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Comparative transcriptional analysis provides insights of possible molecular mechanisms of wing polyphenism induced by postnatal crowding in *Aphis gossypii*

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Abstract

Background: *Aphis gossypii* is a worldwide sap-sucking pest with a variety of hosts and a vector of more than 50 plant viruses. The strategy of wing polyphenism, mostly resulting from population density increasing, contributes to the evolutionary success of this pest. However, the related molecular basis remains unclear. Here, we identified the effects of postnatal crowding on wing morph determination in cotton aphid, and examined the transcriptomic differences between wingless and wing morphs.

Results: Effect of postnatal crowding on wing determination in *A. gossypii* was evaluated firstly. Under the density of 5 nymphs·cm^{-2}, no wing aphids appeared. Proportion of wing morphs rised with the increase of density in a certain extent, and peaked to 56.1% at the density of 20 nymphs·cm^{-2}, and reduced afterwards. Then, transcriptomes of wingless and wing morphs were assembled and annotated separately to identify potentially exclusively or differentially expressed transcripts between these two morphs, in which 3 126 and 3 392 unigenes annotated in Nr (Non-redundant protein sequence) database were found in wingless or wing morphs exclusively. Moreover, 3 187 up- and 1 880 down-regulated genes were identified in wing versus wingless aphid. Pathways analysis suggested the involvement of differentially expressed genes in multiple cellular signaling pathways involved in wing morphs determination, including lipid catabolic and metabolism, insulin, ecdysone and juvenile hormone biosynthesis. The expression levels of related genes were validated by the reverse transcription quantitative real time polymerase chain reaction (RT-qPCR) soon afterwards.

Conclusions: The present study identified the effects of postnatal crowding on wing morphs induction and demonstrated that the critical population density for wing morphs formation in *A. gossypii* was 20 nymphs·cm^{-2}. Comparative transcriptome analysis provides transcripts potentially expressed exclusively in wingless or wing morph, respectively. Differentially expressed genes between wingless and wing morphs were identified and several signaling pathways potentially involved in cotton aphid wing differentiation were obtained.

Keywords: Cotton aphid, Apterous and alate aphids, Wing induction, Phenotypic plasticity, Gene expression profiling

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Background

Polyphenism denotes that multiple phenotypes are produced by a single genotype due to environmental induction (Ogawa and Miura 2014). This phenomenon exists commonly in organisms, especially in insects, such as castes in social insects, gregarious and solitary change of locusts, seasonal morphs of butterflies, and sexually selected phenotypes in beetle horns (Corona et al. 2016; Klein et al. 2016; Pespeni et al. 2017; Wang et al. 2014). Of the various phenotypes observed in the complex life cycle of aphids, the wing polyphenism seen in most species is conspicuous (Ogawa and Miura 2014). This strategy is considered to have contributed to the evolutionary success of aphids. Alate morph, with long wings, flight muscles and flight capability, benefits for searching hosts, escaping from natural enemies and reducing negative density-dependent population effects through dispersal (Martínez and Costamagna 2018). Nonetheless, wingless morph, with absent wings and underdeveloped flight muscles, shows an earlier onset of oviposition and higher reproductive capacity, allowing a rapid increase of the aphid colony size (Castañeda et al. 2010). In wing-dimorphic aphid species, the production of asexual alate morphs resulted from increased population density, poor plant quality and natural enemies (Braendle et al. 2006). Crowding is considered to be one major environmental factor in aphids wing induction, in which the production of wing is triggered by tactile stimuli between individual aphids (Forrest 1974; Kidd and Tozer 1984; Martínez and Costamagna 2018; Purandare et al. 2014). Series of signal pathways were reported to participate in the wing differentiation induced by crowding in several aphid species, including ecdysone, juvenile hormone (JH), octopamine, olfactory receptors co-receptor (Ishikawa et al. 2013; Jia et al. 2015; Vellichirammal et al. 2017; Wang et al. 2016). These studies have greatly increased our knowledges on the relationship between ecological crowding and wing determination in aphids. Less well understood, by contrast, is the molecular basis underlying wing phenotypic plasticity, especially in *Aphis gossypii*, the worldwide destructive agricultural pest.

A. gossypii, also known as the cotton or melon aphid, is a globally sap-sucking pest with a variety of hosts and a vector of more than 50 plant viruses (Dogimont et al. 2015; Nilesh et al. 2014). Mitigation of economic losses caused by this pest has been a challenge worldwide due to their complex phenotypic morphs, particularly wing plasticity (Liu et al. 2014). Wing morphs facilitate population outbreak and plant virus spread by migration during the crop growing seasons in summer (Costamagna et al. 2013; Zhu et al. 2006). The production of winged morphs has been observed due to the increase of population density in this species (Reinhard 1927). However, how crowding works on the wing induction and the related molecular mechanism are still unclear.

Wing morph determination in aphids occurs pre- or postnatally, during embryogenesis or newly born stages, in which postnatal crowding can induce wing development alone (Müller et al. 2001; Martínez and Costamagna 2018; Vellichirammal et al. 2017). In this study, we firstly determined the effects of postnatal crowding on the production of wing morph in *A. gossypii*. Then, differentially expressed genes (DEGs) between wing and wingless morphs were identified by RNA-seq approach, followed by reverse transcription quantitative real time polymerase chain reaction (RT-qPCR). Then the putative roles of selected DEGs and significantly enriched signal pathways potentially involved in the wing morphs formation were discussed. The objectives of this study were 1) to determine the effects of postnatal crowding on wing morph determination, and 2) to reveal gene expression profiles between wingless and winged morphs. Taking all together, this study aims to provide molecular basis that underpins wing ploryphensim in *A. gossypii*. These will facilitate the sustainable management of cotton aphid through disruption of its migratory behavior by the method of RNA interference in future.

Materials and methods

Aphid maintenance

A. gossypii colony, collected originally in Anyang, Henan, China, was reared on cotton plants at Institute of Cotton Research of Chinese Academy of Agricultural Sciences under controlled laboratory conditions (25 ± 1 °C, 75% relative humidity, and 16 L : 8D photoperiod). Aphid population densities and cotton plant growth were monitored routinely to avoid the production of wing aphids before experiments were conducted.

Postnatal crowding experiment

The effects of postnatal crowding on wing determination were evaluated by rearing newly born nymphs at different population densities (1, 5, 10, 15, 20, 25, 30, 40, 50, 60 nymphs· cm^{-2}) in mini clip-cages upon cotton seedling leaves. The proportions of wing morph at each density level were calculated. There were 9–12 biological replicates at each density. Morphological characters of the two morphs including body color and wall, thoraces, legs, wing, antennae in the adult stage of each morph were visualized using a SteREO Discovery V8 microscope (Zeiss, Germany).

Samples preparation

To eliminate the potential influence of embryonic offspring embedded in the ovaries of the adult, wing and wingless adults were collected separately only after their reproductive cycles overed. Wingless or wing morphs were transferred to new host plants once after last molting in adulthood. When the offsprings were given birth up, the whole body of the two wing morphs were

collected, respectively. There were four biological replicates for each morph in RNA-seq by using BGISEQ-500 platform. Each biological replicate contained 50 wingless or wing adults, respectively. Total RNA was extracted from biological replicate using TRIzol® reagent (Promega, USA). The RNA integrity was verified by 1% (mass fraction) agarose gel electrophoresis and the quantity of extracted RNA was assessed using a spectrophotometer Nanodrop 2000 (Thermo, USA). Synthesis of cDNA and Illumina library generation were completed at Beijing Genomics Institute (BGI) (Shenzhen, China).

Transcriptome assembly and gene annotation

Transcriptomes of two morphs were assembled de novo individually using a short reads assembling program, Trinity (Grabherr et al. 2011). Unigene sequences larger than 200 bp were aligned to protein databases, including Nr, Swiss-Prot, KEGG, Pfam, KOG by balstx and to the nucleotide Nt by blastn with the cutoff e-value of 10E-5. Nr genes annotated in wing and wingless morphs exclusively were illustrated by venn diagram (Bardou et al. 2014).

Gene profiling and data processing

Gene expression levels were calculated by fragments per kb per million reads (FPKM) method using Bowtie2 (version 2.2.5, –q --phred64 --sensitive --dpad 0 --gbar 99999999 --mp 1,1 --np 1 --score-min L,0,-0.1 -p 16 -k 200) and RESM (version 1.2.8) with default settings (Langmead and Salzberg 2012; Li and Dewey 2011). Fold change (FC), the relative expression level of an unigene in one morph to another, and *P*-value were used to determine the differentially expressed gene between two morphs by DEGseq (Wang et al. 2010), in which *P*-values in multiple tests were adjusted by false discovery rate (FDR)(Reiner et al. 2003). Ultimately, FC > 2 and adjusted $P < 0.001$ were the thresholds to determine significant differences in gene expression. Gene ontology (GO) enrichment was performed by Blast2GO suites at the criteria of adjusted $P < 0.05$ in Fisher's exact test (Conesa et al. 2005). Significantly enriched KEGG pathways were identified by hypergeometric tests at the cutoff criteria of adjusted $P < 0.05$ (Xie et al. 2011).

RT-qPCR validation

Single-stranded cDNA was synthesized using 1 μg of RNA from various samples with a reverse transcription system (Promega, USA) according to the manufacturer's recommendations. RT-qPCR was performed on Mastercycler ep realplex detection system (Eppendorf, Germany) using GoTaq® qPCR Master Mix (Promega, USA): initiated at 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s, 60 °C for 20 s, and 72 °C for 30 s. Melting curve analysis was conducted to verify the specificity of amplification. The relative expression levels were calculated using the $2^{-\Delta\Delta C}{}_t$ method (Livak and Schmittgen 2001) and normalized by the housekeeping gene *GAPDH* (Gao et al. 2017). Primers used for RT-qPCR validation were showed in Additional file 1: Table S1. There were four biological replicates for each morph in RT-qPCR. Each biological replicate contained 50 wingless or wing adults, respectively.

Statistic analysis

Statistical analyses were performed by Student's *t*-test using SPSS 19.0 software. Significant differences were considered at $P < 0.05$. Values were reported as mean ± SE.

Results

Effects of postnatal crowding on wing determination in *A. gossypii*

Under crowding conditions, most aphid species produce fully winged migratory forms that can fly to new hosts. By raising newly born nymphs at different population densities in laboratory, the effects of postnatal crowding on the production of alate morphs in the cotton aphid were found. No winged aphids were induced when the population density was lower than 5 nymphs·cm^{-2} (Fig. 1a). The proportion of wing aphids rised with the increasing population densities in a certain extent, and peaked to 56.1% at the density of 20 nymphs·cm^{-2}. However, the production of wing aphids reduced gradually later (Fig. 1a). Except for wing, the two morphs were distinguishable in sclerotization of head and thorax, melanism of body, hind tibiae color (Fig. 1b, c).

Transcriptome assembly of wingless and wing aphid individually

Transcriptomes of wingless and wing aphids were sequenced using Illumina technology independently. A total of 246.06 M and 244.48 M clean reads were obtained with the Q30 of 90.48% and 91.08%, the GC contents of 43.0% and 43.1% in wingless and wing aphids, respectively (Table 1). The clean reads were then assembled into 27 585 unigenes with the N50 length of 2 580 bp in wingless aphid and 29 763 unigenes with the N50 length of 2 609 bp in wing aphid, respectively (Table 1). All the unigenes matched previously described reads with more than 91.81% coverage, including no less than 47.60% of uniquely mapped reads (Table 1). All the raw reads have been submitted to the NCBI SRA under the accession number PRJNA510737.

Functional annotation of cotton aphid transcriptome

Assembled unigenes in the two morphs were firstly searched against public databases individually, including Nr, Nt, Swiss-Prot, KEGG, KOG, Pfam, GO. Using this approach, 22 776 (82.57%) and 24 180 (81.24%) unigenes returned a significant result at least in one of the

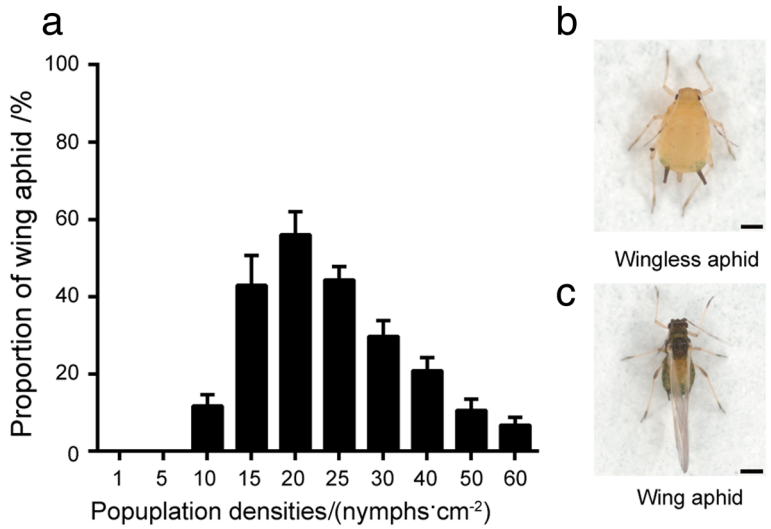

Fig. 1 Effects of postnatal crowding on wing morph switch in *Aphis gossypii*. **a** Proportion of wing cotton aphids induced by postnatal crowding at different population densities in laboratory. **b** Morphology of wingless cotton aphid. **c** Morphology of wing cotton aphid. Scale bars, 0.5 mm

searched databases in the two morphs, respectively (Table 1). Interestingly, there were much more unigenes mapped to the seven databases in wing aphid than in wingless aphid (Fig. 2a). 20 783 and 22 068 unigenes in the two morphs were found to be homologic in the Nr database (Fig. 2a). By filtering redundant data, a total of 18 409 Nr genes were returned, including 11 891 Nr genes annotated in both morphs, 3 126 and 3 392 Nr genes annotated in wingless or wing morphs exclusively (Fig. 2b). The genes exclusively returned in wingless aphid were enriched in the pathway of renin secretion, phagosome, ribosome, while those uniquely returned in wing aphid were clustered in phototransduction, gastric acid and salivary secretion, estrogen, oxytocin, pancreatic secretion, oocyte meiosis (Fig. 2c). Besides, the annotated unigenes sequences had the greatest homology with those in *Myzus persicae*, followed by *Acyrthosiphon pisum*, *Diuraphis noxia*, *A. gossypii*, *Bemisia tabaci* (Additional file 2: Fig. S1).

Table 1 Summary of transcriptome data of wingless and wing cotton aphids

Groups	Wingless aphid	Wing aphid
Clean reads	246 058 970	244 483 952
Q30 /%	90.48	91.08
GC content /%	43.00	43.10
Assembled unigenes	27 585	29 763
N50 /bp	2 580	2 609
Mapped reads	226.35	224.47
Mapped ratios /%	91.99	91.81
Uniquely mapped reads	120.93	116.38
Uniquely mapped ratio /%	49.15	47.60
Annotated unigenes	22 776	24 180
Annotated ration /%	82.57	81.24

DEGs between wingless and wing aphids

Based on the cutoff criteria (FC > 2, $P < 0.001$), 5 067 DEGs including 3 187 up-regulated (FC =2–9 953) and 1 880 down-regulated (FC =25–497) genes were identified in wing aphid compared with wingless aphid (Fig. 3a). The variability pattern was displayed by volcano plot which revealed large number of DEGs (Fig. 3b). The number of DEGs with low (2 < FC < 10), medium (10 < FC < 100), high (100 < FC < 1 000) fold changes were 3 781 (up, 2 497; down, 1 284), 891 (up, 488; down, 403), 363 (up, 178; down, 185), respectively. Besides, the fold changes of 32 DEGs (up, 24; down, 8) were more than 1 000 (Fig. 3c). Details in all DEGs with different fold changes levels were listed in Additional file 3: Table S3.

GO category enrichment analysis of DGEs

To uncover the functions of DEGs between the two morphs, we initially analyzed GO terms with pooled up- and down-regulated DEGs. For wing aphid vs. wingless aphid, 2 239 DEGs were clustered into the category of biological process, 3 005 DEGs to cellular component and 2 111 DEGs to molecular function, respectively (Additional file 4: Figure. S2). To further unveil the functions of DEGs, we performed the multilevel GO enrichment analysis on the up- and down-regulated genes individually. For the up-regulated genes, the most represented GO terms were related to lipid catabolic process, glycolytic process, glycerol-3-phosphate metabolic process, heme binding, activity of monooxygenase, oxidoreductase, transporter and

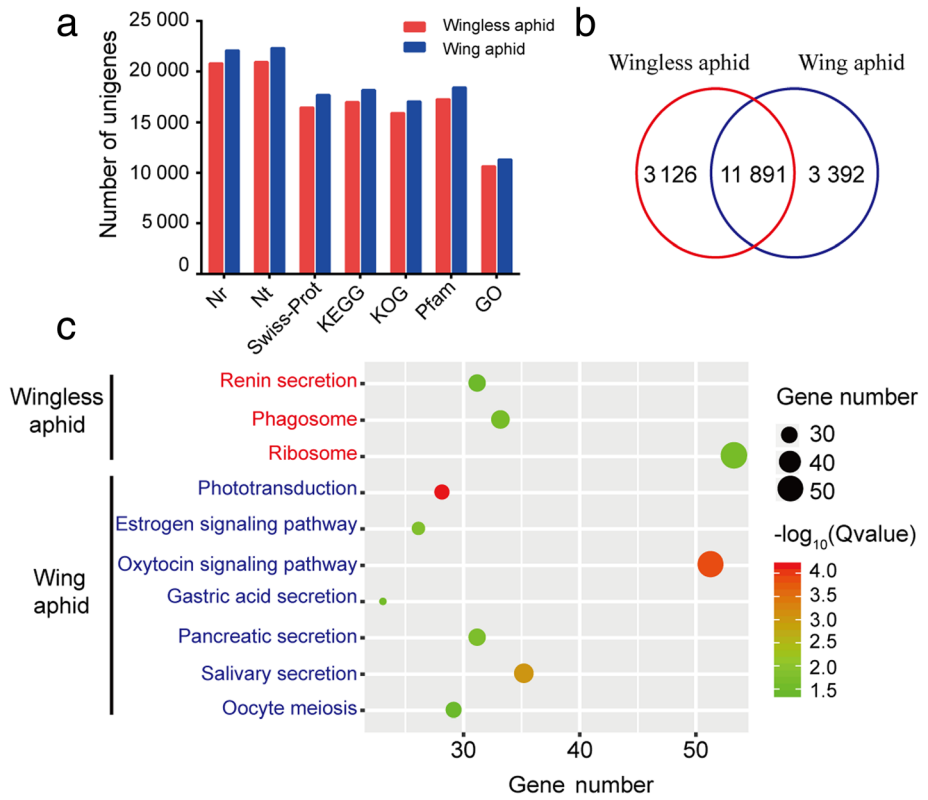

Fig. 2 Characterization of the transcriptomes of the two morphs. **a** Comparative analysis of the two morphs unigenes number annotated in public databases. **b** Venn diagram showing the distribution of genes annotated in Nr database in wingless and wing morphs. **c**. Functional classification of specifical genes that were annotated in Nr database in the two morphs by KEGG enrichment

so on (Fig. 4). For the down-regulated genes, the most represented GO terms were related to serine-type endopeptidase activity, regulation of gene expression, phagocytosis (Fig. 4).

Differentially regulated KEGG pathways between the two morphs

To explore the pathways potentially involved in the wing morphs switch and to distinguish the up- and down-regulated KEGG pathways, we performed KEGG enrichment analysis of up- and down-regulated DEGs separately. Compared with wingless aphid, wing aphid had up-regulated fatty acid biosynthesis and degradation, salivary secretion, biosynthesis of ecdysone and JH, cutin-suberine-wax (Fig. 5). Besides, several pathways were up-regulated including longevity regulating, peroxisome proliferator-activated receptor (PPAR), estrogen, adipocytokine, gonadotropin-releasing hormone (GnRH), insulin, NOD-like receptor,

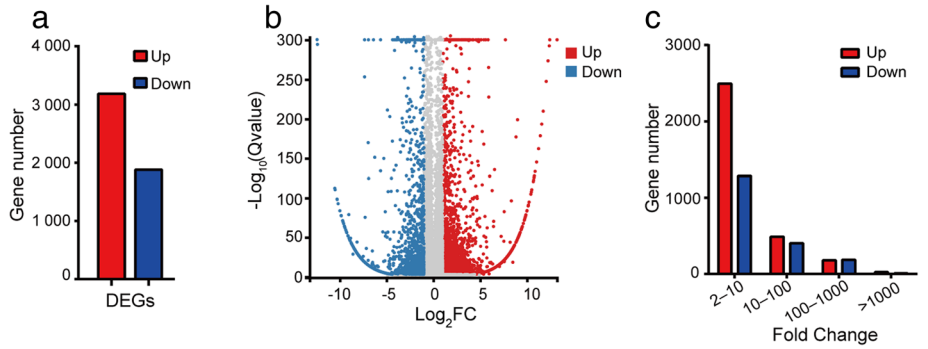

Fig. 3 The distribution of DEGs in wingless and wing aphids. **a** Number of DEGs at FC (fold change) > 2 and $P < 0.001$ in the comparison between wingless and wing cotton aphids. **b** Volcano plot of DEGs. **c** Number of DEGs at different fold change ranges

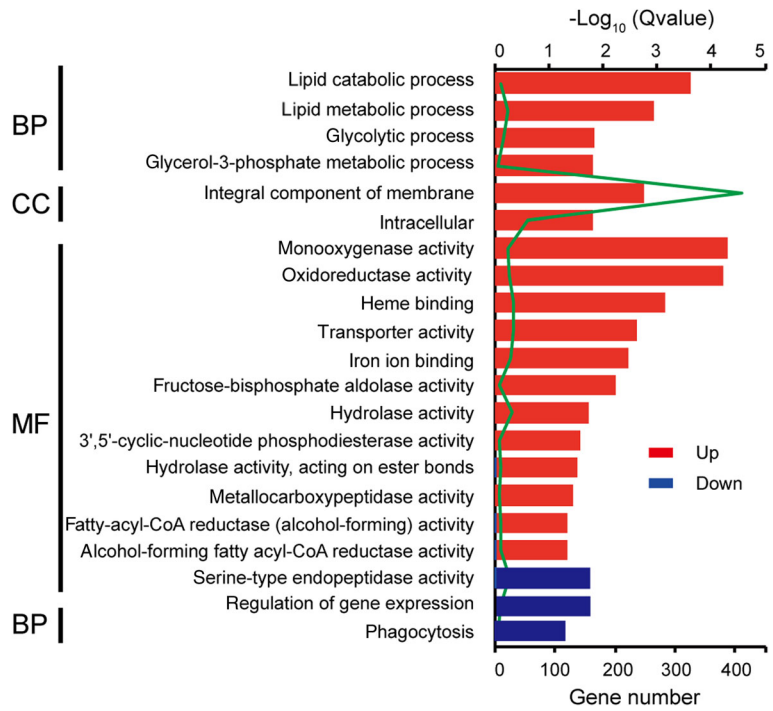

Fig. 4 GO enrichment of the DEGs. GO enrichment of up- and down-regulated DEGs were performed separately. BP, biological process; CC, cellular component; MF, molecular function. Up, up-regulated; Down, down-regulated. The GO term was considered enriched significantly when *P* values of Fisher's exact test were < 0.05

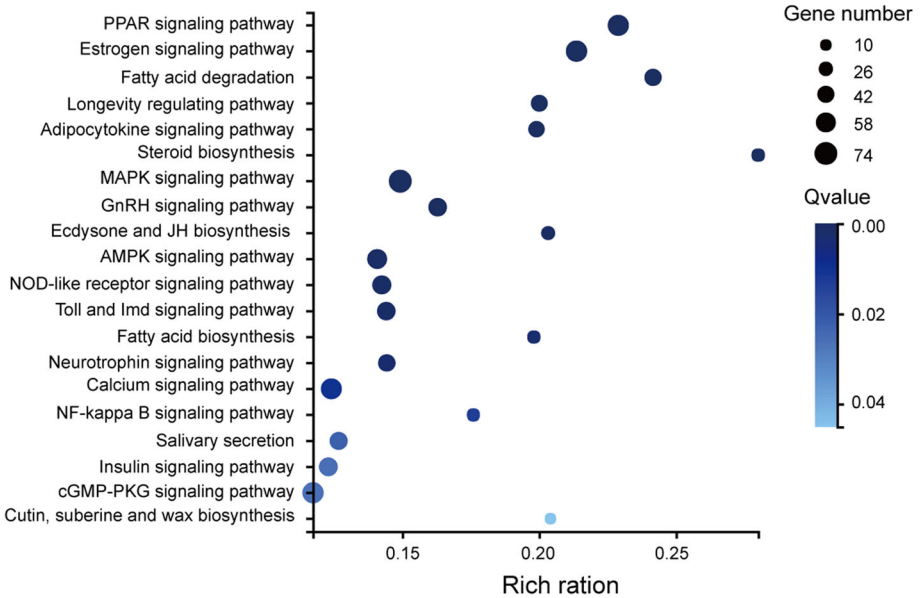

Fig. 5 Significantly enriched up-regulated KEGG pathways. The up- and down-regulated DEGs were performed KEGG pathways enrichment separately. The rich ratio, gene number and Q-value in each enriched up-regulated signaling pathways were shown. Significantly enriched pathways were judged at $P < 0.05$ in hypergeometric test

neurotrophin, MAPK, AMPK, Calcium, NF-kappa B, cGMP-PKG (Fig. 5). In comparison to wingless females, wing aphid had down-regulated signal pathways including RNA polymerase, purine metabolism, pyrimidine metabolism, leukocyte transendothelial migration, vascular smooth muscle contraction (Additional file 5: Table S2).

Validation of selected DEGs by RT-qPCR

Expression levels of 11 out of 14 up-regulated genes of wing morph measured by the transcriptomic method were significantly higher in wing aphid than those in wingless aphid when measured by RT-qPCR, and the other three selected up-regulated genes *ROR1*, *CYP306A1* and *IRS1* were not significantly different (Fig. 6). Expression levels of all four selected down-regulated genes were significantly lower in wing aphid than those in wingless aphid measured by RT-qPCR (Fig. 6). The expression pattern of all these selected genes measured by RT-qPCR were as similar as the RNA-Seq. Therefore, the RNA-Seq data in our study were reliable.

Discussion

Postnatal crowding and wing morphs determination

Wing induction responding to crowding is triggered by tactile stimuli between individual aphids and can occur in pre- or postnatal stages (Forrest 1974; Kidd and Tozer 1984; Müller et al. 2001; Margaritopoulos and Tsitsipis 2002; Martínez and Costamagna 2018). In *A. pisum*, wing morph determination occurs prenatally, during embryogenesis (Sutherland 1969). Once born, a nymph's developmental trajectory, and thus its adult phenotype, is set (Vellichirammal et al. 2017). In this species, embryos receive the maternal signals that determine the wing phenotype through prenatal crowding. However, our results demonstrated that postnatal crowding can also induce wing morph production in *A. gossypii*. Newly born nymphs reared at high densities (≥ 10 nymphs·cm^{-2}) can develop into wing adults (Fig. 1a). Similarly, postnatal crowding effects on wing morphs determination can also be seen in several other aphid species, including *Therioaphis maculata*, *A. fabae*, *M. persicae*, *Sitobion fragariae* (Müller et al. 2001). Besides, none of cotton aphid nymphs reared at low densities (≤ 5 nymphs·cm^{-2}) developed into alate adults (Fig. 1). Similar results were shown in *A. glycines* and densities lower than 3.4 nymphs·cm^{-2} were unlikely to induce wing development in this aphid species as well (Martínez and Costamagna 2018). Wing morph induced by postnatal crowding suggests newly born cotton aphid nymphs own the ability of perceiving oncoming changes such as plant nutrition through population density. Our results indicated that the early nymph stage, especially newly born, may be a developmental switch point for the wing determination in *A. gossypii*.

Comparison of transcriptomes and DEGs in the two morphs

We firstly assembled and annotated transcriptomes in the two morphs independently in aphids. This analysis strategy provided us extra information than previous studies. 3 126 and 3 392 genes in the Nr database were exclusively annotated in wingless and wing aphids,

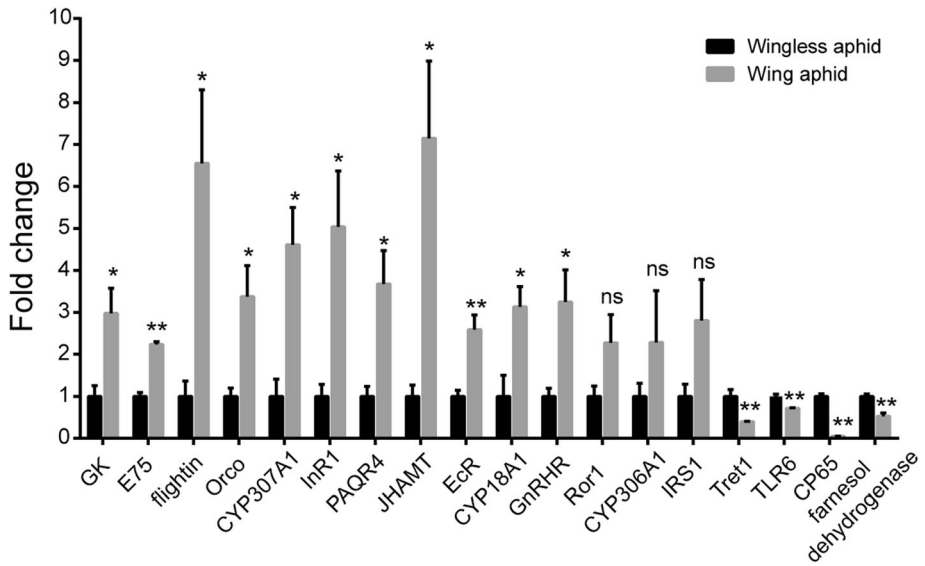

Fig. 6 Relative expression levels of 18 selected genes measured by RT-qPCR method. 18 DEGs potentially involved in the regulation of cotton aphid wing determination were validated by RT-qPCR method. *, $P < 0.05$ and **, $P < 0.01$ compared with the respective wingless aphid levels in wing aphid adult. $n = 4$. ns, no significant differences

respectively (Fig. 2b). These genes were enriched in several signaling pathways including estrogen, oxytocin, phototransduction, secretion of renin and salivary, oocyte meiosis and so on (Fig. 2c). They had never been reported in previous studies of aphid wing polyphenism (Liu et al. 2014; Vellichirammal et al. 2016; Yang et al. 2014). These specific annotated genes and signaling pathways might play an important role in wing plasticity in *A. gossypii.* Through DEGs analysis, we obtained 3 187 up- and 1 880 down-regulated genes in wing versus wingless aphid (Fig. 3a). However, there were only 58 up- and 1 605 down-regulated genes reported in previous researches in cotton aphid (Yang et al. 2014). This may result from different analysis strategies, in which tag-based digital gene expression (DGE) approach was used in Yang's study. In addition, compared with wingless aphid, gynopare (another wing morph in cotton aphid, produced by parthenogenetic females in the fall), owned 741 up- and 879 down-regulated genes (Liu et al. 2014). Taking all together, these results provide comprehensive dataset for gene expression profiles in wing plasticity in cotton aphid, which could facilitate the understanding of molecular mechanisms in the wing mode switch in aphids.

GO category and KEGG pathway enrichment analysis

Transcripts related to lipid metabolism and energy production such as lipid catabolic and metabolic process, fatty-acyl-CoA reductase (alcohol-forming) activity were found at higher expression levels in wing aphid compared with wingless aphid (Fig. 4). Energy allocation is important for trade-off between wing morph (flight capability) and wingless morph (reproduction) in aphids (Yang et al. 2014). The up-regulated lipid metabolism and energy production are consistent with the previous observation of the significantly higher triglyceride content in winged aphid versus the wingless aphid (Shi et al. 2010). Besides, compared with apterous aphid, alate aphid had up-regulated signaling pathway of insulin, biosynthesis of ecdysone and JH (Fig. 5). Insulin signaling had been proved to regulate wing polyphenism in several insects including *Nilaparvata lugens*, *Laodelphax striatellus*, *Sogatella furcifera*, *Blattella germanica* (Abrisqueta et al. 2014; Xu et al. 2015; Xu and Zhang 2017). Ecdysone signaling underlies the pea aphid transgenerational wing polyphenism (Vellichirammal et al. 2017). JH took part in wing morph differentiation in *Megoura crassicauda*, *Gryllus firmus* (Cisper et al. 2000; Ishikawa et al. 2013). These three signaling pathways were all up-regulated in cotton wing aphid. This suggested that the regulation mechanism of wing differentiation in *A. gossypii* is on multi-levels, in which these signaling pathways might work simultaneously, successively or separately.

DEGs potentially involved in wing differentiation in *A. gossypii*

Four up-regulated genes related to ecdysone signaling, including *EcR* (ecdysone receptor), *E75*, *CYP18A1* and *CYP307A1* were validated by RT-qPCR (Fig. 6). Interfering with ecdysone signaling using an ecdysone receptor antagonist or knocking down the *EcR* gene with RNAi resulted in an increased production of winged offspring in pea aphid (Vellichirammal et al. 2017). *CYP307A1* is a regulator for ecdysone synthesis (Namiki et al. 2005; Pondeville et al. 2013); *CYP18A1* is involved in a tissue-specific ecdysone inactivation (Emilie et al. 2011; Li et al. 2014) and *E75* is a stage- and tissue-specific ecdysone primary response gene which mediates steroidogenesis autoregulation and regulate developmental timing (Li et al. 2015; Li et al. 2016). However, they were all up-regulated in wing morph in *A. gossypii* (Fig. 6). This might suggest that ecdysone signaling have a different regulation manner in wing polyphenism in cotton aphid compared with pea aphid. Wing aphid owns flight muscles and flight capability, in which *flightin* is essential for indirect flight muscle development. It was one of the most differentially expressed genes in macropterous and brachypterous *N. lugens* adults (Xue et al. 2013). In this study, *flightin* was increased to 6.6 folds in wing relative to wingless aphid (Fig. 6). This hints the potential importance of *flightin* in flight muscle formation in wing cotton aphid. Besides, odorant receptor co-receptor (*Orco*) involved in wing differentiation in the grain aphid, *Sitobion avenae* (Jia et al. 2015), was also higher expressed in alate cotton aphid (Fig. 6). Two up-regulated genes related to insulin signaling, *PAQR3* and *InR1* were also up-regulated (Fig. 6). *InR1* leads to the long-winged morph if active and the short-winged morph if inactive in planthoppers (Xu et al. 2015). *PAQR3* modulates insulin signaling by shunting phosphoinositide 3-Kinase p110α to the golgi apparatus (Wang et al. 2013). The vital functions of JH in wing polyphenism have been reported in a variety of insect species (Cisper et al. 2000; Ishikawa et al. 2013; Zera and Cisper 2001; Zera et al. 1989; Zera and Tanaka 1996), while JH acid methyltransferase (JHAMT) is the crucial enzyme in JH biosynthesis in insects (Li et al. 2013; Shinoda and Itoyama 2003). In our study, *JHAMT* was increased 7.14 in wing aphid in comparison to wingless aphid (Fig. 6). Taking all together, these up-regulated genes in wing cotton aphid underline the importance of signaling pathways of ecdysone, JH, insulin, Orco, flightin in wing differentiation in aphids yet again. The functions of these genes in wing polyphenism in *A. gossypii* should be confirmed.

Perspectives and future research

This study identified the effects of postnatal crowding on wing morph determination in *A. gossypii* for the first

time. DEGs analyses between the two morphs shed lights on the molecular basis of wing ploypensim in this pest. Compared with wingless aphid, up-regulated DEGs were enriched in several key signaling pathways including insulin, ecdysone and JH biosynthesis and so on in wing aphid. Flight ability of wing aphids depends on the energy supply of lipids. Lipid metabolism, fatty acid biosynthesis and degradation were also up-regulated in wing aphid. The genes expressed levels of *EcR*, *E75*, *CYP307A1*, *CYP18A1*, *InR1*, *Orco*, *flightin* related to these signaling pathways were validated by RT-qPCR. Silencing of these genes using RNA interference (RNAi) will be carried out to identify their functions in the wing ploypensim in cotton aphid.

Conclusion

Wing polyphenism is one of the main reasons contributing to the ecological success of aphids. Crowding in pre- or postnatal nymph stages, was considered to be a key induction factor in this phenotypic plasticity. The present study identified the effects of postnatal crowding on wing morphs induction and identified that the critical population density for wing morph formation in *A. gossypii* was 20 nymphs·cm^{-2}. Comparative transcriptome analysis provides 3 126 and 3 392 transcripts potentially exclusively expressed in wingless or wing morph, respectively. Besides, 3 187 up- and 1 880 down-regulated genes were identified in wing versus wingless aphid. On basis of this, several signaling pathways potentially involved in wing differentiation in the cotton aphid were obtained, including ecdysone, insulin, juvenile hormone, odorant receptor coreceptor, flightin, which confirmed the previous studies of wing polyphenism in other aphids. The expression levels of candidate genes were validated by RT-qPCR soon afterwards. All these discoveries form a basis for deciphering the molecular mechanisms underlying wing determination in cotton aphid. Further studies on the functions of candidate genes by RNAi method will contribute to develop genetic control strategies against this pest by the disruption of its flight behavior.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s42397-019-0036-z.

Additional file 1: Table S1. Primers used in the RT-qPCR.

Additional file 2: Figure S1. Characteristics of homology search of unigenes against the Nr database. Species distribution is shown as percentage of the total homologous gene hits. The results of transcriptome assembled by wingless morph (A) and wing morph (B) were showed individually.

Additional file 3: Table S3. DEGs with different fold change levels between wing morph and wingless morph in cotton aphid.

Additional file 4: Figure S2. Gene ontology classification of DEGs. Gene ontology terms with pooled up- and down-regulated DEGs were summarized as three main categories: biological process (blue panel), cellular component (red panel), molecular function (green panel). The numbers of DEGs were showed in X-axis.

Additional file 5: Table S2. Down-regulated KEGG pathways in wing aphid vs. wingless aphid.

Abbreviations

AMPK: Adenosine 5-monophosphate (AMP)-activated protein kinase signaling pathway; cGMP-PKG: Cyclic GMP dependent protein kinase signaling pathway; CYP306A1: Cytochrome P450 306A1; DEG: Differentially expressed genes; FC: Fold change; FDR: False discovery rate; GnRH: Gonadotropin-releasing hormone receptor signaling pathway; GO: Gene ontology; InR1: Insulin receptor 1; IRS1: Insulin receptor substrate 1; JH: Juvenile hormone; JHAMT: Juvenile hormone acid methyltransferase; KEGG: Kyoto encyclopedia of genes and genomes; KOG: EuKaryotic orthologous groups; MAPK: Mitogen-activated protein kinase cascade signaling pathway; NCBI: National Center for Biotechnology Information; NF-kappa B: Nuclear factor kappa-B signaling pathway; Nr: Non-redundant protein sequence; Nt: Non-redundant nucleotide sequence; Orco: Odorant receptors co-receptor; PAQR3: Progestin and adipoQ receptor 3; Pfam: Protein families database; PPAR: Peroxisome proliferator-activated receptors signaling pathway; ROR1: Receptor tyrosine kinase-like orphan receptor 1; RT-qPCR: Reverse transcription quantitative real time polymerase chain reaction; SRA: Sequence read archive

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Authors' contributions

Cui JJ and Ji JC conceived and designed the research; Zhu XZ and Zhang KX collected the samples. Ji JC conducted experiments. Luo JY, Ji JC and Wang L analyzed the data. Ji JC, Zhang LJ and Zhang S wrote the paper. All authors read and approved the manuscript.

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Availability of data and materials

Insect materials are available from the corresponding author on reasonable request. All data generated or analyzed during this study are included in this published article (and its Additional files).

Ethics approval and consent to participate

We declare that our study was conducted in strict compliance with ethical standards. Collection of *A. gossypii* in this study did not require permits because it is a common cotton pest in China.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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