

# Social insect transcriptomics and the molecular basis of caste diversity

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Studies of gene expression provide fundamentally important information on the molecular mechanisms underlying variation in phenotype. Recent technological advances have allowed for the robust study of gene expression through analysis of whole transcriptomes. Here, we review current advances in social insect transcriptomics and discuss their implications in understanding phenotypic diversity. Recent transcriptomic studies provide detailed inventories of the genes involved in producing distinct phenotypes in social species. These investigations have identified key genes and networks involved in producing distinct social insect castes. Nevertheless, questions concerning the evolution of gene expression patterns remain. We suggest a path forward for studying gene expression in future studies of biological systems.

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## Gene expression and phenotypic diversity in biological systems

A fundamental goal of the study of life is to understand the processes leading to phenotypic diversity. Phenotypic variation within species is remarkable because such variation must arise from a single set of genetic instructions that is shared among conspecifics. This ‘genomic flexibility’ arises from the differential expression of genes [1].

Gene expression is the process of converting information in a DNA sequence into a functional end product, such

as a protein or noncoding RNA. Gene expression can be studied at any number of levels of biological organization. For example, one can consider differences in gene expression between species, between populations within species, between forms within populations (e.g. sexes, castes), between tissues within individuals, or between cells within tissues (Figure 1).

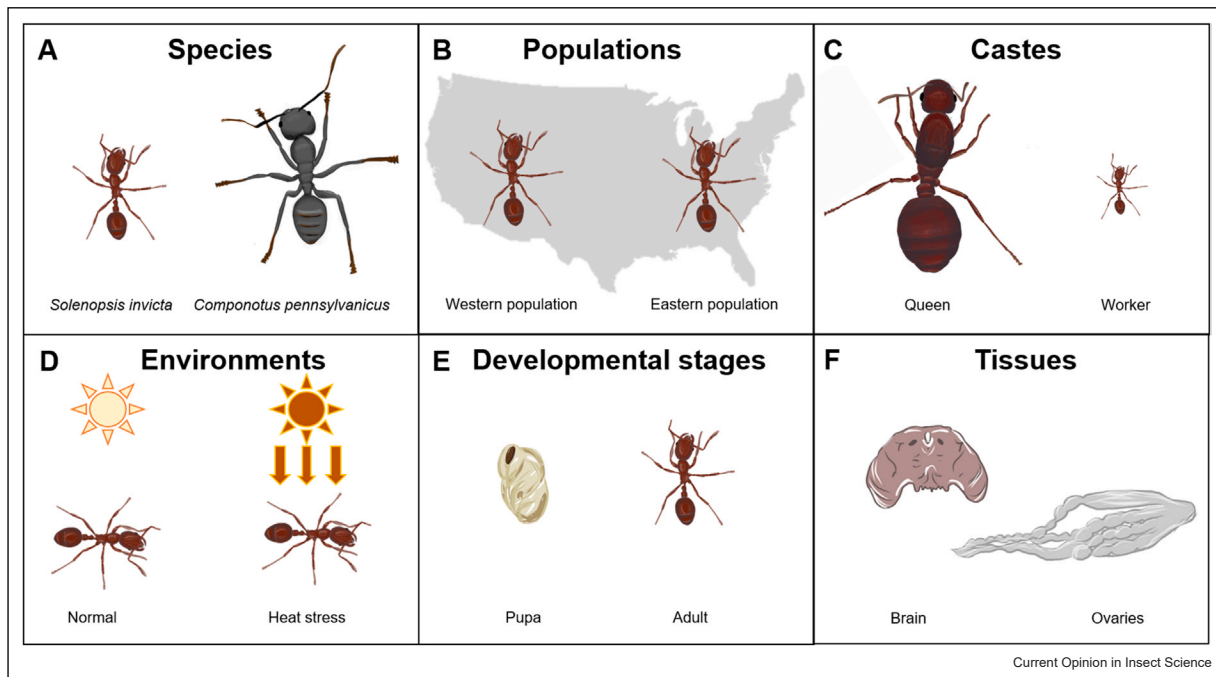
The interpretation of patterns of gene expression rests on the assumption that natural selection is the primary process that has fine-tuned gene regulation; therefore, genes are generally expressed in an adaptive and appropriate manner. In this case, finding a gene expressed in a particular tissue indicates that the gene is involved in the successful function of that tissue, whereas unexpressed genes are not. Recent studies suggest gene expression is subject to stabilizing selection, although the nature of selection acting on expression patterns remains controversial [2,3]. Moreover, a recent study indicated that selection acts to promote highly plastic gene regulation in some species [4].

The regulation of gene function is a complex process. For example, gene expression variation can be associated with ‘cis’ or ‘trans’ components that regulate genes on the same DNA molecule or a distant one, respectively. Cis-variation is usually caused by sequence differences associated with particular alleles, whereas trans-variation arises from diffusible products such as transcription factors. Trans-factors in general have been found to be more influential in affecting gene expression [5], and trans-acting mutations evolve more slowly than cis-acting mutations.

Epigenetic information is a key to understanding how patterns of gene expression are established and maintained. Epigenetics is the study of molecular mechanisms that govern how genes are expressed within and between tissues. Epigenetic information systems are typically viewed as including molecular marks such as DNA methylation and histone modifications, in addition to regulatory RNAs [6]. Epigenetic systems are responsive to environmental signals that lead to a signaling cascade that ultimately triggers various changes in gene function.

There are a great variety of techniques used to profile patterns of gene expression [7]. The earliest techniques of studying gene expression, such as the Northern blot,

Figure 1



Examples of comparative studies of gene expression. Gene expression differences can be explored between (a) species, (b) populations, (c) castes, (d) environments, (e) developmental stages, and (f) tissues.

focused on analyzing one gene at a time. However, the genomics revolution brought about the ability to study expression profiles of many genes at once. Microarrays first offered a scaled-up version of the Northern blot and produced transcriptomic analyses of gene expression patterns. And then, RNA-Seq led to the ability to sequence entire transcriptomes [8,9]. Further, scientists have developed the ability to sequence the transcriptome of single cells and produce remarkably specific biological information [10]. Long-read RNA sequencing represents another developing method that sequences full-length cDNA [11,12]. Overall, the genomics revolution has catapulted the study of gene function across all biological systems.

### The social insects

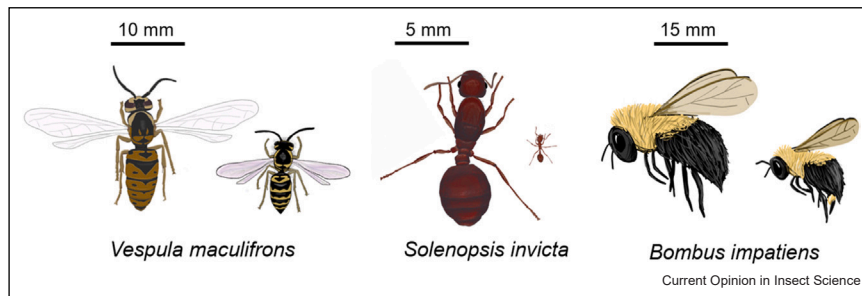
Studies of gene expression are of particular interest to those studying highly social (i.e. eusocial) insects [13]. Social insects are generally defined as living in social groups and displaying a caste system, including a division of individuals into reproductive and non-reproductive phenotypes. Social insects include some

bees, some wasps, and all ants, as well as all termites (Figure 2). In addition, several other insects show sporadic origins of highly social behavior, including some beetles, thrips, and aphids.

Caste differences generally arise through differential gene expression. Studies of gene expression have thus taken particular importance in social insects [14,15]. For example, the study of caste differences allows for insight into the molecular and evolutionary processes that generate biological variation within species. In addition, studying differences in gene expression between phenotypes can help identify caste-biased genes, which are differentially expressed between castes. And so, gene expression studies in social insect systems allow for a greater understanding of how multiple traits become expressed together to generate a particular phenotypic form through developmental plasticity.

This concise review will focus on recent studies of social insect gene expression. We synthesize recent transcriptomics work that has helped unravel important

Figure 2



Remarkable phenotype differences between hymenopteran queen and worker castes of (a) *Vespa maculifrons* wasps, (b) *Solenopsis invicta* ants, and (c) *Bombus impatiens* bees.

genes and pathways responsible for eliciting caste-specific phenotypes. We specifically confine this review to studies using RNA-Seq, which is the most direct way to study patterns of gene expression (Figure 3). RNA-Seq has just begun to be integrated into studies of phenotypic diversity in social systems and has already elucidated many new mechanisms underlying caste differences.

## Gene expression and caste differences in insect societies

### Ants

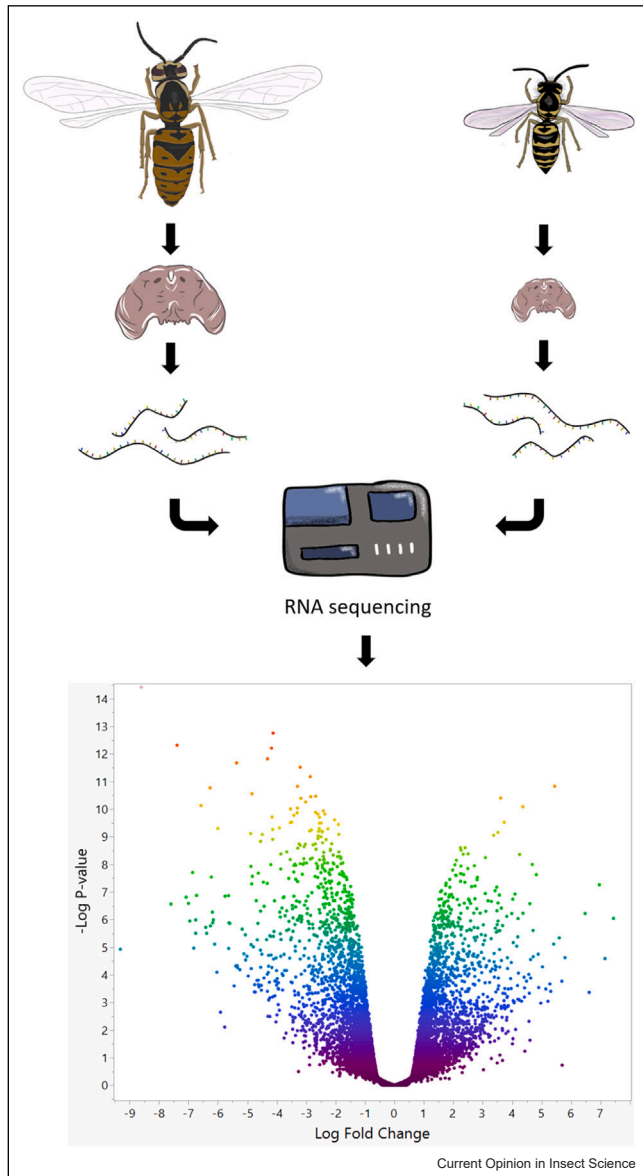
Like most social insects, ants possess fascinating and complex societies that typically feature two distinct female castes: workers and queens. Advances in technology have allowed for more specific approaches to identifying gene expression differences between castes. For example, single-cell transcriptomics was used to explore key caste differentiation pathways in neural circuitry of *Monomorium pharaonis* [17]. In addition, gene expression and chromatin accessibility in brain tissue of *M. pharaonis* castes were explored in a related study that revealed caste-biased expression of *Vitellogenin* (*Vg*) in queens [18]. Workers also expressed *Ras-related and estrogen-regulated growth inhibitor* and displayed a striking downregulation in two vision-related genes. In a study with *Harpegnathos salator* ants, researchers found that a transcriptional repressor, *Kr-h1*, controlled by juvenile hormone and ecdysone, played an essential role in downregulating inappropriate genes for each caste [19].

*Solenopsis invicta* fire ants have also served as important models for studying caste-biased genes. For example, *S. invicta* castes display key expression differences of G proteins and G-protein-coupled receptors in the brains,

which have important implications for many physiological processes [20]. By way of comparison, another recent study in ants focused on the canalization of caste-specific phenotypes and revealed the importance of juvenile hormone and ecdysone pathways; this study also suggested a newly championed gene, *Follicle relative* [gene-] expression in juvenile ants (*freja*) as a possible regulator of queen development [21]. Interestingly, the two taxa in this study, *M. pharaonis* and *Acromyrmex echinator*, displayed a high number of caste-biased and evolutionarily conserved canalized genes.

Queens and workers are the defining caste phenotypes in social insects. But ants sometimes also display worker subcastes. For example, workers may show age polyethism and initially undertake nursing activities but then transition to foraging behaviors as they age. Foraging has been tied to the expression of both *Inotocin* and *Inotocin* receptors in workers of the ant *Camponotus fellah* [22]. *Vg* genes have also been identified as key mediators of tasks in worker *Formica fusca* [23]. For example, *Vg-like-C* was consistently overexpressed in foragers, while *Vg-like-A* had a higher relative expression in nurse ants. Exploration of gene expression in brain tissue of nurses and foragers also revealed that foragers overexpressed oscillating genes relative to nurses and showed distinct circadian rhythms linked to patterns of gene expression [24]. Interestingly, some ant species, such as *Temnothorax rugatulus*, also produce distinct queen morphs (i.e. macrogynes and microgynes). Although the queen morphs are clearly distinct in size, they differentially express metabolic genes in the fat body and ultimately, produce the same number of eggs [25]. Thus, genes controlling metabolism are important candidates for better understanding caste determination in social insects.

Figure 3



Analysis of differential gene expression between castes. In this simulated [16] example, total RNA is extracted from queen (left) and worker (right) *Vespula maculifrons* wasp brain tissue. The transcriptomes are then sequenced, and statistical analyses are used to identify differentially expressed genes between castes. In the resulting volcano plot, genes that show highly negative or positive values of log fold change are more highly expressed in queens or workers, respectively. And genes that show high values of  $-\text{Log}_{10}$  P-values show more statistically significant differences in gene expression.

### Social bees

Bees represent one of the most well-studied groups of social insects [26]. Transcriptomic analyses have

identified several genes as key players in caste differentiation of bees. For example, Vg proteins are essential to egg production and thus are upregulated in queens [27]. Recent gene expression studies in the eusocial sweat bee, *Lasioglossum laevisimum*, have revealed that Vg is indeed more highly expressed in queens than workers, providing a molecular signature to caste differentiation [28]. Similar results emerged from transcriptomic analysis of tissue from *Apis mellifera* [29] and *Bombus terrestris* queens [30]. Notably, many studies have found that queen bees have highly distinct gene expression profiles compared with workers and drones [31].

Another candidate gene putatively involved in caste differentiation is *Foraging* (*For*). The *For* gene codes a cGMP-dependent protein kinase that drives the intensity of foraging behavior and is often associated with worker phenotype in insect societies [27]. Interestingly, *For* did not meet expression predictions among castes in sweat bees. Instead, *For* was upregulated only in workers during eclosion and early-spring foundresses, suggesting that the expression of *For* may stimulate a biochemical cascade to elicit future foraging behavior in sweat bees [32]. Worker bees may also be differentiated further into nursing and foraging subcastes. The sensory-related genes, *Wnt* and *FoxO*, were identified as influencing nursing versus foraging behavior in workers bees [33].

The larval stages of social insects represent an interesting point of development to study caste-biased gene expression. A recent study compared gene expression patterns of *A. mellifera* queen and worker larval gonads, and found thousands of differentially expressed genes from multiple different physiological pathways [34]. Another study in *A. mellifera* revealed unique gene upregulation in the wing buds of workers during the larval-pupal transition [35]. Additionally, the genetic basis of brain diphenism in developing female honeybee larvae was found to be largely influenced by feeding regimes. In response to a diet of royal jelly, future queen bees differentially expressed several genes, including *Takeout*, *Neurotrophin-1*, *Forked*, and *Masquerade* in brain tissues [36].

Isoforms of mRNA transcripts add another layer of complexity to the molecular control of phenotypic expression. He et al. found that honeybee workers and queens displayed thousands of differentially expressed RNA isoforms [37]. Similarly, thousands of genes in the bumblebee, *B. terrestris*, expressed multiple isoforms through differential splicing [38]. Differential gene expression and alternative splicing may be best

represented in prereproductive *A. mellifera* queens. *A. mellifera* gynes showed greater relative expression of genes involved in oxidative reduction, carbohydrate metabolism, and transport compared with workers or drones [39]. *Bombus ignitus* newly emerged queens displayed higher levels of dopamine and a highly expressed suite of nutrition-related genes compared with workers [40].

Some studies suggest chemosensing as an important caste-specific physiological process. In *A. mellifera*, two chemosensory genes, *OBP16* and *CSP4*, were identified as candidates for queen-rearing [41]. Another recent study examined gene expression differences in the antennae of *A. mellifera* nurses and foragers and identified several genes associated with their divergent behaviors [42]. Additionally, pheromone-related cytochrome P450 genes were differentially expressed in the mandibular glands of queen and worker honeybees [43].

Studies in incipiently social bees can provide great insight into the evolution of sociality. For example, transcriptomics in the facultatively social bee, *Ceratina australensis*, revealed important candidate genes that changed in expression in response to encounters with other individuals [44]. Genes involved in metabolism, chemical communication, and neurotransmission were particularly responsive to social interactions. Similarly, *A. mellifera* workers that underwent experimentally induced ovary activation experienced an increase in the expression of oxidoreductases and other stress-resistant genes, comparable to the natural expression in queens [45].

Much may be learned about the evolution of castes by comparing gene expression patterns across distantly related social insect taxa. For example, one investigation found that multiple taxa had correlated genetic modules and pheromone-sensitive genes, including those involved in lipid biosynthesis, olfaction, and oogenesis [46]. Similarly, a core group of reproductive genes was identified as of importance in both *M. phararonis* and *A. mellifera*, although a majority of caste-biased genes appear to be lineage-specific and evolving rapidly [47]. Future studies should focus on identifying key gene ‘toolboxes’ that are similarly responsive across taxa.

### Social wasps

Social wasps are interesting taxa to study from the perspective of social evolution, because they display such great variation in social behavior and caste diversity. However, relatively few RNA-Seq studies of caste differences have been conducted in social wasps. One recent study in *Polistes dominula* paper wasps highlighted how wasps adjust to environmental changes to maximize their fitness. After the loss of a queen, many workers exhibited queen-like phenotypes, including changes to

queen-like gene expression patterns [48]. Another transcriptomic study in *P. dominula* demonstrated the caste-biased expression of several genes involved in immunity, oxidative-reductive processes, carbohydrate and lipid metabolism, and visual stimuli responses [49]. Differentially expressed genes between castes in *P. dominula* appear to be frequently correlated with metabolism and are often worker-biased, indicating the breadth of roles workers take on in societies [50].

### Termites

Termites are distantly related from their hymenopteran social insect counterparts and therefore represent important candidates for expression analyses. Transcriptomic studies, reviewed by Maekawa et al. [51], have revealed caste-specific expression in termites of immune-related genes, including lysozyme [52,53] and chemoreceptor genes [54]. Soldiers, in particular, represent interesting castes to investigate and many genes display soldier-biased expression [43]. For example, increased expression of transforming growth factor beta (*TGFβ*) signaling-related genes played an important role in presoldier phenotypes [55]. Termite larvae that are destined to become soldiers also upregulate several nutritional-sensitive signaling pathways and lipocalin protein in the internal walls of the gut [56,57].

RNA sequencing has also recently revealed the importance of insulin signaling pathway genes that are upregulated in workers compared with king and queen termites [58,59]. Further, molting plays an important role in caste differentiation in termites. Analysis of gene expression profiles of termites at different stages of molting revealed highly differential expression of genes involved in cell proliferation and nutritional status between worker–worker and worker–presoldier transitions [60].

### Conclusions

Taken together, the new era in molecular biology has revolutionized studies of gene expression. Recent findings of candidate genes and pathways involved in caste differentiation in social insects represent an example of the importance of such studies. Although a ‘toolbox’ of genes found across social insect species may be too simplistic [61], there are several genes that often appear crucial to caste differentiation, including *Vitellogenin* and *For*. We predict that transcriptomic data, especially on nonmodel species, will continue to advance our understanding of caste evolution and social insect biology. Further, there are many important unanswered questions regarding phenotype-biased gene expression left unanswered (Box 1). These questions represent multiple avenues for future research in studies of social insects, in particular, and all eukaryotic species, in general. We

**Box 1** The development of technologies to accurately interrogate gene expression patterns at the genomic scale has opened up the possibility of answering a great many important questions related to gene regulation and phenotypic diversity. We outline some key unanswered questions in the study of gene expression that may lead to important insights into the function of biological systems.

- How do patterns of gene expression evolve over time?
- How important is alternative splicing of transcripts to generating phenotypic differences?
- How does epigenetic information affect gene expression and phenotypic variation?
- What regulatory and gene co-expression networks are involved in producing phenotypic differences?
- How does genetic conflict affect the evolution of phenotypes and phenotype-biased expression?
- What is the effect of phenotype-biased expression on gene evolution?
- What genes act as master regulators of phenotype-biased expression?
- What *cis*-regulatory changes have accompanied the evolution of novel phenotypes?
- How has gene duplication affected phenotype-biased gene expression?
- How much is phenotype-biased expression influenced by natural selection versus genetic drift?
- Which phenotype-biased genes actually affect phenotypic differences?

therefore look forward to a bright future where patterns of gene expression will increasingly be used to probe the molecular basis of phenotypic diversity in all biological systems.

## Data Availability

No new data were generated for the research described in the article.

## Declaration of Competing Interest

Nothing declared.

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