Insights into wing dimorphism in the worldwide agricultural pest *Aphis gossypii*, the host-alternating aphid

Jichao Ji
Chinese Academy of Agricultural Sciences Cotton Research Institute
Shuai Zhang
Chinese Academy of Agricultural Sciences Cotton Research Institute
Ningbo Huangfu
Chinese Academy of Agricultural Sciences Cotton Research Institute
Junyu Luo
Chinese Academy of Agricultural Sciences Cotton Research Institute
Lin Niu
Chinese Academy of Agricultural Sciences Cotton Research Institute
Xueke Gao
Chinese Academy of Agricultural Sciences Cotton Research Institute
Jinjie Cui (aycuijinjie@163.com)
Institute of Cotton Research, Chinese Academy of Agricultural Sciences; Zhengzhou Research Base, State Key Laboratory of Cotton Biology, Zhengzhou University https://orcid.org/0000-0001-5435-3985

Research article

Keywords: cotton aphid, wing parthenogenetic female, gynopara, male, wing plasticity

Posted Date: February 26th, 2020

DOI: https://doi.org/10.21203/rs.2.24662/v1

Version of Record: A version of this preprint was published at Journal of Cotton Research on February 23rd, 2021. See the published version at https://doi.org/10.1186/s42397-021-00080-w.

Abstract

Background: Three wing morphs exists in the life cycle of the worldwide pest Aphis gossypii, i.e., wing parthenogenetic female (WPF), gynopara (GP) and male, which were produced mostly by crowding and host quality, photoperiod, loss of X chromosome, respectively. However, the shared molecular mechanism underlying their wing differentiation remains an enigma. Here we firstly induced gynoparae and males indoors and compared the characters of these wing morphs in body, internal genitals and fecundity. Then we identified the shared and separate differentially expressed genes (DEGs) and signaling pathways potentially involved in the wing morphs regulation in WPF, GP and male compared to wingless parthenogenetic female (WLPF).

Results: Newly-born nymphs reared in short photoperiod condition exclusively produce gynoparae and males in adulthood successively, in which the sex ratio is gynoparae biased. Compared with WLPF, three wing morphs have similar morphology in bodies but is obviously discriminated in the reproductive system and fecundity. Built upon our previous study, 37 090 annotated unigenes were obtained from libraries constructed by the four morphs above through RNA-sequencing, in which 10 867, 19 334 DEGs were identified in pairwise comparison of GP vs. WLPF, Male vs. WLPF, respectively. Furthermore, 2 335 shared DEGs including 1 658 up- and 677 downregulated were obtained in these wing morphs compared to WLPF. The 1 658 shared up-regulated DEGs were enriched in multiple signaling pathways including insulin, FoxO, MAPK, strarch and sucrose metabolism, fatty acid biosynthesis and degradation which hint their key roles in the regulation of wing plasticity in cotton aphid. Gene expression levels were validated by using Pearson’s correlation (r) and potential roles of 15 DEGs related to the insulin signaling pathway in cotton wing dimorphism were discussed.

Conclusions: The results of this study establish a solid foundation for deciphering molecular mechanisms underlying the switch between wingless and wing morphs in the cotton aphid and provide valuable resources for future research on the host-alternating aphid species.

Background

Wings are essential organs for insects to find hosts, mating and spawning sites, which play a vital role in their population continuation. In order to adapt the environmental changes, they develop alternative wing dimorphism, including two categories, long or short wing, winged or wingless morphologies [1, 2]. The former is commonly observed in plant hoppers or crickets, in which host plant nutrition affects the wing developmental plasticity and insulin signal pathway participates in the regulation of wing differentiation [3–5]. In contrast, the later mainly exist in aphid species, which consists of fully winged and flight capable versus fully wingless and flight-incapable morphs [6, 7]. However, the molecular mechanism of wing morphs selection in aphids is still not well understood.

Considerable attempts have been made to uncover the molecular mechanism underlying wing morphs switch in Acyrthosiphon pisum, a non-host-alternating aphid species. Two types of wing dimorphisms

exist in this species, including environmental wing polyphenism and genetic wing polymorphism [6, 8]. Environmental induced wing polyphenism occurs among parthenogenetic females and was regulated by ecdysone or octopamine [9, 10]. By contrast, genetically determined wing polymorphism has been found only in males, in which the sex-linked locus on the X-chromosome named aphicarus controls the wing differentiation [11, 12]. These excellent studies in pea aphid have greatly advanced our understanding of the molecular mechanism underlying wing phenotypic plasticity in the non-host-alternating aphid species. However, not all aphid species own these two types of wing dimorphisms, especially dimorphic males. For example, only about 10% of European species have been recorded with winged and wingless males [13]. Furthermore, host-alternating/heteroecious aphids are often more economically important than no-host-alternating species, as the secondary hosts usually herbaceous species, and frequently a crop plant[14]. More distinctive is, males in all host-alternating aphids are exclusively winged [14] .

In addition to the fully winged male, another exclusively winged morph gynopara, capable of giving birth to sexual female offspring, exists in host-alternating aphid species [2, 15–18]. In other words, three wing morphs exists in these species, namely wing parthenogenetic female (WPF), gynopara (GP) and male, which is extremely different from the non-host-alternating aphid species. Several shared or separate mechanism probably participate in wing differentiation of these three wing morphs. To date, it is well known that WPF, GP and male was induced by crowding, poor plant nutrition, shortened photoperiod, lower temperature [18–20]. However, less well known is the molecular mechanism underlying wing differentiation regulation of these three wing morphs in host-alternating aphids.

The representative of host-alternating aphids, Aphis gossypii Glover, also known as cotton aphid or melon aphid, caught our attention. This aphid species is the top 10 agricultural pests which distributes in 171 countries and damage various crops including those in Cucurbitaceae, Malvaceae, Solanaceae, and Rutaceae [21]. In this species, WPFs appearing in spring and summer facilitate population expansion among various host plants and different regions, while gynoparae and males produced in autumn can fly from second hosts to seek winter hosts and promote gene communication of colonies from different secondary hosts or regions [17, 18]. As an economically important destructive pest in agriculture, horticulture and forestry, several induction strategies for WPF, gynopara and male in this species have been obtained so far [18, 20, 22, 23]. In a word, cotton aphid is a compelling laboratory model to study the molecular mechanism of wing dimorphism phenomenon in host-alternating aphids.

Recently, transcriptome sequencing has offered powerful tools for genes expression profiles analysis, differentially expressed genes (DEGs) selection, function genes mining [24]. In our previous study, the effects of postnatal crowding on wing morphs induction was identified and several signaling pathways potentially involved the wing differentiation of WPF were obtained in cotton aphid [25]. Here, in order to identify the shared important genes and signaling pathways probably involved in wing differentiation of the three wing morphs in cotton aphids, we induced gynoparae and males in the laboratory and employed a comparative transcriptome analysis approach to identify differentially expressed genes (DEGs) in gynoapra, male and wingless parthenogenetic female. Moreover, through a conjoint analysis with DEGs identified in the comparison of WPF vs. WLPF [25], thousands of shared DEGs between these three wing

morphs and WLPF were obtained. On the basis, the putative roles of these shared DEGs and significantly enriched signal pathways potentially involved in the wing morphs switch of cotton aphid were discussed. Taken together, our results provide the most valuable resources to date for gene expression profiles in three wing morphs of the host-alternating aphid, A. gossypii. Our finding also could benefit the understanding of molecular mechanisms in wing mode switch of aphid species.

Results

The production of gynoparae and males

The annual life cycle of cotton aphid is characterized by three representative wing morphs including wing parthenogenetic female, gynopara and male (Fig. 1). Wing morphs of aphids were mostly induced by density, host plant quality, temperature and photoperiod. Here we used low temperature and short day as wing-inducing stimulation to produce gynoaprae and males in cotton aphid. When newly-born nymphs (G1) were reared under the SD conditon (18 ± 1 ℃, 75% relative humidity, 8L:16D photoperiod), they exclusively produced two wing morphs, gynoparae and males, in adulthood (Fig. 2A; Fig. 3A). In other words, 100% of their offspring (G2) were winged. Besides, our experiments confirm that gynoparae precede males among the progeny of an individual G1 mother aphid (Fig. 2A) and show that the sex ratio in the offspring is gynoparae biased (Fig. 2B). Specifically, about 14 gynoparae were frequently produced at the first half of the reproductive time of G1 mother, while 9 males were born subsequently during the second half period of G1 aphid (Fig. 2B).

Morphology Of Three Wing Morphs Of Cotton Aphid

Compared with WLPF, the WPF, GP and male have similar morphology in bodies, including fully wings, heavier melanization of head and thorax (Fig. 3A). However, the reproductive system of these three wing morphs is obviously discriminated. In comparison to WLPF, number of embryos in WPF and gynopara were both fewer (Fig. 3A). Besides, embryos in the ovaries of gynopara are green while those in WPF and WLPF are yellow (Fig. 3A). In contrast, testes and accessory gland exist in the abdomen of male (Fig. 3A). There are no significant differences between WLPF, WPF and gynopara in body length with the exception of male (Fig. 3B). In addition, WLPF give birth to most offspring with the number of 53, followed by WPF with the number of 27 and gynopara with the number of 8 (Fig. 3C). Internal genitals characters of gynopara and male induced in controlled indoor were the same as those captured in the wild field (Fig. S1: A, gynoapra; B, male). Anyway, though these wing morphs were induced by different environmental factors with different reproductive system and fecundity, they probably were regulated by several shared genes or signaling pathways in wing differentiation process.

Transcriptome Assembly Of Cotton Aphid

cDNA libraries of these four morphs were sequenced using Illumina technology and 984.87 M clean reads were obtained with the Q30 of 90.91%, the GC contents of 42.20%. The clean reads were assembled into 46 501 unigenes with the N50 length of 2 876 bp (Table 1). All unigenes matched previously described

reads with more than 92.27% coverage, including no less than 31.25% of unique mapped reads (Table 1). The raw reads have been submitted to the NCBI SRA under the accession number PRJNA544300. Assembled unigenes were searched against public databases individually, including Nr, Nt, Swiss-Prot, KEGG, KOG, Pfam, GO, and 33 998, 33 217, 26 857, 27 779, 25 938, 28 069, 16 554 unigenes were annotated in above database, respectively (Fig. S1). Taken together, a total of 37 090 (79.76%) unigenes returned a significant result at least in one of the searched databases using this approach (Table 1).

DEGs In Winged And Wingless Morphs

Based on the cutoff criteria (FC > 2, P < 0.001), 10 867 DEGs including 7 270 upregulated and 3 597 downregulated genes were identified in GP compared to WLPF (Fig. 4A). When male was compared to WLPF, 11 137 upregulated and 8 197 downregulated DEGs were identified (Fig. 4A). For WPF vs. WLPF, 5 067 DEGs including 3 187 upregulated and 1 880 downregulated genes were identified in our recently study [25], which was showed here as well. In order to identify the shared or separate genes potentially involved in wing differentiation of the three wing morphs in cotton aphids, DEGs above were analyzed by Venn diagram. Interestingly, 769, 1951, 5 822 genes were up-regulated exclusively in WPF, GP and male compared to WLPF, respectively (Fig. 4B). 701, 468, 4 922 genes were downregulated exclusively in WPF, GP and male compared to WLPF, respectively (Fig. 4B). Above all, 2 335 shared DEGs including 1 658 up- and 677 downregulated were identified in all these three wing morphs compared to WLPF (Fig. 4B). These shared DEGs probably contribute to the wing dimorphism regulation in cotton aphid.

KEGG enrichment analysis of shared DGEs in three wing morphs

To explore the pathways potentially involved in the wing differentiation regulation in all three wing morphs in cotton aphid, the shared up- and downregulated DEGs above were performed KEGG enrichment analysis separately. Compared to WLPF, several pathways were up-regulated in all wing morphs, including insulin, FoxO, fatty acid biosynthesis and degradation, metabolism of glycerophospholipid, fructose and mannose, starch and sucrose, steroid biosynthesis and so on (Fig. 5). These up-regulated pathways were clustered into categories of signal transduction, lipid metabolism, carbohydrate metabolism, endocrine system (Fig. 5). By contrast, several pathways related to nucleotide metabolism and transcription were downregulated in these wing morphs compared to WLPF, including RNA polymerase, purine and pyrimidine metabolism (Fig. 5). Though the up-and downregulated signaling pathways potentially involved in wing mode plasticity in cotton aphid were identified, how they work in wing dimorphism is needed to be elucidated in further studies yet. All significantly enriched KEGG pathways performed on shared DEGs were listed in Table S2.

RT-qPCR Validation

To confirm the results of gene expression profiling, we chose 32 up- and 12 downregulated genes that spanned the range of differential expression to validate our statistical analysis with RNA-Seq by RT-qPCR method (Table S3). Fold changes of all these selected genes (either up- or down-regulated) in RT-qPCR were consistent with the results in RNA-seq (Table S3). Pearson correlation coefficient analysis by SPSS

19.0 software indicated a significant positive correlation ($P < 0.0001$) between the results of RT-qPCR and RNA-seq in all these three wing morphs compared to WLPF (Fig. 6). Therefore, the RNA-Seq data in our study were reliable.

DEGs Related To Insulin Signaling Pathway

Insulin signaling pathway participates in wing differentiation and development in several insects [4, 26]. Interestingly, it was highly expressed as well in all these three wing morphs compared to WLPF in cotton aphid (Fig. 5), which implies the importance of insulin signaling in wing dimorphism in A. gossypii. Thus, 15 insulin signaling related genes which were upregulated in all these three wing aphids in the RNA-seq data were analyzed through using the method of RT-qPCR. These genes were significantly high expressed in all these three wing morphs with the fold change ranges of 1.37 ~ 17.46 in WPF vs. WLPF, 1.96 ~ 9.02 in GP vs. WLPF, 3.51 ~ 87.20 in male vs. WLPF (Fig. 7). Next, silencing of these candidate genes potentially involved in wing differentiation by using RNA interference (RNAi) will be carried out to identify their function in the regulation of wing plasticity in all these three wing morphs in cotton aphid.

Discussion

Exclusively winged gynopara and male in A. gossypii

Insects produce alternative wing morphs in response to environment changing [1], in which winged or wingless morphs were mostly observed in aphids species. Considerable studies on wing phenotypic plasticity mostly have been carried out on pea aphid, the non-host-alternating species, in which two wing morphs environmentally induced or genetically determined, namely WLPF and male, existed [8, 27]. However, three wing morphs existed in cotton aphid, the host-alternating species, in which males were exclusively winged and an extra special wing morph, gynopara, were reported in this species [18]. Distinction of wing dimorphism between host-alternating and no-host-alternating aphid species probably result from their diverse survival strategies. The former always alternates between primary (usually woody) host plants and secondary (herbaceous) hosts, while the latter were usually monophagous [28]. Hence, in host-alternating species A. gossypii, gynoparae and males are exclusively winged to migrate between the primary and secondary hosts. By contrast, not all males were winged in Acyrthosiphon pisum, possibly because this species was not entirely necessary to migrate in winter [8]. Certainly, winged parthenogenetic females were produced for population expansion in both host-alternating and non-host-alternating species in summer [28].

Besides, male wing differentiation in pea aphid were determined by a single locus on the X chromosome called aphicarus [8]. However, molecular mechanism about wing differentiation of gynopara and male in cotton aphid were still not well known. Species exhibiting three alternative wing morphs provide valuable models for studying wing differentiation in aphids and some shared physiological or molecular baselines probably exist. Here, methods for gynopara and male induction in laboratory were established by shortening photoperiod in cotton aphid (Fig. 2). Compare to previous studies [18, 29], one generation induction was enough to produce these two wing morphs with a high effectiveness. Furthermore,

morphological characters in body and internal genitals were firstly described and discriminated in three wing morphs (Fig. 3). All these results broaden our understanding about wing phenotypic plasticity in aphid species.

Transcriptomes Comparison And DEGs Analysis

We firstly assembled transcriptomes by WLPF, WPF, gynopara and male in cotton aphid and obtained a total of 46 501 unigenes (Table 1), which was approximated to the number of 44 310 in previous study [20]. After filtering out the repetitive genes and genes without annotation, 37 090 unigenes yielded in our study (Table 1), while only 11 350 unigenes were obtained in Liu's study. This may result from the difference in wing morphs selection strategies. Only WLPF, gynopara and sexual female were sequenced in Liu's study, while extra male and WPF were collected in our study meanwhile. Several previously unreported gene transcripts or isoforms probably existed in male and WPF in cotton aphid. In addition, compared to WLPF, gynopara owned 7 270 up-regulated and 3 597 down-regulated DEGs while there were only 741 up- and 879 down-regulated genes in previous study [20]. Different strategies were adopted to eliminate the potential influence of embryos in the mother's ovaries: embryos were manually removed in Liu's study while all offspring were born before adult cotton aphid were collected in our study. Compared to these excellent studies performed on cotton aphid wing differentiation, we firstly identified the shared and exclusively DEGs in WPFs, GP, male compared to WLPFs, respectively (Fig. 4).

Pathways potentially involved in wing differentiation of three wing morphs

Several transcriptional studies have focused on aphids to seek signal pathways involved in the wing differentiation. 1 663 DEGs were identified in WPF compared to WLPF in cotton aphid, which were significantly enriched in ribosome, pyruvate metabolism, proteasome, lipid metabolism, protein synthesis and degradation, RNA transport, antigen processing and presentation [29]. Our results were consistent with those finding, in which pyruvate metabolism, antigen processing and presentation were up-regulated in all these three wing morphs compared to WLPF as well (Table S2). 1 620 DEGs were identified in gynoparae compared to WLPF, in which 6 up- and 7 downregulated signaling pathways were enriched, including starch and sucrose metabolism, phototransduction, dorso-ventral axis formation, Wnt, Notch [20]. Likewise, pathway of starch and sucrose metabolism were up-regulated in these three wing morphs (Table S2). This probably results from the indispensability of energy for flight apparatus and flight behavior.

Those studies advanced our understanding of wing dimorphic in cotton aphid. However, Liu's study mostly was highlighted on reproductive polyphenism while Yang's study only performed on WPF [20, 29]. In this study, three wing morphs in cotton aphid were firstly compared with each other or to wingless parthenogenetic female. 2 335 shared DEGs including 1 658 up- and 677 downregulated were identified in all three wing morphs compared to WLPF (Fig. 4), which were significantly enriched in 49 up- and 7 downregulated KEGG pathways (Table S2). The upregulated pathways were clustered into categories of signal transduction, lipid metabolism, carbohydrate metabolism, endocrine system (Fig. 5). Energy allocation is important for trade-off between wing morph and wingless morph in aphids [29]. Thus, the

upregulated lipid and carbohydrate metabolism in three wing morphs is consistent with the previous observation of the significantly higher triglyceride content in winged morph versus the wingless morph [23]. Besides, compared to WLPFs, all these three alate morphs had up-regulated insulin signaling pathway (Fig. 5), which had been proved regulating wing differentiation and development in several insects including Nilaparvata lugens, Laodelphax striatellus, Sogatella furcifera, Blattella germanica [4, 30, 31]. Considering the importance of insulin in wing determination, relative expression levels of 15 related DEGs in RNA-seq data were validated in all three wing morphs compared to WLPF (Fig. 7), which implied insulin is potentially involved in the wing differentiation of three wing morphs in cotton aphid.

DEGs Associated With Insulin, Flight Muscle And Energy

Insulin receptor 1 (InR1) leads to long-winged morph if active and short-winged morph if active in three planthoppers [4]. Besides, silencing of InR1 disrupts nymph-adult transition of alate viviparous females in A. (Toxoptera) citricidus [26]. InR1 were increased significantly to 6.03, 2.23, 6.70 folds in WPF, gynopara, male compared to WLPF, respectively (Fig. 7, Table S3). This hints the potential importance of InR1 in wing regulation of three wing morphs in cotton aphid. Flightin, a phosphorylated myofibrillar protein essential for thick filament assembly and sarcomere stability in insects flight muscles [32], exhibits the higher transcript accumulation in winged parthenogenetic morphs and male in A. pisum [33], and in winged parthenogenetic females of A. gossypii [29] and Rhopalosiphum padi [34]. The disproportionately high levels of flightin transcript in winged versus wingless morphs likely results from the presence or absence of indirect flight muscles in winged and wingless morphs, which plays a causal role in the morphological divergence of the wing morphs [33]. Particularly, flightin were increased to 2.32, 6.50, 11.75 folds in WPF, gynopara, male compared to WLPF in our results likewise, respectively (Table S3). This imply the potential importance of flightin in flight muscle formation or wing differentiation in three wing morphs in cotton aphid. Besides, phosphoenolpyruvate carboxykinase [GTP]-like (PEPCK) and glycogen phosphorylase-like (glgP) were expressed highly in alate A. citricida adults, and silencing of these two genes individually can all resulted in under-developed wings in WPFs at the rates of 58–79% [35]. Likewise, these two genes were significantly upregulated in all three wing morphs compared to WLPF in cotton aphid (Fig. 7). In addition, odorant receptor co-receptor (Orco) which mediates winged morph differentiation of parthenogenetic female in Sitobion avenae [36], was also higher expressed in all three alate morphs compared to WLPF in cotton aphid (Table. S3).

Taken together, these shared upregulated genes in three wing morphs underline the importance of signaling pathways of insulin, energy generation, orco, flightin in wing differentiation in cotton aphid. The functions of these genes in wing dimorphism in A. gossypii will be confirmed by RNAi strategy in our future study. To our knowledge, this is the first study examining transcriptome-wide patterns of differential transcript accumulation associated with three wing morphs in cotton aphid, which will provide a baseline for future studies on molecular basis of wing differentiation in A. gossypii.

Conclusion

A. gossypii is the top 10 worldwide destructive sap-sucking pest which distributes in 171 countries with various hosts and vector of more than 50 plant viruses[21]. The ecological success of this pest was mostly due to three wing morphs in the life cycle, namely WPF, gynopara and male. In this study, we induced gynoparae and male successfully indoors and compared the morphological characters of these three wing morphs in body and reproduction organ for the first time. Then comparative transcriptomic analysis were employed and the shared 2 335 DEGs were identified in all these three wing morphs compared to parthenogenetic wingless female. At last, potentially signaling pathways involved in the regulation of wing differentiation in all these wing morphs of cotton aphid were achieved by KEGG enrichment analysis. Besides, the annual life cycle of cotton aphid was described and morphological characters of different morphs captured in the wild field were visualized clearly. Hence discoveries above in this study will advance our knowledge about wing dimorphism in cotton aphid and provide basis for developing genetic control strategies against this pest through the disruption of its migratory behavior.

Methods

Induction of gynoparae and males

A. gossypii colony, collected originally in Anyang, Henan, China, was reared on cotton seedlings at Institute of Cotton Research of China Academy Agricultural Sciences under controlled laboratory conditions for more than 50 generations before the start of all experiments (25 ± 1 ℃, 75% relative humidity, 16L:8D photoperiod). Gynoparae and males were induced by rearing newly-born viviparous nymphs (the first generation, G1) at short day (SD) conditions (18 ± 1 ℃, 75% relative humidity, 8L:16D photoperiod). Offspring (the second generation, G2) produced by G1 aphids were translated to a separate cotton seedling daily and their morphs were identified in adulthood. Progeny numbers of G1 mothers were counted every day.

Morphological Characters And Fecundity

WLPFs were obtained by rearing aphids solitarily, while WPFs were generated by rearing aphids in crowding condition [25]. Then, morphological characters of the WLPF, WPF, GP and male in body and internal genitals at their adult stages were visualized using a SteREO Discovery V8 microscope (Zeiss, Germany). In addition, the body length (from head to abdomen, antennae and wings were not included) of each morph was measured and the offspring numbers of WLPF, WPF, GP were counted. Furthermore, internal genitals characters of gynopara and male captured in the wild field were visualized as well to use as a reference.

Sample Preparation For RNA-seq

Gynoparae and males used for RNA sequencing were selected in adulthood, in which adults of gynoparae were gathered individually after they produced all offspring to eliminate the potential influence of embryos embedded in their ovaries. There were four biological replicates for each morph. Each biological

replicate contained 50 gynoparae or male adults, respectively. The samples were immediately frozen in liquid nitrogen and stored at -80℃.

Transcriptome Assembly And Gene Annotation

Total RNA was isolated from the pooled whole body of aphid samples using TRIzol reagent (Promega, USA) according to the manufacturer's instructions. The purity and integrity of RNA were assessed using an Agilent 2100 Bioannlyzer (Agilent, America). cDNA libraries were sequenced in paired-end modes on BGISEQ-500 system at Beijing Genomics Institute (BGI) (Shenzhen, China). 8 libraries were constructed with four biological replicates per morph. After sequencing, the raw reads were obtained and subsequently the adapter sequences and low-quality reads were filtered to acquire clean reads. Then, clean reads from libraries above, libraries of WPFs and WLPFs which were obtained in our previous study [25], were assembled de novo together using software Trinity [37]. Unigene sequences larger than 200 bp were aligned to protein databases, including Nr, Swiss-Prot, KEGG, Pfam, KOG by blastx and to the nucleotide Nt by blastn with the cutoff E-value of 10E-5 [38].

Identification Of Differentially Expressed Genes

Gene expression levels in each library were calculated by fragments per kilobase per million reads (FPKM) method using Bowtie2 and RESM [39, 40]. Pairwise comparisons were made between GP and WLPF, Male and WLPF, respectively. Fold changes (FCs), the relative expression level of a gene in one morph to another, and P-value were used to determine the DEGs between two morphs by DEGseq [41], in which the P-values in multiple tests were adjusted by false discovery rate (FDR) [42]. Ultimately, $FC > 2$ and adjusted $P < 0.001$ were the thresholds to determine significant differences in gene expression. Gene ontology (GO) enrichment and significantly enriched KEGG pathways were identified through hypergeometric tests at the criteria of $P < 0.05$ by function phyper in R software.

RT-qPCR For Validation Of RNA-seq Data

Single-stranded cDNA was reverse-transcribed synthesized using 1 μg of RNA from each sample with PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, Japan) according to the manufacturer's recommendations, respectively. RT-qPCR was performed on ABI StepOnePlus Real-Time PCR System (Thermo, America) using GoTaq® qPCR Master Mix (Promega, USA), initiated at 95 °C for 5 min, followed by 40 cycles of 95 °C for 5 s, 60 °C for 30 s. Melting curve analysis was conducted to verify the specificity of amplification. The relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method [43] and normalized by the housekeeping gene GAPDH [44]. Primers used for RT-qPCR validation were showed in Table S1. Statistical analyses were performed by Student's t-test using SPSS 19.0 software. Significant difference was considered at $P < 0.05$. Values were reported as mean ± SE.

List Of Abbreviations

WLPFs, wingless parthenogenetic females; WPF, wing parthenogenetic females; GP, gynopara; DEGs, different expressed genes; FoxO, the forkhead box O; MAPK, mitogen-activated protein kinase cascade signaling pathway; Nr, non-redundant protein sequence; Nt, non-redundant nucleotide sequence; Pfam, protein families database; FC, fold change; GO, gene ontology; InR1, insulin receptor 1; KEGG, Kyoto encyclopedia of genes and genomes; KOG, EuKaryotic orthologous groups; NCBI, national center for biotechnology information; SRA, sequence read archive; RT-qPCR, reverse transcription quantitative real time polymerase chain reaction; SD, short day; PEPCK, phosphoenolpyruvate carboxykinase [GTP]-like; glgP, glycogen phosphorylase-like; Orco, odorant receptor co-receptor

Declarations

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.

Availability of data and materials

All data generated or analyzed during this study are included in this published article (and its Supplementary Information files). Transcriptome datasets in this study are available at NCBI project PRJNA544300 with accession number SRP199362, and SRA with accession number SRS4812382.

Competing Interests

All authors declare that they have no conflict of interest.

Funding

This research was funded by National Natural Science Foundation of China (No. 31572015) and Special Fund for Basic Public Welfare Research of Institute of Cotton Research of CAAS (No. 1610162019020604).

Author contribution

JJC and JCJ conceived and designed research; NBHF and XKG collected the samples. JCJ and XKG conducted the experiments. LN, JCJ and SZ analyzed the data. JCJ and SZ wrote the paper. All authors have read and approved the manuscript.

Acknowledgments

We thank the editors and reviewers for all their constructive comments on our work. Studies were carried out in laboratories at the Institute of Cotton Research of CAAS, Anyang, China.

References

1. Zhang CX, Brisson JA, Xu HJ: **Molecular mechanisms of wing polymorphism in insects**. *Annu Rev Entomol* 2019, **64**:297–314.
2. Campbell CAM, Tregidga EL: **Photoperiodic determination of gynoparae and males of damson-hop aphid *Phorodon humuli***. *Physiol Entomol* 2005, **30**(2):189–194.
3. Cisper G, Zera AJ, Borst DW: **Juvenile hormone titer and morph-specific reproduction in the wing-polymorphic cricket, *Gryllus firmus***. *J Insect Physiol* 2000, **46**(4):585–596.
4. Xu HJ, Xue J, Lu B, Zhang XC, Zhuo JC, He SF, Ma XF, Jiang YQ, Fan HW, Xu JY: **Two insulin receptors determine alternative wing morphs in planthoppers**. *Nature* 2015, **519**(7544):464–467.
5. Zhao Z, Zera AJ: **Differential lipid biosynthesis underlies a tradeoff between reproduction and flight capability in a wing-polymorphic cricket**. *Proc Natl Acad Sci U S A* 2002, **99**(26):16829–16834.
6. Brisson JA: **Aphid wing dimorphisms: linking environmental and genetic control of trait variation**. *Philos Trans R Soc London, Ser B* 2010, **365**(1540):605–616.
7. Ogawa K, Miura T: **Aphid polyphenisms: trans-generational developmental regulation through viviparity**. *Front Physiol* 2014, **5**:1.
8. Ogawa K, Ishikawa A, Kanbe T, Akimoto SI, Miura T: **Male-specific flight apparatus development in *Acyrthosiphon pisum* (Aphididae, Hemiptera, Insecta): comparison with female wing polyphenism**. *Zoomorphology* 2012, **131**(3):197–207.
9. Wang XX, Zhang Y, Zhang ZF, Tian HG, Liu TX: **Deciphering the function of octopaminergic signaling on wing polyphenism of the pea aphid *Acyrthosiphon pisum***. *Front Physiol* 2016, **7**:603.
10. Vellichirammal NN, Gupta P, Hall TA, Brisson JA: **Ecdysone signaling underlies the pea aphid transgenerational wing polyphenism**. *Proc Natl Acad Sci U S A* 2017, **114**(6):1419–1423.
11. Braendle C, Caillaud MC, Stern DL: **Genetic mapping of aphicarus – a sex-linked locus controlling a wing polymorphism in the pea aphid (*Acyrthosiphon pisum*)**. *Heredity* 2005, **94**(4):435–442.
12. Caillaud MC, Boutin M, Braendle C, Simon JC: **A sex-linked locus controls wing polymorphism in males of the pea aphid, *Acyrthosiphon pisum* (Harris)**. *Heredity* 2002, **89**(5):346–352.
13. Smith MAH, Mackay PA: **Genetic variation in male alary dimorphism in populations of pea aphid, *Acyrthosiphon pisum***. *Entomol Exp Appl* 1989, **51**:125–132.
14. Hardie J: **Life Cycles and Polyphenism**. In: *Aphids as Crop Pests.* Edited by Helmut F. van Emden, Harrington R, 2nd edn. Oxfordshire, UK: CABI; 2017: 81–97.
15. Hardie J: **Reproductive, morphological and behavioural affinities between the alate gynopara and virginopara of the aphid, *Aphis fabae***. *Physiol Entomol* 1980, **5**(4):385–396.
16. Hong J, Boo KS: **Artificial production of sexual morphs in *Aphis spiraecola***. *J Asia-Pacif Entomol* 1998, **1**(2):171–176.

17. Hales DF, Wellings PW, Parkes RA: **Investment in gynoparae and males by *Myzus persicae* (Sulzer)**. *Funct Ecol* 1989, **3**:727–734.
18. Kwon SH, Kim DS: **Effects of temperature and photoperiod on the production of sexual morphs of *Aphis gossypii* (Hemiptera: Aphididae) in Jeju, Korea**. *J Asia-Pacif Entomol* 2017, **20**:53–56.
19. Hardie J: **Reproductive, morphological and behavioural affinities between the alate gynopara and virginopara of the aphid, Aphis fabae**. *Physiol Entomol* 2010, **5**(4):385-396.
20. Liu LJ, Zheng HY, Jiang F, Guo W, Zhou ST: **Comparative transcriptional analysis of asexual and sexual morphs reveals possible mechanisms in reproductive polyphenism of the cotton aphid**. *PLoS One* 2014, **9**(6):e99506.
21. Willis KJ: **Introduction to the state of the world's plant 2017**. *State of the World's Plant* 2017:66-71.
22. Takada H: **Interclonal variation in the photoperiodic response for sexual morph production of Japanese *Aphis gossypii* Glover (Hom., Aphididae)**. *J Appl Entomol* 1988, **106**:188–197.
23. Shi SL, Liu XX, Zhang QW, Zhao ZW: **Morph-specific differences in metabolism related to flight in the wing-dimorphic *Aphis gossypii***. *Insect Sci* 2010, **17**(6):527–534.
24. Zeng W, Sun Z, Cai Z, Chen H, Lai Z, Yang S, Tang X: **Comparative transcriptome analysis of soybean response to bean pyralid larvae**. *BMC Genomics* 2017, **18**:871.
25. Ji J, Zhang S, Luo J, Wang L, Zhu X, Zhang K, Zhang L, Cui J: **Comparative transcriptional analysis provides insights of possible molecular mechanisms of wing polyphenism induced by postnatal crowding in *Aphis gossypii***. *Journal of Cotton Research* 2019, **2**:17.
26. Ding BY, Shang F, Zhang Q, Xiong Y, Yang Q, Niu JZ, Smagghe G, Wang JJ: **Silencing of two insulin receptor genes disrupts nymph-adult transition of alate brown citrus aphid**. *Int J Mol Sci* 2017, **18**(2):357.
27. Ogawa K, Miura T: **Two developmental switch points for the wing polymorphisms in the pea aphid *Acyrthosiphon pisum***. *EvoDevo* 2013, **4**:30.
28. Braendle C, Davis GK, Brisson JA, Stern DL: **Wing dimorphism in aphids**. *Heredity* 2006, **97**:192–199.
29. Yang X, Liu X, Xu X, Li Z, Li Y, Song D, Yu T, Zhu F, Zhang Q, Zhou X: **Gene expression profiling in winged and wingless cotton aphids, *Aphis gossypii* (Hemiptera: Aphididae)**. *Int J Biol Sci* 2014, **10**:257–267.
30. Abrisqueta M, Süren-Castillo S, Maestro JL: **Insulin receptor-mediated nutritional signalling regulates juvenile hormone biosynthesis and vitellogenin production in the German cockroach**. *Insect Biochem Mol Biol* 2014, **49**:14–23.
31. Xu HJ, Zhang CX: **Insulin receptors and wing dimorphism in rice planthoppers**. *Philos Trans R Soc London, Ser B* 2017, **372**:20150489.
32. Reedy MC, Bullard B, Vigoreaux JO: **Flightin is essential for thick filament assembly and sarcomere stability in Drosophila flight muscles**. *J Cell Biol* 2000, **151**(7):1483–1499.
33. Brisson JA, Davis GK, Stern DL: **Common genome-wide patterns of transcript accumulation underlying the wing polyphenism and polymorphism in the pea aphid (*Acyrthosiphon pisum*)**. *Evol*

Dev 2007, **9**(4):338–346.

34. Zhang RJ, Chen J, Jiang LY, Qiao GX: **The genes expression difference between winged and wingless bird cherry-oat aphid *Rhopalosiphum padi* based on transcriptomic data**. *Sci Rep* 2019, **9**:4754.
35. Shang F, Ding BY, Xiong Y, Dou W, Wei D, Jiang HB, Wei DD, Wang JJ: **Differential expression of genes in the alate and apterous morphs of the brown citrus aphid, *Toxoptera citricida***. *Sci Rep* 2016, **6**:32099.
36. Jia F, Yong Z, Frédéric F, Dengfa C, Jingrun S, Julian C: **Orco mediates olfactory behaviors and winged morph differentiation induced by alarm pheromone in the grain aphid, *Sitobion avenae***. *Insect Biochem Mol Biol* 2015, **64**:16–24.
37. Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Xian A, Fan L, Raychowdhury R, Zeng Q: **Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data**. *Nat Biotechnol* 2011, **29**(7):644–652.
38. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ: **Basic local alignment search tool**. *J Mol Biol* 1990, **215**(3):403–410.
39. Li B, Dewey CN: **RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome**. *BMC Bioinformatics* 2011, **12**:323.
40. Langmead B, Salzberg SL: **Fast gapped-read alignment with Bowtie 2**. *Nat Methods* 2012, **9**(4):357–359.
41. Wang L, Feng Z, Wang X, Wang X, Zhang X: **DEGseq: an R package for identifying differentially expressed genes from RNA-seq data**. *Bioinformatics* 2010, **26**(1):136–138.
42. Reiner A, Yekutieli D, Benjamini Y: **Identifying differentially expressed genes using false discovery rate controlling procedures**. *Bioinformatics* 2003, **19**(3):368–375.
43. Livak KJ, Schmittgen TD: **Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method**. *Methods* 2001, **25**(4):402–408.
44. Gao XK, Zhang S, Luo JY, Wang CY, Lü LM, Zhang LJ, Zhu XZ, Wang L, Cui JJ: **Identification and validation of reference genes for gene expression analysis in *Aphidius gifuensis* (Hymenoptera: Aphidiidae)**. *PLoS One* 2017, **12**(11):e0188477.

Table

Table 1 Summary of transcriptome data of cotton aphid

Groups	*Aphis gossypii*
Clean reads	984 865 702
Q30 (%)	90.91
GC content (%)	42.20
Assembled unigenes	46 501
N50 (bp)	2 876
Mapped reads (M)	908 778 671
Mapped ratios (%)	92.27
Unique mapped reads	307 801 309
Unique mapped ratio (%)	31.25
Annotated unigenes	37 090
Annotated ration (%)	79.76%

Supplementary Figure & Table Captions

Figure S1 Internal genitals of gynopara (A) and male (B) captured in the wild field

Figure S2 Unigenes number returned at the different searched databases

Table S1 Primers used in the RT-qPCR

Table S2 Lists of significantly enriched KEGG pathways from the shared DEGs in wing morphs

Table S3 Fold changes of selected DEGs validated by RT-qPCR

Figures

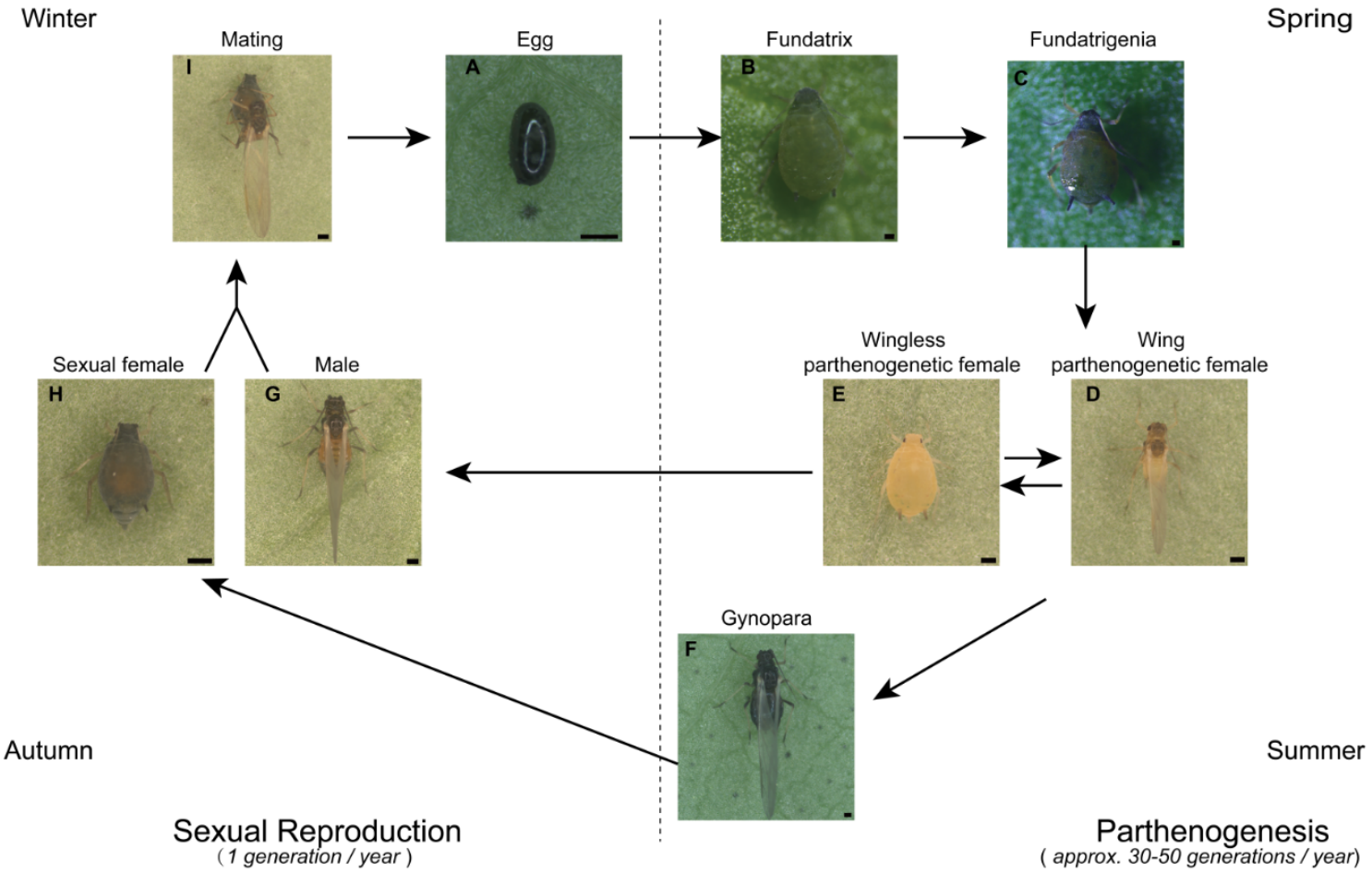

Figure 1

The annual life cycle of cotton aphid. Three representative wing morphs exist in the life cycle of cotton aphid. In spring, the overwintering egg (A) hatches and develops to fundatrix (B) which gives birth to fundatrigena (C) on the primary host plants. The fundatrigena undergoes several generations and then produces wing parthenogenetic female (D) which migrates to the second hosts such as the cotton in late spring. During the summer, cotton aphid alternative morphs between wing or wingless parthenogenetic females (E) over a dozen of generations to adapt to changing conditions such as population density and host plant quality. In the autumn, the exclusively winged gynopara (F) and male (G) are produced successively and migrate to the primary hosts. Sexual female (H) was produced by gynopara and oviposits overwintering fertilized eggs after mating with the male (I). All these morphs above were captured in the wild field. Scale bars, 0.2 mm.

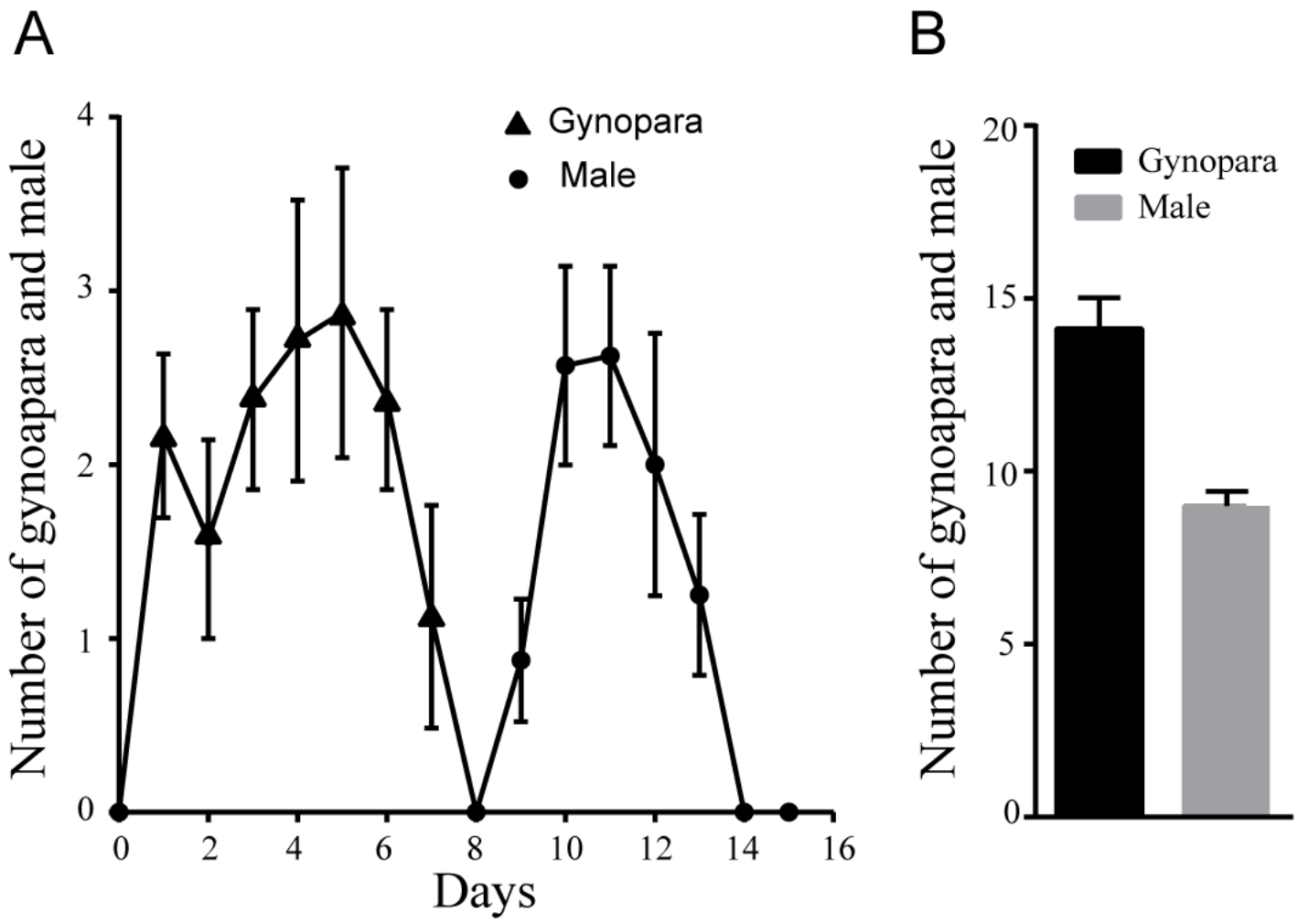

Figure 2

Reproductive schedules of cotton aphid under short day condition. (A) Daily number of gynoparae and males produced by parthenogenetic females which were reared under short day conditions from newly-born stage at 18℃ and photoperiod of 8L:16D. (B) Number of gynoparae and male produced by each parthenogenetic female at 18℃ and photoperiod of 8L:16D. n=8.

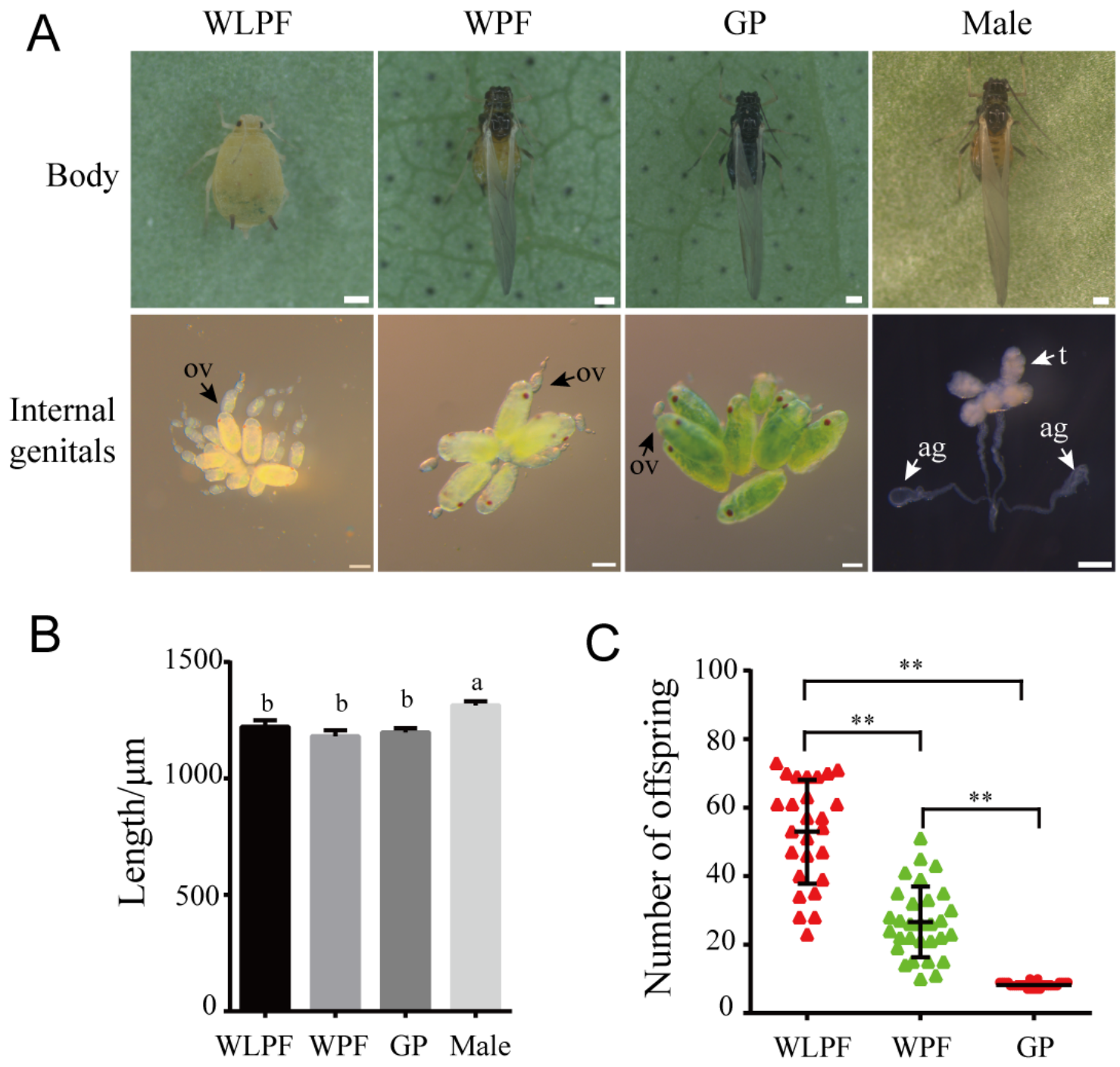

Figure 3

Bodies, internal genitals, fecundity of cotton aphid four morphs. (A) Bodies and internal genitals morphology of WLPF, WPF, GP and male obtained in laboratory condition. ov, ovarioles; ag, accessory glands; t, testes. Scale bars, 0.2 mm. (B) Body length of WLPF, WPF, GP and male with the exception of antennae and wings. More than 25 individual adults for each morph were measured by using a SteREO Discovery V8 microscope. Different letters indicate significant differences ($p < 0.05$) using Tukey's multiple range tests. (C) Fecundity of WLPF, WPF, GP. Offspring of individual WLPF, WPF, GP were counted respectively. Asterisk indicates significant differences between two morphs (Student's t test, $^{**}p < 0.01$).

More than 30 individual adults for each morph were observed daily until death. Values in all panels represent mean ± SE. WLPF, wingless parthenogenetic female; WPF, wing parthenogenetic female; GP, gynopara.

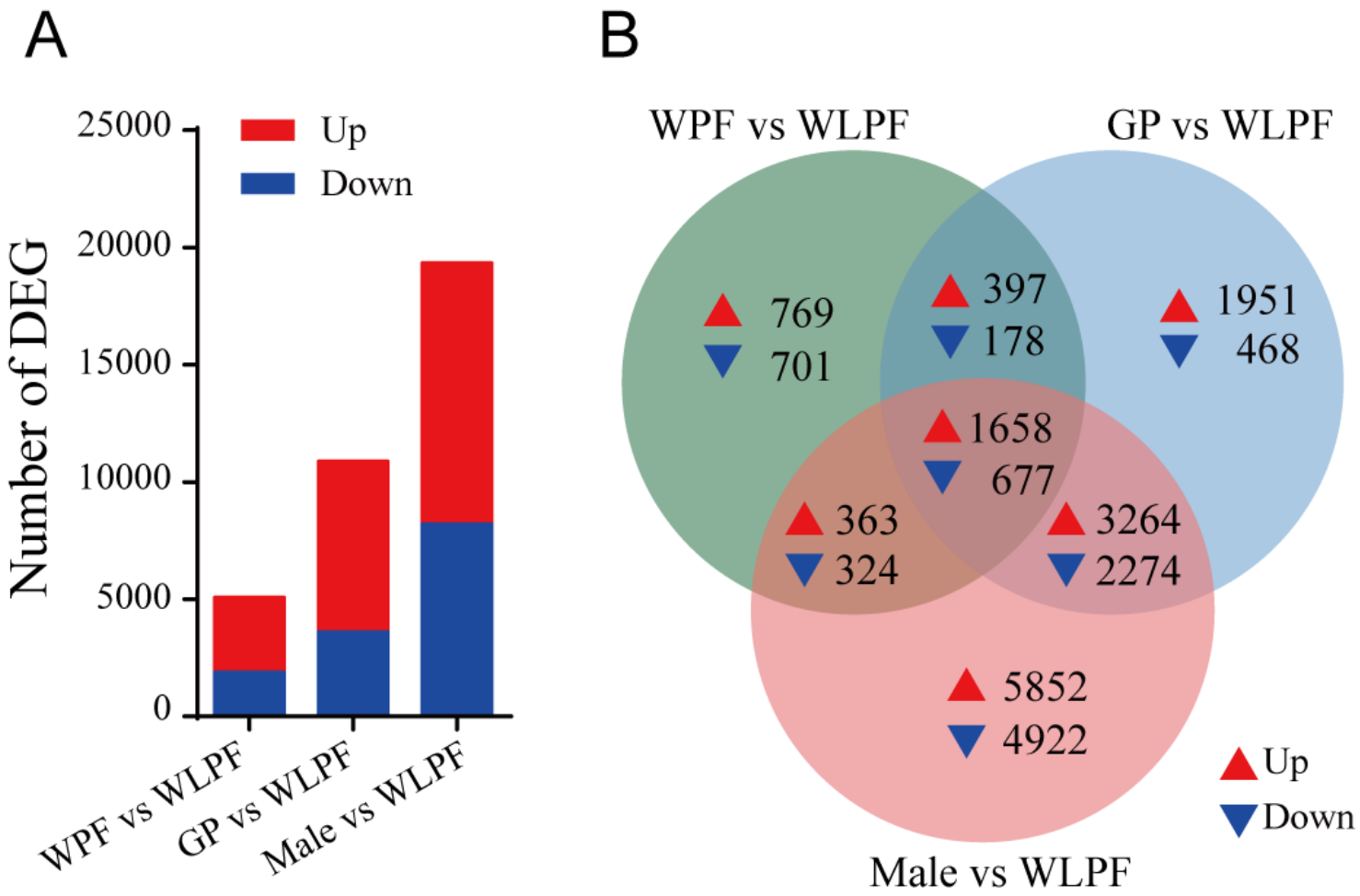

Figure 4

Pairwise comparison of DEGs between wing and wingless morphs. (A) Number of DEGs in the paired comparison between wing morphs and wingless parthenogenetic female. DEGs between WPF and WLPF were from our recent study [25]. (B) The distribution of DEGs in the pairwise comparison between wing and wingless morphs. WLPF, wingless parthenogenetic females; WPF, wing parthenogenetic female; GP, gynopara; Up, up-regulated; Down, down-regulated.

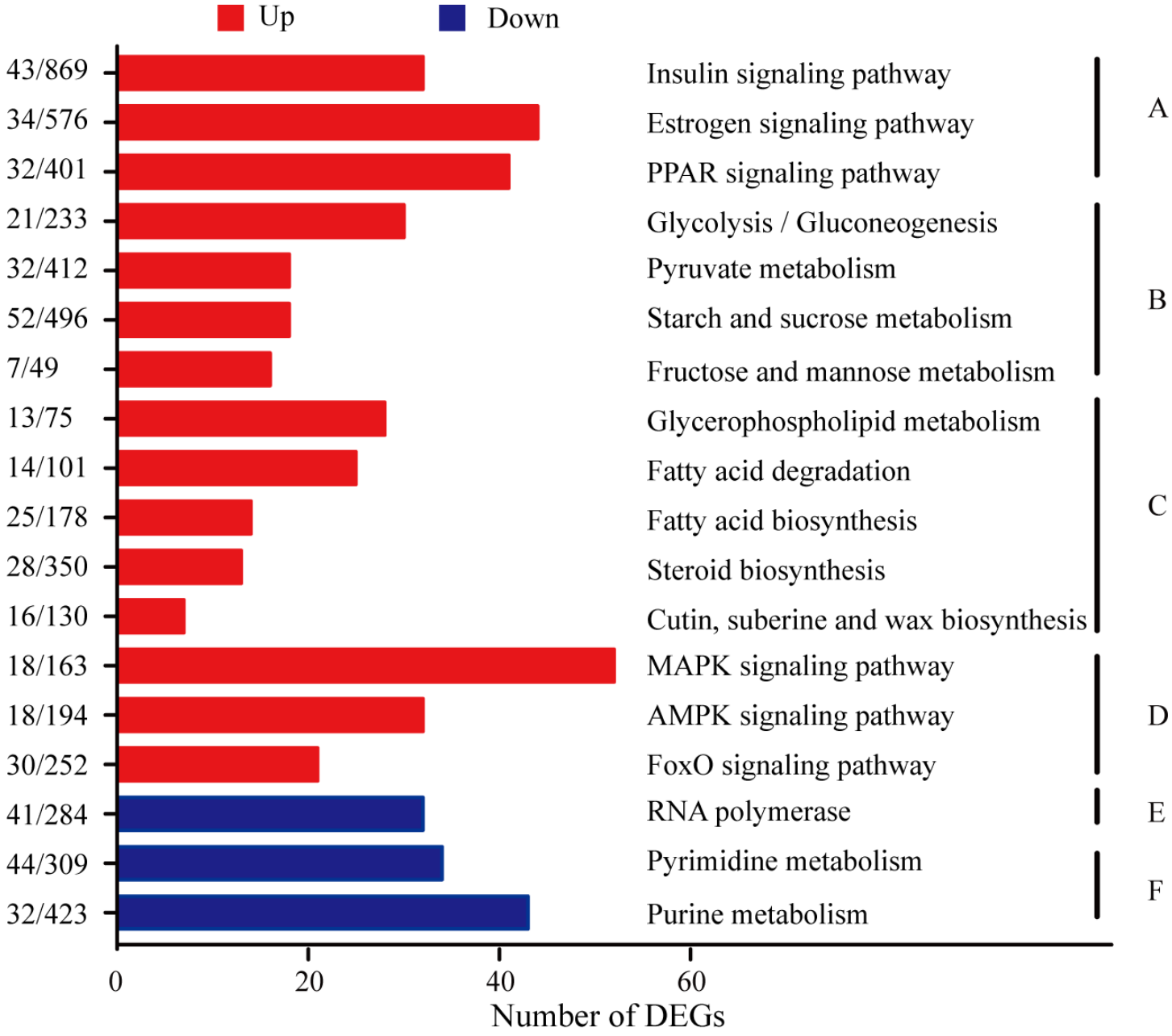

Figure 5

Functional classification of KEGG pathways for the shared DEGs in wing morphs. Compared to wingless parthenogenetic female, 2 335 DEGs including 1 658 up-regulated and 677 down-regulated genes were identified in all three wing morphs. The functional involvement of shared DEGs was analyzed by KEGG enrichment analysis, in which the shared up- and downregulated DEGs were enriched separately. Numerators and denominators of the fractions indicate the number of DEGs and the total number of genes in an enriched KEGG pathway, respectively. Significantly enriched pathways were judged at $P < 0.05$ in hypergeometric test. A, Endocrine system; B, Carbohydrate metabolism; C, Lipid metabolism; D, Signal transduction; E, Transcription; F, Nucleotide metabolism. Up, up-regulated; Down, down-regulated.

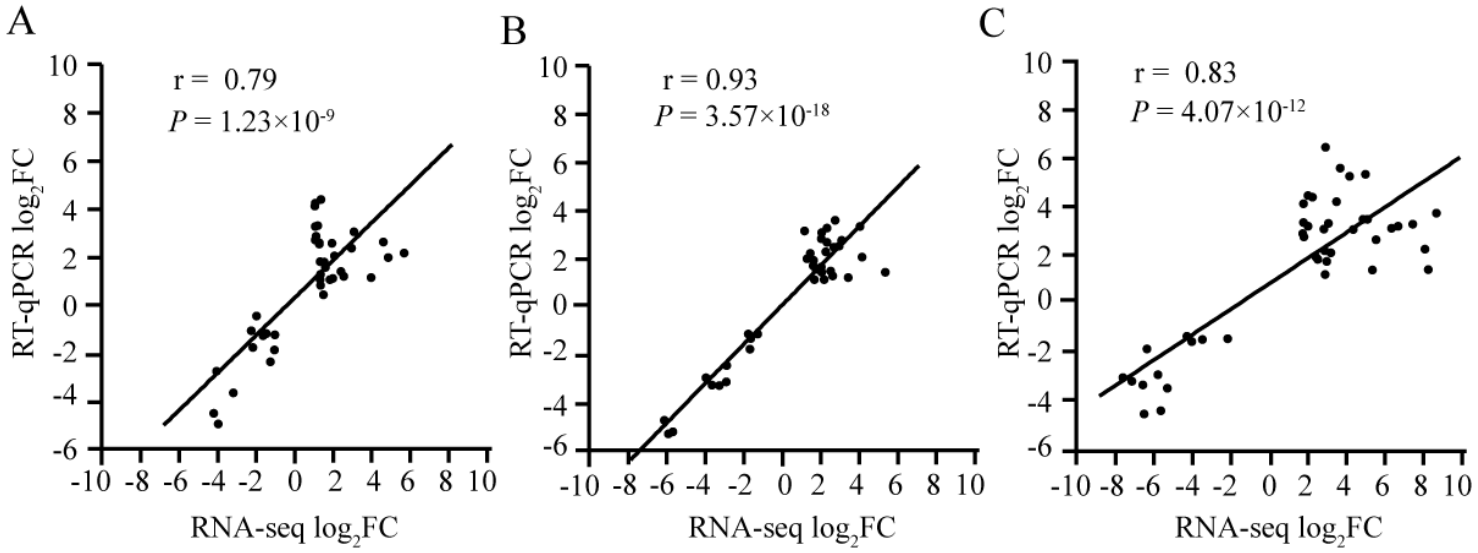

Figure 6

RT-qPCR validation of RNA-Seq results. Validation of gene expression levels (44 selected genes) using Person's correlation (r) between fold changes (log2 scale) observed in RT-qPCR and RNA-seq results in the pairwise comparison between wing morphs and wingless morphs, respectively. (A) WPF vs WLPF; (B) GP vs WLPF; (C) Male vs WLPF. FC, fold change, the relative expression level of a gene in one morph to another.

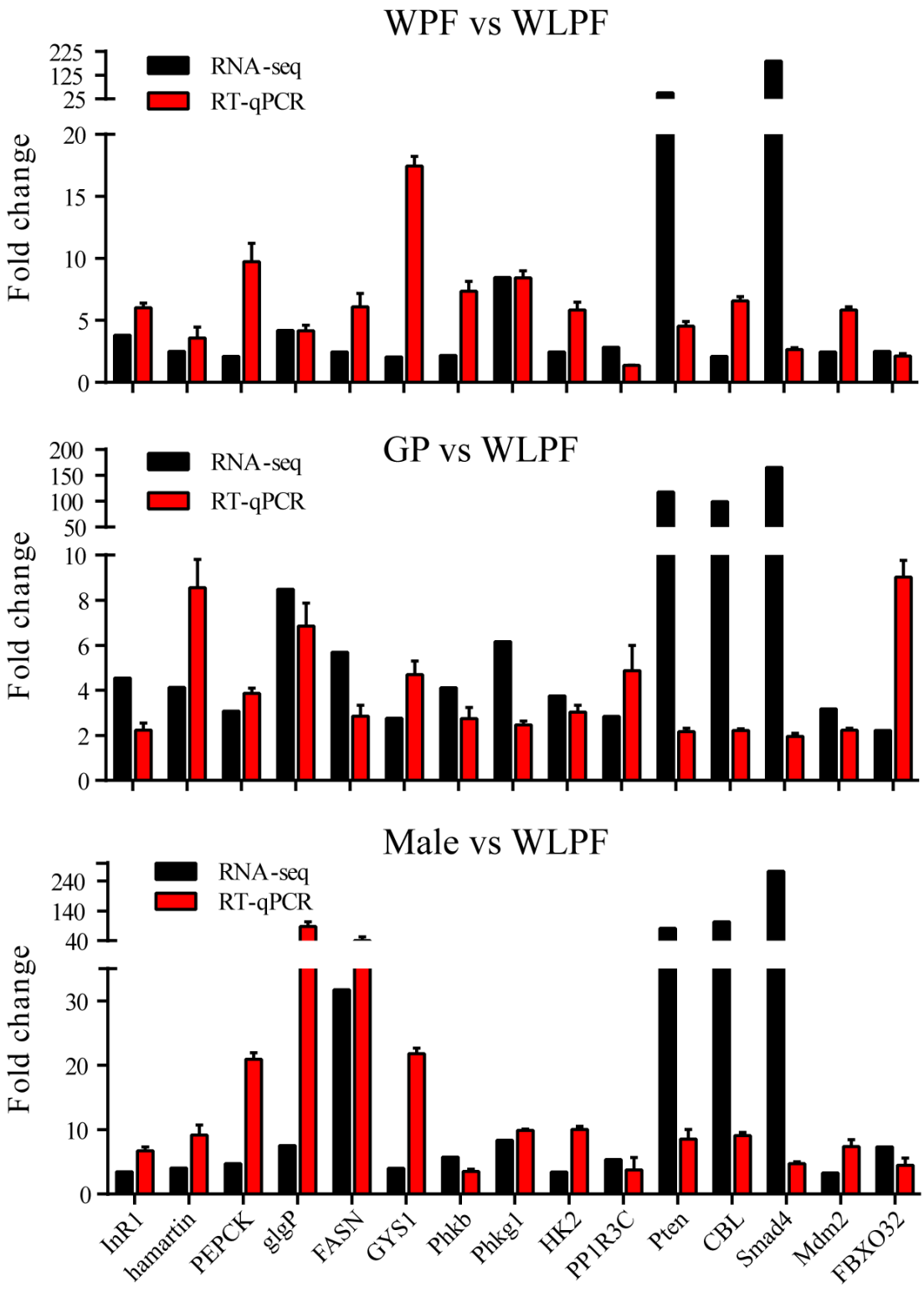

Figure 7

7 Upregulated DEGs associated with insulin signaling pathway in the wing morphs. 15 up-regulated genes related to insulin signaling pathway were observed with the similar expression profiles between RT-qPCR and RNA-seq results in the pairwise comparison between wing morphs and wingless morph. Each value was the mean of four replicates, and error bars indicated SEs. InR1, insulin receptor 1; PEPCK, phosphoenolpyruvate carboxykinase, cytosolic [GTP]-like; glgP, glycogen phosphorylase-like; FASN, fatty

acid synthase; GYS1, glycogen synthase; Phkb, phosphorylase b kinase regulatory subunit beta; Phkg1, phosphorylase b kinase gamma catalytic chain; HK2, hexokinase-2; PP1R3C, protein phosphatase 1 regulatory subunit 3C; Pten, phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase; CBL, calcineurin B-like; Smad4, mothers against decapentaplegic homolog 4; Mdm2, E3 ubiquitin-protein ligase Mdm2; FBXO32, F-box only protein 32. Fold change, the relative expression level of a gene in one morph to another.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureAbstract.tif
- Figure.S2.tif
- TableS3.docx
- TableS1.docx
- TableS2.xlsx
- Figure.S1.tif