Brain Gene Expression of Foraging Behavior and Social Environment in *Ceratina calcarata*

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Abstract

Rudimentary social systems have the potential to both advance our understanding of how complex sociality may have evolved and our understanding of how changes in social environment may influence gene expression and cooperation. Recently, studies of primitively social Hymenoptera have greatly expanded empirical evidence for the role of social environment in shaping behavior and gene expression. Here, we compare brain gene expression profiles of foragers across social contexts in the small carpenter bee, *Ceratina calcarata*. We conducted experimental manipulations of field colonies to examine gene expression profiles among social contexts including foraging mothers, regular daughters, and worker-like dwarf eldest daughters in the presence and absence of mother. Our analysis found significant differences in gene expression associated with female age, reproductive status, and social environment, including circadian clock gene *dyw*, hexamerin, and genes involved in the regulation of juvenile hormone and chemical communication. We also found that candidate genes differentially expressed in our study were also associated with division of labor, including foraging, in other primitively and advanced eusocial insects. Our results offer evidence for the role of the regulation of key developmental hormones and circadian rhythms in producing cooperative behavior in rudimentary insect societies.

Key words: transcriptomics, social environment, foraging behavior, cooperation, behavioral genetics, social evolution.

Significance

Division of foraging and reproductive labor is a hallmark of eusociality. Much is known about obligately eusocial species, but little is known about facultatively and rudimentary social insects. Here, we characterize brain gene expression in a facultatively social bee examining reproductive division of labor among female foraging classes. We not only identify conserved genes for reproductive division of labor and foraging behavior across Hymenoptera but also highlight the role of circadian rhythms underlying nonreproductive foraging physiology.

Introduction

Sociogenomic study of animal societies is critical to understanding the evolution of social behavior (Robinson et al. 2005; Rehan and Toth 2015; Shell and Rehan 2018a). Studies of gene expression in complex insect societies have provided insights into how changes in expression of conserved genes are associated with cooperative task specialization (Ament et al. 2012; Berens et al. 2015; Khamis et al. 2015; Morandin et al. 2016; Sun et al. 2019). These studies suggest that multiple phenotypes involved in task specialization share genes that are coopted for novel social functions (Toth and Robinson 2007; Rittschof and Robinson 2016). Within Hymenoptera, cooperative roles are not only linked to the expression of key candidate genes (Toma et al. 2000; Ben-Shahar 2003; Ingram et al. 2016) but are also associated with genes coexpressed in modules (Chandrasekaran et al. 2011; Khamis et al. 2015). The expression of genes associated with particular cooperative

roles and behavioral states is also sensitive to changes in the social environment (Arsenault et al. 2018; Manfredini et al. 2021). Although these studies of complex societies suggest the importance of social environment to gene expression and associated behaviors, study of simple animal societies, lacking castes, is critical to understanding the evolution of insect social behavior in its rudimentary forms.

The diversity of social behaviors displayed by Hymenoptera has made them valuable models for study of the molecular mechanisms underlying social traits (Michener 1974; Robinson et al. 2005; Schwarz et al. 2007). Social behavior in Hymenoptera ranges from solitary to obligately eusocial species (Michener 1974). The role of social environment in producing cooperation in facultatively social species is critical to understanding how cooperation may have evolved, as these species lack preimaginal determination of caste roles (Michener 1974; Strassmann 1981; Kukuk and May 1991). Facultatively social species are capable of solitary and social organization and have begun to advance our understanding of the proximate causes of cooperation (Rehan and Toth 2015; Kronauer and Libbrecht 2018; Shell and Rehan 2018a). Transcriptomic studies of facultatively social bees and wasps have revealed significant differences in gene expression between individuals exhibiting different behavioral states, suggesting highly distinct genetic regulation of social traits preceding the evolution of obligate eusociality (*Polistes canadensis*, Ferreira et al. 2013; *Megalopta genalis*, Jones et al. 2017; *Ceratina australensis*, Rehan et al. 2018).

Understanding differential expression of genes associated with the social environment of parental care, ancestral to many eusocial lineages, and cooperation, including sibling care among rudimentary social groups, is critical to understanding the evolution of sociality at its onset (Alexander 1974; West-Eberhard 1996). Research in the field of evolutionary development (evo-devo), seeking to understand the molecular basis of the diversity of complex forms and behaviors on which evolution acts, has been highly informative for understanding the proximate causes of social behavior (Toth and Robinson 2007; Toth and Rehan 2017). From an evo-devo perspective, the close association of parents and offspring presents an opportunity for the production of phenotypic plasticity through parental control of development, nutrition, and social environment. The social environment produced by mothers is particularly influential on offspring behavior (Michener 1974; Kukuk and May 1991; Hogendoorn and Velthuis 1999; Kapheim et al. 2011). For instance, maternal manipulation of offspring nutrition has been found to be a means of eliciting cooperation in facultatively social Hymenoptera (Lawson et al. 2017; Kapheim et al. 2016).

An intensive study of well-described molecular pathways, particularly in *Apis mellifera*, has suggested several candidate genes and gene networks associated with key differences in social behavior (Fitzpatrick et al. 2005; Robinson et al. 2005; Toth and Robinson 2005; Smith et al. 2008). While representing a rich resource for understanding the molecular basis of behavior, these studies are less informative of the proximate causes of social group formation than simple social systems (Rehan and Toth 2015; Shell and Rehan 2018a). However, regulation of key genes related to reproduction and development, including the production vitellogenin, ecdysteroids, and juvenile hormone (JH), has also been associated with changes in social environment and cooperative behaviors in simple social systems (Daugherty et al. 2011; Tibbetts and Sheehan 2012; Bloch, Borst, et al. 2000; Bloch, Hefetz, and Hartfelder 2000). Genes involved in the regulation of circadian rhythms, such as *period* and *Shaggy* have also been documented as influential in foraging and intranidal behaviors (Toma et al. 2000; Bloch et al. 2001).

Previous transcriptomic studies have revealed a broad range of genes and gene regulator networks involved in reproduction, development, and circadian rhythms (Khamis et al. 2015; Saleh and Ramírez 2019; Das and de Bekker 2022). Studies of facultatively social species have documented major differences in gene expression among social roles, including reproductive status and cooperative foraging behaviors (Jones et al. 2017; Rehan et al. 2018; Saleh and Ramírez 2019). These studies support the hypothesis that genes associated with solitary behaviors may be coopted and differentially expressed among the specialized roles in social systems including foraging, nest care, and reproductive roles. Recurrent candidate genes associated with foraging and reproductive roles include insulin-like peptides and hexamerins, respectively (Jones et al. 2017; Rehan et al. 2018; Saleh and Ramírez 2019).

The small carpenter bee, *Ceratina calcarata*, displays cooperative social behaviors, as mothers frequently produce a dwarf eldest daughter who engages in sibling care (Rehan and Richards 2010). This species of bee emerges from overwintering to mate in the spring, producing a single brood in its lifetime and thus possesses a univoltine life cycle (Rehan and Richards 2010). The dwarf eldest daughter is the first of the brood to emerge in the late summer and forages to feed her adult siblings who remain within the nest (Rehan et al. 2014). Increased foraging behavior of dwarf eldest daughters in orphaned nests of *C. calcarata* is well established (Mikát et al. 2017) and presents an opportunity to investigate the proximate mechanisms of cooperation, by comparing brain gene expression among foraging females across social contexts. Dwarf eldest daughters are thus thought to act as insurance against loss of mothers, providing a second round of feeding required for overwinter survival of adult offspring until the spring dispersal period (Mikát et al. 2017; Shell and Rehan 2018b). These dwarf eldest daughters never take on a reproductive role, as they rarely, if ever, survive to the following spring (Rehan

and Richards 2010). The smaller size of the worker-like dwarf eldest daughters indicates that maternal manipulation through nutritional deprivation is critical in inducing cooperative behavior (Lawson et al. 2017, 2016). While the smaller size of dwarf eldest daughters is determined by larval provisioning, the proximate mechanism of increased cooperative behavior of dwarf eldest daughters in the absence of mothers is not well known (Alexander 1974; Michener and Brothers 1974; Lawson et al. 2017). Maternal presence in *C. calcarata* nests has been shown to have significant effects on both offspring behavior and brain gene expression (Rehan and Richards 2013; Arsenault et al. 2018). Social experience has also been well documented with both dominant and subordinate status predicted by former interactions, and each has signature brain gene expression patterns in this species (Withee and Rehan 2017). Critically, division of labor in *C. calcarata* is associated with distinct gene regulatory modules and highly conserved genes associated with caste differentiation in eusocial insects (Shell and Rehan 2019).

Here, we examine the effects of social environment on brain gene expression in *C. calcarata* through removal of nest mothers and dwarf eldest daughters to elicit foraging behavior across female classes. This study has two aims: first, to determine the influence of social environment on brain gene expression. Here, we compared foraging mothers and regular daughters to dwarf eldest daughters in the presence and absence of mothers to identify differences in gene expression between reproductive and nonreproductive classes. Second, we identify conserved genes underlying reproductive physiology and foraging behavior across three independent origins of eusociality in bees. Taken together, these results reveal the role of social environment in eliciting division of labor and determine the underlying genes and regulatory pathways regulating foraging and reproductive roles.

Results

Brain Gene Expression and GO Analyses

Comparison of gene expression among all four groups (mothers, dwarf eldest daughters in the presence and absence of mothers, and regular daughters) resulted in 1,094 differentially expressed genes (DEGs) in total (false discovery rate < 0.05; supplementary table S2, Supplementary Material online). Multivariate analysis accounted for 74% of variation in the data across four principle components (PCs; fig. 1). Regular daughters were strongly differentiated by PC1 (38%), while dwarf eldest daughters with mothers removed were most unique across PC2 (16%). Dwarf eldest daughters in the presence of mothers were most unique in PC3 (12%). Mothers and regular daughters group closely after PC1 (fig. 1). Dwarf

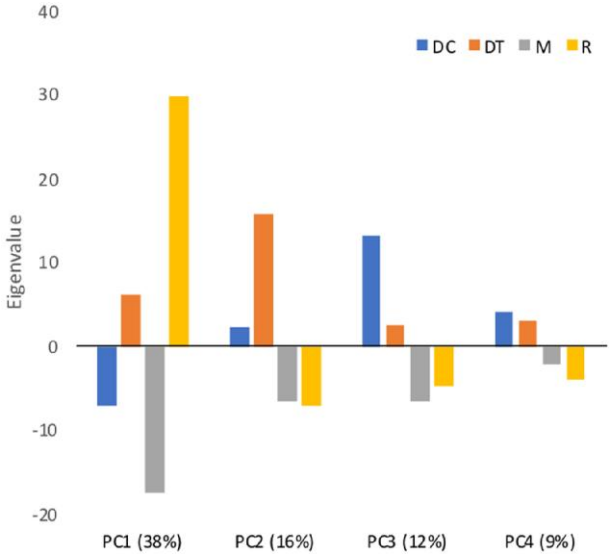

Fig. 1.—PCA of gene expression in foraging females of each behavioral class, across the first four PCs (PC1–PC4). Values in brackets represent percentage of total variation explained by each PC. DC, control dwarf eldest daughters with mother present; DT, dwarf eldest daughters with mother removed; M, mothers; R, regular daughters.

eldest daughters in both the presence and absence of mothers shared similar eigenvalues versus mothers and regular daughters in PC4 (9%) (fig. 1). Interestingly, mothers and dwarf eldest daughters in the presence of mothers group most closely in PC1 whereas mothers and regular daughters group most closely across PCs 3 and 4 (fig. 1). Dwarf eldest daughter in both the presence and absence of mothers group most closely across PCs 3 and 4 (fig. 1).

Across all DEGs, a subset was uniquely expressed in each of the four categories of foraging females (fig. 2). Regular daughters showed the highest number of unique DEGs, followed by mothers, dwarf eldest daughters with mothers removed, and dwarf eldest daughters in the presence of mother (fig. 2). Regular daughters and dwarf eldest daughters with mothers removed showed the greatest overlap in DEGs followed by mothers and regular daughters (fig. 2). Dwarf eldest daughters in the presence of mothers were distinguished by 244 DEGs compared with all other groups (supplementary table S2, Supplementary Material online). Overall, dwarf eldest daughters in the presence of mothers were enriched for gene ontology (GO) terms (supplementary table S3, Supplementary Material online) including RNA-dependent DNA biosynthetic process (GO: 0006278) and DNA metabolic process (GO: 0006259; fig. 2). Weighted gene coexpression network analysis (WGCNA) coexpression analyses associated dwarf eldest daughters in the presence of mothers with a single module underrepresented for circadian sleep/wake cycle (GO: 0042745; supplementary table S4, Supplementary

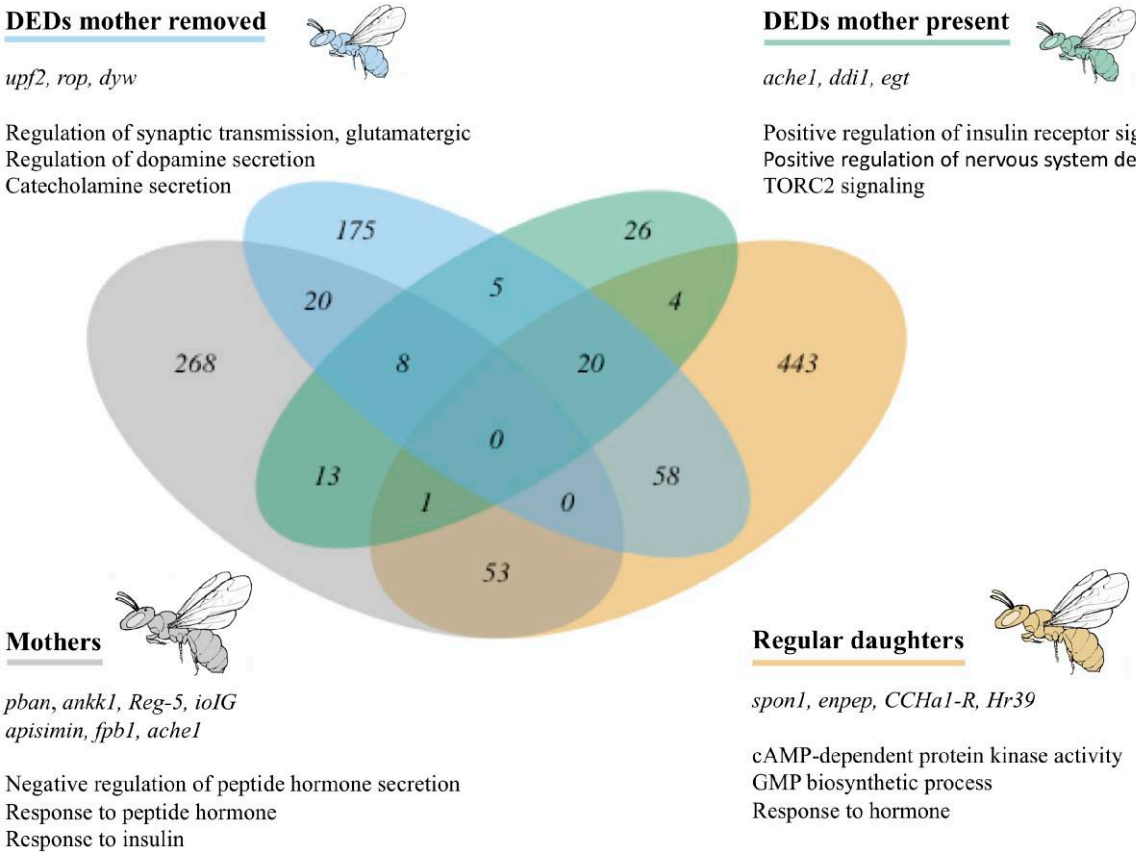

Fig. 2.—Counts of DEGs across each female behavioral category, with associated annotated genes (italicized), followed by enriched GO terms. Full list of DEGs and GO terms is available in supplementary table S3, Supplementary Material online. Illustration by Jesse Huisken.

Material online). This included hub gene Poly(U)-specific endoribonuclease homolog (*ENDOU*) (supplementary table S5, Supplementary Material online). Comparison of dwarf eldest daughters with mothers present and dwarf eldest daughters absent showed the least differential expression, with only seven genes differentially expressed. These genes were as follows: L trypsin-1 (*tryp1*), retrovirus-related polyprotein from transposon 17.6 (*pol*) and ecdysteroid UDP-glucosyltransferase (*egt*), and four proteins of unknown function (fig. 3 and supplementary table S2, Supplementary Material online). These genes were upregulated in dwarf eldest daughters with mothers present, with the exception of *egt*, which was downregulated in the absence of mothers (fig. 3).

Dwarf eldest daughters with mother removed were distinguished by 584 DEGs compared with other groups, including upregulated rhythmically expressed gene 5 (*Reg-5*), circadian clock-controlled protein daywake (*dyw*), and *egt* (figs. 1 and 2 and supplementary table S2, Supplementary Material online). Dwarf eldest daughters with mother removed had GO enrichment for the largest number of terms, including methylation (GO: 0032259), DNA integration (GO: 0015074), generation of precursor metabolites and energy (GO: 0006091), and ATP biosynthetic processes (GO: 0006754; supplementary table S3, Supplementary Material online). Dwarf eldest daughters in the absence of mothers were also associated with a larger number of gene coexpression modules (supplementary table S4, Supplementary Material online). These include positive association with the modules enriched for response to light stimulus (GO: 0009416) and response to external stimulus (GO: 0009605; table 1 and supplementary table S4, Supplementary Material online), including the hub genes phospholipase DDHD1 (*DDH1*) and retrotransposable element R1DM (*R1A1-element \ORF2*) (supplementary table S6, Supplementary Material online).

Mothers were distinguished by 874 DEGs compared with other groups (supplementary table S2, Supplementary Material online). Upregulated genes included royal jelly protein apisimin and AMP phosphotransferase AK3 (AK3) (fig. 2). DEGs in mothers were enriched for GO terms including transmembrane transport (GO: 0055085), establishment of localization (GO: 0051234), cation transport (GO: 0006812), carboxylic acid metabolic process (GO: 0019752), and oxoacid metabolic processes (GO: 0043436; supplementary table S3,

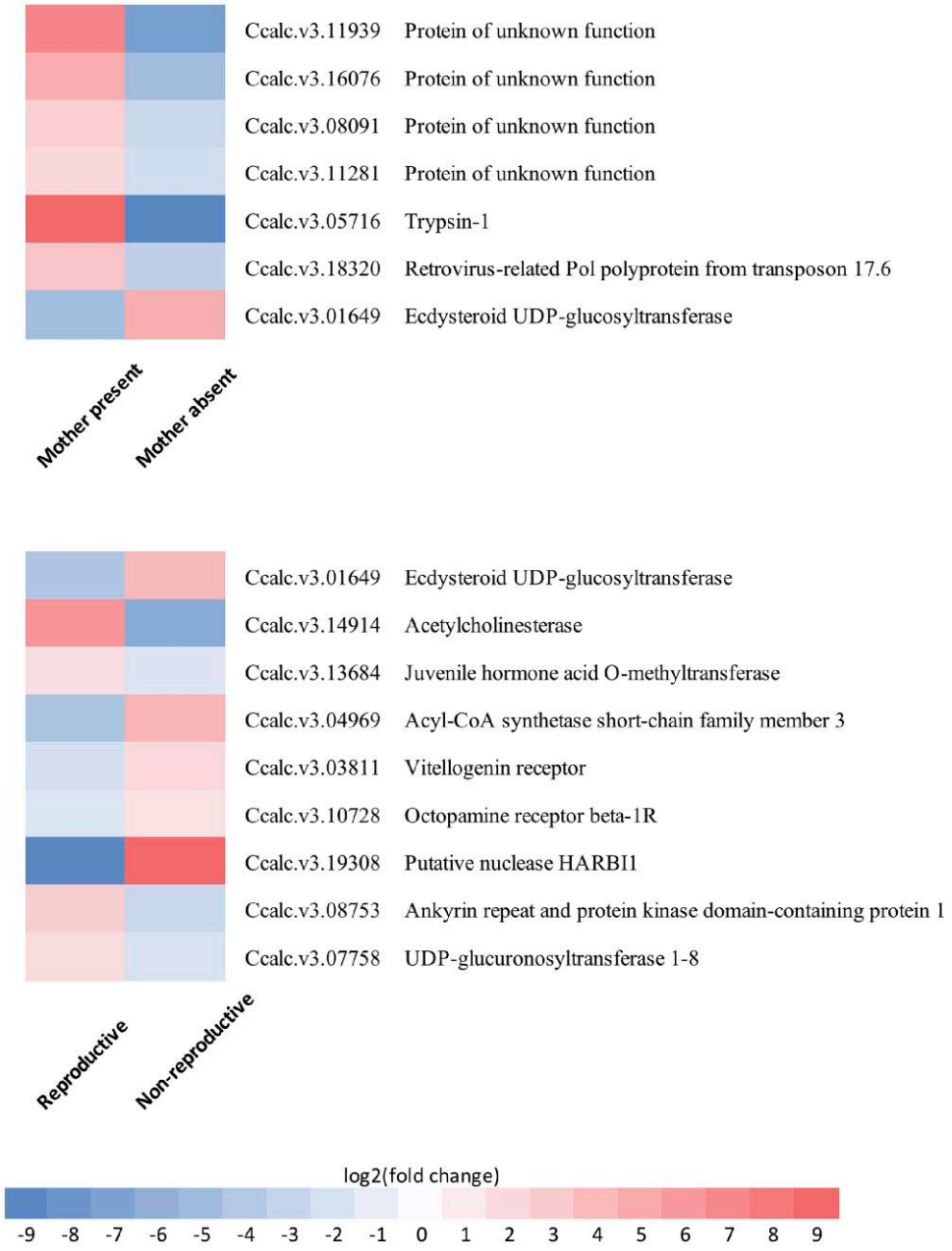

Fig. 3.—Candidate DEGs with annotations for social environment, dwarf eldest daughters with mother present/absent, and for reproductive status, reproductive (regular daughters and mothers) and nonreproductive (dwarf eldest daughters). Full list of DEGs found in supplementary table S1, Supplementary Material online.

Supplementary Material online). Mothers were associated with four gene coexpression modules enriched for response to carbohydrate (GO: 0009743; table 1 and supplementary table S5, Supplementary Material online) and with hub genes smg8 (*smg-8*) and cyclin G-associated kinase (*gak*) (supplementary tables S6, Supplementary Material online).

Regular daughters were distinguished by 941 DEGs relative to other groups, the most dissimilar to other female foraging classes (supplementary table S2, Supplementary Material online). Upregulated DEGs included harbinger transposase derived (*harbi1*) and cell surface glycoprotein 1 (*olpB*) (supplementary table S2, Supplementary Material online). Regular daughter DEGs were enriched for cellular protein metabolic process (GO: 0044267) and carbohydrate metabolic processes (GO: 0005975), as well as macromolecule modification (GO: 0043412; supplementary table S3, Supplementary Material online). Regular daughters were associated with the most gene

coexpression modules (5), including hub genes trafficking protein particle complex subunit 13 (*trappc13*), zinc finger BED domain-containing protein 4 (*zbed4*), and neurofibromin (*nf1*) (supplementary tables S4 and S6, Supplementary Material online). These modules were enriched for sensory perception of smell and taste (GO: 0007608; GO: 0050909) and peptide pheromone export (GO: 0000770; table 1 and supplementary table S7, Supplementary Material online).

Table 1
Gene Coexpression Modules

Comparison	Correlation of Module	GO Terms
Mother removed	Positive	Response to external stimulus (GO: 0009605) Response to light stimulus (GO: 0009416)
Mother present	Negative	Circadian sleep/wake cycle (GO: 0042745)
Reproductive	Positive	Ecdysteroid secretion (GO: 0045457) Response to peptide hormone (GO: 0043434) Sensory perception of taste (GO: 0050909) Response to nutrient levels (GO: 0031667) cGMP metabolic process (GO: 0046068)
Non-reproductive	Positive	Negative regulation of peptide hormone secretion (GO: 0090278)
	Negative	Mating behavior, sex discrimination (GO: 0048047)

Note.—Modules significantly correlated with reproductive status and social environment with selected GO enrichment terms. Full list of genes and GO terms is available in supplementary table S5, Supplementary Material online.

Comparison between reproductive bees, mothers and regular daughters, with nonreproductive dwarf eldest daughters, revealed 394 DEGs (supplementary table S2, Supplementary Material online). Genes highly expressed in reproductive bees included JH acid O-methyltransferase (*jhamt*), acetylcholinesterase (*ache1*), and ankyrin repeat and protein kinase domain containing 1 (*ankk1*) (fig. 3 and supplementary table S2, Supplementary Material online). Those upregulated in nonreproductive dwarf eldest daughters included *egt* and octopamine receptor beta-1 R (*Octbeta1R*) (fig. 3 and supplementary table S2, Supplementary Material online).

Cross Species Analysis

Comparisons with transcriptomic studies across four bee species found overlapping expression patterns and candidate genes (table 2 and supplementary table S9, Supplementary Material online). Genes upregulated in *C. calcarata* mothers including insulin-like peptide (*ilp*) and PBAN-type neuropeptides (*pban*) were similarly upregulated in predispersal and social primaries in *C. australensis*, reproductive dominant *Euglossa dilemma*, and nurses and foragers of *A. mellifera* (table 2 and supplementary table S10, Supplementary Material online). In contrast, upregulated genes shared among *C. calcarata* reproductive mothers and regular daughters overlapped primarily with

Table 2
Selected Annotated Genes

Group	Gene ID	Comparative Gene Expression	Species			
			Ceratina australensis	*Megalopta genalis*	*Euglossa dilemma*	*Apis mellifera*
Reproductive	Ccalc.v3.02948	UVOP: Opsin				
	Ccalc.v3.04673	Krueppel-like factor 10				
	Ccalc.v3.11259	Insulin-like peptide				
	Ccalc.v3.08753	Ankyrin repeat and protein kinase domain-containing 1				
	Ccalc.v3.02213	Nuclear hormone receptor FTZ-F1 beta				
	Ccalc.v3.02206	Innexin				
	Ccalc.v3.07881	Neuroblastoma suppressor of tumorigenicity 1				
	Ccalc.v3.12614	Patched domain-containing protein 3				
	Ccalc.v3.14412	Glucose dehydrogenase				
Nonreproductive	Ccalc.v3.03234	Purine nucleoside phosphorylase				
	Ccalc.v3.03136	G-protein coupled receptor moody				
	Ccalc.v3.00470	Transcription factor hamlet				

Note.—Putative orthologs (>65% identity and $<1 \times 10^{-15}$ e-value) from the four groups examined in *C. calcarata* from this study, with differential expression patterns in relevant groups in considered studies. Solid blue indicates the presence of a putative ortholog found differentially expressed in species in the corresponding column. For full list, see supplementary table S10, Supplementary Material online.

genes upregulated in nonreproductive social secondaries of *C. australensis* and foragers of *A. mellifera*, including spondin 1 (*spon1*) and transcription factor hamlet (*hamlet*) (table 2 and supplementary table S8, Supplementary Material online). Upregulated genes of dwarf eldest daughters with mothers present included *ankk1* (table 2), which were also upregulated in social secondaries of *C. australensis* (table 2 and supplementary table S8, Supplementary Material online). Upregulated genes in foraging dwarf eldest daughters including insulin-like growth factor binding protein acid labile subunit (*igfal*) and *moody* (table 2) were also upregulated in both predispersal and social secondaries of *C. australensis*. Among cross species comparisons, we found significant overlap in DEGs found in this study of *C. calcarata* and those reported in former studies of foraging behavior in *C. australensis* (hypergeometric means test, $P < 0.0001$; supplementary table S10, Supplementary Material online) but not with the remaining three more distantly related taxa (supplementary table S9, Supplementary Material online).

Comparison of gene expression patterns of prereproductive regular daughters versus nonreproductive dwarf eldest daughters using rank–rank hypergeometric overlap (RRHO) found significant overlap with homologs found in studies of all three species compared (fig. 4 and supplementary table S11, Supplementary Material online). In particular, *C. calcarata* regular daughters showed the strongest overlap with reproductive dominants of *E. dilemma*, whereas dwarf eldest daughters tended to overlap with *M. genalis* workers and *C. australensis* nonreproductive secondaries (fig. 4). We found 956 significantly overlapping upregulated genes with *E. dilemma* orthologs, including period circadian protein (*per*), insulin-like receptor (*InR*), and ecdysone receptor (*EcR*) (supplementary table S11, Supplementary Material online). Between *C. calcarata* and *C. australensis*, we found seven significantly overlapping upregulated genes, consisting of two unknown proteins and five genes of known function, including hymenoptaecin and defensin-1 (*Def-1*) (supplementary table S11, Supplementary Material online). Seven upregulated genes significantly overlapped with *M. genalis* gene expression included three proteins of unknown function and four genes of known function: two histone-coding genes for histone H1.3, and H2B.3, as well as alpha amylase (*amy-p*) and chymotrypsin-like elastase family member 2A (*cela2a*) (supplementary table S11, Supplementary Material online).

Discussion

Through experimental manipulation of social environment, this study revealed that females engaging in foraging behavior have significant differences in gene expression attributable to both social environment and reproductive role. Postreproductive and prereproductive bees, mothers and regular daughters respectively, had the largest compliment of DEGs, and nonreproductive dwarf eldest daughters showed the lowest compliment of DEGs. Our study found differential expression of multiple genes related to division of labor in obligately eusocial species. This included myo-inositol (*ioG*), hexamerin, *ilp*, and a putative gene coding for a royal jelly protein, apisimin, all involved in queen versus worker differentiation in *A. mellifera* (Evans and Wheeler 1999; Bíliková et al. 2002; Wheeler et al. 2006; Begna et al. 2011; Wang et al. 2013). Similarities in patterns of gene expression were found not only between reproductive and worker relationships but also among particular worker roles. These included upregulation of *ache1* in control dwarf eldest daughters, associated with the transition from nursing to foraging in *A. mellifera* (Shapira et al. 2001), and PBAN-type neuropeptides (*pban*) upregulation in mothers, coding for a pheromone regulating neuropeptide associated with increased foraging in *A. mellifera* (Brockmann et al. 2009). Candidate genes associated with division of labor tended to be expressed in *C. calcarata* mothers, including expression of genes related to foraging castes in *A. mellifera* (Shapira et al. 2001; Brockmann et al. 2009). *Ceratina calcarata* mothers also showed upregulation of *jhamt*, involved in JH synthesis. JH is of particular interest as JH titers are known to vary with social hierarchy and reproductive dominance in many facultatively and obligately social Hymenoptera (Robinson et al. 1992; Tibbetts and Huang 2010; Tibbetts et al. 2011). Hexamerin, signaling potential reproductive dominance in *E. dilemma*, was found to be most upregulated in both groups of dwarf eldest daughters, relative to both mothers and regular daughters (Saleh and Ramírez 2019). Hexamerin thus appears to have a function similar to that in advanced eusocial species, where it is associated with reduced JH efficacy producing worker castes (*Solenopsis invicta*, Zhou et al. 2007; *Reticulitermes flavipes*, Hawkings et al. 2019). In our study, foraging postreproductive mothers also showed greater expression of Krueppel-like factor 10 (*klf10*), a family of transcription factors associated with delay of transition from nurse to worker in *A. mellifera* by queen mandibular pheromone (Grozinger et al. 2003). Though this pheromone has not been found outside of *Apis* bees to date (Le Conte and Hefetz 2008), higher levels genes coding for Krueppel homologs are associated with reproductive status or reproductive dominance in many solitary and some facultatively social bees (Shpigler et al. 2014). Rhythmic expression of Krueppel-like factors has also been for to synchronize circadian rhythms with metabolism in mice (Ruberto et al. 2021).

Regular daughters were the most distinct from other groups and upregulated DEGs related to reproduction, feeding, olfaction, protein, and carbohydrate metabolism.

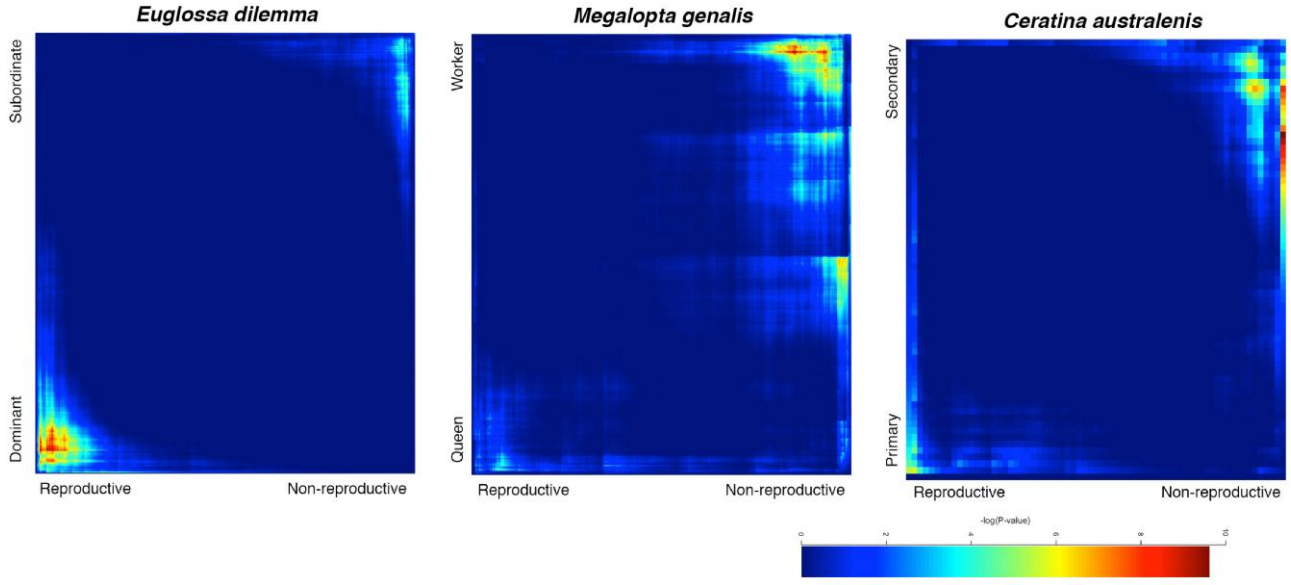

Fig. 4.—Heat map of RRHO visualizing correlations between gene expression profiles of *C. calcarata* reproductive (regular daughters) and nonreproductive (dwarf eldest daughters), on the *x*-axis, compared with reproductive and nonreproductive phenotypes in three species, on the *y*-axis. Scale represents relative scale of Log_{10} transformed values from RRHO analysis.

This is in keeping with their preparation for overwintering diapause and future reproduction, as well as their foraging for self-feeding, in contrast to the altruistic provisioning of dwarf eldest daughters and mothers (Rehan et al. 2014). Regular foraging daughters were upregulated for *GnRH-R* and *Hr39*, genes involved in neuropeptide and hormone signaling, including JH and ecdysteroid (Loof et al. 2001). Upregulated DEGs also included those related to olfaction and hunger, neuropeptide receptor *CCHa1-R*, signaling between brain and gut and olfaction during starvation in *Drosophila melanogaster* (Farhan et al. 2013; Li et al. 2013). Previous studies of *C. calcarata* have suggested a possible role for chemical communication in sibling and maternal care involving *obp* biosynthesis (Rehan et al. 2014), as well as cuticular hydrocarbons in division of labor (Pizzi and Rehan 2021). This study reveals that genes associated with foraging in regular daughters are distinct from those of mothers. This is corroborated by regular daughters having a greater number of significantly coexpressed modules, including those related sensory perception of taste, ecdysteroid, and peptide hormone.

Dwarf eldest daughters in the absence of mothers showed unique upregulation of JH-binding circadian rhythm gene *dyw*, known to be regulated by the cyclic expression of the *period* (*per*) gene in *D. melanogaster* (Van Gelder and Krasnow 1996). The regulation of circadian rhythms by the *per* gene network is highly conserved, also found in mammals including humans (Panda et al. 2002). This gene regulatory network is also known to play a role in sociality, regulating division of labor in *A. mellifera*, with differential expression of *per* being associated with differences between synchronous foragers, and asynchronous nurses, who remain in nests (Bloch et al. 2001). In association with *per*, which primarily responds to photoperiod, cycles of JH-binding *dyw* expression respond to changes in temperature through the sensory system, suppressing siesta behavior in *D. melanogaster* (Prestwich et al. 1996; George and Stanewsky 2021). The importance of circadian rhythms and response to light and temperature in dwarf eldest daughters in the absence of mothers is further supported by their association with enriched GO terms related to response to light and external stimulus (table 1). The upregulation of these genes suggests that foraging of dwarf eldest daughters in the absence of mothers may be related to changes in the regulation of circadian rhythms and response to temperature. Accordingly, dwarf eldest daughters in the presence of mothers were negatively associated with a module enriched for circadian rhythms (table 1). The association of *dyw* with dwarf eldest daughters in the absence of mothers and downregulation of a gene expression module related to circadian rhythms with regular daughters suggests that differences in gene regulation may underlie differences in foraging patterns in these two groups. Future studies are needed to determine if foraging of dwarf eldest daughters in the presence of mothers and of regular daughters, as compared with the frequent foraging trips of dwarf eldest daughters in orphaned nests, may be asynchronous and more related to self-feeding, rather than periodic worker-like sibling provisioning.

Interestingly, dwarf eldest daughters in the presence/absence of mothers were distinguished from one another by only seven genes, including the downregulation of a single gene, *egt*, a suppressor of ecdysteroids, in dwarf eldest daughters in the absence of mothers (Ahn et al. 2012). Though not as well studied as JH, as key components of the insect endocrine system, ecdysteroids have been the subject of investigation for their potential role in division of labor in eusocial insects, with results varying drastically by species (Robinson et al. 1991; Röseler et al. 1985; Hartfelder et al. 2002; Bloch, Hefetz, and Hartfelder 2000). In honey bees, *A. mellifera,* ecdysteroid titers are detectable only in egg-laying workers, thus likely playing some role in reproduction, but not division of labor or castes per se (Robinson et al. 1991). Ecdysteroid levels also appear to be unresponsive to changes in social environment in some eusocial insects, including *A. mellifera* and stingless bee *Melipona quadrifasciata*, where it remains unaltered by queen removal (Hartfelder et al. 2002). Within the primitively eusocial paper wasp *Polistes gallicus*, ecdysteroid is associated with fertility but not social role (Röseler et al. 1985). In contrast, our results are more consistent with those found in *Bombus terrestris*, where ecdysteroid not only plays a role in reproduction, with higher titers being found in queens, but also responds to social environment and status, with titers gradually increasing in workers after queen removals and being associated with dominance (Bloch, Hefetz, and Hartfelder 2000). Thus, the role of *egt* in the suppression of ecdysteroids and ecdysteroid suppression in general are important topics for further study in simple social systems.

Cross Species Conserved Genes for Foraging, Chemical Communication, and Circadian Rhythms

Comparative analysis revealed *C. calcarata* reproductive, and foraging classes shared candidate genes and expression patterns across other social insects. Interestingly, many DEGs upregulated in mothers and regular daughters are found to play a critical role in foraging behavior and caste differentiation in eusocial honey bees, *A. mellifera*, and the ants, *S. invicta* and *Formica exsecta* (Brockmann et al. 2009; Choi and Meer 2012). Pheromone-induced neuropeptide (*pban*) was upregulated in *C. calcarata* mothers and is not only associated with increased foraging in *A. mellifera* (Brockmann et al. 2009) but also with the synthesis of ant trail pheromones in *S. invicta* (Choi and Meer 2012). This suggests a possible role for chemical communication in division of labor in *C. calcarata* foraging behavior. This is corroborated by gene coexpression modules enriched for GO terms related to peptide pheromone export (GO: 0000770; table 1). Regular daughters also showed upregulation of *GnRH-R* and *Hr39* involved in neuropeptide and hormone signaling, including JH and ecdysteroid, and *A. mellifera* reproductive status and behavior-associated gene networks (Loof et al. 2001).

Unexpectedly, both dwarf eldest daughters, regardless of social context, expressed fewer behaviorally characterized DEG orthologs in comparison with the existing eusocial insect literature than mothers or regular daughters (table 2). Foraging dwarf eldest daughters in the presence of mother showed upregulation of *ache1*, also upregulated in foraging *C. calcarata* mothers, which is similarly associated with the transition from nursing to foraging in *A. mellifera* (Shapira et al. 2001). The foraging behavior of dwarf eldest daughters thus appears to be associated not only with genes known to play a conserved role in eusocial lineages but also with highly conserved circadian rhythms known from a diversity of species, including both insects and mammals (Van Gelder and Krasnow 1996; Tei et al. 1997; Bloch et al. 2001).

Conclusion

Polyphenism in foraging behavior represents a distinct opportunity to characterize the influence of social environment on division of labor and the gene regulatory networks underpinning social traits. Our results suggest that foraging behavior can be experimentally induced in any female in this facultatively social bee, but that distinct patterns of gene expression underlie this trait depending on reproductive role and social environment. We further found that genes related to foraging may be similarly associated with those related to foraging and reproductive roles in eusocial species, including obligately eusocial Hymenoptera, but many are broadly conserved across insects and even mammals. We found evidence for regulation of circadian rhythms in nonreproductive foraging and a possible role for chemical communication between mothers and offspring. Differential gene expression underlying key developmental hormones, including JH and ecdysteroid, is evidenced between reproductive and nonreproductive *C. calcarata*. These results highlight the role of social environment on behavior and associated gene expression in facultatively social systems. Regulatory networks underlying circadian rhythms, sensory response, and appetite are identified here as strong candidate genes for future functional validation and cross species comparisons to differentiate social roles in simple social systems.

Materials and Methods

Sample Collection and Foraging Data

In April of 2020, artificial nests of cut raspberry branches (*Rubus idaeus*) mounted to bamboo stakes with zip ties were deployed at two sites: 1) the Langstaff Eco Park (43° 48′21.9″N 79°29′03.1″W) and 2) Tommy Thompson Park (43°39′02.2″N 79°19′16.6″W) in Toronto, Canada.

Foraging behavior was observed across the season to compare control and removal nests. Four plots of 30 nests were established at the two sites ($n = 240$). Beginning June 14, sites were attended sequentially from 8 AM to 4 PM. Nests were monitored once a week at each site, until August when sites were attended more frequently, using all days with suitable weather (>15 °C with clear skies, no rain, or overcast cloud cover). Beginning August 13, treatments were applied by collecting marked and identified bees in the field sequentially as they emerged to forage. Foraging bees were captured by securing clear plastic cups over openings of occupied nests before 8 AM. Captured bees were anesthetized on ice and marked on the thorax with an enamel paint pen, identified to sex, head width was measured to identify dwarf eldest daughters, and their wing wear was scored to determine relative age of mothers versus daughters (mother wing wear ≥ 2 and newly emerged daughters <2 during late summer; Rehan and Richards 2010). Adult females were captured from their nests while foraging for flash freezing in liquid nitrogen and brought back to the lab for subsequent analysis. All nests were collected and dissected and any remaining nest occupants recorded and preserved in 100% EtOH for other research in the Rehan Lab at York University.

RNA Extraction and Differential Gene Expression

RNA was extracted from brains of individual bees using the QIAGEN RNeasy Kit and standard protocol. A total of five mothers, three foraging dwarf eldest daughters from control nests, three from removal nests, and three foraging regular daughters (a total of 14) were sent to Genome Québec for library preparation and Illumina NovaSeq 6000 sequencing using 150 base paired-end reads, with an average coverage of 54 M reads per library (supplementary table S1, Supplementary Material online). All raw reads are available from NCBI SRA Bioproject PRJNA915459.

All analyses were conducted in R 3.6.2 and using Blast+ (Camacho et al. 2009). Reads were aligned to the *C. calcarata* genome (NCBI Bioproject PRJNA791561) using STAR with default settings (Dobin et al. 2013) with an average of 93% reads uniquely mapped. Analysis of DEGs between foraging mothers, dwarf eldest daughters both in presence and absence of mothers, and regular daughters, as well as between pooled mothers and regular daughters (prereproductive and postreproductive) and dwarf eldest daughters (nonreproductives), was performed using DESeq2 (Love et al. 2014). Our two models for this analysis were to, firstly, consider the four conditions as predictor (regular daughter, mothers, dwarf eldest daughters with mothers, and dwarf eldest daughters without mothers) and, secondly, consider reproductive status, with reproductive (mothers and regular daughters) and nonreproductive (all dwarf eldest daughters) as predictors. A principal component analysis (PCA) was performed on data transformed using the vst function in base R and the default plotPCA function in DESeq2 (Love et al. 2014; R Core Team 2021). A complementary WGCNA was also performed to identify modular gene coexpression correlated with each class of foraging females (Langfelder and Horvath 2008). A soft threshold power of 6 was chosen by visually inspecting plots of soft threshold and scale-free topology model fit, and a cut height of 0.20 was chosen. Hub genes were identified with the chooseTopHubInEachModule function in the WGCN R package (Langfelder and Horvath 2008).

For cross species analysis, DEGs were compared with the current social insect literature for association with foraging and caste differentiation. GO term enrichment was determined in topGO v2.44.0 (Alexa and Rahnenfuhrer 2016) with GO annotation using Blast2GO for all genes in *C. calcarata* genome as a gene universe (Götz et al. 2008). This gene universe was chosen over the shorter list of GO annotations for DEGs found in the present study because gene expression is restricted to a specific season and behavioral state, and DEGs thus do not represent an exhaustive list of brain tissue specific genes. While differences in gene universe change results considerably, a complete list is the more unbiased option in the case of this nonmodel organism (Yon Rhee et al. 2008; Wijesooriya et al. 2022). Lists of DEGs were compared with previously published studies of four bee species were included (*A. mellifera*, Khamis et al. 2015; *M. genalis*, Jones et al. 2017; *C. australensis*, Rehan et al. 2018; *E. dilemma*, Saleh and Ramírez 2019), using Blast+ with >65% identity and $<1 \times 10^{-15}$ *e*-value matches retained (Camacho et al. 2009). Significance of overlap in gene lists with these studies was tested using a hypergeometric test with the phyper function in base R (3.6.2). Further, RRHO was used to identify overlapping patterns in overall gene expression between reproductive and nonreproductive groups where available (*M. genalis*, Jones et al. 2017; *C. australensis*, Rehan et al. 2018; *E. dilemma*, Plaisier et al. 2010; Saleh and Ramírez 2019).

Supplementary Material

Supplementary data are available at *Genome Biology and Evolution* online (http://www.gbe.oxfordjournals.org/).

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Data Availability

The data underlying this article are available in NCBI at https://www.ncbi.nlm.nih.gov/, and all raw reads are available from SRA Bioproject PRJNA915459. Reads were aligned to the *C. calcarata* genome (NCBI Bioproject PRJNA791561). Code for this research is represented at https://github.com/huisken/Brain-gene-expression-foraging-Ceratina-calcarata.

Literature Cited

Ahn S-J, Vogel H, Heckel DG. 2012. Comparative analysis of the UDP-glycosyltransferase multigene family in insects. Insect Biochem Mol Biol. 42:133–147.

Alexa A, Rahnenfuhrer J. 2016. topGO: Enrichment analysis for gene ontology. R package version 2.28.0.

Alexander RD. 1974. The evolution of social behavior. Annu Rev Ecol Evol Syst. 5:325–383.

Ament SA, et al. 2012. The transcription factor ultraspiracle influences honey bee social behavior and behavior-related gene expression. PLoS Genet. 8:e1002596.

Arsenault SV, Brendan HG, Rehan SM. 2018. The effect of maternal care on gene expression and DNA methylation in a subsocial bee. Nat Commun. 9:1–9.

Begna D, Fang Y, Feng M, Li J. 2011. Mitochondrial proteins differential expression during honeybee (*Apis mellifera* L.) queen and worker larvae caste determination. J Proteome Res. 10: 4263–4280.

Ben-Shahar Y. 2003. cGMP-dependent changes in phototaxis: a possible role for the foraging gene in honey bee division of labor. J Exp Biol. 206:2507–2515.

Berens AJ, Hunt JH, Toth AL. 2015. Comparative transcriptomics of convergent evolution: different genes but conserved pathways underlie caste phenotypes across lineages of eusocial insects. Mol Biol Evol. 32:690–703.

Bíliková K, et al. 2002. Apisimin, a new serine–valine-rich peptide from honeybee (*Apis mellifera* L.) royal jelly: purification and molecular characterization. FEBS Lett. 528:125–129.

Bloch G, Borst DW, et al. 2000. Juvenile hormone titers, juvenile hormone biosynthesis, ovarian development and social environment in *Bombus terrestris*. J Insect Physiol. 46:47–57.

Bloch G, Hefetz A, Hartfelder K. 2000. Ecdysteroid titer, ovary status, and dominance in adult worker and queen bumble bees (*Bombus terrestris*). J Insect Physiol. 46:1033–1040.

Bloch G, Toma DP, Robinson GE. 2001. Behavioral rhythmicity, age, division of labor and period expression in the honey bee brain. J Biol Rhythms. 16:444–456.

Brockmann A, et al. 2009. Quantitative peptidomics reveal brain peptide signatures of behavior. PNAS 106:2383–2388.

Camacho C, et al. 2009. Blast+: architecture and applications. BMC Bioinformatics 10:421.

Chandrasekaran S, et al. 2011. Behavior-specific changes in transcriptional modules lead to distinct and predictable neurogenomic states. PNAS 108:18020–18025.

Choi M-Y, Meer RKV. 2012. Ant trail pheromone biosynthesis is triggered by a neuropeptide hormone. PLoS One 7:e50400.

Das B, de Bekker C. 2022. Time-course RNASeq of *Camponotus floridanus* forager and nurse ant brains indicate links between plasticity in the biological clock and behavioral division of labor. BMC Genomics 23:57.

Daugherty THF, Toth AL, Robinson GE. 2011. Nutrition and division of labor: effects on foraging and brain gene expression in the paper wasp *Polistes metricus*. Mol Ecol. 20:5337–5347.

Dobin A, et al. 2013. STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29:15–21.

Evans JD, Wheeler DE. 1999. Differential gene expression between developing queens and workers in the honey bee, *Apis mellifera*. PNAS 96:5575–5580.

Farhan A, et al. 2013. The CCHamide 1 receptor modulates sensory perception and olfactory behavior in starved *Drosophila*. Sci Rep. 3:2765.

Ferreira PG, et al. 2013. Transcriptome analyses of primitively eusocial wasps reveal novel insights into the evolution of sociality and the origin of alternative phenotypes. Genome Biol. 14:R20.

Fitzpatrick MJ, et al. 2005. Candidate genes for behavioural ecology. Trends Ecol Evol. 20:96–104.

George R, Stanewsky R. 2021. Peripheral sensory organs contribute to temperature synchronization of the circadian clock in *Drosophila melanogaster*. Front Physiol. 12:622545.

Götz S, et al. 2008. High-throughput functional annotation and data mining with the Blast2GO suite. Nucleic Acid Res. 36:3420–3435.

Grozinger CM, Sharabash NM, Whitfield CW, Robinson GE. 2003. Pheromone-mediated gene expression in the honey bee brain. PNAS 100:14519–14525.

Hartfelder K, Bitondi MMG, Santana WC, Simões ZLP. 2002. Ecdysteroid titer and reproduction in queens and workers of the honey bee and of a stingless bee: loss of ecdysteroid function at increasing levels of sociality? Insect Biochem Mol Biol. 32:211–216.

Hawkings C, Calkins TL, Pietrantonio PV, Tamborindeguy C. 2019. Caste-based differential transcriptional expression of hexamerins in response to a juvenile hormone analog in the red imported fire ant (*Solenopsis invicta*). PLoS One 14:e0216800.

Hogendoorn K, Velthuis HHW. 1999. Task allocation and reproductive skew in social mass provisioning carpenter bees in relation to age and size. Insectes Soc. 46:198–207.

Ingram KK, et al. 2016. Context-dependent expression of the foraging gene in field colonies of ants: the interacting roles of age, environment and task. Proc R Soc Lond Ser B. 283:20160841.

Jones BM, Kingwell CJ, Wcislo WT, Robinson GE. 2017. Caste-biased gene expression in a facultatively eusocial bee suggests a role for genetic accommodation in the evolution of eusociality. Proc R Soc Lond Ser B. 284:20162228.

Kapheim KM, Bernal SP, Smith AR, Nonacs P, Wcislo WT. 2011. Support for maternal manipulation of developmental nutrition in a facultatively eusocial bee, *Megalopta genalis* (Halictidae). Behav Ecol Sociobiol. 65:1179–1190.

Kapheim KM, Chan T-Y, Smith AR, Wcislo WT, Nonacs P. 2016. Ontogeny of division of labor in a facultatively eusocial sweat bee *Megalopta genalis*. Insect Soc. 63:185–191.

Khamis AM, et al. 2015. Insights into the transcriptional architecture of behavioral plasticity in the honey bee *Apis mellifera*. Sci Rep. 5: 11136.

Kronauer DJ, Libbrecht R. 2018. Back to the roots: the importance of using simple insect societies to understand the molecular basis of complex social life. Curr Opin Insect Sci. 28:33–39.

Kukuk PF, May BP. 1991. Colony dynamics in a primitively eusocial halictine bee *Lasioglossum* (*Dialictus*) *zephyrum* (Hymenoptera: Halictidae). Insectes Soc. 38:171–188.

Langfelder P, Horvath S. 2008. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics 9:559.

Lawson SP, Ciaccio KN, Rehan SM. 2016. Maternal manipulation of pollen provisions affects worker production in a small carpenter bee. Behav Ecol Sociobiol. 70:1891–1900.

Lawson SP, Helmreich SL, Rehan SM. 2017. Effects of nutritional deprivation on development and behavior in the subsocial bee *Ceratina calcarata* (Hymenoptera: Xylocopinae). J Exp Biol. 220: 4456–4462.
Le Conte Y, Hefetz A. 2008. Primer pheromones in social Hymenoptera. Annu Rev Entomol. 53:523–542.
Li S, et al. 2013. Expression patterns of the *Drosophila* neuropeptide CCHamide-2 and its receptor may suggest hormonal signaling from the gut to the brain. PLoS One 8:e76131.
Loof AD, et al. 2001. Gonadotropins in insects: an overview. Arch Insect Biochem Physiol. 47:129–138.
Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15:550.
Manfredini F, Martinez-Ruiz C, Wurm Y, Shoemaker DW, Brown MJF. 2021. Social isolation and group size are associated with divergent gene expression in the brain of ant queens. Genes Brain Behav. 21: e12758.
Michener CD. 1974. The social behaviour of the bees: a comparative study. MA: Harvard University Press: Cambridge.
Michener CD, Brothers DJ. 1974. Were workers of eusocial Hymenoptera initially altruistic or oppressed? PNAS 71:671–674.
Mikát M, Franchino C, Rehan SM. 2017. Sociodemographic variation in foraging behavior and the adaptive significance of worker production in the facultatively social small carpenter bee, *Ceratina calcarata*. Behav Ecol Sociobiol. 71:135.
Morandin C, et al. 2016. Comparative transcriptomics reveals the conserved building blocks involved in parallel evolution of diverse phenotypic traits in ants. Genome Biol. 17:43.
Panda S, Hogenesch JB, Kay SA. 2002. Circadian rhythms from flies to human. Nature 417:329–335.
Pizzi NJ, Rehan SM. 2021. Characterization of cuticular hydrocarbons in a subsocial bee, *Ceratina calcarata*. Insectes Soc. 68:351–358.
Plaisier SB, Taschereau R, Wong JA, Graeber TG. 2010. Rank–rank hypergeometric overlap: identification of statistically significant overlap between gene-expression signatures. Nucleic Acids Res. 38: e169.
Prestwich GD, Wojtasek H, Lentz AJ, Rabinovich JM. 1996. Biochemistry of proteins that bind and metabolize juvenile hormones. Arch Insect Biochem Physiol. 32:407–419.
R Core Team. 2021. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. Available from: https://www.R-project.org/.
Rehan SM, et al. 2018. Conserved genes underlie phenotypic plasticity in an incipiently social bee. Genome Biol Evol. 10:2749–2758.
Rehan SM, Berens AJ, Toth AL. 2014. At the brink of eusociality: transcriptomic correlates of worker behaviour in a small carpenter bee. BMC Evol Biol. 14:260.
Rehan SM, Richards MH. 2010. Nesting biology and subsociality in *Ceratina calcarata* (Hymenoptera: Apidae). Can Entomol. 142: 65–74.
Rehan SM, Richards MH. 2013. Reproductive aggression and nestmate recognition in a subsocial bee. Anim Behav. 85:733–741.
Rehan SM, Toth AL. 2015. Climbing the social ladder: the molecular evolution of sociality. Trends Ecol Evol. 30:426–433.
Rittschof CC, Robinson GE. 2016. Behavioral genetic toolkits: toward the evolutionary origins of complex phenotypes. Curr Top Dev Biol. 119:157–204.
Robinson GE, Grozinger CM, Whitfield CW. 2005. Sociogenomics: social life in molecular terms. Nat Rev Genet. 6:257–270.
Robinson GE, Strambi C, Strambi A, Feldlaufer MF. 1991. Comparison of juvenile hormone and ecdysteroid haemolymph titres in adult worker and queen honey bees (*Apis mellifera*). J Insect Physiol. 37:929–935.
Robinson GE, Strambi C, Strambi A, Huang Z-Y. 1992. Reproduction in worker honey bees is associated with low juvenile hormone titers and rates of biosynthesis. Gen Comp Endocrinol. 87:471–480.
Röseler P-F, Röseler I, Strambi A. 1985. Role of ovaries and ecdysteroids in dominance hierarchy establishment among foundresses of the primitively social wasp, *Polistes gallicus*. Behav Ecol Sociobiol. 18: 9–13.
Ruberto AA, et al. 2021. KLF10 Integrates circadian timing and sugar signaling to coordinate hepatic metabolism. eLife 10:e65574.
Saleh NW, Ramírez SR. 2019. Sociality emerges from solitary behaviours and reproductive plasticity in the orchid bee *Euglossa dilemma*. Proc R Soc Lond Ser B. 286:20190588.
Schwarz MP, Richards MH, Danforth BN. 2007. Changing paradigms in insect social evolution: insights from halictine and allodapine bees. Annu Rev Entomo. 52:127–150.
Shapira M, Thompson CK, Soreq H, Robinson GE. 2001. Changes in neuronal acetylcholinesterase gene expression and division of labor in honey bee colonies. J Mol Neurosci. 17:1–12.
Shell WA, Rehan SM. 2018a. Behavioral and genetic mechanisms of social evolution: insights from incipiently and facultatively social bees. Apidologie 49:13–30.
Shell WA, Rehan SM. 2018b. The price of insurance: costs and benefits of worker production in a facultatively social bee. Behav Ecol. 29:204–211.
Shell WA, Rehan SM. 2019. Social modularity: conserved genes and regulatory elements underlie caste-antecedent behavioural states in an incipiently social bee. P Roy Soc B-Bio. 286:20191815.
Shpigler H, et al. 2014. Gonadotropic and physiological functions of juvenile hormone in bumblebee (*Bombus terrestris*) workers. PLoS One 9:e100650.
Smith CR, Toth AL, Suarez AV, Robinson GE. 2008. Genetic and genomic analyses of the division of labour in insect societies. Nat Rev Genet. 9:735–748.
Strassmann JE. 1981. Wasp reproduction and kin selection: reproductive competition and dominance hierarchies among *Polistes annularis* foundresses. Fla Entomol. 64:74–88.
Sun P, et al. 2019. Transcriptomic and functional analyses of phenotypic plasticity in a higher termite, *Macrotermes barneyi* Light. Front Genet. 10:964.
Tei H, et al. 1997. Circadian oscillation of a mammalian homologue of the *Drosophila* period gene. Nature 389:512–516.
Tibbetts EA, Huang ZY. 2010. The challenge hypothesis in an insect: juvenile hormone increases during reproductive conflict following queen loss in *Polistes* wasps. Am Nat. 176:123–130.
Tibbetts EA, Izzo A, Huang ZY. 2011. Behavioral and physiological factors associated with juvenile hormone in *Polistes* wasp foundresses. Behav Ecol Sociobiol. 65:1123–1131.
Tibbetts EA, Sheehan MJ. 2012. The effect of juvenile hormone on *Polistes* wasp fertility varies with cooperative behavior. Horm Behav. 61:559–564.
Toma DP, Bloch G, Moore D, Robinson GE. 2000. Changes in period mRNA levels in the brain and division of labor in honey bee colonies. PNAS 97:6914–6919.
Toth AL, Rehan SM. 2017. Molecular evolution of insect sociality: an eco-evo-devo perspective. Annu Rev Entomol. 62:419–442.
Toth AL, Robinson GE. 2005. Worker nutrition and division of labour in honeybees. Anim Behav. 69:427–435.
Toth AL, Robinson GE. 2007. Evo-devo and the evolution of social behavior. Trends Genet. 23:334–341.
Van Gelder R, Krasnow M. 1996. A novel circadianly expressed *Drosophila melanogaster* gene dependent on the period gene for its rhythmic expression. EMBO J. 15:1625–1631.
Wang Y, Azevedo SV, Hartfelder K, Amdam GV. 2013. Insulin-like peptides (AmILP1 and AmILP2) differentially affect female caste development in the honey bee (*Apis mellifera* L). J Exp Biol. 216:4347–4357.

West-Eberhard MJ. 1996. Wasp societies as microcosms for the study of development and evolution. In: West-Eberhard MJ, Turillazzi S, editors. Natural history and evolution of paper-wasps. New York: Oxford University Press. p. 254–255.
Wheeler DE, Buck N, Evans JD. 2006. Expression of insulin pathway genes during the period of caste determination in the honey bee, *Apis mellifera*. Insect Mol Biol. 15:597–602.
Wijesooriya K, Jadaan SA, Perera KL, Kaur T, Ziemann M. 2022. Urgent need for consistent standards in functional enrichment analysis. PLoS Comput Biol. 18:e1009935.
Withee JR, Rehan SM. 2017. Social aggression, experience, and brain gene expression in a subsocial bee. Integr Comp Biol. 57:640–648.
Yon Rhee S, Wood V, Dolinski K, Draghici S. 2008. Use and misuse of the gene ontology annotations. Nat Rev Genet. 9: 509–515.
Zhou X, Tarver MR, Scharf ME. 2007. Hexamerin-based regulation of juvenile hormone-dependent gene expression underlies phenotypic plasticity in a social insect. Development 134:601–610.

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