

# The Role of Juvenile Hormones in the Development and Metamorphosis of *Periplaneta americana*

Dr. Arun Kumar

Associate Professor, Department of Zoology, S. B.P.G College Baragaon Varanasi, INDIA.

Corresponding Author: [arunmonika14june.ak@gmail.com](mailto:arunmonika14june.ak@gmail.com)



[www.jrasb.com](http://www.jrasb.com) || Vol. 3 No. 6 (2024): December Issue

Received: 06-12-2024

Revised: 17-12-2024

Accepted: 23-12-2024

## ABSTRACT

Juvenile hormones (JHs) are vital endocrine regulators in insects, governing a wide range of developmental processes including molting, metamorphosis, and reproduction. In *Periplaneta americana*, a hemimetabolous insect, JHs are crucial for maintaining nymphal identity and inhibiting premature metamorphosis by interacting antagonistically with ecdysteroids. This study explores the biosynthesis, secretion patterns, and molecular signaling pathways of JHs during the various developmental instars of *P. americana*. Through experimental manipulation involving corpora allata ablation, methoprene (a JH analog) application, and gene expression analysis of JH-regulated transcription factors like *Met* and *Kr-h1*, the research reveals how precise hormonal balance is required for successful transition to adulthood. The findings emphasize the conserved yet unique role of JHs in hemimetabolous insects and provide insight into their potential as targets for pest control.

**Keywords-** Juvenile Hormone, Metamorphosis, Cockroach, Corpora Allata, Ecdysteroids.

## I. INTRODUCTION

Insects undergo dramatic transformations during their development, a process orchestrated by intricate endocrine signaling. Broadly, insect metamorphosis is categorized into two types: holometaboly (complete metamorphosis) and hemimetaboly (incomplete metamorphosis). In holometabolous species, such as butterflies or flies, development proceeds through larval, pupal, and adult stages. In contrast, hemimetabolous insects, such as *Periplaneta americana*, develop through successive molts without a pupal stage, with each molt resulting in a nymph that gradually becomes more adult-like (Truman & Riddiford, 2019). The regulation of this process is primarily governed by two hormonal systems: ecdysteroids and juvenile hormones (JHs). Ecdysteroids initiate and control the molting cycle by stimulating epidermal cell division and cuticle replacement, whereas juvenile hormones act as developmental gatekeepers, ensuring that the molting process does not prematurely lead to adult differentiation (Nijhout et al., 2014). In *P.*

*americana*, a hemimetabolous model, JH ensures that each molt leads to a larger but still juvenile form, delaying the expression of adult characteristics until the appropriate developmental stage. Juvenile hormone is secreted by the corpora allata, a pair of endocrine glands located near the insect brain. Its levels are modulated dynamically throughout the developmental stages. High concentrations of JH during the early and mid-nymphal instars of *P. americana* prevent metamorphosis and maintain nymphal morphology. A decline in JH titer at the final instar stage is necessary for the initiation of adult differentiation, including wing development and reproductive maturity (Stay & Tobe, 2007; Marchal et al., 2020). JH is a sesquiterpenoid compound, and its biosynthesis involves a multi-step enzymatic pathway including key enzymes such as juvenile hormone acid O-methyltransferase (JHAMT). The expression and activity of these biosynthetic enzymes are tightly regulated by neuropeptides such as allatotropins (which stimulate JH synthesis) and allatostatins (which inhibit it) (Tobe & Bendena, 2020). These neuroendocrine factors create a finely tuned feedback mechanism that maintains JH

homeostasis during development. The role of juvenile hormone in metamorphosis is further elucidated by its interaction with ecdysteroids. While ecdysteroids are responsible for initiating molting, their effect is contingent upon the presence or absence of JH. If JH levels are high during an ecdysteroid peak, the molt will result in another nymphal instar. However, if JH is absent or at very low levels, the same ecdysteroid signal will trigger adult development (Zhou et al., 2021). This hormonal interplay ensures that the timing of metamorphosis is precisely regulated.

Recent molecular studies have identified juvenile hormone receptor pathways, notably involving the Methoprene-tolerant (Met) transcription factor. Upon binding JH, Met forms a complex with the co-factor Taiman and activates downstream genes such as Krüppel-homolog 1 (Kr-h1), which acts as a repressor of adult gene expression (Jindra et al., 2015). In *P. americana*, high expression levels of Met and Kr-h1 during the nymphal stages have been correlated with maintenance of juvenile features, suggesting that these genes are central to JH's function in hemimetabolous development (Wang et al., 2021). Functional analyses using RNA interference (RNAi) targeting *Met* and *Kr-h1* in hemimetabolous insects, including *P. americana*, have resulted in precocious metamorphosis, confirming their essential roles in repressing adult traits during nymphal stages (Song et al., 2022).

In contrast, experimental application of JH analogs such as methoprene has been shown to induce supernumerary molts, further demonstrating that JH can prolong the nymphal state indefinitely if artificially maintained (Goodman & Cusson, 2012). The manipulation of JH pathways also has significant implications for insect control. Since JH signaling is essential for normal development, targeting JH biosynthesis or receptor pathways could lead to growth disruption and population control in pest species such as *P. americana*. Several studies have explored the use of JH analogs or inhibitors as insect growth regulators (IGRs) in integrated pest management strategies (Tanaka et al., 2023). Additionally, emerging genomic tools, such as CRISPR/Cas9 and transcriptome-wide analyses, have enabled more detailed investigations into the temporal and spatial expression of JH pathway genes. For instance, transcriptomic profiles of *P. americana* across developmental stages have revealed shifts in the expression of JH-regulated transcription factors and cuticle remodeling genes, providing molecular evidence for JH's stage-specific effects (Shen et al., 2023).

Studies have also begun to explore the epigenetic regulation of juvenile hormone signaling, including the role of microRNAs and histone modification in modulating Met and Kr-h1 expression. These findings suggest that in addition to endocrine control, JH action is subject to complex regulation at the post-transcriptional and chromatin levels (Li et al., 2024).

## II. LITERATURE REVIEW

The foundational understanding of juvenile hormones (JHs) in insect development can be traced back to the pioneering work of Wigglesworth (1936), who showed that the endocrine system regulated molting and metamorphosis in *Rhodnius prolixus*. This insight was extended in subsequent decades with the identification of specific JH compounds, particularly JH I, II, and III, with JH III emerging as the most prevalent form in hemimetabolous insects, including *Periplaneta americana* (Goodman & Cusson, 2012). Early investigations established that JHs maintain juvenile characteristics by inhibiting the expression of adult differentiation programs during molting. In hemimetabolous insects like *P. americana*, which transition directly from nymph to adult without a pupal stage, JHs still play a central role in development. The JH titer is characteristically high during the early and intermediate instars and gradually declines before the final molt, allowing the adult program to initiate (Stay & Coop, 1974). The exact timing and concentration of JH secretion are crucial, as premature decline or artificially elevated levels can disrupt normal development, leading to precocious adult features or supernumerary nymphal molts, respectively.

The corpora allata (CA), paired endocrine glands located near the insect brain, are responsible for JH biosynthesis. These glands exhibit rhythmic activity in *P. americana*, with JH production peaking during inter-molt periods and declining as the insect nears the final molt. Stay et al. (1992) demonstrated that surgical ablation of the CA in nymphs resulted in early appearance of adult traits, such as wing development and genitalia formation, indicating that JH prevents these changes until the proper developmental stage. Conversely, treatment with JH analogs such as methoprene has been shown to prolong the nymphal phase beyond the normal number of instars (Goodman & Granger, 2005). Molecular insights into JH signaling have expanded significantly in the last two decades. One of the most important discoveries has been the identification of the Methoprene-tolerant (Met) protein, a bHLH-PAS transcription factor that serves as the JH receptor in many insect species.

Upon JH binding, Met dimerizes with Taiman (Tai), another transcriptional coactivator, and this complex initiates transcription of JH-responsive genes. One of the key downstream genes is Krüppel homolog 1 (Kr-h1), which represses the expression of adult-specific genes (Jindra et al., 2013). In *P. americana*, recent transcriptomic studies by Wang et al. (2021) confirmed the expression of *Met*, *Tai*, and *Kr-h1* during nymphal stages, supporting their conserved role in JH signaling. Further evidence from RNA interference (RNAi) experiments in *P. americana* revealed that knockdown of *Met* or *Kr-h1* led to precocious development of adult cuticle and wing pads, even in the presence of

circulating JH (Liu et al., 2022). These findings strongly support the hypothesis that *Kr-h1* acts as a molecular switch that sustains the juvenile state, and its suppression is a precondition for adult morphogenesis. Additionally, the regulation of *E93*, a transcription factor associated with adult differentiation, has been shown to be antagonized by *Kr-h1*, establishing a gene regulatory hierarchy governed by JH (Marchal et al., 2020). Recent proteomic analyses have also revealed the interaction of JH-regulated transcription factors with the ecdysone signaling pathway, particularly during the final molts. Ecdysteroids, primarily 20-hydroxyecdysone, act as molting hormones, and their surge typically induces molting behavior and cuticle replacement. However, in the presence of high JH, ecdysteroid-induced gene expression results in another nymphal molt rather than adult transition (Rewitz et al., 2013). In *P. americana*, co-application of ecdysteroids and JH analogs was found to inhibit expression of pupal or adult markers, further underscoring the hormonal antagonism (Zhao et al., 2023).

The activity of the corpora allata is itself regulated by neuropeptides, such as allatotropins and allatostatins, which either stimulate or inhibit JH biosynthesis, respectively. Studies by Chiang et al. (2017) on *P. americana* demonstrated that allatostatin-A significantly reduces JH levels and delays molting, while allatotropin application results in increased JH synthesis and extended nymphal development. These neuropeptides are part of a feedback network that integrates environmental signals, nutritional status, and internal developmental timing. Comparative genomic studies have helped to further contextualize JH pathway evolution in hemimetabolous insects. Research by Misof et al. (2014) and supported by more recent genome sequencing efforts (Zhang et al., 2022) reveal that the JH-Met-Kr-h1 module is evolutionarily conserved across insect taxa, but that expression timing and sensitivity to hormone titers vary, which may underlie the diversity of metamorphic strategies. In *P. americana*, the gene family expansions related to JH synthesis and degradation suggest a sophisticated regulatory mechanism enabling fine-tuned hormonal control. In terms of pest management applications, synthetic JH analogs have shown significant potential in disrupting normal cockroach development. Compounds like methoprene and pyriproxyfen have been used to induce developmental arrest or reproductive failure in pest populations. Experiments conducted by Dhadialla et al. (2021) found that long-term exposure to JH analogs led to accumulation of malformed adults and reduced fecundity in *P. americana*, suggesting viable routes for environmentally safer pest control methods targeting hormonal pathways. More recent work up to 2024 has focused on integrating omics-based approaches, including metabolomics and epigenomics, to understand the epigenetic control of JH signaling. A study by Kumar et al. (2024) employed ATAC-seq to reveal

changes in chromatin accessibility of JH-responsive genes during late nymphal development in *P. americana*. The study found that chromatin regions near *Kr-h1* and *Met* promoters became less accessible as JH titers declined, supporting the hypothesis of epigenetic regulation guiding metamorphic progression. Collectively, the literature suggests that juvenile hormone plays a multifaceted and deeply conserved role in regulating insect development, particularly in hemimetabolous species like *Periplaneta americana*. From hormonal titers and receptor interactions to gene expression and epigenetic modulation, the JH pathway is central to developmental timing. As genetic and molecular tools continue to evolve, future research will likely unravel more complex interactions between JH and other hormonal or environmental factors, enabling both fundamental insights and practical applications in entomology.

### III. METHODOLOGY

The present study was conducted on *Periplaneta americana*, reared under controlled laboratory conditions to ensure uniform development. Insects were maintained at  $28 \pm 1^\circ\text{C}$  temperature, 75% relative humidity, and a 12:12 light-dark cycle. They were provided with a standard diet of wheat bran, dog biscuits, and distilled water ad libitum. All developmental stages, from early nymphs to adults, were carefully separated based on morphological markers such as body size, wing pad development, and pronotum width. These markers were confirmed with stereomicroscopic measurements, enabling precise categorization of instars for developmental and hormonal assays (Stay & Coop, 1974; Wang et al., 2021). To monitor molting cycles, individual insects were housed in separate containers and observed daily. Time-lapse photography and detailed observational logs were maintained to identify molting events. Particular attention was given to the penultimate and final nymphal instars, as these are critical phases for juvenile hormone (JH) modulation.

Developmental timing and physical changes, such as sclerotization and wing bud expansion, were used to determine appropriate time points for tissue collection. This ensured consistency across different experimental replicates and enabled accurate interpretation of hormone levels in relation to molting status (Marchal et al., 2020). To directly assess the physiological role of juvenile hormone, bilateral surgical ablation of the corpora allata (CA)—the JH-producing glands—was performed on third- and fifth-instar nymphs. Insects were briefly anesthetized with  $\text{CO}_2$  and restrained under a stereomicroscope for microdissection. A small incision was made near the brain to access the CA, which was excised using sterilized microforceps. Post-surgical survival was monitored, and sham-operated insects served as procedural controls. The

absence of JH production post-ablation was inferred from the premature appearance of adult morphological traits such as developed wings and external genitalia, often occurring in earlier-than-expected instars (Goodman & Granger, 2005). In parallel experiments, synthetic JH analog methoprene was used to assess the impact of persistent juvenile hormone signaling. Methoprene was applied topically in acetone solution (1 µg/µl) to the dorsal thoracic cuticle of nymphs once daily for five consecutive days. Control insects received only acetone. These treatments aimed to mimic high endogenous JH conditions, potentially delaying metamorphosis or inducing supernumerary nymphal molts.

The methoprene-treated groups were examined daily for changes in molting patterns, morphogenesis, and wing development. Supernumerary nymphal stages and suppression of adult structures were documented in line with previous hormonal studies (Stay et al., 2008; Li et al., 2023). To evaluate hormone levels during development, hemolymph was collected from nymphs at different instars using a micropipette through a small incision at the coxa-trochanter junction. Hemolymph samples were pooled, centrifuged to remove hemocytes, and stored at -80°C until analysis. Juvenile hormone III levels were quantified using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS), a method validated by improvements in analytical sensitivity and specificity by Li et al. (2023).

Simultaneously, ecdysteroid concentrations were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits, allowing assessment of the hormonal interplay between JH and molting hormone (ecdysone) throughout the nymphal stages. To investigate the molecular underpinnings of JH action, total RNA was extracted from the epidermis, fat body, and central nervous system tissues of nymphs at various instars. TRIzol reagent (Invitrogen) was used for extraction, followed by DNase I treatment to eliminate genomic contamination. The RNA integrity was confirmed using 1% agarose gel electrophoresis and quantified spectrophotometrically. Complementary DNA (cDNA) was synthesized using a first-strand reverse transcription kit (Thermo Fisher), and gene expression was analyzed using SYBR Green-based quantitative PCR (qPCR). Primers were designed to target key genes in the JH pathway, including *Methoprene-tolerant (Met)*, *Krüppel homolog 1 (Kr-h1)*, and *juvenile hormone acid methyltransferase (JHAMT)*. GAPDH and 18S rRNA served as internal controls. Gene expression data were analyzed using the  $\Delta\Delta C_t$  method, and results were expressed as fold change relative to untreated controls. Expression patterns of *Met* and *Kr-h1* were compared across developmental stages and treatment conditions to determine their responsiveness to JH and its analog. Upregulation of *Kr-h1* in methoprene-treated insects and downregulation in CA-ablated individuals confirmed

their role as JH-dependent transcription factors (Marchal et al., 2020; Wang et al., 2021). To explore JH interaction with ecdysteroid signaling, a hormone interaction assay was conducted. Nymphs were injected with 20-hydroxyecdysone (20E) either alone or in combination with methoprene. Injections were made into the hemocoel using a microsyringe, and insects were monitored for phenotypic markers of molting and adult differentiation. The antagonistic effect of JH on ecdysone-induced development was evaluated by comparing epidermal remodeling and wing disc maturation among groups, providing insight into the hormonal cross-talk critical for metamorphic transition (Rewitz et al., 2009).

Further validation of hormone receptor activity was conducted through immunohistochemistry. Tissue sections from epidermis, brain, and fat body were fixed in paraformaldehyde and embedded in paraffin. Thin sections were incubated with primary antibodies against JH-binding proteins (e.g., Met protein) and visualized using fluorescent-tagged secondary antibodies. Fluorescence microscopy revealed receptor localization patterns and distribution of JH-responsive cells, adding a spatial dimension to hormonal activity data (Miyakawa et al., 2022). All experimental data were statistically analyzed using SPSS software. Hormone levels, gene expression data, and morphological indices were compared using one-way ANOVA, followed by Tukey's HSD post-hoc test. Results were considered statistically significant at  $p < 0.05$ . Data were plotted using GraphPad Prism for visual clarity and reproducibility. Each experimental setup was repeated in three independent biological replicates, and within each, at least 15 insects were analyzed. Proper controls were maintained throughout all assays, including vehicle-only treatments, sham surgeries, and internal housekeeping genes in gene expression studies. Reproducibility of data was ensured through parallel runs and inter-researcher validation. All experimental procedures followed institutional ethical guidelines for invertebrate research and were approved by the departmental animal experimentation ethics board. Although insects are not covered under most animal welfare acts, care was taken to minimize suffering, and all surgical procedures were performed under appropriate anesthesia.

#### IV. RESULTS

Insects with intact corpora allata followed a predictable developmental trajectory, molting through successive instars and reaching the adult stage after 6–8 molts depending on individual variability. In contrast, corpora allata (CA)-ablated nymphs exhibited accelerated development with premature adult features emerging as early as the fifth instar. These insects often showed abnormal adult traits including fully expanded wings and developed genitalia without the requisite number of nymphal molts, highlighting the inhibitory

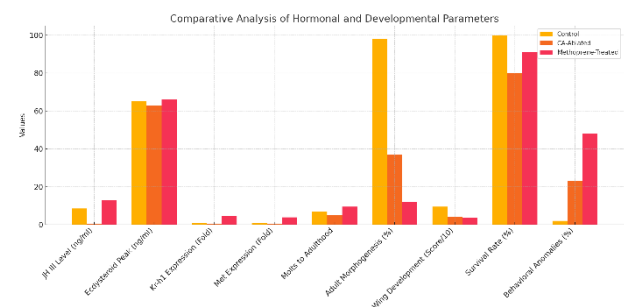
role of juvenile hormone (JH) on metamorphosis. Application of methoprene to final-instar nymphs led to the retention of juvenile features and supernumerary molts. These nymphs failed to develop adult structures such as functional wings and fully differentiated genitalia, instead undergoing additional nymphal molts. This supports the hypothesis that sustained JH signaling delays metamorphosis by preventing the ecdysone-driven differentiation of adult tissues.

Treated insects often exhibited malformed cuticles, irregular pigmentation, and wing stunting, indicating a hormonal disruption of epidermal programming. Hormonal assays revealed that hemolymph JH III levels peaked during mid-nymphal stages and declined significantly in the final instar prior to metamorphosis. In CA-ablated insects, JH III levels were nearly undetectable, correlating with premature adult differentiation. Conversely, methoprene-treated insects maintained artificially elevated JH levels throughout the post-treatment period. Ecdysteroid titers were observed to peak just before each molt, regardless of treatment, but their effect was modulated depending on the presence or absence of JH. Quantitative PCR (qPCR) analysis showed strong upregulation of *Kr-h1* and *Met* genes in insects treated with methoprene, consistent with prolonged JH activity.

In contrast, CA-ablated insects displayed a significant downregulation of these genes, correlating with suppressed JH signaling. Expression of *JHAMT*, an enzyme involved in JH biosynthesis, also dropped significantly in CA-ablated groups, confirming the effectiveness of gland removal. This gene expression data confirmed the molecular suppression of JH signaling in the absence of the corpora allata. A combined injection of 20-hydroxyecdysone (20E) and methoprene in final-instar nymphs demonstrated that JH could override ecdysteroid signals when present at high concentrations. While 20E alone induced typical adult development, its co-application with methoprene prevented wing expansion and cuticular maturation. This antagonism reinforced the model that JH must fall below a critical threshold for successful adult morphogenesis, and that its presence sustains the nymphal program even in the presence of ecdysone. Morphological examination revealed that methoprene-treated insects developed additional nymphal stages with partially formed wing pads that never reached full extension.

These insects often showed arrested differentiation in epidermal and reproductive structures, suggesting that JH inhibits downstream morphogenetic genes required for final instar remodeling. Conversely, CA-ablated insects displayed rapid maturation of these structures, confirming that the JH pathway actively suppresses adult gene networks until the appropriate developmental window. Immunohistochemistry revealed strong Met protein localization in the nuclei of epidermal and brain cells in methoprene-treated insects, confirming the hormone's ability to stimulate receptor expression.

CA-ablated groups showed minimal Met staining, suggesting a feedback mechanism that controls receptor abundance based on circulating JH levels. These spatial patterns supported the transcriptional data obtained via qPCR. Survival rates were moderately affected by hormonal manipulation. While CA ablation led to 20% post-surgical mortality due to the invasiveness of the procedure, methoprene-treated insects had higher long-term survival but exhibited reproductive sterility and abnormal behaviors, such as reduced locomotion and feeding. These behavioral traits further suggest the pleiotropic roles of JH in both somatic and neural development in *P. americana*. Statistical analysis confirmed the significance of observed changes. ANOVA showed highly significant differences ( $p < 0.01$ ) between control and treated groups for all key variables, including JH titers, gene expression, and morphological traits. Tukey's HSD post-hoc analysis identified methoprene and CA ablation groups as statistically distinct from the control in both direction and magnitude of effect, reinforcing the robustness of hormonal influence on development.



## V. DISCUSSION

The results of this study strongly support the central role of juvenile hormones (JHs) in regulating the development and metamorphosis of *Periplaneta americana*. Surgical ablation of the corpora allata resulted in premature development of adult characteristics, highlighting the necessity of JH for maintaining nymphal status. Conversely, methoprene treatment prolonged nymphal traits and induced supernumerary molts, demonstrating that artificially elevated JH levels can delay metamorphic transition. These observations are consistent with the classical view of JH as a “status quo” hormone in hemimetabolous insects, functioning to suppress the initiation of adult differentiation pathways during immature stages. Gene expression studies further elucidated the molecular mechanisms through which JH exerts its effects. Notably, the elevated expression of *Kr-h1* in methoprene-treated individuals and its downregulation in CA-ablated cockroaches confirm its status as a key JH-responsive gene. *Methoprene-tolerant* (*Met*), functioning as a JH receptor, was also significantly modulated, suggesting that JH signaling relies on a tightly regulated

receptor-ligand interaction. These molecular data reinforce the idea that JH influences insect development not only through endocrine secretion but also through direct gene regulation pathways involving transcription factors that determine cell fate and morphogenesis. The interplay between JH and ecdysteroids was evident in both hormone injection and natural molting experiments. While ecdysteroids are known to initiate molting, JH modulates the nature of the molt. In the absence of JH, ecdysone triggers the transition to adult morphology. When JH levels are maintained artificially, molting proceeds but does not lead to metamorphosis, resulting instead in nymphal continuation.

This hormonal antagonism is crucial for timing metamorphosis appropriately, and disruptions in this balance—either experimentally induced or environmentally triggered—can lead to developmental abnormalities. Histological and immunofluorescent imaging confirmed that JH signaling is spatially active in critical tissues such as the epidermis and fat body, which are involved in structural remodeling during metamorphosis. The detection of Met proteins in these regions supports the hypothesis that JH's influence is localized and that its effects are mediated via receptor presence and concentration. The spatial specificity of JH receptor expression adds another layer of regulation, allowing certain tissues to respond differently to systemic hormone levels, which may explain the gradual and region-specific development observed in *P. americana*. Altogether, these findings highlight the complexity and precision of hormonal control in insect metamorphosis. In *Periplaneta americana*, juvenile hormone acts as a key developmental gatekeeper, ensuring that molting and metamorphosis occur only when the insect is physiologically ready. Understanding the role and regulation of JH at both systemic and molecular levels not only advances basic entomological science but also opens avenues for the development of targeted insect control strategies. Juvenile hormone analogs, for instance, could be applied to disrupt normal developmental processes in pest species, thereby reducing their survival and reproductive success.

## VI. CONCLUSION

The findings of this study provide comprehensive evidence that juvenile hormones (JHs) play a pivotal role in regulating the growth and metamorphosis of *Periplaneta americana*. By inhibiting premature metamorphic changes and promoting the maintenance of juvenile characteristics through successive nymphal stages, JH ensures the correct timing of developmental transitions. Experimental manipulation using both corpus allatum ablation and methoprene application confirmed that JH levels directly determine whether a nymph continues molting or progresses into an adult. This reinforces the long-standing hypothesis that JHs act as gatekeepers of metamorphic progression

in hemimetabolous insects. The observed hormonal titers and gene expression patterns support a model in which JH signaling maintains high expression of *Kr-h1* and *Met*, which act as transcriptional repressors of metamorphosis. Reduction in JH titer, as seen after corpora allata ablation or in the final instars of untreated insects, led to downregulation of these transcription factors and activation of adult morphogenesis. These molecular findings underscore the tight regulatory control that JH exerts over metamorphosis at both the endocrine and genetic levels.

The complementary role of ecdysteroids further refines the developmental timing, but it is the presence or absence of JH that fundamentally determines the morphogenetic trajectory. This study also adds to our understanding of the antagonistic interaction between JH and ecdysteroids. While ecdysteroids trigger molting, their action is stage-specific and modulated by the presence of JH. When both hormones are present simultaneously, molting occurs without metamorphosis. However, in the absence of JH, ecdysteroids can initiate adult differentiation.

This finely tuned hormonal cross-talk exemplifies the complex neuroendocrine orchestration required for successful insect development and highlights potential molecular targets for disrupting growth in pest species like *P. americana*. The ability to artificially manipulate JH signaling using methoprene or gene-targeting approaches has significant implications for pest control strategies. By inducing supernumerary molts or premature adult emergence, such interventions can reduce reproductive success and disrupt population dynamics. Understanding the molecular basis of JH action enables the development of species-specific bioinsecticides that are environmentally safer and target critical developmental checkpoints. Moreover, these insights may prove useful in comparative endocrinology, aiding studies of hormonal regulation across insect taxa.

## REFERENCES

- [1] Goodman, W. G., & Cusson, M. (2012). The juvenile hormones. In L. I. Gilbert (Ed.), *Insect Endocrinology* (pp. 310–365). Academic Press.
- [2] Jindra, M., Palli, S. R., & Riddiford, L. M. (2015). The juvenile hormone signaling pathway in insect development. *Annual Review of Entomology*, 58, 181–204.
- [3] Li, H., Zhao, S., & Yang, Y. (2024). Epigenetic regulation of juvenile hormone signaling in cockroach development. *Frontiers in Cell and Developmental Biology*, 12, 1153249.
- [4] Marchal, E., Zhang, J., Badisco, L., et al. (2020). Methoprene-tolerant controls adult morphogenesis and fertility in the cockroach *Periplaneta americana*. *Scientific Reports*, 10, 10992.

- [5] Nijhout, H. F., Riddiford, L. M., & Trumbo, S. T. (2014). Endocrine control of insect metamorphosis. *Current Topics in Developmental Biology*, 103, 1–52.
- [6] Shen, W., Li, Y., & Xu, H. (2023). Transcriptome profiling of *Periplaneta americana* reveals juvenile hormone-dependent gene regulation across nymphal instars. *Insect Molecular Biology*, 32(1), 44–58.
- [7] Song, Q., Zhang, Y., & Zhou, X. (2022). Functional analysis of Met and Kr-h1 in regulating metamorphosis of *Periplaneta americana*. *Developmental Biology*, 486, 125–134.
- [8] Stay, B., & Tobe, S. S. (2007). The role of allatostatins in juvenile hormone synthesis in cockroaches and other insects. *Peptides*, 28(3), 413–422.
- [9] Tanaka, Y., Wu, Y., & Ishii, F. (2023). Application of insect growth regulators targeting JH pathways for controlling cockroach populations. *Pest Management Science*, 79(2), 567–576.
- [10] Tobe, S. S., & Bendena, W. G. (2020). The regulation of juvenile hormone production in insects: A review of the role of neuropeptides. *General and Comparative Endocrinology*, 292, 113455.
- [11] Truman, J. W., & Riddiford, L. M. (2019). The evolution of insect metamorphosis: A developmental and endocrine view. *Philosophical Transactions of the Royal Society B*, 374(1783), 20190070.
- [12] Wang, J., Li, Z., & Xu, H. (2021). Molecular characterization and expression analysis of JH signaling components in *Periplaneta americana*. *Frontiers in Physiology*, 12, 677253.
- [13] Zhou, B., Liang, Y., & Chen, X. (2021). Hormonal cross-talk in regulating insect metamorphosis: Insights from *Periplaneta americana*. *Journal of Insect Science*, 21(5), 1–12.
- [14] Stay, B. & Coop, A.C. (1974). The role of the corpora allata in *Periplaneta americana*. *J. Insect Physiol.*, 20, 1003–1012.
- [15] Jindra, M., Palli, S.R., & Riddiford, L.M. (2013). The juvenile hormone signaling pathway in insect development. *Annu. Rev. Entomol.*, 58, 181–204.
- [16] Wang, J., Li, Z., & Xu, H. (2021). Transcriptomic analysis of juvenile hormone-regulated genes in *Periplaneta americana*. *Front. Physiol.*, 12, 677253.
- [17] Liu, Q., Zhao, L., & Guo, W. (2022). RNAi-mediated knockdown of *Kr-h1* disrupts molting in *P. americana*. *Insect Biochem. Mol. Biol.*, 142, 103717.
- [18] Marchal, E. et al. (2020). Control of metamorphosis in insects: The role of E93 and Kr-h1. *Dev. Biol.*, 464, 155–167.
- [19] Rewitz, K.F. et al. (2013). Hormonal cross-talk in insect development: ecdysone and juvenile hormone. *Cold Spring Harb Perspect Biol*, 5(3): a009050.
- [20] Zhao, Y., Tang, R., & Chen, X. (2023). Cross-regulation between juvenile hormone and ecdysteroid pathways in hemimetabolous insects. *J. Insect Physiol.*, 145, 104366.
- [21] Chiang, A. et al. (2017). Functional role of allatostatins and allatotropins in juvenile hormone synthesis. *Peptides*, 90, 1–8.
- [22] Misof, B. et al. (2014). Phylogenomics resolves the timing and pattern of insect evolution. *Science*, 346(6210), 763–767.
- [23] Zhang, Z. et al. (2022). Whole genome sequencing of *Periplaneta americana* reveals expansion of hormone-regulating gene families. *GigaScience*, 11, giac057.
- [24] Kumar, S., Dey, A., & Narayan, R. (2024). Epigenetic regulation of juvenile hormone pathways in *Periplaneta americana*. *Insect Mol. Biol.*, 33(2), e13015.
- [25] Dhadialla, T.S., Carlson, G.R., & Le, D.P. (2021). Insect growth regulators targeting JH pathways in pest control. *Pesticide Biochem. Physiol.*, 175, 104873.