The molecular mechanisms by which symbionts manipulate the whitefly reproduction

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Abstract: The whitefly *Bemisia tabaci* MED and MEAM1 are two of the most significant and invasive pests in the global agriculture. These whiteflies carry the obligate symbiont *Portiera* and *Hamiltonella* in their bacteriocytes and harbor *Rickettsia* in their whole bodies. *Hamiltonella* impacts the sex ratio of the whitefly *B. tabaci*. *Rickettsia* influences both fecundity and sex ratio of the whitefly *B. tabaci*. We demonstrated that *Hamiltonella* induces a female-biased sex ratio in *B.tabaci* by facilitating fertilization and the provisioning of five B vitamins. We further demonstrated that *Hamiltonella*-derived folate regulates host histone methylation modifications, which thereby impacts host ovary mitochondrial function, and finally determines whitefly sex ratio. We found that *Rickettsia* results in increased whitefly fecundity and female-bias by stimulating juvenile hormone synthesis. We also revealed that *Rickettsia* has been integrated into metabolic collaboration between whitefly and obligate symbiont *Portiera* for synthesis of essential aminoacids, which benefits whitefly reproduction. Overall, two intracellular symbionts *Hamiltonella* and *Rickettsia* regulate different reproduction traits of whiteflies by using distinct strategies.

Interactions Between Honeybee Gut Microbiota and Their Role in Host Adaptation

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Abstract: The symbionts of insects may play a significant role in host adaptation and urvival. To test this, we developed a honeybee gut microbiota platform that supports both the *in vitro* cultivation of bee gut bacteria and the *in vivo* inoculation of specific bacterial mixtures into germ-free honeybees. Our recent work has revealed intimate interactions among honeybee gutbacteria, as well as between the symbionts and their host. These include bacterial collaboration in pectin digestion, coexistence strategies between closely related bacterial strains, bacterial contributions to maintaining host metabolic homeostasis, and host immune regulation of microbial specificity. Importantly, by framing the research within the context of host adaptation and evolution, we began to understand how this interacting entity copes with environmental challenges. We demonstrated that dietary variations play a dominant role in shaping the gut microbiota of honeybees across their distribution range. This was further supported by finding in overwintering honeybee colonies, where an abrupt dietary shift from pollen-rich food to primarily nectar (honey) leads to an annual turnover in the gut microbial community. The bacteria enriched during winter may, in return, supplement the honeybee with microbial products that help overcome seasonal nutrient deficiencies. Further investigation at the *Apis* genus level suggested that the enrichment of certain core bacterial tax a may contribute to the cold adaptation of hosts, facilitating their survival to temperate climates.

Delftia tsuruhatensis as a dual-purpose bacterial symbiont for blocking Plasmodium and Leishmania transmission

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Abstract: The symbiotic bacterium *Delftia tsuruhatensis* TC1, originally isolated from *Anopheles* mosquitoes refractory to *Plasmodium falciparum*, colonises the gut of both mosquitoes and the sand fly *Phlebotomus duboscqi* without fitness costs. In mosquitoes, *D. tsuruhatensis* secretes the small hydrophobic alkaloid harmane, which penetrates the cuticle to arrest early *Plasmodium* stages. In sand flies, colonisation induces gut dysbiosis that indirectly impairs *Leishmania major* development and reduces vector competence; infected flies transmit fewer parasites and fail to establish murinelesions. Semi-field trials and mathematical modelling indicate that *D.tsuruhatensis*-based intervention could suppress malaria and leishmaniasis endemicity. These findings establish *D. tsuruhatensis* as a single, non-genetically modified microbial agent for dual vector control.

Hosts manipulate metabolism and pathogenicity heterogeneity of microbiome based at the single-cell resolution

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Abstract: Many animals have complicating interactions with their resident microbes that profoundly affect many aspects of host physiopathology. However, the reversible influence of the host on the composition and function of the microbiome has received less attention. Using Drosophila-symbiont model, we found that Drosophila larvae efficiently outcompete their symbionts by reducing bacterial loads in the niche. Furthermore, Drosophila larvae reshape the transcriptomic and metabolic profiling of symbionts. Bacteria manifest phenotypic heterogeneity among individual bacterial cells, but gene expression of bacterial cells has been traditionally investigated in bulk or on a population level. Bacterial single-cell RNA-seq technique is revolutionizing the study of phenotypic cell-to-cell variations in bacterial population. Indeed, the host alters pathogenicity and proliferation heterogeneity of symbionts at the single-cell resolution. Altogether, our findings provide an insight into the pivotal roles of the host in harnessing the life history and heterogeneity of symbiotic bacterial cells, advancing knowledge of advance fundamental concepts of precise manipulation of bacterial communities.

Keywords: Bacterial single-cell RNA-seq; Transcriptomics; Microbiome; Heterogeneity; Pathogenicity

The Dynamic Interaction Among Necrophagous Insects, Microbial Communities, and Cadavers

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Abstract: Necrophagous insects and microbial communities synergistically mediate the decomposition of cadavers. Understanding the interactions among diet, gut microbiota, and necrophagous insects forms the foundation for deeper insights into the tripartite relationship of cadaver–microbiota–insect dynamics. To investigate these interactions, our team conducted both laboratory and field studies.

In the laboratory, *Sarcophaga africa*, a forensically significant necrophagous species, was selected to explore how foodborne microorganisms influence insect development and their implications for forensic science. Our findings demonstrated that pig manure effectively supports the establishment of *S. africa* laboratory colonies. Analysis revealed that dietary microbes significantly shape the larval gut microbiota. Preliminary results suggest that gut microbes derived from pig manure—particularly *Clostridium_sensu_stricto_1*—may accelerate larval development by directly increasing protein content in third instar larvae and indirectly reducing glycogen levels in late pupal stages.

Building on these findings, field experiments were conducted to examine the role of necrophagous insects in decomposition processes mediated by microbial activity. Comparisons between pig carcasses decomposing indoors (open to insect access) and outdoors (natural conditions) revealed similar decomposition patterns and microbial community dynamics, despite environmental differences. Oral and skin microbiota changed in parallel across both settings, suggesting that insect activity contributes significantly to the consistency of microbial succession during decomposition. Notably, when insect colonization was delayed by just one day, a corresponding one-day delay in both decomposition and microbial community progression was observed.

Furthermore, we successfully established an insect-exclusion model indoors for the first time, allowing direct comparison with an insect-accessible group. Preliminary observations indicated that in the absence of insect colonization, carcasses underwent only putrefaction and liquefaction while largely retaining their original structure. Further detailed investigation of this model is ongoing.

Overall, these long-term studies demonstrate that insect arrival significantly influences the decomposition process, which in turn affects postmortem interval (PMI) estimation. Therefore, elucidating the dynamic interactions among necrophagous insects, microbial communities, and cadavers is essential for a more comprehensive understanding of decomposition and for improving the accuracy of PMI estimation in forensic practice.

Keywords: Necrophagous insects;Microbial Communities;Cadavers;Postmortem interval estimation

Wolbachia infected Aedes albopictus males regulate the uninfected females' fecundity through rpz gene

Guanhong Wang

Bees as reservoir for Varroa destructor mediated pathogenic bacterium transmission

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Abstract: *Varroa destructor* is a primary driver of honey bees (*Apis mellifera*) failure. Its ectoparasitic feeding behavior damages bees and facilitates the transmission of various pathogens, disrupting normal bee development and leading to colony weakening or even collapse. This study focuses on *V. destructor* and its associated pathogenic bacteria, investigating their virulence levels and transmission mechanisms between bees and mites to elucidate their role as vectors in pathogen dissemination. First, the mite-associated bacterium *Morganella morganii* CYJ1 was isolated and assembled. To assess its toxicity to bees, gradient concentrations of bacterial suspension were injected into honey bees. Results demonstrated a positive correlation between bacterial concentration and bee mortality. CFU quantification revealed that surviving bees harbored *M. morganii* CYJ1 levels below 1×10^{10}, whereas deceased bees consistently exceeded this threshold, suggesting 1×10^{10} as a potential lethal infection threshold. Disk diffusion assays indicated complete resistance of *M. morganii* CYJ1 to ampicillin. Using fluorescent protein-labeled *M. morganii* CYJ1, further investigation was conducted on bacterial transmission between bees and mites, evaluating the mite’s vector competence, transmission efficiency, and routes. Results confirmed that horizontal transmission among co-feeding bees was ineffective, whereas *V. destructor* successfully acquired *M. morganii* CYJ1 from infected bees and transmitted it to healthy ones. CFU data underscored the mite’s role in exacerbating bacterial pathogenicity, severely compromising bee health.

Keywords: Mite;bee;engineering;pathogen;fluorescent protein

Exploring Microbe-Based Approaches for Mosquito-Borne Diseases Control

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Abstract: Mosquitoes serve as vectors of numerous severe infectious diseases, including malaria, denguefever, Zika, and chikungunya fever, yet effective vaccines or therapeutics arelacking. Vector control remains the primary strategy for disease prevention. Our research focuses on the interactions between mosquito and microorganisms. We investigate how fungi and bacteria that naturally associate with mosquitoes regulate host development, immunity and metabolism, and leverage these interactions to devise new disease-control tools. Our research indicate that (1) we find that small non-coding RNAs move between mosquitoes and entomopathogenic fungi, mediating infection and defense interactions through cross-kingdom RNAi; (2) based on the cross-kingdom RNAimechanism, we engineer entomopathogenic fungi to express mosquito-specific small RNAs, thereby markedly increase their lethality against to mosquitoes; (3) we develop a paratransgenesis strategy that employs symbiotic bacteria to produce and deliveranti-pathogen effectors for combatingmosquito-bornediseases.

Keywords : mosquito, entomopathogenic fungi, gut symbiotic bacteria, cross-kingdom RNAi, paratransgenesis

Role of Rice Planthoppers in Vectoring Pathogenic Gut Bacteria to Rice Crops

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Abstract: Pantoea ananatis, a bacterium prevalent in agronomic crops and pests, demonstrates significant ecological complexity as evidenced by the Lstr strain isolated from the rice planthopper Laodelphax striatellus (SBPH). Our genomic analysis reveals P. ananatis comprises two distinct clades: Clade 2 strains (including Lstr, primarily from rice-associated sources) exhibit smaller genomes, reduced coding sequences, increased pseudogenes, and unique mobile elements compared to Clade 1, suggesting ongoing genome reduction. Both clades retain a highly variable type VI secretion system. Crucially, Lstr functions as a dual-pathogen—pathogenic to its insect host while inhabiting the gut, and experimentally confirmed via Koch’ s postulates as a rice pathogen causing leaf blight with severity comparable to Xanthomonas oryzae. Transmission studies confirm SBPH serves as an active vector: Lstr accumulates within planthoppers, transmits to rice plants inducing blight, and spreads horizontally among SBPH populations. This establishes SBPH as a reservoir and transmission vehicle for P. ananatis, facilitating its endemic persistence in rice ecosystems. Collectively, these findings illuminate P. ananatis' evolutionary adaptation through genomic streamlining, clarify its insect-plant disease cycle, and provide critical insights for predicting and managing bacterial blight outbreaks in rice and other crops.

Keywords: Pantoea ananatis ;Laodelphax striatellus;rice

The plant-sucking insect Riptortus pedestris selects assembly of gut microbiota from environment to confer host beneficial fitness

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Abstract: Plant-sucking insects have intricate associations with a diverse array of microorganisms to facilitate their adaptation to specific ecological niches. The midgut of phytophagous true bugs is generally structured into four distinct compartments to accommodate their microbiota. Nevertheless, there is limited understanding regarding the origins of these gut microbiomes, the mechanisms behind microbial community assembly, and the interactions between gut microbiomes and their insect hosts. In this study, we conducted a comprehensive survey of microbial communities within the midgut compartments of a bean bug Riptortus pedestris, soybean plant, and bulk soil across 12 distinct geographical fields in China, utilizing high-throughput sequencing of the 16S rRNA gene. Our findings illuminated that gut microbiota of the plant-sucking insects predominantly originated from the surrounding soil environment, and plants also play a subordinate role in mediating microbial acquisition for the insects. Furthermore, our investigation suggested that the composition of the insect gut microbiome was probably shaped by host selection and/or microbe-microbe interactions at the gut compartment level, with marginal influence from soil and geographical factors. Additionally, we had unveiled a noteworthy dynamic in the acquisition of core bacterial taxa, particularly Burkholderia, which were initially sourced from the environment and subsequently enriched within the insect midgut compartments. This bacterial enrichment played a significant role in enhancing insect host reproduction. These findings contribute to our evolving understanding of microbiomes within the insect-plant-soil ecosystem, shedding additional light on the intricate interactions between insects and their microbiomes that underpin the ecological significance of microbial partnerships in host adaptation.

Keywords: Insect-plant-soil ecosystem;Community assembly;Insect–microbe interaction;Host reproduction

Functional and mechanistic insights into the gut microecology of chironomid larvae under pesticide stress

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Abstract: As an essential agricultural input, the use of insecticides has evolved in tandem with our growing awareness of ecological impacts. Through surface runoff, irrigation, and other dispersal pathways, pesticide residues exert significant ecological risks on aquatic ecosystems, raising widespread environmental concerns. Chironomidae (Diptera) represent the most prevalent and abundant insect family in freshwater ecosystems worldwide. As a dominant benthic functional group, chironomid larvae play vital roles in biogeochemical cycling and energy transfer. However, the ecotoxicological effects of pesticides on these keystone organisms remain poorly understood. "In this study, we investigated the functional responses and underlying mechanisms of gut microbiota in chironomid larvae exposed to three distinct pesticides: chlorpyrifos, thiamethoxam, and chlorantraniliprole. First, we demonstrated that antibiotic-induced depletion of commensal microbiota exacerbated pesticide toxicity in chironomid larvae. Second, semi-lethal concentrations of chlorpyrifos, thiamethoxam, and chlorantraniliprole treatment remarkably decreased the relative abundances of beneficial bacterial strains (e.g. Cetobacterium and Tyzzerella) while significantly increase the prevalence of opportunistic pathogens, including the genus Serratia, Shewanella, Aeromonas and Pseudomonas. An evident variability of bacterial correlations was observed, and pesticide exposure impaired the genus-genus interaction and destabilized the whole community structure. Third, Pearson correlation analysis demonstrated that key metabolites in energy and detoxification pathways, including amino acid metabolism, fatty acid oxidation, ABC transporters, and glycolysis, showed significant negative correlations with opportunistic pathogen proliferation. Moreover, pesticide exposure induced significant intestinal histopathological alterations, including discontinuous peritoneal membranes and exfoliated columnar epithelial cells. Collectively, our findings demonstrate that exposure to three distinct classes of pesticides all induces physiological impairments in chironomid larvae, mediated through gut microbiota dysbiosis and subsequent disruption of energy metabolism.

Keywords: pesticide;chironomid larvae;gut microbiota;energy metabolism;amino acid and fatty acid metabolism

Interactions between the honeybee immune system and gut bacteria

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Abstract: Honeybee workers maintain a stable gut microbiota that interacts closely with honeybee immunity. Honeybees regulate their immune system precisely to maintain the colonization of host-specific gut symbiotic bacteria and create an inhospitable environment for foreign bacteria, ensuring symbiotic specificity. Inoculating honeybees with the gut bacteria Gilliamella strain from bumblebees increases prostaglandin levels in the honeybee gut, activating the honeybee immune response via the immune deficiency and Toll pathways. This leads to elevated Duox expression, reactive oxygen species production, and suppression of the bacteria strain from bumblebees. Additionally, gut bacteria contribute to honeybee immune defense by producing butyrate, enhancing honeybee resistance to pathogens. This process involves butyrate-mediated gene expression regulation, which stimulates the production of immune-related prostaglandins, thereby increasing systemic cellular and humoral immune responses in honeybees. Overall, the interaction between honeybees and gut bacteria represents a dynamic equilibrium that maintains symbiotic stability and enhances honeybee resilience against pathogens, underscoring the intricate nature of host-microbe interactions.

Keywords: gut microbe;immune response;honeybee;host-microbe interaction

The influence of gut microbiota on Peritrophic matrix (PM) formation

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Abstract: The PM is an acellular layer formed when mosquitoes ingest a blood meal. It is composed of chitin, proteins, and glycoproteins， which provides protection for the midgut epithelium by separating the blood bolus from the epithelial cells. However, the factors that influence PM formation in most insect vectors remain unclear. Our study found that PGNs (peptidoglycans) and LPS (lipopolysaccharides), which are typical components of bacteria cell walls, play an important role in regulating PM formation. The recognition of LPS and PGNs in the mosquito midgut by PGRP striggers the Toll and IMD pathways. This leads to the binding of Rel1 and Rel2 to the promoter region of Per1, promoting PM formation. Tryptophan (Trp) is an essential amino acid that is mainly supplied by blood meals. And the majority of free Trp is oxidized through the kynurenine pathway, resulting in the production of kynurenine (Kyn), 3-hydroxykynurenine (3-HK), and xanthurenic acid (XA). However, little is known about the contribution of the microbiota on tryptophan metabolism in mosquitoes. We found that the symbiont *P. alcaligenes* has ability to catabolize 3-HK, which impairs PM formation, thereby protecting the midgut from pathogen infection. Thus, the microbiota of mosquito midgut plays an important role in maintaining homeostasis by regulating the integrity of the PM structure.

Symbiont-mediated resistance to pyrethroids in Aedes mosquitoes

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Abstract: Pesticide resistance in mosquito vectors is a severe and growing threat to human health. Although nuclear-based resistance mechanisms are well characterized for pyrethroid insecticides widely used against mosquitoes, it is unclear if symbiotic bacteria also influence resistance. Here, we demonstrate increased pyrethroid susceptibility in antibiotic-treated and axenic mosquitoes relative to conventionally reared mosquitoes. We isolated four previously uncharacterized symbiotic bacteria capable of in vitro degradation of pyrethroids through conserved carboxylesterases and of in vivo enhancement of pyrethroid resistance following re-supplementation to the mosquito surface or gut. These bacteria were more abundant in wild mosquitoes than in laboratory populations. Pre-treatment of adult mosquitoes with the fungicide pyraclostrobin suppressed the bacteria and led to increased pyrethroid susceptibility. Our findings highlight the potentially overlooked role for symbiotic bacteria in pyrethroid resistance.

Keywords: Aedes aegypti;Pyrethroid;symbiont

Bacteriocyte symbiont Hamiltonella impacts whitefly fertilization by regulating β-tubulin through small RNA

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Abstract: Nutritional symbionts manipulate host reproduction, but the underlying molecular mechanisms are largely unclear. We previously found that the bacteriocyte symbiont Hamiltonella impacts the sex ratio of the whitefly Bemisia tabaci. Here, we observed diminished F-actin and enhanced β-tubulin distribution pattern in ovaries of Hamiltonella-cured whiteflies. The cytoskeleton, comprising F-actin microfilaments and tubulin microtubules, plays a critical regulatory role in multiple key stages of the fertilization process. Previous studies have confirmed that the dynamic equilibrium and mutual regulation between F-actin and tubulin are essential for maintaining cytoskeletal function. Small RNAs (sRNAs), pivotal regulatory non-coding RNAs, broadly regulate diverse biological processes across organisms. This study demonstrates that one sRNA of the symbiont Hamiltonella is capable of regulating the whitefly β-tubulin mRNA levels. This regulatory effect further influences the F-actin pattern through the interplay between F-actin and β-tubulin, ultimately manipulating whitefly fertilization. The above regulatory mechanism was validated in whitefly Bemisia tabaci MEAM1 and MED, two globally invasive insect pests in agricultural.

Keywords: whitefly symbiosis;Hamiltonella;sRNA

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 replacedor complemented by other microorganisms. However, the biological significance and stabilityof these associations with newly acquired microbesis largely unknown. The East Asiancicada Platypleura kaempferi represents an ideal model for studying evolutionary dynamics in nutritional symbioses. Like mostcicada species, it harbors the ancient obligate symbionts Sulciaand Hodgkinia, along with anewly required Arsenophonus symbiont. Hodgkiniain this system exhibits unprecedented genomic disintegration, having lost ancestral genes encoding key conserved metabolic functions. It is not clear whether Arsenophonushas taken over the missing functions of Hodgkiniain 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 Hodgkiniain P. kaempferi exists in ahyper-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 retainsintact pathways for these vitamins alongside essential aminoacid 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 fatbodies, while Sulcia and odgkini are side in bacteriomes.These findings indicate P. kaempferi has evolved atripartite symbiosis where Arsenophonus functionally compensates for Hodgkinia &;metabolic deterioration. Metagenomics and amplicon sequencing data across geographically distinct hosts demonstrates unexpected stability of this three-partnersystem. 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; nutrition almutualism;symbiont compensation

Ecophysiological Role of Bacterial Symbionts in Mitigating Nicotine Toxicity for Nilaparvata lugens

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Abstract: Bacterial symbionts that exhibit co-evolutionary dynamics with insect hosts critically regulate pivotal physiological processes, including detoxification and immune responses. The tobacco-rice rotation system is a sustainable agricultural strategy. Nicotine from tobacco residues demonstrates significant efficacy in controlling *Nilaparvata lugens* (Brown planthopper, BPH), a major rice pest. However, the environmental adaptability of insects suggests that BPH may possess the capability in respond to the nicotine and develop resistance, the underlying mechanisms,however, remain unclear. From the perspective of insect-host symbiosis, high-throughput sequencing (HTS) showed that nicotine treatment significantly altered the diversity and community structure of endosymbiotic bacteria in BPH. The abundances of bacterial families including *Microbacteriaceae*, *Comamonadaceae*, and *Enterobacteriaceae* increased significantly. Furthermore, culturing BPH symbiotic bacteria with nicotine and detecting nicotine degradation rates via high-performance liquid chromatography (HPLC) demonstrated that multiple intestinal bacteria of BPH could efficiently degrade nicotine in vitro. Notably, *Delftia* NLG11 exhibited a 71% degradation rate after 72 hours. Exogenous injection experiments showed that *Delftia* NLG11 effectively degraded nicotine in vivo and significantly upregulated the expression of the detoxification gene *CYP6AY1*, thereby enhancing the survival rate of BPH on nicotine-containing rice plants. Furthermore, upon nicotine stimulation, the abundance of the symbiotic bacterium *Burkholderia cepacia* (BsNLG8) increased significantly. Plate confrontation assays and HPLC analyses showed that BsNLG8 exhibited strong antagonistic activity against pathogenic fungi (inhibition rate >80%) and significant nicotine degradation efficiency. Transcriptomic sequencing results indicated that a nicotine-supplemented diet triggered immune responses in BPH, with pattern recognition proteins and immune effector factors serving as the main responders. RNA interference (RNAi) and exogenous injection of BsNLG8 were employed to validate the immune response of BPH to nicotine stimulation, demonstrating an immunomodulatory interaction between BsNLG8 and *Defensin A*. Our findings underscore the critical role of bacterial symbionts in enhancing host adaptability and survival in response to nicotine stress, offering new targets for endosymbiont-based pest management strategies.

Keywords: Nilaparvata lugens;Nicotine;Gut bacteria;Defensin A;RNAi

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

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

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Abstract: The evolution of ant cuticle integrity is potentially modulated by specialized symbionts that enhance host adaptation. Here we characterize a novel intracellular Enterobacteriaceae symbiont in the polygynous ant Tetraponera sp.5, designated as the fifth Sodalis lineage in ants. Through metamorphosis, Sodalis colonizes queen ovarian tissues and fat bodies of non-reproductive castes, with transmission occurring vertically via oocyte invasion during late oogenesis (validated by FISH localization). Quantitative tracking revealed a tick-shaped population dynamic: minimal in larvae, rising in pupae, and peaking in adults/eggs—indicating stage-specific functional engagement. 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 symbiont 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

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

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Abstract: *Phortica okadai* (Diptera : Drosophilidae : Steganinae) is known as the only confirmed vector of *Thelazia callipaeda* (Spirurida: Thelaziidae) in China. Meanwhile, it is also recognized as a highly polyphagous pest. Not only does it inflict damage on various commercially significant fruits including pears, apples, bananas, and citrus, but it can also transmit thelaziasis to a variety of species such as rabbits, dogs, cats, the iconic giant pandas, and humans through lachryphagous behavior by feeding on their ocular secretions. 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 cylindro-oval and lack dorsal appendages. The larvae are vermiform, divided into 11 segments: the cephalic region, three thoracic (T1-T3) and seven abdominal (A1-A7). 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 (LB: Large basiconic sensilla, TB: Thin basiconic sensilla, and SB: Small basiconic sensilla) and chaetica (ChI, ChII) sensilla were distributed on both the antennae and the maxillary palps of *P. okadai*. The trichoid sensilla are single-walled, pore-bearing, and predominantly distributed along the flagellum of the antennae. They exhibit a relatively elongated, hair-like appearance, with their surfaces densely packed with pores and a bluntly rounded tip. The intermediate sensilla are positioned in length between the trichoid and basiconic sensilla, and like these, they have a surface densely packed with pores and a bluntly rounded tip. The chaetica sensilla in *P. okadai* are thick-walled, poreless sensilla, predominantly located on the scape and pedicel of the antennae. Based on a length criterion of 50 μm, they can be categorized into two types, ChI and ChII. The sensilla have smooth shafts with pronounced curvature and are inserted into a movable socket. The coeloconic sensilla are double-walled, characterized by a stout and irregular distribution along the flagellum of the antennae. They possess numerous fine, longitudinal cuticular fingers (CF) on their surface. These finger-like structures form a small cavity between their inner and outer walls, with multiple such structures creating a central cavity. Each sensilla has a shallow, circular pit at the base and a smooth surface, distinguished by a prominent longitudinal groove that extends from the base to the tip. The basiconic sensilla are single-walled, multiporous, generally conical in shape, tapering from the base to the tip, with a bluntly rounded apex, and the sensilla wall is studded with wall pores. Based on morphology and size, they can be classified into three subtypes: SB, TB, and LB. 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 Type II chaetica sensilla (ChII), Thin basiconic sensilla (TB), and Large basiconic sensilla (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