Open Access Library Journal
2025, Volume 12, e14693
ISSN Online: 2333-9721
ISSN Print: 2333-9705

Epigenetic Mechanisms That Interact in the Development of Social Insects

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How to cite this paper: Castellanos-Zacarías, C., Domínguez-Rebolledo, A., Us-Camas, R., Baeza-Rodríguez, J., Zamora-Bustillos, R., Rodríguez-Salinas, A., Vivas-Rodríguez, J., Ramón-Ugalde, J. and Loeza-Concha, H. (2025) Epigenetic Mechanisms That Interact in the Development of Social Insects. *Open Access Library Journal*, **12**: e14693.
https://doi.org/10.4236/oalib.1114693

Received: November 30, 2025
Accepted: December 26, 2025
Published: December 29, 2025

Abstract

Epigenetic mechanisms regulate and stabilize a broad range of biological processes without altering the primary DNA sequence. This regulation is achieved through DNA methylation, post-translational histone modifications (PTMs), and non-coding RNAs (ncRNAs). These mechanisms facilitate rapid and flexible changes in gene expression in response to environmental cues such as temperature, humidity, nutrition, and chemical exposure. Upon detection of these signals, the neuroendocrine system relays the information to target tissues, where it initiates the reprogramming of gene expression and the subsequent generation of phenotypic changes. Among the most extensively studied phenomena are development, sex and caste determination, seasonal effects, dispersal, and behavioral polyphenisms. These diverse phenotypes result from the epigenetic modulation of a shared genome, enabling genetically identical individuals to produce distinct "morphs." This review summarizes current understanding of the core epigenetic mechanisms including DNA methylation, histone modifications, and non-coding RNAs and elucidates their pivotal role in the gene regulation of polyphenism in social insects.

Subject Areas

Reproductive Biotechnology

Keywords

Epigenetics, Methylation, Polyphenism, Social Insects

1. Introduction

Throughout their development, organisms have the intrinsic capacity to adjust their life history trajectory to adapt to specific environmental conditions or respond to changes in the surrounding environment. However, this flexibility can potentially lead to a phenotype-environment mismatch due to environmental variation, which may have significant implications for individual survival and reproductive success [1].

In social insects, these organisms possess complex systems of gene regulation that allow for the organization of tens of thousands of individuals, of which only a small fraction is reproductive [2]. Gene regulation in these organisms is highly sensitive to nutritional changes [3], a factor that can trigger specific physiological responses within the colony.

Epigenetic regulatory mechanisms, such as DNA methylation, Post-Translational Histone Modifications (PTMs), and non-coding RNAs (ncRNAs), are fundamental in regulating metabolism and RNA processing. These mechanisms profoundly influence phenotypic plasticity, enabling organisms with the same genotype to respond adaptively to variations in biotic and abiotic environments [4].

Among insect models and social insect models for the study of epigenetics are the fruit fly (*Drosophila melanogaster*) [5], the honey bee (*Apis mellifera*) [6], the bumblebee (*Bombus terrestris*), the yellow meadow ant (*Lasius flavus*), the black garden ant (*Lasius niger*) [7], the Nevada dampwood termite (*Zootermopsis nevadensis*) [8], and the Florida carpenter ant (*Camponotus floridanus*) [9], among others.

In the majority of Hymenoptera, nutritional stimuli, such as royal jelly, significantly influence phenotypic plasticity and DNA methylation, crucially determining the development of female castes (queen or worker). Furthermore, these stimuli affect pheromones, thereby impacting the behavior and physiology of adult workers [10] [11].

Less research has focused on the male caste (drone), where it is commonly assumed that sperm solely contributes DNA to the fertilized egg. However, studies have demonstrated that drones undergo both DNA methylation loss and gain during their development and spermatogenesis [12]. This process restricts the mating range of the newly inseminated queen [13], enhances the reproductive status of her daughters [14], and increases the probability of them being reared as reproductive queens [15].

Overall, eusocial insects like *Apis mellifera* provide a valuable model for understanding the role of epigenetics in phenotypic plasticity, demonstrating how they can produce distinct caste phenotypes in response to environmental variations. This not only provides insight into the mechanisms underlying these changes but also sheds light on the evolutionary origins of eusociality, reproduction, and biodiversity.

Therefore, the main objective of this review is to provide crucial information on epigenetic mechanisms including DNA modification, histone alterations, and non-coding RNAs and their fundamental role in the transcriptional regulation of

key genes that influence the reproductive behavior and phenotypic plasticity of social insects.

2. Epigenetic Transcriptional Mechanisms Involved in Sex Determination in Insects

Within the insect kingdom, sets of genetic regulators possess the capacity to adapt and be modified in response to a variety of environmental, physiological, and social stimuli. This plasticity enables them to fine-tune their reproduction and pheromone production according to surrounding conditions [16].

A clear example of this modulation involves the variation in nutritional resource availability, which can induce changes in gene expression and the activity of the biochemical pathways that govern these processes [17].

In this context, orthologous genes are noteworthy. These are genes that share a common ancestor and a similar function across different species, such as bees and ants. These genes exhibit comparable levels of sensitivity to pheromones, reflecting evolutionary convergence in their adaptation to shared environmental factors [7] (See **Figure 1**).

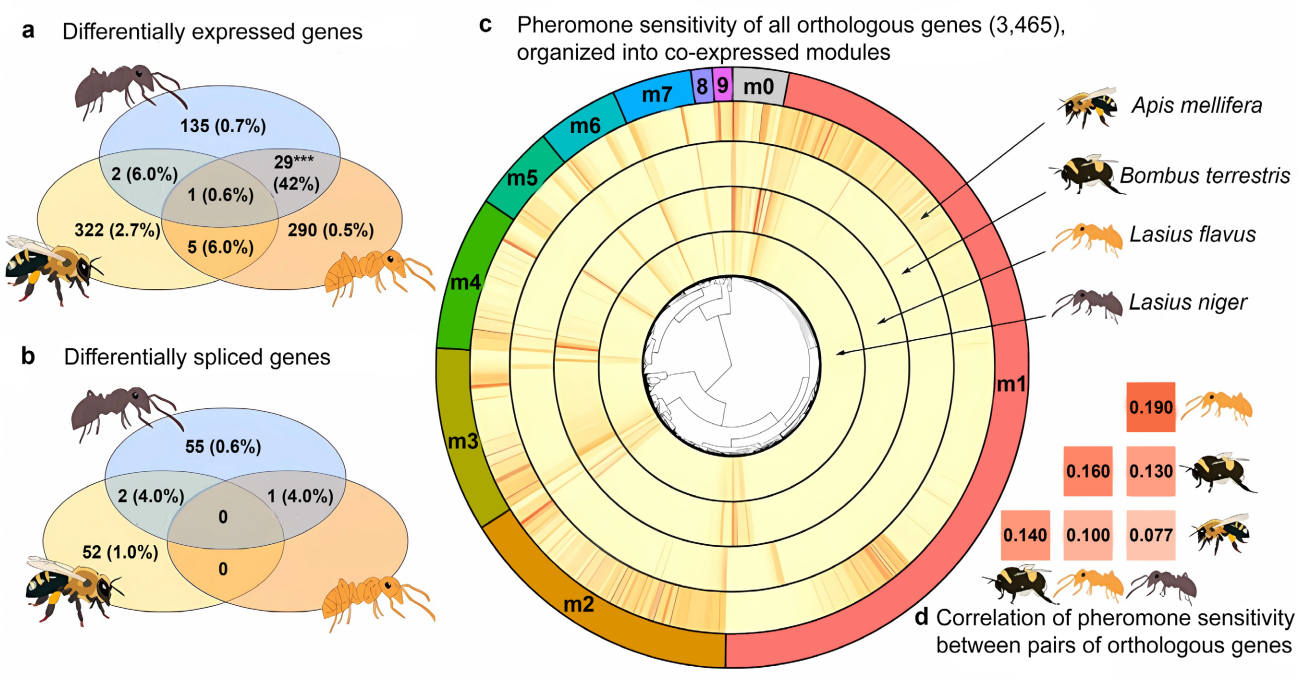

Figure 1. Effects of Queen Pheromone on Gene Expression and Splicing Overlap Among Bee and Ant Species. The analysis focuses on four key aspects related to the queen pheromone: (a)-(b) Differentially spliced or expressed genes per species, visualized using Venn diagrams. (**c**) Similarity in pheromone sensitivity across 3465 orthologous genes. (d) Correlation of queen pheromone sensitivity between pairs of species. From: [7].

2.1. Conserved Orthologous Gene Modules and Pheromone Response

The honey bee (*Apis mellifera*), the bumblebee (*Bombus terrestris*), and the ants (*Lasius flavus* and *Lasius niger*) share nine orthologous gene modules that are co-

expressed. Crucially, three of these modules (Modules 1, 4, and 9) respond specifically and uniformly to queen pheromones across all species studied. This particular sensitivity to pheromones within gene expression modules underscores the conservation of chemical communication mechanisms among diverse Hymenopteran species (See Figure 2).

Module 1: Epigenetic regulation and transcriptional machinery, clusters genes associated with the cell cycle, DNA repair, transcription, RNA splicing, and ribosome formation. It includes key genes such as DNA methyltransferase 3 (*Dnmt3*) and various histone deacetylases and methyltransferases, which are essential for epigenetic regulation and the modulation of gene expression.

Module 4: Cellular metabolism and pheromone precursors, focuses on cellular metabolism, including the pentose phosphate pathway, the biosynthesis of fatty acids and amino acids, and lipid metabolism. It also includes genes of the

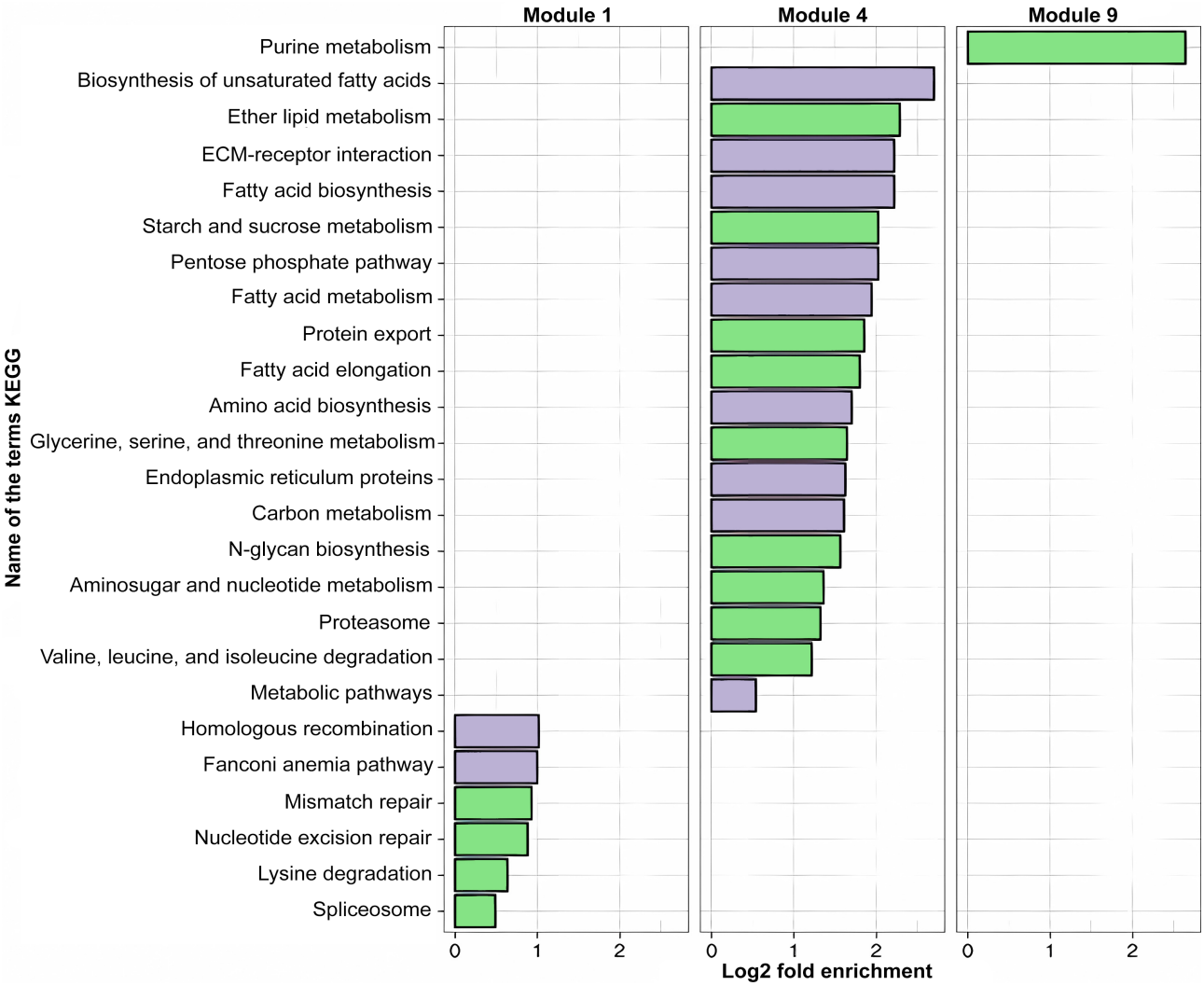

Figure 2. KEGG Pathway Enrichment Analysis of Genes within the Three Transcriptional Modules (1, 4, and 9) exhibiting specific sensitivity to queen pheromones. The figure illustrates the enriched biological pathways associated with the genes clustered in each module. From: [7].

endoplasmic reticulum (ER) involved in the synthesis of long-chain fatty acids and Acetyl Coenzyme A (Acetyl-CoA). These compounds are precursors to cuticular hydrocarbons (CHCs) and are key components of the Queen Mandibular Pheromone (QMP).

Module 9: Purine metabolism and energy production encompasses genes related to purine metabolism, which is necessary for cell division and transcription. Furthermore, this module includes genes involved in the synthesis of crucial biomolecules such as adenosine triphosphate (ATP), reduced nicotinamide adenine dinucleotide (NADH), and Coenzyme A. These are fundamental for maintaining cellular functions and energy production [7].

These findings highlight the specificity and complexity of the molecular mechanisms that respond to pheromones in Hymenoptera. The conservation of these transcriptional modules underscores their crucial role in regulating essential biological processes, such as reproduction and social behavior.

2.2. Epigenetic and Transcriptional Mechanisms in Insect Sex Determination

In both the honey bee (*Apis mellifera*) and the yellow meadow ant (*Lasius flavus*), queen pheromones induce significant changes in alternative splicing across multiple gene loci. This mechanism is fundamental for the generation of polyphenisms and enables the emergence of distinct phenotypes from a shared genome. Similar phenomena have been documented in other insects, such as the small brown planthopper (*Nilaparvata lugens*) [15] and the fruit fly (*Drosophila melanogaster*) [18].

2.3. Alternative Splicing and Phenotypic Diversity

Alternative splicing plays an essential role in generating phenotypic diversity in insects. In Nilaparvata lugens, the knockdown of the Transformer-2 (Tra-2) gene using RNA interference (RNAi) during the nymph stage prevents ovarian development, leading to the production of long-winged adults. This process also decreases the expression of key genes, such as the transcription factor FoxO (NlFoxO), and genes involved in insulin signaling and nutrition, including phosphatidylinositol-3-OH kinase (PI3K) and protein kinase B (Akt) [19].

2.4. The Doublesex (dsx) Pathway

In the majority of insects, sex determination is mediated by the production of the Doublesex (dsx) protein. The doublesex pre-messenger RNA (pre-mRNA) undergoes alternative splicing, generating three female-specific isoforms (*dsxf1*, *dsxf2*, and *dsxf3*) and one male-specific isoform (*dsxm*). These isoforms function as transcription factors that regulate somatic sex determination and differentiation, often interacting with juvenile hormone signaling pathways. This hormone acts as a key intermediary linking nutrition to the development of structures, such as mandibles, in beetles like *Cyclommatus metallifer*, *Onthophagus taurus* [20], and

Tribolium castaneum [21].

In species lacking sex chromosomes, the determination of secondary sexual characteristics varies significantly. In these cases, sexual differentiation is controlled by the autosomic gene Sex lethal (*Sxl*), which encodes an RNA-binding protein that regulates the expression of genes producing the TRA and TRA-2 proteins. These proteins modulate the alternative splicing of the doublesex mRNA (*dsx*), generating either female-specific (*dsxf*) or male-specific (*dsxm*) isoforms. The *dsx* isoforms subsequently function as transcription factors that regulate the expression of sex phenotype-associated genes. This mechanism has been documented in species like *Drosophila melanogaster* and *Bombyx mori* [22].

2.5. Sex Determination in Bees

In bees, sex determination is controlled by the feminizer (*fem*) gene, which undergoes sex-specific splicing during embryonic development to determine the individual's sex [23]. Furthermore, the expression of this gene varies specifically according to sex and caste during larval development, as observed in the Jandaíra stingless bee (*Melipona interrupta*) [24].

2.6. Epigenetic Regulation of Metabolism and Caste

In *Apis mellifera*, gene methylation regulates various essential metabolic pathways crucial for cellular maintenance, including the Tricarboxylic Acid (TCA) cycle, the ubiquitin-proteasome pathway, the inositol phosphate/TOR (Target of Rapamycin) pathway, the cell division spliceosome, and the cytoskeletal network [25].

The TCA cycle plays a crucial role in generating metabolic fluxes associated with insulin signaling in the brain of worker bees, both in their nurse and foraging stages. The regulation of TCA routes and the Insulin/TOR signaling is mediated by phosphoenolpyruvate carboxykinase (PEPCK), which has two isoforms: a mitochondrial and a cytoplasmic one. The latter is regulated by insulin and participates in the gluconeogenic pathway, integrating metabolic signals into these biological processes.

The TOR/PI3K pathway constitutes a complex multigenic network that links nutrient detection with the regulation of cellular growth and metabolism, generating signals that promote systemic growth. This pathway is crucial for development and cellular homeostasis, especially in response to nutrient availability. In *Apis mellifera*, differences in gene expression associated with cerebral energy metabolism and insulin signaling have been observed between nurse and forager bees. These variations allow bees to adapt efficiently to their specific roles within the colony, optimizing foraging and the transition to brood care behaviors [17].

2.7. Evolutionary Diversity of Sex Determination

Finally, the great diversity of sex determination mechanisms observed across insects reflects the rapid evolution of the genes involved in this process. These mech-

anisms can vary considerably among species and are influenced by both genetic and epigenetic factors.

3. Epigenetic Mechanisms Involved in Development and Caste Determination in Insects

3.1. Non-Coding RNAs (ncRNAs)

MicroRNAs (miRNAs) are small, endogenous non-coding RNAs that play an essential role in the post-transcriptional regulation of messenger RNA (mRNA), modulating its stability, transcription, and translation [26]. These miRNAs participate in diverse biological processes, including RNA-directed DNA methylation, translational activation, and alternative splicing [27].

In social insects, miRNAs are key regulators of critical cellular processes such as neuronal differentiation, cellular signaling, and the formation of caste-specific structures. This highlights their importance in phenotypic plasticity and the adaptive capacity within a bee colony [28].

3.2. Genetic and Epigenetic Basis of Caste

In most social insects, such as bees, both queens and workers develop from genetically identical eggs. However, the type and quantity of larval diet play a fundamental role in DNA methylation patterns, thereby determining caste differentiation [29]. Fertilized eggs, which have the potential to develop into workers or queens, are diploid (containing two alleles per gene). In contrast, unfertilized eggs, which give rise to drones, are haploid (possessing a single allele per gene). This interplay between nutrition and genetics demonstrates how epigenetic and genetic factors collaborate to determine the development and specialized functions of the different castes within the colony.

During the first 24 hours of larval development in social insects, no major differences in overall miRNA abundance or transcription are typically detected. However, subtle but functionally significant variations in the profiles of small RNAs particularly piRNAs and miRNAs begin to emerge and contribute to early caste specific trajectories [30]. While miRNAs primarily regulate post-transcriptional gene expression by promoting mRNA degradation or translational repression, piRNAs play a central role in transposon silencing and the preservation of genomic integrity in the germline [31].

In *Apis mellifera*, caste bias arises as these small RNA classes begin to diverge: miRNAs such as miR-133 and miR-375 modulate larval growth, nutrient dependent signaling, and ovary primordia development, thereby contributing to the differential reproductive potential of queens versus workers [28]. In parallel, piRNAs act as guardians of genomic stability by suppressing transposable elements during early larval stages, helping maintain the epigenetic fidelity necessary for stable caste specific gene expression programs [31].

piRNAs are associated with PIWI-family proteins and act as guides in the silencing of transposons through various epigenetic mechanisms, including histone

modification, DNA methylation, and post-transcriptional processes like RNA degradation and cleavage [32]. In addition to their role in gene silencing within insect germline cells [33], piRNAs also participate in DNA damage repair and fundamental processes like sex determination and development, as shown in the silkworm (*Bombyx mori*) [31].

3.3. The Role of Royal Jelly and Dietary ncRNAs

In *Apis mellifera*, the miRNAs present in royal jelly a secretion from the nurse bees' hypopharyngeal glands play an essential role in caste differentiation. Royal jelly contains specific miRNAs and is produced via pathways related to protein synthesis and energy metabolism [34].

When newly hatched larvae receive an abundant diet of royal jelly, it reduces the expression of the Dnmt3 gene, which encodes a DNA methyltransferase. This, in turn, decreases global DNA methylation, favoring the development of the queen phenotype over the worker phenotype [29].

In contrast, bee bread (a mixture of honey and plant pollen) contains higher levels of plant-derived miRNAs that significantly influence larval development by delaying growth, reducing body size, and affecting ovary development in worker bees [35].

3.4. Signaling Pathways Integrating Nutrition and Caste

In *Apis mellifera*, feeding activates several essential signaling pathways associated with the Epidermal Growth Factor Receptor (Egfr) gene. These pathways include the Mammalian Target of Rapamycin (mTOR) pathway, the Juvenile Hormone (JH) pathway, and insulin signaling routes [36]. These molecular networks regulate critical processes such as cell proliferation, growth, and larval development.

A key component in this process is Royalactin (MRJP-1), a protein present in royal jelly. Royalactin plays a determining role in caste differentiation by directly interacting with the mTOR gene network, stimulating cell proliferation and modulating Egfr gene expression. Through these signaling pathways, Royalactin promotes the development of queen-specific characteristics, such as larger body size and fully developed ovaries, which are essential for her reproductive role within the colony [37].

In the carpenter ant (*Camponotus floridanus*), the transcription of the Egfr gene is associated with variations in larval size, highlighting the importance of this pathway in regulating growth and development across social insects [8].

Another fundamental pathway for caste differentiation in *Apis mellifera* is the Insulin signaling pathway. The transcription of insulin-like peptides, such as Honeybee Insulin-Like Peptide 2 (AmILP2), plays a crucial role in the development of specific organs (e.g., ovaries) and in determining body mass [38]. This pathway also modulates the response to dietary carbohydrates, regulating the availability and use of energy resources. These energetic adjustments have a direct impact on differential caste development, allowing larvae designated as queens to

develop distinctive characteristics such as greater body size and functional ovaries.

3.5. DNA Methylation: Epigenetic Control of Caste in Social Insects

DNA methylation is an essential epigenetic process mediated by DNA methyltransferases (DNMT). This mechanism initiates when the DNMT3 methyltransferase introduces de novo methylation marks onto specific DNA regions, particularly at 5'-CG-3' dinucleotides, known as CpG islands. Once established, these epigenetic patterns are maintained during mitotic cell divisions by the action of maintenance DNA methyltransferase (DNMT1). DNMT1 ensures the stability and heritability of gene expression programs, playing a critical role in development and cellular differentiation [39] [40].

Nutrient sensing pathways such as Insulin/TOR and EGFR signaling interface directly with the epigenetic machinery to modulate chromatin accessibility and caste-biased gene expression. Royalactin mediated activation of mTOR enhances the activity of histone acetyltransferases such as p300, increasing H3K27ac deposition at the promoter regions of queen associated developmental genes, thereby promoting a transcriptionally permissive chromatin state [28]. Likewise, juvenile hormone (JH) signaling recruits DNA methyltransferases, including DNMT3 for de novo methylation and DNMT1 for maintenance methylation, to establish differential methylation patterns across caste related loci. This mechanistic linkage between hormonal cues and DNMT mediated epigenetic reprogramming provides a functional axis through which nutritional environment and endocrine status shape long-term developmental trajectories in *Apis mellifera* [41] [42].

3.6. Role of DNMT in Honey Bee Caste Determination

In the sequenced genome of *Apis mellifera*, differential DNA methylation mediated by DNMT enzymes plays a central role in caste development. This epigenetic process regulates the phenotypic differentiation between queens and workers by influencing the biosynthesis of juvenile hormone (JH), a crucial component in caste determination [41].

Research has demonstrated that DNA methylation is also closely linked to the alternative splicing of caste-specific genes. For instance, the knockdown of the methylating enzyme Dnmt3 in the fat body tissue of adult bees significantly affects alternative splicing, thereby altering gene expression and, consequently, cellular functions [43]. Furthermore, the deletion of the Dnmt3 gene in newly emerged larvae produces effects similar to those induced by royal jelly, suggesting a shift in development towards queen-like morphological characteristics [44]. These findings reinforce the importance of differential DNA methylation as a key regulatory mechanism in phenotypic plasticity and caste development in social insects.

3.7. Caste-Specific Gene Regulation

In *Apis mellifera* larvae, those destined to become queens predominantly regulate genes related to physio-metabolic processes, while worker larvae activate genes

associated with the development of specific structures, such as pollen baskets [41].

During female caste differentiation, modifications in gene regulation can generate significant developmental changes. These modifications, even incremental ones, affect both general growth and the development of specific organs, such as the ovaries. This directly impacts the size and functionality of certain structures, a phenomenon also observed in other social species, such as the Pharaoh ant (*Monomorium pharaonis*) [45].

3.8. Contrasting Mechanisms in Social Insects

Unlike *Apis mellifera*, in the stingless bee *Melipona scutellaris*, sex and caste cannot be morphologically distinguished during the larval stages [46]. In this species, caste differentiation is strongly influenced by specific compounds present in the larval diet. One of the most significant is geraniol, a pheromonal compound secreted by the labial glands of nurse bees that directly inhibits queen development by suppressing ovarian differentiation and lowering juvenile hormone titers [47].

Beyond this immediate physiology effect, geraniol also modulates the expression of key reproductive and metabolic genes, including vitellogenin and insulin-like peptides, thereby steering larvae toward worker specific developmental trajectories. Experimental exposure to geraniol reduces ovary size and shifts metabolic pathways toward worker phenotypes, phenotypes, notably enhancing the expression of genes associated with foraging related functions [47].

In contrast, caste differentiation in *Apis mellifera* depends on an interaction between genetic and environmental factors. These factors directly affect larval nutrition and, collectively, regulate the transcriptional control of juvenile hormone esterase (JHE), a key enzyme in the regulation of JH biosynthesis, which plays a vital role in this process [48].

3.9. Methylation, Alternative Splicing, and CTCF

In *Apis mellifera* larvae, DNA methylation plays a crucial role in regulating the alternative splicing of genes, a process that occurs co-transcriptionally. This mechanism is mediated by the interaction of protein complexes that influence both the elongation kinetics of RNA polymerase II and the RNA structure.

A key aspect of this process is the ability of DNA methylation to inhibit the function of the CCTC-binding factor (CTCF) protein. CTCF typically facilitates the inclusion of exons at the 5' end of the gene by pausing RNA polymerase II, thereby promoting alternative splicing [49]. This mechanism regulates the expression of specific genes and plays an essential role in phenotypic differentiation and caste development in social insects.

3.10. Dietary Components and Signaling Pathways

p-Coumaric acid, a plant derived phenolic compound present in honey, pollen, and consequently in the larval diet of *Apis mellifera*, plays a key regulatory role in caste differentiation. This molecule modulates the expression of genes involved in larval development, transcriptional control, and the Hippo signaling pathway

[50]. Biologically, *p*-coumaric acid reinforces the worker developmental trajectory by suppressing queen biased molecular programs, acting through two major nutrient sensing pathways: the Insulin/Target of Rapamycin (IIS/TOR) pathway and the Epidermal Growth Factor Receptor (EGFR) [37].

Exposure to p-coumaric acid downregulates growth promoting genes and delays development, resulting in reduced body size and the stabilization of worker phenotypes. Additionally, its presence in bee bread counteracts royal jelly induced epigenetic shifts, thereby preventing the activation of queen specific pathways and ensuring the maintenance of worker specific growth trajectories [35].

3.11. The ALK Gene and Nutrient-Independent Growth

Among the Differentially Methylated Genes (DMG) in *Apis mellifera* is the gene encoding the Anaplastic Lymphoma Kinase (ALK) protein. This protein plays a crucial role in diet-independent growth by directly activating the PI3K pathway, independently of the Insulin Receptor (InR) and TOR. The ALK gene exhibits 27 exons that are alternatively transcribed and spliced, generating numerous functional isoforms. Furthermore, ALK regulates PI3-kinase signaling based on nutritional input in both adult and larval bees [41].

In *Drosophila melanogaster*, ALK has a protective function in the growth of neuronal progenitors under conditions of nutrient restriction. This mechanism operates via two main pathways:

1. Suppression of amino acid sensing requirements, acting on the Slimfast/Rheb/TOR complex.
2. Activation of the PI3-kinase pathway, where ALK acts as an insulin-like receptor, facilitating cell survival and growth under nutritional stress [51].

3.12. Histone Modifications: Regulators of Caste Differentiation

The Post-Translational Histone Modifications (PTMs) play a fundamental role in caste differentiation in both *Melipona scutellaris* and *Apis mellifera* by influencing chromatin structure and regulating gene expression during larval development.

PTMs in *Melipona scutellaris*

In the stingless bee *Melipona scutellaris*, these epigenetic modifications specifically affect chromatin dynamics, marking significant differences between newly emerged queens and workers. Notable modifications include:

- Phosphorylation of Threonine 3 on Histone H3 (H3T3-P).
- Monomethylation of Lysine 4 on Histone H3 (H3K4-Me) [52].

PTMs in *Apis mellifera*

In *Apis mellifera*, specific patterns of histone modifications have been identified that are closely associated with caste differentiation:

1. **Lysine Methylation:** Methylation patterns at H3K27 and H3K36 are more prevalent in histones extracted from the ovaries of queens compared to larvae. These marks are related to the regulation of key genes that control the queen's reproductive development and functions [53].

2. **Acetylation and Metabolic Integration:** Histone acetylation in *Apis mellifera* is linked to the activity of ATP Citrate Lyase (ATPCL). This essential enzyme converts mitochondrial citrate into Acetyl-CoA (Acetyl Coenzyme A). This process is crucial for integrating growth signals, as Acetyl-CoA serves as the substrate for histone acetylation, thereby directly connecting nutrient metabolism with epigenetic regulation and gene expression [54].

4. Epigenetic Mechanisms Interacting in the Insect Endocrine and Reproductive System

In Hymenoptera, the endocrine system regulates key developmental and physiological processes, including metabolism, metamorphosis, growth, molting, and reproduction [55]. Within this system, the Juvenile Hormone (JH), produced by the corpora allata (CA), plays a fundamental role in development and reproduction, as seen in insects like the mosquito (*Aedes aegypti*) [56].

4.1. JH, Epigenetics, and Reproductive Organ Development

The epigenetic regulation of essential genes, such as msl-1 and msl-2, is influenced by JH and affects the development of reproductive organs and fertility. In *Drosophila melanogaster*, JH regulates the levels of the steroid Ecdysone and the Insulin/TOR pathways, directly impacting the growth and size of reproductive organs [57].

In males, the msl-1 and msl-2 genes are essential for testicular formation and participate in the Dosage Compensation Complex (DCC), which balances gene expression between the sex chromosomes [58] [59]. Although the epigenetic regulation of these genes in Hymenoptera is not yet fully understood, JH is recognized to play a crucial role in the reproductive and endocrine processes of insects.

4.2. Endocrine-Epigenetic Control of Caste in *Apis mellifera*

In *Apis mellifera*, the larval diet is crucial for caste determination, as it activates signaling pathways and endocrine processes that regulate differential gene expression and ensure the correct differentiation between queens and workers. Two key components of this process are:

1. **Juvenile Hormone (JH):** JH acts as a crucial cellular messenger, promoting larval developmental plasticity. This hormone is capable of transforming larvae into queens by modifying the expression of specific genes that regulate processes like growth and reproductive development [60].
2. **Insulin-Like Peptides (AmILP1 and AmILP):** AmILP1 prevents programmed cell death (apoptosis) in the ovaries, preserving their reproductive capacity. AmILP2 is essential for ovarian development, ensuring the formation of functional reproductive organs in future queens. The inhibition of these peptides via RNA interference (RNAi) not only reduces JH levels but also significantly decreases ovary size, highlighting their importance in endocrine regulation and caste determination [38] [50] [61].

During queen development in *Apis mellifera*, the differential methylation of genes in the TOR/PI3K/Insulin cascade plays a fundamental role by influencing the expression of key genes for growth and development [42]. This process regulates cell proliferation and the differentiation of essential organs, such as the ovaries.

In *Drosophila melanogaster*, nutrient availability and Insulin/TOR signaling also have a significant impact on ovary size and structure. These mechanisms activate processes like autophagy in the fat body, an essential process for maintaining energy homeostasis and favoring cellular development under stress or nutrient restriction [62]. Although autophagy in the *Apis mellifera* fat body has not been extensively investigated, similar mechanisms are presumed to be involved in regulating ovarian development in response to nutritional and hormonal signals. This process could act as a crucial regulator for balancing energetic resources during critical phases of larval and reproductive development, especially in queens.

4.3. Reproductive Transition in Worker Bees

The transition of a worker bee from nurse to forager involves coordinated physiological and molecular adjustments that extend beyond reproductive development. This shift is shaped by larval nutrition, endocrine regulation, and a suite of epigenetic mechanisms that reprogram neural and metabolic pathways.

1. **Ovarian Apoptosis:** Worker bees undergo ovarian apoptosis primarily in the 2a/2b region, a process determined by nutritional inputs during the fourth and fifth larval instars. This early suppression of ovarian development establishes their lifelong reproductive constraint and differentiates them functionally from queens.

2. **Hormonal Regulation:** The behavioral transition later in life is accompanied by pronounced endocrine shifts. During the nurse to forager switch, the synthesis of methyl farnesoate epoxidase (*mfe*) increases in the corpora allata and prothoracic glands, initiating a rise in juvenile hormone (JH) production [63]. JH levels surge during the fifth larval instar and again in adults preparing to transition to foraging, linking hormonal state to task allocation [64].

Beyond these developmental and endocrine processes, epigenetic regulation provides the mechanistic bridge to behavioral polyphenism. Dynamic DNA methylation changes at loci such as Amfor and AmDopR modulate neuromodulator pathways, influencing responsiveness to sensory cues and task specialization [43]. Complementarily, histone modifications including H3K4me3 and H3K27ac reshape chromatin structure in brain regions like the mushroom bodies, activating gene networks involved in circadian regulation, learning, and sensory processing required for foraging [65]. Small RNAs further fine-tune this regulatory axis by targeting transcripts associated with JH metabolism and vitellogenin levels, thereby integrating endocrine state with behavioral plasticity [66]. These epigenetic responses are highly sensitive to social cues, pheromonal environment, and nutritional status, enabling workers to rapidly transition between tasks in accordance with colony demands.

4.4. Epigenetics in Male Spermatogenesis and Development

In Drosophila, the activation of genes necessary for meiosis and spermiogenesis is mediated by the testis specific meiotic arrest complex (TMAc) and the TBP-associated factors (tTAF) [67]. In *Bombyx mori*, the proteins bmpRMT5 and bmvasa form an essential regulatory module for spermatogenesis, highlighting their key role in germline cell development [40]. Furthermore, the genes Bcl2 and Bcl-xL are fundamental for the regulation of apoptosis and mitochondrial homeostasis, critical processes for maintaining cell viability during development and spermatogenesis [68].

In *Apis mellifera*, embryogenesis in unfertilized eggs begins with a physical compression during the egg's passage through the oviduct. CpG methylation in intragenic regions of the genome regulates the activity of genes essential for various biological processes in all cells [12] (See **Figure 3**).

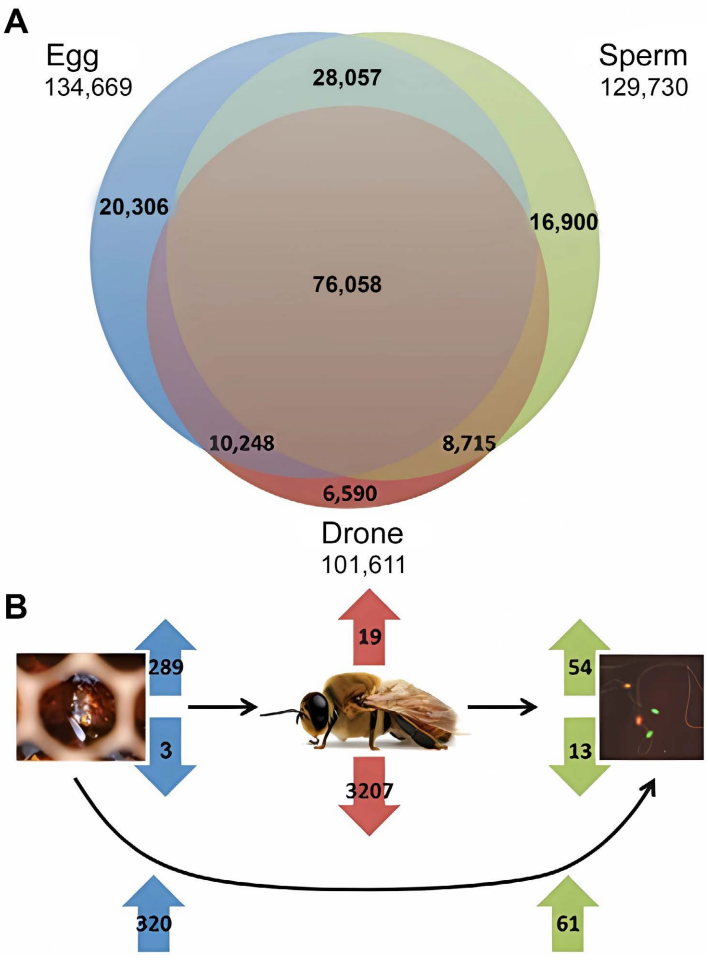

Figure 3. CpG Methylation Counts in *Apis mellifera* Oocytes, Drones, and Spermatozoa. (**A**) Total number of Differentially Methylated Genes (DMG) identified in each of the three sample types. (**B**) Top: Number of hyper- and hypo-methylated DMGs in trilateral comparisons among oocytes, drones, and spermatozoa (blue, red, and green arrows, respectively). Bottom: Number of hyper-methylated DMGs in bidirectional comparisons between oocytes and spermatozoa (blue and green arrows, respectively). From: [69].

During drone development and spermatogenesis, a dynamic cycle of DNA methylation and demethylation is observed. The enzyme Dnmt1, responsible for adding methyl groups to DNA, plays a fundamental role in spermatogenesis, facilitating the transformation of precursor cells into mature spermatozoa. This enzyme is also involved in the genetic pathway associated with meiosis in the milkweed bug (*Oncopeltus fasciatus*) [70].

The DNA methylation in *Apis mellifera* spermatozoa shows a bimodal pattern, with variable levels in CpG islands depending on the gene, suggesting a diversity in the mechanisms of epigenetic regulation in invertebrates. In contrast to *Bombyx mori*, where DNA methylation is less frequent [71], *Apis mellifera* exhibits a more complex methylation system, possibly associated with mutagenic effects in the germline [69].

Beyond its role during drone development, paternal epigenetic information carried in sperm has profound consequences for colony level phenotypes and evolutionary dynamics in social insects. In *Apis mellifera*, sperm DNA methylation patterns can influence caste trajectories in the offspring, acting as paternal regulatory cues that modulate the balance between queen and worker development. Experimental and theoretical evidence suggests that hypermethylated sperm may bias daughters toward worker phenotypes, whereas hypomethylated sperm may favor queen differentiation, forming a paternal "epigenetic toolkit" that optimizes colony fitness under variable environmental conditions [69] [72].

In parallel, piRNAs transmitted through sperm contribute to intergenerational genome stability, guiding transposable element silencing during early embryogenesis and ensuring faithful epigenetic inheritance across castes [73]. These paternal epigenetic mechanisms spanning DNA methylation to small RNA mediated regulation have been proposed as drivers of polyandry, reproductive skew, and adaptive colony level responses in eusocial lineages, highlighting their role in shaping the evolutionary conflicts and cooperation inherent to insect societies [11].

4.5. DNA Methylation Patterns in *Apis mellifera* Gametes and Drones

In *Apis mellifera*, DNA methylation is predominantly concentrated in the CpG islands of exons present in oocytes, spermatozoa, and the drone thorax.

Developmental and Tissue-Specific Dynamics

- **Development and Spermatogenesis:** A decrease in methylation levels is observed during general development, while these levels increase during spermatogenesis.
- **Drone Thorax:** The highest methylation values are found in the drone thorax, likely due to the cellular diversity present in this region, which results in more varied methylation patterns [69].

Paternal Inheritance and Evolutionary Divergence

- **Gamete Differences:** More Differentially Methylated Genes (DMG) are identified in oocytes compared to spermatozoa, suggesting the existence of specific

paternal marks in the gametes.

- **Paternal Effects:** These methylation differences could explain the significant paternal effects observed in crosses between the Cape honey bee (*Apis mellifera* capensis) and the African honey bee (*Apis mellifera* scutellata) [70].

Contrast with Vertebrates

The mechanisms of methylation in *Apis mellifera* contrast sharply with those in vertebrates:

- **Gamete Differences:** In vertebrates, paternal DNA methylation patterns remain stable during embryonic development, whereas the maternal patterns are largely reprogrammed to align with the paternal ones [74].

These differences in methylation mechanisms between invertebrates (like the honey bee) and vertebrates highlight the evolutionary diversity in the epigenetic regulation of genes.

5. Conclusions and Future Perspectives

In the majority of insects, DNA methylation, histone modifications, and non-coding RNAs (ncRNAs) play fundamental roles in phenotypic plasticity, encompassing processes such as sex determination, caste differentiation, and the development of the endocrine and reproductive systems.

These processes are tightly regulated by epigenetic markers that respond to environmental factors, including temperature, humidity, nutrition, and physicochemical conditions. These external stimuli alter the DNA structure within nucleosomes, thereby modulating the expression of key genes associated with phenotypic plasticity. Consequently, factors like pathogens, parasites, chemical substances, and environmental changes have the potential to affect complex functional aspects, such as fecundity, longevity, and disease resistance, impacting individuals even across successive generations.

6. Future Research Directions

Despite the significant progress made in the field of insect social epigenetics, key questions persist:

- **Environmental-Germline Interface:** It is not yet fully understood how the external environment induces modifications in the epigenome of the reproductive tract or the sperm. These epigenomic alterations could significantly influence essential sperm variables, such as sperm survival and functionality.
- **Molecular Mechanisms:** The precise molecular mechanisms underlying these epigenetic transitions remain an area of intense and emerging scientific interest.

Therefore, future research should aim to provide crucial information on the epigenetic mechanisms that govern these processes, as well as shed light on the evolutionary origins of eusociality and the potential implications arising from the complexity and potential absence of a direct paternal genome contribution in these biological systems. A particularly promising avenue is to investigate the crosstalk

between histone modifications, DNA methylation, and non-coding RNAs at key developmental loci in response to royal jelly. For instance, integrating ChIP-seq, whole genome bisulfite sequencing, and small RNA-seq in larvae exposed to royal jelly would allow researchers to determine how activating histone marks such as H3K27ac, CpG methylation states, and caste biased miRNAs converge to regulate developmental regulators such as Dnmt3, Egfr, and ILP2. This multiomic approach would provide mechanistic insight into how a single nutritional stimulus orchestrates coordinated epigenetic landscapes that ultimately drive caste differentiation.

Funding

This work was supported by the TecNM (Project No. 14555.22-P) and carried out within the framework of the project "Small-Scale Queen Rearing Model for High-Value Genetic *Apis mellifera*" (PROTTINS_2025_01_01).

Conflict of Interest

The authors declare no conflicts of interest.

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