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Meta-analysis of public RNA sequencing data of queens and workers in social Hymenoptera and termites

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Simple Summary: Social insects form complex, sophisticated societies in which members work cooperatively. Ants, bees, wasps, and termites are examples of social insects. The key characteristic of their structured societies is the reproductive division of labor. The queens reproduce within the nests, whereas workers engage in other tasks for colony development. Although gene expression profiles associated with the reproductive division of labor have been revealed for each social species, the existence of core regulatory genes commonly shared by all or multiple social insects is largely unknown. To address this issue, we conducted a meta-analysis by collecting and reanalyzing massive amounts of data from social bees, social wasps, ants, and termites. We identified 20 genes with queen- and worker-biased expression. Twelve of these genes have not been previously associated with reproductive division of labor. The expression of these genes is likely necessary for social insects to exhibit reproductive division of labor.

Abstract: Eusociality in insects has evolved independently many times. One of the most notable characteristics of eusociality is the reproductive division of labor. In social insects, the reproductive division of labor is accomplished by queens and workers. Transcriptome analyses of queens and workers have been conducted for various eusocial species. However, the genes that regulate the reproductive division of labor across all or multiple eusocial species have not yet been fully elucidated. Therefore, we conducted a meta-analysis using publicly available RNA-sequencing data from four major groups of social insects. In this meta-analysis, we collected 258 pairs (queen vs. worker) of RNA-sequencing data from 34 eusocial species. The meta-analysis identified a total of 20 genes that were differentially expressed in queens or workers. Out of these, 12 genes have not previously been reported to be involved in the reproductive division of labor. Functional annotation of these 20 genes in other organisms revealed that they could be regulators of behaviors and physiological states related to the reproductive division of labor. These 20 genes, revealed using massive datasets of numerous eusocial insects, may be key regulators of the reproductive division of labor.

Keywords: Division of labor; eusociality; social insect; meta-analysis; RNA sequencing

1. Introduction

Organisms have undergone several evolutionary transitions from cellular to multicellular organisms and their organized societies [1]. Sociogenomics aims to comprehensively understand social life from a molecular perspective [2]. Eusociality is the most advanced social form, characterized by a high level of cooperation among members of society. An essential characteristic of eusociality is the reproductive division of labor, which is accomplished by reproductive and non-reproductive castes. Reproductive castes are physiologically and behaviorally specialized in reproduction, whereas non-reproductive castes engage in brood care or foraging. The division of labor is believed to increase the overall productivity of eusocial colonies [3].

Eusociality, which is based on the reproductive division of labor, has evolved independently in several insect lineages [4,5]. Eusocial Hymenoptera (bees, ants, and wasps) and termites are extensively studied insects that have complex and sophisticated societies. The reproductive and non-reproductive castes of these social insects are referred to as queen/king and workers/soldiers, respectively [6]. The investigation of genomic changes underlying the convergent evolution of eusociality among several lineages (bees and ants) has identified the genes that molecular evolution has occurred with increased social complexity [7–10]. Transcriptome analysis using the pharaoh ant *Monomorium pharaonis* and the honeybee *Apis mellifera* revealed that convergent eusocial evolution is based on the expression of highly conserved reproductive and lineage-specific genes [11]. In addition, many transcriptome analyses of queens and workers have been conducted for each species of bee, ant, wasp, and termite [11–32]. However, no comprehensive transcriptome analysis has been performed to understand the regulatory mechanisms of reproductive division of labor across a wide range of eusocial species.

A meta-analysis that combines transcriptomic data obtained from multiple studies is effective identifying novel gene expression associated with specific biological processes [33–35]. Additionally, it is possible to identify master regulatory genes by combining transcriptome data from a wide range of species. We developed meta-analysis methods for publicly available transcriptome data from humans, plants, and insects [33,36–39]. In this study, we used a meta-analysis method to identify regulatory genes related to the reproductive division of labor in ants, bees, wasps, and termites. We collected RNA sequencing data for 258 pairs (queens vs. workers) from 34 eusocial species. Meta-analysis detected 212 genes that were conserved in all species examined in this study, 20 of these 212 genes were significantly differentially expressed between queens and workers.

2. Materials and Methods

2.1. Curation of public RNA sequencing data

Accession IDs (Project ID) related to eusociality were searched from the public databases All Of gene Expression (AOE) [40] and DBCLS SRA (https://sra.dbcls.jp/ (accessed on 11 May 2022)) using the keywords caste, eusocial, eusociality, and sociality. Metadata was obtained using NCBI SRA Run Selector (https://www.ncbi.nlm.nih.gov/Traces/study/ (accessed on 12 May 2022)). The acquisition of RNA sequencing data was limited to species for which the genome or reference transcriptome was available. RNA sequencing data from each species were obtained in pairs (queen and worker) to investigate gene expression related to the reproductive division of labor.

2.2. RNA-seq data retrieval, processing, and quantification

SRA retrieval and conversion to FASTQ files were performed using the prefetch and fasterq-dump programs in the SRA Toolkit (v3.0.0) in the Docker environment (v3.3.1) [41]. To decrease the disk space usage, the obtained FASTQ files were immediately compressed using pigz (v. 2.4). For trimming and quality control, Trim Galore! (v.0.6.6) [42] and Cutadapt (v.3.4) [43] software were used. Trim Galore! was run with the parameter fastqc --trim1--paired (if data were paired-end). Trim Galore! included Cutadapt (v.3.4) to trim low-quality base calls. Because all the data passed through this quality check, low-quality data were not included in this study. Biological replicates were treated as one experiment, and technical replicates were combined into one expression dataset.

The files of the reference genome and transcripts used for quantifying gene expression levels are listed in Table S1 (https://doi.org/10.6084/m9.figshare.21524286.v1). If available, these data were obtained from the NCBI Reference Sequence Database (RefSeq) or open data repositories used in the cited literature (accessed on 5 Aug 2022).

Transcript expression was quantified using Salmon (v.1.5.0) [44] with the parameters -i index -l IU. Gene expression levels were quantified using transcripts per million (TPM). For species with only genome sequences, RNA sequencing reads were mapped to the reference genome using HISAT2 (v.2.2.1) [45] with the parameters -q --dta -x, and then quantification of the mapped data was performed using StringTie (2.1.5) [46]. TPM was used as a quantitative value of the gene expression level.

2.3. The detection of genes related to the reproductive division of labor

A calculation method developed for the meta-analysis of RNA sequencing data was employed [33,36–39] as described below. Pairs of the queen and worker RNA sequencing data (Table S2: https://doi.org/10.6084/m9.figshare.21524286.v1) were used to calculate the queen/worker ratio of TPM for each transcript (referred to as the QW ratio). The QW ratio for each transcript was calculated using Equation (1).

$$\text{QW ratio} = \log_2\left(\text{TPM}_{queen} + 0.01\right) - \log_2(\text{TPM}_{worker} + 0.01), \quad (1)$$

This pairwise calculation was performed for all the pairs described in Table S2 (https://doi.org/10.6084/m9.figshare.21524286.v1). The value "0.01" was added to TPM to convert zero into a logarithm. QW ratios were classified as "upregulated," "downregulated," or "unchanged" according to the thresholds. Transcripts with a QW ratio greater than 0.58496250072116 (1.5-fold expression change) were treated as "upregulated." Transcripts with QW ratios less than –0.58496250072116 (1/1.5-fold expression changes) were treated as "downregulated." Others were treated as "unchanged." The QW score was calculated to identify the genes involved in the reproductive division of labor. The QW score was calculated by subtracting the number of pairs with "downregulated" from those with "upregulated, " as shown in Equation (2):

$$\text{QW score} = \text{count number}_{\text{upregulated}} - \text{count number}_{\text{downregulated}}, \quad (2)$$

Python codes for calculating QW-ratios and QW-scores were obtained from a previous study[36](https://github.com/no85j/hypoxia_code/blob/master/CodingGene/HN-score.ipynb (accessed on 13 Aug 2022)).

2.4. Aannotation for transcripts and gene set enrichment analysis (GSEA)

To determine homologous relationships, the protein BLAST (BLASTP) program in the NCBI BLAST software package (v2.6.0) was used with parameters -evalue 1e-10 -outfmt 6 -max_target_seqs 1. The transcript files used for protein translation are listed in Table S1 (https://doi.org/10.6084/m9.figshare.21524286.v1). For species with only genome sequences, GFFread (v0.12.1) [47] was used to extract transcript sequences from the genome sequence. TransDecoder (v5.5.0) (https://transdecoder.github.io/ (accessed on 22 Jun 2021)) was used to identify and translate the coding regions into amino acid sequences. Open reading frames were extracted using TransDecoder.Longorfs with parameters -m 100, and coding regions were predicted using TransDecoder.Predict with the default parameters. As a result of the above processes, the transcript IDs of all species were converted to the protein IDs of *A. mellifera*. The protein IDs of *A. mellifera* were then converted to those of other species using the annotation table for transcripts of *A. mellifera* [48]. Transcripts and amino acid sequences created in this study can be downloaded from figshare (Supplemental File S1). GSEA was performed using the Metascape software [49].

3. Results

3.1. Data collection of transcriptomes related to queens and workers

RNA sequencing data of queens and workers have been collected from many social insects to identify the genes underlying the reproductive division of labor. Consequently, 258 pairs (queen vs. worker) of RNA sequencing data were collected from 34 eusocial in-

sect species (Figure 1A, Table S2; https://doi.org/10.6084/m9.figshare.21524286.v1). Ants (*Harpegnathos saltator* and *Monomorium pahraonis*), termites (*Cryptotermes secundus*), honeybees (*A. mellifera*), and wasps (*Polistes dominula*) were among the higher ranked species, indicating that RNA sequencing data from a wide range of eusocial species were included in the dataset. When compared among the tissues, brain-derived RNA sequencing data were the most abundant (Figure 1B).

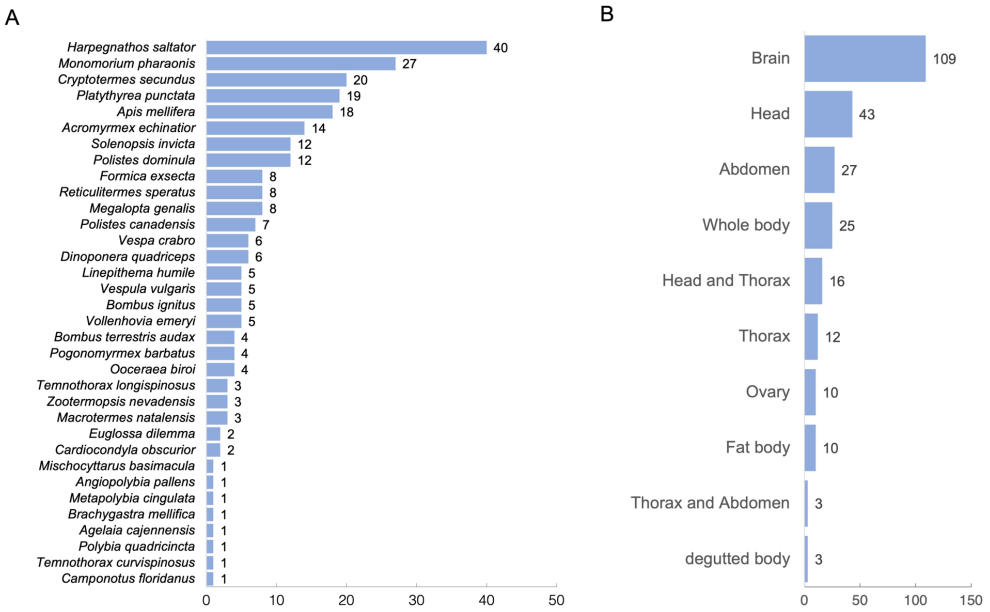

Figure 1. Number of RNA sequencing data compared across species (A) and tissues (B).

3.2. Meta-analysis of RNA sequencing data in social insects

The expression ratio (queen vs. worker) (ratio) was calculated for each species. At this point, the transcript IDs in the QW ratio table differ from species to species, even for homologs. Therefore, we converted the transcript IDs of each species to the protein IDs of *A. mellifera*, and then merged the QW-ratio tables of each species. We obtained QW-ratio values for 212 genes in 258 pairs of RNA sequencing data (Table S3: https://doi.org/10.6084/m9.figshare.21524286.v1). The QW score for each gene was then calculated based on the QW ratio table. The QW score for each gene was calculated by subtracting the number of pairs with downregulated (less than 1/1.5-fold change) gene expression from the number of pairs with upregulated (more than 1.5-fold change) gene expression. This method provides the overall trends of gene expression changes in 258 pairs of RNA sequencing data. High scores indicate highly expressed genes in the queen, while low scores indicate highly expressed genes in the workers. The 212 identified genes were ranked based on their QW-score values (Table S4: https://doi.org/10.6084/m9.figshare.21524286.v1). These genes were annotated by the corresponding homologs of other species (*D. melanogaster*, *H. sapiens*, *Mus musculus*, *Bombyx mori*, *Bombus terrestis*, and *Nasonia vitripennis*) using the annotation table created by Yokoi et al 2022 [48] (Table S4: https://doi.org/10.6084/m9.figshare.21524286.v1). We focused on the top 10 and bottom 10 genes in Table S4 because they showed abrupt score changes (Figure 2 and Table 1). Abrupt score changes indicated that their expression was remarkably variable in the dataset used in this study.

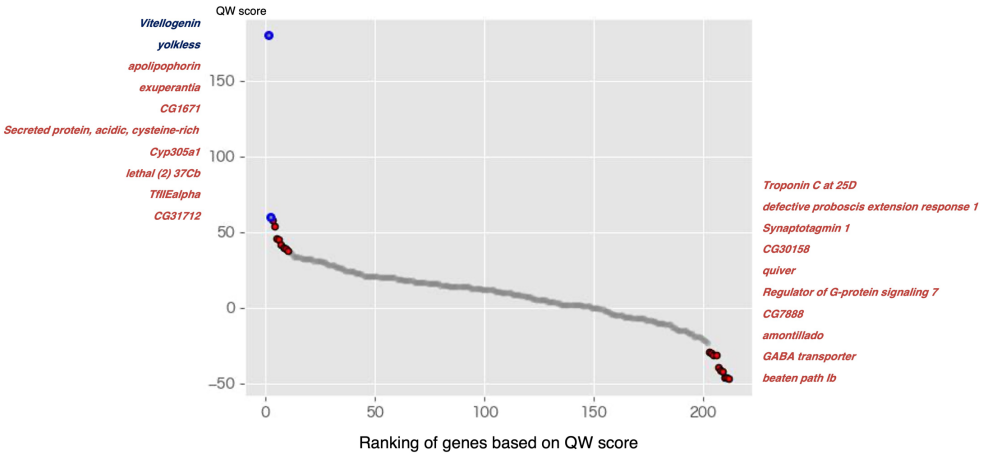

Figure 2. Gene ranking based on QW score values. The gene names follow those of *D. melanogaster*. Red and blue dots indicate the top 10 best-ranked and worst-ranked genes, respectively. The blue dots indicate *Vitellogenin* and *yorkless* (Vitellogenin receptor), which are significantly expressed in queens of many eusocial species. Gene names indicate the top 10 and bottom 10 genes.

Table 1. Top 10 best-ranked and worst-ranked genes based on QW-score values.

RANK	QW score	Gene ID of *A. mellifera*	Gene symbol of *D.melanogaster*	Gene symbol *of H. sapiens*	Tissue-expression profile in female adult *D. melanogaster*	Caste with high expression that were reported in previous study	Biological/molecular function
1	180	NP_001011578.1 (Vitellogenin)	-	-	-	Queen in many species [53-57]	Oogenesis [51, 52]
2	60	XP_026295652.1	yl	LRP2	ovary	Queen in *Diacamma sp.*; *A. mellifera*; *M. pharaonis*; *T. longispinosus* [11, 58, 59]	Oogenesis [51, 52]
3	58	XP_026298285.1	apolpp	GTPBP2	fat body, spermatheca, head, eye	Queen in *R. speratus* [31]	JH binding [66]
4	54	XP_623977.2	exu	-	ovary	Queen in *A. mellifera*; *T. longispinosus* [59, 70]	Oogenesis [69]
5	46	XP_001120493.3	CG1671	TBL3	relatively high in ovary	-	Ribosome biogenesis [71]
6	45	XP_623079.2	SPARC	SPARC	heart, eye, head thoracicoabdominal ganglion	-	Insulin secretion [74]
7	42	NP_001314895.1	Cyp305a1	CYP2A13	spermatheca, head	-	JH synthesis [65]
8	40	XP_026300350.1	l(2)37Cb	DHX16	relatively high in heart	-	Splicesome [78]
9	39	XP_395253.2	TfIIEalpha	GTF2E1	relatively high in ovary	-	Ribosome biogenesis [72]
10	38	XP_395437.4	CG31712	GKAP1	brain, thoracicoabdominal ganglion,head	-	Splicesome [79]

203	-29	XP_026299328.1	TpnC25D	CALM3	head, eye, gut	-	A calcium signal transduction pathway (Uniprot)
204	-30	XP_026300722.1	dpr1	CNTN4	brain, thoracicoabdominal ganglion, head	-	Immunoglobulin superfamily [80,81]
205	-31	XP_026295297.1	Syt1	SYT1	thoracicoabdominal ganglion, brain, head	worker (forager) in *A. mellifera*; *P. metricus*; *S. invicta* [83]	Neurotransmitter release at synapses [83]
206	-31	XP_026298569.1	CG30158	RASD1	brain, head, thoracicoabdominal ganglion	-	Circadian entrainment [91, 92]
207	-39	XP_026302000.1	qvr	LYPD6	thoracicoabdominal ganglion, head, eye	worker (forager) in *B. terrestris* [87]	Sleep [87]
208	-41	XP_393403.3	RSG7	RGS7	brain, thoracicoabdominal ganglion, head	-	Insulin secretion [74]
209	-42	XP_393138.2	CG7888	SLC36A1	hindgut, thoracicoabdominal ganglion, brain	-	Amino acid transport (Uniprot)
210	-46	XP_392366.2	amon	PCSK2	brain, thoracicoabdominal ganglion, head	-	The production of bioactive neuropeptide hormones [86]
211	-46	XP_006571520.1	Gat	SLC6A1	thoracicoabdominal ganglion, brain, head	Worker in A. mellifera [70]	regulation of circadian sleep [89]
212	-47	XP_026298737.1	beat	PSG4	brain, thoracicoabdominal ganglion, head	-	Immunoglobulin superfamily [80, 82]

To estimate the function of these genes, the information of tissue-expression profile in *D. melanogaster* were added (Table 1). Nine out of ten highly expressed genes in workers

were found primarily in the brain and thoracoabdominal ganglia in *D. melanogaster*, while highly expressed genes in queens were found in various tissues. If high expression has already been reported in queens or workers, caste and species names are noted. In addition, we added the biological/molecular functions predicted by the cited literature.

3.3. GSEA

We selected the top 30 best-ranked and worst-ranked genes based on the QW score, and then performed GSEA using the corresponding IDs of *D. melanogaster* and *H. sapiens* (Figure 3). No GO terms with low p-values were detected when *D. melanogaster* IDs were used (Figure 3AB). However, when *H. sapiens* IDs were used, rRNA processing was significantly enriched in the top 30 genes (Figure 3C), whereas the positive regulation of ion transport and modulation of chemical synaptic transmission were enriched in the last 30 genes (Figure 3D).

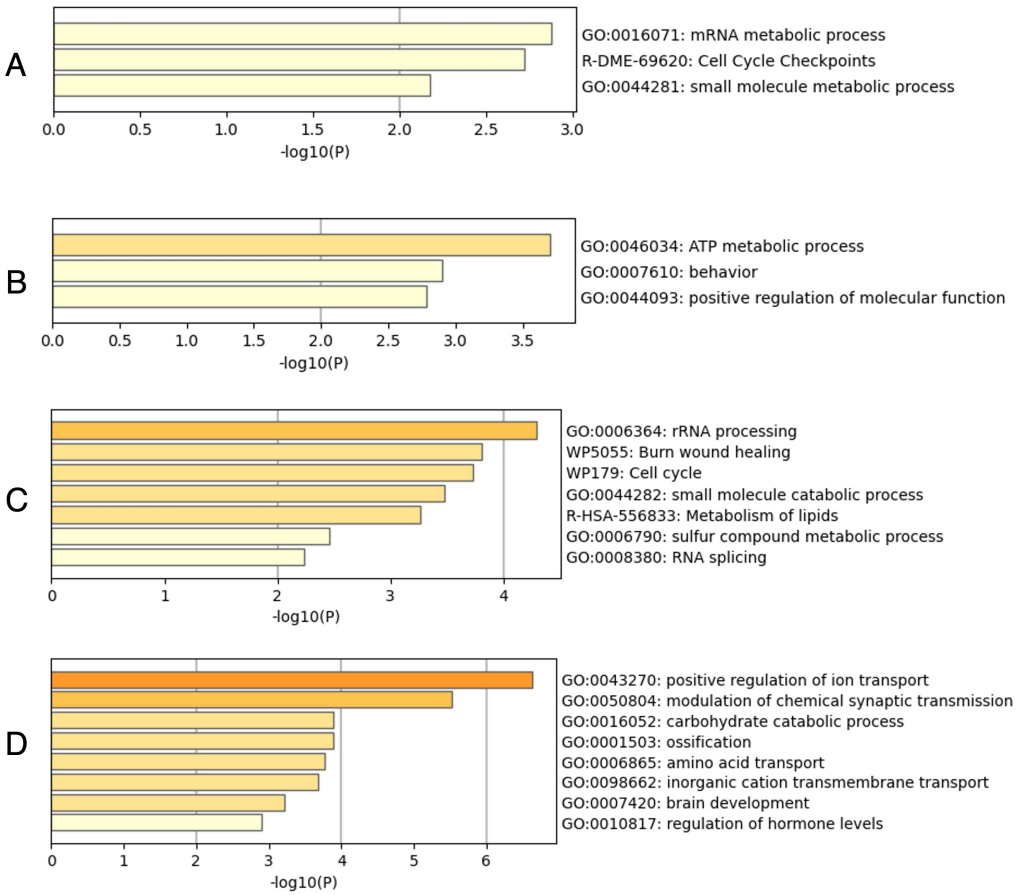

Figure 3. Results of GSEA using *D. melanogaster* IDs (A, B) and *H. sapiens* IDs (B, C).

4. Discussion

In this study, we aimed to identify genes that regulate the reproductive division of labor across all or multiple eusocial insects by performing a meta-analysis of RNA-seq data. Meta-analysis revealed 20 genes with differential expression between queens and workers. Among these genes, vitellogenin and vitellogenin receptors, which are highly expressed in queens across many social insects [29,50–56], showed the highest QW score (Figure 1). This suggests that the current meta-analysis method is suitable for identifying differences in gene expression between queens and workers.

Among the genes identified in this study, we focused on 20 genes with the highest or lowest QW scores (Table 1). As the results of this study represent a vast amount of da-

ta from a wide variety of eusocial species, it is likely that these genes are the key regulators underlying the reproductive division of labor in many social insects. In addition, several of the 20 genes were not previously implicated in the reproductive division of labor, providing novel insights into the regulatory mechanisms of the reproductive division of labor. In the following sections, we summarize the biological and molecular functions of the corresponding homologs of these 20 genes in other organisms and discuss their role in the regulatory mechanisms of the reproductive division of labor.

4.1. Oogenesis, Juvenile hormone (JH) binding, and synthesis (Vitellogenin, yolkless, Cyp305a1, apolipophorins, and exuperantia)

Vitellogenin is a precursor protein of the egg yolk that is taken up by oocytes via receptor-mediated endocytosis [57,58]. These genes are highly expressed in the reproductive castes of various insects [29,50–56]. JH generally acts as a gonadotropic hormone in eusocial and solitary insects [59–63]. *Cyp305a1*, which encodes JH epoxidase, is required for JH biosynthesis in *D. melanogaster* [64]. *Apolpp* is a JH-binding protein in the cockroach *Blattella germanica* [65], and is upregulated in the termite queens, *Reticuliterms speratus* [30]. These genes may contribute to queen ovarian development via an upregulated JH titer. However, in the honeybees *A. mellifera* and the ant *H. saltator,* the gonadotropic function of JH was lost [66,67]. The gonadotropic effects of these genes may depend on species.

Exuperantia is a maternal factor that is involved in oocyte formation[68]. In social insects, it is reported to be expressed in the reproductive workers of the honeybee *A. mellifera* [69] and queens of the ant *Temnothorax longispinosus* [70]. This gene is likely involved in the formation of normal oocytes in both social and solitary insects.

4.2. Ribosome biogenesis (CG1671 and TfIIEalpha)

CG1671 was orthologous to human transducin beta-like 3 (TBL3/UTP13). UTP-13, which is a component of the UTP-B complex, binds to pre-rRNA to form 90S preribosomes, followed by pre-RNA cleavage [71]. TFIIE, a general transcription factor, is required for initiation of transcription by RNA polymerase II. Additionally, TFIIE affects ribosomal assembly and function [72]. Defects in ribosomal synthesis or function are implicated in various disease states known as 'ribosomopathies' in humans [73]. Consequently, *CG1671* and *TFIIEalpha* may affect ribosomal synthesis and performance, which may influence the physiological state of the queen.

Genes involved in rRNA processing were enriched in the results of GSEA, supporting the importance of rRNA processing as a regulator of the reproductive division of labor.

4.3. Insulin secretion SPARC (secreted protein acidic and cysteine rich) and RSG7 (regulator of G protein signaling 7)

In this study, *SPARC* was highly expressed in queens, and *RSG7* was highly expressed in workers. In mice, *SPARC* promotes insulin secretion via downregulation of *regulator of G protein 4* (*RGS4*) expression in pancreatic β cells [74]. It also may regulate insulin secretion in eusocial insects via downregulation of *RSG7* expression. The expression of insulin-like peptide (ILP) is upregulated in the queens of several ants and in the termite *Macrotermes natalensis* [75,76]. In *A. mellifera*, however, *ILP* expression is lower in the old queen than in the old workers [77]. Therefore, the relationship between *SPARC* and *RGS* may possibly not apply to all eusocial species.

4.4. Spliceosome (l(2)37Cb and CG31712)

l(2)37Cb is orthologous to human DEAH-Box Protein 16 (DHX16), which encodes an ATP-dependent RNA helicase that is recruited to Complex B in the spliceosome [78]. The *CG31712* protein interacts with the spliceosome complex, including *l(2)37Cb* [79].

These genes may be related to differences in alternative splicing patterns between queens and workers.

4.5. Immunoglobulin superfamily (beat and dpr1)

Immunoglobulin superfamily proteins have been implicated in neuron-neuron and neuron-muscle interactions [80]. *dpr1 (*defective proboscis extension response 1*)* is required for salt aversion in *D. melanogaster* [81]. *beat* (*beaten path Ib*) is necessary for the formation of normal nerve branches in the muscle [82]. In contrast to queens, workers actively engage in foraging and brood care. These genes may be involved in modifying the nervous system required for workers to engage in the colony task.

4.6. Neurotransmitter and neuro peptide Syt1 (Synaptotagmin 1) and amon (amontillado)

Syt1, which encodes a synaptic vesicle calcium-binding protein, is required for synaptic vesicles to interact with the plasma membrane [83]. $Ca2^{+}$ ions act on molecular complexes, including *Syt1*, triggering rapid exocytosis at the synapse. *Syt1* is highly expressed in ants (*S. invicta*), honeybees (*A. mellifera*), and wasps (*P. metricus*) [84]. *Syt1* might regulate the secretion of neurotransmitters that cause foraging and brood care. Serotonin secretion regulates worker responsiveness to trail pheromones in the ant *Pheidole dentata*, leading to cooperative foraging behavior [85].

Amon, which encodes a member of the prohormone convertase family, is required for propeptide processing to form bioactive neuropeptides [86]. In *D. melanogaster*, amon protein is an important enzyme that catalyses many neuropeptides in the larval corpora cardiaca and perineuronal organs of the thorax and abdomen. Corazonin is a neuropeptide whose production is dependent on ammonia catalysis [86]. Corazonin is a neuropeptide that is highly expressed in the workers of three ants (*Harpegnathos saltator*, *Camponotus floridanus* and *Monomorium pharaonis*) and one wasp (*Poistes canadensis*) [15]. In addition to corazonin, *amon* may regulate the production of neuropeptides that regulate worker behavior.

4.7. Regulation of circadian sleep (Gat (*GABA transporter*)*, quiver (qvr), and CG30158)*

qvr, which encodes a glycosylphosphatidylinositol (GPI)-anchored membrane protein, is a sleep-promoting factor in *D. melanogaster* [87]. *Gat* (*GABA transporter*) encodes a protein belonging to the solute carrier transporter family. Mutations in *Gat* cause excessive sleep because of the reduced uptake of GABA (a sleep inhibitor) from synapses to astrocytes in *D. melanogaster* [88]. *Gat* and *quiver* are reported to be highly expressed in worker foragers of the bumblebees *B. terrestris* and *A. mellifera*, respectively [69,89]. In honeybees, *A. mellifera*, sleep deprivation negatively affects the accuracy of the waggle dance, which informs colony members of the location of food resources [90]. It is possible that the amount of sleep of workers was optimized by *Gat* and *quiver*. It is unknown what effect excessive sleep has on the task performance of workers, but it is believed that the longer workers sleep, the less time they spend foraging and providing brood care for the colony.

CG30158 is homologous to mammalian *Dexras1*, which encodes a Ras-like G protein. *Dexras1* is required for entrainment of circadian rhythms in the environmental light cycle in mice [91,92]. In worker foragers of the honeybee, *A. cerana japonica*, the circadian rhythm of locomotion activity is synchronized with light and temperature [93]. The *A. mellifera* foragers are often inactive at night [94]. In addition, the sleep rhythms of *A. mellifera* foragers are also known to be synchronized with the timing of the food available [95]. In social insects, the homolog of *CG30158* may play an important role in synchronizing foraging timing with environmental cycles.

4.8 Characteristics of genes highly expressed in workers

Tissue expression profiles in *D. melanogaster* were referred for 20 listed genes using FlyAtras2 [96] (accessed on 14 Oct 2022). Unlike the genes significantly expressed in queens, many of the gene sets upregulated in workers were expressed in the nervous system of adult *D. melanogaster* (Table 1). Enrichment analysis showed that genes involved in the modulation of chemical synaptic transmission (GO:0050804) were enriched in the gene set that is highly expressed in workers, providing evidence for active gene expression in the nervous system of workers.

5. Conclusions

This study is the first meta-analysis conducted on massive datasets of numerous eusocial species in two phylogenetically distant lineages, social Hymenoptera and termites, and provides two novel findings. First, the meta-analysis found 12 genes that had not been reported to be associated with the reproductive division of labor. This is because meta-analysis removes the data bias that occurs in the results of individual studies. Second, the 20 genes retrieved from massive datasets of a large number of eusocial species may be key regulators of the reproductive division of labor. Functional analyses of the genes identified in this study are expected in the future. As there is increase in the publicly available RNA sequencing data, meta-analyses should prove useful in providing new insights into targeted biological phenomena, despite the fact that meta-analysis of RNA sequencing data is hardly widespread in entomology. The present study demonstrated the effectiveness of a meta-analysis of RNA sequencing data.

Supplementary Materials: Supporting information can be downloaded from figshare (https://doi.org/10.6084/m9.figshare.21524286.v1). Table S1: Reference genome and transcriptome used for quantifying gene expression levels (DOI: https://doi.org/10.6084/m9.figshare.21524286.v1). Table S2: Data sets for meta-analysis of public RNA sequencing data in eusocial insects (DOI: https://doi.org/10.6084/m9.figshare.21524286.v1). Table S3: Gene expression ratios when comparing queens and workers (QW ratio) (DOI: https://doi.org/10.6084/m9.figshare.21524286.v1). Table S4: QW score calculated based on the QW ratio. The protein ID of *Apis mellifera* was functionally annotated using homologs of other species (DOI: https://doi.org/10.6084/m9.figshare.21524286.v1). Information from Unigene and Pham has also been added to this table. Supplemental File S1: Amino acid sequence newly created for the present study (peptide.tar.gz). The files of transcript sequence newly created for the present study (transcript.tar.gz) (DOI: https://doi.org/10.6084/m9.figshare.21524286.v1).

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