll signaling pathway, thereby inhibiting the phosphorylation and nuclear translocation of the transcription factor Dorsal-related immunity factor (DIF). This immune suppression downregulates the *E*-β-farnesene-responsive odorant receptor Or86 while upregulating the β-caryophyllene-responsive receptor Or127, shifting the olfactory preference of virus-carrying planthoppers toward uninfected plants. Knocking down Or86 or Or127 abolished this behavioral shift, reducing viral transmission rates in both plants and vectors. Our findings uncover a tripartite mechanism wherein a viral effector co-opts conserved immune signaling to rewire chemosensory perception in insect vectors, promoting virus spread. This work advances insight into the molecular arms race between viruses and vectors, offering novel targets for disrupting viral transmission in agricultural ecosystems.

Keywords: Sogatella furcifera;Southern rice black-streaked dwarf virus;Olfactory

DNA methylation modulates jasmonate signaling to govern direct and indirect defenses against Phthorimaea operculella in Solanum tuberosum

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Abstract: DNA methylation serves as a pivotal epigenetic mechanism in plant defense responses, yet its molecular role in insect resistance remains poorly understood. Here, we induced genome-wide DNA hypomethylation in potato plants using the DNA methyltransferase inhibitor 5-azacytidine (5-azaC) to investigate its effects on resistance against the *Phthorimaea operculella*. Foliar application of 100 μM 5-azaC significantly reduced global DNA methylation levels. Hypomethylated plants exhibited compromised resistance phenotypes: larvae fed treated leaves showed accelerated weight gain with enhanced feeding preference, while PTM adults displayed increased oviposition preference toward hypomethylated plants. This dual resistance impairment was associated with substantial reductions in trypsin protease inhibitor (TPI) content and diminished emission of β-caryophyllene, a key anti-herbivore volatile, accompanied by transcriptional downregulation of their biosynthetic pathway genes. Subsequent analysis revealed significantly decreased jasmonic acid (JA) levels and suppressed expression of JA biosynthesis genes in hypomethylated plants. Given the established regulatory role of JA signaling in both TPI production and β-caryophyllene synthesis, methyl jasmonate (MeJA) complementation experiments were conducted. Exogenous MeJA application restored PTM resistance by rescuing the accumulation of TPI and β-caryophyllene, demonstrating that genome-wide DNA hypomethylation compromises JA-dependent dual defense mechanisms against PTM. Our findings establish that DNA methylation dynamics govern both direct (TPI-mediated) and indirect (volatile-mediated) JA-dependent defenses in potato, providing a molecular foundation for epigenetic strategies to enhance crop resistance against insect herbivores.

Keywords: DNA methylation;jasmonate;Phthorimaea operculella;Solanum tuberosum

HaβGRP4 regulates larval growth in Helicoverpa armigera by modulating gut bacterial homeostasis

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Abstract: The gut microbiota is essential for insect growth and development, particularly during the larval feeding stage. However, the immune mechanisms that maintain microbial homeostasis in insects remain poorly understood. Here, we identify HaβGRP4, a β-1,3-glucan-binding protein gene highly expressed in the midgut of *Helicoverpa armigera* larvae, as a key regulator of gut microbiota balance. HaβGRP4 encodes a protein with glucanase activity that facilitates the degradation of fungal β-1,3-glucans. Silencing HaβGRP4 elevates antimicrobial peptide (AMP) expression, reduces the abundance of beneficial Acinetobacter, and lowers levels of the tryptophan metabolite indole-3-carboxaldehyde (3-IAld). This downregulates protocadherin expression, compromises intestinal integrity, and increases gut permeability, allowing microbial components to enter the hemocoel and trigger systemic immune responses. The resulting immune overactivation leads to stunted growth and delayed pupation.
These findings demonstrate that HaβGRP4 is essential for maintaining gut microbial homeostasis and normal larval development in *H. armigera*, highlighting a novel link between immune recognition, microbiota regulation, and developmental processes in insects.

Keywords: β-1;3-glucan-binding protein; microbiota; homeostasis; development

Low-Temperature Phosphine Fumigation Is Effective Against Drosophila suzukii in Sweet Cherry

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Abstract: *Drosophila suzukii*, classified as a quarantine pest in some countries, poses a serious threat to global trade due to its ability to damage berries and cherries. Recent studies indicate that low-temperature phosphine (PH_3) fumigation effectively controls this pest without compromising fresh produce quality. However, the specific protocol for managing *D. suzukii* larvae in cherries using this method remains underexplored. This study evaluates the efficacy of low-temperature PH_3 fumigation in controlling *D. suzukii* larvae across three cherry varieties at 3 °C and investigates potential effects on key quality metrics. Tolerance assessments revealed that 3rd instar larvae exhibit the highest resistance to PH_3 among all developmental stages. A concentration of 800 mL/m^3 PH_3 for 84 h at 3 °C achieved phytosanitary efficacy for 0.99997 with no negative effect on the quality attributes of the tested cherry varieties. These results support low-temperature PH_3 fumigation as a viable postharvest treatment for D. suzukii management in sweet cherries.

Keywords: toxicity; large-scale verification; postharvest quality; efficacy

Single-cell transcriptome maps of hemocytes in the butterfly Pieris rapae and their response mechanisms to parasitization

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Abstract: Parasitoid wasps significantly modulate hemocyte-mediated immune defenses in host insects, yet their regulatory mechanisms governing dynamic changes in host hemocyte types and molecular interactions remain undefined. Here, we performed single-cell RNA sequencing (scRNA-seq) of hemocytes in *Pieris rapae* pupae to comprehensively identify the hemocyte subsets and determine specific molecular and cellular characteristics in each hemocyte subset before and after parasitization by *Pteromalus puparum*, a natural enemy of Lepidoptera on vegetable pests. Comparative analysis identified 6 hemocyte types (8,389 cells total) with 14 functional subtypes in controls, including granulocytes, plasmatocytes, oenocytoids, spherulocytes, prohemocytes and coagulocytes. In parasitized hosts, only 3 types (2,561 cells total) with 6 subtypes were retained: plasmatocytes, granulocytes, and prohemocytes. We also proposed marker genes for these cell types. Interestingly, parasitization caused great changes in the distribution of hemocyte types. Plasmatocytes substantially increased, granulocytes markedly decreased, prohemocytes increased, while oenocytoids, coagulocytes, and spherulocytes were nearly depleted. Furthermore, functional analysis revealed that encapsulation-associated subtypes (granulocytes gr2, gr3 and plasmatocyte pl3) were reduced, suggesting immune exhaustion. Catabolic granulocytes (gr5 and gr6) expanded and showed enrichment in catabolic process and vesicular transport pathways, potentially accelerating foreign substance clearance. Plasmatocyte subtype pl1 (melanization-associated) and prohemocytes (proliferation-associated) increased, indicating compensatory immune mechanisms. In summary, we constructed the first single-cell transcriptomic atlas of *P. rapae* hemocytes, revealing their diversity characteristics and observing that parasitization significantly alters hemocyte type distribution. This work provides a deeper understanding of *P. rapae* hemocyte functionality and their response mechanisms to parasitization.

Keywords: Pieris rapae;Parasitoid wasp;Pteromalus puparum;scRNA-seq;Hemocyte;Cellular immunity

A single-nucleus transcriptomic atlas of the venom gland in the parasitoid wasp Pteromalus puparum

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Abstract: The venom gland is a remarkable organ of the parasitoid wasps, which produces venom that controls the host and is important for the survival and reproduction of the parasitoid. But little is known about how venom gland cells precisely regulate venom protein synthesis. In this study, we used single nucleus RNA sequencing (snRNA-seq) to construct the cell atlas of the venom gland and ovary of the *Pteromalus puparum*, a natural enemy of vegetable pests. The cells in the venom gland were clustered into six cell subsets, and the main cell types in the ovary were terminal filament, follicle cells, germline cells, nurse cells, muscle sheath cells and oviduct cells. The cellular function of venom gland clusters named VgC1 to VgC6 was enriched in the production and secretion of venom components, confirming that they are specialized secretory cells, except that VgC5 is similar to oviduct cells with indicated function of protein transport. In addition, the expression pattern of identified venom genes that 77 genes expressed across all six VgCs and 46 genes in five VgCs, demonstrated that most venom genes are widely expressed in VgCs, while specific cell types synthesize certain specialized venom proteins. Finally, WGCNA analysis of venom gland cells showed a set of co-expressed venom and non-venom genes consisting of putative regulators of venom production in *P. puparum*. By predicting binding sites for transcription factors (TFs) within the venom modules, ten TF families, including TFs like AP-1, sox9b, E2F3, SHR2 and EHF, existed potential regulatory relationships with venom genes. Taken together, this work reveals the heterogeneity of venom gland cells, the expression patterns of venom genes and regulatory role of TFs in *P. puparum*, leading to a deeper understanding of the venom-producing organs at the single-cell level, providing key data for future studies of venom system evolution in animals.
Keywords: parasitoid wasp;Pteromalus puparum;snRNA-seq;venom gland;secretory cell;venom gene

Genome-wide identification and analysis of the odorant-binding protein gene family in the Pteromalus puparum (Hymenoptera: Pteromalidae)

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Abstract: Odorant-binding proteins (OBPs) serve as key transporters of odor molecules and play a vital role in host location for insects. *Pteromalus puparum* (Hymenoptera: Pteromalidae) is a dominant pupal parasitoid of *Pieris rapae* (Lepidoptera: Pieridae), a major global pest of cruciferous vegetables. Despite the crucial functional roles of OBPs, the expression patterns and mechanisms of action in *P. puparum* remain incompletely understood. Based on homology searches (BLAST and HMMER), we identified 52 OBPs in the *P. puparum* genome. The chromosomal location showed that these OBPs were widely distributed across 12 scaffolds. Phylogenetic analysis was constructed to infer the evolutionary relationships among these OBP genes within hymenopterans. Analysis of gene and protein structural characteristics demonstrated substantial diversity within the OBP gene family. Furthermore, transcriptomic data analysis revealed differential expression of OBP genes, with the majority highly expressed in olfactory sensilla-rich tissues like the head, suggesting their importance in olfactory recognition during host-seeking and mating. Notably, only *Ppup173360.1* exhibited high expression in the venom gland. This tissue-specific expression pattern was confirmed by real-time quantitative PCR, indicating its potential role in modulating host immune responses. This study provides a systematic, genome-wide identification of the OBP gene family in *P. puparum*, integrating transcriptomic data. We analyzed the structural features, evolutionary relationships, and expression patterns of OBP family members, establishing a foundation for future functional studies on olfactory-related proteins in this parasitoid wasp.

Keywords: Pteromalus puparum; odorant-binding proteins; genome-wide identification; phylogeny; gene characteristics; expression profile

Energy reserve allocation in the trade-off between migration and reproduction in fall armyworm

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Abstract: Striking a trade-off between migration and reproduction becomes imperative during long-range migration to ensure proper energy allocation. However, the mechanisms involved in this trade-off remain poorly understood. Here, we used a takeoff assay to distinguish migratory from non-migratory individuals in the fall armyworm, a major migratory insect worldwide. Migratory females displayed delayed ovarian development and flew further and faster than non-migratory females during tethered flight. Transcriptome analyses demonstrated enrichment of fatty acid genes across successive levels of ovarian development and different migratory behaviors. Additionally, genes with roles in phototransduction and carbohydrate digestion along with absorption function were enriched in migratory females. Consistent with this, we identified increased abdominal lipids in migratory females that were mobilized to supply energy to the flight muscles in the thorax. Our study reveals that fall armyworm face a trade-off in allocating abdominal triglycerides between migration and reproduction during flight. The findings provide valuable insights for future research on this trade-off and highlight the key energy components involved in this strategic balance.

Keywords: migration and reproduction; transcriptomics; Spodoptera frugiperda; energy reallocation; flight capacity

The evolution of genome size in different life-forms of Acridoidea insects and its correlation with morphological traits

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Abstract: To elucidate the evolutionary patterns of genome size (GS, measured as C-value) within Acridoidea (grasshoppers) and its potential correlations with life form, body size (body length and mass), flight capacity (relative wing length and relative flight muscle mass), and reproductive investment (gonadosomatic index), we conducted comparative statistical analyses of GS and morphological trait data across different life forms. We reconstructed a robust phylogeny of Acridoidea based on mitochondrial genomes. Using this phylogeny, we performed evolutionary analyses of GS and morphological traits, estimated divergence times, and reconstructed ancestral states to infer the evolutionary history and trajectory of GS in these insects. Our results revealed significant variation in GS among Acridoidea lineages. Female genomes were significantly larger than those of males ($p < 0.05$). While GS exhibited a positive correlation with body size in both sexes ($r > 0$), its relationships with flight capacity and reproductive investment varied across lineages and showed no consistent evolutionary correlation. Life form did not exert a significant influence on GS evolution. Both GS and morphological traits displayed significant phylogenetic signal. Furthermore, GS and flight capacity demonstrated significant phylogenetic dependence ($p < 0.005$). Throughout their evolutionary history (spanning approximately 91.37 million years), Acridoidea experienced multiple independent episodes of genome size expansion and contraction, although the overall trend was towards genome enlargement. This study provides fundamental insights into the evolutionary dynamics of genome size within Orthoptera. Our findings establish a crucial foundation for future genomic research in this ecologically and economically significant insect order.

Keywords: Insect;Genome size;Evolution;Acridoidea

The odorant receptor co-receptor gene contributes to host preference behaviors in Meteorus pulchricornis

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Abstract: Background: Successful host recognition behaviors of parasitoid wasps are important for biological control efficiency and are often depend on a highly sensitive olfactory system. Central to this olfactory molecular mechanism is the odorant receptor co-receptor (ORco), which is essential for the detection of environmental chemical cues. However, the specific function of ORco in mediating host recognition and preference behaviors in parasitoid wasps remains poorly characterized.

Results: Genomic and transcriptomic analyses revealed that MpulORco is predominantly expressed in the antennae, suggesting its pivotal role in olfactory perception. Host preference test revealed that M. pulchricornis exhibits a strong preference for S. frugiperda, with this behavior being genetically ingrained rather than learned. Furthermore, comparative fitness analyses indicated that S. frugiperda-reared wasps exhibited superior biological performance, evidenced by significantly greater body mass, larger body size, and enhanced fecundity compared to those reared on alternative hosts. The marked decline in parasitism efficiency following MpulORco knockdown underscore volatile organic compounds (VOCs) mediated host discrimination.

Conclusion: Our study established the essential role of MpulORco in host recognition, expanding our knowledge of chemoreception in parasitoid wasps and paving the way for innovative pest management approaches.

Keywords: parasitoid wasps;odorant receptor co-receptor (Orco);RNA interference; biological control;behavior assays

Roles of Volatile Terpenoid DMNT in the Interaction among Cowpea, Leafminer, and Parasitoid Wasp

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Abstract: Plants release herbivore-induced plant volatiles (HIPVs) after attack by herbivores, which can repel pests or attract natural enemies. However, the responses of insects to the volatiles of cowpea (*Vigna unguiculata* L.) remain insufficiently understood. Here, we demonstrated that the leafminer *Liriomyza sativae* shows a preference for healthy cowpeas, whereas the parasitoid wasp *Hemiptarsenus varicornis* is more attracted to infested cowpeas. Through gas chromatography-mass spectrometry (GC-MS) analysis and Y-tube olfactometer assays on healthy and herbivore-damaged cowpeas, we identified the substance responsible for these behaviors: (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT). Transcriptome analysis, combined with prokaryotic expression and enzyme activity assays, confirmed that VuNES catalyzes the conversion of farnesyl pyrophosphate (FPP) to nerolidol, the precursor of DMNT in cowpeas. Spraying healthy cowpeas with methyl jasmonate (MeJA) resulted in increased *VuNES* expression and elevated DMNT content. Exogenous supplementation of DMNT to healthy cowpeas activated the jasmonic acid (JA)-related synthesis pathway, leading to increased levels of JA and jasmonoyl-isoleucine (JA-Ile). This study revealed that when cowpeas are infested by leafminers, they enhance JA levels, which stimulates the upregulation of *VuNES* expression, induces the production of nerolidol, and thereby promotes the massive synthesis of DMNT. Meanwhile, the large-scale release of DMNT can induce neighboring healthy cowpeas to preemptively activate defense responses. The results of this study illustrate the effects of terpenoid in cowpeas on the behaviors of pests and their natural enemies, and also lay a foundation for regulating terpenoid biosynthesis for application in agricultural pest control.

Keywords: Leafminer;HIPVs;DMNT;Cowpea ;Parasitoid wasp

Transcription factor, CncC and Maf regulate the UDP family to participate in the insecticide detoxification of Locusta migratoria

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Abstract: The transcription factors CncC and LmMaf play pivotal roles in regulating metabolic detoxification enzyme systems in *Locusta migratoria*, a globally destructive agricultural pest species. Imidacloprid has been established as an effective insecticide for locust control.

In this study, we employed RNA interference (RNAi)-mediated silencing of *LmCncC* and *LmMaf* coupled with insecticide bioassays, which revealed significantly increased susceptibility to both deltamethrin and imidacloprid in treated locusts. Comparative transcriptomic profiling following gene knockdown identified four downstream *UDP-glycosyltransferase* (*UGT*) genes under transcriptional regulation, among which *LmUGT392C1* showed predominant midgut-specific expression in third-instar nymphs. RNAi targeting *LmUGT392C1* substantially enhanced nymphal sensitivity to imidacloprid, suggesting its functional involvement in detoxification. Dual-luciferase reporter assays confirmed that the CncC/Maf heterodimer potently activates the *LmUGT392C1* promoter. CRISPR/Cas9-mediated generation of LmCncC and LmUGT392C1 knockout mutants consistently exhibited hypersensitive phenotypes to imidacloprid, providing genetic evidence for their roles in insecticide metabolism.

Our findings elucidate a regulatory cascade wherein *LmCncC*/*LmMaf* modulates *LmUGT392C1*-mediated detoxification pathways, offering mechanistic insights for developing sustainable pest management strategies against *L. migratoria*.

Keywords: LmCncC;LmMaf;UDP-glycosyltransferase;Locust migratoria;Imidacloprid

Structural Diversity and Function of Phosphosphingolipids in Insects

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Abstract: Phosphosphingolipids (PSLs) are a specialized group of lipids essential to the composition of eukaryotic cell. In previous studies, we found that there may be significant differences in the composition of sphosphingolipids among different insects. Flies contains ceramide phosphoethanolamine (CPE) as the major sphingolipid, whereas we did not detect a large amount of CPE in rice planthopper; and sphingomyelin (SM) is present in its place. In this study, Analysis of the composition of sphingolipids in 19 insect species by LC-MS and phylogenetic analysis of SM synthase (SMS) and CPE synthase (CPES). We described the differences in phosphosphingolipid composition and their synthases among different insects at the class Insecta level, and can classify insects into three major categories: the first category is hemipteran insects lacking CPES, with very low CPE content; the second category is dipteran flies lacking SMS2, thus containing no SM; and the third category includes most insects that possess both SMS2 and CPES, with substantial amounts of SM and CPE. We further focused on Tribolium castaneum, which possesses both SM and CPE, knock down SMS and CPES respectively by RNAi. The results indicated that SMS and CPES may exert similar effects on insect oviposition and have a synergistic regulatory role. Our research enriches the studies on insect sphingolipids and also provides potential targets for green pest control.

Keywords: Sphingolipids;Insects;Ceramide phosphoethanolamine;Sphingomyelin

Providing aged parasitoids can enhance the mass-rearing efficiency of Telenomus remus, a dominant egg parasitoid of Spodoptera frugiperda, on Spodoptera litura eggs

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Abstract: *Telenomus remus* (Nixon) is an egg parasitoid of several *Spodoptera* spp. insects, especially the destructive agricultural pest *Spodoptera frugiperda* (J. E. Smith). Studies showed that this parasitoid can be efficiently reared on *Spodoptera litura* (Fabricius) eggs. Understanding the relationship among parasitoid age, host egg age, and parasitism efficiency is an important part of mass-rearing biological control agents. To this end, we measured the impacts of female *T. remus* age (1-, 2-, 3-, and 4-day-old), *S. litura* egg age (1-, 2-, and 3-day-old), and their interactions on parasitism capacity, developmental time, offspring fitness, and oviposition behavior. Results indicated that the 3-day-old and 4-day-old parasitoids had higher parasitism performance on all age eggs than 1-day-old and 2-day-old parasitoids, especially on 3-day-old eggs. The number of parasitized eggs decreased as egg age increased, and the developmental time of the progeny increased. The emergence rate and percentage of females were hardly affected. For oviposition behavior, 4-day-old parasitoids showed the same drumming and oviposition time on different age eggs, while for others the drumming, drilling, oviposition, and total time increased with increasing *S. litura* egg age. In summary, the optimal combinations were 3-day-old or 4-day-old female parasitoids and 1-day-old or 2-day-old *S. litura* eggs. The findings presented in this study can be employed to enhance *T. remus* mass-rearing efficiency and availability.

Keywords: Biological control;Fall armyworm;Host egg age;Parasitoid age;Mass-rearing efficiency;Oviposition behavior

Serial amber fossils unveiling the innovation process of mouthparts in water boatmen (Hemiptera: Corixoidea) in the phylogenetic context of total evidence

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Abstract: Insects are the most species-rich group of animals, and the high morphological complexity and spectacular diversification of mouthparts among insects are crucial to their prosperity. Currently, the knowledge on insect mouthpart types remains largely stationary, yet few evidence has been documented to illustrate the transitional forms. In this study, we report for the first time a series of fossil specimens which reveal the successive process between two types of insect mouthparts. Correspondingly, three new families including six new species are established based on such mid-Cretaceous Kachin ambers to place these new fossil records. Starting from the morphological differences in the mouthparts of the newly defined families and the extant water boatmen families, we further deduce the structural homology between different mouthpart types and find the coevolution between the mouthparts and the forelegs in the phylogenetic context of total evidence. Our discovery provides direct evidence of the transformation process of the water boatmen’s mouthparts from sucking to triangular type. This study provides new evidence and perspectives for in-depth understanding of the evolutionary process of insect mouthparts.

Keywords: Coevolution; Phylogeny;Aquatic bugs; Kachin amber

Coordinated Metabolomic and Microbiome Responses of Camellia sinensis var. assamica cv. Hainan Dayezhong to Empoasca onukii Feeding and Their Impacts on Black Tea Flavor

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Abstract: The tea green leafhopper (*Empoasca onukii*) is a major piercing-sucking pest in southern China's tea plantations. Its feeding behavior can induce profound physiological changes in tea plants, including metabolic reprogramming and reshaping of the microbial community, which ultimately affect tea quality. In this study, we investigated the multi-level responses of *Camellia sinensis* var. assamica cv. Hainan Dayezhong to natural *E. onukii* infestation by integrating non-targeted metabolomics, microbial profiling, and flavor compound analysis.

Tea leaves from healthy plants and naturally infested plants were processed into black tea using traditional methods (natural withering, hand rolling, and spontaneous fermentation). Metabolomic analysis (LC-MS) revealed a significant upregulation of flavonoid glycosides (e.g., mauritianin, robinin, grosvenorine) and lipid-related compounds (e.g., 10-oxodecanoate) in response to insect feeding. Concurrently, the levels of key volatile aroma precursors such as 2-methylfuran, butanal, and ethyl octanoate were markedly reduced in the insect-stressed group, suggesting an alteration in tea aroma profile.

16S rRNA gene sequencing showed that the dominant bacterial genera during fermentation, including *Paucibacter*, *Aureimonas*, and *Chryseobacterium*, were strongly correlated with these altered metabolites (Spearman $|r| > 0.9$). Specifically, flavonoid glycosides were positively associated with *Paucibacter*, while short-chain fatty acid derivatives such as 10-oxodecanoate were correlated with *Aureimonas* and *Acidovorax*. These results suggest that *E. onukii*-induced metabolic shifts may drive microbial community restructuring, which in turn modulates secondary metabolite transformation and flavor development during fermentation.

Overall, this study highlights a multi-omics interaction framework linking herbivorous insect feeding with plant metabolism, microbial dynamics, and final tea quality. Our findings offer novel insights into the ecological roles of herbivore-induced changes in tea plants and their associated microbiota, and provide a theoretical basis for both high-quality tea production and ecologically informed pest management strategies.

Keywords: Empoasca onukii;Camellia sinensis var. assamica cv. Hainan Dayezhong;Flavonoid glycosides;Non-targeted metabolomics;16S rRNA gene sequencing;Volatile flavor compounds

The interaction between GCN2 and eIF2 mediates the resistance of cotton bollworm to the Bacillus thuringiensis Cry1Ac toxin

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Abstract: Deciphering the molecular mechanisms underlying insect resistance to Cry toxins produced by the soil bacterium Bacillus thuringiensis (Bt) is crucial for the sustainable utilization of Bt-based products. We investigated HaeIF2's role in Cry1Ac resistance. Quantitative PCR (qPCR) conffrmed that HaeIF2 expression was signiffcantly downregulated in Cry1Ac-resistant H. armigera(BtR). Overexpression and RNAi in midgut cells and larvae showed that HaeIF2's expression affects susceptibility to Cry1Ac by modulating the expression of receptors CAD, ABCC2, and ABCC3. Further studies demonstrated that HaeIF2 activates receptor expression by binding to eIF2 sites in the promoter regions. The downregulated three receptors in the BtR consistent with reduced HaeIF2 levels suggest HaeIF2 is involved in Cry1Ac resistance. Further, we found that an eIF2 alpha kinase, GCN2, is highly conserved in Lepidoptera insects. Its inhibitor GCN2iB (ATP-competitive inhibitor of serine/threonine protein kinase, stress-responsive kinase) could decrease the toxicity of Cry1Ac, and the GCN2 enzyme activities decreased after larvae fed Cry1Ac. The expression level of the HaGCN2 gene and the enzymatic activity of its corresponding protein were significantly down-regulated in the BtR resistant strain. Moreover, both BiFC and Y2H assays demonstrated that eIF2 could interact with GCN2. Finally, the silencing of GCN2 expression not only decreased the protein and phosphorylation levels of eIF2α but also reduced the expression of HaCAD, HaABCC2, and HaABCC3. This consequently led to a decrease in the toxicity of Cry1Ac toward Helicoverpa armigera. These results indicate that GCN2 conferring Cry1Ac resistance in H. armigera through regulating the expression and phosphorylation of eIF2. This finding deepens our understanding of the transcriptional regulation of midgut Cry receptor genes and the molecular basis of insect resistance to Bt Cry toxins.

Keywords: Cry1Ac resistance; Helicoverpa armigera; eIF2; GCN2; midgut receptor; interaction

Development of Simple Sequence Repeat of Monochamus alternatus (Coleoptera: Cerambycidae) Based on Restriction Site-Associated DNA Sequencing

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Abstract: Monochamus alternatus, a pest posing a serious threat to coniferous species, such as Pinus massoniana, has had devastating effects on pine forests due to its association with Bursaphelenchus xylophilus. The creation of unique simple sequence repeat (SSR) primers for M. alternatus is crucial, as there has been little study of the species' phylogeography. The aim of this study was to identify and create polymorphic SSR primers by sequencing samples of M. alternatus obtained from three different sampling points using the restriction site-associated DNA sequencing (Red-seq) approach. Subsequently, supplementary samples were integrated, and genetic typing was performed utilizing the identified polymorphic primers. Through comprehensive analysis, a total of 95,612 SSR loci were identified. Among these, mononucleotide repeats (51.43%), dinucleotide repeats (28.79%), and trinucleotide repeats (16.74%) predominated among the SSR motif types. Ultimately, 18 pairs of SSR primers were screened out, demonstrating stable amplification and high polymorphism. Genetic typing revealed that the mean number of alleles (Na) for these primer pairs ranged from 3 to 8, observed heterozygosity (Ho) ranged from 0.133 to 0.733, polymorphic information content (PIC) ranged from 0.294 and 0.783, and Shannon's index (I) ranged from 0.590 to 1.802. This study effectively produced 16 pairs of SSR primers that can be applied to different populations of M. alternatus. As a result, important tools for furthering studies on the phylogeography of pine wood nematodes, creating genetic maps, gene mapping, and carrying out in-depth investigations into gene function have been made available.

Keywords: Monochamus alternatus; genetic diversity; restriction site-associated DNA sequencing; simple sequence repeat.

The species and abundance of gut bacteria impact Phortica okadai behavior

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Abstract: *Phortica okadai*, a vector of the zoonotic nematode *Thelazia callipaeda* and a polyphagous pest of commercial fruits, poses significant threats to public health and agriculture in Asia. Current reliance on insecticides underscores the urgent need for sustainable pest management strategies. Growing evidence indicates that insect gut bacteria critically influence host physiology, development, and environmental adaptation. Notably, these bacteria can attract hosts and modulate feeding behavior, suggesting potential for pest control applications. Using 16S rRNA sequencing, we analyzed gut bacterial diversity and identified core taxa. Germ-free (GF), single-bacterium-colonized, and wild-type flies were then used in behavioral assays to assess the attractiveness of specific bacterial cultures and explore underlying mechanisms. We hypothesize that gut bacteria impact host behavior not only through species identity but also via their abundance within the gut. Sequencing revealed 54 bacterial genera (>99.9% total abundance), with 11 core genera present across all developmental stages—five *Firmicutes*, five *Proteobacteria*, and one unclassified bacterium. Gut bacterial composition in adults exhibited greater variability compared to other stages. Adults showed higher abundances of *Acetobacter*, *Lactiplantibacillus*, *Liquorilactobacillus*, and *Leuconostoc*, with sex-specific differences observed in *Acetobacter* and *Lactiplantibacillus* abundance. Based on sequencing results, *L. argentoratensis*, *L. citreum*, *L. brevis*, *L. fusiformis*, and *A. tropicalis* were selected for attraction experiments. Behavioral assays demonstrated that cultures of *L. citreum*, *L. brevis*, *L. argentoratensis*, and *A. tropicalis* significantly attracted *P. okadai*, with *L. argentoratensis* exhibiting the strongest attraction effect. Notably, the attraction of *L. argentoratensis* culture was comparable to that of naturally fermented fruits. Screening among these four bacteria confirmed *L. argentoratensis* as the most potent attractant. To assess the influence of abundance, GF P. okadai were mono-colonized with each bacterium. Results demonstrated that mono-colonized hosts preferentially consumed food containing their specific bacterial strain, indicating abundance-dependent behavioral modulation. GC-MS analysis was performed on volatile compounds produced by *L. argentoratensis* at its peak attraction day (day 3 of culture), using the basal medium as a control. Based on GC-MS quantitative results and literature review, 13 volatile metabolites produced at relatively high concentrations were selected as candidate semiochemicals. Subsequent behavioral assays using standards of these metabolites against *P. okadai* revealed eight compounds with attraction activity: 2,5-dimethylpyrazine, acetic acid, tert-amyl alcohol, isophorone, 3,4-dimethylbenzaldehyde, benzaldehyde, isoamyl alcohol, and isovaleric acid. Overall, this study revealed that *P. okadai* harbors consistently high abundances of Acetobacteraceae and Lactobacillaceae bacteria across all developmental stages. Adult *P. okadai* exhibited higher gut bacterial diversity and abundance compared to immature stages. *L. argentoratensis* demonstrated potent foraging attraction towards *P. okadai*, and the flies showed a preference for food containing bacterial species dominant within their gut microbiota. Finally, eight volatile metabolites derived from *L. argentoratensis* were identified as effective attractants. These findings provide novel insights into host-microbe interactions and support the development of targeted biocontrol strategies against this significant agricultural and medical pest.

Keywords: Phortica okadai;Gut bacteria;Abundance;Host behavior;Thelazia callipaeda

The mechanism of Emamectin Benzoate regulates the reproductive capacity of brown planthoppers and Drosophila melanogaster

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Abstract: Pesticide-induced resurgence, increases in pest insect populations following pesticide application, is a serious threat to the sustainable control of many highly damaging crop pests. Resurgence can result from pesticide-enhanced pest reproduction, however, the molecular mechanisms mediating this process remain unresolved. Here we show that brown planthopper (BPH) resurgence in rice crops following exposure to sublethal doses of the pesticide emamectin benzoate (EB) results from the coordinated action of a suite of genes that regulate juvenile hormone (JH) levels, resulting in increased JH titer in adult females and enhanced fecundity. We demonstrate that EB treatment at sublethal levels results in profound changes in female BPH fitness including increased egg maturation and oviposition. This enhanced reproductive fitness results from the EB-mediated upregulation of key genes involved in the regulation of JH, including JHAMT and Kr-h1 and the downregulation of allatostatin (AstA) and allatostatin receptor (AstAR) expression. AstA signaling is known to inhibit the production of JH in the corpora allata and hence EB exposure diminishes this inhibitory action. We find that the changes in gene expression following EB exposure are caused by the action of this insecticide on its molecular target, the glutamate-gated chloride channel (GluCl). Collectively, these results provide mechanistic insights into the regulation of negative pesticide-induced responses in insects and reveal some key actors involved in the JH-signaling pathway that underpin pesticide resurgence.In addition, we have discovered completely opposite phenotypes in fruit flies. Subsequently, we will provide insights for agricultural pest control by analyzing the phenotypes in fruit flies.

Keywords: resurgence;reproduction;ovulation;pesticide;brown planthopper

Effects of tebuconazole on insecticidal activity and symbionts in brown planthopper, Nilaparvata lugens (Hemiptera: Delphacidae)

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Abstract: Harnessing symbionts as targets for pest management is an emerging and promising strategy that can contribute to sustainable agriculture and environmental protection. Brown planthopper (BPH), a major rice pest, significantly threatens crop yields and quality. In this study, we discovered that BPHs exhibited a significant increase in mortality after consuming the fungicide tebuconazole, indicating its direct toxic effect. Tebuconazole negatively impacts the body weight, digestive enzyme activity, and reproductive capacity in BPHs, and it also leads to a significant downregulation of the expression levels of the ecdysteroid biosynthetic genes. The number of symbionts and the expression level of Noda in the BPH treated with tebuconazole was significantly reduced. Sequencing results showed that tebuconazole had a significant effect on the richness of symbiotic fungi and bacteria in BPH. As a fungicide, tebuconazole can offer new approaches and insights for managing resistance and integrated pest control.

Keywords: Brown planthopper;Tebuconazole;Symbionts;Insecticidal activity;Reproducibility

Effects of imidacloprid combined with validamycin on the population fitness and symbiotic of Nilaparvata lugens (Hemiptera: Delphacidae)

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Abstract: Using a high-efficiency insecticide in combination with fungicides that have different mechanisms of action is a conventional method in the current management of brown planthopper (BPH) resistance. In this study, we investigate the separate and combined effects of the low-toxicity fungicide validamycin and the non-cross- resistant insecticide imidacloprid on the fitness and symbiosis of BPH. These research results indicate that when the proportion of active ingredients in validamycin is combined with imidacloprid at a ratio of 1:30, the toxicity ratio and co-toxicity coefficient are 1.34 and 691.73, respectively, suggesting that the combination has a synergistic effect on the control of BPH. The number of yeast-like symbiotic (YLS) and dominant symbiotic (Noda) in the imidacloprid + validamycin groups were significantly lower than the other three treatment groups (validamycin, imidacloprid, and water). The results of the study on population fitness show that the lifespan of the BPH population in validamycin, imidacloprid, and imidacloprid + validamycin was shortened. Notably, the BPH populations in the imidacloprid + validamycin groups were significantly lower than other groups in terms of average generation cycle, intrinsic growth rate, net reproduction rate, finite rate of increase, and fitness. The Real-time quantitative PCR showed that validamycin and imidacloprid + validamycin can significantly inhibit the expression of the farnesyl diphosphate farnesyl transferase gene (EC2.5.1.21) and uricase gene (EC1.7.3.3), with imidacloprid + validamycin demonstrating the most pronounced inhibitory effect. Our research results can provide insights and approaches for delaying resistance and integrated management of BPH.

Keywords: Nilaparvata lugens ;Biological control ;Synergistic effect ;Fitness costs ;Life table

Differentially spliced mitochondrial CYP419A1 contributes to ethiprole resistance in Nilaparvata lugens

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Abstract: The brown planthopper Nilaparvata lugens is one of the most economically important pests of cultivated rice in Southeast Asia. Extensive use of insecticide treatments, such as imidacloprid, fipronil and ethiprole, has resulted in the emergence of multiple resistant strains of N. lugens. Previous investigation of the mechanisms of resistance to imidacloprid and ethiprole demonstrated that overexpression and qualitative changes in the cytochrome P450 gene CYP6ER1 lead to enhanced metabolic detoxification of these compounds. Here, we present the identification of a secondary mechanism enhancing ethiprole resistance mediated by differential splicing and overexpression of CYP419A1, a planthopper-specific, mitochondrial P450 gene. Although metabolic resistance to insecticides is usually mediated by overexpression of P450 genes belonging to either CYP 3 or 4 clades, we validate the protective effect of over-expression of CYP419A1, in vivo, using transgenic Drosophila melanogaster. Additionally, we report some unusual features of both the CYP419A1 gene locus and protein, which include, altered splicing associated with resistance, a non-canonical heme-binding motif and an extreme 5′ end extension of the open reading frame. These results provide insight into the molecular mechanisms underpinning resistance to insecticides and have applied implications for the control of a highly damaging crop pest.

Keywords: Nilaparvata lugens; Resistance mechanism; cytochrome P450

Essential Role and Universal Presence of Recently Acquired Co-obligate Symbionts

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Abstract: Nutritional endosymbioses have enabled insects to exploit nutrient-poor diets. Though some of these symbioses have been stable for hundreds of millions of years, in many lineages of insects the ancient symbionts show extreme genome erosion and have gotten replaced or complemented by other microorganisms. However, the biological significance and stability of these associations with newly acquired microbes is largely unknown. The East Asian cicada *Platypleura kaempferi* represents an ideal model for studying evolutionary dynamics in nutritional symbioses. Like most cicada species, it harbors the ancient obligate symbionts *Sulcia* and *Hodgkinia*, along with a newly required *Arsenophonus* symbiont. *Hodgkinia* in this system exhibits unprecedented genomic disintegration, having lost ancestral genes encoding key conserved metabolic functions. It is not clear whether *Arsenophonus* has taken over the missing functions of *Hodgkinia* in *P. kaempferi* and how stable this tripartite symbiotic association is. Using a combination of microscopy, amplicon and metagemonic sequencing on *Platypleura kaempferi* from 17 populations, we show that *Hodgkinia* in *P. kaempferi* exists in a hyper-fragmented state, with preliminary data suggesting the rRNA operon is the only element that seems to be retained across the many putative genomes.. This radical fragmentation coincides with complete erosion of folate (B9) and riboflavin (B2) biosynthesis pathways - functions conserved in other *Hodgkinia* lineages. Crucially, the co-occurring *Arsenophonus* symbiont retains intact pathways for these vitamins alongside essential amino acid production. Phylogenetically, cicada-associated *Arsenophonus* clusters with obligate nutritional symbionts from other insects, exhibiting hallmark genomic features of mutualism: reduced genome size (~1.8 Mb), low GC content (23%), and retention of host-beneficial metabolic genes. Spatial mapping via FISH confirms *Arsenophonus* predominantly colonizes fat bodies, while *Sulcia* and *Hodgkinia* reside in bacteriomes. These findings indicate *P. kaempferi* has evolved a tripartite symbiosis where *Arsenophonus* functionally compensates for *Hodgkinia*'s metabolic deterioration. Metagenomics and amplicon sequencing data across geographically distinct hosts demonstrates unexpected stability of this three-partner system. This system provides a unique model to investigate how hosts sustain symbioses amid escalating genomic instability, offering insights into the mechanisms underpinning symbiont complementarity and replacement during nutritional mutualism breakdown.

Keywords: genome fragmentation;metabolic complementarity;nutritional mutualism;symbiont compensation

Salivary Protein Cyclin-Dependent Kinase-like from Grain Aphid Sitobion avenae Suppresses Wheat Defense Response and Enhances Aphid Adaptation

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Abstract: Aphids are insect pests that suck phloem sap and introduce salivary proteins into planttissues through saliva secretion. The effector of salivary proteins plays a key role in the modulation ofhost plant defense responses and enhancing aphid host adaptation. Based on previous transcriptomesequencing results, a candidate effector cyclin-dependent kinase-like (CDK) was identiffed from thegrain aphid Sitobion avenae. In this study, the function of SaCDK in wheat defense response and theadaptation of S. avenae was investigated. Our results showed that the transient overexpression of SaCDK in tobacco Nicotiana benthamiana suppressed cell death triggered by mouse pro-apoptotic protein-BAX or Phytophthora infestans PAMP-INF1. SaCDK, delivered into wheat cells through aPseudomonas ffuorescens-mediated bacterial type III secretion system, suppressed callose deposition inwheat seedlings, and the overexpression of SaCDK in wheat signiffcantly decreased the expressionlevels of salicylic acid and jasmonic acid signaling pathway-related genes phenylalanine ammonialyase (PAL), pathogenesis-related 1 protein (PR1), lipoxygenase (LOX) and Ω-3 fatty acid desaturase(FAD). In addition, aphid bioassay results showed that the survival and fecundity of S. avenae weresigniffcantly increased while feeding on the wheat plants carrying SaCDK. Taken together, ourffndings demonstrate that the salivary protein SaCDK is involved in inhibiting host defense responseand improving its host adaptation, which lays the foundation to uncover the mechanism of theinteraction of cereal aphids and host plants.

Keywords: Sitobion avenae;salivary protein;defense response;aphid adaptation; bacterial type III secretion system

Functional dissection of conserved RNAi pathways reveals Dicer2-independent antiviral defense against RNA viruses in Hemiptera insects

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Abstract: The RNA interference (RNAi) pathway is a crucial post-transcriptional gene silencing mechanism involved in antiviral defense, gene regulation, and transposon suppression. In insects, RNAi operates through three main pathways: the microRNA (miRNA) pathway, the Piwi-interacting RNA (piRNA) pathway, and the small interfering RNA (siRNA) pathway. Compared to well-studied model insects of the order Diptera, such as mosquitoes and fruit flies, RNAi pathways in non-model insects remain largely unexplored. In this study, we conducted a comprehensive functional screening of core RNAi-related genes in the small brown planthopper (Laodelphax striatellus), a Hemiptera insect. Our results demonstrated that genes in the miRNA pathway (AGO1 and DCL1) and the piRNA pathway (Piwi and AGO3) play essential roles in the survival, development, and fecundity of L. striatellus but are not involved in the antiviral response to RNA virus infection. Interestingly, knockdown of the key siRNA pathway gene DCL2 had no effect on RNA virus replication or the abundance of virus-derived siRNAs (vsiRNAs), suggesting that the siRNA pathway in L. striatellus might function independently of DCL2. This finding was further confirmed in three other Hemiptera insects (Sogatella furcifera, Nilaparvata lugens, and Bemisia tabaci). Additionally, CRISPR/Cas9-mediated DCL2 knockout resulted in embryonic lethality in two homozygous mutant strains, while heterozygous mutants exhibited significantly prolonged developmental duration of L. striatellus, indicating that DCL2 might play an essential role in Hemiptera insect development. In summary, our findings provide valuable insights into the RNAi mediated antiviral pathways in hemipteran insects and broaden our understanding on the novel functional role of DCL2 in non-model insects.

Keywords: RNAi; DICER2; Laodelphax striatellus; Hemiptera insect

Insect Cytochrome P450 Database: An Integrated Resource of Genetic Diversity, Evolution and Function

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Abstract: Insects, the most numerous and diverse group of animal species on Earth, have important interactions with humans through providing resources, transmitting diseases and damaging agricultural cultivars. Cytochrome P450 monooxygenases (P450s) are one of the most important protein families in insects implicated in the endogenous metabolism and detoxification of xenobiotics, including allelochemicals, insecticides and environmental pollutants. To better understand the evolution and function of insect P450s and support the development and application of insecticides for pest control, an integrated bioinformatics platform is highly desirable. Here, we present the Insect Cytochrome P450 database (ICPD, http://www.insectp450.net/), which contains 66,477 P450s collected from public databases and predicted from the genomes of 682 insect species using a standardised bioinformatics protocol. Phylogenetic relationships between P450 genes are constructed for each species. The structures of all P450 proteins in the database are predicted using ESMFold, then visualised using WeView. Web services, such as BLAST, homogeneous modelling and molecular docking, are provided for determining the catalytic activities of P450 proteins. The ICPD will facilitate systematic investigations of the evolution and functions of the complete insect P450 complement, and represents a powerful tool for guiding insecticide design and application.

Keywords: cytochrome P450;database;insect;molecular docking;phylogeny;protein structure

Diaphorina citri E3 ubiquitin ligase FANCL promotes CLas bacterial proliferation by targeting to the host FHL2

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Abstract: Diaphorina citri, serves as the primary vector for *Candidatus* Liberibacter asiaticus (*C*Las), the pathogen responsible for citrus Huanglongbing (HLB). In this study, a adaptor protein FHL2 was identified from database of ubiquitylome, and showed upregulation after *C*Las infection. Ubiquitination of FHL2 was confirmed by immunoprecipitation, western blotting and LC-MS/MS. Silencing of FHL2 via RNAi significantly reduced *C*Las bacterial content in *D. citri*. Furthermore, interactions between FHL2 and *D. citri* E3-FANCL were confirmed using yeast two-hybrid assay and Co-IP. However, inhibition of E3-FANCL significantly increased *C*Las bacterial content. These findings suggest that *C*Las infection may inhibit E3-FANCL expression in *D. citri*, leading to subsequent interactions with FHL2 that facilitate the ubiquitination modification of FHL2, ultimately resulting in reduced expression levels and promoting *C*Las proliferation within *D. citri*. This study establishes a foundation for further investigation into the role of FHL2 ubiquitination modification during CLas bacterial infection.

Keywords: Huanglongbing;Diaphorina citri;FHL2;Ubiquitination

Chemosensory protein 8 enhances thiamethoxam resistance in Diaphorina citri

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Abstract: The Asian citrus psyllid (*Diaphorina citri*) serves as the primary vector of the bacterium *Candidatus* Liberibacter asiaticus (*C*Las), which is responsible for causing citrus Huanglongbing. Neonicotinoid pesticides such as thiamethoxam are frequently utilized for *D. citri* management worldwide, but their use have resulted in considerable development of insecticide resistance within *D. citri* populations. Recent reports have revealed that chemosensory proteins (CSPs)-mediated resistance represents a novel insecticide resistance mechanism in insects. In this finding, we found that exposure to thiamethoxam at different concentrations resulted in significant upregulation of transcriptional levels of *DcitCSP8* . The expression profiles among different tissues showed that *DcitCSP8* highly occurred in the legs and wings, and its expression being significantly induced by thiamethoxam with 2.40-fold increase specifically in the legs. The purified recombinant protein DcitCSP8, derived from *Escherichia coli* expression, present strong *in vitro* binding affinity (Ki = 4.74 μM) to thiamethoxam. Furthermore, the nanocarrier star polycation (SPc) was used to enhance silencing efficiency, resulting in a significant reduction of *DcitCSP8* by 85.37% and 91.80% at 24 and 48 h post application of SPc+ds DcitCSP8. Knockdown of *DcitCSP8* transcription significantly increased the susceptibility of *D. citri* adults in response to thiamethoxam, while reinjection of DcitCSP8 protein could restore the resistance in DcitCSP8-silenced individuals. Our findings highlight the association between DcitCSP8 and thiamethoxam resistance via high binding affinity that mitigates toxicity in *D. citri*, shedding light on CSP-mediated insecticide resistance and potentially contributing to the development of novel strategies targeting CSPs for managing resistance in *D. citri*.

Keywords: Diaphorina citri; Chemosensory protein; Thiamethoxam; Insectiside resistance; RNAi and rescue

Evolution and functions of sex-biased gene expression across in insects

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Abstract: Although males and females share a genome, sex-biased gene expression is thought to drive most sexually dimorphic traits. In this study, we systematically analyzed the evolution and expression patterns of sex-biased genes across 108 insect species, identifying 847,312 sex-biased genes. Our findings reveal that male-biased genes generally exhibit lower expression bias and slower evolutionary rates compared to female-biased genes. By analyzing newly generated transcriptome data from 280 samples spanning five tissues in ten representative species, we demonstrate that reproductive tissues are the primary organs to sex-biased gene expression. Furthermore, tracking expression patterns through developmental stages, we found that female- and male-biased genes display contracting mode of regulatory trajectories. Finally, we provide experimental evidence that gene duplication—where one paralog evolves female-biased expression and the other male-biased expression—resolves sexual antagonism. This study advances our understanding of the mechanisms governing sex-biased gene expression and its role in shaping sexual dimorphism.

Keywords: Sex-biased gene expression;Sexually dimorphic;Insecta

GhHAM regulates GoPGF-dependent gland development and contributes to broad-spectrum pest resistance in cotton

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Abstract: Cotton is a globally cultivated crop, producing 87% of the natural fiber used in the global textile industry. The pigment glands, unique to cotton and its relatives, serve as a defense structure against pests and pathogens. However, the molecular mechanism underlying gland formation and the specific role of pigment glands in cotton’s pest defense are still not well understood. In this study, we cloned a gland-related transcription factor GhHAM and generated the GhHAM knockout mutant using CRISPR/Cas9. Phenotypic observations, transcriptome analysis, and promoter-binding experiments revealed that GhHAM binds to the promoter of GoPGF, regulating pigment gland formation in cotton’s multiple organs via the GoPGF GhJUB1 module. The knockout of GhHAM significantly reduced gossypol production and increased cotton’s susceptibility to pests in the field. Feeding assays demonstrated that more than 80% of the cotton bollworm larvae preferred ghham over the wild type. Furthermore, the ghham mutants displayed shorter cell length and decreased gibberellins (GA) production in the stem. Exogenous application of GA3 restored stem cell elongation but not gland formation, thereby indicating that GhHAM controls gland morphogenesis independently of GA. Our study sheds light on the functional differentiation of HAM proteins among plant species, highlights the significant role of pigment glands in influencing pest feeding preference, and provides a theoretical basis for breeding pest-resistant cotton varieties to address the challenges posed by frequent outbreaks of pests.

Keywords: Gossypium; defense; feeding behavior;terpene

Coordinated Detoxification by Cytochrome P450 and UDP-Glycosyltransferase Underpins Polyphagy in Cotton Bollworm

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Abstract: Polyphagous insects exhibit remarkable adaptability to a wide range of host plants, primarily due to the expansion and diversification of detoxification enzyme gene families involved in phase I (functionalization), phase II (conjugation), and phase III (excretion) pathways. Although the functions of these detoxification systems have been extensively studied individually, the synergistic interactions between distinct enzyme families remain largely uncharacterized. Here, we show that cytochrome P450 monooxygenases and UDP-glucosyltransferases (UGTs) act synergistically to detoxify furanocoumarin-type plant secondary metabolites in *Helicoverpa armigera*. Comparative genomic analysis across multiple geographical populations reveals a substantial expansion of the UGT gene repertoire, particularly within the UGT33 family, forming a phase II detoxification superfamily. CRISPR-Cas9–mediated deletion of the UGT33 cluster demonstrates its critical role in the detoxification of xanthotoxin and imperatorin. Untargeted and targeted metabolomics identify xanthotoxol, 5-hydroxyxanthotoxin, and their corresponding glucosides as the main metabolic products. Functional assays further confirm that the phase I enzyme CYP6AE19 oxidizes xanthotoxin into xanthotoxol and 5-hydroxyxanthotoxin, which are subsequently glucosylated by the phase II enzyme UGT33B3. These findings define a stepwise, assembly line–like metabolic process wherein CYP6AE19 and UGT33B3 cooperatively convert toxic phytochemicals into less harmful glucosides. This work provides direct evidence for cross-phase coordination between detoxification systems, offers mechanistic insights into the host plant adaptability of polyphagous insects, and highlights new molecular targets for pest control strategies.

Keywords: Helicoverpa armigera; UDP- glycosyltransferases; Cytochrome P450s; Xenobiotic detoxification; Xanthotoxin

NPF1 and sNPF synergistically regulate Starvation-induced hyperactivity behavior of Locusta migratoria

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Abstract: Behavioral priority enables animals to adapt actions based on internal needs for survival and reproduction. Starvation often induces hyperactivity (starvation-induced hyperactivity, SIH) to enhance foraging success. This study investigated how developmental stage influences this response in female migratory locusts (*Locusta migratoria*). We found that starvation robustly induced SIH during the vegetative growth stage, characterized by intense foraging and preparation for migration, but not during the reproductive growth stage, where energy is prioritized for ovarian development. Neuropeptide F (NPF1) and Short Neuropeptide F (sNPF) exhibited stage-specific expression patterns in the nervous system in response to starvation. Functional analyses revealed that both neuropeptides promote post-starvation food intake. Crucially, NPF1 primarily regulated the differential SIH response: interfering with NPF1 significantly increased hyperactivity in vegetative-stage locusts by elevating brain nitric oxide (NO) levels, while its expression and effect remained unchanged in reproductive-stage locusts. In conclusion, sNPF and NPF1 jointly regulate feeding, but NPF1 is the key mediator of stage-specific differences in SIH. This differential regulation by NPF1 provides significant insights into the neurobiological mechanisms underlying how insects efficiently allocate energy between locomotion and reproduction to optimize reproductive success under nutritional stress.

Keywords: starvation; neuropeptide; behavior; priority

Estrogen-Related Receptor Potential Target Genes in Silkworm (Bombyx mori): Insights into Metabolic Regulation

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Abstract: Estrogen-related receptors (ERRs) are important transcription factors within the nuclear receptor family that regulate cellular energy storage and consumption by binding to estrogen-related receptor response elements (ERREs) on gene promoters. While ERRs’ role in vertebrates is well-studied, their molecular mechanisms in insect metabolism and development remain unclear. This study systematically summarizes the functions of ERRs in insects, focusing on silkworms by analyzing gene functions and comparing databases. ERRE-like elements were identified in the 2000 bp upstream promoter regions of 69 metabolism-related silkworm genes. Furthermore, electrophoretic mobility shift assays (EMSAs) revealed that ERREs within the promoters of 15 genes related to sugar, fat, and protein metabolism specifically bind to ERR. Notably, an ERRE in the promoter of the trehalose transporter 1 gene (BmTret1), crucial for trehalose homeostasis in insect hemolymph, exhibited significantly enhanced activity in ERR-overexpressing cells. These findings suggest that ERR is a potential regulatory factor in silkworm metabolism and refine its metabolic regulatory network. This study highlights the broader and more critical role of ERR in insects than that previously recognized, contributing to a deeper understanding of insect metabolism and its potential applications in related fields.

Keywords: estrogen-related receptor; estrogen-related receptor response element; metabolic regulation; physiological function; silkworm

The application of SPP in insects: Resistance Breeding and Biological Control

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Abstract: Signal peptide peptidase (SPP) is an intracellular lysate aspartate protease with significant evolutionary conservation and a broad spectrum of biological functions. It regulates normal immune surveillance of cells, hydrolyzes signal peptides, promotes the release of epitope-containing fragments, and prevents natural killer cells (NK) from erroneously damaging normal cells. Additionally, it interacts with glycoproteins on the virus surface, influencing its processing and reproduction. It can also be utilized by the virus to evade the killing of the host immune system. SPP plays an important role in human immunity, but its role in silkworms remains unclear. Here, we obtained the *BmSPP*^{KO} knockout strain using CRISPR/Cas9-based gene editing. We found that the DNA content of *Bombyx mori* nucleopolyhedrovirus (BmNPV) was significantly decreased in *BmSPP*^{KO} silkworms compared to the control, resulting in an 12.4% lower mortality. It indicates that *BmSPP* plays a negative regulatory role in silkworm resistance to BmNPV infection and is a target gene of BmNPV virus resistance in silkworm. In addition, we also found that *BmSPP*^{KO}silkworms showed a susceptibility phenotype to *Beausteria bassiana*. This was caused by the inhibition of chitin synthesis in the epidermis of *Bombyx mori*, leading to a reduction in the thickness of the chitin layer in the epidermis. These results indicate that *SPP* can serve as an important target gene, it can not only play a significant role in the breeding of BmNPV resistance in silkworms, but also act as a target gene to improve the biological control effect of *Beausteria bassiana* and establish a solid research foundation for the broad-spectrum synergism of *Beausteria bassiana* in pest control.

Keywords: SPP;antiviral;BmNPV;Beauveria bassiana;biological control

lncRNA regulates caste differentiation in ants

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Abstract: Caste differentiation in ants represents one of the major evolutionary transitions and provides a valuable model for studying developmental canalization, where genetically identical individuals follow divergent epigenetically-regulated trajectories to produce morphologically distinct phenotypes. While long non-coding RNAs (lncRNAs) are emerging as key epigenetic regulators across diverse biological systems, their specific contributions to caste development and developmental canalization remain largely unexplored. Here, we conducted comprehensive transcriptomic analyses across major developmental stages in two ant species, Monomorium pharaonis and Acromyrmex echinatior, identifying over 10,000 lncRNAs in each species. We demonstrate that lncRNAs exhibit dynamic, caste-specific expression patterns throughout development, with juvenile hormone treatment capable of redirecting worker lncRNA expression profiles toward gyne-like patterns, linking lncRNA regulation to established hormonal pathways controlling caste fate. Critically, we identified a subset of lncRNAs displaying canalized expression patterns, characterized by progressively increasing caste bias and decreasing within-caste variation as development proceeds. These canalized lncRNAs show striking tissue-specific enrichment, with gyne-biased canalized lncRNAs predominantly expressed in ovaries and flight muscle tissues, while worker-biased canalized lncRNAs are concentrated in brain tissues. Functional validation through RNA interference revealed that canalized lncRNAs directly regulate caste-specific traits. Knockdown of an ovary-specific lncRNA lncov impaired oogenesis in reproductive gynes, while suppression of ASfln, an antisense lncRNA regulating the flight muscle gene flightin, disrupted wing development and flight muscle formation. Co-expression analyses further revealed that canalized lncRNAs are functionally linked to key developmental regulators, including the master caste regulator Freja and core juvenile hormone signaling components. Our findings establish lncRNAs as active architects of developmental canalization in social insects, demonstrating that these rapidly-evolving regulatory molecules contribute directly to the evolution and maintenance of complex social phenotypes through tissue-specific regulation of caste-associated developmental programs.

Keywords: Social insects; caste differentiation; epigenomics; long non-coding RNAs (lncRNAs); gene regulation; canalization; evo-devo