**Supplementary Material for** Yang, Wen-Jia, Kang-Kang Xu, Feng Shang, Wei Dou, and Jin-Jun Wang—Identification and characterization of three juvenile hormone genes from *Bactrocera dorsalis* (Diptera: Tephritidae).

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**Abstract**

Juvenile hormone (JH) plays an important role in regulating growth, development, and reproduction of insects. Three key enzymes, namely, JH esterase (JHE), JH epoxide hydrolase (JHEH), and JH diol kinase (JHDK), are involved in JH degradation. In this study, we identified the full-length cDNAs of the 3 genes *BdJHEH2*, *BdJHEH3*, and *BdJHDK* encoding JHEH and JHDK from the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). We used quantitative real-time polymerase chain reaction to investigate mRNA expression profiles of these 3 genes in various development stages and tissues, and in response to both 20-hydroxyecdysone (20E) and starvation. Both *BdJHEH2* and *BdJHDK* were highly expressed during the larval–pupal transition, whereas *BdJHEH3* was mainly expressed in the early 3rd instars. All 3 genes were highly expressed in 7-d-old and 10-d-old adults, but exhibited sex-specific expression patterns. *BdJHEH2* was highly expressed in fat body, whereas *BdJHEH3* and *BdJHDK* were most abundant in Malpighian tubules. In response to 20E exposure, the 3 genes were significantly up-regulated at various time points compared with the control. However, the transcript levels of *BdJHDK* decreased significantly during the initial exposure to 20E. After starvation treatment, expression of *BdJHEH2* and *BdJHEH3* significantly decreased, whereas *BdJHDK* was up-regulated. No significant change was observed after feeding resumption. These 3 genes have distinct roles in regulating growth and development of *B. dorsalis*.

**Resumen**

La hormona juvenil (JH) juega un papel importante en la regulación de crecimiento, el desarrollo y la reproducción de los insectos. Tres enzimas clave, JH esterasa (JHE), JH epóxido hidrolasa (JHEH), y JH diol quinasa (JHDK), están implicadas en la degradación de JH. En este estudio, hemos identificado los ADNc de longitud completa de los 3 genes *BdJHEH2, BdJHEH3* y BdJHDK que codifican JHEH y JHDK de la mosca oriental de la fruta, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). Se utilizó PCR cuantitativa en tiempo real para investigar los perfiles de expresión de mRNA de estos 3 genes en diferentes estadios de desarrollo y tejidos, y en respuesta a tanto 20-hidroxiecdisona (20E) y la inanicion. Tanto *BdJHEH2* y *BdJHDK* fueron altamente expresado durante la transición larval-pupal, mientras que *BdJHEH3* se expresó principalmente en el 3er estadio temprano. Todos los 3 genes fueron altamente expresado en los adultos de 7 días de edad y de 10 días de edad, pero exhiben patrones de expresión específicos al sexo. *BdJHEH2* fue altamente expresado en la grasa corporal, mientras que *BdJHEH3* y *BdJHDK* fueron más abundantes en los túbulos de Malpighi. En respuesta a la exposición 20E, los 3 genes fueron significativamente hasta reguladas en varios puntos de tiempo en comparación con el control. Sin embargo, los niveles de transcripción de *BdJHDK* disminuyeron significativamente durante la exposición inicial a 20E. Después del tratamiento de inanicion, la expresión de *BdJHEH2* y *BdJHEH3* disminuyó significativamente, mientras que BdJHDK su regulacion aumento. No se observó ningún cambio significativo después de la reanudación de la alimentación. Estos 3 genes tienen funciones distintas en la regulación del crecimiento y el desarrollo de *B. dorsalis*.

**Key Words:**

juvenile hormone; JH epoxide hydrolase; JH diol kinase; expression pattern; 20-hydroxyecdysone; starvation

1 **ATG**GGCACCTTGTTGCGTATTACATTCGTGGTCCTAGCGATTGTCGTAGGTCTGTGTGTGCATAAATATCAGGAACTC

1 M G T L L R I T F V V L A I V V G L C V H K Y Q E L

79 ACAAGCTCAGCGCCAATTCCCCAACTGAACGATGCGGAATATTGGGGACCCGGCAGCGCCGCTAAATATAAGGAGAAC

27 T S S A P I P Q L N D A E Y W G P G S A A K Y K E N

157 ACTGCCGTTAAAGCTTTCGATATAAGCGCCAAACCGGAGTTAATTGAAGATCTGAAAACGCAGCTGTCGCGGCCGCTG

53 T A V K A F D I S A K P E L I E D L K T Q L S R P L

235 GTGCTCACCGAACCCCTCGAAGGTGTGGGATTCCAATACGGCTTCAATTCTAAGTACTTGAAAGAGGTGGTGGCATAC

79 V L T E P L E G V G F Q Y G F N S K Y L K E V V A Y

313 TGGCGCGACACTTACCTGCCCAAGTGGGGTGAGCGCGAGGCCTTTCTCAAACAGTTCCCGCATTTTGAAACACAGATT

105 W R D T Y L P K W G E R E A F L K Q F P H F E T Q I

391 CAAGGTTTACGAATACACTTCATTCATGTGAAGCCCAAGTCCACCGAAGGTAAAAAGGTGGTGCCTCTGTTACTGATC

131 Q G L R I H F I H V K P K S T E G K K V V P L L L I

469 CACGGTTGGCCAGGATCAGTGCGCGAATTCTATAGGCTAATACCACTGCTGACGAAACCAAACCCCAAAAGTGAATAT

157 H G W P G S V R E F Y R L I P L L T K P N P K S E Y

547 GTCTTCGAAGTGATAGCACCCAGCTTGCCCGGTTATGGTTGGTCTCAGGGGGCCTCAAAGGTAAACTTTGGACCCGCA

183 V F E V I A P S L P G Y G W S Q G A S K V N F G P A

625 CAGATGTCGTTAGTGTTGCGCAATTTGATGCTGCGTTTGGGTCATGAGAAATTCCTAATACAGGGTGGTGATTGGGGC

209 Q M S L V L R N L M L R L G H E K F L I Q G G D W G

703 TCTGTACTCGGTGCAAACATCGTTACTTTATCGCCACAGAATGTGCTCGGTTATCATTCGAATCTTTGCTTTGTTCTT

235 S V L G A N I V T L S P Q N V L G Y H S N L C F V L

781 AGTCATCCAATGATTCACCTTCATAAATTATTGCGAAATTGGTTCCCGAGCTTCTTTATAAAAGAAGAAAATAGAATT

261 S H P M I H L H K L L R N W F P S F F I K E E N R I

859 TTCTTTAAACCATTCAGCAAAGAGTTAAGCGAAACGTTAGAGGAGTCTGGCTACATGCATATTCAAGCATCCAAACCA

287 F F K P F S K E L S E T L E E S G Y M H I Q A S K P

937 GACACAATTGGCACAACGCTCACACAGAATCCTGTCGGTTTGGCCGCATACATCTTGGAGAAATTCTCCACATGGACC

313 D T I G T T L T Q N P V G L A A Y I L E K F S T W T

1015 AATCCTGCATATAAGCAACTCGAGGATGGTGGCCTCACCAAACGCTTCACTTTAGACGAGCTTTTGGATAACATTATG

339 N P A Y K Q L E D G G L T K R F T L D E L L D N I M

1093 ATTTATTACACGACAAACTCGATAACTACGTCTCAGCGCTTGTACTCGGAAGGGTTCAATTTCGCTTACTTTGCTATG

365 I Y Y T T N S I T T S Q R L Y S E G F N F A Y F A M

1171 AATTTGGACGGGACGCATATAAATGTTCCTACCGGTTGTGCACGTTTTCTGCATGACTTATTGCATTTGACGGACTCC

391 N L D G T H I N V P T G C A R F L H D L L H L T D S

1249 GAGCTTGGATTGAAATTCAAAAATATTGTGCACAGTACCTACCATAAGGAAGGTGGTCATTTTGCAGCAATGGAGGTT

417 E L G L K F K N I V H S T Y H K E G G H F A A M E V

1327 CCACATATTCTTTACAGCGATTTCGTCGAATTTGTCGCTAAAGTATTCAGGAAGCCACAACCAAAGCAA**TAG**

443 P H I L Y S D F V E F V A K V F R K P Q P K Q \*

**Suppl. Fig. 1.** Nucleotide and deduced amino acid sequence of *BdJHEH2* cDNA in *Bactrocera dorsalis*. The start codon is indicated with bold and the stop codon is indicated with both bold and an asterisk. The predicted signal peptide is underlined.

1 acatgggagtaggtgactggcaatcagcgcaagtggacgcaatttcggcaggttcttcttcgctacgcatattcaagt

79 agcaattcgagaaaaaagtaacgttaattatttctgttgcgttagtttcgggcaatagaagtgctgagtgctgtgtgt

157 tagactttgttcaat**ATG**AAGGCAATTATATTTATTGTCATATTGGCCATTTCGTGGTTTTGGCTTTACCGCGGGGTC

1 M K A I I F I V I L A I S W F W L Y R G V

235 AACGAATTCATTAAGCCGCTACCGAAGCCAGAAATCAGCAATAACACCTACTGGGGACCCGGCAAACCTGTAAACTAC

22 N E F I K P L P K P E I S N N T Y W G P G K P V N Y

313 GAAGCGCCAACAGATATTGTGCCTTTCGAAATCAAATACGATCAAACGATTATTGACGATTTGCGCACACAATTCAAT

48 E A P T D I V P F E I K Y D Q T I I D D L R T Q F N

391 CGTACTTGGAAATTCACTGCGCCGTTGGAAAATATCAAATTCGAATATGGTTTCAATCCAGAAGCGCTGAGGCACATT

74 R T W K F T A P L E N I K F E Y G F N P E A L R H I

469 GTCGAATATTGGCGCGACTACTACTTGCTCAAATGGGGAGCACATGAAGATTACCTGAATTCGCTGCCGCACTTTAAA

100 V E Y W R D Y Y L L K W G A H E D Y L N S L P H F K

547 ACCGAGATACAAGGGCTGAAGATTCACTTCATACACGCAAAGCCGTCAGAGGAGGCGCGTAAAGAGAAGAAAGTGGTG

126 T E I Q G L K I H F I H A K P S E E A R K E K K V V

625 CCGATGTTATTGCTGCACGGTTGGCCGGGTTCTGTGCGTGAATTTTACGAGTTCATAAAGCTTTTGGTGGAGGTGTCC

152 P M L L L H G W P G S V R E F Y E F I K L L V E V S

703 GATGTCAATGATTATGTGTTCGAAGTTATTGCACCCTCTCTAGTCGGATATGGGTTCTCGGACGCTGCCACCAGACCC

178 D V N D Y V F E V I A P S L V G Y G F S D A A T R P

781 GGTTTCGATTCGCTAGAGATAGCTGTTGTCATGCGGAATCTAATGTTACGTATTGGTTATGACAAATTTCTGGTACAG

204 G F D S L E I A V V M R N L M L R I G Y D K F L V Q

859 GGTGGCGATTGGGGCTCCATCATTGGCAGCGCCATTTCCACGCTCTTCCCTGAGAATGTGCTCGGCTTTCATTCTAAT

230 G G D W G S I I G S A I S T L F P E N V L G F H S N

937 ATGTGTGTGTTGAACACACCGCTAGCTACGATAAAATCGTACATTGCCAGTTGGATGCCAGAGCGCTTTATACCAGCG

256 M C V L N T P L A T I K S Y I A S W M P E R F I P A

1015 CGTTTCTTCTACAATCATCATTTTCCACTAAAGGATAAATATAAGTTCCTAATCGTTGAGTCCGGTTACTTCCGCATA

282 R F F Y N H H F P L K D K Y K F L I V E S G Y F R I

1093 CAAGCAACAAAGCCGGACACCATTGGCATTGCACTGGAGGCTAGTCCCATTGCCTTGGCCGCATACATACTGGAGAAA

308 Q A T K P D T I G I A L E A S P I A L A A Y I L E K

1171 TTCCAATTGGCAACTGGTGCTGGTCGGAATCAGGAATTCAATGCTATGGACAGAACCTATAAATTAGATGCTATATTA

334 F Q L A T G A G R N Q E F N A M D R T Y K L D A I L

1249 GATAATCTCATGATACACTATCTCACCGGCACAGTCACGACAGCAGGACGGTTCTACGCCGAAAATCTCGCCAGTGAC

360 D N L M I H Y L T G T V T T A G R F Y A E N L A S D

1327 TCTAAGGCTCTGAAAATGGATCGCGTGCCGACGACTGTGCCTATGGGTTGTGCTCGCTTCCAGTTCGACTTGCCTCCT

386 S K A L K M D R V P T T V P M G C A R F Q F D L P P

1405 GCCATTGACTGGGCGCTCAAAGACAAGTTTCCGAATCTGGTTCACAGTACATACTTCAATCAAGGTGGTCATTTCGCT

412 A I D W A L K D K F P N L V H S T Y F N Q G G H F A

1483 GCCTTGGAACTGCCTGGTATGTTGTATATTAACTTCCAAGAGTTTGTGAAGAAAGCCATTGCTGAG**TAA**cttatgatt

438 A L E L P G M L Y I N F Q E F V K K A I A E \*

1561 ttgtattctaaatgaataatttaagtaataagtaaagctggaaaatacacaatatagtcactgcgattttttactaga

1639 aaaaaaaaaaaaa

**Suppl. Fig. 2.** Nucleotide and deduced amino acid sequence of *BdJHEH3* cDNA in *Bactrocera dorsalis*. The start codon is indicated with bold and the stop codon is indicated with both bold and an asterisk. The predicted signal peptide is underlined. Potential *N*-glycosylation sites are boxed.

1 cacgcgcaaccagccacaagcaacttgcaacagtcgcagtcgcagtcagccaaccgaaggcacagtgttctaacgaaa

79 atccgttgatttccaaattaaaggccgcaggagtagcaaaatttcacttaattgcagaagcgattaataatttgcatt

157 ccagaaacttttggcgctcaaagaagttactgcagcgctacgacatttgtcaatagtttttctacagttaacgcagtt

235 acaacagcaacaacaacaacagtatc**ATG**TCGATCTCCGACTTCCGTAAGAAGAAATTGCTCTTCCTGTTCAACGTTT

1 M S I S D F R K K K L L F L F N V

313 TCTTTGATGTCAATCAGAGCGGCGAAATCGACGTTAAAGACTTTGAGTTGGCCATTGAGCGCGTCTGCAAACTGCGCG

18 F F D V N Q S G E I D V K D F E L A I E R V C K L R

391 GCTGGGCAGAGAACACACCCAAGAACAAGGAGATCCACGCGGTCATGCTGGAGATCTGGGAGGGCCTGCGCTCCAAGG

44 G W A E N T P K N K E I H A V M L E I W E G L R S K

469 CCGACAAGGACAATGATGGGCAGGTTTCCGTCGACGAGTGGTGCAACATGTGGGACGCCTACGCCAAGGATCCCAGCA

70 A D K D N D G Q V S V D E W C N M W D A Y A K D P S

547 GCGTTATGGACTGGCAAACCAAATACATGAACTTCATGTTCGACCTGGAGGACGCCTCGACTGACGGCAGCATCGACG

96 S V M D W Q T K Y M N F M F D L E D A S T D G S I D

625 CGGAGGAGTTCACATTGGTCTGCTCCAGCTATGGCCTGGACAAGGCCGAATGCCAAGACGCCTTCCAGAAAATGGCGC

122 A E E F T L V C S S Y G L D K A E C Q D A F Q K M A

703 AGGGCAATTCCGAGGTCACTCGCGACCAGTTCGCCGCCTTATGGAAGGAATACTTCGCAGCAGAGGATGTGAATGCGC

148 Q G N S E V T R D Q F A A L W K E Y F A A E D V N A

781 CCGGCAATTTCATTTTCGGCAAAAATGCATTT**TAA**cgctctaaaatttgctcagctggctaaggcgctccgcgtgggc

174 P G N F I F G K N A F \*

859 ccacagccaggcttaaattttcaagacgtttttgtggtctgattgcaagtgaagtatgcacgctttgtgaagtgaagg

937 ggtgaagtgtgttggtgtgctgaggcatacattttgaagctggcatatgcaactattaatgaaatgtttgagcataat

1015 aaagaaacatatattaataagaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa

**Suppl. Fig. 3.** Nucleotide and deduced amino acid sequence of *BdJHDK* cDNA in *Bactrocera dorsalis*. The start codon is indicated with bold and the stop codon is indicated with both bold and an asterisk. Potential *N*-glycosylation sites are boxed.