Supplementary Material for YUAN, XIN, WEN-WU ZHOU, YING ZHOU, SU LIU, FANG LU, MAO-FA YANG, JIAAN CHENG, GEOFF M GURR AND ZENG-RONG ZHU−**Composition and Expression of Heat Shock Proteins in an Invasive Pest, the Rice Water Weevil (Coleoptera: Curculionidae).** Florida Entomologist 97(1) (March, 2014) at http://purl.fcla.edu/fcla/entomologist/browse

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ABSTRACT

Many factors affect the distribution of species in nature and temperature is one of the most profound. For poikilothermic groups such as insects, the capacity to adapt to different temperature regimes is particularly important for invasive species. To investigate the possible role of heat shock proteins (Hsps) in the success of the invasive pest, the rice water weevil (*Lissorhoptrus oryzophilus* Kuschel, Coleoptera: Curculionidae), we first analyzed the composition and expression profile of Hsp families under sub-lethal temperatures of 0 °C and 43 °C, using the quantitative real-time polymerase chain reaction. Eight genes coding Hsp90, Hsp70, and small Hsps were up-regulated under heat stress, while only 1 Hsp70 gene and 1 Hsp90 gene were up-regulated under cold stress. Results indicate that Hsps from all families except Hsp60 are responsible for the capacity of *L. oryzophilus* to tolerate temperature stress though more genes were up-regulated, and more rapidly, under heat stress than under cold stress. A second study compared Hsp expression patterns in diapausing and non-diapausing female adults. Insects in diapause up-regulated no Hsp gene but 4 small Hsps, 2 Hsp90, 1 Hsp70 and 1 Hsp60 genes were down-regulated.

Key Words: diapause, heat shock protein, invasive species, thermal tolerance

RESUMEN

Muchos factores afectan la distribución de especies en la naturaleza y la temperatura es uno de los más profundos. Para grupos de poikilotérmicos como los insectos, la capacidad de adaptación

a diferentes regímenes de temperatura es para especies invasoras especialmente importante. Para investigar el posible papel de proteínas de choque térmico (Pcts) en el éxito de la plaga invasiva, analizamos primeramente el perfil de la composición y la expresión de las familias de Pcts bajo temperaturas sub-letales de 0 ° C y 43 ° C en el gorgojo del agua de arroz (*Lissorhoptrus oryzophilus* Kuschel, Coleoptera : Curculionidae), utilizando la reacción en cadena de la polimerasa cuantitativa en tiempo real. Ocho genes codificantes de Pct90, Pct70, y pequeños Pcts fueron incrementados bajo condiciones de estrés térmico, mientras que sólo 1 gen Pct 70 y 1 gen Pct 90 fueron incrementados bajo condiciones de estrés por frío. Los resultados indican que las Hsps de todas las familias menos la Pct 60 son responsables por la capacidad de *L. oryzophilus* para tolerar el estrés de temperatura, aunque más genes fueron incrementados más rápidamente bajo condiciones de estrés por calor que bajo estrés por frío. Un segundo estudio comparó los patrones de expresión de Pct en hembras adultas en diapausa y no diapausa. Los insectos en diapausa no incrementaron el gen Hsp pero si redujeron 4 genes pequeños de Pcts - 2 Pct 90, 1 Pct 70 y 1 Pct6.

Palabras Clave: diapausa, proteína de choque térmico, especies invasoras, tolerancia térmica

Suppl. Fig. 1. Alignment comparison of deduced amino acid sequences of heat shock protein families of the rice water weevil, *L. oryzophilus*.

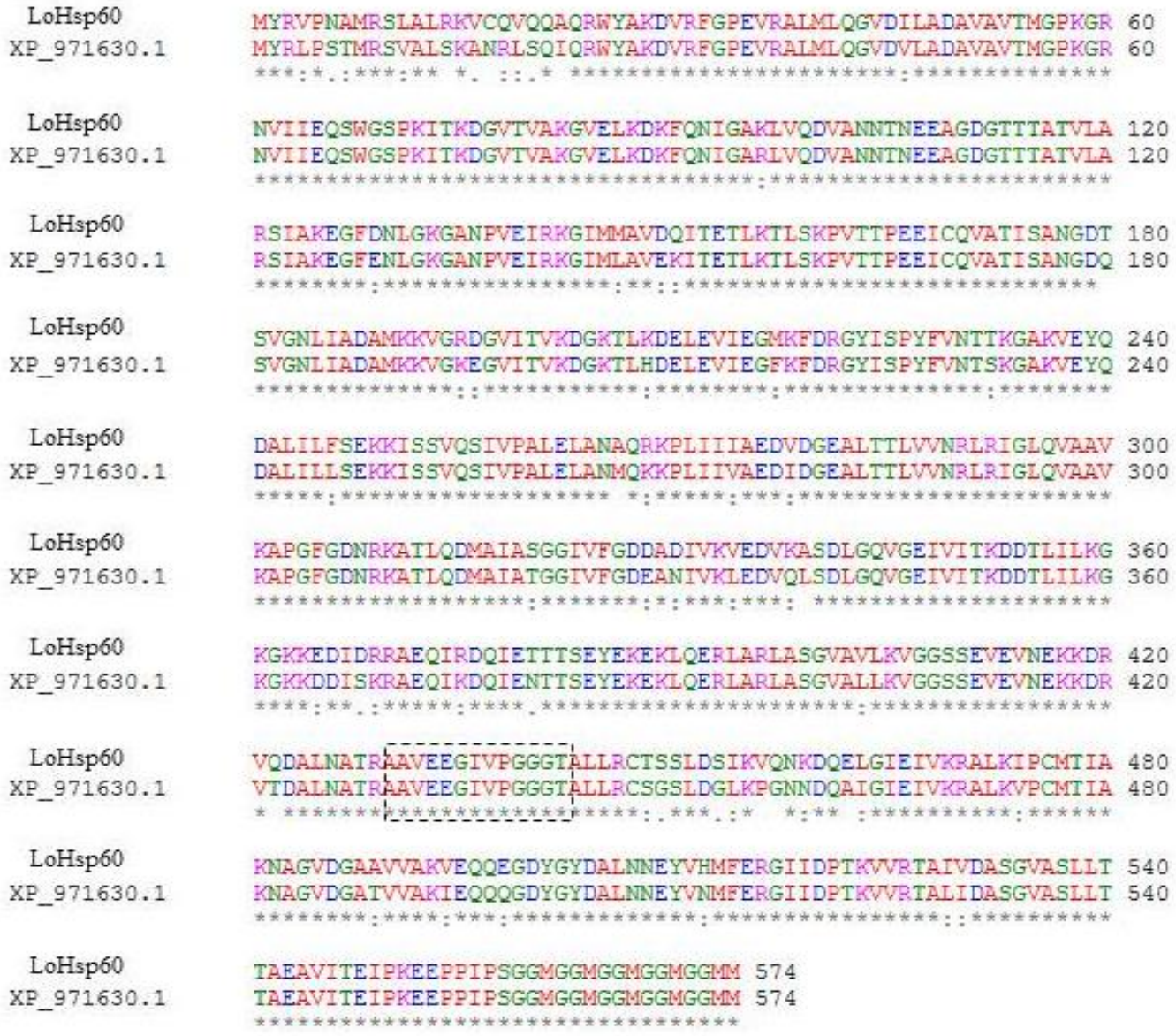

Fig. 1a

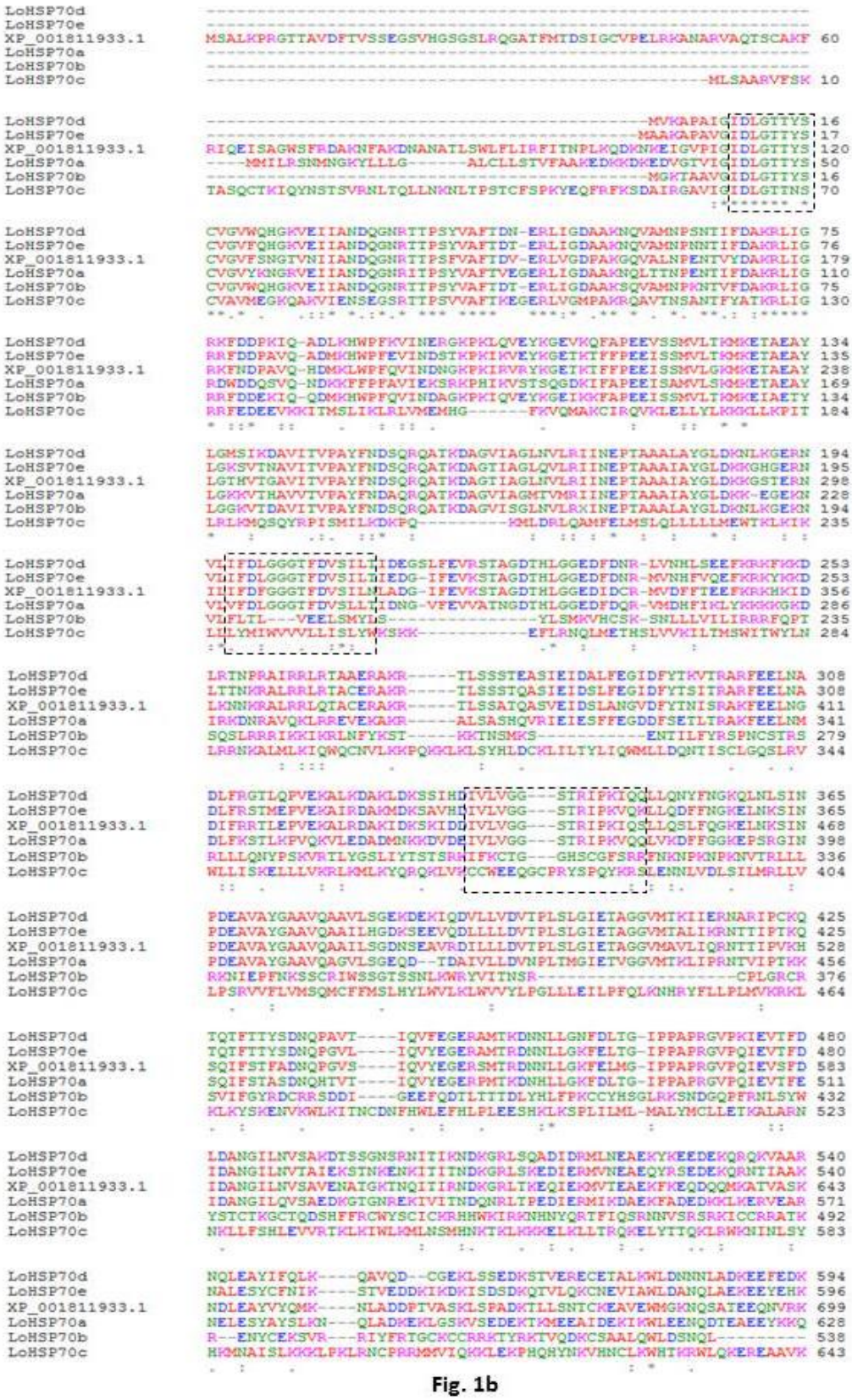
LoHSP70d
LoHSP70e
XP_001811933.1
LoHSP70a
LoHSP70b
LoHSP70c

Fig. 1b

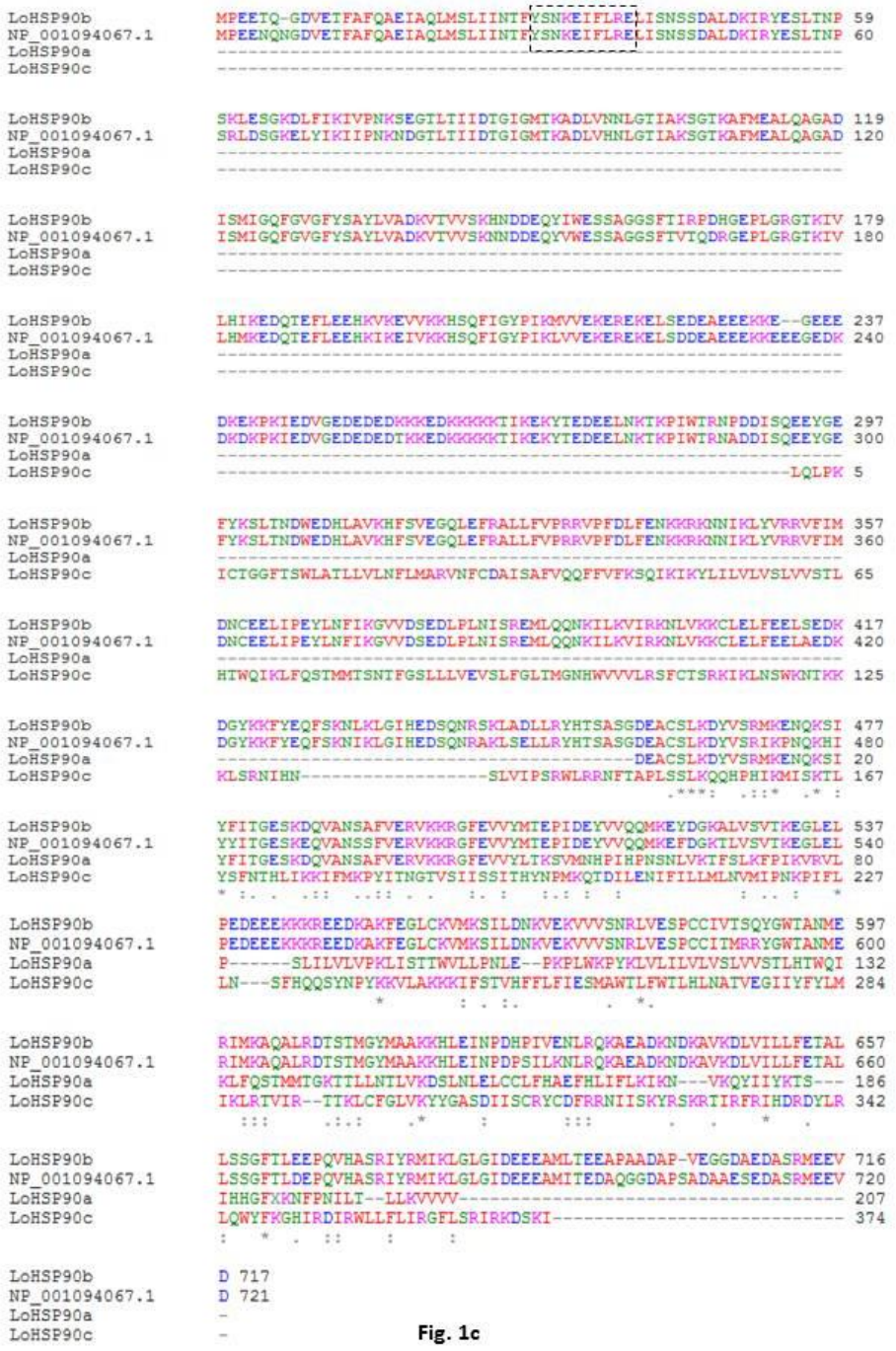

Fig. 1c

Suppl. Fig. 1. Alignment comparison of deduced amino acid sequences of heat shock protein families of the rice water weevil, *L. oryzophilus*. (a) Hsp60; (b) Hsp70; (c) Hsp90. Hsp60 of *Tribolium castaneum* (XP_971630.1), Hsp70 of *Tribolium castaneum* (XP_001811933.1) and Hsp90 of *Tribolium castaneum* (NP_001094067.1) were used as the reference genes. Symbols below the alignment indicate the same residue (*), a strong positive residue (:) and a weaker positive residue (.), respectively. The last residue in each line is assigned a nnumber. The motif sequences are indicated by dot-line boxes.

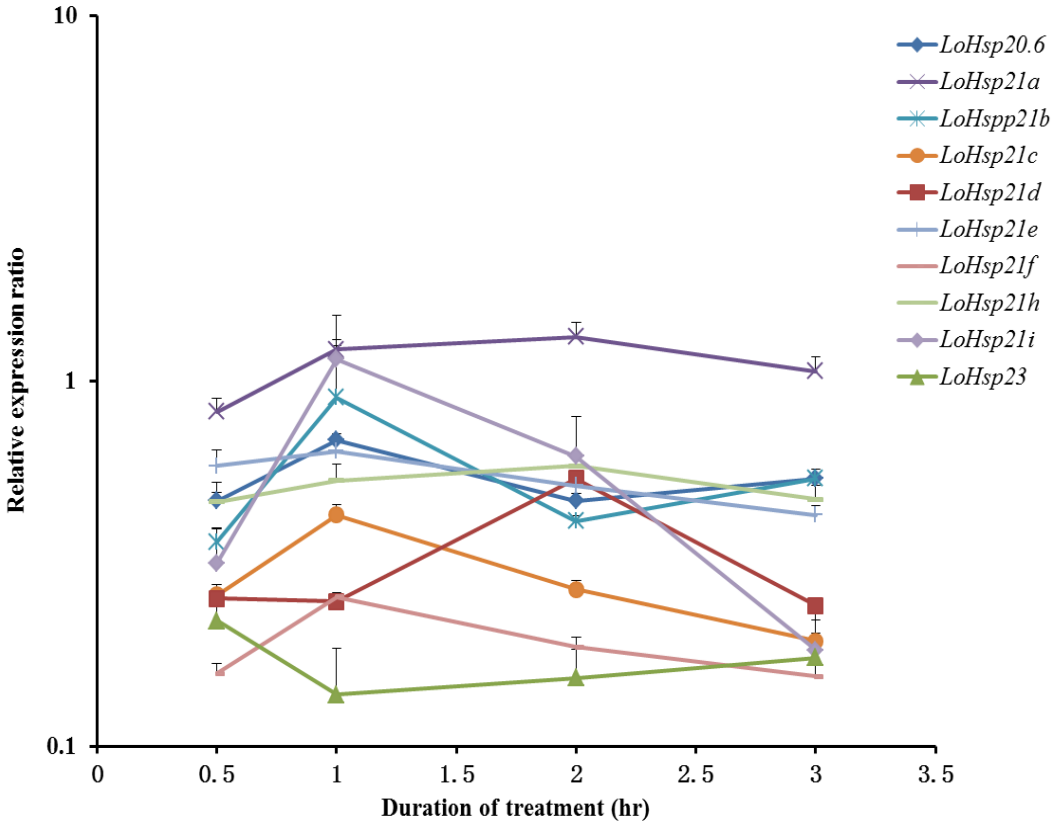

Fig. 1. Relative expression of *L. oryzophilus* small Hsps under cold stress (0 ℃) compared to non-stressed controls.

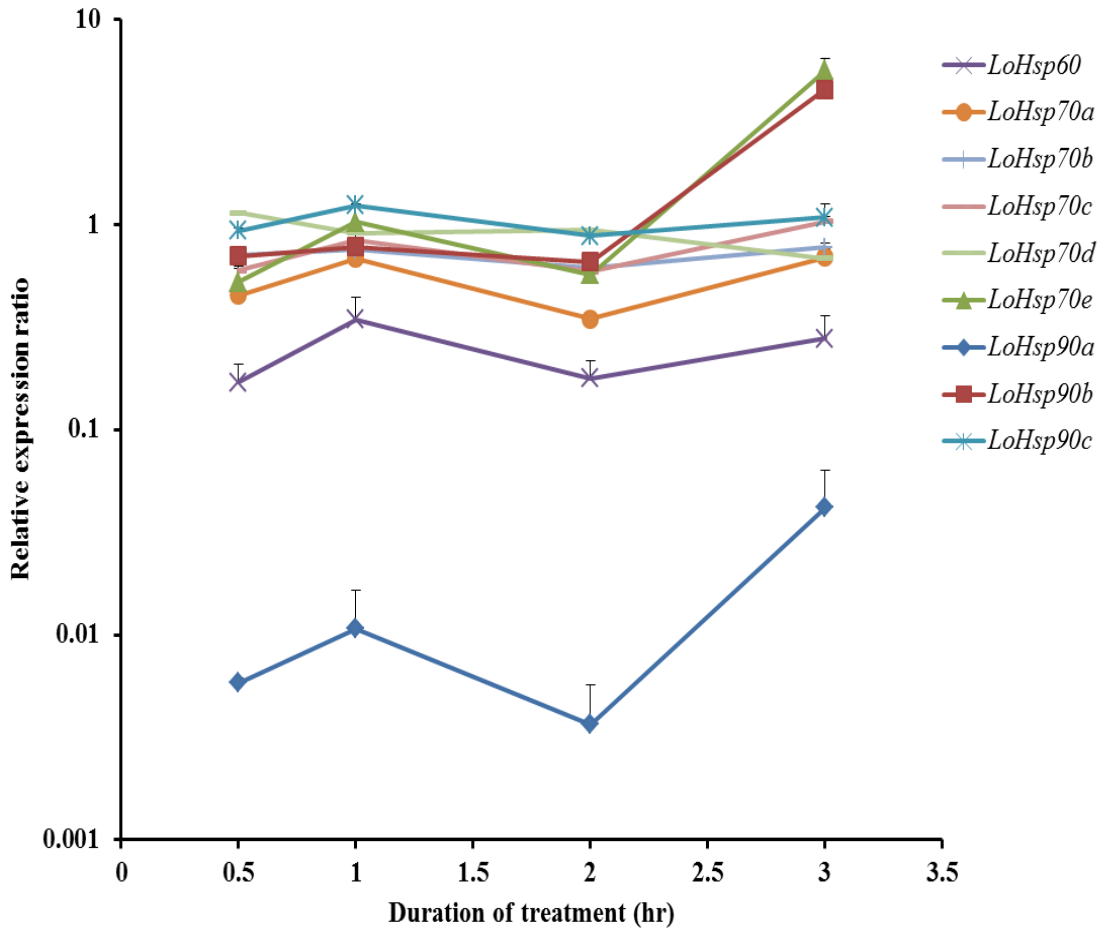

Fig. 2. Relative expression of *L. oryzophilus* Hsp60, Hsp70 and Hsp90 genes under cold stress compared to non-stressed controls.

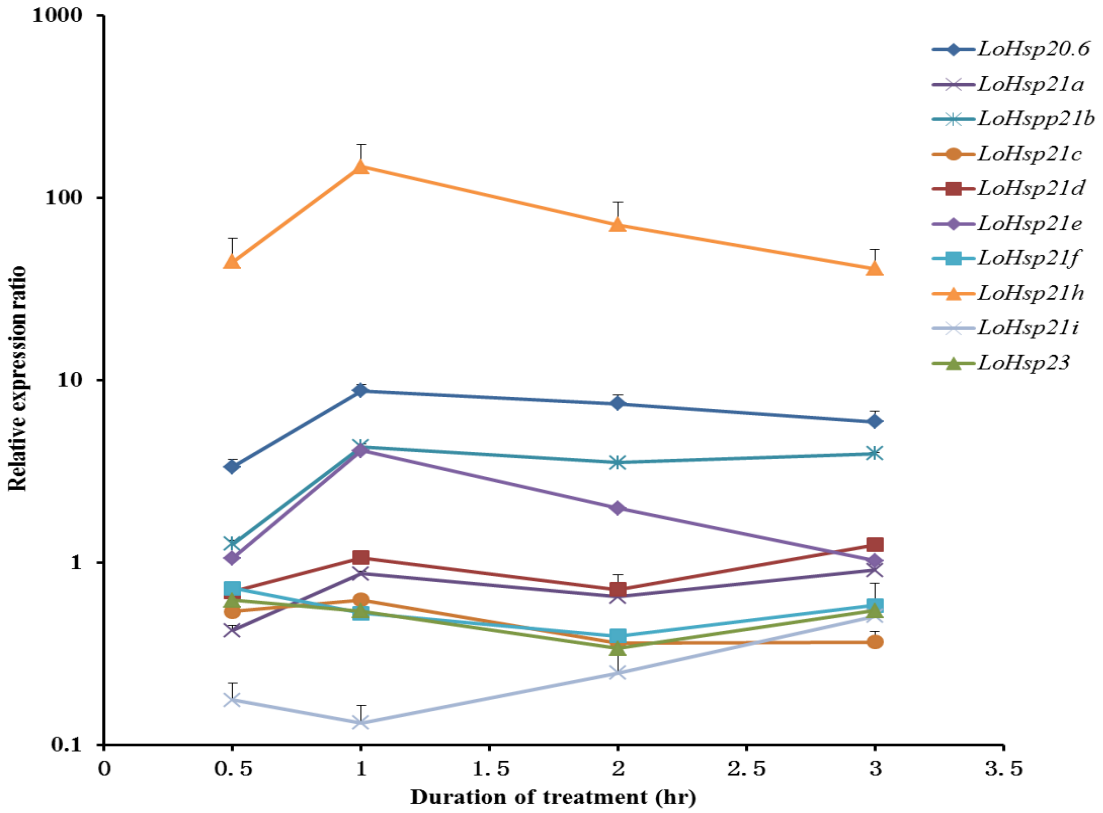

Fig. 3. Relative expression of *L. oryzophilus* small Hsps under heat stress compared to non-stressed controls.

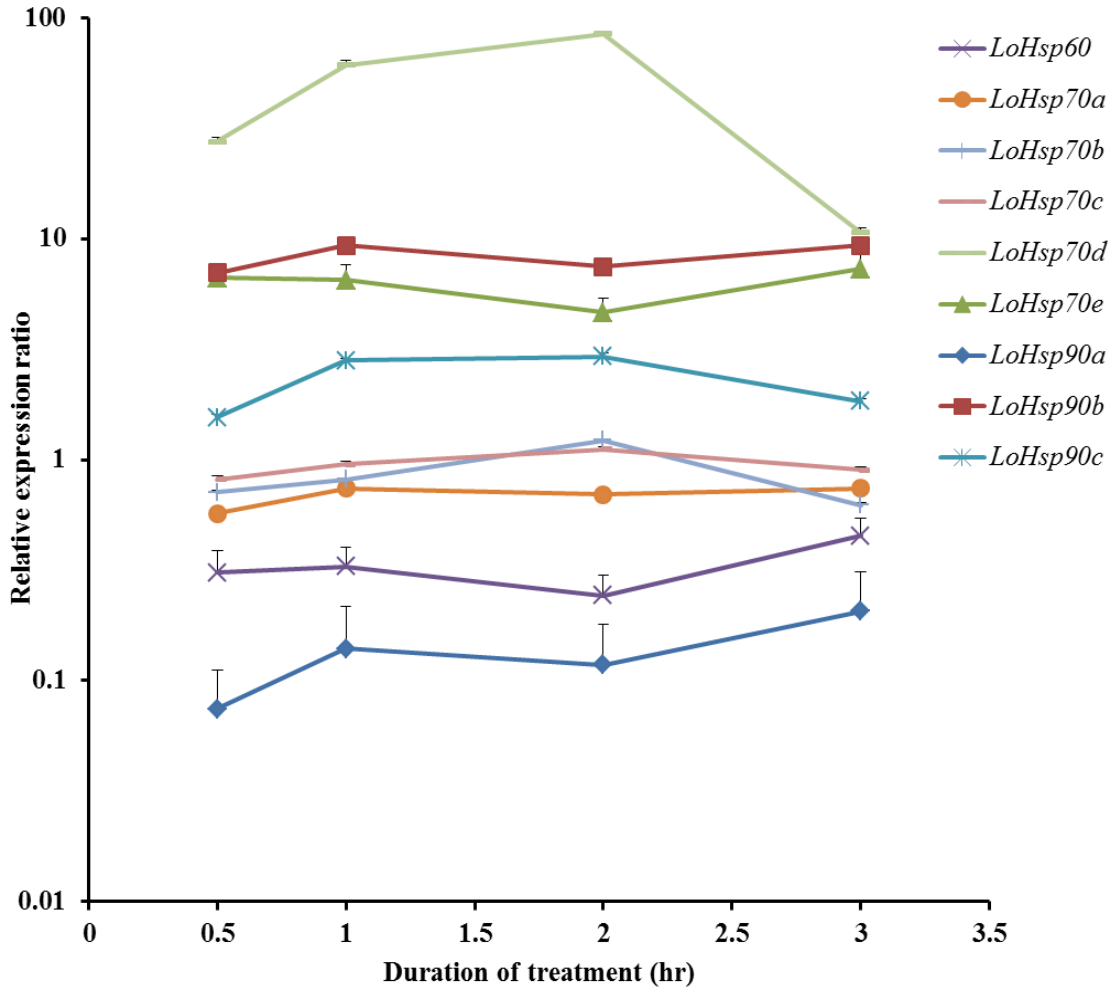

Fig. 4. Relative expressions of *L. oryzophilus* Hsp60, Hsp70 and Hsp90 genes under heat stress compared to non-stressed controls.

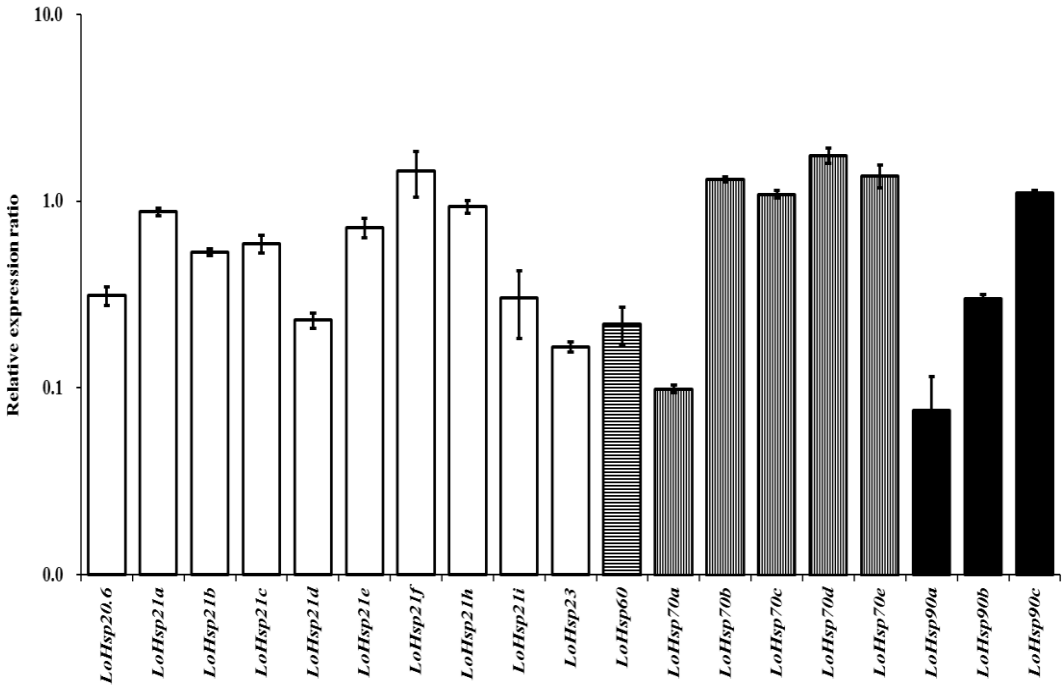

Fig. 5. Relative expression of Hsp genes in diapauseing adult females compared to non-diapause controls.