Gene Expression Profiles of Heat Shock Proteins 70 and 90 from Empoasca onukii (Hemiptera: Cicadellidae) in Response to Temperature Stress

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Gene Expression Profiles of Heat Shock Proteins 70 and 90 From *Empoasca onukii* (Hemiptera: Cicadellidae) in Response to Temperature Stress

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ABSTRACT. *Empoasca onukii* Matsuda is a worldwide pest that causes great economic loss in tea growing areas and is significantly affected by temperatures. Heat shock protein (Hsp) genes are important in insects' response to temperature stress. In this study, two full-length Hsp genes, *Eohsp90* and *Eohsp70*, were cloned from *E. onukii* using rapid amplification of complementary DNA ends. The open reading frames of *Eohsp90* and *Eohsp70* were 2,172 bp and 2,016 bp in length, respectively. Their deduced amino acid sequences of *Eohsp90* and *Eohsp70* showed high homology with other species. Subsequently, the transcriptional expression of *Eohsp90* and *Eohsp70* in *E. onukii* adults exposed to various temperatures (−5, 0, 10, 15, 20, 25, 30, 35, 38, 41 and 44°C) for 1 h, and at extreme temperatures (0°C and 41°C) for various time duration (0, 20, 40, 60, 80, 100, and 120 min) were investigated via real-time quantitative polymerase chain reaction. The relative expression levels of both *Eohsp90* and *Eohsp70* in *E. onukii* adults were upregulated as the temperature rises or falls over time, except in the −5°C or 44°C temperature groups. Moreover, the expression level in the temperature elevated groups was higher than that of the lower temperature groups. In addition, the *Eohsp70* generally demonstrated a higher transcriptional level than *Eohsp90*, and both genes had a higher expression profile in female adults compared with the males. The expression profiles indicated that *Eohsp90* and *Eohsp70* may play important roles in *E. onukii* adult responses to ecologically relevant environmental temperature threat.

Key Words: *Empoasca onukii* Matsuda, heat shock protein, temperature stress, sex difference

When organisms are exposed to heat, cold, or some other stresses, they synthesize a small set of proteins called heat shock proteins (Hsps) (Parsell and Lindquist 1994, Sørensen et al. 2003, Arya et al. 2007), which participate in folding and unfolding, aggregation, degradation, and transport of proteins induced by the stress (Hartl 1996, Beissinger and Buchner 1998). The Hsps are a ubiquitous group of highly conserved proteins (Denlinger and Yocum 1998, Sørensen et al. 2003) that are organized into several families based on their molecular mass (kDa), with *Hsp90* and *Hsp70* proteins belonging to the two major families. *Hsp90* is one of the most abundant proteins, accounting for 1–2% of all cell proteins under nonstressed conditions (Csermely et al. 1998). *Hsp70* originates through the gene duplication process (Bettencourt and Feder 2001) and exhibits differential gene expression profiles under various stress conditions. Conserved structures of *Hsp90* and *Hsp70* proteins are characterized by the N-terminal ATPase domain and the C-terminal polypeptide-binding domain (Young et al. 2004). Consequently, the role of *Hsp90* and *Hsp70* as stress markers has been well recognized in many organisms.

Insects are small-bodied poikilotherms that are relatively easily affected by heat or cold stress, which can quickly increase or decrease their body temperatures to lethal levels (Denlinger and Yocum 1998, Rinehart et al. 2007). Insects have evolved many behavioral and physiological strategies to avoid temperature impairments, and production of Hsps is one of them (Feder and Hofmann 1999, Sørensen et al. 2003). Inducible Hsps can be synthesized when temperatures increase to levels substantially above normal, and one of their best known functions is to increase heat tolerance of organisms to protect them from thermal injury and death (Gehring and Wehner 1995, Dahlgaard et al. 1998). It has been proposed recently that Hsps also contribute to the cold tolerance of insects (Chen et al. 2005, Rinehart et al. 2007).

The green tea leafhopper *Empoasca onukii* (*E. onukii*) Matsuda (Hemiptera: Cicadellidae), is a major pest in many Asian tea plantations (Feng et al. 2010, Wang et al. 2010, Jin et al. 2012). Both adults and nymphs actively move around to feed on fresh tender leaves causing characteristic symptoms referred to as "hopperburn" (Backus et al. 2005). The average tea loss is about 10–15% each year. This insect can also cause a dramatic decrease in tea quality and a loss of over 50% in some temperate years (Peng et al. 2010). In mid-south of China, *E. onukii* are active from mid-March to early December, and peak numbers occur in July and October. Populations sharply decline in later November with decreasing temperature. In the summer, this insect usually spends its life on deciduous trees and in the winter on conifers or evergreens adjacent to tea fields. It is now apparent that there is no physiological diapause in this pest and the optimal temperature range for survival, development, and reproduction of *E. onukii* is 20–25°C (Yukio 2010). Therefore, *E. onukii* is frequently exposed to high or low temperature stress in nature. The temperature is normally above 20°C during this period in China, occasionally with a temperature higher than 39°C or lower than 0°C (Cui et al. 2008). In previous studies, we have investigated the biological parameters of *E. onukii* under high- and low-temperature conditions and found that adults had the greatest thermal and cold tolerance among all developmental stages in natural environments, and with the higher or lower temperature conditions, there were more female than male adults in tea plantations (L.Q., unpublished data). This may be related to the expression of Hsp genes.

Comprehensive information about the changes in the level of Hsp genes from *E. onukii* in the response to temperature stress is not available yet. To explore changes of *E. onukii* to thermal and cold stress conditions at the molecular level, here in this article, we cloned two full-length genes encoding *Hsp90* and *Hsp70*, respectively, and

analyzed their transcriptional expressions under various temperature stress scenarios and investigated their expression differences between females and males to elucidate the gene expression profiles.

Materials and Methods

Insects. *E. onukii* were originally collected from tea plantations in the Maan Mountains (113° 45′–115° 55′ E, 30° 23′–32° 27′ N, elevation 120 m; tea cultivar belongs to the Xinyang colony species), which is part of the Academy of Agricultural Sciences in Xinyang City, Henan Province, China. Adults and nymphs were reared on biennial tea trees in plastic flowerpots (15 cm in diameter) covered with 60 mesh filter gauze. The phytotron with *E. onukii* colonies was maintained at $20 \pm 1°C$, with a relative humidity of $80 \pm 5\%$ and a photoperiod of 14:10 (L:D). The experimental adults were collected from the fifth generation reared in phytotron.

Heat and Cold Treatments. Five adults (1-day-old adults) were randomly selected for the experiment and placed in a 1.5-ml centrifuge tube blocked with cotton, and heated at 35°C for 1 h in a dry bath incubator (Allsheng Instruments, Hangzhou, China) to provide material for carrying out Hsp transcripts cloning. The temperature treatment levels chosen for these experiments were selected based on data from a prior pilot experiment. Before conducting real-time quantitative polymerase chain reaction (RT-qPCR), adults were exposed to various temperature treatments of −5, 0, 10, 15, 20, 25, 30, 35, 38, 41, and 44°C for 1 h and at two selected extreme temperatures of low 0°C and high 41°C for various time treatments (0, 20, 40, 60, 80, 100, and 120 min). After each stress temperature treatment, all adults were immediately snap-frozen in liquid nitrogen and stored at −80°C prior to processing. Five female and five male adults were included in each treatment. To observe the survival rates of *E. onukii* under various temperatures, 30 female and 30 male adults were exposed to above temperatures for 1 h. The survival rates were assessed after the treated insects were allowed to recover at $20 \pm 1°C$ for 24 h. Adults set at $20 \pm 1°C$ were regarded as the control group. Each treatment was repeated three times.

RNA Extraction and First Strand cDNA Synthesis. Total RNA was extracted using RNAiso Plus (TaKaRa, Dalian, China) and treated with *Dnase I* (MBI Fermentas, Hanover, MD) to remove any contaminating DNA (per the manufacturer's instructions). The RNA quality was determined by using a spectrophotometer (Nanodrop2000c, Thermo Fisher Scientific, West Palm Beach, FL) and gel electrophoresis, respectively. To generate the first strand of complementary DNA (cDNA), 0.5 μg of total RNA was used as template, and oligo dT primers and the PrimeScript RT reagent kit (TaKaRa, Dalian, China) were used for reverse transcription and cDNA synthesis.

Cloning of Hsp Fragments. To amplify partial cDNA fragments of *Hsp90* and *Hsp70*, degenerate primers (Table 1) were designed based on sequences from other insect species. PCR reactions were performed as follows: 3 min at 95°C; 35 cycles of 30 s at 95°C, 30 s at 50°C for *Hsp90,* or 30 s at 48°C for *Hsp70* and 72°C for 75 s; 10 min at 72°C.

The PCR products obtained were separated on a 1.5% agarose gel. After being purified by a universal DNA purification kit, bands with expected sizes (Tiangen Biotech, Beijing, China) were cut and then cloned into a pGEM-T vector (Promega, Madison, WI) and transformed into DH5α competent cells (Transgen Biotech, Beijing, China). Positive products were determined based on blue–white screening and then cultured on liquid Luxol blue (LB) culture medium. Plasmids were extracted by using a plasmid mini kit (Tiangen Biotech) and sequenced (AuGCT DNA-SYN Biotechnology, Beijing, China).

Based on the sequence information of partial fragments, 5′ and 3′ regions of the corresponding cDNAs were obtained by 5′ and 3′ RACE (rapid amplification of cDNA ends) using designed gene-specific primers (Table 1), and the 5′-full RACE kit and 3′-full RACE core set (ver. 2.0, TaKaRa). The nested PCR reaction for 3′ RACE was 3 min at 94°C; 30 cycles of 30 s at 94°C, 30 s at 50°C for Hsp90 or 30 s at 58°C for Hsp70 and 72°C for 1 min; and 10 min at 72°C. The nested PCR reaction for 5' RACE was 3 min at 94°C; 30 cycles of 30 s at 94°C, 30 s

Table 1. Primers used in transcript cloning and real-time quantitative PCR

Primer name	Primer sequence from 5′ to 3′
PCR *Hsp90*	CATGACCGAACCAATTGAYGARTAYGT
	TCAGGTCTTCGCAGTTRTCCATDAT
PCR *Hsp70*	CAACATGGTAAAGTGGAAATTATTGCNAAYGAYCA
	GCTTGCACGGCGGCNCCRTANGC
3′RACE *Hsp90* outer	GGACCCGAAACCCTGAT
	TACCGTCGTTCCACTAGTGATTT
3′RACE *Hsp90* inner	TCATCTGGCTGTCAAAC
	CGCGGATCCTCCACTAGTGATTTCACTATAGG
3′RACE *Hsp70* outer	TTCCTGCCTATCTCAATG
	TACCGTCGTTCCACTAGTGATTT
3′RACE *Hsp70* inner	GGCAGATGAGTTCAAGAGG
	CGCGGATCCTCCACTAGTGATTTCACTATAGG
5′RACE *Hsp90* outer	GTAGACCACTTCAAATCCG
	CATGGCTACATGCTGACAGCCTA
5′RACE *Hsp90* inner	GTTATTCTTACGCTTCTTGT
	CGCGGATCCACAGCCTACTGATGATCAGTCGATG
5′RACE *Hsp70* outer	CGAAGTCCTCACCTCCC
	CATGGCTACATGCTGACAGCCTA
5′RACE *Hsp70* inner	GGAACCGTGATGACTGC
	CGCGGATCCACAGCCTACTGATGATCAGTCGATG
β-actin	GAGGTAGCCGCCCTTGTGGT
	CGGACTCGTCGTATTCTTG
Hsp90 full validation	GAAGGAGCGTGATAAGGA
	ATGGAGTGGTCAGGGTTG
Hsp70 full validation	TCATGCCGTCGAAAATGCCTGCTAT
	GTCAATCTTGGGTACGCCGCGAGCA
RT-PCR *Hsp90*	TTATCGGCTATCCTATCAA
	TTCCTTCTTCTCCTCTTC
RT-PCR *Hsp70*	GACTGCGGGTGGAGTAATG
	AATGTGCCCAGGAGGTTGT
RT-PCR β-actin	GTATGGAATCTTGCGGTATC
	CAGGTCCTTACGGATGTC

at 50°C, and 1 min at 72°C, with a final extension phase at 72°C for 10 min for *Hsp90* and *Hsp70*, followed by sample storage at −20°C. Distinct bands with expected sizes were recovered from the gel, and then cloned and sequenced as above. Finally, based on the preliminary sequence of the obtained cDNA, full sequences validation of *Hsp90* and *Hsp70* were implemented using designed gene-specific primers (Table 1). The reaction for full sequences validation was 3 min at 95°C; 35 cycles of 30 s at 95°C, 30 s at 55°C and 72°C for 75 s; 10 min at 72°C, respectively.

Gene Sequence Analysis. Analogs of the obtained cDNA sequences were searched using the Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi). Partial sequences were assembled using CAP3 sequence assembly program (http://pbil.uni-vlyon1.fr/cap3.php). The sequence alignment and identity analysis were carried out using the DNAMAN software package (Lynnon Corporation, Pointe-Claire, Quebec, Canada). Open reading frames (ORF) were identified with the aid of the ORF Finder software (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The molecular weights of predicted proteins were calculated by the SWISS-PROT (ExPASy server) program "Compute pI/Mw" (http://au.expasy.org/tools/pi_tool.html).

Real-Time qPCR. RT-qPCR reactions were performed in 25 μl total reaction volume including 12.5 μl of 2×SYBR Green Mix (Hangzhou Bloer Technology Co., Ltd, China), 1 μl of gene-specific primers (Table 1) each, 2 μl of cDNA template, and 8.5 μl of ddH₂O. Samples without cDNA template were used as negative controls. Reactions were carried out on the iQ5 (Bio-Rad, Hercules, CA) according to the operation manual. PCR conditions were initial step at 95°C for 3 min, followed by 40 cycles of 95°C 10 s, 55°C 15 s and 72°C 30 s, then 15 s at 95°C, 30 s at 55°C, and 15 s at 55°C (gradient rising to 95°C, at an interval of 0.5°C) to generate the melting curve. All reactions were carried out in three biological and technical replicates.

The expression levels of targeted *Hsp90* and *Hsp70* genes were quantified according to geNorm directions (Vandesompele et al. 2002).

The expression of each gene was determined from the Ct (cycles threshold) value that corresponded to a number of cycles required for the PCR amplification to reach a fixed threshold in the exponential phase (Walker 2002). The real-time dissociation curve was used to determine the presence of a unique PCR product. Following the normalization strategy outlined in Vandesompele et al. (2002), we tested the stability of both β-*actin* and α-*tubulin* as reference genes (GenBank accession number: KJ476139). β-*actin* was selected and used as the final reference gene, because its expression was stable and had less change than α-*tubulin* under different temperature treatments based on analyses with Normfinder and Genorm. For all reactions, melting analysis confirmed that only a single amplicon was generated.

Statistical Analysis. Transcript levels under different temperature treatments were determined by qPCR and the result analysis using $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). β-*actin* of *E. onukii* was used as a reference gene (Table 1) and data collected at the treatment of 20°C or 0 min was used as the calibrator. All test and calibration samples were normalized to the ΔCt value ($\Delta Ct_{(test)} = Ct_{(target\ test)} - Ct_{(reference\ test)}$, $\Delta Ct_{(calibrator)} = Ct_{(target\ calibrator)} - Ct_{(reference\ calibrator)}$) and then to the $\Delta\Delta Ct$ value ($\Delta\Delta Ct = \Delta Ct_{(test)} - \Delta Ct_{(calibrator)}$). The final relative expression level was calculated using the formula of $F = 2^{-\Delta\Delta Ct}$.

Significant differences among treatments were analyzed using a one-way analysis of variance to compare means of three samples and followed by a Student–Newman–Keuls test for multiple comparisons (means for groups in homogeneous subsets) using SPSS 16.0 software (SPSS Inc., Chicago, IL). The significance level was 0.05. Values throughout the manuscript are means ± standard deviation.

Results

Cloning and Characterization of Hsps. Partial sequences of *Hsp90* and *Hsp70* obtained from *E. onukii* were 378 bp and 1,070 bp, respectively. Based on BLAST searches, a high homology to the Hsps of other insects was discovered, and we further performed RACE and obtained their full-length sequences and designated them as *Eohsp90* (GenBank accession number: KF730250) and *Eohsp70* (GenBank accession number: KF730249). Their complete nucleotide and deduced amino acid sequences are shown in Figs. 1 and 2, respectively.

The ORF of *EoHsp90* was 2,172 bp, encoding 723 amino acids, with a predicted molecular mass of 83.34 kDa, and the theoretical isoelectric point (pI) 4.96. All five conserved amino acid motifs characteristic of the *Hsp90* protein family (Gupta 1995) were discovered in *E. onukii EoHsp90* including a C-terminal MEEVD motif found in the cytosolic *EoHsp90* members (Fig. 1). The ORF of *EoHsp70* was 2,016 bp, encoding 671 amino acids with predicted molecular mass of 73.36 kDa and a pI of 5.65. Within the predicted *E. onukii EoHsp70* protein sequence, three *EoHsp70* protein family signatures (Sonoda et al. 2006) were identified: IDLGTTYS, IFDLGGGTFDVSIL, and VVLVGGSTRIPKIQ, including the cytosolic EEVD signal at the C-terminus (Fig. 2). The termination codon (TAA) occurred at nucleotide 2,169 in *Eohsp90* and 2,013 in *Eohsp70*. The possible polyadenylation signal (AAUAAA) was present at 164 and 31 nucleotides downstream from the termination codon in *Eohsp90* and *Eohsp70*, respectively.

The deduced amino acid sequences of *Eohsp90* and *70* Hsp genes and a range of highly homologous Hsp genes from other Hemiptera insect species (selected from BLAST analysis) were used for the construction of alignments (Figs. 3 and 4). These sequences proved to be highly conservative. The *Eohsp90* of our insect displayed 86.50%, 86.33%, 87.97%, 91.38%, and 91.26% identity with that of *Trialeurodes vaporariorum* (FJ457626), *Bemisia tabaci* (HM367080), *Lygus hesperus* (JX627809), *Laodelphax striatella* (KF660250), and *Nilaparvata lugens* (GU723300), respectively. The *Eohsp70* displayed 81.82%, 79.64%, 74.07%, 76.30%, and 81.22% identity compared with *B. tabaci* (HM367079), *Corythucha ciliata* (KF018929), *Ericerus pela* (KC161300), *N. lugens* (JQ782193), and *T. vaporariorum*

(EU934244), respectively. For the identified *Hsp90* amino acid sequence, the greatest similarity was found to be with other Hemiptera insect species.

Survival Rates After Temperature Treatments. As shown in Fig. 5, after different temperature treatments for 1 h, the survival rates of *E. onukii* adults were at the similar level at 5–35°C, both males and females. The survival rates of males were gradual declining starting at 38°C, were significantly different from other treatments, but females undifferentiated at 38°C. As the temperature rose (>38°C) or fell (<5°C), the survival rates declined significantly. A large number of *E. onukii* were unable to survive above the 44°C and under −5°C treatment points, and the survival rates of females were higher than males (females: 48% under 44°C and 52% under −5°C; males: 20% under 44°C and 39% under −5°C).

Expression of Hsps at Heat and Cold Shock Temperature Stress. The expressions of *Eohsp90* and *Eohsp70* were induced by both high (25°C to 44°C) or low (−5°C to 15°C) temperatures. The higher (25°C to 44°C) or the lower (−5°C to 15°C) the temperature, the higher the expression level of both *Eohsp90* and *Eohsp70*, except for the −5°C and 44°C temperature points (Fig. 6). Moreover, the expression level of *Eohsp90* and *Eohsp70* at the high temperature points was higher than that of the low temperature points, and the expression level of female was higher than that of the male as the temperature was raised or lowered beyond the normal physiological range (15–25°C). The expression of *Eohsp90* and *Eohsp70* remained constant between 15°C and 25°C but significantly differed when the temperature rose above 25°C or dropped below 15°C.

In heat treatments, the expression level of *Eohsp90* increased dramatically at 30°C compared with 20°C in both males and females (Fig. 6A). The maximal expression value was found to be approximately 4.53-fold higher in males and approximately 4.87-fold higher in females when the temperature of the *E. onukii* increased from 30°C up to 41°C. In cold treatment, the expression was increased dramatically at the 0°C temperature point. The maximal expression value was found to be approximately 3.05-fold in males and approximately 4.16-fold in females when the temperature of the *E. onukii* decreased from 10°C down to 0°C.

In heat treatments, the expression level of *Eohsp70* (Fig. 6B) increased dramatically at 30°C compared with that of 20°C and reached a peak at 41°C. The maximal expression value was found to be approximately 8.72-fold in males and approximately 7.90-fold in females when the temperature of the *E. onukii* increased from 30°C up to 41°C. In cold treatment, the expression was also increased dramatically at the 0°C temperature point. The maximal expression value was increased substantially to approximately 5.93-fold in males and approximately 5.10-fold in females when the temperature of the *E. onukii* decreased from 10°C down to 0°C. The expression of *Eohsp90* or *Eohsp70* was lower (−5°C and 44°C) than that at 35–41°C and 0–5°C but were still elevated relative to those under 10–30°C temperature points.

Expression of Hsps at Different Time Duration. The expression of *Eohsp90* and *Eohsp70* of females was higher than that of males during both heat and cold treatments at all checked time points except that at 0 min, and furthermore, *Eohsp70* expression was higher than the expression of *Eohsp90*. Within the 20-min duration, the *Eohsp90* and *Eohsp70* had similar expression profiles (Fig. 7). In male and female adults, the increase in expression of *Eohsp90* and *Eohsp70* was highly significant after 40 min heat treatment compared with that at 0 min. At 41°C, the expression of *Eohsp90* increased maximum with the increase of treatment time for about 120 min; however, there was no significant difference of the expression levels between the 100 and the 120 min treatments (Fig. 7A). *Eohsp70* expression was the highest at 80 min and leveled off to 100 min, after that there was a significant decrease at 120 min treatment (Fig. 7B).

The increase of expression level of *Eohsp90* in both males and females was clearly visible after 20 min of cold exposure and continued to increase for up to 40 min in males or 60 min in females at which time,

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1      TCATTGTTACCAAGTGCCGTTCGGAGCAGTACTTGTGGTTTTTGTGATTTCGAAGTTCTC
61     ATTCATTTCTACTAGCAACTGAAACTAAAGATGCCTGAAGATATCGATATGAAGGAAGAA
1                               M   P   E   D   I   D   M   K   E   E
121    GTCGAGACCTTCGCCTTCCAGGCAGAGATCGCTCAGTTGATGAGCTTGATCATCAACACC
11     V   E   T   F   A   F   Q   A   E   I   A   Q   L   M   S   L   I   I   N   T
181    TTCTACTCCAACAAGGAAATCTTCCTCCGAGAATTGATCTCCAACTCATCAGACGCTTTG
31     F   Y   S   N   K   E   I   F   L   R   E   L   I   S   N   S   S   D   A   L
241    GACAAGATTCGCTACGAGTCGCTGACAGATGCGTCCAAGCTGGAGTCTGGCAAGGAGCTT
51     D   K   I   R   Y   E   S   L   T   D   A   S   K   L   E   S   G   K   E   L
301    TACATCAAGATCATCCCCAACAAGAACGACAGGACTCTCACCATCATTGACACTGGAATT
71     Y   I   K   I   I   P   N   K   N   D   R   T   L   T   I   I   D   T   G   I
361    GGTATGACAAAGGCTGACCTCGTGAACAACTTGGGAACCATCGCCAAGTCTGGAACTAAG
91     G   M   T   K   A   D   L   V   N   N   L   G   T   I   A   K   S   G   T   K
421    GCTTTCATGGAAGCACTGCAGGCCGGTGCCGACATCTCCATGATCGGTCAGTTTGGTGTG
111    A   F   M   E   A   L   Q   A   G   A   D   I   S   M   I   G   Q   F   G   V
481    GGTTTCTACTCTGCCTATCTGGTCGCCGACAAGGTAACAGTCACGTCCAAGCACAACGAT
131    G   F   Y   S   A   Y   L   V   A   D   K   V   T   V   T   S   K   H   N   D
541    GACGAGCAGTATCTCTGGGAGTCTTCTGCCGGAGGTTCCTTCACCGTTCGCACTGACCCC
151    D   E   Q   Y   L   W   E   S   S   A   G   G   S   F   T   V   R   T   D   P
601    GGCGAGCCCCTGGGTCGCGGAACCAAGATTGTCCTTTTCATCAAGGAGGATCAAGCGGAG
171    G   E   P   L   G   R   G   T   K   I   V   L   F   I   K   E   D   Q   A   E
661    TTCTTGGAGGAGAGGAAGGTCAAAGAGATCGTCAAGAAGCACTCTCAGTTTATCGGCTAT
191    F   L   E   E   R   K   V   K   E   I   V   K   K   H   S   Q   F   I   G   Y
721    CCTATCAAGCTGGTCGTCGAGAAGGAGCGTGATAAGGAACTGAGCGACGAGGAAGTGGAA
211    P   I   K   L   V   V   E   K   E   R   D   K   E   L   S   D   E   E   V   E
781    GAGGAAGAAGAAGGAGAAGGTGACAAGGAGGAAGACATGACCGAACCAATTGATGAG
231    E   E   K   E   G   E   G   D   K   E   E   D   M   T   E   P   I   D   E
841    TATGTAGGTGAAGATGAGGATGAAGACAAGGAAGAGAAATCTAAGAAGAAAAAGAAGACA
251    Y   V   G   E   D   E   D   E   E   K   S   K   K   K   K   T
901    GTTAAAGAAAAATACACGAAGATGAAGAGCTCAACAAAACTAAACCTATTTGGACCCGA
271    V   K   E   K   Y   T   E   D   E   L   N   K   T   K   P   I   W   T   R
961    AACCCTGATGACATCAGTCAAGAAGAATATGGAGAGTTTTACAAGTCTTTAACAAACGAT
291    N   P   D   D   I   S   Q   E   E   Y   G   E   F   Y   K   S   L   T   N   D
1021   TGGGAAGATCATCTGGCTGTCAAACATTTCTCTGTTGAAGGTCAGCTTGAGTTCAGAGCG
311    W   E   D   H   L   A   V   K   H   F   S   V   E   G   Q   L   E   F   R   A
1081   CTTTTTGTTCATTCCTCGTCGAGCACCATTTGATCTGTTTGAAAACAAGAAGCGTAAGAAT
331    L   L   F   I   P   R   R   A   P   F   D   L   F   E   N   K   K   R   K   N
1141   AACATCAAATTGTATGTTCGAAGGGTGTTTATTATGGATAACTGCGAAGACCTGAACCCA
351    N   I   K   L   Y   V   R   R   V   F   I   M   D   N   C   E   D   L   N   P
1201   GAGTACCTGAACTTCATTAAGGGAGTAGTCGACAGTGAGGATCTTCCTCTGAACATCTCC
371    E   Y   L   N   F   I   K   G   V   V   D   S   E   D   L   P   L   N   I   S
1261   CGAGAAATGCTTCAACAGAACAAGATCCTGAAGGTTATCCGCAAGAACCTGGTGAAGAAG
391    R   E   M   L   Q   Q   N   K   I   L   K   V   I   R   K   N   L   V   K   K
1321   TGCTTGGAACTGTTTGACGAGATCGCAGAGGACAAGGAGAGCTACAAGAAGTACTACGAA
411    C   L   E   L   F   D   E   I   A   E   D   K   E   S   Y   K   K   Y   Y   E
1381   CAGTTCAGCAAGAACCTGAAGCTGGGAATCCACGAGGACAGCCAGAACAGGAAGAAGCTC
431    Q   F   S   K   N   L   K   L   G   I   H   E   D   S   Q   N   R   K   K   L
1441   TCTGACCTGCTGAGGTACAACACATCCGCCTCCGGAGATGAAGTTTGCTCTCTGAAGGAC
451    S   D   L   L   R   Y   N   T   S   A   S   G   D   E   V   C   S   L   K   D
1501   TATGTCGGACGTATGAAGGAGAATCGGAAACACATCTACTACATCACCGGTGAGAACAAG
471    Y   V   G   R   M   K   E   N   R   K   H   I   Y   Y   I   T   G   E   N   K
1561   GACCAAGTAGCCAACTCTTCCTTCGTAGAGCGAGTGAAGAAGCGCGGATTTGAAGTGGTC
491    D   Q   V   A   N   S   S   F   V   E   R   V   K   K   R   G   F   E   V   V
1621   TACATGACAGAGCCGATCGATGAGTATGTAGTACAGCAGATGAAGGAGTACGACGGCAAG
511    Y   M   T   E   P   I   D   E   Y   V   V   Q   Q   M   K   E   Y   D   G   K
1681   CAGCTGGTGTCCGTCACCAAGGAGGGTCTGGAGCTGCCGGAGGACGATGCCGAGATGAAG
531    Q   L   V   S   V   T   K   E   G   L   E   L   P   E   D   D   A   E   M   K
1741   AAGCGAGAGGAAGACAAGGCCAAGTACGAGAACCTGTGCAAGGTCATGAAGGACATCCTG
551    K   R   E   E   D   K   A   K   Y   E   N   L   C   K   V   M   K   D   I   L
1801   GACAAGAAGGTGGAGAAGGTGGTCGTGAGCAACAGGCTGGTCGAGTCACCCTGCTGTATC
571    D   K   K   V   E   K   V   V   V   S   N   R   L   V   E   S   P   C   C   I
1861   GTCACATCGCAGTATGGCTGGACCGCCAACATGGAGAGGATCATGAAGGCGCAAGCCCTG
591    V   T   S   Q   Y   G   W   T   A   N   M   E   R   I   M   K   A   Q   A   L
1921   AGAGACACCTCCACCATGGGCTACATGGCGGCCAAGAAACACCTCGAGATCAACCCTGAC
611    R   D   T   S   T   M   G   Y   M   A   A   K   K   H   L   E   I   N   P   D
1981   CACTCCATCATGGACACCCTGAGACAGAAGGCCGACGTGGACAAGAACGACAAGGCCGTC
631    H   S   I   M   D   T   L   R   Q   K   A   D   V   D   K   N   D   K   A   V
2041   AAGGACTTGGTCATGTTGCTATTCGAGACTGCTCTCCTGTCCTCTGGCTTCGCCCTGGAG
651    K   D   L   V   M   L   L   F   E   T   A   L   L   S   S   G   F   A   L   E
2101   GATCCCCAGATCCACGCAGCTAGGATCCACCGCATGATCAAGCTCGGTCTGGGAATTGAC
671    D   P   Q   I   H   A   A   R   I   H   R   M   I   K   L   G   L   G   I   D
2161   GAGGATGATCCGGTTCCTCAGGAGGATGAGAAGGTTGACGCTGAAATGCCTCCACTGGAA
691    E   D   D   P   V   P   Q   E   D   E   K   V   D   A   E   M   P   P   L   E
2221   GGAGACGGTGAGGACGCTTCTAGGATGGAGGAGGTTGATTAAATTAATTGTGTATTTTTA
711    G   D   G   E   D   A   S   R   M   E   E   V   D   *
2281   ACAAACTAAGACTGTTTATGTACAGACTGTTGATTTTGTAATCTTGTACAGAGTTACATG
2341   TAAGACGACTAGGTTT AATAA CTTTTAATAGTTATTAATTTATTTCAATTGCAACAATTG
2401   TATTTCCAACCGTGTTTTGTATTTACTTGTGATCAAAACCAAATATCGTTCCTGTTCTTT
2461   TGTACATTTGTTTAATTTTAAGTAAATTGAATTAAATGCAATTCTTGTTTCTAAAAAAAA
2521   AAAA
```

Fig. 1. Nucleotide and deduced amino acid sequences of *Hsp90* from *E. onukii*. Five highly conserved motifs of the *Hsp90* family are underlined. The cytoplasm *Hsp90* C-terminal region (MEEVD) is emphasized with double underlines. A possible consensus signal sequence for polyadenylation is indicated with a box. The asterisk indicates the translational termination codon. Bold numbers indicate amino acid residue positions and nonbold numbers indicate nucleotide residue positions.

```
   1   CGTAACAAGTGACACAAGCGACTTGCTAGTGCGATACTTTTACTCAGTGCGATTATACGA
  61   GTGTTGTTATTGTGGAATTGTGTTTAGTGTATACTGTGAGTGGATATCATCATGCCGTCG
   1                                                        M   P   S
 121   AAAATGCCTGCTATTGGAATCGACCTGGGGACGACCTACTCCTGCGTCGGCGTCTGGCAA
   4     K   M   P   A   I   G   I   D   L   G   T   T   Y   S   C   V   G   V   W   Q
 181   CATGGAAAAGTGGAAATTATTGCTAACGATCACGGAAACAGGACAACTCCAAGCTATGTC
  24     H   G   K   V   E   I   I   A   N   D   H   G   N   R   T   T   P   S   Y   V
 241   GCCTTCACCGACACAGAACGATTGATCGGGGATGCCGCAAAGAATCAGGTAGCACTAAAT
  44     A   F   T   D   T   E   R   L   I   G   D   A   A   K   N   Q   V   A   L   N
 301   CCACAAAATACAGTTTTTGATGCTAAAAGACTGATTGGCAGAAAATATGATGATCCTAAA
  64     P   Q   N   T   V   F   D   A   K   R   L   I   G   R   K   Y   D   D   P   K
 361   ATTCAAGATGATATTAAACACTGGCCTTTCAAAGTAACTAATGATTGTACCAAACCAAAA
  84     I   Q   D   D   I   K   H   W   P   F   K   V   T   N   D   C   T   K   P   K
 421   ATTCAAGTTGAGTTTAAAGGGGAAACCAAAACCTTTGCTCCTGAAGAAATAAGCTCAATG
 104     I   Q   V   E   F   K   G   E   T   K   T   F   A   P   E   E   I   S   S   M
 481   GTATTGACTAAAATGAAAGAGATTGCCAGTGCTTACCTAGGAGCCCCTGTGAAAGATGCA
 124     V   L   T   K   M   K   E   I   A   S   A   Y   L   G   A   P   V   K   D   A
 541   GTCATCACGGTTCCTGCCTATCTCAATGATTCTCAAAGACAGGCCACTAAAGATGCAGGG
 144     V   I   T   V   P   A   Y   L   N   D   S   Q   R   Q   A   T   K   D   A   G
 601   GCAATCGCAGGTCTGAATGTACTCAGAATTATCAATGAACCTACGGCTGCTGCTTTAGCC
 164     A   I   A   G   L   N   V   L   R   I   I   N   E   P   T   A   A   A   L   A
 661   TATGGTCTTGACAAAAATCTGAAGGGTGAAAAAAATGTTTTGATTTTTGATCTTGGTGGT
 184     Y   G   L   D   K   N   L   K   G   E   K   N   V   L   I   F   D   L   G   G
 721   GGAACTTTTGATGTATCTATACTAAGCATCAGCGAGGGTTCCCTGTTTGAAGTCAAGTCA
 204     G   T   F   D   V   S   I   L   S   I   S   E   G   S   L   F   E   V   K   S
 781   ACAGCTGGTGACACACACTTGGGAGGTGAGGACTTCGATAACCGCCTGGTCAACCATTTG
 224     T   A   G   D   T   H   L   G   G   E   D   F   D   N   R   L   V   N   H   L
 841   GCAGATGAGTTCAAGAGGAAGTTTAAAAAGGACTTGAAAAGTAACTCAAGAGCTCTTCGC
 244     A   D   E   F   K   R   K   F   K   K   D   L   K   S   N   S   R   A   L   R
 901   AGATTGAGAACAGCAGCAGAGCGTGCCAAGAGAACTTTATCTTCAAGTACTGAAGCTGGA
 264     R   L   R   T   A   A   E   R   A   K   R   T   L   S   S   S   T   E   A   G
 961   ATCGAAATTGATGCTTTGATAGATGGCATTGACTTCTACACCAAGGTAACTCGAGCTCGT
 284     I   E   I   D   A   L   I   D   G   I   D   F   Y   T   K   V   T   R   A   R
1021   TTTGAAGAACTTTGTTCAGATCTTTTCCGTAGCACTCTTCAGCCTGTAGAGAAGGCGCTT
 304     F   E   E   L   C   S   D   L   F   R   S   T   L   Q   P   V   E   K   A   L
1081   ACTGATGCAAAGTTGGATAAAGCTTCAATTCATGATGTAGTTTTGGTCGGAGGTTCTACT
 324     T   D   A   K   L   D   K   A   S   I   H   D   V   V   L   V   G   G   S   T
1141   CGAATTCCAAAAATCCAAAGCTTACTGCAGAATTTCTTCTCAGGCAAACCGCTTAACCTT
 344     R   I   P   K   I   Q   S   L   L   Q   N   F   F   S   G   K   P   L   N   L
1201   TCTATCAACCCTGATGAAGCAGTAGCCTATGGAGCCGCCGTGCAAGCTGCCATCCTGAAC
 364     S   I   N   P   D   E   A   V   A   Y   G   A   A   V   Q   A   A   I   L   N
1261   GGAGACCAGAGCTCAGCCATCCAAGATGTCCTTCTGGTGGATGTCACCCCACTGTCCCTT
 384     G   D   Q   S   S   A   I   Q   D   V   L   L   V   D   V   T   P   L   S   L
1321   GGCATCGAGACTGCGGGTGGAGTAATGGCCAAGATCATCGAGCGCAACGCCAGGATTCCT
 404     G   I   E   T   A   G   G   V   M   A   K   I   I   E   R   N   A   R   I   P
1381   TGCAAGCAGAGTCAAACCTTCACCACCTACGCAGACAACCAGCCAGCTGTTACCATCCAG
 424     C   K   Q   S   Q   T   F   T   T   Y   A   D   N   Q   P   A   V   T   I   Q
1441   GTGTTCGAGGGAGAGCGAGCCATGACCAAGGACAACAACCTCCTGGGCACATTTGACCTG
 444     V   F   E   G   E   R   A   M   T   K   D   N   N   L   L   G   T   F   D   L
1501   ACAGGAATACCACCAGCTGCTCGCGGCGTACCCAAGATTGACGTCACGTTTGACCTTGAC
 464     T   G   I   P   P   A   A   R   G   V   P   K   I   D   V   T   F   D   L   D
1561   GCCAACGGCATCTTGAATGTGACCGCCAAGGATACAAGCTCTGGCAAATCAAGAGTATA
 484     A   N   G   I   L   N   V   T   A   K   D   T   S   S   G   K   S   K   S   I
1621   ACCATCCAAAACAACAAAGGAAGACTTTCCAAAGAAGAAATTGAAAGGATGGTCAATGAA
 504     T   I   Q   N   N   K   G   R   L   S   K   E   E   I   E   R   M   V   N   E
1681   GCTGAGAAGTACAAGGAAGAAGATGACAAGCAGAGGGAGAAGATTGCTGCAAGAAATCAA
 524     A   E   K   Y   K   E   E   D   D   K   Q   R   E   K   I   A   A   R   N   Q
1741   TTGGAGAGTTACATCTTCAGCGTGAAGCAGGCCGGTGGAAGAGTGTGGCGATAAACTGAAT
 544     L   E   S   Y   I   F   S   V   K   Q   A   V   E   E   C   G   D   K   L   N
1801   GACAGTGACAAATCTACAGTGAAGAGGGAATGTGAGGAGACCTTGAGATGGCTGGAGAAC
 564     D   S   D   K   S   T   V   K   R   E   C   E   E   T   L   R   W   L   E   N
1861   AACTCACTAGCTGATAAGGAGGAGTTGGAACACCGACTGCAGGAGTTGCAGAAGAAGTGC
 584     N   S   L   A   D   K   E   E   L   E   H   R   L   Q   E   L   Q   K   K   C
1921   ACTCCTTTCATGTCGAAGCTGCATGGAGCTGGCAACCCTGGTCAAGGACAAGGCTTCAAC
 604     T   P   F   M   S   K   L   H   G   A   G   N   P   G   Q   G   Q   G   F   N
1981   CATGAACAAGGCTTTAACCCTGGACAAGGCTTCAACCCTGGACAAGGCTTTAACCCTGGA
 624     H   E   Q   G   F   N   P   G   Q   G   F   N   P   G   Q   G   F   N   P   G
2041   CAAGGCTTCAACCCTGGACAAGGCTTCAACCCGGGCCAAGGCTTCAACCCAGGATACCAA
 644     Q   G   F   N   P   G   Q   G   F   N   P   G   Q   G   F   N   P   G   Y   Q
2101   GGACCTACAGTGGAAGAAGTTGACTGAATTCATTTGTAAAGATTTATTGATTTTTATTTA
 664     G   P   T   V   E   E   V   D   *
2161   AGACTGAATTGTATCATGTGAAAATTGTAAATATGTATAGTTTTTGACTGTTGAATTTAT
2221   TTTTTATTCACACAAAAGAAATGTAAAGTAATGTTCCTATTATGTACATAGTATAATGTA
2281   TTATTACTTTTGTGATCAATATTTGTTTTTACTTATGAAAATAAACACTCAATTTGTTTT
2341   AACAAAAAAAAAAAA
```

Fig. 2. Nucleotide and deduced amino acid sequences of *Hsp70* from *E. onukii*. Three highly conserved motifs of *Hsp70* family are underlined. The cytoplasm *Hsp70* C-terminal region (V/IEEVD) is emphasized with double underlines. A possible consensus signal sequence for polyadenylation is indicated with a box. The asterisk indicates the translational termination codon. Bold numbers indicate amino acid residue positions and nonbold numbers indicate nucleotide residue positions.
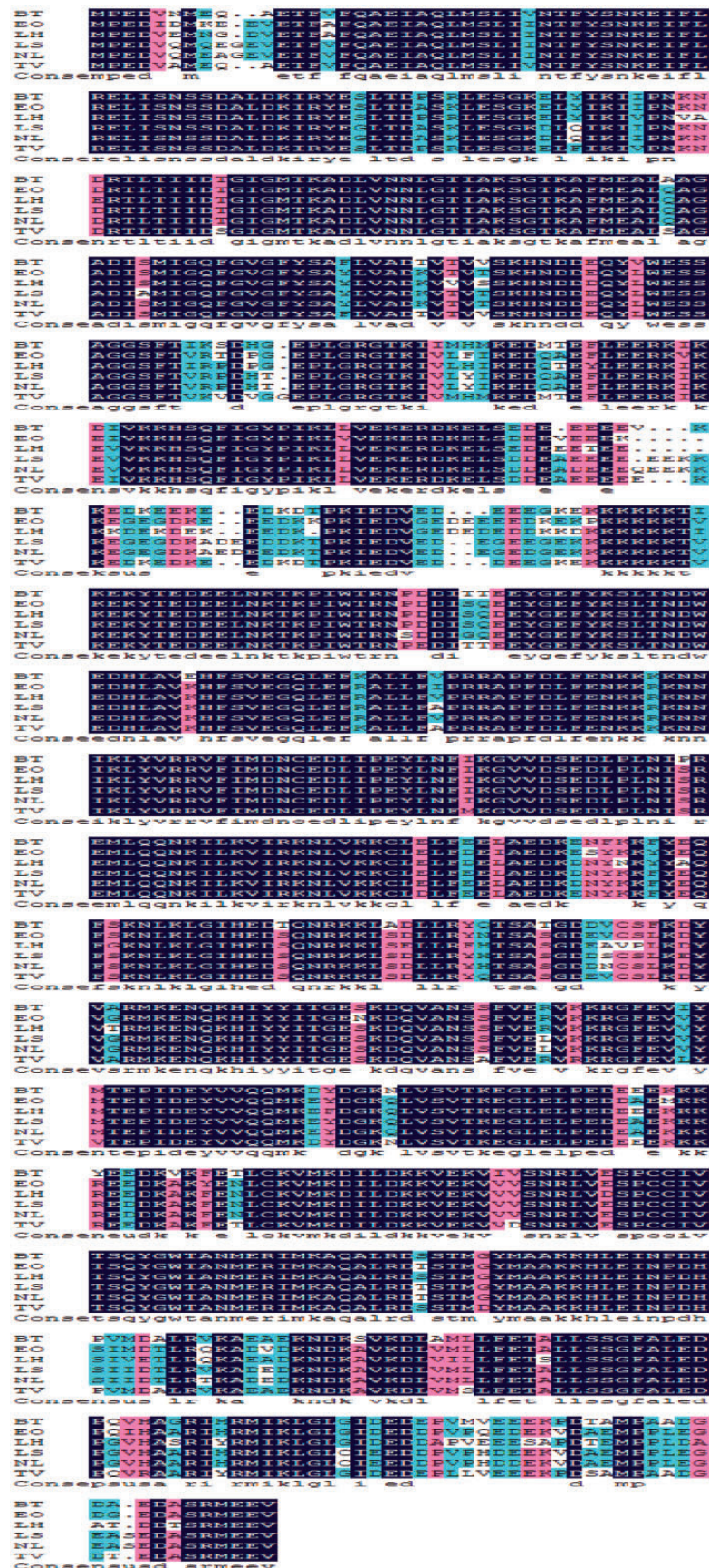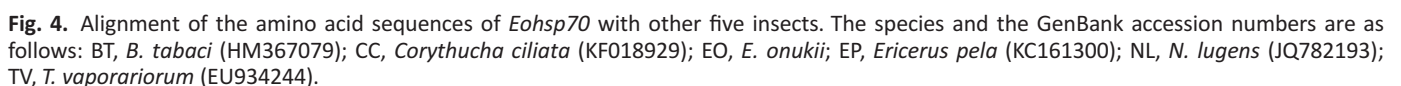
Fig. 3. Alignment of the amino acid sequences of *Eohsp90* with other five insects. The species and the GenBank accession numbers are as follows: TV, *T. vaporariorum* (FJ457626); BT, *B. tabaci* (HM367080); LH, *Lygus hesperus* (JX627809); EO, *E. onukii*; LS, *Laodelphax striatella* (KF660250); NL, *N. lugens* (GU723300).
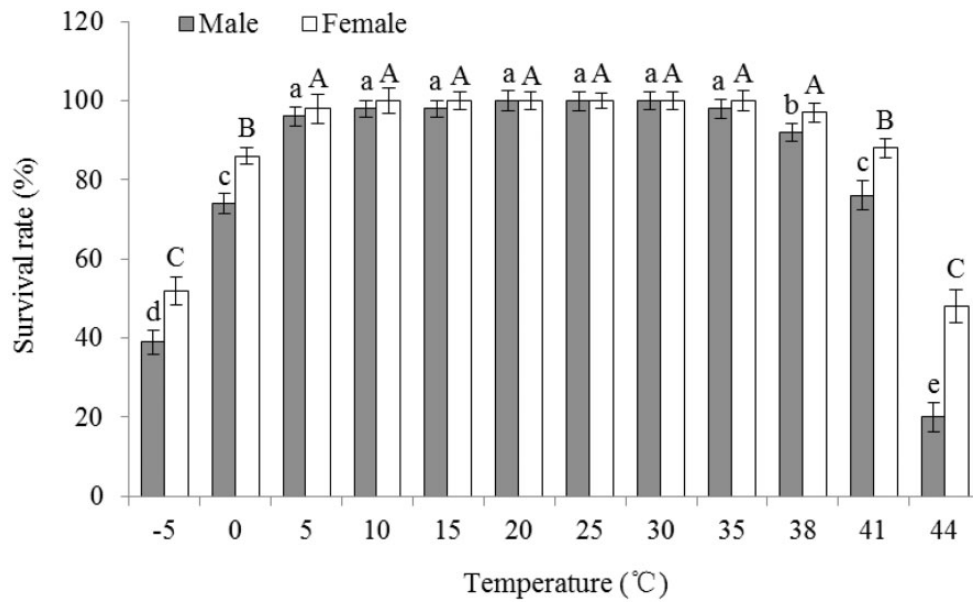
Fig. 4. Alignment of the amino acid sequences of *Eohsp70* with other five insects. The species and the GenBank accession numbers are as follows: BT, *B. tabaci* (HM367079); CC, *Corythucha ciliata* (KF018929); EO, *E. onukii*; EP, *Ericerus pela* (KC161300); NL, *N. lugens* (JQ782193); TV, *T. vaporariorum* (EU934244).

Fig. 5. Survival rates of *E. onukii* adults under different temperature treatments. Males and females of *E. onukii* were exposed to each temperature ranging from −5°C to 44°C for 1 h. Different letters on the error bars indicate significant differences among the different temperature treatments, and the lower case letters are used for males and the capital letters for females ($P < 0.05$).
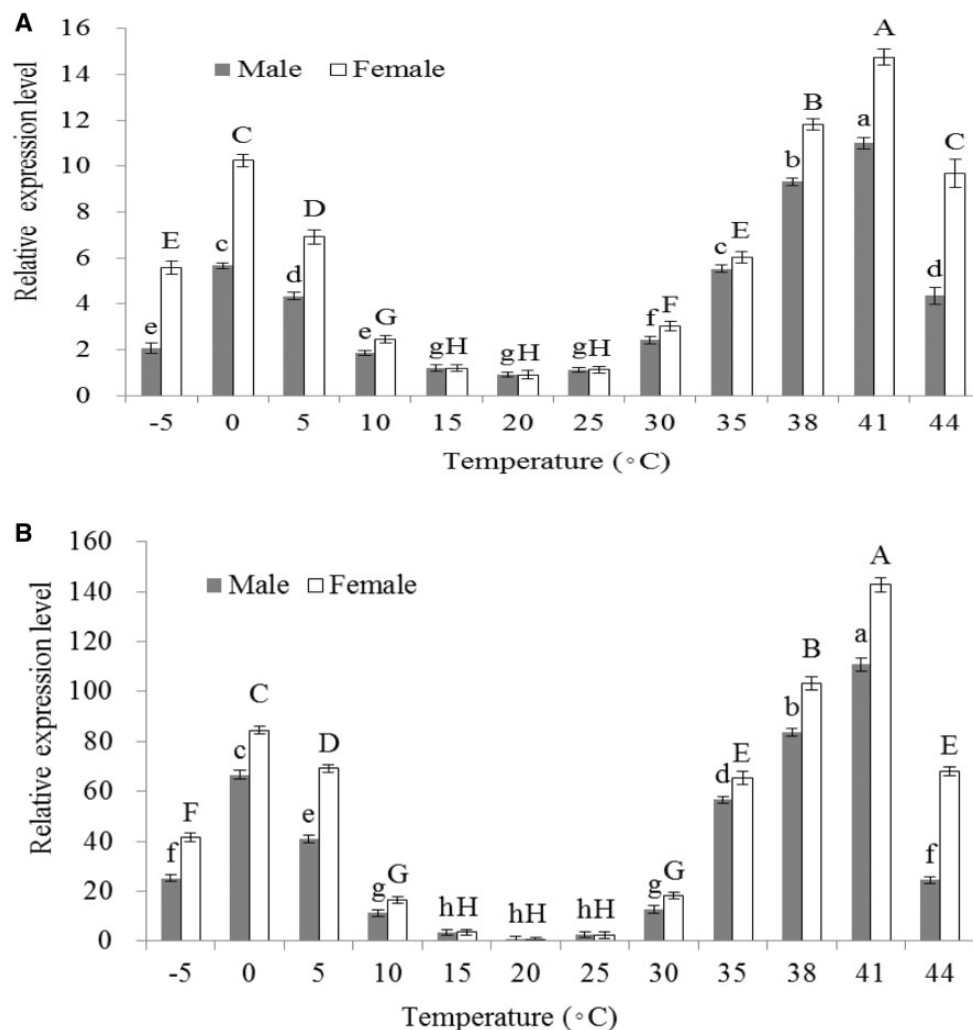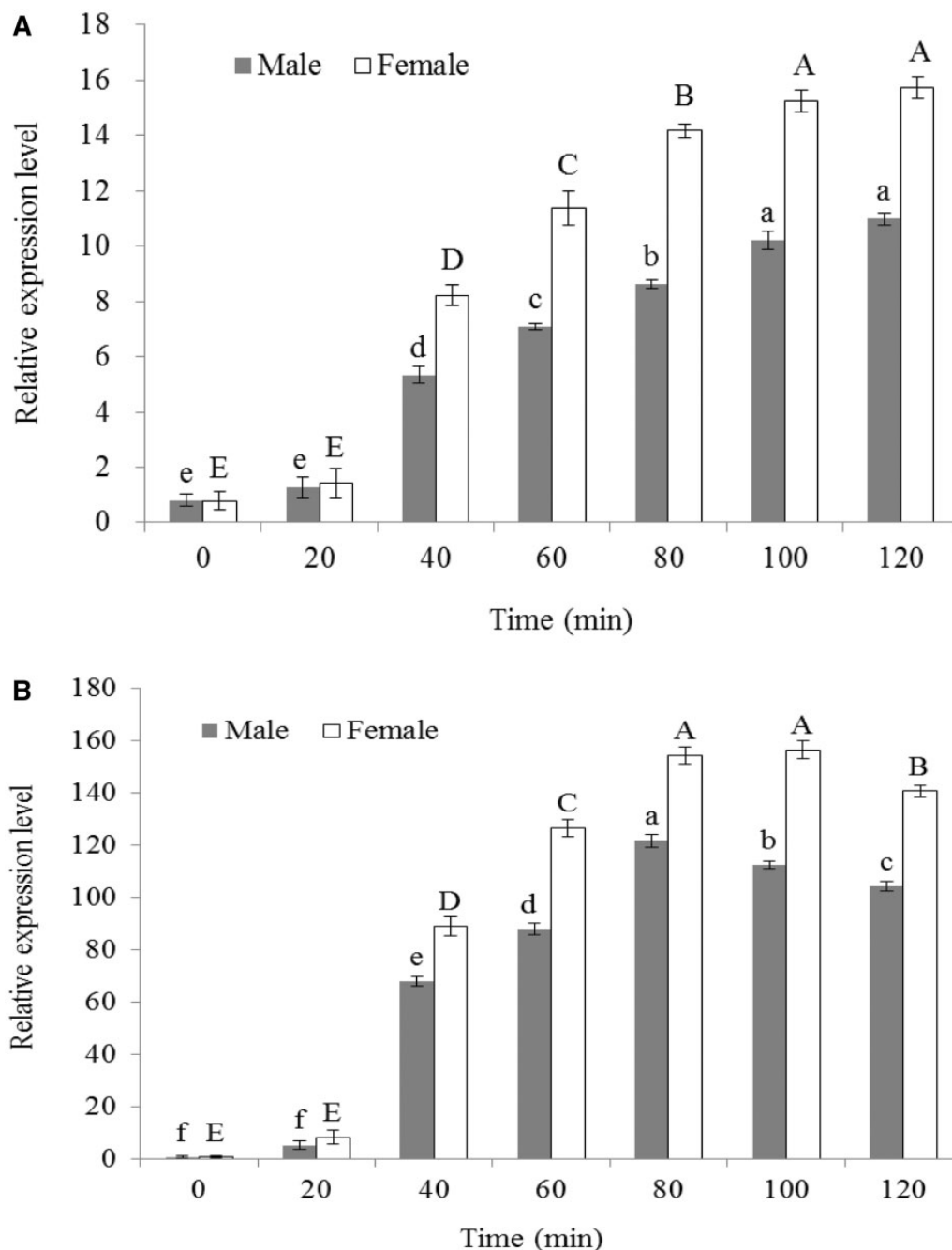

Fig. 6. Relative expression of *Eohsp90* (A) and *Eohsp70* (B) in males and females of *E. onukii* adults under different temperature treatments. The fold changes of each temperature treatment were relative to the calibrator (20°C treatment). Different letters on the error bars indicate significant differences among the different temperature treatments, and the lower case letters are used for males and the capital letters for females ($P < 0.05$).

Fig. 7. Relative expression of *Eohsp90* (A) and *Eohsp70* (B) transcripts subjected at 41°C heat treatment for different time durations in *E. onukii* adults. The fold changes of each time treatment were relative to the calibrator (0 min). Different letters on the error bars indicate significant differences between the sexes, and the lower case letters are used for males and the capital letters for females ($P < 0.05$).

there was a leveling off of expression for both sexes (Fig. 8A). The expression of *Eohsp70* showed a similar expression pattern as that of *Eohsp90* with a significant increase for both females and males in 20 min, and a continue increase to 80 min for males and 100 min for females, after that there were no further statistically significant increases in expression in 120 min (Fig. 8B).

Discussion

Understanding how *E. onukii* responds to heat and cold stress is crucially important to understand how they withstand extreme environmental temperatures. Hsps represent a class of molecular chaperones that are well known for their quick responses to environmental stress (Sørensen et al. 2003). This study focused on the full length of an ORF

and the encoding sequences for members of two major Hsp families, genes *Hsp90* and *Hsp70*, and the gene expression level in *E. onukii* Hsps in response to both heat and cold stress conditions.

Hsps are highly conserved in their coding regions (Feder and Hofmann 1999, Sørensen et al. 2003) and our results confirmed this high homology. The deduced amino acid sequences of two full-length transcripts of *Hsps* in *E. onukii* showed high similarity compared with their counterparts in other insect species. The identity range was 86–91% for *Eohsp90* and 74–81% for *Eohsp70*. The typical signature structures of the Hsp family and a conserved MEEVD or V/IEEVD motif at the C-terminals was also found in the deduced amino acid sequences. The conserved C-terminal motif MEEVD is suggested to bind to many cochaperones with small helical TRP domains (Pearl and
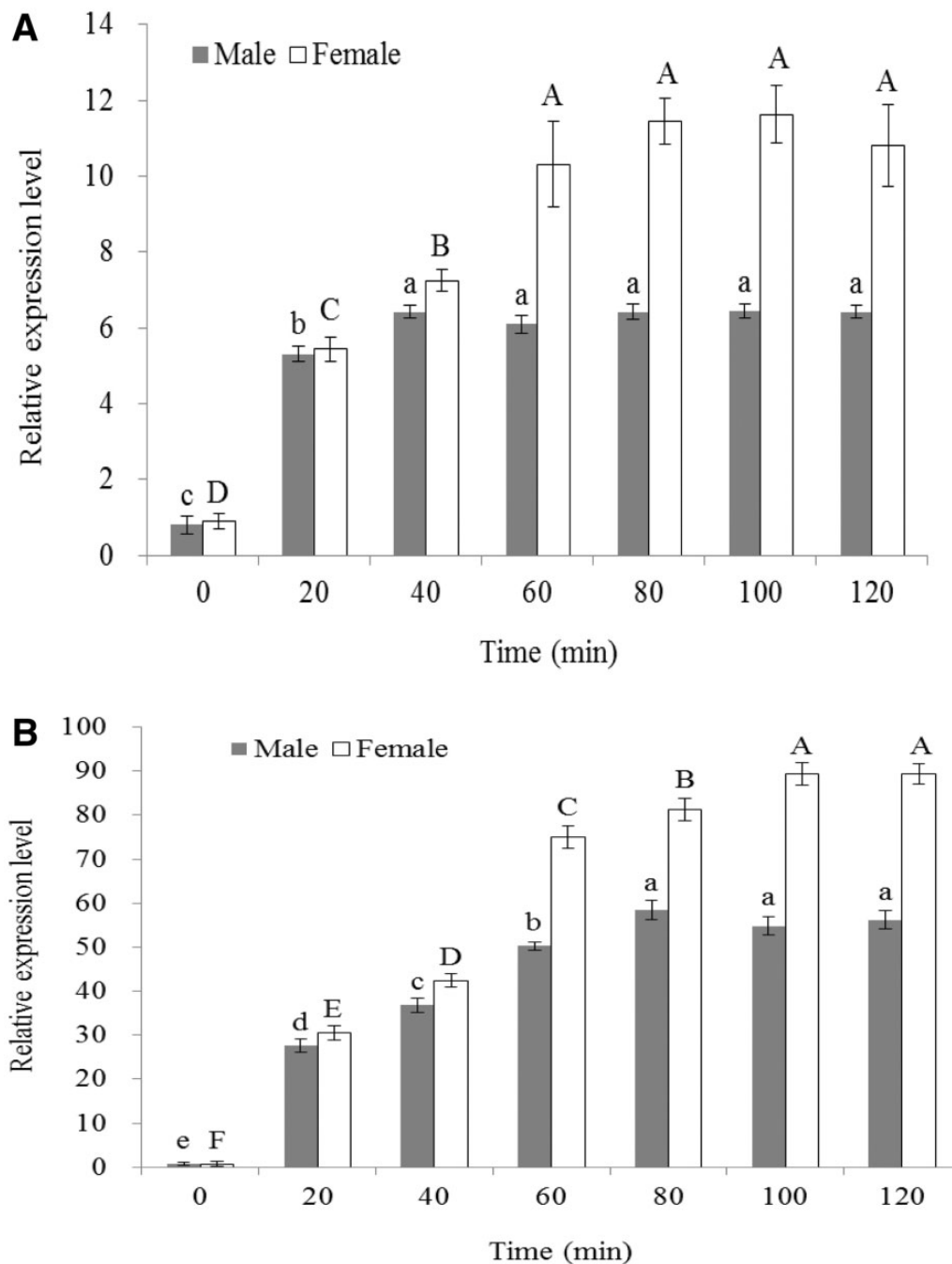
Fig. 8. Relative expression of *Eohsp90* (A) and *Eohsp70* (B) transcripts subjected at 0°C low temperature treatment for different time durations in *E. onukii* adults. The fold changes of each time treatment were relative to the calibrator (0 min). Different letters above the error bars indicate significant differences between the sexes, and the lower case letters are used for males and the capital letters are used for females ($P < 0.05$).

Prodromou 2006, Ramya et al. 2006), and this indicates that *Eohsp90* is a cytosolic Hsp (Gupta 1995). The conserved V/IEEVD motif enabled *Eohsp70* to bind with other cochaperones (Daugarrd et al. 2007). Synthesis of Hsps in response to cold and heat stress is well documented in many organisms (Parsell and Lindquist 1994, Dahlgaard et al. 1998, Sørensen et al. 1999, Kim et al. 2008, Chen et al. 2013, Zhu et al. 2013). However, in eukaryotes, Hsp families comprise multiple members that differ in inducibility and function (Feder and Hofmann 1999, Huang et al. 2009, Garczynski et al. 2011).

In this study, the relative expressions of *Eohsp90* and *Eohsp70* were upregulated following an increase or decrease of temperature except at the extreme −5°C and 44°C temperature points, and a general increase in expression was observed following prolonged exposure to high or low temperatures. The results suggested that *Eohsp90* and *Eohsp70* had a relationship with heat and cold temperature changes and perhaps play important roles in surviving temperatures stress for *E. onukii* adults. The relative expression of *Eohsp70* was about one order of magnitude higher than that of *Eohsp90* at high and low temperatures and even after exposure to extreme temperatures. For example, the maximum expression of *Eohsp70* could reach 8.72-fold increase, but it had only 4.87-fold increase for *Eohsp90*. This finding is consistent with the earlier study by Zhang and Denlinger (2010) in *Helicoverpa zea*. The *Hsp70*

family appears to be the most prominent contributor to temperature tolerance in insects, and other members of family, while not as highly expressed, are still a conspicuous element in temperature response (Denlinger and Yocum 1998).

Experiments proved that cold stress induces some Hsp genes in insects. For example, in the flesh fly, *Sarcophaga crassipalpis*, cold shock at either $-10°C$ or $0°C$ stimulated the synthesis of several Hsps (Joplin et al. 1990). Cold stress also upregulated expression levels of *Hsp90* and *Hsp70* (Rinehart and Denlinger 2000). In our study, the expression of Hsps increased as the cold temperature except decreased at the $-5°C$ temperature point, and the expression level when exposed to the high temperature was higher than the expression at the low temperature points. More interestingly, we found that expression levels of both *Eohsp90* and *Eohsp70* also varied in different sexes of *E. onukii*. The level of expression in females was much higher than that in males at moderately heat and cold temperatures and at unfavorable extreme temperatures after prolonged (at least 80 min) exposure. These results indicated that induced expression of Hsps in females of *E. onukii* is perhaps more sensitive than in males. However, the expression of Hsps in *E. onukii* under different environmental temperatures at the molecular level has not been reported.

Regarding to the survival rates of *E. onukii*, as the temperature rose ($>38°C$) or fell ($<5°C$), the survival rates of females were higher than males, which indicated that female adult is more thermal and cold tolerance than male. The result is consistent with the females adult number are higher than males, whether in hot summer or cold winter in tea plantation (L.Q., unpublished data).

Our results suggested that there was a maximal threshold level for the expression of Hsps. For example, the expression levels of *Eohsp90* and *Eohsp70* reached a peak at $0°C$ and $41°C$ and declined beyond those temperatures (e.g., $-5°C$ and $44°C$). The expression reached the highest level at $41°C$ after 100 min exposure for *Eohsp90* and 80 min for *Eohsp70* of in both females and males. The *Eohsp90* expression reached the highest level at $0°C$ after 40 min of exposure in males and 60 min of exposure in females. The *Eohsp70* expression reached the highest level at $0°C$ after 80 min of exposure in males and 100 min of exposure in females. It is impossible for the genes encoding Hsps to undergo unlimited amplification in an organism, and Hsps are therefore subjected to strict regulation by multiple molecular mechanisms (Feder and Hofmann 1999). Therefore, the ability of Hsps as molecular chaperones to completely mitigate the damage resulting from extreme thermal or cold stress and increase temperature tolerance and survival rates of insects is limited. To some extent, the death of adults at $-5°C$ and $44°C$ within 1 h indicated that Hsps expression cannot prevent death in severe temperature stresses, even for a short period of time in either male or female *E. onukii* adults.

In conclusion, Hsps of *E. onukii* could be induced by temperature stress, especially heat stress as supported by the dramatic increase in expression for *Eohsp90* and *Eohsp70*, at 35, 38, and 41°C temperatures. Both Hsps in this study were more sensitive to heat than cold based on differential gene expression between heat and cold temperatures. *Eohsp90* and *Eohsp70* showed different transcriptional expression profiles in response to temperatures stress in *E. onukii* adults and varied in induction between sexes. The induced expressions of *Eohsp90* and *Eohsp70* in female were at earlier time than male, which showed that female was more sensitive to high or low temperature than male. Our comprehension of Hsps in response to temperatures stress is crucial for understanding the capability of *E. onukii* to tolerate future changes to the environmental temperature. Ultimately, these novel molecular findings may aid in the development of protocols to control the *E. onukii* and other related pests thereby increasing tea yield.

Acknowledgments

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