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SYMPOSIUM

Evaluation of Possible Proximate Mechanisms Underlying the Kinship Theory of Intragenomic Conflict in Social Insects

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Synopsis Kinship theory provides a universal framework in which to understand the evolution of altruism, but there are many molecular and genetic mechanisms that can generate altruistic behaviors. Interestingly, kinship theory specifically predicts intragenomic conflict between maternally-derived alleles (matrigenes) and paternally-derived alleles (patrigenes) over the generation of altruistic behavior in cases where the interests of the matrigenes and patrigenes are not aligned. Under these conditions, individual differences in selfish versus altruistic behavior are predicted to arise from differential expression of the matrigenes and patrigenes (parent-specific gene expression or PSGE) that regulate selfish versus altruistic behaviors. As one of the leading theories to describe PSGE and genomic imprinting, kinship theory has been used to generate predictions to describe the reproductive division of labor in social insect colonies, which represents an excellent model system to test the hypotheses of kinship theory and examine the underlying mechanisms driving it. Recent studies have confirmed the predicted differences in the influence of matrigenes and patrigenes on reproductive division of labor in social insects, and demonstrated that these differences are associated with differences in PSGE of key genes involved in regulating reproductive physiology, providing further support for kinship theory. However, the mechanisms mediating PSGE in social insects, and how PSGE leads to differences in selfish versus altruistic behavior, remain to be determined. Here, we review the available supporting evidence for three possible epigenetic mechanisms (DNA methylation, piRNAs, and histone modification) that may generate PSGE in social insects, and discuss how these may lead to variation in social behavior.

Introduction

The evolution and regulation of altruistic behavior—in which one individual forgoes or reduces its own reproduction to help another reproduce—has long puzzled biologists (Darwin 1859). The development of kin selection theory by W.D. Hamilton provided a framework for understanding the evolution of these traits (Hamilton 1964). Genes can ensure their transmission to the next generation by generating traits that increase the reproductive output of their host (improving their host's direct fitness) or by generating traits that increase the reproductive output of relatives that contain the same alleles of the gene (improving their host's indirect fitness). Kin selection theory predicts that genes/traits that improve an individual's inclusive fitness (both direct and indirect) will be favored, and thus can lead to elaborate cooperative social behaviors. With the increasing power of and access to genomic technologies, it is now possible to probe the proximate genetic and molecular mechanisms underpinning cooperative and altruistic behaviors, and it is clear that multiple avenues can lead to social behavior through the co-option of different sets of genes and phenotypes (Robinson et al. 2005; West et al. 2007; Wilson 2008; Thompson et al. 2013).

Kin selection theory predicts that, in some cases, intragenomic conflict between alleles inherited from the mother (matrigenes) and the father (patrigenes) may arise in sexually reproducing organisms

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(Haig 2002). In most sexually reproducing organisms, the interests of the matrigenes and patrigenes are aligned, such that they promote the fitness of their host and close relatives. However, when the distribution of matrigenes and patrigenes across group members is unequal, conflict can arise. Classic examples include conflict over offspring growth in mammals and plants, in cases where the mother can mate multiply (Reik et al. 2003). In these cases, the matrigenes favor even distribution of maternal resources across all offspring, while patrigenes favor traits that exploit maternal resources. This intragenomic conflict is mediated by differential expression of matrigenes and patrigenes associated with the phenotype of interest (Cosmides and Tooby 1981; Haig 1992).

In 2003, David Queller developed detailed predictions for how intragenomic conflict could operate in haplodiploid social insect societies (Haig 2002; Queller 2003). Haplodiploidy (where females are diploid and males are typically haploid), complex mating patterns, and nuanced social behaviors in these societies allows for the development of sophisticated predictions about the role of intragenomic conflict in shaping social behaviors in different species. In particular, kin selection theory predicts intragenomic conflict over worker reproduction in honey bees. Honey bee colonies consist of a single reproductive female (the queen) and thousands of facultatively sterile females (workers) that rear the queen's offspring and perform other colony tasks (Winston 1991). The queen mates multiply (with an average of 12 males [Tarpy et al. 2004]), thus producing multiple worker "patrilines" in the colony. If the queen is lost, workers can activate their ovaries and lay unfertilized eggs which develop into haploid males (drones) (Winston 1991). Because half-sisters share matrigenes but no patrigenes, patrigenes are predicted to favor worker reproduction/competition. Because matrigenes gain fitness from rearing the offspring of both sisters and half-sisters, they are predicted to favor the altruistic behavior of remaining sterile and rearing males produced by other workers (Queller 2003).

Recently, it has been demonstrated that differential expression of patrigenes and matrigenes in honey bees (*Apis mellifera*) is wide-spread, and that parent-specific gene expression (PSGE) patterns follow the predictions of kin selection theory (Kocher et al. 2015; Galbraith et al. 2016). Expression of patrigenes (many of which have known roles in regulated reproductive physiology in honey bees) in reproductive tissues (fat bodies and ovaries) increased significantly in reproductive workers, while expression of matrigenes remained unchanged (Galbraith et al. 2016). Furthermore, physiological assessments (evaluation of ovary size and activation rates) demonstrated that the paternal genome plays a greater role in shaping the reproductive physiology of worker honey bees than the maternal genome (Oldroyd et al. 2014; Galbraith et al. 2016). These results revealed an additional layer of regulation (PSGE) in the modulation of reproductive division of labor and the associated social and altruistic behaviors, which can now be readily probed across a wide array of systems.

Kinship theory provided a fairly nuanced prediction for interactions between parent-specific alleles in shaping altruistic behaviors in haplodiploid social insect societies, and these predictions have been supported by two recent studies (Kocher et al. 2015; Galbraith et al. 2016). However, the underlying molecular mechanisms regulating PSGE patterns in these systems remain to be determined. In Kocher et al. (2015) and Galbraith et al. (2016), differences in expression patterns of patrigenes and matrigenes were based entirely on the parental source of the allele, not on the allele sequence itself (lineage-of-origin effects were separated from parent-of-origin effects in the analysis) and thus the underlying mechanism is likely to be epigenetic in nature. Here, we discuss three epigenetic mechanisms that may drive the PSGE bias predicted by kin selection theory, and highlight results from recent studies that provide some support for the role of these different mechanisms. Understanding the proximate mechanisms that drive the behavioral and physiological traits associated with kin selection theory can provide an important framework for understanding how these traits evolved.

DNA methylation

One of the most heavily studied epigenetic mechanisms is DNA methylation (for a comprehensive review of this process, please see (Suzuki and Bird 2008; Jones 2012; Mendizabal et al. 2014). DNA methylation involves the addition of a methyl group (CH_3) to the fifth carbon in the cytosine pyrimidine ring. It primarily occurs within CpG (cytosine phosphate guanine) dinucleotides, but it also occurs to a lesser extent in CHG and CHH sequence contexts (H = A, C, or T) (Klose and Bird 2006). The addition of a methyl group is executed by a family of DNA methyltransferase (Dnmt) enzymes. Dnmt1 is considered to be involved in maintenance methylation, ensuring that DNA methylation patterns persist across cell divisions, while Dnmt3 is implicated in *de novo* methylation and the establishment of new DNA

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methylation patterns (Goll and Bestor 2005; Wang et al. 2006).

In vertebrates, DNA methylation is found extensively throughout the genome. DNA methylation in the promoter region of a gene has been linked to transcriptional repression at the gene level, although this repression is by no means absolute (Lou et al. 2014; Mendizabal and Yi 2015). This transcriptional repression is likely caused by repressor complexes that are recruited by a family of proteins (methyl-CpG binding domain proteins) that bind to DNA methylation near the transcription start site (Wade 2001). DNA methylation is also associated with other regulatory processes including X-chromosome inactivation in females (Riggs 1975) and genomic imprinting (a heritable expression pattern where one of the two inherited alleles is silenced) (Li et al. 1993).

The discovery and characterization of insect DNA methylation have expanded our understanding of DNA methylation dramatically. Initial studies of DNA methylation in insects focused on *Drosophila melanogaster* (Urieli-Shoval et al. 1982). These studies concluded that *Drosophila* does not possess the full suite of enzymes required for DNA methylation and that the genome exhibited almost undetectable levels of DNA methylation (Urieli-Shoval et al. 1982). However, the discovery of a full suite of DNA methylation enzymes in honey bees caused a resurgence in interest in DNA methylation in insects (Wang et al. 2006). Since then, studies have continued to examine DNA methylation in insect systems and determined that this epigenetic mark is found in many insect taxa, although it has been lost in some species (Glastad et al. 2011). However, the functional role of DNA methylation in insects remains unclear. In honey bees, differential DNA methylation patterns have been associated with queen-worker caste differentiation (Elango et al. 2009; Lyko et al. 2010), task differentiation among workers (Herb et al. 2012), and learning and memory (Biergans et al. 2012). Interestingly, two paper wasp species (*Polistes dominula* and *Polistes canadensis*), hymenopteran species which also display caste differences and reproductive division of labor, do not appear to have a functional *de novo* DNA methylation system (Patalano et al. 2015; Standage et al. 2016). Thus, similar phenotypes in different species may be regulated via different mechanisms; similarly the function of DNA methylation may vary across species.

Changes in DNA methylation can have different effects depending on the genomic context in which it occurs, and the effects vary between insects and vertebrates. As noted above, in vertebrates, DNA methylation is typically found throughout the genome, but if DNA methylation occurs within or near the transcription start site, it can suppress transcription (Klose and Bird 2006). Unlike vertebrates, DNA methylation in insects is highly localized in the genome, and is typically concentrated in coding sequences, or "gene bodies" (Suzuki and Bird 2008; Sarda et al. 2012; Keller et al. 2016). This "gene body" DNA methylation has been proposed to function in alternative splicing (Flores et al. 2012; Foret et al. 2012; Herb et al. 2012) and in the suppression of transcriptional noise (Bird 1995; Huh et al. 2013). Previous genome-wide analyses of gene body DNA methylation and gene expression found at most weak correlation in honey bees and other insects (Zemach et al. 2010; Wang et al. 2013; Galbraith et al. 2015). However, recent studies suggest that DNA methylation of regulatory regions, such as promoters, may also regulate expression of adjacent genes in insects (Riviere et al. 2013; Olson and Roberts 2014; Saint-Carlier and Riviere 2015; Keller et al. 2016). Thus, the effects of DNA methylation on the regulation of gene expression in insect genomes merit further studies that evaluate DNA methylation patterns beyond the intragenic regions.

In well-studied mammalian systems, DNA methylation plays a crucial role in PSGE (Bartolomei and Tilghman 1997; Wood and Oakey 2006; Ferguson-Smith 2011). Regions responsible for PSGE, so-called imprinting control regions (ICRs), are differentially methylated in the maternal and paternal germlines, and are then maintained across subsequent cell divisions (Wood and Oakey 2006; Ferguson-Smith 2011). Differential DNA methylation of ICRs can generate PSGE via several different coordinated epigenetic regulatory mechanisms. For example, some ICRs occur in intergenic regions and control access of transcription factors to enhancer elements (Bartolomei and Tilghman 1997; Bell and Felsenfeld 2000). Alternatively, the ICR may encode a non-coding RNA, which affects parent-origin specific expression of several nearby genes through interactions with specific chromatin domains and repressive histone modifying enzymes (e.g., [Sleutels et al. 2002; Nagano et al. 2009]). In these cases, DNA methylation affects PSGE in –cis (nearby genic regions are affected).

Thus far, the association between allele specific expression and allele specific methylation in insects has not been broadly evaluated. In two previous studies, one in ants (Bonasio et al. 2012) and one in bumble bees (Lee et al. 2015), monoallelic DNA methylation was correlated with allele specific expression differences at a handful of genes. However, these

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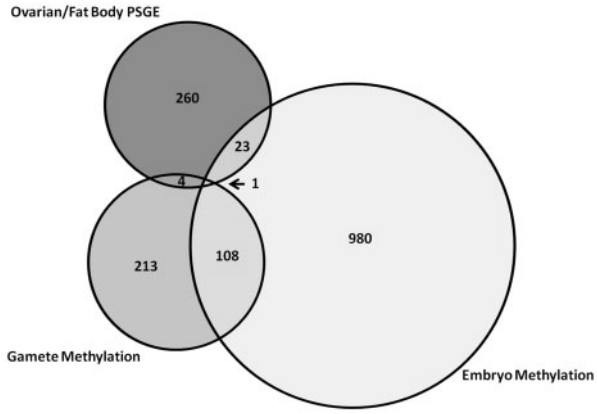

Fig. 1 There is minimal overlap between genes exhibiting a parental specific gene expression pattern and allele specific DNA methylation. Venn diagram showing the overlap between a study examining the PSGE patterns associated with worker reproduction (Galbraith et al. 2016) and two studies that observed allele specific DNA methylation patterns in developing embryos (Remnant et al. 2016) and in sperm and unfertilized eggs (Drewell et al. 2014). There is little overlap among the genes showing PSGE and allele specific methylation. See Supplementary Table S1 for a full listing of the genes. Venn diagram was generated using eulerAPE (Micallef and Rodgers 2014).

experiments did not use individuals generated by a reciprocal cross breeding scheme, and thus it is unclear if the differential expression and methylation was due to parent-of-origin or lineage-of-origin effects. In a recent study evaluating the transcriptomes and methylomes of reciprocal crosses of two species of the nonsocial parasitoid wasp *Nasonia*, increased methylation of an allele correlated with increased expression of the allele, but this correlation was not consistent across all genes and no parent-of-origin effects were observed (Wang et al. 2016).

Given the importance of DNA methylation in regulating PSGE in mammalian systems, it seems likely that it could also serve as a mechanism to generate PSGE in insect systems. If this were the case, it would be predicted that differential parent-of-origin methylation patterns would be established in the germline and maintained in the offspring. Indeed, previous studies have demonstrated that DNA in honey bee eggs and sperm (Drewell et al. 2014) and developing embryos (Remnant et al. 2016) is methylated. There is minimal overlap between the differentially methylated genes identified in these studies (Drewell et al. 2014) and the PSGE patterns found in reproductive workers (Galbraith et al. 2016) (Fig. 1 and Supplementary Table S1). However, it is possible that PSGE and parent-specific methylation patterns (PSMPs) are variable across genotypes, and thus it would be best to conduct these studies using matched drone sperm and offspring. Alternatively, PSGE and PSMP may vary according to tissue and developmental stage (e.g., initially a large number of genes are methylated and subsequently these are reduced to different subsets of methylated genes in different tissues/time points). Indeed, different sets of genes could function in different tissues to promote selfish or cooperative behaviors, and thus confirming tissue-specific PSGE/PSMP patterns would provide even greater support for kin selection theory. Furthermore, it is possible that the same gene functions to differentially regulate selfish versus altruistic behavior depending on which tissue it is expressed in. For example, vitellogenin, a major yolk precursor in most oviparous species, has multiple functions throughout a honey bee, including as a yolk precursor in the ovaries (a "selfish" behavior), the production of brood food in nurse bees (an "altruistic" behavior), and behavioral maturation from nursing behavior through hormonal regulation (Page et al. 2012). Interestingly, this gene was found to exhibit significant PSGE effects in the ovaries and fat bodies of reproductive honey bee workers. Evaluating if and how PSGE/PSGM varies across developmental stages and tissues will provide great insights into the plasticity of these processes, and how these can be fine-tuned by evolutionary mechanisms.

Piwi-interacting RNAs

Because *Drosophila* lack *de novo* DNA methylation systems, it has been suggested that Piwi-interacting RNAs (piRNAs) may function to regulate differential allelic expression (for a comprehensive review of this process, please see Erwin et al. 2015; Huang et al. 2014). The piRNA pathway and piRNAs are found broadly across insect species. piRNAs are small RNAs (25–30 nt in length) that are thought to be generated from precursors derived from endogenous loci, primarily from transposable elements, but can also be found in intergenic regions and protein-coding genes. These small RNAs are generally thought to protect the genome from transposable elements, and maintain genomic integrity and stability (Klattenhoff and Theurkauf 2008). Unlike other small RNAs (siRNAs and miRNAs), the biogenesis of piRNAs is independent of the Dicer protein, but instead uses a separate enzyme called Zucchini, a single strand specific nuclease (Vagin et al. 2006). These small RNAs then form a complex with other proteins, including Aubergine (*Aub*), Argonaute3 (*Ago3*), or Piwi, that then directs the cleavage and subsequent degradation of a target sequence

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(Gunawardane et al. 2007). Interestingly, the antisense-stranded piRNAs will preferentially bind to *Aub* and Piwi, while the sense-strand will preferentially bind to *Ago3*, allowing the process to generate more piRNAs and cleave the target RNA simultaneously (Brennecke et al. 2007).

In Drosophilids, piRNAs are generally found in the germline and function by suppressing transposon activity in the gametic tissue (Brennecke et al. 2007). Mutations in the piRNA pathway results in a disruption of oogenesis due to increased transposon activity (Gillespie and Berg 1995). Furthermore, there is evidence to suggest that piRNA clusters have coevolved with the transposons that they target (Malone et al. 2009). Crosses between different *Drosophila* strains may result in sterile offspring due to defects in gametogenesis, a phenomenon known as hybrid dysgenesis (Castro and Carareto 2004). Hybrid dysgenesis is due to the inheritance of paternal transposons without the associated piRNAs to suppress their expression. To overcome this defect, maternal piRNAs are passed on to the offspring to suppress the expression of these rogue transposons (Brennecke et al. 2008).

Recent studies suggest that piRNAs may be involved in parent-of-origin specific transcriptional activity in *Drosophila* (Erwin et al. 2015). These piRNA complexes bind to highly specific complementary sequences within the genome to recruit histone modifying proteins resulting in transcriptional repression through histone methylation (Huang et al. 2014). Additionally, in mice, it is suggested that piRNAs can direct *de novo* DNA methylation in germ cells (Aravin et al. 2008), resulting in differential DNA methylation of inherited alleles (Watanabe et al. 2011).

Interestingly, several genes that interact with the piRNA pathway display signatures of rapid evolution in primitively eusocial bee species, indicating that they may function in the evolution of social behavior (Woodard et al. 2011). Functional Piwi genes have also been identified in honey bees (Liao et al. 2010); however, their role in oogenesis and reproduction remains unclear. Kocher et al. (2015) and Gibson et al. (2015) suggested that the piRNA pathway may play a role in mediating biased parental expression in honey bees in their studies, though there was no supporting evidence for this hypothesis. Galbraith et al. provided preliminary evidence to suggest that the piRNA pathway is involved in mediating PSGE, because genes *aubergine* and *capsuleen* exhibit a paternal expression bias. However, levels of individual paternally- and maternally-derived piRNAs associated with worker reproduction were not assessed in this study and thus further analyses are necessary. For piRNAs to function in this system, it would be necessary for them to be activated specifically in the male or female germline and for them to be closely linked (to reduce recombination) with their targets such that they specifically modulate expression of the matrigene or patrigene.

Histone modifications

Histone proteins are a critical component of DNA packaging in eukaryotic cells, thereby modulating transcription levels (for a comprehensive review of this process, please see Suganuma and Workman 2011). An octamer composed of four core-histone proteins (H2A, H2B, H3, and H4), collectively called a nucleosome, functions as a spool for DNA to be wrapped around. This constriction of the DNA is a necessary process to ensure that all of the genomic material can fit into the nucleus of the cell. Post-translational modifications of the histone proteins provide specific "marks" that are recognized by chromatin binding proteins (Suganuma and Workman 2011). Each histone protein possesses an N-terminal tail that can be the subject of several types of post-translational modification, including methylation, phosphorylation, acetylation, and many others, each with potential to alter the way the histones regulate gene expression. These modifications are typically added to a lysine or arginine amino acid residue in the N-terminal tail of the histone proteins, but other amino acid residues may also be targeted, albeit less frequently. Histone modifications often interact with other epigenetic processes, such as DNA methylation. For example, preservation of specific histone modification across cell divisions can be facilitated by cellular memory encoded in DNA methylation, and DNA methylation can be recruited to particular loci by histone modifications (Cedar and Bergman 2009).

Histone modifications have the ability to alter the chemical and physical properties of the histone proteins themselves. For example, adding methyl groups to histone proteins increases the pH level of the protein, making it more basic, which increases the affinity for negatively charged molecules, such as DNA (Rice and Allis 2001). This process likely alters the nucleosome/DNA/chromatin interaction. The resulting modification has the potential to either activate or suppress gene expression depending on the type and location of the modification (Barski et al. 2007), lending support to the hypothesis of a "histone code". Specifically, distinct combinations of histone modifications are required for transcriptional

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activation or suppression, representing a fundamental regulatory mechanism at the chromatin level (Jenuwein and Allis 2001). While histone modifications likely play a general role in regulating gene expression in social insects, recent studies have demonstrated a strong link between caste differentiation and differential histone modifications. In honey bees, a histone deacetylase inhibitor has been found in the royal jelly that is specifically fed to developing queen larvae (Spannhoff et al. 2011). Histone modification has also been linked to caste differentiation in ants (*C. floridanus*), and in conjunction with DNA methylation, suggests a role for chromatin structure in modulating phenotypic plasticity (Hunt et al. 2013; Simola et al. 2013). On a genome-wide scale, histone modification patterns can distinguish between major and minor female workers, as well as males in *C. floridanus* (Simola et al. 2013).

There is a growing body of evidence that histone modification plays significant roles in genomic imprinting and PSGE (Weaver and Bartolomei 2014). Polycomb Group (PcG) proteins have the ability to modify histone proteins, which subsequently alters the chromatin structure, leading to transcriptional repression (Sauvageau and Sauvageau 2010). Repressive histone modifications mediated by PcGs have been linked to parent-specific allelic silencing that is associated with genomic imprinting (Lewis et al. 2004; Umlauf et al. 2004; Singh et al. 2011). There is also evidence that PcG protein complexes interact with non-coding RNA to target specific regions of the genome for repressive histone methylation (Rinn et al. 2007), suggesting a potential RNA-directed specificity mechanism to distinguish between parental alleles. In well-studied mammalian model systems, specific histone modifications are linked to regulation of genomic imprinting, in conjunction with DNA methylation. For example, H3K4 methylation may directly interact with DNMTs to regulate imprinting (Ciccone et al. 2009). In addition, proteins that modify specific histone residues, or ''histone modifiers'', may interact with DNA methylation machineries and regulate DNA methylation (Viré et al. 2005). In fact, regulation of DNA methylation through histone modifiers is observed in diverse taxa including animals, plants, and fungi (Jackson et al. 2002; Freitag et al. 2004).

The effects of histone modification in genomic imprinting have not been examined in social insects. However, interestingly, Galbraith et al. (2016) found that several histone modifiers (including homologs of *D. melanogaster Hmt4-20*, *brahma*, SET and MYND domain-containing protein 4 (SMYD4), and *enhancer of zeste*) show evidence of parent-specific expression in reproductive honey bee workers. It is possible that specific histone modifications in insect germlines directly modulate DNA methylation, which then coordinates PSGE. Alternatively, the interaction between histone modifiers and DNA methylation may regulate parent-of-origin specific gene expression. Characterizing epigenetic states of PSGE genes in germlines throughout development will provide much needed insights into the role of histone modifications on the regulation of PSGE.

Conclusions

The kinship theory of intragenomic conflict generates numerous detailed predictions of the role of matrigenes and patrigenes in mediating an array of social behaviors in different social contexts (Queller 2003). Previous studies validated the prediction that paternal alleles will favor worker reproduction in haplodiploid social insect colonies that are headed by a single multiply mated queen, providing support for the theory at the physiological and molecular level (Oldroyd et al. 2014; Galbraith et al. 2016). However, additional studies are necessary to determine the breadth and nuance of the predictions of this theory: for example, are different suites of genes differentially regulated in different tissues? Galbraith et al. evaluated reproductive tissues and found PSGE associated with genes involved in reproductive processes; would genes associated with sensory processing and behavior be differentially regulated in brain tissues, for example? Furthermore, the mechanisms regulating PSGE in this context remain to be determined. In mammals, differential allelic methylation, coupled with differential histone modifications, has been strongly linked to differential allelic transcription. In insects, the association of methylation and transcription is not linear, at least in terms of gene body methylation, which is the predominant form of methylation observed in insect species, though there is some evidence that allelic expression bias is associated with monoallelic methylation (Bonasio et al. 2012; Lee et al. 2015). Furthermore, Piwi-interacting RNAs may function, perhaps via interactions with chromatin remodeling enzymes, to regulate PSGE even in the absence of DNA methylation. Interestingly, genes encoding proteins involved in histone modifications and the Piwi pathways have been found to show PSGE in honey bees workers, but how this translates to PSGE in genes that directly function in regulate reproductive physiology remains to be determined. In this review, we have presented three possible mechanisms underlying PSGE as it relates to kinship theory (DNA methylation, histone

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modification, and the Piwi pathway), however, each of these mechanisms have the ability to interact with each other, and thus it is possible that the PSGE patterns observed by Galbraith et al. and Kocher et al. are the result of a combination, or all three of the mechanisms discussed here. To further characterize the underlying mechanisms driving PSGE, future studies should examine PSGE and PSMP to determine if these two mechanisms are correlated with the predictions generated by kinship theory, identify piRNA's and their associated targets in relation to PSGE, and elucidate histone modification patterns in close proximity to genes exhibiting PSGE effects. In conclusion, social insects provide an excellent system to examine the mechanisms that mediate intragenomic conflict, and further research will provide us with important new insights into the evolution of social behavior, as well as the myriad of mechanisms employed to fine-tune gene expression patterns across tissues, developmental stages, environmental, and social contexts.

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Supplementary data

Supplementary data available at *ICB* online.

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