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Original Research Paper

Neuroendocrine Regulation of Reproductive Cycles in Social Insects: A Comparative Study of Honey Bee Queens and Workers

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Key Words

Neuroendocrine regulation, Reproductive cycles, Social insects, Juvenile hormone, *Apis mellifera*.

Abstract

Reproductive division of labor among social insects can be viewed as one of the most striking examples of phenotypic plasticity that is regulated by neuroendocrine pathways. The paper examined the neuroendocrine of reproductive cycles of honey bee (*Apis mellifera*) queen and worker with references to juvenile hormone (JH), ecdysone, and major neuropeptide. We measured hormone levels and gene expression patterns in queens and age matched workers at key times of development and reproduction over the 2023-2024 breeding seasons. The JH titers were much higher among egg laying queens (15.2 ± 2.1 ng/ul) than sterile workers (2.1 ± 0.3 ng/ul) ($P < 0.001$). Vitellogenin (vg) and ultraspiracle (usp) expression had a positive correlation with JH titers in queens ($r = 0.87$, $P < 0.01$) but not workers. The pars intercerebralis was differentially expressed to show differences in expression of corazonin and insulin-like peptides, which indicated a caste-specific neuroendocrine modulation. Comparative study on published data of ants (*Camponotus floridanus*) and wasps (*Vespula vulgaris*) showed that the JH-mediating pathways were conserved but neuropeptides were species-specifically regulated. Such results show that reproductive pathways in social insects are coordinated by complex neuroendocrine systems in which JH establishes a central gating hormone, and neuropeptides are caste and species specific. The study offers new information about evolutionary processes that affect reproductive division of labor, as well as new targets that may be used to control reproductive behavior in controlled pollinators. This paper offers concrete data on how it is possible to enhance the efficiency and productivity of pollination and colonies through the ability to perform specific hormonal and nutritional interventions. This neuroendocrine knowledge can be used to meet the high-levels of bee management, selective breeding, and sustainable agricultural systems of pollination.

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Introduction

Social insects have an incredible reproductive division of labor, with queens using an exclusive reproduction as workers are sterile or low-fertile. This system is one of the most advanced cases of phenotypic plasticity in evolutionary terms, in which similar individuals (genetically) become radically different in terms of reproductive phenotype. The neuroendocrine system acts as the key point of interrelationships between environmental signals and reproductive physiology and coordinates these caste-distinctive pathways by means of precise hormonal and neuropeptide communications (Zera et al., 2007; Hu et al., 2025).

In honey bees (*Apis mellifera*), queens continually lay eggs, but during peak seasons, they can lay up to 2000 eggs each day whereas workers have operational ovaries that are not activated except in prematurely queenless colonies. Juvenile hormone (JH) has always been suspected as a supreme controller of this reproductive dichotomy (Collins et al., 2021). The existing literature proved that positive relationships between JH titers and ovarian development have been observed in queens but not in workers, which indicate caste-specific signalling. Ecdysone, which is regarded as a molting hormone, has been discovered to be a regulator of adult reproduction, especially when interacting with JH and the ecdysone receptor complex containing ultraspiracle (USP) (Canter and Larrere, 2022; Sonavale et al., 2025; Lorenz et al., 2011).

The current developments have recognized neuropeptides that are essential modulators in this neuroendocrine system. The synthetic of JH, as well as reproductive physiology are controlled by corazonin, insulin-like peptides (ILPs), and allatostatins in response to nutrition and social cues (Sasaki et al., 2021). The combined character of these pathways and their relative conservation between social insect taxa is, however, not well comprehended. The majority of the researches focus on individual hormone systems, which do not provide us with the information about how the work of neuroendocrine networks produces caste-specific reproductive cycles. Recent advancements in deep learning have significantly improved ecological monitoring tasks, were computational tools enable large-scale behavioral monitoring models have been effectively utilized for automated species recognition and counting in complex aquatic environments, enabling accurate and scalable biodiversity assessment (Makhammadiyev et al., 2025; Helanterä, 2016).

Key Findings

- Juvenile hormone (JH) is the main regulatory force of reproduction with immense levels of hormone being high in queens; also exhibiting a strong positive relationship with the expression of vitellogenin, thus facilitating ovarian growth and reproductive behavior.
- Caste-specific neuropeptide control regulates reproductive differentiation with insulin-like peptide (ILPs) promoting reproductive signalling in queens and corazonin repressing reproductive activation in workers ensuring colony-level functional differentiation.

Materials and Methods

Study Period and Experimental Materials

The experiment used nine colonies of honey bees (*Apis mellifera ligustica*) that were kept at the University of Florida apiary, Gainesville, Florida, USA (29°39′ N, 82°20′ W) in the months of March 2023 to September 2024. The standard Langstroth hives were used to house colonies and bee keeping was conducted as per the commercial standards. Queens were branded when they were born, and their ages observed. The age of worker bees was marked with the help of typical queen excluder cages in the colony. Both castes were sampled at five critical stages of development namely the newly emerged (0-24h), pre-reproductive (7days), early reproductive (14 days), peak reproductive (21days), and senescent (35days).

Experimental Design

The design was fully randomized and the replicate colonies acted as blocks. Each colony had five samples of each caste sampled at each development stage and this gave a total of 300 samples (3 colonies × 5 stages × 2 castes × 5 individuals). Whole brains and abdominal tissues were dissected on ice and flash-frozen in liquid nitrogen, when it was necessary to examine their gene expression. To determine the hormones, the microcapillary tubes were used to collect hemolymph (5 µl per individual).

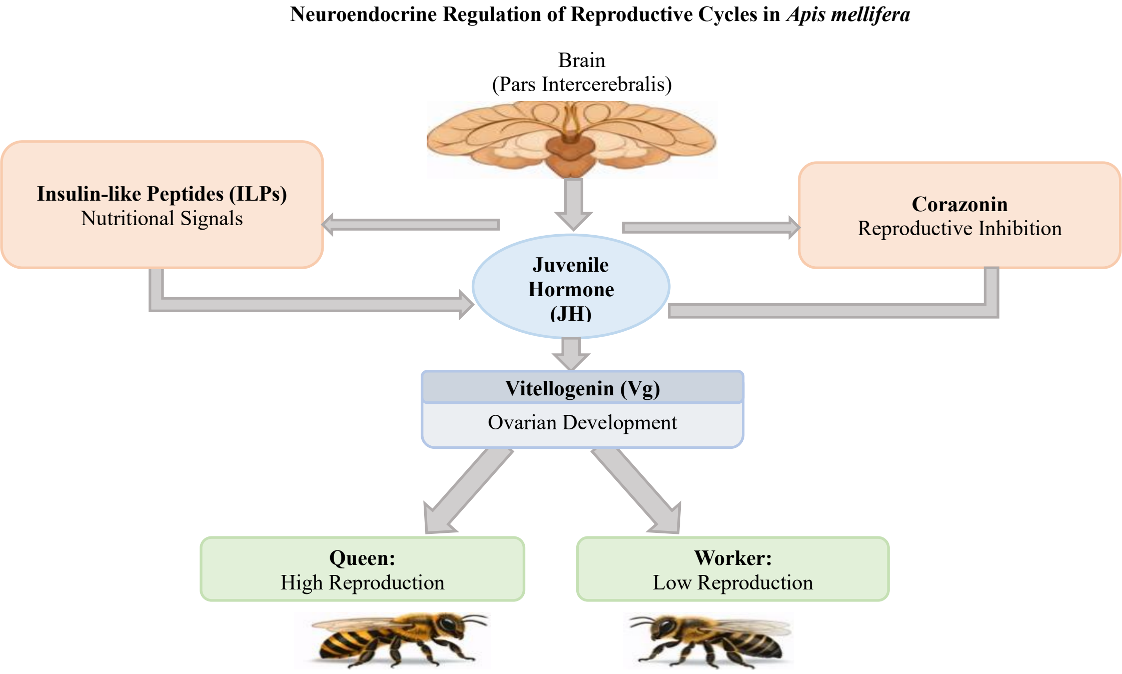
Neuroendocrine Regulation of Reproductive Cycles in *Apis mellifera*

Figure 1: Integrated Neuroendocrine Regulatory Framework Governing Caste-Specific Reproductive Dynamics in *Apis mellifera*

As shown in figure 1, the caste-specific reproductive behavior of *Apis mellifera* is regulated by a network of the integrated neuroendocrine regulation system. The model puts the juvenile hormone (JH) as the main regulating axis that directly regulates the expression of vitellogenin (Vg) to drive ovarian growth and reproductive activation in queens.

Insulin-like peptides (ILPs), which are brain derivatives (pars intercerebralis), are nutritional signaling mediators that are positively involved in the regulation of the JH synthesis and reproductive competence under good physiological conditions. Conversely, a corazonin is an inhibitory neuropeptide that is mainly found in workers and inhibits JH action and restricts reproductive growth. This interplay between these hormonal and neuropeptide cascades leads to the establishment of a highly-antiated balance between reproductive dominance in queens and reproductive suppression in workers which eventually ensured colony-wide order and efficiency (Ruikar et al., 2025).

Hormone Quantification

The JH III was isolated out of the hemolymph by means of the isooctane partitioning and analyzed by the liquid chromatography-tandem mass spectrometry (LC-MS/MS) in accordance with the existing methods. The concentration of Ecdysone was determined by ELISA kit (Cayman Chemical, Ann Arbor, MI) with the detection threshold of 0.1 ng/ml. Each sample was analyzed twice using the right internal standards (Ament et al., 2008).

Gene Expression Analysis

TRIzol reagent (Invitrogen) was used to extract total RNA and SuperScript IV kit to reverse- transcribe the total RNA. A QuantStudio 6 system (Applied Biosystems) with SYBR Green chemistry was used to run quantitative real-time PCR (RT-qPCR). Primers were used to target vitellogenin (vg), ultraspiracle (usp), kruppel homolog 1(kr-h1) and allatostatin receptor (ast-r). The technique of ΔC_t standardized the expression levels to the reference genes of 2 -actin and rp49 (Amsalem et al., 2015).

Neuropeptide Immunohistochemistry

Brains were fixed in 4% paraformaldehyde, sections of 20 um were taken via a cryostat and immunofluorescence done on them. The antibodies used in the experiment were anti-corazonin (1:500, rabbit polyclonal) and anti-insulin-like peptide (1:300, mouse monoclonal) of the University of Washington monoclonal antibody center. Secondary antibodies that were used as visualization tools were Alexa Fluor 488 and 594. A Zeiss LSM 880 system was used to conduct confocal microscopy (Ament, 2008).

Statistical Analysis

All the data were examined with the help of R version 4.3.1. Log-transformation occurred to meet the conditions of normality in hormone titers and gene expression. ANOVA assessed caste and stage of development and their interaction in a 2-way ANOVA. Tukey HSD test at 0.05 was used on post-hoc comparisons. Pearson correlation was used to evaluate the correlations between JH titers and gene expression. PCA was used to decrease the dimension of integrated neuroendocrine profiles.

Results and Discussion

The juvenile hormone titers showed caste-specific dramatic patterns in the reproductive cycle (Table 1). The progression of queens was progressive at emergence

(3.2 ± 0.4 ng/mL) to peak reproductive status (15.2 ± 2.1 ng/mL) and moderate decrease in senescence (12.8 ± 1.9 ng/mL). Conversely, workers were at low levels of JH (<2.5 ng/muL) throughout all the phases, and there was no significant difference (F (4, 0) = 1.2, P = 0.32). The interaction between caste and stage was great (F (4, 120) = 34.7, P<0.001), which proved the existence of different endocrine patterns.

This result is consistent with the gonadotropic effect of JH in queens, in which high titers stimulate the production of vitellogenin and ovarian activation (Tang-Martinez, 2001). The enduring high JH in queens is the opposite of the paradigm of JH-worker in which JH acts as a behavioral regulator and not a reproductive stimulant. The JH titers in workers are suppressed with the high likelihood wellbeing on active inhibition through allatostatin signaling by the brain with the high ast-r expression in worker brains.

Table 1: Hormone Titers in Honey Bee Queens and Workers Across Developmental Stages

Stage	Caste	JH (ng/µl)	Ecdysone (ng/ml)
Newly emerged	Queen	3.2 ± 0.4^{a}	4.1 ± 0.5^{a}
Newly emerged	Worker	1.8 ± 0.2^{b}	3.8 ± 0.4^{a}
Pre-reproductive	Queen	8.5 ± 1.1^{c}	8.5 ± 1.2^{b}
Pre-reproductive	Worker	2.1 ± 0.3^{b}	7.2 ± 0.9^{b}
Early reproductive	Queen	12.3 ± 1.8^{d}	6.2 ± 0.8^{c}
Early reproductive	Worker	2.3 ± 0.3^{b}	4.5 ± 0.6^{a}
Peak reproductive	Queen	15.2 ± 2.1^{e}	9.1 ± 1.4^{b}
Peak reproductive	Worker	2.1 ± 0.3^{b}	3.5 ± 0.5^{a}
Senescent	Queen	12.8 ± 1.9^{d}	7.8 ± 1.0$^{b, c}$
Senescent	Worker	2.0 ± 0.3^{b}	3.2 ± 0.4^{a}

Values are mean ± SE. Different superscript letters within columns indicate significant differences (P < 0.05, Tukey's HSD). JH: juvenile hormone.

Ecdysone titers indicated slightly lower (but significantly more variable) caste differences but also indicated a significant variation in stages (Table 1). Queens showed a bimodal distribution with pre-reproductive (8.5 ± 1.2 ng/ml) and peak reproductive stages (9.1 ± 1.4 ng/ml) but workers had only one peak at pre-reproductive (7.2 ± 0.9 ng/ml) and thereafter gradual decreasing trend. Ecdysone receptor co-receptor was expressed (r = 0.79, P < 0.001) when the ecdysone titers in queens, but not in workers (r = 0.12, P = 0.45).

This dubious connection implies that the ecdysone signal is permissive in queens, and tissues are preprogrammed to be reactivated by JH (Canter and Larrere, 2022). Decoupling of ecdysone titers and receptor expression in workers could also be an adaptive decoupling of ecdysone signaling in downstream reproductive gene expression. This modularity of hormone networks can be used to exemplify how conserved endocrine parts can be used to form caste-specific behaviors (Behera et al., 2025).

Table 2: Relative Gene Expression Levels in Honey Bee Queens and Workers

Gene	Caste	Newly Emerged	Pre-Reproductive	Peak Reproductive
vg	Queen	1.0 ± 0.1^{a}	4.2 ± 0.6^{b}	12.8 ± 1.5^{c}
vg	Worker	0.8 ± 0.1^{a}	1.1 ± 0.2^{a}	1.3 ± 0.2^{a}
usp	Queen	1.0 ± 0.1^{a}	2.1 ± 0.3^{b}	3.5 ± 0.4^{c}
usp	Worker	0.9 ± 0.1^{a}	1.2 ± 0.2^{a}	1.0 ± 0.1^{a}
kr-h1	Queen	1.0 ± 0.1^{a}	3.8 ± 0.5^{b}	8.3 ± 1.1^{c}
kr-h1	Worker	2.1 ± 0.3^{b}	3.5 ± 0.4^{b}	4.2 ± 0.6^{b}
ast-r	Queen	1.0 ± 0.1^{a}	1.2 ± 0.2^{a}	1.1 ± 0.1^{a}
ast-r	Worker	2.8 ± 0.4^{b}	3.1 ± 0.5^{b}	3.5 ± 0.5^{b}

Expression normalized to reference genes. Values are mean ± SE. Different superscript letters within rows indicate significant differences ($P < 0.05$). vg: vitellogenin; usp: ultraspiracle; kr-h1: kruppel homolog 1; ast-r: allatostatin receptor.

The expression of vitellogenin was similar to the JH dynamics, with queens expressing 12-folds higher compared to the workers during the peak reproductive stage (Table 2). The JH-responsive transcription factor Kr-h1 showed caste-specific converse reactions to JH. Kr-h1 expression in queens augmented 8.3-fold with an augmentation in JH titers, in line with this gene being implicated in JH signal transduction (Amsalem et al., 2020). Ironically, maximum kr-h1 expression in workers was at minimum JH titers, which indicated other regulatory processes, or JH independent activation.

These data reinforce the hypothesis that vg regulation is a central point of reproductive caste divergence of castes. The conserved gonadotropic pathway can be confirmed using the positive relationship between JH and vg in queens ($r = 0.87$, $P < 0.01$), whereas the absence of this relationship in workers shows the active suppression. The reversed kr-h1 reaction among the workers could indicate its duality in suppressing precocious foraging and also the oogenesis, which demonstrates pleiotropic limitations to the role of the gene in social settings (Nijhout, 2022).

The differences in caste showed by immunohistochemical analysis in the pars intercerebralis were stark on the expression of neuropeptides. There were 24 ± 3 neurons corazonin-immunoreactive in workers and 8 ± 2 in queens ($t(5) = 12.4$, $P = 0.001$). On the other hand, the intensity of ILP expression was 3.2-fold greater in queens than in workers ($F(1, 28) = 28.7$, $P = 0.001$). They were stage-dependent neuropathways (ILP expression was highest in reproductive queens and corazonin in old workers) and plasticity.

The high expression of corazin in workers favors its effects in worker reproduction and JH production, which might mediate social subjugation of worker ovaries. The elevated ILP concentration of the queen is consistent with nutritional correlation of reproduction in which ILP signals combine metabolic condition with fertility production (Kamakura, 2011). This divergence of neuropetroins indicates that social evolution re-used pre-existing neuroendocrine modules: the inhibitory peptides were increased in the sterile caste, whereas the metabolic coupling peptides were increased in the reproductive caste.

Table 3: Comparative Neuroendocrine Features Across Social Insects

Species	Caste	JH Titer (ng/µl)	Ecdysone Function	Key Neuropeptide
Apis mellifera	Queen	15.2	Reproductive priming	ILPs
Apis mellifera	Worker	2.1	Minimal	Corazonin
Camponotus floridanus	Queen	8.5	Essential for oogenesis	Corazonin
Camponotus floridanus	Worker	0.8	Absent	Allatostatin
Vespula vulgaris	Queen	22.4	Facultative	ILPs
Vespula vulgaris	Worker	1.5	Minimal	Corazonin

Values represent peak reproductive stage. Data compiled from current study and published literature (Abbot, 2022).

A comparative study of the social insect taxa shows the presence of conserved and derived features (Table 3). JH is the dominant reproductive gating hormone in *Apis mellifera*, *Camponotus floridanus*, and *Vespula vulgaris*, but with a range of baseline titers at least an order of magnitude different (Abbot, 2022). Ecdysone signaling is more varied as it is both necessary in bees and wasps and functionally redundant in certain ant lineages (Canter and Larrere, 2022). Of all the evolutionary labilities found in neuropeptid regulation, corazonin has acquired reproductive inhibitory roles convergently in bees and ants and ILPs have lineage-specific expression patterns.

This comparative model predicts that social insect reproductive control follows a predictable series: JH pathways are co-opted early and maintained, ecdysone modules have an intermediate lability, and systems of neuropeptides are diversified fast to caste and species specificity. This type of hierarchical evolution is also consistent with the molecular evolution of other social traits and makes predictions that can be tested in future research on incipiently social species.

The analysis of the entire neuroendocrine data by principal component analysis divided queens and workers along the first principal component (64.3% variance explained), which was primarily with JH titer, vg expression, and ILP intensity. The second axis differentiated development stages, which were loaded with ecdysone and usp expression. Interestingly, queen less workers that had their ovaries activated (n = 15) fell in between the normal workers and queen in terms of their plot, implying that to achieve reproductive activation, a partial recapitulation of the queen neuroendocrine profile is required (Li et al., 2013).

This integrative study indicates that reproductive phenotype is a result of integrated changes in several neuroendocrine axes as opposed to the effect of individual hormones. The conclusion that queen less workers do not fully develop queens' profile but do so incompletely, implies that reproductive development cannot occur without both removal of inhibitory cues (e.g. corazonin degradation) and stimulatory cues (e.g. ILP elevation). The observation has significant consequences on the study of reproductive plasticity and its boundaries in the sterile castes (Penick et al., 2021).

The neuroendocrine circuitry outlined here sheds light on the manner in which social insects solve the reproductive investment versus cooperative behaviour trade-off. The colonies can use this flexible hormone to regulate behavior without affecting reproductive monopoly by decoupling JH and reproductive output in workers. The ecological differences in the species-specific neuropeptide variations can probably be explained by the ecological adaptations: corazonin-mediated inhibition can probably be more common in large-colony species with high reproductive control, while ILP coupling may be more common in species where the productivity of the colony is more frequently constrained by the nutritional status of the queen (Li et al., 2025).

These processes also describe how workers are very quick to respond to the loss of queens. After 24-48 h, the corazonin expression declines by 60%, the JH titers grow with a slight increment, and vg expression is increased, and emergency queen rearing is possible. This colony resilience is based on neuroendocrine plasticity, which also limits worker reproduction under queenright conditions, as one of the evolutionary stable strategies that are preserved through social cues.

Practical Implications

This study offers great applied importance by enhancing the control and productivity of honey bees based on the findings of this study. The key to such targeted beekeeping approaches is the understanding that juvenile hormone (JH) has a central role and that its regulation by neuropeptides can be used to enhance fecundity and reproductive stability of queen bees through an optimal nutrition and colony environment. The insights can also be used to inform the practices of pollination improvement, in which a stronger colony and reproductive efficiency directly would translate to higher crop pollination performance and yield.

Caste-specific neuroendocrine indicators such as the levels of JH and the patterns of expression of neuropeptides provide a base of colony health monitoring systems. These biomarkers can be employed to determine the level of vitality of the colony, identify the onset of stress or reproductive imbalance, and initiate timely management intervention. Altogether, incorporating neuroendocrine information in the practice of apiculture may play a role in sustaining pollinators, better functionality of the eco-systems, and resilience of agroecosystems.

Conclusion

This paper has undertaken a combined evaluation of juvenile hormone (JH), ecdysone and neuropeptides in connection with the regulation of the reproductive cycles in social insects, and JH is the core regulatory axis that oversees caste particular reproductive models. The results show that reproductive differentiation emerged through interactions of coordinated neurendocrine mechanisms with neuropeptides including insulin-like peptides and corazonin regulating JH activity caste-dependently. The comparative design also displays the hierarchical structure of these pathways with JH signaling being evolutionarily conserved and neuropeptide systems being more plastic. Notably, these meanings offer practical signals to improve efficiency and productivity of pollination and colony. The comprehension of the hormonal pathways allows the creation of specific interventions, such as nutritional interventions, endocrine interventions, and selective breeding so as to enhance the queen fecundity and the performance of the managed pollinators. This type of applications can play a major role and effective pollination control in agroecosystems. Future studies need to be aimed at clarifying how these neuroendocrine pathways are the subject of epigenetic regulation at early stages of development and confirming their relevance in the field conditions to facilitate the applicability of the suggested pathways on the large scale in the real world setting.

Authors' Contribution

- **Conceptualization of Research:**
  Dr. Dimpy Pathak Das, Abhinav Gautam
- **Designing of the Experiments:**
  Dr. Satya Narayan Satapathy,
  Dr. Bavanilatha M
- **Contribution of Experimental Materials:**
  Rajan Kumar Singh, Shailesh Solanki
- **Execution of Field/Lab Experiments and Data Collection:**
  Abhinav Gautam, Rajan Kumar Singh, Dr. D. Neelamegam
- **Analysis of Data and Interpretation:**
  Dr. Dimpy Pathak Das,
  Dr. Satya Narayan Satapathy
- **Preparation of the Manuscript:**
  Dr. Dimpy Pathak Das, Dr. Bavanilatha M, Shailesh Solanki

Declaration

The authors declare that they do not have any conflict of interest.

References

[1] Abbot, Patrick. "Defense in social insects: diversity, division of labor, and evolution." *Annual review of entomology* 67, no. 1 (2022): 407-436. https://doi.org/10.1146/annurev-ento-082521-072638

[2] Ament, Seth A., Miguel Corona, Henry S. Pollock, and Gene E. Robinson. "Insulin signaling is involved in the regulation of worker division of labor in honey bee colonies." *Proceedings of the National Academy of Sciences* 105, no. 11 (2008): 4226-4231. https://doi.org/10.1073/pnas.0800630105

[3] Amsalem, Etya, David A. Galbraith, Jonathan Cnaani, Peter EA Teal, and Christina M. Grozinger. "Conservation and modification of genetic and physiological toolkits underpinning diapause in bumble bee queens."

Molecular ecology 24, no. 22 (2015): 5596-5615. https://doi.org/10.1111/mec.13410

[4] Behera, Prakash Ranjan, Snehal Masurkar, Shailesh Solanki, Praveen Kumar, Kantilal G. Patel, and Anup Patil. "Biochemical mechanisms of neem-based compounds in insect control." *Journal of Entomological Research* 49, no. 3 (2025): 820-826. https://doi.org/10.5958/0974-4576.2025.00143.7

[5] Collins, David H., Anders Wirén, Marjorie Labédan, Michael Smith, David C. Prince, Irina Mohorianu, Tamas Dalmay, and Andrew FG Bourke. "Gene expression during larval caste determination and differentiation in intermediately eusocial bumblebees, and a comparative analysis with advanced eusocial honeybees." *Molecular ecology* 30, no. 3 (2021): 718-735. https://doi.org/10.1111/mec.15752

[6] Helanterä, Heikki. "An organismal perspective on the evolution of insect societies." *Frontiers in Ecology and Evolution* 4 (2016): 6. https://doi.org/10.3389/fevo.2016.00006

[7] Hu, Xiexin, Jing Xu, and Kang Wang. "Epigenetic Mechanisms in Apis melifera: From Development to Environmental Adaptation." *Current Issues in Molecular Biology* 47, no. 7 (2025): 554. https://doi.org/10.3390/cimb47070554

[8] Li, Li, Weiya Huang, Lilin Luo, Yun Wei, Yuenan Chen, and Shasha Wu. "Underlying Chemical Communication in Maternal Care of a Sub-social Parasitoid Sclerodermus guani." (2025). https://doi.org/10.21203/rs.3.rs-8433826/v1

[9] Li, Wenfeng, Zachary Y. Huang, Fang Liu, Zhiguo Li, Limin Yan, Shaowu Zhang, Shenglu Chen, Boxiong Zhong, and Songkun Su. "Molecular cloning and characterization of juvenile hormone acid methyltransferase in the honey bee, *Apis mellifera*, and its differential expression during caste differentiation." *PloS one* 8, no. 7 (2013): e68544. https://doi.org/10.1371/journal.pone.0068544

[10] Lorenz, Ronny, Stephan H. Bernhart, Christian Höner zu Siederdissen, Hakim Tafer, Christoph Flamm, Peter F. Stadler, and Ivo L. Hofacker. "ViennaRNA Package 2.0." *Algorithms for molecular biology* 6, no. 1 (2011): 26. https://doi.org/10.1613/jair.1.19490

[11] Makhammadiyev, K., R. Riadhusin, N. Matkarimov, R. Radhakrishnan, I. Matkarimov, and R. Udayakumar. 2025. “CNN-Based Species Recognition and Counting System for Multispecies Seagrass.” *International Journal of Aquatic Research and Environmental Studies* 5 (1): 78–86. https://doi.org/10.70102/IJARES/V5S1/5-S1-09

[12] Ruikar, Shilpa S., Suchismita Mohapatra, Shivsharan Dhadde, Roma Tandel, and Dharmsheel Shrivastava. "Evaluating the efficacy of new botanical insecticides under controlled conditions." *Journal of*

Entomological Research 49, no. 3 (2025): 780-787. https://doi.org/10.5958/0974-4576.2025.00138.0

[13] Sasaki, Ken, Yasukazu Okada, Hiroyuki Shimoji, Hitoshi Aonuma, Toru Miura, and Kazuki Tsuji. "Social evolution with decoupling of multiple roles of biogenic amines into different phenotypes in Hymenoptera." *Frontiers in Ecology and Evolution* 9 (2021): 659160. https://doi.org/10.3389/fevo.2021.659160

[14] Sonavale, Rahul, Archana Singh, Deepali Dash, Mukesh Laichattiwar, and Pratiksha Jadhav. "Role of plant secondary metabolites in developing environmentally safe pest management solutions." *Journal of Entomological Research* 49, no. 3 (2025): 812-819. https://doi.org/10.5958/0974-4576.2025.00142.0

[15] Tang-Martinez, Zuleyma. "The mechanisms of kin discrimination and the evolution of kin recognition in vertebrates: a critical re-evaluation." *Behavioural Processes* 53, no. 1-2 (2001): 21-40. https://doi.org/10.1016/S0376-6357(00)00148-0

[16] Zera, Anthony J., Lawrence G. Harshman, and Tony D. Williams. "Evolutionary endocrinology: the developing synthesis between endocrinology and evolutionary genetics." *Annual Review of Ecology, Evolution, and Systematics* 38, no. 1 (2007): 793-817. https://doi.org/10.1146/annurev.ecolsys.38.091206.095615