

The role of epigenetics in insects in changing environments

The special issue of *Insect Molecular Biology* on 'The role of epigenetics in insects in changing environments' has arrived. The field of epigenetics is concerned with how different cellular phenotypes arise from the same genotype (Nicoglu & Merlin, 2017). Understanding the nature and operation of epigenetic information is of great contemporary importance and interest (Allis & Jenuwein, 2016; Cavalli & Heard, 2019). Epigenetic information serves as a molecular intermediate that helps translate environmental signals received by the cell into changes in gene expression that allow the cell, and consequently, the organism, to produce a phenotype better suited to its surroundings. Epigenetic information affects gene function, can be passed on through cell divisions and impacts evolutionary processes (Fitz-James & Cavalli, 2022; Yi & Goodisman, 2021). Two of the most important epigenetic information systems are the methylation of DNA and the modification of histone proteins, both of which may affect gene expression in eukaryotic systems (Almouzni & Cedar, 2016).

Insects constitute the majority of animal species on Earth and display remarkable interspecific and intraspecific phenotypic diversity. This diversity arises, in part, from the operation of epigenetic information (Glastad et al., 2019). Epigenetic information is believed to play a significant role in enabling insects to develop phenotypes appropriate to the environment (Duncan et al., 2022; Villagra & Frías-Lasserre, 2020). Insects show interesting variation in epigenetic systems, are important ecologically and economically and are amenable to experimentation (Verlinden, 2017). Thus, insects have emerged as key systems for studying the function of epigenetic information. In this special issue, we present nine papers investigating a diversity of insect species that cover topics on epigenetic regulation during development, under conditions of environmental stress and across generations.

A good entrée to this special issue is the review by Maleszka (2024). This 'reminiscence' uses the historically important benchmark of the sequencing of the honeybee genome as a jumping off point to consider the state of insect molecular biology, in general, and insect epigenetics, in particular. Maleszka discusses how the field of social behaviour was advanced by the sequencing of the honeybee genome. He also promotes the idea of metabolo-epigenetics, which considers how energy metabolites influence epigenetic regulation. Maleszka continues by discussing the epigenomic revolution, whereby the epigenetic state of the entire genome can now be assessed. He concludes by noting that researchers should focus on the idea that 'epigenetics is about bridging the gap between the genotype and the phenotype'. We hope that the articles provided in this special issue will help bridge this gap and further our understanding of epigenetic information in insects.

Several studies in this special issue provide new insight into the importance of DNA methylation. DNA methylation in insects was first discovered in honeybees but has since been identified in a wide variety of holometabolous and hemimetabolous insects (Bewick et al., 2017). DNA methylation is believed to occur through the operation of the family of DNA methyltransferase (*Dnmt*) genes. Studies in mammalian systems suggest that the enzyme DNA methyltransferase 3 is responsible for de novo DNA methylation, whereas DNA methyltransferase 1 maintains patterns of DNA methylation (Lyko, 2018). Interestingly, however, these *Dnmt* genes show gains and losses across the insect phylogeny (Bewick et al., 2017). In addition, different insect species show great variation in the levels of DNA methylation (Thomas et al., 2020). These findings have led to questions about the actual function of DNA methylation in insect genomes. Therefore, research on the nature and function of DNA methylation in insects continues to be an area of high priority.

Yoon et al. (2024) investigate the evolution and function of DNA methylation in the pea aphid, *Acyrthosiphon pisum*. Yoon et al. find that an important gene duplication in *Dnmt3* occurred 150 million years ago. They demonstrate the crucial role of the *Dnmt3* gene for early development in the pea aphid. They then show that experimental treatment of developing aphids with chemicals that affect DNA methylation leads to defects in reproductive function. Yoon et al. thus suggest that DNA methylation genes are important for early development and successful reproduction in insects.

Cunningham et al. (2024) also study the function of *Dnmt* genes. However, they study DNA methylation in the whitefly, *Bemisia tabaci*. They specifically investigate the function of the gene *Dnmt1* and find that this gene influences specific molecular pathways. Cunningham et al. use experimental techniques, including RNA interference, to probe the function of *Dnmt1*. Consistent with prior findings, they find that knock-down of *Dnmt1* affects gametogenesis. Surprisingly, however, their experimental treatment does not result in large, wholesale changes in the expression of genes. The research team also examines patterns of DNA methylation in *B. tabaci*. They uncover higher levels of DNA methylation around exons and for genes that are highly and uniformly expressed. Such findings are consistent with previous observations in insect taxa and further support the associations between DNA methylation and gene expression.

Tang et al. (2024) investigate the importance of the gene *Dnmt1* in the tomato leafminer, *Tuta absoluta*. They find that *Dnmt1* is a key regulator of temperature stress responses, phenotypic plasticity and development. The research team investigates the expression of the *Dnmt1* gene in different developmental stages. They also use RNA

interference to knock down the expression of *Dnmt1*. These experiments produce changes in the temperature tolerances of *T. absoluta*. Thus, the study of Tang et al. identifies the functional effects of *Dnmt* genes in insects in response to changing environments.

Epigenetic mechanisms play a crucial role in responding to environmental signals. McCaw et al. (2024) report that epigenetic mechanisms related to DNA repair and recycling pathways modulate insect life history responses to temperature changes. McCaw et al. identify variation in the levels of expression of *Dnmt1* in the seed beetle, *Callosobruchus maculatus*. The research team uses chemical treatments to experimentally change levels of DNA methylation in *C. maculatus*. These experiments result in changes in development, fecundity and viability of individuals under some conditions. Importantly, however, these effects are often temperature dependent, indicating the importance of environmental context in modulating epigenetic changes.

Hunt et al. (2024) investigate the role of DNA methylation during the development of the bumblebee *Bombus terrestris*. They use whole-genome bisulphite sequencing, a technique that measures methylation at each nucleotide throughout the genome, to study DNA methylation patterns in *B. terrestris*. The research team finds differences in methylation among *B. terrestris* tissues. Specifically, levels of DNA methylation are particularly low in worker ovaries and high in male sperm. Variations in levels of DNA methylation are also identified across different developmental stages of the bee. Importantly, Hunt et al. suggest that DNA methylation marks may be erased during oogenesis, thereby allowing for epigenetic reprogramming each generation.

DNA methylation in animals almost always occurs on cytosine bases followed by a guanine base (so-called CpG dinucleotide sites). However, evidence suggests that methylation of DNA may occur in other contexts in animal genomes. Royle et al. (2024) study non-CpG DNA methylation in the cotton bollworm, *Helicoverpa armigera*, using whole-genome bisulphite sequencing. They find that levels of cytosine methylation differ among life stages. Further analysis indicates that much of this variation arises from variation in methylation of cytosine bases in non-CpG contexts. Genes differentially methylated in these nonstandard contexts are enriched in functions related to key signalling pathways and chromatin remodelling. Thus, Royle et al. suggest that nonstandard DNA methylation should be considered in future investigations of epigenetic inheritance.

Post-translational modifications to histone proteins represent a second major class of epigenetic information. Histones are an ancient and fundamentally important family of genes found throughout eukaryotic organisms. The core role of histone proteins is to aid in the packaging of DNA. In addition, histone proteins can be chemically modified in a variety of ways. These chemical modifications represent important sources of epigenetic information (Stewart-Morgan et al., 2020). The so-called histone code affects the expression and function of genes by altering the properties of chromatin and influencing which regulatory proteins are recruited to gene regions (Talbert & Henikoff, 2010; Zentner & Henikoff, 2013). Histone modifications are of wide importance across insect taxa.

However, research on histone post-translational modifications has been conducted in relatively few insect species.

Wei et al. (2024) investigate specific histone modifications in the northern house mosquito, *Culex pipiens*. They first compare the protein sequences of the core histone protein H3 among invertebrate taxa. They confirm the high protein sequence conservation of H3 across the Diptera. They then investigate variation in the epigenetic mark of a specific H3 lysine methylation. Wei et al. uncover differences in the frequency of this epigenetic mark across diapausing and nondiapausing *C. pipiens*. Specifically, histone methylation levels show different patterns in different tissues when comparing diapausing to nondiapausing mosquitoes. They suggest that these epigenetic differences may be involved in diapause phenotypes.

This special issue on insect epigenetics may best be concluded by reading the commentary by Gunn et al. (2024), who discuss the importance of epigenetic information in the context of agriculturally important insect pests. They are particularly interested in highlighting how pest insects can adapt and acclimate to environmental changes and stressors. Gunn et al. review how adaptive changes occur through various genetic mechanisms and stress-induced epigenetic modifications, both of which contribute to heritable resistance traits. They suggest that insecticide resistance is a key environmental stressor that leads to the acclimation and adaptation of insects. They conclude by suggesting that agricultural pest insects offer valuable insights into rapid adaptation and stress-induced evolutionary processes.

Overall, this special issue of *Insect Molecular Biology* provides new insight into the area of epigenetics in insects, particularly in the context of changing environmental conditions. These studies help advance our understanding of epigenetic processes operating at the molecular level. However, there is still a great deal to be learned about epigenetic information in insects (Maleszka & Kucharski, 2022). As highlighted in this special issue, the function and nature of DNA methylation are still unclear. More investigations on the molecular mechanisms operating on histone modifications are needed across diverse insect taxa. Thus, we see a bright future for research in insect epigenetics.

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