

Regulation of lipid metabolism in *Spodoptera frugiperda* by the symbiotic bracovirus of the gregarious parasitoid *Cotesia ruficrus*

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Abstract: Parasitoids alter host energy homeostasis to create a favorable environment for their own development. However, the mechanisms underlying this process remain largely unexplored, especially for gregarious parasitoids. *Cotesia ruficrus*, a gregarious endoparasitoid native to China, targets the invasive pest *Spodoptera frugiperda* (fall armyworm, FAW) and has been shown to effectively control FAW populations. This study investigates the role of the polydnavirus (PDV) produced by *C. ruficrus* in regulating lipid metabolism of FAW larvae. The results demonstrated that, following PDV injection for 5 days, both triglyceride concentrations and lipid droplet diameters in the fat bodies of FAW larvae significantly increased. RNA interference (RNAi) targeting the PDV gene CrBV3-31 led to a reduction in triglyceride concentrations and lipid droplet size, along with an upregulation of the LSD1 gene. Furthermore, silencing CrBV3-31 decreased triglyceride levels in *C. ruficrus* pupae and lowered its eclosion rate. These findings suggest that the PDV gene CrBV3-31 plays a crucial role in enhancing lipid accumulation in FAW larvae, thereby supporting the survival of *C. ruficrus* offspring. This study uncovers a novel mechanism by which gregarious endoparasitoids exploit symbiotic bracovirus genes to regulate host energy metabolism, increasing lipid levels to meet the developmental needs of their multiple offspring.

Keywords: *Cotesia ruficrus*; *Spodoptera frugiperda*; Polydnavirus; CrBV genome; lipid metabolism

The pseudogerm in polyembryonic *M. cingulum* functions to modulate host physiology

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Abstract: Endoparasitoid wasps have evolved diverse strategies to counter host immune defenses. While the functional mechanisms of certain parasitic factors have been well characterized, the broader strategies employed by most endoparasitoids remain largely unexplored. Here, we report a novel parasitic factor—the pseudogerm, a syncytial structure likely derived from the polar body in the polyembryonic wasp *Macrocentrus cingulum* and its function.

We first examined DAPI staining during cleavage and early embryogenesis. The results showed that the polar bodies are retained and contribute to the formation of both embryos and pseudogermes. The syncytial nuclei of the pseudogerm are noticeably larger than the egg nuclei and divide more slowly during early cleavage. Secondary embryonic cells containing only polar body nuclei develop into syncytial pseudogermes, whereas cells containing both polar body nuclei and egg nuclei give rise to embryos. In this process, the egg nucleus directs the formation of the embryonic body, while the polar body nuclei guide the development of the syncytial extraembryonic membrane.

The cytoplasm of the pseudogerm is densely packed with endoplasmic reticulum and mitochondria and covered externally with microvilli, indicating strong secretory and absorptive capacities. In vitro culture and ultrastructural analyses indicate that pseudogermes can secrete exosomes. Sequencing of microRNAs from pseudogermes, embryos, and host hemolymph at different time points after parasitization shows that both pseudogermes and embryos release large amounts of microRNAs into the host hemolymph, among which 37 microRNAs are significantly upregulated post-parasitization and 26 are detected within exosomes secreted by pseudogermes. Proteomic and transcriptomic analyses further confirm that pseudogermes express a wide range of secretory proteins.

Embryo transplantation experiments demonstrate that pseudogermes can inhibit host cellular immune responses against wasp larvae. When five pseudogermes were co-transplanted with wasp embryos into naïve larvae of *Ostrinia furnacalis*, the encapsulation rate of the hatched wasp larvae decreased from 99% to 0%, furthermore, the pupation rate of host larvae decreased to 0 too, indicating that pseudogerm can inhibit host immune attack to wasp larvae and modulate the development of host larva to promote wasp larval survival within the host hemocoel. Further investigations revealed that miR-281-5p can suppress the expression of its target gene Apolipoprotein in the host across species boundaries, thereby reducing its regulatory effect on hemocyte adhesion and inhibiting hemocyte encapsulation of wasp larvae. Additionally, pseudogermes can disrupt the host prothoracic gland, leading to decreased 20E production and suppression of host pupation (Zhang et al., 2025). miR-281-5p also downregulates E93 expression in the host, further interfering with pupation (under revision). These results indicate that microRNAs secreted by pseudogermes play an important role in manipulating host physiological processes, although the functions of many other microRNAs remain to be elucidated. Moreover, during the late developmental stages of the wasp larvae, pseudogermes can serve as a nutritional resource when consumed by the larvae, providing supplemental nourishment (Hu et al., 2015).

Our findings uncover a unique strategy that polar body originate parasitic factor, pseudogerm, manipulates host immunity and development to promote the survival of wasp larvae in host. These results provide further insights into the co-evolutionary mechanisms between parasitoid wasps and their hosts.

Keywords: Parasitoid; parasitic factor; pseudogerm; microRNAs; modulation

Parasitoid wasps recruited a bacterial gene that governs venom against host defence

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Genetic Improvement of Fecundity Traits and Molecular Mechanisms in *Trichogramma dendrolimi*

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Abstract: *Trichogramma dendrolimi* is an egg parasitoid and natural enemy widely employed for the biological control of various lepidopteran pests. The Wolbachia-induced thelytokous reproductive strain of *T. dendrolimi* exhibits significant pest control potential and holds promising applications in biological control. The reproductive traits of *Trichogramma* serve as crucial indicators for assessing its effectiveness in field pest control. Therefore, elucidating the molecular regulatory mechanisms underlying fecundity in *Trichogramma* is of paramount importance. Additionally, achieving targeted genetic enhancement of fecundity in the thelytokous strains of the pine caterpillar red-eye wasp is crucial for improving the efficacy of *Trichogramma* biocontrol in field conditions. This study compares the reproductive capacities of *T. dendrolimi* from different geographical populations to identify high-fertility (TdH) and low-fertility (TdL) strains. Hybridization and backcrossing experiments were conducted to determine the genetic inheritance patterns of reproductive traits in *T. dendrolimi*. BSA-seq analysis was employed to identify candidate genes regulating the reproductive traits in *T. dendrolimi*. RNAi technology was applied to silence the expression of candidate genes associated with reproductive capacity, thereby assessing their effects on fertility and exploring the regulatory roles of JH and 20E in the reproductive capacity of *T. dendrolimi*. Gradual introgression hybridization and molecular marker-assisted selection were employed to achieve targeted genetic improvement of the reproductive capacity in the thelytokous strain of *T. dendrolimi*. This thesis provides a theoretical foundation for the genetic improvement research on other natural enemy insects.

Keywords: *Trichogramma dendrolimi*; Fecundity; Genetic improvement

The Dual Regulation of Insulin Signaling and Juvenile Hormone Pathway in Reproductive Diapause of the Natural Enemy, Seven-Spotted Ladybeetle (*Coccinella septempunctata*)

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Abstract : Understanding the regulation of diapause in natural enemy insects is crucial for improving the long-term storage, transportation, and field deployment of biological control agents. This study investigates the dual regulation of insulin signaling (IIS) and juvenile hormone (JH) pathways in reproductive diapause of the seven-spotted ladybeetle (*Coccinella septempunctata*), a key aphid predator. We demonstrate that enhanced JH degradation plays a critical role in diapause induction. Applying methoprene (a JH analog) to pre-diapause females elevated JH signaling and reversed the diapause program, confirming that low JH levels are essential for diapause induction. Conversely, RNAi knockdown of JH degradation genes increased expression of JH-inducible gene (*Kr-h1*), reduced diapause characteristics, and promoted reproduction (accelerated ovary growth, yolk deposition, suppressed lipid accumulation). Further, we show that IIS regulates diapause via the transcription factor FoxO. In diapausing beetles, exogenous insulin application or RNAi knockdown of *CsFoxo* terminated diapause. Conversely, in non-diapausing beetles, RNAi knockdown of the insulin receptor (*CsInR*) arrested ovarian development and decreased JH levels, inducing a diapause-like state. Collectively, these results reveal that shut down of insulin signaling activates *CsFoxO*, driving the diapause phenotype, while reduced JH titer facilitates reproductive arrest. This dual regulatory mechanism provides fundamental insights into *C. septempunctata* diapause control.

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

Nanocarrier-mediated transdermal delivery of *Lmidgf4* dsRNA expedites biological control of locusts by *Beauveria bassiana*

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Abstract: Locusts have been a major global agricultural pest that poses a serious threat to crop and livestock production. Entomopathogenic fungi (EPF) provide an eco-friendly control method; however, their efficacy usually takes slow and is unstable. To achieve an enhancement of the biocontrol efficacy of *Beauveria bassiana* (*B. bassiana*) against locusts, we developed a new strategy by which *B. bassiana* and nanocarrier-mediated dsRNA are co-applied across the locust cuticle. The nanocarrier star polycation (SPc) effectively delivers *Lmidgf4* dsRNA (ds*Lmidgf4*) into the locust, which targets *Locusta migratoria* *imaginal disc growth factor 4* (*Lmidgf4*). SPc protects ds*Lmidgf4* from degradation by the hemolymph and enables efficient gene silencing. Furthermore, SPc has no adverse effects on *B. bassiana* spore germination and growth. *Lmidgf4* interference leads to a thinner layer of endocuticle, thus facilitates infection of *B. bassiana*, and finally reduces the median lethal time of locusts infected with *B. bassiana*. In conclusion, the combination of *B. bassiana* and dsRNA/SPc complex overcomes the slow action of fungi, providing a novel strategy for field control of locusts.

Keywords: Nanocarrier; Transdermal delivery; Fungi; Imaginal disc growth factor 4; DsRNA; Locust

Effects of host age, parasitoid age, and superparasitism on reproductive fitness of *Macrocentrus cingulum* (Hymenoptera: Braconidae), a larval polyembryonic parasitoid

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Abstract: The Asian corn borer, *Ostrinia furnacalis* (Guenée), is an important pest of maize that is found throughout Asia, Australia, Africa, and the Western Pacific Islands. Larvae of *O. furnacalis* feed all parts of corn plants, and boring in ears and stalks can cause severe damage. Several reports indicate this pest has caused 40-60% yield losses in the Philippines and 6-9 million tons of lost yield annually in China. *Macrocentrus cingulum* Brischke (Hymenoptera: Braconidae) is a polyembryonic endoparasitoid that is a promising species for control of *O. furnacalis* larvae. *M. cingulum* can deposit more than one egg in one host larvae, producing several hundred embryos and early-instar larvae through clonal division inside the host, but only 40-90 progenies emergence from the host eventually. Thus, intraspecific competition among immature larvae for host resources occurred frequently.

We investigated the main factors likely to influence progeny production by *M. cingulum* when reared in *O. furnacalis*, i.e., host age and parasitoid age, focus on effects on the parasitism rate, brood size, and progeny sex ratio. We also examined the influence of superparasitism on brood size during both the larval and adult periods. These findings facilitate the mass rearing of *M. cingulum*, and suggest conditions useful to maximize production of this polyembryonic parasitoid for releases against *O. furnacalis*.

Keywords: biological control; reproductive efficiency; polyembryonic parasitoid

Multi-level Investigation of Diapause in *Arma chinensis*: Transcriptomics, Metabolomics, and Functional Studies

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Abstract: The predatory stinkbug *Arma chinensis* is an important biological control agent whose diapause mechanisms are crucial for storage and field application. We conducted an integrated multi-omics study combining transcriptomic, metabolomic, and functional analyses to elucidate diapause regulation in this species. Transcriptomic and metabolomic profiling revealed 9,254 differentially expressed genes and 493 altered metabolites across diapause stages. Major metabolic pathways including glutathione metabolism, TCA cycle, glycolysis, and lipid metabolism underwent significant reorganization. Pre-diapause was characterized by increased lipid accumulation and energy consumption, while maintenance phase showed amino acid and glucose metabolism restructuring. We identified the dopamine D2-like receptor (AcDD2R) as a key neuroendocrine regulator. AcDD2R expression peaked in head tissue during pre-diapause. RNA interference demonstrated that AcDD2R negatively regulates lipid accumulation by suppressing lipogenic genes (acetyl-CoA carboxylase and fatty acid synthase). DD2R knockdown not only increased lipid storage but also enhanced post-diapause reproductive fitness, shortening pre-oviposition period and increasing egg production and hatch ability. This comprehensive characterization reveals coordinated metabolic reprogramming and neuroendocrine control of diapause in *A. chinensis*. The identification of DD2R as a regulator of both lipid metabolism and reproductive fitness provides potential targets for optimizing diapause manipulation in biological control programs.

Sorbitol Suppression in *Spodoptera frugiperda* Enhances Parasitoid Development and Fitness

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Abstract: Parasitoid parasitism reprograms host metabolism to nourish the developing offspring, yet its impact on alternative carbohydrate pathways, such as the polyol pathway, is poorly understood. Here, we demonstrate that a generalist parasitoid wasp *Meteorus pulchricornis* suppresses sorbitol contents in its host *Spodoptera frugiperda* larvae, by downregulating polyol metabolism in the fat body. Two aldose reductase genes, *AKR1B1-1* and *AKR1B1-2*, responsible for catalyzing glucose-to-sorbitol conversion, are identified as key mediators of this metabolic shift. Furthermore, sorbitol restoration in hosts disrupts parasitoid larval development and reduces its fitness, including a decrease in pupation rate, reduced cocoon weight, smaller adult size and lower parasitism rates. Transcriptomic analysis and the fluctuating wasp ROS level suggest that the elevated host circulating sorbitol may disrupt redox homeostasis in developing wasp larvae. These findings provide new insights into host-parasite metabolic interactions centered on polyol metabolism and lay the groundwork for enhancing parasitoid-based biological control strategies.

Keywords: Host-parasite interaction; sorbitol; polyol pathway; aldose reductase; parasitoid fitness