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Impact of mild temperature hardening on thermotolerance, fecundity, and Hsp gene expression in *Liriomyza huidobrensis*

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Abstract

The pea leafminer, *Liriomyza huidobrensis*, is one of the most important economic insect pests around the world. Its population fluctuates greatly with seasonal change in China, and temperature was thought to be one of the important reasons. In attempt to further explore the impact of disadvantageous temperature on *L. huidobrensis*, 1-day-old adults were shocked at various temperatures (10, 25, 32, and 35 °C, respectively) for 4 h, and the effects on thermotolerance, feeding, and fecundity were studied. Meanwhile the expression of five heat shock genes (*hsp90*, *70*, *60*, *40*, and *20*) was examined by real-time quantitative PCR. Our results showed that both 32 and 35 °C hardenings remarkably increased adult heat resistance, whereas cold tolerance was not improved accordingly. No cross resistance in response to cold and heat stresses was observed. Both adult feeding and fecundity were dramatically reduced, but no effect was observed on egg hatching, larval survival, pupal eclosion, or sex ratio. The results indicate that the deleterious effect on fecundity is the result of direct cessation of oviposition during the period of stress. Simultaneously, the mRNA levels of *hsp70* and *hsp20* significantly increased upon thermal hardening. Taken together, our results suggest that mild heat hardening improves thermotolerance of *L. huidobrensis* at the cost of impairment on fecundity, and the induced expression of *hsp70* and *hsp20* may play an important role in balancing the functional tradeoff.
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Keywords: Heat shock proteins; Thermotolerance; Fecundity; Tradeoff; Cross resistance

1. Introduction

The pea leafminer, *Liriomyza huidobrensis*, is one of the most important economic insect pests on vegetables and ornamentals. Female adults puncture the leaf surface with their ovipositors, feed on the leaf tissue, and insert their eggs into leaves. When the eggs hatch, the larvae mine within the leaf tissue, forming serpentine tunnels. The leafminers can severely reduce plant yield, transport viral and fungal diseases (Civelek and Önder, 1999), and even kill the plants at high fly density (Spencer and Steyskal, 1986). First found in China in 1993, they have spread to more than 15 provinces, and can occur throughout the year (Kang, 1996; Chen and Kang, 2002). The populations of *L. huidobrensis* fluctuate seasonally in nature, and dominate their congeners such as *Liriomza sativae* in late spring and autumn, whereas decrease their abundance in hot summer (Chen and Kang, 2002). Recent studies have shown that thermotolerance plays an important role in determining seasonal abundance and geographic distribution in these *Liriomyza* species (Chen and Kang, 2002, 2005).

Temperature is one of the most important variables that affect insects (Cossins and Bowler, 1987; Worner, 1998; Bale et al., 2002). The disadvantageous temperatures can significantly affect development. Leibee (1984) found that high temperature (35 °C) decreased pupal viability of *Liriomza trifolii*. At extreme temperatures, individual development of *Drosophila* would not proceed through the whole life cycle (Chakir et al., 2002). Another important effect of temperature is to decrease fecundity. The threshold temperatures generally limited reproduction (Schnebel and Grossfield, 1984) in most tropical and temperate species when they are continuously exposed to

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the disadvantageous temperatures. In *Drosophila* males, sterility was induced at temperatures above 30 °C and below 12 °C (David and Clavel, 1969).

During evolutionary history, insects have evolved many behavioral and physiological strategies such as seeking shelters, changing the fluidity of cell membranes, and accumulating sugars, polyols, antifreeze proteins and amino acids (Storey, 1997; Duman, 2001; Wang and Kang, 2005a) to avoid temperature impairments. One of the most important physiological adaptations to temperature stress is to induce the expression of heat shock proteins (Hsps). When organisms are exposed to a variety of stress factors such as cold, heat, CO_2, heavy metal, and various chemicals (Lindquist, 1986; Hoffmann and Parsons, 1991; Krishna et al., 1992; Ferrando et al., 1995), they synthesize a set of Hsps, which usually act as molecular chaperones, and play diverse roles in transporting, folding, assembling of degraded or misfolded proteins (Johnston et al., 1998; Sørensen et al., 2003). Hsps can be divided into several families including Hsp90, 70, 60, 40 and small Hsps (sHsps) on the basis of molecular weight and homology of amino acid sequences (Feder and Hofmann, 1999; Sørensen et al., 2003). The sHsps contain an α-crystalline domain with molecular weights of 12–43 kDa due to the variable N- and C-terminal extensions (MacRae, 2000; Taylor and Benjamin, 2005). The Hsps have been shown to provide protection from thermal killing (Gehring and Wehner, 1995), whereas overexpression brings disadvantages in cell division (Feder et al., 1992), development (Krebs and Feder, 1997), and reproduction (Krebs and Loeschcke, 1994; Silbermann and Tatar, 2000).

The thermotolerance and distribution of *L. huidobrensis* have been clearly revealed after they invaded China in 1993 (Chen and Kang, 2002, 2004, 2005). However, the impact of disadvantageous temperature on the population remains unclear. In this study, we investigated the effects of mild temperature hardening on adult thermotolerance, feeding, and fecundity. Meanwhile, the mRNA levels of five *hsp* genes were examined. We attempt to address the following questions: (1) How does mild temperature hardening affect the pea leafminer? and (2) what kinds of *hsps* are induced during the process? The answers may help elucidate the mechanism of seasonal population dynamics of *L. huidobrensis*.

2. Materials and methods

2.1. Insect samples

The laboratory-reared population of *L. huidobrensis* was originally collected on celery in Beijing in 2001, and maintained at 25–26 °C on the bean plant Phaseolus vulgaris following protocols outlined by Chen and Kang (2002). Beans were seeded by 2–3 plants per pot (12 cm in diameter). Ten days after germination, eight pots of plants about 20 cm tall were put into a wood cage (40 × 40 × 40 cm) with mesh Saran screening draped over it. About 100 adults (1:1 sex ratio) were raised per cage. The detailed procedure was described by Chen and Kang (2005). The pupae were kept in a glass tube and blocked with cotton. A piece of filter paper saturated with honey water was put in the tube to provide adults with food. The 1-day-old adults were used in the test.

2.2. Thermotolerance

Forty adults (1:1 sex ratio) were collected in a 5 mL cryogenic tube, exposed to the target temperatures (10, 25, 32, and 35 °C) for 4 h, then allowed to recover at 25 °C for 1 h. Recovery adults were kept at 41 °C for 1 h and −12 °C for 4 h to examine the heat or cold tolerance, respectively. Flies were tallied as surviving if they responded to gentle prodding after 1 h recovery at 25 °C. The temperature alterations were achieved by submersing the tubes into a glycol bath (Programmable Temperature Controller, Polyscience®). Each treatment was repeated four times.

2.3. Adult feeding and fecundity

Forty adults (1:1 sex ratio) were respectively exposed to 10, 25, 32, and 35 °C for 4 h, and then raised with a pot of plants in a 20 × 20 × 34 cm (length × weight × height) cage. A fresh plant was supplied every day until the flies died. Fecundity is often indicated by the amount of eggs laid (Leibee, 1984; Sæthre and Hofsvang, 2002). An oviposition puncture was taken to indicate the presence of an egg (Facknath, 2005). The numbers of feeding and oviposition punctures were counted by examining leaves under a stereomicroscope according to the method of Reitz and Trumble (2002). The feeding and oviposition punctures can be distinguished from each other by the fact that most of the feeding punctures are located on the upside of leaves, while the oviposition ones are mainly present on the underside. Moreover, the feeding punctures are much bigger than the oviposition ones, and the former are usually visible by the naked eyes (Luo et al., 2001). The number of 1-instar larvae was checked according to their tunnels 4 days after the plant was inoculated. Pupae were collected, and the adult amount and sex ratio (male: female) were recorded 10 days after eclosion. The 25 °C hardening samples were used as the control, and each treatment was repeated four times.

2.4. Real-time quantitative PCR

Forty adults (sex ratio 1:1) were treated at different temperatures as above, recovered for 1 h at 25 °C, and then frozen quickly in liquid nitrogen and stored at −70 °C. Each treatment was repeated three times. The total RNAs were isolated using the RNeasy® Mini Kit (Qiagen), and 2 μg RNAs were used to generate the cDNAs. The mRNA amounts of Hsps were quantified by real-time quantitative PCR. The PCR reactions were performed in a 20 μL total reaction volume including 10 μL of 2 × SYBR® Premix EX

Table 1
Primer sequences used in the real-time quantitative PCR

Gene	Primer sequence (5′→3′)	Fragment length (bp)
hsp90	CATCACAATACGGTTGGTCTGC CTTGCCACTCATGTAGCCCAT	92
hsp70	CTTTGACTTGGGTGGCGGTA GACGCAAGGCTCTGGGATT	197
Hsp60	ATTCGTCGTGGTGTCATGTTGG GCTGAGATGGTGGCTACTTGAG	110
Hsp40	ATTAGGCGGTGGTGCTTTTCG GAGCCAAGGACATGCGTGAGA	167
hsp20	AGTAGAGGGGAAGCACGAGGA CTTCATAGGGGCACGCACA	154
β-actin	TGACTGAAGCCCCATTGAACC GCGACCAGCCAAGTCCAAAC	236

Taq™ (TaKaRa) master mix, 5 μM each of gene-specific primers (Table 1), and 1 μL cDNA templates. They were carried out on the Mx 3000P detection system (Stratagene), and the parameters were as follow: 10 s at 95 °C, then 40 cycles of 5 s at 95 °C, 20 s at 58 °C and 20 s at 72 °C, then one cycle of 30 s at 95 °C, 30 s at 58 °C and 30 s at 95 °C in order to produce the melting curves, which can be used to judge the specificity of PCR products. A standard curve was derived from the serial dilutions to quantify the copy numbers of target mRNAs. The relative level of each *hsp* was defined as the increased folds compared with the amount of *β*-actin. The *hsp70* mRNAs of the control were examined in every PCR plate to eliminate the systematic error.

2.5. Statistical analysis

Differences between treatments were compared either by *t*-test (for comparison of two means), or by one-way analysis of variance (ANOVA; Systat, Inc.) followed by a Tukey's test for multiple comparisons. The data were denoted as means ± SE (Standard Error of Mean), and analyzed using SPSS 11.0 software.

3. Results

3.1. Adult thermotolerance

Heat resistance was greatly elevated by a 4 h exposure to 32 or 35 °C (except for 10 °C). After hardenings, adult survival at 41 °C changed from 49.8% (25 °C) to 45.6% (10 °C, $t = 1.04$, $P = 0.34$), 77.8% (32 °C, $t = 8.1$, $P < 0.001$) and 93.8% (35 °C, $t = 17.3$, $P < 0.001$), respectively (Fig. 1A). However, these temperature hardenings did not improve cold tolerance (Fig. 1B). After 4 h exposures to 10, 32, and 35 °C, respectively, the adults did not show significant improvement to survive extreme cold treatment (−12 °C, 4 h) (ANOVA, $F_{3,\ 15} = 0.17$, $P = 0.91$).

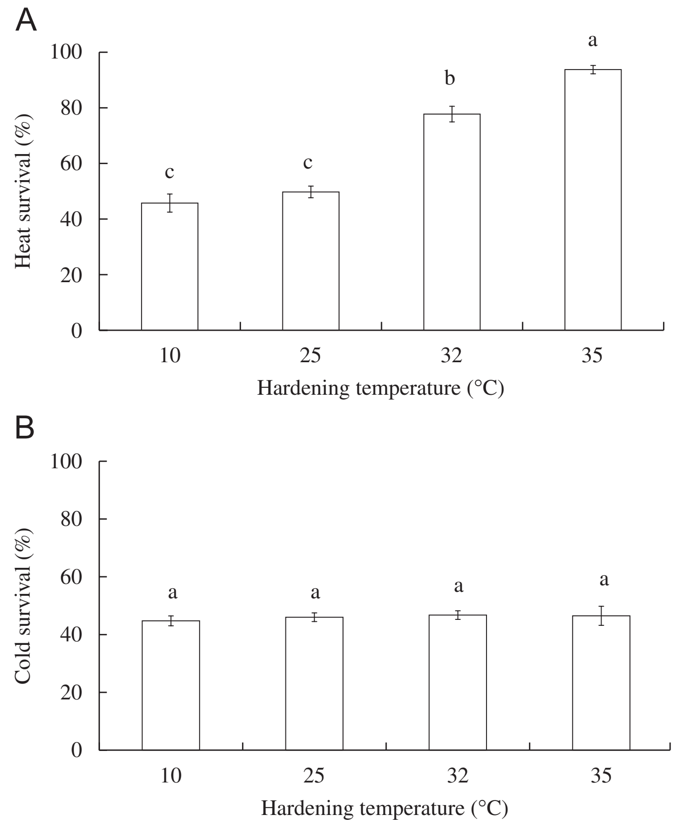

Fig. 1. Survival experiment. Adult *L. huidobrensis* were exposed for 4 h to 10, 25, 32, and 35 °C, respectively. After 1 h recovery at 25 °C, the adults were exposed to 41 °C for 1 h (A), or −12 °C for 4 h (B). Survival rate was presented as mean ± SE.

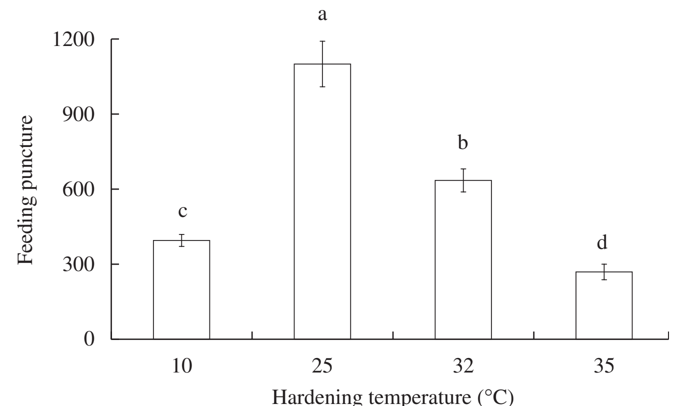

Fig. 2. Feeding punctures of adult *L. huidobrensis* at different temperature hardenings. Forty adults (sex ratio 1:1) were exposed for 4 h to 10, 25, 32, and 35 °C, respectively, and then raised on fresh leaves at 25 °C. The number of feeding punctures during their lives was summed. Columns topped by different letters had significantly different means (ANOVA, $F_{3,15} = 44.86$, $P < 0.001$).

3.2. Adult feeding

Mild temperature hardenings dramatically reduced the number of feeding punctures (Fig. 2). After 4 h hardenings at 10, 32, and 35 °C, the numbers of average feeding punctures produced by 40 adults during their lives were

395, 635, and 269, respectively, which were significantly lower than that (1100 punctures) at 25 °C (10° C, $t = 7.47$, $P < 0.001$; 32 °C, $t = 4.55$, $P = 0.004$; 35 °C, $t = 8.62$, $P < 0.001$). The 35 °C hardening had the greatest effect on adult feeding.

3.3. Adult fecundity

The amount of eggs deposited remarkably decreased during the mild temperature hardenings (ANOVA, $F_{3,15} = 55.90$, $P < 0.001$) (Fig. 3). For example, the average egg amount was 111 at 25 °C. However, it dropped to 38 ($t = 10.8$, $P < 0.001$) after 4 h exposure to 10 °C. The exposure to 35 °C reduced the amount to 4 ($t = 18.2$, $P < 0.001$), and almost stopped egg deposition.

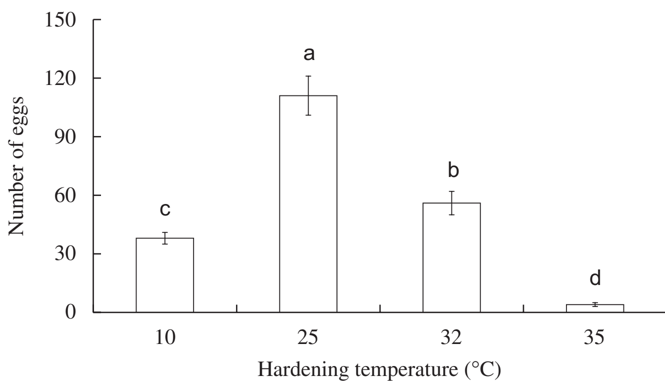

Fig. 3. Egg production by adults of *L. huidobrensis* under different temperature hardenings. An oviposition puncture was taken to indicate the presence of an egg. The number of oviposition punctures was counted by examining leaves under a stereomicroscope. Refer to Fig. 2 for the treatment method. Columns topped by different letters had significantly different means (ANOVA, $F_{3,15} = 55.90$, $P < 0.001$).

3.4. Offspring fitness

Egg hatchability, larval survival, emergence rate of pupae, and sex ratio were investigated to assess the effect of mild temperature hardening on offspring fitness. None of these elements changed, no matter how the parents were stressed by the mild temperature hardening (Fig. 4). The sex ratio of the offspring remained 1:1, and there was no significant difference among the treatments and control (ANOVA, $F_{3,15} = 0.66$, $P = 0.59$) (Fig. 4D).

3.5. Expression of five hsp genes

In order to know which kind of *hsp* is involved in the mild temperature hardening, we examined the relative levels of five *hsps*. Both *hsp70* and *hsp20* were significantly induced (Fig. 5). The relative levels of *hsp70* mRNA were 5.27, 3.92, and 3.8×10^{-2} at 10, 32, and 35 °C, respectively. These amounts were remarkably higher than that (1.49×10^{-2}) at 25 °C (10 °C, $t = 5.79$, $P = 0.004$; 32 °C, $t = 3.59$, $P = 0.023$; 35 °C, $t = 5.90$, $P = 0.004$). As compared with the control (25 °C), the relative levels of *hsp20* increased to 3.8, 3.0, and 2.6 folds at 10, 32, and 35 °C, respectively. However, the relative levels of *hsp90*, *hsp60*, and *hsp40* did not change under the temperature

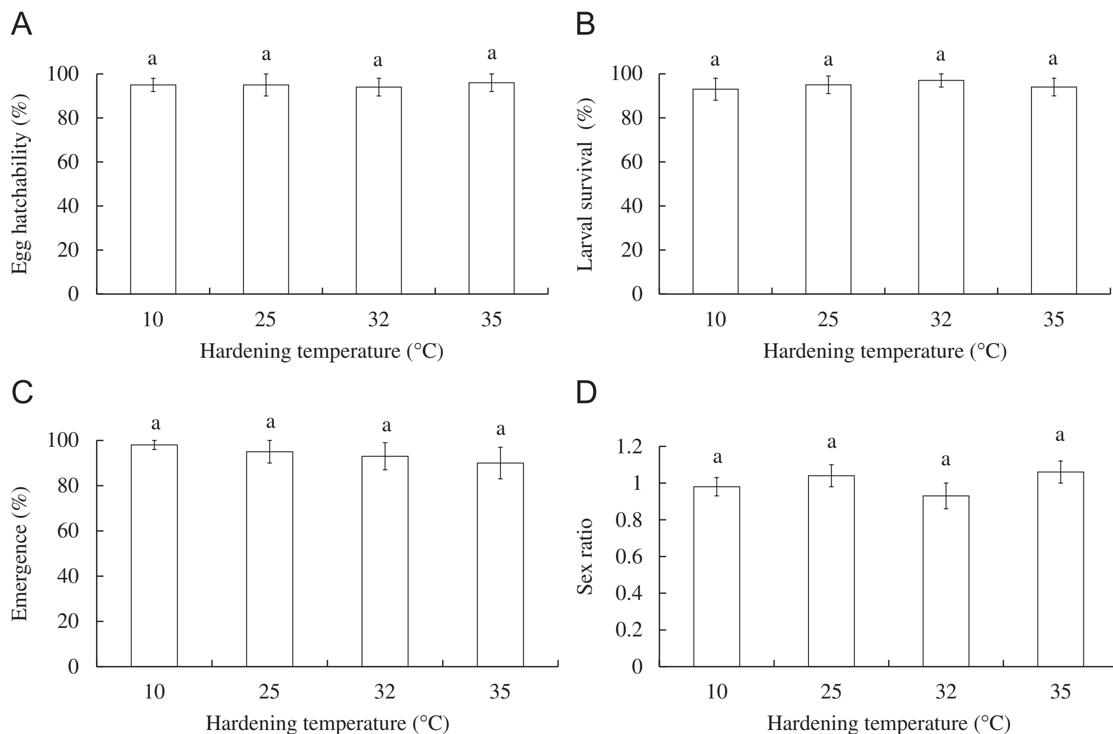

Fig. 4. Effect of mild temperature hardening on offspring fitness: egg hatchability (A), larval survival (B), emergence rate of pupae (C), and sex ratio (male:female) (D).

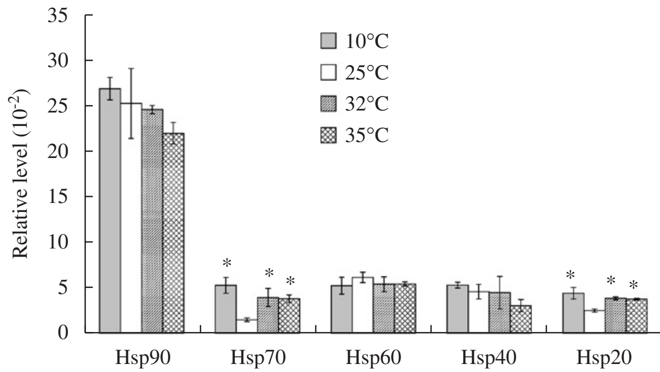

Fig. 5. Expression levels of five *hsps* under different temperature hardenings. The treatment was carried out as described for Fig. 2. The mRNA level was determined by real-time quantitative PCR. The asterisk on the columns indicates a significant difference from the controls (25 °C) ($P<0.05$).

hardenings. Former studies have shown that Hsps have a basal expression at the normal environment (Foster and Brown, 1996; Rossi et al., 2002). We found the basal mRNA levels of both *hsp70* and *hsp20* were about 1.5×10^{-2}, which were significantly lower than those of *hsp90* (28.97×10^{-2}), *hsp60* (7.03×10^{-2}), and *hsp40* (5.26×10^{-2}) (ANOVA, $F_{4,14}=26.31$, $P<0.001$).

4. Discussion

Insects are usually exposed to various stresses such as heat, cold, desiccation, and chemical poison in nature, so they have to cope with the associated effects. In some situations, one stressor can enhance tolerance to other stressors. This phenomenon of cross resistance is well known for chemical resistance. However, whether cross resistance applies to thermal acclimation is less understood (Hoffmann et al., 2003). Our results showed that mild heat hardening (4 h exposure to 32 or 35 °C) significantly increased heat resistance of adult leafminers, but it was unable to enhance cold survival. Moreover, 10 °C cold hardening could not increase heat survival. There seems to be no cross resistance in the thermotolerance of *L. huidobrensis*. This phenomenon is partially consistent with a previous study by Bubliy and Loeschcke (2005), which showed that the cold hardening was unable to increase heat resistance of *Drosophila melanogaster*, while heat hardening could elevate cold tolerance. However, in the same species Sejerkilde et al. (2003) found that heat hardening had a negative effect on cold tolerance. These results indicate a possibility of different multiple-stress-resistance mechanisms for stress resistances (Bubliy and Loeschcke, 2005). Perhaps heat and cold hardenings have a different physiological basis.

Although temperature hardening can improve thermotolerance of *L. huidobrensis*, it has a negative impact on fecundity. We found that the egg amount remarkably decreased after 4 h exposure to 10, 32, or 35 °C, and the 35 °C exposure almost completely halted egg deposition. Similar results have been reported in other insects such as *Eurosta solidaginis* (Irwin and Lee Jr., 2000), *Drosophila melanogaster* (Hercus et al., 2003), and *Diplolepis spinosa* (Wiliams et al., 2003). Although the mild temperature is non-lethal to adults, it may produce negative effects for many physiological functions, especially reproductive systems. The work on *Scathophaga stercoraria* indicated that warmer temperatures resulted in smaller ovarioles and testes (Blanckenhorn and Henseler, 2005). We also found that egg hatchability, larval survival, emergence rate of pupae, and sex ratio were not affected by treatments of their parents. This implies that the deleterious effect on fecundity may be the result of direct cessation of egg deposition during the period of stress.

Another consequence resulting from temperature hardening is to depress adult feeding. An earlier study on the leafminer *L. sativae* showed that a mild temperature (28 °C) treatment decreased the food intake of larvae (Hao and Kang, 2001). In fact, mild temperature also affects adult feeding. For instance, the number of feeding punctures remarkably declined after adults of *L. huiborensis* were stressed by mild temperature hardenings. Feeding is very important for insect reproduction. Supplementary food during the early adult stage has been reported to increase female survival and egg deposition (Hou and Sheng, 2000). On the other hand, exposure of premature adults to low food rations during vitellogenesis led to a sharp drop in mean ovary weight, and was associated with poor growth of vitellogenic oocytes (Bromley et al., 2000). These findings suggest that mild temperature hardenings can result in depressed adult feeding which may cause a subsequent impairment on fecundity.

A correlation has been shown between Hsp expression and the consequential phenotypic variations such as stress tolerance, fecundity, and longevity (Feder and Hofmann, 1999; Hoffmann et al., 2003; Vermeulen and Loeschcke, 2007). For example, a non-lethal cold hardening elevated cold tolerance of *Drosophila melanogaster* and induced Hsp70 expression (Sejerkilde et al., 2003). In this study, we found that both *hsp70* and *hsp20* were induced after mild temperature hardenings. By contrast, thermotolerance of *L. huidobrensis* was greatly improved, whereas the numbers of eggs significantly declined. This phenomenon was in agreement with the earlier study that overexpression of *hsp26* and *hsp27* enhanced *Drosophila* resistance to stress as well as induced a decline in fecundity (Wang et al., 2004). These results indicate that the overexpression of heat shock proteins highly relates to the tradeoff between thermal protection and fecundity. Tradeoffs between thermotolerance and other consequences (such as fecundity and longevity) have been widely revealed in various organisms (Hoffmann et al., 2003; Sørensen et al., 2003), but the underlying mechanisms are less understood. The synthesis of heat shock protein has been proved to be a process of energy consumption (Koehn and Bayne, 1989; Hoffmann, 1995) and usually results in a concomitant reduction in the synthesis of other proteins (Parsell and Lindquist, 1994).

Therefore, the tradeoff between thermotolerance and fecundity in *L. huidobrensis* is possibly due to a reduction of energy support, which has been consumed to synthesize stress proteins (probably Hsp70 and Hsp20) to cope with thermal stresses. In *L. huidobrensis*, the induced synthesis of Hsp70 and Hsp20 may play an important role in balancing the functional tradeoff (thermotolerance vs. fecundity) to ensure their survival at disadvantageous temperatures. The other *hsp* members (*hsp90*, *hsp60*, and *hsp40*) were not induced by mild temperature hardenings in this study. We found that their basal levels were significantly higher than both *hsp70* and *hsp20*. It appears that the *hsp* genes with lower basal expression are more easily induced. This is in accord with our previous study showing that *hsp70* and *hsp20* were more susceptive to thermal stress than the other *hsps* (Huang and Kang, 2007). One possible explanation for this phenomenon is that these *hsp* genes with high basal levels may mainly play housekeeping functions in the cell physiology of *Liriomya*.

Mild temperature hardening is one of the most frequent stresses confronting insects in nature. In most insect species, populations fluctuate with seasonal change. The population of *L. huidobrensis* reaches its climax in cool seasons, and drops greatly in summer. Low heat resistance was thought to be one of the important reasons (Chen and Kang, 2002). Our study further demonstrated that poor heat resistance would make them suffer from constant heat stress in hot weather and result in overexpression of both *hsp70* and *hsp20*. Although the expression of heat shock proteins will provide thermal protection (Gehring and Wehner, 1995), it is at the cost of reproductive impairment. This may be attributable to the population reduction of *L. huidobrensis* in summer.

Thermal selection can make organisms produce genetic variation and adaptive evolution. Leafminer populations in high latitudes are more cold tolerant than those in low latitudes (Chen and Kang, 2004). The connections between climatic parameters and stress resistance suggest that climatic selection positively contributes to the evolution of thermotolerance in insects. The inheritance of thermotolerance has been observed in *Drosophila melanogaster* (Guerra et al., 2000) and *Locusta migratoria* (Wang and Kang, 2005b). In general, the tolerance of F1 generation was approximately intermediate between their parental stocks, but with a bias towards their mother, indicating a possible maternal inheritance. Our results have shown that the short-term mild temperature hardening has no effect on offspring fitness. However, whether the long-term repeated stress in nature has produced a heritable effect on offspring needs further study.

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References

Bale, J.S., Masters, G.J., Hodkinson, I.D., Awmak, C., Bezemer, T.M., Brown, V., Butterfield, J., Buse, A., Coulson, J.C., Farrar, J., Good, J.E.G., Hartley, R., Jones, T.H., Lindroth, R.L., Press, M.C., Symrnioudis, I., Watt, A., Whittaker, J.B., 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. Global Change Biology 8, 1–16.

Blanckenhorn, W.U., Henseler, C., 2005. Temperature-dependent ovariole and testis maturation in the yellow dung fly. Entomologia Experimentalis et Applicata 116, 159–165.

Bromley, P.J., Ravier, C., Witthames, P.R., 2000. The influence of feeding regime on sexual maturation, fecundity and atresia in first-time spawning turbot. Journal of Fish Biology 56, 264–278.

Bubliy, O.A., Loeschcke, V., 2005. Correlated responses to selection for stress resistance and longevity in a laboratory population of *Drosophila melanogaster*. Journal of Evolutionary Biology 18, 789–803.

Chakir, M., Chafik, A., Moreteau, B., Gibert, P., David, J.R., 2002. Male sterility thermal thresholds in Drosophila: *D. simulans* appears more cold-adapted than its sibling *D. melanogaster*. Genetica 114, 195–205.

Chen, B., Kang, L., 2002. Cold hardiness and supercooling capability in the pea leafminer *Liriomyza huidobrensis*. CryoLetters 23, 173–182.

Chen, B., Kang, L., 2004. Variation in cold hardiness of *Liriomyza huidobrensis* (Diptera: Agromyzidae) along latitudinal gradients. Environmental Entomology 33, 155–164.

Chen, B., Kang, L., 2005. Implication of pupal cold tolerance for the northern over-wintering range limit of the leafminer *Liriomyza sativae* (Diptera: Agromyzidae) in China. Applied Entomology and Zoology 40, 437–446.

Civelek, H.S., Önder, F., 1999. Investigations on determining of natural enemies of leafminers (Diptera: Agromyzidae) in Izmir province. In: Proceedings of the Fourth Turkish Biological Control Congress, Adana, Turkey, pp. 26–29.

Cossins, A.R., Bowler, K. (Eds.), 1987. Temperature Biology of Animals. Chapman and Hall, New York, pp. 125–157.

David, J.R., Clavel, M.F., 1969. Influence de la température surle nombre, le pourcentage d' éclosion et la taille des œufs pondus par Drosophila melanogaster. Annales de la Société Entomologique de France 5, 161–171.

Duman, J.G., 2001. Antifreeae and ice nucleator proteins in terrestrial arthropods. Annual Review of Physiology 63, 327–357.

Facknath, S., 2005. Leaf age and life history variables of a leafminer: the case of *Liriomyza trifolii* on potato leaves. Entomologia Experimentalis et Applicata 115, 79–87.

Feder, M.E., Hofmann, G.E., 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annual Review of Physiology 61, 243–282.

Feder, J.H., Rossi, J.M., Solomon, J., Solomon, N., Lindquist, S., 1992. The consequences of expressing Hsp70 in Drosophila cells at normal temperatures. Genes and Development 6, 1402–1413.

Ferrando, R.E., Schuschereba, S.T., Quong, J.A., Bowman, P.D., 1995. Carbon dioxide laser induction of heat shock protein 70 synthesis: comparison with high temperature treatment. Lasers in Medical Science 10, 207–212.

Foster, J., Brown, I., 1996. Basal expression of stress-inducible Hsp70 mRNA detected in hippocampal and cortical neurons of normal rabbit brain. Brain Research 724, 73–83.

Gehring, W.J., Wehner, R., 1995. Heat shock protein synthesis and thermotolerance in Cataglyphis, an ant from the Sahara desert. Proceedings of the National Academy of Sciences of the United States of America 92, 2994–2998.

Guerra, D., Loeschcke, V., Cavicchi, S., 2000. Chromosomal and cytoplasmic analysis of heat shock resistance in natural population of *Drosophila melanogaster*. Hereditas 132, 143–149.

Hao, S.G., Kang, L., 2001. Effects of temperature and relative humidity on development, survivorship and food intake of *Liriomyza sativae*. Acta Entomological Sinica 44, 332–336.

Hercus, M.J., Loeschcke, V., Rattan, S.I.S., 2003. Life span extension of *Drosophila melanogaster* through hormesis by repeated mild stress. Biogerontology 4, 149–156.

Hoffmann, A.A., 1995. Acclimation: increasing survival at a cost. Trends in Ecology and Evolution 10, 1–2.

Hoffmann, A.A., Parsons, P.A., 1991. Evolutionary Genetics and Environmental Stress. Oxford University Press, New York.

Hoffmann, A.A., Sørensen, J.G., Loeschcke, V., 2003. Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. Journal of Thermal Biology 28, 175–216.

Hou, M.L., Sheng, C.F., 2000. Effects of adult feeding on reproduction of female cotton bollworm. Acta Ecological Sinica 20, 601–605.

Huang, L.H., Kang, L., 2007. Cloning and interspecific altered expression of heat shock protein's genes in the two leafminer species in response to thermal stress. Insect Molecular Biology, in press, doi:10.1111/j.1365-2583.2007.00744x.

Irwin, J.T., Lee Jr., R.E., 2000. Mild winter temperatures reduce survival and potential fecundity of the goldenrod gall fly, *Eurosta solidaginis* (Diptera: Tephritidae). Journal of Insect Physiology 46, 655–661.

Johnston, J.A., Ward, C.L., Kopito, R.R., 1998. Aggresomes: a cellular response to misfolded proteins. Journal of Cell Biology 143, 1883–1898.

Kang, L. (Ed.), 1996. Ecology and Sustainable Control of Serpentine Leafminers. Science Press, Beijing, 254pp.

Koehn, R.K., Bayne, B.L., 1989. Towards a physiological and genetical understanding of the energetics of the stress response. Biological Journal of the Linnean Society (London) 37, 157–171.

Krebs, R.A., Feder, M.E., 1997. Deleterious consequences of Hsp70 overexpression in *Drosophila melanogaster* larvae. Cell Stress and Chaperones 2, 60–71.

Krebs, R.A., Loeschcke, V., 1994. Costs and benefits of activation of the heat-shock response in *Drosophila melanogaster*. Functional Ecology 8, 730–737.

Krishna, P., Felsherim, R.F., Larkin, J.C., Das, A., 1992. Structure and light-induced expression of a small heat-shock protein gene of *Pharbitis nil*. Plant Physiology 100, 1772–1779.

Leibee, G.L., 1984. Influence of temperature on development and fecundity of *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) on celery. Environmental Entomology 13, 497–501.

Lindquist, L., 1986. The heat-shock response. Annual Review of Biochemistry 55, 1151–1191.

Luo, K.J., Chen, A.D., Chen, Z.Q., Miao, S., 2001. The feeding and oviposition behavior of *Liriomyza huidobrensis*. Entomological Knowledge 38, 372–373.

MacRae, T.H., 2000. Structure and function of small heat shock/α-crystallin proteins: established concepts and emerging ideas. Cellular and Molecular Life Sciences 57, 899–913.

Parsell, D.A., Lindquist, S., 1994. Heat shock proteins and stress tolerance. In: Morimoto, R.I., Tissieres, A., Georgopoulos, C. (Eds.), The Biology of Heat Shock Proteins and Molecular Chaperones. Cold Spring Harbor Laboratory Press, Plainview, NY, pp. 457–494.

Reitz, S.R., Trumble, J.T., 2002. Interspecific and intraspecific differences in two *Liriomyza* leafminer species in California. Entomologia Experimentalis et Applicata 102, 101–113.

Rossi, M.R., Somji, S., Garrett, S.H., Sens, M.A., Nath, J., Sens, D.A., 2002. Expression of Hsp27, Hsp60, hsc70, and Hsp70 stress response genes in cultured human urothelial cells (UROtsa) exposed to lethal and sublethal concentrations of sodium arsenite. Environmental Health Perspectives 110, 1225–1232.

Schnebel, E.M., Grossfield, J., 1984. Mating-temperature range in *Drosophila*. Evolution 38, 1296–1307.

Sejerkilde, M., Sørensen, J.G., Loeschcke, V., 2003. Effects of cold- and heat hardening on thermal resistance in *Drosophile melanogaster*. Journal of Insect Physiology 49, 719–726.

Silbermann, R., Tatar, M., 2000. Reproductive costs of heat shock protein in transgenic *Drosophila melanogaster*. Evolution 54, 2038–2045.

Spencer, K.A., Steyskal, G.C., 1986. Manual of the Agromyzidae (Diptera) of the United States. United States Department of Agriculture, Washington.

Storey, K.B., 1997. Organic solutes in freezing tolerance. Comparative Biochemistry and Physiology A 117, 319–326.

Sørensen, J.G., Kristensen, T.N., Loeschcke, V., 2003. The evolutionary and ecological role of heat shock proteins. Ecology Letters 6, 1025–1037.

Sæthre, M.G., Hofsvang, T., 2002. Effect of temperature on oviposition behavior, fecundity, and fertility in two Northern European populations of the codling moth (Lepidoptera: Tortricidae). Environmental Entomology 31, 804–815.

Taylor, R.P., Benjamin, I.J., 2005. Small heat shock proteins: a new classification scheme in mammals. Journal of Molecular and Cellular Cardiology 38, 433–444.

Vermeulen, C.J., Loeschcke, V., 2007. Longevity and the stress response in *Drosophila*. Experimental Gerontology 42, 153–159.

Wang, H., Kazemi-Esfarjani, P., Benzer, S., 2004. Multiple-stress analysis for isolation of Drosophila longevity genes. Proceedings of the National Academy of Sciences of the United States of America 101, 12610–12615.

Wang, H.S., Kang, L., 2005a. Effect of cooling rates on the cold hardiness and cryoprotectant profiles of locust eggs. Cryobiology 51, 220–229.

Wang, X.H., Kang, L., 2005b. Differences in egg thermotolerance between tropical and temperate populations of the migratory locust *Locusta migratoria* (Orthoptera: Acridiidae). Journal of Insect Physiology 51, 1277–1285.

Wiliams, J.B., Shorthouse, J.D., Lee Jr., R.E., 2003. Deleterious effects of mild simulated overwintering temperatures on survival and potential fecundity of rose-galling Diplolepis wasps (Hymenoptera: Cynipidae). Journal of Experimental Zoology Part A: Comparative Experimental Biology 298, 23–31.

Worner, S.P., 1998. Ecoclimatic assessment of potential establishment of exotic pests. Journal of Economic Entomology 81, 973–983.