Bioscience Methods 2024, Vol.15, No.6, 302-314
http://bioscipublisher.com/index.php/bm

Research Insight Open Access

Innate Immune Response and Pathogen Defense Mechanisms in Earwigs: A Comprehensive Molecular Biology Analysis

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Bioscience Methods, 2024, Vol.15, No.6 doi: 10.5376/bm.2024.15.0030
Received: 20 Sep., 2024
Accepted: 31 Oct., 2024
Published: 21 Nov., 2024

Preferred citation for this article:
Xu J., and Xu Q.B., 2024, Innate immune response and pathogen defense mechanisms in earwigs: a comprehensive molecular biology analysis, Bioscience Methods, 15(6): 302-314 (doi: 10.5376/bm.2024.15.0030)

Abstract Earwigs have emerged as valuable model organisms for studying innate immunity in invertebrates, providing insights into the complex defense mechanisms against pathogens. This study focuses on the immune components of earwigs, including immune cells, organs, and pattern recognition receptors (PRRs), which play a critical role in pathogen detection and immune activation; identifies and characterized antimicrobial peptides (AMPs) in earwigs, exploring their mechanisms of action against bacterial, fungal, and viral pathogens. Additionally, the RNA interference (RNAi) pathway was examined for its role in viral suppression, highlighting its molecular regulation in earwig immunity. Comparative analysis of immune responses across various pathogens—bacteria, fungi, and viruses—was conducted to reveal unique immune features and evolutionary adaptations in earwigs. A case study of *Forficula auricularia* under pathogen stress provided further molecular insights into immune-related gene expression. The findings contribute to a broader understanding of invertebrate immunity, with potential applications in developing biopesticides and enhancing pest management strategies. This study underscores the significance of earwig immune studies for evolutionary biology and disease control.

Keywords Earwig immunity; Antimicrobial peptides; RNA interference; Pathogen defense; Immune regulation

1 Introduction

Earwigs, belonging to the order Dermaptera, are a fascinating group of insects known for their distinctive forceps-like appendages and complex social behaviors, including maternal care (Liu et al., 2022; Meunier, 2023). Despite their relatively small order size, earwigs exhibit a wide range of biological characteristics that make them an intriguing subject for scientific study. Recent advances in molecular tools and imaging techniques have highlighted earwigs as a valuable model for understanding various biological processes, including development and reproduction (Núñez-Pascual et al., 2022). The genetic diversity and evolutionary history of earwigs have been explored through phylogenetic and mitogenomic studies, revealing significant insights into their molecular characteristics and evolutionary relationships (Naegle et al., 2016; Wipfler et al., 2020). These attributes, combined with their ecological roles and behaviors, position earwigs as a promising model organism for studying innate immunity.

Innate immunity represents the first line of defense against pathogens in all animals, including invertebrates. Unlike vertebrates, invertebrates lack an adaptive immune system and rely solely on innate immune mechanisms to combat infections (Kangale et al., 2021). This type of immunity involves a range of physical barriers, cellular responses, and humoral factors that work together to recognize and eliminate pathogens (Romo et al., 2016; Yu et al., 2022). Studying innate immunity in invertebrates not only provides insights into the fundamental aspects of immune responses but also helps to understand the evolutionary origins of immune mechanisms that are conserved across different species (Guryanova and Ovchinnikova, 2022b). Invertebrates, such as earwigs, offer a unique perspective on innate immunity due to their diverse ecological niches and evolutionary adaptations.

This study conducts a comprehensive molecular biology analysis of the innate immune response and pathogen defense mechanisms in earwigs. By focusing on earwigs, this study elucidates the genetic and molecular basis of their immune responses, identifies key signaling pathways and immune-related genes, and understand how these mechanisms contribute to their ability to resist infections. This study will not only enhance our knowledge of earwig biology but also provide broader insights into the evolution and function of innate immunity in invertebrates. This study aims to uncover novel aspects of earwig defense mechanisms that could have implications for pest control and the development of new antimicrobial strategies.

2 Earwig Immune System Components

2.1 Immune cells and organs in earwigs

The immune system of earwigs, like other insects, comprises both cellular and humoral components. The primary immune cells involved are haemocytes, which play crucial roles in various immune responses such as phagocytosis, nodulation, and encapsulation. These cells are essential for limiting pathogen movement and replication within the insect body (Eleftherianos et al., 2021a). Additionally, the fat body, analogous to the liver in vertebrates, is a significant organ in the humoral immune response, producing antimicrobial peptides that are secreted into the hemolymph to combat infections (Eleftherianos et al., 2021b).

2.2 Role of pattern recognition receptors (PRRs) in pathogen detection

Pattern recognition receptors (PRRs) are critical for the detection of pathogens in earwigs. These receptors identify pathogen-associated molecular patterns (PAMPs), which are conserved molecular signatures found on pathogens. The recognition of PAMPs by PRRs triggers the activation of various immune responses. In insects, PRRs such as Toll-like receptors and other signaling molecules are involved in initiating immune responses against bacterial, fungal, and viral infections (Smith et al., 2019). The activation of these receptors leads to the production of antimicrobial peptides and other defensive molecules that help in neutralizing the pathogens (Yu et al., 2022).

2.3 Signaling pathways involved in immune response

Several signaling pathways are involved in the immune response of earwigs. The Toll pathway, Imd pathway, JNK pathway, and JAK/STAT pathway are some of the key signaling mechanisms that regulate the immune responses in insects (Figure 1). These pathways coordinate the production of antimicrobial peptides and other immune effectors in response to pathogen detection (Romo et al., 2016). The Toll pathway, for instance, is primarily activated by fungal and Gram-positive bacterial infections, leading to the production of specific antimicrobial peptides. The Imd pathway, on the other hand, is activated by Gram-negative bacterial infections and also results in the production of antimicrobial peptides. Additionally, the phenoloxidase (PO) cascade is another crucial component of the insect immune system, involved in melanization and the production of reactive intermediates that are toxic to pathogens (González-Santoyo and Córdoba-Aguilar, 2012).

The study of Yu et al. (2022) illustrates the complex interaction between key immune pathways in *Drosophila*'s innate immunity system, specifically the Toll, Imd, JNK, and JAK/STAT pathways. These pathways are shown to regulate immune responses such as antimicrobial peptide production, melanization, cell proliferation, and apoptosis. The final immunological functions, ranging from antimicrobial defense to anti-tumor activity and wound healing, highlight the broad protective roles of these pathways. This detailed network underscores the sophisticated nature of immune regulation and its various physiological impacts in *Drosophila*.

By understanding these components and mechanisms, we can gain insights into the complex and efficient immune system of earwigs, which allows them to effectively defend against a wide range of pathogens.

3 Antimicrobial Peptides (AMPs)

3.1 Types of AMPs identified in earwigs

Antimicrobial peptides (AMPs) are a diverse group of molecules that play a crucial role in the innate immune system of earwigs. These peptides are typically short, cationic, and AMPhipathic, allowing them to interact with microbial membranes effectively. In earwigs, several types of AMPs have been identified, including defensins, cecropins, and attacins. Defensins are characterized by their cysteine-stabilized α/β structure, while cecropins are linear peptides known for their potent antibacterial activity. Attacins, on the other hand, are glycine-rich peptides that exhibit a broad spectrum of antimicrobial properties (Ageitos et al., 2017; Stączek et al., 2023).

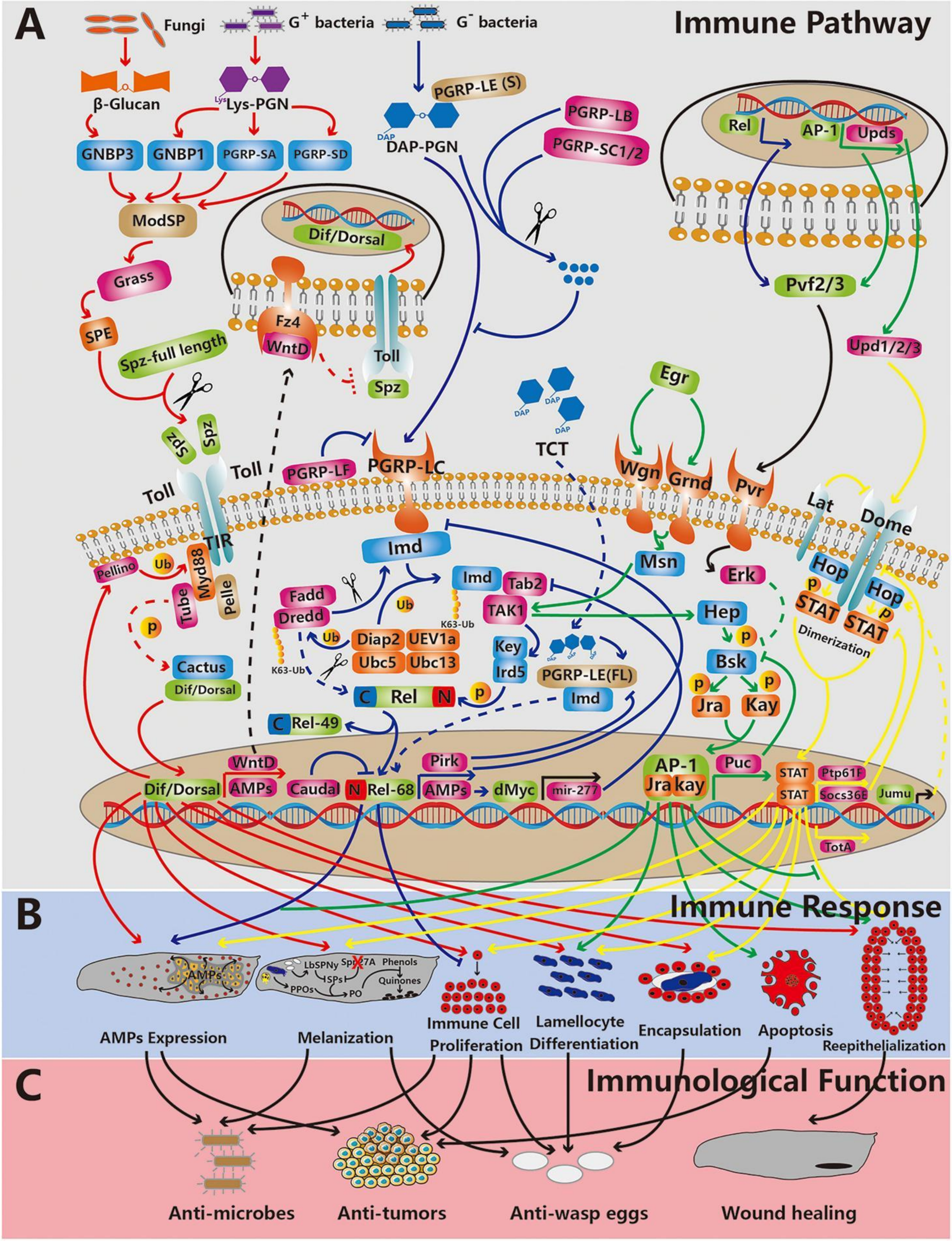

Figure 1 The relationship between main immune pathways, immune responses and immunological functions in *Drosophila* innate immunity. From top to bottom, the three rows represent the immune pathway (the Toll, Imd, JNK and JAK/STAT pathways are depicted,from left to right) (A), the immune response (B) and immunological functions (C). The red, blue, green and yellow lines indicate that the processes are related to the Toll, Imd, JNK and JAK/STAT pathways, respectively. The black lines indicate that the processes are related to other pathways. The dotted lines represent processes waiting to be confirmed. The arrows and “T” indicate promoting and inhibitory effects, respectively. The red cross indicates depletion. The scissors represent cleavage. Abbreviation: p, phosphorylation; Ub, ubiquitination; (s), short form; and (f), full length (Adopted from Yu et al., 2022)

3.2 Mechanism of action of AMPs against bacteria, fungi, and viruses

AMPs employ various mechanisms to combat bacteria, fungi, and viruses. The primary mode of action involves the disruption of microbial membranes. This can occur through several mechanisms, including the barrel-stave, carpet, and toroidal pore models. In the barrel-stave model, AMPs insert themselves into the membrane, forming a pore that disrupts membrane integrity. The carpet model involves AMPs covering the membrane surface, leading to membrane disintegration. The toroidal pore model combines elements of both, where AMPs induce curvature in the membrane, forming transient pores (Pasupuleti et al., 2012; Corrêa et al., 2019).

In addition to membrane disruption, AMPs can interfere with intracellular targets. For instance, they can bind to nucleic acids, inhibiting DNA, RNA, and protein synthesis. Some AMPs also generate reactive oxygen species (ROS), leading to oxidative stress and cell death. These multifaceted mechanisms make it difficult for pathogens to develop resistance against AMPs (Huan et al., 2020; Dijksteel et al., 2021).

3.3 Gene regulation and expression of AMPs in earwigs

The expression of AMPs in earwigs is tightly regulated by the immune system. Upon infection, pattern recognition receptors (PRRs) detect pathogen-associated molecular patterns (PAMPs), triggering signaling pathways that lead to the activation of AMP genes. Key signaling pathways involved include the Toll and Imd pathways, which are analogous to the Toll-like receptor and TNF receptor pathways in mammals. These pathways activate transcription factors such as NF-κB, which bind to the promoter regions of AMP genes, initiating their transcription (Zhang and Gallo, 2016; Shanmugaraj et al., 2021).

Environmental factors, such as microbial exposure and stress, can also influence AMP expression. For example, earwigs exposed to bacterial infections show a rapid upregulation of AMP genes, enhancing their ability to combat the invading pathogens. Additionally, epigenetic modifications, such as DNA methylation and histone acetylation, play a role in the long-term regulation of AMP gene expression, ensuring a swift and robust immune response upon subsequent exposures (Patel and Akhtar, 2017; Guryanova and Ovchinnikova, 2022a).

In summary, AMPs in earwigs are a vital component of their innate immune system, providing a robust defense against a wide range of pathogens through diverse mechanisms and tightly regulated gene expression.

4 RNA Interference (RNAi) Mechanisms in Pathogen Defense

4.1 Overview of RNAi in invertebrate immunity

RNA interference (RNAi) is a crucial antiviral defense mechanism in invertebrates, providing RNA-based protection against viral infections. Unlike vertebrates, which rely on the interferon (IFN) system for antiviral defense, invertebrates utilize RNAi to degrade viral RNAs in a sequence-specific manner. This mechanism is activated by the recognition of viral double-stranded RNA (dsRNA), which is produced during viral replication. The RNAi pathway then processes these dsRNAs into small interfering RNAs (siRNAs) that guide the degradation of viral RNA, thereby restricting viral replication and dissemination (Nayak et al., 2013; Wang and He, 2019).

4.2 RNAi pathways in earwigs and their role in viral suppression

In earwigs, as in other invertebrates, the RNAi pathway plays a pivotal role in antiviral immunity. The pathway involves several key steps: the recognition of viral dsRNA, its processing into siRNAs by the enzyme Dicer, and the incorporation of these siRNAs into the RNA-induced silencing complex (RISC). The RISC, guided by the siRNAs, targets and degrades complementary viral RNAs, effectively suppressing viral replication. This mechanism has been observed to be highly effective against various types of viruses, including negative-strand RNA viruses, which do not produce easily detectable amounts of dsRNA but still trigger a potent RNAi response (Mueller et al., 2010; Guryanova and Ovchinnikova, 2022b).

4.3 Molecular regulation of RNAi-mediated defense

The regulation of RNAi-mediated defense in earwigs involves a complex interplay of molecular components. Key regulatory proteins include Dicer, which processes viral dsRNA into siRNAs, and Argonaute, a core component of the RISC that mediates the cleavage of viral RNA. Additionally, other small RNA pathways, such as those involving microRNAs (miRNAs), may also contribute to the antiviral response. Viruses, in turn, have evolved various strategies to evade RNAi, including the production of RNAi suppressors that inhibit the host's RNAi machinery. Understanding the molecular regulation of RNAi in earwigs can provide insights into the coevolutionary arms race between hosts and pathogens and highlight potential targets for enhancing antiviral defenses (Nakad et al., 2016; Berkhout, 2018; Lazzaro, 2018).

5 Response to Fungal, Bacterial, and Viral Infections

5.1 Immune responses to fungal pathogens

Earwigs, like other insects, possess a robust innate immune system that provides the first line of defense against fungal pathogens. The immune response to fungal infections involves several key components, including pattern recognition receptors (PRRs) that detect fungal cell wall components such as beta-glucan and chitin. These PRRs trigger signaling pathways that lead to the production of antimicrobial peptides and other immune effectors (Silipo et al., 2010). Additionally, the immune response includes the activation of cellular mechanisms such as phagocytosis by hemocytes, which engulf and destroy fungal cells.

Recent studies have highlighted the role of regulated cell death (RCD) in fungal defense. RCD is a genetically controlled process that eliminates infected cells, thereby preventing the spread of the pathogen. This mechanism is similar to the hypersensitive response observed in plants and involves several distinct pathways characterized by different biochemical and morphological features (Gaspar and Pawlowska, 2022). Furthermore, the immune response to fungal pathogens can vary depending on the virulence of the fungal strain, with different genes being expressed in response to high- and low-virulence strains (Wang et al., 2020).

5.2 Defense mechanisms against bacterial infections

Earwigs employ multiple strategies to defend against bacterial infections. One of the primary mechanisms involves the recognition of bacterial microbe-associated molecular patterns (MAMPs) such as lipopolysaccharides (LPS) and peptidoglycan (PGN) by PRRs. This recognition triggers a cascade of immune responses, including the production of antimicrobial peptides that target and kill bacteria. The innate immune system also involves the activation of signaling pathways such as the Toll and Imd pathways, which regulate the expression of immune-related genes and coordinate the overall immune response (Yu et al., 2022).

In addition to these molecular mechanisms, earwigs utilize physical barriers such as the cuticle to prevent bacterial entry. Hemocytes play a crucial role in the immune response by phagocytosing bacteria and releasing reactive oxygen species (ROS) to kill the pathogens. The interplay between immune cells and non-immune cells, such as epithelial cells, further enhances the defense against bacterial infections (Netea et al., 2015).

5.3 Viral immune suppression and molecular pathways

The immune response to viral infections in earwigs involves both RNA silencing and innate immunity. RNA silencing is a sequence-specific mechanism that targets viral RNA for degradation, thereby preventing viral replication. This process is mediated by small RNAs such as microRNAs (miRNAs) and short interfering RNAs (siRNAs), which are produced by Dicer-like proteins. In addition to RNA silencing, earwigs utilize innate immune pathways to combat viral infections.

Viruses have evolved various strategies to suppress the host immune response, including the production of viral suppressors that inhibit RNA silencing and other immune pathways. These viral effectors can interfere with the recognition of viral components by PRRs and inhibit the signaling pathways that lead to the production of antiviral molecules (Zvereva and Pooggin, 2012). The interplay between viral suppression mechanisms and the host immune response is a critical area of research, as it provides insights into the molecular pathways involved in viral defense and the evolution of host-pathogen interactions.

In summary, earwigs employ a combination of RNA silencing and innate immune responses to defend against viral infections. The ability of viruses to suppress these immune mechanisms highlights the ongoing evolutionary arms race between pathogens and their hosts. Understanding these interactions at the molecular level is essential for developing strategies to enhance immune defenses in earwigs and other insects.

6 Molecular Regulation of Immune Responses

6.1 Transcriptional regulation of immune genes

Transcriptional regulation plays a pivotal role in the innate immune response of earwigs, as it does in other organisms. Key transcription factors such as NF-κB, ATF-7, and TFEB/TFE3 are central to this process. NF-κB, activated through pathways like Toll and IMD, regulates the expression of various immune effectors, including antimicrobial peptides and cytokines, which are crucial for pathogen defense (Aalto et al., 2023). In Caenorhabditis elegans, the PMK-1 p38 MAPK pathway phosphorylates ATF-7, which then binds to the regulatory regions of pathogen-induced genes, orchestrating a comprehensive transcriptional response to infection (Fletcher et al., 2019). Similarly, TFEB and TFE3, regulated by the AMPK-FLCN axis, control the expression of antimicrobial genes and pro-inflammatory cytokines, linking metabolic status to immune function (El-Houjeiri et al., 2019).

6.2 Role of epigenetics in immune modulation

Epigenetic mechanisms, including DNA methylation, histone modification, and non-coding RNAs, significantly influence the modulation of immune responses. These mechanisms ensure that immune genes are expressed at the right time and place, providing a rapid and reversible means of regulating gene activity. In fish, for instance, various immunoregulatory factors, including transcription factors and hormone receptors, have been identified, highlighting the conservation of these regulatory mechanisms across vertebrates (Rebl and Goldammer, 2018). The role of microRNAs and long non-coding RNAs in regulating immune responses is also increasingly recognized, as they can modulate the stability and translation of mRNAs encoding immune-related proteins.

6.3 Post-transcriptional and post-translational regulation

Post-transcriptional and post-translational mechanisms add additional layers of control to the immune response. Post-transcriptional regulation involves processes such as mRNA splicing, polyadenylation, and stability, which can fine-tune the expression of immune genes in response to pathogens (Carpenter et al., 2014). For example, in Anopheles mosquitoes, splicing factors regulated by the IMD and Toll pathways determine the production of pathogen-specific splice variants of the pattern recognition receptor AgDscam, providing a tailored immune response to different pathogens (Dong et al., 2012). Post-translational modifications, such as ubiquitination, also play a critical role in immune signaling. In *Drosophila*, ubiquitination regulates the degradation of signaling molecules, ensuring a balanced immune response and preventing chronic inflammation. These regulatory mechanisms are crucial for maintaining immune homeostasis and preventing excessive immune activation that could lead to tissue damage or autoimmune diseases (Luecke et al., 2021).

By understanding these molecular regulatory mechanisms, we can gain insights into the sophisticated control of immune responses in earwigs and other organisms, potentially leading to novel therapeutic strategies for managing infections and immune-related disorders.

7 Comparative Analysis of Earwig Immune Mechanisms

7.1 Comparison with other insects' immune systems

The immune system of earwigs, like other insects, relies heavily on innate immunity, which includes both cellular and humoral responses. Cellular responses are mediated by haemocytes, which perform functions such as phagocytosis, nodulation, and encapsulation, similar to other insects (Figure 2) (Eleftherianos et al., 2021a). The humoral response involves the production of antimicrobial peptides (AMPs) from the fat body, analogous to the liver in vertebrates, which are crucial for combating pathogens (Eleftherianos et al., 2021b). Insects, including earwigs, also utilize pathways such as the Imd, Toll, and Jak-STAT pathways to mount antiviral responses (Kingsolver et al., 2015). These pathways are conserved across many insect species, indicating a shared evolutionary strategy for pathogen defense.

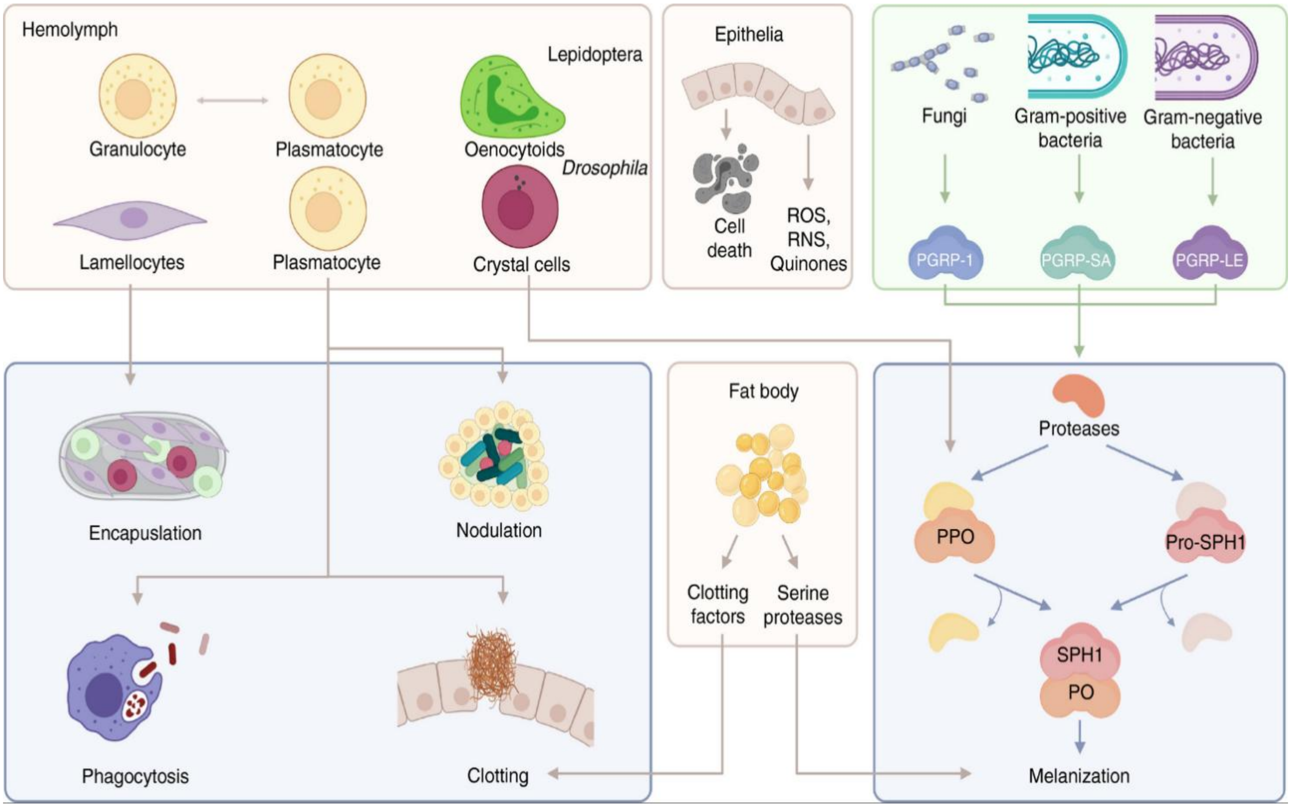

Figure 2 A simplified overview of haemocytes and their contribution to insect immunity and wound healing. Haemocytes include plasmatocytes, granulocytes, lamellocytes and oenocytoids/crystal cells. Cellular immune responses to microbial or parasitic infection are graphically depicted. These include encapsulation by lamellocytes, nodulation, phagocytosis and haemolymph clotting by plasmatocytes, and melanization by crystal cells and oenocytoids. The contribution of haemocytes and fat body in the prophenoloxidase (PPO) cascade activation is also portrayed, in addition to role of epithelia in immunity. Cytotoxic species released in epithelia include Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS) and quinones. Upon infection, the fat body induces the production of clotting factors and serine proteases. The latter regulates the melanization response. Microbial recognition induces the expression of peptidoglycan recognition protein (PGRP), which regulate the activity of proteases that control the prophenoloxidase (PPO) cascade and melanization response (Adopted from Eleftherianos et al., 2021a)

The study of Eleftherianos et al. (2021a) outlines the role of different hemocytes, such as plasmatocytes, granulocytes, and crystal cells, in insect immunity. These cells contribute to various immune responses like phagocytosis, encapsulation, nodulation, and clotting. The process of melanization, vital for defense against pathogens, is regulated by proteases and involves the activation of prophenoloxidase (PPO). Hemocytes work in conjunction with the fat body, which produces clotting factors and serine proteases, to combat infections. The image also highlights the role of epithelial cells, which produce reactive species as a part of the immune response to microbial threats.

7.2 Unique immune features in earwigs

While earwigs share many common immune mechanisms with other insects, they also exhibit unique features. One notable aspect is the potential for immune priming, where prior exposure to a pathogen enhances the immune response upon subsequent encounters. This phenomenon, although observed in other insects, may have unique regulatory mechanisms in earwigs (Cooper and Eleftherianos, 2017; Sheehan et al., 2020). Additionally, earwigs might possess specific antimicrobial peptides or proteins that are tailored to their ecological niches and specific pathogens they encounter, which could differ from those found in other insects (Wojda, 2017).

7.3 Evolutionary adaptation of immune responses

The immune responses in earwigs have likely evolved to address specific ecological challenges and pathogen pressures. The ability to mount a rapid and effective immune response, including the production of reactive oxygen and nitrogen species during melanization, is a critical adaptation. The evolutionary conservation of immune pathways such as RNAi and Jak-STAT in earwigs and other insects underscores the importance of these mechanisms in providing a robust defense against a wide array of pathogens (Kingsolver and Hardy, 2012). Furthermore, the integration and communication between different immune pathways, as seen in other insects, suggest that earwigs may also possess a highly coordinated and adaptable immune system (Zhang et al., 2021).

In summary, while earwigs share many immune mechanisms with other insects, they also exhibit unique features and adaptations that reflect their specific ecological contexts and evolutionary history. Understanding these nuances can provide deeper insights into the diversity and evolution of insect immune systems.

8 Case Study

8.1 Examination of immune responses in Forficula auricularia

The European earwig, Forficula auricularia, serves as an intriguing model for studying immune responses due to its ecological role and interactions with pathogens. Recent advancements in transcriptomic analysis have provided a comprehensive understanding of the genetic basis underlying these immune responses. A de novo transcriptome assembly of F. auricularia has revealed significant insights into the species' molecular biology, including the identification of over 8,800 contigs with significant similarity to insect-specific proteins. This extensive dataset has facilitated the assignment of Gene Ontology terms and the establishment of quantitative PCR tests for expression stability, which are crucial for studying immune-related gene expression (Roulin et al., 2014).

8.2 Pathogen challenges and immune activation

Earwigs, like other insects, encounter a variety of pathogens in their natural habitats, necessitating robust immune defense mechanisms. Studies on *Drosophila melanogaster* have shown that infection with diverse pathogens induces the expression of numerous genes, many of which are involved in immune responses. For instance, a meta-analysis identified 62 genes significantly induced by infection, including those encoding known immune response factors and novel genes not previously associated with immunity. This highlights the complexity and specificity of immune responses to different pathogens (Waring et al., 2021). In the context of earwigs, similar pathogen challenges likely trigger the activation of immune pathways, although specific studies on earwig immune activation are still emerging.

8.3 Molecular analysis of immune-related gene expression under pathogen stress

The molecular mechanisms underlying immune responses in earwigs involve the regulation of gene expression in response to pathogen exposure. In apple orchards, earwigs exposed to different pest management strategies exhibit variations in the expression of resistance-associated genes. For example, earwigs from organic orchards showed higher expression levels of acetylcholinesterase 2, a gene associated with resistance. Additionally, genes involved in detoxification, such as cytochromes P450, esterases, and glutathione S-transferases, were over-expressed in earwigs subjected to various management strategies (Figure 3). These findings underscore the impact of environmental factors on the molecular resistance mechanisms in earwigs and their potential role in biocontrol (Fricaux et al., 2023).

The study of Fricaux et al. (2023) shows the relative expression levels of detoxification genes (*CYP*, *CCE*, and *GST*) in earwigs collected from various orchard treatments (no treatment, organic, integrated pest management, and conventional). The results indicate variable gene expression across different orchard treatments, with higher expression levels in treated orchards compared to non-treated ones. Significant increases in the expression of certain detoxification genes, such as *CYP6NW1* and *CYP6NQ1*, are observed in treated groups, particularly in organic and integrated pest management conditions, indicating a response to pesticide exposure or other environmental factors.

Furthermore, the role of Toll-like receptors (TLRs) in immune signaling pathways is well-documented in other organisms. TLRs detect conserved microbial structures and initiate immune responses, which include the induction of gene expression and coordination of gene regulation and metabolism. These mechanisms are likely conserved in earwigs, contributing to their ability to mount effective immune responses against pathogens (Fitzgerald and Kagan, 2020).

In summary, the examination of immune responses in *Forficula auricularia*, pathogen challenges, and the molecular analysis of immune-related gene expression provide a comprehensive understanding of the innate immune mechanisms in earwigs. These insights are crucial for developing strategies to enhance the role of earwigs in biological control and pest management.

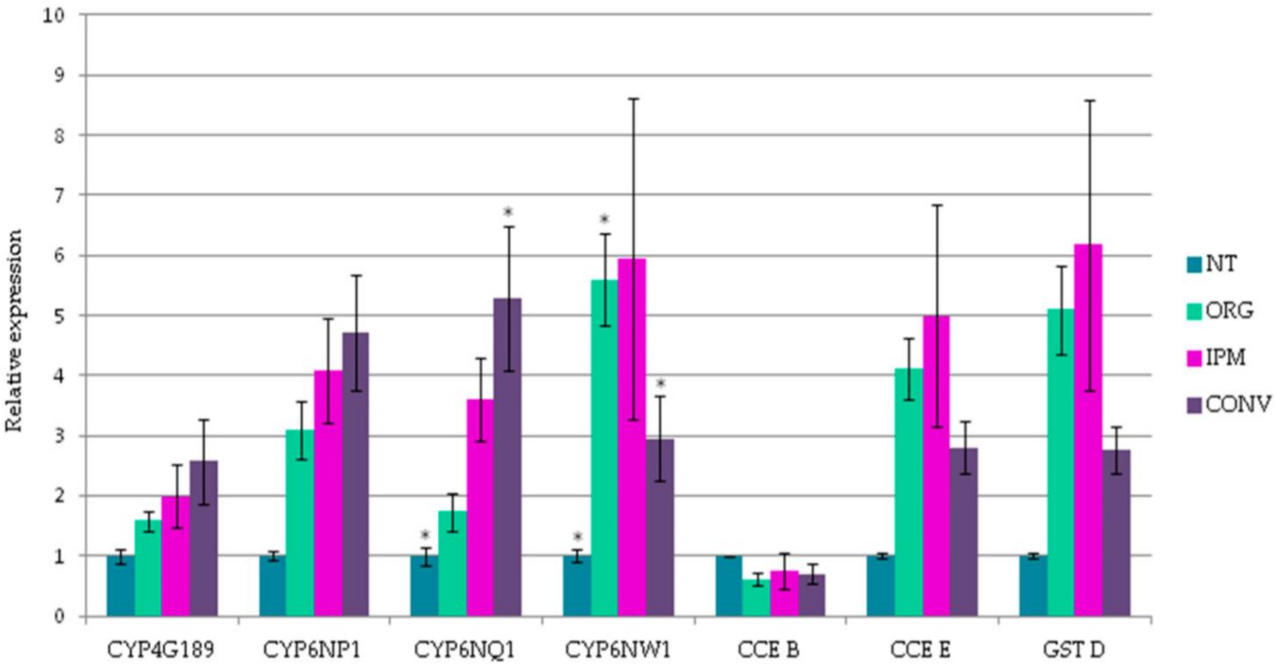

Figure 3 Level of expression of detoxification genes. Gene expression was normalized using the expression of three reference genes (*actin*, *EF1* and *GAPDH*) and shown as fold-change relative to the expression of earwigs collected in no treated orchard. *CYP* (cytochromes P450); *CCE* (carboxylesterases); *GST* (glutathione-S-transferases). Data are mean values of three biological replicates±SEM, comparison between samples was performed using one-way ANOVA followed by Tukey test (* $p<0.05$), indicating significant difference between earwigs from non-treated orchards versus treated orchards (Adopted from Fricaux et al., 2023)

9 Potential Applications of Earwig Immune Studies

9.1 Development of biopesticides based on earwig immune proteins

The study of earwig immune proteins, particularly antimicrobial peptides (AMPs), offers promising avenues for the development of biopesticides. AMPs are a crucial component of the innate immune response in insects, providing a robust defense against pathogenic microbes and parasites (Lin et al., 2020). These peptides can be harnessed to create environmentally friendly biopesticides that target specific agricultural pests without harming beneficial insects or the broader ecosystem. The evolutionary plasticity of insect immunity, including the expansion and functional diversification of AMPs, underscores their potential utility in pest management strategies (Vilcinskas, 2013). Additionally, the structure-activity relationships of these peptides can be optimized to enhance their efficacy and selectivity against target pests, providing a sustainable alternative to traditional chemical pesticides (Ciulla and Gelain, 2023).

9.2 Insights into evolutionary biology and immune system adaptation

Research on earwig immune mechanisms contributes significantly to our understanding of evolutionary biology and the adaptation of immune systems. The innate immune response is highly conserved across different species, and studying earwigs can reveal how immune genes evolve in response to environmental pressures (Shokal and Eleftherianos, 2017; Guryanova and Ovchinnikova, 2022b). Comparative analyses of immunity-related genes across various insect species have demonstrated significant evolutionary plasticity, with the emergence of novel proteins and protein domains (Xu, 2024). These studies highlight the dynamic nature of immune system evolution and the trade-offs involved in maintaining and deploying immune responses. Insights gained from earwig immune studies can inform broader evolutionary theories and enhance our understanding of host-pathogen interactions and coevolution (Yu et al., 2022).

9.3 Applications in disease control and agricultural pest management

The knowledge gained from earwig immune studies can be applied to disease control and agricultural pest management. Understanding the molecular mechanisms underlying earwig immunity can lead to the development of new strategies for controlling insect-borne diseases and managing pest populations. For instance, the identification of key immune signaling pathways and effector molecules in earwigs can inform the design of targeted interventions to disrupt pathogen transmission or enhance pest resistance (Fitzgerald and Kagan, 2020). Additionally, the study of insect-derived antimicrobial peptides and proteins offers potential applications in developing novel antimicrobial agents for use in medicine, veterinary science, and biotechnology (Romo et al., 2016; Wojda, 2017). By leveraging the innate immune mechanisms of earwigs, researchers can develop innovative solutions to address pressing challenges in public health and agriculture.

10 Concluding Remarks

The study of innate immune responses in earwigs has revealed several critical insights into their pathogen defense mechanisms. Earwigs, like other insects, utilize a combination of cellular and humoral responses to combat infections. Hemocytes play a pivotal role in cellular immunity through processes such as phagocytosis, nodulation, and encapsulation, which are essential for limiting pathogen spread and replication. Additionally, the production of antimicrobial peptides by the fat body, analogous to the liver in vertebrates, is a significant aspect of the humoral immune response, providing a robust defense against microbial invaders. The research also highlights the importance of pattern recognition receptors (PRRs) in detecting pathogen-associated molecular patterns (PAMPs) and initiating immune responses. Furthermore, the study of earwig immune responses to different fungal strains has shown that pathogen virulence can influence the expression of specific immune-related genes, indicating a sophisticated level of immune regulation.

Future research should focus on several key areas to further our understanding of earwig immune defense mechanisms. Firstly, there is a need to explore the genetic and molecular basis of immune responses in earwigs, particularly the signaling pathways involved in PRR-mediated recognition and response to pathogens. Comparative studies with other insects, such as *Drosophila*, could provide valuable insights into conserved and unique aspects of insect immunity. Additionally, investigating the role of environmental factors, such as pathogen exposure and maternal care, on the development and modulation of immune responses in earwigs could reveal important adaptive strategies. Another promising area of research is the potential application of earwig antimicrobial peptides in developing new antimicrobial agents for medical and biotechnological use.

The study of immune defense mechanisms in earwigs is of significant importance for several reasons. Firstly, it enhances our understanding of the evolutionary conservation and diversification of innate immunity across different species, providing a broader perspective on the fundamental principles of immune defense. Secondly, earwigs serve as a valuable model for studying the interplay between cellular and humoral immune responses, offering insights that could be applicable to other insects and even vertebrates. Finally, the knowledge gained from earwig immune studies has practical implications, including the potential development of novel antimicrobial compounds and the improvement of pest management strategies. Overall, earwig immune defense studies contribute to the broader field of immunology and have the potential to impact various scientific and practical domains.

Acknowledgments

BioSci Publisher thanks the anonymous reviewers for their contributions.

Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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