



Research

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Evolutionary biology

Queen pheromones modulate DNA methyltransferase activity in bee and ant workers

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DNA methylation is emerging as an important regulator of polyphenism in the social insects. Research has concentrated on differences in methylation between queens and workers, though we hypothesized that methylation is involved in mediating other flexible phenotypes, including pheromone-dependent changes in worker behaviour and physiology. Here, we find that exposure to queen pheromone affects the expression of two DNA methyltransferase genes in *Apis mellifera* honeybees and in two species of *Lasius* ants, but not in *Bombus terrestris* bumblebees. These results suggest that queen pheromones influence the worker methylome, pointing to a novel proximate mechanism for these key social signals.

1. Introduction

Epigenetic marks such as DNA methylation and histone modification serve to modulate gene expression, helping genetically identical tissues to produce a variable phenotype. Epimarks are instrumental in the regulation of development in early life [1], and there is growing evidence that they can also change dynamically throughout life in response to external stimuli [2,3]. Epigenetic processes have also been implicated in mediating environmentally induced polyphenisms in diverse insects, including the gregarious/solitary forms of locusts [4].

The social insects are characterized by the presence of two 'castes': queens and workers. Caste fate is typically environmentally determined [5] and may be regulated epigenetically, for example by histone modification [6] and DNA methylation [7]. Unlike some insect taxa, many social Hymenoptera possess a full set of genes for DNA methylation and demethylation, including one or more orthologues of the 'maintenance' DNA methyltransferase *dnmt1* and the 'de novo' DNA methyltransferase *dnmt3* [8]. Insect DNA methylation has been linked to alternative splicing [9] and is mostly found in the exons of highly expressed genes [8], implying that it affects both the type and amount of transcript produced. In honeybees, DNA methylation was found to be caste-specific in initial studies [10,11], though not in a subsequent study [2]. Moreover, knockout of *dnmt3* caused worker-destined larvae to develop queen-like traits [7] and affected the expression of 17% of the transcriptome [9], consistent with a role for methylation in polyphenism. In bumblebees (*Bombus terrestris*), experimentally induced DNA demethylation produced queen-like traits, and there is some evidence for a difference in methylation between reproductive and non-reproductive workers [12]. DNA methylation was also found to be caste-specific in some ants [13]. Finally, there is some evidence that honeybee workers that switch task from nurse to forager, and back again, undergo changes in their methylome [2], which would indicate that DNA methylation can be modified reversibly in response to external cues.

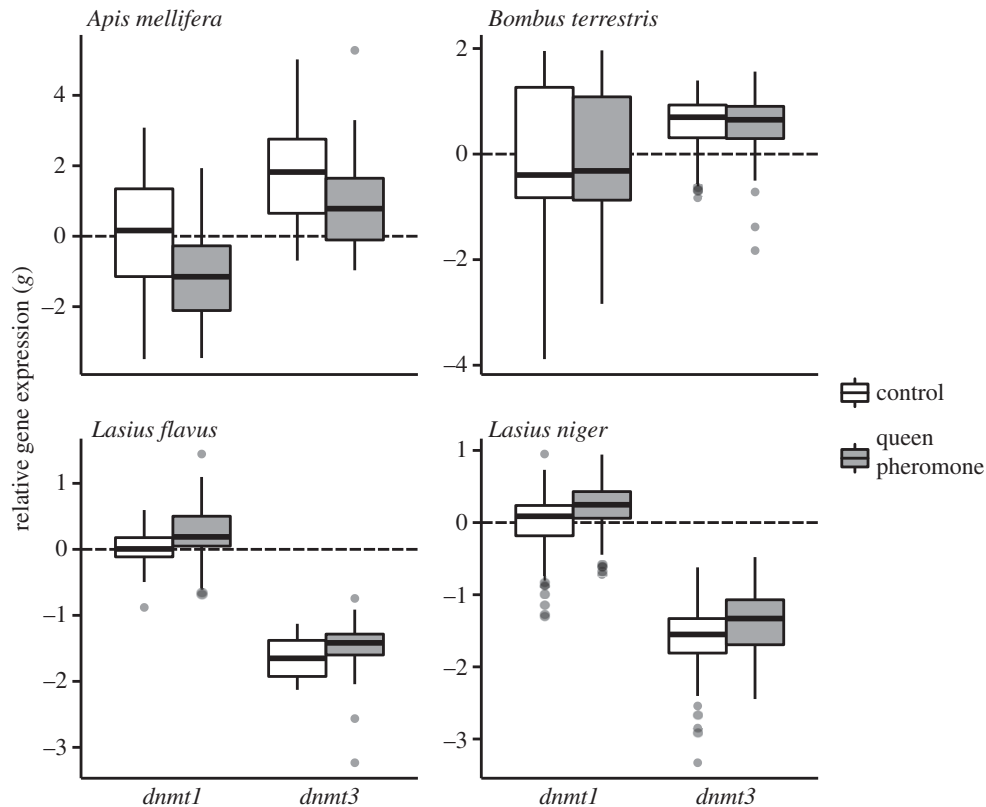


Figure 1. Expression of *dnmt1* and *dnmt3* in each treatment, relative to the mean expression level of *dnmt1* in the control group for the focal species (g). Positive values of g indicate higher expression than this reference point, while negative values indicate lower expression. The boxes show the median and interquartile range (IQR), and the whiskers show the farthest points that are within $1.5 \times \text{IQR}$ of the bounds of the IQR.

Here, we focus on the previously untested hypothesis that the worker methylome is affected by exposure to queen pheromones. Queen pheromones are chemical signals that characterize queens and other reproductive individuals; they have multiple important functions, and likely exist in all eusocial insects [14]. Queen pheromones have long-lasting ‘primer’ effects on recipients’ physiology, such as rendering them sterile [14]. Primer effects might involve epigenetic changes, allowing individuals that have detected queen pheromone to record this information into their genomic hardware, and thereby express a long-lasting transcriptomic response.

Although the effect of pheromones on methylation is unstudied, previous data point to a connection. The most direct evidence is that the set of genes whose expression was affected by knockout of *dnmt3* in [9] overlapped significantly with the set of genes showing queen pheromone-sensitive expression in [15]. Additionally, workers not exposed to queen pheromone become fertile [14], and fertile workers have a queen-like transcriptome [16–18]. Thus, it is possible that lack of queen pheromone causes the methylome to become more queen-like, i.e. hypomethylated [7,12,13], in turn leading to queen-like gene expression. We therefore hypothesize that queen pheromone stimulates DNA methyltransferase activity in workers.

2. Material and methods

We experimentally treated groups of workers with synthetic queen pheromone, and then used quantitative PCR to measure the expression of two DNA methyltransferase genes, *dnmt1* and *dnmt3*, relative to control groups of workers that did not receive pheromone. We studied four species, spanning two

evolutionarily independent origins of sociality (namely ants and corbiculate bees), for which queen pheromones that affect worker fecundity have been isolated [14,19–21]. We used RNA extracts from individual whole bodies (or half-bodies for the larger *B. terrestris*). See electronic supplementary material for detailed methods and a breakdown of sample sizes (table S1; total $n = 1113$ individuals from 32 colonies).

3. Results

In *Apis mellifera* honeybees, queen pheromone treatment reduced the expression of both DNA methyltransferase genes to less than half of control levels, but in the ants *Lasius niger* and *L. flavus*, both genes showed 16–30% higher expression in pheromone-treated workers (figure 1; electronic supplementary material, table S2). These treatment effects were significant (all $p < 0.017$) and were very consistent across colonies (electronic supplementary material, figure S1 and table S2). Pheromone treatment had no significant effect on the expression of either gene in the bumblebee *B. terrestris* (electronic supplementary material, table S2). Additionally, *dnmt3* showed higher expression than *dnmt1* in the two bee species, while in the two ant species, *dnmt1* was expressed more than *dnmt3* (figure 1).

4. Discussion

We found that queen pheromone treatment lowered DNA methyltransferase expression in *A. mellifera* honeybees, elevated expression in *Lasius niger* and *L. flavus* ants, and had no effect on expression in *B. terrestris* bumblebees. We also replicated recent findings [8] that *dnmt1* shows higher expression than

dnmt3 in ants, though interestingly we found the opposite pattern in bees.

Experimental manipulation of *dnmt3* expression has been shown to strongly influence genome-wide rates of DNA methylation in honeybees [9], suggesting that the gene expression changes that we observed probably affected the methylome. Thus, our results suggest that the presence or absence of queen pheromone produces differences in DNA methylation. In the two ant species, the effect of queen pheromone agreed with our predictions based on previous data implying that infertile workers show hypermethylation relative to fertile workers or queens [7,12,13]. That is, our data suggest that a queen pheromone causing worker sterility stimulates transcription of the genes that control DNA methylation. In honeybees, the effect of queen pheromone was opposite to our prediction: workers deprived of queen pheromone likely have more methylation. These results beg follow-up work, for example the use of bisulfite sequencing to identify specific loci whose methylation status is affected by queen pheromone.

In bumblebees, there was no detectable effect of queen pheromone on DNA methylation. *Bombus terrestris* was the only species in which we did not use a split colony design, so it is conceivable that between-colony variance in gene expression overshadowed the effect of treatment. We also used different durations of pheromone treatment for each species, matching the durations over which pheromones have been demonstrated to have an effect in previous work (see the electronic supplementary material), and it is possible that pheromone-mediated effects on *dnmt* expression vary temporally or between tissues. Additionally, we note that bumblebees have comparatively primitive sociality relative to honeybees or *Lasius* ants, with annual colonies and comparatively fertile workers. It is thus tempting to suggest that more 'socially advanced' species may have more pheromone-dependent DNA methylation, though a formal test of this prediction would require similar data on multiple species from across the social insects.

Herb *et al.* [2] found some evidence that worker honeybees that changed task specialization, from nurse to forager and

back again, undergo changes in DNA methylation. Their study provides the only other evidence (to our knowledge) that DNA methylation is plastic and sensitive to external cues in adult insects. In aphids, differences in DNA methylation have been hypothesized to underlie the polyphenism of winged and wingless forms, which are induced depending on the level of crowding. Walsh *et al.* [22] therefore compared the activity of DNA methyltransferases between aphids raised in crowded and uncrowded conditions. They found no effect of crowding on DNA methyltransferase expression, though the experiment's sample size seems inadequate to draw a firm conclusion ($n = 6$). We suggest that DNA methylation may mediate many other inducible polyphenisms, and we encourage research into DNA methylation dynamics in adult insects.

Our data provide evidence that adult *A. mellifera*, *L. niger* and *L. flavus* workers change their DNA methylation profile in response to queen pheromones. Queen pheromones are thought to honestly signal the presence and quality of reproductive(s) in the colony. These pheromones are thus central to many inclusive fitness 'decisions' facing workers, e.g. relating to worker reproduction, worker policing and the rearing of replacement queens [23]. Pheromone-dependent methylation may thus be an important proximate mechanism linking the social environment to adaptive phenotypic plasticity.

Data accessibility. The data are archived at Data Dryad: <http://dx.doi.org/10.5061/dryad.b952b>.

Authors' contributions. All authors performed experiments and co-wrote the paper, approved the final version for publication and agree to be held accountable for its content.

Competing interests. We have no competing interests.

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