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Effects of high temperature on life history traits and heat shock protein expression in chlorpyrifos-resistant *Laodelphax striatella*

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ABSTRACT

The resistance of the small brown planthopper (SBPH), *Laodelphax striatella*, to insecticides has been widely found in China, and has posed serious problems to efforts to control the pest. To determine the costs and benefits of resistance, the life tables of chlorpyrifos-resistant and -susceptible strains were constructed at 24 and 30 °C. The results showed the resistant SBPH (YN-CPF) had lower fitness at 24 °C, but slightly higher fitness at 30 °C compared to the susceptible SBPH. Transcriptomic analysis showed there are five heat shock protein genes changed their expression, and the up-regulated genes are *LsHsc70-1* and *LsHsc70-2*. The deduced amino acid sequences of *LsHsc70-1* and *LsHsc70-2* include three heat shock protein 70 (HSP70) family signatures, but LsHSC70-1 has the conserved HSP70 carboxyl terminal region of the "EEVD" motif, while LsHSC70-2 has the endoplasmic reticulum (ER) retention signal of the "KDEL" motif. The phylogenetic tree further identified *LsHsc70-1* has closer evolutionary distances to cytoplasmic/nuclear HSP70s from human and *Drosophila melanogaster*, while *LsHsc70-2* has closer evolutionary distances to HSP70s localized to ER. After treatment at 30–44°C, the expression of *LsHsc70-1* and *LsHsc70-2* was slightly increased in YN-CPF. These results suggested that *LsHsc70-1* and *LsHsc70-2* are members of *Hsc70* family, localized to the cytosol/nucleus and ER, respectively. The up-regulated expression of these genes may protect the chlorpyrifos-resistant pest against damage under high temperatures, increasing its relative fitness, but the lower relative fitness of this population under optimal temperature may be the trade-off.

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1. Introduction

Temperature has important effects on insect life history traits. Fitness is often decreased when the temperature of an insect's environment exceeds the range of suitable temperature [1–5]. Resistance to insecticides is another factor that determines the fitness of insects. In general, resistant populations have lower fitness at optimal temperatures [6–9] and experience greater fitness costs at suboptimal temperatures [10–13]. But some pests, such as permethrin-resistant *Culex pipiens* and acetamiprid-resistant *B. tabaci*, did not show increased fitness costs at high temperatures [14,15].

The small brown planthopper (SBPH), *Laodelphax striatella*, is mainly distributed in temperate zones [16]. It damages plants by sucking juices and transmitting viruses, such as the rice stripe virus and the maize rough dwarf virus [17–19]. Chlorpyrifos, an organophosphorus pesticide, was widely used to control SBPH in China. However, the resistance of SBPH to chlorpyrifos has been reported in the Chinese provinces of Shandong, Jiangsu, Fujian and Guangzhou [20]. Chlorpyrifos-resistant SBPH experiences a significant fitness costs at optimal temperatures [21]. But little is known about what fitness costs the resistant insects experiences at high temperatures.

Heat shock proteins are a set of conserved polypeptides that respond to abiotic stresses such as elevated temperature and chemical pesticides [22]. Heat shock proteins include HSP110, HSP90, HSP70, HSP60 and sHSP by molecular weight. The expression of heat shock proteins is induced by stressors such as temperature and insecticides [23–25], and the up-regulated expression of these genes is known to contribute to the insect's heat tolerance [25,26]. However, the expression profiles of heat shock protein genes in resistant insects and the relationship of these genes to fitness costs has not been well documented. To explore the effects and mechanisms of chlorpyrifos resistance on the adaptation of SBPH to the environment, the life history traits of chlorpyrifos-resistant and -susceptible SBPH at 24 and 30 °C was constructed, and the members of heat shock protein genes that have been involved in the

Abbreviations: SBPH, small brown planthopper; YN-CPF, chlorpyrifos-resistant *Laodelphax striatella*; YN, chlorpyrifos-susceptible *L. striatella*; *hsp*, gene encoding heat shock protein; HSP, heat shock protein; *Hsc70*, gene encoding heat shock cognate protein 70; HSC70, heat shock cognate protein 70; *Grp78*, gene encoding glucose-regulated protein 78; GRP78, glucose-regulated protein 78; ER, endoplasmic reticulum; qPCR, real-time quantitative PCR; ORF, open reading frame.

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evolution of chlorpyrifos resistance were screened. Then the characteristics and expression profiles of up-regulated heat shock protein genes were investigated.

2. Materials and methods

2.1. Insects

The YN strain of *L.striatella* was collected from Yunnan, China, in July 2001 and reared without exposure to any insecticides. The YN-CPF strain was developed from the YN strain by continuous selection with chlorpyrifos in the laboratory until the resistant level was >100 fold [27], then the resistant level was maintained. All insects were reared on rice seedlings at 27 ± 1 °C under a 14:10 h light:dark regime.

2.2. Life history traits

2.2.1. Larval survival and development period

The newly hatched larvae were transferred within 24 h to new glass tubes with rice seedlings, each tube with one larva, and these tubes were placed in artificial climate chambers (RH = 60%; light:dark = 14:10 h) at 24 and 30 °C. The number and stage of larvae that survived were recorded daily until adult emergence, and about 60 larvae were observed for every sample.

2.2.2. Adult longevity and fertility

The freshly emerged adults were collected and transferred to new glass tubes (one female and two males per tube with seedlings), which were placed in climate chambers at 24 and 30 °C. The seedlings were replaced daily for the first seven days, and then were replaced every five days until all adults were dead. The seedlings replaced were collected, and the number of hatched larvae was recorded daily until no larvae hatched for three consecutive days. The longevity was dated from adult emergence to death. Fertility was measured as the number of larvae that hatched.

2.2.3. Relative fitness

The relative fitness of the resistant strain was evaluated with the population trend index of the resistant strain divided by that of the susceptible strain. The formula for the population trend index is N_{n+1}/N_n [28], where N_n is the number of insects in the n generation and N_{n+1} is the number of insects in the n + 1 generation. When the value of relative fitness was >1, the resistant population had higher fitness than the susceptible population. By contrast, the resistant population had lower fitness than the susceptible population.

2.3. Transcriptomic sequencing for screening heat shock protein genes associated with chlorpyrifos resistance

2.3.1. Sample preparation for sequencing

The heat shock protein genes (*hsps*) related to chlorpyrifos resistance were screened by transcriptomic analyses, which were performed by Novogene Co., Ltd. Total RNA was extracted using Trizol Reagent (Invitrogen, Carlsbad, CA, USA). RNA quality and concentration were determined by 1% agarose gel electrophoresis and a NanoDrop spectrophotometer. A total of 1.5 μg RNA per sample was used as input material for the RNA sample preparations. Sequencing libraries were generated using NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, Ipswich, MA, USA) following manufacturer's recommendations, and index codes were added to attribute sequences to each sample. The clustering of the index-coded samples was performed on a cBot Cluster Generation System using TruSeq PE Cluster Kit v3-cBot-HS (Illumia), then the library preparations were sequenced on an Illumina HiSeq4000 platform and 150 bp paired-end reads were generated.

2.3.2. Screening heat shock protein genes associated with chlorpyrifos resistance

The differential expression analysis of resistant and susceptible strain was performed using the DESeq R package. DESeq provides statistical routines for determining differential expression in digital gene expression data using a model based on the negative binomial distribution. The resulting *P* values were adjusted using Benjamini and Hochberg's approach for controlling the false discovery rate [29]. Genes with an adjusted *P*-value <0.05 found by DESeq were assigned as differentially expressed. Then the *hsps* associated with chlorpyrifos resistance were screened and resequenced.

2.4. Real-time quantitative PCR

RNA was extracted from adults of the resistant and susceptible strains using the SV Total RNA Isolation System (Promega, Madison, WI, USA). Three replications of each treatment were carried out, and each replication contained either six females or ten males. When the induced expression profiles of LsHsc70-1 and LsHsc70-2 by heat treatment were examined, the adults were collected and transferred to 30, 33, 36, 39, 42 and 44 °C thermostat for 1 h, and allowed to recover at 26 °C for another 1 h; finally, the adults were collected for RNA extraction.

The first-strand cDNA was synthesized from 500 ng of total RNA using PrimeScript™ RT Master Mix (Perfect Real Time) (Takara, Japan). The relative expression levels of the genes were determined with real-time quantitative PCR (qPCR) using an ADP-ribosylation factor-like protein (GenBank accession number JF728807.1) as the reference gene [30,31]. The primer sequences are shown in Table 1. qPCR was performed on an Applied Biosystems 7300 thermocycler, and reactions contained 10 μl of SYBR® Premix Ex Taq™ (Takara, Japan), 1.0 μl of cDNA, 0.4 μl of ROX Reference Dye (50×), and 0.4 μl of 10 μM sense and antisense primers in a 20 μl total volume. The optimized cycling program was 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s and at 60 °C for 31 s; a final disassociation stage was automatically added with the 7300 System SDS software. Relative expression was calculated using the $2^{-\Delta\Delta CT}$ method [32].

2.5. Statistical analysis

Differences of larval development period and survival, adult longevity and fertility between the strains of YN and YN-CPF were calculated by SPSS 19.0. The means and differences were evaluated by ANOVA followed by the LSD test. For the relative expressions of *hsps* in YN-CPF and the mRNA expression of LsHsc70s under different temperatures, the means were also calculated by SPSS 19.0 with ANOVA. The differences were considered significant when the *P* value was <0.5.

3. Results

3.1. Effects of high temperature on life history traits

3.1.1. Effects of high temperature on larval survival and development period

High temperatures had a significant effect on larval survival, with the average survival rate being 48.5% at 30 °C and 92.8% at 24 °C in

Table 1
Sequences of the primers for real-time quantitative PCR.

Primers	Forward (5′-3′)	Reverse (5′-3′)
LsHsc70-1	GATGCCAAGATTGACAAGAGC	ATAGAATAGCAGCCTGGACGG
LsHsc70-2	TGGTGCTAAGGTGAGCGATTC	CAGCTTGGCGATGATTGG
LsHsp70-1	TCGTGTGTTGGAGTGTGGCA	GTTTTTCGGGTTCATGGCTA
LsHsp70-3	GACGAAGCAGGAAGTAAG	ATTGTTGGTCCATTGTGA
LsHsp4	GAGGAGAAGAAGGACGAACAC	TTTGAATGATTGGGATGGAC
Ref	TTGGACAGTATCAAGACCCATC	GCAGCAATGTCATCAATAAGC

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