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Evolutionary insights into DNA methylation in insects

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Epigenetic information affects gene function and plays a critical role in development. DNA methylation is one of the most widespread epigenetic marks and has been linked to developmental plasticity in insects. Here, we review the patterns and functions of DNA methylation in insects. We specifically focus on how the application of an evolutionary framework has led to important insights into the role of DNA methylation. We discuss the importance of evolutionary variation in DNA methylation among insect taxa and show how comparative analyses have revealed conservation in targets of DNA methylation. We then show how the distribution of DNA methylation in insect genomes has been linked to evolutionary conserved patterns of histone modifications and variants. We conclude by discussing how the evolutionary conservation and variability of DNA methylation in insects can provide insight into the function of DNA methylation across eukaryotic systems.

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Introduction to epigenetic inheritance

Many organisms exhibit developmental responsiveness to their environments. Polyphenisms, wherein distinct phenotypes arise from a common genotype, are among the most remarkable examples of developmental plasticity and are particularly common in insects. For instance, aphids grow wings in response to crowded conditions, dung beetles produce horns for competition, and social insects develop distinct castes to fulfill social functions [1,2].

Successful organismal development often relies on epigenetic information. Epigenetic information refers to stable alterations made to DNA and chromatin that are not directly linked to changes in DNA sequence. Epigenetic information affects gene function and, therefore, plays a critical role in allowing a single genotype to produce distinct phenotypes [3].

One of the most widespread and important types of epigenetic information is the methylation of DNA [4,5]. DNA methylation has been studied extensively in mammalian and plant systems [5]. However, the study of DNA methylation in insects is relatively new.

DNA methylation was first identified in insects approximately 20 years ago [6]. Methylation has now been found at appreciable levels in the genomes of many, but not all, insects [7]. The importance of DNA methylation in insect development has most strikingly been demonstrated in the honeybee, where experimental alternations in levels of DNA methylation led to changes in the frequency with which different social castes developed [8]. This result suggested that DNA methylation may be directly involved in the generation of different phenotypes in insects [9,10]. The apparent phenotypic importance of DNA methylation helped to launch a great deal of research into the question of how DNA methylation operates in insect taxa [11,12•,13,14•,15,16••,17•,18•,19,20,21••,22••,23,24].

Here, we discuss recent progress in understanding DNA methylation in insects. We focus on understanding the function of DNA methylation during development but do not address transgenerational epigenetic inheritance [25]. We specifically demonstrate how evolutionary thinking has been useful in providing insights into the function of DNA methylation in insects. Evolutionary thinking can be of great utility in deciphering the function of biological structures, physiological patterns, behavioral processes, and molecular mechanisms. DNA methylation in insects, in particular, shows remarkable evolutionary patterns, which encourages the application of evolutionary approaches to studying the nature of epigenetic information. Such analyses have helped propel our understanding of DNA methylation and how it affects gene function in diverse insect taxa.

The phylogenetic distribution of enzymes involved in DNA methylation

DNA methylation in animals is mostly confined to cytosine bases followed by guanine bases (i.e., CpG dinucleotides [5]). The enzymes responsible for DNA methylation across animals are thought to be a family of DNA methyltransferases (DNMTs), primarily

DNMT1 and DNMT3 [26]. It has been assumed that insect DNMTs have the same function as their functionally characterized, mammalian orthologs [27]. Consequently, DNMT1 is thought to be the maintenance methyltransferase, which is responsible for maintaining already-present methylation patterns across cell divisions. In contrast, DNMT3 is thought to be the *de novo* methyltransferase, which is responsible for establishing new methylation patterns [26].

The presence of these key genes has generally served as an important signal that the genome of a particular insect was methylated. However, two insects with empirically established DNA methylation, the silkworm *Bombyx mori* and the locus *Schistocerca gregaria*, apparently lack a DNMT3 gene [11,28•]. This suggests that our understanding of the DNA methylation toolkit is incomplete, and that a functional DNA methylation system cannot be supported or refuted based on the presence of the DNMT1 and DNMT3 genes alone. It is thus possible that a single DNMT gene can produce enzymes that possess both *de novo* and maintenance methylation functions or that other uncharacterized genes are involved in the process of DNA methylation in insects.

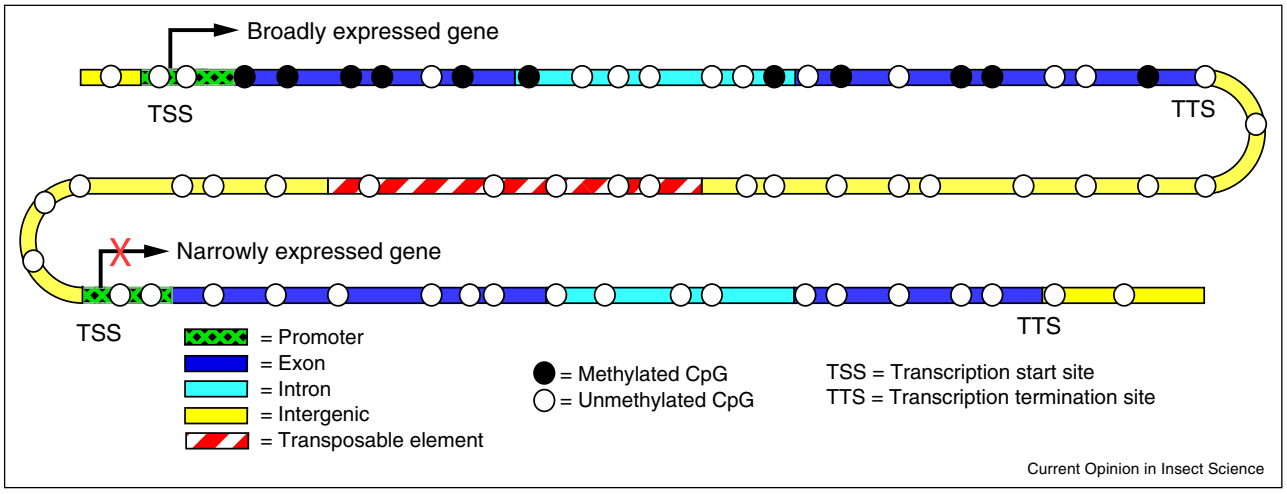
Evolutionary patterns of DNA methylation in insects

The levels of genome methylation show great variation among different insect species. For example, both the model organism *Drosophila melanogaster* and red flour beetle *Tribolium castaneum* lack substantial levels of DNA methylation in their genomes [29–31], suggesting that many species within the Diptera and Coleoptera may not display DNA methylation at appreciable levels. However, other holometabolous insects, such as species within the Hymenoptera [32•,33] and Lepidoptera [11], do show DNA methylation within the genome. In addition, DNA methylation is present in several hemimetabolous insects [12•,13,28•,34]. Thus, DNA methylation appears to be ancestral to insects, but has apparently been lost in some of the most successful holometabolous insect orders [7].

Comparative analyses have also revealed that methylation levels in insects are relatively low compared to other animals. For example, roughly 70% of all CpG dinucleotides in the human genome were methylated in greater than 80% of cells from human brain samples [35]. In contrast, DNA methylation is targeted to less than 1% of CpG dinucleotides in the honeybee genome, with roughly 45% of those CpG dinucleotides methylated in greater than 80% of cells from honeybee brain samples [15]. Insects with functional DNA methylation systems also possess lower levels of DNA methylation than the more basal invertebrates *Nematostella vectensis* and *Ciona intestinalis* [14•]. This suggests that DNA methylation may have undergone a reduction in the ancestors of insects, which may have ultimately led to the complete loss of genome methylation in certain insect lineages.

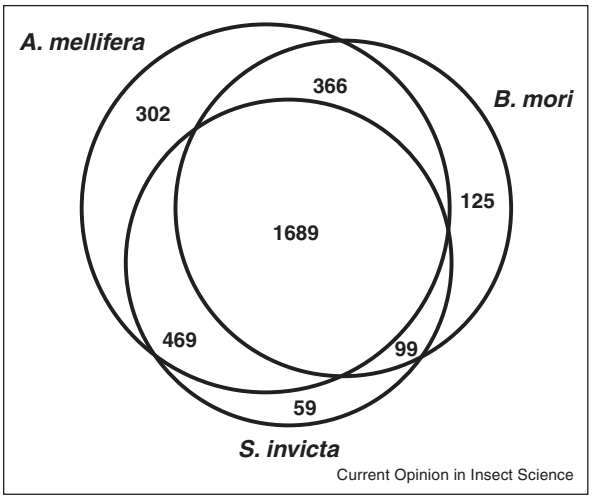
Insects that do possess DNA methylation show a high concordance in patterns of methylation. Specifically, DNA methylation in holometabolous insects is largely targeted to gene bodies (exons + introns) and primarily to exons [15,16••,36] (Figure 1). On a finer scale, DNA methylation is preferentially found in exons in the 5′ region of genes [10,16••,17•,37]. Moreover, DNA meth-

Figure 1



Targets of DNA methylation in insects. DNA methylation is found predominantly in the gene body (exons + introns). Exons in the 5′ regions of genes show higher levels of methylation than those in the 3′ regions. In addition, introns show lower levels of DNA methylation than exons in holometabolous insects, but methylation levels in introns are higher near exon–intron boundaries. Other regions of the genome, such as intergenic regions, promoters, and transposable elements, are generally not as highly methylated in insect genomes. Methylated genes tend to be broadly expressed, whereas genes that are narrowly expressed tend to be unmethylated.

Figure 2



Evolutionary conservation of patterns of DNA methylation in insects. The Venn diagram shows the overlap in genes that are significantly methylated in the honeybee *Apis mellifera* (Hymenoptera), the silkworm *Bombyx mori* (Lepidoptera), and the fire ant *Solenopsis invicta* (Hymenoptera). Most genes that are methylated in one species are also methylated in the others, illustrating that patterns of DNA methylation are conserved among insect taxa. This result is particularly notable as the Hymenoptera and Lepidoptera diverged ~300 MYA.

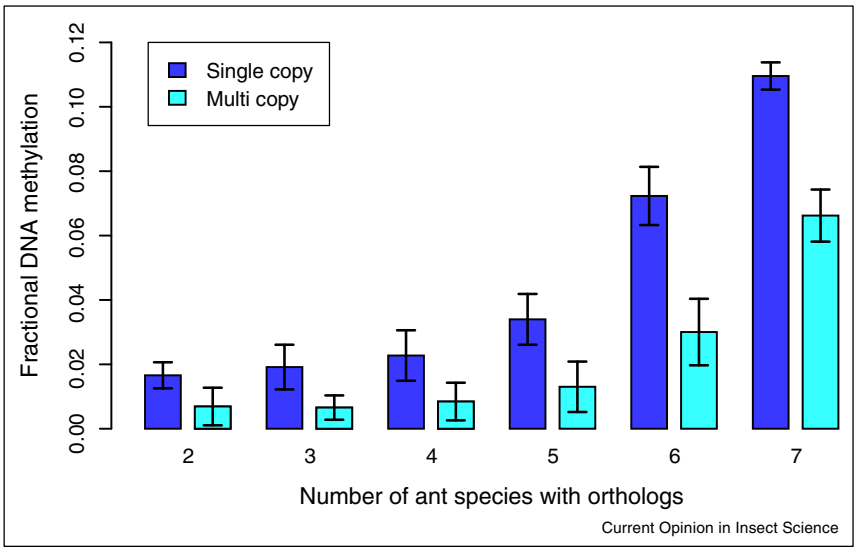
ylation targets largely overlapping sets of orthologs [14•,17•], indicating that similar genes are methylated in distinct insect taxa (Figure 2). Thus, insect species with functional DNA methylation systems exhibit remarkable conservation in the patterns of genomic methylation overall [7,11,14•,15,16••,17•]. The patterns of DNA methylation in insects contrast with those found in mammals, for example, where most of the genome, and not just the genes, is heavily methylated. In plants, DNA methylation is targeted to only a subset of genomic elements. Like insects, plants exhibit DNA methylation in actively expressed genes, but unlike most insects investigated to date, plants also exhibit DNA methylation in transposons [5,38•].

Functional associations of DNA methylation in insects

Studies examining insect DNA methylation using both empirical [15,16••,17•,18•] and computational [12•,19,20] approaches have observed a striking similarity in the patterns of gene expression of genes targeted by DNA methylation. Specifically, methylated genes tend to be ubiquitously expressed among cell types or phenotypes [12•,16••,17•,18•,19,20]. These 'housekeeping' genes are also generally conserved at the sequence level [11,14•,15,19,20]. Thus methylated genes also tend to have orthologs in many species (Figure 3). In contrast, genes that are differentially expressed across cell types tend to be unmethylated in insects (Figure 1). Notably, however, direct analyses of DNA methylation and gene expression at the tissue or cell level are still lacking in many insect species. Investigation of DNA methylation on this fine scale is needed to provide further information on the relationship between gene expression and DNA methylation.

Recent studies in the honeybee have uncovered evidence linking DNA methylation to alternative splicing

Figure 3



DNA methylation and the taxonomic prevalence of orthologs in seven ant species. Fractional DNA methylation (mCG/CG, where mCG indicates methylated cytosines in CpG context) of genes in the fire ant *Solenopsis invicta* versus taxonomic prevalence of orthologs among seven ants (modified from Simola *et al*. [32•]). Notably, genes with orthologs in all seven ant genomes exhibit the highest methylation levels among both single-copy and multi-copy orthologs, while universal single copy genes exhibit the highest methylation levels overall. Means and 95% confidence intervals are plotted.

[15,21••,22••,23] (but see [18•]). DNA methylation may directly affect the binding and function of proteins that affect splicing [4,39] or otherwise help define exon–intron boundaries, thereby affecting the production of splice variants [40]. For example, experiments in human cell systems demonstrated that DNA methylation can *impede* the inclusion of an alternative exon by affecting the binding of a transcription factor [39]. Remarkably, DNA methylation in humans has also been shown to *facilitate* the inclusion of alternative exons through the recruitment of a methyl-binding domain protein [41]. Thus DNA methylation can apparently lead to exclusion or inclusion of alternative exons in eukaryotic systems. If such contrasting mechanisms are evolutionary conserved in insects, then the variable effects of DNA methylation on alternative splicing may complicate global analyses of DNA methylation and alternative splicing, and could explain why previous studies in insects have not revealed a strong association between levels of DNA methylation and alternative splicing events [15,18•,21••,22••,23].

Conservation of epigenetic states across taxa

Methylation of gene bodies is found in plants [36,37], mammals [36,37], possibly fungi [42], and insects [7,36,37]. This broad phylogenetic distribution suggests that gene body methylation is one of the oldest conserved features of eukaryotic DNA methylation systems. Thus, it is likely that much insight can be gained into the function of insect DNA methylation by evaluating findings in other eukaryotic taxa displaying gene body methylation.

In mammals and plants, gene body methylation has been linked to the alteration of local transcription factor binding [39,43], regulation of alternative intragenic promoters [44,45], and positioning of nucleosomes [38•,46]. However, how DNA methylation mediates these diverse functions at the molecular level remains poorly understood.

One potential link arises from recent studies which have discovered that DNA methylation may be associated with histones displaying particular post-translational modifications in insects [17•,24,47]. Importantly, patterns of histone modifications in *Drosophila melanogaster* show strong concordance with orthologous patterns of DNA methylation in fire ants and honeybees [17•]. Specifically, the orthologs of genes that are methylated in fire ants and honeybees tend to be associated with histone proteins possessing active post-translational modifications, such as H3K4me3 and H3K36me3, in *D. melanogaster*. These taxa are diverged by some 350 Myr, suggesting that transcriptionally active epigenetic states show deep conservation, despite the loss of DNA methylation in some lineages.

One of the more compelling proposed functions for DNA methylation comes from studies of the histone variant H2A.Z. H2A.Z is a chemically modified variant of the canonical histone H2. High levels of H2A.Z within the gene body are negatively correlated with gene expression level and breadth [37,48]. Intragenic DNA methylation has been shown to exhibit an antagonistic spatial relationship with H2A.Z profiles in vertebrates and plants [37,49]. Notably, experimental reduction of DNA methylation levels have been shown to result in increased local H2A.Z levels in both mammals [50] and plants [49], but alteration of H2A.Z does not likewise affect DNA methylation [51]. This suggests that DNA methylation prevents the incorporation of H2A.Z-containing nucleosomes within gene bodies, which is in turn linked to the stable maintenance of active gene expression. Thus, it is possible that the exclusion of H2A.Z by DNA methylation is a conserved function in insects. This may explain the highly conserved patterns of insect DNA methylation targeting, as constitutively expressed genes are those where H2A.Z would be most detrimental in the gene body.

Conclusions

Insects are remarkable because of their diversity of form and function. This evolutionary variation makes insects particularly useful for studying DNA methylation. Comparative analyses have demonstrated that insects show widely varying levels of DNA methylation. However, insects that have DNA methylation show conserved patterns in terms of which genes are methylated and how these genes are expressed. Thus evolutionary approaches have helped identify major features of insect methylation systems.

We suggest that continued comparative analysis of patterns of DNA methylation among insect lineages will provide important information on the function of DNA methylation. For example, in order to better understand the developmental significance of DNA methylation, it will be essential to determine the targets and levels of *de novo* DNA methylation during post-embryonic development in different species. These data would provide insight into patterns of DNA methylation during periods of development critical to the mediation of phenotypic plasticity. Studies within specific insect tissues would also help advance our understanding of DNA methylation by providing important information on how methylation patterns vary among cell types. In addition, it will be useful to directly determine the relative importance of DNMT1 and DNMT3 to the methylation process in different insect taxa.

Future studies may also explore the diversity of epigenetic marks found in insects in order to better inform our understanding of the broader epigenetic context of DNA methylation and to provide a more comprehensive view of gene regulation [52••]. Indeed, addressing the crucial and perplexing question of why some insects have lost the ability to methylate their genomes and how epigenetic systems compensate for this loss cannot be easily answered without such information. Thus insects will

continue to provide an important arena for understanding how DNA methylation and other epigenetic information contribute to phenotypic variation.

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