

DNA methylation and sexual dimorphism: New insights from mealybugs

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DNA methylation is an ancient epigenetic pathway found across eukaryotes. Nevertheless, the targets of DNA methylation within genomes evolve extremely rapidly. Arthropods display many such examples. The mealybug *Planococcus citri* has evolved methylation at promoter sequences, associated with gene silencing just as in mammals. In this issue of *Molecular Ecology*, Bain et al. (2021), thoroughly characterise mealybug methylation, exploring its potential functions in gene expression and the spectacular sexual dimorphism that is a characteristic of this species. Their results provide new insights into the complex relationship between DNA methylation and gene expression and highlight how rapidly different methylation systems can evolve.

KEYWORDS

conservation genetics, DNA methylation, epigenetics, evolution of sex, gene structure and function, insects, life history evolution

1 | INTRODUCTION

Epigenetic gene regulation is key to development as it enables different gene expression profiles to be controlled by the same genome sequence in different cell types. DNA methylation is one such epigenetic modification where cytosine bases in DNA, usually in the CG sequence context, acquire a methyl group at the 5 position (Jeltsch, 2006; Law and Jacobsen, 2010). In mammals, DNA methylation is enriched at transposable elements, where it is associated with silencing (Bird, 2002). Additionally, DNA methylation controls the expression of genes: DNA methylation of CG-rich sequences within promoters turns genes off whereas unmethylated promoters are associated with active genes (Bird, 2002). However, methylation is very different in different animal species across evolution (Feng et al., 2010; Suzuki & Bird, 2008; Zemach et al., 2010). In most arthropods there is no evidence of promoter methylation and instead DNA methylation is found at a subset of genes with moderate levels of gene expression (Lewis et al., 2020). Recently it was discovered that the mealybug *Planococcus citri* is a key exception as it has

promoter methylation which is associated with reduced levels of expression of the nearby gene (Lewis et al., 2020). This methylation pattern has evolved independently from mammalian methylation, raising the question of which aspects of the genome or ecology of this species are responsible.

Planococcus citri males and females display extremely distinct morphology (See Figure 1 of Bain et al., 2021). An intriguing possibility is that the evolution of promoter methylation is related to the distinct patterns of gene expression required to maintain these states. Bain et al. (2021) set out to test this hypothesis using high resolution bisulphite sequencing to track methylation at single nucleotide resolution in males and females. Using RNA sequencing to map gene expression they could then compare to test how DNA methylation differences are related to gene expression differences. This offers a really important “natural experiment” to test whether DNA methylation changes could be responsible for changes in gene expression.

The authors find that gene expression shows marked differences between male and female mealybugs, including a substantial number of genes with extremely sex biased expression. Interestingly,

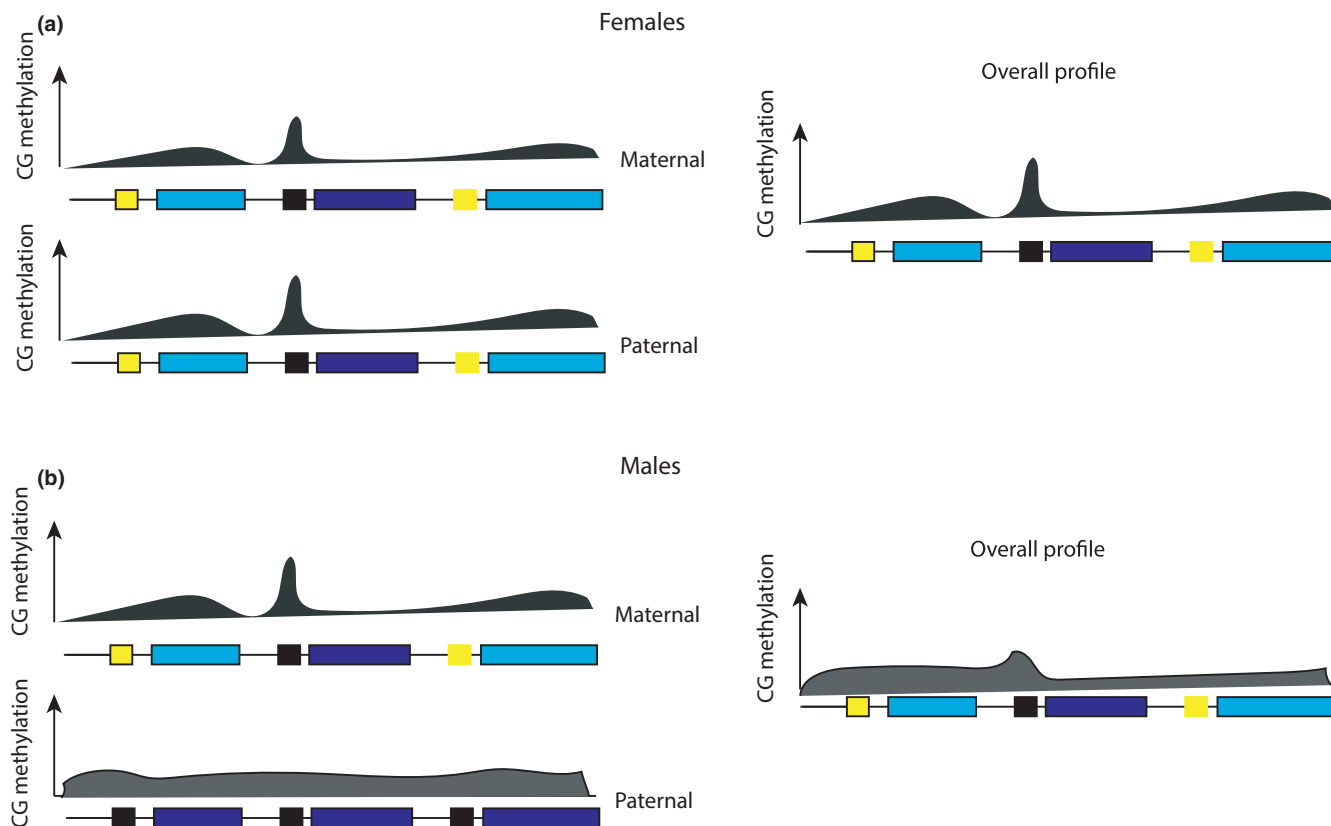


FIGURE 1 Hypothetical methylation profiles in *Planococcus citri*. (a) In females, expressed genes (light blue) correlate to unmethylated promoters (yellow) and silent genes (dark blue) correlate to methylated promoters (black). This occurs on both chromosomes leading to a clear overall methylation profile from genome-wide bisulphite sequencing. (b) In males, the paternal chromosome is condensed and silent, corresponding to uniform methylation. The overall profile from bisulphite signalling is therefore smoothed out so that peaks and troughs are less obvious

a large number of genes show differential alternative splicing between males and females. Bain et al. (2021) also find several genes that show differential methylation between males and females. Moreover, many promoters show significantly different levels of methylation between sexes. Furthermore they confirm that in both males and females promoter methylation is generally negatively correlated with gene expression. However, Bain et al. (2021) show rigorously that there is no significant association between changes in methylation, either at promoters or within genes, and the expression of the same gene.

The result that DNA methylation can change significantly without affecting gene expression is very important in interpreting the function of promoter methylation in *P. citri*. It clearly suggests that sex biased gene expression does not result from switches in promoter methylation. Why then, is there a correlation between DNA methylation and low expression of the nearby gene? It could be that both DNA methylation and gene expression are weakly associated with a third molecular factor, for example chromatin structure such as nucleosome positioning or histone modifications, or the binding of a specific transcription factor. Alterations in this hypothetical factor could lead either to DNA methylation or transcription changes but rarely to both at the same locus. It is also possible that differences in DNA methylation can drive changes in gene expression within sexes

but not between them, potentially due to differential expression of an effector binding protein.

In regards to this latter possibility, it is notable that Bain et al. (2021) discovered a striking difference in the genome-wide pattern of DNA methylation between males and females. DNA methylation in males was found at a higher proportion of CG sequences (9.5% vs. 8.4%) but methylation at individual features was generally lower. This suggests that female methylation is more concentrated at specific locations whereas male methylation is more uniformly distributed. This might suggest that the functions of methylation in affecting gene regulation are different in male and female. Intriguingly the authors speculate that the more uniform distribution of methylation in male might be linked to the fact that the paternal chromosomes in males are shut down completely corresponding to a highly condensed state. The condensed chromatin may have high uniform methylation, which would mean that the average methylation across both chromosomes would be more uniform with both peaks and troughs reduced in size relative to females (Figure 1). In contrast, the authors speculate that the more focussed regions of methylation in females are important in regulating genes so that the overall expression of most genes is similar between males and females.

An important caveat to this study and the hypotheses generated is that the methylation and expression profiles were conducted from

whole animals. It is possible that some of the differences in methylation patterns, as well as the inability to detect changes in gene expression associated with differential methylation, could be due to the fact that methylation is only found in specific cell types, and that the distribution of cell types may differ between male and females. New techniques to analyse methylation patterns as well as gene expression in single cells could be useful ways to tackle this issue in the future. Nevertheless, the possibility that methylation differs on different alleles in male *P. citri* is a fascinating prospect for future study which could be addressed by analysing methylation profiles from crosses between genetically diverse individuals to track methylation levels of maternal and paternal chromosomes.

Overall, the study by Bain et al. is an excellent example of how novel molecular functions for highly conserved epigenetic pathways can evolve surprisingly rapidly. It firmly establishes the mealybug system as a superb model to understand how epigenetics is involved in the striking diversity of developmental systems across animals.

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AUTHOR CONTRIBUTIONS

PS wrote the article.

DATA AVAILABILITY STATEMENT

No primary data was generated.

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