Regulation of Hormone-Related Genes in *Ericerus pela* (Hemiptera: Coccidae) for Dimorphic Metamorphosis

Liu PengFei,1,2 Wang Weiwei,1 Ling Xiaofei,1 Lu Qin,1 Zhang Jinwen,1 He Rui,1,3 and Chen Hang1,3,4

1Research Institute of Resources Insect, Chinese Academy of Forestry, Kunming 650224, China, 2Nanjing Forestry University, Nanjing 210000, China, 3The Key Laboratory of Cultivating and Utilization of Resources Insects, State Forestry Administration, Kunming 650224, China, and 4Corresponding author, e-mail: stuchen6481@gmail.com

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Abstract

Insect hormones regulate metamorphosis including that leading to sexual dimorphism. Using RNA-Seq, we discovered that the second-instar male larva (SM) of the white wax insect, *Ericerus pela*, have 5,968 and 8,620 differentially expressed transcripts compared with the second-instar female larva (SF) and the first-instar male larva (FM), respectively. The expression levels of genes involved in the apoptosis of old tissues and the reconstruction of new ones in the SM significantly enhanced, while the SF mainly has enhanced expression levels of anabolic genes such as chitin. We predicted that the second-instar larvae are the developmental origin of sexual dimorphic metamorphosis. Meanwhile, in the juvenile hormone (JH) metabolic pathway, *CYP15A1* and JH esterase (*JHE*) are differentially expressed; and in the 20-hydroxyecdysone (20E) metabolic pathway, *CYP307A1*, *CYP314A1*, and *CYP18A1* are differentially expressed. In the SM, the expression levels of *CYP307A1* and *CYP314A1* are significantly increased, whereas the expression level of *CYP18A1* is significantly decreased; in the SF, the expression levels of the above genes are opposite to that of the SM. Expression trends of RNA-seq is consistent with the expression level of qRT–PCR, and seven of them are highly correlated (*R* ≥ 0.610) and four are moderately correlated (0.588 ≥ *R* ≥ 0.542).

Key words: transcriptome, dimorphic, metamorphosis, hormone

The white wax scale insect *Ericerus pela* (Chavannes) (Hemiptera: Coccidae) is distributed in northern subtropical, subtropical and temperate regions. It is a vital resource insect with over a thousand years usage history in China. The second-instar male larvae secrete a large amount of white wax whose main component is ceryl cerotate, a 26-acid and 26-lipid (C_{25}H_{51}COOC_{25}H_{53}) compound, with a high nutritional, health, economic, and ecological value. It is widely used in printing, machinery, medicine, foodstuff, cosmetics, and related fields (Chen 2011).

*Ericerus pela* shows a unique sexual dimorphic metamorphosis in its life cycle. The female develops from an egg (E), to a first-instar larvae (FF), a second-instar larvae (SF), an early female adult (EA), through to a late female adult (LA) in a hemimetabolous development mode. In contrast, the male develops from an egg (E), to a first-instar larvae (FM), a second-instar larvae (SM), a pre-pupal stage (PP), a pupal stage (P), through to an adult (MA) in holometabolous development mode (Fig. 1a). The life cycles also differ—the female up to 1 yr, with a main purpose of laying eggs in the adult stage, while the male life cycle is only about 5 mo. In males, the SM produce white wax which they use to cover themselves and then they complete the pupation process under the wax layer. The adult male dies after mating with the female on the 10th to 15th day (Chen 2011).

It was previously reported that the sexual dimorphism of *E. pela* was an ecological phenomenon of insect sex separation and differentiation caused by differential mating processes or sexual selection (Yang et al. 2015), and which was jointly regulated by juvenile hormone (JH) and molting hormone (20-hydroxyecdysone [20E]) (Röller et al. 2010). JH, a sesquiterpenoid hormone, has the functions of maintaining the growth characteristics of larvae: regulating growth and development, promoting ovarian maturation, and preventing larvae from prematurely entering the next instar (Qu et al. 2018). 20E, a steroid hormone, regulates the metamorphosis process of the insect body, including molting, tissue apoptosis, and reconstruction (Mai et al. 2017). When the concentration of JH in the larvae is high, the insect larvae state is maintained, and the metamorphosis is prevented. At the end of each larval instar, the concentration of JH decreases and 20E increases, promoting pupation or metamorphosis into adulthood (Rachinsky et al. 1990, Sakurai and Satake 1998, Truman and Riddiford 1999). JH and 20E play major regulatory roles in the...
development of the unique sexual dimorphic metamorphosis in E. pela (hemimetabolous of the female and holometabolous of the male) (Yang et al. 2015), but their regulatory mechanisms are not completely understood.

We explore differential expression of transcripts using transcriptome data, with a particular emphasis on the second-instar larvae. We use KEGG metabolic pathway to identify genes related to JH and 20E metabolism, analyze the expression levels of ones, and predict the key genes involved in regulating dimorphic metamorphosis. We also preliminarily illustrate the differences phenomenon in sexual dimorphic metamorphosis and establish the development patterns of sexual dimorphic of E. pela.

Materials and Methods

Sample Collection

_Ericerus pela_ samples were collected from _Ligustrum lucidum_ by the Research Institute of Resource Insect, Chinese Academy of Forestry, Kunming, China (25° 3’ 20″N, 102° 45’ 16″E, 1,950 m above sea level), and the eggs come from Zhaotong, Sichuan. Samples of FF, SF, EA, LA, FM, and SM were collected in three sets of each group, and were stored at −80°C.

RNA-seq

Hundred milligrams of _E. pela_ tissues of each developmental stage were used to isolate total RNA separately using an EZ-10 Total RNA Mini-Preps Kit (Sangon Biotech, Shanghai, China) according to the manufacturer’s protocols. RNA purity and integrity were assessed using a RNA Nano 6000 Assay Kit (Agilent Technologies, CA).

To prepare for RNA-seq analysis, the mRNA was enriched with Oligo (dT) magnetic beads, broken into short fragments with a fragmentation buffer, and the first strand cDNA was synthesized with random hexamer primers using mRNA as a template. The second strand of cDNA was then synthesized by adding buffer, dNTPs, DNA polymerase I, and RNase H. The double-stranded cDNA was purified with AMPure XP beads, the ends were repaired (the poly A tail and the sequencing linker were ligated), and the fragment size was selected using AMPure XP beads. Finally, PCR amplification was performed and the PCR product was purified using AMPure XP beads to obtain the final cDNA library.

After the library was constructed, preliminary quantification was performed using Qubit2.0, and the library was diluted to 1.5 ng/μl. The insert size of the library was detected using an Agilent 2100. As the insert size was as expected, the effective concentration of the library was determined by qPCR; accurate quantification (effective
library concentration > 2 nM) was performed to ensure the quality of the library. The different libraries were then pooled according to the effective concentration and the target data volume, and sequenced with Illumina HiSeq.

Nonparametric Transcriptome Assembly and Annotation
RNA-seq reads were filtered to remove adaptors and low-quality sequences to obtain clean reads. The clean reads were spliced using Trinity (Grabherr et al. 2011) to obtain transcript files stored in Fasta format. The transcripts were then hierarchically clustered using the number of reads and expression patterns of the aligned transcripts on Corset's official website at https://code.google.com/p/corset-project/ (Davidson and Oshlack 2014). The hierarchical clustering analysis (HCA) was performed to obtain the longest Cluster sequence for subsequent gene function annotation and analysis.

Gene Expression Profiles and Annotations
Gene function annotations were performed using seven databases: NR, NT, KO, SwissProt, PFAM, GO, and KOG. Differential expression analysis between samples was performed using DEG seq (v.1.12.0), and genes with q-value <0.005 and log2FoldChange >1 were selected as differentially expressed genes.

Data Analysis
HCA and principal component analysis (PCA) of differential genes were performed in Rstudio (R*64 3.5.1). Using prism 6.0 software analyzes qPCR results and ‘t.test’. Statistical analysis of data was run in MS Excel.

qRT–PCR
Total RNA was reverse transcribed using PrimerScript RT reagent Kit with gDNA Eraser (Takara). qPCR was performed using SYBR Premix Ex Tap II (Tli RNaseH Plus) Kit; 11 transcripts were selected to validate RNA-seq data with the beta-actin gene as a reference. qPCR was carried out in a 25 μl reaction containing 1 μl of 5 ng/μl cDNA, 12.5 μl SYBR Premix Ex Tap II (Tli RNaseH Plus) (Takara), and 5 μM each of the forward and reverse primers. Gene-specific primers were designed using Primer 6.0, and the primer sequences are shown in Supp Table S1 (online only). The qPCR reaction was carried out using a BioRad CFX Real-Time PCR System (Applied Biosystems) with the following program: pre-denaturation at 95°C for 30 s, followed by 39 cycles of denaturation at 95°C for 5 s and annealing at 60°C for 40 s. The gene Ct values were normalized with beta-actin Ct values.

Data Availability
All final data and the de novo assembled transcript sequences were submitted to GenBank with the project accession number of PRJNA551363. Nucleotide sequences have been deposited in the Sequence Read Archive (SRA) of NCBI under accession codes SRR9617904, SRR9617908, and SRR9617915 (E. pela: FF); SRR9617916, SRR9617917, and SRR9617918 (E. pela: SF); SRR9617902, SRR9617905, and SRR9617914 (E. pela: EA); SRR9617901, SRR9617903, and SRR9617909 (E. pela: LA); SRR9617906, SRR9617907, and SRR9617912 (E. pela: FM); SRR9617910, SRR9617911 and SRR9617913 (E. pela: SM).

Results
In order to analyze the transcriptome characteristics during the development of the sexually dimorphic metamorphosis of E. pela, we obtained transcripts of the FF, SF, EA, and LA of the female, and the FM and SM of the male. After Trinity splicing, there were 528,309 transcripts (Supp Fig. S1 [online only]). The longest cluster sequence obtained by HCA was used in subsequent analysis, and we obtained 249,288 longest transcripts (unigenes) (Fig. S2 [online only]). The 249,288 transcripts were then annotated with seven major databases: NR, NT, KO, SwissProt, PFAM, GO, and KOG; there were 79,710, 56,789, 90,672, 97,375, 98,216, 59,154, and 16,273 transcripts annotations, respectively; and all the transcripts were successfully annotated (Fig. 1b and Supp Fig. S3 [online only]). In total, 158,968 (63.76%) transcripts were expressed in the FM, 149,025 (59.78%) transcripts in the SM, 162,651 (65.25%) transcripts in the SF, 182,538 (73.22%) transcripts in the FF, 120,901 (48.50%) transcripts in the EA, and 118,539 (47.55%) transcripts in the LA (Fig. S4 [online only]). The transcripts of the second-instar male and second-instar female larvae were significantly differently expressed, with smaller differences observed between the male and female first-instar larvae. Once the female enters the adult stage, transcript expression tends to be stable (Fig. 1c).

Transcript Differences in Different Insect Stages
The PCA shows that the expression results were similar between FM and FF, FM and SM, and FM and FF (Supp Fig. S5 [online only]), and that the expression difference between EA and LA was small and stable. The results of the HCA (FPKM ≥ 1) show that the gene expression profile is clustered into five groups: FM, SF, FF, SM, and EA-LA (Fig. 2a). The EA and LA expression profiles were very similar, while the larvae expression profiles were more different from the adults. Within the larvae, about 20% of the gene expression of FM is significantly different from that of FF, and about 50% of the gene expression of SM is significantly different from that of SF, indicating that a large number of genes which regulate the development direction of the male and female dimorphism of E. pela are differentially expressed during the second-instar larva stage. Meanwhile, we found that the expression profile of EA1 samples was significantly different from other samples (Fig. 2a and Supp Fig. S5 [online only]), which should be mainly caused by the contamination of samples during sample collection or sequencing, so EA1 outliers were removed during data analysis.

On the basis of differentially expressed transcript analysis, the results revealed that SF versus LA has 20,923 transcripts differentially expressed (17,549 upregulated and 3,374 downregulated), SM versus FM has 17,296 (14,403 upregulated and 2,893 downregulated), and SF versus SM has 16,308 (10,118 upregulated and 6,190 downregulated). However, EA versus LA has only 54 differentially expressed transcripts (27 upregulated and 27 downregulated), FF versus FM has 1,488 (893 upregulated and 595 downregulated) and FF versus SF has 3,176 (233 upregulated and 2,943 downregulated) (Fig. 2b). Meanwhile, the results indicate that the number of differentially expressed transcripts which are only expressed between SM and FM, and between SF and SM, are 8620 and 5968, respectively (Fig. 2c). Therefore, the second-instar larvae of E. pela showed greater changes in different directions of physiological and biochemical aspects. It is possible that the regulatory factors of the metamorphosis of E. pela begin to express in large numbers in the second-instar larvae, which shows extremely significant differences.

Differences in Internal Metabolism
GO enrichment analysis indicated that a large number of enrichment genes in the SM stage (Fig. 2d), including 12,318 in SM versus FM and 11,717 in SF versus SM, were mainly related to biological
process and molecular function (Fig. 2e). In the comparison of SF and SM, we found that the chitin metabolic process, glucosamine-containing compound metabolic process, chitin binding, amino sugar metabolic process, structural constituent of cuticle, and other related genes are enriched in the SF stage, while catalytic activity, metabolic process, biosynthetic process, organic substance biosynthetic process, and other related genes are enriched in the SM stage. In the comparison of SM and FM, we found that catalytic activity, metabolic process, RNA polymerase II transcription factor activity, sequence-specific DNA binding, and other related genes are enriched in the SM stage, while 3-beta-hydroxy-delta5-steroid dehydrogenase activity, C21-steroid hormone metabolic process, androgen metabolic process, estrogen metabolic process, and other related genes are enriched in the FM stage (Fig. 2f and Supp Table S2 [online only]). Overall, there are decreased hormone metabolism, enhanced catalytic activity, metabolism, and biosynthesis transcripts in the SM stage, which prepare for the male E. pela holometabolous development, while enhanced metabolic processes of chitin binding, glucosamine-containing compounds, and the structural components of cuticle in the SF stage, which prepare for the female E. pela hemimetabolous development.

Differentially Expressed Genes Related to JH and 20E

From the number of differentially expressed transcripts, we predict that the starting point of sexual dimorphism in development is the second larvae stage. JH and 20E have shown significant regulatory effects on the metamorphosis of E. pela as metabolism of both is intimately related to the synthesis of the terpene compounds (Supp Fig. S3 [online only]). Terpene compounds are precursors of both JH and 20E, with the triterpenes of 20E synthesis only coming from plants (Lafont and Koolman 1984). By analyzing the KEGG pathway, we found that the genes involved in insect hormone and terpene compound synthesis are differentially expressed much more in SF versus SM and SM versus FM than in FF versus FM and FF versus SF (Fig. 3a).

In the process of terpene synthesis, 35 transcripts were differentially expressed between SF and SM, including hydroxymethylglutaryl-CoA reductase (NADPH) (HMGCR), acetyl-CoA C-acetyltransferase(atoB), hydroxymethylglutaryl-CoA synthase (HMGR), farnesyl diposphate synthase (FDFS), geranylgeranyl diposphate synthase, type III (GGPS1), protein farnesyltransferase subunit beta (FNTB), ditrans, polycis-polyprenyl...
diphosphate synthase (DHDDS), decaprenyl-diphosphate synthase subunit 1 (PDS11), mevalonate kinase (MVK), STE24 endopeptidase, diphostemonevalone decarboxylase (MVD), isopentenyl-diphosphate Delta-isomerase (IDI), and prenyl protein peptidase (Fig. 3b; Suppl Fig. S7-1 and Table S3). Thirty-nine transcripts were differentially expressed between SM and FM, including HMGR, atoB, HMGR, FDFS, GGPS1, ENTRB, DHDDS, PDS11 (upregulated: 2), MVK, STE24 endopeptidase, MVD, IDI, prenyl protein peptidase, and phosphomevalonate kinase (mvaK2) (Fig. 3c; Supp Fig. S7-2 and Table S4 [online only]), supporting the idea that terpene compound genes are closely related to JH synthesis.

In the process of JH and 20E metabolism, JH esterase (JHE) and cytochrome oxidase-related genes (CYP15A1, CYP307A1, CYP314A1, CYP18A1) are differentially expressed. In SF and SM (Fig. 3c; Supp Fig. S6-1 and Table S5 [online only]), differentially expressed include the eight upregulated transcripts of CYP15A1 that catalyze the synthesis of JH (Minakuchi et al., 2015), the two downregulated transcripts of JHE that hydrolyze JH (Wyatt and Davey 1999), the one upregulated and three downregulated transcripts of CYP307A1 that catalyze 20E synthesis (Rewitz et al., 2006), the one upregulated transcript of CYP314A1 that catalyzes 20E synthesis (Rewitz et al., 2006), and the one upregulated transcript of CYP15A1 that hydrolyzes 20E (Guitard et al. 2011). In SM versus FM (Fig. 3c; Supp Fig. S6-2 and Table S6 [online only]), CYP15A1 expresses one downregulated transcript, JHE expresses three upregulated transcripts, CYP307A1 expresses five upregulated and one downregulated transcripts, CYP314A1 expresses one upregulated transcript, and CYP18A1 expresses one upregulated transcript. On the basis of HCA (Fig. 3f), the one upregulated transcript expresses one upregulated transcript of CYP18A1, the one upregulated transcript of CYP307A1 expresses five upregulated transcripts, and the one downregulated transcript of CYP314A1 is differentially expressed (Fig. 3b; Suppl Fig. S7-1 and Table S3). Thirty-nine transcripts were differentially expressed between SF and SM, including HMGCR, atoB, HMGR, FDFS, GGPS1, ENTRB, DHDDS, PDS11 (upregulated: 2), MVK, STE24 endopeptidase, MVD, IDI, prenyl protein peptidase, and phosphomevalonate kinase (mvaK2) (Fig. 3c; Supp Fig. S7-2 and Table S4 [online only]), supporting the idea that terpene compound genes are closely related to JH synthesis.

qRT–PCR Validation
The qRT verification showed that CYP15A1, JHE, CYP307A1, CYP314A1, and CYP18A1 were significantly differentially expressed. In SM versus SF, JHE, CYP307A1, and CYP314A1 were highly expressed in SF; and CYP15A1 and CYP18A1 are highly expressed in SM (Fig. 4a). In SM versus FM, JHE was highly expressed in SM; the expression level was 154 times that of FM; and CYP314A1 was highly expressed in SM; and CYP15A1, CYP307A1, and CYP18A1 were highly expressed in FM (Fig. 4b). Moreover, qPCR results of the above key genes were basically consistent with the expression profile found with RNA-seq. We selected 11 transcripts related to the metabolism of terpenes and insect hormones to confirm that the expression level of RNA-seq was consistent with the expression level of quantitative PCR by RT–PCR (Fig. 3b–e and Supp Table S1 [online only]), and seven of them are highly correlated (R ≥ 0.610) and four are moderately correlated (0.588 ≥ R ≥ 0.542) (Fig. 4c).

Discussion
This paper analyzes the expression characteristics of sexual dimorphism of E. pela, based on transcriptome data analysis, revealing a suggested mechanism of the hemimetabolous metamorphosis of the female and the holometabolous metamorphosis of the male. We specify the putative genes of the JH and 20E metabolic pathways which affect the metamorphosis of sexually dimorphism of E. pela, demonstrate the regulation effect of insect hormones on the metamorphosis of sexual dimorphism in the insect, and provide a research basis and ideas for follow-up studies of sexual dimorphism in E. pela.

By comparing SF and SM, we discovered that genes related to biosynthesis and metabolism are significantly enriched in SM, including metabolism, biosynthesis of organic substances, ribosome, peptide biosynthesis, and hormone synthesis. However, genes related to biological processes of the chitin shell are significantly enriched in SF, including those involved in the metabolism of chitin, the compound metabolism of glucosamine, the binding of chitin, the metabolism of amino sugar, and the structure and composition of the cuticle. By comparing the SM and FM, we found that catalytic activity, metabolic processes, and other related genes are enriched in SM, but androgen metabolism, steroid estrogen metabolic process, and other related genes are enriched in FM. It was found that genes enriched in the SM, which are related to the apoptosis of old tissues and the re-structure of the early female adult and regulated the female larva to start the hemimetabolous development. Genes enriched in the SF, which are associated with the structure and composition of cuticle, amino sugar, and the cuticle, provided a material basis for entering the early female adult and regulated the second female larva to start the hemimetabolous development. This supports the prediction that the E. pela metamorphosis divergence begins in the second-instar larvae.

The JH- and 20E-related genes were differentially expressed in the SM, SF, and FM, including JHE, CYP15A1, CYP307A1, CYP314A1, and CYP18A1.

CYP15A1 is a highly specific epoxidase, which takes part in JH terminal synthesis (Marchal et al. 2011). In the corpora allata of cockroaches, CYP15A1 was identified as a gene encoding a cytochrome P450 enzyme which catalyzes the epoxidation of methyl farnesote to JH, with a catalytic rate as high as 98% (Helvig et al. 2004). CYP15C1, which epoxidizes farnesyl to JH acid, was found in Bombyx (Daimon et al. 2012). The CYP15A1 gene is highly conserved in insects except for the higher Diptera such as Drosophila melanogaster, which does not encode the CYP15A1 gene (Daimon and Shinoda, 2013). In the red flour beetle (Tenebrio), the transcriptional level of the CYP15A1 peaks in the embryonic stage, and the transcriptional level of the JH methytransferase (JHAMT) mRNA simultaneously increased, indicating that CYP15A1 is involved in JH biosynthesis (Minakuchi et al. 2013).

JHE is a carboxyl esterase produced in epithelial, midgut, and fat cells (Shuhua et al. 2008). JHE can specifically isolate JHBP-JH complex and degrade JH into JHA (Hammock and Sparks, 1977), and can also degrade the product of JH epoxidase (JHEH) into JHAD (Share and Roe 1988) (Fig. 3f). Therefore, JHE plays a vital role in controlling JH titer in insects (ElSheikh et al. 2011). During insect metamorphosis, JHE plays an important role in larvae pupation and insect behavior. In the early stage of pupation, inhibiting the biological activity of JHE leads to the failure of pupation; the larvae will continue to feed, and abnormal development will result in hypertrophy larvae in the over-age stage (Abdel-Aal and Hammock 1986). Before the pupation of Anoplophora nobilis, the biological activity of JHE was significantly enhanced and the titer of JH was significantly decreased; the activity of JHE reached its highest levels in the pre-pupal stage, and the delay of pupation was related to the activity of JHE (Munyiri and Ishikawa 2004). Mackert et al. found that when the JH titer in worker bees increased, the expression level of JH lipase gene (AmJHE-like) decreased, and the behavior of worker bees changed from nursing to foraging. It was suggested that expression of the AmJHE-like gene regulated the change of this behavior (Mackert et al. 2008). Therefore, it is possible that JHE directly affects insect pupation...
behavior and regulates the metamorphosis process by regulating the JH titer.

CYP307A1 may be a significant part of the oxidative process of 20E, which oxidizes 7-dehydrocholesterol to diketone (Zhou et al. 2013). In 1986, Abdel-aal and Hammock found that CYP307A1 was only expressed in embryonic and follicular cells, but not in the larval stage. However, the homologous CYP307A2 is only expressed in prothorax gland (PG) cells at the larval stage, and the decreased expression level of CYP307A2 by RNA interference leads to the cessation of development at the first larval stage. The addition of ketotriol and ketodiol can make the knockdown CYP307A2 larvae and CYP307A1 mutant embryos follow normal development, respectively (Abdel-Aal and Hammock 1986). In 1995, Warren et al. identified spo (CYP307A1) and spok (CYP307A2) in Schistocerca gregaria using molecular genetics (Warren et al. 1995). In 2005, Namiki et al. found that the cytochrome P450 gene CYP307A1 in Bombyx was only expressed in PG, and the transcription level was positively correlated to the change in 20E titer (Namiki et al. 2005). In 2013, Emilie et al. found that the expression level of CYP307A1 in Anopheles gambiae was similar to that of the steroid gene. In addition, the RNAi targeted CYP307A1 experiment further proved that the loss of CYP307A1 function interfered with the production of exocrine hormone, suggesting that this gene is necessary for the synthesis of exocrine hormone by An. gambiae (Emilie et al. 2013).
CYP314A1 catalyzes 20E to produce the bioactive 20-hydroxyl ecdysone, the mitochondrial enzyme that catalyzes the final step in the synthesis of 20E (Petryk et al. 2003, Gilbert 2004, Rewitz et al. 2006).

CYP18A1 is involved in the degradation and inactivation of 20E. In 1993, Hurban and Thummel discovered eig-17, a gene associated with ecdysone metabolism in D. melanogaster (Hurban and Thummel 1993). In 1997, Bassett et al. noted that the eig-17 gene in S. gregaria was similar to the full-length cytochrome oxidase CYP18A1, so much so that CYP18A1 was considered as ecdysteroids 26-hydroxylase (Bassett et al. 1997). In 2006, Davies et al. discovered the expression level of CYP18A1 changed in the 20E, which was able to maintain the balance of 20E in vivo (Davies et al. 2006). In 2011, Guittard et al. found that as the expression level of CYP18A1 decreased, the hydrolysis of 20E slowed down resulting in pupal death, further supporting that CYP18A1 in S. gregaria catalyzes the 26-hydroxylation of ecdysteroids and oxidizes them into a 26-carbon carboxylic acid (Guittard et al. 2011). In 2014, Li et al. demonstrated that CYP18A1 takes part in the inactivation process of steroid hormones in Bombyx (Li et al. 2014).

Fig. 4. qPCR validation. (a) results of insect hormone metabolism pathway SM versus SF stage differentially expressed genes; (b) results of insect hormone metabolism pathway SM versus FM stage differentially expressed genes; (c) RT-PCR was used to verify the results of RNA-seq sequencing, the x-axis represents the different periods of insects and the y-axis represents the expression quantity.
In summary, in the progression of JH metabolism, CYPI15A1 oxidizes methyl farnesolate to generate JH, and JHE hydrolyzes JH. In the process of 20E metabolism, CYP307A1 catalyzes 7-dehydrocholesterol to bilestones, CYP314A1 catalyzes molting ketone to 20E, and CYPI18A1 hydrolyzes 20E into a 20- or 26-carbon carboxylic acid. Insect hormones play a vital regulatory role in insect metamorphosis.

By analyzing the transcriptome, we found that CYPI15A1 was upregulated in SF relative to SM, and JHE and CYP307A1 were upregulated in SM over SF. CYPI15A1 was upregulated in FM compared to SM, and JHE and CYP307A1 were upregulated in SM relative to FM. qPCR further showed that CYPI15A1 and CYPI18A1 were upregulated in SF relative to SM, and JHE, CYP307A1, and CYP314A1 were upregulated in SM relative to SF; CYPI15A1, CYP307A1, and CYPI18A1 were upregulated in FM over SM, and JHE and CYP314A1 were upregulated in SM relative to FM; the differences in expression were significant. This suggests that the genes mentioned above play a significant part in the sexually dimorphic metamorphosis of E. pela.

In summary, we speculate that the starting point of the dimorphic development of E. pela is the second-instar larvae. In the SF stage, the expression levels of CYP307A1 and CYP314A1, the key genes involved in the synthesis of 20E, and of JHE, the key gene involved in the hydrolysis of JH, were reduced, while the expression levels of CYPI18A1, the key gene involved in the hydrolysis of 20E, and CYPI15A1, the key gene involved in JH synthesis, were higher than that in the SM. Meanwhile, the metabolism of chitin, amino sugar and cuticle was increased in the SF, providing a material basis for entering the EA stage. In the SM stage, the expression levels of CYP307A1, CYP314A1, and JHE were increased, and those of CYPI18A1 and CYPI15A1 were decreased. In the SM stage, the apoptosis of old organization and the rebuilding of new organization were increased, providing a material basis for entering the pre-pupal stage. Ultimately, we predicted that CYPI15A1, JHE, CYP307A1, CYP314A1, and CYPI18A1 regulate the sexually dimorphic metamorphosis of E. pela. Firstly, the gene expression levels map to insect hormone synthesis level, in the SM stage: 20E concentration increases and JH concentration decreases, which weakens their interaction, so that the SM begins to go into holometabolous development and enter the pre-pupal stage. In the SF, 20E concentration decreases and JH concentration increases, enhancing their interaction and pushing the SF into hemimetabolous development and the early female adult. Secondly, these five genes have been shown to directly regulate the metamorphosis in other regulation ways. Future studies will continue to explore the inter-regulation of these genes.

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

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