Highlights

- Transcriptome of poultry red mites (PRMs) are greatly altered by blood feeding.
- In starved PRMs, biological processes are widely suppressed.
- Energy synthesis and metabolic processes are highly active in blood-fed PRMs.

Abstract

Poultry red mites (*Dermanyssus gallinae*, PRMs) are noxious, blood-feeding ectoparasites that threaten laying hens' farming worldwide. As reported in other arthropods, such as ticks, their gene expression patterns are drastically changed by blood feeding. Thus, it is important to compare the transcriptomes of blood-fed and starved states in order to better understand the biological features of blood-feeding parasites. Here, we examined the gene expression patterns of blood-fed and starved PRMs, and investigated the dynamics of their physiologies based on their transcriptomes. We found that the expressions of 2,154 genes were markedly upregulated after blood feeding, while those of 4,175 genes were downregulated. Gene ontology and metabolic pathway enrichment analyses revealed that the genes associated with the regulation of biological processes were highly expressed in starved PRMs, while their expression was reduced in a blood-fed state. In contrast, the expression of genes involved in adenosine triphosphate synthetic processes was much higher in blood-fed PRMs, suggesting that blood feeding promotes several metabolic processes, especially energy synthesis, to facilitate molting and reproduction. This study may help better understand the biological characteristics associated with the blood feeding stages of PRMs and be applicable to the development of novel PRMs control strategies.

Keywords

Transcriptomics; *Dermanyssus gallinae*; Blood feeding

Original Article

Transcriptome dynamics of blood-fed and starved poultry red mites, *Dermanyssus gallinae*

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Running head: Transcriptome analysis of the poultry red mites

Abbreviations

PRM, Poultry red mite; Contigs, Contiguous sequences; FPKM, fragments per kilobase of exon per million reads mapped; FDR, False discovery rate; log_2CPM, Logarithmic values of counts per millions; GO, Gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; IPR, InterProScan; CC, Cellular component; MF, Molecular function; BP, Biological process.

1. Introduction

Poultry red mites (*Dermanyssus gallinae*, PRMs) are one of the most harmful ectoparasites of the avian species, including chickens. Currently, PRMs are found across various countries and regions, especially in European countries, Asian countries such as China, Iran, and Japan, and Brazil [1-3]. Mass infestation by PRMs causes various harmful effects in chickens, such as anemia, hyposthenia, reduction in egg production and egg quality, sometimes resulting in death. Furthermore, it has been suggested that the PRM may be a vector for several pathogens, such as the Avipox virus, the avian influenza virus, *Salmonella* Gallinarum, and *Erysipelothrix rhusiopathiae* [4-7]. The annual economic burden linked to production loss and associated with the potential control of PRMs was estimated to be €130 million in the EU in 2005 [8], and €66.85 million in Japan [9, 10]. In addition, it has also been found that PRMs sometimes infest mammalian species, including humans, causing pruritus, skin lesions, and dermatitis [11, 12]. Therefore, the invasion of PRMs is a serious issue in both the spheres of public health and veterinary hygiene. The current preventive strategies against PRMs mainly depend on the thorough cleaning and use of acaricides, such as organic phosphorus, carbamates, and pyrethroids. However, as PRMs are nocturnal and hide in the cracks of cages during the daytime [13], they may survive acaricide treatment. Moreover, the emergence of acaricide-resistant PRMs has been reported [14-16], indicating that the establishment of an alternative control method for the management PRMs is desperately required.

PRMs require blood feeding for development in the latter three stages of their lifecycles, namely, protonymph, deutonymph, and adult [17]. In addition, female PRMs feed on blood repeatedly throughout their lives for egg development [13], evoking the idea that blood feeding may change PRMs' physiologies, including their metabolism. Importantly, for ticks, which are biologically related to PRMs, blood feeding has been shown to dramatically alter their gene expression patterns. In the salivary gland and the midgut of *Ixodes ricinus*, gene expression patterns were greatly changed after blood feeding [18]. Moreover, in the midgut of *Ornithodoros erraticus* and *Haemaphysalis flava*, the expression of genes associated with the metabolic processes of several molecules, as well as with cell growth, were significantly upregulated after blood feeding, while gene transcription and translation were suppressed [19, 20]. Thus, the comparative analysis of the transcriptomes of blood-fed and starved states is necessary to further understand the biological characteristics of hematophagous parasites. In a previous study, researchers have performed a transcriptome analysis of PRMs by RNA sequencing, using a mixture of RNA samples, regardless of stages, sexes, and feeding states, revealing that some metabolic pathways were highly active [21]. However, the difference in gene expression patterns between blood-fed and starved PRMs has yet to be elucidated, despite its importance.

In this study, to examine the dynamics of gene expression patterns in response to blood feeding, we performed an RNA sequencing analysis in each of the PRM's states in order to compare their gene

expression patterns and predict physiological states such as metabolism. We found that the transcriptomic profile was greatly altered by blood feeding, and gene ontology (GO) and metabolic pathway enrichment analyses revealed that blood feeding promotes some metabolic processes, especially energy synthesis, to promote growth and reproduction in PRMs. This study may inform our understanding of the biological characteristics of the blood feeding stages of PRMs and be applicable to the development of novel PRM control strategies.

2. Materials and methods

2.1. PRMs collection and total RNA isolation

Mixed growth stages and sexes of PRMs were obtained from an egg-laying farm in Japan. PRMs were kept in a TubeSpin Bioreactor 600 (TPP Techno Plastic Products AG, Trasadingen, Switzerland). Some of the dark red, round PRMs were designated as “blood-fed PRMs” on morphological observation and were collected in 1,200 μL extra-long filter tips (WATSON Bio Lab, Tokyo, Japan) within 2 days of sample collection. The residual PRMs were maintained at 25 °C in 70 % humidity for a 2-week period, and designated “starved PRMs”. Starved PRMs were collected in the same manner as blood-fed PRMs. Both mite-samples included the blood feeding stages of PRMs, namely protonymphs, deutonymphs, and adults, and did not include eggs and larvae. All collected PRMs were stored at -80 °C until use.

About 500 PRMs from each sample were suspended with 1 mL of phosphate-buffered saline and homogenized thoroughly using a 1.5-mL Homogenization Pestle for a 1.5-mL Microcentrifuge Tube (Scientific Specialties, Inc., Lodi, CA, USA) in 600 μL of Buffer RLT Plus (RNeasy Plus Mini Kit, Qiagen, Hilden, Germany). Total RNA was isolated using an RNeasy Plus Mini Kit according to the manufacturer’s instructions.

2.2. RNA-Seq

RNA sequencing was performed using the total isolated RNA samples, as described above. All the following procedures were conducted at Hokkaido System Science Co., Ltd. (Hokkaido, Japan).

2.2.1. Preparation of cDNA library and sequencing

The qualities of the total RNA samples were confirmed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Böblingen, Germany). Next, a cDNA library was constructed using a TruSeq Stranded mRNA Sample Prep Kit (Illumina, Inc., San Diego, CA, USA), according to the standard protocol. Paired-end sequencing was performed using the HiSeq™ (Illumina, Inc., San Diego, CA, USA), according to the manufacturers' instructions.

2.2.2. Sequence assembly

The adaptor sequences which were added to construct the cDNA library (5´-AGA TCG GAA GAG CAC ACG TCT GAA CTC CAG TCA C-3´, 5´-AGA TCG GAA GAG CGTCGT GTA GGG AAA GAG TGT-3´) were trimmed from raw reads using cutadapt software v1.1 (https://cutadapt.readthedocs.org/en/stable/). The read sequences which indicated low quality values (QV) were further trimmed using Trimmomatic software v0.32 (http://www.usadellab.org/cms/index.php?page=trimmomatic). The regions in which QVs were lower than 20 were trimmed, and the read sequences that had more than 50 bp were used for further

101 analyses. The trimmed reads were assembled to create contiguous sequences (contigs) using Trinity
102 software v2.4.0 (https://github.com/trinityrnaseq/trinityrnaseq/wiki). *De novo* assembly was
103 performed using the read sequences obtained from both mite-samples. Raw sequence data were
104 deposited in DDBJ/ENA/GeneBank (BioSample accessions: SAMD00228960, SAMD00229086)
105
106 2.2.3. Expression analysis
107 The expression intensities of each contig were analyzed and compared between blood-fed and
108 starved PRMs based on the Trinity assembly and analysis pipeline [22]. The trimmed reads were
109 mapped to the contigs described above using Bowtie2 software v2.3.2 (http://bowtie-
110 bio.sourceforge.net/bowtie2/index.shtml). In this process, the trimmed reads were also mapped to the
111 genome sequence of the chicken (*Gallus gallus*, NCBI:txid9031), and chicken-derived contigs were
112 excluded. Next, the expected counts and expression intensities (fragments per kilobase of exon per
113 million reads mapped; FPKM) of these contigs were calculated both in blood-fed and starved PRMs
114 using RSEM package v1.3.0 for R (http://deweylab.biostat.wisc.edu/rsem/). These expression
115 intensities were compared between blood-fed and starved PRMs were conducted using edgeR
116 package v3.8.6 for R (http://bioinf.wehi.edu.au/edgeR/). The *P*-value and false discovery rate (FDR)
117 were adjusted using the edgeR package to estimate statistical significances.
118

2.2.4. Functional annotation

The contigs were translated into amino acid sequences using the ‘getorf’ program from the EMBOSS package v6.6.0 (http://emboss.sourceforge.net/). These amino acid sequences were applied to the InterProScan program v5.32-71.0 (http://bioinf.wehi.edu.au/edgeR/), and functional annotations based on their amino acid motifs and domains were added to each contig. The contigs, which could be considered as virus-derived products based on the InterProScan (IPR) annotation, were then excluded.

2.3. Bioinformatic analysis

To minimize the rate of false positives, the genes with a logarithmic value of their counts per millions (log_2CPM, outputted by edgeR package) less than 1.0 were excluded from analyses. A Gene ontology (GO) enrichment and metabolic pathway enrichment analyses were performed using the contigs, except for virus-derived genes and ribosomal RNA genes, in accordance with Kyoto Encyclopedia of Genes and Genomes (KEGG) [23] and Shiny GO v0.61 (http://bioinformatics.sdstate.edu/go/), based on each transcript’s IPR annotation, respectively.

3. Results and Discussion

3.1. Sequencing and *de novo* assembly

The numbers of reads obtained by sequencing are summarized in Supplemental Table 1. In the present study, 168,996,962 reads from blood-fed PRMs and 165,252,914 reads from starved PRMs were obtained. After read trimming, 167,594,546 reads (99.2 %) from blood-fed PRMs and 163,337,310 reads (98.8 %) from starved PRMs were acquired. To prevent contamination from host-derived genes, the trimmed reads of blood-fed PRMs were mapped to the chicken genome sequence, and 86,194 reads (0.05 %) were excluded, considered chicken-derived transcripts. *De novo* sequence assembly using the trimmed reads from both states of PRMs yielded 162,263 contiguous sequences (contigs). The average size of contigs was 1,013 bp (Supplemental Table 2), and 92.12 % in the raw and 87.75 % were mapped to the assembled contigs, respectively (Supplemental Table 1).

3.2. Expression analysis and functional annotation

Among the obtained contigs, 40,642 of the acquired contigs (24.9 %) indicated intensities, of which logarithmic values of counts per millions ($\log_2$CPM) were no less than 0 (Supplemental Table 3). In blood-fed PRMs, 37,900 genes were highly expressed, while the expression of 36,632 genes was observed in starved PRMs. Among them, 1,268 genes (3.1 %) were expressed only in blood-fed PRMs, whereas 2,742 genes (6.7 %) were only detected in starved PRMs. All contigs were translated

153 into their deduced amino acid sequences and functionally annotated based on their amino acid motifs
154 and domains. In total, 36,785 contigs (22.7 %) were characterized for their functions, and 19,666
155 contigs (53.3 %) were expressed with measurable intensities ($\log_2$CPM $\geq$ 0). After removing the
156 contigs derived from viruses and ribosomal RNA genes, 19,114 genes were acquired and designed
157 for further analyses. The comparison of gene expression between blood-fed and starved PRMs
158 revealed that the expressions of 2,154 genes were significantly upregulated in blood-fed PRMs,
159 whereas the expression intensities of 4,175 genes were much higher in starved PRMs (Figure 1).
160
161 3.3. GO and metabolic pathway enrichment analysis
162 The PRM genes with significant differences in expression intensities between each state were
163 applied to GO and metabolic pathway enrichment analyses. Each gene was classified into three GO
164 categories, consisting of cellular components (CC), molecular function (MF), and biological
165 processes (BP). Within the CC and MF GO terms, the GO terms annotated with high frequency were
166 found to be similar between each PRM state, although there were some differences in the order of
167 enrichment false discovery rate (FDR, Tables 1 and 2). The top 5 CC terms were ‘Intracellular
168 organelle’, ‘Organelle’, ‘Membrane-bounded organelle’, and ‘Cytoplasm’ in blood-fed PRMs. These
169 were identical to those in starved PRMs, except for the difference in the order of the top two (Table
170 1). In blood-fed PRMs, the majority of annotated MF terms corresponded to ‘Ion binding’,

171 ‘Heterocyclic compound binding’, ‘Organic cyclic compound binding’, ‘Protein binding’ and
172 ‘Transferase activity’, whereas the top category was ‘Protein binding’ in starved PRMs, followed by
173 ‘Ion binding’, ‘Organic cyclic compound binding’, ‘Heterocyclic compound binding’, and ‘Anion
174 binding’ (Table 2). Consistent with the results of a previous study on the midgut transcriptome of
175 *Haemaphysalis flava* [20], the genes associated with metal ion binding were more frequently
176 expressed in blood-fed PRMs, suggesting either that the transport or uptake of host-derived metal
177 ions, such as ferrous or copper ion, were facilitated, or that PRMs responded to pro-oxidant
178 molecules, such as iron, which is released during blood digestion to reduce oxidative tissue damage
179 [24].
180 Outstanding differences were found in the BP category (Table 3). In blood-fed PRMs, the top 3
181 terms were ‘Cellular nitrogen compound metabolic process’, ‘Heterocycle metabolic process’, and
182 ‘Organic cyclic compound metabolic process’. Meanwhile, in starved PRMs, the terms ‘Biological
183 regulation’, ‘Regulation of biological process’, and ‘Regulation of cellular process’ comprised the
184 majority of the annotated terms. In addition, within the top 30 terms in starved PRMs, the next 5
185 terms which were associated with the regulation of metabolism and other biological activities were
186 included, while these were not assigned to the terms in blood-fed PRMs (Table 3). Although PRMs
187 are dependent on nutrition for the development and reproduction exclusively on blood meal [17],
188 they can survive for long periods without blood feeding [25]. Our data suggest that in a starved state,

189 biological processes, including metabolism, are restrained in order to save energy to maintain life in
190 the absence of sufficient nutrition. We also performed a metabolic pathway enrichment analysis by
191 mapping the contigs to the KEGG pathways [23]. The most major pathway was that of ‘Metabolic
192 pathways’, both in blood-fed and starved PRMs, followed by ‘Oxidative phosphorylation’ and
193 ‘Carbon metabolism’ in blood-fed PRMs, and ‘Endocytosis’ and ‘Purine metabolism’ in starved
194 PRMs (Table 4). The contigs classified into ‘Metabolic pathways’ in Table 4 were ranked according
195 to their expression intensities, and the motifs/domains in the top 15 contigs with high expression
196 intensities were predicted based on the IPR IDs (Supplemental Table 4). In blood-fed PRMs, the
197 contigs involved in the phosphorylation of nucleotide diphosphates and adenosine triphosphate
198 synthesis were found to strongly expressed, suggesting that energy synthesis is highly active in
199 blood-fed PRMs. In contrast, starved PRMs strongly expressed genes associated with
200 gluconeogenesis and the β-oxidation of fatty acids (Supplemental Table 4).
201 To confirm the differences in expression intensities of some genes involved in energy synthesis,
202 gluconeogenesis, and β-oxidation of fatty acids, the expression profiles of some contigs were
203 summarized in Table 5 and visualized in the scatter plot showing the relationship between statistical
204 significance and relative changes in expression intensities (Figure 2). In blood-fed PRMs, the
205 significant upregulation in the expression of contigs involved in energy synthesis was confirmed,
206 whereas the expression of the contigs involved in gluconeogenesis and β-oxidation of fatty acids was

found to be downregulated. In this study, the possibility that environmental contaminants or dirt on the mite surface may have resulted in biased results could not be excluded. Although the expression intensities of these genes derived from such contaminants was not significant (such as that of genes listed in Supplemental Table 4), we should be aware of this possibility for the application of our data. In addition, our data may include the transcriptomes from microbial symbionts. The population of these symbionts would also be altered by blood feeding, and these symbionts play important roles in arthropods' physiology [26, 27]. However, we applied Poly (A) – RNA for the RNA-Seq analysis; the transcripts derived from prokaryotes, including bacteria, were then largely removed from our data. Therefore, the transcripts derived from prokaryotes will not significantly alter the results, although they cannot be completely excluded. In addition, we analyzed single samples from both starved and blood-fed PRMs in this study. To validate our data, analyses using multiple samples would be desirable, and the establishment of a quantitative reverse transcription polymerase chain reaction (qRT-PCR) method to quantify the expression levels of the PRM transcripts is required. Taken together, physiological conditions for the growth of PRMs, especially energy synthesis and metabolic pathways, were found to be drastically different between different feeding states.

4. Conclusion

Blood meal is the only source of nutrients for the development and reproduction of PRMs.

Therefore, the dynamics of their biological status in response to blood feeding is crucial information for better understanding their physiology. In this study, the comprehensive PRMs' transcriptome in each feeding state was analyzed and compared. Here, we revealed that the expression of genes related to metabolism and energy synthesis were significantly higher in blood-fed PRMs. On the contrary, genes involved in the regulation of biological processes, gluconeogenesis, and fatty acid oxidation were markedly expressed in starved PRMs, which were not described in the previous report [21]. These data provide valuable information for investigating the targets of alternative PRM control strategies, as well as for exploring novel molting, blood digestion, and embryogenesis pathways.

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CRediT authorship contribution statement

Sotaro Fujisawa: Methodology, Investigation, Writing - original draft. **Shiro Murata:** Methodology, Investigation, Writing - review & editing, Resources, Project administration, Funding acquisition. **Masayoshi Isezaki:** Investigation. **Eiji Oishi:** Writing - review & editing. **Akira Taneno:** Resources, Writing - review & editing. **Naoya Maekawa:** Investigation, Resources. **Tomohiro Okagawa:** Investigation, Resources. **Satoru Konnai:** Resources, Writing - review & editing. **Kazuhiko Ohashi:** Resources, Writing - review & editing, Funding acquisition.

Declaration of interest

The authors have no conflicts of interest to declare.

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Data availability statement

The datasets supporting the conclusions of this article are available from the corresponding author upon reasonable request.

Appendix A. Supplemental data

The following are the supplemental data to this article:

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Fig. 1. Differences in gene expression patterns between blood-fed and starved poultry red mites (PRMs)

The differences in gene expression pattern between blood-fed and starved PRMs were visualized, by displaying the fold change versus the averages of expression intensities (MA plot). The orange dots indicate the genes expressed in blood-fed PRMs while the blue dots show those expressed in starved PRMs, representing a total of 2,154 and 4,175, respectively. The false discovery rate cut-off value was set to 0.01.

Fig. 2. Dynamics of gene expression patterns in response to blood feeding

The relationship between the statistical significances (false discovery rate, FDR) and the fold change was visualized. The gray dots indicate the genes with significant differences in gene expression. The orange dots indicate the representative genes involved in energy synthesis, whereas the blue dots reflect the representative genes associated with gluconeogenesis and b-oxidation. The FDR cut-off value was set to 0.01. NDKB: nucleoside diphosphate kinase B, ATPSG: ATP synthase subunit g, ATPSCF: ATP synthase-coupling factor, UCCR: ubiquinol-cytochrome C reductase, GAPDH: glyceraldehyde-3-phosphate dehydrogenase, PPCK: phosphoenolpyruvate carboxykinase, IBCD: isobutyryl-CoA dehydrogenase, I5MD1: inosine-5'-monophosphate dehydrogenase 1.

Fig. 1

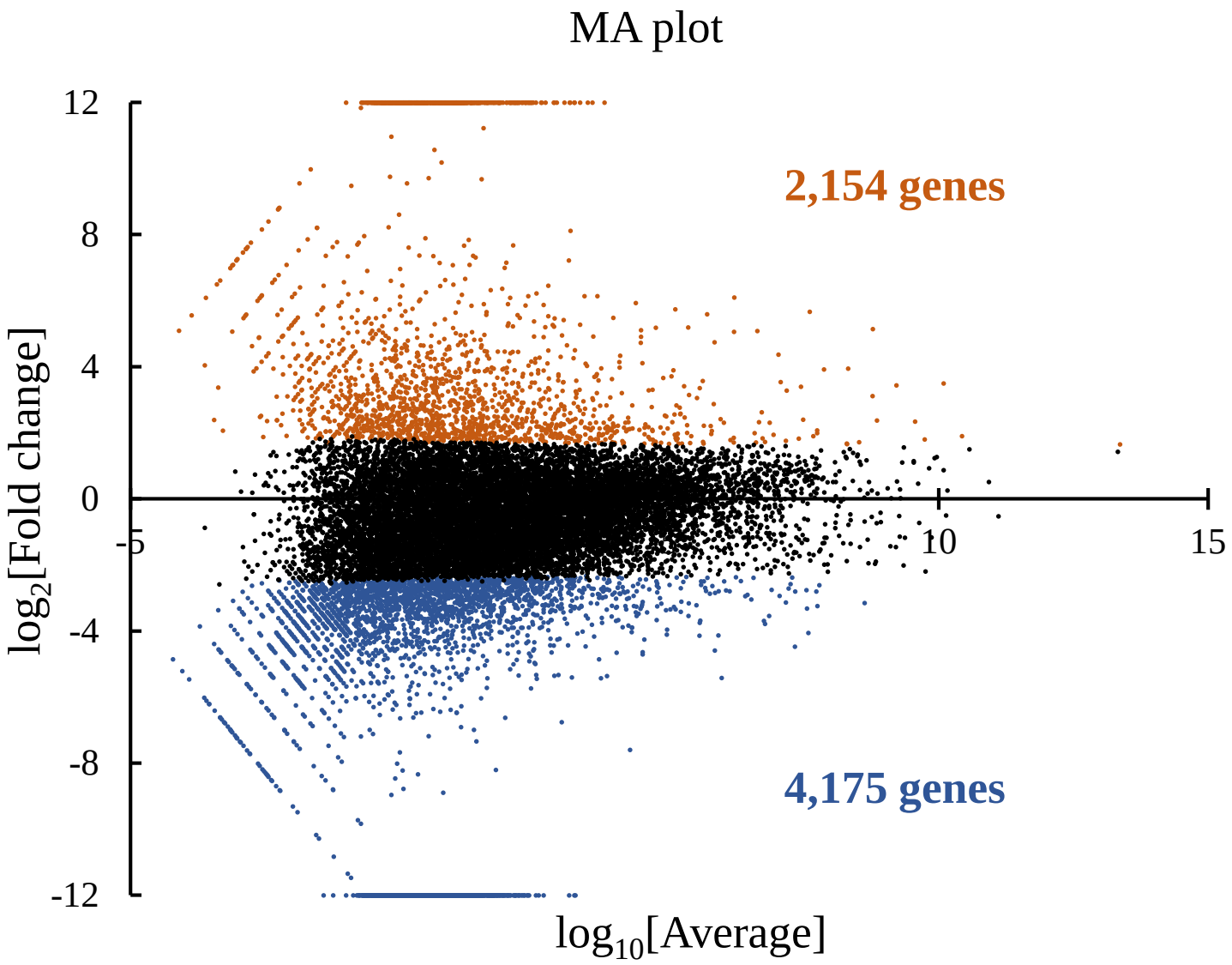
MA plot
2,154 genes
4,175 genes
12
8
4
0
-4
-8
-12
-5
10
15
log2[Fold change]
log10[Average]

Fig. 2

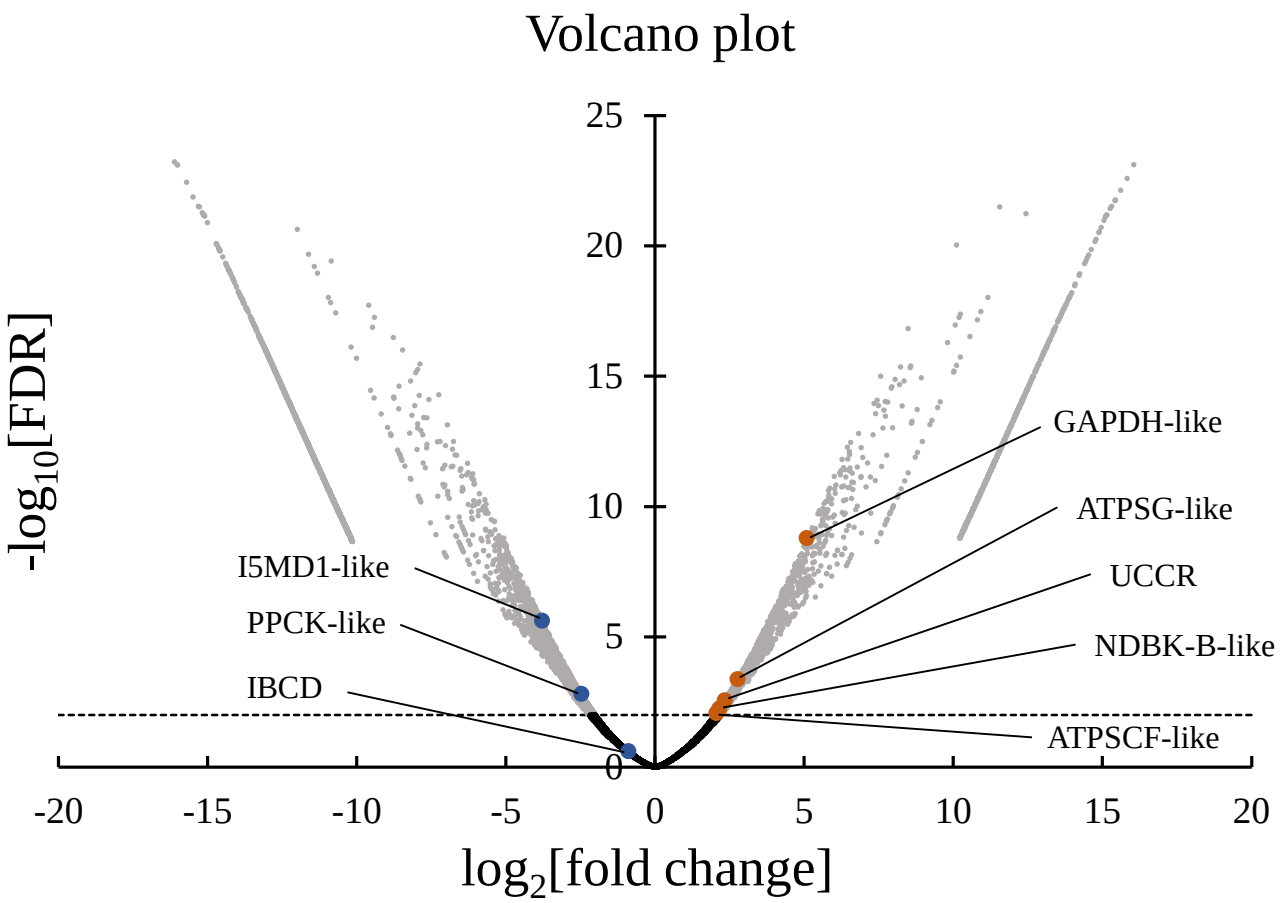
Volcano plot
25
20
15
10
5
0
-20
-15
-10
-5
0
5
10
15
20
-log10[FDR]
log2[fold change]
GAPDH-like
ATPSG-like
UCCR
NDBK-B-like
ATPSCF-like
I5MD1-like
PPCK-like
IBCD

Table 1. Top 10 GO categories with respect to “Cellular component”

	Blood-fed mites				Starved mites			
	Enrichment FDR	No. of GO terms associated with each category	Total No. of GO terms classified into each category	Functional Category	Enrichment FDR	No. of GO terms associated with each category	Total No. of GO terms classified into each category	Functional Category
1	7.16E-95	572	4051	Intracellular organelle	1.67E-101	762	4082	Organelle
2	7.66E-95	574	4082	Organelle	1.67E-101	758	4051	Intracellular organelle
3	8.17E-93	533	3645	Membrane-bounded organelle	1.04E-97	701	3645	Membrane-bounded organelle
4	1.34E-91	521	3534	Intracellular membrane-bounded organelle	3.34E-91	674	3534	Intracellular membrane-bounded organelle
5	6.61E-87	499	3380	Cytoplasm	7.22E-88	648	3380	Cytoplasm
6	7.35E-70	375	2361	Cytoplasmic part	7.15E-63	466	2361	Cytoplasmic part
7	7.70E-59	336	2166	Intracellular organelle part	9.11E-58	422	2110	Protein-containing complex
8	1.62E-57	338	2215	Organelle part	1.91E-56	403	1989	Nucleus
9	6.79E-54	321	2110	Protein-containing complex	1.98E-43	396	2166	Intracellular organelle part
10	1.17E-37	276	1989	Nucleus	1.31E-42	400	2215	Organelle part

*GO terms were listed in descending order of statistical significance (FDR value).

Table 2. Top 10 GO categories with respect to “Molecular function”

	Blood-fed mites				Starved mites			
	Enrichment FDR	No. of GO terms associated with each category	Total No. of GO terms classified into each category	Functional Category	Enrichment FDR	No. of GO terms associated with each category	Total No. of GO terms classified into each category	Functional Category
1	1.32E-28	302	2506	Ion binding	5.07E-72	493	2405	Protein binding
2	2.20E-23	346	3251	Heterocyclic compound binding	8.12E-45	444	2506	Ion binding
3	2.74E-23	346	3262	Organic cyclic compound binding	6.43E-33	499	3262	Organic cyclic compound binding
4	8.41E-21	270	2405	Protein binding	8.37E-33	497	3251	Heterocyclic compound binding
5	1.11E-19	247	2168	Transferase activity	1.25E-30	257	1323	Anion binding
6	6.08E-16	166	1342	Small molecule binding	4.46E-30	258	1342	Small molecule binding
7	6.08E-16	161	1284	Metal ion binding	1.87E-28	357	2168	Transferase activity
8	7.30E-16	163	1312	Cation binding	3.10E-24	228	1228	Nucleotide binding
9	2.56E-15	96	609	RNA binding	3.10E-24	228	1228	Nucleoside phosphate binding
10	4.81E-15	153	1228	Nucleotide binding	1.41E-23	237	1312	Cation binding

*GO terms were listed in descending order of statistical significance (FDR value).

Table 3. Top 30 GO categories with respect to "Biological process"

	Blood-fed mites				Starved mites			
	Enrichment FDR	No. of GO terms associated with each category	Total No. of GO terms classified into each category	Functional Category	Enrichment FDR	No. of GO terms associated with each category	Total No. of GO terms classified into each category	Functional Category
1	1.20E-73	407	2601	Cellular nitrogen compound metabolic process	3.45E-88	608	3035	**Biological regulation**
2	1.07E-66	355	2191	Heterocycle metabolic process	6.87E-74	533	2681	**Regulation of biological process**
3	6.05E-66	357	2229	Organic cyclic compound metabolic process	1.68E-65	496	2534	**Regulation of cellular process**
4	3.20E-65	352	2193	Cellular aromatic compound metabolic process	3.10E-65	594	3330	Cellular macromolecule metabolic process
5	6.31E-65	345	2128	Nucleobase-containing compound metabolic process	1.07E-63	500	2601	Cellular nitrogen compound metabolic process
6	3.91E-51	518	4498	Macromolecule metabolic process	1.07E-63	725	4498	Macromolecule metabolic process
7	5.42E-42	403	3330	Cellular macromolecule metabolic process	1.66E-61	442	2191	Heterocycle metabolic process
8	3.88E-41	270	1826	Nucleic acid metabolic process	4.31E-61	446	2229	Organic cyclic compound metabolic process
9	3.96E-39	261	1776	Gene expression	5.09E-61	441	2193	Cellular aromatic compound metabolic process
10	6.27E-39	298	2186	Biosynthetic process	6.25E-60	430	2128	Nucleobase-containing compound metabolic process
11	3.23E-38	293	2149	Organic substance biosynthetic process	2.72E-53	423	2186	Biosynthetic process
12	2.95E-37	286	2096	Cellular biosynthetic process	2.38E-52	416	2149	Organic substance biosynthetic process
13	1.22E-35	404	3554	Organonitrogen compound metabolic process	5.29E-51	406	2096	Cellular biosynthetic process
14	1.65E-32	220	1495	RNA metabolic process	7.53E-49	312	1434	**Regulation of metabolic process**
15	1.20E-28	156	929	Small molecule metabolic process	3.58E-48	317	1480	Cellular component organization
16	4.59E-27	220	1629	Cellular component organization or biogenesis	4.07E-47	355	1776	Gene expression
17	4.72E-27	214	1564	Cellular nitrogen compound biosynthetic process	7.64E-45	297	1387	**Regulation of macromolecule metabolic process**
18	2.65E-25	78	307	Nucleobase-containing small molecule metabolic process	1.06E-44	321	1564	Cellular nitrogen compound biosynthetic process
19	1.97E-24	211	1602	Macromolecule biosynthetic process	1.06E-44	356	1826	Nucleic acid metabolic process
20	4.14E-24	208	1578	Cellular macromolecule biosynthetic process	2.85E-44	270	1206	Organic cyclic compound biosynthetic process
21	3.51E-22	97	498	RNA processing	5.15E-44	310	1495	RNA metabolic process
22	3.63E-22	257	2210	Cellular protein metabolic process	5.94E-44	328	1629	Cellular component organization or biogenesis
23	1.14E-21	76	332	NcRNA metabolic process	1.22E-43	264	1174	Aromatic compound biosynthetic process
24	2.61E-21	67	268	Nucleoside phosphate metabolic process	2.18E-43	562	3554	Organonitrogen compound metabolic process
25	2.70E-21	165	1185	Heterocycle biosynthetic process	2.22E-43	265	1185	Heterocycle biosynthetic process
26	2.70E-21	205	1629	Macromolecule modification	6.23E-43	258	1143	Nucleobase-containing compound biosynthetic process
27	2.70E-21	167	1206	Organic cyclic compound biosynthetic process	1.38E-42	245	1059	Organelle organization
28	1.77E-20	86	430	Organophosphate metabolic process	7.00E-41	279	1321	**Regulation of cellular metabolic process**
29	1.92E-20	65	263	Nucleotide metabolic process	1.17E-40	231	990	**Regulation of gene expression**
30	2.48E-20	37	87	Nucleoside triphosphate metabolic process	3.15E-39	274	1311	**Regulation of primary metabolic process**

*GO terms were listed in descending order of statistical significance (FDR value)..

Table 4. Top 30 functional categories of the predicted KEGG pathways

	Blood-fed mites				Starved mites			
	Enrichment FDR	No. of GO terms associated with each category	Total No. of GO terms classified into each category	Functional Category	Enrichment FDR	No. of GO terms associated with each category	Total No. of GO terms classified into each category	Functional Category
1	5.82E-37	181	1022	Metabolic pathways	1.51E-29	213	1022	Metabolic pathways
2	9.25E-10	25	83	Oxidative phosphorylation	1.89E-09	39	134	Endocytosis
3	2.63E-05	22	109	Carbon metabolism	2.35E-07	28	92	Purine metabolism
4	0.000106	23	129	Protein processing in endoplasmic reticulum	2.31E-06	21	63	FoxO signaling pathway
5	0.000178	6	10	Thiamine metabolism	1.58E-05	29	119	RNA transport
6	0.000231	15	68	Ribosome biogenesis in eukaryotes	1.71E-05	11	22	Starch and sucrose metabolism
7	0.000294	12	47	RNA degradation	1.74E-05	27	109	Carbon metabolism
8	0.000726	10	37	Nucleotide excision repair	1.74E-05	18	56	mRNA surveillance pathway
9	0.000954	11	46	Aminoacyl-tRNA biosynthesis	2.00E-05	11	23	Notch signaling pathway
10	0.001248	9	33	Basal transcription factors	0.000116	20	76	Wnt signaling pathway
11	0.001405	16	92	Purine metabolism	0.000168	12	33	Basal transcription factors
12	0.0017	9	35	Fanconi anemia pathway	0.000168	21	85	Autophagy
13	0.001835	11	52	Pyrimidine metabolism	0.000286	10	25	Galactose metabolism
14	0.001835	8	29	RNA polymerase	0.000642	6	10	Thiamine metabolism
15	0.001835	8	29	Homologous recombination	0.000912	24	117	Peroxisome
16	0.002168	8	30	DNA replication	0.001482	9	25	Base excision repair
17	0.003236	7	25	Galactose metabolism	0.001778	10	31	Propanoate metabolism
18	0.003236	7	25	Base excision repair	0.001973	12	43	Lysine degradation
19	0.005515	17	119	RNA transport	0.002006	18	82	MTOR signaling pathway
20	0.006342	6	21	Terpenoid backbone biosynthesis	0.002881	6	13	One carbon pool by folate
21	0.007869	6	22	Starch and sucrose metabolism	0.003313	12	46	Aminoacyl-tRNA biosynthesis
22	0.007951	10	55	Biosynthesis of amino acids	0.003562	10	35	Citrate cycle (TCA cycle)
23	0.008149	5	16	Fructose and mannose metabolism	0.003562	12	47	Valine, leucine and isoleucine degradation
24	0.008149	7	30	Fatty acid degradation	0.003562	12	47	RNA degradation
25	0.008149	10	56	mRNA surveillance pathway	0.003562	21	109	Spliceosome
26	0.008149	6	23	Notch signaling pathway	0.004292	13	55	Biosynthesis of amino acids
27	0.009113	13	87	Glutathione metabolism	0.004292	10	36	AGE-RAGE signaling pathway in diabetic complications
28	0.009113	11	67	Amino sugar and nucleotide sugar metabolism	0.004952	7	20	Autophagy
29	0.009584	15	109	Spliceosome	0.004952	10	37	TGF-beta signaling pathway
30	0.009584	13	88	Ubiquitin mediated proteolysis	0.005481	11	44	Alanine, aspartate and glutamate metabolism

*GO terms were listed in descending order of statistical significance (FDR value).

Table 5. The expression profiles of the representative genes associated with energy synthesis, gluconeogenesis, and the β-oxidation of fatty acid

FPKM value		Contig No.	$\log_2$[fold change] (Blood-fed/Starved)	$-\log_{10}$[FDR]	BLAST top hit (Accession No.)
Blood-fed	Starved				
1591.02	458.05	TRINITY_DN45047_c0_g1_i1	2.15	2.24	nucleoside diphosphate kinase B-like (NDKB-like) [Varroa destructor] (XP_022653377.1)
1051.95	202.66	TRINITY_DN42035_c4_g2_i2	2.77	3.38	ATP synthase subunit g, mitochondrial-like (ATPSG-like) [Varroa destructor] (XP_022653165.1)
559.59	175.48	TRINITY_DN43086_c0_g2_i1	2.05	2.08	ATP synthase-coupling factor 6, mitochondrial-like (ATPSCF6-like) [Varroa destructor] (XP_022643748.1)
427.30	107.62	TRINITY_DN40018_c2_g1_i1	2.34	2.57	ubiquinol-cytochrome C reductase complex (UCCR) [Ixodes scapularis] (AAY66915.1)
295.71	11.05	TRINITY_DN42158_c2_g1_i1	5.08	8.79	glyceraldehyde-3-phosphate dehydrogenase-like (GAPDH-like) [Varroa destructor] (XP_022655133.1)
80.18	558.95	TRINITY_DN41863_c2_g3_i3	-2.47	2.81	phosphoenolpyruvate carboxykinase, cytosolic [GTP]-like (PPCK-like) [Varroa destructor] (XP_022663562.1)
119.74	280.24	TRINITY_DN39874_c1_g1_i1	-0.89	0.62	isobutyryl-CoA dehydrogenase (IBCD) [Tropilaelaps mercedesae] (OQR74842.1)
14.38	251.53	TRINITY_DN44967_c0_g1_i3	-3.79	5.61	inosine-5'-monophosphate dehydrogenase 1-like isoform X1 (I5MD1-like) [Varroa destructor] (XP_022660176.1)