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Molecular cloning and characterization of a juvenile hormone esterase gene from brown planthopper, *Nilaparvata lugens*

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ABSTRACT

Juvenile hormone (JH) plays key roles in the regulation of growth, development, diapause and reproduction in insects, and juvenile hormone esterase (JHE) plays an important role in regulating JH titers. We obtained a full-length cDNA encoding JHE in *Nilaparvata lugens* (NlJHE), the first JHE gene cloned from the hemipteran insects. The deduced protein sequence of *Nljhe* contains the five conserved motifs identified in JHEs of other insect species, including a consensus GQSAG motif that is required for the enzymatic activity of JHE proteins. *Nljhe* showed high amino acid similarities with *Athalia rosae* JHE (40%) and *Apis mellifera* JHE (39%). Recombinant NlJHE protein expressed in the baculovirus expression system hydrolyzed [^{3}H] JH III at high activity and yielded the specificity constants ($k_{cat}/K_M = 4.28 \times 10^6\ M^{-1}\ s^{-1}$) close to those of the validated JHEs from other insect species, indicating that *Nljhe* cDNA encodes a functional JH esterase. The *Nljhe* transcript was expressed mainly in the fat body and the expression level reached a peak at 48 h after ecdysis of the 5th instar nymphs. In the 5th instar, macropterous insects showed significantly higher *Nljhe* mRNA levels and JHE activities, but much lower JH III levels, than those detected in the brachypterous insects soon after ecdysis and at 48 h after ecdysis. These data suggest that NlJHE might play important roles in regulation of JH levels and wing form differentiation.

1. Introduction

Juvenile hormone (JH) plays major roles in the control of growth, development, metamorphosis, diapause and reproduction in insects (Riddiford et al., 2003). The regulation of JH titers is thus critical in the entire life of the insect. One key event is the clearing of JH that generally precedes the molt from the last larval stage to the pupal stage of holometabolous insects (Campbell et al., 2001). The very low JH titer at this time is generally achieved by the combined effect of reduced JH synthesis and scavenging by JH degrading enzymes (Roe and Venkatesh, 1990). Neurosecretory cells in the brain release allatotropic and allatostatic factors that regulate the synthesis and secretion of JH (Stay, 2000). Additionally, some important enzymes also play key roles in the synthesis and regulation of JH, such as JH methyl transferase and JH epoxidase (for a review, see Bellés et al., 2005). Degradation of JH in the tissues and in the hemolymph is another major way in which hormone titers are regulated, and JH esterase (JHE) has been thought to play key roles in the metabolism of JH (de Kort and Granger, 1996).

Some progress had been achieved in insect JHEs, such as JHE protein purification, JHE cDNAs cloning, recombinant JHE protein studies and JHE protein structure. JHE proteins have been purified from *Heliothis virescens* (Hanzlik et al., 1989), *Manduca sexta* (Venkatesh et al., 1990), *Trichoplusia ni* (Hanzlik and Hammock, 1987), *Drosophila melanogaster* (Campbell et al., 1998), *Tenebrio moliter* (Thomas et al., 2000) and *Bombyx mori* (Shiotsuki et al., 2000). Cloning of JHE cDNAs has been performed for two dipteran species *D. melanogaster* (Campbell et al., 2001) and *Aedes aegypti* (Bai et al., 2007), for two coleopteran species *T. moliter* (Hinton and Hammock, 2003b) and *Psacothea hilaris* (Munyiri and Ishikawa, 2007), and for several lepidopteran species *H. virescens* (Harshman et al., 1994), *T. ni* (Jones et al., 1994), *Choristoneura fumiferana* (Feng et al., 1999), *M. sexta* (Hinton and Hammock, 2001) and *B. mori* (Hirai et al., 2002). Recombinant JHEs have been expressed and biochemically characterized in *H. virescens* (Harshman et al., 1994), *C. fumiferana* (Feng et al., 1999), *B. mori* (Hirai et al., 2002), *M. sexta* (Hinton and Hammock, 2003a), *T. molitor* (Hinton and Hammock, 2003b) and *A. aegypti* (Bai et al., 2007). In order to perform detailed studies on the interaction between JHE and its substrates or inhibitors, the crystal structure of the JHE protein from *M. sexta* has

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been solved, which showed that JHE contains a long hydrophobic binding pocket with solvent-inaccessible catalytic triad (Wogulis et al., 2006). However, no information about JHE gene or protein from hemipteran insects has been obtained.

The brown planthopper (*Nilaparvata lugens*), a hemipteran insect, is a major rice pest in many parts of Asia. Wing polymorphism in this species is known to be a common and ecologically important trait. Although the genetic basis of wing polymorphism in insects generally is not well understood, it is presumed to be under polygenic control in this group (Denno and Roderick, 1990). It is assumed that these genes determine the level of juvenile hormone so that it is present above or below a certain threshold, leading to the production of short or long wing forms, respectively (Roff, 1986). In 4th and 5th instar nymphs of *N. lugens*, the brachypterous individuals (short wing) showed significantly higher JH titers and lower JHE activity than the macropterous (long wing) (Dai et al., 2001). Application of exogenous chemicals, such as JH, its agonists and antagonists, could regulate JH titers and JHE activity in *N. lugens*, which resulted in the production of short or long wing forms in different percentages (Ayoade et al., 1999). Here, as part of the study on the basic mechanism of wing polymorphism in *N. lugens*, we report the cloning of full-length cDNA encoding a functional JHE, tissue and developmental expression of the mRNA and its response to the exogenous JH application. Expression levels for both macropterous and brachypterous nymphs have been determined, together with JHE activity and JH titer.

2. Materials and methods

2.1. Experimental insects

Insects, *N. lugens*, were kept in laboratory cages at 25 ± 1 °C, humidity 70–80% and 16 h light/8 h dark photoperiod. The developmental stages were synchronized at each larval molt. Fat body, midgut, and other tissues were dissected from the 5th instar larvae in phosphate buffered saline (PBS) treated with 0.1% diethylpyrocarbonate (DEPC) and stored at −70 °C prior to use.

2.2. Amplification of a JHE cDNA fragment

Total RNA was isolated from 10 individuals of the 5th instar female using a Trizol kit (Invitrogen). Synthesis of first-strand cDNAs was carried out according to the reverse transcriptase XL (AMV) (TaKaRa) protocol with oligo dT_{18}. The first strand cDNA (1 μL) was used as a template for PCR. Degenerate primers, BP1 (ATHCCNTAYGCNAARCCNCC) and BP2 (GCNSCNCCNGCNYWY TGNCC), were designed from the conserved regions of insect JHEs, which were IPYAKPP (BP1) and GQSAGG(A)A (BP2) respectively. The components of PCR were PCR reaction buffer containing 0.1 mM dNTP, 5 μM each primer, and 1.0 unit of Ex-Taq DNA polymerase (Promega) in a total volume of 20 μL. Thermal cycling conditions were 95 °C for 5 min followed by 35 cycles of 94 °C for 45 s, 50 °C for 1 min and 72 °C for 1 min. The last cycle was followed by final extension at 72 °C for 10 min. The amplified product was separated onto agarose gel and purified using the Wizard PCR Preps DNA Purification System (Promega). Purified DNA was ligated into the pGEM-T easy vector (Promega) and several independent subclones were sequenced from both directions. The full-length cDNA was obtained by the rapid amplification of cDNA ends (RACE) according to the Smart Race kit (Clontech) protocol with gene-specific primers (GSPs) for 5′-RACE (5′-GSP1: CCAAATACCTGTAGATGCTAAGC; 5′-GSP2: CTGTCACATTCCTAGGTTCACTCC) and 3′-RACE (3′-GSP1: GTTGTTGTCAACTATAGACTCG; 3′-GSP2: GGAGACTCGAATATGGTGTCATTGG).

2.3. Quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR)

mRNA levels were measured by qRT-PCR using the One Step SYBR PrimeScript RT-PCR Kit (Takara). Total RNAs were treated by DNase I (Sigma). qRT-PCR was performed in a 25 μL total reaction volume containing 5 ng of total RNA, 0.5 μL primer mix containing 10 μM each of forward and reverse gene specific primers, 0.5 μL of Ex TaqTM HS (5 U/μL), 0.5 μL of PrimeScript RT Enzyme Mix, 12.5 μL of 2× One Step SYBR RT-PCR Buffer and 8.5 μL of H_2O. Two kinds of negative controls were set up: non-template reactions (replacing total RNA by H_2O) and minus reverse transcriptase controls (replacing PrimeScript RT Enzyme Mix by H_2O). qRT-PCR was done with the following cycling regime: initial incubation of 42 °C for 5 min and 95 °C for 10 s; 40 cycles of 95 °C for 5 s, 60 °C for 20 s and 72 °C for 15 s. Standard curves were obtained using a ten-fold serial dilution of pooled total RNAs from 20 individuals. β-Actin (EU179846) was used as an internal control, which had been recognized as a suitable normalization gene by Northern blotting test (data not shown). mRNA levels of *Nljhe* were quantified in relation to the expression of β-actin. The primer pair of each gene was designed to amplify about 200 bp PCR products, which were verified by nucleotide sequencing. In order to avoid genomic DNA contamination, the specific primers for JHE gene were designed to span an intron region (1154 bp). Only data that showed good efficiency (≥85%) and correlation coefficient (≥95%) were included in the analysis. Means and standard errors for each time point were obtained from the average of three independent sample sets. Gene specific primers for JHE and β-actin were listed as: JHE-F: AAGTAACTGGCAGATTCAACC; JHE-R: CTCGAATAGATGTGCTGCAGG; β-F: TGGACTTCGA GCAGGAAATGG; β-R: ACGTCGCACTTCATGATCGAG.

2.4. JH titer determination

Tang et al. (2001) used GC–MS to show that JH III was the main JH in *N. lugens*, so JH III levels were determined as the JH titers here according to the GC–MS method. The GC–MS system consisted of a Hewlett Packard HP6890 series II gas chromatograph and a mass selective detector (model 5973MS). 10 mg (about 6 individuals of the 5th instar female) *N. lugens* whole bodies were dried in Modulyod-230 Freeze Dryer (Thermo Electron) and were homogenized in 0.5 mL Hexane. The contents were centrifuged at 13,000 × *g* and 4 °C for 10 min. The supernatant was dried using a stream of N_2 gas and diluted to 25 μL in hexane. GC operating conditions: column HP-5, 25 m × 0.2 mm i.d., film thickness 0.2 μm; column temperature programmed from 120 °C (isothermal for 2 min) to 230 °C (15 °C/min); carrier gas helium, flow rate 40 mL/min; injector temperature 250 °C; volume injected 1 μL. The standard JH III was purchased from Sigma–Aldrich.

2.5. JHE activity determination

[10-^{3}H (N)]-JH III (17.5 Ci/mmol) was purchased from PerkinElmer Life Sciences. Juvenile hormone esterase assay was performed by the partition method of Hammock and Sparks (1977) as modified by Lefevere (1989). The substrate [^{3}H] JH III was dissolved in ethanol and stored at −20 °C prior to assay. 30 mg (about 18 individuals of the 5th instar female) *N. lugens* whole bodies were homogenized in 1 mL 0.1 mol/L phosphate buffer (PH 7.0) at 0 °C. The contents were centrifuged at 6000 × *g* and 4 °C for 10 min and the supernatant was collected for JHE solution. 100 μL aliquot of whole body supernatant were incubated with [^{3}H] JH III (130,000 DPM) and unlabeled JH III (final JH III concentration was 5×10^{-4} M) for 30 min at 30 °C. The reaction was terminated by

adding 50 μL of methanol: water: ammonium hydroxide solution (10:9:1, v/v/v). The solution was extracted with 250 μL isooctane. The lower phase (aqueous) and the upper phase (organic) were counted for radioactivity to estimate the degradation of JH III.

2.6. Baculovirus expression of recombinant NlJHE

Recombinant baculovirus expressing NlJHE was constructed using the BAC-TO-BAC™ baculovirus expression system (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instruction. *Nljhe* full-length cDNA was PCR amplified from pGEM-T-subunit plasmid using the gene-specific primers including SalI and XbaI specific regions. PCR product and the expression vector pFASTBAC were treated by SalI and XbaI, and purified by Wizard PCR Preps DNA Purification System (Promega, USA). Then the treated and purified PCR product was subcloned into vector pFASTBAC. The plasmid was transfected with DH10 Bac cells and the recombinant bacmid was then isolated. Sf9 cells were cultured in SF-900 SFM (Gibco BRL). The recombinant bacmid (300 ng) was transfected with 1×10^6 Sf9 cells and 6 μL of Cellfectin reagent. 3 days after the infection, the cell culture medium was collected and used to assay JHE activity as described above. Kinetic parameters

```
   1 GAAGTTTAGGTTTTTTAATCAGTTCTAGTCATGCAGTATTCTTAGTCTACAGTTTTTTACTAAACATTAACGGCCAAATTCTAAGTAGAC
  91 GAGTTGATGTTGGGAATGGCATCCGCTATCTGTGGGCCTAGAACCATGTGGTGTGTGGTGGCCGTGACGCTGGTATTGCTAGTGCTAGTG
                                                  M  W  C  V  V  A  V  T  L  V  L  L  V  L  V
 181 GCGCAGGTGTATGGTCTAGGAAAGTTCAAAGACCTCGATTTAGATGATGTAATCAATGCCAAAACGGTGATCAGATCTGGAATTGTGTAT
      A  Q  V  Y  G  L  G  K  F  K  D  L  D  L  D  D  V  I  N  A  K  T  V  I  R  S  G  I  V  Y
 271 GGAAACATATTCAAATTTGTTGGCGATGACATATTCCAGGCCTGGACCGACATTCCATATGCCAAACCTCCCACCGGTGACCAAAGATAT
      G  N  I  F  K  F  V  G  D  D  I  F  Q  A  W  T  D  I  P  Y  A  K  P  P  T  G  D  Q  R  Y
 361 AGGGAGGCGGATTCAGAAGTAACGTGGAGTGAACCTAGGAATGTGACAGCGAAGAAGCCTGAGCGATGTCTTCAATACAACTTTATATCA
      R  E  A  D  S  E  V  T  W  S  E  P  R  N  V  T  A  K  K  P  E  R  C  L  Q  Y  N  F  I  S
 451 GATAAATACAATCGAGTTGAGGGTACAGAAGAAGCATGTCTCATGCTTAGCATCTACAGGTATTTGGGGATGGTAAGGCCGCGATTGGGT
      D  K  Y  N  R  V  E  G  T  E  E  A  C  L  M  L  S  I  Y  R  Y  L  G  M  V  R  P  R  L  G
 541 CCGGTCATAGTGCTGCTGCATCCAGGCACCTACATGTACGACTTTCCGTTTGATCCTCGAAGGTTCGCTGCCAACCTCTGCTGCGATTTC
      P  V  I  V  L  L  H  P  G  T  Y  M  Y  D  F  P  F  D  P  R  R  F  A  A  N  L  C  C  D  F
 631 TTTGTCACTGTTGTTGTCAACTATAGACTCGCACATACGGGTTTCCTGTCAATGCATAAAAATGGCGTGAAAGCGAACCTAGGCCTATTA
      F  V  T  V  V  V  N  Y  R  L  A  H  T  G  F  L  S  M  H  K  N  G  V  K  A  N  L  G  L  L
 721 GACCAAGTGGCCGCCCTCAAGTGGGTTCAGCGGAACATCGAAGCATTCTATGGAGACTCGAATATGGTGTCATTGGTGGGTCAGAGTGCG
      D  Q  V  A  A  L  K  W  V  Q  R  N  I  E  A  F  Y  G  D  S  N  M  V  S  L  V  G  Q  S  A
 811 GGTGCTGCTGCTGTACAACTACATCTCCTGTCATCCATGTCCAGGGGCCTGTTCCGCAGTGCAGTCTCGTACAGCGGCTCAGCACTGGTG
      G  A  A  A  V  Q  L  H  L  L  S  S  M  S  R  G  L  F  R  S  A  V  S  Y  S  G  S  A  L  V
 901 CCGTGGGCGATCGGAGGCGATGTCGGTAAGAAGAGCTTGAACTTTGTCCGCCATAACCTCAACTGCACTTCGCTGGCAATTACGGAAATT
      P  W  A  I  G  G  D  V  G  K  K  S  L  N  F  V  R  H  N  L  N  C  T  S  L  A  I  T  E  I
 991 GCCGAATGTTTGCGTTCCATCTCTCTCAACGCATTGTTCAAAGAAGTAACTGGCAGATTCAACCATCCCTTGTTCCATGACTTCCAGAAC
      A  E  C  L  R  S  I  S  L  N  A  L  F  K  E  V  T  G  R  F  N  H  P  L  F  H  D  F  Q  N
1081 ATGCCGTTGAGCCCTCTGGGTCCTGTCGTGGATAACAACTTCCTTGATGATGACCCTCTGATCCTACTGAGGGCGGGAGCTGTCACCCAA
      M  P  L  S  P  L  G  P  V  V  D  N  N  F  L  D  D  D  P  L  I  L  L  R  A  G  A  V  T  Q
1171 ATGCCCTATCTCTTCTCCTTCACTGATTCGGAAGGCATACTTCCTGCAGCACATCTATTCGAGAAGGAAGTTCCTGAGACCAGAGTTGAG
      M  P  Y  L  F  S  F  T  D  S  E  G  I  L  P  A  A  H  L  F  E  K  E  V  P  E  T  R  V  E
1261 AATGAATGGAATACCTTGGCACCAACCATTCTCGACTACAACTACACAATGCACCATCTGTTGCATGAAAAGTTTGCTGAGCGTGCTCGC
      N  E  W  N  T  L  A  P  T  I  L  D  Y  N  Y  T  M  H  H  L  L  H  E  K  F  A  E  R  A  R
1351 AAGTTCTACTATCCGGATTACGAAAACGGTACTAAACCGCCCTTGTTCGACAATTATGGGCGTTTTATCAAGATGCTAACTGACCGTCTG
      K  F  Y  Y  P  D  Y  E  N  G  T  K  P  P  L  F  D  N  Y  G  R  F  I  K  M  L  T  D  R  L
1441 TACACAACGGGCATTGTTGAGGGGGCTCGGCTGCAGGGAGTTGCTACCAAAAATGATTCTGTCTATTTGTACAGATATTCCTATCAGGGT
      Y  T  T  G  I  V  E  G  A  R  L  Q  G  V  A  T  K  N  D  S  V  Y  L  Y  R  Y  S  Y  Q  G
1531 GAATTCAGTTTTTCTGATCAGATTTTTGGATCTCGCCAACTTTTTGGGCCAGCGCATGCAGATGACTTCATGACCTACATAGCCAATCAA
      E  F  S  F  S  D  Q  I  F  G  S  R  Q  L  F  G  P  A  H  A  D  D  F  M  T  Y  I  A  N  Q
1621 TTTCCTGTGCGAAACTTCGCAGATCGTGTCCAGAAACAAATGCTGACACATATTTTTCTGGGATTCATAGAATTTGGGTGGGCAGACCTA
      F  P  V  R  N  F  A  D  R  V  Q  K  Q  M  L  T  H  I  F  L  G  F  I  E  F  G  W  A  D  L
1711 CCGCAGGGGCATACGATCAATGATACGTTTGGTTTGCATTTCTTAGTGCCAATGGCGACGACAAAGCAATATTTCAAAGGAGCCTCACAT
      P  Q  G  H  T  I  N  D  T  F  G  L  H  F  L  V  P  M  A  T  T  K  Q  Y  F  K  G  A  S  H
1801 CCACAGCAAAAACGCAATGCACGCGGCTCTCGCATTGTATCATACAACCACTTACCTCCAAGTCACTTAGTGGCAGGCAGCAAAAAGCAC
      P  Q  Q  K  R  N  A  R  G  S  R  I  V  S  Y  N  H  L  P  P  S  H  L  V  A  G  S  K  K  H
1891 GAGTCGTACACCTAAAGCACTCGAATGACCTTGGGAAGGCCAAGTTTTGGCGTAGGGCTCCCATTTAGCATCCCACAATACGTTATCGGG
      E  S  Y  T  *
1981 ACCGCGGCCTCTATCCAGAAATCCTCAACGATGAAGCTTGGGATGACCATCCTCATATCGATGATGTTCTTAATGAACTCAGGGGTATCG
2071 AAGCACCAAAACATAATAAGCCAGAACCAAAAATCAGTTCCAGATTAGTAATGACTAATCAAATTTATAATCAAAATCTTTATGATCTGAA
2161 TATAAATACTTATGAGTGTTTTAATTTAAGTAAGATTTGAATTGAAATTGAATTGTATTTGTGTGGCACTCCTACACAAAATTGAGGAAA
2251 TAATAAATTCATGTTGTGTGATTTGAAAATAAATTAGGGTACATTTGAAAAAAAAAAAAAAAAAAAAA
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Fig. 1. Nucleotide and deduced amino acid sequence of *Nljhe*. The putative signal peptide is indicated by the thin line under the amino acid sequence. The positions of the primers used in the initial degenerate RT-PCR are shown by thin lines under the nucleotide sequence. The stop codon is indicated by an asterisk. Catalytic domains, including the GQSAG motif within which the catalytic serine is contained are indicated by thick lines under the amino acid sequence.

were calculated by hyperbolic regression analysis using the Hyper32 computer program (Easterby, 2008). When it was apparent that K_M exceeded the solubility limit of a substrate, the specificity constant (k_{cat}/K_M) was taken from the slope of the linear part of Michaelis–Menten kinetic plots.

2.7. JH III treatment

JH III was dissolved in methanol to make final concentration of 0.5, 2 and 8 ng/μL. The treatment method followed the micro-topical application technique reported by Nagata (1982) with some modification. Under carbon dioxide anesthesia, a droplet (0.1 μL) of JH III solution was applied topically to the abdominal sternites of 5th instar female (soon after ecdysis) with a hand microapplicator (Burkard Manufacturing Co. Ltd., Rickmansworth, UK). Controls used methanol alone. In 12, 24 and 48 h after the topical application of JH III, the insects were collected and JHE mRNA level were determined as the above description.

3. Results

3.1. Nljhe cDNA

RT-PCR and RACE techniques were used to clone the full-size *N. lugens* JHE (*Nljhe*) cDNA. Fig. 1 shows the full-length cDNA sequence together with the deduced amino acid sequence (Genbank accession number, EU380769). The sequence has an open reading frame (ORF) of 1770 bp and 589 deduced amino acids. All five catalytic motifs, known to be essential for the catalytic activity of JHE (Ward et al., 1992), are very well conserved in *Nljhe*. The deduced protein sequence of *Nljhe* shows 40–37% similarities to the hymenopteran *Athalia rosae* JHE, hymenopteran *Apis mellifera* JHE, coleopteran *P. hilaris* JHE and Dipteran *D. melanogaster* JHE (Fig. 2). *Nljhe* also shows more than 25% similarities with other insect JHEs, including *B. mori* (AY489292), *C. fumiferana* (AF153367), *Harmonia axyridis* (AB201554), *H. virescens* (AF037197), *M. sexta* (AF327882) and *T.*

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NlJHE: MWCVVAVTLVLLVLVAQVYGLGKFKDLDLDDVINAKTVIRSGIVYGNIFKFVGDDIFQAWTDIPYAKPPTGDQRY  75
ArJHE: ------MQLPRL-IYA-LIILLSGVALCLADESLPKVVTSLGIIEGYFKTSNAGRKYEAYEGIPYARPPVGEHRF  67
AmJHE: ----------MKL-LF--LVLLSSLVTFGWTLEDAPRVKTPLGAIKGYYKISGNGKQYEAYEGIPYALPPVGKFRF  63
PhJHE: --------MCTCKIIS-VKALLVLCN-AVFAKEDPIVETKYGIVEGKTAYSISGRPFYSFQGIPYAKPPLDNLRF  65
DmJHE: -----MLQLLLLGQL--LAGPGPFCAALATVDQLTVCPPSVGCLKGTNLQGYQSERFEAFMGIPYALPPIGDLRF  68
                                                *   *                 **** **    *

NlJHE: READSEVTWSEPRNVTAKKPERCLQYN-FISDKYNRVEGTEEACLMLSIYRYLGMVR-PR-----LG-PVIVLLH 142
ArJHE: KVPKSISSWTGTLMAKKYSSL-CLQYNHFPLDPNNRVEGSED-CLYLNVYAA--IKTQS--SKNDL-LPVIFYIH 135
AmJHE: KAPQKIPAWIGELSATKFGFP-CLQYTQLPVNPRDKIEGAED-CLYLNVYVP--ADRTP--SQS---LPVIFWIH 129
PhJHE: KAPVEPNKWPDIMKTKDNAPH-CLQKNYLFSNP--KVIGSED-CLYLNVYSP--KLRARRHARKSL-LPVMVFIH 133
DmJHE: SNPKVMPKLLGMYDASAPKMD-CIQKNYLLPTP--VVYGDED-CLYLNVYRP--EIR------KSA-LPVMVYIH 130
                          * *                 * *  ** *  *              **       *

NlJHE: PGTYNYDFPFDPRRFAANLCCDF--FVTVVVNYRLAHTGFLSMHKNGVKANLGLLDQVAALKWVQRNIEAFYGDS 215
ArJHE: -GGAFT-FG-SGIFYGSKYLLDN-DVIFVTINYRLGPLGFLSTEDETVPGNMGLKDQSMALRWVKDNILYFGGDP 206
AmJHE: -GGAFQ-FG-SGIPMGAKYLMDS-DVIFVTINYRLGILGFLSTEDEVVPGNMGLKDQSMALRWVSENIEWFGGNP 200
PhJHE: WGGFFTGFS-SSDYLGPEYIMDK-NVVLVTFNYRLGILGFFSTNDDAAPGNYGLKDQVAALKWVQSNIEYFGGDN 206
DmJHE: GGGFFGGSA-GPGVTGPEYFMDSGEVILVTNAYRLGPFGFLSTQDAVMSGNFGLKDQNLALRWVQRNIRFFGGDP 204
         *                 *       *   ***   ** *        * ** **  ** **  **  * *

NlJHE: NMVSLVGQSAGAAAVQLHLLSSMSRGLFRSAVSYSGSALVPWAIGGDVGKKSLNFVRHNL-NCTS---LAITEIA 286
ArJHE: EKITIFGQSAGGASVHYHYMSKLSAGLFRGGWSLSGCALECWPQTEGALEK--AKKLANIVGCPS-D--NVKTMV 276
AmJHE: KRITLIGLSAGGASVHYHYLSPLSAGLFQGGISISGTALNCWTQTENSLEK--AKQVGAFMGCP-TR--NVKEMI 270
PhJHE: EKVTIFGQSAGGASVNLHMFSPESKDLFHQGISQSGTSLALWAKPLSETQLILARTQATFVGCK--DLENTTILV 279
DmJHE: QRVTIFGQSAGGVAAHMHLLSPRSHGLFHRVISMSGTANVPFAIAEQPLEQ--ARLLAEFADVPDARNLSTVKLT 277
              * ***      *  *  *  **    * **

NlJHE: ECLRSISLNALFKEVTGRFNHPLFHDFQNMPLSPLGPVVDNN---FLDDDPLILLRAGAVTQMPYLFSFTDSEGI 358
ArJHE: KCLRSRPAHGIVQAVGN-FMPWLYNPYTPFGPVVEKGGSDGTT--FIDRSPIEIINSGDIMDVPWITGITSEEGL 348
AmJHE: RCLRYRPARAIVETLAN-FMRFYYNPFTPFGPVTEKVNNDSNSLPFIDRTPIEIINSGDVQDVPWVTGVTSEEGL 344
PhJHE: TCLRNKTAENLVESGDK-FKFFSIDPLNVYLPSIEQ-KTESNPNPFITKQPIDYIRSKEFYKVPWILGLVTDEGL 352
DmJHE: KALRRINATKLLNAGDG-LKYWDVDHMTNFRPVVEEG---LEVDAFLNAHPMDMLAQGMPTSIPLLLGTVPGEGA 348
         **                          *   *          *       **

NlJHE: LPAAHLFEKEVPETRVENEWNTLAPTILDYNYTMHHLLHEKFAERARKFYYPDYENGTKPPLFDNYGRFIKMLTD 433
ArJHE: YPAADFVGNEKLLEELRNNVETFAPHLLDFNVTIPKTDHAKVANLIKEHYLGS--SPIGKS---TVNRLVQMIGD 418
AmJHE: YPVAEFIAKPEALKLLDENVDLIAPYFLDYNYTIPKEKHVEVARLIRNYYFES--NKIDET---TLKHLIDVASD 414
PhJHE: IRAEAILRQPTTRTALNKDFNKLMPELIALSMSTPSSNISRIWSKIKEYYFKS-LHEINITEPRNMKALINLYSD 426
DmJHE: VRVVNILGNETLRQSFNLRFDELLQELLEFPASFSQDRREKMMDLLVEVYFQG-QHEVNEL---TVQGFMNLISD 419
                                                          *                     *

NlJHE: RLYTTGIVEGARLQGVATKNDSVYLYRYSYQGEFSFSDQIFGSRQL----FGPAHADDFMTYIANQFPVRNFADR 504
ArJHE: RLFVYDSEEAARLQARVTRSP-VRYYYFTYRGAHSLSEEMSGTTED----FGVSHADDLAYVIDVL-F-DSSTTQ 486
AmJHE: RFFITDGEKAARMQAKVNRQP-VWFYYYTYKGAHSISEIMSGTSNK----YGVCHADDAYMVVDTP-FLASTTTT 483
PhJHE: RSFSYSTYQAALFHNLKGHDD-IWFYNFNYTGQYSYGDVFAATKEDINYDWGVSHCDDLLYLFNSSALFPEFENN 500
DmJHE: RGFKQPLYNTIHKNVCHTPNP-VYLYSFNYQGPLSYASAY--TSANVTGKYGVVHCDDLLYLFRSPLLFPDFQRN 491
         *                          *   * *  *                 *  * **

NlJHE: VQKQNLTHIFLGFIEFGWADLPQGHTINDTFGLHFLVPMATTKQYFKGASHPQQKRNARGSRIVSYNHLPPSHLV 579
ArJHE: K-DRDMQKLLGNLWVSFATKGSPD-L-GIEWPKVSPT------SNAFPYLYIAG------PTEIEIKDTLEIGEK 546
AmJHE: N-DIKMQKVLIDFWVSFVNNGVPN-VNSVQWPRLNPN------EKSLHYLHIAG------PGKIQMDSSTNFGRE 544
PhJHE: N-DLIMIQTMTDLWTNFAIYGHPTPSKTVQWPKLD-NLSHGMLDKNIYYMNINGSYKDSNPPSLKMEKGFYKERF 573
DmJHE: STEAKVIHSFVDYFVHFAKFGKPRNSESLT-P-CSIEVLQSRPDGICDYHEFANAPDAYQGFEVHVASEFQTDRV 564

NlJHE: AGSKKHESYT---------- 589
ArJHE: KFWRSIDFRENSFNPNR--KSEL 567
AmJHE: DFWNSINFNENKLHTSDTLKEEL 567
PhJHE: KFWEDIPLVEN-IEELKQMHIEM 595
DmJHE: NLWSHI-LNEK---------- 574
```

Fig. 2. The alignment of amino acid sequence of NlJHE with the sequences of other insect JHEs: numbers on the right side of the alignment indicate the position of residues in the sequence of each protein. The five catalytic motifs which are conserved in insect JHEs are indicated by thick underlines. Identical amino acid residues are indicated by asterisks. NlJHE (*N. lugens*, EU380769), ArJHE (*A. rosae*, AB208650), AmJHE (*A. mellifera*, NM_001011563), PhJHE (*P. hilaris*, AB259898) and DmJHE (*D. melanogaster*, NM_079034) are used in the alignment.

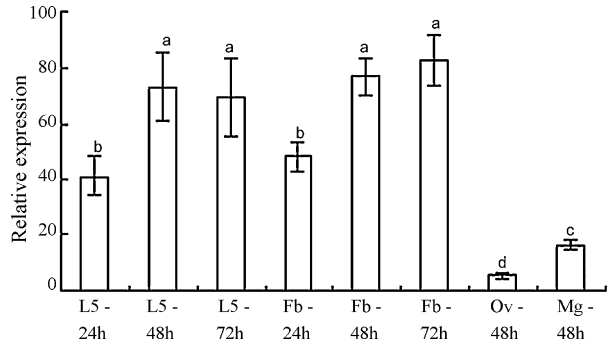

Fig. 3. Tissue and developmental expression of *Nljhe* mRNA detected by the quantitative real-time RT-PCR. L5, whole body of 5th instar macropterous female nymphs; Fb, fat body; Ov, ovary; Mg, midgut. L5-24 h, L5-48 h and L5-72 h showed the samples were from the whole body of 5th instar macropterous female nymphs at 24, 48 and 72 h after the molting, which showed the similar indication for Fb-24 h, Fb-48 h and Fb-72 h. The data represent mean values ± S.E. of at least three repeats, normalized relative to β-actin transcript levels. Different lowercase letters above the columns indicate significant differences at $P < 0.05$ level (t test).

molitor (AF448479), but only 15% similarity and 5% identity to *L. decemlineata* (AF035423).

3.2. Tissue and developmental expression of the Nljhe mRNA

The *Nljhe* mRNA levels of whole body of 5th instar macropterous nymphs (24, 48 and 72 h after the molting) and of different tissues (fat body, midgut and ovary) were determined by qRT-PCR (Fig. 3). The level was lowest at 24 h after ecdysis and was high at 48 and 72 h in the samples from the whole bodies. High levels of the transcript were detected in the fat body, but only low levels in the midgut and negligible levels in the ovary. The levels in the fat body had the same developmental expression pattern with the whole body samples. This result showed the whole body expression level could represent that in the fat body and in the rest of the work whole body expression levels were determined instead of fat body.

3.3. Expression of NlJHE protein by baculovirus expression system

To confirm that the cDNA isolated here encodes a functional JHE, we expressed a recombinant NlJHE protein using the baculovirus system and assayed this protein for enzymatic activity against [^{3}H] JH III. Cell culture medium from recombinant virus infected cells hydrolyzed [^{3}H] JH III with an activity of 0.64 ± 0.05 pmol/mg protein/min. Cell culture medium from uninfected samples degraded [^{3}H] JH III at only 0.03 ± 0.01 pmol/mg protein/min, which was significantly lower than that of virus infected cells. Kinetic parameters, K_M (514.4 ± 46.7 nM) and k_{cat} (2.2 ± 0.3 s^{-1}), towards JH III were calculated by hyperbolic regression analysis; the specificity constant (k_{cat}/K_M) calculated from the linear part of Michaelis–Menten plots was 4.28×10^6 M^{-1} s^{-1}.

3.4. Different expression levels in 5th instar macropterous and brachypterous nymphs

Different levels of NlJHE expression were observed in 5th instar macropterous and brachypterous nymphs (Fig. 4B). In 5th instar macropterous female nymphs, two peaks of *Nljhe* mRNA level were detected at 12 h and at 48–72 h after ecdysis, and the lowest level was observed at 24 h. After 48 h, *Nljhe* mRNA levels remained high until the time of emergence. In 5th instar brachypterous female nymphs, a peak of *Nljhe* mRNA was detected at 12 h, but levels of this transcript then decreased gradually after 48 h. The differences in mRNA levels between macropterous and brachypterous female nymphs were statistically significant ($P < 0.05$) at 0 h (soon after ecdysis of 5th instar nymphs) and at times after 48 h.

The JHE activities of 5th instar nymphs (whole bodies) were also determined (Fig. 4A). The patterns of JHE activity in 5th instar nymphs of both macropterous and brachypterous insects were similar to those of *Nljhe* mRNA levels. Peak JHE activities occurred at 12 and 48–72 h in 5th instar macropterous nymphs. The peak activity was also detected at 12 h and the lowest at 24 h in 5th instar brachypterous nymphs. Significant differences ($P < 0.05$) were observed between the JHE activities of 5th instar macropterous and brachypterous nymphs at 0 h (soon after ecdysis of 5th instar nymphs) and at times after 48 h.

In order to evaluate the possible relationship between the JH titer and *Nljhe* mRNA level or JHE activity in 5th instar nymphs, JH titers were also determined (Fig. 4C). In 5th instar macropterous nymphs, a peak of JH titer was found at 24 h, corresponding to the lowest level of *Nljhe* mRNA and the lowest JHE activity. However, the peak of JH titer in 5th instar brachypterous nymphs occurred at 84 h, the last time point measured in 5th instar, in which *Nljhe* mRNA level and JHE activity were the lowest. JH titers of 5th instar brachypterous nymphs were significantly higher than those of 5th instar macropterous nymphs at 0 h and at all time points in the range of 48–84 h. These were also the time points at which *Nljhe* mRNA levels and JHE activities of 5th instar brachypterous nymphs were significantly lower than those of 5th instar macropterous nymphs.

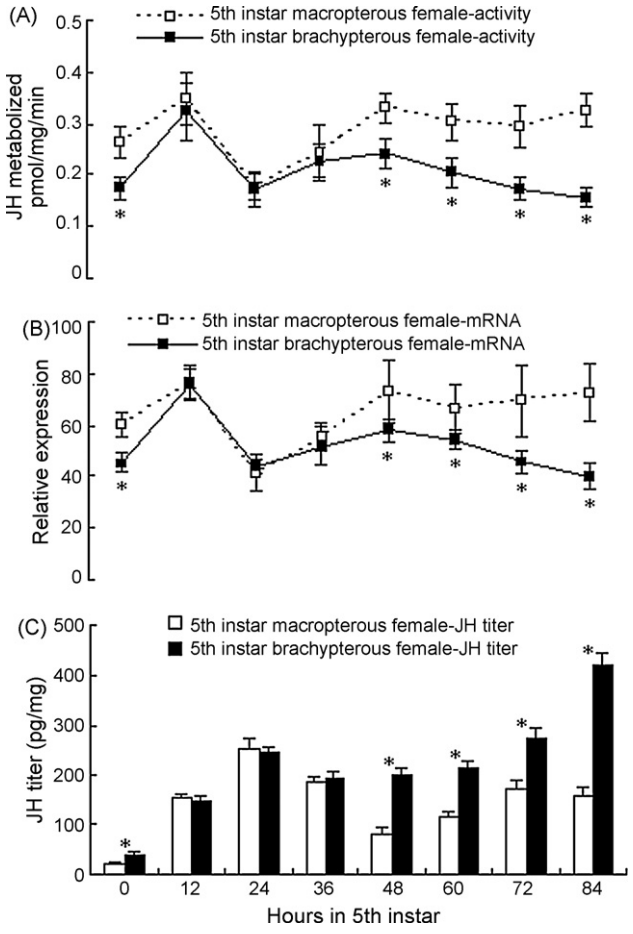

Fig. 4. The dynamics of the relative *Nljhe* transcript levels (B), JHE activities (A) and JH titers (C) in 5th instar macropterous and brachypterous female nymphs. Values are plotted as means ± S.E. of at least three repeats. * Indicates the significant differences at $P < 0.05$ level (t test).

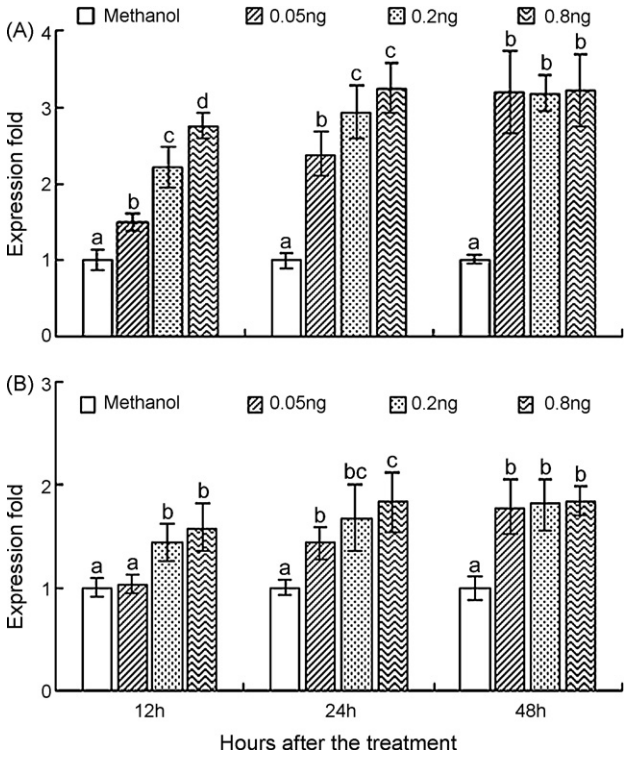

Fig. 5. *Nljhe* mRNA expression induced by JH III in 5th instar macropterous (A) and brachypterous (B) females. The transcript levels were normalized to β-actin transcript levels first, and in each group (12, 24 and 48 h) the lowest expression value (methanol treated samples) was converted to 1. The data represent mean values ± S.E. of at least three repeats. Different lowercase letters above the columns in each group indicate significant differences at $P < 0.05$ level (t test).

3.5. Response of the NlJHE expression to the application of JH III

In order to evaluate the effect of the exogenous JH on *Nljhe* mRNA levels, three different concentrations of JH III were applied to 5th instar nymphs (soon after ecdysis) with methanol as the control. Fig. 5 shows the application of JH III induced expression of *Nljhe* mRNA. The level of *Nljhe* mRNA increased in a concentration- and time-dependent manner in both 5th instar macropterous and brachypterous nymphs. By 48 h, treatments with all three concentrations of JH had caused similar induction of *Nljhe* mRNA expression, which appeared to reach a maximum. However, the applied JH III showed smaller induction in 5th instar brachypterous nymphs (maximum induction fold is 1.85 ± 0.14; Fig. 5B) than in 5th instar macropterous nymphs (maximum induction fold is 3.22 ± 0.46; Fig. 5A).

4. Discussion

Using RT-PCR and RACE techniques, a full-length cDNA coding a putative JHE (NlJHE) was cloned from *N. lugens*. The deduced amino acid sequence of *Nljhe* cDNA contains the five motifs conserved in JHEs of other species, and includes the catalytic triad members, Ser-His-Glu, that are reported as essential for the catalytic activity of JHE (Ward et al., 1992). The catalytic serine residue in *Nljhe* cDNA is situated within a consensus GQSAG motif that is unique among the JHE proteins and only one such motif was present in *Nljhe* cDNA. The deduced protein sequence shows a high degree of similarity to other insect JHEs, with 40–37% similarities to JHEs from *A. rosae*, *A. mellifera*, *P. hilaris* and *D. melanogaster*. These results suggest that *Nljhe* cDNA encodes a JHE or JHE-like protein.

The consensus GQSAG motif may be not enough to identify whether a candidate gene really encodes a protein with JHE activity, as has been found in *A. mellifera*, *D. melanogaster* and *A. aegypti* (Claudianos et al., 2006; Crone et al., 2007b; Bai et al., 2007). In *D. melanogaster* and *A. aegypti*, only one JHE gene was identified by expression *in vitro* from four and three JHE-like genes containing the GQSAG active site motif (Crone et al., 2007b; Bai et al., 2007). In *A. aegypti*, the correlation between the mRNA expression profiles and the developmental expression profiles of JHE enzyme activity was also thought as the indirect evidence for a functional JHE (Bai et al., 2007). Although only one functional JHE was identified in *D. melanogaster* (i.e. a protein that displayed physiologically significant JH III degradation), all three (JHEdup, EST-R and EST-Q) of the other expressed esterases also had measurable JH III esterase activity (Crone et al., 2007b). These three proteins (HEdup, EST-R and EST-Q) are encoded by genes found in three distinct clades of insect carboxylesterases (Oakeshott et al., 2005), implying that JH III hydrolyzing activity may be widespread throughout the insect carboxylesterase family (Crone et al., 2007b). In *A. mellifera*, no one protein possesses the active site motif GQSAG, even in the putative JHE GB18660, in which GHSAG exists instead of GQSAG (Claudianos et al., 2006). Recently, another JHE-like gene GB15327 (*AmJHE* in Fig. 2, NM_001011563) has been identified as the specific JH metabolizing enzyme in *A. mellifera*; the evidence for this included an appropriate pattern of developmental expression correlated with JH titers, which was strongly supported by gene silencing data that showed down regulation of GB15327 led to an increase in JH titer (Mackert et al., 2008). GB15327 (*AmJHE*) also included a GLSAG motif instead of GQSAG. These results show *in vitro* JH activity alone cannot serve as a diagnostic for physiologically relevant JH esterases. Identification of JH-selective esterases requires characterization of JH hydrolytic activity to ensure that the affinity and turnover rate are physiologically significant towards the forms of JH present in the insect (Crone et al., 2007b). In the present study, high activity against [^{3}H] JH III was observed in the recombinant NlJHE protein. NlJHE protein showed high affinity and moderate turnover towards JH III and yielded a specificity constant (k_{cat}/K_M), which was close to the specificity constant of JHEs from other insects (Crone et al., 2007b). These results constitute strong evidence that the cDNA (*Nljhe*) isolated here encodes a functional JHE. Moreover, the significant correlation between *Nljhe* mRNA levels and JHE enzyme activities in 5th instar *N. lugens* nymphs also provided the indirect evidence that the predicted JHE gene (EAT43357) from *A. aegypti* (Bai et al., 2007) may also be a functional JHE sequence.

The *Nljhe* transcript was abundant in the fat body of the 5th instar nymphs, low in the midgut and negligible in the ovary. There has been evidence showing the fat body to be a major source of hemolymph JHE (Wing et al., 1981), although other tissues may also be important depending on the species (Klages and Emmerich, 1979). This result is consistent with previous findings in *T. ni* (Wing et al., 1981), *C. fumiferana* (Feng et al., 1999) and *P. hilaris* (Munyiri and Ishikawa, 2007). The dynamics of *Nljhe* mRNA levels in whole body samples and fat body samples were similar, although levels in fat body were a little, but not significantly, higher than those in whole body (Fig. 3).

The degradation of JH is one key factor for JH regulation at certain stages of development, although the synthesis and release of JH by the CA are known to be the major regulatory factors for the hemolymph JH titers (de Kort and Granger, 1996). In *N. lugens* nymphs (3rd–5th instar), the JHE activity and JH titer were negatively correlated, which indicates that JHE is an important factor in the regulation of JH titer in these insects (Dai et al., 2001). In 5th instar female nymphs, macropterous insects showed significantly higher *Nljhe* mRNA levels (and JHE activities) and

much lower JH titers than brachypterous insects after the middle stage (48 h) of this instar (Fig. 4), which indicates that JHE plays a key role in regulating the different JH titers in these two morphs. The positive correlation between *Nljhe* mRNA levels and the changes in JHE activity (Fig. 4) of 5th instar nymphs suggests that regulation of JHE activity during the final larval instar of *N. lugens* occurs mainly at the level of gene transcription. A similar correlation between JHE mRNA levels and JHE activities has also been observed in *B. mori* (Hirai et al., 2002), *P. hilaris* (Munyiri and Ishikawa, 2007) and *Gryllus assimilis* (Crone et al., 2007a). The negative correlation between *Nljhe* mRNA levels (JHE enzyme activities) and JH titers, found in the 5th instar macropterous nymphs, indicates that NlJHE is a key factor in regulating JH titer. However, a similar negative correlation was not observed in 5th instar brachypterous nymphs, suggesting an increase in JH titer results in a decrease in the expression of *Nljhe* (Fig. 4).

In *C. fumiferana*, the JHE gene (*Cfjhe*) was identified as a primary JH-response gene (Feng et al., 1999). Cloning and characterization of the promoter region of *Cfjhe* identified a 30 bp region that is sufficient to support both JH (JH I) induction and 20-hydroxyecdysone (20E) suppression (Kethidi et al., 2004). In *D. melanogaster*, JH III induced *Dmjhe* mRNA but not *DHR3* mRNA, and 20E induced *DHR3* mRNA and suppressed JH III induction of *DmJhe* mRNA (Kethidi et al., 2005). These studies show that induction of JHE mRNA by JH is a common trait. Here we showed that externally applied JH III induced *Nljhe* expression in both macropterous and brachypterous 5th instar nymphs. The extent of induction of *Nljhe* expression by applied JH III in 5th instar brachypterous nymphs was much smaller than that in 5th instar macropterous nymphs (Fig. 5), however, which is not sufficient to explain the difference in the relationship between *Nljhe* mRNA levels and JH titers in the 5th instar nymphs. Although this difference was also observed by Dai et al. (2001), we have no rational explanation of this difference at present. It is possible that another factor contributes importantly to the regulation of JH titer in 5th instar brachypterous nymphs, or that another factor inhibits the induction of JHE expression (JHE activity) by endogenous JH in 5th instar brachypterous nymphs. These possibilities need further studies.

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References

Ayoade, O., Morooka, S., Tojo, S., 1999. Enhancement of short wing formation and ovarian growth in the genetically defined macropterous strain of the brown planthopper, *Nilaparvata lugens*. Journal of Insect Physiology 45, 93–100.

Bai, H., Ramaseshadri, P., Palli, S.R., 2007. Identification and characterization of juvenile hormone esterase gene from the yellow fever mosquito, *Aedes aegypti*. Insect Biochemistry and Molecular Biology 37, 829–837.

Bellés, X., Martín, D., Piulachs, M.D., 2005. The mevalonate pathway and the synthesis of juvenile hormone in insects. Annual Review of Entomology 50, 181–199.

Campbell, P.M., Oakeshott, J.G., Healy, M.J., 1998. Purification and kinetic characterisation of juvenile hormone esterase from *Drosophila melanogaster*. Insect Biochemistry and Molecular Biology 28, 501–515.

Campbell, P.M., Harcourt, R.L., Crone, E.J., Claudianos, C., Hammock, B.D., Russell, R.J., Oakeshott, J.G., 2001. Identification of a juvenile hormone esterase gene by matching its peptide mass fingerprint with a sequence from the *Drosophila genome* project. Insect Biochemistry and Molecular Biology 31, 513–520.

Claudianos, C., Ranson, H., Johnson, R.M., Biswas, S., Schuler, M.A., Berenbaum, M.R., Feyereisen, R., Oakeshott, J.G., 2006. A deficit of detoxification enzymes: pesticide sensitivity and environmental response in the honeybee. Insect Molecular Biology 15, 615–636.

Crone, E.J., Zera, A.J., Anand, A., Oakeshott, J.G., Sutherland, T.D., Russell, R.J., Harshman, L.G., Hoffmann, F.G., Claudianos, C., 2007a. Jhe in *Gryllus assimilis*: cloning, sequence-activity associations and phylogeny. Insect Biochemistry and Molecular Biology 37, 1359–1365.

Crone, E.J., Sutherland, T.D., Campbell, P.M., Coppin, C.W., Russell, R.J., Oakeshott, J.G., 2007b. Only one esterase of *Drosophila melanogaster* is likely to degrade juvenile hormone in vivo. Insect Biochemistry and Molecular Biology 37, 540–549.

Dai, H., Wu, X., Wu, S., 2001. The change of juvenile hormone titer and its relation with wing dimorphism of brown planthopper, *Nilaparvata lugens*. Acta Entomologica Sinica 44, 27–32.

de Kort, C.A.D., Granger, N.A., 1996. Regulation of JH titers: the relevance of degradative enzymes and binding proteins. Archives of Insect Biochemistry and Physiology 33, 1–26.

Denno, R.F., Roderick, G.K., 1990. Population biology of planthoppers. Annual Review of Entomology 35, 489–520.

Easterby, J.S., 2008. Hyper32-Version of Hyper for 32 bit operating systems: hyperbolic regression analysis of enzyme kinetic data. http://homepage.ntlworld.com/john.easterby/hyper32.html.

Feng, Q.L., Ladd, T.R., Tomkins, B.L., Sundaram, M., Sohi, S.S., Retnakaran, A., Davey, K.G., Palli, S.R., 1999. Spruce budworm (*Choristoneura fumiferana*) juvenile hormone esterase: hormonal regulation, developmental expression and cDNA cloning. Molecular and Cellular Endocrinology 148, 95–108.

Hammock, B.D., Sparks, T.C., 1977. A rapid assay for insect juvenile hormone esterase activity. Analytical Biochemistry 82, 573–579.

Hanzlik, T.N., Hammock, B.D., 1987. Characterization of affinity purified juvenile hormone esterase from *Trichoplusia ni*. Journal of Biological Chemistry 262, 13584–13591.

Hanzlik, T.N., Abdelaal, Y.A.I., Harshman, L.G., Hammock, B.D., 1989. Isolation and sequencing of cDNA clones coding for juvenile hormone esterase from *Heliothis virescens*. Evidence for a catalytic mechanism for the serine carboxylesterases different from that of the serine proteases. Journal of Biological Chemistry 264, 12419–12425.

Harshman, L.G., Ward, V.K., Beetham, J.K., Grant, D.F., Grahan, L.J., Zraket, C.A., Heckel, D.G., Hammock, B.D., 1994. Cloning, characterization, and genetics of the juvenile hormone esterase gene from *Heliothis virescens*. Insect Biochemistry and Molecular Biology 24, 671–676.

Hinton, A.C., Hammock, B.D., 2001. Purification of juvenile hormone esterase and molecular cloning of the cDNA from *Manduca sexta*. Insect Biochemistry and Molecular Biology 32, 57–66.

Hinton, A.C., Hammock, B.D., 2003a. In vitro expression and biochemical characterization of juvenile hormone esterase from *Manduca sexta*. Insect Biochemistry and Molecular Biology 33, 317–329.

Hinton, A.C., Hammock, B.D., 2003b. Juvenile hormone esterase (JHE) from *Tenebrio molitor*: full-length cDNA sequence, in vitro expression, and characterization of the recombinant protein. Insect Biochemistry and Molecular Biology 33, 477–487.

Hirai, M., Kamimura, M., Kikuchi, K., Yasukochi, Y., Kiuchi, M., Shinoda, T., Shiotsuki, T., 2002. cDNA cloning and characterization of *Bombyx mori* juvenile hormone esterase: an inducible gene by the imidazole insect growth regulator KK-42. Insect Biochemistry and Molecular Biology 32, 627–635.

Jones, G., Venkataraman, V., Ridley, B., O'Mahony, P., Turner, H., 1994. Structure, expression and gene sequence of a juvenile hormone esterase-related protein from metamorphosing larvae of *Trichoplusia ni*. Biochemical Journal 302, 827–835.

Kethidi, D.R., Perera, S.C., Zheng, S., Feng, Q.L., Krell, P., Retnakaran, A., Palli, S.R., 2004. Identification and characterization of a juvenile hormone (JH) response region in the JH esterase gene from the spruce budworm, *Choristoneura fumiferana*. Journal of Biological Chemistry 279, 19634–19642.

Kethidi, D.R., Xi, Z., Palli, S.R., 2005. Developmental and hormonal regulation of juvenile hormone esterase gene in *Drosophila melanogaster*. Journal of Insect Physiology 51, 393–400.

Klages, G., Emmerich, H., 1979. Juvenile hormone metabolism and juvenile hormone esterase titer in hemolymph and peripheral tissues of *Drosophila hydei*. Journal of Comparative Physiology 132, 319–325.

Lefevere, K.S., 1989. Endocrine control of diapause termination in the adult female Colorado potato beetle *Liptinotarsa decemlineata*. Journal of Insect Physiology 35, 197–203.

Mackert, A., do Nascimento, A.M., Bitondi, M.M.G., Hartfelder, K., Simões, Z.L.P., 2008. Identification of a juvenile hormone esterase-like gene in the honey bee, *Apis mellifera* L.—expression analysis and functional assays. Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology 150, 33–44.

Munyiri, F.N., Ishikawa, Y., 2007. Molecular cloning and developmental expression of the gene encoding juvenile hormone esterase in the yellow-spotted longicorn beetle, *Psacothea hilaris*. Insect Biochemistry and Molecular Biology 37, 497–505.

Nagata, T., 1982. Insecticide resistance and chemical control of the rice planthopper, *Nilaparvata lugens* Stål. Bulletin of Kyushu National Agricultural Experiment Station 22, 49–164.

Oakeshott, J.G., Claudianos, C., Campbell, P.M., Newcomb, R.D., Russell, R.J., 2005. Biochemical genetics and genomics of insect esterases. In: Gilbert, L.I., Iatrou,

K., Gill, S.S. (Eds.), Comprehensive Molecular Insect Science, vol. 5, Pharmacology. Elsevier Science BV, Oxford, pp. 309–381.

Riddiford, L.M., Hiruma, K., Zhou, X., Nelson, C.A., 2003. Insights into the molecular basis of the hormonal control of molting and metamorphosis from *Manduca sexta* and *Drosophila melanogaster*. Insect Biochemistry and Molecular Biology 33, 1327–1338.

Roe, R.M., Venkatesh, K., 1990. Metabolism of juvenile hormones: degradation and titer regulation. In: Gupta, A.P. (Ed.), Morphogenic Hormones of Arthropods, vol. 1. Rutgers University Press, New Brunswick, pp. 125–180.

Roff, D.A., 1986. The evolution of wing dimorphism in insects. Evolution 4, 1009–1020.

Shiotsuki, T., Bonning, B.C., Hirai, M., Kikuchi, K., Hammock, B.D., 2000. Characterization and affinity purification of juvenile hormone esterase from *Bombyx mori*. Bioscience Biotechnology and Biochemistry 64, 1681–1687.

Stay, B., 2000. A review of the role of neurosecretion in the control of juvenile hormone synthesis: a tribute to *Berta Scharrer*. Insect Biochemistry and Molecular Biology 30, 653–662.

Tang, J., Ping, X., Tang, F., Yang, B., 2001. Determination of juvenile hormone in brown planthopper (*Nilaparvata lugens*) by GC/MS. Chinese Journal of Rice Science 15, 142–144.

Thomas, B.A., Hinton, A.C., Moskowitz, H., Severson, T.F., Hammock, B.D., 2000. Isolation of juvenile hormone esterase and its partial cDNA clone from the beetle, *Tenebrio molitor*. Insect Biochemistry and Molecular Biology 30, 529–540.

Venkatesh, K., Abdelaal, Y.A.I., Armstrong, F.B., Roe, R.M., 1990. Characterization of affinity purified juvenile hormone esterase from the plasma of the tobacco hornworm, *Manduca sexta*. Journal of Biological Chemistry 265, 21727–21732.

Ward, V.K., Bonning, B.C., Huang, T.L., Shiotsuki, T., Griffeth, V.N., Hammock, B.D., 1992. Analysis of the catalytic mechanism of juvenile hormone esterase by site-directed mutagenesis. International Journal of Biochemistry 24, 1933–1941.

Wing, K.D., Sparks, T.C., Lovell, V.M., Levinson, S.O., Hammock, B.D., 1981. The distribution of juvenile hormone esterase and its interrelationship with other proteins influencing JH metabolism in the cabbage looper, *Trichoplusia ni*. Insect Biochemistry 11, 473–485.

Wogulis, M., Wheelock, C.E., Kamita, S.G., Hinton, A.C., Whetstone, P.A., Hammock, B.D., Wilson, D.K., 2006. Structural studies of a potent insect maturation inhibitor bound to the juvenile hormone esterase of *Manduca sexta*. Biochemistry 45, 4045–4057.