Molecular mechanisms underlying chromatin remodeling during spermatogenesis in an endoparasitic wasp, Pteromalus puparum

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Abstract: Insect spermatogenesis is a remarkably sophisticated and precisely regulated biological process. Male germ cells undergo a highly ordered series of proliferation and differentiation events to ultimately form mature sperm. During the spermiogenesis phase, the nuclear chromatin condensed, with its structural and spatial reorganization, which are collectively called chromatin remodeling. This intricate process involves several key molecular events: the replacement of canonical histones by histone variants, histone hyperacetylation, coordinated DNA damage repair, replacement of histone variants by transition protein, and final substitution of transition proteins by protamine. To elucidate the mechanisms underlying spermiogenesis chromatin remodeling during spermiogenesisin parasitic wasps, particularly the associated signaling pathways and gene regulatory networks, we conducted an in-depth investigation using an endoparasitic wasp, *Pteromalus puparum*, which is a predominant pupal parasitoid of several lepidopteran pests.

The developmental processes of testis and spermatogenesis in *P. puparum* were investigated using light microscopy, transmission electron microscopy and immunofluorescence. Our observations revealed that the germ cell division within different cysts occurs asynchronously during spermatogenesis in *P. puparum*. As spermiogenesis progressed, the spermatid nuclear chromatin condensed into thick fibrillar structures, histones were progressively eliminated from the elongated spermatid nuclei, providing evidence of active chromatin remodeling in this stage. To explore the molecular mechanisms underlying chromatin remodeling, we characterized two testis-specifiche histone H1 variants (PpH1V1 and PpH1V2) in this wasp. Evolutionary analyses indicate that these H1 variants exhibit rapid evolutionary rates, and are typically maintained as single-copy genes in Hymenoptera. Functional studies using RNA interference yielded distinct phenotypes: (1) *PpH1V1* knockdown did not disrupt spermatogenesis in pupal testesis, but significantly impaired sperm fertility in the adult seminal vesicle and altered offspring sex ratio, indicating that PpH1V1 is associated with male fertility. (2) *PpH1V2* knockdown showed no observable effects on spermatogenesis or male fertility. Further investigation identified two sperm nuclear basic proteins (PpPrtl1 and PpPrtl2) involved in histone replacement during spermiogenesis in this wasp. RNAi-mediated knockdown experiments revealed that PpPrtl1 knockdown led to male sterility, with spermiogenesis process blocked. PpH1V1retention in the nuclei and failure to produce mature sperm indicated PpPrtl1 was essential in histone replacement and chromatins. *PpPrtl2* knockdown has no detectable effect on male fertility or spermatogenesis. Our study provides significant insights into chromatin remodeling during *P. puparum* spermatogenesis: PpH1V1 regulates chromatin structure and sperm functionality without being essential for spermatogenesis completion; PpPrtl1, acting downstream of PpH1V1in thechromatin remodeling cascade, is indispensable for both spermatid development and chromatin compaction, mediating the critical transition from histones to protamine-like proteins.

These findings advance our understanding of reproductive biology in parasitic wasps and provide a foundation for further studies on chromatin dynamics in insect spermatogenesis.

Keywords: Parasitoid wasp;Pteromalus puparum;spermatogenesis;chromatin remodeling;histone H1 variant;sperm nuclear basic protein

Regulation of phase separation and antiviral activity of Cactin by glycolytic enzyme PGK in insects

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Abstract: Liquid–liquid phase separation (LLPS) plays a vital role in numerous biological processes in eukaryotic organisms, including immune responses in mammals. Cactin, a highly conserved eukaryotic protein, undergoes LLPS to form dynamic, droplet-like condensates. This process is primarily driven by its intrinsically disordered regions (IDRs), arginine/serine-rich (RS) domain, and coiled-coil (CC) domain, and is essential for its antiviral function. Notably, this phase behavior is finely regulated by phosphorylation mediated by the glycolytic enzyme phosphoglycerate kinase (PGK). This conserved but adaptable mechanism enables defense against a range of viruses, including Southern rice black-streaked dwarf virus (SRBSDV) in the white-backed planthopper (WBPH) and Drosophila C virus (DCV) in fruit flies. In both systems, PGK interacts with Cactin and modulates its phosphorylation within specific IDRs. PGK-mediated phosphorylation promotes the transition of Cactin from stable aggregates to dynamic liquid droplets, thereby significantly enhancing its antiviral activity. Together, the PGK–Cactin axis represents a critical and tunable component of insect antiviral immunity.
Keywords: Cactin;Liquid-liquid phase separation (LLPS);SRBSDV; DCV; Phosphoglycerate kinase (PGK); Antiviral immunity

Decoding 4-vinylanisole biosynthesis and pivotal enzymes in locusts

Xiaojiao Guo

Tissue-Specific CLOCK Isoforms Modulate Circadian Feedback Loops to Govern Reproductive Fitness in *Drosophila*

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Abstract: Circadian rhythms, conserved across life, are governed by molecular feedback loops in *Drosophila* clock neurons, though how these loops adapt in peripheral tissue clocks remains unclear. In this study, we discovered that in reproductive tissues, the expression profile of *Clock* is notably different. It is expressed at very low levels in the ovary, while a shorter isoform of *Clock* is particularly abundant in the testis. Downregulation of these isoforms impaired fertility in both male and female flies. The short isoform of CLOCK inhibited the binding of the longer isoform to CYCLE, leading to a reduction in CLOCK / CYCLE co-binding in the testis. Using ChIP-seq to identify CLOCK and CYCLE binding sites in the head, ovary and testis, we found that the binding profile of CLOCK / CYCLE was distinct between these tissues, in which CLOCK / CYCLE targets to genes involve in spermatogenesis specifically in testes. Immunostaining revealed knock down of CLOCK short form results in defects in spermatogenesis. This study reveals the expression profiles and functional mechanisms of specific *Clock* isoforms in reproductive tissues, which highlighted the modification of clock regulatory loop and essential role of *Clock* in reproductive organs.

Dumpless1 regulates mature follicle rupture by integrating ecdysone and octopamine signaling in *Drosophila*

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Abstract: Ecdysone signaling is known to regulate octopamine (OA)-induced a matrix metalloproteinase 2 (Mmp2)-dependent mature follicle rupture to promote ovulation; However, the specific mechanisms through which it modulates OA signaling remain unclear. Here, we identify Dumpless1, a ZAD-C_2H_2 zinc-finger transcription factor, as a critical bridge connecting ecdysone and OA signaling. Mutations in *Dumpless1* led to the impeded rupture of mature follicles and a consequent reduction in egg-laying. Moreover, the diminished Mmp2 activity and reduced expression of the OA receptor *oamb* in *Dumpless1*$^{-/-}$ ovaries were detected. Notably, mature follicles in these mutants exhibited defects in OA-induced follicle rupture. Furthermore, Dumpless1 was unregulated in mature stage-14 posterior follicle cells. In addition, disruption of the ecdysone receptor B1 (EcRB1) in mature follicle cells decreased Dumpless1 expression. Our data suggest that *Dumpless1* is a key regulator by mediating ecdysone and octopamine signaling, thereby participating in mature follicle rupture and ensuring proper ovulation. This study provides a novel insight into the molecular mechanisms of ecdysone-OA communication in *Drosophila* ovulation.

Functional roles of SRPK in the sperm development of insects

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Abstract: SRPK (Serine/arginine protein-specific kinase) is a kinase that specifically catalyzes the phosphorylation of SR proteins, serving as a key enzyme family regulating pre-mRNA splicing activity in cells. Through knockdown and overexpression of the SRPK gene in Bombyx mori, we found that SRPK participates in spermatid dimorphism and sperm maturation. Overexpression of SRPK increased the number of apyrene bundle. The reduced egg production in offspring indicates that the SRPK gene is involved in sperm development in Lepidoptera insects.
Keywords: Silkworm;SRPK;Sperm development

Probing the optimal architecture and molecular mechanism of insect odorant receptor heteromeric channels

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Abstract: Insects have a powerful olfactory system that is far more selective and sensitive than artificial detectors. Insect odorant receptors (ORs) are key components of the system, which are ligand-gated ion channels comprising a specific odorant-sensing OR and a highly conserved odorant receptor co-receptor (Orco). However, the stoichiometric ratios of the heterotetramers remain inconclusive, and the molecular mechanism by which the ligand initiates channel opening is still not fully understood. The present study is based on the technical approach of molecular dynamics (MD) simulation. We predicted the spatial structures of locust LmOR35-Orco heterotetramer under various stoichiometric ratios, constructed it within a membrane environment, and compared the structural changes of LmOR35-Orco before and after ligand binding. Furthermore, we analyzed the molecular mechanism of LmOR35-Orco across different architectures. Our findings propose an optimal architecture (1OR:3Orco) for insect heteromeric odorant receptors, elucidate the molecular mechanism underlying receptor activation due to ligand-induced ion channel opening, and identify critical residues involved in ligand recognition and ion channel gating. This study provides valuable insights into the regulatory mechanism of insect olfaction and has significant implications for function modification and the development of bionic electronic nose.
Keywords: odorant receptors; ligand-gated ion channels; stoichiometric ratio; odorant recognition; molecular dynamics

Ccdc13 is essential for the assembly of ciliary central microtubules in *Drosophila*

Zhimao Wu

Tryptophan catabolism reprograms insect immunity during hemolymph bacterial infection

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Abstract: Background: Although insect hemolymph¾the functional equivalent of vertebrate blood¾has traditionally been regarded as sterile, it can harbor translocated gut-derived bacteria due to its rich nutrient content. Uncontrolled bacterial proliferation in the hemolymph poses a significant threat to insect development and survival, underscoring the necessity for precise bacterial homeostasis. However, the underlying regulatory mechanisms remain poorly understood.
Results: This study demonstrates that the Spätzle gene cluster (HaSpz3-6) in the cotton bollworm (Helicoverpa armigera) plays a pivotal role in preserving hemolymph bacterial balance during molting. Depletion of HaSpz3-6 downregulates the expression of antimicrobial peptides, facilitating the overgrowth of Bacillus spp. and impairing larval fitness. Elevated bacterial loads subsequently induce tryptophan (Trp) catabolism, resulting in increased synthesis of serotonin (5-hydroxytryptamine, 5-HT) and N-acetylserotonin (NAS). 5-HT-mediated phagocytosis, along with bactericidal reactive oxygen species (ROS), restores bacterial homeostasis in HaSpz3-6-depleted larvae. Additionally, NAS scavenges excess ROS to limit oxidative damage.
Conclusions: Our findings uncover a compensatory immune strategy wherein Trp catabolism bridges humoral and cellular responses. This metabolic-immune crosstalk illustrates the dynamic adaptability of the insect immune system in maintaining bacterial homeostasis within the hemolymph.
Keywords: Serotonin; N-acetylserotonin; Spätzle; Phagocytosis; Microbiota

USP8 regulates endoreplication by promoting Fzr deubiquitination and stabilization

Wenliang Qian

The role of RpUGT344J7 in the reproduction switch of Rhopalosiphum padi with holocyclic life cycle

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Abstract: Many aphid species exhibit both cyclical parthenogenesis (CP) and the obligate parthenogenesis (OP) life history, which are genetically determined. In CP aphid lineages, the parthenogenetic individuals can switch from asexual to sexual reproduction quickly in response to environmental factors such as changes in photoperiod and temperature. However, the OP aphid lineages do not undergo sexual reproduction under any conditions. So far, mechanisms underlying the reproduction switch in CP aphids have not been fully elucidated. Rhopalosiphum padi, a serious worldwide insect pest of wheat, has both CP and OP lineages. Uridine diphosphate-glycosyltransferases (UGTs), which participate in the metabolic detoxification of xenobiotics, play an important role in the response of insect to adverse conditions. In this study, we identified all RpUGT genes from R. padi genome sequences. The results showed that the RpUGT344J7 may be involved in the reproductive transformation process of R. padi through the analysis of the UGTs activities and expression patterns of RpUGT genes. RNAi and injection of inhibitor further revealed that RpUGT344J7 can influence the critical time points and total numbers of CP lineages to produce virginoparae, gynoparae, and males, for the first time. The analysis of the expression regulation mechanism of RpUGT344J7 revealed that novel_38 can regulate the expression of RpUGT344J7, thereby affecting the reproduction switch of of R. padi with holocyclic life cycle. The findings contribute to our understanding of the molecular mechanisms underlying the quick shift from asexual to sexual reproduction in aphid species.
Keywords: Aphids;reproduction switch;life cycle

Aspects of wing differentiation in insects

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Abstract: As the flying organs of insects, wings play an important role in foraging, avoiding enemies, reproduction and migration of insects. There have been many studies on the development of wing imaginal disc, but the wing differentiation from pupal stage to adult stage has not been extensively studied. Based on RNA-seq data and RNAi interference screening, we revealed the functions of four key genes in wing differentiation and maturation, including the role of chitinase Cht6, Cht10 in the maturation of the apicellular matrix, and Osiris17 in the formation of adhesive connections at the basal site of epithelial cells. Cyp311a1 may participate in wing development by regulating microvilli formation. With these studies, we contribute to the advanced understanding of action network of wing differentiation and maturation.

Keywords: Wing; differentiation; maturation; network

Molecular Mechanisms of Summer Diapause Regulation in the Adult Galeruca daurica

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Abstract: Galeruca daurica is a new pest with great outbreaks in the Inner Mongolia grasslands in recent years, and mainly feeds on Allium plants, such as Allium mongolium, A. polyrhizum, and A. ramosum. This pest has not only caused serious economic losses to grassland animal husbandry but also caused grassland degradation, which seriously threatened the ecological security in north China.G. daurica, an obligatory diapause insect, occurs one generation each year, and oversummers as diapausing adults and overwinters as diapausing eggs. Our study elucidated that lipids and glycogen are the primary energy substances for G. daurica adults to survive the summer. Juvenile hormone analog methoprene or ecdysteroid can inhibit lipid accumulation and promote ovarian development, thereby delaying the entry of adults into diapause. Decreased expression levels of juvenile hormone tolerance protein GdMet, ecdysteroid receptor GdEcR, nuclear hormone receptor GdHR3, and lipoprotein receptor GdLpR can all promote the expression of fatty acid synthase GdFAS while inhibiting the expression of vitellogenin GdVg. This leads to a significant increase in total lipid content, impeded ovarian development, and consequently, premature entry of adults into diapause. Therefore, the juvenile hormone signaling pathway and the ecdysteroid signaling pathway play crucial regulatory roles in the summer diapause process of G. daurica adults. miRNAs also exert significant regulatory effects during insect diapause. Specifically, miRNA let-7-5p regulates summer diapause in G. daurica adults by targeting Kr-h1, while miR-2765-3p regulates it by targeting FoxO.
Keywords: Galeruca daurica; Summer diapause; Juvenile hormone; microRNA regulation

The Central Role of BmCENPT in Cell Cycle Regulation, Chromosome Segregation and Microtubule Organization in the Silkworm, Bombyx mori

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Abstract: The kinetochore plays a pivotal role in cell division by ensuring the proper attachment of chromosomes to the spindle microtubules. The assembly and function of the kinetochore are particularly intriguing in the holocentric Bombyx mori. The inner kinetochore protein CENPT is indispensable for microtubule assembly in this organism. Lepidoptera CENPTs, closely related to vertebrates, exhibit a variable MDN1 domain unlike conserved human/yeast CENPT-T. BmCENPT is highly expressed in silkworm gonads, low in epidermis on day 3 of the fifth larval instar (L5D3), with variable patterns in ovary development through qRT-PCR examination. BmCENPT localizes to the nucleus during interphase and subsequently to chromosomes, kinetochores, and the spindle midzone during mitosis. Its broad distribution along the chromosomes compensates for the absence of CENH3 and CENPC, providing multiple sites for microtubule attachment. Overexpression of BmCENPT promotes cell proliferation by enhancing DNA replication, while its knockdown inhibits these processes. Flow cytometry analysis reveals that BmCENPT knockdown increases the proportion of cells in the S phase, suggesting a stall in DNA replication, whereas overexpression arrests cells in the G1 phase. Additionally, BmCENPT depletion leads to abnormal spindle positioning and multipolar spindle formation, while overexpression results in chromatin bridges and cytokinesis defects. Our study underscores BmCENPT's crucial role in chromosome segregation, microtubule organization, and cell cycle progression in silkworm cells. Its impact on cell cycle checkpoints and DNA replication highlights its importance for genomic integrity in holocentric chromosomes. Future work should explore the molecular mechanisms regulating these processes.

Keywords: Silkworm; CENPT; cell cycle; Chromosome Segregation; microtubule organization

The Toll pathway regulated by PGRP-S2 mediates defensin-induced apoptosis to inhibit Israeli acute paralysis virus in insect

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Abstract: Honey bees, the important pollinators, provide a crucial ecosystem service that is significant for sustaining both wild flowering plant diversity and many agricultural crops. Despite the extensive studies on the infection characterization of honey bee virus, the activation mechanism of immune pathways has not yet been defined, and how honey bee recognizes viral infection remains unclear. Here, we identified Toll immune pathway as primary antiviral immune responses responsible for inducing antimicrobial peptides. Moreover, we found that Israeli acute paralysis virus capsid protein VP3 directly interacted with short-type peptidoglycan recognition protein (PGRP-S2) to activate Toll pathway. Interference of PGRP-S2 enhanced the viral titers while reduced the expression of antimcrobial peptides including defensin1. The yeast two-hybrid analysis showed that defensin1 bound to insulin receptor substrate (IRS) to induce apoptosis to limit viral infection. This work identified the new function of defensin1 in invertebrates. Our findings revealed the defensin1’ s role in inititating the apoptosis in insects. Especially interaction between defensin1 and IRS might be a new mechanism as tissue-specific immune signalling on the fat body, and offering potential therapeutic strategy for virus disease.

Keywords: PGRP-S2; IAPV;Toll pathway; Apis mellifera; AMPs; Apoptosis

Duplication of a conserved mitochondrial enzyme gene arms parasitoid wasps with venom cytotoxicity and oogenesis regulation

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Abstract: Gene duplication, followed by neofunctionalization, is a key mechanism driving the emergence of evolutionary novelties. Despite its significance, the molecular and functional processes underlying this phenomenon remain incompletely understood. By tracing the evolutionary history of cysteine-S-conjugate beta-lyase (CCBL) genes within the kynurenine aminotransferase family, we identified a gene duplication event in parasitoid wasps of the Chalcidoidea superfamily. Notably, a single-copy, highly conserved mitochondria-localized physiological gene underwent a significant duplication, resulting in one copy being recruited into the venom system and acquired cytotoxicity against wasps' hosts. Through this neofunctionalization process, we observed several key evolutionary changes, including loss of ancestral mitochondrial localization and enzyme activity, acquisition of a secretory signal peptide, shift in expression pattern, positive selection, and the establishment of novel protein-protein interactions. Additionally, we found that another duplicate copy was specialized in wasps' ovary and repurposed for oogenesis regulation. Our study offers a detailed insight into the genetic and molecular mechanisms that drive functional diversification during the evolution of gene families.

Keywords: Gene duplication; neofunctionalization; venom evolution; cytotoxicity; ovarian development

A Moonlighting Function of NudC in Insect Polyploid Cells: The Key Regulator of Ribosome Homeostasis

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Abstract: Ribosomes, the essential machinery for protein synthesis, are fundamental across all kingdoms of life, including insects. The proper regulation of ribosome biogenesis is especially critical during the development of insect polyploid cells—large cells that undergo rounds of DNA replication without cell division and therefore require elevated protein synthesis to support their growth and function. Disruption in ribosome production can cause severe cellular dysfunction and impair insect development, underscoring the importance of understanding its regulatory mechanisms.

In this study, we identified the gene *NudC* (*nuclear distribution C, a dynein complex regulator*) as a key regulator of ribosome biogenesis in polyploid cells, using the larval salivary gland (SG) of *Drosophila melanogaster* as a model. RNA interference (RNAi) targeting *NudC* in SG cells led to a significant reduction in ribosome abundance, loss of rough endoplasmic reticulum membranes (the sites of ribosome attachment), and a marked decrease in protein synthesis accompanied by messenger RNA degradation. This decline in ribosome abundance was attributed to a substantial drop in nucleolar ribosomal RNA precursor (pre-rRNA) levels during ribosome biogenesis. Conversely, however, *NudC* RNAi in SG cells resulted in a pronounced upregulation, rather than downregulation, of the transcription and translation of ribosome biogenesis factors (RBFs) and ribosomal proteins, resembling the compensatory responses seen upon RBF depletion. These results suggest that NudC and RBFs may cooperate to maintain ribosome homeostasis in polyploid cells.

Other molecular responses in *NudC*-deficient cells also mirrored those seen in RBF-deficient cells, including autophagy in polyploid tissues and apoptosis in diploid tissues, processes likely mediated by c-Jun N-terminal kinase (JNK) signaling. Additional phenomena, such as the accumulation of virus-like particles and abnormal chromosome structure, were also observed in *NudC* RNAi SGs. Notably, our data indicate that NudC's function in ribosome biogenesis is independent of its known role in dynein regulation, suggesting that this protein has additional "moonlighting" functions.

In summary, our study reveals a critical role for NudC in maintaining the homeostasis of ribosome biogenesis in the polyploid SG cells of *Drosophila* larvae, providing new insights that may extend to ribosome regulation across a broad range of insect species.

Keywords: Ribosome biogenesis;Homeostasis;NudC;Polyploid cells;Drosophila

High-Value Valorization of Proteins and Lipids from Black Soldier Fly Larvae

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Abstract: Facing converging global threats, including population growth, climate change, resource depletion and biodiversity loss, the exploration of new biological resources has become imperative. Insects, the most abundant animal group in Earth, represent a vital and nutrient-rich resource. Black soldier fly larvae (BSFL, *Hermetia illucens* L.), an emerging resource insect, efficiently converts organic waste into valuable protein and lipids. BSFL larvae protein exhibited a balanced amino acid profile, high nutritional value, and superior digestibility compared to plant proteins. BSFL lipids, rich in lauric, linoleic, oleic and palmitoleic acids, showed antibacterial activity and could beneficially modulate gut microbiota by increasing diversity and reducing inflammation. Additionally, BSFL produced bioactive compounds, such as antimicrobial peptides and lysozymes, which enhance immunity and demonstrated potential in preventing diseases including hyperglycemia, hyperlipidemia, hypertension and tumors. Thus, we develop valorization methods for BSFL derived proteins and lipids to create applicable high-value products.

Keywords: black soldier fly larvae;proteins;lipids;valorization method

Mutation in Resilin reveals attachment impairment in Bombyx mori

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Abstract: Insects can effortlessly walk on various substrate surfaces using attachment pads on their feet, even upside down on smooth surfaces. These pads undergo frequent deformation throughout the lifecycle of insects to achieve stable adhesion. Interest in insect adhesion spans 100s of years; studies have indicated the special mechanical properties and intricate structural design of insect attachment pads. However, the genetic basis of their function remains unclear. Resilin, an elastic protein widely distributed in insect exoskeletons, plays a crucial role in many physiological activities. Here, we identified a resilin-like protein in the Lepidopteran insect Bombyx mori (BmResilin), which is predominantly composed of glycine and features short motifs such as "PxSxYGxP" and "GYPxGGP", and contains a hydrophilic-hydrophobic-hydrophilic module, suggesting it may have dynamic conformation and elasticity. To investigate BmResilin's function, we established a mutant line using the CRISPR/Cas9 system. The results showed that adults displayed a conspicuous "slipping" phenotype on smooth surfaces but behaved normally on rough ones, and quantitative friction force measurements showed a reduction in adhesive capacity compared to wild-type individuals. We also investigated the courtship behavior, wingbeat frequency, and wing flapping amplitude of adults, finding that BmResilin mutation had no effect on courtship behavior and wing flapping amplitude but slightly reduced the wingbeat frequency. Further behavioral investigations, microscopic observations and mechanical performance tests confirmed that the mutation stiffened the attachment pads. Notably, distinct darkening occurred at the arolium edge, and the mutants' arolium appeared thinner and more shriveled compared to the plump wild-type arolium. Additionally, the rod layer fibers were unevenly and disorderly distributed, with reduced gaps between bundles, thereby reducing flexibility and effective contact area, which ultimately led to decreased adhesive ability in adults. Molecular-level analyses revealed that the resilin-like mutation led to differential expression of melanin metabolism-related genes, potentially explaining the hardening and abnormal pigmentation of the attachment pads. These findings establish a mechanistic link between resilin deficiency, altered cuticular mechanics, and impaired adhesion, demonstrating that resilin maintains arolium flexibility by modulating melanin-related sclerotization. Our study provides the first genetic evidence for resilin's essential role in insect attachment systems, with implications for understanding evolutionary adaptations in Lepidoptera and biomimetic material design.
Keywords: Lepidoptera; attachment pads; biological adhesion system; cuticle protein; resilin; silkworm.

Radiation enhances cellular plasticity of enteroendocrine cells in the Drosophila midgut

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Abstract: The insect gut epithelium is a dynamic tissue that must preserve its integrity despite constant exposure to physiological stresses. This demands tightly regulated mechanisms for cell turnover and regeneration. *Drosophila melanogaster* serves as a powerful model to study intestinal epithelial homeostasis. Within the adult *Drosophila* midgut epithelium, intestinal stem cells (ISCs) produce committed progenitors that differentiate into absorptive enterocytes (ECs) or secretory enteroendocrine cells (EEs).

While ISCs typically serve as the primary source of new cells, recent evidence suggests that differentiated cells regain plasticity and contribute to regeneration under stress. The mechanism by which insect guts utilize cellular plasticity to respond to genotoxic stress, such as radiation —a type of stress gaining relevance across both practical and experimental contexts —remains largely unknown.

Here, we provided a comprehensive analysis of radiation epithelial cell plasticity. Upon exposure to 100 Gy of X-rays, we observed a reduction in ISC numbers, while EE populations remained largely unaffected. With EE-specific lineage tracing, we found that a subset of EEs gained plasticity in response to radiation. These cells reacquired progenitor-like features and subsequently redifferentiated into ECs.

We further identified a stress-responsive transcription factor *Xrp1* as a key regulator of radiation-induced EE plasticity. Xrp1 protein was produced in EEs and EE-derived cells following irradiation, while EE-specific knockdown of *Xrp1* abolished radiation-induced EE plasticity. These findings establish *Xrp1* as a critical factor enabling radiation-induced cellular plasticity in the insect gut.

By uncovering how differentiated epithelial cells respond to radiation, our study contributes to a deeper understanding of gut resilience and epithelial plasticity in insects facing environmental or artificial genotoxic stress.

Keywords: Drosophila;radiation;enteroendocrine cell;intestinal stem cell

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

MicroRNAs analyses provide insights into mechanisms of Wolbachia-induced paternal defects

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Abstract: The obligate endosymbiont Wolbachia can modulate insect reproduction with the most common phenotype being cytoplasmic incompatibility (CI), which results in embryonic lethality when sperm from Wolbachia-infected (wMel+) males fertilize eggs from uninfected females (wMel-), indicating Wolbachia-inducing defects are mainly in paternal side. Our previous study found that Wolbachia infection promotes the biogenesis of novel microRNAs (miRNAs) in the testes of Drosophila melanogaster, among which nov-miR-6~18 are specifically expressed in Wolbachia-infected testes. Here, we demonstrated that nov-miR-11 was significantly upregulated in the testes of wMel+. Through the RNAhybird, we predicted the potential target genes of nov-miR-11. Dual-luciferase reporter assays showed that eif4h2, exhibiting extremely significant downregulation in wMel+ testes, is a direct target of nov-miR-11. nov-miR-11 negatively regulated eif4h2 through binding to its 3’ UTR region. Furthermore, knockdown of eif4h2 in the testis caused male sterility with absence of mature sperm in the seminal vesicles. Immunofluorescence staining revealed that eif4h2 knockdown caused disorganized sperm bundles, disrupted elongation caps, and scattered individualization complexes. Similar defects were observed in eif4h2 knockout fly testis. These results indicate that Wolbachia negatively regulates eif4h2 transcription by promoting nov-miR-11 biogenesis, thereby mediating male host reproduction.
Keywords: Drosophila melanogaster;Wolbachia ;microRNA;eif4h2;spermatogenesis

ApoLp-II/I mediates parasitoid larvae encapsulation by enhancing the adhesion of host hemocytes and binding to larval surfaces

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Abstract: Insects primarily defend against parasitoid wasps via encapsulation. However, limited understanding of this host response hinders elucidating parasitoid counter-strategies. Based on our previous research, we hypothesized that Apolipophorin-II/I in Ostrinia furnacalis (ApoLp-II/I) plays a role in the encapsulation of Macrocentrus cingulum larvae. ApoLp-II/I was expressed in the fat body and secreted into the hemolymph, then cleaved by Flynn protease into ApoLp-II (76.97 kDa) and ApoLp-I (288.56 kDa). The encapsulation of O. furnacalis hemocytes against wasp larvae was reduced when the ApoLp-I protein was blocked with anti-ApoLp-I antibody, the expression of the apoLp-II/I gene was interfered with dsRNA, or the apoLp-II/I gene was knocked out via CRISPR/Cas9. These findings suggest that ApoLp-II/I plays a central role in the encapsulation process. The expression of apoLp-II/I can be induced at the mRNA level within the host fat body by wasp larvae. Although protein abundance did not significantly increase, ApoLp-I and ApoLp-II were transferred from plasma to hemocytes. Immunofluorescence revealed that ApoLp-I could bind to the surface of specific hemocytes and enter these cells via endocytosis. Notably, hemocytes rich in ApoLp-I demonstrated strong adhesion properties. Aggregation of ApoLp-I protein was also detected on the surface of wasp larvae following incubation with 50% naïve O. furnacalis larvae plasma. Further investigation into the binding protein of ApoLp-I in hemocytes showed that ApoLp-I and UGT2 (UDP-glycosyltransferase) proteins were co-localized, and the expression of UGT2 in the hemocytes of O. furnacalis larvae could be induced by wasp larvae. Knock down the expression of UGT2 significantly impeded the encapsulation ability of O. furnacalis against wasp larvae. These findings indicated that ApoLp-II/I entered hemocytes and activated the downstream UGT2 protein. This process could enhance the adhesion ability of hemocytes, thereby promoting the encapsulation of wasp larvae. Additionally, ApoLp-II/I might function as an immune recognition factor for wasp larvae. These results clarify the potential mechanisms underlying the encapsulation of closely related species by insects and deepen our understanding of parasitoid-host coevolution.

Keywords: ApoLp-II/I;Encapsulation;Hemocytes;Ostrinia furnacalis

Immulectin-3 mediates parasitoid larvae encapsulation by binding to host hemocytes and larval surfaces

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Abstract: Parasitoids evade host immunity, ultimately causing host death. However, the limited understanding of the mechanisms underlying the host's encapsulation response to parasitoids hinders our ability to comprehend the strategies parasitoids use to overcome host immune defenses. Based on our previous study, immulectin-3 in *Ostrinia furnacalis* (*Of*IML-3) was speculated to function in encapsulating the larvae of *Macrocentrus cingulum*. *Of*IML-3 was expressed mainly in the fat body and secreted into the plasma, and its expression could be induced at both the messenger RNA (mRNA) and protein levels by wasp larvae. Approximately 88% of wasp larvae were completely encapsulated by hemocytes after transplantation into naive *O. furnacalis* larvae; however, this percentage significantly decreased to 37% after the expression of *Of*IML-3 was knocked down by RNA interference. In the homozygous mutant strain of IML-3 (*Of*IML-$3^{-/-}$), the proportion of encapsulated wasp larvae further decreased to 7%. This indicates that *Of*IML-3 plays a core role in encapsulation. Furthermore, higher levels of *Of*IML-3 were detected on hemocytes after wasp larvae were transplanted into naive *O. furnacalis* larvae, and on the surface of the wasp larvae. In the *Of*IML-$3^{-/-}$ strain, less *Of*IML-3 was detected on both surfaces, indicating that the binding of *Of*IML-3 to both hemocytes and wasp larvae is a crucial step in the encapsulation process. Our findings suggest that *Of*IML-3 likely acts as a recognition factor to promote encapsulation of parasitoids by the host. This elucidates the potential mechanisms of encapsulation of closely related species by insects and enhances our insight into parasitoid–host coevolution.

Keywords: C-type lectin; encapsulation; hemocytes; CRISPR/Cas9; Ostrinia furnacalis

Isolation and identification of a Pantoea agglomerans PxG45 from Plutella xylostella (Lepidoptera: Plutellidae) gut and its antifungal activity

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Abstract: [Aim] Gut symbiotic bacteria in insects, the main members of the gut microbial community, play important roles in the growth and development, nutrient absorption, and immune system of insects. In this study, a gut symbiotic bacterium of *Plutella xylostella* was isolated and cultured, and its function was identified to explore the influence of the gut symbiotic bacteria on the adaptability of *P. xylostella* and the broad-spectrum inhibitory activities against fungi. **[Methods]** The gut bacterium, PxG45, was isolated and purified from the 3rd instar larva of *P. xylostella* by plate culture in vitro. Morphological characterization, 16S rDNA gene sequencing, and physiological and biochemical characterization were used to identify the bacterial species. The effects of PxG45 on the survival rate of the 3rd instar larva of *P. xylostella* and the effects of the *Beauveria bassiana* infection on the survival rate of the 3rd instar larva of *P. xylostella* were detected by bioassay. The inhibition of PxG45 on *B. bassiana* was defined by plate confrontation method to further detect the broad-spectrum resistance of PxG45 to the plant pathogenic fungi *Colletotrichum higginsianum*, *C. camelliae*, *Fusarium oxysporum* f. sp. cubense race 4, *Alternaria solani*, *Magnaporthe oryzae*, and *Pestalotiopsis versicolor* pv. myricae. **[Results]** PxG45 in the gut of the 3rd instar larva of *P. xylostella* was identified as *Pantoea agglomerans*. Compared with the control group which was not fed with PxG45, supplemental PxG45 not only significantly increased the survival rate of the healthy 3rd instar larva of *P. xylostella*, but also improved the survival rate of the 3rdinstar larva infected by *B. bassiana*. The colonial diameters of *B. bassiana* and six plant pathogenic fungi in antagonistic culture with PxG45 were significantly smaller than those cultured alone of *B. bassiana* and six plant pathogenic fungi, and the growth of fungi was inhibited after antagonistic culture with PxG45. **[Conclusion]**PxG45 affects the adaptability of *P. xylostella* by direct and broad-spectrum antibacterial effects, and can significantly inhibit the growth of plant pathogenic fungi. This study demonstrates that PxG45 possesses the potential as a biocontrol bacterium and provides a new way to use the gut symbionts of pest.

Keywords: Plutella xylostella;Pantoea agglomerans PxG45;improved survival rate;antifungal activity

PsmFAR-W9, a fatty acyl-CoA reductase gene that affects wax secretion of Paurocephala sauteri Enderlein in mulberry tree

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Abstract: Paurocephala sauteri Enderlein (Hemiptera: Psyllidae), an important pest of mulberry trees in the tropical and subtropical regions, it secreted wax and honeydew fell onto the lower leaves in mulberry tree, which significantly affected photosynthesis of mulberry tree and the quality of mulberry leaves for silkworm cultivation. The fatty acyl-coenzyme A reductase (FAR) gene *PsmFAR-W9* was 1713 bp long. Expression of PsmFAR-W9 had a significant difference between male and female adults and nymphs. Baculovirus expression vector pFastBac-F9-E-mid could stably express in sf9 cells, expression of pFastBac-F9-E-mid was down-regulated by 95.9% after 48 hours of RNA interference. Feeding 5th instar nymphs with 50 nmol/L siRNA showed 88.87% of the nymphs stopped secreting wax in experimental group, however all nymphs in the control group can secrete wax normally, *PsmFAR-W9* expression was down-regulated by 77.88%. Interestingly, the activity of nymphs in the experimental group were gradually decreased, and the mortality rate of them reached to 30.60% 24 hours after feeding. Those findings suggested that Ss not only can reduce the wax secretion but affect the mortality of P. sauteri.
Keywords: Paurocephala sauteri;Wax secretion;fatty acyl-CoA reductase (FAR)