Importance of Taiman in Larval-Pupal Transition in *Leptinotarsa decemlineata*

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Insect Taiman (Tai) binds to methoprene-tolerant to form a heterodimeric complex, mediating juvenile hormone (JH) signaling to regulate larval development and to prevent premature metamorphosis. Tai also acts as a steroid receptor coactivator of 20-hydroxyecdysone (20E) receptor heterodimer, ecdysone receptor (EcR) and Ultraspiracle (USP), to control the differentiation of early germline cells and the migration of specific follicle cells and border cells in ovaries in several insect species. In holometabolous insects, however, whether Tai functions as the coactivator of EcR/USP to transduce 20E message during larval-pupal transition is unknown. In the present paper, we found that the *LdTai* mRNA levels were positively correlated with circulating JH and 20E titers in *Leptinotarsa decemlineata*; and ingestion of either JH or 20E stimulated the transcription of *LdTai*. Moreover, RNA interference (RNAi)-aided knockdown of *LdTai* at the fourth (final) instar stage repressed both JH and 20E signals, inhibited larval growth and shortened larval developing period. The knockdown caused 100% larval lethality due to failure of larval-pupal ecdysis. Under the apolysed larval cuticle, the *LdTai* RNAi prepupae possessed pupal thorax. In contrast, the process of tracheal ecdysis was uncompleted. Neither JH nor 20E rescued the aforementioned defectives in *LdTai* RNAi larvae. It appears that Tai mediates both JH and 20E signaling. Our results uncover a link between JH and 20E pathways during metamorphosis in *L. decemlineata*.

Keywords: *Leptinotarsa decemlineata*, *LdTai*, metamorphosis, trachea, fat body

INTRODUCTION

Insect development and reproduction are essentially governed by the steroid 20-hydroxyecdysone (20E) and the sesquiterpenoid juvenile hormone (JH) (Jindra et al., 2013, 2015a; Santos et al., 2019). During larval development in holometabolous insects, the presence of JH suppresses the expression of metamorphosis-initiation genes and 20E can only induce the larval-larval molt (Riddiford, 1994; Ureña et al., 2014). At the late stage of the final instar larva, JH titer drops substantially, to terminate the suppression effects on metamorphosis-initiation genes (Smykal et al., 2014b). As a result, 20E induces larval-pupal and pupal-adult molts (Jindra et al., 2013; Santos et al., 2019).

JH acts through its receptor methoprene-tolerant (Met) (Ashok et al., 1998; Charles et al., 2011; Jindra et al., 2015b), a basic helix-loop-helix (bHLH)/Per-Arnt-Sim (PAS) transcription...
factor (Partch and Gardner, 2010). After binding of JH, Met recruits another bHLH/PAS protein Taiman (Tai) to form an active receptor complex. The binding of Met/Tai heterodimer to JH response element (JHRE) triggers the transcription of Krüppel-homolog 1 (Kr-h1) and Hairy to repress metamorphosis (Saha et al., 2016; Wu et al., 2016), partially by inhibition of prothoracicotropic hormone (PTTH) signaling (PTTH-Torso-MAPK) to repress 20E biosynthesis and release in Coleopteran for instance the Colorado potato beetle Leptinotarsa decemlineata, the most destructive insect defoliator of potatoes (Meng et al., 2018, 2019), and Lepidopteran such as the tobacco hornworm Manduca sexta (Rountree and Bollenbacher, 1986; Watson and Bollenbacher, 1988).

In insects, Tai also acts as a steroid receptor coactivator of the functional 20E receptor heterodimer, ecdysone receptor (EcR) and Ultraspiracle (USP) in adults (Yao et al., 1993; Bai et al., 2000). In the common fruit fly Drosophila melanogaster, 20E-dependent binding of Tai to EcR/USP is crucial for the migration of specific follicle cells and border cells in ovaries, and for the differentiation of early germline cells (Bai et al., 2000; König et al., 2011; König and Scherbata, 2015). In the yellow fever mosquito Aedes aegypti (Liu et al., 2018) and the firebug Pyrrhocoris apterus (Smykal et al., 2014a), depletion of Tai impairs oocyte development.

Is Tai involved in the development of juvenile insects, as a coactivator of EcR/USP? In the German cockroach Blattella germanica (Lozano and Belles, 2014) and P. apterus (Smykal et al., 2014b), knockdown of Met in nymphaal instars triggers precocious metamorphosis, whereas depletion of Tai causes nymphal lethality (Lozano et al., 2014; Smykal et al., 2014a). The different negative effects between Met- and Tai-depleted insects suggest that Tai may be an important component in 20E transduction cascade regulating insect development in hemimetabolan insects.

In holometabolans, whether Tai functions as a coactivator of EcR/USP during larval-pupal-adult transition is unknown. In the oriental fruit fly Bactrocera dorsalis, depletion of Tai in larval stage produces no mortality but causes precocious pupal development (Liu et al., 2017), a typical phenotype in JH deficient insects (Jindra et al., 2013, 2015a). In the red flour beetle Tribolium castaneum (Bitra et al., 2009) and D. melanogaster (Perrimon et al., 1996; Liu et al., 2009; Abdou et al., 2011; König et al., 2011; Jindra et al., 2015b; Wang et al., 2016), RNAi of or mutation in either Met or Tai causes nymphal lethality (Lozano et al., 2014; Smykal et al., 2014a). The different negative effects between Met- and Tai-depleted insects suggest that Tai may be an important component in 20E transduction cascade regulating insect development in holometabolan insects.

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In L. decemlineata, the main genes that are involved in ecdysteroidogenesis (Wan et al., 2013; Kong et al., 2014), JH biosynthesis (Fu et al., 2016; Li et al., 2016), 20E (Liu et al., 2014; Guo et al., 2015, 2016; Xu et al., 2018a,b, 2019a,c) and JH (Meng et al., 2018, 2019) signaling have been identified. Knockdown of LdMet reduces the body size and accelerates larval development (Meng et al., 2018, 2019), whereas 20E deficient (Kong et al., 2014) or repression of 20E signaling (knockdown of LdEcr, LdUSP, LdHR3, LdHR4, LdE74, LdE75, or LdFTZ-F1) (Liu et al., 2014; Guo et al., 2015; Shi et al., 2016; Xu et al., 2018a,b, 2019a,c) causes failure of larval-pupal-adult ecdisis and lethality. The distinct phenotypes between disturbance of JH and 20E signals will facilitate us to determine the roles of Tai during larval-pupal development. In the present paper, we first measured the transcription patterns of LdTai at whole development excursion. We then examined the defects after silence of LdTai using RNAi, and found that the silence repressed both JH and 20E signals and brought about lethality due to failure of larval-pupal ecdisis. Moreover, the process of tracheal ecdisis was uncompleted in L. decemlineata. Our results unveil, for the first time in a holometabolous insect species, that Tai mediates both JH and 20E signaling pathways during larval-pupal transition.

**MATERIALS AND METHODS**

**Insect Rearing**

The L. decemlineata beetles were kept in an insectary according to a previously described method (Shi et al., 2013), with potato foliage at the vegetative growth or young tuber stages in order to assure sufficient nutrition. At this feeding protocol, the larvae progressed through four distinct instars, with approximate periods of the first-, second-, third-, and fourth-instar stages of 2, 2, 2, and 4 days, respectively. Upon reaching full size, the fourth larval instars stops feeding, dropped to the ground, burrowed to the soil and entered the prepupal stage. The prepupae spent an approximately 3 days to pupate. The pupae lasted about 5 days and the adults emerged.

**Preparation of dsRNAs**

Specific primers used to clone the fragments of dsRNAs derived from LdTai and enhanced green fluorescent protein (egfp) were listed in **Supplementary Table S1**. These dsRNAs were individually expressed using Escherichia coli HT115 (DE3) competent cells lacking RNase III following an established method (Kong et al., 2014). Individual colonies were inoculated, and grown until cultures reached an OD600 value of 1.0. The colonies were then induced to express dsRNA by addition of isopropyl β-D-1-thiogalactopyranoside to a final concentration of 0.1 mM. The expressed dsRNA was extracted and confirmed by electrophoresis on 1% agarose gel. Bacteria cells were centrifuged at 5000 × g for 10 min, and resuspended in an equal original culture volume of 0.05 M phosphate buffered saline (PBS, pH 7.4). The bacterial solutions (at a dsRNA concentration of about 0.5 μg/ml) were used for experiment.

**Bioassay**

20E (Sigma-Aldrich, United States) or JH (Sigma-Aldrich, United States) was dissolved in distilled water with added surfactant (T’ween 20, 1 g/L) to give stock solutions of 100 ng/ml. It was diluted ten folds with distilled water before bioassay.

Two independent bioassays were carried out as previously described (Kong et al., 2014) using newly ecdysed fourth-instar larvae. For each bioassay, seven treatments were set: PBS,
with a 10-fold logarithmic dilution of a cDNA mixture to generate by gel electrophoresis. The primer pair for each gene was tested in 2009), the generation of specific PCR products was confirmed by reverse transcriptase reactions, respectively. DNA and to check for primer-dimer or contamination in the included for each primer set to confirm the absence of genomic results (Shi et al., 2013). An RT negative control (without listed in the day 4 fourth-instar larvae. For analysis of the effects of Malpighian tubules, hemocytes, fat body, and epidermis of derived from the brain-corpora cardiaca-corpora allata complex, the day 4 fourth-instar larvae. Integument and tracheae were observed and photographed under a light microscope. Dissection and observation were performed from day 4 (continuously feeding dsRNA for 3 days) to day 15 after ec dysis to fourth-instar larvae at an interval of 2 days. For each treatment, 3–4 larvae were dissected at each time point. For extraction of 20E, JH and total RNA for gene expression analysis, a total of nine replicates were collected after continuously fed on treated foliage for 3 days.

Quantitative Determination of JH and 20E

Hemolymph was collected from the treated larvae. JH was extracted following the methods described previously (Zhou et al., 2013). Its content (ng per ml hemolymph) was quantified by liquid chromatography tandem mass spectrometry system (Cornette et al., 2008).

20E was extracted according to an ultrasonic-assisted extraction method (Liu et al., 2014), and its titer (ng per g body weight) was analyzed by a liquid chromatography tandem mass spectrometry-mass spectrometry (LC-MS/MS) system using a protocol the same as described (Zhou et al., 2011).

Data Analysis

We used SPSS for Windows (Chicago, IL, United States) for statistical analyses. The averages (± SE) were submitted to analysis of variance with the Tukey-Kramer test. Since no significant differences in phenotypes between dsRNAs targeting two different regions of LdTai gene (dsTai-1 and dsTai-2) were found, we used the results from dsTai-1 (hereafter referred as dsTai) for detailed analyses.

RESULTS

Identification of LdTai

By mining the L. decemlineata transcriptome and genome data, five LdTai transcripts were found (XM_023164758.1, XM_023164759.1, XM_023164764.1, XM_023164763.1, and XM_023164765.1). LdTai gene contains 14 exons. E11, E12, and E13 are alternatively spliced to form the five isoforms. LdTai-A (XM_023164758.1) includes all the 14 exons, whereas LdTai-B (XM_023164763.1), LdTai-C (XM_023164759.1), LdTai-D (XM_023164764.1) and LdTai-E (XM_023164765.1), respectively, lack E11, E13, E11 and E13, and E12 and E13 (Figure 1A).

Evolutionary relationship of Tai-like proteins derived from 13 species was evaluated. An unrooted phylogenetic tree revealed that Tai-like proteins formed two clades: one from insects, and the other from Arachnida and Crustacea. In insect group, two from Coleopterans, two from Hymenopterans, two from Diptera and two from Lepidopterans, respectively, clustered together with 100% of bootstrap support. Obviously, LdTai-A (XM_023164758.1) belonged to Coleopteran cluster (Figure 1B).

Taiaman contains four major domains: a bHLH domain, a PAS domain, LxxLL motifs and domain containing several polyglutamine stretches (PolyQ) (Wang et al., 2016). The bHLH, template concentration], which was used to calculate the primer pair efficiency. All primer pairs amplified a single PCR product with the expected sizes, showed a slope less than −3.0, and exhibited efficiency values ranging from 2.4 to 2.7. Data were analyzed by the $2^{-\Delta\Delta CT}$ method, using the geometric mean of the four internal control genes for normalization. Relative transcripts are the ratios of relative copy numbers in treated individuals to control (CK), which are set as 1.
FIGURE 1 | Scheme of the LdTai isoforms from Leptinotarsa decemlineata (A) and phylogenetic analysis of Taiman-like proteins of representative Arthropoda species (B). (A) The constitutive exons are shown as boxes. E11, E12 and E13 are alternatively spliced in the isoforms. Black lines point to the region where dsRNA is designed to knock down all LdTai isoforms. Blue line indicates the locations used in qRT-PCR. (B) The Taiman-like proteins are from two Coleopterans Leptinotarsa decemlineata (Lde) and Tribolium castaneum (Tca), two Hymenopterans Apis mellifera (Ame) and Camponotus floridanus (Cfl), two Dipterans Drosophila melanogaster (Dme) and Aedes aegypti (Aae), two Lepidopterans Bombyx mori (Bmo) and Danaus plexippus (Dpl), an Orthopteran Locusta migratoria (Lmi), a Blattodean Blattella germanica (Bge), a Hemipteran Acyrthosiphon pisum (Api), an Arachnida Parasteatoda tepidariorum (Pte) and a Crustacea Daphnia pulex (Dpu). The tree is constructed using the neighbor-joining method based on the full-length protein sequence alignments. Bootstrap analyses of 1000 replications are carried out and bootstrap values > 50% are shown on the tree.

PAS-A, and PAS-B domains are conserved among insect species (Supplementary Figure S1).

Expression Pattern of LdTai

In the present paper, we designed a pair of primers in E7 to measure the expression of all splicing isoforms (hereafter LdTai) by qRT-PCR (Figure 1A). The temporal expression pattern analysis revealed that LdTai mRNA was detectable throughout all larval stages. Within the first, second and third larval instars, the expression levels were higher just before and right after the molt than those in the intermediate instar. In the fourth larval instar, the level of LdTai gradually rose and a peak occurred 112 h after ecdysis (Figure 2A).

The tissue expression profiles of LdTai were also determined. LdTai was widely expressed in the brain-corpora cardiaca-corpora allata complex, VG, muscle, trachea, foregut, midgut, ileum, rectum, MT, hemocytes, FB, and epidermis of the day 4 fourth-instar larvae. Its levels were higher in the cardiaca-corpora allata complex, VG, ileum and MT, moderate in the trachea, foregut, midgut, rectum, FB and epidermis, and low in the muscle and hemocytes (Figure 2B).

Effect of dsTai, Hormone and Their Combination on Larval Performance

To dissect the physiological roles of LdTai, we dietarily introduced each of the two dsRNAs targeting different regions (Figure 1A) of the LdTai mRNA (dsTai-1 and dsTai-2) into newly molted fourth-instar larvae, and observed the impacts on larval performance. As no significant differences between dsTai-1 and dsTai-2 were found, we used the results from dsTai-1 (hereafter referred as dsTai) for detailed analyses (Figure 3).
After the fourth larval instars continuously exposed to dsTai-immersed foliage for 3 days, LdTai mRNA abundance in the treated larvae significantly decreased, comparing with PBS- and dsegfp-exposed larvae (Figures 3A,E). As a result, the average weights of the fully grown larvae were reduced (Figures 3B,F), and the developing periods of the larvae (from the initiation of the bioassay to the occurrence of soil-digging behavior) were shortened (Figures 3C,G). Moreover, all the dsTai-exposed larvae did not normally pupate (Figures 3D,H, 4D). While the PBS- and dsegfp-exposed larvae became prepupae, pupae and adults 5, 8, and 13 days after initiation of bioassay, all LdTai RNAi larvae showed arrested development (Figure 3I). Eight days after initiation of the experiment, these LdTai RNAi beetles were still wrapped in the old larval cuticles (Figure 3J). After removal of the apolysed larval cuticle, the LdTai RNAi prepupae possessed pupal prothorax, mesothorax and metathorax (Figure 3K). Thirteen days after initiation of the experiment, the LdTai depleted prepupae were somewhat withered, dried and darkened (Figure 3K), and finally died. After PBS- and dsegfp-fed beetles emerged to adults, the normal tracheae were formed (Figure 3L). In LdTai knockdown larvae, the old tracheal linings still remained in tracheal trunks, some tracheae became brown (Figure 3M).

Ingestion of JH or 20E by the PBS-fed fourth-instar larvae activated the expression of LdTai (Figures 3A,E). JH ingestion increased the average weight of the fully grown larvae and lengthened the developing period (Figures 3B,C), whereas 20E feeding reduced the average weight of the fully grown larvae and shortened the developing period of the PBS-exposed larvae (Figures 3D,H, 4D). Ingestion of either JH or 20E by the LdTai RNAi larvae did not rescue the decreased expression level of LdTai (Figures 3A, 4E), the lowered average weight of the fully grown larvae (Figures 3B,F), the shortened developing period (Figures 3C,G), and the reduced pupation rate (Figures 3D,H).

Knockdown of LdTai Disturbs Hormonal Signals, Non-rescuable by JH or 20E

LdIDI and LdJHAMT are two biosynthesis genes that encode isopentenyl-diphosphate isomerase and JH acid methyltransferase (Fu et al., 2016; Li et al., 2016), and LdKr-h1 (Krüppel homolog 1) and LdHairy are two JH signaling genes (Meng et al., 2018). Knockdown of LdTai enhanced JH biosynthesis but reduced JH signaling. Ingestion of JH by PBS-fed larvae decreased the expression levels of LdIDI and LdJHAMT, raised the JH titer and intensified the expression of LdKr-h1 and LdHairy. In contrast, feeding of 20E by PBS-exposed larvae decreased both JH biosynthesis and JH signaling. However, the reduction of JH signal in the LdTai RNAi larvae was non-rescuable by either JH or 20E (Figure 4).

According to the expression peaks (Zhu et al., 2015; Shi et al., 2017), the expression levels of PTTH gene (LdPTTH),
Ingestion of dsTai by the final instar larvae affects larval performance in *Leptinotarsa decemlineata*. The newly ecdysed final instar larvae had ingested PBS (CK)-, ds*egfp*- , JH-, dsTai- and dsTai+JH-dipped leaves, or PBS (CK)-, ds*egfp*- , 20E-, dsTai- and dsTai+20E-immersed foliage for 3 days, and untreated foliage for several days if necessary. The expression levels of *LdTai* were tested. Relative transcripts are the ratios of relative copy numbers in treated individuals to PBS-fed controls (CK), which is set as 1. The larvae were weighed at an interval of 12 h after treatment to evaluate the maximum weight. Developing periods were calculated from initiation of bioassay to occurrence of soil-digging behavior. The pupation rates were recorded during a 4-week trial period. The bars represent values ($\pm$ SE). Different letters indicate significant difference at *P*-value $< 0.05$. The blank controls and ds*egfp*-exposed larvae became prepupae, pupae and adults 5, 7, and 12 days after experiment. Eight days after initiation of the experiment, these *LdTai* RNAi beetle were still wrapped in the old larval cuticles. After removal of the apolysed larval cuticle, the *LdTai* RNAi prepupae possess pupal prothorax (PT), mesothorax (MST) and metathorax (MTT). Thirteen days after initiation of the experiment, the *LdTai* depleted prepupae are somewhat withered, dried and darkened. The normal tracheae were formed after PBS- and *ds*egfp-*fed beetles emerged to adults. In contrast, the old tracheal linings still remained in tracheal trunks, some tracheae became brown in *LdTai* knockdown larvae.

Figure 3 | Ingestion of dsTai by the final instar larvae affects larval performance in *Leptinotarsa decemlineata*. The newly ecdysed final instar larvae had ingested PBS (CK)-, ds*egfp*- , JH-, dsTai- and dsTai+JH-dipped leaves, or PBS (CK)-, ds*egfp*- , 20E-, dsTai- and dsTai+20E-immersed foliage for 3 days, and untreated foliage for several days if necessary. The expression levels of *LdTai* were tested. Relative transcripts are the ratios of relative copy numbers in treated individuals to PBS-fed controls (CK), which is set as 1. The larvae were weighed at an interval of 12 h after treatment to evaluate the maximum weight. Developing periods were calculated from initiation of bioassay to occurrence of soil-digging behavior. The pupation rates were recorded during a 4-week trial period. The bars represent values ($\pm$ SE). Different letters indicate significant difference at *P*-value $< 0.05$. The blank controls and ds*egfp*-exposed larvae became prepupae, pupae and adults 5, 7, and 12 days after experiment. Eight days after initiation of the experiment, these *LdTai* RNAi beetle were still wrapped in the old larval cuticles. After removal of the apolysed larval cuticle, the *LdTai* RNAi prepupae possess pupal prothorax (PT), mesothorax (MST) and metathorax (MTT). Thirteen days after initiation of the experiment, the *LdTai* depleted prepupae are somewhat withered, dried and darkened. The normal tracheae were formed after PBS- and *ds*egfp-*fed beetles emerged to adults. In contrast, the old tracheal linings still remained in tracheal trunks, some tracheae became brown in *LdTai* knockdown larvae.

Receptor tyrosine kinase gene *Torso* (*LdTorsa*) and a MAPK gene (*LdRas*) were measured after the animals have ingested dsRNA for 3 days. The transcript levels of the three genes were significantly raised in the specimens having ingested dsTai or dsTai+JH (Figures 5A–C), or dsTai or dsTai+20E (Figures 5G–I), whereas their mRNA levels were significantly reduced in the larvae having fed on JH but upregulated in the larvae having fed on 20E, compared with those in the control specimens (Figures 5A–C,G–I).

Influence on PTTH signaling in treated larvae may change ecdysteroidogenesis. Therefore, we tested the expression levels of two ecdysteroidogenesis genes *Ldphm* and *Lddib* (Wan et al., 2013; Kong et al., 2014) and measured 20E titers in the treated larvae. As expected, ecdysteroidogenesis was intensified in the samples having ingested dsTai or dsTai+JH (Figures 5D–F), or dsTai or dsTai+20E (Figures 5J–L).

Subsequently, we determined the expression levels of 20E signaling transcripts *LdEcRA* (Figures 6A,I), *LdEcRB1*...
Deletion of LdTai disturbs JH signal in Leptinotarsa decemlineata. The newly ecdysed final instar larvae had ingested PBS (CK)-, ds egfp-, JH-, ds Tai- and ds Tai+JH-dipped leaves, or PBS (CK)-, ds egfp-, 20E-, ds Tai- and ds Tai+20E-immersed foliage for 3 days. The expression levels of two JH biosynthesis genes LdIDI (A,G) and LdJHAMT (B,H), three JH signaling genes LdMet (D,J), LdKr-h1 (E,K) and LdHairy (F,L) were measured using qRT-PCR. JH titer was tested (C,I). The bars represent average values (± SE). Different letters indicate significant difference at P-value < 0.05.

**DISCUSSION**

Based on findings obtained from RNAi and hormone rescuing experiments, we established in the present paper that Tai was critical for the regulation of metamorphosis by mediating both JH and 20E signals in L. decemlineata.

**LdTai Mediates JH Signaling**

In L. decemlineata, the expression levels of LdTai were higher right after the molt than those in the intermediate instar within the first, second and third larval instars (Figure 2A). Comparably, within each instar the JH titers appear to rise shortly before or right after the molt, and then drop sharply in L. decemlineata (Meng et al., 2015) and the drywood termite Cryptotermes secundus (Korb et al., 2012). It appears that the mRNA levels of LdTai are positively correlated with circulating JH titers in L. decemlineata. Moreover, JH ingestion enhanced the transcription of LdTai in L. decemlineata (Figure 3A), as well as in B. germanica (Lozano et al., 2014) and B. dorsalis (Liu et al., 2017). Upregulation of Tai transcription in response to JH in aforementioned insect species indicates that Tai is the functional receptor of JH.
Our findings in this study revealed that knockdown of Tai inhibited larval growth and shortened larval developing period in *L. decemlineata* (Figures 3B,C,F,G). Similar phenotypes have been documented in *T. castaneum* Tai RNAi larvae (Bitra et al., 2009). Moreover, we found that dietary supplement with JH failed to rescue the lowered average weight and the shortened developing period in *LdTai* RNAi larvae, even though JH ingestion increased the average weight and lengthened the developing period in control *L. decemlineata* larvae. In agreement with these results, our previous results disclose that knockdown of *LdMet* reduces body size, the reduction cannot be restored by JH in *LdMet* RNAi larvae (Meng et al., 2018). The similar regulation of Met (Meng et al., 2018) and Tai (this study) on larval growth indicates that the two bHLH-PAS transcription factors form a heterodimer to mediate JH signaling.

We directly analyzed the influences of *LdTai* silencing on JH signaling in the present paper, and found that silencing *LdTai* decreased the expression of *Met*. It is suggested that Tai may mediate JH to trigger its receptor expression. Moreover, RNAi *LdTai* repressed JH signal. The repression was non-rescuable by JH in the *LdTai* RNAi larvae (Figure 4). Similarly, RNAi of Tai significantly reduced the mRNA levels of *Met*, *Kr-h1* and Broad complex (*BrC*) in *B. germanica* (Lozano et al., 2014), and *Kr-h1* in

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**FIGURE 5** | Silence of *LdTai* affects ecdysosteroneogenesis in *Leptinotarsa decemlineata*. The newly ecdysed final instar larvae had ingested PBS (CK)-, ds*egfp*-, JH-, ds*Tai*- and ds*Tai*+JH-dipped leaves, or PBS (CK)-, ds*egfp*-, 20E-, ds*Tai* and ds*Tai*+20E-immersed foliage for 3 days. The mRNA levels of *LdPTTH* (A,G), *LdToro* (B,H), *LdRas* (C,I), two Halloween genes *Ldphm* (D,J) and *Lddib* (E,K) were tested using qRT-PCR. 20E titer was determined (F,L). The bars represent average values (± SE). Different letters indicate significant difference at *P*-value < 0.05.
**FIGURE 6** Knockdown of *LdTai* disrupts 20E signaling in *Leptinotarsa decemlineata*. The newly ecdysed final instar larvae had ingested PBS (CK)-, ds*egfp*-, JH-, ds*Tai*- and ds*Tai*+JH-dipped leaves, or PBS (CK)-, ds*egfp*-, 20E-, ds*Tai*- and ds*Tai*+20E-immersed foliage for 3 days. The mRNA levels of eight 20E signaling transcripts *LdEcRA* (A, I), *LdEcRB1* (B, J), *LdUSP* (C, K), *LdE74* (D, L), *LdE75* (E, M), *LdHR3* (F, N), *LdHR4* (G, O) and *LdFTZ-F1* (H, P) were tested using qRT-PCR. The bars represent average values (± SE). Different letters indicate significant difference at *P*-value < 0.05.

**P. apterus** (Smykal et al., 2014a). This result again supports that Tai mediates JH signaling.

Our previous results show that knockdown of *LdMet* enhances the transcription of *LdPTTH*. As a result, ecdysteroidogenesis is activated (Meng et al., 2018, 2019). In the present paper, we found that silence of *LdTai* also raised the expression levels of three PTTH signaling genes. As a result, ecdysteroidogenesis was intensified in *LdTai* RNAi larvae (Figures 5, 6).

All these findings in this study support that Tai mediates JH signaling in *L. decemlineata*.

**LdTai Transduces 20E Signaling**

The high *LdTai* levels just before the molt within young larval instars and the peak at 112 h after third-fourth instar ecdysis (Figure 2A) are positively correlated with circulating 20E titers in *L. decemlineata* (Smagghe et al., 1995). Moreover, ingestion of 20E stimulated the transcription of *LdTai* (Figure 3E). It can accordingly be postulated that Tai is the transducer of 20E.

In this study, we found that all *LdTai* RNAi larvae were wrapped in the old larval exuviae in *L. decemlineata*, with abnormal pupation and uncompleted trachea ecdysis (Figures 3H–M). Likewise, some Tai mutants in *D. melanogaster* are lethal at larval stages (Perrimon et al., 1996; König et al., 2011; Wang et al., 2016). In *T. castaneum*, metamorphosis fails and all beetles eventually die when Tai is depleted at penultimate and final instar larval stages (Bitra et al., 2009). In hemimetabolous insects *B. germanica* (Lozano et al., 2014) and *P. apterus* (Smykal et al., 2014a), removal of Tai disrupts nymphal ecdysis, and causes 100% mortality. The pharate animals of *P. apterus* undergo apolysis but are unable to shed the old cuticle (Smykal et al., 2014a). Accordingly, disrupted ecdysis in Tai RNAi larvae reflects that Tai may transduce 20E message in *L. decemlineata* (this study), as well as other holometabolous and hemimetabolous insect species (Perrimon et al., 1996; Bitra et al., 2009; König et al., 2011; Lozano et al., 2014; Smykal et al., 2014a; Wang et al., 2016).

Moreover, we unveiled that silencing *LdTai* intensified ecdysteroidogenesis (Figure 5) and the expression of EcR,
but declined 20E signaling. The decline could not restore by 20E ingestion in LdTai RNAi larvae (Figure 6). Since nuclear receptor coactivators are essential for steroid-dependent transactivation of genes in addition to the availability of the hormone and the expression of its receptor (Tetel, 2009), our results strongly support that Tai acts as a coactivator of ecdysone receptor components during larval-pupal metamorphosis of L. decemlineata. Similarly, RNAi of BgTai significantly reduced the mRNA levels of EcR, USP and E75A in B. germanica (Lozano et al., 2014). In A. aegypti, knockdown of Tai reduces the transcript levels of four 20E responsible genes E75A, E74B, vitellogenin, vitellogenic carboxypeptidase in the FB of adult females (Zhu et al., 2006; Liu et al., 2018). Thus, our data afford an indication that Tai mediates 20E pathway.

It is well known that a high level of 20E in Holometabolan insects such as M. sexta, D. melanogaster, Bombyx mori, and T. castaneum stimulates the production but prevents the release of ecdysis triggering hormone (ETH). On the other hand, the high 20E level sensitizes the central nervous system to ETH by expressing the ETH receptor (ETHR) (Adams and Žitnán, 1997; Žitnán et al., 2002, 2003, 2007; Kim et al., 2006; Arakane et al., 2008; Bai et al., 2011). However, acquisition of Inka cell competence to release ETH requires a decline of 20E level and timely FTZ-F1 expression a few hours prior to ecdysis (Žitnán et al., 2007). In the present paper, however, 20E titers were higher and expression levels of FTZ-F1 were lower in LdTai RNAi larvae, this may inhibit ETH release and impair pupation.

Tai May Be Involved in Other Signaling Pathways

In A. aegypti, using a yeast two-hybrid system, it has been found that Tai interacts with the transcription factor FTZ-F1. Therefore, AaTai may serve as a coactivator of FTZ-F1, and is named as FTZ-F1-interacting steroid receptor coactivator (FISC) (Zhu et al., 2006). Moreover, Tai promotes Drosophila adult intestinal stem cell proliferation as a coactivator of Yorkie in the Hippo pathway (Ren et al., 2010; Shaw et al., 2010; Wang et al., 2016). In B. germanica, knockdown of some Tai isoforms significantly reduces the expression of an insulin gene BgILP-1 (Lozano et al., 2014), suggesting that Tai may be involved in the insulin signaling pathway. Our results in the present paper do not exclude that Tai may act as a player in other signaling pathways.

CONCLUSION

Our results indicate that Tai mediates both JH and 20E signaling pathways during larval-pupal-adult transition in L. decemlineata, perhaps acting as transcription factor and transcription cofactor, respectively. Therefore, our results uncover a link between JH and 20E pathways in metamorphosis. Compatibly, a bHLH-PAS family protein aryl hydrocarbon receptor nuclear translocator protein (ARNT) can also function as either transcription factors or transcription cofactors, depending on the circumstances. Upon binding of environmental pollutants, a bHLH-PAS domain protein aryl hydrocarbon receptor (AHR) translocates from the cytoplasm to the nucleus to form the ARNT-AHR heterodimer. The dimer binds directly to the xenobiotic response element and activates expression of proteins involved in xenobiotic metabolism (Swanson et al., 1995). ARNT can also serve as a coactivator of estrogen receptor (ER)-dependent transcription, by recruitment to ER target gene promoters and physical interaction with ER (Brunnerberg et al., 2003).

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The research project was conducted on invertebrate species that are not subjected to any specific ethical issue and legislation.

AUTHOR CONTRIBUTIONS

Q-YX and G-QL designed the research. Q-YX, J-LD, and L-LM performed all of the experiments. W-CG provided the materials. W-CG and G-QL wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2019.00724/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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