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RNA-Seq Analysis Revealed Differential Gene Expression and Exon-Specific Effects Associated with the Mating Process in Honey Bee Queens

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ABSTRACT

Mating in honeybees causes dramatic changes not only in its behavior and physiology but in genes' transcriptional level. To determine the molecular mechanisms regulating post-mating behavioral changes, we examined gene expression modification and exon-specific expression of the virgin queen versus the queen injected with semen in hemocoel. The DESeq2 package of R was used to identify the Differentially Expressed Genes (DEGs). The DEGs were selected for functional enrichment analysis and Protein-Protein Interaction (PPI) network construction. We also performed differential exon usage analysis using the DEXseq R package. Results identified a significant expression (FDR< 0.05) of a total of 971 genes between two groups of insects. The mating process produced significant changes in the expression of cell surface receptor signaling pathway, innate immune response, extracellular region, proteinaceous extracellular matrix, nucleous, G-protein coupled receptor activity, heme binding, and transmembrane transporter activity genes. Protein-Protein Interaction (PPI) network shows that LOC552504 (titin-like) could be considered as a super-hub gene in the mating process of queens. In addition, we identified exons that were differentially expressed in two groups of honeybee queens. At 10% FDR, we found significant differential exon usage in 79 genes. Among them, GB55396 gene had the most differences in exon usage and could be the best candidate gene for mating and reproductive activation in queens.

Keywords: *Apis mellifera*, Hemocoel, Mating behavior, Protein-protein interaction.

INTRODUCTION

In addition to producing various crops, bees play their most important role in nature by intervening in pollination practices, increasing agricultural production, and revitalizing the environment. Bees are known as one of the most important pollinating groups worldwide, decrease of this pollination services could potentially reduce yields by about 40% (Stein *et al*., 2017). Therefore, it is important to study the biological process of this useful insect from different aspects of physiology, behavior and genetics.

Colonies usually consist of a single reproductive queen and thousands of worker bees, and the queen mates with several males (an average of 12 males) in a short period in early adulthood (Tarpy and Nielsen, 2002). The female honeybee ontogenies are governed by the same genomes (Macedo *et al.*, 2016). However, differences in the quality of the food provided to young larvae lead to the growth of certain pathways, producing highly specialized female phenotypes: queens and workers (Macedo *et al.*, 2016). Honeybee queens are equipped with large ovaries (150–200 ovarioles on average).

After mating, queens undergo profound behaviors and physiological changes to prepare for oviposition; a prominent change in behavior is that the queen becomes an

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egg-laying machine (Manfredini *et al.*, 2015). Physiologically, the queen's activated ovary becomes larger to ensure that she lays approximately 1500 eggs a day in ideal conditions (Macedo *et al.*, 2016). The pheromone profile of the queen also changes dramatically (Richard *et al.*, 2007). Major changes also occur in the brain as well, causing workers to surround the queen in a retinue response, antennating and licking her. The queens' ovarian activation process is so rapid that queens begin laying eggs about three days after the mating (Gary, 1992). Virgin queens to mating are photophilic and eager to fly (Kocher *et al.*, 2010), and are more aggressive than other non-mating virgin queens (Pflugfelder and Koeniger, 2003). In contrast, mated queens are photophobic, unlikely to engage in fighting with other queens.

It is interesting to know that physiological and behavioral changes after queen mating are similar to changes after queen anesthesia with carbon dioxide (Vergoz *et al.*, 2012; Koywiwattrakul *et al.*, 2005; Berger *et al.*, 2005). Such studies have shown that treatment of honeybee queens with carbon dioxide stops mating flights and activates ovaries (Vergoz *et al.*, 2012). The queen's behavior is also influenced by the instrumental insemination process itself, where insemination with saline solution at a high volume or co treatment of CO_2 and physical manipulation terminate mating flights and activate the ovaries (Niño, 2011, 2013). Injecting semen into the hemocoel is a well-proven method for examining post-mating changes in many insects (Baumann, 1974; Helinski *et al.*, 2012).

Advances in RNA sequencing technologies enable the rapid detection of protein-coding and non-coding RNAs in the vertebrate and invertebrate genomes. Brain transcription can be used to relate complex behaviors such as mating to genome-scale gene expression patterns (Hitzemann *et al.*, 2013; Harris and Hofmann, 2014) and to identify conserved molecular pathways across species. Alternative splicing is an essential biological mechanism that controls gene expression and increases protein diversity. Alternative splicing can be generally categorized into four major groups: (1) Exon skipping, (2) Alternative 5' splicing, (3) Alternative 3' splicing, and (4) Intron Retention (IR) (Black, 2003; Pan *et al.*, 2008; Sammeth *et al.*, 2008).

Here we examine the effects of the mating process on gene expression and exon expression in the brains of queen honeybees from an *Apis mellifera carnica* population using RNA-sequencing data. For this purpose, we used the RNA-Seq data from the study of Jasper *et al.* (2020), to examine gene expression in the honeybee queen's brain after mating. These data consist of the brains of virgin queens and queens injected with semen in hemocoel. We expected gene expression patterns to differ between virgin queens and queens injected with semen into hemocoel. Our study is an important step forward in understanding the relationship between semen injection into hemocoel and gene expression in honeybee's brain. It enables us to dissect how the mating process changes gene expression.

MATERIALS AND METHODS

In Silico Analysis of RNA-Seq Data

Transcriptome data of honeybee queen's brain of *Apis mellifera carnica* were downloaded from the National Center of Biotechnology Information GEO database (https://www.ncbi.nlm.nih.gov/gds/, SRX7735038, SRX7735039, SRX7735040, SRX7735041, SRX7735042, SRX7735048, SRX7735049, SRX7735050, SRX7735051, SRX7735052). These data included 10 samples of brain tissue of honeybee queens: 5 samples of virgin queen and 5 samples of queens injected with semen in hemocoel. The details are described by Jasper *et al*. (2020).

The RNA-Seq files were filtered for quality using FastQC v0.11.5. Then, the

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honeybee genome (version Amel_4.5, ftp://ftp.ensemblgenomes.org/pub/metazoa/release-48/fasta/apis_mellifera/dna/) was indexed using STAR v2.5.3 and the filtered reads were mapped using STAR v2.5.3.

Differentially Expressed Genes (DEG) Identification

The statistical analysis of differential gene expression was performed with the DESeq2 package of R (Love *et al.*, 2014). Briefly, genes that had fewer than 8 aligned reads in each sample were filtered from analysis (Min. count= 8). Genes were considered significant if their Bonferroni corrected P-value was < 0.05 (corrected for the number of differentially expressed genes). Then, obtained data were normalized by the DESeq2 package. We performed MA plot using the DESeq2 package. These plots show the log2 fold changes from the treatment over the mean of normalized counts, i.e. the average of counts normalized by size factors. Subsequently, we used Principal Component Analysis (PCA) to screen all samples using the R stats package (version 4.0.3).

Differential Exon Usage (DEU) Analysis

Arguably, the most widely used differential exon usage detection method is implemented in the DEXSeq package of R (Anders *et al.*, 2012). We tested for DEU between virgin queen and semen queen using the DEXSeq R package. Since DEXSeq infers differential exon usage, it is left to the user to interpret which transcripts are differentially used, given the evidence for a particular exon bin–condition interaction. However, already knowing which exons are affected can lead to biologically meaningful interpretation of the functional impact of their differential usage (Anders *et al.*, 2012). DEXSeq fits generalized linear models to test for the presence of an interaction between the virgin queen vs semen queen. Exons with a DEXSeq P-value < 0.05 were defined as DEU. The MA plots are commonly used to represent log fold-change versus mean expression between two treatments.

Functional Enrichment Analysis

DEGs and DEUs were uploaded as a background list to DAVID Bioinformatics Resources (Huang *et al.*, 2009a, b). The overrepresented Gene Ontology (GO) terms of DEGs were determined using the BEEBASE_ID identifier option (honeybee gene model) in the DAVID online software. To identify the biological functions associated with the DEGs and DEUs, the potential functions of differentially expressed gene were analyzed by Gene Ontology (GO) terms (Biological Process, Cellular Components and Molecular Functions) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway functional annotation. Significant gene sets were defined as those with a Benjamini corrected P value < 0.05.

Co-Expression Network Analysis

A co-expression network identifies which genes have a tendency to show a coordinated expression pattern across a group of samples. We used genes identified to build networks using the String database (https://string-db.org/), which integrates both known and predicted PPIs, and can be applied to predict functional interactions of proteins (Szklarczyk *et al.*, 2015). Cytoscape software version 3.8.2 was used to visualize the PPI network (Smoot *et al*., 2011). In the networks, the nodes correspond to the proteins and the edges represent the interactions. The inclusion criteria of the hub genes are as follows: the genes with the highest degree and betweenness centrality by Cytoscape.

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RESULTS

Differentially Expressed Genes Identification

The average number of reads aligned to the *Apis mellifera* genome was 73% in samples. Statistical analysis revealed that, out of 13,862 transcripts included in the analysis, 971 transcripts were differentially expressed (FDR< 0.05) in the brain between the queen groups (virgin queens group and semen injected in the hemocoel of queens group) (Supplementary File 1). The results revealed 674 genes were up-regulated and 297 genes were down-regulated in virgin queens.

PCA analysis was performed with the normalized counts (based on the DESeq2 method) to investigate if samples from the same group cluster together. As a result, the first two Principal Components (PCs) explained the variability among the samples and both of the groups were grouped in distinct clusters (Figure 1). This finding indicated a clear difference between the transcriptome profiles of two groups (virgin queen and queen injected with semen into hemocoel). The resulting log2 fold changes of all samples were then plotted in an MA plot, which shows the fold-change values of genes against the mean of normalized counts of all samples. The differentially expressed genes were highlighted as red points in the plot (Figure 2). The GB51759 gene has the most difference in the expressed genes, however, the function of which is unknown. GB41428 gene (defensin 1) that was significantly differentially expressed in this study (Supplementary File 1) has already been reported in mating queen of honeybees (Kocher *et al*., 2008 and 2010; Manfredini *et al*., 2015).

Differential Exon Usage Analysis

One of the strengths of the RNA-Seq data is that it allows the analysis of differential expression at the level of independent exons. For identification of exons that are differentially expressed in virgin queen honeybees vs. semen injected queen honeybees at 10% FDR, DEXSeq package found significant differential exon usage in 79 genes, 50 genes were identified by DAVID database (Table 1). The most differences of exon usage were seen in GB55396 gene, the function of which is unknown. The results are in Supplementary File 2. Consistently, we did not find significant differential expression of gene transcript isoforms. MA plot produced using DESeq2 for differential expression analysis of exon usage in two groups of queen honeybee is shown in Figure 3 (a, b).

Functional Enrichment Analysis

Gene Ontology (GO) plays an important role in the annotation and categorization of sequences, including unidentified and unannotated sequences. GO enrichment analysis provides all GO terms enriched significantly in the Differentially Expressed Genes (DEGs) compared to the genome background, and filters the DEGs that correspond to biological functions.

Results of Gene Ontology (GO) terms are shown in Figure 4 (Supplementary File 3). There is a significant overrepresentation of genes involved in biological process: Cell surface receptor signaling pathway (P< 0.00029), innate immune response (P< 0.036), Cellular components: Extracellular region (P< 0.016), proteinaceous extracellular matrix (P< 0.053), nucleus (P< 0.072) and Molecular functions: G-protein coupled receptor activity (P< 0.000034), heme binding (P< 0.025) and transmembrane transporter activity (P< 0.076). KEGG pathway analysis is shown in Figure 5. Several of these genes were part of pathways: other glycan degradation, glycosphingolipid biosynthesis-ganglio series, sphingolipid metabolism, glycosaminoglycan degradation, and lysosome.

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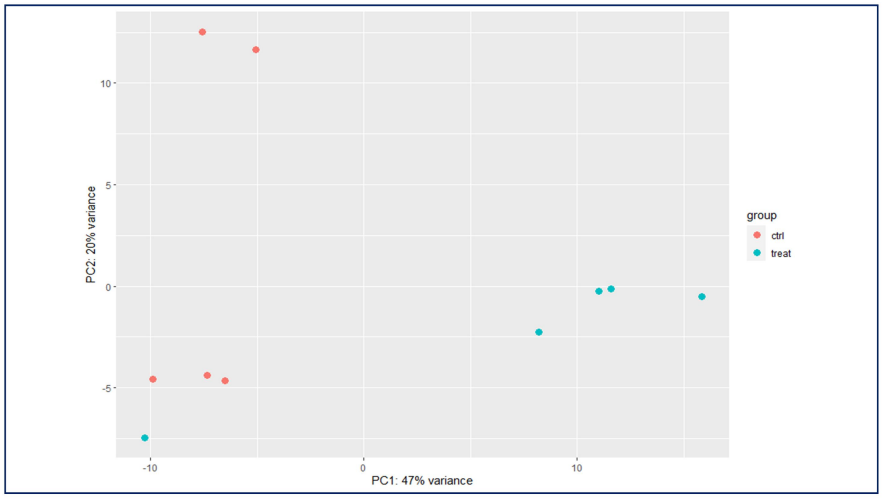

Figure 1. Principal Component analysis (PCA) in two groups of queen honeybee. Through the matrix decomposition method, we can obtain the distribution of the samples on the principal component axis. Experimental group in blue represents the treated samples (queens injected with semen into hemocoel) and control group in red represents the virgin queen samples.

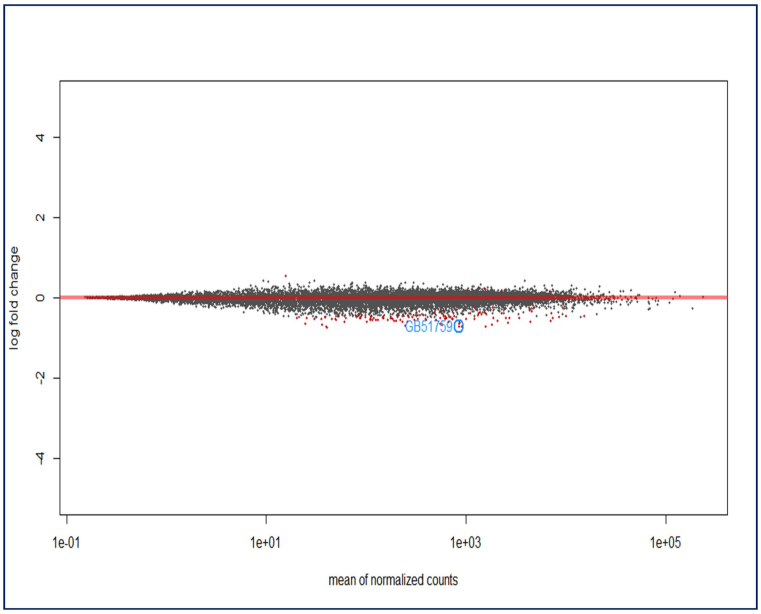

Figure 2. MA plot produced using DESeq2 for differential gene expression analysis in two groups of queen honeybee. The X-axis shows the mean normalized read counts and the Y-axis shows log 2 fold changes. Points in red show significant DEGs (Padj< 0.05).

Co-Expression Network Analysis

The PPI network of DEGs was established based on the String online database and Cytoscape software. There were 971 DEGs in the two groups of queen honeybee. Nodes with large degree and high BC represent the key genes. Titin-like (LOC552504), Unc-89, Mhc1, GB51652-PA, GB43198-PA, Troponin I (410815), paramyosin and long

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form-like (LOC409787) were the key nodes (hub genes), displaying the highest connectivity within the network. Of these genes, only three were identified by DAVID database (Figure 6). In total, 5 genes were finally selected having high degree and high

Table 1. DEUs in the brains of mated (injected with semen) *vs* virgin queens.

BeeBase	Gene ID	Gene description
GB49078	411564	Nose resistant to fluoxetine protein 6-like (LOC411564)
GB48983	410740	RING finger protein 121 (LOC410740)
GB46564	409051	Microtubule-associated protein futsch (LOC409051)
GB54460	725017	Uncharacterized LOC725017 (LOC725017)
GB47977	551259	Titin (LOC551259)
GB46915	409443	Serine/Threonine-protein phosphatase 2A activator (LOC409443)
GB43459	408716	Histone-lysine N-methyltransferase trithorax (LOC408716)
GB42489	724660	Protein furry (LOC724660)
GB50853	410887	Myotubularin-related protein 13 (LOC410887)
GB44183	726254	E3 ubiquitin-protein ligase HECTD1 (LOC726254)
GB48530	551566	Uncharacterized LOC551566 (LOC551566)
GB47045	551408	Uncharacterized LOC551408 (LOC551408)
GB47046	551347	UPF0605 protein CG18335-like (LOC551347)
GB46795	413021	Papilin (LOC413021)
GB50772	100578420	Uncharacterized LOC100578420 (LOC100578420)
GB55387	724254	Lilliputian (Lilli)
GB49902	725099	Spermidine synthase (LOC725099)
GB55781	551170	dumpy(LOC551170)
GB50690	726456	Unconventional myosin-Va (LOC726456)
GB40498	552358	Low-density lipoprotein receptor-related protein 2 (LOC552358)
GB40010	551319	Titin-like (LOC551319)
GB54649	725503	Uncharacterized LOC725503(LOC725503)
GB52346	724599	Chromodomain-helicase-DNA-binding protein 1 (LOC724599)
GB47113	724402	Uncharacterized LOC724402 (LOC724402)
GB54288	412508	Nucleoprotein TPR(LOC412508)
GB46345	409032	Peptide-N(4)-(N-acetyl-beta-glucosaminyl)asparagine amidase (LOC409032)
GB42787	100576271	Dentin sialophosphoprotein-like(LOC100576271)
GB43238	408335	Nuclear pore complex protein Nup98-Nup96 (LOC408335)
GB53908	100576425	Uncharacterized LOC100576425 (LOC100576425)
GB43234	408331	Histone deacetylase 5 (LOC408331)
GB43870	409722	Basement membrane-specific heparan sulfate proteoglycan core protein (LOC409722)
GB42021	409456	AT-rich interactive domain-containing protein 4B (LOC409456)
GB51646	725485	Uncharacterized LOC725485 (LOC725485)
GB47022	406074	Complementary sex determiner (Csd)
GB47584	409655	Sorbin and SH3 domain-containing protein 1 (LOC409655)
GB55646	410831	Casein kinase I (LOC410831)
GB47100	413372	putative glutamate synthase [NADPH] (LOC413372)
GB52253	409242	Protein PRRC2C-like (LOC409242)
GB50357	550716	Clathrin heavy chain (LOC550716)
GB48355	410237	Uncharacterized LOC410237 (LOC410237)
GB47146	413382	SAM and SH3 domain-containing protein 1 (LOC413382)
GB50510	409674	Uncharacterized LOC409674 (LOC409674)
GB47026	408733	Uncharacterized LOC408733 (LOC408733)
GB54593	726262	Adenylate cyclase type 8-like (LOC726262)
GB55483	409821	Twitchin (LOC409821)
GB54198	409902	Liprin-alpha-1 (LOC409902)
GB46614	413310	Protein-methionine sulfoxide oxidase Mical (LOC413310)
GB40504	408957	Uncharacterized LOC408957 (LOC408957)
GB41680	724159	Histone acetyltransferase KAT6B (LOC724159)
GB54507	408961	Apolipophorins (LOC408961)

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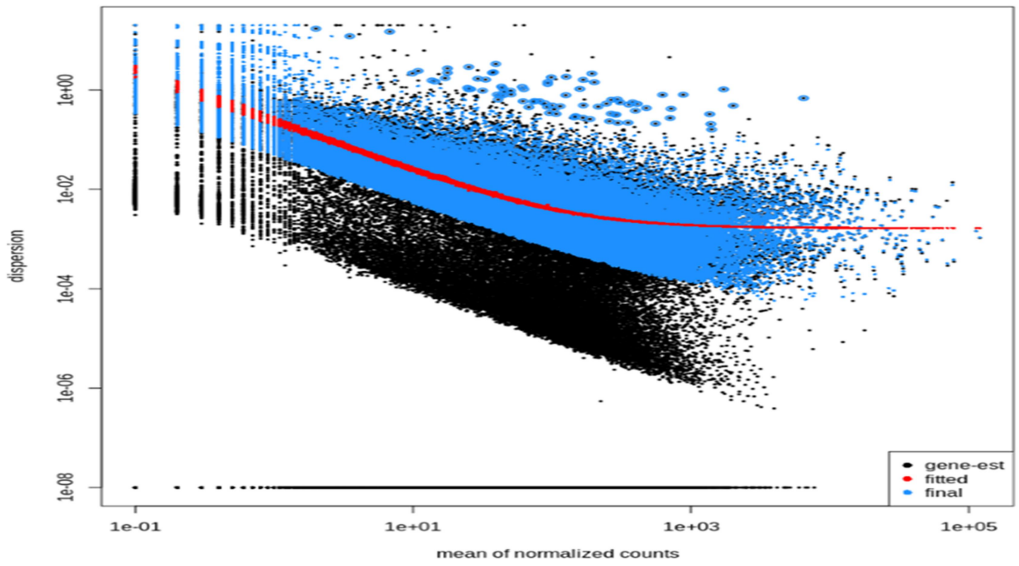
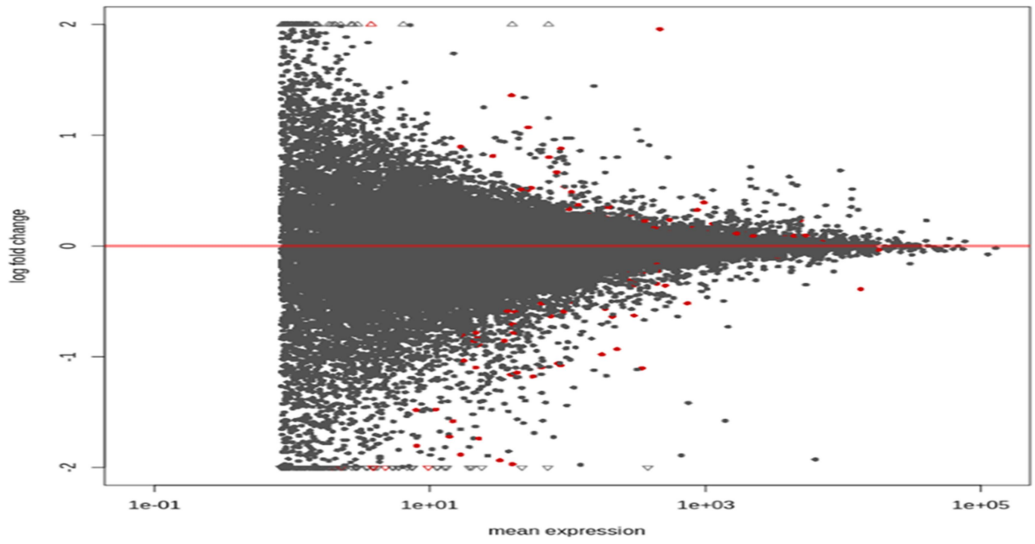


(A)



(B)

Figure 3. MA plot produced for exon usage differences between these two groups of queen honeybee. (A) Shrinkage estimation of dispersion plots provided a visual means of examining dispersion estimates of genomic features relative to average expression strength. The x-axis shows the mean normalized read counts and the y-axis shows log 2 fold changes. Black points represent per gene gene-wise maximum likelihood estimates. The red curve represents the overall trend of the dispersion to mean dependence. Blue points represent gene-wise estimates following shrinkage towards the mean. (B) The X-axis shows the mean expression and the Y-axis shows log 2 fold changes. Points in red show significant DEUs. (Padj< 0.05).

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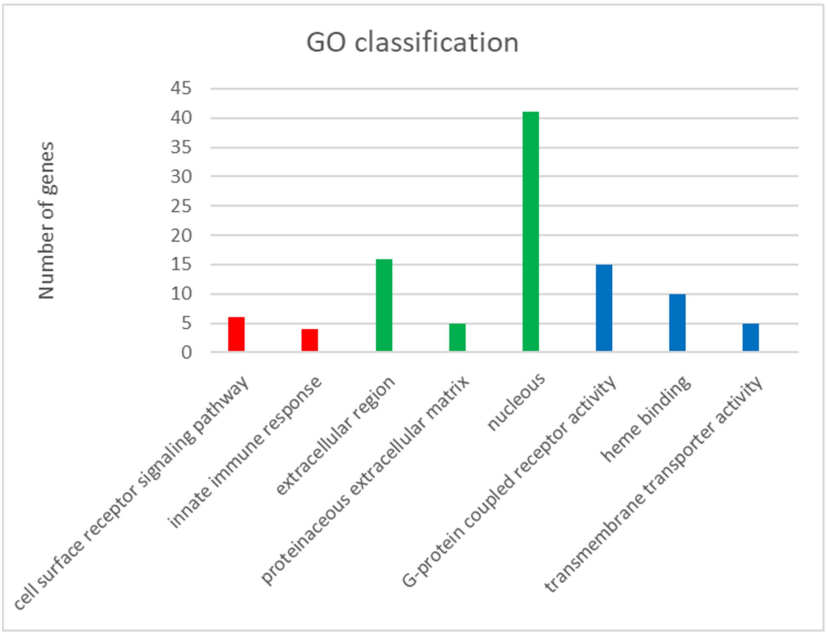

Figure 4. Summary of Gene Ontology (GO) enriched terms are such as biological processes, cellular components, and molecular functions (medium stringency, P-value< 0.05) are shown in red, green, and blue color, respectively.

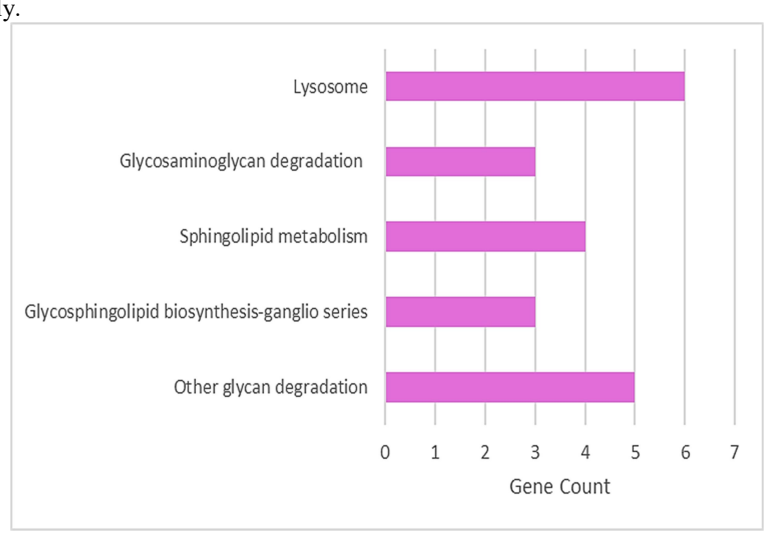

Figure 5. KEGG pathway enrichment analysis of DEGs in two groups of queen honey bees.

BC values as shown in Table 2. Here, LOC552504 (titin-like) occupied the center of the backbone network, having the largest degree and highest BC, which suggests that LOC552504 (titin-like) could be considered as a super-hub gene (Node degree= 64, Betweenness centrality= 0.33).

DISCUSSION

Mating is well known to induce extensive behavioral and physiological changes in animals of many taxa. RNA sequencing (RNA-Seq) is one of the most commonly used techniques in life sciences. The aim of

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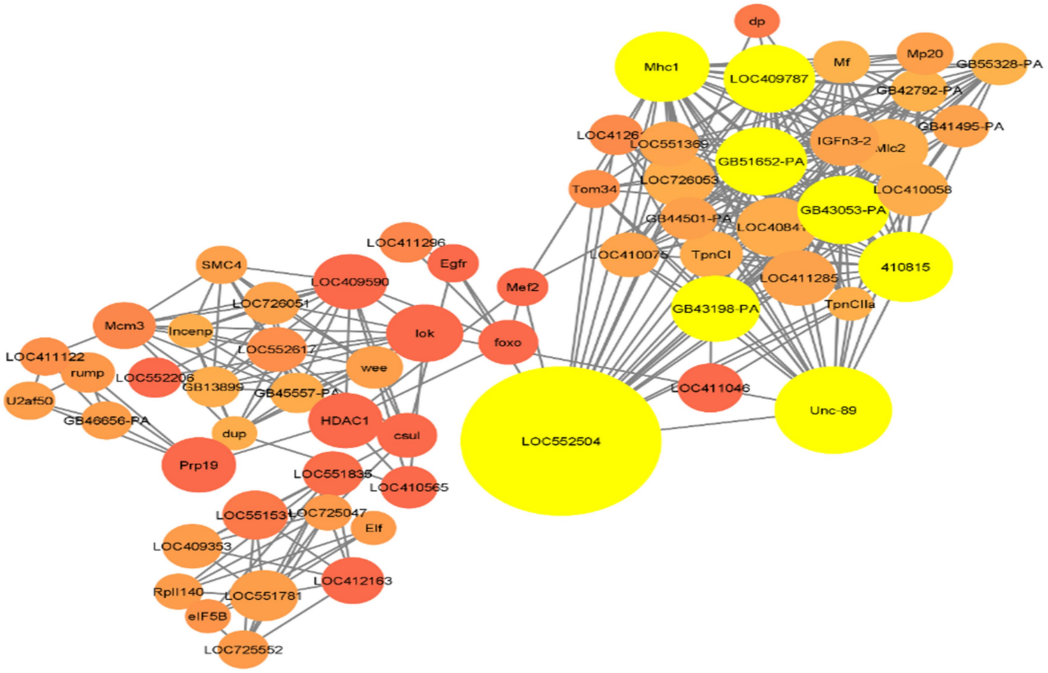

Figure 6. Overview of PPI Network constructed using Cytoscape. The size of the nodes refers to the gene degree and their color from orange to red, indicates an increase in their value in betweenness centrality (The depth of the color denotes the change degree). The node with yellow colors represents the key genes in the network with cut off value node degree > 25. Among key genes, *LOC552504* gene is superhub gene with highest betweenness centrality and node degree.

Table 2. Key genes selected based on topological parameters like BC and degree (with cut off value node degree > 25 and cut off value node betweenness centrality > 0.01).

Gene	Degree	Betweenness centrality
LOC552504 (Titin-like)	64	0.33
Unc-89	35	0.059
Mhc1	27	0.03
GB51652-PA	26	0.013
GB43198-PA	25	0.015

this study was to identify differentially expression genes and differentially expression exons related to mating process in honeybee queen brain. We tried to investigate key DEGs and pathways associated with mating process from brain tissue. We used RNA-Seq data to analyze transcriptome differences in the brain tissue of *Apis mellifera carnica*. Our study cataloged the collection of expressed genes in brain tissue of queen honeybee and reported significant changes among the queen bee injected with semen *vs* virgin queen from each other, following GO classification, KEGG pathway enrichment and network construction analysis. We tested major prediction that are significant changes to the expression of genes following mating in honeybee queen injected with semen vs virgin queen. Gene expression in the brains of queens injected with semen differs strongly from that seen in virgins.

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The mode and nature of gene expression profiles in the two groups are markedly different. Overall, we found a significant degree of overlap between this study and previous studies of transcriptional changes associated with mating in *Apis mellifera* (Kocher *et al*., 2008 and 2010; Manfredini *et al*., 2015). GB41428 (Def1) that was significantly differentially expressed in our study and in Kocher *et al.* (2008); Kocher *et al.* (2010) and Manfredini *et al.* (2015), can be the best candidate gene for mating process and reproductive activation. The defensin gene was up-regulated in virgin queens, like in other studies (Kocher *et al.*, 2008; Manfredini *et al.*, 2015), although it was up-regulated in mated queens in the study of Kocher *et al.* (2010).

Honeybee defensins have enough broad-spectrum antibacterial activity. The cytotoxic activity of defensins against gram-positive bacteria and several species of gram-negative bacteria is known. Defensin 1, contained in royal jelly and honey and synthesized in the salivary glands, is involved in the formation of the social immunity of colonies (Ilyasov *et al*., 2013).

In insects, the biological activity of defensin is directed toward the protection against infectious diseases. The high expression of defensin in leaf-cutting ants indicated that these ants invest in specific immune defenses for pathogen protection in organs that store sperm (Chérasse and Aron 2018).

The expression pattern of defensin differed across studies; in our study, defensin was reduced after mating, so that some energy could be diverted from the immune system to reproductive activity.

GO and KEGG pathway enrichment analysis was performed by DAVID online software to explore the biological functions of DEGs. GO enrichment analysis indicated that the identified DEGs were mainly enriched in the GPCRs and immune process. GO terms related to immune functions and response to other organism are overrepresented across studies (Kocher *et al.*, 2008 and 2010; Niño *et al.*, 2011; Manfredini *et al.*, 2015). Importantly, changes in pathways related to metabolism and immunity match expectations from known behavioral changes. These results partially corroborate previous studies, and provide new insight into the molecular regulation of key behavioral transitions in honeybee queens. In our study, about 15 genes were associated with G Protein-Coupled Receptors (GPCRs). Of all insect GPCRs, the neurohormone (neuropeptide, protein hormone, biogenic amine) GPCRs are especially important, because they, together with their ligands, occupy a high hierarchic position in the physiology of insects and steer crucial processes such as development, reproduction, and behavior (Hauser *et al.*, 2006). We identified candidate GPCRs involved in mating, for example: D2-like Dopamine receptor (Dop3). In our study, the expression of this gene was higher in the virgin queens. In queens, dopamine and reproductive status are negatively correlated and dopamine levels decrease after mating (K-i *et al.*, 2005). Our study provides further support for this reversed relationship in queens, as Dop3 was down regulated in queens injected with semen. Dopamine signaling pathways are positively associated with reproductive status in workers (Harris *et al.*, 1996; Koywiwattrakul *et al.*, 2005; Vergoz *et al.*, 2012).

Alternative Splicing (AS) is a means of expressing several or many different transcripts from the same genomic DNA and results from the inclusion of a subset of the available exons for a particular protein. By excluding one or more exons, certain protein domains may be lost from the encoded protein, which can result in protein function damage or gain. In our study, GB55396 gene has the most differences exon usage, the function of which is unknown. We identified 79 genes (Supplementary File 2) with significant differential exon usage related to mating process. Most of these genes are unknown and need further study. Consistently, we did not find significant

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differential expression of gene transcript isoforms.

In summary, mating in queen honeybee induces rapid and permanent changes in behavior, physiology, and gene expression. In these early post-mating stages, there are no significant differences in flight behavior, pheromone production or ovary activation between saline and semen inseminated queens (Kocher *et al*., 2010), suggesting that insemination volume, not substance, is the main factor that triggers these post-mating changes. However, we did find that 971 genes were expressed at significantly different levels in the brains of injected queens with semen into hemocoel, suggesting that the insemination substance into hemocoel does effect brain gene expression, and may have subtle effects on behavior and physiology. Finally, a number of other factors like injecting semen into a hemocell may explain the low-level of congruence between our results and earlier studies. However, our study overlapped a number of genes with previous studies.

CONCLUSIONS

In conclusion, the present study is the first to elucidate a gene expression and exon expression profile in honeybee queen's brain injected with semen into hemocoel. Overall, 13862 differential gene expression (Mean count< 8) were detected between virgin queens and semen queens. Of these, 971genes (P-value< 0.05) were detected to show markedly differential expression in mating process and 79 differentially expressed exon were identified in mating process too, indicating potentially substantial effects in honeybee mating. Next, GO and KEGG pathway analyses revealed that numerous genes of differentially expressed genes were involved in biological regulation and mating related processes. Our study helps to understand the changes in gene expression post-mating, as well as our understanding of the mechanical processes of mating and reproduction of the queen bee, and provides a basis for future studies. This study focused on injecting semen into the hemocoel, which examines post-mating changes of queen bees on *Apis mellifera carnica*, and may be a promising field of research in other species. Future studies need to focus on determining the mechanisms how semen injection into insect hemocoel alters gene expression like natural mating.

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تجزیه و تحلیل RNA-Seq بیان ژن افتراقی و اثرات خاص اگزون مرتبط با فرآیند جفت‌گیری در زنبور عسل ملکه

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چکیده

فرآیند جفت‌گیری در زنبورهای عسل نه تنها در رفتار و فیزیولوژی آن‌ها، بلکه در سطح رونویسی ژن‌ها نیز تغییرات چشمگیری ایجاد می‌کند. برای تعیین مکانیسم‌های مولکولی تنظیم‌کننده تغییرات رفتاری پس از

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جفت‌گیری، الگوی بیان ژن و بیان خاص اگزون ملکه زنبور عسل باکره را در مقابل ملکه زنبور عسل تزریق شده با مایع منی داخل هموسل بررسی کردیم. از بستهDESeq2 نرم افزار R برای شناسایی ژن‌هایی با بیان متفاوت (DEGs)استفاده شد. DEGها برای تجزیه و تحلیل غنی‌سازی عملکردی و ساخت شبکه تعامل پروتئین-پروتئین (PPI) انتخاب شدند. ما همچنین تجزیه و تحلیل کاربرد افتراقی اگزون را با استفاده از بسته DEXseqنرم افزار R انجام دادیم. نتایج یک بیان معنی‌دار (FDR <0.05) از مجموع ۹۷۱ ژن را بین دو گروه از حشرات شناسایی کرد. فرآیند جفت‌گیری تغییرات قابل‌توجه‌ای در بیان مسیر سیگنال‌دهی گیرنده سطح سلول، پاسخ ایمنی ذاتی، ناحیه خارج سلولی، ماتریکس خارج سلولی پروتئینی، هسته، فعالیت گیرنده جفت‌شده با پروتئینG ، اتصال به هم و ژن‌های فعالیت ناقل بین غشایی ایجاد کرد. شبکه تعامل پروتئین-پروتئین (PPI) نشان داد که ژن LOC552504 (شبه تیتین) می‌تواند به عنوان یک ژن سوپرهاب در فرآیند جفت‌گیری ملکه زنبور عسل در نظر گرفته شود. علاوه بر این، ما اگزون‌هایی را شناسایی کردیم که به طور متفاوت در دو گروه ملکه زنبور عسل بیان شدند. در FDR ۱۰ درصد ، ما کاربرد افتراقی اگزون را در ۷۹ ژن پیدا کردیم. در این میان ژن GB55396 بیشترین تفاوت را در استفاده از اگزون دارد و می‌تواند بهترین ژن کاندیدا برای جفت‌گیری و فعال‌سازی تولید مثل در ملکه زنبور عسل باشد.

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