REVIEW ARTICLE

Epigenetic changes in eusocial insects which affect age and longevity

Srđana Đorđievski*, Tatjana V. Čelić, Elvira L. Vukašinović, Danijela Kojić and Jelena Purać

Faculty of Sciences, Department of Biology and Ecology, University of Novi Sad, Trg Dositeja Obradovića 3, Serbia

Ageing is a complex process common to all living organisms, influenced by different environmental and genetic factors which are difficult to understand. Epigenetic modifications such as DNA methylation, histone post-translational modification and non-coding RNA affect ageing. Eusocial insects provide an ideal platform for analysing the impact of epigenetic changes on ageing due to their phenotypic plasticity. This study summarizes most of the data published so far on epigenetic changes during ageing in eusocial insects.

Keywords: DNA methylation, histone modification, invertebrates, non-coding RNA.

AGEING is a well-known complex process common to all living organisms. It is associated with a progressive decrease and loss of normal physiological functions and cell senescence, culminating in the onset of diseases and, finally, death of an organism[1,2]. This process depends on the interaction of various environmental factors and genetic mechanisms, which are still not fully understood. At the molecular level, ageing is influenced by energy metabolism, which impacts cellular senescence, telomere shortening and autophagy. Recently, however, much progress has been made in characterizing the epigenetic mechanisms that underpin ageing[2–4]. The role of epigenetics in ageing is being actively studied, and the important model organisms for these studies are eusocial insects (such as honey bees, wasps, bumble bees and ants) due to their phenotypic plasticity, which is an important feature of eusociality[4–7]. Social insects are a good example of polyphenism, a unique subtype of phenotypic plasticity, where two or more distinct phenotypes arise from the same genotype, and genetically identical individuals express evident differences in behaviour and longevity[8]. Phenotypic plasticity is also associated with division of labour, which is important for development of the caste system, with lifespan divergence between castes[5,9,10].

The term 'epigenetics' comes from the Greek word 'epigenesis' (*epi*: above and *genesis*: generation). It was first used by the British scientist C. H. Waddington in 1942, who defined epigenetics as 'the branch of biology which studies the casual interaction between genes and their products which bring the phenotype into being'[11]. Epigenetic modifications play a major role in modulating gene expression and thus establish, maintain and change the phenotype, affecting behaviour and longevity. Although the chromosomes in our genome contain many genes, the basic physical and functional unit of heredity, the epigenome, is responsible for the functional use and stability of that valuable information; that is, it connects the genotype with the phenotype[2,12]. So far, several different mechanisms of epigenetic modification of gene expression have been identified, including DNA methylation, post-translational modification of histones and the influence of regulatory non-coding RNA (ncRNA). This article presents some key experiments, summed up in Table 1, that introduce us to the connection between epigenetics and ageing, as well as the mechanism potentially underlying ageing and longevity in eusocial insects.

DNA methylation

DNA methylation is a covalent chemical modification of the fifth carbon atom of cytosine to form 5-methyl-cytosine. The donor of the methyl group is S-adenosyl methionine (SAM), and the reaction is catalysed by the enzyme DNA methyltransferase (DNMT). DNA methylation often occurs where cytosine and guanine are next to each other in the nucleotide sequence, known as the CpG islands[13–15]. DNA methylation at the CpG islands is associated with inhibition of gene expression, and thereby, transcriptional silencing[16]. In most insect studies, methylation was predominately restricted to coding exons and absent in promotor regions[16–18]. This epigenetic modification is the most studied, as it has several benefits over other modifications, including the fact that DNA methylation is inherited throughout cell division, which is enabled by the action of the most abundant methyltransferase, DNMT1 (refs 13–15). In addition to DNMT1, DNMT2, 3 and 4, are referred to as *de novo* methyltransferases[19–21]. DNMT3 is important as it introduces new patterns of methylation and performs a role in the development of castes in social insects[22]. DNMT2 was previously linked to cytosine methylation, but now it is known to methylate transfer RNA (tRNA)[23]. It thus performs an important role in protecting insects

*For correspondence. (e-mail: srdjana@dbe.uns.ac.rs)

Table 1. Summary of processes in which epigenetic regulations play an important role in eusocial insects

Family	Species	Epigenetic modification	Process regulation
Apidae	*Apis mellifera*	DNM	Caste development[22]
Apidae	*Apis mellifera*	DNM	Not yet defined[38,39]
Apidae	*Apis mellifera*	DNM	Caste behaviour[28]
Apidae	*Apis mellifera*	DNM	Caste determination[37]
Apidae	*Apis mellifera*	DNM	Caste-specific gene expression[41]
Apidae	*Bombus terrestris*	DNM	Ageing[6]
Vespidae	*Polistes dominula*	DNM	Physiology and behaviour[42]
Formicidae	*Linepithema humile*	DNM	Reproductive development[54]
Formicidae	*Camponotus floridanus*	DNM	Caste-specific gene expression[44]
Pteromilidae	*Nasonia vitripennis*	DNM	Genes for cellularization and gastrulation of embryo[43]
Apidae	*Apis mellifera*	HPTM	Caste development[53]
Formicidae	*Camponotus floridanus*	HPTM	Potentially ageing[54]
	Harpegnathos venator		
Apidae	*Apis mellifera*	HPTM	Caste development[57]
Formicidae	*Camponotus floridanus*	HPTM	Caste identity[58]
Formicidae	*Temnothorax rugatulus*	HPTM	Downregulates genes with immunity, ageing and longevity functions[59]
Apidae	*Apis mellifera*	ncRNA	Ageing and longevity[60]
Apidae	*Apis mellifera*	ncRNA	Development of different phenotypes[69–71]
Formicidae	*Camponotus floridanus*		
Formicidae	*Harpegnathos saltator*		

DNM, DNA methylation; HPTM, Histone post-translation modification; ncRNA, Non-coding RNA.

from harmful environments, as has been shown in studies with the fruit fly *Drosophila melanogaster*[19,20,24]. In different species, ageing is associated with methylation at the CpG islands[25]. Pathways that control DNA methylation, unlike histone modification, are not conserved across taxa. Global abundance of methylated CpG islands is present between different species[26,27]. Insects have a relatively low level of DNA methylation (0–3%); for example, CpG methylation is absent in *D. melanogaster*[28] and beetles *Tribolium castaneum*[29], but it is common among social insects[30]. Humans and birds have 5% of methylated DNA, while in plants, more than 30% of the DNA is methylated[13]. Also, methylated CpG contents varied during insect development. For instance, in honey bee, *Apis meliffera*, the first species found to have a fully functioning methylation system, embryos had the highest level of methylation in comparison with adults[31,32].

Apis mellifera L. has a complete DNA methylation system[33] and represents a good example of phenotypic plasticity, where a fertile queen develops from larvae fed with royal jelly while workers are fed with jelly that lacks in sugars and some other important ingredients[34]. Such nutritional differences may affect age and longevity in honey bees as well as DNA methylation[35]. Hence this species is one of the most studied with regard to epigenetic modifications. Experiments on the honey bee, one of the most studied social insects, have introduced the special role of DNMT3 in caste development. Kucharski *et al.*[22] showed that silencing of the gene for DNMT3 using simple small interfering RNA (siRNA) in newly hatched larvae led to the development of more queens (72%) than worker bees (28%). In addition, comparing the heads of destinated queens and those that emerged from larvae with the silenced *DNMT3* gene showed a decreased level of DNA methylation. Numerous studies have been conducted in order to better understand DNA methylation. It has been shown that methylation is important for key biological processes such as development, caste determination, behaviour and, above all, ageing and longevity[24]. Lyko *et al.*[28] performed high-resolution bisulphite sequencing on whole-brain genome of queen and worker honey bees, where they discovered more than 550 genes showing different patterns of DNA methylation between them, which potentially affects the differences in their behaviour. This method analyses the DNA treated with bisulphite, which converts unmethylated cytosine to uracil, which can then be detected and compared to the reference genome[36]. Foret *et al.*[37] sequenced methylomes in larvae and queen heads, discovering 2399 methylated genes that were significantly different with respect to methylation. They showed that several highly conserved signalling and metabolic pathways, such as juvenile hormone and insulin, are enriched in methylated genes, previously shown to regulate caste determination. The gene *hexamerin 110* encoding storage protein[38] and *dynactin p62*, the conserved gene responsible for feeding changes[39], displayed different levels of DNA methylation on comparing queen and worker bees. There was a significantly different global level of DNA methylation in bees of different ages, which can be a good basis for understanding and explaining DNA methylation and how it affects ageing[40]. Wang *et al.*[41] compared the genomic methylation of the queen and worker larvae at three, four and five days old, using the whole-genome bisulphite sequencing technique. They concluded that the fundamental traits of methylation are equal among them. However, the methylation levels of queen and worker larvae showed differences and varied with age of the organism. Wang *et al.*[41] additionally singled out ten methylated

differentially genes (DMGs) and 13 cast-specific genes that could be possible molecular markers for selective breeding of this species to improve fecundity, production of royal jelly, body size and foraging.

Similar mechanisms have been observed in other social insects such as ants, wasps and bumble bees, showing significant changes in DNA methylation during ageing and caste-specific methylation patterns. In bumble bee *Bombus terrestris* workers, significant age-related increase in DNMT3 expressed in fat body was observed, suggesting a novel association between ageing and methylation[6]. In an experiment with primitively social paper wasp *Polisters dominula*, Weiner *et al*.[42] showed surprisingly increased overall DNA methylation and caste-related differences in site-specific methylation, suggesting the role of DNA methylation in physiological and behavioural regulation. Recent studies in jewel wasp *Nasonia vitripenis* have shown that knocking out the *DNMT1* gene leads to failure in cellularization and gastrulation of the embryo, which demonstrates that reduction in DNA methylation is associated with decreased gene expression[43]. Evolutionarily conserved DNA methyltransferase has an important role in the reproductive caste development in social insects like ants[23]. It regulates phenotypic plasticity of size in carpenter ants and is correlated with age-related and caste-specific gene expression. However, patterns of DNA methylation also change during development and caste-specific gene expression in ants[44,45].

DNA methylation is reversible and this can be done in two ways: (1) passive demethylation – by blocking DNMT1 maintenance process during DNA replication and (2) active demethylation – by the enzyme family TET (ten–eleven translocation) dioxygenases, which oxidize the methyl group on cytosine to form 5-hydroxymethyl-cytosine, which can, in mammals, be oxidized to 5-formyl-cytosine and 5-carboxyl-cytosine, and finally converted into cytosine. 5-Hydroxymethyl-cytosine is present in insects, but there are no reports about the presence of the other two forms in insects[36,46,47]. Studies on *A. mellifera* have proven that this species, like many different insects, including *B. terrestris* and *N. vitripennis*[23], has only one type of TET dioxygenase, while in vertebrates, three types of this enzyme have been discovered[47,48]. These enzymes were found in different tissues and developmental stages in the honey bee[49,50]. In addition, TET dioxygenases are mostly expressed in the brain of honey-bee workers. However, their expression level does not correspond to the level of 5-hydroxymethyl-cytosine, which implies that these enzymes possibly have other roles as well[15].

Post-translational histone modification

Post-translational modification of histone is a type of epigenetic modification that involves structural changes in the chromatin, which is responsible for the packaging and organization of DNA in the nucleus, and affects various important biological processes[4,24]. The DNA is wrapped around small, positively charged proteins called histones (H1, H2a, H2b, H3 and H4), with amino acid tails subjected to various changes. Chromatin manages DNA availability for processes such as transcription, replication and DNA reparation, so it plays a major role in the developmental trajectories of phenotype construction[24]. Post-translational modifications of histone tails operate together with DNA methylation in regulating gene expression[3]. These modifications include methylation, acetylation, phosphorylation, ubiquitination, ADP-ribosylation, etc., which aim to activate or repress transcription to regulate gene expression. The configuration of chromatin, which can be condensed or relaxed, depends on these modifications. More than 160 histone modifications have been detected in insects, but most mechanisms are still not fully understood[13].

During histone methylation, methyl groups are relocated to lysine or arginine side chains, altering the DNA packaging, which is proven to regulate insect development and longevity[24,51]. Two enzymes, histone methyltransferase (HMT) and histone demethylase (HDM) play important roles in these modifications[24,52]. Methylation of H3K9, H3K27 and H4K20 is associated with transcription repression, while methylation of H3K4, H3K36 and H3K79 is associated with active chromatin[3]. Studies conducted on honey-bee worker and queen larvae 96 h after hatching showed a well-preserved methylation pattern of H3K4 and H3K36, indicating an important role in caste development[53]. Bonasio *et al*.[54] identified 27 proteins containing the conserved SET histone methyltransferase domain (proteins that methylate histone on lysine) in ants *Camponotus floridanus* and 22 in *Harpegnathos saltator* but crucial results are missing on how this affects ageing. There is little work done in this field of study, so additional research is required to better understand the mechanism of action of histone methylation in other social insects such as ants and wasps.

Histone acetylation, the first discovered modification, is based on the addition of an acetyl group from acetyl-CoA to the amino group of lysine by the action of the enzyme histone acetyltransferase (HAT). Histone tails undergo rapid acetylation and deacetylation by histone deacetylase (HDAC), and the half-life of the acetylated histones is only a few minutes. HAT and HADC are often part of larger enzyme complexes with different activities, and each has a coordinated function, recognizes specific regions of chromatin and makes the necessary modifications. Histone acetylation can activate transcription by reducing histone–DNA interactions, recruiting transcription factors and remodelling the chromatin structure[52,55,56]. Dickman *et al*.[57] identified 23 post-translational modifications in 96-h-old honey bee larvae, an important time point for key genetic modifications triggered by consumption of royal jelly, and acetylation (two), in particular H3K9 and H3K14, which were shown to be mutually dependent. Simola *et al*.[58]

conducted a similar experiment with *C. floridanus* and found that gene changes in histone modification, especially H3K27 acetylation, could be a powerful predictor of caste identity.

The latest study by Choppin *et al*.[59] on ant *Temnothorax rugatulus* showed that feeding workers with chemical inhibitors for histone acetylation is C646 and inhibitor for histone deacetylation is trichostatin A (TSA) affect gene expression, especially by downregulating genes with immunity, ageing and longevity functions. They concluded the importance of histone acetylation in phenotypic plasticity of this species. In contrast, Hu *et al*.[60] showed that treatment of honey bees with Na-butyrate, a well-known HDAC inhibitor, led to increased histone acetylation and prolonged their life span compared to untreated bees. HDAC inhibitors commonly present in royal jelly are necessary for the development of queen bees, which may indicate that royal jelly directly influences the histone acetylation level and thereby caste determination[61]. Numerous studies have been conducted on histone modification in insects, but it is still unclear how they affect ageing and longevity. Therefore, additional experiments are required.

Non-coding RNA

ncRNAs are DNA transcripts, but rather than being translated into proteins, they play an essential role in gene expression regulation at the post-translational stage, affecting gene expression and chromatin remodelling by binding to their targets[52]. There are several distinct ncRNAs: micro RNA (miRNA), small interfering RNA (siRNA), PiWi interacting RNA (piRNA), long non-coding RNA (lncRNA) and circular RNA (circRNA)[9,62–65]. Along with DNA methylation, they play a crucial role in paramutations, which occur when one allele affects another from the same locus and causes hereditary modifications in that allele, but are also involved in protecting insects from viral infections[12,13]. These ncRNAs play an important role in epigenetic modifications by affecting age and longevity, as has been shown in an experiment with *C. elegans*, where total miRNA and piRNA decreased with ageing[66]. Genetic modulations of certain ncRNAs affect the life span of insects, in addition to the modulation of protein factors that alter ageing[52,66]. CircRNA increases in an age-dependent manner. In general, miRNA affects age and longevity by interaction with different genes, thus altering the gene expression, but also targets 3′-UTR of mRNA affecting transcriptional repression[52]. Several experiments on social insects such as *A. mellifera*, ant *C. floridanus* and *H. saltator* showed the differences in miRNA expression, suggesting its role in the development of different phenotypes. Experiments conducted on honey bees have shown that royal jelly, the primary food for larvae from which queens develop, contains numerous ncRNAs, including piRNA, siRNA[67] and miRNA[68], that play a role in post-translational silencing of genes, providing, therefore, an additional level of epigenetic regulation[12,69]. miRNAs are much more common in jelly-feeding larvae, while reproductive queens have significantly higher levels of piRNA. So these RNAs are considered to play a role in the development of specific phenotypes in the caste[69–71]. Honey bee is the most popular model organism for studying different biological processes. However, information is lacking on how ncRNA affects ageing in honey bees, as well as in other social insects such as ants, wasps and bumble bees.

Conclusion

Epigenetics and ageing are extremely popular areas of research, particularly as they are inter-related. Social insects hold great promise in epigenetic studies. They play a crucial role as model organisms due to their phenotypical plasticity, flexible ageing and longevity. It is fascinating how the division in the castes works and how much individuals differ significantly in morphology and behaviour as well as in life expectancy, regardless of a similar genotype. In addition, social insects are simple to maintain in laboratory conditions. Therefore, research on epigenetic changes and ageing in social insects is essential for understanding the mechanisms behind these processes and how they can be influenced. Even though several studies, especially in DNA methylation, have been done, there is a lack of detailed knowledge on epigenetic changes. Further analysis on histone post-translational modifications is particularly required to understand better which genes are affected by these modifications and the way these changes affect ageing or caste formation. In recent years, RNA research has become popular, especially since it has been established that ncRNAs impact the epigenetics and ageing of an organism. Additional research is needed to better explain how certain ncRNAs affect the genome and cause epigenetic changes. Understanding the link between ageing and epigenetics is challenging in any model organism. Social insects provide a more manipulable system, but difficulties do exist. To understand this link, we may need to look at the cellular level to understand what is happening in different cell types and how that affects phenotype. Future studies could potentially identify therapeutics that may be used to treat ageing-related disorders and slow down the ageing process of organisms.

1. Moskalev, A. A., Aliper, A. M., Smit-McBride, Z., Buzdin, A. and Zhavoronkov, A., Genetics and epigenetics of aging and longevity. *Cell Cycle*, 2014, **13**, 1063–1077.
2. Pal, S. and Tyler, J. K., Epigenetics and aging. *Sci. Adv.*, 2016, **2**, 1–19.
3. Kozeretska, I. A., Serga, S. V., Koliada, A. K. and Vaiserman, A. M., Epigenetic regulation of longevity in insects. In *Advances in Insect Physiology*, Elsevier, 2017, 1st edn.
4. Vaiserman, A. M., Lushchak, O. V. and Koliada, A. K., Epigenetics of longevity in social insects. *Epigenet. Aging Longev.*, 2018, **4**, 271–289.

5. Corona, M., Libbrecht, R. and Wheeler, D. E., Molecular mechanisms of phenotypic plasticity in social insects. *Curr. Opin. Insect Sci.*, 2016, **13**, 55–60.
6. Lockett, G. A., Almond, E. J., Huggins, T. J., Parker, J. D. and Bourke, A. F. G., Gene expression differences in relation to age and social environment in queen and worker bumble bees. *Exp. Gerontol.*, 2016, **77**, 52–61.
7. Berens, A. J., Hunt, J. H. and Toth, A. L., Nourishment level affects caste-related gene expression in *Polistes* wasps. *BMC Genomics*, 2015, **16**, 1–12.
8. Yan, H., Bonasio, R., Simola, D. F., Liebig, J., Berger, S. L. and Reinberg, D., DNA methylation in social insects: how epigenetics can control behavior and longevity. *Annu. Rev. Entomol.*, 2015, **60**, 23.1–23.18.
9. Glastad, K. M., Chau, L. M. and Goodisman, M. A. D., Epigenetics in social insects. *Adv. Insect Physiol.*, 2015, **48**, 227–269.
10. Yang, C. H. and Pospisilik, J. A., Polyphenism – a window into gene–environment interactions and phenotypic plasticity. *Front. Genet.*, 2019, **10**.
11. Waddington, C. H., The epigenotype. 1942. *Int. J. Epidemiol.*, 2012, **41**, 10–13.
12. Cridge, A., Harrop, T., Lovegrove, M., Remnant, E. and Dearden, P., Nutrition and epigenetic change in insects: evidence and implications. *Adv. Insect Physiol.*, 2017, **53**, 31–34.
13. Burggren, W. W., Epigenetics in insects: mechanisms, phenotypes and ecological and evolutionary implications. In *Advances in Insect Physiology*, 2017, 1st edn.
14. Ciechomska, M., Roszkowski, L. and Maslinski, W., DNA methylation as a future therapeutic and diagnostic target in rheumatoid arthritis. *Cells*, 2019, **8**, 1–16.
15. Wedd, L. and Maleszka, R., DNA methylation and gene regulation in honeybees: from genome-wide analyses to obligatory epialleles. *Adv. Exp. Med. Biol.*, 2016, **945**, 193–211.
16. Klose, R. J. and Bird, A. P., Genomic DNA methylation: the mark and its mediators. *Trends Biochem. Sci.*, 2006, **31**, 89–97.
17. Maleszka, R., Epigenetic code and insect behavioural plasticity. *Curr. Opin. Insect Sci.*, 2016, **15**, 45–52.
18. Patalano, S., Hore, T. A., Reik, W. and Sumner, S., Shifting behaviour: epigenetic reprogramming in eusocial insects. *Curr. Opin. Cell Biol.*, 2012, **24**, 367–373.
19. Goll, M. G. and Bestor, T. H., Eukaryotic cytosine methyltransferases. *Annu. Rev. Biochem.*, 2005, **74**, 481–514.
20. Marbaniang, C. N. and Vogel, J., Emerging roles of RNA modifications in bacteria. *Curr. Opin. Microbiol.*, 2016, **30**, 50–57.
21. Goll, M. G. *et al*., Methylation of tRNAAsp by the DNA methyltransferase homolog Dnmt2. *Science*, 2006, **311**, 395–398.
22. Kucharski, R., Maleszka, J., Foret, S. and Maleszka, R., Nutritional control of reproductive status in honeybees via DNA methylation. *Science*, 2008, **319**, 1827–1830.
23. Li-Byarlay, H., The function of DNA methylation marks in social insects. *Front. Ecol. Evol.*, 2016, **4**, 1–8.
24. Villagra, C. and Frías-Lasserre, D., Epigenetic molecular mechanisms in insects. *Neotrop. Entomol.*, 2020, **49**, 615–642.
25. Christensen, B. C. *et al.*, Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CPG island context. *PLoS Genet.*, 2009, **5**, e1000602.
26. Campos, E. I. and Reinberg, D., Histones: annotating chromatin. *Annu. Rev. Genet.*, 2009, **43**, 559–599.
27. Suzuki, M. M. and Bird, A., DNA methylation landscapes: provocative insights from epigenomics. *Nature Rev. Genet.*, 2008, **9**, 465–476.
28. Lyko, F., Foret, S., Kucharski, R., Wolf, S., Falckenhayn, C. and Maleszka, R., The honey bee epigenomes: differential methylation of brain DNA in queens and workers. *PLoS Biol.*, 2010, **8**, e1000506.
29. Richards, S. *et al*., The genome of the model beetle and pest *Tribolium castaneum*. *Nature*, 2008, **452**, 949–955.
30. Kronforst, M. R., Gilley, D. C., Strassmann, J. E. and Queller, D. C., DNA methylation is widespread across social Hymenoptera. *Curr. Biol.*, 2008, **18**, 287–288.
31. Drewell, R. A. *et al*., The dynamic DNA methylation cycle from egg to sperm in the honey bee *Apis mellifera*. *Develop*, 2014, **141**, 2702–2711.
32. Wang, Y. *et al*., Functional CpG methylation system in a social insect. *Science*, 2006, **314**, 645–647.
33. Lockett, G. A., Helliwell, P. and Maleszka, R., Involvement of DNA methylation in memory processing in the honey bee. *Neuroreport*, 2010, **21**, 812–816.
34. Wang, Y., Ma, L., Zhang, W., Cui, X., Wang, H. and Xu, B., Comparison of the nutrient composition of royal jelly and worker jelly of honey bees (*Apis mellifera*). *Apidologie*, 2016, **47**, 48–56.
35. Maleszka, R., Beyond royalactin and a master inducer explanation of phenotypic plasticity in honey bees. *Commun. Biol.*, 2018, **1**, 1–7.
36. Duncan, E. J., Cunningham, C. B. and Dearden, P. K., Phenotypic plasticity: what has DNA methylation got to do with it? *Insects*, 2022, **13**, 1–17.
37. Foret, S. *et al.*, DNA methylation dynamics, metabolic fluxes, gene splicing, and alternative phenotypes in honey bees. *Proc. Natl. Acad. Sci. USA*, 2012, **109**, 4968–4973.
38. Ikeda, T., Furukawa, S., Nakamura, J., Sasaki, T. and Sasaki, M., CpG methylation in the hexamerin 110 gene in the European honeybee, *Apis mellifera*. *J. Insect Sci.*, 2011, **11**, 1–11.
39. Shi, Y. Y., Huang, Z. Y., Zeng, Z. J., Wang, Z. L., Wu, X. B. and Yan, W. Y., Diet and cell size both affect queen–worker differentiation through DNA methylation in honey bees (*Apis mellifera*, Apidae). *PLoS ONE*, 2011, **6**, 1–6.
40. Shi, Y. Y., Yan, W. Y., Huang, Z. Y., Wang, Z. L., Wu, X. B. and Zeng, Z. J., Genomewide analysis indicates that queen larvae have lower methylation levels in the honey bee (*Apis mellifera*). *Naturwissenschaften*, 2013, **100**, 193–197.
41. Wang, H., Liu, Z., Wang, Y., Ma, L., Zhang, W. and Xu, B., Genome-wide differential DNA methylation in reproductive, morphological, and visual system differences between queen bee and worker bee (*Apis mellifera*). *Front. Genet.*, 2020, **11**, 1–15.
42. Weiner, S. A. *et al.*, A survey of DNA methylation across social insect species, life stages, and castes reveals abundant and caste-associated methylation in a primitively social wasp. *Naturwissenschaften*, 2013, **100**, 1–21.
43. Arsala, D., Wu, X., Yi, S. V. and Lynch, J. A., Dnmt1a is essential for gene body methylation and the regulation of zygotic genome activation in the wasp. *BioRxiv*, 2021, 1–24.
44. Smith, C. D. *et al.*, Draft genome of the globally widespread and invasive Argentine ant (*Linepithema humile*). *Proc. Natl. Acad. Sci. USA*, 2011, **108**, 5673–5678.
45. Libbrecht, R., Oxley, P. R., Kronauer, D. J. C. and Keller, L., Ant genomics sheds light on the molecular regulation of social organization. *Genome Biol.*, 2013, **14**, 136–143.
46. Piccolo, F. M. and Fisher, A. G., Getting rid of DNA methylation. *Trends Cell Biol.*, 2014, **24**, 1–8.
47. Kohli, R. M. and Zhang, Y., TET enzymes, TDG and the dynamics of DNA demethylation. *Nature*, 2013, **502**, 472–479.
48. Bogdanović, O., TET proteins: master regulators of vertebrate body plan formation? *Epigenomics*, 2017, **9**, 93–96.
49. Wojciechowski, M. *et al*., Insights into DNA hydroxymethylation in the honeybee from in-depth analyses of TET dioxygenase. *Open Biol.*, 2014, **4**, 1–9.
50. Rasmussen, E. M. K. and Amdam, G. V., Cytosine modifications in the honey bee (*Apis mellifera*) worker genome. *Front. Genet.*, 2015, **6**, 1–6.
51. Suzuki, R., Yaguchi, H. and Maekawa, K., Histone modifying genes are involved in the molting period during soldier differentiation in *Zootermopsis nevadensis*. *J. Insect Physiol.*, 2019, **117**, 103892.

52. Yu, G., Wu, Q., Gao, Y., Chen, M. and Yang, M., The epigenetics of aging in invertebrates. *Int. J. Mol. Sci.*, 2019, **20**, 1–17.
53. Wojciechowski, M., Lowe, R., Maleszka, J., Conn, D., Maleszka, R. and Hurd, P. J., Phenotypically distinct female castes in honey bees are defined by alternative chromatin states during larval development. *Genome Res.*, 2018, **28**, 1–11.
54. Bonasio, R. *et al*., Genome-wide and caste-specific DNA methylomes of the ants *Camponotus floridanus* and *Harpegnathos saltator*. *Curr. Biol.*, 2012, **22**, 1755–1764.
55. Bannister, A. J. and Kouzarides, T., Regulation of chromatin by histone modifications. *Cell Res.*, 2011, **21**, 381–395.
56. Blakey, C. A. and Litt, M. D., Histone modifications – models and mechanisms. In *Epigenetic Gene Expression and Regulation*, Elsevier, 2015, pp. 21–42.
57. Dickman, M. J., Kucharski, R., Maleszka, R. and Hurd, P. J., Extensive histone post-translational modification in honey bees. *Insect Biochem. Mol. Biol.*, 2013, **43**, 125–137.
58. Simola, D. F. *et al*., A chromatin link to caste identity in the carpenter ant *Camponotus floridanus*. *Genome Res.*, 2013, **23**, 486–496.
59. Choppin, M., Feldmeyer, B. and Foitzik, S., Histone acetylation regulates the expression of genes involved in worker reproduction in the ant *Temnothorax rugatulus*. *BMC Genomics*, 2021, **22**, 1–13.
60. Hu, Y. T., Wu, T. C., Yang, E. C., Wu, P. C., Lin, P. T. and Wu, Y. L., Regulation of genes related to immune signaling and detoxification in *Apis mellifera* by an inhibitor of histone deacetylation. *Sci. Rep.*, 2017, **7**, 1–14.
61. Spannhoff, A. *et al*., Histone deacetylase inhibitor activity in royal jelly might facilitate caste switching in bees. *EMBO Rep.*, 2011, **12**, 238–243.
62. Asgari, S., MicroRNA functions in insects. *Insect Biochem. Mol. Biol.*, 2013, **43**, 388–397.
63. Abu, N. and Jamal, R., Circular RNAs as promising biomarkers: a mini-review. *Front. Physiol.*, 2016, **7**, 1–6.
64. Holoch, D. and Moazed, D., RNA-mediated epigenetic regulation of gene expression. *Nature Rev. Genet.*, 2015, **16**, 71–84.
65. Marco, A., Regulatory RNAs in the light of *Drosophila* genomics. *Brief. Funct. Genomics*, 2012, **11**, 356–365.
66. Kim, S. S. and Lee, S. J. V., Non-coding RNAs in *Caenorhabditis elegans* aging. *Mol. Cells*, 2019, **42**, 379–385.
67. Carthew, R. W. and Sontheimer, E. J., Origins and mechanisms of miRNAs and siRNAs. *Cell*, 2009, **136**, 642–655.
68. Ashby, R., Forêt, S., Searle, I. and Maleszka, R., MicroRNAs in honey bee caste determination. *Sci. Rep.*, 2016, **6**, 1–15.
69. Guo, X. *et al.*, Receipe for a busy bee: microRNAs in honey bee caste determination. *PLoS ONE*, 2013, **8**, e81661.
70. Behura, S. K. and Whitfield, C. W., Correlated expression patterns of microRNA genes with age-dependent behavioural changes in honeybee. *Insect Mol. Biol.*, 2010, **19**, 431–439.
71. Bonasio, R. *et al*., Genome comparison of the ants *Camponotus floridanus* and *Harpegnathos saltator*. *Science*, 2010, **329**, 1068–1071.

ACKNOWLEDGEMENTS. We acknowledge financial support from the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-9/2022-14/200125) and Science Fund of the Republic of Serbia (Program IDEAS, Grant No. 7721972, Implication of dietary and endogenous polyamines for the health and longevity of honey bees-B-HEALTH).

Received 7 February 2022; revised accepted 30 May 2022

doi: 10.18520/cs/v123/i2/154-159