Analysis of Differentially Expressed Genes of *Chrysoperla sinica* Related to Flight Capacity by Transcriptome

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Abstract

The lacewing *Chrysoperla sinica* (Tjeder) is a common natural enemy of many insect pests in China and is frequently employed for biological control programs. Adults make migratory flights after emergence, which reduces their effectiveness as biological control agents. Previously, we proved that 2-d-old unmated females exhibited significantly stronger flight ability than 3-d-old ones. Meanwhile, 3-d-old unmated adults flew significantly longer distances than mated ones. In this study, Illumina RNA sequencing was performed to characterize differentially expressed genes (DEGs) between virgin and mated adults of different ages in a single female strain of *C. sinica*. In total, 713,563,726 clean reads were obtained and de novo assembled into 109,165 unigenes with an average length of 847 bp (N50 of 1,754 bp), among which 4,382 (4.01%) unigenes matched known proteins. Based on these annotations, many putative transcripts were related to *C. sinica*’s flight capacity and muscle structure, energy supply, growth, development, environmental adaptability, and metabolism of nutritional components and bioactive components. In addition, the differential expression of transcripts between different ages and mating status were analyzed, and DEGs participating in flight capacity and muscles were detected, including glutathione hydrolase, NAD-specific glutamate dehydrogenase, aminopeptidase, and acidic amino acid decarboxylase. The DEGs with functions associated with flight capacity and muscles exhibited higher transcript levels for younger (2 d–old) virgins. This comprehensive *C. sinica* transcriptomic data provide a foundation for a better understanding of the molecular mechanisms underlying the flight capacity to meet the physiological demands of flight muscles in *C. sinica*.

Key words: *Chrysoperla sinica* (Tjeder), flight capacity, age, mating, differentially expressed genes

The lacewing *Chrysoperla sinica* (Tjeder) (Neuroptera: Chrysopidae) is a common insect species throughout China (Xu et al. 1999) and has been widely recognized as an important natural predator of many pests involved in agriculture and forestry, including aphids, whiteflies, thrips, lepidopterans, and mites (Senior and McEwen 2001, Wang and He 2006, Ragsdale et al. 2011). Adult lacewings are not preaceous and feed on plant-derived food, such as nectar and pollen, as well as insect honeydew (Xu et al. 2004, Villenave et al. 2005). However, *C. sinica* larvae are commonly known as aphid lions since they voraciously attack aphids by seizing them with large, piercing mandibles and inject a paralyzing venom. Therefore, the lacewing *C. sinica* is considered to be a potentially good biological control agent for pests (Liu et al. 2011a, Pappas et al. 2011), particularly for aphids in crop fields, orchards, and greenhouses with the greatest versatility in integrated pest management (IPM). In nature, adults lacewings *C. sinica* demonstrate the ability to initiate and sustain periods of long-distance flight for reproduction and food following emergence. These migratory and dispersal abilities enable *C. sinica* to avoid unfavorable conditions for survival but also reduce the efficacy and effectiveness of biological control programs (Liu et al. 2011b). A better understanding of the regulatory mechanisms underlying *C. sinica* migration and dispersal habits is essential in order to use them more effectively and incorporate them in integrated pest management to improve biological control effectiveness in different crop systems.

The behavioral, physiological, and morphological adaptations that are necessary to insect migration have been widely investigated, including the mobilization of lipids as flight fuel (Lorenz and Gäde 2009, Arrese and Soulages 2010), the development of flight muscle structure (Iwamoto 2011, Lu et al. 2020), and the regulation of hormones (Lorenz and Gäde 2009) that influence migratory physiology. Juvenile hormones (JHs) play an important role in the trade-off between reproductive and migratory physiology (Zera 2003, Zera and Zhao 2004, Roff and Fairbairn 2007, McCulloch et al. 2019). Usually, low levels of JH titers triggered migratory behavior of insects, and the JH titers would stop the migration and promote the development of ovaries when they were higher than a specific threshold (Zera 2006). The JH analog, Methoprene, promoted the...
in-colony male movement of *Apis mellifera* Linnaeus from the center to the periphery (Harano 2013) and inhibited the growth and differentiation of wing primordia in silkworms (*Bombyx mori* Linnaeus) (Hu et al. 2017). Transcriptomic and genomic analyses have been used to uncover the genes involved in insect migration and flying capacity (Yang et al. 2014, Jones et al. 2015, Xiao et al. 2017, Guo et al. 2018, Sun et al. 2018, DeAguero et al. 2019, Hunt et al. 2019, McCulloch et al. 2019).

Migratory populations of *C. sinica* demonstrated adaptations to long-distance flights with significant phenotypic and genetic variation in the individual traits that comprise flight capacity (Liu et al. 2011a, 2011b). The flight ability of the lacewing *C. sinica* individuals was determined by sex, mating status (virgin vs mated), and age. Liu et al. (2011a, 2011b) characterized the flying performance of *C. sinica* using flight mills, showing significant effects of age on the flying distance, continuous flight time, and flight speed. Two-day-old males and females had the highest total flight activity levels and the longest individual flight bouts. Compared with the mated individuals, 3-d-old unmated *C. sinica* individuals showed significantly longer flight distance and greater continuous flight time and flight speed. Five-day-old females in the preoviposition period flew over the longest distance; however, the flight ability of the 5-d-old individuals was not affected by mating status (Liu et al. 2011a). The sarcomere length and myofibril diameter of flying muscles in *C. sinica* females and males significantly changed with age. The longest sarcomeres were observed in 3-d-old females and 10-d-old males. The genes and associated biochemical and signaling pathways underlying the flight capacity in *C. sinica*, however, remain poorly understood.

The flight behavior of *C. sinica* is particularly important not only for their survival and colonization but also for efficient utilization in integrated pest management (IPM). In the present study, we tested the flight performance of *C. sinica* of different age and mating status to reveal differences in their flight capacity. Subsequent Illumina RNA sequencing (RNA-seq) was performed to characterize differentially expressed genes (DEGs) to better understand the molecular mechanisms underlying the differences in flight capacity. Among the identified DEGs, candidate genes involved in flight muscle structure, energy metabolism, signal transduction, and metabolic pathways play important roles in flight capacity. The key genes associated with flight capacity and muscles were further validated by real-time quantitative reverse transcription PCR (qRT-PCR). These findings provide novel insights into a deeper understanding of the molecular mechanisms underlying flight performance in *C. sinica*.

### Materials and Methods

**Establishment and Rearing of a Single Female Strain of *C. sinica***

A laboratory colony of *C. sinica* was initiated with adults collected from apple orchards in August 2012 at Wanrong City, Shanxi Province, China. Larvae were reared in Petri dishes (90 mm ID) containing *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) eggs. Adults were provided with an artificial diet as described by Zhou et al. (1981).
The adult males and females were raised individually or in pairs within 24 h after emergence for sampling of different ages and mating status. For each age group, a total of 20–30 unmated females or males were separately sampled at the same time at 2 and 3 d following emergence (F2N: 2-d-old unmated females; M2N: 2-d-old unmated males; F3N: 3-d-old unmated females; and M3N: 3-d-old unmated males). For the mating status groups, 20–30 virgin males or females and 20–30 mated males or females were separately sampled at 3 d after emergence (F3N: 3-d-old unmated females; M3N: 3-d-old unmated males; F3Y: 3-d-old mated females; and M3Y: 3-d-old mated males). To minimize biological variance, three independent biological replicates were performed for each sample group.

Flight Capacity Tests
Experiments testing the flight capacity of 2 and 3-d-old unmated and mated adults were conducted for 8 h using tethered flight assays under a temperature maintained at 23°C and a relative humidity (RH) of 75% as previously described (Liu et al. 2011a). For each group, 30 or more healthy adults were employed for the flight capacity tests. Unhealthy insects and those with damaged wings were not used in experiments. Each test was started at 20:00 h for an 8-h period under a 0L: 24D photoperiod. During flight, neither moisture nor the nutrition source was replenished. The flight parameters, including the average cumulative flight distance (km/insect), the average cumulative flight duration (h/insect), and the flight speed (m/s), were recorded.
RNA Extraction and Sequencing
Total RNA was extracted from whole individual adults per sample using the RNeasy Mini Kit (QIAGEN, Hilden, Germany) in accordance with the manufacturer’s instructions. Genomic DNA was digested by RNase-Free DNase Set (QIAGEN, Hilden, Germany). The quality and quantity of RNA were determined using a Nano Drop 2000 (Thermo Scientific, Wilmington, DE, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA); the RNA was then diluted to 1 ng/µl for library construction. Briefly, mRNA was broken into short fragments (about 300 bp) in length by ion interruption and reverse transcribed to the first strand of cDNA by 6 bp random primers. Then, the first strand of cDNA was used as a template to synthesize the second strand of cDNA. Library fragments were enriched by PCR amplification to select the fragment size of 450 bp. Equal amounts of libraries with different Index sequences were pooled prior to sequencing and diluted to 2 nM for paired-end sequencing on the IlluminaHiSeq 2500 platform (Illumina Inc., San Diego, CA).

Read Mapping and Transcript Profiling
The adapter and low-quality sequences were removed from the raw RNA-seq reads to generate high-quality clean reads. At the same time, the Q20, Q30, and GC content were calculated. The clean reads from all libraries were de novo assembled using Trinity v2.0.6 software (Grabherr et al. 2011) without a reference genome (parameter settings: -min_contig_length 150 - CPU 8 -min_kmer_cov 3 -min_glims 3 -bfly_opts “-V 5-edge-thr=0.1 -stderr”) to generate transcripts for clustering gene family, from which the longest transcript under each gene was used for the representative sequence of the unigenes.

All unigenes were aligned using BLAST v2.2.23 (Altschul et al. 1997) (e-value ≤ 10^{-5}) to public databases including NR (NCBI nonredundant protein sequences, ftp://ftp.ncbi.nih.gov/blast/db/29-02-2015), GO (Gene Ontology), KEGG (Kyoto Encyclopedia of Genes and Genome), eggNOG (evolutionary genealogy of genes: Nonsupervised Orthologous Groups), Swiss-Prot, and Pfam (Nr, COG, KEGG). The best hits from these databases were employed to determine the sequence orientations and coding sequences (CDSs) of the unigenes. The GO annotations for unigenes were annotated with the NR database using Blast2GO v2.5.0 (Conesa et al. 2005). The generated transcriptome data were deposited under NCBI BioProject (Accession Number: PRJNA656886).

Identification of Differentially Expressed Genes (DEGs)
The counts for each gene were normalized as fragments per kilobase of transcript per million mapped reads (FPKM) (Trapnell et al. 2010).
Fig. 3. Differentially expressed genes between F2N and F3N (A), GO terms significantly related to flight capacity (B), and KEGG enrichment pathways significantly related to flight capacity (C).
Principal component analysis (PCA) was performed to compare the log2-transformed FPKM values of the expressed gene profiles among treatments using the prcomp function in R program (https://www.rproject.org). A hierarchical clustering of samples was performed using pheatmap in R. Read coverage over gene body, was calculated by RSeQC (Wang et al. 2012), and the corresponding plot figure was made using ggplot2 with R script.

DEGs between groups were identified using the statistical package DEGseq with the MA-plot-based method (Wang et al. 2010) in R version 3.0.3, where genes were considered differentially expressed if llog2FoldChange > 1, and an adjusted P-value using the Benjamini–Hochberg procedure (false discovery rate (FDR)) was <0.05.

Gene Annotation (GO) and Functional Enrichment Analysis
The GO enrichment analysis for biological processes, molecular functions, and cellular components was performed using TopGo (Alexa and Rahnenfuhrer 2020) with a P-value of <0.05 being considered statistically significant. KEGG (Kyoto Encyclopedia of Genes and Genome) pathway enrichment analysis was performed on all DEGs in the KEGG platform (http://www.genome.jp/kegg/) (Kanehisa et al. 2008), and an adjusted P-value of <0.05 was considered statistically significant.

Validation of DEGs by Quantitative Real-time PCR (qRT-PCR)
The expression levels of a set of randomly selected 10 DEGs were validated by a qRT-PCR assay. Total RNA was extracted using an RNAprep Pure Kit (Tiangen, Beijing, China) in accordance with the manufacturer’s instructions, and then treated with RNase-free DNase I (New England Biolabs, Ipswich, MA, USA) to eliminate all contaminating DNA. 1000 ng total RNA was used for the reverse transcription with PrimeScript first stand cDNA Synthesis Kit. qRT-PCR was performed with SYBR Premix Ex Taq (TaKaRa, Dalian, China) on ABI Step One RT–PCR system according to the manufacturer’s instructions (20 µl reaction mix: 1 µl cDNA, 10 µl 2xSYBR real-time PCR premixture, 0.4 µl each 10 µM primer, and 8.2 µl distilled water). Three biological replicates with two technical replicates were made for each sample. The gene IDs and the primer sequences are listed in Supp Table 1 (online only). The PCR program was as follows: 95°C for 5 min, followed by 40 cycles of 95°C for 15 s, and 60°C for 30 s. Relative expression was normalized to the internal control gene GAPDH with the 2−ΔΔCT method (Livak and Schmittgen 2001). Pearson’s correlation was performed to determine the correlation of gene expression between qRT-PCR and transcriptomic data.

Statistical Analysis
Traits were expressed as the mean ± standard error of three independent biological replicates and subjected to one-way analysis of
Fig. 4. Differentially expressed genes between F3N and F3Y (A), GO terms significantly related to flight capacity (B), and KEGG enrichment pathways significantly related to flight capacity (C).
variance (ANOVA) followed by Duncan’s Multiple Range post-hoc testing and Student t-test (SPSS 17, IBM Corporation, Armonk, NY); the highly significant and, marginally significant levels were set to $P < 0.01$ and $P < 0.05$, respectively.

**Results**

**Flight Capacity Differs Significantly Between Ages and Mating Statuses**

Tethered flight assays for flight speed, flight duration, and flight distance showed that 2-d-old unmated females exhibited significantly stronger flight ability than 3-d-old ones, and 3-d-old unmated adults flew significantly longer distances than mated ones, consistent with our previous results (Liu et al. 2011a) (Supplementary Note (online only)). In the present study, therefore, comparisons of F2N versus F3N and F3N versus F3Y were employed for further transcriptomic analysis.

**Qualitative Description for Assembly and Annotation of Transcripts**

The RNA extracted from F2N versus F3N and F3N versus F3Y samples were analyzed by RNA-seq, with three replicates per tissue sample. In total, 753,563,850 reads were generated from 18 libraries (Supp Table 2 [online only]), from which 713,563,726 clean reads were obtained by removing the adapter, poly-N, and low-quality sequences. The Q30 percentage of bases was over 93%, indicating that the sequencing data were reliable. The clean reads were assembled into 316,222 contigs, with mean contig sizes of 371.51 bp. The contigs from all three libraries were combined and assembled into a reference unigene database, thereby yielding 109,165 unigenes with an average length of 847.83 bp and an N50 of 1,754 (Supp Table 3 [online only]). The length distribution of transcripts and unigenes is shown in Supp Fig. 1 (online only). Principal component analyses (PCA) revealed that the three replicates of each treatment were located nearest to each other (Fig. 1), demonstrating the reliability of our datasets.

All unigenes were annotated to six databases: NR (nonredundant), Swiss-Prot, GO (Gene Ontology Consortium), eggNOG (evolutionary genealogy of genes: Nonsupervised Orthologous Groups), Pfam (Nr, COG, KEGG), and KEGG (Kyoto Encyclopedia of Genes and Genomes) (Table 1). As shown in Table 1, 4382 unigenes (4.01% of all distinct unigenes) were successfully annotated, and 10,466 unigenes were matched to one or more of the databases. According to all alignments, 25,153 (23.04 %) unigenes were homologous in the NR database. Of these unigenes, 65.14% were similar to others and 15.57% were significantly similar to sequences of *Tribolium castaneum* (Supp Fig. 2 [online only]).

The unigenes were assigned to one or more GO terms for cellular component (CC), molecular function (MF), and biological process (BP) (Fig. 2A). Among them, unigenes were mainly assigned to ‘cellular process’, ‘metabolic process’, and ‘single-organism process’
in the BP category. In the CC category, unigenes were mainly enriched in 'Cell', 'cell part', and 'organelle'. 'Catalytic activity' and 'binding' were the most abundant terms in the MF category. The unigenes were assigned to 5 KEGG pathways with five main clusters, including metabolism, genetic information processing, organismal systems, cellular processes, and environmental information processing (Fig. 2B). The top five pathways were 'signal transduction', 'translation', 'carbohydrate metabolism', 'transport and catabolism', and 'endocrine system'.

DEGs Annotation in GO and KEGG Databases
Based on the DEGSeq results, the clustering analysis showed evidence of the overall aggregation clustering and the expression patterns of the remarkably regulated genes between different ages and mating statuses (Supp Fig. 3 [online only]). In total, 4,086 unigenes were differentially expressed between F2N (2-d-old unmated females) vs F3N (3-d-old unmated females), among which 1,405 were upregulated and 2,681 were downregulated (Fig. 3A). As evidenced by the functional annotation (Fig. 3B), DEGs were mainly annotated by GO to be significantly associated with flight capacity and physiology such as the chitin metabolic process, glucosamine-containing compound metabolic process, amino sugar metabolic process, and glucosamine-containing compound metabolic process (Fig. 3B). The KEGG pathway enrichment analysis showed that DEGs were mainly enriched into metabolism and pathways directly associated with flight physiology and energy such as peroxisome, pentose, and gluconate interconversions, glutathione metabolism, steroid hormone biosynthesis, and ascorbate and aldare metabolic process, among which taurine metabolism is the most significant pathway (Fig. 3C).

In total, 2,211 DEGs were identified between F3N (3-d-old unmated females) and F3Y (3-d-old mated females), among which 798 DEGs were upregulated and 1,413 DEGs were downregulated (Fig. 4A). DEGs were mainly annotated by GO to be significantly associated with flight capacity and physiology such as the chitin metabolic process, glucosamine-containing compound metabolic process, amino sugar metabolic process, and aminoalgalan metabolic process (Fig. 4B). The significantly enriched KEGG pathways were mainly associated with energy supply (pentose and gluconate interconversions, renin-angiotensin system pathway), sex reproductive physiology and mating (steroid hormone biosynthesis), xenobiotic biodegradation, and metabolism (drug metabolism-cytochrome P450, drug metabolism of other enzymes, and metabolism of xenobiotics by cytochrome P450), central nervous system morphogenesis (neuroactive ligand-receptor interaction), and cofactor and vitamin metabolism (ascorbate and aldare metabolic process and retinol metabolism), among which the renin-angiotensin system pathway is the most significant pathway (Fig. 4C).
Candidate Genes Involved in Flight Capacity

Several gene sequences that are putatively involved in flight muscle structure and flight capacity were annotated in the *C. sinica* transcriptome. Four DEGs encoding glutathione hydrolase, NAD-specific glutamate dehydrogenase (GDH), and acidic amino acid decarboxylase were significantly involved in the taurine and hypotaurine pathway; all four were downregulated (Fig. 5A, Supp Table 4 [online only]), including the glutathione hydrolase (GGT) family (TRINITY_DN45272_c2_g8, TRINITY_DN45272_c2_g7, TRINITY_DN37304_c0_g2, TRINITY_DN36809_c3_g3, TRINITY_DN36809_c3_g4, TRINITY_DN35099_c1_g2, TRINITY_DN36809_c3_g1, and TRINITY_DN36809_c3_g2), NAD-specific GDH (TRINITY_DN44674_c2_g1), acidic amino acid decarboxylase (CDL1) (TRINITY_DN40641_c4_g1), and a gene with an unknown function (TRINITY_DN45272_c2_g4). DEGs encoding GGT1, NAD-specific GDH, and acidic amino acid decarboxylase are the key down-regulated genes in flight muscles from 2 to 3-d-old unmated females.

Eight DEGs between 3-d-old unmated and mated females were significantly involved in the renin–angiotensin system pathway (Fig. 5B, Supp Table 5 [online only]), all of which were downregulated, including the aminopeptidase N (APN) family (TRINITY_DN40589_c3_g4, TRINITY_DN43099_c4_g3, TRINITY_DN44278_c0_g1, TRINITY_DN42800_c2_g2, TRINITY_DN43099_c4_g2, TRINITY_DN42800_c1_g1, TRINITY_DN42800_c2_g6, and TRINITY_DN34778_c0_g1), angiotensin-converting enzyme (ACE) (TRINITY_DN44586_c1_g1, TRINITY_DN45140_c0_g1, and TRINITY_DN42216_c0_g3), probable phosphoenolpyruvate synthase (ppsA) (TRINITY_DN44940_c1_g1), and chymotrypsin (TRINITY_DN44563_c6_g4 and TRINITY_DN43329_c3_g3). Aminopeptidase family genes are the key downregulated genes after mating.

Validation of RNA-Seq Gene Expression Data

qRT-PCR was employed for 10 selected DEGs participating in the taurine and hypotaurine pathway and renin–angiotensin system pathway to confirm the reliability of the RNA-Seq data. Overall, consistent expression patterns for these 10 genes were obtained between the qRT-PCR and RNA-Seq analyzes (Fig. 6, Supp Table 6 [online only]), indicating that the transcriptome sequencing results were reliable and would enable us to make reasonable deductions based on the functional enrichment analysis of the DEGs.

Discussion

The green lacewing, *Chrysoperla sinica* (Tjeder), is a polyphagous natural enemy attacking several pests that feed on various crops and has been widely employed in China for biological control of many important agricultural pests, such as aphids, leafhoppers, thrips, mites, and Lepidopterans (Crabtree and Newsholme 1970, Wang and He 2006, Ragsdale et al. 2011, Khuhro et al. 2012). However,
following emergenc, *C. sinica* adults possess sufficient flight capacity for dispersal, thereby significantly limiting its value for biological control programs. An extensive understanding of molecular mechanisms regulating flight performance of *C. sinica* may allow us to develop more sustainable and environmentally-friendly integrated pest management by manipulating their migratory behavior. Surprisingly, BLASTx annotation of *C. sinica* transcriptome sequences revealed the highest similarity with others (65.14%), while demonstrating a lower identity match (1.61%) with *Athalia rosae* Linnaeus, a representative of the order Hymenoptera. The protein sequences available in the database are clearly limited for *C. sinica*. To the best of our knowledge, the *C. sinica* transcriptomic and DEG profiling data may serve as a reference for the efficient complementation and enrichment of the *C. sinica* database, thereby potentially facilitating the characterization of novel genes, gene functional analysis, and developmental molecular mechanisms of *C. sinica*.

*Chrysoperla sinica* migration is regulated not only by environmental conditions but also by its own physiological factors, with molecular modulation playing an important role in this process. Numerous genes, signals, and metabolic pathways are involved in the complex developmental dynamics of flight muscle structure and flight capacity (Yang et al. 2014, Jones et al. 2015, Xiao et al. 2017, Guo et al. 2018, Sun et al. 2018, DeAguero et al. 2019, Hunt et al. 2019, McCulloch et al. 2019). The results of flight testing confirmed the significant differences in flight capacity of 2 and 3-d-old unmated females, and 3-d-old unmated and mated females, consistent with previous reports (Liu et al. 2011a, 2011b). Compared with 3-d-old unmated females, 2-d-old unmated females exhibited greater endurance. The 3-d-old unmated females showed better flight performance than the 3-d-old mated ones. In order to understand molecular mechanisms underlying the flight performance in *C. sinica*, the flight capacity and transcriptome were compared between groups of different age and mating status from a single female strain of *C. sinica*. In total, 109,165 unigenes were detected, of which many putative transcripts relate to regulating the flight activity, growth, development, and environmental adaptability of *C. sinica*. In total, 4,086 and 2,211 DEGs were identified in comparisons of 2-d-old versus 3-d-old unmated females and 3-d-old unmated versus 3-d-old mated females, respectively, presenting valuable information on genes’ expression profiles. These DEGs were significantly enriched in pathways associated with energy supply (pentose and gluconate interconversions, the renin–angiotensin system pathway), sex reproductive physiology and mating (steroid hormone biosynthesis), and the digestive and excretory systems (ascorbate and aldarate metabolism and retinol metabolism), among which the renin–angiotensin system pathway and the taurine and hypotaurine pathway were the most significant pathways. Our analysis provides insights into the whole picture of gene expression of *C. sinica* at different ages and mating statuses and in their various biosynthetic pathways.

Insect flight is one of the fastest, most intense, and energy-demanding motor activities, requiring metabolic rate increases of 50- to 100-fold (Arrese and Soulages 2010). Jones et al. (2015) revealed
a set of expressed candidate genes important to the mobilization of lipids as flight fuel, the development of flight muscle structure, and the regulation of hormones. Transcripts related to lipid metabolism and energy production were found at higher expression levels in migratory morphs (Yang et al. 2014). We discovered that DEGs were significantly enriched in several functional GO terms and KEGG pathways related to energy production and flight muscle structure. Several proteins, including flight muscle GDH in the taurine and hypotaurine pathway and APN in the renin–angiotensin system pathway, were involved in the oxidation of proline to generate glutamate and energy, including adenosine triphosphate (ATP) and lipids, as a fuel for flight (Crabtree and Newsholme 1970, Male and Storey 1983), thus allowing enzyme activity in response to the energy status of the cell at the initiation of flight. High ATP turnover and lipid-fueled flight activity causes oxidative damage (Magwere et al. 2006). GGT1 in the taurine and hypotaurine pathway initiates extracellular glutathione (GSH) breakdown, provides cells with a local cysteine supply, and contributes to maintaining intracellular GSH levels, thereby protecting insects from oxidative stress and damage from prolonged flights. ACE in the renin–angiotensin system pathway was differentially expressed between 3-d-old unmated and 3-d-old mated females. ACE is a zinc metallopeptidase capable of inactivating a variety of small- to medium-size peptide hormones by cleavage of C-terminal dipeptides and dipeptide amides, thereby playing an important physiological role for the metabolism of bioactive peptides (Isaac et al. 2007) in insect reproduction (Xu et al. 2013, Kumar et al. 2016). Insect ACE is susceptible to inhibitors such as captopril, lisinopril, fosinoprilat, enalapril, and trandolaprilat (Williams et al. 1996). Inhibition of these protein expressions may degrade the flight ability of C. sinica for dispersal, thereby enhancing its value for a biological control program.

In conclusion, we, for the first time, performed de novo transcriptome sequencing analysis of C. sinica. The reads were assembled without a reference genome to generate 109,165 unigenes with an average length of 847 bp. A large number of candidate genes potentially involved in flight capacity and flight muscles were profiled. The transcriptomic and DEG data provide the fundamentals for understanding the molecular mechanisms underlying flight capacity and physiological demands of flight muscles, knowledge which could be used for the future development and implementation of environmentally sustainable integrated pest management.

Supplementary Data
Supplementary data are available at Journal of Insect Science online.

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Authors Contributions
ZL contributed to the design of experiments, data collection and analysis, and was primarily responsible for figure development and manuscript writing. RFand YG conceived and obtained funding for the research. PZ and JF helped design and interpret experimental results and edited the manuscript.YL and XS performed phenotyping and sampling in collaboration with ZL. JY helped with identification of candidate genes and aided in the designing of experiments. All authors read and approved the final manuscript.

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