Lipidomics and RNA-Seq Study of Lipid Regulation in *Aphis gossypii* parasitized by *Lysiphlebia japonica*

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The cotton–melon aphid, *Aphis gossypii* Glover, is a major insect pest worldwide. *Lysiphlebia japonica* (Ashmead) is an obligate parasitic wasp of *A. gossypii*, and has the ability to regulate lipid metabolism of the cotton-melon aphid. Lipids are known to play critical roles in energy homeostasis, membrane structure, and signaling. However, the parasitoid genes that regulate fat metabolism and lipid composition in aphids are not known. 34 glycerolipids and 248 glycerophospholipids were identified in this study. We have shown that a 3-day parasitism of aphids can induce significant changes in the content and acyl chain composition of triacylglycerols (TAGs) and subspecies composition of glycerophospholipids content and acyl chains. It also upregulate the expression of several genes involved in triacylglycerol synthesis and glycerophospholipid metabolism. Pathway analysis showed that a higher expression of genes involved in the tricarboxylic acid cycle and glycolysis pathways may contribute to TAGs synthesis in parasitized aphids. Interestingly, the higher expression of genes in the sphingomyelin pathway and reduced sphingomyelin content may be related to the reproductive ability of *A. gossypii*. We provide a comprehensive resource describing the molecular signature of parasitized *A. gossypii* particularly the changes associated with the lipid metabolism and discuss the biological and ecological significance of this change.

The cotton-melon aphid, *Aphis gossypii* Glover, is an important agricultural pest, and is a major pest of cotton in China. It can cause serious damage to cotton seedlings during the early growing season, by sucking on host plants. The natural enemy of cotton aphid is the wasp, *Lysiphlebia japonica* (Ashmead), which is the predominant parasitoid of cotton–melon aphids and is a dominant species in the fields of northern China. This wasp can effectively control cotton aphid populations in early summer, thus projecting a promising future in the control of aphids by biological control strategy.

It has been reported that parasitoids can modify host metabolism such that the host nutrition sustains the wasp's growth than the host themselves. Particularly, parasitoids are known to regulate the hosts' fatty acid composition, which is a major factor influencing host suitability to parasitoids. For example, *Pseudaletia separata* parasitized by *Euplectrus separatens* for 3 to 8 days had significantly higher fat content in the hemolymph than non-parasitized *P. separata*. A number of studies have shown that parasitoids regulate the metabolism in fat body to increase the content of triacylglycerol and fatty acids in the hemolymph. Triglycerides (TAGs) in the fat body cells are the main energy source for insects at different developmental stages. Other lipids such as phospholipids (PLs) function as structural components of cell membranes. The fluctuation of the composition of these lipids provide new insights into the physiological state of insects.

Parasitoids have been reported to affect lipid synthesis in cotton–melon aphids. However, it is not known how these changes in lipid synthesis affect the cellular and physiological processes of the cotton–melon aphid. In this study, we investigated the effects of *L. japonica* parasitism on the lipid metabolism of *A. gossypii*. RNA-Seq combined with ultra-high performance liquid chromatography (UHPLC) coupled with hybrid quadrupole time-of-flight mass spectrometry (Q-TOF-MS) based metabolomics was applied to determine the effects of parasitism on the gene expression and probe content-dependent alterations in the metabolic profiles of parasitized and non-parasitized control aphids. Our results showed that parasitism resulted in marked changes in the lipid metabolism, and composition and remodeling of glycerolipids and glycerophospholipids. These results provide...
novel insights into the lipid metabolism of parasitized aphids and will enable further characterization of the lipid synthesis pathway and its relationship with the tricarboxylic acid (TCA) cycle and glycolysis.

**Materials and Methods**

**Insects and Parasitoids.**  Mumified *A. gossypii* were collected from a cotton field in the Institute of Cotton Research at the Chinese Academy of Agricultural Sciences (36°5′34.8″N, 114°31′47.19″E). A single population of *A. gossypii* that reproduced by parthenogenesis was used in the study. Aphids were reared on cotton leaves at 26 ± 1 °C, 65 ± 5% relative humidity and a 14:10 h light:dark photoperiod. To rear *L. japonica*, they were placed together with the aphids and maintained at 24 ± 1 °C, 75 ± 5% relative humidity and a 14:10 h light:dark photoperiod. To generate parasitized aphids, third instar cotton-melon aphids were exposed to wasps until parasitism was observed. Both parasitized and non-parasitized aphids were reared for 3 days on cotton leaves in incubators. Aphids were collected and dissected under the microscope to separate the parasitized from non-parasitized aphids and to remove the wasp larvae.

**Lipid extraction.**  We maintained two groups of aphids; non-parasitized and parasitized (after removing *L. japonica*) with six biological replicates per group with 12 samples/group. To extract lipid metabolites from tissues, each sample was weighed and 20 mg of the tissue was homogenized in ball mill after addition of 5 balls and 100 µL water, at 4 °C and 30 Hz for 3 min. The homogenate was then transferred to a glass tube, mixed with 400 µL CHCl₂:MeOH (2:1, v/v) and vortexed for 30 s. The mixture was then centrifuged for 15 min at 2500 rpm and the organic layer (~200 µL) was transferred to a clean glass vial and evaporated to dryness; the dried extract was reconstituted in 100 µL CHCl₂:MeOH (1:1, v/v) and used in UHPLC-QTOFMS analyses.

**Lipid Analysis—Mass Spectroscopy.**  The following reagents were used in the experiments: ACN (Honeywell, Japan), HCOONH₄ (Thermo, USA), and IPA (Thermo, USA). The TripleTOF mass spectrometer was used for its ability to acquire MS/MS spectra on an information-dependent basis (IDA) during an LC/MS experiment. In this mode, the acquisition software (Analyst TF 1.7, AB Sciex) continuously evaluates the full scan survey MS data as it collects and triggers the acquisition of MS/MS spectra depending on preselected criteria. In each cycle, 6 precursor ions whose intensity greater were higher than 100 were chosen for fragmentation at collision energy (CE) of 35 V (15 MS/MS events with product ion accumulation time of 50 msec each). ESI source conditions were set as following: Ion source gas 1 as 60, Ion source gas 2 as 60, Curtain gas as 30, source temperature 550 °C, Ion Spray Voltage Floating (ISVF) 5500 V or −4500 V in positive or negative modes, respectively. Lipids were tested on UHPLC-QTOFMS (Agilent 1290 UHPLC + AB Triple TOF 6600 +), Chromatographic column was from Kinetex (Phenomenex, USA; 1.7 μ C18 100 Å 100 × 2.1 mm). The TripleTOF mass spectrometer was used for its ability to acquire MS/MS spectra on an information-dependent basis (IDA) during an LC/MS experiment. In this mode, the acquisition software (Analyst TF 1.7, AB Sciex) continuously evaluates the full scan survey MS data as it collects and triggers the acquisition of MS/MS spectra depending on preselected criteria. Mobile phase settings were: A: 10 mM HCOONH₄ in ACN/H₂O (v/v, 6:4), B: 10 mM HCOONH₄ in ACN/IPA (v/v, 1:9); Program: 0:00 min, 400 µL/min, 60% A, 40% B; 9:00 min, 400 µL/min, 0% A, 100% B; 10:00 min, 400 µL/min, 0% A, 100% B; 10:20 min, 400 µL/min, 60% A, 40% B; 13:00 min, 400 µL/min, 60% A, 40% B. MS parameter settings were: GS1: 60 psi; GS2: 60 psi; CUR: 30 psi; TEM: 600 °C; ISVF: 5500 V; TOF Masses (Da): Min = 200.0000, Max = 1200.0000.

**Statistical Analysis.**  Raw data were converted to mzXML format using ProteoWizard, and further analyzed by XCMS. Metabolites were identified by accurate mass search (<30 ppm) and MS/MS spectral match using an in-house standard MS/MS library.

Peaks were detected and metabolites could be left through interquartile range denoising method, and then, missing values of raw data were filled up by half of the minimum value. In addition, overall normalization method was employed in this data analysis. The resulting three-dimensional data containing the peak number, sample name, and normalized peak area were fed to the SIMCA14 software package (Umetrics, Umea, Sweden) for principal component analysis (PCA) and orthogonal projections to latent structures-discriminate analysis (OPLS-DA). In order to obtain a higher level of group separation and to better understand the variables responsible for classification, supervised OPLS-DA was applied. Then, parameters for the classification of R²Y and Q²Y were obtained from the software and checked for stability and good to fitness prediction. To establish robustness and the predictive ability of our model we used the 7-fold cross validation method with permutations. R² and Q² intercept values were obtained after 200 permutations. Low values of the Q² intercept show the robustness of the models, and thus indicate a low risk for over fitting and reliability. Based on OPLS-DA, a loading plot was constructed, and it showed the contribution of variables to differences between the groups (Figure S2). It also showed the important variables that were situated far away from the origin. However, the loading plot is complex because of many variables.

Heatmap (R package, Version 3.2.3) was used for a more intuitive analysis of the differences among these components. The relative difference (percent) between the two groups was estimated using the Hodges-Lehmann estimator, X axis stand relative difference (%). To refine the analysis, variable importance projection (VIP) was the first principal component measured. VIP values exceeding 1.0 were first selected as changed metabolites. The remaining variables were then evaluated by Student’s t-test (p > 0.05) and those without significant differences were discarded. Moreover, we used fold change as another criterion to assess differences in the levels of compounds (fold change > 1.5 or <0.5 and p < 0.05). In addition, commercial databases, including NIST (http://www.nist.gov/index.html) and KEGG (http://www.genome.jp/kegg/) were used for qualitative analysis and to search for the metabolites in the lipid biosynthesis pathway. The metabolites number is MTBLS410.
Extraction of RNA and RNA-Seq data analysis. Total RNA was extracted from both parasitized (after removing L. japonica) and non-parasitized aphids using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer’s instructions. Each of the parasitized and non-parasitized groups had three biological replicates. Illumina sequencing of the samples was performed at the Beijing Genomics Institute (Shenzhen, China). After filtering the raw reads by removing the adapter, poly-N and low quality sequences, we used Trinity to perform de novo assembly of the clean reads. Then, TAGicl was used to cluster transcripts to obtain unigenes. TAGicl was applied to each sample individually and repeatedly to obtain the final unigenes for downstream analyses. Unigenes larger than 150 bp were first aligned by BLASTN to the NCBI-NT database (e-value < 10^-5) and by BLASTX to protein databases including NCBI-Nr, Swiss-Prot, KEGG and COG (e-value < 10^-5) to retrieve proteins along with their functional annotations. DEseq2, based on the negative binomial distribution, was performed as described previously24. Differential expression was determined using a cutoff significance level of false discovery rate (FDR) < 0.05. Functional enrichment analysis was performed with HOMER25 using pathways related to metabolism from the KEGG database annotation26-28. Expression abundance analysis of the unigenes was calculated based on the reads per kilobase per million mapped reads (RPKM) method, which eliminated the influence of different gene lengths and sequencing discrepancies while calculating expression abundance. The clean reads and computationally assembled sequences were submitted to the NCBI/SRA database and NCBI/TSA repository. The ArrayExpress accession number is MTAB-5228.

Results

Mass Spectra and Pattern Recognition Analysis of Lipids Extracts. Each positive mode and negative mode received 12 samples and 5 QC samples. Among these, 2056 peaks were positive, and 3757 peaks were negative. Mass spectra of lipid extracts from the control and parasitized aphids were dominated by signals of mass lipids. The resonance of lipids was assigned based on the literature and results from the lipid map. Mass spectra were mainly composed of various phospholipids (PLs) and triacylglycerols (TAGs). A complete list of the lipidomics data is provided in Table S1.

We identified a total of 34 different TAGs and 248 PLs (POS and NEG) from the D and F samples. To evaluate whether parasitism affects lipid synthesis and composition, 70 kinds of phospholipids were selected for further analysis. In the lipidomics analysis, six independent pairwise comparisons were performed to eliminate false positives and negatives, to view only the robust alterations in lipid composition after parasitism. Principle component analysis (PCA) was initially applied to the spectra to visualize inherent clustering between control and parasitized classes. As shown in Fig. 1(A,B), unsupervised PCA revealed noticeable separation between Non-parasitized and parasitized. There was an obvious difference in the lipid composition between two groups. In order to obtain a higher level of group separation and for a better understanding of the variables responsible for classification, supervised OPLS-DA was applied. Characteristics of the models generated are summarized in Table 1. Good separation of the lipid extracts between Non-parasitized and parasitized groups were achieved as shown in the OPLS-DA scores plots (Fig. 1C–F).

Parasitism Drastically Changes the Expression of Genes Related to Lipid Metabolism. Lipidomics changed significantly in parasitized aphids and was evidenced as changes in the expression of genes involved in the lipid-associated pathway between parasitized and non-parasitized insects. Both parasitized (after removing L. japonica) and non-parasitized aphids were tested to map the transcriptional changes in A. gossypii in response to parasitism. The A. gossypii sequences revealed substantial (48.65%) matches with Acrystosiphon pisum. Analysis of the unigenes resulted in the identification of differentially expressed genes (DEGs) using DEseq2. Using a significance level of p < 0.05, we found a total of 14,455 DEGs among which 13,015 were up-regulated and 1,440 were down-regulated (Fig. 2A).

We then processed the DEGs to obtain their functional classification using the Gene Ontology (GO) and KEGG databases. We found that some DEGs in the metabolic pathways and signal transduction were significantly enriched, which included glycerolipid metabolism, glycerophospholipid metabolism, and sphingomyelin (Fig. 2). Several genes involved in these pathways had increased expression in parasitized aphids and included the fatty acyl chain elongase. Particularly, Elovl3 (the gene encoding the main enzyme involved in elongation of saturated and monounsaturated C18-C22 fatty acid substrates29) and Elovl6 (elongation of very long chain fatty acids protein 6) (Fig. 2B) displayed significantly higher expression levels after parasitism. We also detected a marked increase in the expression of Psd (phosphatidylserine decarboxylase), Agpat1/3 (1-acyl-sn-glycerol-3-phosphate acyltransferase), Pgs1 (phosphatidyglycerophosphate synthase 1), Pla2g2e (secretory phospholipase A2) and Cds1 (CDP-diacylglycerol synthase 1), which are involved in the glycerophospholipid metabolism pathway (Fig. 2B). Up-regulated genes related to the glycerolipid metabolism included LPL (lipoprotein lipase) and cs (citrate synthase) while atpcd (adenosine triphosphate (ATP) citrate lyase) was significantly down-regulated after exposure to parasitoids (Fig. 2B) indicating an inhibitory feedback circuit. In addition, genes related to the MAPK signaling pathway, and antigen processing and presentation also increased significantly after parasitism by L. japonica (Figure S1). Together, these results indicate that a 3-day parasitism induces extensive changes in the transcriptome of A. gossypii when compared to non-parasitized aphids. These changes may contribute significantly to the growth and development of L. japonica and may eventually lead to the death of A. gossypii. Different expressed genes in some related pathway were showed in Table S2.

Parasitism Affects the Composition of Fatty Acyl Chains but not the Lipid Class of TAG in A. gossypii. To determine how parasitism affects the composition and distribution of TAGs and glycerophospholipid in A. gossypii we quantified the lipid classes in the control and parasitized aphids. Parasitism significantly influenced the TAG and PL metabolite profiles in A. gossypii. Relative difference among the lipid classes revealed significant parasitism-induced changes in the abundance of lipid species in most of the analyzed lipid classes.
Although parasitism did not alter the lipid class of different triacylglycerol and glycerophospholipid in *A. gossypii*, it increased the levels of numerous triacylglycerols and decreased the levels of many glycerophospholipid species to below the limit of detection (Fig. 4). Note that there were many unidentified lipids (ULP) that are not listed in this study.

In insects, TAGs are stored in specialized lipid droplets. Studies have shown that lipid droplets not only assume the role of passive storage, but also actively participate in fat and energy metabolism. In our analysis, we found that monoacylglycerols (MGs) and diacylglycerols (DGs), which are the intermediate products in lipid synthesis, were unstable. All TAGs (Fig. 3) (TAG (18:1/18:2/18:2), 46.5-fold; TAG (18:2/18:2/18:2), 38.3-fold; TAG (18:0/18:1/18:1), 20.1-fold) differed markedly between two groups. After parasitism, the TAGs content was significantly increased relative to the non-parasitized aphids. Consistent with this, the expression levels of several genes involved in the synthesis of TAG were up-regulated; *Ppop2a* (phosphatidic acid phosphatase type 2A) and

Table 1. Summary for the OPLS-DA Model-Based Discrimination between parasitized and non-parasitized (parasitized VS non-parasitized) aphids Using Cross-Validation. $R^2_X$ and $R^2_Y$ values indicate the total number of variations in the X and Y matrix explained by the model, respectively. $Q^2$ represents the predictability of the models and relates to its statistical validity.

| Model | Type       | A    | N  | $R^2_X$ (cum) | $R^2_Y$ (cum) | $Q^2$ (cum) | Title |
|-------|------------|------|----|---------------|---------------|-------------|-------|
| M3    | OPLS-DA    | 1 + 1 + 0 | 12 | 0.936         | 1             | 0.997       | POS   |
| M3    | OPLS-DA    | 1 + 1 + 0 | 12 | 0.858         | 1             | 0.995       | NEG   |
Gpat1 (glycerol-3-phosphate O-acyltransferase 1) in glycerolipid metabolism pathway were 7.91- and 7.80-fold higher in parasitized aphids than in non-parasitized aphids, respectively (Fig. 3D).

When analyzing the fatty acyl chains associated with triacylglycerols, we detected a marked increase in the levels of the acyl chains, C18:1 and C18:2 in major and middle TAGs of parasitized *A. gossypii* when compared to non-parasitized aphids (Fig. 3E,F). Interestingly, we observed that the saturated odd-numbered acyl chains (C17:0) in the middle TAG (Fig. 3F) were affected only marginally by parasitism while some very-long-chain fatty acyls (C ≥ 20) increased robustly in aphids after parasitism (e.g., C20:0, C20:1, C20:2). The increased abundance of fatty acyls may have resulted from increased activity of the glycerolipid metabolism pathway. The relative peak area of quantified lipid classes in minor TAG is shown in Figure S3.

**Evaluating the Effects of *L. japonica* Parasitism on the *A. gossypii* Sphingomyelin Metabolic Pathway.** Analysis of sphingomyelin (SM) species revealed no changes between the parasitized and non-parasitized aphids (Fig. 3). However, numerous changes were observed in the composition of these species. All SM species (e.g., SM (15:0/24:1), 0.1-fold) and Glycosphingolipids (GlcCer) (e.g., GlcCer (d17:0/20:0), 0.3-fold) were decreased in parasitized aphids compared to non-parasitized aphids, while ceramide (e.g., cer
(d17:1/20:0), 8.2-fold) was highly enriched after parasitism. Moreover, almost all genes in the SM metabolic pathway were up-regulated (Fig. 5C, Table S2). For example, Ppap2a, LAG1 (acyl-CoA-dependent ceramide synthase) and cers (ceramide synthetase) were up-regulated consistent with the marked SM remodeling in response to parasitism. Analysis of SM fatty acyl chains, which mainly contains the sphingoid base, d15 and d17, revealed a high abundance of d15 and d17 indicating that parasitism induces changes in the relative proportions of the various forms of fatty acyl chains of SM (Fig. 5).

The Glycerophospholipid Pathway is Stimulated by Parasitism. Glycerophospholipid metabolism was the most significantly altered parasitized-induced gene pathway in previous studies\(^{20,21}\). In contrast, parasitism did not alter the abundance of the glycerophospholipid classes (Fig. 6, Table S1). When compared with the control group, as many as 32 PLs (Phosphatidylcholines, PC(16:0/18:1), 0.2-fold; Phosphatidylethanolamines, PE(18:0/18:0), Phosphatidyllysinolipid, PI (18:0/18:2), 0.03-fold; Phosphatidylglycerols, PG (18:2/18:1), 0.07-fold;
Figure 4. Heatmaps of the integration of intermatrix Lipids correlation between D vs F (A - POS, B - NEG). Heat maps were applied to display the quantitative expression of lipids with a red color gradient representing high expression and a green color gradient representing low expression. Lipid synthesis metabolites with significant changes in the non-parasitized aphids and parasitized aphids groups from UHPLC-Q-TOF-MS was analyzed in the heatmap (VIP > 1, p value < 0.05). Scale bars on the top right of each heatmap represent the degree of gene expression with red showing high expression and green indicating low expression.

Figure 5. Changes in the gene expression and lipid composition in the sphingomyelin metabolism. (A,B) Quantification of the relative peak area of major sphingomyelin (A) and middle sphingomyelin (B) lipid species in parasitized and control aphids. Data are means ± SEM; n = 6/group. **p < 0.01. (C) The sphingomyelin metabolism is induced in A. gossypii after exposure to cold conditions. Select sphingomyelin metabolites in the KEGG pathway, were significantly regulated in parasitized aphids. Red boxes indicate increased gene expression levels in parasitized insects and blue boxes indicate the high expression levels of sphingomyelin components. Cers, ceramide synthetase; KDSR, 3-dehydrosphinganine reductase; SGMS, shingomyelin synthase; Lacz, beta-galactosidase; GBA, glucosylceramidase. (D) Sphingomyelin base chemistry and fatty acyl chains in parasitized and control aphids. Data are means ± SEM (n = 6/group).
Phosphatidylserines, GPSer(20:0/18:2), 0.05-fold) were decreased significantly in the parasitized group. In addition, a few Lysophosphatidylcholines (LPC), Lysophosphatidylethanolamines (LPE), Alkenyl-linked PC (PC-P) and Alkenyl-linked PE (PE-P) of PLs (LPC(16:0/0:0), LPE(16:0/0:0), LPC(18:2/0:0), LPE(19:0/0:0)) were increased in the parasitized group (Fig. 6). The increased levels of LPC and LPE, and the changes in their compositions indicate a marked glycerophospholipid remodeling in response to parasitism. This is also consistent with the result that parasitism leads to an increased expression of several genes involved in the synthesis and

Figure 6. Changes in the lipid composition and fatty acyl chains of phospholipids in parasitized aphids. (A, B) Quantification of the relative peak area of major phospholipids (A) and middle phospholipids (B) in parasitized and control aphids. Data are means ± SEM; n = 6/group. *p < 0.05, **p < 0.01. N.D, not detected. (C–H) Changes in the glycerophospholipid base chemistry and fatty acyl chains in the phospholipids of parasitized and control aphids (PC, C; PE, D; PG, E; PI, F; LPC, G; LPE, H). Data are means ± SEM (n = 6/group).
remodeling of LPC and LPE. Moreover, alkenyllinked PC and PE were only detected in the control or parasitized aphids, respectively (Fig. 6).

In this study, we detected a marked reduction in C18:2 (except PG) (Fig. 6) and only a marginal reduction in C16:0 and C16:1 (Fig. 6) in parasitized aphids. This may have resulted from the reduction in the levels of PC, PE, and PI species (Fig. 6) that contained these acyl chains. However, quantification of the base chemistry and fatty acyl chains for the individual glycerophospholipid subclasses in parasitized and non-parasitized aphids revealed a marked reduction in the PG species C18:1 (Fig. 6E) similar to the result observed in the LPE subclasses (Fig. 6H). These changes confirmed that parasitism not only changed the content of glycerophospholipid subclasses, but also resulted in the remodeling of fatty acyl chains of glycerophospholipid. This clearly indicated that when parasitized, the aphid metabolism is altered by the parasitoid so as to provide for the demands of the parasitoid itself.

In addition, we demonstrated that the increased levels of LPC and LPE were accompanied by the increased levels of C16:0 and C18:0 (Fig. 6G,H). Consistent with these increases, the expression of several genes involved in the synthesis and remodeling of PC, PE, Phosphatidylinositols(PI) and PG were also increased (Fig. 6). The relative peak area of quantified lipid classes in additional middle PL and minor PL is shown in Figure S3.

Interestingly, the high expression of genes such as Cds1, Pgo1, Agapt3 and Gpm (glycerol-3-phosphate acyltransferase) (Fig. 7, Table S3) involved in the glycerophospholipid metabolism did not contribute to the increase in PLs. Conversely, the reduction in PLs did not weaken signal transmission (Figure S1A,B). Therefore, we presume that the increases in the PL levels could be likely used by the parasitoid larvae for self-growth and tissue remodeling. Besides, some genes involved in the TCA cycle and glycolysis were also up-regulated in parasitized aphids (Figure S1).

Discussion
Metabolites participate in multiple cellular reactions, connecting different pathways that mediate and perform several cell functions. Therefore, metabolite profiling may show changes in biological functions or phenotypes in response to parasitic31–34. With the exception of studies in lepidopterans35–38 and other model insects39, not much is known about how changes induced by parasitoids at the transcriptional and lipidomic levels in aphids can affect parasitoid-host relationship. In order to understand parasitism-induced changes in the composition of the different lipids classes and the expression of genes involved in the lipid metabolism we applied
mass-spectrometry-based lipidomics and RNA-Seq in an attempt to provide a resource describing the molecular signature of parasitized aphids at the lipidomic and transcriptomic levels. It is known that the host aphid is killed on day 6–7 after parasitism and that adult parasitoids emerge a week later40–42. We had observed that L. japonica with their 2nd–3rd instar larval stages developing within 72 h after oviposition. Moreover, wasp mortality in highly resistant aphids typically occurs prior to 72 h43. So, we choose 3 d as the point time for analysis the regulation of L. japonica.

Studies reported that parasitoids can eat the host fat and modify host fat metabolism1–9. In this study, we analyzed 282 lipid metabolites in both parasitized and non-parasitized aphids, and found that parasitism affected the composition of lipids and remodeled them Moreover, the contents and fatty acyl chains of PCs, PEs, Psls, PGSs, SMs, LPCs, LPEs in A. gossypii were altered after parasitism. A number of studies have demonstrated the inability of parasitoid species to synthesize lipids during their life14, 43–45, it is evident that it must obtain exogenous sources to complete life cycle. Our data showed a sharp increase in the TAG levels and the downregulated of the tgl1 in A. gossypii after parasitism by L. japonica. In all eukaryotic organisms, TAG synthesis and mobilization play important roles in energy homeostasis46. The main function of fat cells is stored energy in the form of triacylglycerol, and released to supply to the body when the body needs. The higher levels of TAGs could likely meet the demand of lipid and energy of L. japonica that is required for flight, mating activities, and oviposition47 in the adult stage.

Lipid reserves play a key role in both survival and reproduction48. Sphingolipids comprise a complex and ubiquitous class of membrane lipids49, which are recognized as key regulators of cell cycle progression as well as growth and cellular metabolism50. In our data, among the major lipid classes analyzed, sphingolipids were more abundant in the non-parasitized group (e.g., di5:0/24:1, except Cer) although we cannot exclude direct germline effects because gonads were included in the parasitized aphid samples. SM metabolites have been implicated in brain development and neuronal function in Drosophila spp and sphingolipid metabolism was reported to elicit apoptosis-associated reproductive defects51–53. Additionally, we found high levels of Cer after parasitism suggesting a potential link between parasitism-associated biochemical alterations and the direct cytopathic effect observed in the host system54. Ceramides and other sheath phospholipids act as second messengers, regulating the action of many target proteins by inducing cascade amplification of enzyme activity. Cer may play a critical role in L. japonica development although these are minor PL species. Alternatively, the reduced levels of SM species and GlcCer could be necessary for aphid growth and reproduction.

Our data demonstrated that a significant remodeling of glycerolipid and glycerophospholipid in response to parasitism. In the L. japonica – A. gossypii association, parasitism induced differential changes in the different subclasses of acyl chains and subspecies of TAG and PL. This highly specific change may be a selective regulation by L. japonica; alternatively, it could be the coping strategy of A. gossypii when parasitized. The highly selective remodeling of lipids subspecies is likely to have significant functional implications on L. japonica growth and reproduction.

Host development is regulated by factors that are hereditary55, 56 or are injected by the parasitoids during oviposition57. Based on the effects of parasitism on lipidomics, we focused our analyses on glycerolipid, glycerophospholipid and SM metabolic pathways in parasitized aphids. Our study indicated that the expression of a large number of genes in these pathways was induced significantly in parasitized aphids. An interesting finding from our study is the down-regulation of tgl1 mRNA (2.7-fold) in parasitized aphids, tgl plays a negative role in the TAG metabolism, but the other way of TAG metabolism was induced, resulted from the upregulated of LPL. In addition, Gpam is increased 7.8-fold in parasitized aphids, this indicated that the increased TAG were mainly used to synthesize DG which may contributed to synthesize Lyso phosphatidyl. Moreover, Agpat3, Gds1, Lpgat1, Pgs1 and Plat2g2c that regulate Lyso phosphatidyl phospholipid synthesis genes were induced after parasitism for 3 days. Lyso phosphatidyl display the role of neurotoxicity and can make cells dissolve in low dose58. Parasitoids regulate the physiological milieu of the host to facilitate utilization of host nutrients by the parasitoids51, 32, 59, 60, it could be seen that aphids were controled to lysis cells and emit energy after parasitized by regulating the target genes which contributed to synthesize Lyso phosphatidyl. The biological and physiological importance of the up- and down-regulation of these genes should be investigated further using knockout models.

Major metabolic pathways associated with sugar and lipid metabolism are typically highly conserved across taxa61. Studies had reported that all lipid metabolism related genes in glycolysis pathway were expressed in high levels in parasitized aphids20, 21. Furthermore, parasitism was also shown to enhance glycolysis and the TCA cycle62, 63. Our data demonstrated that genes involved in TCA cycle and glycolysis pathway had increased expression. Typically, A. gossypii feeds on plant phloem, an abundant source of carbon and energy. Sucrose carbons in aphid tissues are mainly converted into lipids64. Previous genomic analysis indicated that TAG is synthesized by the glycerol 3-phosphate (G3P) pathway, which is the sole pathway for TAGs synthesis in Rhodinus prolitus65, and when A. gossypii was parasitized by Pratovolucra, glycolysis was galvanized, likely to favor polyols biosynthesis66. Our data showed that some genes involved in the TCA cycle and glycolysis (e.g., atpcl, gk, gpdh (glycerol-3-phosphate dehydrogenase) and tin (triosephosphate isomerase)) were up-regulated consistent with previous reports67. We presume that glycerophospholipid metabolism was accompanied by TCA and glycolysis pathway, and the induced TCA and glycolysis pathway was necessary for the synthesis of TAGs and PLs. What more, more metabolic changes may occur during the parasitized stage to adapt to the changing environment of aphids.

Our study has provided insights on the TAG and PL diversity and biochemistry during parasitism. Our integrated results indicate that parasitism may activate the transcriptional program of glycerolipid and glycerophospholipid metabolism in aphids, enhance the expression of genes involved in SM metabolism as well as the TCA cycle and glycolysis pathway and may be accompanied by remodeling of the triacylglycerol and glycerophospholipid composition. These changes are highly specific to this species. The biochemical information we described here will help future studies investigating the regulation of host lipid metabolism by parasitoids.
Conclusion

In this study, we have reported changes in the lipidomic and transcriptional profiles of parasitized cotton-melon aphids for the first time. Our comprehensive analysis shows that parasitism can markedly enhance the expression of genes involved in the glycerolipids and glycerophospholipids pathways and also results in the remodeling of triacylglycerol and glycerophospholipids. These results show that L. japonica can influence the hosts’ nutritional physiology to benefit its own growth while deterring A. gossypii development. These data may serve as a valuable resource for studies on parasitoid-insect interactions. Such a comprehensive study on the lipidomics of parasitoids and its host species would help us not only to determine their dietary requirements but also help improve parasitoid mass rearing for use in biological control programs.

References

1. Li, Z. Q. et al. Ecological Adaption Analysis of the Cotton Aphid (Aphis gossypii) in Different Phenotypes by Transcriptome Comparison. Plos One 8, doi: ARTN e8318010.1371/journal.pone.0083180 (2013).
2. Wu, K. & Guo, Y. Influences of Bacillus thuringiensis Berliner cotton planting on population dynamics of the cotton aphid, Aphis gossypii Glover, in northern China. Environmental Entomology 43, 312–318, doi:10.1603/0046-225X.32.2.312 (2003).
3. Hou, Z., Chen, X., Zhang, Y., Guo, B. & Yan, F. EAG and orientation tests on the parasitoid Lysiphleba japonica (Hym., Aphididae) to volatile chemicals extracted from host plants of cotton aphid Aphis gossypii (Hom., Aphididae). Journal of Applied Entomology 121, 495–500, doi:10.1111/j.1439-0469.1997.121.issue-1-3 (1997).
4. Cui, J. J., Luo, J. Y., Van der Werf, W., Ma, Y. & Xia, J. Y. Effect of Pyramiding Bt and CpTI Genes on Resistance of Cotton to Helicoverpa armigera (Lepidoptera: Noctuidae) Under Laboratory and Field Conditions. J Econ Entomol 104, 673–684, doi:10.1603/E09228 (2011).
5. Bae, S. & Kim, Y. Host physiological changes due to parasitism of a brood ant wasp, Cotesia plutellae, on diamondback moth, Plutella xylostella. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 138, 39–44, doi:10.1016/j.cbpa.2004.02.018 (2004).
6. Beckage, N. E. & Gelman, D. B. WASP Parasitoid Disruption of Host Development: Implications for New Biologically Based Strategies for Insect Control*. Annual Reviews in Entomology 49, 299–330, doi:10.1146/annurev.ento.49.061802.123234 (2004).
7. Christe, P., Arlettaz, R. & Vogel, P. Variation in intensity of a parasitic mite (Spinturnix myoti) in relation to the reproductive cycle and immunocompetence of its host (Myotis myotis). Ecology Letters 3, 207–212, doi:10.1111/j.1461-0248.2000.00142.x (2000).
8. Engelstadter, J. & Hurst, G. D. The ecology and evolution of microbes that manipulate host reproduction. Annual Review of Ecology, Evolution, and Systematics 40, 127–149, doi:10.1146/annurev.ecolsys.110308.120206 (2009).
9. Barts, M., S., & Sasaki, A. The evolutionary dynamics of local infection and global reproduction in parasitoid-insect interactions. Ecology Letters 3, 181–185, doi:10.1111/j.1461-0248.2000.00199.x (2000).
10. Thompson, S. & Barlow, J. The fatty acid composition of parasitic Hymenoptera and its possible biological significance. Annals of the Entomological Society of America 67, 627–632, doi:10.1093/aesa/67.4.627 (1974).
11. Nakamatsu, Y. & Tanaka, T. Venom of Euplectrus separatae causes hyperlipidemia by lysis of host fat body cells. Journal Of Insect Physiology 50, 267–275, doi:10.1016/j.jinsphys.2003.12.005 (2004).
12. Kaeslin, M., Pfister-Wilhelm, R., Molina, D. & Lanzrein, B. Changes in the haemolymph proteome of parasitoids and its host species would help us not only to determine their dietary requirements but also help improve parasitoid mass rearing for use in biological control programs.
13. Alves-Bezerra, M. & Gondim, K. C. Triacylglycerol biosynthesis occurs via the glycerol-3-phosphate pathway in the insect Melanogryllus desertus (Orthoptera: Gryllidae). J Biol Chem 275, 14778–8015, doi:10.1074/jbc.275.11.8007 (2000).
14. Rivers, D. B. & Denlinger, D. L. Venom-Induced Alterations In Fly Lipid-Metabolism And Its Impact on Larval Development Of the Ectoparasitoid Nasonia-Vitripennis (Walker) (Hymenoptera, Pteromalidae). J Invertebr Pathol 66, 104–110, doi:10.1006/jipj.1995.1071 (1995).
15. Visser, B. & Ellers, J. Lack of lipogenesis in parasitoids: A review of physiological mechanisms and evolutionary implications. Journal Of Insect Physiology 53, 1315–1322, doi:10.1016/j.jinsphys.2008.07.014 (2008).
16. Allmann, S. et al. Triacylglycerol Storage in Lipid Droplets in Procyctic Trypanosoma brucei. Plos One 9, e114628, doi:10.1371/journal.pone.0114628 (2014).
17. Guerra, A. A. & Robacker, D. C. Effects of sex, age, and diet on the triacylglycerol fatty acid composition of subtropical boll weevils, Anthonomus grandis Boheman (Coleoptera: Curculionidae). Journal of agricultural and food chemistry 37, 796–799, doi:10.1021/jf00087a050 (1989).
18. Cohen, W. M. & Levinthal, D. A. Absorptive capacity: A new perspective on learning and innovation. Administrative Science Quarterly 38, 1–21 (2014).
19. Storey, J. D. & Tibshirani, R. Statistical significance for genomewide studies. PNAS 100, 9440–9445, doi:10.1073/pnas.1530509100 (2003).
20. Alves-Bezerra, M. & Gondim, K. C. Triacylglycerol biosynthesis occurs via the glycerol-3–phosphate pathway in the insect Rhodnius prolixus. Bba-Mol Cell Biol L 1821, 1462–1471, doi:10.1016/j.bbalip.2012.08.002 (2012).
21. Zhang, S. et al. Effects of Lysiphleba japonica (Ashmead) on cotton-melon aphid Aphis gossypii Glover lipid synthesis. Insect Mol Biol 24, 348–357, doi:10.1111/1365-2027.12162 (2015).
22. Storey, J. D. & Tibshirani, R. Statistical significance for genomewide studies. Proceedings of the National Academy of Sciences 100, 9440–9445, doi:10.1073/pnas.1530509100 (2003).
23. Kind, T. et al. FiehnLib: mass spectral and retention index libraries for metabolomics based on quadrupole and time-of-flight gas chromatography/mass spectrometry. Analytical chemistry 81, 10038–10048, doi:10.1021/ac9019522 (2009).
24. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome biology 15, 1–21 (2014).
25. Heinz, S. et al. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Molecular cell 38, 576–589, doi:10.1016/j.molcel.2010.05.004 (2010).
26. Kanekane, M. et al. Data, information, knowledge and principle: back to metabolism in KEGG. Nucleic acids research 42, D199–D205, doi:10.1093/nar/gkt1076 (2014).
27. Ogata, H. et al. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic acids research 27, 29–34, doi:10.1093/nar.27.1.29 (1999).
28. Kanesaka, M. & Goto, S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic acids research 28, 27–30, doi:10.1093/nar.28.1.27 (2000).
29. Guilhou, H., Zadrazev, D., Martin, P. G. & Jacobsson, A. The key roles of elongases and desaturases in mammalian fatty acid metabolism: Insights from transgenic mice. Progress in lipid research 49, 186–199, doi:10.1016/j.plipres.2009.12.002 (2010).
30. Wältcher, T. C. & Farese, R. V. The life of lipid droplets. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids 1791, 459–466, doi:10.1016/j.bbalip.2008.10.009 (2009).
31. Alleyne, M. & Beckage, N. E. Parasitism-induced effects on host growth and metabolic efficiency in tobacco hornworm larvae parasitized by Cotesia congregata. Journal of Insect Physiology 43, 407–424, doi:10.1016/S0022-1910(96)00086-8 (1997).
32. Bischof, C. & Ortel, J. The effects of parasitism by Glyptapanteles liparidis (Braconidae: Hymenoptera) on the hemolymph and total body composition of gypsy moth larvae (Lymantria dispar, Lepidoptera: Lasiocampidae). *Parasitology Research* **82**, 687–692, doi:10.1007/s004360050186 (1996).

33. Horwood, M. A. & Hales, D. F. Fat body changes in a locust, *Chortoicetes terminifera* (Walker) (Orthoptera: Acrididae), parasitized by a nemestrinid fly. *Archives of Insect Biochemistry and Physiology* **17**, 53–63, doi:10.1002/(ISSN)1520-6237 (1991).

34. Salvador, G. & Consonni, F. L. Changes in the hemolymph and fat body metabolites of *Diaurica soychurai* (Fabricius) (Lepidoptera: Crambidae) parasitized by *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae). *Biological Control* **45**, 103–110, doi:10.1016/j.bioc.2007.12.007 (2008).

35. Mapelli, V., Olsson, L. & Nielsen, J. Metabolic footprinting in microbiology: methods and applications in functional genomics and biotechnology. *Trends in biotechnology* **26**, 490–497, doi:10.1016/j.tibtech.2008.05.008 (2008).

36. Villas-Boas, S. G., Mas, S., Åkesson, M., Smedsgaard, J. & Nielsen, J. Mass spectrometry in metabolome analysis. *Mass spectrometry reviews* **24**, 613–646, doi:10.1002/mscr.20032 (2005).

37. Yang, C., Hua, Q., Baba, T., Mori, H. & Shimizu, K. Analysis of Escherichia coli anaerobic metabolism and its regulation mechanisms from the metabolic processes to altered dilution rates and phosphoenoxypropane carboxyl kinase knockout. *Biotechnology and Bioengineering* **84**, 129–144, doi:10.1002/bt.10692 (2003).

38. Shi, Y. et al. The Energy Metabolism in Caenorhabditis elegans under The Extremely Low-Frequency Electromagnetic Field Exposure. *Scientific reports* **5**, 10.1038/srep09471 (2015).

39. Fougeron, A.-S., Farine, J.-P., Flaven-Pouchon, J., Everaerts, C. & Ferveur, J.-F. Fatty-acid preference changes during development in Drosophila melanogaster. *PloS One* **6**, e26899, doi:10.1371/journal.pone.0026899 (2011).

40. Danyk, T. P. & Mackauer, M. An Extraserosal Envelope in Eggs of *Praon pequodorum* (Hymenoptera, Aphidiidae), A Parasitoid of *Plathypene plathypenae*. *Journal of Insect Physiology* **49**, 141–147, doi:10.1016/S0022-1910(99)00122-7 (2003).

41. Nakamatsu, Y. & Tanaka, T. Venom of ectoparasitoid, *Glyptapanteles liparidis* (Braconidae: Hymenoptera). *Biological Control* **1**, 41–65, doi:10.1016/0990-3349(92)90266-4 (1992).

42. Thompson, S. Parasitism enhances the induction of glucogenesis by the insect. *Journal of Insect Physiology* **49**, 141–147, doi:10.1016/S0022-1910(99)00122-7 (2003).

43. Nakamura, Y., Goto, T. & Tanaka, T. The endoparasitoid *Cotesia kariyi* (Ck) regulates the growth and metabolic efficiency of *Pseudaletia separata* larvae by venom and Ck polydnavirus. *Journal of Insect Physiology* **47**, 573–584, doi:10.1016/S0022-1910(99)00147-5 (2001).

44. Nakamatsu, Y. & Tanaka, T. Venom of ectoparasitoid, *Euplectra sp. near plathypene* (Hymenoptera: Eulophidae) regulates the physiological state of *Pseudaletia separata* (Lepidoptera: Noctuidae) host as a food resource. *Journal of Insect Physiology* **49**, 149–159, doi:10.1016/S0022-1910(98)00261-5 (2003).

45. Turkish, A. R. & Starley, S. L. The genetics of neutral lipid biosynthesis: an evolutionary perspective. *American Journal of Physiology-Endocrinology and Metabolism* **297**, E19–E27, doi:10.1152/ajpendo.90898.2008 (2009).

46. Thompson, S. Parasitism enhances the induction of gluconeogenesis by the insect. *Manduca sexta*. *The international journal of Developmental biology* **49**(1), 41–65, doi:10.2210/kb-1991-0494.ch004 (1991).

47. Dahlman, D. & Vinson, S. Teratocytic developmental and biochemical characteristics. *Parasites and pathogens of insects* **1**, 145–165, doi:10.1016/S0098-780-08-091649-150012-8 (1993).

48. Hochuli, A. & Lanzrein, B. Characterization of a 212 kD protein, released into the host by the larva of the endoparasitoid *Chelonus inanitus* (Hymenoptera, Braconidae). *Journal of Insect Physiology* **47**, 1313–1319, doi:10.1016/S0965-1850(02)00006-4 (1999).

49. Fueller, M., Wang, D. A., Tügì, G. & Siess, W. Activation of human monocytic cells by lysophosphatidic acid and sphingosine-1-phosphate. *Cellular Signalling* **15**, 367–375, doi:10.1016/S0898-6568(02)00117-1 (2003).

50. Nakamura, Y., Goto, T. & Tanaka, T. The endoparasitoid *Cotesia kariyi* (Ck) regulates the growth and metabolic efficiency of *Pseudaletia separata* larvae by venom and Ck polydnavirus. *Journal of Insect Physiology* **47**, 573–584, doi:10.1016/S0022-1910(99)00147-5 (2001).

51. Nicholas J. Colinet, H., Renault, D., Chary-Geulé, B. & Com, E. Metabolic and proteomic profiling of diapause in the aphid parasitoid Praon volucris. *PloS One* **7**, e32606, doi:10.1371/journal.pone.0032606 (2012).
Author Contributions
Z.S., G.X.K., and C.J.J. conceived and designed the experiments; Z.S., and G.X.K. performed the experiments; G.X.K., Z.S., L.J.Y., L.L.M. and Z.L.J. analyzed the data; Z.L.J. and Z.S. evaluated the conclusions; and G.X.K., Z.S. and C.J.J. wrote the paper. All authors read and approved the final manuscript.

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